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1

SOLICITUD DE



Patente de Invención



Patente de Modelo de Utilidad



Capítulo I



Capítulo II

2

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sociedad constituida y existente bajo las leyes de Canadá

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IDENTIFICACION

C C



NIT



C E



Otro
Cual



Número

3

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O APODERADO

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6

Título (54) INMUNIZACION DE GANADO LECHERO CON PROTEINA GapC CONTRA LA INFECCION DEL ESTREPTOCOCOS

7

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8

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Anexos

Comprobante de pago de la tasa de presentación de la solicitud por \$ 378 000 oo



Comprobante de pago por reivindicación de prioridad



Documento que acredita la existencia y representación legal cuando el solicitante sea persona jurídica



Poderes si fuere el caso



Documento de cesión del inventor al solicitante o a su causante



Declaraciones



Copia de la solicitud internacional (Resumen descripción reivindicaciones dibujos)



Traducción de la solicitud internacional al castellano (Resumen descripción reivindicaciones dibujos)



Copia del examen preliminar efectuado por la IPEA.



Traducción del examen preliminar efectuado por la IPEA



De ser el caso copia del contrato de acceso



De ser el caso documento que acredite la licencia o autorización de uso de conocimientos tradicionales de las comunidades indígenas



De ser el caso certificado de depósito del material biológico



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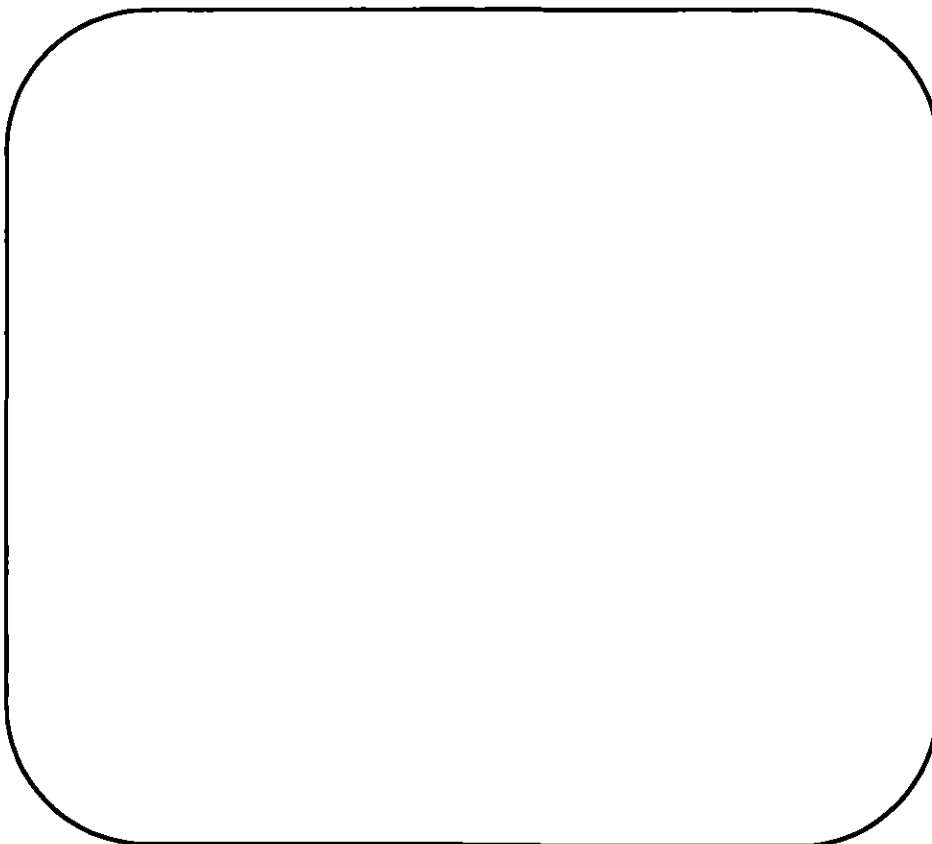


De ser el caso información sobre otras solicitudes de patente o títulos obtenidos en el extranjero por el mismo titular o su causante relacionadas parcial o totalmente con la invención de esta solicitud



Otros

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FIGURA CARACTERISTICA.

12

Solicito la concesión de la patente

NOMBRE EMILIO FERRERO

FIRMA 
C C 438 019 de Usaquén T P 31 929

JBP/mrm-ld 1



Presentation

Front

Image

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1 of 1

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(54) IMMUNIZATION OF DAIRY CATTLE WITH GapC PROTEIN AGAINST
Title STREPTOCOCCUS INFECTION

(57) Abstract

The GapC plasmin binding protein genes of *Streptococcus dysgalactiae* (*S. dysgalactiae*), *Streptococcus agalactiae* (*S. agalactiae*), *Streptococcus uberis* (*S. uberis*), *Streptococcus parauberis* (*S. parauberis*), and *Streptococcus iniae* (*S. iniae*) are described, as well as the recombinant production of the GapC proteins therefrom. Also described is the use of the GapC proteins from those species in vaccine compositions to prevent or treat bacterial infections in general, and mastitis in particular.



IMMUNIZATION OF DAIRY CATTLE WITH GapC PROTEIN AGAINST STREPTOCOCCUS INFECTION TECHNICAL FIELD

The present invention relates generally to bacterial antigens and genes encoding the same. More particularly, the present invention pertains to the cloning, expression and characterization of the GapC plasmin-binding proteins from *Streptococcus dysgalactiae*, *Streptococcus agalactiae*, *Streptococcus uberis* [STREPTOCOCCUS PARAUBERIS] and *Streptococcus* [INIAE] and the use of the same in vaccine compositions.

BACKGROUND Mastitis is an infection of the mammary gland usually caused by bacteria or fungus.

The inflammatory response following infection results in decreased milk yield as well as quality and causes major annual economic losses to the dairy industry.

Among the bacterial species most commonly associated with mastitis are various species of the genus *Streptococcus*, including *Streptococcus aureus*, *Streptococcus uberis* (untypeable), *Streptococcus agalactiae* (Lancefield group B), *Streptococcus dysgalactiae* (Lancefield group C), *Streptococcus* [ZOOEPIDEMICUS] and the Lancefield groups D, G, L and N streptococci. Some of those species are contagious (e.g., *S. agalactiae*) while others are considered environmental pathogens (e.g., *S. dysgalactiae* and *S. uberis*).

The environmental pathogen *S. [UBERIS]* is responsible for about 20% of all clinical cases of mastitis (Bramley A. J. and Dodd F. H. (1984) J. [DAIRYRES] 51: 481-512; Bramley A. J. (1987) Animal Health Nutrition 42: 12-16; Watts J. L. (1988) J. Dairy Sci. 71: 1616-1624). It is the predominant organism isolated from mammary glands during the non-lactating period (Bramley A. J. (1984) Br. Vet. J. 140: 328-335; Bramley and Dodd (1984) J. Dairy Res. 51: 481-512; Oliver S. P. (1988) Am. J. Vet. Res. 49: 1789-1793).

Mastitis resulting from infection with *S. uberis* is commonly [SUBCLINICAL] characterized by apparently normal milk with an increase in somatic cell counts due to the influx of leucocytes. The chemical composition of milk is changed due to suppression of secretion with the transfer of sodium chloride and bicarbonate from blood to milk, causing a shift of pH to a more alkaline level. *S. uberis* mastitis may also take the form of an acute clinical condition with obvious signs of disease such as clots or discoloration of the milk and swelling or hardness of the mammary gland. Some cases of the clinical disease can be severe and pyrexia may be present. For a review of the clinical manifestations [OF *S. UBERIS*] mastitis, see Bramley (1991) Mastitis: physiology or pathology, p. 3-9. In C. Burvenich, G. Vandeputte-van Messom and A. W. Hill (ed.), New insights into the pathogenesis of [MASTITIS RIJKSUNIVERSITEIT] Gent, Belgium, and Schalm et al. (1971) The mastitis complex-A brief summary, p. 1-3. In Bovine Mastitis, Lea & Febiger, Philadelphia. Conventional antibacterial control methods such as teat dipping and antibiotic therapy are effective in the control of many types of contagious mastitis, but the environmental organisms typically found in all dairy barns are often resistant to such measures. Vaccination is therefore an attractive strategy to prevent infections of the mammary glands and has been shown to be beneficial in the case of some contagious mastitis pathogens.

The literature is limited regarding vaccination studies with *S. dysgalactiae* and [*S. uberis*] and variable results have been observed. In some cases, immunization has resulted in increased sensitivity to the specific organism and in other cases, strain-specific protection has been obtained.

For example, previous studies have shown that primary infection with *S. [UBERIS]* can considerably reduce the rate of infection following a second challenge with the same strain (Hill A. W. (1988) Res. Vet. Sci. 44: 386-387). Local vaccination with killed *S. [UBERIS]* protects the bovine mammary gland against intramammary challenge with the homologous strain (Finch et al. (1994) Infect. Immun. 62: 3599-3603). Similarly, subcutaneous vaccination with live *S. [UBERIS]* has been shown to cause a dramatic modification of the pathogenesis of mastitis with the same strain (Hill et al. (1994) [FEMS IMMUNOL. MED.]

Microbiol. 8: 109-118). Animals vaccinated in this way shed fewer bacteria in their milk and many quarters remain free of infection. Nonetheless, vaccination with live or attenuated bacteria can pose

risks to the recipient Further it is clear that conventional killed vaccines are in general largely ineffective against S [UBERIS] and S agalactiae either due to lack of protective antigens on in vitro-grown cells or masking of these antigens by molecular mimicry

The current lack of existing mastitis vaccines against [S] agalactiae or the contagious streptococcus strains is due at least in part to a lack of knowledge regarding the virulence determinants and protective antigens produced by those organisms which are involved in invasion and protection of the mammary gland (Collins et al (1988) [J] Dairy Res 55 25-32 Leigh et al (1990) Res Vet Sci 49 85-87 Marshall et al (1986) J Dairy Res 53 507- 514) S dysgalactiae is known to bind several extracellular and plasma-derived proteins such as fibronectin fibrinogen collagen alpha-II-macroglobulin IgG albumin and other compounds The organism also produces hyaluronidase and fibrinolysin and is capable of adhering to and invading bovine mammary epithelial cells However the exact roles of the bacterial components responsible for these phenotypes in pathogenesis is not known

Similarly the pathogenesis of S uberis infection is poorly understood

Furthermore the influence of S [UBERIS] virulence factors on host defense mechanisms and mammary gland physiology is not well defined Known virulence factors associated with S [UBERIS] include a hyaluronic acid capsule (Hill A W (1988) Res [VET SCI] 45 400-404) hyaluronidase [(SCHAUFUSS] et al (1989) [ZENTRALBL] Bakteriologie Ser [A] 271 46-53) [R-LIKE] protein (Groschup M H and Timoney J F (1993) Res Vet Sci 54 124-126) and a cohemolysin the CAMP factor also known as UBERIS factor (Skalka B and Smola J [(1981) ZENTRALBL BAKTERIOLOGIE SER A] 249 190-194) R-like protein plasminogen activator and CAMP factor However very little is known of their roles in pathogenicity

The use of virulence determinants from Streptococcus as immunogenic agents has been proposed For example the CAMP factor of [S UBERIS] has been shown to protect vertebrate subjects from infection by that organism (Jiang U S Patent No 5 863 543)

The [Y] antigen of the group B Streptococci strain A909 (ATCC No 27591) is a component of the c protein marker complex which additionally comprises an a and (3 subunit (Boyle U S Patent No 5 721 339) Subsets [OF SEROTYPE IA II] and virtually all serotype Ib cells of group B streptococci have been reported to express components of the c protein Use of the subunit as an immunogenic agent against infections by Lancefield Group B Streptococcus infection has been proposed However its use to prevent or treat bacterial infections in animals including mastitis in cattle has not been studied

The group A streptococcal M protein is considered to be one of the major virulence factors of this organism by virtue of its ability to impede attack by human phagocytes (Lancefield R C (1962) J Immunol 89 307-313) The bacteria persist in the infected tissue until antibodies are produced against the M molecule Type-specific antibodies to the M protein are able to reverse the antiphagocytic effect of the molecule and allow efficient clearance of the invading organism

M proteins are one of the key virulence factors [OF STREPTOCOCCUS PYOGENES] due to their involvement in mediating resistance to phagocytosis (Kehoe M A [(1991) VACCINE] 9 797-806) and their ability to induce potentially harmful host immune responses via their [SUPERANTIGENICITY] and their capacity to induce host-cross-reactive antibody responses (Bisno A L (1991) [NEW ENGL J MED] 325 783-793 Froude et al (1989) [CURRZ] Top [MICROBIOL IMMUNOL] 145 5-26 Stollerman G H (1991) Clin [IMMUNOL IMMUNOPATHOL] 61 131-142)

However obstacles exist to using intact M proteins as vaccines The protein s opsonic epitopes are extremely type-specific resulting in narrow type-specific protection

Further some M proteins appear to contain epitopes that cross react with tissues of the immunized subject causing a harmful autoimmune response (See e g Dale J L and Beached G H (1982) J Exp Med 156 1165-1176 Dale J L and Beached G H (1985) [J] [EXP] Med 161 113-122 Baird R W Bronze M S Drabs W Hill H R Veasey L G and Dale J L (1991) [J IMMUN] 146 3132-3137 Bronze M S and Dale J L (1993) J Immun 151 2820-2828 Cunningham M W and Russell S M (1983) Infect

Immun

42 531-538)

[CHIMERIC] proteins containing three different fibronectin binding domains [(FNBDs)] derived from fibronectin binding proteins [OF S DYSALACTIAE] and [STAPHYLOCOCCUS AUREUS] have been expressed on the surface [OF STAPH CARNOSUS] cells. In the case of one of these proteins intranasal immunizations with live recombinant [STAPH] [CARNOSUS] cells expressing the chimeric protein on their surface resulted in an improved antibody response to a model immunogen present within the chimeric surface protein.

A GapC plasmin binding protein from a strain of Group A

Streptococcus has previously been identified and characterized and its use in thrombolytic therapies has been described (Boyle et al U S Patent No 5 237 050 Boyle et al U S Patent No 5 328 996)

However until now the protective capability of GapC has not been studied nor have the GapC proteins [OF STREPTOCOCCUS DYSALACTIAE STREPTOCOCCUS AGALACTIAE] Streptococcus uberis Streptococcus parauberis or Streptococcus [INIAE] been isolated or characterized SUMMARY OF THE INVENTION Accordingly the present invention provides novel Streptococcus GapC proteins and vaccine compositions

comprising the same. In one embodiment the invention is directed to an isolated GapC protein selected from the group consisting [OF]

(a) an isolated Streptococcus dysgalactiae GapC protein comprising the amino acid sequence shown at amino acid positions 1 to 336 inclusive of Figures [1A-1B] (SEQ ID NO 4) (b) an isolated Streptococcus agalactiae GapC protein comprising the amino acid sequence shown at amino acid positions 1 to 336 inclusive of Figures 2A-2B (SEQ ID NO 6) (c) an isolated Streptococcus uberis GapC protein comprising the amino acid sequence shown at amino acid positions 1 to 336 inclusive of Figures 3A-3B [(SEQ ID] NO [8)

] (d) an isolated Streptococcus parauberis GapC protein comprising the amino acid sequence shown at amino acid positions 1 to 336 inclusive of Figures 4A-4B (SEQ ID NO 10) (e) an isolated Streptococcus iniae GapC protein comprising the amino acid sequence shown at amino acid positions 1 to 336 inclusive of Figures [5A-5B] [(SEQ ID] NO 12) and [(F)] immunogenic fragments of (a) (b) (c) (d) and (e) comprising at least about 5 amino acids

In another embodiment the invention is directed to isolated polynucleotides comprising coding sequences for the above-described GapC proteins or complements thereof recombinant vectors comprising the polynucleotides host cells comprising the recombinant vectors and methods of recombinantly producing the GapC proteins

In yet other embodiments the subject invention is directed to vaccine compositions comprising a [PHARMACEUTICALLY] acceptable vehicle and a GapC protein as described above methods of producing the vaccine compositions as well as methods of treating or preventing bacterial infections in a vertebrate subject comprising administering to the subject a therapeutically effective amount of the vaccine composition. The bacterial infection is for example a streptococcus infection and may cause mastitis. The vaccine compositions may further comprise an adjuvant.

In other embodiments the invention is directed to antibodies directed against the isolated GapC proteins. The antibodies may be polyclonal or monoclonal.

In still further embodiments the invention is directed to methods of detecting Streptococcus antibodies in a biological sample comprising (a) reacting the biological sample with an isolated GapC protein under conditions which allow said Streptococcus antibodies when present in the biological sample to bind to the GapC protein to form an antibody/antigen complex and (b) detecting the presence or absence of the complex thereby detecting the presence or absence of Streptococcus antibodies in said sample.

In another embodiment the invention is directed to a method of detecting a GapC protein in a biological sample comprising (a) reacting the sample with antibodies directed against the GapC protein under conditions which allow the antibodies to bind to the GapC protein when present in the sample to form an antibody/antigen complex and (b) detecting the presence or absence of the complex thereby detecting the presence or absence of a GapC protein in the sample.

In another embodiment the invention is directed to an [IMMUNODIAGNOSTIC] test kit for detecting Streptococcus infection the test kit comprising a GapC protein or antibodies directed against a GapC protein and instructions for conducting the

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immunodiagnostic test

These and other embodiments of the subject invention will readily occur to those of skill in the art in view of the disclosure herein

BRIEF DESCRIPTION OF THE FIGURES Figures 1A-1B depict the isolated nucleotide sequences and deduced amino acid sequences of the gapC gene for *S. dysgalactiae* (SEQ ID NO 3 and SEQ ID NO 4) Figures 2A-2B depict the isolated nucleotide sequences and deduced amino acid sequences of the [GAPC] gene for [S] *agalactiae* (SEQ ID NO 5] and SEQ ID NO 6) Figures 3A-3B depict the isolated nucleotide sequences and deduced amino acid sequences of the [GAPC] gene for *S. uberis* (SEQ ID NO 7 and SEQ ID NO 8] Figures 4A-4B depict the isolated nucleotide sequences and deduced amino acid sequences of the [GAPC] gene for *S. parauberis* (SEQ ID NO 9 and SEQ ID NO 10) Figures 5A-5B depict the isolated nucleotide sequences and deduced amino acid sequences of the [GAPC] gene for *S. [INIAE]* (SEQ ID NO 11 and SEQ ID NO 12) Figures 6A-6E shows a DNA alignment created by PileUp and displayed by Pretty software (a component of the GCG Wisconsin Package version 10 provided by the SeqWeb sequence analysis package version 1.1 of the Canadian [BIOINFORMATICS] Resource) The figure depicts the isolated nucleotide sequences of the [GAPC] genes from *S. dysgalactiae* (DysGapC Check 9344) *S. agalactiae* (AgalGapC Check 2895) [S] [UBERIS] (UberGapC Check 5966) *S. [PARAUBERIS]* (PUberGapC Check 9672) and *S. [INIAE]* (IniaeGapC Check 990) The previously known sequences of *S. equisimilis* [(SEQGAPC] Check 5841) [S] [PYOGENES] (SpyGapC Check 4037) and a bovine GAPDH protein [(BOVGAPC] check 5059) are also included The length and weight parameters were the same for all sequences (1018 and 100 respectively) The parameters used in the DNA sequence comparison were as follows Plurality--200 Threshold-1 [AWEWEIGHT--1] 00 [AWEWEIGHT--1] 00 AvMisMatch-- 000 Symbol comparison [TABLE--PILEUPDNA] cmp [COMPCHECLC--6876] [GAPWEIGHT--5] [GAPLENGTHWEIGHT--L] PileUp MSF--1018 Type--N [CHECK--3804] In the figure dashes represent identical nucleotides dots represent gaps introduced by the software used to generate the alignment chart and tildes represent regions not included in the overall alignment due to differences in the length of the gene sequences Figures 7A-7B shows an amino acid sequence alignment created by PileUp and displayed by Pretty (as above) that depicts the deduced amino acid sequences of the GapC proteins from *S. dysgalactiae* (DysGapC Check [6731] *S. AGALACTIAE*] (AgalGapC Check 1229) *S. uberis* (UberGapC Check 8229) *S. parauberis* [(PUBERGAPC] Check [8889]) and *S. [INIAE]* (IniaeGapC check [8785]) The previously known sequences of *S. equisimilis* (SeqGapC Check 8252) *S. [PYOGENES]* (SpyGapC Check 6626) and a bovine GAPDH protein [(BOVGAPC] Check 8479) are also included In the figure dashes represent identical amino acid residues dots represent gaps introduced by the PileUp software and tildes represent regions not included in the overall alignment due to differences in the length of the gene sequences Figure 8 shows Kyte-Doolittle hydropathy plots (averaged over a window of 7) Emini surface probability plots [KARPLUS-SCHULZ] chain flexibility plots [JAMESON-WOLF] antigenic index plots and both Chou-Fasman and Garnier-Osguthorpe-Robson secondary structure plots for the GapC protein isolated from *S. dysgalactiae* Figure 9 shows [KYTE-DOOLITTLE] hydropathy plots (averaged over a window of 7) Emini surface probability plots [KARPLUS-SCHULZ] chain flexibility plots Jameson-Wolf antigenic index plots and both Chou-Fasman and Garnier-Osguthorpe-Robson secondary structure plots for the GapC protein isolated from [S] *agalactiae* Figure 10 shows Kyte-Doolittle hydropathy plots (averaged over a window of 7) Emini surface probability plots [KARPLUS-SCHULZ] chain flexibility plots Jameson-Wolf antigenic index plots and both Chou-Fasman and Garnier-Osguthorpe-Robson secondary structure plots for the GapC protein isolated from [S] [UBERIS] Figure 11 shows Kyte-Doolittle hydropathy plots (averaged over a window of 7) Emini surface probability plots [KARPLUS-SCHULZ] chain flexibility plots Jameson-Wolf antigenic index plots and both Chou-Fasman and [GARNIER-OSGUTHORPE-ROBSON] secondary structure plots for the GapC protein isolated from *S. [PARAUBERIS]* Figure 12 shows Kyte-Doolittle hydropathy plots (averaged over a window of 7) Emini surface probability plots [KARPLUS-SCHULZ]

chain flexibility plots Jameson-Wolf antigenic index plots and both Chou-Fasman and [GARNIER-OSGUTHORPE-ROBSON] secondary structure plots for the GapC protein isolated from *S. iniae*

Figure 13 is a diagrammatic representation of the Chou-Fasman secondary structure plots for the GapC protein isolated from [*S. dysgal*]

Figure 14 is a diagrammatic representation of the Chou-Fasman secondary structure plots for the GapC protein isolated from *S. agalactiae*

Figure 15 is a diagrammatic representation of the [CHOU-FASMAN] secondary structure plots for the GapC protein isolated from *S. [UBERIS]*

Figure 16 is a diagrammatic representation of the Chou-Fasman secondary structure plots for the GapC protein isolated from *S. [PARAUBERIS]*

Figure 17 is a diagrammatic representation of the [CHOU-FASMAN] secondary structure plots for the GapC protein isolated from *S. iniae*

Figure 18 shows the results of SDS-polyacrylamide gel electrophoresis of recombinant *S. dysgalactiae* GapC produced in [*E. COLI* DE3] Lane 1 molecular weight markers (20 5-103 kDa BioRad Emeryville CA) lane 2 soluble recombinant *S. dysgalactiae* GapC purified by [NI-NTA] affinity chromatography Numbers on the left of the figure indicate the positions of the molecular weight markers (in kDa)

Figure 19 is a histogram which compares the enzymatic activity of bovine plasmin bound to intact *S. dysgalactiae* cells bovine plasmin bound to affinity-purified recombinant *S. dysgalactiae* GapC protein intact *S. dysgalactiae* cells alone affinity-purified recombinant *S. dysgalactiae* GapC protein alone and bovine plasmin alone Activity was determined by an increase in the absorbance at [405NM] following release of paranitroanalide from the synthetic substrate [CHROMOZINE-PL] (Roche Diagnostics Laval Quebec Canada)

The data represents the mean of three individual assays

Figure 20 compares the change in the percentage of udder quarters infected with *S. dysgalactiae* over a 7 day period among three experimental groups (1) unvaccinated control animals (2) animals vaccinated with the Mig Fc binding protein and (3) animals vaccinated with GapC Infection was defined as recovery [OF > 500 CFU OF THE *S. DYSGALACTIAE*] per ml of milk secretions

Figure 21 plots the maximum number of *S. dysgalactiae* in any udder quarter against serum anti-GapC antibody titre (expressed as the reciprocal of the dilution showing activity above background levels) Serum anti-GapC titers were shown to correlate with the maximum number [OF CFU/ML] recovered from the mammary glands [(D) = 0.74 as determined using GraphPad Prism software v 2.01 from GraphPad Software Inc San Diego CA]

Figure 22 plots cumulative number of infected mammary quarters against serum antibody titer (again expressed as the reciprocal of the dilution showing activity above background levels) A strong correlation between the anti-GapC antibody serum antibody level and the total number of infected quarters was observed

Figure 23 illustrates the recovery of bacteria from GapC-immunized animals data points are plotted as mean bacterial recoveries/ml milk vs time (in days post-challenge) In the figure diamonds [(-+)] represent non-vaccinated animals squares [(-)] represent low titer animals (i.e. animals exhibiting the poorest response against GapC in terms of antibody titre) and triangles represent high titer animals (i.e. the remaining animals)

Figure 24 shows recovery of *S. dysgalactiae* from GapC-immunized animals plotted as the percent of mammary quarters infected vs time in days post-challenge In the figure the stippled bars represent non-vaccinated animals the cross-hatched bars represent low-titer animals (i.e. animals exhibiting the poorest response against GapC in terms of antibody titre) and the unshaded bars represent high-titer animals (i.e. the remaining animals)

Figure 25 depicts the observed inflammatory response to infection with *S. dysgalactiae* plotted as mean somatic cell counts (SCC) for each experimental group versus time in days post challenge In the figure diamonds [(-+)] represent unchallenged unvaccinated quarters squares [(-)] represent challenged unvaccinated animals triangles [(-)] represent challenged Mig-vaccinated animals and x's [(-X)] represent challenged GapC-vaccinated animals

Figure 26 illustrates somatic cell counts per mammary quarter on day 1 post-challenge In the figure the bar represents the mean for

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each group Squares [(- ◀-)] represent unvaccinated animals
triangles [(- ^-)] represent GapC-vaccinated animals and inverted
triangles [(- S-)] represent Mig-vaccinated animals
Figure 27 depicts the somatic cell counts of non-vaccinated
non-challenged and GapC-immunized high-titer animals (i.e. the
four animals exhibiting the highest antibody titres of the eight
animals in the particular group) for seven days post challenge
plotted as the [LOG] of the mean somatic cell count/ml milk
against time in days post challenge
Diamonds [(- + -)] represent unchallenged unvaccinated animals
squares [(- -)] represent challenged unvaccinated animals and
triangles [(- A-)] represent challenged GapC- vaccinated animals
DETAILED DESCRIPTION OF THE INVENTION The practice of the present
invention will employ unless otherwise indicated conventional
techniques of molecular biology microbiology recombinant DNA
technology and immunology which are within the skill of the art
Such techniques are explained fully in the literature See e.g.
Sambrook Fritsch & Maniatis Molecular Cloning A Laboratory
Manual Vols I II and III Second Edition (1989) Perbal B A
Practical Guide to Molecular Cloning (1984) the series Methods In
Enzymology (S
[COLOWICK] and N Kaplan eds Academic Press Inc) and Handbook
of Experimental Immunology Vols [I-IV] (D M Weir and C C
Blackwell eds 1986 Blackwell Scientific Publications)
The following amino acid abbreviations are used throughout the text
Alanine Ala (A) Arginine Arg (R) Asparagine Asn [(N)] Aspartic
acid Asp (D) Cysteine Cys (C) Glutamine Gln (Q) Glutamic acid
Glu (E) Glycine Gly (G) Histidine His (H) Isoleucine Ile [(I)]
Leucine Leu (L) Lysine Lys (K) Methionin Met (M) Phenylalanine
Phe [(F)] Proline Pro (P) Serine Ser (S) Threonine Thr (T)
Tryptophan Trp [(W)] Tyrosine Tyr [(Y)] Valine Val (V) 1
DEFINITIONS In describing the present invention the following terms
will be employed and are intended to be defined as indicated below

It must be noted that as used in this specification and the
appended claims the singular forms a an and the include plural
referents unless the content clearly dictates otherwise Thus for
example reference to a Streptococcus GapC protein includes a
mixture of two or more such proteins and the like
The terms GapC [PROTEIN"AND"GAPC] plasmin binding protein" (used
interchangeably herein) or a nucleotide sequence encoding the same
intends a protein or a nucleotide sequence respectively which is
derived from a GapC gene found in a variety of Streptococcus
species including without limitation certain strains of group A
streptococci (Lottenbery R et al (1987) Infect Immun 55
[1914-1918]) The nucleotide sequence of representative
Streptococcus [GAPC] genes and the corresponding amino acid
sequence of the GapC proteins encoded by these genes are depicted
in the Figures In particular Figures 1 through 5 depict the
isolated nucleotide sequences and isolated amino acid sequences [OF
S] dysgalactiae (SEQ ID NO 3 and SEQ ID NO 4 respectively) S
agalactiae (SEQ ID NO 5 and SEQ ID NO 6 respectively) [S]
uberis (SEQ ID NO 7 and SEQ ID NO 8 respectively) S
parauberis (SEQ ID NO 9 and SEQ ID NO 10 respectively) and
[S] [INIAE] (SEQ ID NO 11 and SEQ ID NO 12 respectively)
However a GapC protein as defined herein is not limited to the
depicted sequences as subtypes of each of these Streptococcus
species are known and variations in GapC proteins will occur between
them

Representative [GAPC] genes derived from S dysgalactiae S
[AGALACTIAE S UBERIS] and S parauberis are found in the
plasmids pET15bgapC (ATCC No) pMF521c (ATCC No) [PMF521A] (ATCC
No) [PMF521D] (ATCC No) and [PMF521E] (ATCC No) respectively
Furthermore the derived protein or nucleotide sequences need not be
physically derived from the gene described above but may be
generated in any manner including for example chemical synthesis
isolation (e.g. from S dysgalactiae) or by recombinant
production based on the information provided herein Additionally
the term intends proteins having amino acid sequences substantially
homologous (as defined below) to contiguous amino acid sequences
encoded by the genes which display immunological and/or
plasmin-binding activity
Thus the terms intend full-length as well as immunogenic
truncated and partial sequences and active analogs and precursor
forms of the proteins Also included in the term are nucleotide
fragments of the gene that include at least about 8 contiguous base

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pairs more preferably at least about 10-20 contiguous base pairs and most preferably at least about 25 to 50 or more contiguous base pairs of the gene or any integers between these values. Such fragments are useful as probes and in diagnostic methods discussed more fully below.

The terms also include those forms possessing as well as lacking a signal sequence if such is present as well as the nucleic acid sequences coding therefore. Additionally, the term intends forms of the GapC proteins which lack a membrane anchor region and nucleic acid sequences encoding proteins with such deletions. Such deletions may be desirable in systems that do not provide for secretion of the protein. Furthermore, the plasmin-binding domains of the proteins may or may not be present. Thus, for example, if the GapC plasmin-binding protein will be used to purify plasmin, the plasmin-binding domain will generally be retained. If the protein is to be used in vaccine compositions, immunogenic epitopes which may or may not include the plasmin-binding domain will be present. The terms also include proteins in neutral form or in the form of basic or acid addition salts depending on the mode of preparation. Such acid addition salts may involve free amino groups and basic salts may be formed with free carboxyls. [PHARMACEUTICALLY] acceptable basic and acid addition salts are discussed further below. In addition, the proteins may be modified by combination with other biological materials such as lipids (both those occurring naturally with the molecule or other lipids that do not destroy immunological activity) and saccharides or by side chain modification such as acetylation of amino groups, phosphorylation of hydroxyl side chains, oxidation of sulfhydryl groups, glycosylation of amino acid residues as well as other modifications of the encoded primary sequence.

The term therefore intends deletions, additions and substitutions to the sequence so long as the polypeptide functions to produce an immunological response as defined herein.

In this regard, particularly preferred substitutions will generally be conservative in nature, i.e., those substitutions that take place within a family of amino acids. For example, amino acids are generally divided into four families: (1) acidic--aspartate and glutamate; (2) basic--lysine, arginine, histidine; (3) non-polar--alanine, valine, leucine, isoleucine, proline, [PHENYLALANINE], methionine, tryptophan; and (4) uncharged polar--glycine, asparagine, glutamine, cystine, serine, threonine, tyrosine. Phenylalanine, tryptophan, and tyrosine are sometimes classified as aromatic amino acids. For example, it is reasonably predictable that an isolated replacement of leucine with isoleucine or valine or vice versa, an aspartate with a glutamate or vice versa, a threonine with a serine or vice versa, or a similar conservative replacement of an amino acid with a structurally related amino acid will not have a major effect on the biological activity. Proteins having substantially the same amino acid sequence as the reference molecule but possessing minor amino acid substitutions that do not substantially affect the [IMMUNOGENICITY] and/or plasmin-binding affinity of the protein are therefore within the definition of the reference polypeptide.

For example, the polypeptide of interest may include up to about 5-10 conservative or non-conservative amino acid substitutions or even up to about 15-25 or 20-50 conservative or non-conservative amino acid substitutions or any integer between these values so long as the desired function of the molecule remains intact.

In this regard, GapC proteins isolated from streptococci exhibit several variable regions in their amino acid sequences located at amino acid positions 62 to 81, 102 to 112, 165 to 172, 248 to 271 and 286 to 305. These regions, which in [S] dysgalactiae, S [AGALACTIAE], S UBERIS, S PARAUBERIS and S INIAE exhibit from 1 to 9 amino acid substitutions, are likely to be amenable to variation without substantially affecting immunogenic or enzymatic function.

Similarly, substitutions occurring in the transmembrane binding domain, if present, and the signal sequence, if present, normally will not affect [IMMUNOGENICITY]. One of skill in the art may readily determine other regions of the molecule of interest that can tolerate change by reference to the protein structure data shown in Figures [8-17] herein.

The term streptococcal GapC protein intends a GapC plasmin-binding protein as defined above, derived from a streptococcal species that produces the same, including but not limited to S dysgalactiae, S agalactiae, S uberis, S parauberis and S iniae. For example,

[A S] dysgalactiae GapC protein is a GapC plasmin-binding protein as defined above derived from S dysgalactiae Similarly an S agalactiae GapC protein"intends a [GAPC] binding protein derived from S agalactiae

Wild type or native"proteins or polypeptides refer to proteins or polypeptides isolated from the source in which the proteins naturally [OCCUR RECOMBINANT POLYPEPTIDES] refer to polypeptides produced by recombinant DNA techniques i e produced from cells transformed by an exogenous DNA construct encoding the desired polypeptide Synthetic polypeptides are those prepared by chemical synthesis

An isolated protein or polypeptide is a protein or polypeptide molecule separate and discrete from the whole organism with which the molecule is found in nature or a protein or polypeptide devoid in whole or part of sequences normally associated with it in nature or a sequence as it exists in nature but having heterologous sequences (as defined below) in association therewith The [TERM"FUNCTIONALLY] equivalent intends that the amino acid sequence of a GapC plasmin-binding protein is one that will elicit a substantially equivalent or enhanced immunological response as defined above as compared to the response elicited by a GapC plasmin-binding protein having identity with the reference GapC plasmin-binding protein or an immunogenic portion thereof

The term epitope"refers to the site on an antigen or hapten to which specific B cells and/or T cells respond The term is also used interchangeably with antigenic determinant or antigenic determinant site Antibodies that recognize the same epitope can be identified in a simple immunoassay showing the ability of one antibody to block the binding of another antibody to a target antigen

The terms"immunogenic protein or polypeptide refer to an amino acid sequence which elicits an immunological response as described above An immunogenic protein or polypeptide as used herein includes the full-length sequence of the GapC plasmin-binding protein in question with or without the signal sequence membrane anchor domain and/or plasmin-binding domain analogs thereof or immunogenic fragments thereof By immunogenic fragment is meant a fragment of a GapC plasmin-binding protein which includes one or more epitopes and thus elicits the immunological response described above

Such fragments can be identified using any number of epitope mapping techniques well known in the art See e g Epitope Mapping Protocols in Methods in Molecular Biology Vol 66 (Glenn E Morris Ed 1996) Humana Press Totowa New Jersey For example linear epitopes may be determined by e g concurrently synthesizing large numbers of peptides on solid supports the peptides corresponding to portions of the protein molecule and reacting the peptides with antibodies while the peptides are still attached to the supports Such techniques are known in the art and described in e g U S Patent No

4 708 871 Geysen et al (1984) [PROC] Natl Acad Sci USA 81 [3998-4002] Geysen et al (1986) [MOLEC] Immunol 23 709-715 Similarly [CONFORMATIONAL] epitopes are readily identified by determining spatial conformation of amino acids such as by e g x-ray crystallography and 2-dimensional nuclear magnetic resonance See e g Epitope Mapping Protocols supra Antigenic regions of proteins can also be identified using standard antigenicity and hydropathy plots such as those calculated using e g the Omega version 1 0 software program available from the Oxford Molecular Group This computer program employs the Hopp/Woods method Hopp et al Proc [NATL] Acad Sci USA (1981) 78 [3824-] 3828 for determining antigenicity profiles and the Kyte-Doolittle technique Kyte et [AL] J Mol Biol (1982) 157 105-132 for hydropathy plots Figures 8 to 12 herein depict Kyte- Doolittle profiles for representative proteins encompassed by the invention

[IMMUNE GENIE] fragments for purposes of the present invention will usually include at least about 3 amino acids preferably at least about 5 amino acids more preferably at least about 10-15 amino acids and most preferably 25 or more amino acids of the parent GapC plasmin-binding-binding protein molecule There is no critical upper limit to the length of the fragment which may comprise nearly the full-length of the protein sequence or even a fusion protein comprising two or more epitopes of GapC

An immunogenic composition"is a composition that comprises an antigenic molecule where administration of the composition to a subject results in the development in the subject of a humoral

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and/or a cellular immune response to the antigenic molecule of interest

By subunit vaccine composition is meant a composition containing at least one immunogenic polypeptide but not all antigens derived from or homologous to an antigen from a pathogen of interest. Such a composition is substantially free of intact pathogen cells or particles or the lysate of such cells or particles. Thus a "subunit vaccine composition is prepared from at least partially purified (preferably substantially purified) immunogenic polypeptides from the pathogen or recombinant analogs thereof. A subunit vaccine composition can comprise the subunit antigen or antigens of interest substantially free of other antigens or polypeptides from the pathogen.

[BY PHARMACEUTICALLY] acceptable or pharmacologically acceptable is meant a material which is not biologically or otherwise undesirable i.e. the material may be administered to an individual in a formulation or composition without causing any undesirable biological effects or interacting in a deleterious manner with any of the components of the composition in which it is contained. An immunological response to a composition or vaccine is the development in the host of a cellular and/or antibody-mediated immune response to the composition or vaccine of interest. Usually [AN IMMUNOLOGICAL] response includes but is not limited to one or more of the following effects: the production of antibodies, B cells, helper T cells, suppressor T cells, and/or cytotoxic T cells and/or [Y5] T cells directed specifically to an antigen or antigens included in the composition or vaccine of interest. Preferably the host will display either a therapeutic or protective immunological response such that resistance of the mammary gland to new infection will be enhanced and/or the clinical severity of the disease reduced. Such protection will be demonstrated by either a reduction or lack of symptoms normally displayed by an infected host and/or a quicker recovery time.

By nucleic acid [IMMUNIZATION] IS meant the introduction of a nucleic acid molecule encoding one or more selected antigens into a host cell for the in vivo expression of an antigen, antigens, an epitope, or epitopes. The nucleic acid molecule can be introduced directly into a recipient subject such as by injection, inhalation, oral, intranasal and mucosal administration or the like, or can be introduced ex vivo into cells which have been removed from the host. In the latter case, the transformed cells are reintroduced into the subject where an immune response can be mounted against the antigen encoded by the nucleic acid molecule.

The term treatment as used herein refers to either (1) the prevention of infection or reinfection (prophylaxis) or (2) the reduction or elimination of symptoms of the disease of interest (therapy).

By mastitis is meant an inflammation of the mammary gland in mammals including in cows, ewes, goats, sows, mares, and the like caused by the presence of S. [UBERIS]. The infection manifests itself by the infiltration of phagocytic cells in the gland. Generally 4 clinical types of mastitis are recognized: (1) peracute associated with swelling, heat, pain, and abnormal secretion in the gland and accompanied by fever and other signs of systemic disturbance such as marked depression, rapid weak pulse, sunken eyes, weakness, and complete anorexia; (2) acute with changes in the gland similar to those above but where fever, anorexia, and depression are slight to moderate; (3) subacute where no systemic changes are displayed and the changes in the gland and its secretion are less marked; and (4) subclinical where the inflammatory reaction is detectable only by standard tests for mastitis. Standard tests for the detection of mastitis include but are not limited to the California Mastitis Test, the Wisconsin Mastitis Test, the Nagase test, the electronic cell count, and somatic cell counts used to detect a persistently high white blood cell content in milk. In general, a somatic cell count of about 300,000 to about 500,000 cells per ml or higher in milk will indicate the presence of infection. Thus a vaccine is considered effective in the treatment and/or prevention of mastitis when, for example, the somatic cell count in milk is retained below about 500,000 cells per ml. For a discussion of mastitis and the diagnosis thereof, see e.g. The [MERCK] Veterinary Manual: A Handbook of Diagnosis, Therapy, and Disease Prevention and Control for the Veterinarian, Merck and Co., Rahway, New Jersey, 1991.

By the [TERMS VERTEBRATE SUBJECT AND VERTEBRATE] [SUBJECT ARE] meant any member of the subphylum Chordata including without

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limitation mammals such as cattle sheep pigs goats horses and humans domestic animals such as dogs and cats and birds including domestic wild and game birds such as cocks and hens including chickens turkeys and other gallinaceous birds and fish The term does not denote a particular age Thus both adult and newborn animals as well as fetuses are intended to be covered A nucleic acid molecule can include but is not limited to procaryotic sequences eucaryotic [MRNA] cDNA from eucaryotic [MRNA] genomic DNA sequences from eucaryotic (e g mammalian) DNA and even synthetic DNA sequences The term also captures sequences that include any of the known base analogs of DNA and RNA

An isolated nucleic acid molecule is a nucleic acid molecule separate and discrete from the whole organism with which the molecule is found in nature or a nucleic acid molecule devoid in whole or part of sequences normally associated with it in nature or a sequence as it exists in nature but having heterologous sequences (as defined below) in association therewith The [TERM"ISOLATED IN] the context of a polynucleotide intends that the polynucleotide is isolated from the chromosome with which it is normally associated and is isolated from the complete genomic sequence in which it normally occurs

Purified polynucleotide refers to a polynucleotide of interest or fragment thereof which is essentially free e g contains less than about 50% preferably less than about 70% and more preferably less than about 90% of the protein with which the polynucleotide is naturally associated Techniques for purifying polynucleotides of interest are well-known in the art and include for example disruption of the cell containing the polynucleotide with a chaotropic agent and separation of the polynucleotide (s) and proteins by ion-exchange chromatography affinity chromatography and sedimentation according to density

A "coding sequence" or a nucleotide sequence encoding particular protein is a nucleotide sequence which is transcribed and translated into a polypeptide in vitro or in vivo when placed under the control of appropriate regulatory elements The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus A coding sequence can include but is not limited to procaryotic sequences cDNA from eucaryotic [MRNA] genomic DNA sequences from eucaryotic (e g mammalian) DNA and even synthetic DNA sequences A transcription termination sequence will usually be located [3&APOS TO] the coding sequence A complementary sequence is one in which the nitrogenous base at a given nucleotide position is the complement of the nitrogenous base appearing at the same position in the reference sequence To illustrate the complement of adenosine is tyrosine and vice versa similarly cytosine is complementary to guanine and vice versa hence the complement of the reference sequence 5'-ATGCTGA-3' would be 5'-TACGACT-3'

A wild-type or native sequence as used herein refers to polypeptide encoding sequences that are essentially as they are found in nature e g the S dysgalactiae GapC protein encoding sequences depicted in Figures 1A-1B (SEQ ID NO 4)

"Recombinant as used herein to describe a nucleic acid molecule means a polynucleotide of genomic [cDNA] semisynthetic or synthetic origin which by virtue of its origin or manipulation (1) is not associated with all or a portion of the polynucleotide with which it is associated in nature and/or (2) is linked to a polynucleotide other than that to which it is linked in nature The term recombinant as used with respect to a protein or polypeptide means a polypeptide produced by expression of a recombinant polynucleotide

Recombinant host cells "host cells "cells "cell lines cell cultures and other such terms denoting procaryotic microorganisms or eucaryotic cell lines cultured as unicellular entities are used interchangeably and refer to cells which can be or have been used as recipients for recombinant vectors or other transfer DNA and include the progeny of the original cell which has been transfected It is understood that the progeny of a single parental cell may not necessarily be completely identical in morphology or in genomic or total DNA complement to the original parent due to accidental or deliberate mutation

Progeny of the parental cell which are sufficiently similar to the parent to be characterized by the relevant property such as the presence of a nucleotide sequence encoding a desired peptide are included in the progeny intended by this definition and are covered

by the above terms

'Homology refers to the percent identity between two polynucleotide or two polypeptide moieties. Two DNA or two polypeptide sequences are substantially homologous to each other when the sequences exhibit at least about 80%-85% preferably at least about 90% and most preferably at least about 95%-98% sequence identity over a defined length of the molecules. As used herein substantially homologous also refers to sequences showing complete identity to the specified DNA or polypeptide sequence.

In general identity refers to an exact nucleotide-to-nucleotide or amino acid-to-amino acid correspondence of two polynucleotides or polypeptide sequences respectively.

Percent identity can be determined by a direct comparison of the sequence information between two molecules by aligning the sequences counting the exact number of matches between the two aligned sequences dividing by the length of the shorter sequence and multiplying the result by 100. Readily available computer programs can be used to aid in the analysis such as ALIGN Dayhoff M O in Atlas of Protein Sequence and Structure M O Dayhoff ed 5 Suppl 3 353-358 National Biomedical Research Foundation Washington DC which adapts the local homology algorithm of Smith and Waterman [(1981)] Advances [IN APPL MATH 2] 482-489 for peptide analysis. Programs for determining nucleotide sequence identity are available in the Wisconsin Sequence Analysis Package Version 8 (available from Genetics Computer Group Madison WI) for example the BESTFIT FASTA and GAP programs which also rely on the Smith and Waterman algorithm. These programs are readily utilized with the default parameters recommended by the manufacturer and described in the Wisconsin Sequence Analysis Package referred to above. For example percent identity of a particular nucleotide sequence to a reference sequence can be determined using the homology algorithm of Smith and Waterman with a default scoring table and a gap penalty of six nucleotide positions.

Another method of establishing percent identity in the context of the present invention is to use the MPSRCH package of programs copyrighted by the University of Edinburgh developed by John F Collins and Shane S Sturrock and distributed by [INTELLIGENETICS] Inc (Mountain View CA). From this suite of packages the Smith-Waterman algorithm can be employed where default parameters are used for the scoring table (for example gap open penalty of 12 gap extension penalty of one and a gap of six).

From the data generated the Match value reflects sequence identity. Other suitable programs for calculating the percent identity or similarity between sequences are generally known in the art for example another alignment program is BLAST used with default parameters. For example BLASTN and BLASTP can be used using the following default parameters: genetic code = standard filter = none strand = both cutoff = 60 expect [-10] Matrix = BLOSUM62 Descriptions = 50 sequences sort by = HIGH SCORE [DATABASES =] non-redundant GenBank + EMBL + DDBJ + PDB + GenBank CDS translations + Swiss protein + Spupdate + PIR. Details of these programs can be found at the following internet address [http://www\[NCBI\] nlm gov/cgi-bin/BLAST](http://www[NCBI] nlm gov/cgi-bin/BLAST)

Alternatively homology can be determined by hybridization of polynucleotides under conditions which form stable duplexes between homologous regions followed by digestion with single-stranded-specific nuclease (s) and size determination of the digested fragments. DNA sequences that are substantially homologous can be identified in a Southern hybridization experiment under for example stringent conditions as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. See e.g. Sambrook et al supra DNA Cloning supra Nucleic Acid Hybridization supra.

By the term degenerate [VARIANT IS] intended a polynucleotide containing changes in the nucleic acid sequence thereof that encodes a polypeptide having the same amino acid sequence as the polypeptide encoded by the polynucleotide from which the degenerate variant is derived.

Techniques for determining amino acid sequence similarity are well known in the art. In general "similarity" means the exact amino acid to amino acid comparison of two or more polypeptides at the appropriate place where amino acids are identical or possess similar chemical and/or physical properties such as charge or hydrophobicity. A so-termed percent [SIMILARITY] THEN can be determined between the compared polypeptide sequences.

Techniques for determining nucleic acid and amino acid sequence

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identity also are well known in the art and include [DETERMINING] the nucleotide sequence of the [MRNA] for that gene (usually via a cDNA intermediate) and determining the amino acid sequence encoded thereby and comparing this to a second amino acid sequence. In general "identity refers to an exact nucleotide to nucleotide or amino acid to amino acid correspondence of two polynucleotides or polypeptide sequences respectively

[A HETEROLOGOUS REGION] of a DNA construct is an identifiable segment of DNA within or attached to another DNA molecule that is not found in association with the other molecule in nature. Thus when the heterologous region encodes a bacterial gene the gene will usually be flanked by DNA that does not flank the bacterial gene in the genome of the source bacteria. Another example of the heterologous coding sequence is a construct where the coding sequence itself is not found in nature (e.g. synthetic sequences having codons different from the native gene). Allelic variation or naturally occurring mutational events do not give rise to a heterologous region of DNA as used herein.

A vector is a replicon such as a plasmid, phage or cosmid to which another DNA segment may be attached so as to bring about the replication of the attached segment. A vector is capable of transferring gene sequences to target cells (e.g. bacterial plasmid vectors, viral vectors, non-viral vectors, particulate carriers and liposomes).

Typically the terms vector, construct, expression vector, gene expression vector, gene delivery vector, gene transfer vector and expression cassette all refer to an assembly which is capable of directing the expression of a sequence or gene of interest.

Thus the terms include cloning and expression vehicles as well as viral vectors.

These assemblies include a promoter which is operably linked to the sequences or gene(s) of interest. Other control elements may be present as well. The expression cassettes described herein may be contained within a plasmid construct. In addition to the components of the expression cassette the plasmid construct may also include a bacterial origin of replication, one or more selectable markers, a signal which allows the plasmid construct to exist as a single-stranded DNA (e.g. [G] a [M13] origin of replication), a multiple cloning site and [A MAMMALIAN ORIGIN] of replication (e.g. a SV40 or adenovirus origin of replication).

[DNA "CONTROL"] elements refers collectively to transcription promoters, transcription enhancer elements, transcription termination sequences, polyadenylation sequences (located 3' to the translation stop codon), sequences for optimization of initiation of translation (located 5' to the coding sequence), translation termination sequences, upstream regulatory domains, ribosome binding sites and the like which collectively provide for the transcription and translation of a coding sequence in a host cell. See e.g. McCaughan et al.

(1995) PNAS USA 92: 5431-5435; Kochetov et al. (1998) [FEBS LETTERS] 440: 351-355. Not all of these control sequences need always be present in a recombinant vector so long as the desired gene is capable of being transcribed and translated.

"Operably [LINKED] REFERS" to an arrangement of elements wherein the components so described are configured so as to perform their usual function. Thus control elements operably linked to a coding sequence are capable of effecting the expression of the coding sequence. The control elements need not be contiguous with the coding sequence so long as they function to direct the expression thereof. Thus for example intervening untranslated yet transcribed sequences can be present between a promoter and the coding sequence and the promoter can still be considered "operably [LINKED TO]" the coding sequence.

Similarly control elements compatible with expression in a subject are those which are capable of effecting the expression of the coding sequence in that subject.

A control element such as a promoter "directs" the [TRANSCRIPTION] OF a coding sequence in a cell when RNA polymerase will bind the promoter and transcribe the coding sequence into [MRNA] which is then translated into the polypeptide encoded by the coding sequence.

A "host cell" is a cell which has been transformed or is capable of transformation by an exogenous nucleic acid molecule.

A cell has [BEEN TRANSFORMED] BY exogenous DNA when such exogenous DNA has been introduced inside the cell membrane. Exogenous DNA may

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or may not be integrated (covalently linked) into chromosomal DNA making up the genome of the cell. In procaryotes and yeasts, for example, the exogenous DNA may be maintained on an episomal element such as a plasmid. With respect to eucaryotic cells, a stably transformed cell is one in which the exogenous DNA has become integrated into the chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eucaryotic cell to establish cell lines or clones comprised of a population of daughter cells containing the exogenous DNA.

As used herein, a "biological sample" refers to a sample of tissue or fluid isolated from a subject, including but not limited to, for example, blood, plasma, serum, fecal matter, urine, bone marrow, bile, spinal fluid, lymph fluid, samples of the skin, external secretions of the skin, respiratory, intestinal, and genitourinary tracts, tears, saliva, milk, blood cells, organs, biopsies, and also samples of in vitro cell culture constituents, including but not limited to conditioned media resulting from the growth of cells and tissues in culture medium, e.g., recombinant cells, and cell components.

As used herein, the terms "label" and "detectable label" refer to a molecule capable of detection, including but not limited to, radioactive isotopes, fluorescers, chemiluminescers, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors, chromophores, dyes, metal ions, metal salts, ligands (e.g., biotin or haptens), and the like.

The term "fluorescer" refers to a substance or a portion thereof which is capable of exhibiting fluorescence in the detectable range.

Particular examples of labels which may be used under the invention include fluorescein, rhodamine, dansyl, umbelliferone, Texas red, [LUMINOL], NADPH, and [A-P-GALACTOSIDASE].

2. MODES OF CARRYING OUT THE INVENTION. Before describing the present invention in detail, it is to be understood that this invention is not limited to particular formulations or process parameters, as such may of course vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only and is not intended to be limiting.

Although a number of methods and materials similar or equivalent to those described herein can be used in the practice of the present invention, the preferred materials and methods are described herein.

Central to the present invention is the discovery that the GapC protein is capable of eliciting an immune response in a vertebrate subject. In particular, the genes for the GapC protein in *S. dysgalactiae*, *S. agalactiae*, *S. uberis*, *S. parauberis*, and *S. [INIAE]* have been isolated, sequenced, and characterized, and the protein sequences for those genes have been deduced therefrom. The complete DNA sequences for those genes and the corresponding amino acid sequences are shown in Figures 1 through 5.

As described in the examples, the full-length *S. dysgalactiae* [GAPC] gene, depicted at nucleotide positions 1-1011 inclusive of Figures 1A-1B, encodes the full-length *S. dysgalactiae* GapC protein of 336 amino acids, shown as amino acids 1-336 inclusive of the same figure. *S. dysgalactiae* GapC has a predicted molecular weight of about 36 kDa.

(calculated using the Peptide Sort program of the GCG Wisconsin Package, version provided by the SeqWeb sequence analysis package, version [1] [1] of the Canadian Bioinformatics Resource).

Similarly, the gapC genes isolated from *S. agalactiae*, *S. uberis*, *S. parauberis*, and *S. [INIAE]* are depicted in Figures 2 through 5, each encoding a full-length GapC protein also of 336 amino acids, each also having a predicted molecular weight of about 36 kDa. None of the full-length sequences appear to include a signal peptide or a membrane anchor region.

Figures 6 and 7 show an alignment of DNA and amino acid sequences, respectively, showing regions of homology and variability that exist among GapC proteins from various streptococci strains. In particular, several variable regions are located at amino acid positions 62 to 81, 102 to 112, 165 to 172, 248 to 271, and 286 to 305. Such variable regions are typically more amenable to change. Hence, amino acid changes in these regions, such as substitutions, additions, and deletions, are likely tolerated. Figures 8 through 12 show plots of the following for each of the GapC proteins of the present invention: Kyte-Doolittle hydrophathy, averaged over a window of 7; surface probability according to Emini

chain flexibility according to Karplus-Schulz antigenicity index according to Jameson-Wolf secondary structure according to Garnier-Osguthorpe-Robson secondary structure according to Chou-Fasman and predicted glycosylation sites

Figures 13 through 17 show plots of secondary structure according to Chou-Fasman for each of the GapC proteins of the present invention. One of skill in the art can readily use the information presented in Figures 8 to 17 to determine immunogenic regions in the protein for use in vaccine compositions.

[S] dysgalactiae GapC plasmin-binding protein immunogenic fragments thereof or chimeric proteins including the same can be provided in subunit vaccine compositions. In addition to use in vaccine compositions, the proteins or antibodies thereto can be used as diagnostic reagents to detect the presence of infection in a vertebrate subject. Similarly, the genes encoding the proteins can be cloned and used to design probes to detect and isolate homologous genes in other bacterial strains. For example, fragments comprising at least about 15-20 nucleotides, more preferably at least about 20-50 nucleotides, and most preferably about 60-100 nucleotides, or any integer between these values, will find use in these embodiments.

The vaccine compositions of the present invention can be used to treat or prevent a wide variety of bacterial infections in vertebrate subjects. For example, vaccine compositions including GapC plasmin-binding proteins from S [DYS GALACTIAE S UBERIS S] parauberis [S] iniae and/or group B streptococci (GBS) such as S agalactiae can be used to treat streptococcal infections in vertebrate subjects that are caused by these or other species. In particular, S uberis and S agalactiae are common bacterial pathogens associated with mastitis in bovine, equine, ovine and goat species. Additionally, group B streptococci such as S agalactiae are known to cause numerous other infections in vertebrates, including septicemia, meningitis, bacteremia, impetigo, arthritis, urinary tract infections, abscesses, spontaneous abortion, etc. Hence, vaccine compositions containing streptococcal GapC plasmin-binding proteins will find use in treating and/or preventing a wide variety of streptococcal infections.

Similarly, GapC plasmin-binding proteins derived from other bacterial genera such as Staphylococcus, Mycobacterium, Escherichia, Pseudomonas, Nocardia, Pasteurella [CLOSTRIDIUM AND MYCOPLASMA] will find use for treating bacterial infections caused by species belonging to those genera. Thus, it is readily apparent that GapC plasmin-binding proteins can be used to treat and/or prevent a wide variety of bacterial infections in numerous species.

The streptococcal GapC plasmin-binding proteins of the present invention can be used in vaccine compositions either alone or in combination with other bacterial, fungal, viral or protozoal antigens. These antigens can be provided separately or even as fusion proteins comprising one or more epitopes of a GapC plasmin-binding protein fused to one or more of these antigens. For example, other immunogenic proteins from [S] uberis such as the CAMP factor, hyaluronic acid capsule, hyaluronidase, R-like protein and plasminogen activator can be administered with the GapC protein. Additionally, immunogenic proteins from other organisms involved in mastitis such as from the genera Staphylococcus [CORYNEBACTERIUM PSEUDOMONAS NOCARDIA CLOSTRIDIUM MYCOBACTERIUM MYCOPLASMA] Pasteurella, Prototheca, other streptococci [COLIFORM] bacteria as well as yeast can be administered along with the GapC plasmin-binding proteins described herein to provide a broad spectrum of protection. Thus, for example, immunogenic proteins from [STAPHYLOCOCCUS AUREUS STR AGALACTIAE STR DYS GALACTIAE STR ZOEPIDEMICUS] Corynebacterium [PYOGENES] [PSEUDOMONAS AERUGINOSA NOCARDIA ASTEROIDES CLOSTRIDIUM] [PEIFRINGENS ESCHERICHIA COLI EITEROBACTER AEROGENES AND KLEBSIELLA SPP] can be provided along with the GapC plasmin-binding proteins of the present invention. Additionally, GapC proteins from different streptococcal species may be used together in the vaccine compositions of the present invention. In this embodiment, the multiple GapC proteins may be provided as fusion proteins or as discrete antigens in the same or different vaccine compositions.

Production of GapC Plasmin-Binding Proteins The above-described plasmin-binding proteins and active fragments, analogs and chimeric proteins derived from the same can be produced by variety of methods.

Specifically, GapC plasmin-binding proteins can be isolated directly from bacteria which express the same. This is generally accomplished

by first preparing a crude extract which lacks cellular components and several extraneous proteins. The desired proteins can then be further purified from the cell lysate fraction by e.g. column chromatography, HPLC, [IMMUNOADSORBENT] techniques or other conventional methods well known in the art.

More particularly, techniques for isolating GapC plasmin-binding proteins have been described. For example, the GapC protein [OF S. PYOGENES] was purified from a crude cell extract by precipitation with ammonium sulfate, followed by two cycles of chromatography through a Mono FPLC column and single cycles through Superose 12 FPLC and TSK-phenol HPLC columns (Pancholi V. and [FISCHETTI] VA (1992) JExptl

Med 76 415-426). Another technique involves the use of a [NAD⁺-AGAROSE] affinity column to purify GapC from lysed protoplasts of S. [PYOGENES] strain 64/14 [(WINRAM) SB and Lottenberg R (1996) Microbiol 142 2311-2320).

Alternatively, the proteins can be recombinantly produced as described herein. As explained above, these recombinant products can take the form of partial protein sequences, full-length sequences, precursor forms that include signal sequences, mature forms without signals, or even fusion proteins (e.g. with an appropriate leader for the recombinant host or with another subunit antigen sequence for Streptococcus or another pathogen).

In one embodiment of the present invention, the GapC proteins are fused to a histidine tag produced by recombinant means and are then purified from a cell lysate fraction using affinity chromatography. See Example [1A-E] infra.

The GapC plasmin-binding proteins of the present invention can be isolated based on the ability of the protein products to bind plasmin using plasmin-binding assays as described below. See e.g. the method described in section F 3 of Example 1 infra. Thus, gene libraries can be constructed and the resulting clones used to transform an appropriate host cell. Colonies can be pooled and screened for clones having plasmin-binding activity.

Colonies can also be screened using polyclonal serum or monoclonal antibodies to the plasmin-binding protein.

Alternatively, once the amino acid sequences are determined, oligonucleotide probes which contain the codons for a portion of the determined amino acid sequences can be prepared and used to screen genomic or cDNA libraries for genes encoding the subject proteins. The basic strategies for preparing oligonucleotide probes and DNA libraries, as well as their screening by nucleic acid hybridization, are well known to those of ordinary skill in the art. See e.g. DNA Cloning Vol. 11 supra; Nucleic Acid Hybridization supra; Oligonucleotide Synthesis supra; Sambrook et al. supra. Once a clone from the screened library has been identified by positive hybridization, it can be confirmed by restriction enzyme analysis and DNA sequencing that the particular library insert contains GapC plasmin-binding protein gene or a [HOMOLOG] thereof. The genes can then be further isolated using standard techniques and, if desired, PCR approaches or restriction enzymes employed to delete portions of the full-length sequence.

Similarly, genes can be isolated directly from bacteria using known techniques, such as phenol extraction and the sequence further manipulated to produce any desired alterations. See e.g. Sambrook et al. supra for a description of techniques used to obtain and isolate DNA.

Alternatively, DNA sequences encoding the proteins of interest can be prepared synthetically rather than cloned. The DNA sequences can be designed with the appropriate codons for the particular amino acid sequence. In general, one will select preferred codons for the intended host if the sequence will be used for expression. The complete sequence is assembled from overlapping oligonucleotides prepared by standard methods and assembled into a complete coding sequence. See e.g. Edge (1981) Nature 292 756; [NAMBAIR] et al.

(1984) Science 223 1299; Jay et al. (1984) [J] Biol Chem 259 [6311].

Once coding sequences for the desired proteins have been prepared or isolated, they can be cloned into any suitable vector or replicon. Numerous cloning vectors are known to those of skill in the art, and the selection of an appropriate cloning vector is a matter of choice. Examples of recombinant DNA vectors for cloning and host cells which they can transform include the bacteriophage [K] (E. coli), pBR322 (E. coli), pACYC177 (E. coli), [PKT230] (gram-negative bacteria), [PGV1106] (gram-negative bacteria), [PLAFRL].

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(gram-negative bacteria) pME290 (non-E coli gram-negative bacteria) pHV14 (E coli and Bacillus subtilis) pBD9 (Bacillus pIJ61 [(STEPTO2YCES)] pUC6 [(STREPTOMYCES)] YIp5 [(SACCHAROMYCES)] [YCPL9 (SACCHAROMYCES)] and bovine papilloma virus (mammalian cells)
See Sambrook et al supra DNA Cloning supra B Perbal supra

The gene can be placed under the control of a promoter ribosome binding site (for bacterial expression) and optionally an operator (collectively referred to herein as "control elements") so that the DNA sequence encoding the desired protein is transcribed into RNA in the host cell transformed by a vector containing this expression construction. The coding sequence may or may not contain a signal peptide or leader sequence. If a signal sequence is included it can either be the native homologous sequence or a heterologous sequence.

For example the signal sequence for *S. dysgalactiae* GapC plasmin-binding protein can be used for secretion thereof as can a number of other signal sequences well known in the art. Leader sequences can be removed by the host in post-translational processing. See e.g. U.S. Patent Nos. 4,431,739; 4,425,437; 4,338,397.

Other regulatory sequences may also be desirable which allow for regulation of expression of the protein sequences relative to the growth of the host cell. Regulatory sequences are known to those of skill in the art and examples include those which cause the expression of a gene to be turned on or off in response to a chemical or physical stimulus including the presence of a regulatory compound. Other types of regulatory elements may also be present in the vector for example enhancer sequences. The control sequences and other regulatory sequences may be ligated to the coding sequence prior to insertion into a vector such as the cloning vectors described above.

Alternatively the coding sequence can be cloned directly into an expression vector which already contains the control sequences and an appropriate restriction site. In some cases it may be necessary to modify the coding sequence so that it may be attached to the control sequences with the appropriate orientation i.e. to maintain the proper reading frame. It may also be desirable to produce mutants or analogs of the GapC plasmin-binding protein. Mutants or analogs may be prepared by the deletion of a portion of the sequence encoding the protein by insertion of a sequence and/or by substitution of one or more nucleotides within the sequence. Techniques for modifying nucleotide sequences such as site-directed mutagenesis are described in e.g. Sambrook et al supra DNA Cloning supra Nucleic Acid Hybridization supra.

The expression vector is then used to transform an appropriate host cell. A number of mammalian cell lines are known in the art and include immortalized cell lines available from the American Type Culture Collection (ATCC) such as but not limited to Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g. Hep G2), Madin-Darby bovine kidney ["MDBK"] cells as well as others. Similarly bacterial hosts such as *E. coli*, *Bacillus subtilis* and *Streptococcus* [SPP] will find use with the present expression constructs. Yeast hosts useful in the present invention include inter alia *Saccharomyces cerevisiae*, *Candida* [ALBICANS], *Candida maltosa*, *Hansenula polymorpha*, *Kluyveromyces* [

 FRAGILIS, *KLUYVEROMYCES LACTIS PICHIA GUILLERIMONDII*, *PICHIA PASTORIS*, *SCHIZOSACCHAROMYCES*] [POMPE] and [YARROWIA LIPOLYTICA]. Insect cells for use with baculovirus expression vectors include inter alia *Aedes aegypti*, *Autographa* [CALIFORNICA], *Bombyx mori*, *Drosophila* [MELANOGASTER], [SPODOPTERA FRUGIPERDA] and *Trichoplusia ni*.

Depending on the expression system and host selected the proteins of the present invention are produced by culturing host cells transformed by an expression vector described above under conditions whereby the protein of interest is expressed. The protein is then isolated from the host cells and purified. If the expression system secretes the protein into the growth media the protein can be purified directly from the media. If the protein is not secreted it is isolated from cell lysates. The selection of the appropriate growth conditions and recovery methods are within the skill of the art.

The proteins of the present invention may also be produced by

chemical synthesis such as solid phase peptide synthesis using known amino acid sequences or amino acid sequences derived from the DNA sequence of the genes of interest. Such methods are known to those skilled in the art. See e.g. J. M. Stewart and J. D. Young, *Solid Phase Peptide Synthesis*, 2nd Ed., Pierce Chemical Co. [ROCKFORD] IL (1984) and G. Barany and R. B. Merrifield [SHE] *Peptides: Analysis, Synthesis, Biology*, editors E. Gross and J.

Meienhofer, Vol. 2, Academic Press, New York (1980), pp. 3-254 for solid phase peptide synthesis techniques, and M. Bodansky, *Principles of Peptide Synthesis*, Springer-Verlag, Berlin (1984) and E. Gross and J. Meienhofer, Eds., *The Peptides: Analysis, Synthesis, Biology*, supra, Vol. 1 for classical solution synthesis. Chemical synthesis of peptides may be preferable if a small fragment of the antigen in question is capable of raising an immunological response in the subject of interest.

The GapC plasmin-binding proteins of the present invention or their fragments can be used to produce antibodies, both polyclonal and monoclonal. If polyclonal antibodies are desired, a selected mammal (e.g. mouse, rabbit, goat, horse, etc.) is immunized with an antigen of the present invention or its fragment or a mutated antigen. Serum from the immunized animal is collected and treated according to known procedures. See e.g. Jurgens et al. (1985) [J] *Chrom.* 348: 363-370. If serum containing polyclonal antibodies is used, the polyclonal antibodies can be purified by immunoaffinity chromatography using known procedures.

Monoclonal antibodies to the GapC plasmin-binding proteins and to the fragments thereof can also be readily produced by one skilled in the art. The general methodology for making monoclonal antibodies by using hybridoma technology is well known. Immortal antibody-producing cell lines can be created by cell fusion and also by other techniques such as direct transformation of B lymphocytes with oncogenic DNA or transfection with Epstein-Barr virus. See e.g. M. Schreier et al., *Hybridoma Techniques* (1980), Hammering et al., [MONOCLONAL ANTIBODIES AND T-CELL HYBRIDOMAS] (1981) [KENNETT] et al., *Monoclonal Antibodies* (1980), see also U.S. Patent Nos. 4,341,761; 4,399,121; 4,427,783; 4,444,887; 4,452,570; 4,466,917; 4,472,500; 4,491,632; and 4,493,890. Panels of monoclonal antibodies produced against the GapC plasmin-binding proteins or fragments thereof can be screened for various properties, i.e. for isotype, epitope, affinity, etc.

Monoclonal antibodies are useful in purification using immunoaffinity techniques of the individual antigens which they are directed against. Both polyclonal and monoclonal antibodies can also be used for passive immunization or can be combined with subunit vaccine preparations to enhance the immune response. Polyclonal and monoclonal antibodies are also useful for diagnostic purposes. Vaccine Formulations and Administration: The GapC plasmin-binding proteins of the present invention can be formulated into vaccine compositions, either alone or in combination with other antigens for use in immunizing subjects as described below. Methods of preparing such formulations are described in e.g. Remington's *Pharmaceutical Sciences*, Mack Publishing Company, Easton, Pennsylvania, 18th Edition, 1990. Typically, the vaccines of the present invention are prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in or suspension in liquid vehicles prior to injection may also be prepared. The preparation may also be emulsified or the active ingredient encapsulated in liposome vehicles. The active immunogenic ingredient is generally mixed with a compatible pharmaceutical vehicle such as, for example, water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. In addition, if desired, the vehicle may contain minor amounts of auxiliary substances such as wetting or emulsifying agents and pH buffering agents.

Adjuvants which enhance the effectiveness of the vaccine may also be added to the formulation. Adjuvants may include, for example, [MURAMYL] dipeptides, avridine, aluminum hydroxide, dimethyldioctadecyl ammonium bromide (DDA), oils, oil-in-water emulsions, saponins, [CYTOKINES] and other substances known in the art.

The GapC plasmin-binding protein may be linked to a carrier in order to increase the [IMMUNOGENICITY] thereof. Suitable carriers include large, slowly metabolized macro-molecules such as proteins, including serum albumins, keyhole limpet [HEMOCYANIN], immunoglobulin molecules, thyroglobulin, ovalbumin, and other

proteins well known to those skilled in the art polysaccharides such as sepharose agarose cellulose cellulose beads and the like polymeric amino acids such as polyglutamic acid polylysine and the like amino acid copolymers and inactive virus particles The GapC plasmin-binding proteins may be used in their native form or their functional group content may be modified by for example [SUCCINYLATION] of lysine residues or reaction with Cys-thiolactone A [SULFHYDRYL] group may also be incorporated into the carrier (or antigen) by for example reaction of amino functions with [2-IMINOTHIOLANE] or the [N-HYDROXYSUCCINIMIDE] ester of 3-(4-dithiopyridyl) propionate Suitable carriers may also be modified to incorporate spacer arms (such as hexamethylene diamine or other bifunctional molecules of similar size) for attachment of peptides Other suitable carriers for the GapC plasmin-binding proteins of the present invention include VP6 polypeptides of rotaviruses or functional fragments thereof as disclosed in U S Patent No 5 071 651 Also useful is a fusion product of a viral protein and the subject immunogens made by methods disclosed in U S Patent No 4 722 840 Still other suitable carriers include cells such as lymphocytes since presentation in this form mimics the natural mode of presentation in the subject which gives rise to the immunized state Alternatively the proteins of the present invention may be coupled to erythrocytes preferably the subject's own erythrocytes Methods of coupling peptides to proteins or cells are known to those of skill in the art

Furthermore the GapC plasmin-binding proteins (or complexes thereof) may be formulated into vaccine compositions in either neutral or salt forms [PHARMACEUTICALLY] acceptable salts include the acid addition salts (formed with the free amino groups of the active polypeptides) and which are formed with inorganic acids such as for example hydrochloric or phosphoric acids or such organic acids as acetic oxalic tartaric mandelic and the like Salts formed from free carboxyl groups may also be derived from inorganic bases such as for example sodium potassium ammonium calcium or ferric hydroxides and such organic bases as isopropylamine trimethylamine 2-ethylamino ethanol histidine procaine and the like

Vaccine formulations will contain a therapeutically effective amount of the active ingredient that is an amount capable of eliciting an immune response in a subject to which the composition is administered In the treatment and prevention of mastitis for example a "therapeutically effective amount" would preferably be an amount that enhances resistance of the mammary gland to new infection and/or reduces the clinical severity of the disease Such protection will be demonstrated by either a reduction or lack of symptoms normally displayed by an infected host a quicker recovery time and/or a lowered somatic cell count in milk from the infected quarter For example the ability of the composition to retain or bring the somatic cell count (SCC) in milk below about 500 000 cells per ml the threshold value set by the International Dairy Federation above which animals are considered to have clinical mastitis will be indicative of a therapeutic effect The exact amount is readily determined by one skilled in the art using standard tests

The GapC plasmin-binding protein concentration will typically range from about [1%] to about 95% (w/w) of the composition or even higher or lower if appropriate With the present vaccine formulations 5 to [500 UG] of active ingredient per ml of injected solution should be adequate to raise an immunological response when a dose of 1 to 3 ml per animal is administered

To immunize a subject the vaccine is generally administered parenterally usually by intramuscular injection Other modes of administration however such as subcutaneous intraperitoneal and intravenous injection are also acceptable The quantity to be administered depends on the animal to be treated the capacity of the animal's immune system to synthesize antibodies and the degree of protection desired Effective dosages can be readily established by one of ordinary skill in the art through routine trials establishing dose response curves The subject is immunized by administration of the vaccine in at least one dose and preferably two doses Moreover the animal may be administered as many doses as is required to maintain a state of immunity to infection Additional vaccine formulations which are suitable for other modes of administration include suppositories and in some cases aerosol intranasal oral formulations and sustained release formulations For suppositories the vehicle composition will include traditional

binders and carriers such as polyalkaline glycols or [TRIGLYCERIDES] Such suppositories may be formed from mixtures containing the active ingredient in the range of about 0.5% to about 10% (w/w) preferably about 1% to about 2%. Oral vehicles include such normally employed excipients as for example pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, cellulose, magnesium carbonate and the like. These oral vaccine compositions may be taken in the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain from about 10% to about 95% of the active ingredient, preferably about 25% to about 70%. Intranasal formulations will usually include vehicles that neither cause irritation to the nasal mucosa nor significantly disturb ciliary function. Diluents such as water, aqueous saline or other known substances can be employed with the subject invention. The nasal formulations may also contain preservatives such as but not limited to chlorobutanol and benzalkonium chloride. A surfactant may be present to enhance absorption of the subject proteins by the nasal mucosa.

Controlled or sustained release formulations are made by incorporating the protein into carriers or vehicles such as liposomes, [NONRESORBABLE] impermeable polymers such as [ETHYLENEVINYL] acetate copolymers and [HYTREL (G)] copolymers, swellable polymers such as hydrogels or resorbable polymers such as collagen and certain polyacids or polyesters such as those used to make resorbable sutures. The GapC plasmin-binding proteins can also be delivered using implanted mini-pumps, well known in the art. The GapC plasmin-binding proteins of the instant invention can also be administered via a carrier virus which expresses the same. Carrier viruses which will find use with the instant invention include but are not limited to the vaccinia and other pox viruses, adenovirus and herpes virus. By way of example, vaccinia virus recombinants expressing the novel proteins can be constructed as follows. The DNA encoding the particular protein is first inserted into an appropriate vector so that it is adjacent to a vaccinia promoter and flanking vaccinia DNA sequences such as the sequence encoding thymidine kinase [(TK)]. This vector is then used to transfect cells which are simultaneously infected with vaccinia. Homologous recombination serves to insert the vaccinia promoter plus the gene encoding the instant protein into the viral genome. The resulting TIC recombinant can be selected by culturing the cells in the presence of 5-bromodeoxyuridine and picking viral plaques resistant thereto.

An alternative route of administration involves gene therapy or nucleic acid immunization. Thus, nucleotide sequences (and accompanying regulatory elements) encoding the subject GapC plasmin-binding proteins can be administered directly to a subject for *in vivo* translation thereof. Alternatively, gene transfer can be accomplished by transfecting the subject's cells or tissues *ex vivo* and reintroducing the transformed material into the host. DNA can be directly introduced into the host organism, i.e., by injection (see International Publication No. WO/90/11092 and Wolf et al. (1990) Science 247: 1465-1468). Liposome-mediated gene transfer can also be accomplished using known methods.

See e.g., [HAZINSKI] et al. [(1991) AM J RESPIR CELL MOL BIOL] 4: 206-209; Brigham et al. (1989) Am J Med [SCI] 298: 278-281; Canonico et al. (1991) Clin Res 39: 219A; [AND NABEL] et al. (1990) Science 249: 1285-1288. Targeting agents such as antibodies directed against surface antigens expressed on specific cell types can be [COVALENTLY] conjugated to the liposomal surface so that the nucleic acid can be delivered to specific tissues and cells susceptible to infection.

Diagnostic Assays As explained above, the GapC plasmin-binding proteins of the present invention may also be used as diagnostics to detect the presence of reactive antibodies of streptococcus, for example [S.] dysgalactiae, in a biological sample in order to determine the presence of streptococcus infection. For example, the presence of antibodies reactive with GapC plasmin-binding proteins can be detected using standard electrophoretic and [IMMUNODIAGNOSTIC] techniques including immunoassays such as competition, direct reaction or sandwich type assays. Such assays include but are not limited to Western blots, agglutination tests, enzyme-labeled and mediated immunoassays such as [ELISAS], biotin/avidin type assays, radioimmunoassays, immunoelectrophoresis, immunoprecipitation, etc. The reactions generally include revealing

labels such as fluorescent chemiluminescent radioactive enzymatic labels or dye molecules or other methods for detecting the formation of a complex between the antigen and the antibody or antibodies reacted therewith

The aforementioned assays generally involve separation of unbound antibody in a liquid phase from a solid phase support to which antigen-antibody complexes are bound

Solid supports which can be used in the practice of the invention include substrates such as nitrocellulose (e g in membrane or microtiter well form) polyvinylchloride (e g sheets or microtiter wells) polystyrene latex (e g beads or microtiter plates) polyvinylidene fluoride diazotized paper nylon membranes activated beads magnetically responsive beads and the like Typically a solid support is first reacted with a solid phase component (e g one or more GapC plasmin-binding proteins) under suitable binding conditions such that the component is sufficiently immobilized to the support Sometimes immobilization of the antigen to the support can be enhanced by first coupling the antigen to a protein with better binding properties Suitable coupling proteins include but are not limited to macromolecules such as serum albumins including bovine serum albumin (BSA) keyhole limpet hemocyanin immunoglobulin molecules thyroglobulin ovalbumin and other proteins well known to those skilled in the art Other molecules that can be used to bind the antigens to the support include polysaccharides polylactic acids polyglycolic acids polymeric amino acids amino acid copolymers and the like Such molecules and methods of coupling these molecules to the antigens are well known to those of ordinary skill in the art See e g Brinkley M A Bioconjugate Chem (1992) 3 2-13 Hashida et [AL] J [APPL]

[BIOCHEM] (1984) 6 56-63 and [ANJANEYULU] and Staros International J of Peptide and Protein Res (1987) 30 117-124

After reacting the solid support with the solid phase component any non-immobilized solid-phase components are removed from the support by washing and the support-bound component is then contacted with a biological sample suspected of containing ligand moieties (e g antibodies toward the immobilized antigens) under suitable binding conditions After washing to remove any non-bound ligand a secondary binder moiety is added under suitable binding conditions wherein the secondary binder is capable of associating selectively with the bound ligand The presence of the secondary binder can then be detected using techniques well known in the art

More particularly an ELISA method can be used wherein the wells of a microtiter plate are coated with a GapC plasmin-binding protein A biological sample containing or suspected of containing anti-GapC plasmin-binding protein immunoglobulin molecules is then added to the coated wells After a period of incubation sufficient to allow antibody binding to the immobilized antigen the plate (s) can be washed to remove unbound moieties and a detectably labeled secondary binding molecule added The secondary binding molecule is allowed to react with any captured sample antibodies the plate washed and the presence of the secondary binding molecule detected using methods well known in the art

Thus in one particular embodiment the presence of bound anti-GapC plasmin-binding antigen ligands from a biological sample can be readily detected using a secondary binder comprising an antibody directed against the antibody ligands A number of anti-bovine immunoglobulin (Ig) molecules are known in the art which can be readily conjugated to a detectable enzyme label such as horseradish peroxidase alkaline phosphatase or urease using methods known to those of skill in the art An appropriate enzyme substrate is then used to generate a detectable signal In other related embodiments competitive-type ELISA techniques can be practiced using methods known to those skilled in the art

Assays can also be conducted in solution such that the GapC plasmin-binding proteins and antibodies specific for those proteins form complexes under precipitating conditions In one particular embodiment GapC plasmin-binding proteins can be attached to a solid phase particle (e g an agarose bead or the like) using coupling techniques known in the art such as by direct chemical or indirect coupling The antigen-coated particle is then contacted under suitable binding conditions with a biological sample suspected of containing antibodies for the GapC plasmin-binding proteins Cross-linking between bound antibodies causes the formation of particle-antigen-antibody complex aggregates which can be precipitated and separated from the sample using washing and/or

centrifugation The reaction mixture can be analyzed to determine the presence or absence of antibody-antigen complexes using any of a number of standard methods such as those [IMMUNODIAGNOSTIC] methods described above

In yet a further embodiment an immunoaffinity matrix can be provided wherein a polyclonal population of antibodies from a biological sample suspected of containing anti-GapC plasmin-binding molecules is immobilized to a substrate In this regard an initial affinity purification of the sample can be carried out using immobilized antigens The resultant sample preparation will thus only contain anti-streptococcus moieties avoiding potential nonspecific binding properties in the affinity support A number of methods of immobilizing immunoglobulins (either intact or in specific fragments) at high yield and good retention of antigen binding activity are known in the art Not being limited by any particular method immobilized protein A or protein G can be used to immobilize immunoglobulins

Accordingly once the immunoglobulin molecules have been immobilized to provide an immunoaffinity matrix labeled GapC plasmin-binding proteins are contacted with the bound antibodies under suitable binding conditions After any non-specifically bound antigen has been washed from the immunoaffinity support the presence of bound antigen can be determined by assaying for label using methods known in the art

Additionally antibodies raised to the GapC plasmin-binding proteins rather than the GapC plasmin-binding proteins themselves can be used in the above-described assays in order to detect the presence of antibodies to the proteins in a given sample These assays are performed essentially as described above and are well known to those of skill in the art

The above-described assay reagents including the GapC plasmin-binding proteins or antibodies thereto can be provided in kits with suitable instructions and other necessary reagents in order to conduct immunoassays as described above The kit can also contain depending on the particular immunoassay used suitable labels and other packaged reagents and materials (i.e. wash buffers and the like) Standard immunoassays such as those described above can be conducted using these kits

Deposits of Strains Useful in Practicing the Invention A deposit of biologically pure cultures of the following strains was made with the American Type Culture Collection 10801 University Boulevard Manassas Virginia under the provisions of the Budapest Treaty The accession number indicated was assigned after successful viability testing and the requisite fees were paid The designated deposits will be maintained for a period of thirty (30) years from the date of deposit or for five (5) years after the last request for the deposit whichever is longer Should a culture become nonviable or be inadvertently destroyed or in the case of plasmid-containing strains lose its plasmid it will be replaced with a viable culture (s) of the same taxonomic description

Should there be a discrepancy between the sequence presented in the present application and the sequence of the gene of interest in the deposited plasmid due to routine sequencing errors the sequence in the deposited plasmid controls

Bacterial Plasmid Gene Deposit Date ATCC No

Strain E coli [PET15BGAPC] [GAPC] May 31 2000 PTA-1976 BL21 DE3 [(S) dysgalactiae] E coli pMF521c [GAPC] May 31 2000 PTA-1975 BL21 DE3 [(S) agalactiae] E coli pMF521a gapC May 31 2000 PTA-1973 BL21 DE3 [(S) uberis] E [COLI PMF521E GAPC] May 31 2000 PTA-1972 BL21 DE3 [(S) ini] 3

EXPERIMENTAL Below are examples of specific

embodiments for carrying out the present invention The examples are offered for illustrative purposes only and are not intended to limit the scope of the present invention in any way Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts temperatures etc.) but some experimental error and deviation should of course be allowed for

Materials and Methods Enzymes were purchased from commercial sources and used according to the manufacturers directions

In the isolation of DNA fragments except where noted all DNA manipulations were done according to standard procedures See Sambrook et al supra Restriction enzymes T4 DNA ligase [E] coli DNA polymerase [II] Klenow fragment and other biological reagents can be purchased from commercial suppliers and used according to the manufacturers' directions Double stranded DNA fragments were separated on agarose gels

Sources for chemical reagents generally include Sigma Chemical

Company St

Louis MO Alrich Milwaukee WI Roche Molecular Biochemicals
Indianapolis IN

EXAMPLE 1 Preparation Amplification [SEQUENCING] Expression
Purification and Characterization of [THE S DVS GALACTIAE GAPC]
Plasmin Binding Protein [ZALACTIAE] Chromosomal DNA A clinical [S]
dysgalactiae isolate from a case of bovine mastitis (ATCC Accession
No ATCC43078) was obtained from the American Type Culture
Collection (10801 University Boulevard Manassas VA 20110-2209)
and was used as a source of DNA The organism was routinely grown on
TSA sheep blood agar plates (PML Microbiologicals Mississauga
Ontario) at [37°] C for [18] hours or in [TODD-HEWITT] broth (Oxoid
Ltd Hampshire England) supplemented with 0.3% yeast extract
(THB-YE) at 37° C 5% CO₂

Chromosomal DNA was prepared from [S] dysgalactiae grown in 100 ml
[OF THB-YE] supplemented with 20 mM glycine for approximately 6
hours until an [AGOO] of 0.8 to 1.0 was reached Cells were
harvested and re-suspended in 50 mM EDTA 50 mM [TRIS-HCL] 0.5%
[TWEEN-20&COMMAT] (Sigma St Louis MO) and supplemented with
RNase A (200 mg/ml) proteinase K (20 mg/ml) lysozyme (100 mg/ml)
and mutanolysin (100 mg/ml) (SIGMA St Louis MO) Following
bacterial lysis for 30 minutes at 37° C with vigorous shaking
guanidine hydrochloride and [TWEEN-2&COMMAT] pH 5.5 were mixed
with the lysate to give a final concentration of 0.8 M and 5%
respectively This mixture was incubated at [50° C] for 30 minutes
The chromosomal DNA was then purified using a Qiagen genomic-tip
100g (Qiagen Santa Clarita CA) and precipitated using 0.7 volumes
of isopropanol The resulting pellet was washed in 70% ethanol and
re-suspended in 0.5 ml 10 mM [TRIS-HCL] pH 8.8

B Amplification and Cloning of the [S] [DVS GALACTIAE GAPC GENE]

The [GAPC] gene was amplified by PCR (See Mullis et al U S
Patent No 4 683 195 Mullis U S Patent No 4 683 202) The
forward primer gaps contained an NdeI restriction (SEQ ID NO
[1] shown in Table 1) and the reverse primer gapClr contained a
[BAMHI] site (SEQ ID NO 2 shown in Table 1) In the preceding
primer sequences depicted in Table 1 underlining denotes
nucleotides added to the original sequence and bolding indicates
the location of restriction endonuclease recognition sites
PCR was carried out using Vent DNA polymerase (New England Biolabs
Mississauga ON Canada) 0.7 [JUG] of S dysgalactiae chromosomal
DNA was incubated in a reaction mixture containing [L] LM] of each
of the preceding primers [200 UM] each [OF DATP] [DTP] [DCTP]
and [DGTP] [3MM MGSO₄ LX] concentration [OF THERMOPOL] buffer (New
England Biolabs Mississauga ON Canada) and 2 units Vent DNA
polymerase This mixture was incubated for 3 amplification cycles of
1 minute at [94° C] 3 minutes at [50° C] and minute 10 seconds at
[72° C] then for 27 amplification cycles at 15 seconds at [95° C] 30
seconds at [55° C] and 1 minute at [72° C] and finally for 1 cycle
of 5 minutes at [72° C]

TABLE 1 Sequence Identification Numbers and Corresponding Nucleotide
and Amino Acid Sequences SEQ ID NO Name Sequence 1 Primer gapCl
5 -GG CGG CGG CAT ATG GTA GTT AAA GTT GGT ATT AAC GG-3' 2 Primer
gapClr 5 -GC GGA TCC TTA TTT AGC GAT TTT TGC AAA GTA CTC-3 3
Streptococcus dysgalactiae gapC gene (see Figure 1) 4 Streptococcus
dysgalactiae GapC protein 5 Streptococcus agalactiae gaps gene (see
Figure 2) 6 Streptococcus agalactiae GapC protein 7 Streptococcus
uberis gapC gene (see Figure 3) Streptococcus uberis (see Figure 3)
GapC protein 9 Streptococcus parauberis gapC gene (see Figure 4) 10
Streptococcus parauberis GapC protein 11 Streptococcus iniae gapC
gene (see Figure 5) 12 Streptococcus iniae GapC protein The [GAPC
PCR] product was cloned into the expression vector [PET15B]
(Novagen Madison WI) which had been digested with [BAMHI AND
NDEI] Cloning of the PCR product into this site results in the
addition of an in-frame coding sequence for a hexahistidyl tag to
the [GAPC] coding sequence Subsequent expression yields a
full-length protein with an attached histidine tag which permits
purification of the protein under non-denaturing conditions using
metal chelate chromatography

This construct was used to transform E coli BL21 DE3 (Life
Technologies Gaithersburg MD) This transformed strain was
designated BL21 DE3 [(PET15BGAPC)] (ATCC No)

C Isolation of Chromosomal DNA and Amplification and Cloning [OF
THE ZAPC] Gene from [S] [AGALACTIAE S UBERIS S PARAUBERIS] and
S [INIAE] The [GAPC] gene were prepared from other isolates
essentially as described above

Chromosomal DNA from S [AGALACTIAE S UBERIS] and [S]

[PARAUBERIS] was isolated from strains obtained from the American Type Culture Collection [(10801) University Boulevard Manassas VA 20110-2209 strains designated ATCC 27541 9927 and (13386) respectively) Chromosomal DNA from *S. iniae* was isolated from a strain designated 9117 obtained from Mount Sinai Hospital University of Toronto

The primers used to amplify the [GAPC] genes from the *Streptococcus* strains listed above were the same as those used in the case of *S. dysgalactiae* i.e. primer [GAPCL] (SEQ ID NO 1) and primer [GAPCLR] (SEQ ID NO 2)

After amplification the PCR product in each case was cloned into pPCR-Script using the cloning protocol described in the PCR-Script Amp cloning Kit (Stratagene La Jolla CA) The PCR product insert was then excised using [NDEI] and [BA7NHI] and re-cloned into those sites in [PE15B] using conventional cloning protocols (See e.g. Sambrook et al. [SUPRA]) The plasmids containing the *S. agalactiae* *S. uberis* *S. parauberis* and *S. iniae* were designated [PMF521C] (ATCC No) [PMF521A] (ATCC No) PMF521d (ATCC No) and PMF521e (ATCC No) respectively

D Nucleotide Sequence of the [GAPC] Gene and Deduced Amino Acid Sequences Sequences homologous to [GAPC OF *S. EQUISIMILIS*] homolog (Gase et al. (1996) European [J OF BIOCHEM 239 42-51]) were originally identified while sequencing a linked but unrelated gene of *S. dysgalactiae* To obtain the complete sequence of the *S. dysgalactiae* gapC gene PCR was employed using the primers described above i.e. primer gapCL [ANDPRIMERGAPCLR]

The sequence was determined using fluorescence tag terminators on an ABI 373 DNA automatic sequencer (Applied Biosystems Emeryville CA) at the Plant Biotechnology Institute [(PBI) Saskatoon Canada] both 1 depicts the coding sequence of the gapC gene from *S. dysgalactiae* (DysGapC) (SEQ ID NO 3) and the deduced amino acid sequence (SEQ ID NO 4)

The sequences of the GapC proteins isolated from *S. agalactiae* *S. uberis* *S. parauberis* and *S. iniae* were determined by the same method

Figures 2 through 5 depict both the nucleotide sequences and the predicted amino acid sequences for the *S. dysgalactiae* GapC protein (DysgalGapC) (SEQ ID NO 3 and SEQ ID NO 4) as well as for the GapC proteins of *S. agalactiae* (AgalGapC) (SEQ ID NO 5) and SEQ ID NO 6) *S. uberis* (UberGapC) (SEQ ID NO 7 and SEQ ID NO 8) [S] [PARAUBERIS] [(PUBERGAPC)] (SEQ ID NO 9 and SEQ ID NO 10) and *S. iniae* (IniaeGapC) (SEQ ID NO 11 and SEQ ID NO 12) respectively

The *S. dysgalactiae* GapC protein gene depicted in Figures [1A-1B] codes for a 336 amino acid protein which does not appear to contain either a signal sequence or membrane anchor domain A search of the GenBank database using the BLASTX program revealed that the open reading frame was 95.5% homologous to GapC of *S. [EQUISIMILIS]* (GenBank Accession No X97788) and 99.4% homologous to GapC [OF *S. PYOGENES* (GENBANK) Accession No M95569) The predicted amino acid sequence of the GapC protein also exhibited 43% amino acid identity to bovine glyceraldehyde-3-phosphate dehydrogenase (GenBank Accession No [U85042])

Similarly for the [S] [AGALACTIAE *S. UBERIS* *S. PARAUBERIS* AND *S. INIAE*] GapC protein sequences neither signal sequences nor membrane anchor domains appear to be present

Sequence homologies are tabulated in Table 2

TABLE 2 Sequence Homologies Between Various GapC Protein Sequences
S. equisimilis *S. pyogenes* Bovine GAPDH *S. dysgalactiae* 95.5% 94.4% 43%
S. agalactiae 87.02% 91.07% *S. parauberis* 86.31% 90.77% (not determined)
S. uberis 88.39% 92.26% *S. iniae* 86.31% 89.88%
 Expression and Purification of the Recombinant [S] [D] Binding Protein The Hexahistidyl-tagged GapC protein was expressed and purified under non-denaturing conditions using metal chelate (Ni-NTA) affinity chromatography

E. coli BL21 DE3 containing the recombinant plasmid was grown in Luria Broth containing [100 UG/ML] ampicillin to an [A600 OF] approximately 0.5 Expression of the GapC protein was then induced by the addition of 1 mM isopropyl- β -D-thiogalactoside (IPTG) (Sigma St Louis MO) Following three hours incubation at [37°C] cells were harvested washed in column buffer (50 mM sodium phosphate buffer pH8.0 0.3 M [NaCl] 10 mM imidazole) and lysed by sonication

Approximately 40% of the recombinant protein was in the soluble fraction of the cell [SONICATE] with a yield of approximately 50 mg of the recombinant protein per litre of culture volume determined

with a DC Protein Assay Kit (Bio-Rad Laboratories Mississauga ON Canada) using bovine serum albumin (Pierce Rockford IL) as a standard

The lysate was cleared by centrifugation and the soluble fraction was applied to a [NI-NTA] column (Qiagen) which was subsequently washed with 10 column volumes of column buffer (as above except containing 20 mM imidazole) The [HEXAHISTIDYL-TAGGED] GapC was eluted using column buffer (as above except containing 250 mM imidazole) yielding a homogenous protein fraction having a GapC concentration of 10-15 mg/ml That fraction was dialyzed against 2000 volumes [OF PBSA] (136mM sodium chloride 2.6mM potassium chloride 8 [1mM] sodium phosphate dibasic 1.46 mM potassium phosphate monobasic)

F Expression and Purification of Recombinant GapC Protein from *S* [AGALACTIAE] *S* [UBERIS] *S* [PARAUBERIS] and *S* [INIAE] Expression and purification of the recombinant proteins from these streptococcus species is accomplished by the same methods described in Example 1E above The transformed bacterial strains used to express the *S* [AGALACTIAE] *S* [UBERIS] *S* [PARAUBERIS] AND [*S*] [INIAE] recombinant GapC proteins were designated BL21 DE3 (pMF521c) (ATCC No) BL21 DE3 [(PMF521A)] (ATCC No) BL21 DE3 [(PMF521D)] (ATCC No) and BL21 DE3 (pMF521e) (ATCC No) respectively

G Characterization of the Recombinant [*S*] [DLS ALACTIAE GUC] Protein 1 SDS-Page Analysis SDS-polyacrylamide gel electrophoresis was performed on a sample of the eluted protein using the method described by Laemmli (Laemmli U K (1970) Nature 227 680-685) The results are presented in Figure 18 In the figure lane 1 molecular weight markers (20.5 to 103 kDa range BioRad Laboratories Emeryville CA) lane 2 soluble recombinant [*S*] dysgalactiae GapC protein purified by [NI-NTA] affinity chromatography

These results demonstrate that purification by affinity chromatography on a [NI-NTA] column yielded a homogenous protein fraction

2 [GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE (GAPDH) ACTIVITY OF] recombinant GapC and [*S*] [DVSGALACTIAE WHOLE CELLS] GAPDH catalyzes the oxidative phosphorylation of D-glyceraldehyde-3-phosphate to 1,3-diphosphoglycerate in the presence of NAD and inorganic phosphate The high degree of homology of GapC to streptococcal glyceraldehyde-3-phosphate dehydrogenase suggested that GapC may exhibit this enzyme activity

The GAPDH activity of *S* dysgalactiae whole cells [(101°) [CFIJ]] and the recombinant GapC protein was determined by measuring the reduction of NAD to NADH The assay buffer was composed of 40 mM triethanolamine 50 mM Na₂ [HOP04 AN] 5 mM EDTA pH 6.8 *S* dysgalactiae cells or 5 mg of purified recombinant protein were incubated in assay buffer containing 7 ml glyceraldehyde-3-phosphate (49 [MG/ML] Sigma Chemical Company) 75 microliters [NAD+] (15 mM Sigma Chemical Company) in a final volume of 1 ml

Negative controls consisted of samples which did not contain glyceraldehyde-3-phosphate or the recombinant GapC molecule/*S* dysgalactiae cells The reduction [OF NAD+ TO] NADH was monitored spectrophotometrically at an Absorbance of 340 nanometers

The results indicated that both the recombinant protein as well as intact wild-type [*S*] dysgalactiae cells had enzymatic activity (not shown) Furthermore when *S* dysgalactiae cells were treated with Trypsin to digest surface proteins GAPDH activity disappeared Thus the enzymatic activity observed for the intact wild type cells was not due to intracellular GAPDH

This data suggests that the GapC protein is localized on the cell surface despite the apparent lack of either a signal sequence or a membrane anchoring region in either the nucleotide or amino acid

3 Plasmin-Binding Activity of Recombinant [*S*] [DESGALACTIAE] GapC Plasmin [BINDING PROTEIN AND *S* DVSGALACTIAE] whole cells

A [MICROPLATE] assay was used to determine if the recombinant GapC protein was capable of binding bovine plasmin and if so whether the bound plasmin was in an enzymatically active form

Ninety-six-well microtiter plates were coated with 5 mg of purified recombinant GapC protein washed 3 times with 0.1% gelatin-PBSA with 0.05% TWEEN-20 (PBSGT)

The wells were blocked for one hour at 37° C in the same buffer washed and incubated with 200 ml of bovine plasmin (0.25mg/ml

[BOEHRINGER] Mannheim Indianapolis IN) for 1 hr at [37° C] The wells were then washed 8 times with PBSGT 200 ml of the synthetic substrate chromazine-PL (Tos-Gly-Pro-Lys-4-NA 0.3 mg/ml) were added to the wells and incubated at 37° C for one hour The presence of

associated plasmin activity was determined by measuring the level of paranitroanilide (4-nitraniline) released into the supernatant and detected based on an Absorbance of 405 nanometers. A similar procedure was used to measure plasmin-binding activity of *S. dysgalactiae* whole cells with the exception that [101° CELLS] were washed with PBSGT and re-suspended in [400] [UL] chromazine- PL (0.3 mg/ml) and incubated for 1 hour at [37°C].

The results shown in Figure 19 demonstrate that the purified recombinant protein was capable of binding enzymatically active bovine plasmin. Likewise, when [S] *S. dysgalactiae* whole cells were utilized, similar results were obtained. In the figure, the data represents the mean of three individual assays.

Thus, the plasmin-receptor is located on the surface of *S. dysgalactiae* and the purified protein retains biological activity.

EXAMPLE 2 [IMMUNIZATION WITH *S. DYSGALACTIAE* GAPC] and experimental infection of cattle vaccines were formulated in such a fashion that they contained 50 mg/ml of affinity purified recombinant GapC in the oil-based adjuvant VSA3 (VIDO, Saskatoon, Saskatchewan, Canada). VSA3 is a combination [OF EMULSIGEN PLUS] (MVP Laboratories, Ralston, Nebraska) and Dimethyldioctadecyl ammonium bromide (Kodak, Rochester, New York). The affinity-purified recombinant GapC protein used for the vaccine preparation is shown in Figure 18.

Twenty-four non-lactating Holsteins with no history of *S. dysgalactiae* infection were obtained from various farms in Saskatchewan, Canada. One week prior to vaccination, all animals were treated with [CEPHA-DRY] (Ayerst Laboratories, Montreal, Canada) (300 mg per quarter) in order to clear any infection of the udders prior to the vaccination step.

Groups of 8 animals were immunized [SUBCUTANEOUSLY] with two doses of vaccines containing [S. DYSGALACTIAE] GapC-Mig (an Fc receptor protein isolated from *S. dysgalactiae* which was evaluated simultaneously) or a placebo with a three-week interval between immunizations. Two weeks following the second immunization, animals were exposed to 650 colony forming units of [S] *S. dysgalactiae* delivered into three quarters with an udder infusion canula. The fourth quarter on each animal served as an un-infective control. All animals were examined daily for clinical signs of disease and samples from all udder quarters were collected on each day. Samples were observed for consistency and somatic cell counts as well as bacterial numbers were determined.

EXAMPLE 3 Determination of GapC-specific Antibodies GapC-specific antibodies in bovine serum were measured using an enzyme-linked immunosorbent assay (ELISA). Briefly, microtiter plates (NUNC, Naperville, Illinois) were coated by adding 1 microgram per well purified recombinant antigen in 50mM sodium carbonate buffer, pH 9.6, and incubated overnight at [4°C]. The liquid was removed and the wells were blocked with 3% bovine serum albumin for 1 hr at [37°C]. Serial dilutions of bovine serum (from 1 in 4 to 1 in 6400) were then added to the wells and incubated for 2 hours at room temperature. The wells were aspirated, washed, and incubated with 100 µl of alkaline phosphatase-conjugated goat anti-bovine IgG (Kirkgaard & Perry Laboratories Inc., Gaithersburg, MD) for 1 hr at room temperature. The wells were washed again and 100 [U L OFF-NITROPHENOL] phosphate (SIGMA, St. Louis, MO) was added as a substrate to detect alkaline phosphatase activity. The absorbance at 405 nanometers was recorded following 1 hr incubation with the substrate at room temperature.

Where referred to in the figures, the specific antibody titer is expressed as the reciprocal of the dilution showing activity above background levels.

EXAMPLE 4 Bacterial Colonization Bacteria were enumerated by spreading serial dilutions [(10° TO 10-3)] directly onto TSA sheep blood agar plates followed by overnight incubation at [37°C] 5% CO₂.

Colonization is defined as >500 [CFU/ML] of the challenge organism recovered.

To confirm that the bacteria recovered from milk secretions were [S] *S. dysgalactiae*, selected colonies recovered from each animal were tested using an API Strep-20 test [(BIOMERIEUX), St. Louis, Missouri] according to the manufacturer's instructions. This test is a standardized method combining 20 biochemical tests for the determination of enzymatic activity and fermentation of sugars. The reactions are read according to a reading table and the identification is obtained by either referring to the analytical profile index or using identification software.

Following challenge, animals from all groups were shown to be

colonized by *S. dysgalactiae* (Figure 20) Only the GapC-immunized cows had a statistically significant reduction in the number of infected quarters and total numbers of bacteria isolated per quarter. Therefore immunization with GapC reduced bacterial colonization following challenge with *S. dysgalactiae*. The relationship between anti-GapC titer and bacterial colonization is shown in Figures 21 and 22. There was a strong correlation between anti-GapC serum antibody level and the maximum number of bacteria (expressed in [CFU] [(LOG10)/ML] milk) found in any quarter $r = 0.74$ (Figure 21) as well as the total number of infected quarters [(R = 0.74)] (Figure 22). Correlation was calculated using GraphPad Prism software version 2.01 (GraphPad Software Inc. San Diego, California).

This correlation is also illustrated in Figure 23 and 24 where the GapC-immunized group is subdivided into high titer and low titer responders. In these figures "low titer responders" refer to the four animals with the poorest response against GapC while high titer responders refer to the remainder of the group. No colonization occurred in the high titer group while even the low titer group showed reduced numbers of bacteria recovered after day 3.

EXAMPLE 5 Determination of inflammatory response. Inflammatory response was measured as a function of somatic cell count (i.e. lymphocytes, neutrophils and monocytes). Somatic cell counts were measured in a Coulter counter using standard techniques as recommended by Agriculture and Agri-Food Canada Pamphlet IDF50B (1985) [MILK AND MILKPRODUCTS--METHODS OF SAMPLING SAMPLES WERE] always read within 48 hours of collection and fixation at days 1 through 7 post challenge.

The numbers of somatic cells present in the gland was [DETERMINED] on each day post challenge. Numbers from the unchallenged quarter remained constant throughout the trial while on day 1 the GapC group was lower than the placebo-immunized group (Figure 25).

The difference between the GapC and the placebo groups was statistically significant. The individual data from day 1 is shown in Figure 26. Data for GapC-treated animals over a 7 day period post-challenge is shown in Figure 27. Samples from the quarters of GapC-immunized animals were indistinguishable from unchallenged quarters.

Therefore immunization with GapC reduced the inflammatory response following challenge with *S. dysgalactiae*.

EXAMPLE 6 Cross-protection against [*S.*] [UBERIS] infection. During the last 3 days of the vaccine trial a mixed population of bacteria were recovered from the mammary gland secretions. Further analysis with the API 20 Strep test confirmed the identity of the strains present in the mixture as *S. dysgalactiae* and [*S.*] uberis, the latter representing a natural infection which occurred during the trial. The group which was immunized with GapC appeared to be significantly cross-protected against [*S.*] [UBERIS] colonization (see Figure 20) indicating that GapC may be a broadly cross-protective antigen capable of protecting against infection by multiple Streptococcal species.

Thus vaccination with *S. dysgalactiae* GapC is therefore capable of providing cross-protection against *S. uberis* infection.

***** Thus the cloning, expression and characterization of various GapC plasmin binding proteins is disclosed as are methods of using the same. Although preferred embodiments of the subject invention have been described in some detail, it is understood that obvious variations can be made without departing from the spirit and the scope of the invention as defined by the appended claims.

Description Claims

SDOCL LA- EN >

What is claimed is: 1. An isolated GapC protein selected from the group consisting of: (a) an isolated *Streptococcus dysgalactiae* GapC protein comprising the amino acid sequence shown at amino acid positions 1 to 336 inclusive of Figure [IA-IB] (SEQ ID NO. 4); (b) an isolated *Streptococcus agalactiae* GapC protein comprising the amino acid sequence shown at amino acid positions 1 to 336 inclusive of Figures 2A-2B (SEQ ID NO. 6); (c) an isolated *Streptococcus uberis* GapC protein comprising the amino acid sequence shown at amino acid positions 1 to 336 inclusive of Figures 3A-3B (SEQ ID NO. 8); (d) an isolated *Streptococcus parauberis* GapC protein comprising the amino acid sequence shown at amino acid

positions 1 to 336 inclusive of Figures 4A-4B (SEQ ID NO 10) (e) an isolated Streptococcus [INIAE] GapC protein comprising the amino acid sequence shown at amino acid positions 1 to 336 inclusive of Figures [5A-5B] (SEQ ID NO 12) and [(F)] immunogenic fragments of (a) (b) (c) (d) and (e) comprising at least about 5 amino acids

2 The isolated GapC protein of claim 1 wherein the protein is a Streptococcus dysgalactiae GapC protein comprising the amino acid sequence shown at amino acid positions 1 to 336 inclusive of Figures [1A-1B] (SEQ ID NO 4)

3 The isolated GapC protein of claim 1 wherein the protein is a Streptococcus agalactiae GapC protein comprising the amino acid sequence shown at amino acid positions 1 to 336 inclusive of Figures 2A-2B (SEQ ID NO 6)

4 The isolated GapC protein of claim 1 wherein the protein is a Streptococcus uberis GapC protein comprising the amino acid sequence shown at amino acid positions 1 to 336 inclusive of Figures 3A-3B (SEQ ID NO [8])

5 The isolated GapC protein of claim [1] wherein the protein is a Streptococcus parauberis GapC protein comprising the amino acid sequence shown at amino acid positions 1 to 336 inclusive of Figures 4A-4B (SEQ ID NO 10)

6 The isolated GapC protein of claim [1] wherein the protein is a Streptococcus [I7ZIAE] GapC protein comprising the amino acid sequence shown at amino acid positions 1 to [336] inclusive of Figures [5A-5B] (SEQ ID NO 12)

7 An isolated polynucleotide comprising a coding sequence for an isolated GapC protein according to any of claims 1-6 or complements thereof

8 A recombinant vector comprising (a) the isolated polynucleotide of claim 7 and (b) at least one heterologous control element operably linked to said isolated polynucleotide whereby said coding sequence can be transcribed and translated in a host cell and at least one of said control elements is heterologous to said coding sequence

9 A host cell comprising the recombinant vector of claim 8

10 A method for producing a GapC protein said method comprising culturing the cells of claim 9 under conditions for producing said protein

11 A vaccine composition comprising a [PHARMACEUTICALLY] acceptable vehicle and a GapC protein wherein said GapC protein is selected from the group consisting of (a) a Streptococcus dysgalactiae GapC protein comprising the amino acid sequence shown at amino acid positions 1 to 336 inclusive of Figures [1A-1B] (SEQ ID NO 4)

(b) a Streptococcus agalactiae GapC protein comprising the amino acid sequence shown at amino acid positions 1 to 336 inclusive of Figures 2A-2B (SEQ ID NO 6) [(C)] a Streptococcus uberis GapC protein comprising the amino acid sequence shown at amino acid positions 1 to 336 inclusive of Figures 3A-3B (SEQ ID NO 8) (d) a Streptococcus parauberis GapC protein comprising the amino acid sequence shown at amino acid positions 1 to [336] inclusive of Figures 4A-4B (SEQ ID NO 10) (e) a Streptococcus iniae GapC protein comprising the amino acid sequence shown at amino acid positions [1] to [336] inclusive of Figures [5A-5B] (SEQ ID NO 12) [(F)] a Streptococcus GapC protein having at least about 70% sequence identity to (a) (b) (c) (d) and (e) and (g) immunogenic fragments of (a) (b) (c) (d) (e) and [(F)] said fragments comprising at least about 5 amino acids

12 The vaccine composition of claim 11 wherein said GapC protein comprises the amino acid sequence of the Streptococcus dysgalactiae GapC protein depicted at amino acid positions 1 to 336 inclusive of Figures [1A-1B] (SEQ ID NO 4) or an immunogenic fragment thereof said fragment comprising at least about 5 amino acids

13 The vaccine composition of claim 12 wherein said GapC protein comprises the amino acid sequence depicted at amino acid position 1 to 336 inclusive of Figures [1A-1B] (SEQ ID NO 4)

14 The vaccine composition of claim 11 wherein said GapC protein comprises the amino acid sequence of the Streptococcus agalactiae GapC protein depicted at amino acid positions 1 to 336 inclusive of Figures [2A-2B] (SEQ ID NO 6) or an immunogenic fragment thereof said fragment comprising at least about 5 amino acids

15 The vaccine composition of claim 14 wherein said GapC protein comprises the amino acid sequence depicted at amino acid positions 1 to [336] inclusive of Figures 2A-2B (SEQ ID NO 6)

16 The vaccine composition of claim 11 wherein said GapC protein comprises the amino acid sequence of the Streptococcus uberis GapC

protein depicted at amino acid positions 1 to 336 inclusive of Figures 3A-3B (SEQ ID NO 8) or an immunogenic fragment thereof said fragment comprising at least about 5 amino acids

17 The vaccine composition of claim 16 wherein said GapC protein comprises the amino acid sequence depicted at amino acid positions 1 to 336 inclusive of Figures 3A-3B (SEQ ID NO 8)

18 The vaccine composition of claim 11 wherein said GapC protein comprises the amino acid sequence of the Streptococcus parauberis GapC protein depicted at amino acid positions 1 to 336 inclusive of Figures 4A-4B (SEQ ID NO 10) or an immunogenic fragment thereof said fragment comprising at least about 5 amino acids

19 The vaccine composition of claim 18 wherein said GapC protein comprises the amino acid sequence depicted at amino acid positions 1 to 336 inclusive of Figures 4A-4B (SEQ ID NO 10)

20 The vaccine composition of claim 11 wherein said GapC protein comprises the amino acid sequence of the Streptococcus iniae GapC protein depicted at amino acid positions 1 to [336] inclusive of Figures [5A-5B] (SEQ ID NO 12) or an immunogenic fragment thereof said fragment comprising at least about 5 amino acids

21 The vaccine composition of claim 20 wherein said GapC protein comprises the amino acid sequence depicted at amino acid positions 1 to 336 inclusive of Figures [5A-5B] (SEQ ID NO 12)

22 The vaccine composition according to any of claims 11-21 further comprising an adjuvant

23 A method of producing a vaccine composition comprising the steps of (1) providing a GapC protein or an immunogenic fragment thereof said fragment comprising at least about 5 amino acids and (2) combining said protein with a pharmaceutically acceptable vehicle

24 The method of claim 23 wherein the GapC protein is the protein of any of claims 1-6

25 Antibodies directed against the isolated GapC protein of any of claims 1-6

26 The antibodies of claim 25 wherein said antibodies are polyclonal

27 The antibodies of claim 25 wherein said antibodies are monoclonal

28 Use of a GapC protein according to any of claims 1-6 in the manufacture of a vaccine composition useful for treating or preventing a bacterial infection in a vertebrate subject

29 The use of claim 28 wherein said bacterial infection is a streptococcus infection

30 The use of claim 29 wherein said bacterial infection causes mastitis

[31] Use of a polynucleotide according to claim 7 in the manufacture of a vaccine composition for treating or preventing a bacterial infection in a vertebrate subject

32 The use of claim [31] wherein said bacterial infection is a streptococcal infection

33 The use of claim 32 wherein said bacterial infection causes mastitis

34 Use of a GapC protein in the manufacture of a diagnostic reagent for detecting Streptococcus antibodies in a biological sample

35 Use of a GapC protein according to any of claims 1-6 in the manufacture of a diagnostic reagent for detecting Streptococcus antibodies in a biological sample

36 Use of antibodies according to any of claims 25-27 in the manufacture of a diagnostic reagent for detecting a GapC protein in a biological sample

37 An immunodiagnostic test kit for detecting Streptococcus infection said test kit comprising a GapC protein and instructions for conducting the [IMMUNODIAGNOSTIC] test

[38] An [IMMUNODIAGNOSTIC] test kit for detecting Streptococcus infection said test kit comprising antibodies directed against a GapC protein and instructions for conducting the [IMMUNODIAGNOSTIC] test

39 A method of treating or preventing a bacterial infection in a vertebrate subject comprising administering to said subject a therapeutically effective amount of a vaccine composition according to any of claims 11-22

40 The method of claim 39 wherein said bacterial infection is a streptococcus infection

41 The method of claim 40 wherein said bacterial infection causes mastitis

42 A method of treating or preventing a bacterial infection in a vertebrate subject comprising administering to said subject a therapeutically effective amount of a polynucleotide according to

claim 7

43 The method of claim 42 wherein said bacterial infection is a streptococcal infection

44 The method of claim 43 wherein said bacterial infection causes mastitis

45 A method of detecting Streptococcus antibodies in a biological sample comprising (a) reacting said biological sample with an isolated GapC protein under conditions which allow said Streptococcus antibodies when present in the biological sample to bind to said GapC protein to form an antibody/antigen complex and (b) detecting the presence or absence of said complex thereby detecting the presence or absence of Streptococcus antibodies in said sample

46 A method of detecting Streptococcus antibodies in a biological sample comprising (a) reacting said biological sample with an isolated GapC protein according to any of claims 1-6 under conditions which allow said Streptococcus antibodies when present in the biological sample to bind to said GapC protein to form an antibody/antigen complex and (b) detecting the presence or absence of said complex thereby detecting the presence or absence of Streptococcus antibodies in said sample

47 A method of detecting a GapC protein in a biological sample comprising (a) reacting said sample with antibodies directed against the GapC protein under conditions which allow said antibodies to bind to said GapC protein when present in the sample to form an [ANTIBODY/ANTIGEN] complex and (b) detecting the presence or absence of said complex thereby detecting the presence or absence of a GapC protein in said sample

Description Claims

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty

PCT / CA 01/00838	
International Application No.	
11 JUNE 2001 (11 06 01)	
International Filing Date	
RO/CA	
Name of receiving Office and PCT International Application	
Applicant's or agent's file reference (if different) (12 characters maximum) 08 891814WO	

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Box No. I TITLE OF INVENTION Immunization of Dairy Cattle with GapC Protein Against Streptococcus Infection	
Box No. II APPLICANT ☐ This person is also inventor	
Name and address: (Family name followed by given name, for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this box is the applicant's State (that is, country) of residence if an State of residence is indicated below.)	Telephone No. 306 - 996-7465
University of Saskatchewan 120 Veterinary Road Saskatoon Saskatchewan CANADA S7N 5E3	Facsimile No. 306 - 996-7478
	Teleprinter No.
	Applicant's registration No. with the Office
State (that is, country) of nationality CA	State (that is, country) of residence. CA
This person is applicant for the purposes of ☒ all designated States ☐ all designated States except the U.S. and States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box	
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State (that is, country) of nationality	State (that is, country) of residence
This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.) POTTER Andrew A 521 Dalhousie Crescent Saskatoon Saskatchewan CANADA S7H 3S5	This person is: ☐ applicant only ☐ applicant and inventor ☒ inventor only (If this check-box is marked, do not fill in below.) Applicant's registration No. with the Office
State (that is, country) of nationality	State (that is, country) of residence
This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)	This person is: ☐ applicant only ☐ applicant and inventor ☐ inventor only (If this check-box is marked, do not fill in below.) Applicant's registration No. with the Office
State (that is, country) of nationality	State (that is, country) of residence
This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box	

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet

Box No V DESIGNATION OF STATES Mark the applicable check-boxes below at least one must be marked

The following designations are hereby made under Rule 4.9(a)

Regional Patent

- ☐ AP ARIPO Patent GH Ghana GM Gambia KE Kenya LS Lesotho MW Malawi MZ Mozambique SD Sudan SL Sierra Leone SZ Swaziland TZ United Republic of Tanzania UG Uganda ZW Zimbabwe and any other State which is a Contracting State of the Harare Protocol and of the CT
- ☐ EA Eurasian Patent AM Armenia AZ Azerbaijan BY Belarus KG Kyrgyzstan KZ Kazakhstan MD Republic of Moldova RU Russian Federation TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- ☒ EP European Patent AT Austria BE Belgium, CH & LI Switzerland and Liechtenstein, CY Cyprus DE Germany DK Denmark ES Spain FI Finland FR France GB United Kingdom GR Greece, IE Ireland, IT Italy LU Luxembourg, MC Monaco NL Netherlands PT Portugal SE Sweden, TR Turkey and any other State which is a Contracting State of the European Patent Convention and of the PCT
- ☐ OA OAPI Patent BF Burkina Faso, BJ Benin, CF Central African Republic CG Congo CI Côte d'Ivoire CM Cameroon, GA Gabon, GN Guinea GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger SN Senegal TD Chad TG Togo and any other State which is a member State of OAPI and a Contracting State of the PCT (If other kind of protection or treatment desired, specify on dotted line)

National Patent (If other kind of protection or treatment desired, specify on dotted line):

- | | | |
|--|---|---|
| ☐ AE United Arab Emirates | ☐ GE Georgia | ☐ MW Malawi |
| ☐ AG Antigua and Barbuda | ☐ GH Ghana | ☐ MX Mexico |
| ☐ AL Albania | ☐ GM Gambia | ☐ MZ Mozambique |
| ☐ AM Armenia | ☐ HR Croatia | ☐ NO Norway |
| ☐ AT Austria | ☐ HU Hungary | ☐ NZ New Zealand |
| ☐ AU Australia | ☐ ID Indonesia | ☐ PL Poland |
| ☐ AZ Azerbaijan | ☐ IL Israel | ☐ PT Portugal |
| ☐ BA Bosnia and Herzegovina | ☐ IN India | ☐ RO Romania |
| | ☐ IS Iceland | ☐ RU Russian Federation |
| ☐ BB Barbados | ☐ JP Japan | |
| ☐ BG Bulgaria | ☐ KE Kenya | ☐ SD Sudan |
| ☐ BR Brazil | ☐ KG Kyrgyzstan | ☐ SE Sweden |
| ☐ BY Belarus | ☐ KP Democratic People's Republic of Korea | ☐ SG Singapore |
| ☐ BZ Belize | ☐ KR Republic of Korea | ☐ SI Slovenia |
| ☒ CA Canada | ☐ KZ Kazakhstan | ☐ SK Slovakia |
| ☐ CH & LI Switzerland and Liechtenstein | ☐ LC Saint Lucia | ☐ SL Sierra Leone |
| ☐ CN China | ☐ LK Sri Lanka | ☐ TJ Tajikistan |
| ☒ CO Colombia | ☐ LR Liberia | ☐ TM Turkmenistan |
| ☐ CR Costa Rica | ☐ LS Lesotho | ☐ TR Turkey |
| ☐ CU Cuba | ☐ LT Lithuania | ☐ TT Trinidad and Tobago |
| ☐ CZ Czech Republic | ☐ LU Luxembourg | ☐ TZ United Republic of Tanzania |
| ☐ DE Germany | ☐ LV Latvia | ☐ UA Ukraine |
| ☐ DK Denmark | ☐ MA Morocco | ☐ UG Uganda |
| ☐ DM Dominica | ☐ MD Republic of Moldova | ☐ US United States of America |
| ☐ DZ Algeria | | |
| ☐ EE Estonia | ☐ MG Madagascar | ☐ UZ Uzbekistan |
| ☐ ES Spain | ☐ MK The former Yugoslav Republic of Macedonia | ☐ VN Viet Nam |
| ☐ FI Finland | ☐ MN Mongolia | ☐ YU Yugoslavia |
| ☐ GB United Kingdom | | ☐ ZA South Africa |
| ☐ GD Grenada | | ☐ ZW Zimbabwe |
| ☐ UNRESERVED FOR DESIGNATION BY THE APPLICANT | | |
| ☐ | ☐ | ☐ |
| ☐ | ☐ | ☐ |

Precautionary Designation Statement In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that these additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that date limit. (Confirmation (including fees) must reach the receiving Office within the 15-month time limit.)

Box No. VI PRIORITY CLAIM

The priority of the following earlier application(s) is hereby claimed.

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is.		
		national application country	regional application: regional Office	international application: receiving Office
Item (1) 12 June 2000 (12.06.00)	60/211,022	US		
Item (2)				
Item (3)				
Item (4)				
Item (5)				

☐ Further priority claims are indicated in the Supplemental Box.

The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) (only if the earlier application was filed with the Office which for the purposes of this international application is the receiving Office) identified

☐ all items ☐ item (1) ☐ item (2) ☐ item (3) ☐ item (4) ☐ item (5) ☐ other, see Supplemental Box

116. The applicant is an EPC applicant, indicating at least one country party to the Paris Convention for the Protection of Industrial Property or one Member of the World Trade Organization for which this international application was filed (PCT/CA 01/00858)

Choice of International Searching Authority (ISA) (if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used)

ISA / EPO

Request to use results of earlier search reference to that search (if an earlier search has been carried out by or requested from the International Searching Authority)

Date (day/month/year)

Number

Country (or regional Office)

Box No. VIII DECLARATIONS

The following declarations are contained in Boxes Nos. VIII (i) to (v) (mark the applicable check-boxes below and indicate in the right column the number of each type of declaration):

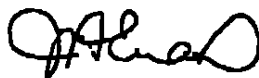
Number of
declarations

- ☐ Box No. VIII (i) Declaration as to the identity of the inventor
- ☐ Box No. VIII (ii) Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent
- ☐ Box No. VIII (iii) Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application
- ☐ Box No. VIII (iv) Declaration of ownership (only for the purposes of the designation of the United States of America)
- ☐ Box No. VIII (v) Declaration as to non-prejudicial disclosures or exceptions to lack of novelty

Box No. IX CHECK LIST LANGUAGE OF FILING		
This international application contains: (a) the following number of sheets in paper form: request (including declaration sheets) 5 description (excluding sequence listing part) 53 claims 7 abstract 1 drawings -37 - Sub-total number of sheets 103 sequence listing part of description (actual number of sheets if filed in paper form, whether or not also filed in computer readable form, see (b) below) 17 Total number of sheets 120 (b) sequence listing part of description filed in computer readable form: (i) ☐ only (under Section 801(a)(i)) (ii) ☐ in addition to being filed in paper form (under Section 801(a)(ii)) Type and number of carriers (diskette, CD-ROM, CD-R or other) on which the sequence listing part is contained (additional copies to be indicated under item 9(ii) in right column):		This international application is accompanied by the following item(s) (mark the applicable check-boxes below and indicate in right column the number of each item): 1 ☐ fee calculation sheet 2 ☐ original separate power of attorney 3 ☐ original general power of attorney 4 ☐ copy of general power of attorney reference number if any 5 ☐ statement explaining lack of signature 6 ☐ priority document(s) identified in Box No. VI as item(s) 7 ☐ translation of international application into (language): 8 ☐ separate indications concerning deposited microorganism or other biological material 9 ☒ sequence listing in computer readable form (indicate also type and number of carriers (diskette, CD-ROM, CD-R or other)): (i) ☐ copy submitted for the purposes of international search under Rule 13ter only (and not as part of the international application) (ii) ☐ (only where check-box (b)(i) or (b)(ii) is marked in left column) additional copies including, where applicable, the copy for the purposes of international search under Rule 13ter (iii) ☐ together with relevant statement as to the identity of the copy or copies with the sequence listing part mentioned in left column 10 ☐ other (specify):
Figure of the drawings which should accompany the abstract: 19	Language of filing of the international application: ENGLISH	

Box No. X SIGNATURE OF APPLICANT AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).



Judy A. Erratt

For receiving Office use only	
1 Date of actual receipt of the purported international application: 11 JUNE 2001 (11 06 01)	2 Drawings: ☒ received. ☐ not received:
3 Corrected date of actual receipt due to later but timely received papers in connection with the purported international application.	
4 Date of timely receipt of the required corrections under PCT Article 11(2)	
5 International Searching Authority (if two or more are competent) ISA /	6 ☒ Transmittal of search copy delayed until search fee is paid
For International Bureau use only	
Date of receipt of the record copy by the International Bureau	

PATENT COOPERATION TREATY

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PCT

From the INTERNATIONAL BUREAU

NOTIFICATION CONCERNING SUBMISSION OR TRANSMITTAL OF PRIORITY DOCUMENT

(PCT Administrative Instructions Section 411)

To

ERRATT Judy A.
Gowlings Lafleur Henderson LLP
Suite 2800
160 Elgin Street
Ottawa Ontario K1P 1C3
CANADA

RECEIVED

NOV 08 2001

GOWLINGS

Date of mailing (day/month/year) 29 October 2001 (29 10 01)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference 08-891814WO	
International application No. PCT/CA01/00838	International filing date (day/month/year) 11 June 2001 (11 06 01)
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 12 June 2000 (12 06 00)
Applicant UNIVERSITY OF SASKATCHEWAN	

- 1 The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) relating to the earlier application(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, or by the letters "NR" in the right-hand column, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17 1(a) or (b).
- 2 This updates and replaces any previously issued notification concerning submission or transmittal of priority documents.
- 3 An asterisk(*) appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17 1(a) or (b). In such a case, the attention of the applicant is directed to Rule 17 1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity upon entry into the national phase to furnish the priority document within a time limit which is reasonable under the circumstances.
- 4 The letters "NR" appearing in the right-hand column denotes a priority document which was not received by the International Bureau in which the applicant did not request the relevant Office to forward and transmit to the International Bureau as provided by Rule 17 1(a) or (b), respectively. In such a case, the attention of the applicant is directed to Rule 17 1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity upon entry into the national phase to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
12 June 2000 (12 06 00)	60/211 022	US	02 Octo 2001 (02 10 01)

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. (41-22) 740.14.35	Authorized officer  Beatriz LARGO Telephone No. (41-22) 338.83.38
--	--

Form PCT/IB/304 (July 1998)

004488578

PATENT COOPERATION TREATY

70

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

RECEIVED

JAN 29 2002

To:

Erratt, Judy A
GOWLING LAFLEUR HENDERSON LLP
160 Elgin Street, Suite 2600
Ottawa, Ontario K1P 1C3
CANADA

NOTIFICATION OF RECEIPT
OF DEMAND BY COMPETENT INTERNATIONAL
PRELIMINARY EXAMINING AUTHORITY

(PCT Rules 59.3(e) and 61.1(b), first sentence
and Administrative Instructions, Section 601(a))

GOWLINGS

Date of mailing
(day/month/year)

18.01.02

Applicant's or agent's file reference

08-891814WU

IMPORTANT NOTIFICATION

International application No.

PCT/CA 01/00838

International filing date (day/month/year)

11/06/2001

Priority date (day/month/year)

12/06/2000

Applicant

UNIVERSITY OF SASKATCHEWAN

1 The applicant is hereby notified that this International Preliminary Examining Authority considers the following date the date of receipt of the demand for international preliminary examination of the international application.

10/01/2002

2 This date of receipt is:

- ☒ the actual date of receipt of the demand by this Authority (Rule 61.1(b)).
- ☐ the actual date of receipt of the demand on behalf of this Authority (Rule 59.3(e)).
- ☐ the date on which this Authority has, in response to the invitation to correct defects in the demand (Form PCT/IPEA/404), removed the required corrections.

3 ☐ ATTENTION: This date of receipt is AFTER the expiration of 18 months from the priority date. Consequently, the election(s) made in the demand does (do) not have the effect of postponing the entry into the national phase until 30 months from the priority date (or later in some Offices) (Article 39(1)). Therefore the acts for entry into the national phase must be performed within 20 months from the priority date (or later in some Offices) (Article 32). For details, see the PCT Applicant's Guide Volume II.

☐ (If applicable) This notification confirms the information given by telephone, facsimile transmission or in person.

4 Only where paragraph 3 applies, a copy of this notification has been sent to the International Bureau.

Name and mailing address of the IPEA/

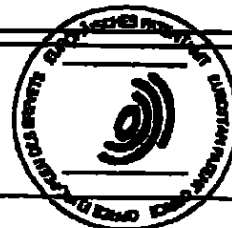


European Patent Office
D-80298 Munich
Tel. (+49-89) 2399-0 Tx 533636 eptou d
Fax. (+49-89) 2399-4465

Authorized officer

SENKARA A

Tel. (+49-89) 2399-4465



Form PCT/IPEA/402 (July 1998) P20452

(15/D1/2002)

PATENT COOPERATION TREATY

✓

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

To
GOWLING LAFLEUR HENDERSON LLP
Attn: Erratt, Judy A
160 Elgin Street, Suite 2600
Ottawa Ontario K1P 1C3
CANADA

Date of mailing
(day/month/year) 01/03/2002

Applicant or agent's file reference
08-891814W0

FOR FURTHER ACTION See paragraphs 1 and 4 below

International application No.
PCT/CA 01/00838

International filing date
(day/month/year) 11/06/2001

Applicant

UNIVERSITY OF SASKATCHEWAN

- 1 ☒ The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:

The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46)

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No. (41-22) 740.14.36

For more detailed instructions see the notes on the accompanying sheet.

- 2 ☐ The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

- 3 ☐ With regard to the protest against payment of (an) additional fee(s) under Rule 40.2 the applicant is notified that:

☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

- 4 Further action(s): The applicant is reminded of the following:

Shortly after 18 months from the priority date the International application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication a notice of withdrawal of the International application or of the priority claim must reach the International Bureau as provided in Rules 50bis.1 and 50bis.3, respectively before the completion of the technical preparations for international publication.

Within 18 months from the priority date, a request for international preliminary examination must be filed if an applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within 20 months from the priority date the applicant must perform the prescribed acts for entry into the national phase before all designated Offices where entry is not automatic in the case of a PCT application. Failure to do so may result in the application being deemed withdrawn or could not be entered because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority



European Patent Office P.S. 5518 Patentstein 2
NL-2280 HV Rijswijk
Tel (+31-70) 340-2040 Tx 31 851 epo nl
Fax (+31-70) 340-3016

INTERNATIONAL OFFICE

Barbara Klaver

Form PCT/ISA/220 (July 1998)

NOTES TO FORM PCT/ISA/220

X2

These Notes are intended to give the basic instructions concerning the filing of amendments under Article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and these requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, Article, Rule, and Section refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g., the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 48.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 48.2).

Where a demand for international preliminary examination has been filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 285(p)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 285(b)).

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the Statement under Article 19(1) (see below under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English. If the language of the international application is French, the letter must be in French.

V3

NOTES TO FORM PCT/ISA/220 (continued)

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

- (i) the claim is unchanged;
- (ii) the claim is cancelled;
- (iii) the claim is new;
- (iv) the claim replaces one or more claims as filed;
- (v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."
2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."
3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added. or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."
4. [Where various kinds of amendments are made]:
"Claims 1, 10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 18(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 18(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief: not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading preferably by using the words "Statement under Article 18(1)".

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. References to citations relevant to a given claim contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If at the time of filing any amendments under Article 18, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's amendments or claims in the form filed, as well as the amendments or claims as amended under Article 18 may have to be furnished to the designated/selected Office, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/selected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 08-891814W0	FOR FURTHER ACTION see Notification of Transmission of International Search Report (Form PCT/ISA/220) as well as where applicable, item 5 below	
International application No. PCT/CA 01/00838	International filing date (day/month/year) 11/06/2001	(Earliest) Priority Date (day/month/year) 12/06/2000
Applicant UNIVERSITY OF SASKATCHEWAN		

This International Search Report has been prepared by the International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the language, the International search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item

☐ the International search was carried out on the basis of a translation of the international application furnished to the Authority (Rule 23.1(b)).

b. With regard to any nucleotide and/or amino acid sequence identified in the international application, the International search was carried out on the basis of the sequence listing

☒ contained in the international application in written form.

☒ filed together with the international application in computer readable form.

☐ -- -- -- -- --

☐ furnished subsequently to the Authority in computer readable form

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2 ☒ Certain claims were found unsearchable (See Box I)

3 ☐ Unity of invention is lacking (see Box II)

4. With regard to the title,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows.

5. With regard to the abstract,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(p) by the Authority as it appears in Box III. The applicant may within one month from the date of mailing of this International search report, submit comments to this Authority

6. The figure of the drawings to be published with the abstract is Figure No

☒ as suggested by the applicant.

☐ because the applicant asked to suggest a figure.

☐ because this figure better characterizes the invention.

19

☐ None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 01/00838

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K14/315 C12N15/31 C12N15/70 C12N1/21 A61K39/09
C07K16/12 A61P31/04 A61K31/7088 G01N33/569 G01N33/577
G01N33/68

According to the rules of Patent Cooperation Treaty (PCT) with national law and PCT

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K

Documentation searched other than minimum documentation to the extent that each document is included in the fields searched

Electronic data bases consulted during the international search (name of data base and, where practical, search terms used)

BIOISIS, EMBASE, WPI Data, PAJ, EPO-Internal, SEQUENCE SEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim no.
A	WO 98 18930 A (HUMAN GENOME SCIENCES) 7 May 1998 (1998-05-07) seq id no 54	1-47
A	K GASE ET AL "Cloning, sequencing and multifunctional overexpression of the Streptococcus equisimilis H46A gapC gene encoding a glyceraldehyde-3-phosphate dehydrogenase that also functions as a plasmin(ogen)-binding protein Purification and biochemical characterization of the protein EUROPEAN JOURNAL OF BIOCHEMISTRY vol 238 no 1, 1 July 1996 (1996-07-01), pages 42-51, XP001053701 Berlin, Germany abstract	1-47

☒ Further documents are cited in the continuation of box C.

☒ Patent family members are cited in annex.

Special categories of cited documents

- A document defining the general state of the art which is not considered to be of particular relevance
- E earlier document but published on or after the international filing date
- L document which may throw doubts on priority claims or which is cited to establish the publication date of another claim or other special reason (as specified)
- C document relating to an oral disclosure, use exhibition or other means
- T document published prior to the international filing date but later than the priority date claimed

- T later document published after the international filing date or priority date and not in conflict with the application but cited to substantiate the principle or theory underlying the invention
- X document of particular relevance the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- Y document of particular relevance the claimed invention cannot be considered to involve an inventive step when the document is considered with one or more other such documents, each combination being obvious to a person skilled in the art.
- A document member of the same patent family

Date of the actual completion of the international search

19 February 2002

Date of mailing of the international search report

01/03/2002

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No. of pages, F

Form PCT/CA/210 (previously issued July 1992)

INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 01/00838

✓6

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication where appropriate, of the relevant passages	Relevance to claim No.
A	US 5 328 996 A (BOYLE ET AL) 12 July 1994 (1994-07-12) cited in the application examples	1-47
A	WO 96 41879 A (UNIVERSITY OF SASKATCHEWAN) 27 December 1996 (1996-12-27) cited in the application examples claims	1-47
A	WO 99 42588 A (BIOCHEM VACCINS INC ET AL) 26 August 1999 (1999-08-26) examples claims	1-47
A	J FINCH ET AL "Further studies on the efficacy of a live vaccine against mastitis caused by Streptococcus uberis " VACCINE, vol 15 no 10, July 1997 (1997-07) pages 1138-1143, XP004085975 Guildford GB the whole document	1-47

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 01/00838

V7

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons.

1. ☒ Claims Nos.
because they relate to subject matter not required to be searched by this Authority namely
Although claims 39-44 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition
2. ☐ Claims Nos.
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out specifically
3. ☐ Claims Nos.
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in the International application as follows.

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, the Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.
4. ☐ No required additional search fees were timely paid by the applicant. Consequently this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INFORMATION ON PATENT FAMILY MEMBERS
 Information on patent family members

International Application No
 PCT/CA 01/00838

✓8

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WU 951893U	07-03-1998	AU 6909098 A EP 0942983 A2 EP 0941335 A2 JP 2001505415 T JP 2001501833 T WO 9818930 A2 WO 9818931 A2 US 6159469 A	22-05-1998 22-09-1999 15-09-1999 24-04-2001 13-02-2001 07-05-1998 07-05-1998 12-12-2000
US 5328996	12-07-1994	US 5237050 A US 6136323 A	17-08-1993 24-10-2000
WO 9641879	27-12-1996	US 5863543 A AU 709920 B2 AU 5889996 A CA 2222701 A1 WO 9641879 A1 CN 1191569 A EP 0832239 A1 NZ 308991 A	26-01-1999 09-09-1999 09-01-1997 27-12-1996 27-12-1996 26-08-1998 01-04-1998 29-04-1999
WO 9942588	26-08-1999	AU 2505999 A WO 9942588 A2 CN 1297482 T EP 1054971 A2 HU 0102304 A2 NO 20004161 A TR 200002437 T2 ZA 9901325 A	06-09-1999 26-08-1999 30-05-2001 29-11-2000 28-10-2001 19-10-2000 21-11-2000 20-08-1999

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PATENT COOPERATION TREATY

44

GOWLINGS

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From the INTERNATIONAL BUREAU

To

22301

INFORMATION CONCERNING ELECTED
OFFICES NOTIFIED OF THEIR ELECTION

(PCT Rule 61.3)

ERRATT, Judy A
Gowlings Lafleur Henderson LLP
Suite 2600
160 Elgin Street
Ottawa Ontario K1P 1C3
CANADA

Date of mailing (day/month/year)

30 January 2002 (30 01 02)

Applicant's or agent's file reference

08-891814WO

IMPORTANT INFORMATION

International application No.

PCT/CA01/00838

International filing date (day/month/year)

11 June 2001 (11 06.01)

Priority date (day/month/year)

12 June 2000 (12.06 00)

Applicant

UNIVERSITY OF SASKATCHEWAN

- 1 The applicant is hereby informed that the International Bureau has, according to Article 31(7), notified each of the following Offices of its election:

EP AT BE CH CY,DE,DK ES FI FR GB,GR IE IT LU,MC NL PT SE TR
National CA

- 2 The following Offices have waived the requirement for the notification of their election, the notification will be sent to them by the International Bureau only upon their request:

National CO

- 3 The applicant is reminded that he must enter the national phase before the expiration of 30 months from the priority date before each of the Offices listed above. This must be done by paying the national fee(s) and furnishing, if prescribed, a translation of the international application (Article 39(1)(a)), as well as, where applicable, by furnishing a translation of any annexes of the international preliminary examination report (Article 35(3)(b) and Rule 74.1).

Some offices have fixed time limits expiring later than the above-mentioned time limit. For detailed information about the applicable time limits and the acts to be performed upon entry into the national phase before a particular Office, see Volume II of the PCT Applicant's Guide.

The entry into the European regional phase is postponed until 31 months from the priority date for all States designated for the purposes of obtaining a European patent.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Authorized officer

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PATENT COOPERATION TREATY

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EXAMINING AUTHORITY

PCTOWLINGS

To:

Erratt, Judy A
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NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing
(day/month/year) 22/05/2002

Applicant's or agent's file reference
08-891814W0

IMPORTANT NOTIFICATION

International application No.

PCT/CA 01/00838

International filing date (day/month/year)

11/06/2001

Priority date (day/month/year)

12/06/2000

Applicant

UNIVERSITY OF SASKATCHEWAN

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes if any established on the international application.
2. A copy of the report and its annexes, if any is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.
4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office the translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

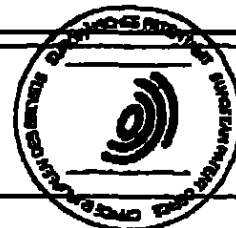
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Form PCT/IPEA/416 (July 1992) P20473

PCT

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 08-891814W0	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/16)	
International application No. PCT/CA 01/ 00838	International filing date (day/month/year) 11/06/2001	Priority date (day/month/year) 12/06/2000
International Patent Classification (IPC) or national classification and IPC C07K14/195		
Applicant UNIVERSITY OF SASKATCHEWAN		

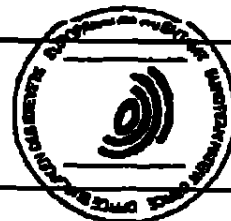
- 1 This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 16
- 2 This REPORT consists of a total of 2 sheets, including this cover sheet.
- ☐ This report is also accompanied by ANNEXES i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70 16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of _____ sheets.

- 3 This report contains indications relating to the following items:
- I ☒ Basis of the report
 - II ☐ Priority
 - III ☒ Non-establishment of opinion with regard to novelty inventive step and industrial applicability
 - IV ☐ Lack of unity of invention
 - V ☒ Reasoned statement under Article 35(2) with regard to novelty inventive step or industrial applicability (clauses and explanations supporting such statement)
 - VI ☐ Certain documents cited
 - VII ☐ Certain defects in the international application
 - VIII ☐ Certain observations on the international application

Date of submission of the demand 10/01/2002	Date of completion of this report 16/05/2002
Name and mailing address of the IPBA/  European Patent Office D-80399 Munich Tel (+49-89) 2399-0 Tlx 523656 epeu d Fax (+49-89) 2399-4465	Authorized officer BOUTRUCHE J E E Tel. (+49-89) 2399 2828

Form PCT/IPEA/409 (cover sheet) (July 1998)



52

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/CA 01/00898

I Basis of the report

The basis of this international preliminary examination is the application as originally filed

III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The question of whether the claimed invention appears to be novel to involve an inventive step or to be industrially applicable has not been the subject of the international preliminary examination in respect of the claims which have not been searched (Article 17(2)(a) or (3) and Rule 66 1(e) PCT) see also international search report)

V Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability

To the extent that the international preliminary examination has been carried out (see item III above) the following is pointed out

In light of the data indicated in the international search report it is concluded that the invention as defined in the claims which have been the subject of an international search report, meets the criteria mentioned in Article 33(1) PCT i.e. appears to be novel to involve an inventive step and to be industrially applicable

(19) World Intellectual Property Organization
International Bureau



INTERNATIONAL PATENT COOPERATION TREATY (PCT)

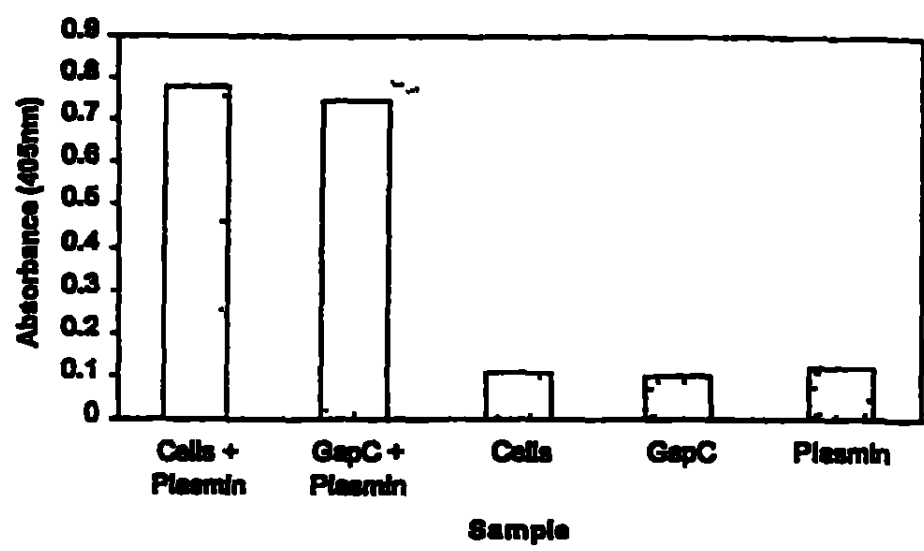
(43) International Publication Date
20 December 2001 (20 12 2001)

PCT

(10) International Publication Number
WO 01/96381 A2

- (51) International Patent Classification⁷ C07K 14/195
- (21) International Application Number PCT/CA01/00838 ✓
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- (30) Priority Data 60/211,022 12 June 2000 (12.06.2000) US
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- (74) Agents: ERRATT Judy A. et al., Gowlings Lafleur Henderson LLP Suite 2500, 160 Elgin Street, Ottawa, Ontario K1P 1C3 (CA).
- (81) Designated States (national) CA, CO ✓
- (84) Designated States (regional) European patent (AT BE, CH CY DE, DK, ES, FI, FR, GB GR, IE, IT LU MC, NL, PT SE, TR).
- Published: — without international search report and to be republished upon receipt of that report
- For two-letter codes and other abbreviations refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title IMMUNIZATION OF DAIRY CATTLE WITH GapC PROTEIN AGAINST *STREPTOCOCCUS* INFECTION



(57) Abstract The GapC plasmin binding protein genes of *Streptococcus dysgalactiae* (*S. dysgalactiae*) *Streptococcus agalactiae* (*S. agalactiae*) *Streptococcus uberis* (*S. uberis*) *Streptococcus parauberis* (*S. parauberis*) and *Streptococcus iniae* (*S. iniae*) are described as well as the recombinant production of the GapC proteins therefrom. Also described is the use of the GapC proteins from these species in vaccine compositions to prevent or treat bacterial infections in general and mastitis in particular

WO 01/96381 A2

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5 IMMUNIZATION OF DAIRY CATTLE WITH GapC PROTEIN AGAINST
 STREPTOCOCCUS INFECTION

TECHNICAL FIELD

10 The present invention relates generally to bacterial antigens and genes encoding the same. More particularly, the present invention pertains to the cloning, expression and characterization of the GapC plasmid-binding proteins from *Streptococcus dysgalactiae*, *Streptococcus agalactiae*, *Streptococcus uberis*, *Streptococcus parauberis* and *Streptococcus iniae*, and the use of the same in vaccine compositions

15 BACKGROUND

 Mastitis is an infection of the mammary gland usually caused by bacteria or fungus. The inflammatory response following infection results in decreased milk yield as well as quality, and causes major annual economic losses to the dairy industry.

20 Among the bacterial species most commonly associated with mastitis are various species of the genus *Streptococcus*, including *Streptococcus aureus*, *Streptococcus uberis* (untypeable), *Streptococcus agalactiae* (Lancefield group B), *Streptococcus dysgalactiae* (Lancefield group C), *Streptococcus zooepidemicus* and the Lancefield groups D, G, L and N streptococci. Some of those species are contagious (e.g. *S. agalactiae*), while others are considered environmental pathogens (e.g. *S. dysgalactiae* and *S. uberis*).

25 The environmental pathogen *S. uberis* is responsible for about 20% of all clinical cases of mastitis (Bramley, A.J. and Dodd, F.H. (1984) *J. Dairy Res.* 51: 481-512, Bramley A.J. (1987) *Animal Health Nutrition* 42: 12-16, Watts J.L. (1988) *J. Dairy Sci.* 71: 1616-1624), it is the predominant organism isolated from mammary glands during the non-lactating period (Bramley, A.J. (1984) *Br. Vet. J.* 140: 328-335, Bramley and Dodd (1984) *J.*
30 *Dairy Res.* 51: 481-512, Oliver, S.P. (1988) *Am. J. Vet. Res.* 49: 1789-1793).

 Mastitis resulting from infection with *S. uberis* is commonly subclinical, characterized by apparently normal milk with an increase in somatic cell counts due to the influx of leukocytes. The chemical composition of milk is changed due to suppression of

SUBSTITUTE SHEET (RULE 26)

secretion with the transfer of sodium chloride and bicarbonate from blood to milk, causing a shift of pH to a more alkaline level. *S. uberis* mastitis may also take the form of an acute clinical condition, with obvious signs of disease such as clots or discoloration of the milk and swelling or hardness of the mammary gland. Some cases of the clinical disease can be severe and pyrexia may be present. For a review of the clinical manifestations of *S. uberis* mastitis, see, Bramley (1991) Mastitis physiology or pathology p 3-9 In C. Burvench, G Vandeputte-van Messom, and A. W Hill (ed), *New insights into the pathogenesis of mastitis* Rijksuniversiteit Gent, Belgium, and Schalm et al (1971) The mastitis complex-A brief summary p 1-3 In *Bovine Mastitis* Lea & Febiger, Philadelphia

Conventional antibacterial control methods such as teat dipping and antibiotic therapy are effective in the control of many types of contagious mastitis, but the environmental organisms typically found in all dairy barns are often resistant to such measures. Vaccination is therefore an attractive strategy to prevent infections of the mammary glands, and has been shown to be beneficial in the case of some contagious mastitis pathogens

The literature is limited regarding vaccination studies with *S. dysgalactiae* and *S. uberis*, and variable results have been observed. In some cases, immunization has resulted in increased sensitivity to the specific organism and in other cases strain-specific protection has been obtained.

For example, previous studies have shown that primary infection with *S. uberis* can considerably reduce the rate of infection following a second challenge with the same strain (Hill, A.W (1988) *Res Vet. Sci.* 44 386-387). Local vaccination with killed *S. uberis* protects the bovine mammary gland against intramammary challenge with the homologous strain (Fmch et al. (1994) *Infect. Immun.* 62 3599-3603). Similarly, subcutaneous vaccination with live *S. uberis* has been shown to cause a dramatic modification of the pathogenesis of mastitis with the same strain (Hill et al (1994) *FEMS Immunol. Med. Microbiol.* 8 109-118). Animals vaccinated in this way shed fewer bacteria in their milk and many quarters remain free of infection.

Nonetheless, vaccination with live or attenuated bacteria can pose risks to the recipient. Further, it is clear that conventional killed vaccines are in general largely ineffective against *S. uberis* and *S. agalactiae*, either due to lack of protective antigens on *in vitro*-grown cells or masking of these antigens by molecular mimicry.

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The current lack of existing mastitis vaccines against *S. agalactiae* or the contagious streptococcus strains is due at least in part to a lack of knowledge regarding the virulence determinants and protective antigens produced by those organisms which are involved in invasion and protection of the mammary gland (Collins et al (1988) *J Dairy Res* 55 25-32, Leigh et al. (1990) *Res Vet. Sci.* 49 85-87, Marshall et al. (1986) *J Dairy Res* 53 507-514)

S. dysgalactiae is known to bind several extracellular and plasma-derived proteins such as fibronectin, fibrinogen, collagen, alpha-II-macroglobulin, IgG, albumin and other compounds. The organism also produces hyaluronidase and fibrinolysin and is capable of adhering to and invading bovine mammary epithelial cells. However, the exact roles of the bacterial components responsible for these phenotypes in pathogenesis is not known.

Similarly, the pathogenesis of *S. uberis* infection is poorly understood. Furthermore, the influence of *S. uberis* virulence factors on host defense mechanisms and mammary gland physiology is not well defined. Known virulence factors associated with *S. uberis* include a hyaluronic acid capsule (Hill, A.W (1988) *Res Vet. Sci.* 45 400-404), hyaluronidase (Schaufuss et al (1989) *Zentralbl. Bakteriologie. Ser. A* 271 46-53), R-like protein (Groschup, M.H. and Timoney, J.F (1993) *Res Vet. Sci.* 54 124-126), and a cohemolysin, the CAMP factor, also known as UBERIS factor (Skalka, B and Smola, J (1981) *Zentralbl. Bakteriologie. Ser. A* 242 190-194), R-like protein, plasminogen activator and CAMP factor. However, very little is known of their roles in pathogenicity.

The use of virulence determinants from *Streptococcus* as immunogenic agents has been proposed. For example, the CAMP factor of *S. uberis* has been shown to protect vertebrate subjects from infection by that organism (Jiang, U S Patent No 5,863,543)

The γ antigen of the group B *Streptococci* strain A909 (ATCC No 27591) is a component of the c protein marker complex, which additionally comprises an α and β subunit (Boyle, U S Patent No 5,721,339). Subsets of serotype Ia, II, and virtually all serotype Ib cells of group B streptococci, have been reported to express components of the c protein. Use of the γ subunit as an immunogenic agent against infections by Lancefield Group B *Streptococcus* infection has been proposed. However, its use to prevent or treat bacterial infections in animals, including mastitis in cattle, has not been studied.

The group A streptococcal M protein is considered to be one of the major virulence factors of this organism by virtue of its ability to impede attack by human phagocytes (Lancefield, R.C (1962) *J Immunol.* 89 307-313). The bacteria persist in the infected

tissue until antibodies are produced against the M molecule. Type-specific antibodies to the M protein are able to reverse the antiphagocytic effect of the molecule and allow efficient clearance of the invading organism.

M proteins are one of the key virulence factors of *Streptococcus pyogenes* due to their involvement in mediating resistance to phagocytosis (Kehoe, M.A. (1991) *Vaccine* 9 797-806) and their ability to induce potentially harmful host immune responses via their superantigenicity and their capacity to induce host-cross-reactive antibody responses (Bisno, A.L. (1991) *New Engl J Med.* 325 783-793, Froude et al. (1989) *Curr Top Microbiol Immunol.* 145 5-26, Stollerman, G.H. (1991) *Clin. Immunol. Immunopathol.* 61 131-142)

However, obstacles exist to using intact M proteins as vaccines. The protein's opsonic epitopes are extremely type-specific, resulting in narrow, type-specific protection. Further, some M proteins appear to contain epitopes that cross react with tissues of the immunized subject, causing a harmful autoimmune response (See e.g., Dale, J.L. and Beached, G.H. (1982) *J Exp Med* 156 1165-1176, Dale, J.L. and Beached, G.H. (1985) *J Exp Med* 161 113-122, Baird, R.W., Bronza, M.S., Drabs, W., Hill, H.R., Veasey, L.G. and Dale, J.L. (1991) *J Immunol.* 146 3132-3137, Bronza, M.S. and Dale, J.L. (1993) *J Immunol.* 151 2820-2828, Cunningham, M.W. and Russell, S.M. (1983) *Infect. Immunol.* 42 531-538)

Chimeric proteins containing three different fibronectin binding domains (FNBDs) derived from fibronectin binding proteins of *S. dysgalactiae* and *Staphylococcus aureus* have been expressed on the surface of *Staph. carnosus* cells. In the case of one of these proteins, intranasal immunizations with live recombinant *Staph. carnosus* cells expressing the chimeric protein on their surface resulted in an improved antibody response to a model immunogen present within the chimeric surface protein.

A GapC plasmin binding protein from a strain of Group A *Streptococcus* has previously been identified and characterized, and its use in thrombolytic therapies has been described (Boyle, et al., U.S. Patent No. 5,237,050, Boyle, et al., U.S. Patent No. 5,328,996)

However, until now, the protective capability of GapC has not been studied, nor have the GapC proteins of *Streptococcus dysgalactiae*, *Streptococcus agalactiae*, *Streptococcus uberis*, *Streptococcus parauberis* or *Streptococcus iniae* been isolated or characterized.

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SUMMARY OF THE INVENTION

Accordingly, the present invention provides novel *Streptococcus* GapC proteins and vaccine compositions comprising the same. In one embodiment, the invention is directed to an isolated GapC protein selected from the group consisting of

(a) an isolated *Streptococcus dysgalactiae* GapC protein comprising the amino acid sequence shown at amino acid positions 1 to 336, inclusive, of Figures 1A-1B (SEQ ID NO 4),

(b) an isolated *Streptococcus agalactiae* GapC protein comprising the amino acid sequence shown at amino acid positions 1 to 336, inclusive, of Figures 2A-2B (SEQ ID NO 6),

(c) an isolated *Streptococcus uberis* GapC protein comprising the amino acid sequence shown at amino acid positions 1 to 336, inclusive, of Figures 3A-3B (SEQ ID NO 8),

(d) an isolated *Streptococcus parauberis* GapC protein comprising the amino acid sequence shown at amino acid positions 1 to 336, inclusive, of Figures 4A-4B (SEQ ID NO 10),

(e) an isolated *Streptococcus iniae* GapC protein comprising the amino acid sequence shown at amino acid positions 1 to 336, inclusive, of Figures 5A-5B (SEQ ID NO 12), and

(f) immunogenic fragments of (a), (b), (c), (d) and (e) comprising at least about 5 amino acids

In another embodiment, the invention is directed to isolated polynucleotides comprising coding sequences for the above-described GapC proteins, or complements thereof, recombinant vectors comprising the polynucleotides, host cells comprising the recombinant vectors, and methods of recombinantly producing the GapC proteins

In yet other embodiments, the subject invention is directed to vaccine compositions comprising a pharmaceutically acceptable vehicle and a GapC protein as described above, methods of producing the vaccine compositions, as well as methods of treating or preventing bacterial infections in a vertebrate subject comprising administering to the subject a therapeutically effective amount of the vaccine composition. The bacterial infection is, for example, a streptococcus infection and may cause mastitis. The vaccine compositions may further comprise an adjuvant

In other embodiments, the invention is directed to antibodies directed against the isolated GapC proteins. The antibodies may be polyclonal or monoclonal.

In still further embodiments, the invention is directed to methods of detecting *Streptococcus* antibodies in a biological sample, comprising

5 (a) reacting the biological sample with an isolated GapC protein under conditions which allow said *Streptococcus* antibodies, when present in the biological sample, to bind to the GapC protein to form an antibody/antigen complex, and

(b) detecting the presence or absence of the complex, thereby detecting the presence or absence of *Streptococcus* antibodies in said sample

10 In another embodiment, the invention is directed to a method of detecting a GapC protein in a biological sample, comprising

(a) reacting the sample with antibodies directed against the GapC protein under conditions which allow the antibodies to bind to the GapC protein, when present in the sample, to form an antibody/antigen complex, and

15 (b) detecting the presence or absence of the complex, thereby detecting the presence or absence of a GapC protein in the sample.

In another embodiment, the invention is directed to an immunodiagnostic test kit for detecting *Streptococcus* infection, the test kit comprising a GapC protein, or antibodies directed against a GapC protein, and instructions for conducting the immunodiagnostic test.

20 These and other embodiments of the subject invention will readily occur to those of skill in the art in view of the disclosure herein.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A-1B depict the isolated nucleotide sequences and deduced amino acid sequences of the *gapC* gene for *S. dysgalactiae* (SEQ ID NO 3 and SEQ ID NO 4)

Figures 2A-2B depict the isolated nucleotide sequences and deduced amino acid sequences of the *gapC* gene for *S. agalactiae* (SEQ ID NO 5 and SEQ ID NO 6)

Figures 3A-3B depict the isolated nucleotide sequences and deduced amino acid sequences of the *gapC* gene for *S. uberis* (SEQ ID NO 7 and SEQ ID NO 8)

30 Figures 4A-4B depict the isolated nucleotide sequences and deduced amino acid sequences of the *gapC* gene for *S. parauberis* (SEQ ID NO 9 and SEQ ID NO 10)

Figures 5A-5B depict the isolated nucleotide sequences and deduced amino acid sequences of the *gapC* gene for *S. miae* (SEQ ID NO 11 and SEQ ID NO 12)

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Figures 6A-6E shows a DNA alignment created by PileUp and displayed by Pretty software (a component of the GCG Wisconsin Package, version 10, provided by the SeqWeb sequence analysis package, version 1.1, of the Canadian Bioinformatics Resource). The figure depicts the isolated nucleotide sequences of the *gapC* genes from *S. dysgalactiae* (DysGapC, Check 9344), *S. agalactiae* (AgalGapC, Check 2895), *S. uberis* (UberGapC, Check 5966), *S. parauberis* (PUberGapC, Check 9672), and *S. iniae* (IniaeGapC, Check 990). The previously known sequences of *S. equisimilis* (SeqGapC, Check 5841), *S. pyogenes* (SpyGapC, Check 4037), and a bovine GAPDH protein (BovGapC, check 5059) are also included. The length and weight parameters were the same for all sequences (1018 and 100, respectively). The parameters used in the DNA sequence comparison were as follows: Plurality=2.00, Threshold=1, AveWeight=1.00, AveMatch=1.00, AvMismatch=0.00, Symbol comparison table=pileupdna.cmp, CompCheck=6876, GapWeight=5, GapLengthWeight=1, PileUp MSF=1018, Type=N, Check=3804. In the figure, dashes represent identical nucleotides, dots represent gaps introduced by the software used to generate the alignment chart, and tildes represent regions not included in the overall alignment due to differences in the length of the gene sequences.

Figures 7A-7B shows an amino acid sequence alignment created by PileUp and displayed by Pretty (as above) that depicts the deduced amino acid sequences of the GapC proteins from *S. dysgalactiae* (DysGapC, Check 6731), *S. agalactiae* (AgalGapC, Check 1229), *S. uberis* (UberGapC, Check 8229), *S. parauberis* (PUberGapC, Check 8889) and *S. iniae* (IniaeGapC, check 8785). The previously known sequences of *S. equisimilis* (SeqGapC, Check 8252), *S. pyogenes* (SpyGapC, Check 6626) and a bovine GAPDH protein (BovGapC, Check 8479) are also included. In the figure, dashes represent identical amino acid residues, dots represent gaps introduced by the PileUp software, and tildes represent regions not included in the overall alignment due to differences in the length of the gene sequences.

Figure 8 shows Kyte-Doolittle hydropathy plots (averaged over a window of 7), Emami surface probability plots, Karplus-Schulz chain flexibility plots, Jameson-Wolf antigenic index plots, and both Chou-Fasman and Garnier-Osguthorpe-Robson secondary structure plots for the GapC protein isolated from *S. dysgalactiae*.

Figure 9 shows Kyte-Doolittle hydropathy plots (averaged over a window of 7), Emami surface probability plots, Karplus-Schulz chain flexibility plots, Jameson-Wolf

antigenic index plots, and both Chou-Fasman and Garner-Osguthorpe-Robson secondary structure plots for the GapC protein isolated from *S. agalactiae*.

Figure 10 shows Kyte-Doolittle hydropathy plots (averaged over a window of 7), Emami surface probability plots, Karplus-Schulz chain flexibility plots, Jameson-Wolf antigenic index plots, and both Chou-Fasman and Garner-Osguthorpe-Robson secondary structure plots for the GapC protein isolated from *S. uberis*

Figure 11 shows Kyte-Doolittle hydropathy plots (averaged over a window of 7), Emami surface probability plots, Karplus-Schulz chain flexibility plots, Jameson-Wolf antigenic index plots, and both Chou-Fasman and Garner-Osguthorpe-Robson secondary structure plots for the GapC protein isolated from *S. parauberis*

Figure 12 shows Kyte-Doolittle hydropathy plots (averaged over a window of 7), Emami surface probability plots, Karplus-Schulz chain flexibility plots, Jameson-Wolf antigenic index plots, and both Chou-Fasman and Garner-Osguthorpe-Robson secondary structure plots for the GapC protein isolated from *S. iniae*.

Figure 13 is a diagrammatic representation of the Chou-Fasman secondary structure plots for the GapC protein isolated from *S. dysgal*.

Figure 14 is a diagrammatic representation of the Chou-Fasman secondary structure plots for the GapC protein isolated from *S. agalactiae*.

Figure 15 is a diagrammatic representation of the Chou-Fasman secondary structure plots for the GapC protein isolated from *S. uberis*

Figure 16 is a diagrammatic representation of the Chou-Fasman secondary structure plots for the GapC protein isolated from *S. parauberis*

Figure 17 is a diagrammatic representation of the Chou-Fasman secondary structure plots for the GapC protein isolated from *S. iniae*.

Figure 18 shows the results of SDS-polyacrylamide gel electrophoresis of recombinant *S. dysgalactiae* GapC produced in *E. coli* DE3 Lane 1, molecular weight markers (20.5 - 103 kDa, BioRad, Emeryville, CA), lane 2, soluble recombinant *S. dysgalactiae* GapC purified by Ni-NTA affinity chromatography. Numbers on the left of the figure indicate the positions of the molecular weight markers (in kDa)

Figure 19 is a histogram which compares the enzymatic activity of bovine plasmin bound to intact *S. dysgalactiae* cells, bovine plasmin bound to affinity-purified recombinant *S. dysgalactiae* GapC protein, intact *S. dysgalactiae* cells alone, affinity-purified recombinant *S. dysgalactiae* GapC protein alone; and bovine plasmin alone. Activity was

determined by an increase in the absorbance at 405nm following release of paranitroanalide from the synthetic substrate chromozine-PL (Roche Diagnostics, Laval, Quebec, Canada) The data represents the mean of three individual assays.

5 Figure 20 compares the change in the percentage of udder quarters infected with *S dysgalactiae* over a 7 day period among three experimental groups (1) unvaccinated control animals, (2) animals vaccinated with the Mig Fc binding protein, and (3) animals vaccinated with GapC Infection was defined as recovery of >500 cfu of the *S dysgalactiae* per ml of milk secretions

10 Figure 21 plots the maximum number of *S. dysgalactiae* in any udder quarter against serum anti-GapC antibody titre (expressed as the reciprocal of the dilution showing activity above background levels) Serum anti-GapC titers were shown to correlate with the maximum number of cfu/ml recovered from the mammary glands $R = 0.74$, as determined using GraphPad Prism software, v 2.01, from GraphPad Software, Inc., San Diego, CA)

15 Figure 22 plots cumulative number of infected mammary quarters against serum antibody titer (again expressed as the reciprocal of the dilution showing activity above background levels) A strong correlation between the anti-GapC antibody serum antibody level and the total number of infected quarters was observed

20 Figure 23 illustrates the recovery of bacteria from GapC-immunized animals, data points are plotted as mean bacterial recoveries/ml milk vs time (in days post-challenge) In the figure, diamonds (-♦-) represent non-vaccinated animals, squares (-■-) represent low titer animals (i.e. animals exhibiting the poorest response against GapC in terms of antibody titre), and triangles (-Δ-) represent high titer animals (i.e., the remaining animals)

25 Figure 24 shows recovery of *S dysgalactiae* from GapC-immunized animals, plotted as the percent of mammary quarters infected vs time in days post-challenge In the figure, the stippled bars represent non-vaccinated animals, the cross-hatched bars represent low-titer animals (i.e., animals exhibiting the poorest response against GapC in terms of antibody titre) and the unshaded bars represent high-titer animals (i.e., the remaining animals)

30 Figure 25 depicts the observed inflammatory response to infection with *S. dysgalactiae* plotted as mean somatic cell counts (SCC) for each experimental group versus time in days post challenge. In the figure, diamonds (-♦-) represent unchallenged, unvaccinated quarters, squares (-■-) represent challenged, unvaccinated animals, triangles(-

Δ-) represent challenged, Mig-vaccinated animals, and x's (-X-) represent challenged, GapC-vaccinated animals

Figure 26 illustrates somatic cell counts per mammary quarter on day 1 post-challenge. In the figure, the bar represents the mean for each group. Squares (■-) represent unvaccinated animals, triangles (-Δ-) represent GapC-vaccinated animals, and inverted triangles (-▽-) represent Mig-vaccinated animals.

Figure 27 depicts the somatic cell counts of non-vaccinated, non-challenged and GapC-immunized, high-titer animals (i.e., the four animals exhibiting the highest antibody titres of the eight animals in the particular group) for seven days post challenge, plotted as the log₁₀ of the mean somatic cell count/ml milk against time in days post challenge. Diamonds (-♦-) represent unchallenged, unvaccinated animals, squares (■-) represent challenged, unvaccinated animals, and triangles (-Δ-) represent challenged, GapC-vaccinated animals.

DETAILED DESCRIPTION OF THE INVENTION

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA technology, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook, Fritsch & Maniatis, *Molecular Cloning: A Laboratory Manual*, Vols. I, II and III, Second Edition (1989), Perbal, B., *A Practical Guide to Molecular Cloning* (1984), the series, *Methods In Enzymology* (S Colowick and N Kaplan eds., Academic Press, Inc.), and *Handbook of Experimental Immunology*, Vols. I-IV (D.M. Weir and C.C. Blackwell eds., 1986, Blackwell Scientific Publications).

The following amino acid abbreviations are used throughout the text

Alanine Ala (A)	Arginine Arg (R)
Asparagine Asn (N)	Aspartic acid Asp (D)
Cysteine Cys (C)	Glutamine Gln (Q)
Glutamic acid Gln (E)	Glycine Gly (G)
Histidine His (H)	Isoleucine Ile (I)
Leucine Leu (L)	Lysine Lys (K)
Methionine Met (M)	Phenylalanine Phe (F)

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Proline Pro (P)	Serine Ser (S)
Threonine Thr (T)	Tryptophan Trp (W)
Tyrosine Tyr (Y)	Valine Val (V)

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1 DEFINITIONS

In describing the present invention, the following terms will be employed, and are intended to be defined as indicated below

It must be noted that, as used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "a *Streptococcus* GapC protein" includes a mixture of two or more such proteins, and the like

The terms "GapC protein" and "GapC plasmin binding protein" (used interchangeably herein) or a nucleotide sequence encoding the same, intends a protein or a nucleotide sequence, respectively, which is derived from a GapC gene found in a variety of *Streptococcus* species, including, without limitation certain strains of group A streptococci (Lottenbery, R., et al., (1987) *Infect. Immun.* 55 1914-1918). The nucleotide sequence of representative *Streptococcus gapC* genes, and the corresponding amino acid sequence of the GapC proteins encoded by these genes, are depicted in the Figures. In particular, Figures 1 through 5 depict the isolated nucleotide sequences and isolated amino acid sequences of *S. dysgalactiae* (SEQ ID NO 3 and SEQ ID NO 4, respectively), *S. agalactiae* (SEQ ID NO 5 and SEQ ID NO 6, respectively), *S. uberis* (SEQ ID NO 7 and SEQ ID NO 8, respectively), *S. parauberis* (SEQ ID NO 9 and SEQ ID NO 10, respectively), and *S. miae* (SEQ ID NO 11 and SEQ ID NO 12, respectively). However, a GapC protein as defined herein is not limited to the depicted sequences as subtypes of each of these *Streptococcus* species are known and variations in GapC proteins will occur between them.

Representative *gapC* genes, derived from *S. dysgalactiae*, *S. agalactiae*, *S. uberis* and *S. parauberis* are found in the plasmids pET15bgapC (ATCC No), pMF521c (ATCC No), pMF521a (ATCC No), pMF521d (ATCC No), and pMF521e (ATCC No), respectively

Furthermore, the derived protein or nucleotide sequences need not be physically derived from the gene described above, but may be generated in any manner, including for example, chemical synthesis, isolation (e.g. from *S. dysgalactiae*) or by recombinant

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production, based on the information provided herein. Additionally, the term intends proteins having amino acid sequences substantially homologous (as defined below) to contiguous amino acid sequences encoded by the genes, which display immunological and/or plasmin binding activity

5 Thus, the terms intend full-length, as well as immunogenic, truncated and partial sequences, and active analogs and precursor forms of the proteins. Also included in the term are nucleotide fragments of the gene that include at least about 8 contiguous base pairs, more preferably at least about 10-20 contiguous base pairs, and most preferably at least about 25 to 50, or more, contiguous base pairs of the gene, or any integers between these
10 values. Such fragments are useful as probes and in diagnostic methods, discussed more fully below.

The terms also include those forms possessing, as well as lacking, a signal sequence, if such is present, as well as the nucleic acid sequences coding therefore. Additionally, the term intends forms of the GapC proteins which lack a membrane anchor region, and nucleic
15 acid sequences encoding proteins with such deletions. Such deletions may be desirable in systems that do not provide for secretion of the protein. Furthermore, the plasmin-binding domains of the proteins, may or may not be present. Thus, for example, if the GapC plasmin-binding protein will be used to purify plasmin, the plasmin-binding domain will generally be retained. If the protein is to be used in vaccine compositions, immunogenic
20 epitopes which may or may not include the plasmin-binding domain, will be present.

The terms also include proteins in neutral form or in the form of basic or acid addition salts depending on the mode of preparation. Such acid addition salts may involve free amino groups and basic salts may be formed with free carboxyls. Pharmaceutically acceptable basic and acid addition salts are discussed further below. In addition, the
25 proteins may be modified by combination with other biological materials such as lipids (both those occurring naturally with the molecule or other lipids that do not destroy immunological activity) and saccharides, or by side chain modification, such as acetylation of amino groups, phosphorylation of hydroxyl side chains, oxidation of sulfhydryl groups, glycosylation of amino acid residues, as well as other modifications of the encoded primary
30 sequence.

The term therefore intends deletions, additions and substitutions to the sequence, so long as the polypeptide functions to produce an immunological response as defined herein. In this regard, particularly preferred substitutions will generally be conservative in nature,

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1 e., those substitutions that take place within a family of amino acids. For example, amino acids are generally divided into four families (1) acidic — aspartate and glutamate, (2) basic — lysine, arginine, histidine, (3) non-polar — alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan, and (4) uncharged polar — glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine. Phenylalanine, tryptophan, and tyrosine are sometimes classified as aromatic amino acids. For example, it is reasonably predictable that an isolated replacement of leucine with isoleucine or valine, or vice versa, an aspartate with a glutamate or vice versa, a threonine with a serine or vice versa, or a similar conservative replacement of an amino acid with a structurally related amino acid, will not have a major effect on the biological activity. Proteins having substantially the same amino acid sequence as the reference molecule, but possessing minor amino acid substitutions that do not substantially affect the immunogenicity and/or plasma-binding affinity of the protein, are therefore within the definition of the reference polypeptide.

For example, the polypeptide of interest may include up to about 5-10 conservative or non-conservative amino acid substitutions, or even up to about 15-25 or 20-50 conservative or non-conservative amino acid substitutions, or any integer between these values, so long as the desired function of the molecule remains intact.

In this regard, GapC proteins isolated from streptococci exhibit several variable regions in their amino acid sequences, located at amino acid positions 62 to 81, 102 to 112, 165 to 172, 248 to 271, and 286 to 305. These regions, which in *S. dysgalactiae*, *S. agalactiae*, *S. uberis*, *S. parauberis* and *S. iniae* exhibit from 1 to 9 amino acid substitutions, are likely to be amenable to variation without substantially affecting immunogenic or enzymatic function.

Similarly, substitutions occurring in the transmembrane binding domain, if present, and the signal sequence, if present, normally will not affect immunogenicity. One of skill in the art may readily determine other regions of the molecule of interest that can tolerate change by reference to the protein structure data shown in Figures 8-17 herein.

The term "streptococcal GapC protein" intends a GapC plasma-binding protein, as defined above, derived from a streptococcal species that produces the same, including, but not limited to *S. dysgalactiae*, *S. agalactiae*, *S. uberis*, *S. parauberis* and *S. iniae*. For example, a "*S. dysgalactiae* GapC protein" is a GapC plasma-binding protein as defined above, derived from *S. dysgalactiae*. Similarly, an "*S. agalactiae* GapC protein" intends a gapC binding protein derived from *S. agalactiae*.

"Wild type" or "native" proteins or polypeptides refer to proteins or polypeptides isolated from the source in which the proteins naturally occur. "Recombinant" polypeptides refer to polypeptides produced by recombinant DNA techniques, i.e., produced from cells transformed by an exogenous DNA construct encoding the desired polypeptide. "Synthetic" polypeptides are those prepared by chemical synthesis.

An "isolated" protein or polypeptide is a protein or polypeptide molecule separate and discrete from the whole organism with which the molecule is found in nature, or a protein or polypeptide devoid, in whole or part, of sequences normally associated with it in nature, or a sequence, as it exists in nature, but having heterologous sequences (as defined below) in association therewith.

The term "functionally equivalent" intends that the amino acid sequence of a GapC plasmin-binding protein is one that will elicit a substantially equivalent or enhanced immunological response, as defined above, as compared to the response elicited by a GapC plasmin-binding protein having identity with the reference GapC plasmin-binding protein, or an immunogenic portion thereof.

The term "epitope" refers to the site on an antigen or hapten to which specific B cells and/or T cells respond. The term is also used interchangeably with "antigenic determinant" or "antigenic determinant site." Antibodies that recognize the same epitope can be identified in a simple immunoassay showing the ability of one antibody to block the binding of another antibody to a target antigen.

The terms "immunogenic" protein or polypeptide refer to an amino acid sequence which elicits an immunological response as described above. An "immunogenic" protein or polypeptide, as used herein, includes the full-length sequence of the GapC plasmin-binding protein in question, with or without the signal sequence, membrane anchor domain and/or plasmin-binding domain, analogs thereof, or immunogenic fragments thereof. By "immunogenic fragment" is meant a fragment of a GapC plasmin-binding protein which includes one or more epitopes and thus elicits the immunological response described above. Such fragments can be identified using any number of epitope mapping techniques, well known in the art. See, e.g., *Epitope Mapping Protocols* in *Methods in Molecular Biology*, Vol. 66 (Glenn B. Morris, Ed., 1996) Humana Press, Totowa, New Jersey. For example, linear epitopes may be determined by e.g., concurrently synthesizing large numbers of peptides on solid supports, the peptides corresponding to portions of the protein molecule, and reacting the peptides with antibodies while the peptides are still attached to the

supports Such techniques are known in the art and described in, e.g., U.S. Patent No 4,708,871, Geysen et al. (1984) *Proc. Natl. Acad. Sci. USA* 81 3998-4002, Geysen et al (1986) *Molec. Immunol.* 23 709-715. Similarly, conformational epitopes are readily identified by determining spatial conformation of amino acids such as by, e.g., x-ray crystallography and 2-dimensional nuclear magnetic resonance. See, e.g., *Epitope Mapping Protocols supra*. Antigenic regions of proteins can also be identified using standard antigenicity and hydrophathy plots, such as those calculated using, e.g., the Omega version 1.0 software program available from the Oxford Molecular Group. This computer program employs the Hopp/Woods method, Hopp et al., *Proc. Natl. Acad. Sci. USA* (1981) 78 3824-3828 for determining antigenicity profiles and the Kyte-Doolittle technique, Kyte et al., *J. Mol. Biol.* (1982) 157 105-132 for hydrophathy plots. Figures 8 to 12 herein depict Kyte-Doolittle profiles for representative proteins encompassed by the invention.

Immunogenic fragments, for purposes of the present invention, will usually include at least about 3 amino acids, preferably at least about 5 amino acids, more preferably at least about 10-15 amino acids, and most preferably 25 or more amino acids, of the parent GapC plasmid-binding-binding protein molecule. There is no critical upper limit to the length of the fragment, which may comprise nearly the full-length of the protein sequence, or even a fusion protein comprising two or more epitopes of GapC.

An "immunogenic composition" is a composition that comprises an antigenic molecule where administration of the composition to a subject results in the development in the subject of a humoral and/or a cellular immune response to the antigenic molecule of interest.

By "subunit vaccine composition" is meant a composition containing at least one immunogenic polypeptide, but not all antigens, derived from or homologous to an antigen from a pathogen of interest. Such a composition is substantially free of intact pathogen cells or particles, or the lysate of such cells or particles. Thus, a "subunit vaccine composition" is prepared from at least partially purified (preferably substantially purified) immunogenic polypeptides from the pathogen, or recombinant analogs thereof. A subunit vaccine composition can comprise the subunit antigen or antigens of interest substantially free of other antigens or polypeptides from the pathogen.

By "pharmaceutically acceptable" or "pharmacologically acceptable" is meant a material which is not biologically or otherwise undesirable, i.e., the material may be administered to an individual in a formulation or composition without causing any

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undesirable biological effects or interacting in a deleterious manner with any of the components of the composition in which it is contained

An "immunological response" to a composition or vaccine is the development in the host of a cellular and/ or antibody-mediated immune response to the composition or vaccine of interest. Usually, an "immunological response" includes but is not limited to one or more of the following effects: the production of antibodies, B cells, helper T cells, suppressor T cells, and/or cytotoxic T cells and/or $\gamma\delta$ T cells, directed specifically to an antigen or antigens included in the composition or vaccine of interest. Preferably, the host will display either a therapeutic or protective immunological response such that resistance of the mammary gland to new infection will be enhanced and/or the clinical severity of the disease reduced. Such protection will be demonstrated by either a reduction or lack of symptoms normally displayed by an infected host and/or a quicker recovery time

By "nucleic acid immunization" is meant the introduction of a nucleic acid molecule encoding one or more selected antigens into a host cell, for the *in vivo* expression of an antigen, antigens, an epitope, or epitopes. The nucleic acid molecule can be introduced directly into a recipient subject, such as by injection, inhalation, oral, intranasal and mucosal administration, or the like, or can be introduced *ex vivo*, into cells which have been removed from the host. In the latter case, the transformed cells are reintroduced into the subject where an immune response can be mounted against the antigen encoded by the nucleic acid molecule.

The term "treatment" as used herein refers to either (1) the prevention of infection or reinfection (prophylaxis), or (2) the reduction or elimination of symptoms of the disease of interest (therapy)

By "mastitis" is meant an inflammation of the mammary gland in mammals, including in cows, ewes, goats, sows, mares, and the like, caused by the presence of *S. uberis*. The infection manifests itself by the infiltration of phagocytic cells in the gland. Generally, 4 clinical types of mastitis are recognized. (1) peracute, associated with swelling, heat, pain, and abnormal secretion in the gland and accompanied by fever and other signs of systemic disturbance, such as marked depression, rapid weak pulse, sunken eyes, weakness and complete anorexia, (2) acute, with changes in the gland similar to those above but where fever, anorexia and depression are slight to moderate, (3) subacute, where no systemic changes are displayed and the changes in the gland and its secretion are less

marked, and (4) subclinical, where the inflammatory reaction is detectable only by standard tests for mastitis

Standard tests for the detection of mastitis include but are not limited to, the California Mastitis Test, the Wisconsin Mastitis Test, the Nagase test, the electronic cell count and somatic cell counts used to detect a persistently high white blood cell content in milk. In general, a somatic cell count of about 300,000 to about 500,000 cells per ml or higher, in milk will indicate the presence of infection. Thus, a vaccine is considered effective in the treatment and/or prevention of mastitis when, for example, the somatic cell count in milk is retained below about 500,000 cells per ml. For a discussion of mastitis and the diagnosis thereof, see, e.g., *The Merck Veterinary Manual A Handbook of Diagnosis Therapy and Disease Prevention and Control for the Veterinarian*, Merck and Co., Rahway, New Jersey, 1991

By the terms "vertebrate," "subject," and "vertebrate subject" are meant any member of the subphylum Chordata, including, without limitation, mammals such as cattle, sheep, pigs, goats, horses, and humans, domestic animals such as dogs and cats, and birds, including domestic, wild and game birds such as cocks and hens including chickens, turkeys and other gallinaceous birds, and fish. The term does not denote a particular age. Thus, both adult and newborn animals, as well as fetuses, are intended to be covered.

A "nucleic acid" molecule can include, but is not limited to, procaryotic sequences, eucaryotic mRNA, cDNA from eucaryotic mRNA, genomic DNA sequences from eucaryotic (e.g., mammalian) DNA, and even synthetic DNA sequences. The term also captures sequences that include any of the known base analogs of DNA and RNA.

An "isolated" nucleic acid molecule is a nucleic acid molecule separate and discrete from the whole organism with which the molecule is found in nature, or a nucleic acid molecule devoid, in whole or part, of sequences normally associated with it in nature, or a sequence, as it exists in nature, but having heterologous sequences (as defined below) in association therewith. The term "isolated" in the context of a polynucleotide intends that the polynucleotide is isolated from the chromosome with which it is normally associated, and is isolated from the complete genomic sequence in which it normally occurs

"Purified polynucleotide" refers to a polynucleotide of interest or fragment thereof which is essentially free, e.g., contains less than about 50%, preferably less than about 70%, and more preferably less than about 90%, of the protein with which the polynucleotide is naturally associated. Techniques for purifying polynucleotides of interest are well-known

in the art and include, for example, disruption of the cell containing the polynucleotide with a chaotropic agent and separation of the polynucleotide(s) and proteins by ion-exchange chromatography, affinity chromatography and sedimentation according to density

A "coding sequence" or a "nucleotide sequence encoding" a particular protein, is a nucleotide sequence which is transcribed and translated into a polypeptide *in vitro* or *in vivo* when placed under the control of appropriate regulatory elements. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A coding sequence can include, but is not limited to, procaryotic sequences, cDNA from eucaryotic mRNA, genomic DNA sequences from eucaryotic (e.g., mammalian) DNA, and even synthetic DNA sequences. A transcription termination sequence will usually be located 3' to the coding sequence. A "complementary" sequence is one in which the nitrogenous base at a given nucleotide position is the complement of the nitrogenous base appearing at the same position in the reference sequence. To illustrate, the complement of adenosine is thymine, and vice versa, similarly, cytosine is complementary to guanine, and vice versa, hence, the complement of the reference sequence 5'-ATGCTGA-3' would be 5'-TACGACT-3'.

A "wild-type" or "native" sequence, as used herein, refers to polypeptide encoding sequences that are essentially as they are found in nature, e.g., the *S. dysgalactiae* GapC protein encoding sequences depicted in Figures 1A-1B (SEQ ID NO 4).

"Recombinant" as used herein to describe a nucleic acid molecule means a polynucleotide of genomic, cDNA, semisynthetic, or synthetic origin which, by virtue of its origin or manipulation (1) is not associated with all or a portion of the polynucleotide with which it is associated in nature, and/or (2) is linked to a polynucleotide other than that to which it is linked in nature. The term "recombinant" as used with respect to a protein or polypeptide means a polypeptide produced by expression of a recombinant polynucleotide. "Recombinant host cells," "host cells," "cells," "cell lines," "cell cultures," and other such terms denoting procaryotic microorganisms or eucaryotic cell lines cultured as unicellular entities, are used interchangeably, and refer to cells which can be, or have been, used as recipients for recombinant vectors or other transfer DNA, and include the progeny of the original cell which has been transfected. It is understood that the progeny of a single parental cell may not necessarily be completely identical in morphology or in genomic or total DNA complement to the original parent, due to accidental or deliberate mutation. Progeny of the parental cell which are sufficiently similar to the parent to be characterized

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by the relevant property, such as the presence of a nucleotide sequence encoding a desired peptide, are included in the progeny intended by this definition, and are covered by the above terms

"Homology" refers to the percent identity between two polynucleotide or two polypeptide moieties. Two DNA, or two polypeptide sequences are "substantially homologous" to each other when the sequences exhibit at least about 80%-85%, preferably at least about 90%, and most preferably at least about 95%-98% sequence identity over a defined length of the molecules. As used herein, substantially homologous also refers to sequences showing complete identity to the specified DNA or polypeptide sequence.

In general, "identity" refers to an exact nucleotide-to-nucleotide or amino acid to-amino acid correspondence of two polynucleotides or polypeptide sequences, respectively. Percent identity can be determined by a direct comparison of the sequence information between two molecules by aligning the sequences, counting the exact number of matches between the two aligned sequences, dividing by the length of the shorter sequence, and multiplying the result by 100. Readily available computer programs can be used to aid in the analysis, such as ALIGN, Dayhoff, M.O. in *Atlas of Protein Sequence and Structure* M.O. Dayhoff ed., 5 Suppl. 3 353-358, National biomedical Research Foundation, Washington, DC, which adapts the local homology algorithm of Smith and Waterman (1981) *Advances in Appl. Math.* 2 482-489 for peptide analysis. Programs for determining nucleotide sequence identity are available in the Wisconsin Sequence Analysis Package, Version 8 (available from Genetics Computer Group, Madison, WI) for example, the BESTFIT, FASTA and GAP programs, which also rely on the Smith and Waterman algorithm. These programs are readily utilized with the default parameters recommended by the manufacturer and described in the Wisconsin Sequence Analysis Package referred to above. For example, percent identity of a particular nucleotide sequence to a reference sequence can be determined using the homology algorithm of Smith and Waterman with a default scoring table and a gap penalty of six nucleotide positions.

Another method of establishing percent identity in the context of the present invention is to use the MPSRCH package of programs copyrighted by the University of Edinburgh, developed by John F. Collins and Shane S. Sturrock, and distributed by IntelliGenetics, Inc. (Mountain View, CA). From this suite of packages the Smith-Waterman algorithm can be employed where default parameters are used for the scoring table (for example, gap open penalty of 12, gap extension penalty of one, and a gap of six).

From the data generated the "Match" value reflects "sequence identity." Other suitable programs for calculating the percent identity or similarity between sequences are generally known in the art, for example, another alignment program is BLAST, used with default parameters. For example, BLASTN and BLASTP can be used using the following default parameters: genetic code = standard, filter = none, strand = both, cutoff = 60, expect = 10, Matrix = BLOSUM62, Descriptions = 50 sequences, sort by = HIGH SCORE, Databases = non-redundant, GenBank + EMBL + DDBJ + PDB + GenBank CDS translations + Swiss protein + Spupdate + PIR. Details of these programs can be found at the following internet address: <http://www.ncbi.nlm.gov/cgi-bin/BLAST>

Alternatively, homology can be determined by hybridization of polynucleotides under conditions which form stable duplexes between homologous regions, followed by digestion with single-stranded-specific nuclease(s), and size determination of the digested fragments. DNA sequences that are substantially homologous can be identified in a Southern hybridization experiment under, for example, stringent conditions, as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. See, e.g., Sambrook et al., *supra*, *DNA Cloning, supra*, *Nucleic Acid Hybridization, supra*

By the term "degenerate variant" is intended a polynucleotide containing changes in the nucleic acid sequence thereof, that encodes a polypeptide having the same amino acid sequence as the polypeptide encoded by the polynucleotide from which the degenerate variant is derived.

Techniques for determining amino acid sequence "similarity" are well known in the art. In general, "similarity" means the exact amino acid to amino acid comparison of two or more polypeptides at the appropriate place, where amino acids are identical or possess similar chemical and/or physical properties such as charge or hydrophobicity. A so-termed "percent similarity" then can be determined between the compared polypeptide sequences. Techniques for determining nucleic acid and amino acid sequence identity also are well known in the art and include determining the nucleotide sequence of the mRNA for that gene (usually via a cDNA intermediate) and determining the amino acid sequence encoded thereby, and comparing this to a second amino acid sequence. In general, "identity" refers to an exact nucleotide to nucleotide or amino acid to amino acid correspondence of two polynucleotides or polypeptide sequences, respectively.

A "heterologous" region of a DNA construct is an identifiable segment of DNA within or attached to another DNA molecule that is not found in association with the other molecule in nature. Thus, when the heterologous region encodes a bacterial gene, the gene will usually be flanked by DNA that does not flank the bacterial gene in the genome of the source bacteria. Another example of the heterologous coding sequence is a construct where the coding sequence itself is not found in nature (e.g., synthetic sequences having codons different from the native gene). Allelic variation or naturally occurring mutational events do not give rise to a heterologous region of DNA, as used herein.

A "vector" is a replicon, such as a plasmid, phage, or cosmid, to which another DNA segment may be attached so as to bring about the replication of the attached segment. A vector is capable of transferring gene sequences to target cells (e.g., bacterial plasmid vectors, viral vectors, non-viral vectors, particulate carriers, and liposomes).

Typically, the terms "vector construct," "expression vector," "gene expression vector," "gene delivery vector," "gene transfer vector," and "expression cassette" all refer to an assembly which is capable of directing the expression of a sequence or gene of interest. Thus, the terms include cloning and expression vehicles, as well as viral vectors.

These assemblies include a promoter which is operably linked to the sequences or gene(s) of interest. Other control elements may be present as well. The expression cassettes described herein may be contained within a plasmid construct. In addition to the components of the expression cassette, the plasmid construct may also include a bacterial origin of replication, one or more selectable markers, a signal which allows the plasmid construct to exist as single-stranded DNA (e.g., a M13 origin of replication), a multiple cloning site, and a "mammalian" origin of replication (e.g., a SV40 or adenovirus origin of replication).

DNA "control elements" refers collectively to transcription promoters, transcription enhancer elements, transcription termination sequences, polyadenylation sequences (located 3' to the translation stop codon), sequences for optimization of initiation of translation (located 5' to the coding sequence), translation termination sequences, upstream regulatory domains, ribosome binding sites and the like, which collectively provide for the transcription and translation of a coding sequence in a host cell. See e.g., McCaughan et al (1995) *PNAS USA* 92 5431-5435, Kochetov et al (1998) *FEBS Letts* 440 351-355. Not all of these control sequences need always be present in a recombinant vector so long as the desired gene is capable of being transcribed and translated.

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"Operably linked" refers to an arrangement of elements wherein the components so described are configured so as to perform their usual function. Thus, control elements operably linked to a coding sequence are capable of effecting the expression of the coding sequence. The control elements need not be contiguous with the coding sequence, so long as they function to direct the expression thereof. Thus, for example, intervening untranslated yet transcribed sequences can be present between a promoter and the coding sequence and the promoter can still be considered "operably linked" to the coding sequence. Similarly, "control elements compatible with expression in a subject" are those which are capable of effecting the expression of the coding sequence in that subject.

A control element, such as a promoter, "directs the transcription" of a coding sequence in a cell when RNA polymerase will bind the promoter and transcribe the coding sequence into mRNA, which is then translated into the polypeptide encoded by the coding sequence.

A "host cell" is a cell which has been transformed, or is capable of transformation, by an exogenous nucleic acid molecule.

A cell has been "transformed" by exogenous DNA when such exogenous DNA has been introduced inside the cell membrane. Exogenous DNA may or may not be integrated (covalently linked) into chromosomal DNA making up the genome of the cell. In prokaryotes and yeasts, for example, the exogenous DNA may be maintained on an episomal element, such as a plasmid. With respect to eucaryotic cells, a stably transformed cell is one in which the exogenous DNA has become integrated into the chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eucaryotic cell to establish cell lines or clones comprised of a population of daughter cells containing the exogenous DNA.

As used herein, a "biological sample" refers to a sample of tissue or fluid isolated from a subject, including but not limited to, for example, blood, plasma, serum, fecal matter, urine, bone marrow, bile, spinal fluid, lymph fluid, samples of the skin, external secretions of the skin, respiratory, intestinal, and genitourinary tracts, tears, saliva, milk, blood cells, organs, biopsies and also samples of *in vitro* cell culture constituents including but not limited to conditioned media resulting from the growth of cells and tissues in culture medium, e.g., recombinant cells, and cell components.

As used herein, the terms "label" and "detectable label" refer to a molecule capable of detection, including, but not limited to, radioactive isotopes, fluorescers,

chemiluminescers, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors, chromophores, dyes, metal ions metal sols, ligands (e.g., biotin or haptens) and the like. The term "fluorescer" refers to a substance or a portion thereof which is capable of exhibiting fluorescence in the detectable range. Particular examples of labels which may be used under the invention include fluorescein, rhodamine, dansyl, umbelliferone, Texas red, lumol, NADPH and α - β -galactosidase.

2 MODES OF CARRYING OUT THE INVENTION

Before describing the present invention in detail, it is to be understood that this invention is not limited to particular formulations or process parameters as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting.

Although a number of methods and materials similar or equivalent to those described herein can be used in the practice of the present invention, the preferred materials and methods are described herein.

Central to the present invention is the discovery that the GapC protein is capable of eliciting an immune response in a vertebrate subject. In particular, the genes for the GapC protein in *S. dysgalactiae*, *S. agalactiae*, *S. uberis*, *S. parauberis* and *S. iniae* have been isolated, sequenced and characterized, and the protein sequences for those genes have been deduced therefrom. The complete DNA sequences for those genes and the corresponding amino acid sequences are shown in Figures 1 through 5.

As described in the examples, the full-length *S. dysgalactiae* gapC gene, depicted at nucleotide positions 1-1011, inclusive, of Figures 1A-1B, encodes the full-length *S. dysgalactiae* GapC protein of 336 amino acids, shown as amino acids 1-336, inclusive, of the same figure. *S. dysgalactiae* GapC has a predicted molecular weight of about 36 kDa. (calculated using the Peptide Sort program of the GCG Wisconsin Package, version 10, provided by the SeqWeb sequence analysis package, version 1.1 of the Canadian Bioinformatics Resource). Similarly, the gapC genes isolated from *S. agalactiae*, *S. uberis*, *S. parauberis* and *S. iniae* are depicted in Figures 2 through 5, each encodes a full-length GapC protein also of 336 amino acids, each also having a predicted molecular weight of about 36 kDa. None of the full-length sequences appear to include a signal peptide or a membrane anchor region.

Figures 6 and 7 show an alignment of DNA and amino acid sequences, respectively, showing regions of homology and variability that exist among GapC proteins from various streptococci strains. In particular, several variable regions are located at amino acid positions 62 to 81, 102 to 112, 165 to 172, 248 to 271, and 286 to 305. Such variable regions are typically more amenable to change. Hence, amino acid changes in these regions, such as substitutions, additions and deletions, are likely tolerated.

Figures 8 through 12 show plots of the following for each of the GapC proteins of the present invention: Kyte-Doolittle hydrophathy, averaged over a window of 7, surface probability according to Emini, chain flexibility according to Karplus-Schulz, antigenicity index according to Jameson-Wolf, secondary structure according to Garnier-Osguthorpe-Robson, secondary structure according to Chou-Fasman, and predicted glycosylation sites. Figures 13 through 17 show plots of secondary structure according to Chou-Fasman for each of the GapC proteins of the present invention. One of skill in the art can readily use the information presented in Figures 8 to 17 to determine immunogenic regions in the protein for use in vaccine compositions.

S. dysgalactiae GapC plasmin-binding protein, immunogenic fragments thereof or chimeric proteins including the same, can be provided in subunit vaccine compositions. In addition to use in vaccine compositions, the proteins or antibodies thereto can be used as diagnostic reagents to detect the presence of infection in a vertebrate subject. Similarly, the genes encoding the proteins can be cloned and used to design probes to detect and isolate homologous genes in other bacterial strains. For example, fragments comprising at least about 15-20 nucleotides, more preferably at least about 20-50 nucleotides, and most preferably about 60-100 nucleotides, or any integer between these values, will find use in these embodiments.

The vaccine compositions of the present invention can be used to treat or prevent a wide variety of bacterial infections in vertebrate subjects. For example, vaccine compositions including GapC plasmin-binding proteins from *S. dysgalactiae*, *S. uberis*, *S. parauberis*, *S. iniae* and/or group B streptococci (GBS) such as *S. agalactiae*, can be used to treat streptococcal infections in vertebrate subjects that are caused by these or other species. In particular, *S. uberis* and *S. agalactiae* are common bacterial pathogens associated with mastitis in bovine, equine, ovine and goat species. Additionally, group B streptococci, such as *S. agalactiae*, are known to cause numerous other infections in vertebrates including septicemia, meningitis, bacteremia, impetigo, arthritis, urinary tract

infections, abscesses, spontaneous abortion etc. Hence, vaccine compositions containing streptococcal GapC plasmin-binding proteins will find use in treating and/or preventing a wide variety of streptococcal infections.

Similarly, GapC plasmin-binding proteins derived from other bacterial genera such as *Staphylococcus*, *Mycobacterium*, *Escherichia*, *Pseudomonas*, *Nocardia*, *Pasteurella*, *Clostridium* and *Mycoplasma* will find use for treating bacterial infections caused by species belonging to those genera. Thus, it is readily apparent that GapC plasmin-binding proteins can be used to treat and/or prevent a wide variety of bacterial infections in numerous species.

The streptococcal GapC plasmin-binding proteins of the present invention can be used in vaccine compositions either alone or in combination with other bacterial, fungal, viral or protozoal antigens. These antigens can be provided separately or even as fusion proteins comprising one or more epitopes of a GapC plasmin-binding protein fused to one or more of these antigens. For example, other immunogenic proteins from *S. uberis*, such as the CAMP factor, hyaluronic acid capsule, hyaluronidase, R-like protein and plasminogen activator, can be administered with the GapC protein. Additionally, immunogenic proteins from other organisms involved in mastitis, such as from the genera *Staphylococcus*, *Corynebacterium*, *Pseudomonas*, *Nocardia*, *Clostridium*, *Mycobacterium*, *Mycoplasma*, *Pasteurella*, *Prototheca*, other streptococci, coliform bacteria, as well as yeast, can be administered along with the GapC plasmin-binding proteins described herein to provide a broad spectrum of protection. Thus, for example, immunogenic proteins from *Staphylococcus aureus*, *Str. agalactiae*, *Str. dysgalactiae*, *Str. zooepidemicus*, *Corynebacterium pyogenes*, *Pseudomonas aeruginosa*, *Nocardia asteroides*, *Clostridium perfringens*, *Escherichia coli*, *Enterobacter aerogenes* and *Klebsiella spp.* can be provided along with the GapC plasmin-binding proteins of the present invention.

Additionally, GapC proteins from different streptococcal species may be used together in the vaccine compositions of the present invention. In this embodiment, the multiple GapC proteins may be provided as fusion proteins or as discrete antigens in the same or different vaccine compositions.

Production of GapC Plasmin-Binding Proteins

The above-described plasmin-binding proteins and active fragments, analogs and chimeric proteins derived from the same, can be produced by variety of methods.

Specifically, GapC plasmin-binding proteins can be isolated directly from bacteria which express the same. This is generally accomplished by first preparing a crude extract which lacks cellular components and several extraneous proteins. The desired proteins can then be further purified from the cell lysate fraction by e.g., column chromatography, HPLC

5 immunoadsorbent techniques or other conventional methods well known in the art

More particularly techniques for isolating GapC plasmin-binding proteins have been described. For example, the GapC protein of *S. pyogenes* was purified from a crude cell extract by precipitation with ammonium sulfate, followed by two cycles of chromatography through a Mono FPLC column, and single cycles through superose 12
10 FPLC, and TSK-phenol HPLC columns (Pancholi, V and Fischetti, VA (1992) *J Exptl Med* 76 415-426). Another technique involves the use of a NAD⁺-agarose affinity column to purify GapC from lysed protoplasts of *S. pyogenes* strain 64/14 (Winram, SB and Lottenberg, R (1996) *Microbiol.* 142 2311-2320)

Alternatively, the proteins can be recombinantly produced as described herein. As
15 explained above, these recombinant products can take the form of partial protein sequences, full-length sequences, precursor forms that include signal sequences, mature forms without signals, or even fusion proteins (e.g., with an appropriate leader for the recombinant host, or with another subunit antigen sequence for *Streptococcus* or another pathogen)

In one embodiment of the present invention, the GapC proteins are fused to a
20 histidine tag, produced by recombinant means, and are then purified from a cell lysate fraction using affinity chromatography. See Example 1A-E, *infra*.

The GapC plasmin-binding proteins of the present invention can be isolated based on the ability of the protein products to bind plasmin, using plasmin-binding assays as described below. See, e.g., the method described in section F 3 of Example 1, *infra*. Thus,
25 gene libraries can be constructed and the resulting clones used to transform an appropriate host cell. Colonies can be pooled and screened for clones having plasmin-binding activity. Colonies can also be screened using polyclonal serum or monoclonal antibodies to the plasmin-binding protein.

Alternatively, once the amino acid sequences are determined, oligonucleotide probes
30 which contain the codons for a portion of the determined amino acid sequences can be prepared and used to screen genomic or cDNA libraries for genes encoding the subject proteins. The basic strategies for preparing oligonucleotide probes and DNA libraries, as well as their screening by nucleic acid hybridization, are well known to those of ordinary

skill in the art. See, e.g. *DNA Cloning* Vol. 1I, *supra*, *Nucleic Acid Hybridization*, *supra*,
Oligonucleotide Synthesis, *supra*, Sambrook et al, *supra*. Once a clone from the screened
library has been identified by positive hybridization, it can be confirmed by restriction
enzyme analysis and DNA sequencing that the particular library insert contains GapC
plasmin-binding protein gene or a homolog thereof. The genes can then be further isolated
using standard techniques and, if desired, PCR approaches or restriction enzymes employed
to delete portions of the full-length sequence.

Similarly, genes can be isolated directly from bacteria using known techniques, such
as phenol extraction and the sequence further manipulated to produce any desired
alterations. See, e.g., Sambrook et al., *supra*, for a description of techniques used to obtain
and isolate DNA.

Alternatively, DNA sequences encoding the proteins of interest can be prepared
synthetically rather than cloned. The DNA sequences can be designed with the appropriate
codons for the particular amino acid sequence. In general, one will select preferred codons
for the intended host if the sequence will be used for expression. The complete sequence is
assembled from overlapping oligonucleotides prepared by standard methods and assembled
into a complete coding sequence. See, e.g., Edge (1981) *Nature* 292 756, Nambair et al.
(1984) *Science* 223 1299, Jay et al. (1984) *J Biol Chem.* 259 6311

Once coding sequences for the desired proteins have been prepared or isolated, they
can be cloned into any suitable vector or replicon. Numerous cloning vectors are known to
those of skill in the art, and the selection of an appropriate cloning vector is a matter of
choice. Examples of recombinant DNA vectors for cloning and host cells which they can
transform include the bacteriophage λ (*E. coli*), pBR322 (*E. coli*), pACYC177 (*E. coli*)
pKT230 (gram-negative bacteria), pGV1106 (gram-negative bacteria), pLAFR1
(gram-negative bacteria), pME290 (non-*E. coli* gram-negative bacteria), pHV14 (*E. coli* and
Bacillus subtilis), pBD9 (*Bacillus*), pIJ61 (*Streptomyces*), pUC6 (*Streptomyces*), YIp5
(*Saccharomyces*), YCp19 (*Saccharomyces*) and bovine papilloma virus (mammalian cells)
See, Sambrook et al, *supra*, *DNA Cloning*, *supra*, B. Perbal, *supra*.

The gene can be placed under the control of a promoter, ribosome binding site (for
bacterial expression) and, optionally, an operator (collectively referred to herein as "control"
elements) so that the DNA sequence encoding the desired protein is transcribed into RNA
in the host cell transformed by a vector containing this expression construction. The coding
sequence may or may not contain a signal peptide or leader sequence. If a signal sequence

is included, it can either be the native, homologous sequence, or a heterologous sequence. For example, the signal sequence for *S. dysgalactiae* GapC plasmin-binding protein can be used for secretion thereof, as can a number of other signal sequences, well known in the art. Leader sequences can be removed by the host in post-translational processing. See, e.g.,
5 U S Patent Nos 4,431,739, 4,425,437, 4,338,397

Other regulatory sequences may also be desirable which allow for regulation of expression of the protein sequences relative to the growth of the host cell. Regulatory sequences are known to those of skill in the art, and examples include those which cause the expression of a gene to be turned on or off in response to a chemical or physical stimulus,
10 including the presence of a regulatory compound. Other types of regulatory elements may also be present in the vector, for example, enhancer sequences

The control sequences and other regulatory sequences may be ligated to the coding sequence prior to insertion into a vector, such as the cloning vectors described above. Alternatively the coding sequence can be cloned directly into an expression vector which
15 already contains the control sequences and an appropriate restriction site

In some cases it may be necessary to modify the coding sequence so that it may be attached to the control sequences with the appropriate orientation, i.e., to maintain the proper reading frame. It may also be desirable to produce mutants or analogs of the GapC plasmin-binding protein. Mutants or analogs may be prepared by the deletion of a portion
20 of the sequence encoding the protein, by insertion of a sequence, and/or by substitution of one or more nucleotides within the sequence. Techniques for modifying nucleotide sequences, such as site-directed mutagenesis, are described in, e.g., Sambrook et al., *supra*, *DNA Cloning, supra*, *Nucleic Acid Hybridization, supra*

The expression vector is then used to transform an appropriate host cell. A number
25 of mammalian cell lines are known in the art and include immortalized cell lines available from the American Type Culture Collection (ATCC), such as, but not limited to, Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), Madin-Darby bovine kidney ("MDBK") cells, as well as others. Similarly, bacterial hosts such as *E. coli*,
30 *Bacillus subtilis*, and *Streptococcus spp*, will find use with the present expression constructs. Yeast hosts useful in the present invention include *inter alia*, *Saccharomyces cerevisiae*, *Candida albicans*, *Candida maltosa*, *Hansenula polymorpha*, *Kluyveromyces fragilis*, *Kluyveromyces lactis*, *Pichia guilliermondii*, *Pichia pastoris*, *Schizosaccharomyces*

pombe and *Yarrowia lipolytica* Insect cells for use with baculovirus expression vectors include, *inter alia*, *Aedes aegypti*, *Autographa californica*, *Bombyx mori*, *Drosophila melanogaster*, *Spodoptera frugiperda*, and *Trichoplusia ni*

Depending on the expression system and host selected, the proteins of the present invention are produced by culturing host cells transformed by an expression vector described above under conditions whereby the protein of interest is expressed. The protein is then isolated from the host cells and purified. If the expression system secretes the protein into the growth media, the protein can be purified directly from the media. If the protein is not secreted, it is isolated from cell lysates. The selection of the appropriate growth conditions and recovery methods are within the skill of the art.

The proteins of the present invention may also be produced by chemical synthesis such as solid phase peptide synthesis, using known amino acid sequences or amino acid sequences derived from the DNA sequence of the genes of interest. Such methods are known to those skilled in the art. See, e.g., J. M. Stewart and J. D. Young, *Solid Phase Peptide Synthesis*, 2nd Ed., Pierce Chemical Co., Rockford, IL (1984) and G. Barany and R. B. Merrifield, *The Peptides: Analysis, Synthesis, Biology*, editors E. Gross and J. Meienhofer, Vol. 2, Academic Press, New York, (1980), pp. 3-254, for solid phase peptide synthesis techniques, and M. Bodansky, *Principles of Peptide Synthesis*, Springer-Verlag, Berlin (1984) and E. Gross and J. Meienhofer, Eds. *The Peptides: Analysis, Synthesis, Biology*, *supra*, Vol. 1, for classical solution synthesis. Chemical synthesis of peptides may be preferable if a small fragment of the antigen in question is capable of raising an immunological response in the subject of interest.

The GapC plasmin-binding proteins of the present invention, or their fragments, can be used to produce antibodies, both polyclonal and monoclonal. If polyclonal antibodies are desired, a selected mammal, (e.g., mouse, rabbit, goat, horse, etc.) is immunized with an antigen of the present invention, or its fragment, or a mutated antigen. Serum from the immunized animal is collected and treated according to known procedures. See, e.g., Jurgens et al (1985) *J Chrom.* 348 363-370. If serum containing polyclonal antibodies is used, the polyclonal antibodies can be purified by immunoaffinity chromatography, using known procedures.

Monoclonal antibodies to the GapC plasmin-binding proteins and to the fragments thereof, can also be readily produced by one skilled in the art. The general methodology for making monoclonal antibodies by using hybridoma technology is well known. Immortal

antibody-producing cell lines can be created by cell fusion, and also by other techniques such as direct transformation of B lymphocytes with oncogenic DNA, or transfection with Epstein-Barr virus. See, e.g., M. Schreier et al. *Hybridoma Techniques* (1980), Hammerling et al., *Monoclonal Antibodies and T-cell Hybridomas* (1981), Kennett et al. *Monoclonal Antibodies* (1980), see also U.S. Patent Nos. 4,341,761, 4,399,121, 4,427,783, 4,444,887, 4,452,570, 4,466,917, 4,472,500, 4,491,632, and 4,493,890. Panels of monoclonal antibodies produced against the GapC plasmin-binding proteins, or fragments thereof, can be screened for various properties, i.e., for isotype, epitope, affinity, etc. Monoclonal antibodies are useful in purification, using immunoaffinity techniques, of the individual antigens which they are directed against. Both polyclonal and monoclonal antibodies can also be used for passive immunization or can be combined with subunit vaccine preparations to enhance the immune response. Polyclonal and monoclonal antibodies are also useful for diagnostic purposes.

15 Vaccine Formulations and Administration

The GapC plasmin-binding proteins of the present invention can be formulated into vaccine compositions, either alone or in combination with other antigens, for use in immunizing subjects as described below. Methods of preparing such formulations are described in, e.g., *Remington's Pharmaceutical Sciences*, Mack Publishing Company, Easton, Pennsylvania, 18 Edition, 1990. Typically, the vaccines of the present invention are prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in or suspension in liquid vehicles prior to injection may also be prepared. The preparation may also be emulsified or the active ingredient encapsulated in liposome vehicles. The active immunogenic ingredient is generally mixed with a compatible pharmaceutical vehicle, such as, for example, water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. In addition, if desired, the vehicle may contain minor amounts of auxiliary substances such as wetting or emulsifying agents and pH buffering agents.

Adjuvants which enhance the effectiveness of the vaccine may also be added to the formulation. Adjuvants may include, for example, muramyl dipeptides, avridine, aluminum hydroxide, dimethyldioctadecyl ammonium bromide (DDA), oils, oil-in-water emulsions, saponins, cytokines, and other substances known in the art.

The GapC plasmin-binding protein may be linked to a carrier in order to increase the immunogenicity thereof. Suitable carriers include large, slowly metabolized macromolecules such as proteins, including serum albumins, keyhole limpet hemocyanin, immunoglobulin molecules, thyroglobulin, ovalbumin, and other proteins well known to those skilled in the art, polysaccharides, such as sepharose, agarose, cellulose, cellulose beads and the like, polymeric amino acids such as polyglutamic acid, polylysine, and the like, amino acid copolymers, and inactive virus particles

The GapC plasmin-binding proteins may be used in their native form or their functional group content may be modified by, for example, succinylation of lysine residues or reaction with Cys-thiolactone. A sulfhydryl group may also be incorporated into the carrier (or antigen) by for example, reaction of amino functions with 2-iminothiolane or the N-hydroxysuccinimide ester of 3-(4-dithiopyridyl) propionate. Suitable carriers may also be modified to incorporate spacer arms (such as hexamethylene diamine or other bifunctional molecules of similar size) for attachment of peptides

Other suitable carriers for the GapC plasmin-binding proteins of the present invention include VP6 polypeptides of rotaviruses, or functional fragments thereof, as disclosed in U S Patent No 5,071,651. Also useful is a fusion product of a viral protein and the subject immunogens made by methods disclosed in U S Patent No 4,722,840. Still other suitable carriers include cells, such as lymphocytes, since presentation in this form mimics the natural mode of presentation in the subject, which gives rise to the immunized state. Alternatively, the proteins of the present invention may be coupled to erythrocytes, preferably the subject's own erythrocytes. Methods of coupling peptides to proteins or cells are known to those of skill in the art.

Furthermore, the GapC plasmin-binding proteins (or complexes thereof) may be formulated into vaccine compositions in either neutral or salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the active polypeptides) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed from free carboxyl groups may also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

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Vaccine formulations will contain a "therapeutically effective amount" of the active ingredient, that is, an amount capable of eliciting an immune response in a subject to which the composition is administered. In the treatment and prevention of mastitis, for example, a "therapeutically effective amount" would preferably be an amount that enhances resistance of the mammary gland to new infection and/or reduces the clinical severity of the disease. Such protection will be demonstrated by either a reduction or lack of symptoms normally displayed by an infected host, a quicker recovery time and/or a lowered somatic cell count in milk from the infected quarter. For example, the ability of the composition to retain or bring the somatic cell count (SCC) in milk below about 500,000 cells per ml, the threshold value set by the International Dairy Federation, above which, animals are considered to have clinical mastitis, will be indicative of a therapeutic effect.

The exact amount is readily determined by one skilled in the art using standard tests. The GapC plasmid-binding protein concentration will typically range from about 1% to about 95% (w/w) of the composition, or even higher or lower if appropriate. With the present vaccine formulations, 5 to 500 µg of active ingredient per ml of injected solution should be adequate to raise an immunological response when a dose of 1 to 3 ml per animal is administered.

To immunize a subject, the vaccine is generally administered parenterally, usually by intramuscular injection. Other modes of administration, however, such as subcutaneous, intraperitoneal and intravenous injection, are also acceptable. The quantity to be administered depends on the animal to be treated, the capacity of the animal's immune system to synthesize antibodies, and the degree of protection desired. Effective dosages can be readily established by one of ordinary skill in the art through routine trials establishing dose response curves. The subject is immunized by administration of the vaccine in at least one dose, and preferably two doses. Moreover, the animal may be administered as many doses as is required to maintain a state of immunity to infection.

Additional vaccine formulations which are suitable for other modes of administration include suppositories and, in some cases, aerosol, intranasal, oral formulations, and sustained release formulations. For suppositories, the vehicle composition will include traditional binders and carriers, such as, polyalkaline glycols, or triglycerides. Such suppositories may be formed from mixtures containing the active ingredient in the range of about 0.5% to about 10% (w/w), preferably about 1% to about 2%. Oral vehicles include such normally employed excipients as, for example,

pharmaceutical grades of mannitol, lactose, starch, magnesium, stearate, sodium saccharin cellulose, magnesium carbonate, and the like. These oral vaccine compositions may be taken in the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations, or powders, and contain from about 10% to about 95% of the active ingredient, preferably about 25% to about 70%

Intranasal formulations will usually include vehicles that neither cause irritation to the nasal mucosa nor significantly disturb ciliary function. Diluents such as water, aqueous saline or other known substances can be employed with the subject invention. The nasal formulations may also contain preservatives such as, but not limited to, chlorobutanol and benzalkonium chloride. A surfactant may be present to enhance absorption of the subject proteins by the nasal mucosa.

Controlled or sustained release formulations are made by incorporating the protein into carriers or vehicles such as liposomes, nonresorbable impermeable polymers such as ethylenevinyl acetate copolymers and Hytrel® copolymers, swellable polymers such as hydrogels, or resorbable polymers such as collagen and certain polyacids or polyesters such as those used to make resorbable sutures. The GapC plasmin-binding proteins can also be delivered using implanted mini-pumps, well known in the art.

The GapC plasmin-binding proteins of the instant invention can also be administered via a carrier virus which expresses the same. Carrier viruses which will find use with the instant invention include but are not limited to the vaccinia and other pox viruses, adenovirus, and herpes virus. By way of example, vaccinia virus recombinants expressing the novel proteins can be constructed as follows. The DNA encoding the particular protein is first inserted into an appropriate vector so that it is adjacent to a vaccinia promoter and flanking vaccinia DNA sequences, such as the sequence encoding thymidine kinase (TK). This vector is then used to transfect cells which are simultaneously infected with vaccinia. Homologous recombination serves to insert the vaccinia promoter plus the gene encoding the instant protein into the viral genome. The resulting TK⁺ recombinant can be selected by culturing the cells in the presence of 5-bromodeoxyuridine and picking viral plaques resistant thereto.

An alternative route of administration involves gene therapy or nucleic acid immunization. Thus, nucleotide sequences (and accompanying regulatory elements) encoding the subject GapC plasmin-binding proteins can be administered directly to a subject for *in vivo* translation thereof. Alternatively, gene transfer can be accomplished by

transfecting the subject's cells or tissues *ex vivo* and reintroducing the transformed material into the host. DNA can be directly introduced into the host organism, i.e., by injection (see International Publication No WO/90/11092, and Wolff et al (1990) *Science* 247 1465-1468) Liposome-mediated gene transfer can also be accomplished using known methods See, e.g., Hazinski et al (1991) *Am. J Respir Cell Mol. Biol* 4 206-209, Brigham et al (1989) *Am. J Med Sci* 298 278-281, Canomco et al (1991) *Clin. Res* 39 219A, and Nabel et al (1990) *Science* 249 1285-1288 Targeting agents, such as antibodies directed against surface antigens expressed on specific cell types, can be covalently conjugated to the liposomal surface so that the nucleic acid can be delivered to specific tissues and cells susceptible to infection.

Diagnostic Assays

As explained above, the GapC plasmin-binding proteins of the present invention may also be used as diagnostics to detect the presence of reactive antibodies of streptococcus, for example *S. dysgalactiae* in a biological sample in order to determine the presence of streptococcus infection. For example, the presence of antibodies reactive with GapC plasmin-binding proteins can be detected using standard electrophoretic and immunodiagnostic techniques, including immunoassays such as competition, direct reaction, or sandwich type assays Such assays include, but are not limited to, Western blots, agglutination tests, enzyme-labeled and mediated immunoassays, such as ELISAs, biotin/avidin type assays, radioimmunoassays, immunoelectrophoresis, immunoprecipitation, etc The reactions generally include revealing labels such as fluorescent, chemiluminescent, radioactive, enzymatic labels or dye molecules, or other methods for detecting the formation of a complex between the antigen and the antibody or antibodies reacted therewith.

The aforementioned assays generally involve separation of unbound antibody in a liquid phase from a solid phase support to which antigen-antibody complexes are bound. Solid supports which can be used in the practice of the invention include substrates such as nitrocellulose (e.g., in membrane or microtiter well form), polyvinylchloride (e.g., sheets or microtiter wells), polystyrene latex (e.g., beads or microtiter plates), polyvinylidene fluoride, diazotized paper, nylon membranes, activated beads, magnetically responsive beads, and the like.

Typically, a solid support is first reacted with a solid phase component (e.g., one or more GapC plasmin-binding proteins) under suitable binding conditions such that the component is sufficiently immobilized to the support. Sometimes, immobilization of the antigen to the support can be enhanced by first coupling the antigen to a protein with better binding properties. Suitable coupling proteins include, but are not limited to, macromolecules such as serum albumins including bovine serum albumin (BSA), keyhole limpet hemocyanin, immunoglobulin molecules, thyroglobulin, ovalbumin, and other proteins well known to those skilled in the art. Other molecules that can be used to bind the antigens to the support include polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and the like. Such molecules and methods of coupling these molecules to the antigens, are well known to those of ordinary skill in the art. See, e.g., Brinkley, M.A. *Bioconjugate Chem.* (1992) 3:2-13; Hashida et al., *J. Appl. Biochem.* (1984) 6: 56-63, and Anjaneyulu and Staros, *International J. of Peptide and Protein Res.* (1987) 30: 117-124.

After reacting the solid support with the solid phase component, any non-immobilized solid-phase components are removed from the support by washing, and the support-bound component is then contacted with a biological sample suspected of containing ligand moieties (e.g., antibodies toward the immobilized antigens) under suitable binding conditions. After washing to remove any non-bound ligand, a secondary binder moiety is added under suitable binding conditions, wherein the secondary binder is capable of associating selectively with the bound ligand. The presence of the secondary binder can then be detected using techniques well known in the art.

More particularly, an ELISA method can be used, wherein the wells of a microtiter plate are coated with a GapC plasmin-binding protein. A biological sample containing or suspected of containing anti-GapC plasmin-binding protein immunoglobulin molecules is then added to the coated wells. After a period of incubation sufficient to allow antibody binding to the immobilized antigen, the plate(s) can be washed to remove unbound moieties and a detectably labeled secondary binding molecule added. The secondary binding molecule is allowed to react with any captured sample antibodies, the plate washed and the presence of the secondary binding molecule detected using methods well known in the art.

Thus, in one particular embodiment, the presence of bound anti-GapC plasmin-binding antigen ligands from a biological sample can be readily detected using a secondary binder comprising an antibody directed against the antibody ligands. A number of anti-

bovine immunoglobulin (Ig) molecules are known in the art which can be readily conjugated to a detectable enzyme label, such as horseradish peroxidase, alkaline phosphatase or urease, using methods known to those of skill in the art. An appropriate enzyme substrate is then used to generate a detectable signal. In other related embodiments, competitive-type ELISA techniques can be practiced using methods known to those skilled in the art.

Assays can also be conducted in solution, such that the GapC plasmin binding proteins and antibodies specific for those proteins form complexes under precipitating conditions. In one particular embodiment, GapC plasmin-binding proteins can be attached to a solid phase particle (e.g., an agarose bead or the like) using coupling techniques known in the art, such as by direct chemical or indirect coupling. The antigen-coated particle is then contacted under suitable binding conditions with a biological sample suspected of containing antibodies for the GapC plasmin-binding proteins. Cross-linking between bound antibodies causes the formation of particle-antigen-antibody complex aggregates which can be precipitated and separated from the sample using washing and/or centrifugation. The reaction mixture can be analyzed to determine the presence or absence of antibody-antigen complexes using any of a number of standard methods, such as those immunodiagnostic methods described above.

In yet a further embodiment, an immunoaffinity matrix can be provided, wherein a polyclonal population of antibodies from a biological sample suspected of containing anti-GapC plasmin-binding molecules is immobilized to a substrate. In this regard, an initial affinity purification of the sample can be carried out using immobilized antigens. The resultant sample preparation will thus only contain anti-streptococcus moieties, avoiding potential nonspecific binding properties in the affinity support. A number of methods of immobilizing immunoglobulins (either intact or in specific fragments) at high yield and good retention of antigen binding activity are known in the art. Not being limited by any particular method, immobilized protein A or protein G can be used to immobilize immunoglobulins.

Accordingly, once the immunoglobulin molecules have been immobilized to provide an immunoaffinity matrix, labeled GapC plasmin-binding proteins are contacted with the bound antibodies under suitable binding conditions. After any non-specifically bound antigen has been washed from the immunoaffinity support, the presence of bound antigen can be determined by assaying for label using methods known in the art.

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Additionally, antibodies raised to the GapC plasmin-binding proteins, rather than the GapC plasmin-binding proteins themselves, can be used in the above-described assays in order to detect the presence of antibodies to the proteins in a given sample. These assays are performed essentially as described above and are well known to those of skill in the art.

5 The above-described assay reagents, including the GapC plasmin-binding proteins, or antibodies thereto, can be provided in kits, with suitable instructions and other necessary reagents, in order to conduct immunoassays as described above. The kit can also contain, depending on the particular immunoassay used, suitable labels and other packaged reagents and materials (i.e. wash buffers and the like). Standard immunoassays, such as those
10 described above, can be conducted using these kits.

Deposits of Strains Useful in Practicing the Invention

A deposit of biologically pure cultures of the following strains was made with the American Type Culture Collection, 10801 University Boulevard, Manassas Virginia, under
15 the provisions of the Budapest Treaty. The accession number indicated was assigned after successful viability testing, and the requisite fees were paid. The designated deposits will be maintained for a period of thirty (30) years from the date of deposit, or for five (5) years after the last request for the deposit, whichever is longer. Should a culture become nonviable or be inadvertently destroyed, or, in the case of plasmid-containing strains, lose
20 its plasmid, it will be replaced with a viable culture(s) of the same taxonomic description.

Should there be a discrepancy between the sequence presented in the present application and the sequence of the gene of interest in the deposited plasmid due to routine sequencing errors, the sequence in the deposited plasmid controls.

25	Bacterial Strain	Plasmid	Gene	Deposit Date	ATCC No
	<i>E. coli</i> BL21 DE3	pET15bgapC	<i>gapC</i> (<i>S. dysgalactiae</i>)	May 31, 2000	PTA-1976
30	<i>E. coli</i> BL21 DE3	pMF521c	<i>gapC</i> (<i>S. agalactiae</i>)	May 31, 2000	PTA-1975
	<i>E. coli</i> BL21 DE3	pMF521a	<i>gapC</i> (<i>S. ubers</i>)	May 31, 2000	PTA-1973

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E. coli pMF521e
BL21 DE3

gapC
(*S. mae*)

May 31, 2000 PTA-1972

3 EXPERIMENTAL

Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way

Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperatures, etc.), but some experimental error and deviation should, of course, be allowed for

Materials and Methods

Enzymes were purchased from commercial sources, and used according to the manufacturers' directions

In the isolation of DNA fragments, except where noted, all DNA manipulations were done according to standard procedures. See, Sambrook et al., *supra*. Restriction enzymes, T₄ DNA ligase, *E. coli*, DNA polymerase II, Klenow fragment, and other biological reagents can be purchased from commercial suppliers and used according to the manufacturers' directions. Double stranded DNA fragments were separated on agarose gels

Sources for chemical reagents generally include Sigma Chemical Company, St. Louis, MO, Alrich, Milwaukee, WI, Roche Molecular Biochemicals, Indianapolis, IN

EXAMPLE 1

Preparation, Amplification, Sequencing, Expression, Purification and Characterization of the *S. dysgalactiae* GapC Plasmin Binding Protein

A. Preparation of *S. dysgalactiae* Chromosomal DNA

A clinical *S. dysgalactiae* isolate from a case of bovine mastitis (ATCC Accession No. ATCC43078) was obtained from the American Type Culture Collection (10801 University Boulevard, Manassas, VA 20110-2209), and was used as a source of DNA. The organism was routinely grown on TSA sheep blood agar plates (FML Microbiologicals, Mississauga, Ontario) at 37° C for 18 hours, or in Todd-Hewitt broth (Oxoid Ltd., Hampshire, England) supplemented with 0.3% yeast extract (THB-YE) at 37° C, 5% CO₂

Chromosomal DNA was prepared from *S. dysgalactiae* grown in 100 ml of THB-YE supplemented with 20 mM glycine for approximately 6 hours, until an A_{600} of 0.8 to 1.0 was reached. Cells were harvested and re-suspended in 50 mM EDTA, 50 mM Tris-HCl, 0.5% Tween-20® (Sigma, St. Louis, MO) and supplemented with RNase A (200 mg/ml),
5 proteinase K (20 mg/ml), lysozyme (100 mg/ml) and mutanolysin (100 mg/ml) (SIGMA, St. Louis, MO). Following bacterial lysis for 30 minutes at 37° C with vigorous shaking, guanidine hydrochloride and Tween-20®, pH 5.5, were mixed with the lysate to give a final concentration of 0.8 M and 5%, respectively. This mixture was incubated at 50° C for 30 minutes. The chromosomal DNA was then purified using a Qiagen genomic-tip 100g
10 (Qiagen, Santa Clarita, CA) and precipitated using 0.7 volumes of isopropanol. The resulting pellet was washed in 70% ethanol and re-suspended in 0.5 ml 10 mM Tris-HCl, pH 8.8.

B. Amplification and Cloning of the *S. dysgalactiae* gapC Gene

15 The *gapC* gene was amplified by PCR (See Mullis et al., U.S. Patent No. 4,683,195, Mullis, U.S. Patent No. 4,683,202,). The forward primer, *gapC1*, contained an *NdeI* restriction (SEQ ID NO 1, shown in Table 1) and the reverse primer, *gapC1r*, contained a *BamHI* site (SEQ ID NO 2, shown in Table 1). In the preceding primer sequences, depicted in Table 1, underlining denotes nucleotides added to the original sequence, and bolding
20 indicates the location of restriction endonuclease recognition sites.

PCR was carried out using Vent DNA polymerase (New England Biolabs, Mississauga, ON, Canada). 0.7 µg of *S. dysgalactiae* chromosomal DNA was incubated in a reaction mixture containing 1 µM of each of the preceding primers, 200 µM each of dATP, dTTP, dCTP and dGTP, 3mM MgSO₄, 1x concentration of Thermopol buffer (New
25 England Biolabs, Mississauga, ON, Canada) and 2 units Vent DNA polymerase. This mixture was incubated for 3 amplification cycles of 1 minute at 94°C, 3 minutes at 50°C and 1 minute, 10 seconds at 72°C, then for 27 amplification cycles at 15 seconds at 95°C, 30 seconds at 55°C, and 1 minute at 72°C, and finally for 1 cycle of 5 minutes at 72°C.

TABLE 1
Sequence Identification Numbers
and
Corresponding Nucleotide and Amino Acid Sequences

SEQ ID NO	Name	Sequence
1	Primer gapC1	5' <u>GG CGG CGG CAT</u> ATG GTA GTT AAA GTT GGT ATT AAC GG -3'
2	Primer gapC1r	5'- <u>GC GGA TCC</u> TTA TTT AGC GAT TTT TGC AAA GTA CTC -3'
3	<i>Streptococcus dysgalactiae</i> gapC gene	(see Figure 1)
4	<i>Streptococcus dysgalactiae</i> GapC protein	
5	<i>Streptococcus agalactiae</i> gapC gene	(see Figure 2)
6	<i>Streptococcus agalactiae</i> GapC protein	
7	<i>Streptococcus uberis</i> gapC gene	(see Figure 3)
8	<i>Streptococcus uberis</i> GapC protein	
9	<i>Streptococcus parauberis</i> gapC gene	(see Figure 4)
10	<i>Streptococcus parauberis</i> GapC protein	
11	<i>Streptococcus iniae</i> gapC gene	(see Figure 5)
12	<i>Streptococcus iniae</i> GapC protein	

The *gapC* PCR product was cloned into the expression vector pET15B (Novagen, Madison, WI) which had been digested with *Bam*HI and *Nde*I. Cloning of the PCR product into this site results in the addition of an in-frame coding sequence for a hexahistidyl tag to the *gapC* coding sequence. Subsequent expression yields a full-length protein with an attached histidine tag, which permits purification of the protein under non-denaturing conditions using metal chelate chromatography.

This construct was used to transform *E. coli* BL21 DE3 (Life Technologies, Gaithersburg, MD). This transformed strain was designated BL21 DE3 (pET15bgapC) (ATCC No.)

C. Isolation of Chromosomal DNA and Amplification and Cloning of the *gapC* Gene from *S. agalactiae*, *S. uberis*, *S. parauberis* and *S. miae*

The *gapC* genes were prepared from other isolates essentially as described above. Chromosomal DNA from *S. agalactiae*, *S. uberis* and *S. parauberis* was isolated from strains obtained from the American Type Culture Collection (10801 University Boulevard, Manassas, VA 20110-2209, strains designated ATCC 27541, 9927, and 13386, respectively). Chromosomal DNA from *S. miae* was isolated from a strain designated 9117 obtained from Mount Sinai Hospital, University of Toronto.

The primers used to amplify the *gapC* genes from the *Streptococcus* strains listed above were the same as those used in the case of *S. dysgalactiae*, i.e., primer *gapC1* (SEQ ID NO. 1) and primer *gapC1r* (SEQ ID NO. 2).

After amplification, the PCR product in each case was cloned into pPCR-Script, using the cloning protocol described in the PCR-Script Amp cloning Kit (Stratagene, La Jolla, CA). The PCR product insert was then excised using *Nde*I and *Bam*HI and re-cloned into those sites in pET15b using conventional cloning protocols (See e.g., Sambrook et al., *supra*). The plasmids containing the *S. agalactiae*, *S. uberis*, *S. parauberis* and *S. miae* were designated pMF521c (ATCC No.), pMF521a (ATCC No.), pMF521d (ATCC No.), and pMF521e (ATCC No.), respectively.

D Nucleotide Sequence of the gapC Gene and Deduced Amino Acid Sequences

Sequences homologous to *gapC* of *S. equisimilis* homolog (Gase, et al. (1996) *European J of Biochem.* 239 42-51) were originally identified while sequencing a linked but unrelated gene of *S. dysgalactiae*. To obtain the complete sequence of the *S. dysgalactiae gapC* gene, PCR was employed using the primers described above, i.e., primer *gapC1* and primer *gapC1r*

The sequence was determined using fluorescence tag terminators on an ABI 373 DNA automatic sequencer (Applied Biosystems, Emeryville, CA) at the Plant Biotechnology Institute (PBI, Saskatoon, Canada)

both 1 depicts the coding sequence of the *gapC* gene from *S. dysgalactiae* (DysGapC) (SEQ ID NO 3) and the deduced amino acid sequence (SEQ ID NO 4)

The sequences of the GapC proteins isolated from *S. agalactiae*, *S. uberis*, *S. parauberis* and *S. iniae* were determined by the same method.

Figures 2 through 5 depict both the nucleotide sequences and the predicted amino acid sequences for the *S. dysgalactiae* GapC protein (DysgalGapC) (SEQ ID NO 3 and SEQ ID NO 4), as well as for the GapC proteins of *S. agalactiae* (AgalGapC) (SEQ ID NO 5 and SEQ ID NO 6), *S. uberis* (UberGapC) (SEQ ID NO 7 and SEQ ID NO 8), *S. parauberis* (PUberGapC) (SEQ ID NO 9 and SEQ ID NO 10), and *S. iniae* (IniaeGapC) (SEQ ID NO 11 and SEQ ID NO 12), respectively

The *S. dysgalactiae* GapC protein gene depicted in Figures 1A-1B codes for a 336 amino acid protein which does not appear to contain either a signal sequence or membrane anchor domain. A search of the GenBank database using the BLASTX program revealed that the open reading frame was 95.5% homologous to GapC of *S. equisimilis* (GenBank Accession No. X97788) and 99.4% homologous to GapC of *S. pyogenes* (GenBank Accession No. M95569). The predicted amino acid sequence of the GapC protein also exhibited 43% amino acid identity to bovine glyceraldehyde-3-phosphate dehydrogenase (GenBank Accession No. U85042)

Similarly, for the *S. agalactiae*, *S. uberis*, *S. parauberis* and *S. iniae* GapC protein sequences, neither signal sequences nor membrane anchor domains appear to be present.

Sequence homologies are tabulated in Table 2

TABLE 2
Sequence Homologies Between Various GapC Protein Sequences

	<i>S. equianimilis</i>	<i>S. pyogenes</i>	Bovine GAPDH
<i>S. dysgalactiae</i>	95.5%	94.4%	43%
<i>S. agalactiae</i>	87.02%	91.07%	(not determined)
<i>S. parauberis</i>	86.31%	90.77%	
<i>S. uberis</i>	88.39%	92.26%	
<i>S. infantis</i>	86.31%	89.88%	

E. Expression and Purification of the Recombinant *S. dysgalactiae* GapC Plasmin Binding Protein

The Hexahistidyl-tagged GapC protein was expressed and purified under non-denaturing conditions using metal chelate (Ni-NTA) affinity chromatography

E. coli BL21 DE3 containing the recombinant plasmid was grown in Luria Broth, containing 100 µg/ml ampicillin to an A₆₀₀ of approximately 0.5. Expression of the GapC protein was then induced by the addition of 1 mM isopropyl-β-D-thiogalactoside (IPTG) (Sigma, St. Louis, MO]. Following three hours incubation at 37°C, cells were harvested, washed in column buffer (50 mM sodium phosphate buffer, pH8.0, 0.3 M NaCl, 10 mM imidazole) and lysed by sonication.

Approximately 40% of the recombinant protein was in the soluble fraction of the cell sonicate with a yield of approximately 50 mg of the recombinant protein per litre of culture volume, determined with a DC Protein Assay Kit (Bio-Rad Laboratories Mississauga, ON, Canada) using bovine serum albumin (Pierce, Rockford, IL.) as a standard.

The lysate was cleared by centrifugation and the soluble fraction was applied to a Ni-NTA column (Qiagen), which was subsequently washed with 10 column volumes of column buffer (as above, except containing 20 mM imidazole). The Hexahistidyl-tagged GapC was eluted using column buffer (as above, except containing 250 mM imidazole), yielding a homogenous protein fraction having a GapC concentration of 10-15 mg/ml. That fraction was dialyzed against 2000 volumes of PBSA (136mM sodium chloride, 2.6mM

potassium chloride, 8 mM sodium phosphate dibasic, 1.46 mM potassium phosphate monobasic)

F. Expression and Purification of Recombinant GapC Protein from *S. agalactiae*, *S. uberis*, *S. parauberis*, and *S. iniae*

Expression and purification of the recombinant proteins from these *streptococcus* species is accomplished by the same methods described in Example 1E, above. The transformed bacterial strains used to express the *S. agalactiae*, *S. uberis*, *S. parauberis* and *S. iniae* recombinant GapC proteins were designated BL21 DE3 (pMF521c) (ATCC No.), BL21 DE3 (pMF521a) (ATCC No.), BL21 DE3 (pMF521d) (ATCC No.), and BL21 DE3 (pMF521e) (ATCC No.), respectively.

G. Characterization of the Recombinant *S. dysgalactiae* GapC Protein

1. SDS-Page Analysis

SDS-polyacrylamide gel electrophoresis was performed on a sample of the eluted protein using the method described by Laemmli (Laemmli, U.K. (1970) *Nature* 227: 680-685). The results are presented in Figure 18. In the figure, lane 1, molecular weight markers (20.5 to 103 kDa range, BioRad Laboratories, Emeryville, CA), lane 2, soluble recombinant *S. dysgalactiae* GapC protein purified by Ni-NTA affinity chromatography.

These results demonstrate that purification by affinity chromatography on a Ni-NTA column yielded a homogenous protein fraction.

2. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity of recombinant GapC and *S. dysgalactiae* whole cells

GAPDH catalyzes the oxidative phosphorylation of D-glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate in the presence of NAD^+ and inorganic phosphate. The high degree of homology of GapC to streptococcal glyceraldehyde-3-phosphate dehydrogenase suggested that GapC may exhibit this enzyme activity.

The GAPDH activity of *S. dysgalactiae* whole cells (10^{10} CFU) and the recombinant GapC protein was determined by measuring the reduction of NAD^+ to NADH. The assay buffer was composed of 40 mM triethanolamine, 50 mM Na_2HPO_4 , and 5 mM EDTA, pH 6.8. *S. dysgalactiae* cells or 5 mg of purified recombinant protein were incubated in assay buffer containing 7 mM glyceraldehyde-3-phosphate (49 mg/ml, Sigma Chemical Company),

75 microliters NAD^+ (15 mM, Sigma Chemical Company) in a final volume of 1 ml. Negative controls consisted of samples which did not contain glyceraldehyde-3-phosphate or the recombinant GapC molecule/*S. dysgalactiae* cells. The reduction of NAD^+ to NADH was monitored spectrophotometrically at an Absorbance of 340 nanometers.

5 The results indicated that both the recombinant protein as well as intact wild-type *S. dysgalactiae* cells had enzymatic activity (not shown). Furthermore, when *S. dysgalactiae* cells were treated with Trypsin to digest surface proteins, GAPDH activity disappeared. Thus, the enzymatic activity observed for the intact wild type cells was not due to intracellular GAPDH.

10 This data suggests that the GapC protein is localized on the cell surface despite the apparent lack of either a signal sequence or a membrane anchoring region in either the nucleotide or amino acid.

15 3. Plasmin-Binding Activity of Recombinant *S. dysgalactiae* GapC Plasmin Binding Protein and *S. dysgalactiae* whole cells.

A microplate assay was used to determine if the recombinant GapC protein was capable of binding bovine plasmin, and if so whether the bound plasmin was in an enzymatically active form.

20 Ninety-six-well microtiter plates were coated with 5 mg of purified recombinant GapC protein, washed 3 times with 0.1% gelatin-PBSA with 0.05% TWEEN 20 (PBSGT). The wells were blocked for one hour at 37° C in the same buffer, washed, and incubated with 200 μl of bovine plasmin (0.25mg/ml, Boehringer Mannheim, Indianapolis, IN) for 1 hr at 37° C. The wells were then washed 8 times with PBSGT. 200 μl of the synthetic substrate chromazime-PL (Tos-Gly-Pro-Lys-4-NA, 0.3 mg/ml) were added to the wells and
25 incubated at 37° C for one hour. The presence of associated plasmin activity was determined by measuring the level of paranitroanilide (4-nitraniline) released into the supernatant and detected based on an Absorbance of 405 nanometers. A similar procedure was used to measure plasmin-binding activity of *S. dysgalactiae* whole cells, with the
30 exception that 10^{10} cells were washed with PBSGT and re-suspended in 400 μl chromazime-PL (0.3mg/ml) and incubated for 1 hour at 37° C.

The results, shown in Figure 19, demonstrate that the purified recombinant protein was capable of binding enzymatically active bovine plasmin. Likewise, when *S.*

dysgalactiae whole cells were utilized, similar results were obtained. In the figure, the data represents the mean of three individual assays

Thus, the plasmin-receptor is located on the surface of *S. dysgalactiae* and the purified protein retains biological activity

5

EXAMPLE 2

Immunization with *S. dysgalactiae* GapC and experimental infection of cattle

Vaccines were formulated in such a fashion that they contained 50 mg/ml of affinity
10 purified recombinant GapC in the oil-based adjuvant VSA3 (VIDO Saskatoon,
Saskatchewan, Canada) VSA3 is a combination of Emulsigen Plus™ (MVP Laboratories,
Ralston, Nebraska) and Dimethyldioctadecyl ammonium bromide (Kodak, Rochester, New
York) The affinity-purified recombinant GapC protein used for the vaccine preparation is
shown in Figure 18

15 Twenty-four non-lactating Holsteins with no history of *S. dysgalactiae* infection
were obtained from various farms in Saskatchewan, Canada. One week prior to
vaccination, all animals were treated with Cephadry™ (Ayerst Laboratories, Montreal,
Canada) (300 mg per quarter), in order to clear any infection of the udders prior to the
vaccination step

20 Groups of 8 animals were immunized subcutaneously with two doses of vaccines
containing *S. dysgalactiae* GapC, Mig (an Fc receptor protein isolated from *S. dysgalactiae*
which was evaluated simultaneously), or a placebo with a three-week interval between
immunizations Two weeks following the second immunization, animals were exposed to
650 colony forming units of *S. dysgalactiae* delivered into three quarters with an udder
25 infusion cannula. The fourth quarter on each animal served as an un-infective control

All animals were examined daily for clinical signs of disease and samples from all
udder quarters were collected on each day Samples were observed for consistency and
somatic cell counts as well as bacterial numbers were determined.

30

EXAMPLE 3

Determination of GapC-specific Antibodies

GapC-specific antibodies in bovine serum were measured using an enzyme-linked
immunosorbent assay (ELISA) Briefly, microtiter plates (NUNC, Naperville, Illinois)

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were coated by adding 1 microgram per well purified recombinant antigen in 50mM sodium carbonate buffer, pH 9.6, and incubated overnight at 4° C. The liquid was removed and the wells were blocked with 3% bovine serum albumin for 1 hr at 37° C. Serial dilutions of bovine serum (from 1 in 4 to 1 in 6,400) were then added to the wells and incubated for 2 hours at room temperature. The wells were aspirated, washed and incubated with 100 µl of alkaline phosphatase-conjugated goat anti-bovine IgG (Kirkgaard & Perry Laboratories Inc., Gaithersburg, MD) for 1 hr at room temperature. The wells were washed again, and 100 µl of p-nitrophenol phosphate (SIGMA, St. Louis, MO) was added as a substrate to detect alkaline phosphatase activity. The absorbance at 405 nanometers was recorded following 1 hr incubation with the substrate at room temperature.

Where referred to in the figures, the specific antibody titer is expressed as the reciprocal of the dilution showing activity above background levels.

EXAMPLE 4

Bacterial Colonization

Bacteria were enumerated by spreading serial dilutions (10^0 to 10^{-3}) directly onto TSA sheep blood agar plates followed by overnight incubation at 37°C, 5% CO₂. Colonization is defined as >500 cfu/ml of the challenge organism recovered.

To confirm that the bacteria recovered from milk secretions were *S. dysgalactiae*, selected colonies recovered from each animal were tested using an API strep-20 test (bioMérieux SA, Hazelwood, Missouri) according to the manufacturer's instructions. This test is a standardized method combining 20 biochemical tests for the determination of enzymatic activity and fermentation of sugars. The reactions are read according to a reading table and the identification is obtained by either referring to the analytical profile index or using identification software.

Following challenge, animals from all groups were shown to be colonized by *S. dysgalactiae* (Figure 20). Only the GapC-immunized cows had a statistically significant reduction in the number of infected quarters and total numbers of bacteria isolated per quarter. Therefore, immunization with GapC reduced bacterial colonization following challenge with *S. dysgalactiae*.

The relationship between anti-GapC titer and bacterial colonization is shown in Figures 21 and 22. There was a strong correlation between anti-GapC serum antibody level and the maximum number of bacteria (expressed in CFU ($\log_{10}$)/ml milk) found in any

quarter $r = 0.74$) (Figure 21) as well as the total number of infected quarters ($r^2 = 0.74$) (Figure 22). Correlation was calculated using GraphPad Prism software, version 2.01 (GraphPad Software Inc., San Diego, California).

This correlation is also illustrated in Figure 23 and 24 where the GapC-immunized group is subdivided into high titer and low titer responders. In these figures, "low titer responders" refer to the four animals with the poorest response against GapC while "high titer responders" refer to the remainder of the group. No colonization occurred in the high titer group while even the low titer group showed reduced numbers of bacteria recovered after day 3.

EXAMPLE 5

Determination of inflammatory response

Inflammatory response was measured as a function of somatic cell count (i.e., lymphocytes, neutrophils, and monocytes). Somatic cell counts were measured in a Coulter counter using standard techniques, as recommended by Agriculture and Agri-Food Canada Pamphlet IDF50B (1985) *Milk and Milk products—Methods of Sampling*. Samples were always read within 48 hours of collection and fixation, at days 1 through 7 post challenge.

The numbers of somatic cells present in the gland were determined on each day post challenge. Numbers from the unchallenged quarter remained constant throughout the trial while on day 1, the GapC group was lower than the placebo-immunized group (Figure 25). The difference between the GapC and the placebo groups was statistically significant. The individual data from day 1 is shown in Figure 26, data for GapC-treated animals over a 7 day period post-challenge is shown in Figure 27. Samples from the quarters of GapC-immunized animals were indistinguishable from unchallenged quarters.

Therefore, immunization with GapC reduced the inflammatory response following challenge with *S. dysgalactiae*.

EXAMPLE 6

Cross-protection against *S. uberis* infection

During the last 3 days of the vaccine trial, a mixed population of bacteria were recovered from the mammary gland secretions. Further analysis with the API 20 Strep test confirmed the identity of the strains present in the mixture as *S. dysgalactiae* and *S. uberis*, the latter representing a natural infection which occurred during the trial. The group which

was immunized with GapC appeared to be significantly cross-protected against *S. uberis* colonization (see Figure 20), indicating that GapC may be a broadly cross-protective antigen capable of protecting against infection by multiple Streptococcal species

Thus, vaccination with *S. dysgalactiae* GapC is therefore capable of providing cross-
5 protection against *S. uberis* infection.

Thus, the cloning, expression and characterization of various GapC plasmin binding
proteins is disclosed, as are methods of using the same. Although preferred embodiments of
10 the subject invention have been described in some detail, it is understood that obvious
variations can be made without departing from the spirit and the scope of the invention as
defined by the appended claims

What is claimed is

1 An isolated GapC protein selected from the group consisting of

5 (a) an isolated *Streptococcus dysgalactiae* GapC protein comprising the amino acid sequence shown at amino acid positions 1 to 336, inclusive, of Figure 1A-1B (SEQ ID NO 4),

(b) an isolated *Streptococcus agalactiae* GapC protein comprising the amino acid sequence shown at amino acid positions 1 to 336, inclusive, of Figures 2A-2B (SEQ ID NO 6),

10 (c) an isolated *Streptococcus uberis* GapC protein comprising the amino acid sequence shown at amino acid positions 1 to 336, inclusive, of Figures 3A-3B (SEQ ID NO 8),

(d) an isolated *Streptococcus parauberis* GapC protein comprising the amino acid sequence shown at amino acid positions 1 to 336, inclusive, of Figures 4A-4B (SEQ ID NO 10),

15 (e) an isolated *Streptococcus iniae* GapC protein comprising the amino acid sequence shown at amino acid positions 1 to 336, inclusive, of Figures 5A-5B (SEQ ID NO 12), and

20 (f) immunogenic fragments of (a), (b), (c), (d) and (e) comprising at least about 5 amino acids

25 2 The isolated GapC protein of claim 1, wherein the protein is a *Streptococcus dysgalactiae* GapC protein comprising the amino acid sequence shown at amino acid positions 1 to 336, inclusive, of Figures 1A-1B (SEQ ID NO 4)

3 The isolated GapC protein of claim 1, wherein the protein is a *Streptococcus agalactiae* GapC protein comprising the amino acid sequence shown at amino acid positions 1 to 336, inclusive, of Figures 2A-2B (SEQ ID NO 6)

30 4 The isolated GapC protein of claim 1, wherein the protein is a *Streptococcus uberis* GapC protein comprising the amino acid sequence shown at amino acid positions 1 to 336, inclusive, of Figures 3A-3B (SEQ ID NO 8)

5 The isolated GapC protein of claim 1, wherein the protein is a *Streptococcus parauberis* GapC protein comprising the amino acid sequence shown at amino acid positions 1 to 336, inclusive, of Figures 4A-4B (SEQ ID NO 10)

5 6 The isolated GapC protein of claim 1, wherein the protein is a *Streptococcus miae* GapC protein comprising the amino acid sequence shown at amino acid positions 1 to 336, inclusive, of Figures 5A-5B (SEQ ID NO 12)

10 7 An isolated polynucleotide comprising a coding sequence for an isolated GapC protein according to any of claims 1-6, or complements thereof

8 A recombinant vector comprising
(a) the isolated polynucleotide of claim 7, and
(b) at least one heterologous control element operably linked to said isolated
15 polynucleotide, whereby said coding sequence can be transcribed and translated in a host cell, and at least one of said control elements is heterologous to said coding sequence

9 A host cell comprising the recombinant vector of claim 8

20 10 A method for producing a GapC protein, said method comprising culturing the cells of claim 9 under conditions for producing said protein.

11 A vaccine composition comprising a pharmaceutically acceptable vehicle and a GapC protein, wherein said GapC protein is selected from the group consisting of

25 (a) a *Streptococcus dysgalactiae* GapC protein comprising the amino acid sequence shown at amino acid positions 1 to 336, inclusive, of Figures 1A-1B (SEQ ID NO 4),

(b) a *Streptococcus agalactiae* GapC protein comprising the amino acid sequence shown at amino acid positions 1 to 336, inclusive, of Figures 2A-2B (SEQ ID NO 6),

30 (c) a *Streptococcus uberis* GapC protein comprising the amino acid sequence shown at amino acid positions 1 to 336, inclusive, of Figures 3A-3B (SEQ ID NO 8),

(d) a *Streptococcus parauberis* GapC protein comprising the amino acid sequence shown at amino acid positions 1 to 336, inclusive, of Figures 4A-4B (SEQ ID NO 10),

(e) a *Streptococcus miae* GapC protein comprising the amino acid sequence shown at amino acid positions 1 to 336, inclusive, of Figures 5A-5B (SEQ ID NO 12),

(f) a *Streptococcus* GapC protein having at least about 70% sequence identity to (a), (b), (c), (d) and (e), and

5 (g) immunogenic fragments of (a), (b), (c) (d), (e) and (f), said fragments comprising at least about 5 amino acids

12 The vaccine composition of claim 11, wherein said GapC protein comprises the amino acid sequence of the *Streptococcus dysgalactiae* GapC protein depicted at amino acid positions 1 to 336, inclusive, of Figures 1A-1B (SEQ ID NO 4) or an immunogenic
10 fragment thereof, said fragment comprising at least about 5 amino acids

13 The vaccine composition of claim 12, wherein said GapC protein comprises the amino acid sequence depicted at amino acid position 1 to 336, inclusive, of Figures 1A-1B
15 (SEQ ID NO 4)

14 The vaccine composition of claim 11, wherein said GapC protein comprises the amino acid sequence of the *Streptococcus agalactiae* GapC protein depicted at amino acid positions 1 to 336, inclusive, of Figures 2A-2B (SEQ ID NO 6) or an immunogenic
20 fragment thereof, said fragment comprising at least about 5 amino acids

15 The vaccine composition of claim 14, wherein said GapC protein comprises the amino acid sequence depicted at amino acid positions 1 to 336 inclusive, of Figures 2A-2B (SEQ ID NO 6)
25

16 The vaccine composition of claim 11, wherein said GapC protein comprises the amino acid sequence of the *Streptococcus uberis* GapC protein depicted at amino acid positions 1 to 336, inclusive, of Figures 3A-3B (SEQ ID NO 8) or an immunogenic
fragment thereof, said fragment comprising at least about 5 amino acids
30

17 The vaccine composition of claim 16, wherein said GapC protein comprises the amino acid sequence depicted at amino acid positions 1 to 336, inclusive, of Figures 3A-3B (SEQ ID NO 8)

80

18 The vaccine composition of claim 11, wherein said GapC protein comprises the amino acid sequence of the *Streptococcus parauberis* GapC protein depicted at amino acid positions 1 to 336, inclusive, of Figures 4A-4B (SEQ ID NO 10) or an immunogenic fragment thereof, said fragment comprising at least about 5 amino acids

5

19 The vaccine composition of claim 18, wherein said GapC protein comprises the amino acid sequence depicted at amino acid positions 1 to 336, inclusive, of Figures 4A-4B (SEQ ID NO 10)

10

20 The vaccine composition of claim 11, wherein said GapC protein comprises the amino acid sequence of the *Streptococcus mitis* GapC protein depicted at amino acid positions 1 to 336, inclusive, of Figures 5A-5B (SEQ ID NO 12) or an immunogenic fragment thereof, said fragment comprising at least about 5 amino acids

15

21 The vaccine composition of claim 20, wherein said GapC protein comprises the amino acid sequence depicted at amino acid positions 1 to 336, inclusive, of Figures 5A-5B (SEQ ID NO 12)

20

22 The vaccine composition according to any of claims 11-21, further comprising an adjuvant.

25

23 A method of producing a vaccine composition comprising the steps of
(1) providing a GapC protein or an immunogenic fragment thereof, said fragment comprising at least about 5 amino acids, and
(2) combining said protein with a pharmaceutically acceptable vehicle

30

24 The method of claim 23, wherein the GapC protein is the protein of any of claims 1-6

25 Antibodies directed against the isolated GapC protein of any of claims 1-6

26 The antibodies of claim 25 wherein said antibodies are polyclonal.

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27 The antibodies of claim 25 wherein said antibodies are monoclonal.

28 Use of a GapC protein according to any of claims 1-6 in the manufacture of a vaccine composition useful for treating or preventing a bacterial infection in a vertebrate
5 subject.

29 The use of claim 28, wherein said bacterial infection is a streptococcus infection.

10 30 The use of claim 29, wherein said bacterial infection causes mastitis

31 Use of a polynucleotide according to claim 7 in the manufacture of a vaccine composition for treating or preventing a bacterial infection in a vertebrate subject.

15 32 The use of claim 31, wherein said bacterial infection is a streptococcal infection.

33 The use of claim 32, wherein said bacterial infection causes mastitis

20 34 Use of a GapC protein in the manufacture of a diagnostic reagent for detecting *Streptococcus* antibodies in a biological sample.

35 Use of a GapC protein according to any of claims 1-6 in the manufacture of a diagnostic reagent for detecting *Streptococcus* antibodies in a biological sample.

25 36 Use of antibodies according to any of claims 25-27 in the manufacture of a diagnostic reagent for detecting a GapC protein in a biological sample.

37 An immunodiagnostic test kit for detecting *Streptococcus* infection, said test kit comprising a GapC protein and instructions for conducting the immunodiagnostic test.

30

38 An immunodiagnostic test kit for detecting *Streptococcus* infection, said test kit comprising antibodies directed against a GapC protein and instructions for conducting the immunodiagnostic test.

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39 A method of treating or preventing a bacterial infection in a vertebrate subject comprising administering to said subject a therapeutically effective amount of a vaccine composition according to any of claims 11-22

5 40 The method of claim 39, wherein said bacterial infection is a streptococcus infection.

41 The method of claim 40 wherein said bacterial infection causes mastitis

10 42 A method of treating or preventing a bacterial infection in a vertebrate subject comprising administering to said subject a therapeutically effective amount of a polynucleotide according to claim 7

15 43 The method of claim 42, wherein said bacterial infection is a streptococcal infection.

44 The method of claim 43, wherein said bacterial infection causes mastitis

20 45 A method of detecting *Streptococcus* antibodies in a biological sample, comprising

(a) reacting said biological sample with an isolated GapC protein under conditions which allow said *Streptococcus* antibodies, when present in the biological sample, to bind to said GapC protein to form an antibody/antigen complex, and

25 (b) detecting the presence or absence of said complex, thereby detecting the presence or absence of *Streptococcus* antibodies in said sample.

46 A method of detecting *Streptococcus* antibodies in a biological sample, comprising

30 (a) reacting said biological sample with an isolated GapC protein according to any of claims 1-6 under conditions which allow said *Streptococcus* antibodies, when present in the biological sample, to bind to said GapC protein to form an antibody/antigen complex, and

(b) detecting the presence or absence of said complex, thereby detecting the presence or absence of *Streptococcus* antibodies in said sample.

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47 A method of detecting a GapC protein in a biological sample, comprising

(a) reacting said sample with antibodies directed against the GapC protein under conditions which allow said antibodies to bind to said GapC protein, when present in the
5 sample, to form an antibody/antigen complex, and

(b) detecting the presence or absence of said complex, thereby detecting the presence or absence of a GapC protein in said sample

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atg gta gtt aaa gtt ggt att aac ggt ttc ggt cgt atc gga cgt ctt	48
Met Val Val Lys Val Gly Ile Asn Gly Phe Gly Arg Ile Gly Arg Leu	
1 5 10 15	
gca ttc cgt cgt att caa aat gtt gaa ggt gtt gaa gta act cgt atc	96
Ala Phe Arg Arg Ile Gln Asn Val Glu Gly Val Glu Val Thr Arg Ile	
20 25 30	
aac gac ctt aca gat cca aac atg ctt gca cac ttg ttg aaa tac gat	144
Asn Asp Leu Thr Asp Pro Asn Met Leu Ala His Leu Lys Tyr Asp	
35 40 45	
aca act caa gga cgt ttt gac gga act gtt gaa gtt aaa gaa ggt gga	192
Thr Thr Gln Gly Arg Phe Asp Gly Thr Val Glu Val Lys Glu Gly Gly	
50 55 60	
ttt gaa gta aac gga aac ttc atc aaa gtt tct gct gaa cgt gat cca	240
Phe Glu Val Asn Gly Asn Phe Ile Lys Val Ser Ala Glu Arg Asp Pro	
65 70 75 80	
gaa aac atc gac tgg gca act gac ggt gtt gaa atc gtt ctg gaa gca	288
Glu Asn Ile Asp Trp Ala Thr Asp Gly Val Glu Ile Val Leu Glu Ala	
85 90 95	
act ggt ttc ttt gct aaa aaa gaa gct gct gaa aaa cac tta cat gct	336
Thr Gly Phe Phe Ala Lys Lys Glu Ala Ala Glu Lys His Leu His Ala	
100 105 110	
aac ggt gct aaa aaa gtt gtt atc aca gct cct ggt gga aac gac gtt	384
Asn Gly Ala Lys Lys Val Val Ile Thr Ala Pro Gly Gly Asn Asp Val	
115 120 125	
aaa aca gtt gtt ttc aac act aac cac gac att ctt gac ggt act gaa	432
Lys Thr Val Val Phe Asn Thr Asn His Asp Ile Leu Asp Gly Thr Glu	
130 135 140	
aca gtt atc tca ggt gct tca tgt act aca aac tgt tta got cct atg	480
Thr Val Ile Ser Gly Ala Ser Cys Thr Thr Asn Cys Leu Ala Pro Met	
145 150 155 160	
gct aaa gct ctt cac gat gca ttt ggt atc caa aaa ggt ctt atg act	528
Ala Lys Ala Leu His Asp Ala Phe Gly Ile Gln Lys Gly Leu Met Thr	
165 170 175	
aca atc cac gct tat act ggt gac caa atg atc ctt gac gga cca cac	576
Thr Ile His Ala Tyr Thr Gly Asp Gln Met Ile Leu Asp Gly Pro His	
180 185 190	
cgt ggt ggt gac ctt cgt cgt gct cgt gct ggt got gca aac att gtt	624
Arg Gly Gly Asp Leu Arg Arg Ala Arg Ala Gly Ala Ala Asn Ile Val	
195 200 205	

FIG 1A

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cct aac tca act ggt gct gct aaa gct atc ggt ctt gtt atc cca gaa	672
Pro Asn Ser Thr Gly Ala Ala Lys Ala Ile Gly Leu Val Ile Pro Glu	
210 215 220	
ttg aat ggt aaa ctt gat ggt gct gca caa cgt gtt cct gtt cca act	720
Leu Asn Gly Lys Leu Asp Gly Ala Ala Gln Arg Val Pro Val Pro Thr	
225 230 235 240	
gga tca gta act gag ttg gtt gta act ctt gat aaa aac gtt tot gtt	768
Gly Ser Val Thr Glu Leu Val Val Thr Leu Asp Lys Asn Val Ser Val	
245 250 255	
gac gaa atc aac gct gct atg aaa gct gct tca aac gac agt ttc ggt	816
Asp Glu Ile Asn Ala Ala Met Lys Ala Ala Ser Asn Asp Ser Phe Gly	
260 265 270	
tac act gaa gat cca att gtt tct tca gat atc gta ggc gtg tca tac	864
Tyr Thr Glu Asp Pro Ile Val Ser Ser Asp Ile Val Gly Val Ser Tyr	
275 280 285	
ggt tca ttg ttt gac gca act caa act aaa gtt atg gaa gtt gac gga	912
Gly Ser Leu Phe Asp Ala Thr Gln Thr Lys Val Met Glu Val Asp Gly	
290 295 300	
tca caa ttg gtt aaa gtt gta tca tgg tat gac aat gaa atg tct tac	960
Ser Gln Leu Val Lys Val Val Ser Trp Tyr Asp Asn Glu Met Ser Tyr	
305 310 315 320	
act gct caa ctt gtt cgt aca ctt gag tac ttt gca aaa atc gct aaa	1008
Thr Ala Gln Leu Val Arg Thr Leu Glu Tyr Phe Ala Lys Ile Ala Lys	
325 330 335	
taa	1011

FIG 1B

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atg gta gtt aaa gtt ggt att aac ggt ttc ggt cgt atc ggt cgt ctt	48
Met Val Val Lys Val Gly Ile Asn Gly Phe Gly Arg Ile Gly Arg Leu	
1 5 10 15	
gca ttc cgt cgc atc caa aac gta gaa ggt gtt gaa gtt act cgt atc	96
Ala Phe Arg Arg Ile Gln Asn Val Glu Gly Val Glu Val Thr Arg Ile	
20 25 30	
aac gac ctt aca gat cca aac atg ctt gca cac ttg ttg aaa tat gac	144
Asn Asp Leu Thr Asp Pro Asn Met Leu Ala His Leu Leu Lys Tyr Asp	
35 40 45	
aca act caa ggt cgt ttc gac ggt act gtt gaa gtt aaa gaa ggt gga	192
Thr Thr Gln Gly Arg Phe Asp Gly Thr Val Glu Val Lys Glu Gly Gly	
50 55 60	
ttc gaa gtt aac ggt caa ttt gtt aaa gtt tot gct gaa cgc gaa cca	240
Phe Glu Val Asn Gly Gln Phe Val Lys Val Ser Ala Glu Arg Glu Pro	
65 70 75 80	
gca aac att gac tgg gct act gat ggc gta gaa atc gtt ctt gaa gca	288
Ala Asn Ile Asp Trp Ala Thr Asp Gly Val Glu Ile Val Leu Glu Ala	
85 90 95	
act ggt ttc ttt gca tca aaa gaa aaa gct gga caa cac atc cat gaa	336
Thr Gly Phe Phe Ala Ser Lys Glu Lys Ala Gly Gln His Ile His Glu	
100 105 110	
aat ggt gct aaa aaa gtt gtt atc aca gct cct ggt gga aac gac gtt	384
Asn Gly Ala Lys Lys Val Val Ile Thr Ala Pro Gly Gly Asn Asp Val	
115 120 125	
aaa aca gtt gtt ttc aac act aac cac gat atc ctt gat gga act gaa	432
Lys Thr Val Val Phe Asn Thr Asn His Asp Ile Leu Asp Gly Thr Glu	
130 135 140	
aca gtt atc tca ggt gct tca tgt act aca aac tgt ctt gct cca atg	480
Thr Val Ile Ser Gly Ala Ser Cys Thr Thr Asn Cys Leu Ala Pro Met	
145 150 155 160	
gct aaa gct tta caa gac aac ttt ggt gtt aaa caa ggt ttg atg act	528
Ala Lys Ala Leu Gln Asp Asn Phe Gly Val Lys Gln Gly Leu Met Thr	
165 170 175	
act atc cac gca tac act ggt gac caa atg atc ctt gac gga cca cac	576
Thr Ile His Ala Tyr Thr Gly Asp Gln Met Ile Leu Asp Gly Pro His	
180 185 190	
cgt ggt ggt gac ctt cgt cgt gct cgt gca ggt gct gca aac atc gtt	624
Arg Gly Gly Asp Leu Arg Arg Ala Arg Ala Gly Ala Ala Asn Ile Val	
195 200 205	

FIG. 2A

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cct aac tca act ggt gct gca aaa gct atc gga ctt gtt atc cca gaa Pro Asn Ser Thr Gly Ala Ala Lys Ala Ile Gly Leu Val Ile Pro Glu 210 215 220	672
ttg aac ggt aaa ctt gat ggt gct gca caa cgt gtt cct gtt cca act Leu Asn Gly Lys Leu Asp Gly Ala Ala Gln Arg Val Pro Val Pro Thr 225 230 235 240	720
gga tca gta act gaa ttg gtt gca act ctt gaa aaa gac gta act gtc Gly Ser Val Thr Glu Leu Val Ala Thr Leu Glu Lys Asp Val Thr Val 245 250 255	768
gaa gaa gta aat gca gct atg aaa gca gca gct aac gat tca tac ggt Glu Glu Val Asn Ala Ala Met Lys Ala Ala Ala Asn Asp Ser Tyr Gly 260 265 270	816
tat act gaa gat cca atc gta tca tct gat atc gtt ggt att tca tac Tyr Thr Glu Asp Pro Ile Val Ser Ser Asp Ile Val Gly Ile Ser Tyr 275 280 285	864
ggt tca ttg ttt gat gct act caa act aaa gtt caa act gtt gac ggt Gly Ser Leu Phe Asp Ala Thr Gln Thr Lys Val Gln Thr Val Asp Gly 290 295 300	912
aac caa ttg gtt aaa gtt gtt tca tgg tac gat aac gaa atg tca tac Asn Gln Leu Val Lys Val Val Ser Trp Tyr Asp Asn Glu Met Ser Tyr 305 310 315 320	960
act tca caa ctt gtt cgt aca ctt gag tac ttt gca aaa atc gct aaa Thr Ser Gln Leu Val Arg Thr Leu Glu Tyr Phe Ala Lys Ile Ala Lys 325 330 335	1008
taa	1011

FIG 2B

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atg gta gtt aaa gtt ggt att aac ggt ttc ggt cgt atc gga cgt ctt	48
Met Val Val Lys Val Gly Ile Asn Gly Phe Gly Arg Ile Gly Arg Leu	
1 5 10 15	
gca ttc cgt cgt att caa aac gtt gaa ggt gtt gaa gta act cgt att	96
Ala Phe Arg Arg Ile Gln Asn Val Glu Gly Val Glu Val Thr Arg Ile	
20 25 30	
aac gat ctt act gac coa aat atg ctt gca cac ttg ttg aaa tat gat	144
Asn Asp Leu Thr Asp Pro Asn Met Leu Ala His Leu Leu Lys Tyr Asp	
35 40 45	
aca act caa ggt cgt ttc gac ggt aca gtt gaa gtt aaa gat ggt gga	192
Thr Thr Gln Gly Arg Phe Asp Gly Thr Val Glu Val Lys Asp Gly Gly	
50 55 60	
ttc gaa gtt aac gga aac ttc atc aaa gtt tct gct gaa aaa gat cca	240
Phe Glu Val Asn Gly Asn Phe Ile Lys Val Ser Ala Glu Lys Asp Pro	
65 70 75 80	
gaa aac att gac tgg gca act gac ggt gta gaa atc gtt ctt gaa gca	288
Glu Asn Ile Asp Trp Ala Thr Asp Gly Val Glu Ile Val Leu Glu Ala	
85 90 95	
act ggt ttc ttt gct aaa aaa gca gct gct gaa aaa cat tta cat gct	336
Thr Gly Phe Phe Ala Lys Lys Ala Ala Ala Glu Lys His Leu His Ala	
100 105 110	
aac ggt gct aaa aaa gtt gtt atc aca gct cct ggt gga gat gat gtt	384
Asn Gly Ala Lys Lys Val Val Ile Thr Ala Pro Gly Gly Asp Asp Val	
115 120 125	
aaa act gtt gta ttt aac aca aac cat gac att ctt gac ggt aca gaa	432
Lys Thr Val Val Phe Asn Thr Asn His Asp Ile Leu Asp Gly Thr Glu	
130 135 140	
act gta att tca ggt gct tca tgt act act aac tgt tta gct cca atg	480
Thr Val Ile Ser Gly Ala Ser Cys Thr Thr Asn Cys Leu Ala Pro Met	
145 150 155 160	
gct aaa gct ttg caa gat aac ttt ggt gtt aaa caa ggt ttg atg aca	528
Ala Lys Ala Leu Gln Asp Asn Phe Gly Val Lys Gln Gly Leu Met Thr	
165 170 175	
act atc cac gct tac act ggt gac caa atg atc ctt gac gga cca cac	576
Thr Ile His Ala Tyr Thr Gly Asp Gln Met Ile Leu Asp Gly Pro His	
180 185 190	
cgt ggt ggt gac ctt cgt cgt gct cgt gct ggt gca agc aac att gtt	624
Arg Gly Gly Asp Leu Arg Arg Ala Arg Ala Gly Ala Ser Asn Ile Val	
195 200 205	

FIG 3A

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cct aac tca act ggt gct gct aaa gca atc ggt ctt gta atc cca gaa	672
Pro Asn Ser Thr Gly Ala Ala Lys Ala Ile Gly Leu Val Ile Pro Glu	
210 215 220	
tta aat ggt aaa ctt gac ggt gct gca caa cgt gtt cct gtt cca act	720
Leu Asn Gly Lys Leu Asp Gly Ala Ala Gln Arg Val Pro Val Pro Thr	
225 230 235 240	
gga tca gta act gaa tta gta gca gtt ctt gaa aaa gaa act tca gtt	768
Gly Ser Val Thr Glu Leu Val Ala Val Leu Glu Lys Glu Thr Ser Val	
245 250 255	
gaa gaa atc aac gca gca atg aaa gca gct gca aac gat tca tac gga	816
Glu Glu Ile Asn Ala Ala Met Lys Ala Ala Ala Asn Asp Ser Tyr Gly	
260 265 270	
tac act gaa gac cca atc gta tct tct gat atc atc ggt atg gct tac	864
Tyr Thr Glu Asp Pro Ile Val Ser Ser Asp Ile Ile Gly Met Ala Tyr	
275 280 285	
ggt tca ttg ttt gat gct act caa act aaa gta caa act gtt gat gga	912
Gly Ser Leu Phe Asp Ala Thr Gln Thr Lys Val Gln Thr Val Asp Gly	
290 295 300	
aat caa tta gtt aaa gtt gtt tca tgg tat gac aac gaa atg tct tac	960
Asn Gln Leu Val Lys Val Val Ser Trp Tyr Asp Asn Glu Met Ser Tyr	
305 310 315 320	
act gca caa ctt gtt cgt act ctt gag tac ttt gca aaa atc gct aaa	1008
Thr Ala Gln Leu Val Arg Thr Leu Glu Tyr Phe Ala Lys Ile Ala Lys	
325 330 335	
taa	1011

FIG 3B

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atg gta gtt aaa gtt ggt att aac ggt ttt ggc cgt atc gga cgt ctt	48
Met Val Val Lys Val Gly Ile Asn Gly Phe Gly Arg Ile Gly Arg Leu	
1 5 10 15	
gct ttc cgt cgt att caa aat gta gaa ggt gtt gaa gtt act cgc atc	96
Ala Phe Arg Arg Ile Gln Asn Val Glu Gly Val Glu Val Thr Arg Ile	
20 25 30	
aac gac ctt aca gat cca aat atg ctt gca cac ttg tta aaa tac gat	144
Asn Asp Leu Thr Asp Pro Asn Met Leu Ala His Leu Leu Lys Tyr Asp	
35 40 45	
aca act caa ggt cgt ttt gac ggt act gta gaa gtt aaa gat ggt gga	192
Thr Thr Gln Gly Arg Phe Asp Gly Thr Val Glu Val Lys Asp Gly Gly	
50 55 60	
ttt gac gtt aac gga aaa ttc att aaa gtt tct gct gaa aaa gat cca	240
Phe Asp Val Asn Gly Lys Phe Ile Lys Val Ser Ala Glu Lys Asp Pro	
65 70 75 80	
gaa caa att gac tgg gca act gac ggt gtt gaa atc gtt ctt gaa gca	288
Glu Gln Ile Asp Trp Ala Thr Asp Gly Val Glu Ile Val Leu Glu Ala	
85 90 95	
act ggt ttc ttt gct aaa aaa gca gct gct gaa aaa cat tta cat gaa	336
Thr Gly Phe Phe Ala Lys Lys Ala Ala Ala Glu Lys His Leu His Glu	
100 105 110	
aat ggt gct aaa aaa gtt gtt atc act gct cct ggt gga gat gac gtg	384
Asn Gly Ala Lys Lys Val Val Ile Thr Ala Pro Gly Gly Asp Asp Val	
115 120 125	
aaa aca gtt gta ttt aac act aac cat gat atc ctt gat gga act gaa	432
Lys Thr Val Val Phe Asn Thr Asn His Asp Ile Leu Asp Gly Thr Glu	
130 135 140	
aca gtt att tca ggt gct tca tgt act aca aac tgt tta gct cca atg	480
Thr Val Ile Ser Gly Ala Ser Cys Thr Thr Asn Cys Leu Ala Pro Met	
145 150 155 160	
gct aaa gct tta caa gat aac ttt ggc gta aaa caa ggt tta atg aot	528
Ala Lys Ala Leu Gln Asp Asn Phe Gly Val Lys Gln Gly Leu Met Thr	
165 170 175	
aca atc cac gct tac act ggt gat caa atg ctt ctt gat gga cct cac	576
Thr Ile His Ala Tyr Thr Gly Asp Gln Met Leu Leu Asp Gly Pro His	
180 185 190	
cgt ggt ggt gac tta cgt cgt gcc cgt gct ggt gct aac aat att gtt	624
Arg Gly Gly Asp Leu Arg Arg Ala Arg Ala Gly Ala Asn Asn Ile Val	
195 200 205	

FIG 4A

SUBSTITUTE SHEET (RULE 26)

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cct aac tca act ggt gct gct aaa gca atc ggt ctt gtt atc cct gaa	672
Pro Asn Ser Thr Gly Ala Ala Lys Ala Ile Gly Leu Val Ile Pro Glu	
210 215 220	
tta aat ggt aaa ctt gac ggt gct gca caa cgt gta cca gtt cca aca	720
Leu Asn Gly Lys Leu Asp Gly Ala Ala Gln Arg Val Pro Val Pro Thr	
225 230 235 240	
ggt tca gta aca gaa tta gta gca gtt ctt aat aaa gaa act tca gta	768
Gly Ser Val Thr Glu Leu Val Ala Val Leu Asn Lys Glu Thr Ser Val	
245 250 255	
gaa gaa att aac tca gta atg aaa gct gca gct aat gat tca tat ggt	816
Glu Glu Ile Asn Ser Val Met Lys Ala Ala Ala Asn Asp Ser Tyr Gly	
260 265 270	
tac act gaa gat cca atc gta tca tct gat atc gtt ggt atg tct ttc	864
Tyr Thr Glu Asp Pro Ile Val Ser Ser Asp Ile Val Gly Met Ser Phe	
275 280 285	
ggt tca tta ttc gat gct act caa act aaa gta caa act gtt gat gga	912
Gly Ser Leu Phe Asp Ala Thr Gln Thr Lys Val Gln Thr Val Asp Gly	
290 295 300	
aat caa tta gtt aaa gtt gtt tca tgg tat gac aat gaa atg tct tac	960
Asn Gln Leu Val Lys Val Val Ser Trp Tyr Asp Asn Glu Met Ser Tyr	
305 310 315 320	
act gct caa ctt gat cgt aca ctt gag tac ttt gca aaa atc gct aaa	1008
Thr Ala Gln Leu Asp Arg Thr Leu Glu Tyr Phe Ala Lys Ile Ala Lys	
325 330 335	
taa	1011

FIG 4B

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atg gta gtt aaa gtt ggt att aac ggt ttc gga cgt atc ggt cgt ctt Met Val Val Lys Val Gly Ile Asn Gly Phe Gly Arg Ile Gly Arg Leu 1 5 10 15	48
gca ttc cgt cgt att caa aat gtt gaa ggt gtt gaa gta act cgt atc Ala Phe Arg Arg Ile Gln Asn Val Glu Gly Val Glu Val Thr Arg Ile 20 25 30	96
aat gac ctt aca gat cct aac atg ctt gca cac ttg ttg aaa tat gat Asn Asp Leu Thr Asp Pro Asn Met Leu Ala His Leu Leu Lys Tyr Asp 35 40 45	144
aca act caa ggt cgt ttt gac ggt aca gtt gaa gtt aaa gat ggt gga Thr Thr Gln Gly Arg Phe Asp Gly Thr Val Glu Val Lys Asp Gly Gly 50 55 60	192
ttc gaa gtt aac gga agc ttt gtt aaa gtt tct gca gaa cgc gaa cca Phe Glu Val Asn Gly Ser Phe Val Lys Val Ser Ala Glu Arg Glu Pro 65 70 75 80	240
gca aac att gac tgg gct act gat ggt gta gac atc gtt ctt gaa gca Ala Asn Ile Asp Trp Ala Thr Asp Gly Val Asp Ile Val Leu Glu Ala 85 90 95	288
aca ggt ttc ttc gct tct aaa gca gct gct gaa caa cac att cac gct Thr Gly Phe Phe Ala Ser Lys Ala Ala Ala Glu Gln His Ile His Ala 100 105 110	336
aac ggt gog aaa aaa gtt gtt atc aca gct cct ggt gga aat gac gtt Asn Gly Ala Lys Lys Val Val Ile Thr Ala Pro Gly Gly Asn Asp Val 115 120 125	384
aaa aca gtt gtt tac aac act aac cat gat att ctt gat gga act gaa Lys Thr Val Val Tyr Asn Thr Asn His Asp Ile Leu Asp Gly Thr Glu 130 135 140	432
aca gtt atc tca ggt gct tca tgt act aca aac tgt tta gct cca atg Thr Val Ile Ser Gly Ala Ser Cys Thr Thr Asn Cys Leu Ala Pro Met 145 150 155 160	480
gct aaa gca tta caa gat aac ttt ggt gta aaa caa ggt tta atg act Ala Lys Ala Leu Gln Asp Asn Phe Gly Val Lys Gln Gly Leu Met Thr 165 170 175	528
act atc cat ggt tac act ggt gac caa atg gtt ctt gac gga cca cac Thr Ile His Gly Tyr Thr Gly Asp Gln Met Val Leu Asp Gly Pro His 180 185 190	576
cgt ggt ggt gat ctt cgt cgt gct cgt gca gct gca gca aac atc gtt Arg Gly Gly Asp Leu Arg Arg Ala Arg Ala Ala Ala Asn Ile Val 195 200 205	624

FIG 5A

SUBSTITUTE SHEET (RULE 26)

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cct aac tca act ggt gct gct aaa gca atc ggt ctt gtt atc cca gaa	672
Pro Asn Ser Thr Gly Ala Ala Lys Ala Ile Gly Leu Val Ile Pro Glu	
210 215 220	
tta aat ggt aaa ctt gac ggt gct gca caa cgt gtt cct gtt cca act	720
Leu Asn Gly Lys Leu Asp Gly Ala Ala Gln Arg Val Pro Val Pro Thr	
225 230 235 240	
gga tca gta act gaa tta gta gca gtt ctt gaa aaa gat act tca gta	768
Gly Ser Val Thr Glu Leu Val Ala Val Leu Glu Lys Asp Thr Ser Val	
245 250 255	
gaa gaa atc aat gca gct atg aaa gca gca gct aac gat tca tac ggt	816
Glu Glu Ile Asn Ala Ala Met Lys Ala Ala Ala Asn Asp Ser Tyr Gly	
260 265 270	
tac act gaa gat gct atc gta tca tca gat atc gta ggt att tct tac	864
Tyr Thr Glu Asp Ala Ile Val Ser Ser Asp Ile Val Gly Ile Ser Tyr	
275 280 285	
ggt tca tta ttt gat gct act caa act aaa gta caa act gtt gat gga	912
Gly Ser Leu Phe Asp Ala Thr Gln Thr Lys Val Gln Thr Val Asp Gly	
290 295 300	
aat caa ttg gtt aaa gtt gtt tca tgg tat gac aat gaa atg tct tac	960
Asn Gln Leu Val Lys Val Val Ser Trp Tyr Asp Asn Glu Met Ser Tyr	
305 310 315 320	
act gct caa ctt gtt cgt act ctt gag tac ttt gca aaa atc gct aaa	1008
Thr Ala Gln Leu Val Arg Thr Leu Glu Tyr Phe Ala Lys Ile Ala Lys	
325 330 335	
taa	1011

FIG 5B

SUBSTITUTE SHEET (RULE 26)

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	1					50
DysGapC	ATGGTAGTTA	AAGTTGGTAT	TAACGGTTTC	GGTCGTATCG	GACGTCCTGC	
SpyGapC	-----	-----	-----	-----	-----	
SeqGapC	-----	-----	-----	-----	-----	
ParaUbGapC	-----	-----	-----t	--c-----	-----	
UberGapC	-----	-----	-----	-----	-----	
AgalGapC/DNA	-----	-----	-----	-----	-t-----	
SiniGapC	-----	-----	-----	--a-----	-t-----	
BovGapC	-----	-----	-----	---c----	-g--c--g-t	
	51					100
DysGapC	ATTCCGTCGT	ATTCAAAATG	TTGAAGGTGT	TGAAGTAACT	CGTATCAACG	
SpyGapC	-----c---	-----ca	-c-----	-----	-----t-	
SeqGapC	-----	-----	-----	-----	-----	
ParaUbGapC	t-----	-----	-a-----	-----t--	--c-----	
UberGapC	-----	-----c-	-----	-----	-----t---	
AgalGapC/DNA	-----c	--c-----c-	-a-----	-----t-	-----	
SiniGapC	-----	-----	-----	-----	-----t-	
BovGapC	cac-a-ggc-	gc-ttt---t	c--gcaaa--	g--ca-cgtc	gcc-----t-	
	101					150
DysGapC	ACC	TTAC	AGATCCAAAC	ATGCTTGACAC	ACTTGTTGAA	ATACGATACA
SpyGapC	---	---	-----t	-----	-----	---c-----
SeqGapC	---	---	-----	-----	-----	---c-----
ParaUbGapC	---	---	-----t	-----	-----a-	---c-----
UberGapC	-t-	---	t--c-----t	-----	-----	---t-----
AgalGapC/DNA	---	---	-----	-----	-----	---t--c---
SiniGapC	---	---	-----t	-----	-----	---t-----
BovGapC	---cct-c-t	t--c-ttc--	taca-g-tct	--a---cc-	g--t---t-c	

FIG 6A

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	151				200
DysGapC	ACTCAAGGAC	GTTTTCACGG	AAGTGTTCGAA	GTAAAGAAG	GTCGATTGGA
SpyGapC	-----	-----t--	a--a-----	-----	-----t--
SeqGapC	-----a-	-----	a--t-----	-----	-----t--
ParaUbGapC	-----	-----	---t--a---	-----t-	-----t--
UberGapC	-----	---c----	---a-----	-----t-	-----c--
AgalGapCDNA	-----	---c----	---t-----	-----	-----c--
SiniGapC	-----	-----	---a-----	-----t-	-----c--
BovGapC	--c--c--ca	ag--ca---	c--a--ca-g	-cag-ga-c-	-gaagc-c-t
	201				250
DysGapC	AGTAAACGGA	AACTTCATCA	AAGTTTCTGC	TGAACGTGAT	CCAGAAAACA
SpyGapC	---a-----	-----	-----	-----t---	-----
SeqGapC	---a-----	-----	-----	-----t---	-----
ParaUbGapC	c-----	--a----t-	-----	---aaa---	-----c-a-
UberGapC	-----	-----	-----	---aaa---	-----
AgalGapCDNA	-----t	c-a--tg-t-	-----	-----c--a	---c-----
SiniGapC	-----	-g--tg-t-	-----	a-----c--a	---c-----
BovGapC	ca-c--t---	--ggc-----	cca-c-tcca	g--g--a---	--t-cc----
	251				300
DysGapC	TCGACTGGGC	AACTGACGGT	GTTGAAATCG	TTCTGGAAGC	AACTGGTTTC
SpyGapC	-c-----	-----t--g	-----	-----	-----
SeqGapC	-c-----	-----c--	-----	-----	-----
ParaUbGapC	-t-----	-----c--	-----	-----	-----
UberGapC	-t-----	-----c--	--a-----	-----	-----
AgalGapCDNA	-t-----	t-----t--c	--a-----	-----	-----
SiniGapC	-t-----	t-----t--	--a--c----	-----	---a-----
BovGapC	-ca-g---g	tga--ct---	-c---gtat-	-ag-g--gt-	c-----gg--
	301				350
DysGapC	TTTGCTAAAA	AAGAAGCTGC	TGAAAAACAC	TTACATGCTA	ACGGTGCTAA
SpyGapC	-----	-----a-	-----	-----	-----
SeqGapC	-----	-----	-----c-	-----	-----
ParaUbGapC	-----	---c-----	-----t	-----aa-	-t-----
UberGapC	-----	---c-----	-----t	-----	-----
AgalGapCDNA	-----atc-	-----aaa-	--g-c----	a-c-----aa-	-t-----
SiniGapC	--c---tct-	---c-----	---c-----	a-t-c----	-----g--
BovGapC	--ca---cc-	tg--gaag--	--gggct---	--ga-g-g-	--c--c--
	351				400
DysGapC	AAAAGTTGTT	ATCACAGCTC	CTGGTGGAAA	CGACGTTAAA	ACAGTTGTTT
SpyGapC	-----	-----	-----	---t-----	-----
SeqGapC	-----	-----	-----	-----	-----
ParaUbGapC	-----	---t-----	-----g-	t---g---	-----a-
UberGapC	-----	-----	-----g-	t--t-----	--t-----a-
AgalGapCDNA	-----	-----	-----	-----	-----
SiniGapC	-----	-----	-----	t-----	-----
BovGapC	g-gg--ca-c	---t-t--a-	--tc--	c---t-cccc	-tgt---ga

FIG 6B

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	401				450	
DysGapC	TCAACACTAA	CCACGA	CAT	TCTTGACGGT	ACTGAAACAG	TTATCTCAGG
SpyGapC	-----	-----	---	-----	-----	-----
SeqGapC	-----	-----	---	-----	-----	-----
ParaUbGapC	-t-----	---t--	t--	c-----t--a	-----	---t-----
UberGapC	-t-----a--	---t--	---	-----	--a-----t-	-a--t-----
AgalGapCDNA	-----	-----	t--	c-----t--a	-----	-----
SiniGapC	a-----	---t--	t--	-----t--a	-----	-----
BovGapC	-ggg-gtg--	-----a-g	-	a-a--aac	--cctc-aga	--g--agcaa
	451				500	
DysGapC	TGCTTCATGT	ACTACAAACT	GTTTAGCTCC	TATGGCTAAA	GCTCTTCACG	
SpyGapC	-----	-----	-----	t-----	---c-t--c-	
SeqGapC	-----	-----	-----	t-----	---c-t--c-	
ParaUbGapC	-----	-----	-----	-----	---a---	
UberGapC	-----	---t---	-----	-----	---g---	
AgalGapCDNA	-----	-----	-c-t---	-----	---a---	
SiniGapC	-----	-----	-----	-----	--a--a---	
BovGapC	---c--c--c	--c--c---	-c-g--c-	cc---c--g	-tca-c--t-	
	501				550	
DysGapC	ATGCATTTCG	TATCCAAAAA	GGTCTTATGA	CTACAATCCA	CGCTTATACT	
SpyGapC	--gca--c--	-a--c--a--	---c-----	---a-----	-----	
SeqGapC	--gca-----	-a--c--a--	---c-----	---a-----	-----	
ParaUbGapC	-----	cg-a-----	---t-a---	---a-----	-----	
UberGapC	-----	-g-----	---t-g---	-a-t-----	-----	
AgalGapCDNA	-c-----	-g-----	---t-g---	-t-----	---a-----	
SiniGapC	-----	-g-a-----	---t-a---	---t-----	t-g-----	
BovGapC	-cc-----	ca-cgtgg-g	--ac-----	-c--tg---	---cat---	
	551				600	
DysGapC	GGTGACCAA	TGATCCTTGA	CGGACCACAC	CGTGGTGGTG	ACCTTCGTGG	
SpyGapC	-----	-----	-----	-----	-----	
SeqGapC	-----	---g---	t---ac-gt	g-----	-t-----	
ParaUbGapC	---t---	--c-t---	t---t---	-----	--t-a---	
UberGapC	-----	-----	-----	-----	-----	
AgalGapCDNA	-----	-----	-----	-----	-----	
SiniGapC	-----	--g-t---	-----	-----	-t-----	
BovGapC	-ccac---g-	a--ctg-g--	t--c--ctc-	--gaagc	tgtgg---ga	
	601				650	
DysGapC	TGCTCGTGCT	GGTGCTGCAA	ACATTGTTCC	TAACTCAACT	GGTGCTGCTA	
SpyGapC	---a--c---	-----	-----	-----	-----	
SeqGapC	-----	-----	-----	-----	---cg---	
ParaUbGapC	---c-----	---aac-	-t--t-----	-----	-----	
UberGapC	-----	---aagc-	---t-----	-----	-----	
AgalGapCDNA	-----a	-----	-----	-----	---a---	
SiniGapC	-----a	-c--a---	-----	-----	-----	
BovGapC	c-gc--a-gg	-c---ccag-	-t---a-c--	-gct--t---	--c-----c-	

FIG 6C

SUBSTITUTE SHEET (RULE 26)

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	651				700
DysGapC	AAGCTATCGG	TCTTGTTATC	CCAGAAATTGA	ATGGTAAACT	TGATGGGTGCT
SpyGapC	-----	-----	-----c-t-	-c-----	-----
SeqGapC	-----	-----	-----g-	-c-----	-----
ParaUbGapC	----a-----	-----	--t-----a-	-t-----	-----
UberGapC	----a-----	-----a---	-----a-	-t-----	-----
AgalGapCDNA	-----	a-----	-----g-	-c-----	---t-----
SiniGapC	----a-----	-----	-----a-	-t-----	-----
BovGapC	-g--cg-g--	caag--c---	--t--gc-c-	-c-g-g-g-	cact--catg
	701				750
DysGapC	GCACAACGTG	TTCCTGTTCC	AACTGGATCA	GTAAGTGAGT	TGGTTGTAAAC
SpyGapC	-----	-----	-----	-----g-	-----t---
SeqGapC	-----	-----	-----	-----g-	-----t---
ParaUbGapC	-----	-a-a-----	---a-t---	-----a---	-a-a---gt
UberGapC	-----	-----	-----	-----	-a-a---gt
AgalGapCDNA	-----	-----	-----	-----	-----
SiniGapC	-----	-----	-----	-----	-a-a---gt
BovGapC	--cttc--c-	-c--cac---	c-ac-tg--t	--tgtg--tc	--acctgccg
	751				800
DysGapC	TCTTGATAAA	AACGTTTCTG	TTGACGAAAT	CAACGCTGCT	ATGAAAGCTG
SpyGapC	-----c---	a---t-----	-----c-----	-----t-t---	-----
SeqGapC	-----c---	a---t-----	-----c-----	-----t---	-----
ParaUbGapC	----a-t---	--aac---a-	-a-----	t--t---ta	-----
UberGapC	-----	--aac---a-	-----	-----a	-----a-
AgalGapCDNA	-----	---tsa	-c-----g-	a-t-----	-----a-
SiniGapC	-----	--tac---a-	-a-----	---t-----	-----a-
BovGapC	c--g--g---	cct-ccaagt	a---t-g--	---gaag-tg	g---gcag-
	801				850
DysGapC	CTTCAAACGA	CAGTTTCGGT	TACACTGAAG	ATCCAATTGT	TTCTTCAGAT
SpyGapC	--t-----	-agc-t---	-----	-----	t-----
SeqGapC	--t-----	-agc-t---	-----	-----	t-----
ParaUbGapC	-ag-t--t--	---at---	-----	-----	---a-t---
UberGapC	--g-----	-----a--a	-----	-c-----	-----t---
AgalGapCDNA	-ag-t-----	-----a---	--t-----	-----	---a-t---
SiniGapC	-ag-t-----	-----a---	-----	--g-t-----	---a-----
BovGapC	-gt--g-g-g	cc-tct-aag	gg--t-ct--	gctac-ct-a	ggaccag-t-
	851				900
DysGapC	ATCGTAGGCG	TGTCATA	CGGTTCAATTG	TTTGACGCAA	CTCAAACCTAA
SpyGapC	-----cg	-a-----	-----	-----c--a-	-----
SeqGapC	-----cg	-a-----	-----	-----c--a-	-----
ParaUbGapC	-----t--ta	---t-t	-----a	--c-----	-----
UberGapC	---a-c--ta	-g-t--	-----	-----	-----
AgalGapCDNA	-----t--ta	-t-----	-----	-----	-----
SiniGapC	-----ta	-t--t--	-----a	-----	-----
BovGapC	g--tect-cg	ac-tca-cag	--a-a-tcac	-c-tc-a-ct	tcg-tg--gg

FIG 6D

	901				950
DysGapC	AGTTATGGAA	GTTGACGGAT	CACAATTGGT	TAAAGTTGTA	TCATGGTATG
SpyGapC	---aatggaa	-----c---t	ca-----	-----a	-----
SeqGapC	---tatggaa	-----t---t	ca-----	-----a	-----
ParaUbGapC	---a-----	-----t---	-t---a--	-----	-----
UberGapC	---a-----	-----t---	-t---a--	-----	-----
AgalGapCDNA	---t-----	-----c-t-	-c-----	-----	-----c-
SiniGapC	---a-----	-----t---	-t-----	-----	-----
BovGapC	g-ctggc-t-	-ccctcaacg	-c-c-t--	c-gc-ca--	--c-----c-
	951				1000
DysGapC	ACAATGAAAT	GTCTTACACT	GCTCAACTTG	TTCGTACACT	TGAGTATTTT
SpyGapC	---c-----	-----	-----	-a---t--	-----c
SeqGapC	---c-----	-----	-----	-----	-----
ParaUbGapC	---t-----	-----	-----	a-----	-----
UberGapC	---c-----	-----	-a-----	-----t-	-----
AgalGapCDNA	-t-c-----	---a-----	t-a-----	-----	-----
SiniGapC	---t-----	-----	-----	-----t-	-----
BovGapC	---t---t-	tggc---gc	aaa--gg--	-----	-----
	1001		1018		
DysGapC	GCAAAATCG	CTAAATAA			
SpyGapC	-----t-	-----			
SeqGapC	-----	-----			
ParaUbGapC	-----	-----			
UberGapC	-----	-----			
AgalGapCDNA	-----	-----			
SiniGapC	-----	-----			
BovGapC	-----	-----			

FIG 6E

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	1					50
DysGapC	MVVKVGDNGF	GRIGRIAPRR	IQNVGVEVT	RIND	LTDPN	MLAHLKXDT
SpyGapC	-----	-----	---I-----	----	----	-----
SeqGapC	-----	-----	-----	----	----	-----
PUberGapC	-----	-----	-----	----	----	-----
UberGapC	-----	-----	-----	----	----	-----
AgalGapC	-----	-----	-----	----	----	-----
IniaeGapC	-----	-----	-----	----	----	-----
BovGapC	-----	-----vt-a	af-sgk-div	a---pfi-lh	ymvymfq--s	
	51					100
DysGapC	TQGRFDGIVE	VKEGGFEVNG	NFIKVSARD	PENIDWATDG	VEIVLEATGF	
SpyGapC	-----	-----	-----	-----	-----	
SeqGapC	-----	-----	-----	-----	-----	
PUberGapC	-----	--d--d--	k-----k-	--q-----	-----	
UberGapC	-----	--d-----	-----k-	-----	-----	
AgalGapC	-----	-----	q-v-----e	-a-----	-----	
IniaeGapC	-----	--d-----	s-v-----e	-a-----	-d-----	
BovGapC	-h-k-n--k	aen-klvi--	ka-tifq--	-a--k-gda-	a-y-v-s--v	
	101					150
DysGapC	FAKKEAAERK	LHANGAKKV	ITAPGGHDVK	TVVFTINEDI	LDGTETVISG	
SpyGapC	-----	-----	-----	-----	-----	
SeqGapC	-----p	-----	-----	qlfstltts-	-----	
PUberGapC	---a-----	--e-----	-----d--	-----	-----	
UberGapC	---a-----	-----	-----d--	-----	-----	
AgalGapC	--a--k-gq-	i-e-----	-----	-----	-----	
IniaeGapC	--s-a--q-	i-----	-----	--y-----	-----	
BovGapC	-ttu-k-ga-	-kg---r-i	-s--sa-sp	af-sgv--ek	ym-likiv-n	

FIG 7A

	151				200	
DysGapC	ASCTINCLAP	MAKALHDAFG	IQKGLMTTIE	AYTGDQMILD	GPRGGDLRR	
SpyGapC	-----	-----	-----	-----	-----	
SeqGapC	-----	-----	-----	-----v-	-hrg-----	
PUberGapC	-----	----q-n--	v-----	-----	-----	
UberGapC	-----	----q-n--	v-----	-----	-----	
AgalGapC	-----	----q-n--	v-----	-----	-----	
IniaeGapC	-----	----q-n--	v-----	g-----v-	-----	
BovGapC	-----	l--vih-h--	ive-----v-	-i-at-ktv-	-- s-klw-d	
	201				250	
DysGapC	ARAGAANIVP	NSTGAAGAIG	LVIPELNGKL	DGAAQRVPVP	TGSVTELVVT	
SpyGapC	-----	-----	-----	-----	-----	
SeqGapC	-----	-----r----	-----	-----	-----	
PUberGapC	-----n----	-----	-----	-----	-----av	
UberGapC	-----s----	-----	-----	-----	-----av	
AgalGapC	-----	-----	-----	-----	-----a-	
IniaeGapC	---a-----	-----	-----	-----	-----av	
BovGapC	g-ga-q--i-	a-----v-	k-----	t-m-f---t-	nv--vd-tcr	
	251				300	
DysGapC	LDKNVSVDEI	NAAMKAASND	S	PGYTE	DPIVSSDIVG	VSYGSLFDAT
SpyGapC	-----	-s-----	-	-----	-----	-----
SeqGapC	-----	-----	-	-----	-----	-----
PUberGapC	-n-et--e--	-av---a--	-	y----	-----	m-f-----
UberGapC	-e-et--e--	-----a--	-	y----	-----i-	ma-----
AgalGapC	-e-d-t-e-v	-----a--	-	y----	-----	i-----
IniaeGapC	-e-dt--e--	-----a--	-	y----	-a-----	i-----
BovGapC	-e-paky---	kkvv-q--eg	plkgilg---	-qv--c-fns	dths-t---g	
	301				341	
DysGapC	QTKVMEVDGS	QLVKVSVWYD	NEMSYTAQLV	RTLEYFAKIA	K	
SpyGapC	-----	-----	-----	-----	-	
SeqGapC	-----	-----	-----	-----	-	
PUberGapC	----qt---n	-----	-----d	-----	-	
UberGapC	----qt---n	-----	-----	-----	-	
AgalGapC	----qt---n	-----	-----s---	-----	-	
IniaeGapC	----qt---n	-----	-----	-----	-	
BovGapC	acial	nd hf--li----	--fg-sk---	-----	-	

FIG 7B

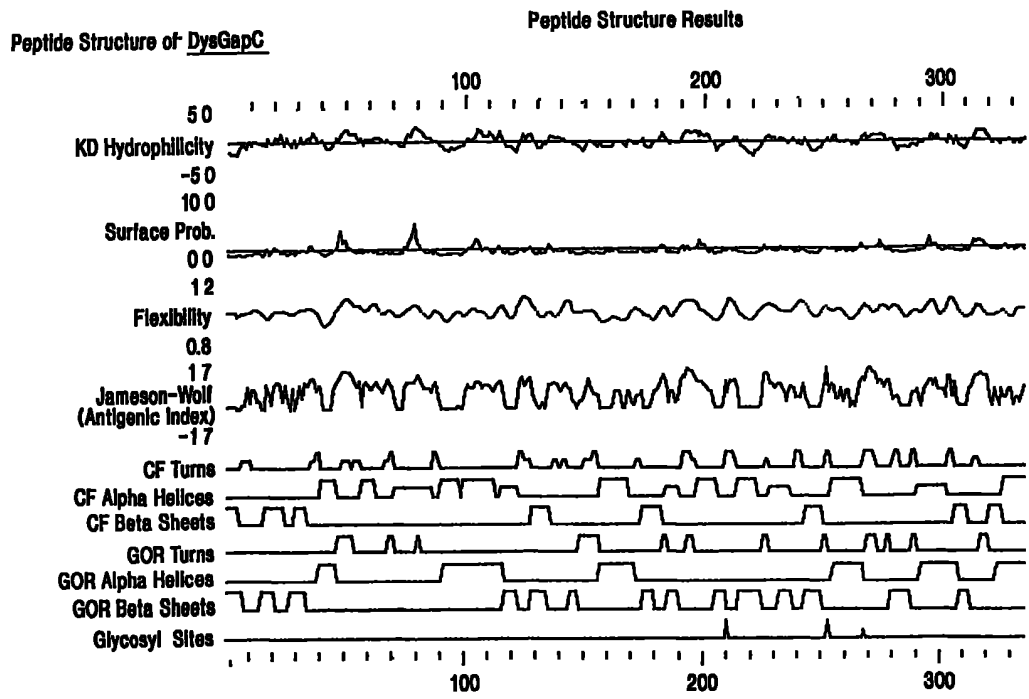


FIG 8

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100

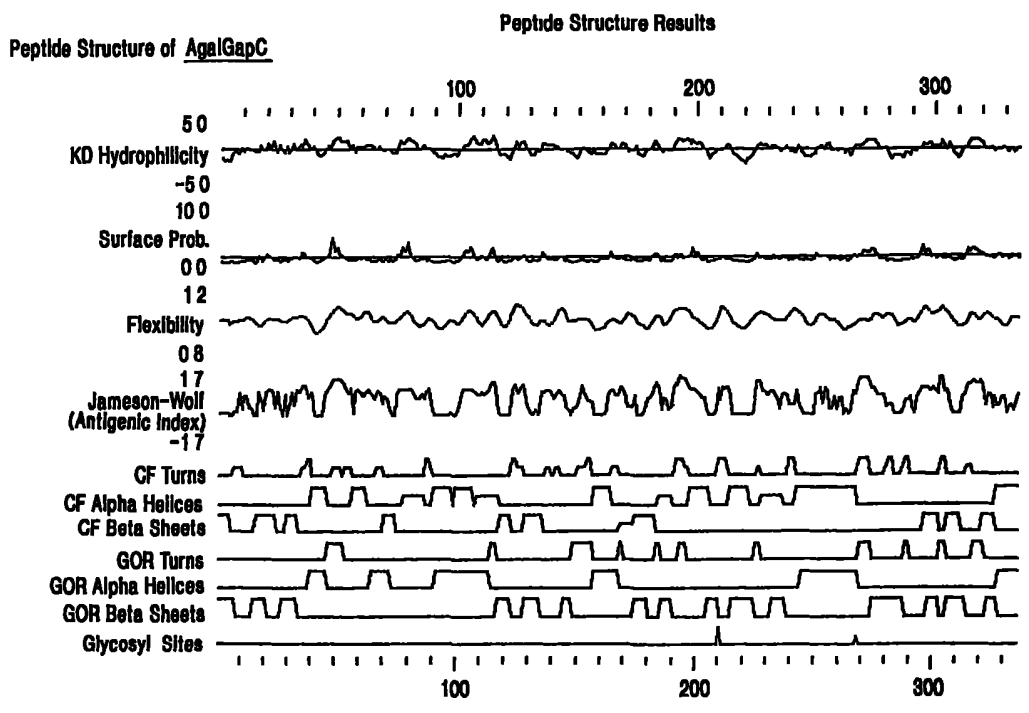


FIG 9

SUBSTITUTE SHEET (RULE 26)

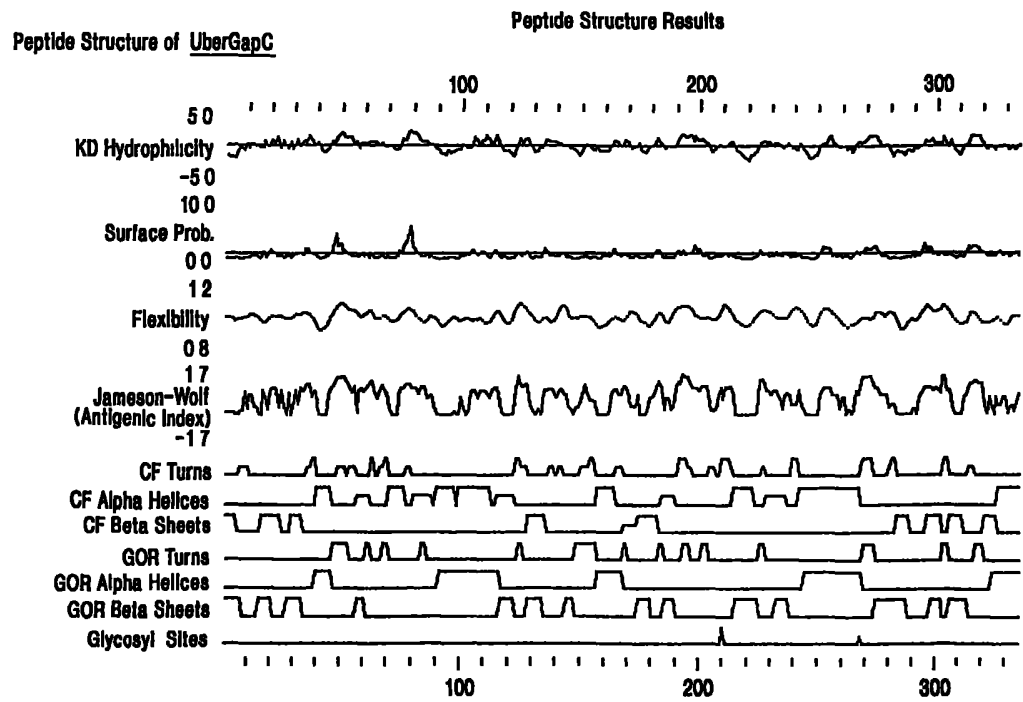


FIG 10

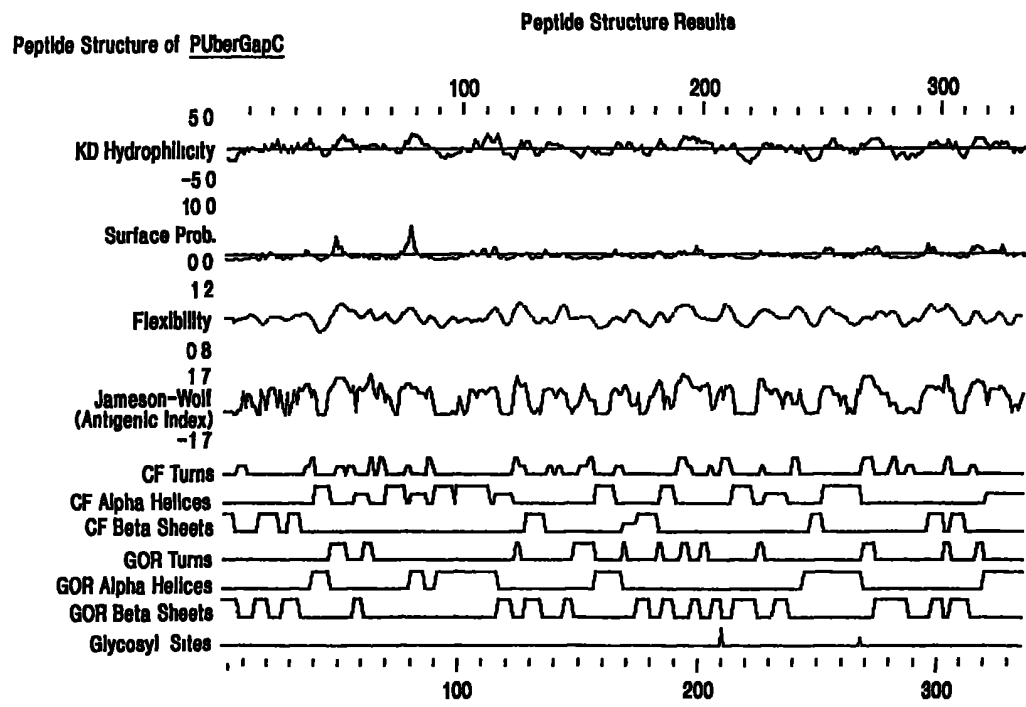


FIG 11

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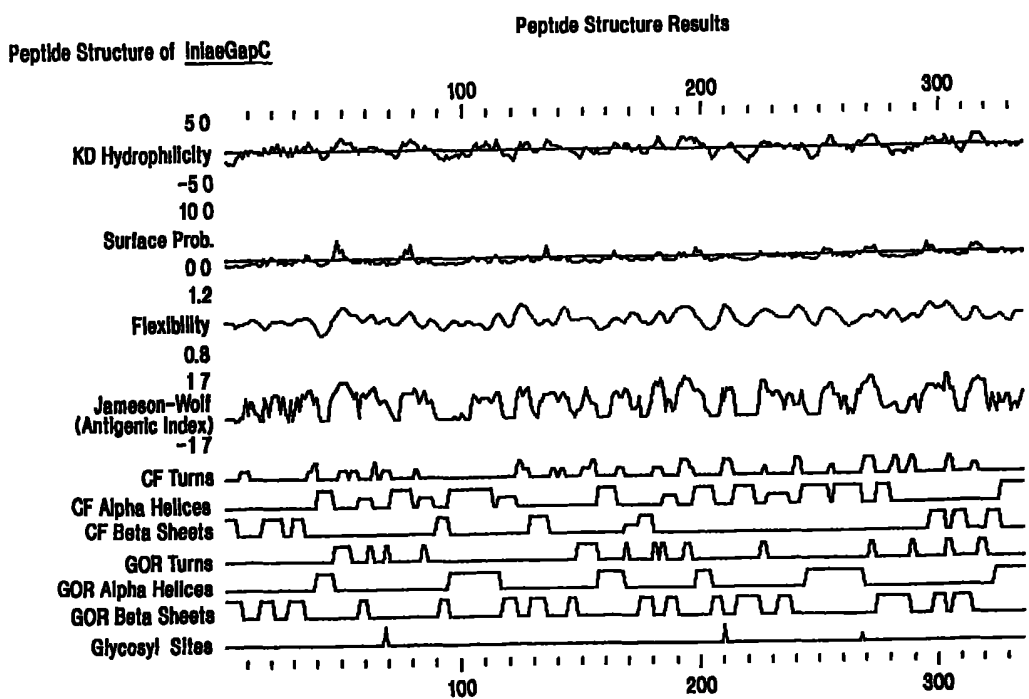


FIG 12

SUBSTITUTE SHEET (RULE 26)

Peptide Structure Results

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Peptide Structure of DysGapC

○ KD Hydrophilicity ≥ 1.3
◇ KD Hydrophobicity ≥ 1.3

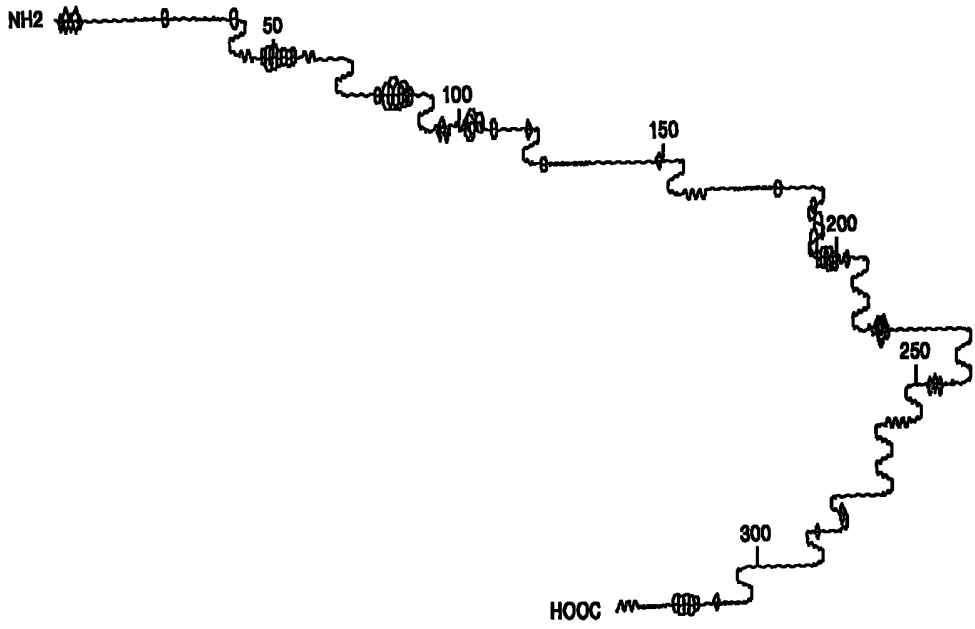


FIG 13

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Peptide Structure Results

Peptide Structure of AgalGapC

○ KD Hydrophilicity ≥ 1.3
◇ KD Hydrophobicity ≥ 1.3

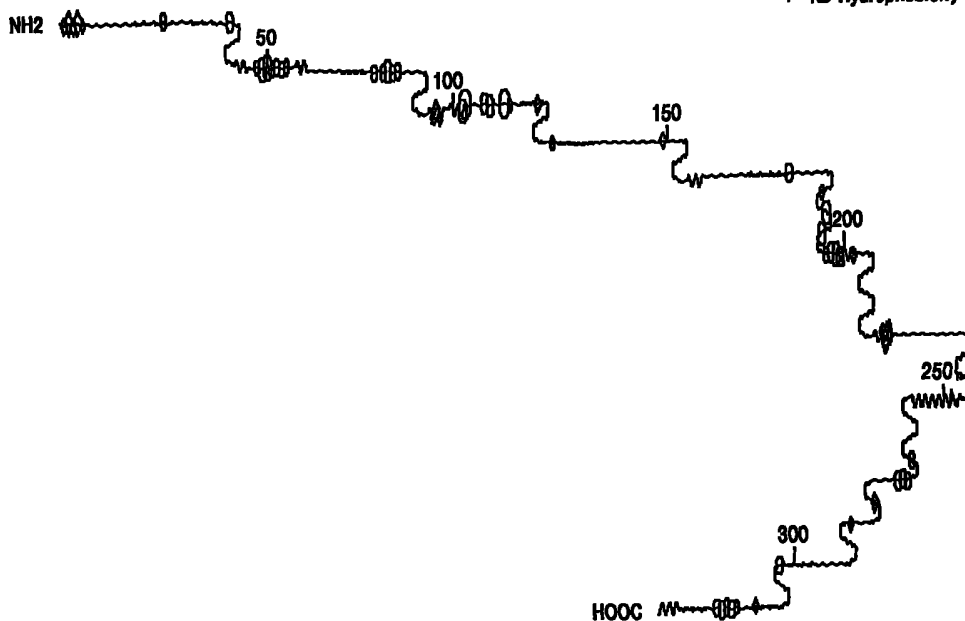


FIG 14

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Peptide Structure Results

Peptide Structure of UberGapC

○ KD Hydrophilicity >=1.3
◇ KD Hydrophobicity >=1.3

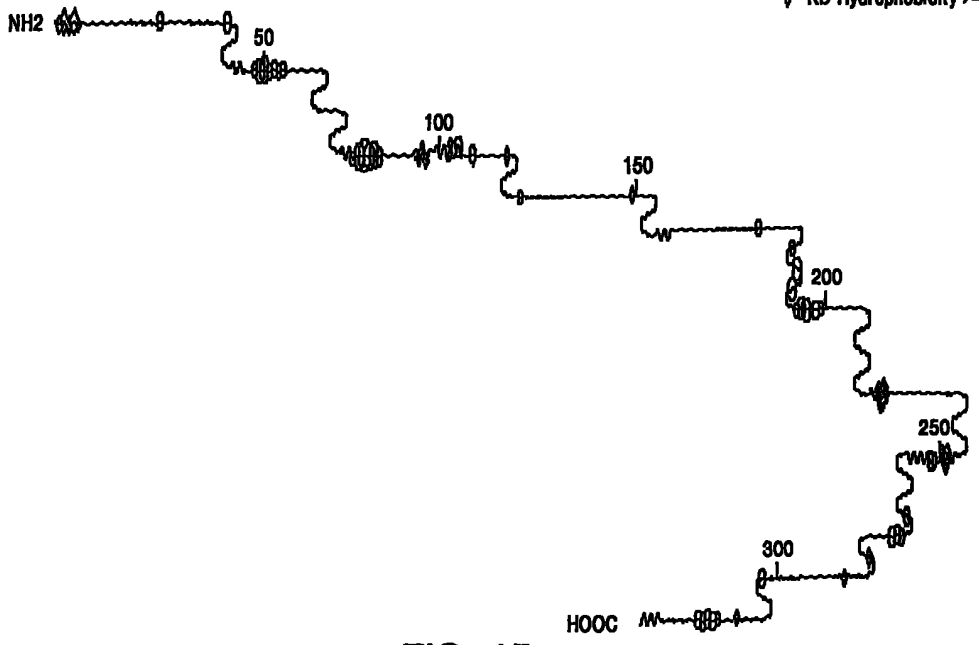


FIG 15

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Peptide Structure Results

Peptide Structure of PUberGapC

- KD Hydrophilicity ≥ 1.3
- ◇ KD Hydrophobicity ≥ 1.3

W/O 01/96381

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PCT/CA01/00838

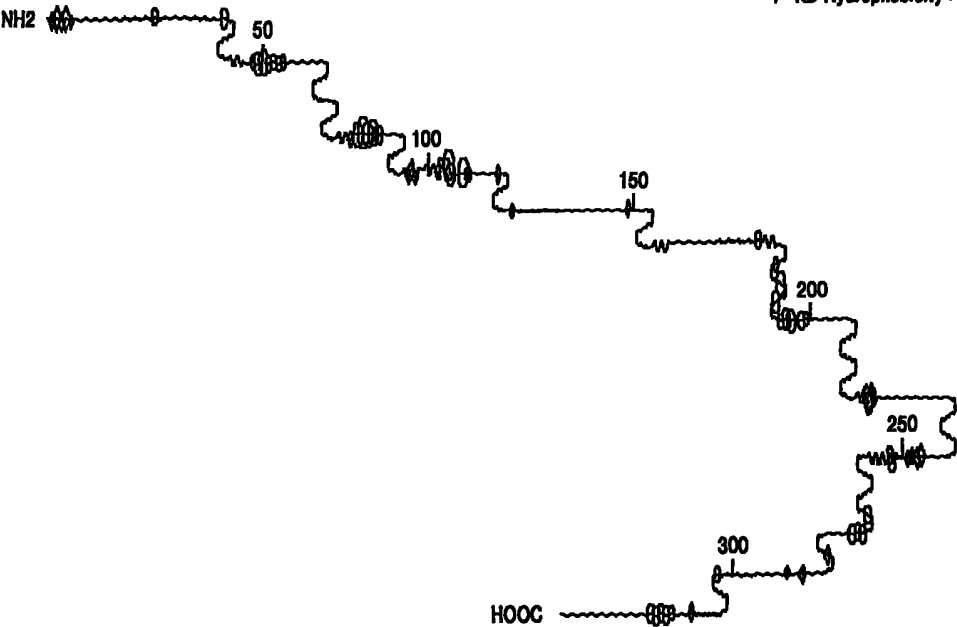


FIG 16

SUBSTITUTE SHEET (RULE 26)

Peptide Structure Results

Peptide Structure of IniaeGapC

⌋ KD Hydrophilicity ≥ 1.3
⌋ KD Hydrophobicity ≥ 1.3

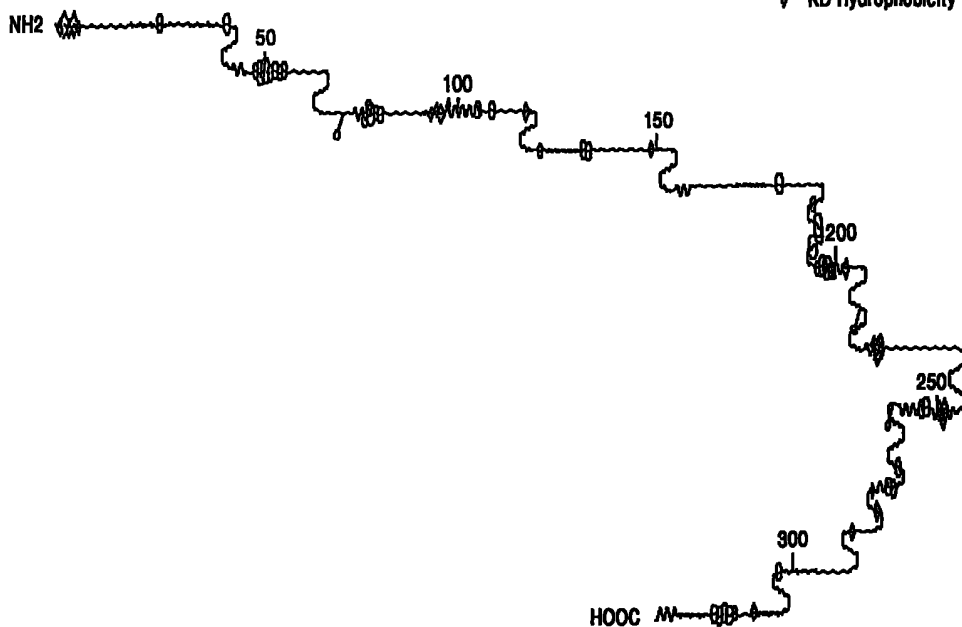


FIG 17

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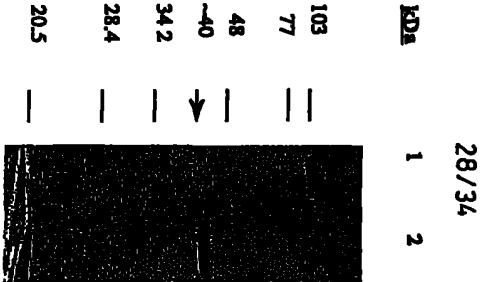


FIG 18

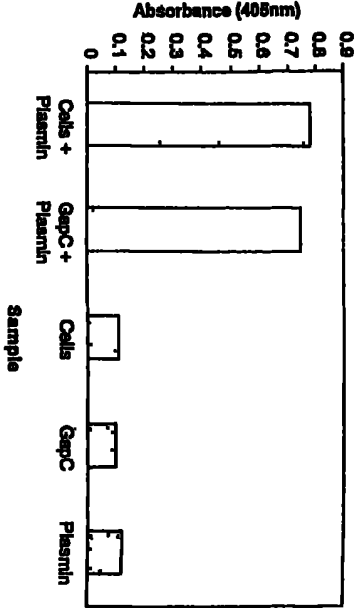


FIG 19

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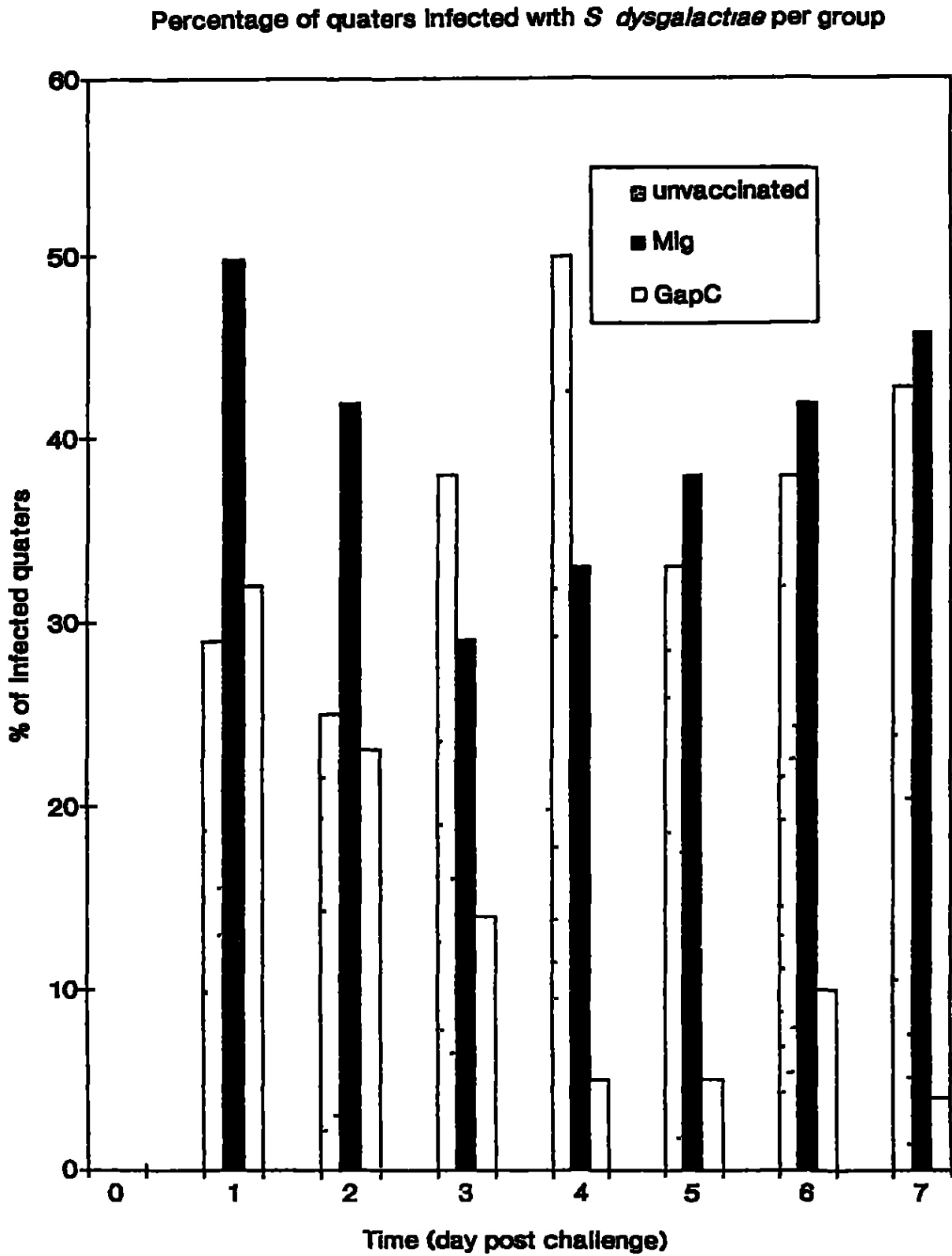


FIG 20

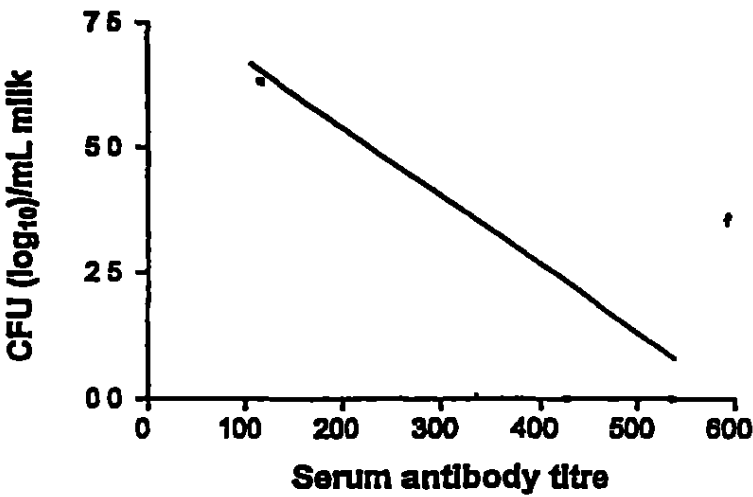


FIG 21

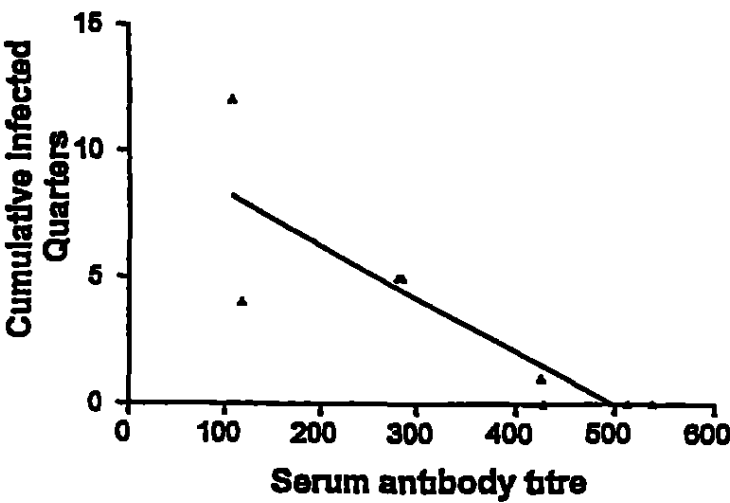


FIG 22

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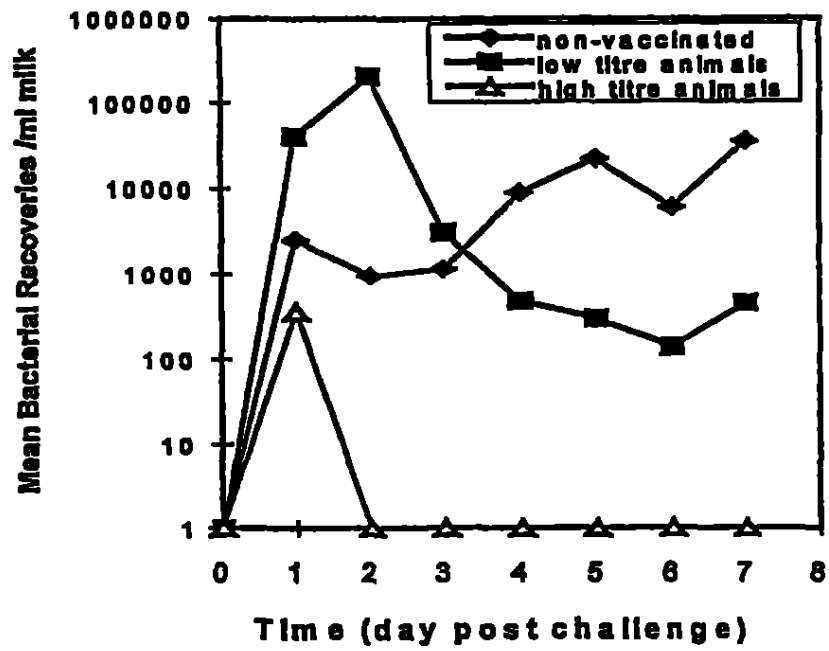


FIG 23

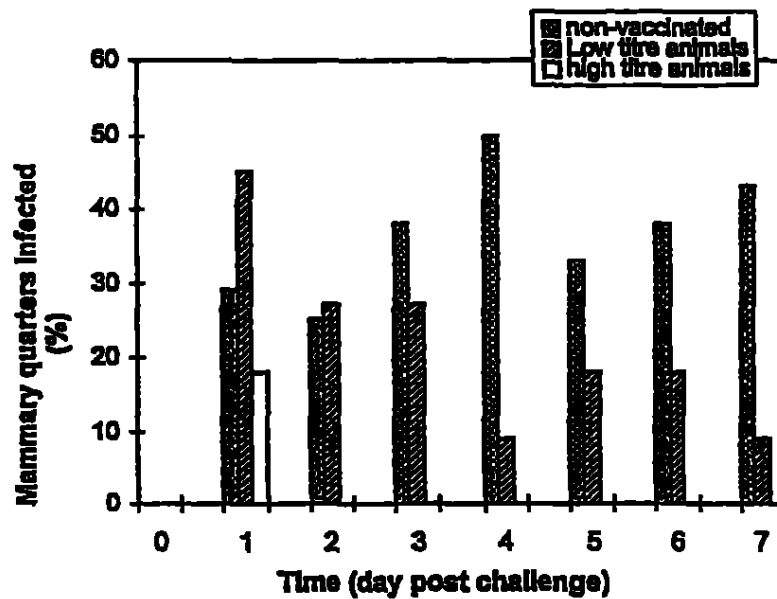


FIG 24

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Mean Somatic Cell Counts (SCC) per group

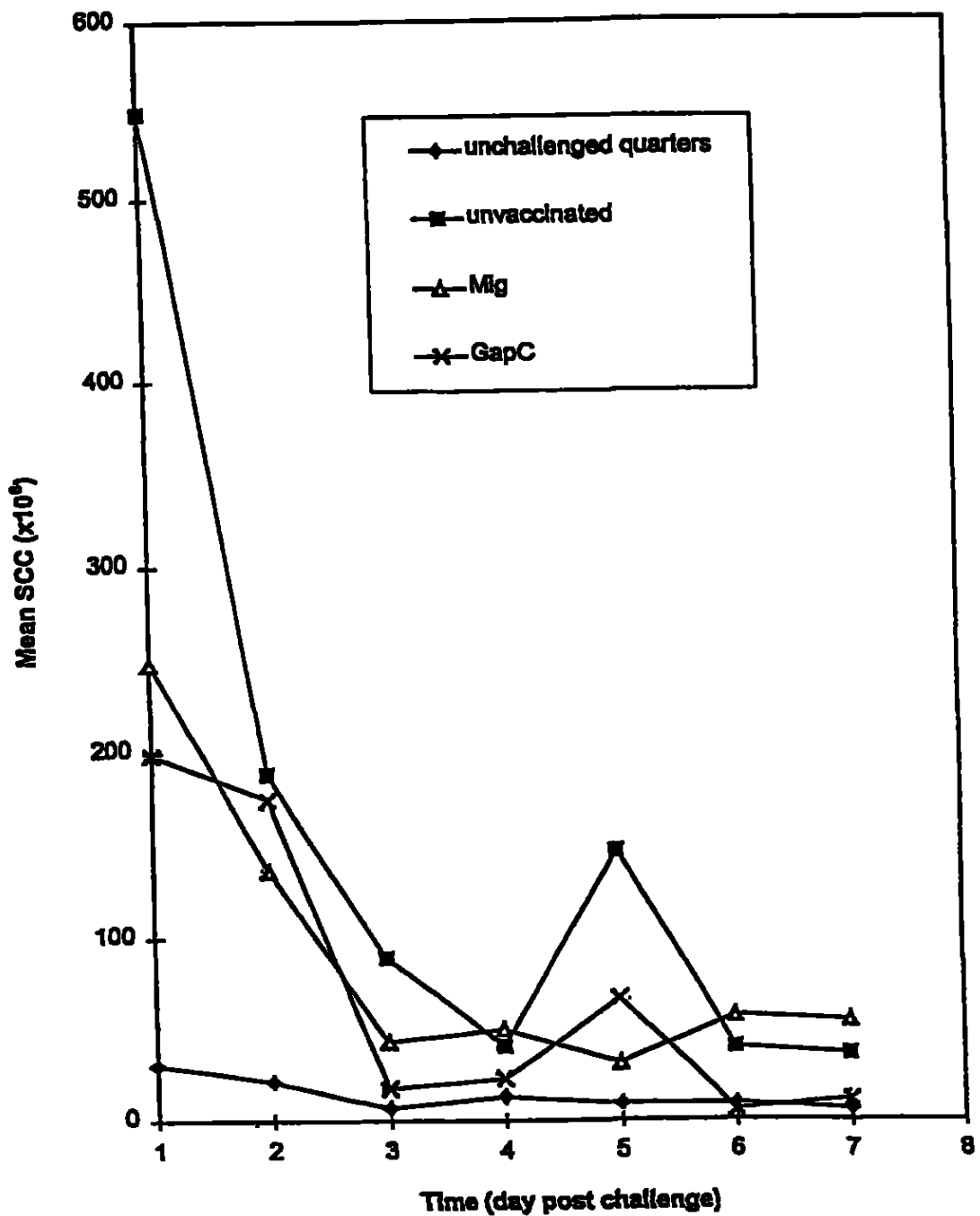


FIG 25

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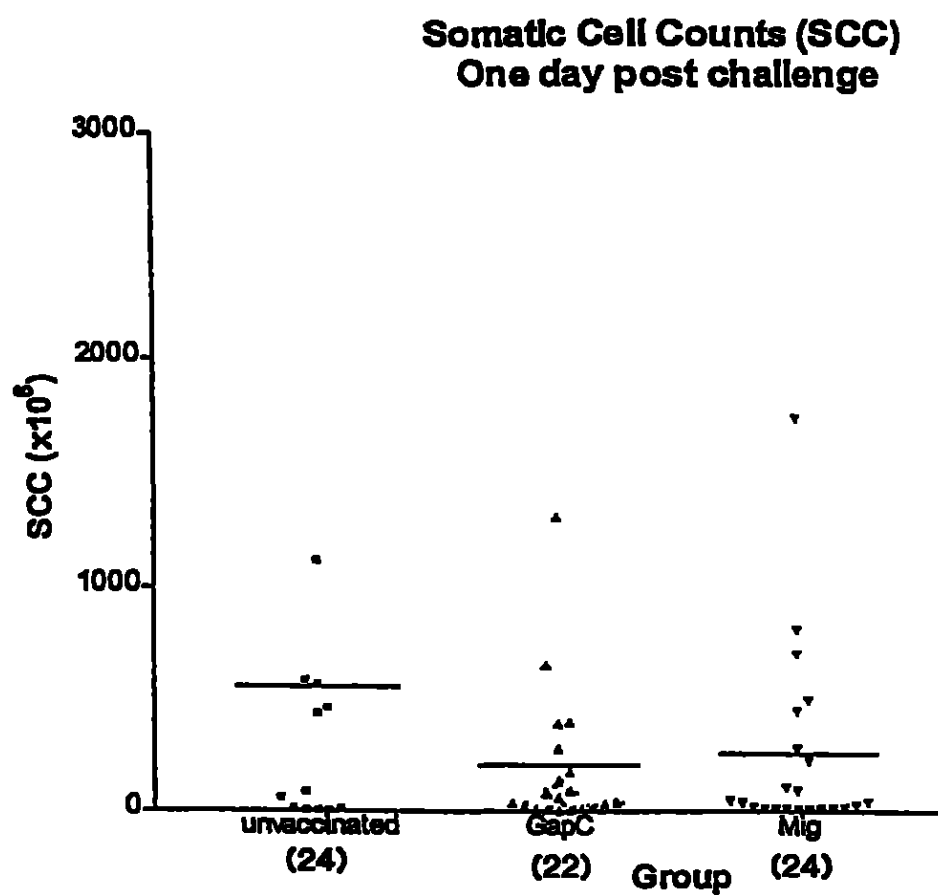


FIG 26

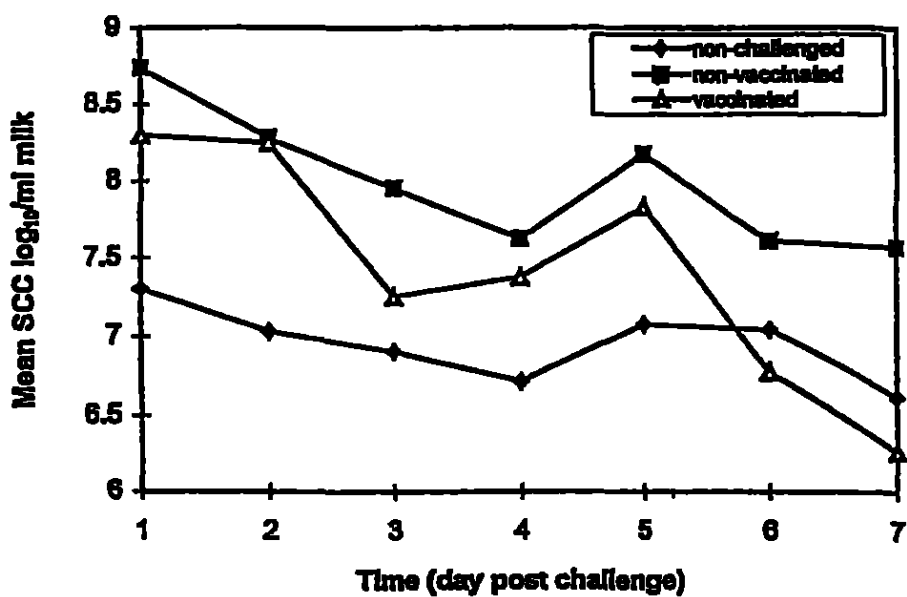


FIG 27

SEQUENCE LISTING

116

<110> University of Saskatchewan
Bolton Alexandra J
Perez-Casal Jose
Fontaine Michael
Potter Andrew A

<120> IMMUNIZATION OF DAIRY CATTLE WITH GapC PROTEIN AGAINST
STREPTOCOCCUS INFECTION

<130> 08-891814WO

<140>

<141>

<160> 12

<170> PatentIn Ver 2 0

<210> 1

<211> 37

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence primer gapC1

<400> 1

ggcggcggca tatggtagtt aaagttggtt ttaacgg

37

<210> 2

<211> 35

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence primer gapC1r

<400> 2

gcggatcctt atttagcgat ttttgcaaag tactc

35

<210> 3

<211> 1011

<212> DNA

<213> Streptococcus dysgalactiae

<220>

<221> CDS

<222> (1) (1011)

<400> 3

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117

atg gta gtt aaa gtt ggt att aac ggt ttc ggt cgt atc gga cgt ctt	48
Met Val Val Lys Val Gly Ile Asn Gly Phe Gly Arg Ile Gly Arg Leu	
1 5 10 15	
gca ttc cgt cgt att caa aat gtt gaa ggt gtt gaa gta act cgt atc	96
Ala Phe Arg Arg Ile Gln Asn Val Glu Gly Val Glu Val Thr Arg Ile	
20 25 30	
aac gac ctt aca gat cca aac atg ctt gca cac ttg ttg aaa tac gat	144
Asn Asp Leu Thr Asp Pro Asn Met Leu Ala His Leu Leu Lys Tyr Asp	
35 40 45	
aca act caa gga cgt ttt gac gga act gtt gaa gtt aaa gaa ggt gga	192
Thr Thr Gln Gly Arg Phe Asp Gly Thr Val Glu Val Lys Glu Gly Gly	
50 55 60	
ttt gaa gta aac gga aac ttc atc aaa gtt tct gct gaa cgt gat cca	240
Phe Glu Val Asn Gly Asn Phe Ile Lys Val Ser Ala Glu Arg Asp Pro	
65 70 75 80	
gaa aac atc gac tgg gca act gac ggt gtt gaa atc gtt ctg gaa gca	288
Glu Asn Ile Asp Trp Ala Thr Asp Gly Val Glu Ile Val Leu Glu Ala	
85 90 95	
act ggt ttc ttt gct aaa aaa gaa gct gct gaa aaa cac tta cat gct	336
Thr Gly Phe Phe Ala Lys Lys Glu Ala Ala Glu Lys His Leu His Ala	
100 105 110	
aac ggt gct aaa aaa gtt gtt atc aca gct cct ggt gga aac gac gtt	384
Asn Gly Ala Lys Lys Val Val Ile Thr Ala Pro Gly Gly Asn Asp Val	
115 120 125	
aaa aca gtt gtt ttc aac act aac cac gac att ctt gac ggt act gaa	432
Lys Thr Val Val Phe Asn Thr Asn His Asp Ile Leu Asp Gly Thr Glu	
130 135 140	
aca gtt atc tca ggt gct tca tgt act aca aac tgt tta gct cct atg	480
Thr Val Ile Ser Gly Ala Ser Cys Thr Thr Asn Cys Leu Ala Pro Met	
145 150 155 160	
gct aaa gct ctt cac gat gca ttt ggt atc caa aaa ggt ctt atg act	528
Ala Lys Ala Leu His Asp Ala Phe Gly Ile Gln Lys Gly Leu Met Thr	
165 170 175	
aca atc cac gct tat act ggt gac caa atg atc ctt gac gga cca cac	576
Thr Ile His Ala Tyr Thr Gly Asp Gln Met Ile Leu Asp Gly Pro His	
180 185 190	
cgt ggt ggt gac ctt cgt cgt gct cgt gct ggt gct gca aac att gtt	624
Arg Gly Gly Asp Leu Arg Arg Ala Arg Ala Gly Ala Ala Asn Ile Val	
195 200 205	
cct aac tca act ggt got gct aaa gct atc ggt ctt gtt atc cca gaa	672
Pro Asn Ser Thr Gly Ala Ala Lys Ala Ile Gly Leu Val Ile Pro Glu	

118

210	215	220	
ttg aat ggt aaa ctt gat ggt gct gca caa cgt gtt cct gtt cca act			720
Leu Asn Gly Lys Leu Asp Gly Ala Ala Gln Arg Val Pro Val Pro Thr			
225	230	235	240
gga tca gta act gag ttg gtt gta act ctt gat aaa aac gtt tct gtt			768
Gly Ser Val Thr Glu Leu Val Val Thr Leu Asp Lys Asn Val Ser Val			
245	250		255
gac gaa atc aac gct gct atg aaa gct gct tca aac gac agt ttc ggt			816
Asp Glu Ile Asn Ala Ala Met Lys Ala Ala Ser Asn Asp Ser Phe Gly			
260	265		270
tac act gaa gat cca att gtt tct tca gat atc gta ggc gtg tca tac			864
Tyr Thr Glu Asp Pro Ile Val Ser Ser Asp Ile Val Gly Val Ser Tyr			
275	280		285
ggt tca ttg ttt gac gca act caa act aaa gtt atg gaa gtt gac gga			912
Gly Ser Leu Phe Asp Ala Thr Gln Thr Lys Val Met Glu Val Asp Gly			
290	295		300
tca caa ttg gtt aaa gtt gta tca tgg tat gac aat gaa atg tct tac			960
Ser Gln Leu Val Lys Val Val Ser Trp Tyr Asp Asn Glu Met Ser Tyr			
305	310	315	320
act gct caa ctt gtt cgt aca ctt gag tac ttt gca aaa atc gct aaa			1008
Thr Ala Gln Leu Val Arg Thr Leu Glu Tyr Phe Ala Lys Ile Ala Lys			
325	330		335
taa			1011

<210> 4

<211> 336

<212> PRT

<213> Streptococcus dysgalactiae

<400> 4

Met Val Val Lys Val Gly Ile Asn Gly Phe Gly Arg Ile Gly Arg Leu			
1	5	10	15
Ala Phe Arg Arg Ile Gln Asn Val Glu Gly Val Glu Val Thr Arg Ile			
20	25		30
Asn Asp Leu Thr Asp Pro Asn Met Leu Ala His Leu Leu Lys Tyr Asp			
35	40		45
Thr Thr Gln Gly Arg Phe Asp Gly Thr Val Glu Val Lys Glu Gly Gly			
50	55		60
Phe Glu Val Asn Gly Asn Phe Ile Lys Val Ser Ala Glu Arg Asp Pro			
65	70	75	80
Glu Asn Ile Asp Trp Ala Thr Asp Gly Val Glu Ile Val Leu Glu Ala			

119

	85	90	95	
Thr Gly Phe Phe Ala Lys Lys Glu Ala Ala Glu Lys His Leu His Ala	100	105	110	
Asn Gly Ala Lys Lys Val Val Ile Thr Ala Pro Gly Gly Asn Asp Val	115	120	125	
Lys Thr Val Val Phe Asn Thr Asn His Asp Ile Leu Asp Gly Thr Glu	130	135	140	
Thr Val Ile Ser Gly Ala Ser Cys Thr Thr Asn Cys Leu Ala Pro Met	145	150	155	160
Ala Lys Ala Leu His Asp Ala Phe Gly Ile Gln Lys Gly Leu Met Thr	165	170	175	
Thr Ile His Ala Tyr Thr Gly Asp Gln Met Ile Leu Asp Gly Pro His	180	185	190	
Arg Gly Gly Asp Leu Arg Arg Ala Arg Ala Gly Ala Ala Asn Ile Val	195	200	205	
Pro Asn Ser Thr Gly Ala Ala Lys Ala Ile Gly Leu Val Ile Pro Glu	210	215	220	
Leu Asn Gly Lys Leu Asp Gly Ala Ala Gln Arg Val Pro Val Pro Thr	225	230	235	240
Gly Ser Val Thr Glu Leu Val Val Thr Leu Asp Lys Asn Val Ser Val	245	250	255	
Asp Glu Ile Asn Ala Ala Met Lys Ala Ala Ser Asn Asp Ser Phe Gly	260	265	270	
Tyr Thr Glu Asp Pro Ile Val Ser Ser Asp Ile Val Gly Val Ser Tyr	275	280	285	
Gly Ser Leu Phe Asp Ala Thr Gln Thr Lys Val Met Glu Val Asp Gly	290	295	300	
Ser Gln Leu Val Lys Val Val Ser Trp Tyr Asp Asn Glu Met Ser Tyr	305	310	315	320
Thr Ala Gln Leu Val Arg Thr Leu Glu Tyr Phe Ala Lys Ile Ala Lys	325	330	335	

<210> 5

<211> 1011

<212> DNA

<213> Streptococcus agalactiae

<220>

<221> CDS

<222> (1) (1011)

120

<400> 5
 atg gta gtt aaa gtt ggt att aac ggt ttc ggt cgt atc ggt cgt ctt 48
 Met Val Val Lys Val Gly Ile Asn Gly Phe Gly Arg Ile Gly Arg Leu
 1 5 10 15

gca ttc cgt cgc atc caa aac gta gaa ggt gtt gaa gtt act cgt atc 96
 Ala Phe Arg Arg Ile Gln Asn Val Glu Gly Val Glu Val Thr Arg Ile
 20 25 30

aac gac ctt aca gat cca aac atg ctt gca cac ttg ttg aaa tat gac 144
 Asn Asp Leu Thr Asp Pro Asn Met Leu Ala His Leu Leu Lys Tyr Asp
 35 40 45

aca act caa ggt cgt ttc gac ggt act gtt gaa gtt aaa gaa ggt gga 192
 Thr Thr Gln Gly Arg Phe Asp Gly Thr Val Glu Val Lys Glu Gly Gly
 50 55 60

ttc gaa gtt aac ggt caa ttt gtt aaa gtt tct gct gaa cgc gaa cca 240
 Phe Glu Val Asn Gly Gln Phe Val Lys Val Ser Ala Glu Arg Glu Pro
 65 70 75 80

gca aac att gac tgg gct act gat ggc gta gaa atc gtt ctt gaa gca 288
 Ala Asn Ile Asp Trp Ala Thr Asp Gly Val Glu Ile Val Leu Glu Ala
 85 90 95

act ggt ttc ttt gca tca aaa gaa aaa gct gga caa cac atc cat gaa 336
 Thr Gly Phe Phe Ala Ser Lys Glu Lys Ala Gly Gln His Ile His Glu
 100 105 110

aat ggt gct aaa aaa gtt gtt atc aca gct cct ggt gga aac gac gtt 384
 Asn Gly Ala Lys Lys Val Val Ile Thr Ala Pro Gly Gly Asn Asp Val
 115 120 125

aaa aca gtt gtt ttc aac act aac cac gat atc ctt gat gga act gaa 432
 Lys Thr Val Val Phe Asn Thr Asn His Asp Ile Leu Asp Gly Thr Glu
 130 135 140

aca gtt atc tca ggt gct tca tgt act aca aac tgt ctt gct cca atg 480
 Thr Val Ile Ser Gly Ala Ser Cys Thr Thr Asn Cys Leu Ala Pro Met
 145 150 155 160

gct aaa gct tta caa gac aac ttt ggt gtt aaa caa ggt ttg atg act 528
 Ala Lys Ala Leu Gln Asp Asn Phe Gly Val Lys Gln Gly Leu Met Thr
 165 170 175

act atc cac gca tac act ggt gac caa atg atc ctt gac gga cca cac 576
 Thr Ile His Ala Tyr Thr Gly Asp Gln Met Ile Leu Asp Gly Pro His
 180 185 190

cgt ggt ggt gac ctt cgt cgt gct cgt gca ggt gct gca aac atc gtt 624
 Arg Gly Gly Asp Leu Arg Arg Ala Arg Ala Gly Ala Ala Asn Ile Val
 195 200 205

cct aac tca act ggt gct gca aaa gct atc gga ctt gtt atc cca gaa 672

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Pro	Asn	Ser	Thr	Gly	Ala	Ala	Lys	Ala	Ile	Gly	Leu	Val	Ile	Pro	Glu	
210						215					220					
ttg	aac	ggt	aaa	ctt	gat	ggt	gct	gca	caa	cgt	gtt	cct	gtt	cca	act	720
Leu	Asn	Gly	Lys	Leu	Asp	Gly	Ala	Ala	Gln	Arg	Val	Pro	Val	Pro	Thr	
225					230				235						240	
gga	tca	gta	act	gaa	ttg	gtt	gca	act	ctt	gaa	aaa	gac	gta	act	gtc	768
Gly	Ser	Val	Thr	Glu	Leu	Val	Ala	Thr	Leu	Glu	Lys	Asp	Val	Thr	Val	
				245					250					255		
gaa	gaa	gta	aat	gca	gct	atg	aaa	gca	gca	gct	aac	gat	tca	tac	ggt	816
Glu	Glu	Val	Asn	Ala	Ala	Met	Lys	Ala	Ala	Ala	Asn	Asp	Ser	Tyr	Gly	
			260					265						270		
tat	act	gaa	gat	cca	atc	gta	tca	tct	gat	atc	gtt	ggt	att	tca	tac	864
Tyr	Thr	Glu	Asp	Pro	Ile	Val	Ser	Ser	Asp	Ile	Val	Gly	Ile	Ser	Tyr	
			275				280						285			
ggt	tca	ttg	ttt	gat	gct	act	caa	act	aaa	gtt	caa	act	gtt	gac	ggt	912
Gly	Ser	Leu	Phe	Asp	Ala	Thr	Gln	Thr	Lys	Val	Gln	Thr	Val	Asp	Gly	
			290			295					300					
aac	caa	ttg	gtt	aaa	gtt	gtt	tca	tggt	tac	gat	aac	gaa	atg	tca	tac	960
Asn	Gln	Leu	Val	Lys	Val	Val	Ser	Trp	Tyr	Asp	Asn	Glu	Met	Ser	Tyr	
305					310					315					320	
act	tca	caa	ctt	gtt	cgt	aca	ctt	gag	tac	ttt	gca	aaa	atc	gct	aaa	1008
Thr	Ser	Gln	Leu	Val	Arg	Thr	Leu	Glu	Tyr	Phe	Ala	Lys	Ile	Ala	Lys	
				325				330						335		
taa																1011

<210> 6
 <211> 336
 <212> PRT
 <213> Streptococcus agalactiae

<400> 6
 Met Val Val Lys Val Gly Ile Asn Gly Phe Gly Arg Ile Gly Arg Leu
 1 5 10 15

Ala Phe Arg Arg Ile Gln Asn Val Glu Gly Val Glu Val Thr Arg Ile
 20 25 30

Asn Asp Leu Thr Asp Pro Asn Met Leu Ala His Leu Leu Lys Tyr Asp
 35 40 45

Thr Thr Gln Gly Arg Phe Asp Gly Thr Val Glu Val Lys Glu Gly Gly
 50 55 60

Phe Glu Val Asn Gly Gln Phe Val Lys Val Ser Ala Glu Arg Glu Pro
 65 70 75 80

(22

Ala Asn Ile Asp Trp Ala Thr Asp Gly Val Glu Ile Val Leu Glu Ala
85 90 95

Thr Gly Phe Phe Ala Ser Lys Glu Lys Ala Gly Gln His Ile His Glu
100 105 110

Asn Gly Ala Lys Lys Val Val Ile Thr Ala Pro Gly Gly Asn Asp Val
115 120 125

Lys Thr Val Val Phe Asn Thr Asn His Asp Ile Leu Asp Gly Thr Glu
130 135 140

Thr Val Ile Ser Gly Ala Ser Cys Thr Thr Asn Cys Leu Ala Pro Met
145 150 155 160

Ala Lys Ala Leu Gln Asp Asn Phe Gly Val Lys Gln Gly Leu Met Thr
165 170 175

Thr Ile His Ala Tyr Thr Gly Asp Gln Met Ile Leu Asp Gly Pro His
180 185 190

Arg Gly Gly Asp Leu Arg Arg Ala Arg Ala Gly Ala Ala Asn Ile Val
195 200 205

Pro Asn Ser Thr Gly Ala Ala Lys Ala Ile Gly Leu Val Ile Pro Glu
210 215 220

Leu Asn Gly Lys Leu Asp Gly Ala Ala Gln Arg Val Pro Val Pro Thr
225 230 235 240

Gly Ser Val Thr Glu Leu Val Ala Thr Leu Glu Lys Asp Val Thr Val
245 250 255

Glu Glu Val Asn Ala Ala Met Lys Ala Ala Ala Asn Asp Ser Tyr Gly
260 265 270

Tyr Thr Glu Asp Pro Ile Val Ser Ser Asp Ile Val Gly Ile Ser Tyr
275 280 285

Gly Ser Leu Phe Asp Ala Thr Gln Thr Lys Val Gln Thr Val Asp Gly
290 295 300

Asn Gln Leu Val Lys Val Val Ser Trp Tyr Asp Asn Glu Met Ser Tyr
305 310 315 320

Thr Ser Gln Leu Val Arg Thr Leu Glu Tyr Phe Ala Lys Ile Ala Lys
325 330 335

<210> 7

<211> 1011

<212> DNA

<213> Streptococcus uberis

<220>

<221> CDS

123

<222> (1) (1011)

<400> 7

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Met Val Val Lys Val Gly Ile Asn Gly Phe Gly Arg Ile Gly Arg Leu	
1 5 10 15	
gca ttc cgt cgt att caa aac gtt gaa ggt gtt gaa gta act cgt att	96
Ala Phe Arg Arg Ile Gln Asn Val Glu Gly Val Glu Val Thr Arg Ile	
20 25 30	
aac gat ctt act gac cca aat atg ctt gca cac ttg ttg aaa tat gat	144
Asn Asp Leu Thr Asp Pro Asn Met Leu Ala His Leu Leu Lys Tyr Asp	
35 40 45	
aca act caa ggt cgt ttc gac ggt aca gtt gaa gtt aaa gat ggt gga	192
Thr Thr Gln Gly Arg Phe Asp Gly Thr Val Glu Val Lys Asp Gly Gly	
50 55 60	
ttc gaa gtt aac gga aac ttc atc aaa gtt tct gct gaa aaa gat cca	240
Phe Glu Val Asn Gly Asn Phe Ile Lys Val Ser Ala Glu Lys Asp Pro	
65 70 75 80	
gaa aac att gac tgg gca act gac ggt gta gaa atc gtt ctt gaa gca	288
Glu Asn Ile Asp Trp Ala Thr Asp Gly Val Glu Ile Val Leu Glu Ala	
85 90 95	
act ggt ttc ttt gct aaa aaa gca gct gct gaa aaa cat tta cat gct	336
Thr Gly Phe Phe Ala Lys Lys Ala Ala Ala Glu Lys His Leu His Ala	
100 105 110	
aac ggt gct aaa aaa gtt gtt atc aca gct cot ggt gga gat gat gtt	384
Asn Gly Ala Lys Lys Val Val Ile Thr Ala Pro Gly Gly Asp Asp Val	
115 120 125	
aaa act gtt gta ttt aac aca aac cat gac att ctt gac ggt aca gaa	432
Lys Thr Val Val Phe Asn Thr Asn His Asp Ile Leu Asp Gly Thr Glu	
130 135 140	
act gta att tca ggt gct tca tgt act act aac tgt tta gct cca atg	480
Thr Val Ile Ser Gly Ala Ser Cys Thr Thr Asn Cys Leu Ala Pro Met	
145 150 155 160	
gct aaa gct ttg caa gat aac ttt ggt gtt aaa caa ggt ttg atg aca	528
Ala Lys Ala Leu Gln Asp Asn Phe Gly Val Lys Gln Gly Leu Met Thr	
165 170 175	
act atc cac gct tac act ggt gac caa atg atc ctt gac gga cca cac	576
Thr Ile His Ala Tyr Thr Gly Asp Gln Met Ile Leu Asp Gly Pro His	
180 185 190	
cgt ggt ggt gac ctt cgt cgt gct cgt gct ggt gca agc aac att gtt	624
Arg Gly Gly Asp Leu Arg Arg Ala Arg Ala Gly Ala Ser Asn Ile Val	
195 200 205	

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124

cct aac tca act ggt gct gct aaa gca atc ggt ctt gta atc cca gaa	672
Pro Asn Ser Thr Gly Ala Ala Lys Ala Ile Gly Leu Val Ile Pro Glu	
210 215 220	
tta aat ggt aaa ctt gac ggt gct gca caa cgt gtt cct gtt cca act	720
Leu Asn Gly Lys Leu Asp Gly Ala Ala Gln Arg Val Pro Val Pro Thr	
225 230 235 240	
gga tca gta act gaa tta gta gca gtt ctt gaa aaa gaa act tca gtt	768
Gly Ser Val Thr Glu Leu Val Ala Val Leu Glu Lys Glu Thr Ser Val	
245 250 255	
gaa gaa atc aac gca gca atg aaa gca gct gca aac gat tca tac gga	816
Glu Glu Ile Asn Ala Ala Met Lys Ala Ala Ala Asn Asp Ser Tyr Gly	
260 265 270	
tac act gaa gac cca atc gta tct tct gat atc atc ggt atg gct tac	864
Tyr Thr Glu Asp Pro Ile Val Ser Ser Asp Ile Ile Gly Met Ala Tyr	
275 280 285	
ggt tca ttg ttt gat gct act caa act aaa gta caa act gtt gat gga	912
Gly Ser Leu Phe Asp Ala Thr Gln Thr Lys Val Gln Thr Val Asp Gly	
290 295 300	
aat caa tta gtt aaa gtt gtt tca tgg tat gac aac gaa atg tct tac	960
Asn Gln Leu Val Lys Val Val Ser Trp Tyr Asp Asn Glu Met Ser Tyr	
305 310 315 320	
act gca caa ctt gtt cgt act ctt gag tac ttt gca aaa atc gct aaa	1008
Thr Ala Gln Leu Val Arg Thr Leu Glu Tyr Phe Ala Lys Ile Ala Lys	
325 330 335	
taa	1011

<210> 8

<211> 336

<212> PRT

<213> Streptococcus uberis

<400> 8

Met Val Val Lys Val Gly Ile Asn Gly Phe Gly Arg Ile Gly Arg Leu
1 5 10 15

Ala Phe Arg Arg Ile Gln Asn Val Glu Gly Val Glu Val Thr Arg Ile
20 25 30

Asn Asp Leu Thr Asp Pro Asn Met Leu Ala His Leu Leu Lys Tyr Asp
35 40 45

Thr Thr Gln Gly Arg Phe Asp Gly Thr Val Glu Val Lys Asp Gly Gly
50 55 60

Phe Glu Val Asn Gly Asn Phe Ile Lys Val Ser Ala Glu Lys Asp Pro
65 70 75 80

Glu	Asn	Ile	Asp	Trp	Ala	Thr	Asp	Gly	Val	Glu	Ile	Val	Leu	Glu	Ala	85	90	95
Thr	Gly	Phe	Phe	Ala	Lys	Lys	Ala	Ala	Ala	Glu	Lys	His	Leu	His	Ala	100	105	110
Asn	Gly	Ala	Lys	Lys	Val	Val	Ile	Thr	Ala	Pro	Gly	Gly	Asp	Asp	Val	115	120	125
Lys	Thr	Val	Val	Phe	Asn	Thr	Asn	His	Asp	Ile	Leu	Asp	Gly	Thr	Glu	130	135	140
Thr	Val	Ile	Ser	Gly	Ala	Ser	Cys	Thr	Thr	Asn	Cys	Leu	Ala	Pro	Met	145	150	155
Ala	Lys	Ala	Leu	Gln	Asp	Asn	Phe	Gly	Val	Lys	Gln	Gly	Leu	Met	Thr	165	170	175
Thr	Ile	His	Ala	Tyr	Thr	Gly	Asp	Gln	Met	Ile	Leu	Asp	Gly	Pro	His	180	185	190
Arg	Gly	Gly	Asp	Leu	Arg	Arg	Ala	Arg	Ala	Gly	Ala	Ser	Asn	Ile	Val	195	200	205
Pro	Asn	Ser	Thr	Gly	Ala	Ala	Lys	Ala	Ile	Gly	Leu	Val	Ile	Pro	Glu	210	215	220
Leu	Asn	Gly	Lys	Leu	Asp	Gly	Ala	Ala	Gln	Arg	Val	Pro	Val	Pro	Thr	225	230	235
Gly	Ser	Val	Thr	Glu	Leu	Val	Ala	Val	Leu	Glu	Lys	Glu	Thr	Ser	Val	245	250	255
Glu	Glu	Ile	Asn	Ala	Ala	Met	Lys	Ala	Ala	Ala	Asn	Asp	Ser	Tyr	Gly	260	265	270
Tyr	Thr	Glu	Asp	Pro	Ile	Val	Ser	Ser	Asp	Ile	Ile	Gly	Met	Ala	Tyr	275	280	285
Gly	Ser	Leu	Phe	Asp	Ala	Thr	Gln	Thr	Lys	Val	Gln	Thr	Val	Asp	Gly	290	295	300
Asn	Gln	Leu	Val	Lys	Val	Val	Ser	Trp	Tyr	Asp	Asn	Glu	Met	Ser	Tyr	305	310	315
Thr	Ala	Gln	Leu	Val	Arg	Thr	Leu	Glu	Tyr	Phe	Ala	Lys	Ile	Ala	Lys	325	330	335

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<210> 9
<211> 1011
<212> DNA
<213> Streptococcus parauberis
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<220>
<221> CDB

126

<222> (1) (1011)

<400> 9

atg gta gtt aaa gtt ggt att aac ggt ttt ggc cgt atc gga cgt ctt	48
Met Val Val Lys Val Gly Ile Asn Gly Phe Gly Arg Ile Gly Arg Leu	
1 5 10 15	
gct ttc cgt cgt att caa aat gta gaa ggt gtt gaa gtt act cgc atc	96
Ala Phe Arg Arg Ile Gln Asn Val Glu Gly Val Glu Val Thr Arg Ile	
20 25 30	
aac gac ctt aca gat cca aat atg ctt gca cac ttg tta aaa tac gat	144
Asn Asp Leu Thr Asp Pro Asn Met Leu Ala His Leu Leu Lys Tyr Asp	
35 40 45	
aca act caa ggt cgt ttt gac ggt act gta gaa gtt aaa gat ggt gga	192
Thr Thr Gln Gly Arg Phe Asp Gly Thr Val Glu Val Lys Asp Gly Gly	
50 55 60	
ttt gac gtt aac gga aaa ttc att aaa gtt tct gct gaa aaa gat cca	240
Phe Asp Val Asn Gly Lys Phe Ile Lys Val Ser Ala Glu Lys Asp Pro	
65 70 75 80	
gaa caa att gac tgg gca act gac ggt gtt gaa atc gtt ctt gaa gca	288
Glu Gln Ile Asp Trp Ala Thr Asp Gly Val Glu Ile Val Leu Glu Ala	
85 90 95	
act ggt ttc ttt gct aaa aaa gca gct gct gaa aaa cat tta cat gaa	336
Thr Gly Phe Phe Ala Lys Lys Ala Ala Ala Glu Lys His Leu His Glu	
100 105 110	
aat ggt gct aaa aaa gtt gtt atc act gct cct ggt gga gat gac gtg	384
Asn Gly Ala Lys Lys Val Val Ile Thr Ala Pro Gly Gly Asp Asp Val	
115 120 125	
aaa aca gtt gta ttt aac act aac cat gat atc ctt gat gga act gaa	432
Lys Thr Val Val Phe Asn Thr Asn His Asp Ile Leu Asp Gly Thr Glu	
130 135 140	
aca gtt att tca ggt gct tca tgt act aca aac tgt tta gct cca atg	480
Thr Val Ile Ser Gly Ala Ser Cys Thr Thr Asn Cys Leu Ala Pro Met	
145 150 155 160	
gct aaa gct tta caa gat aac ttt ggc gta aaa caa ggt tta atg act	528
Ala Lys Ala Leu Gln Asp Asn Phe Gly Val Lys Gln Gly Leu Met Thr	
165 170 175	
aca atc cac gct tac act ggt gat caa atg ctt ctt gat gga cct cac	576
Thr Ile His Ala Tyr Thr Gly Asp Gln Met Leu Leu Asp Gly Pro His	
180 185 190	
cgt ggt ggt gac tta cgt cgt gcc cgt gct ggt gct aac aat att gtt	624
Arg Gly Gly Asp Leu Arg Arg Ala Arg Ala Gly Ala Asn Asn Ile Val	
195 200 205	
cct aac tca act ggt gct gct aaa gca atc ggt ctt gtt atc cct gaa	672

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Pro Asn Ser Thr Gly Ala Ala Lys Ala Ile Gly Leu Val Ile Pro Glu
210 215 220

tta aat ggt aaa ctt gac ggt gct gca caa cgt gta cca gtt cca aca 720
Leu Asn Gly Lys Leu Asp Gly Ala Ala Gln Arg Val Pro Val Pro Thr
225 230 235 240

ggt tca gta aca gaa tta gta gca gtt ctt aat aaa gaa act tca gta 768
Gly Ser Val Thr Glu Leu Val Ala Val Leu Asn Lys Glu Thr Ser Val
245 250 255

gaa gaa att aac tca gta atg aaa gct gca gct aat gat tca tat ggt 816
Glu Glu Ile Asn Ser Val Met Lys Ala Ala Ala Asn Asp Ser Tyr Gly
260 265 270

tac act gaa gat cca atc gta tca tct gat atc gtt ggt atg tct ttc 864
Tyr Thr Glu Asp Pro Ile Val Ser Ser Asp Ile Val Gly Met Ser Phe
275 280 285

ggt tca tta ttc gat gct act caa act aaa gta caa act gtt gat gga 912
Gly Ser Leu Phe Asp Ala Thr Gln Thr Lys Val Gln Thr Val Asp Gly
290 295 300

aat caa tta gtt aaa gtt gtt tca tgg tat gac aat gaa atg tct tac 960
Asn Gln Leu Val Lys Val Val Ser Trp Tyr Asp Asn Glu Met Ser Tyr
305 310 315 320

act gct caa ctt gat cgt aca ctt gag tac ttt gca aaa atc gct aaa 1008
Thr Ala Gln Leu Asp Arg Thr Leu Glu Tyr Phe Ala Lys Ile Ala Lys
325 330 335

taa 1011

<210> 10
<211> 336
<212> PRT
<213> Streptococcus parauberis

<400> 10
Met Val Val Lys Val Gly Ile Asn Gly Phe Gly Arg Ile Gly Arg Leu
1 5 10 15

Ala Phe Arg Arg Ile Gln Asn Val Glu Gly Val Glu Val Thr Arg Ile
20 25 30

Asn Asp Leu Thr Asp Pro Asn Met Leu Ala His Leu Leu Lys Tyr Asp
35 40 45

Thr Thr Gln Gly Arg Phe Asp Gly Thr Val Glu Val Lys Asp Gly Gly
50 55 60

Phe Asp Val Asn Gly Lys Phe Ile Lys Val Ser Ala Glu Lys Asp Pro
65 70 75 80

128

Glu Gln Ile Asp Trp Ala Thr Asp Gly Val Glu Ile Val Leu Glu Ala
85 90 95

Thr Gly Phe Phe Ala Lys Lys Ala Ala Ala Glu Lys His Leu His Glu
100 105 110

Asn Gly Ala Lys Lys Val Val Ile Thr Ala Pro Gly Gly Asp Asp Val
115 120 125

Lys Thr Val Val Phe Asn Thr Asn His Asp Ile Leu Asp Gly Thr Glu
130 135 140

Thr Val Ile Ser Gly Ala Ser Cys Thr Thr Asn Cys Leu Ala Pro Met
145 150 155 160

Ala Lys Ala Leu Gln Asp Asn Phe Gly Val Lys Gln Gly Leu Met Thr
165 170 175

Thr Ile His Ala Tyr Thr Gly Asp Gln Met Leu Leu Asp Gly Pro His
180 185 190

Arg Gly Gly Asp Leu Arg Arg Ala Arg Ala Gly Ala Asn Asn Ile Val
195 200 205

Pro Asn Ser Thr Gly Ala Ala Lys Ala Ile Gly Leu Val Ile Pro Glu
210 215 220

Leu Asn Gly Lys Leu Asp Gly Ala Ala Gln Arg Val Pro Val Pro Thr
225 230 235 240

Gly Ser Val Thr Glu Leu Val Ala Val Leu Asn Lys Glu Thr Ser Val
245 250 255

Glu Glu Ile Asn Ser Val Met Lys Ala Ala Ala Asn Asp Ser Tyr Gly
260 265 270

Tyr Thr Glu Asp Pro Ile Val Ser Ser Asp Ile Val Gly Met Ser Phe
275 280 285

Gly Ser Leu Phe Asp Ala Thr Gln Thr Lys Val Gln Thr Val Asp Gly
290 295 300

Asn Gln Leu Val Lys Val Val Ser Trp Tyr Asp Asn Glu Met Ser Tyr
305 310 315 320

Thr Ala Gln Leu Asp Arg Thr Leu Glu Tyr Phe Ala Lys Ile Ala Lys
325 330 335

<210> 11

<211> 1011

<212> DNA

<213> Streptococcus iniae

<220>

<221> CDS

129

<222> (1) (1011)

<400> 11

atg gta gtt aaa gtt ggt att aac ggt ttc gga cgt atc ggt cgt ctt	48
Met Val Val Lys Val Gly Ile Asn Gly Phe Gly Arg Ile Gly Arg Leu	
1 5 10 15	
gca ttc cgt cgt att caa aat gtt gaa ggt gtt gaa gta act cgt atc	96
Ala Phe Arg Arg Ile Gln Asn Val Glu Gly Val Glu Val Thr Arg Ile	
20 25 30	
aat gac ctt aca gat cct aac atg ctt gca cac ttg ttg aaa tat gat	144
Asn Asp Leu Thr Asp Pro Asn Met Leu Ala His Leu Leu Lys Tyr Asp	
35 40 45	
aca act caa ggt cgt ttt gac ggt aca gtt gaa gtt aaa gat ggt gga	192
Thr Thr Gln Gly Arg Phe Asp Gly Thr Val Glu Val Lys Asp Gly Gly	
50 55 60	
ttc gaa gtt aac gga agc ttt gtt aaa gtt tct gca gaa cgc gaa cca	240
Phe Glu Val Asn Gly Ser Phe Val Lys Val Ser Ala Glu Arg Glu Pro	
65 70 75 80	
gca aac att gac tgg gct act gat ggt gta gac atc gtt ctt gaa gca	288
Ala Asn Ile Asp Trp Ala Thr Asp Gly Val Asp Ile Val Leu Glu Ala	
85 90 95	
aca ggt ttc ttc gct tct aaa gca gct gct gaa caa cac att cac gct	336
Thr Gly Phe Phe Ala Ser Lys Ala Ala Ala Glu Gln His Ile His Ala	
100 105 110	
aac ggt gcg aaa aaa gtt gtt atc aca gct cct ggt gga aat gac gtt	384
Asn Gly Ala Lys Lys Val Val Ile Thr Ala Pro Gly Gly Asn Asp Val	
115 120 125	
aaa aca gtt gtt tac aac act aac cat gat att ctt gat gga act gaa	432
Lys Thr Val Val Tyr Asn Thr Asn His Asp Ile Leu Asp Gly Thr Glu	
130 135 140	
aca gtt atc tca ggt gct tca tgt act aca aac tgt tta gct cca atg	480
Thr Val Ile Ser Gly Ala Ser Cys Thr Thr Asn Cys Leu Ala Pro Met	
145 150 155 160	
gct aaa gca tta caa gat aac ttt ggt gta aaa caa ggt tta atg act	528
Ala Lys Ala Leu Gln Asp Asn Phe Gly Val Lys Gln Gly Leu Met Thr	
165 170 175	
act atc cat ggt tac act ggt gac caa atg gtt ctt gac gga cca cac	576
Thr Ile His Gly Tyr Thr Gly Asp Gln Met Val Leu Asp Gly Pro His	
180 185 190	
cgt ggt ggt gat ctt cgt cgt gct cgt gca gct gca gca aac atc gtt	624
Arg Gly Gly Asp Leu Arg Arg Ala Arg Ala Ala Ala Asn Ile Val	
195 200 205	
cct aac tca act ggt gct gct aaa gca atc ggt ctt gtt atc cca gaa	672

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Pro	Asn	Ser	Thr	Gly	Ala	Ala	Lys	Ala	Ile	Gly	Leu	Val	Ile	Pro	Glu		
210							215				220						
tta	aat	ggt	aaa	ctt	gac	ggt	gct	gca	caa	cgt	gtt	cct	gtt	cca	act	720	
Leu	Asn	Gly	Lys	Leu	Asp	Gly	Ala	Ala	Gln	Arg	Val	Pro	Val	Pro	Thr		
225					230				235					240			
gga	tca	gta	act	gaa	tta	gta	gca	gtt	ctt	gaa	aaa	gat	act	tca	gta	768	
Gly	Ser	Val	Thr	Glu	Leu	Val	Ala	Val	Leu	Glu	Lys	Asp	Thr	Ser	Val		
				245					250					255			
gaa	gaa	atc	aat	gca	gct	atg	aaa	gca	gca	gct	aac	gat	tca	tac	ggt	816	
Glu	Glu	Ile	Asn	Ala	Ala	Met	Lys	Ala	Ala	Ala	Asn	Asp	Ser	Tyr	Gly		
			260					265					270				
tac	act	gaa	gat	gct	atc	gta	tca	tca	gat	atc	gta	ggt	att	tct	tac	864	
Tyr	Thr	Glu	Asp	Ala	Ile	Val	Ser	Ser	Asp	Ile	Val	Gly	Ile	Ser	Tyr		
			275					280					285				
ggt	tca	tta	ttt	gat	gct	act	caa	act	aaa	gta	caa	act	gtt	gat	gga	912	
Gly	Ser	Leu	Phe	Asp	Ala	Thr	Gln	Thr	Lys	Val	Gln	Thr	Val	Asp	Gly		
		290				295					300						
aat	caa	ttg	gtt	aaa	gtt	gtt	tca	tgg	tat	gac	aat	gaa	atg	tct	tac	960	
Asn	Gln	Leu	Val	Lys	Val	Val	Ser	Trp	Tyr	Asp	Asn	Glu	Met	Ser	Tyr		
305					310					315				320			
act	gct	caa	ctt	gtt	cgt	act	ctt	gag	tac	ttt	gca	aaa	atc	gct	aaa	1008	
Thr	Ala	Gln	Leu	Val	Arg	Thr	Leu	Glu	Tyr	Phe	Ala	Lys	Ile	Ala	Lys		
				325					330					335			
taa																1011	

<210> 12

<211> 336

<212> PRT

<213> Streptococcus iniae

<400> 12

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Ala	Phe	Arg	Arg	Ile	Gln	Asn	Val	Glu	Gly	Val	Glu	Val	Thr	Arg	Ile
			20					25					30		

Asn	Asp	Leu	Thr	Asp	Pro	Asn	Met	Leu	Ala	His	Leu	Leu	Lys	Tyr	Asp
		35					40						45		

Thr	Thr	Gln	Gly	Arg	Phe	Asp	Gly	Thr	Val	Glu	Val	Lys	Asp	Gly	Gly
		50				55					60				

Phe	Glu	Val	Asn	Gly	Ser	Phe	Val	Lys	Val	Ser	Ala	Glu	Arg	Glu	Pro
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

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SUBSTITUTE SHEET (RULE 26)

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65	70	75	80
Ala Asn Ile Asp Trp	Ala Thr Asp Gly Val	Asp Ile Val Leu	Glu Ala
85	90	95	
Thr Gly Phe Phe	Ala Ser Lys Ala	Ala Ala Glu Gln	His Ile His Ala
100	105	110	
Asn Gly Ala Lys Lys	Val Val Ile Thr	Ala Pro Gly Gly	Asn Asp Val
115	120	125	
Lys Thr Val Val Tyr	Asn Thr Asn His	Asp Ile Leu	Asp Gly Thr Glu
130	135	140	
Thr Val Ile Ser Gly	Ala Ser Cys Thr	Thr Asn Cys	Leu Ala Pro Met
145	150	155	160
Ala Lys Ala Leu Gln	Asp Asn Phe Gly	Val Lys Gln Gly	Leu Met Thr
165	170	175	
Thr Ile His Gly Tyr	Thr Gly Asp Gln	Met Val Leu	Asp Gly Pro His
180	185	190	
Arg Gly Gly Asp	Leu Arg Arg Ala	Arg Ala Ala Ala	Ala Asn Ile Val
195	200	205	
Pro Asn Ser Thr Gly	Ala Ala Lys Ala	Ile Gly Leu	Val Ile Pro Glu
210	215	220	
Leu Asn Gly Lys Leu	Asp Gly Ala Ala	Gln Arg Val	Pro Val Pro Thr
225	230	235	240
Gly Ser Val Thr Glu	Leu Val Ala Val	Leu Glu Lys	Asp Thr Ser Val
245	250	255	
Glu Glu Ile Asn Ala	Ala Met Lys Ala	Ala Ala Asn	Asp Ser Tyr Gly
260	265	270	
Tyr Thr Glu Asp	Ala Ile Val Ser	Ser Asp Ile	Val Gly Ile Ser Tyr
275	280	285	
Gly Ser Leu Phe Asp	Ala Thr Gln Thr	Lys Val Gln	Thr Val Asp Gly
290	295	300	
Asn Gln Leu Val Lys	Val Val Ser Trp	Tyr Asp Asn	Glu Met Ser Tyr
305	310	315	320
Thr Ala Gln Leu Val	Arg Thr Leu Glu	Tyr Phe Ala	Lys Ile Ala Lys
325	330	335	



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Industria y Comercio
SUPERINTENDENCIA

132

Superalimentación y Alimentos
Radiación: 02112910 00000000 Folios: 132
Fecha (Año): 2012-12-12 16:55:43
Trámite: 011 PCT-PATENT 379 FASE INICI 411 PRESENTA
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

Cl 1357B-B41-003-7

8 de 7 de 89-2

TEL: 312 44 44 44 FAX: 312 44 44 44
E-MAIL: info@sic.gov.co

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CLASIFICACION	TIPO	FECHA	ASO	FECHA	Vr
E 1357B-B41-003-7	COMUNICACION	10/10/12	3 309	07 12 02	1,3 0,000

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1 2 3 4 5 6 7 8 9 10 11 12

1 2 3 4 5 6 7 8 9 10 11 12

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1 2 3 4 5 6 7 8 9 10 11 12

1 2 3 4 5 6 7 8 9 10 11 12

REQUISITOS NECESARIOS PARA PRESENTACION Y AGILIZACION DEL TRAMITE

		USUARIO	RADICADOR
PATENTE DE INVENCION	MODELO DE UTILIDAD	()	(x)
Indicación de que se solicita la concesión de una patente.		()	(x)
- Datos de identificación del solicitante o de la persona que se presenta la solicitud.		()	(x)
Descripción de la invención.		()	(x)
Dibujos de ser estos pertinentes.		()	(x)
Comprobante de Pago de las tasas establecidas.			
COMPLETA	(x)	INCOMPLETA	()
DISEÑO INDUSTRIAL ()			
- Indicación de que se solicita el registro de Diseño Industrial		()	()
- Datos de identificación del solicitante o de la persona que presenta la solicitud.		()	()
- Representación gráfica y fotográfica del Diseño Industrial o muestra del Material que incorpora el diseño.		()	()
Comprobante de pago de las tasas establecidas		()	()
COMPLETA	()	INCOMPLETA	()
ESQUEMA DE TRAZADO ()			
- Indicación de que solicita el registro de un Esquema de trazado		()	()
- Datos de identificación del solicitante o de la persona que presenta la solicitud.		()	()
Representación gráfica del esquema de trazado		()	()
Comprobante de pago de las tasas establecidas		()	()
COMPLETA	()	INCOMPLETA	()

Edgar Navarro
 Firma Responsable:

12-12-2002
 Fecha