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(71) Applicant: **MOLOGIC LTD** [GB/GB]; Bedford Technology Park, Thurleigh Bedfordshire MK44 2YP (GB).

(72) Inventors: **DAVIS, Paul**; c/o Mologic Ltd, Bedford Technology Park, Thurleigh Bedfordshire MK44 2YP (GB).
SCHOUTEN, James; c/o Mologic Ltd, Bedford Technology Park, Thurleigh Bedfordshire MK44 2YP (GB).

(74) Agent: **BOULT WADE TENNANT**; Salisbury Square House, 8 Salisbury Square, London Greater London EC4Y 8AP (GB).

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(54) Title: BACTERIAL VAGINOSIS DIAGNOSTIC

(57) Abstract: The invention provides a sialidase enzyme activity detection kit or device comprising: (i) an indicator molecule comprising a sialylated peptide and a capture site; (ii) a capture zone comprising capture molecules; and (iii) binding molecules capable of binding to the de-sialylated derivative of the indicator molecule. Also provided are methods of using the kits or devices, as well as specific indicator molecules and specific binding molecules.



PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference BNR/P148556WO00	FOR FURTHER ACTION		See Form PCT/IPEA/416
International application No. PCT/GB2019/052101	International filing date (<i>day/month/year</i>) 26.07.2019	Priority date (<i>day/month/year</i>) 27.07.2018	
International Patent Classification (IPC) or national classification and IPC INV. C12Q1/34			
Applicant Mologic Limited			
1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36. 2. This REPORT consists of a total of <u>7</u> sheets, including this cover sheet. 3. This report is also accompanied by ANNEXES, comprising: a. ☒ (<i>sent to the applicant and to the International Bureau</i>) a total of <u>9</u> sheets, as follows: ☒ sheets of the description, claims and/or drawings which have been amended and/or sheets containing rectifications authorized by this Authority, unless those sheets were superseded or cancelled, and any accompanying letters (see Rules 46.5, 66.8, 70.16, 91.2, and Section 607 of the Administrative Instructions). ☐ sheets containing rectifications, where the decision was made by this Authority not to take them into account because they were not authorized by or notified to this Authority at the time when this Authority began to draw up this report, and any accompanying letters (Rules 66.4bis, 70.2(e), 70.16 and 91.2). ☐ superseded sheets and any accompanying letters, where this Authority either considers that the superseding sheets contain an amendment that goes beyond the disclosure in the international application as filed, or the superseding sheets were not accompanied by a letter indicating the basis for the amendments in the application as filed, as indicated in item 4 of Box No. I and the Supplemental Box (see Rule 70.16(b)). b. ☐ (<i>sent to the International Bureau only</i>) a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing, in the form of an Annex C/ST.25 text file, as indicated in the Supplemental Box Relating to Sequence Listing (see paragraph 3ter of Annex C of the Administrative Instructions).			
4. This report contains indications relating to the following items: ☒ Box No. I Basis of the report ☐ Box No. II Priority ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability ☐ Box No. IV Lack of unity of invention ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement ☐ Box No. VI Certain documents cited ☐ Box No. VII Certain defects in the international application ☐ Box No. VIII Certain observations on the international application			
Date of submission of the demand 09.04.2020		Date of completion of this report 13.05.2020	
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Fax: +49 89 2399 - 4465		Authorized officer Hohwy, Morten Telephone No. +49 89 2399-5851	



**INTERNATIONAL PRELIMINARY REPORT
ON PATENTABILITY**

International application No.
PCT/GB2019/052101

Box No. I Basis of the report

1. With regard to the **language**, this report is based on
- ☒ the international application in the language in which it was filed
 - ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of:
 - ☐ international search (under Rules 12.3(a) and 23.1(b))
 - ☐ publication of the international application (under Rule 12.4(a))
 - ☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a) and (b))
2. With regard to the **elements*** of the international application, this report is based on (*replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report*):

Description, Pages

1-57 as originally filed

Sequence listings, SEQ ID NO

1-17 as originally filed

Claims, Numbers

1-26 filed on

09-04-2020

Drawings, Sheets

1-38 as originally filed

- ☒ a sequence listing - see Supplemental Box Relating to Sequence Listing.
3. ☐ The amendments have resulted in the cancellation of:
- ☐ the description, pages
 - ☐ the claims, Nos.
 - ☐ the drawings, sheets/figs
 - ☐ the sequence listing (*specify*):
4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since either they are considered to go beyond the disclosure as filed, or they were not accompanied by a letter indicating the basis for the amendments in the application as filed, as indicated in the Supplemental Box (Rules 70.2(c) and (c-bis)):
- ☐ the description, pages
 - ☐ the claims, Nos.
 - ☐ the drawings, sheets/figs
 - ☐ the sequence listing (*specify*):

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2019/052101

5. ☐ This report has been established:
- ☐ taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rules 66.1(d-*bis*) and 70.2(e)).
 - ☐ without taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91(Rules 66.4*bis* and 70.2(e)).
6. ☒ With regard to top-up searches (Rules 66.1*ter* and 70.2(f)):
- ☒ A top-up search was carried out by this Authority on 30.04.2020 (all discovered documents are listed in the Supplemental Box Relating to Top-up Search).
 - ☐ Additional relevant documents have been discovered during the top-up search.
 - ☐ No top-up search was carried out by this Authority because it would serve no useful purpose.
7. ☐ Supplementary international search report(s) from Authority(ies) has/have been received and taken into account in establishing this report (Rule 45bis.8(b) and (c)).

* If item 4 applies, some or all of those sheets may be marked "superseded".

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	<u>1-26</u>
	No: Claims	
Inventive step (IS)	Yes: Claims	<u>1-26</u>
	No: Claims	
Industrial applicability (IA)	Yes: Claims	<u>1-26</u>
	No: Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed:
 - ☒ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13~~ter~~.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search and/or examination:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13~~ter~~.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13~~ter~~.1(b) and Administrative Instructions, Section 713).
 - d. ☐ furnished to this Authority as an amendment* under PCT Article 34 on :
 - ☐ in the form of an Annex C/ST.25 text file, and preferably identified as "Amended" at the first line of text.
 - ☐ on paper or in the form of an image file.
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

* *If item 4 in Box No. I applies, the sequence listing, which forms part of the basis of the report, may be marked "superseded."*

Reference is made to the following documents:

- D1 NAKANO V ET AL: "A rapid assay of the sialidase activity in species of the Bacteroides fragilis group by using peanut lectin hemagglutination",
ANAEROBE, LONDON, GB,
vol. 12, no. 5-6, 1 October 2006 (2006-10-01), pages 238-241,
XP024917600,
ISSN: 1075-9964, DOI: 10.1016/J.ANAEROBE.2006.07.003
[retrieved on 2006-10-01]
- D2 Gwladys Pourceau ET AL: "Measurement of Enzymatic Activity and Specificity of Human and Avian Influenza Neuraminidases from Whole Virus by Glycoarray and MALDI-TOF Mass Spectrometry",
CHEMBIOCHEM,
vol. 12, no. 13, 7 July 2011 (2011-07-07), pages 2071-2080,
XP55635667,
ISSN: 1439-4227, DOI: 10.1002/cbic.201100128
- D3 TOMIN A ET AL: "Novel assay for direct fluorescent imaging of sialidase activity",
CLINICAL AND BIOMEDICAL SPECTROSCOPY AND IMAGING II, SPIE, 1000 20TH ST. BELLINGHAM WA 98225-6705 USA,
vol. 8087, no. 1, 9 June 2011 (2011-06-09), pages 1-8,
XP060015337,
DOI: 10.1117/12.889172
[retrieved on 1901-01-01]
- D4 US 2005/096263 A1 (KEAY SUSAN K [US] ET AL) 5 May 2005 (2005-05-05)

Re Item V

1 Novelty

- 1.1 The present application describes methods and compositions for detecting the activity of a sialidase by using an indicator molecule comprising a peptide with a Gal-Sial modification, typically on a Ser, and a capture moiety which can be used to immobilize the indicator molecule on a capture zone, further a binding molecule capable of binding to the de-sialylated derivative of the indicator

molecule. The binding molecule is typically an antibody recognizing the Gal-modified peptide. In the experimental examples of the description, five peptides (SEQ. 12-16) are linked to KHL or BSA (check) and used to immunize sheep to produce antibodies that are shown to be able to be used to recognize the peptides and can be used in the sialidase assay. The shorter variant SEQ. 11 was also showed to perform well in ELISA (Example 7).

1.2 Prior art

Document D1 discloses (abstract; p. 239) a neuraminidase (sialidase) assay based on agglutination upon contact with peanut lectin. Inhibition by addition of galactose was observed.

Document D2 discloses (abstract) an assay to assess activity and specificity of neuraminidases using a glycoarray wherein carbohydrate-DNA conjugates labelled with Cy3 are submitted to viral neuraminidase cleavage and monitored by immobilizing on an array and by MALDI-TOF. The oligosaccharide used as substrate is Glc-Gal-NeuAc (a sialic acid).

Document D3 discloses a sialidase activity assay based on the fluorescent substrate 4-MU-NA (2'-(4-Methylumbelliferyl)-alpha-D-N-acetylneuraminic acid).

Document D4 discloses (Fig. 8) glycosylated peptides including NANA-Gal-GlcNAc-SLPAAVVVA and NANA-Gal-GlcNAc-TVPAVVVA including variants wherein D-amino acids are used for stability (par. 129, 160).

1.3 It follows from the comments above, that the present subject matter is novel in the sense of Article 33(2) PCT.

2 Inventive step

2.1 The present application meets the criteria of Article 33(1) PCT, because the present subject-matter involves an inventive step in the sense of Article 33(3) PCT.

2.2 An inventive step can be acknowledged for claim 1 for the following reasons: The prior art does not disclose a sialidase activity assay based on a peptide substrate combined with a recognition molecule (such as an antibody). The mostly used activity assay is the one based on the fluorescent substrate 4-MUNA (*cf.* D3) - it appears to be significantly easier and more convenient. D1-D2 describe other variants, but none which would lead the person skilled in the art to the more involved assay presently described. Hence, claim 1 involves an inventive step.

- 2.3 The subject matter of claims 2-26 involves an inventive step for the same or corresponding reasons.

EUROPEAN PATENT OFFICE (MUNICH)
80298 MUNICH
Germany

9 April 2020

Dear Sir or Madam,

International Patent Application No. PCT/GB2019/052101
In the name of Mologic Ltd
Our Ref: MPS/BNR/OMB/P148556WO00

In response to the International Search Report and Written Opinion, dated 6 November 2019, I hereby file a Demand to request International Examination of the above-identified International patent application.

I also provide payment in respect of the handling and examination fees. If payment does not accompany this letter, or the amount calculated is incorrect, the EPO is hereby authorized to debit or credit my firm's deposit account accordingly.

I provide amended claims 1-26 to replace claims 1-26 currently on file. For the Examiner's convenience, a set of marked-up claims is also provided in order to clearly indicate the changes made at this stage. The amendments are made for expediency and without prejudice; no subject matter is abandoned and the applicant reserves the right to file one or more divisional applications during the national/regional phases. The basis for the amendments as discussed below.

Clarity

Former claims 1-14 have been alleged to lack clarity because they contained references to an "enzyme detection composition of matter" and to a "composition of matter". For expediency, these terms have been deleted from the enclosed claims 1-14.

Former claim 20 has been alleged to lack clarity because it contained the term "consisting essentially of". For expediency, all instances of this term have been removed from the enclosed claims.

The requirements of Article 6 PCT are therefore met.

Novelty

We thank the Examiner for acknowledging the novelty of former claims 1-26. The amendments filed herewith do not broaden the scope of these claims. The requirements of Article 33(1) and (2) PCT are therefore met.

Inventive Step

We thank the Examiner for acknowledging the inventiveness of former claims 1-18 and 20-26.

Former claim 19 has been alleged to lack an inventive step. For expediency, former claim 19 has been deleted.

Former claim 20, which requires the peptide to be as defined in claim 6, has been renumbered as 19 and has been amended explicitly to recite the features of claim 6. The optional feature of former claim 20 has been moved into a new dependent claim (new claim 20).

Since the subject matter of former claim 20 was acknowledged to be inventive, the requirements of Article 33(1) and (3) PCT are met.

Concluding remarks

In light of the amendments and arguments set forth above, we respectfully request reconsideration of the Examiner's outstanding objections. We look forward to receiving a positive IPRP. In the unlikely event that the Examiner has any further objections, he is kindly asked to contact the undersigned by telephone or email, or to issue a further Written Opinion.

Yours faithfully,

RIGBY, Barbara Nicole
For and on behalf of BOULT WADE TENNANT LLP
Association Number 505

CLAIMS

1. An enzyme detection device or enzyme detection kit for detecting the presence in a test sample of cleavage activity of a sialidase enzyme, the device or kit comprising:
 - (i) an indicator molecule comprising
 - (a) a peptide comprising the following sequence:

$$X_1-X_2-X_3[\text{Gal-Sial}]-X_4-X_5 \text{ (SEQ ID NO: 17)}$$
 wherein:
 Sial is a sialyl group;
 X₃ is an amino acid comprising a glycosyl acceptor group and is selected from Ser, Thr, Tyr, Hyl, Hyp, Asn, Arg or phosphoserine (SEP), preferably wherein X₃ is Ser; and
 X₁, X₂, X₄ and X₅ are independently selected from any amino acid provided that at least one, preferably at least two or three, of X₁, X₂, X₄ and X₅ is a D-amino acid and/or a non-standard amino acid or a non-natural amino acid; and
 - b) a capture site which remains intact following cleavage of the sialyl group from the indicator molecule by a sialidase enzyme present in the sample;
 - (ii) a capture zone to receive the test sample, wherein the capture zone comprises capture molecules capable of binding to the capture site of the indicator molecule, irrespective of whether or not the indicator molecule has been cleaved, in order to immobilise the indicator molecule; and
 - (iii) binding molecules capable of binding to the de-sialylated derivative of the indicator molecule, wherein the binding molecules are incapable of binding to the indicator molecule unless and until cleavage of the sialyl group from the indicator molecule by sialidase enzyme present in the sample has occurred.
2. The device or kit of claim 1 wherein at least one of X₁, X₂, X₄ and X₅ is Ala, preferably wherein X₁ and X₂ are both Ala, and/or preferably wherein Ala is DAla or βAla.
3. The device or kit of any one of claims 1-2 wherein at least one of X₁, X₂, X₄ and X₅ is a charged amino acid, optionally wherein each charged amino acid is selected from Arg, DAsp and LOrn.
4. The device or kit of any one of claims 1-3 wherein at least one of X₁, X₂, X₄ and X₅ is a polar amino acid, optionally wherein each polar amino acid is selected from LSer, DSer and Thr.

5. The device or kit of any one of claims 1-4 wherein the peptide comprises the following sequence:



(SEQ ID NO: 10) wherein:

Sial is a sialyl group

X₁ is absent or Thr

X₂ is absent or DAla

X₃ is absent or Nle

X₄ is absent or Glu

X₅ is absent or DAla

X₆ is absent or Arg

X₇ is absent or selected from Glu, Arg, Ser, Nva, βAla

X₈ is absent or selected from DSer, DAla, SEP, Cyc

X₉ is absent or selected from Nva, BIP, DAla, βAla, Orn

X₁₀ is selected from Cyc, Ser, Ile, DAla, DSer

X₁₁ is selected from DAla, Pro, Orn, Nle

X₁₂ is selected from Ser, Thr, Tyr, Hyl, Hyp, Asn, Arg or SEP, preferably Ser

X₁₃ is selected from DAla, BIP, βAla

X₁₄ is selected from Arg, DAsp, Nle, Orn, Nva

X₁₅ is absent or selected from Phe, BIP, Ser, Glu, DAla, DSer

X₁₆ is absent or selected from DSer, Glu

X₁₇ is absent or selected from Val, Ser, Thr

X₁₈ is absent or Cha

X₁₉ is absent or DSer

X₂₀ is absent or Val.

6. The device or kit of any one of claims 1-5 wherein the peptide comprises, or consists of, the following sequence:

- (i) Cyc-DAla-Ser[Gal-Sial]-DAla-Arg (SEQ ID NO: 11)
- (ii) Glu-DSer-Nva-Cyc-DAla-Ser[Gal-Sial]-DAla-Arg-Phe-DSer-Val (SEQ ID NO: 12)
- (iii) Arg-DAla-BIP-Ser-Pro-Ser[Gal-Sial]-DAla-DAsp-Ser (SEQ ID NO: 13)
- (iv) Ser-SEP-DAla-Ile-Orn-Ser[Gal-Sial]-DAla-Nle-Glu (SEQ ID NO: 14)
- (v) DAla-Arg-Nva-DSer-βAla-DAla-Nle-Ser[Gal-Sial]-BIP-Orn-DAla-Glu-Ser (SEQ ID NO: 15); or
- (vi) Thr-DAla-Nle-Glu-DAla-Arg-βAla-Cyc-Orn-DSer-Pro-Ser[Gal-Sial]-βAla-Nva-DSer-Glu-Thr-Cha-DSer-Val (SEQ ID NO: 16).

7. The device or kit of any one of claims 1-6 wherein the peptide is biased for cleavage by one or more specific sialidases, optionally wherein the one or more specific sialidases are of bacterial origin, optionally wherein the bacteria are *Prevotella*, *Bacteroides* and/or *Mobiluncus* species and/or *Gardnerella vaginalis*.
8. The device or kit of any one of claims 1-7 wherein the binding molecule is specific for the de-sialylated form of the peptide defined in claim 6, preferably is specific for an epitope that is present in the peptide motif Cyc-DAla-Ser[Gal]-DAla-Arg and that is absent or cryptic in the corresponding sialylated peptide motif Cyc-DAla-Ser[Gal-Sial]-DAla-Arg.
9. The device or kit of any one of claims 1-8 wherein the binding molecule is an antibody having a heavy chain with 3 CDRs and a light chain with 3 CDRs, wherein the heavy chain CDR1 has SEQ ID NO:1; the heavy chain CDR2 has SEQ ID NO:2; the heavy chain CDR3 has SEQ ID NO:3; the light chain CDR1 has SEQ ID NO: 4; the light chain CDR2 has SEQ ID NO: 5; and the light chain CDR3 has SEQ ID NO: 6, preferably wherein the heavy chain has SEQ ID NO: 7 and/or the light chain has SEQ ID NO: 8.
10. The device or kit of any one of claims 1-9 wherein the binding molecule is labelled with a reporter molecule, preferably a gold particle.
11. The device or kit of any one of claims 1-10 wherein the capture site of the indicator molecule comprises or consists of a biotin molecule or an oxime moiety; and/or is at the N- or C-terminus of the peptide.
12. The device or kit of any one of claims 1-11 wherein the capture site of the indicator molecule is attached to the peptide by a linker, optionally wherein the linker comprises a polyethylene glycol (PEG) moiety, optionally wherein the peptide is linked to a biotin group at its N- or C- terminus via a linker comprising, or consisting of, a polyethylene glycol moiety.
13. The device or kit of any one of claims 1-12 wherein the indicator molecule comprises, or consists of, the following structure:
 - (i) Cyc-DAla-Ser[Gal-Sial]-DAla-Arg-PEG-Biotin
 - (ii) Biotin-PEG-Asp-Glu-DSer-Nva-Cyc-DAla-Ser[Gal-Sial]-DAla-Arg-Phe-DSer-Val
 - (iii) Biotin-PEG-Asp-Arg-DAla-BIP-Ser-Pro-Ser[Gal-Sial]-DAla-DAsp-Ser
 - (iv) Biotin-PEG-Asp-Ser-Ser(PO₃)-DAla-Ile-Orn-Ser[Gal-Sial]-DAla-Nle-Glu

- (v) Biotin-PEG-Asp-DAla-Arg-Nva-DSer-βAla-DAla-Nle-Ser[Gal-Sial]-BIP-Orn-DAla-Glu-Ser; or
- (vi) Biotin-PEG-Asp-Thr-DAla-Nle-Glu-DAla-Arg-βAla-Cyc-Orn-DSer-Pro-Ser[Gal-Sial]-βAla-Nva-DSer-Glu-Thr-Cha-DSer-Val.

14. The device or kit of any one of claims 1-13 wherein the indicator molecule comprises (i) a peptide comprising or consisting of the sequence Cyc-DAla-Ser[Gal-Sial]-DAla-Arg; and (ii) a capture site that comprises or consists of a biotin molecule or an oxime moiety which is optionally linked to the N- or C- terminus of the peptide via a linker comprising or consisting of a polyethylene glycol moiety; and wherein the binding molecule is an antibody as defined in claim 8 or 9 and preferably is labelled with a reporter molecule, most preferably a gold particle.

15. An antibody capable of specifically binding to (i) the de-sialylated derivative of a peptide as defined in any one of claims 1-7; or to (ii) an indicator molecule as defined in any one of claims 1-7 or 11-14, wherein the antibody binds preferentially to the de-sialylated derivative over the sialylated peptide or indicator molecule.

16. The antibody according to claim 15, wherein the antibody is as defined in claim 8, 9 and/or 10.

17. An indicator molecule suitable for use in detecting the presence in a test sample of cleavage activity of a sialidase enzyme, the indicator molecule comprising:

- a) a peptide as defined in any one of claims 1-7; and
- b) a capture site which remains intact following cleavage of the sialyl group from the indicator molecule by a sialidase enzyme present in the sample, wherein the indicator molecule is preferably as defined in any one of claims 1-7 or 11-14.

18. The indicator molecule according to claim 17, wherein the indicator molecule is as defined in claim 13.

19. A peptide comprising or consisting of the following sequence:

- (i) Cyc-DAla-Ser[Gal-Sial]-DAla-Arg (SEQ ID NO: 11)
- (ii) Glu-DSer-Nva-Cyc-DAla-Ser[Gal-Sial]-DAla-Arg-Phe-DSer-Val (SEQ ID NO: 12)
- (iii) Arg-DAla-BIP-Ser-Pro-Ser[Gal-Sial]-DAla-DAsp-Ser (SEQ ID NO: 13)
- (iv) Ser-SEP-DAla-Ile-Orn-Ser[Gal-Sial]-DAla-Nle-Glu (SEQ ID NO: 14)

- (v) $\text{DAla-Arg-Nva-DSer-}\beta\text{Ala-DAla-Nle-Ser[Gal-Sial]-BIP-Orn-DAla-Glu-Ser}$ (SEQ ID NO: 15); or
- (vi) $\text{Thr-DAla-Nle-Glu-DAla-Arg-}\beta\text{Ala-Cyc-Orn-DSer-Pro-Ser[Gal-Sial]-}\beta\text{Ala-Nva-DSer-Glu-Thr-Cha-DSer-Val}$ (SEQ ID NO: 16)

20. The peptide according to claim 19, wherein the peptide

is a peptide comprising or consisting of the sequence $\text{Cyc-DAla-Ser[Gal-Sial]-DAla-Arg}$.

21. A peptide for use in generating an antibody according to claim 15 or 16 wherein the peptide is a de-sialylated derivative of the peptide as defined in any one of claims 1-7.

22. A method for detecting the presence or absence in a test sample of cleavage activity of a sialidase enzyme, the method comprising:

- (i) bringing an indicator molecule as defined in any one of claims 1-7 or 11-14 into contact with the test sample;
- (ii) adding to the test sample binding molecules capable of binding to the de-sialylated derivative of the indicator molecule, wherein the binding molecules are incapable of binding to the indicator molecule unless and until cleavage of the sialyl group from the indicator molecule by sialidase enzyme present in the sample has occurred and wherein the binding molecules are preferably as defined in claims 8, 9 and/or 10;
- (iii) capturing the de-sialylated derivative of the indicator molecule at a capture zone through binding of capture molecules in the capture zone to the capture site, said capture molecules being able to bind to the capture site irrespective of whether or not the indicator molecule has been cleaved; and
- (iv) detecting cleavage of the sialyl group from the indicator molecule by determining binding of the binding molecules to the de-sialylated derivative of the indicator molecule captured in the capture zone.

23. A method for detecting the presence or absence in a test sample of cleavage activity of a sialidase enzyme, the method comprising:

- (i) adding the test sample to a capture zone comprising capture molecules, said capture molecules being bound to the capture site of an indicator molecule as defined in any one of claims 1-7 or 11-14 wherein said capture molecules remain bound to the capture site irrespective of whether or not cleavage of the sialyl group from the indicator molecule by sialidase enzyme present in the sample occurs;
- (ii) adding binding molecules capable of binding to the de-sialylated derivative of the indicator molecule, wherein the binding molecules are incapable of binding to the indicator

molecule unless and until cleavage of the sialyl group from the indicator molecule by sialidase enzyme present in the sample has occurred and wherein the binding molecules are preferably as defined in claims 8, 9 and/or 10;

(iii) detecting cleavage of the sialyl group from the indicator molecule by determining binding of the binding molecules to the de-sialylated derivative of the indicator molecule captured in the capture zone.

24. A method for diagnosing bacterial vaginosis in a test sample by detecting cleavage activity of a sialidase enzyme in the sample, the method comprising:

(i) bringing an indicator molecule as defined in any one of claims 1-7 or 11-14 into contact with the test sample;

(ii) adding to the test sample binding molecules capable of binding to the de-sialylated derivative of the indicator molecule, wherein the binding molecules are incapable of binding to the indicator molecule unless and until cleavage of the sialyl group from the indicator molecule by sialidase enzyme present in the sample has occurred and wherein the binding molecules are preferably as defined in claims 8, 9 and/or 10;

(iii) capturing the de-sialylated derivative of the indicator molecule at a capture zone through binding of capture molecules in the capture zone to the capture site, said capture molecules being able to bind to the capture site irrespective of whether or not the indicator molecule has been cleaved; and

(iv) detecting cleavage of the sialyl group from the indicator molecule by determining binding of the binding molecules to the de-sialylated derivative of the indicator molecule captured in the capture zone wherein an increased level of cleavage compared to a control diagnoses bacterial vaginosis.

25. An enzyme detection kit for detecting the presence in a test sample of cleavage activity of a sialidase enzyme, the kit comprising:

(i) an indicator molecule as defined in any one of claims 1-7 wherein the indicator molecule is preferably freeze-dried onto a carrier;

(ii) capture molecules capable of binding to the capture site of the indicator molecule, irrespective of whether or not the indicator molecule has been cleaved, wherein the capture molecules preferably comprise streptavidin;

(iii) a solid support to which the capture molecules can be attached, or are attached, to form a capture zone to receive the test sample, wherein the solid support preferably comprises nitrocellulose; and

(iv) binding molecules capable of binding to the de-sialylated derivative of the indicator molecule, wherein the binding molecules are incapable of binding to the indicator molecule

unless and until cleavage of the sialyl group from the indicator molecule by sialidase enzyme present in the sample has occurred and wherein the binding molecules are preferably as defined in claims 8, 9 and/or 10; and optionally

(v) an extraction tube and/or a buffer; and/or

(vi) control detection molecules and control detection binders.

26. Use of an enzyme detection device or enzyme detection kit according to any one of claims 1-14, method according to any one of claims 22-24 or the enzyme detection kit according to claim 25 for diagnosing bacterial vaginosis in a test sample.