

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

PCT

To:

see form PCT/ISA/220

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (PCT Rule 43bis.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.
PCT/IB2014/064028

International filing date (day/month/year)
22.08.2014

Priority date (day/month/year)

International Patent Classification (IPC) or both national classification and IPC
INV. A61K39/395 A61K39/42 C07K16/02

Applicant
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1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ 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 Rule 43bis.1(a)(i) with regard to novelty, inventive step and 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

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA:



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Date of completion of
this opinion

see form
PCT/ISA/210

Authorized Officer

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Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:
 - ☒ 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 (Rules 12.3(a) and 23.1 (b)).
2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43bis.1(a))
3. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, 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 13ter.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 only:
 - ☒ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).
4. ☐ 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.
5. Additional comments:

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has, within the applicable time limit:
- ☒ paid additional fees
 - ☐ paid additional fees under protest and, where applicable, the protest fee
 - ☐ paid additional fees under protest but the applicable protest fee was not paid
 - ☐ not paid additional fees
2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is
- ☐ complied with
 - ☒ not complied with for the following reasons:
see separate sheet
4. Consequently, this report has been established in respect of the following parts of the international application:
- ☒ all parts.
 - ☐ the parts relating to claims Nos.

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement
- | | | |
|-------------------------------|-------------|--|
| Novelty (N) | Yes: Claims | <u>3-6, 8-12, 14-18, 22-30, 33-35, 43-45, 47, 48</u> |
| | No: Claims | <u>1, 2, 7, 13, 19-21, 31, 32, 36-42, 46, 49-51</u> |
| Inventive step (IS) | Yes: Claims | <u>33-35, 43-45, 47, 48</u> |
| | No: Claims | <u>1-32, 36-42, 46, 49-51</u> |
| Industrial applicability (IA) | Yes: Claims | <u>1-51</u> |
| | No: Claims | |
2. Citations and explanations
- see separate sheet**

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Reference is made to the following documents:

- D1 US 2009/238816 A1 (LARSEN JANUS BEIERHOLM [DK]) 24 September 2009 (2009-09-24)
- D2 DATABASE WPI
Week 200938
Thomson Scientific, London, GB;
AN 2009-J45395
XP002738891,
& CN 101 428 145 A (BEIJING CENT BIOLOGY CO LTD) 13 May 2009 (2009-05-13)
- D3 PAULY DIANA ET AL: "IgY technology: extraction of chicken antibodies from egg yolk by polyethylene glycol (PEG) precipitation.", JOURNAL OF VISUALIZED EXPERIMENTS : JOVE 2011, no. 51, 2011, - 2011, XP002738892, ISSN: 1940-087X
- D4 YOKOYAMA HIDEAKI ET AL: "A two-step procedure for purification of hen egg yolk immunoglobulin G: Utilization of hydroxypropylmethylcellulose phthalate and synthetic affinity ligand gel (Avid AL)", POULTRY SCIENCE, vol. 72, no. 2, 1993, pages 275-281, ISSN: 0032-5791
- D5 US 4 357 272 A (POLSON ALFRED) 2 November 1982 (1982-11-02)

Re Item IV

Lack of unity of invention

The following separate groups of **inventions** have been identified in the present application:

Invention 1 (Claims 1-32): An emulsified vaccine formulation comprising: (i) antigens, (ii) a light mineral oil and (iii) a particulate adjuvant selected from the group consisting of biodegradable polymer particles, micro-particles or nanoparticles. Use of the emulsified vaccine formulation in preparing a concentrated IgY formulation.

Invention 2 (Claims 33-51): A process for immunoglobulins extraction, delipidation and concentration from egg yolks from hens hiperimmunized with the emulsified vaccine formulation of claims 1 to 21, wherein it comprises the following steps: a) The yolk is diluted 1:4 or 1:8 with 0.01% sodium azide and stored under refrigeration during at least 24h. b) The supernatant is separated, HPMCP is added at 1-15% in a 0.25 ml per each 100 ml of yolk ratio and it is allowed to rest during at least 24h. c) The lipid layer formed in the upper part is separated from the solution and the aquaeous part is filtrated. d) PEG is added nin a 5-30% P/V ratio, mixed and allowed to rest during at least 4-12 hours at 4°C. e) It is centrifuged during 20-30 minutes and the supernatant is removed. f) The resulting tablet is solubilized with PBS 1X or TRIS buffer, at a volume equivalent to 10% of the original volume. An avian derived concentrated IgY immunoglobulins formulation obtained by said process and the use thereof to prepare a medicament.

The above subject-matters are not linked so as to form a single general inventive concept for the following reasons:

Vaccines aimed at producing in chickens immunoglobulins against viral diseases that affect swine, such as PRRS, are known in the art, as it can be seen in D1. Methods for the extraction of chicken antibodies from egg yolk are described in D1 and D3-D5.

In the light of the prior art the present application deals with two different problems and their corresponding solutions:

The first problem can be defined as the further provision of vaccines against swine viral diseases for chickens. The solution herein provided is invention 1.

The second problem here dealt with is the further provision of methods for the extraction of chicken antibodies from egg yolk. The solution herein provided for the second problem is invention 2.

In the view of the fact that both vaccines against swine viral diseases for chickens and methods for the extraction of chicken antibodies from egg yolk are already disclosed in the prior art, due to essential difference in the nature of the two problems and their corresponding solutions and due to the fact that no other technical feature can be distinguished which, in the light of the prior art, could be regarded as special technical feature common to these solutions, there appears to be no common inventive concept linking together said solutions.

Consequently, the present application is considered to relate to two separate inventions which lack unity in the sense of Rule 13.1 PCT.

An opinion with regard to novelty, inventive step or industrial applicability will be given only with respect to the invention first mentioned in the claims, that is, to the following subject-matter: **Claims 1-32:** An emulsified vaccine formulation comprising: (i) antigens, (ii) a light mineral oil and (iii) a particulate adjuvant selected from the group consisting of biodegradable polymer particles, micro-particles or nanoparticles. Use of the emulsified vaccine formulation in preparing a concentrated IgY formulation.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Invention 1:

D2 discloses an emulsion vaccine comprising antigens, light mineral oil and a biodegradable nanoparticle adjuvant (for example chitosan). In the light of D2, claims 1, 2, 7, 13, 19-21, 31 lack novelty. Claim 32 states that the vaccine is "*subcutaneously administrable to the chickens*", what can merely be an inherent property of the composition disclosed in D2, hence the novelty objection (if claim 32 indicated that the vaccine is for administration -and not simply susceptible to be administered- to chickens, then it would be novel over D2).

D1 is regarded as the closest prior art because it discloses a vaccine comprising PRRS peptides and marcol for production of IgY against said virus in chickens.

In view of the formulation of the claims (they are very broad), the requirements of Art. 33(3) PCT are not satisfied, because the skilled person would not have required inventive skills to arrive at the subject-matter claim departing from the prior art.

The difference between the subject-matter of D1 and that of the present application lies in the adjuvant (chitosan in the application instead of $\text{Al}(\text{OH})_3$, CpG, dsRNA, HSP70 or heat-inactivated *B. pertussis*) and the antigen (6 inactivated PRRS strains in the application vs PRRS peptides).

The problem to be solved by the present application may be regarded as the further provision of vaccines against PRRS which raise IgY immunoglobulins against said virus in chickens.

The solution herein disclosed is an emulsified vaccine composition comprising chitosan particles, marcol and 6 inactivated PRRS virus strains, wherein each of the six antigens has a minimum titer of $\text{DIE}_{50\%}$ of $10^4/\text{ml}$.

The specific composition of the vaccine herein disclosed is neither expected nor suggested from the prior art documents available and, in consequence, it involves an inventive step. However, given that the claims are not restricted to the invention, they cannot be regarded as compliant with Art. 33(3) PCT.

Invention 2:

D1 is regarded as the closest prior art, because it also discloses a method for the isolation of immunoglobulins from egg yolk which involves the use of PEG and azide. It mainly differs from the method herein disclosed in the fact that it uses chloroform instead of HPMCP for the delipidation step and in the fact that the addition of preservative (azide) is the last step, while in the present application the azide is added to the yolk after separating it from the white.

The technical problem can be defined as the further provision of methods for the extraction of antibodies from egg yolk and the solution is the process of claim 33.

Although D4 describes the use of HPMCP in a method to purify IgY from egg yolk and D5 describes the addition of azide to the washed egg yolk, the skilled person would have had no incentive to combine all of these features with the teachings of D1 and, furthermore, the combination of features from D1, D4 and D5 would still not render the process for purification of IgY from egg yolk herein described and claimed.

In the light of the above reasoning, claims 33-35 satisfy the requirements of articles 33 (2) and 33(3) PCT.

A product is not rendered novel merely by the fact that it is produced by means of a new process. Claims 36-40 are directed to IgY immunoglobulins which cannot be distinguished from IgY immunoglobulins produced by other methods (see some examples in D1 and D4). Therefore, claims 36-51 cannot be regarded as novel in the sense of Art. 33(2) PCT.

Re Item VIII

Certain observations on the international application

The specific components of the vaccine herein disclosed are regarded as essential technical features: *An emulsified vaccine composition comprising chitosan particles, marcol and 6 inactivated PRRS virus strains, wherein each of the six antigens has a minimum titer of $DIE_{50\%}$ of 10^4 /ml.* Given that none of claims 1-32 contains all those features, the present set of claims does not meet the requirement following from Article 6 PCT taken in combination with Rule 6.3(b) PCT that any independent claim must contain all the technical features essential to the definition of the invention.

Claim 13 is redundant in view of the subject-matter of claims 2, 7 and 10. This contravenes the provisions of Art. 6 PCT.

Thricothecenes are toxins and, in consequence, they do not cause infections. Claim 28 is therefore unclear in the sense of Art. 6 PCT. It has been assumed though that the Applicant refers to infections caused by thricothecenes-producing microorganisms.

In several passages of the description (for example pages 9 and 14) the Applicant refers to "ARN viruses" instead of RNA viruses. This contravenes the provisions of Art. 6 PCT. The same objection applies to the term "*isolation*" on lines 1 and 3 of page 15, which should be "isolate".

The vague and imprecise statements in the description on pages 22 (lines 9-13) and 30 (lines 15-20) imply that the subject-matter for which protection is sought may be different to that defined by the claims, thereby resulting in lack of clarity of the claims (Article 6 PCT) when used to interpret them.