

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: MERCK SHARP & DOHME CORP.
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PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43bis.1)

Date of mailing
(day/month/year)

25 FEB 2019

Applicant's or agent's file reference
24547

FOR FURTHER ACTION

See paragraph 2 below

International application No.

PCT/US 18/63541

International filing date (day/month/year)

03 December 2018 (03.12.2018)

Priority date (day/month/year)

07 December 2017 (07.12.2017)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - A61K 39/12, A61K 47/26, A61K 9/00, A61K 9/14 (2019.01)
CPC - A61K 39/12, A61K 9/19, C12N 7/00

Applicant **MERCK SHARP & DOHME CORP.**

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 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/US
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-8300

Date of completion of this opinion

28 January 2019

Authorized officer

Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

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Box No. I Basis of this 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 43*bis*.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 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 only:

☐ 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).

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:

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Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 4-7, 16, 22, 28-38

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search (*specify*):

☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 4-7, 16, 22, 28-38 are so unclear that no meaningful opinion could be formed (*specify*):

Claims 4-7, 16, 22, 28-38 are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed (*specify*):

☒ no international search report has been established for said claims Nos. 4-7, 16, 22, 28-38

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

☐ furnish a sequence listing in the form of an Annex C/ST.25 text file, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.

☐ furnish a sequence listing on paper or in the form of an image file complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.

☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13ter.1(a) or (b).

☐ See Supplemental Box for further details.

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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:
4. Consequently, this opinion has been established in respect of the following parts of the international application:
- ☐ all parts.
 - ☒ the parts relating to claims Nos. 1-3, 8-15, 17-21, 23-27

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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)	Claims	3, 8-15, 19, 20/19, 21, 23-27	YES
	Claims	1, 2, 17, 18, 20/(17,18)	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-3, 8-15, 17-21, 23-27	NO
Industrial applicability (IA)	Claims	1-3, 8-15, 17-21, 23-27	YES
	Claims	None	NO
2. Citations and explanations:			
Claims 1 and 2 lack novelty under PCT Article 33(2) as being anticipated by US 2016/0296616 A1 to the Board of Regents, the University of Texas System (hereinafter 'UT'). Regarding claim 1, UT teaches a formulation comprising a live attenuated dengue vaccine comprising at least one live attenuated dengue virus (LAV) (para [0067] "the present disclosure provides a vaccine composition comprising an antigen dispersed within an amorphous solid...The antigen may comprise a whole organism, killed, attenuated or live (including killed, attenuated or inactivated bacteria, viruses"; para [0075] "Antigens useful in the present disclosure may include those derived from viruses including, but not limited to...Dengue virus..."), a buffer at pH about 6.5 to 8.5 (para [0006] "the carrier also comprises a pH buffering agent (e.g., phosphate buffered saline). In certain aspects, the carrier has a pH of...between 6.0 and 8.0"; Note, the pH of PBS is 7.4), a sugar (para [0079] "a vaccine composition comprising an amorphous solid may be made by preparing a solution comprising a sugar, sugar derivative or combination of sugars/derivatives in a buffer..."), a glycol, a glycerol (para [0071] "the vaccine compositions of the present disclosure may further comprise one or more oils, polyalcohols, surfactants, permeability enhancers, and/or edible organic acids. ... Examples of suitable polyalcohols may include, but are not limited to, glycerol, polyethylene glycol, propylene glycol, and a combination thereof."), and a cellulose derivative selected from the group consisting of carboxymethyl cellulose, hydroxypropyl cellulose (HPC), hydroxypropyl methylcellulose (HPMC), 2-hydroxyethyl cellulose (2-HEC) (para [0070] "the vaccine compositions of the present disclosure also may comprise a water-soluble polymer including, but not limited to, carboxymethyl cellulose, carboxyvinyl polymers, high amylose starch, hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose"). Regarding claim 2, UT teaches the formulation of claim 1 wherein the buffer is a phosphate buffer (para [0006] "the carrier also comprises a pH buffering agent (e.g., phosphate buffered saline)"). Claim 17, 18, 20/(17,18) lack novelty under PCT Article 33(2) as being anticipated by WO 2017/056101 A1 to Panacea Biotec Limited (hereinafter 'Panacea'). Regarding claim 17, Panacea teaches a formulation that comprises a live attenuated dengue vaccine comprising a live attenuated dengue virus (LAV) at about 2000 PFU/ml (pg 1 line 1-2 "The present invention relates to field of live attenuated recombinant tetravalent dengue vaccines and methods of producing stable compositions."; pg 23 line 8-9 "In preferred embodiments the dengue virus in the vaccine composition is present at a dose of NLT 3.0 log₁₀ PFU per 0.5 ml."), a buffer at pH about 6.5 to 7.5, a sugar at about 20-200 mg/ml, and a carrier selected from the group consisting of HSA or gelatin (pg 22 line 12-16 "The vaccine formulation of the present invention comprises virus and stabilizer components in amounts from about 10 to about 30 mg per 0.5 ml of a protein stabilizer selected from but not restricted to human serum albumin, collagen and gelatin; from about 10 to about 100 mg per 0.5 ml of a sugar stabilizer selected from but not restricted to lactose, sucrose, mannitol and trehalose and an amount of a physiologically active buffer to adjust the pH from about 6.5 to about 7.5"). Regarding claim 18, Panacea teaches the formulation of claim 17 wherein the buffer is a phosphate (pg 21 line 4-6 "In yet another embodiment, the present invention further provides buffering agents for use in the compositions of the invention, which are selected from a group comprising of Potassium dihydrogen phosphate, Di-potassium hydrogen phosphate, monosodium glutamate and the like.>"). Regarding claim 20/(17,18), Panacea teaches the formulations of claim 17 and 18, wherein the sugar is trehalose or sucrose or a combination thereof (pg 20 line 30-31 "In another embodiment, the stabilizer may further comprise lactose, sucrose, mannitol, trehalose, ..., or combinations thereof.>"). -----Please see continuation in supplemental box-----			

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:

Box No. V2 Citations and Explanations:

Claim 23 lacks an inventive step under PCT Article 33(3) as being obvious over Panacea.

Regarding claim 23, Panacea teaches the formulation of claim 17 that comprises a live attenuated dengue vaccine comprising a live attenuated dengue virus (LAV) at about 2000 PFU/ml (pg 1 line 1-2 "The present invention relates to field of live attenuated recombinant tetravalent dengue vaccines and methods of producing stable compositions."; pg 23 line 8-9 "In preferred embodiments the dengue virus in the vaccine composition is present at a dose of NLT 3.0 log₁₀ PFU per 0.5 ml."), a buffer at pH about 6.5-7.5, a combination of sucrose and trehalose (pg 20 line 30-31 "In another embodiment, the stabilizer may further comprise lactose, sucrose, mannitol, trehalose, ..., or combinations thereof."), and about 20-60 mg/ml gelatin (pg 22 line 12-14 "The vaccine formulation of the present invention comprises virus and stabilizer components in amounts from about 10 to about 30 mg per 0.5 ml of a protein stabilizer selected from but not restricted to human serum albumin, collagen and gelatin"). Panacea does not specifically teach wherein the formulation comprises 150-300 mg/ml sugar as a combination of sucrose and trehalose. However, Panacea teaches wherein the formulation comprises 20-200 mg/ml of a sugar stabilizer (pg 22 line 12-15 "The vaccine formulation of the present invention comprises virus and stabilizer components in amounts from about 10 to about 30 mg per 0.5 ml of a protein stabilizer ... from about 10 to about 100 mg per 0.5 ml of a sugar stabilizer selected from but not restricted to lactose, sucrose, mannitol and trehalose."). It would have been obvious to one skilled in the art to have used, for research or therapeutic purposes, a sucrose/trehalose combination of about 150-200 mg/ml in the vaccine formulation made obvious by Panacea, in order to optimize the stability of said formulation.

Claim 3 lacks an inventive step under PCT Article 33(3) as being obvious over UT in view of WO 2017/179017 A1 to Takeda Vaccines (hereinafter 'Takeda').

Regarding claim 3, UT teaches the formulation of claim 1 and 2 but does not teach wherein the formulation comprises an alkali or alkali salt wherein the said alkali or alkali salt is magnesium chloride, calcium chloride, potassium chloride, sodium chloride or a combination thereof.

Takeda teaches a dengue vaccine formulation comprising sodium chloride (para [0005] "...each of the passages provides an attenuated dengue virus contemplated of use in a pharmaceutically acceptable vaccine composition"; para [000106] "formulations of use herein stabilize live, attenuated Flaviviruses for commercial use...the composition further includes a buffer...the buffer can include but is not limited to, phosphate buffered saline (PBS). In accordance with these embodiments, the buffer of the composition can include sodium chloride at a concentration of 25 mM to 200 mM."). It would have been obvious to one skilled in the art to have combined, for research or therapeutic purposes, sodium chloride taught by Takeda with the dengue vaccine formulation made obvious by UT, in order to stabilize the virus in said formulation.

Claims 19 and 20/19 lack an inventive step under PCT Article 33(3) as being obvious over Panacea in view of Takeda.

Regarding claim 19, Panacea teaches the formulation of claim 17, but does not teach wherein said formulation comprises an alkali or alkaline salt wherein said alkali or alkaline salt is magnesium chloride, calcium chloride, potassium chloride, sodium chloride or a combination thereof.

Takeda teaches a dengue vaccine formulation comprising sodium chloride (para [0005] "...each of the passages provides an attenuated dengue virus contemplated of use in a pharmaceutically acceptable vaccine composition"; para [000106] "In other embodiments, formulations of use herein stabilize live, attenuated Flaviviruses for commercial use. In some embodiments, the composition further includes a buffer. In accordance with these embodiments, the buffer can include but is not limited to, phosphate buffered saline (PBS). In accordance with these embodiments, the buffer of the composition can include sodium chloride at a concentration of 25 mM to 200 mM."). It would have been obvious to one skilled in the art to have combined sodium chloride as taught by Takeda with the dengue vaccine formulation made obvious by Panacea, in order to stabilize the virus in said formulation.

Regarding claim 20/19, Panacea and Takeda make obvious the formulation of claim 19. Panacea further teaches wherein the sugar is trehalose or sucrose or a combination thereof (pg 20 line 30-31 "In another embodiment, the stabilizer may further comprise lactose, sucrose, mannitol, trehalose, ..., or combinations thereof.>").

Claim 21/(17,18) lacks an inventive step as being obvious over Panacea in view of WO 2011/098837 A1 to Isis Innovation Limited (hereinafter 'Isis').

Regarding claim 21/(17,18), Panacea teaches the formulations of claim 20/(17,18) but does not teach wherein the sucrose to trehalose ratio is between 1:1 to 1:4.

Isis teaches a virus vaccine formulation wherein the sucrose to trehalose ratio is between 1:1 to 1:9 (pg 2 line 23 -27 "The present invention provides a method of stabilising a virus selected from modified vaccinia Ankara (MVA) virus...wherein said virus retains activity after storage, the method comprising: (a) forming a liquid mixture of the virus with a glass-forming excipient which comprises trehalose and sucrose."; pg 3 line 33 - pg 4 line 1 "The glass-forming excipient consists or comprises two different sugars, namely the first sugar trehalose and the second sugar sucrose. Whilst any particular ratio of the sugars may be employed, the present inventors have identified particularly good stability results when a ratio (first sugar: second sugar)...50:50 (w/w, w/v, or v/v) is employed"). It would have been obvious to one skilled in the art to have combined, for research or therapeutic purposes, the sucrose/trehalose ratios taught by Isis with the vaccine formulation made obvious by Panacea, in order to improve the stability of the virus in said formulation.

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Supplemental Box

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Continuation of:

Previous supplemental box:

Claim 21/19 lacks an inventive step under PCT Article 33(3) as being obvious over Panacea, in view of Takeda and Isis.

Regarding claim 21/19, Panacea and Takeda make obvious the formulation of 20/19 but do not teach wherein the sucrose to trehalose ratio is between 1:1 to 1:4.

Isis teaches a virus vaccine formulation wherein the sucrose to trehalose ratio is between 1:1 to 1:9 (pg 2 line 23 -27 "The present invention provides a method of stabilising a virus selected from modified vaccinia Ankara (MVA) virus ...wherein said virus retains activity after storage, the method comprising: (a) forming a liquid mixture of the virus with a glass-forming excipient which comprises trehalose and sucrose"; pg 3 line 33 L pg 4 line 1 "The glass-forming excipient consists or comprises two different sugars, namely the first sugar trehalose and the second sugar sucrose. Whilst any particular ratio of the sugars may be employed, the present inventors have identified particularly good stability results when a ratio (first sugar: second sugar) of 90:10 through to 50:50 (w/w, w/v, or v/v) is employed. Other preferred ranges include 80:20, 70:30, and 60:40 (w/w, w/v, or v/v), respectively for the first sugar:second sugar."). It would have been obvious to one skilled in the art to have combined, for research or therapeutic purposes, the sucrose/trehalose ratios taught by Isis with the vaccine formulation made obvious by Panacea and Takeda, in order to improve the stability of the virus in said formulation.

Claims 24 and 26 lack an inventive step under PCT Article 33(3) as being obvious over Panacea, in view of Takeda and US 2012/0219588 A1 to Thompson et al. (hereinafter 'Thompson').

Regarding claim 24, Panacea makes obvious the formulation of claim 17 that comprises a live attenuated dengue vaccine comprising a live attenuated dengue virus (LAV) at about 2000 PFU/ml (pg 1 line 1-2 "The present invention relates to field of live attenuated recombinant tetravalent dengue vaccines and methods of producing stable compositions."; pg 23 line 8-9 "In preferred embodiments the dengue virus in the vaccine composition is present at a dose of NLT 3.0 log10 PFU per 0.5 ml."); a phosphate buffer at pH about 6.5-7.5 (pg 22 line 12-16 "The vaccine formulation of the present invention comprises virus and stabilizer components ... and an amount of a physiologically active buffer to adjust the pH from about 6.5 to about 7.5"; pg 21 line 4-6 "In yet another embodiment, the present invention further provides buffering agents for use in the compositions of the invention, which are selected from a group comprising of Potassium dihydrogen phosphate, Di-potassium hydrogen phosphate, monosodium glutamate and the like."), about 20-200 mg/ml sucrose, about 20-200 mg trehalose (pg 22 line 12-15 "The vaccine formulation of the present invention comprises virus and stabilizer components in amounts ... from about 10 to about 100 mg per 0.5 ml of a sugar stabilizer selected from but not restricted to lactose, sucrose, mannitol and trehalose."; pg 20 line 30-31 "In another embodiment, the stabilizer may further comprise lactose, sucrose, mannitol, trehalose, ..., or combinations thereof."), about 20-60 mg/ml gelatin (pg 22 line 12-14 "The vaccine formulation of the present invention comprises virus and stabilizer components in amounts from about 10 to about 30 mg per 0.5 ml of a protein stabilizer selected from but not restricted to human serum albumin, collagen and gelatin"). Panacea does not teach wherein said formulation comprises 5-300 mM of phosphate buffer and 30-90 mM alkali or alkaline salt.

Thompson teaches 11 mM phosphate buffer at pH 7.0-7.4 for stabilizing a virus composition (para [0121] "In a specific embodiment, the viral harvest is stabilized by the addition of a concentrated buffer including but not limited to a sucrose phosphate (SP) buffer, which may be added to obtain a final concentration of about 218 mM sucrose, 11 mM phosphate buffer pH 7.0-7.4 in the stabilized viral harvest."). Takeda teaches a dengue vaccine formulation comprising sodium chloride (para [0005] "...each of the passages provides an attenuated dengue virus contemplated of use in a pharmaceutically acceptable vaccine composition"; para [000106] "In other embodiments, formulations of use herein stabilize live, attenuated Flaviviruses for commercial use. In some embodiments...the buffer of the composition can include sodium chloride at a concentration of 25 mM to 200 mM."). It would have been obvious to one skilled in the art to have combined phosphate buffer as taught by Thompson and sodium chloride as taught by Takeda with the dengue vaccine formulation made obvious by Panacea, in order to stabilize the virus in said formulation.

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:

Previous supplemental box:

Regarding claim 26, Panacea makes obvious the formulation of claim 17 that comprises a live attenuated dengue vaccine comprising a live attenuated dengue virus (LAV) at about 2000 PFU/ml (pg 1 line 1-2 "The present invention relates to field of live attenuated recombinant tetravalent dengue vaccines and methods of producing stable compositions."; pg 23 line 8-9 "In preferred embodiments the dengue virus in the vaccine composition is present at a dose of NLT 3.0 log₁₀ PFU per 0.5 ml."), potassium phosphate at pH about 7.5 (pg 22 line 12-16 "The vaccine formulation of the present invention comprises virus and stabilizer components... and an amount of a physiologically active buffer to adjust the pH from about 6.5 to about 7.5"; pg 21 line 4-6 "the present invention further provides buffering agents for use in the compositions of the invention, which are selected from a group comprising of Potassium dihydrogen phosphate, Di-potassium hydrogen phosphate, monosodium glutamate and the like."), about 20-200 mg of sucrose, about 20-200 mg trehalose (pg 22 line 12-15 "The vaccine formulation of the present invention comprises virus and stabilizer components in amounts ... from about 10 to about 100 mg per 0.5 ml of a sugar stabilizer selected from but not restricted to lactose, sucrose, mannitol and trehalose."; pg 20 line 30-31 "In another embodiment, the stabilizer may further comprise lactose, sucrose, mannitol, trehalose, ..., or combinations thereof."), and about 20-60 mg/ml gelatin (pg 22 line 12-14 "The vaccine formulation of the present invention comprises virus and stabilizer components in amounts from about 10 to about 30 mg per 0.5 ml of a protein stabilizer selected from but not restricted to human serum albumin, collagen and gelatin"). Panacea does not teach wherein said formulation comprises 5-20 mM of phosphate and 30 mM NaCl. However, Thompson teaches an 11 mM phosphate buffer pH 7.0-7.4 for stabilizing a virus composition (para [0121] "In a specific embodiment, the viral harvest is stabilized by the addition of a concentrated buffer including but not limited to a sucrose phosphate (SP) buffer, which may be added to obtain a final concentration of about 218 mM sucrose, 11 mM phosphate buffer pH 7.0-7.4 in the stabilized viral harvest.").

Takeda teaches a dengue vaccine formulation comprising 25-200 mM sodium chloride (para [0005] "...each of the passages provides an attenuated dengue virus contemplated of use in a pharmaceutically acceptable vaccine composition"; para [000106] "In other embodiments, formulations of use herein stabilize live, attenuated Flaviviruses for commercial use. In some embodiments, the composition further includes a buffer. In accordance with these embodiments, the buffer can include but is not limited to, phosphate buffered saline (PBS). In accordance with these embodiments, the buffer of the composition can include sodium chloride at a concentration of 25 mM to 200 mM."). It would have been obvious to one skilled in the art to have combined, for research or therapeutic purposes, phosphate buffer as taught by Thompson, and sodium chloride as taught by Takeda with the dengue vaccine formulation made obvious by Panacea, in order to improve virus stability in said formulation.

Claim 27 lacks an inventive step under PCT Article 33(3) as being obvious over Panacea, in view of US 2015/0246114 A1 to Intervet Inc. (hereinafter 'Intervet'), and in view of US 2003/0180352 A1 to Patel et al. (hereinafter 'Patel').

Regarding claim 27, Panacea makes obvious the formulation of claim 17 that comprises a live attenuated dengue vaccine comprising a live attenuated dengue virus (LAV) at about 2000 PFU/ml (pg 1 line 1-2 "The present invention relates to field of live attenuated recombinant tetravalent dengue vaccines and methods of producing stable compositions."; pg 23 line 8-9 "In preferred embodiments the dengue virus in the vaccine composition is present at a dose of NLT 3.0 log₁₀ PFU per 0.5 ml."), potassium phosphate at pH about 7.5 (pg 22 line 12-16 "The vaccine formulation of the present invention comprises virus and stabilizer components... and an amount of a physiologically active buffer to adjust the pH from about 6.5 to about 7.5"; pg 21 line 4-6 "In yet another embodiment, the present invention further provides buffering agents for use in the compositions of the invention, which are selected from a group comprising of Potassium dihydrogen phosphate, Di-potassium hydrogen phosphate, monosodium glutamate and the like."), sucrose (pg 22 line 12-15 "The vaccine formulation of the present invention comprises virus and stabilizer components in amounts ... from about 10 to about 100 mg per 0.5 ml of a sugar stabilizer selected from but not restricted to lactose, sucrose, mannitol and trehalose."), and PVP (pg 20 line 30-31 "In another embodiment, the stabilizer may further comprise ... polyvinylpyrrolidone"). Panacea does not specifically teach wherein the formulation comprises 5-20 mM potassium phosphate, about 250 mg/ml sucrose, and about 50 mg/ml PVP K12.

Intervet teaches a virus vaccine that comprises about 5-20 mM potassium phosphate, and about 250 mg/ml sucrose (para [0012] "In order to overcome the deficiencies of current vaccines, the present invention provides novel liquid stable, live, attenuated virus vaccines, as well as their corresponding immunogenic compositions."; para [0072] "Because the liquid stable vaccines of the present invention ideally range in pH from pH 6.0 to pH 8.0, the liquid stable vaccines of the present invention can comprise a buffer. Buffers for use in the liquid stable vaccines of the present invention include but are not limited to: ..., potassium and/or sodium phosphate."; para [0017] "the buffer comprises 5 to 25 mM phosphate."; para [0014] "the vaccine comprises 15 to 25 percent sugar additive."; para [0015] "In particular embodiments of the liquid stable virus vaccines of the present invention the sugar additive is sucrose.").

Patel teaches wherein a pharmaceutical viral vaccine composition comprises PVP K12 (para [0045] "The present invention provides solid pharmaceutical compositions for improved delivery of a wide variety of pharmaceutical active ingredients"; para [0111] "Most preferred hydrophilic active ingredients include...influenza virus vaccine"; para [0236] Exemplary additives that are conventionally used in pharmaceutical compositions can be included, and these additives are well known in the art. Such additives include: "; para [0241] "binders (adhesives), i.e., agents that impart cohesive properties to powdered materials through particle-particle bonding, such as ..., film binders (PVP,...)"; para [0437] "Component. Amount (wt/wt). PVP-K12... 5.0"). It would have been obvious to one skilled in the art to have combined, for research or therapeutic purposes, about 5-20 mM potassium phosphate and about 250 mg/ml sucrose as taught by Intervet, and PVP K12 taught by Patel with the formulation made obvious by Panacea. Panacea, Intervet, and Patel do not specifically teach wherein the composition comprises about 50 mg/ml PVP K12. However, one of ordinary skill in the art would have conducted routine experimentations to identify the optimal concentration of PVP K12, and thus would have reached claimed subject wherein said formulation comprises about 50 mg/ml PVP K12.

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Supplemental Box

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Claim 8 lacks an inventive step under PCT Article 33(3) as being obvious over UT, in view of Panacea, US 2016/0324783 A1 to Infectious Disease Research Institute (hereinafter 'IDRI'), and US 2016/0199496 A1 to Arecor Limited (hereinafter 'Arecor').

Regarding claim 8, UT teaches the formulation of claim 1 wherein said formulation comprises a buffer at pH about 6.5 to 8.5 (para [0006] "the carrier also comprises a pH buffering agent (e.g., phosphate buffered saline). In certain aspects, the carrier has a pH of...between 6.0 and 8.0"; Note, the pH of PBS is 7.4). UT does not teach wherein said formulation comprises the live attenuated dengue virus (LAV) at 100 -10,000,000 pfu/ml, about 50-300 mg/ml sugar, about 2.5-10.0 mg/ml propylene glycol (PG) or glycerol, and about 0.3-10 mg/ml sodium carboxymethylcellulose (sodium CMC).

Panacea teaches a dengue virus vaccine composition comprising 2000 PFU/ml (pg 1 line 1-2 "The present invention relates to field of live attenuated recombinant tetravalent dengue vaccines and methods of producing stable compositions."; pg 23 line 8-9 "In preferred embodiments the dengue virus in the vaccine composition is present at a dose of NLT 3.0 log10 PFU per 0.5 ml."), and about 20-200 mg/ml sucrose (pg 22 line 12-15 "The vaccine formulation of the present invention comprises virus and stabilizer components in amounts from ...; from about 10 to about 100 mg per 0.5 ml of a sugar stabilizer selected from but not restricted to lactose, sucrose, mannitol and trehalose").

IDRI teaches wherein a dengue vaccine formulation comprises .5 w/v glycerol (para [0123] "The thermostable lyophilized vaccine compositions further optionally include an antigen and/or an adjuvant ... In some embodiments the composition is formed by the lyophilization of an oil-in water emulsion formulation and the oil-in-water formulation prior to lyophilization or upon reconstitution contains ..., less than or about 0.5 percent (w/v) glycerol,."; para [0230] "An antigen, for use in certain embodiments of the herein described compositions and methods for generating and using such compositions, may be any target epitope, molecule (including a biomolecule), molecular complex (including molecular complexes that contain biomolecules), subcellular assembly, cell or tissue..."; para [0232] "compositions described herein...comprise an antigen or antigenic composition capable of eliciting an immune response against a ...pathogen, which antigen or antigenic composition may include a composition derived from a virus such as from , flaviviruses (e.g., ..., Dengue Virus,)").

Arecor teaches virus vaccine formulations comprising about 0.5-10 mg/ml sodium carboxymethyl cellulose (para [0005] "Recombinant adenovirus vectors are becoming increasingly important for use in vaccine development and gene therapy applications."; para [0073] "The pharmaceutical formulations of the present invention provide long-term storage stability for adenovirus vector compositions at varying degrees of virus concentration"; para [0009] "The present invention is directed to an aqueous formulation comprising an adenovirus vector and at least one anionic polymer."; para [0079] "In some embodiments, the at least one anionic polymer is at a concentration ranging from about 0.5 mg/mL to about 10 mg/mL."; para [0014] "In some embodiments, the at least one anionic polymer is sodium carboxymethyl cellulose. The sodium carboxymethyl cellulose may have an average molecular weight ..., preferably an average molecular weight of about 90000 Da."). It would have been obvious to one skilled in the art to have combined, for research or therapeutic purposes, live-attenuated dengue virus and sucrose as taught by Panacea, glycerol as taught by IDRI, and carboxymethyl cellulose as taught by Arecor with the Dengue vaccine formulation taught by UT, in order to improve the stability of said formulation.

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Claim 25 lacks an inventive step under PCT Article 33(3) as being obvious over Panacea, in view of Thompson, Arecor, and Takeda.

Regarding claim 25, Panacea teaches the formulation of claim 17 wherein that comprises a live attenuated dengue vaccine comprising a live attenuated dengue virus (LAV) at about 2000 PFU/ml (pg 1 line 1-2 "The present invention relates to field of live attenuated recombinant tetravalent dengue vaccines and methods of producing stable compositions."; pg 23 line 8-9 "In preferred embodiments the dengue virus in the vaccine composition is present at a dose of NLT 3.0 log₁₀ PFU per 0.5 ml."), potassium phosphate at pH about 7.5 (pg 22 line 12-16 "The vaccine formulation of the present invention comprises virus and stabilizer components... and an amount of a physiologically active buffer to adjust the pH from about 6.5 to about 7.5"; pg 21 line 4-6 "In yet another embodiment, the present invention further provides buffering agents for use in the compositions of the invention, which are selected from a group comprising of Potassium dihydrogen phosphate, Di-potassium hydrogen phosphate, monosodium glutamate and the like."), about 20-200 mg/ml sucrose, about 20-200 mg trehalose (pg 22 line 12-15 "The vaccine formulation of the present invention comprises virus and stabilizer components in amounts from about 10 to about 100 mg per 0.5 ml of a sugar stabilizer selected from but not restricted to lactose, sucrose, mannitol and trehalose."; pg 20 line 30-31 "In another embodiment, the stabilizer may further comprise lactose, sucrose, mannitol, trehalose, ..., or combinations thereof."). Panacea does not teach wherein said formulation comprises about 5-20 mM of phosphate, 5 mg/ml sodium carboxymethyl cellulose with average molecular weight of about 90,000, and about 30 mM sodium chloride.

Thompson teaches an 11 mM phosphate buffer pH 7.0-7.4 for stabilizing a virus composition (para [0121] "In a specific embodiment, the viral harvest is stabilized by the addition of a concentrated buffer including but not limited to a sucrose phosphate (SP) buffer, which may be added to obtain a final concentration of about 218 mM sucrose, 11 mM phosphate buffer pH 7.0-7.4 in the stabilized viral harvest.").

Arecor teaches virus vaccine formulations comprising about 0.5-10 mg/ml sodium carboxymethyl cellulose with average molecular weight of about 90,000 (para [0005] "Recombinant adenovirus vectors are becoming increasingly important for use in vaccine development and gene therapy applications."; para [0073] "The pharmaceutical formulations of the present invention provide long-term storage stability for adenovirus vector compositions at varying degrees of virus concentration"; para [0009] "an aqueous formulation comprising an adenovirus vector and at least one anionic polymer."; para [0079] "In some embodiments, the at least one anionic polymer is at a concentration ranging from about 0.5 mg/mL to about 10 mg/mL."; para [0014] "The sodium carboxymethyl cellulose may have an average molecular weight ..., preferably an average molecular weight of about 90000 Da.").

Takeda teaches a dengue vaccine formulation comprising 25 mM to 200 mM sodium chloride (para [0005] "...each of the passages provides an attenuated dengue virus contemplated of use in a pharmaceutically acceptable vaccine composition"; para [000106] "In other embodiments, formulations of use herein stabilize live, attenuated Flaviviruses for commercial use. In some embodiments, the composition further includes a buffer. In accordance with these embodiments, the buffer can include but is not limited to, phosphate buffered saline (PBS). In accordance with these embodiments, the buffer of the composition can include sodium chloride at a concentration of 25 mM to 200 mM."). It would have been obvious to one skilled in the art to have combined, for research or therapeutic purposes, potassium phosphate as taught by Thompson, sodium carboxymethyl cellulose with average molecular weight of about 90,000 as taught by Arecor, and sodium chloride as taught by Takeda with the Dengue vaccine formulation made obvious by Panacea, in order to stabilize the virus in said formulation.

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Claims 9 and 14/9 lack an inventive step under PCT Article 33(3) as being obvious over UT, in view of Panacea, Thompson, IDRI, and Arecor.

Regarding claim 9, UT teaches the formulation of claim 1 wherein said formulation comprises a phosphate buffer at pH 7.4 (para [0006] "the carrier also comprises a pH buffering agent (e.g., phosphate buffered saline). In certain aspects, the carrier has a pH of...between 6.0 and 8.0"; Note, the pH of PBS is 7.4). UT does not teach wherein said formulation comprises the live attenuated dengue virus (LAV) at 100 -100,000 pfu/ml, a 5-300 mM phosphate buffer, about 50-300 mg/ml sugar, about 3-10.0 mg/ml propylene glycol (PG) or glycerol, and about 3-10 mg/ml sodium carboxymethylcellulose (sodium CMC).

However, Panacea teaches a dengue virus vaccine composition comprising 2000 PFU/ml (pg 1 line 1-2 "The present invention relates to field of live attenuated recombinant tetravalent dengue vaccines and methods of producing stable compositions."; pg 23 line 8-9 "In preferred embodiments the dengue virus in the vaccine composition is present at a dose of NLT 3.0 log₁₀ PFU per 0.5 ml."), and 20 to 200 mg/mL sucrose (pg 22 line 12-15 "The vaccine formulation of the present invention comprises virus and stabilizer components in amounts from ...; from about 10 to about 100 mg per 0.5 ml of a sugar stabilizer selected from but not restricted to lactose, sucrose, mannitol and trehalose").

Thompson teaches an 11 mM phosphate buffer pH 7.0-7.4 for stabilizing a virus composition (para [0121] "In a specific embodiment, the viral harvest is stabilized by the addition of a concentrated buffer including but not limited to a sucrose phosphate (SP) buffer, which may be added to obtain a final concentration of about 218 mM sucrose, 11 mM phosphate buffer pH 7.0-7.4 in the stabilized viral harvest."; para [0186] "In certain embodiments the Benzonase treated clarified viral harvest is stabilized by the addition of a sucrose phosphate (SP), tris sucrose (TS) or sucrose phosphate glutamate (SPG) buffer. ... In certain embodiments, the stabilization buffer is a SP buffer wherein the buffer comprises sucrose, potassium phosphate (a combination of mono- and dibasic) and potassium chloride.").

IDRI teaches wherein a dengue vaccine formulation comprises .5 % glycerol (para [0123] "The thermostable lyophilized vaccine compositions further optionally include an antigen and/or an adjuvant ... In some embodiments the composition is formed by the lyophilization of an oil-in water emulsion formulation and the oil-in-water formulation prior to lyophilization or upon reconstitution contains ..., less than or about 0.5 percent (w/v) glycerol,"; para [0230] "An antigen, for use in certain embodiments of the herein described compositions and methods for generating and using such compositions, may be any target epitope, molecule (including a biomolecule), molecular complex (including molecular complexes that contain biomolecules), subcellular assembly, cell or tissue..."; para [0232] "In certain embodiments the compositions described herein ... comprise an antigen or antigenic composition capable of eliciting an immune response against a ...pathogen, which antigen or antigenic composition may include a composition derived from a virus such as from ., flaviviruses (e.g., ..., Dengue Virus,...)").

Areacor teaches virus vaccine formulations comprising about 0.5-10 mg/ml sodium carboxymethyl cellulose (para [0005] "Recombinant adenovirus vectors are becoming increasingly important for use in vaccine development and gene therapy applications."; para [0073] "The pharmaceutical formulations of the present invention provide long-term storage stability for adenovirus vector compositions at varying degrees of virus concentration"; para [0009] "The present invention is directed to an aqueous formulation comprising an adenovirus vector and at least one anionic polymer"; para [0079] "In some embodiments, the at least one anionic polymer is at a concentration ranging from about 0.5 mg/mL to about 10 mg/mL."; para [0014] "In some embodiments, the at least one anionic polymer is sodium carboxymethyl cellulose. The sodium carboxymethyl cellulose may have an average molecular weight ..., preferably an average molecular weight of about 90000 Da."). It would have been obvious to one skilled in the art to have combined, for research or therapeutic purposes, the live-attenuated dengue virus and sucrose as taught by Panacea, phosphate buffer as taught by Thompson, glycerol as taught by IDRI, and sodium carboxymethyl cellulose as taught by Arecor with the Dengue vaccine formulation taught by UT, in order to improve the stability of said formulation.

Regarding claim 14/9, UT, Panacea, Thompson, IDRI, and Arecor make obvious the formulation of claim 9. Panacea further teaches wherein the dengue vaccine formulation comprises 20-200 mg/ml trehalose (pg 22 line 12-15 "The vaccine formulation of the present invention comprises virus and stabilizer components in amounts from ...; from about 10 to about 100 mg per 0.5 ml of a sugar stabilizer selected from but not restricted to lactose, sucrose, mannitol and trehalose").

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Claims 10, 11, 14/(10,11) lack an inventive step under PCT Article 33(3) as being obvious over UT, in view of Panacea, Thompson, IDRI, Arecor, and Takeda.

Regarding claim 10, UT teaches the formulation of claim 1. UT does not specifically teach wherein said formulation comprises the live attenuated dengue virus (LAV) at 600-20,000 pfu/ml, about 5-300 mM potassium phosphate buffer at pH 7.0-8.0, about 60-120 mg/ml sucrose, about 3-7 mg/ml propylene glycol (PG) or glycerol, about 3-7 mg/ml sodium carboxymethylcellulose with average molecular weight of about 90,000, and about 30-90 mM NaCl.

However, Panacea teaches a dengue virus vaccine composition comprising 2000 PFU/ml (pg 1 line 1-2 "The present invention relates to field of live attenuated recombinant tetravalent dengue vaccines and methods of producing stable compositions."; pg 23 line 8-9 "In preferred embodiments the dengue virus in the vaccine composition is present at a dose of NLT 3.0 log₁₀ PFU per 0.5 ml."), and 20 to 200 mg/mL sucrose (pg 22 line 12-15 "The vaccine formulation of the present invention comprises virus and stabilizer components in amounts from ...; from about 10 to about 100 mg per 0.5 ml of a sugar stabilizer selected from but not restricted to lactose, sucrose, mannitol and trehalose").

Thompson teaches an 11 mM potassium phosphate buffer pH 7.0-7.4 for stabilizing a virus composition (para [0121] "In a specific embodiment, the viral harvest is stabilized by the addition of a concentrated buffer including but not limited to a sucrose phosphate (SP) buffer, which may be added to obtain a final concentration of about 218 mM sucrose, 11 mM phosphate buffer pH 7.0-7.4 in the stabilized viral harvest."; para [0186] "In certain embodiments the Benzonase treated clarified viral harvest is stabilized by the addition of a sucrose phosphate (SP), tris sucrose (TS) or sucrose phosphate glutamate (SPG) buffer. ... In certain embodiments, the stabilization buffer is a SP buffer wherein the buffer comprises sucrose, potassium phosphate (a combination of mono- and dibasic) and potassium chloride.").

IDRI teaches wherein a dengue vaccine formulation comprises .5 % glycerol (para [0123] "The thermostable lyophilized vaccine compositions further optionally include an antigen and/or an adjuvant ... In some embodiments the composition is formed by the lyophilization of an oil-in water emulsion formulation and the oil-in-water formulation prior to lyophilization or upon reconstitution contains ..., less than or about 0.5 percent (w/v) glycerol,"; para [0230] "An antigen, for use in certain embodiments of the herein described compositions and methods for generating and using such compositions, may be any target epitope, molecule (including a biomolecule), molecular complex (including molecular complexes that contain biomolecules), subcellular assembly, cell or tissue..."; para [0232] "In certain embodiments the compositions described herein ... comprise an antigen or antigenic composition capable of eliciting an immune response against a ...pathogen, which antigen or antigenic composition may include a composition derived from a virus such as from .., flaviviruses (e.g., ..., Dengue Virus,...)").

Areacor teaches virus vaccine formulations comprising about 0.5-10 mg/ml sodium carboxymethyl cellulose with an average molecular weight of about 90,000 (para [0005] "Recombinant adenovirus vectors are becoming increasingly important for use in vaccine development and gene therapy applications."; para [0073] "The pharmaceutical formulations of the present invention provide long-term storage stability for adenovirus vector compositions at varying degrees of virus concentration"; para [0009] "The present invention is directed to an aqueous formulation comprising an adenovirus vector and at least one anionic polymer."; para [0079] "In some embodiments, the at least one anionic polymer is at a concentration ranging from about 0.5 mg/mL to about 10 mg/mL."; para [0014] "In some embodiments, the at least one anionic polymer is sodium carboxymethyl cellulose. The sodium carboxymethyl cellulose may have an average molecular weight ..., preferably an average molecular weight of about 90000 Da.").

Takeda teaches a dengue vaccine formulation comprising 25-200 mM sodium chloride (para [0005] "...each of the passages provides an attenuated dengue virus contemplated of use in a pharmaceutically acceptable vaccine composition"; para [000106] "In other embodiments, formulations of use herein stabilize live, attenuated Flaviviruses for commercial use. In some embodiments, the composition further includes a buffer. In accordance with these embodiments, the buffer can include but is not limited to, phosphate buffered saline (PBS). In accordance with these embodiments, the buffer can include at least one of sodium chloride (NaCl), the buffer of the composition can include sodium chloride at a concentration of 25 mM to 200 mM."). It would have been obvious to one skilled in the art to have combined, for research or therapeutic purposes, live-attenuated dengue virus and sucrose as taught by Panacea, potassium phosphate buffer as taught by Thompson, glycerol as taught by IDRI, sodium carboxymethyl cellulose with an average molecular weight of about 90000 Da as taught by Arecor, and sodium chloride as taught by Takeda with the dengue vaccine formulation made obvious by UT, in order to improve the stability of said formulation.

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Regarding claim 11, UT teaches the formulation of claim 1. UT does not specifically teach wherein said formulation comprises the live attenuated dengue virus (LAV) at 600-20,000 pfu/ml, about 11 mM potassium phosphate buffer at pH about 7.0-8.0, about 90 mg/ml sucrose, about 5 mg/ml propylene glycol (PG) or glycerol, about 5 mg/ml sodium carboxymethylcellulose with average molecular weight of about 90,000, and about 75 mM NaCl.

However, Panacea teaches a dengue virus vaccine composition comprising 2000 PFU/ml (pg 1 line 1-2 "The present invention relates to field of live attenuated recombinant tetravalent dengue vaccines and methods of producing stable compositions."; pg 23 line 8-9 "In preferred embodiments the dengue virus in the vaccine composition is present at a dose of NLT 3.0 log₁₀ PFU per 0.5 ml."), and 20 to 200 mg/mL sucrose (pg 22 line 12-15 "The vaccine formulation of the present invention comprises virus and stabilizer components in amounts from ...; from about 10 to about 100 mg per 0.5 ml of a sugar stabilizer selected from but not restricted to lactose, sucrose, mannitol and trehalose").

Thompson teaches an 11 mM potassium phosphate buffer pH 7.0-7.4 for stabilizing a virus composition (para [0121] "In a specific embodiment, the viral harvest is stabilized by the addition of a concentrated buffer including but not limited to a sucrose phosphate (SP) buffer, which may be added to obtain a final concentration of about 218 mM sucrose, 11 mM phosphate buffer pH 7.0-7.4 in the stabilized viral harvest."; [0186] "In certain embodiments the Benzonase treated clarified viral harvest is stabilized by the addition of a sucrose phosphate (SP), tris sucrose (TS) or sucrose phosphate glutamate (SPG) buffer. ... In certain embodiments, the stabilization buffer is a SP buffer wherein the buffer comprises sucrose, potassium phosphate (a combination of mono- and dibasic) and potassium chloride."). IDRI teaches wherein a dengue vaccine formulation comprises 5 mg/ml glycerol (para [0123] "The thermostable lyophilized vaccine compositions further optionally include an antigen and/or an adjuvant ... In some embodiments the composition is formed by the lyophilization of an oil-in water emulsion formulation and the oil-in-water formulation prior to lyophilization or upon reconstitution contains ..., less than or about 0.5 percent (w/v) glycerol,."; para [0230] "An antigen, for use in certain embodiments of the herein described compositions and methods for generating and using such compositions, may be any target epitope, molecule (including a biomolecule), molecular complex (including molecular complexes that contain biomolecules), subcellular assembly, cell or tissue..."; para [0232] "In certain embodiments the compositions described herein ... comprise an antigen or antigenic composition capable of eliciting an immune response against a ...pathogen, which antigen or antigenic composition may include a composition derived from a virus such as from .., flaviviruses (e.g., ..., Dengue Virus,...)").

Arecor teaches virus vaccine formulations comprising 5 mg/ml sodium carboxymethyl cellulose with average molecular weight of about 90,000 (para [0005] "Recombinant adenovirus vectors are becoming increasingly important for use in vaccine development and gene therapy applications."; para [0073] "The pharmaceutical formulations of the present invention provide long-term storage stability for adenovirus vector compositions at varying degrees of virus concentration"; para [0009] "The present invention is directed to an aqueous formulation comprising an adenovirus vector and at least one anionic polymer."; para [0079] "In some embodiments, the at least one anionic polymer is at a concentration ranging from about 0.5 mg/mL to about 10 mg/mL."; para [0014] "In some embodiments, the at least one anionic polymer is sodium carboxymethyl cellulose. The sodium carboxymethyl cellulose may have an average molecular weight ..., preferably an average molecular weight of about 90000 Da.").

Takeda teaches a dengue vaccine formulation comprising 25-200 mM sodium chloride (para [0005] "...each of the passages provides an attenuated dengue virus contemplated of use in a pharmaceutically acceptable vaccine composition"; para [000106] "In other embodiments, formulations of use herein stabilize live, attenuated Flaviviruses for commercial use. In some embodiments, the composition further includes a buffer. In accordance with these embodiments, the buffer can include but is not limited to, phosphate buffered saline (PBS). In accordance with these embodiments, the buffer can include at least one of sodium chloride (NaCl), the buffer of the composition can include sodium chloride at a concentration of 25 mM to 200 mM."). It would have been obvious to one skilled in the art to have combined, for research or therapeutic purposes, live-attenuated dengue virus and sucrose as taught by Panacea; potassium phosphate buffer as taught by Thompson; glycerol as taught by IDRI; sodium carboxymethyl cellulose with an average molecular weight of about 90000 Da as taught by Arecor, and sodium chloride as taught by Takeda, with the dengue vaccine formulation made obvious by UT, in order to improve the stability of said formulation.

Regarding claim 14/(10,11), UT, Panacea, Thompson, IDRI, Arecor, and Takeda make obvious the formulation of claims 10 and 11. Panacea further teaches wherein the dengue vaccine formulation comprises 20-200 mg/ml trehalose (pg 22 line 12-15 "The vaccine formulation of the present invention comprises virus and stabilizer components in amounts from ...; from about 10 to about 100 mg per 0.5 ml of a sugar stabilizer selected from but not restricted to lactose, sucrose, mannitol and trehalose").

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Claims 12, 13, 14/(12,13), 15 lack an inventive step under PCT Article 33(3) as being obvious over UT, in view of Panacea, Thompson, Arecor, Takeda, and US 2016/0158248 A1 to Hananja Ehf (hereinafter 'Hananja').

Regarding claim 12, UT teaches the formulation of claim 1 but does not teach wherein said formulation comprises the live attenuated dengue virus (LAV) at 600-20,000 pfu/ml, about 11 mM potassium phosphate buffer at pH about 7.5, about 90 mg/ml sucrose, about 5 mg/ml propylene glycol (PG), about 5 mg/ml sodium carboxymethylcellulose with average molecular weight of about 90,000, about 50 mM NaCl, and about 25 mM Leu.

However, Panacea teaches a dengue virus vaccine composition comprising 2000 PFU/ml (pg 1 line 1-2 "The present invention relates to field of live attenuated recombinant tetravalent dengue vaccines and methods of producing stable compositions."; pg 23 line 8-9 "In preferred embodiments the dengue virus in the vaccine composition is present at a dose of NLT 3.0 log10 PFU per 0.5 ml."), and 20 to 200 mg/mL sucrose (pg 22 line 12-15 "The vaccine formulation of the present invention comprises virus and stabilizer components in amounts from ...; from about 10 to about 100 mg per 0.5 ml of a sugar stabilizer selected from but not restricted to lactose, sucrose, mannitol and trehalose").

Thompson teaches an 11 mM potassium phosphate buffer pH 7.0-7.4 for stabilizing a virus composition (para [0121] "In a specific embodiment, the viral harvest is stabilized by the addition of a concentrated buffer including but not limited to a sucrose phosphate (SP) buffer, which may be added to obtain a final concentration of about 218 mM sucrose, 11 mM phosphate buffer pH 7.0-7.4 in the stabilized viral harvest."; [0186] "In certain embodiments the Benzonase treated clarified viral harvest is stabilized by the addition of a sucrose phosphate (SP), tris sucrose (TS) or sucrose phosphate glutamate (SPG) buffer. ... In certain embodiments, the stabilization buffer is a SP buffer wherein the buffer comprises sucrose, potassium phosphate (a combination of mono- and dibasic) and potassium chloride."). Arecor teaches virus vaccine formulations comprising about 0.5-10 mg/ml sodium carboxymethyl cellulose with average molecular weight of about 90,000 and leucine (para [0005] "Recombinant adenovirus vectors are becoming increasingly important for use in vaccine development and gene therapy applications."; para [0073] "The pharmaceutical formulations of the present invention provide long-term storage stability for adenovirus vector compositions at varying degrees of virus concentration"; para [0009] "The present invention is directed to an aqueous formulation comprising an adenovirus vector and at least one anionic polymer.; para [0079] "In some embodiments, the at least one anionic polymer is at a concentration ranging from about 0.5 mg/mL to about 10 mg/mL."; para [0014] "In some embodiments, the at least one anionic polymer is sodium carboxymethyl cellulose. The sodium carboxymethyl cellulose may have an average molecular weight ..., preferably an average molecular weight of about 90000 Da."; para [0056] "Examples of compounds that can be usefully incorporated into the adenovirus formulation as additives and that may, subject to the pH of the formulation, function as displaced buffers are known and include, but are not limited to, ..., leucine").

Takeda teaches a dengue vaccine formulation comprising 25-200 mM sodium chloride (para [0005] "...each of the passages provides an attenuated dengue virus contemplated of use in a pharmaceutically acceptable vaccine composition"; para [000106] "In other embodiments, formulations of use herein stabilize live, attenuated Flaviviruses for commercial use. In some embodiments, the composition further includes a buffer. In accordance with these embodiments, the buffer can include but is not limited to, phosphate buffered saline (PBS). In accordance with these embodiments, the buffer can include at least one of sodium chloride (NaCl), the buffer of the composition can include sodium chloride at a concentration of 25 mM to 200 mM.").

Hananja teaches wherein a vaccine formulation comprises 1-500 mg/ml propylene glycol (para [0054] "The pharmaceutical composition of the invention may comprise one or more therapeutic agents"; para [0056] "In certain embodiments, the therapeutic agent is an analgesic agent, ..., vaccine,."; para [0065] "It is understood that the compositions of the invention, in addition to the alkoxy-polyethylene glycol and the therapeutic agent, can comprise a number of other excipients known to those skilled in the art."; para [0068] "Preferred additional solubilizers include triacetin, ..., and propylene glycol. Typically, the solubilizer, if present, is present in an amount of from about 0.1 percent (w/v) to about 50 percent (w/v)").

It would have been obvious to one skilled in the art to have combined, for research or therapeutic purposes, live-attenuated dengue virus and sucrose as taught by Panacea, potassium phosphate buffer as taught by Thompson, propylene glycol as taught by Hananja, sodium carboxymethyl cellulose with an average molecular weight of about 90000 Da and leucine as taught by Arecor, sodium chloride as taught by Takeda with the Dengue vaccine formulation made obvious by UT, in order to improve the stability of said formulation. UT, Panacea, Thompson, Hananja, Arecor, and Takeda do not specifically teach wherein the formulation comprises about 25 mM leucine. However, one of ordinary skill in the art would have conducted routine experimentations to identify the optimal concentration of leucine, and thus would have reached claimed subject wherein the formulation made obvious by UT, Panacea, Thompson, Hananja, Arecor and Takeda comprises about 25 mM leucine.

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Regarding claim 13, UT makes teaches the formulation of claim 1 but does not teach wherein said formulation comprises the live attenuated dengue virus (LAV) at 600-20,000 pfu/ml, about 11 mM potassium phosphate buffer at pH about 7.5, about 90 mg/ml sucrose, about 5 mg/ml propylene glycol (PG), about 5 mg/ml sodium carboxymethylcellulose with average molecular weight of about 90,000, and about 30 mM NaCl.

However, Panacea teaches a dengue virus vaccine composition comprising 2000 PFU/ml (pg 1 line 1-2 "The present invention relates to field of live attenuated recombinant tetravalent dengue vaccines and methods of producing stable compositions."; pg 23 line 8-9 "In preferred embodiments the dengue virus in the vaccine composition is present at a dose of NLT 3.0 log₁₀ PFU per 0.5 ml."), and 20 to 200 mg/mL sucrose (pg 22 line 12-15 "The vaccine formulation of the present invention comprises virus and stabilizer components in amounts from ...; from about 10 to about 100 mg per 0.5 ml of a sugar stabilizer selected from but not restricted to lactose, sucrose, mannitol and trehalose").

Thompson teaches an 11 mM potassium phosphate buffer pH 7.0-7.4 for stabilizing a virus composition (para [0121] "In a specific embodiment, the viral harvest is stabilized by the addition of a concentrated buffer including but not limited to a sucrose phosphate (SP) buffer, which may be added to obtain a final concentration of about 218 mM sucrose, 11 mM phosphate buffer pH 7.0-7.4 in the stabilized viral harvest."; para [0186] "In certain embodiments the Benzonase treated clarified viral harvest is stabilized by the addition of a sucrose phosphate (SP), tris sucrose (TS) or sucrose phosphate glutamate (SPG) buffer. ... In certain embodiments, the stabilization buffer is a SP buffer wherein the buffer comprises sucrose, potassium phosphate (a combination of mono- and dibasic) and potassium chloride.").

Arecor teaches virus vaccine formulations comprising about 0.5-10 mg/ml sodium carboxymethyl cellulose with average molecular weight of about 90,000 (para [0005] "Recombinant adenovirus vectors are becoming increasingly important for use in vaccine development and gene therapy applications."; para [0073] "The pharmaceutical formulations of the present invention provide long-term storage stability for adenovirus vector compositions at varying degrees of virus concentration"; para [0009] "The present invention is directed to an aqueous formulation comprising an adenovirus vector and at least one anionic polymer."; para [0079] "In some embodiments, the at least one anionic polymer is at a concentration ranging from about 0.5 mg/mL to about 10 mg/mL."; para [0014] "In some embodiments, the at least one anionic polymer is sodium carboxymethyl cellulose. The sodium carboxymethyl cellulose may have an average molecular weight ..., preferably an average molecular weight of about 90000 Da.").

Takeda teaches a dengue vaccine formulation comprising 25-200 mM sodium chloride (para [0005] "...each of the passages provides an attenuated dengue virus contemplated of use in a pharmaceutically acceptable vaccine composition"; para [000106] "In other embodiments, formulations of use herein stabilize live, attenuated Flaviviruses for commercial use. In some embodiments, the composition further includes a buffer. In accordance with these embodiments, the buffer can include but is not limited to, phosphate buffered saline (PBS). In accordance with these embodiments, the buffer can include at least one of sodium chloride (NaCl), the buffer of the composition can include sodium chloride at a concentration of 25 mM to 200 mM.").

Hananja teaches wherein a vaccine formulation comprises 1-500 mg/ml propylene glycol (para [0054] "The pharmaceutical composition of the invention may comprise one or more therapeutic agents"; para [0056] "In certain embodiments, the therapeutic agent is an analgesic agent, ...vaccine,."; para [0065] "It is understood that the compositions of the invention, in addition to the alkoxy-polyethylene glycol and the therapeutic agent, can comprise a number of other excipients known to those skilled in the art."; para [0068] "Preferred additional solubilizers include triacetin,... and propylene glycol. Typically, the solubilizer, if present, is present in an amount of from about 0.1 percent (w/v) to about 50 percent (w/v)").

It would have been obvious to one skilled in the art to have combined, for research or therapeutic purposes, live-attenuated dengue virus and sucrose as taught by Panacea, potassium phosphate buffer as taught by Thompson, propylene glycol as taught by Hananja, sodium carboxymethyl cellulose with an average molecular weight of about 90000 Da as taught by Arecor, and sodium chloride as taught by Takeda, with the dengue vaccine formulation taught by UT, in order to improve the stability of said formulation. UT, Panacea, Thompson, Hananja, Arecor and Takeda do not specifically teach wherein the formulation comprises 11 mM potassium phosphate buffer at pH about 7.5. However, one of ordinary skill in the art would have conducted routine experimentations to identify the optimal pH of said buffer, and thus would have reached claimed subject wherein the formulation made obvious by UT, Panacea, Thompson, Hananja, Arecor and Takeda comprises about 11 mM potassium phosphate buffer at pH about 7.5.

Regarding claim 14/(12,13), UT, Panacea, Thompson, Arecor, Hananja and Takeda make obvious the formulation of claims 12 and 13. Panacea further teaches wherein the dengue vaccine formulation comprises 20-200 mg/ml trehalose (pg 22 line 12-15 "The vaccine formulation of the present invention comprises virus and stabilizer components in amounts from ...; from about 10 to about 100 mg per 0.5 ml of a sugar stabilizer selected from but not restricted to lactose, sucrose, mannitol and trehalose").

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Supplemental Box

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Continuation of:

Previous supplemental box:

Regarding claim 15, UT makes obvious the formulation of claim 1 but does not teach wherein said formulation comprises the live attenuated dengue virus (LAV) at 600-20,000 pfu/ml, about 11 mM potassium phosphate buffer at pH about 7.5-8, about 90 mg/ml sucrose, about 110 mg/ml trehalose, about 5 mg/ml propylene glycol (PG), about 5 mg/ml sodium carboxymethylcellulose with average molecular weight of about 90,000, about 50 mM NaCl, and about 25 mM Leu.

However, Panacea teaches a dengue virus vaccine composition comprising 2000 PFU/ml (pg 1 line 1-2 "The present invention relates to field of live attenuated recombinant tetravalent dengue vaccines and methods of producing stable compositions."; pg 23 line 8-9 "In preferred embodiments the dengue virus in the vaccine composition is present at a dose of NLT 3.0 log₁₀ PFU per 0.5 ml."), about 20 to 200 mg/mL sucrose, and about 20 to 200 mg/mL trehalose (pg 22 line 12-15 "The vaccine formulation of the present invention comprises virus and stabilizer components in amounts ...; from about 10 to about 100 mg per 0.5 ml of a sugar stabilizer selected from but not restricted to lactose, sucrose, mannitol and trehalose."; pg 20 line 30-31 "In another embodiment, the stabilizer may further comprise lactose, sucrose, mannitol, trehalose, ..., or combinations thereof.").

Thompson teaches an 11 mM potassium phosphate buffer for stabilizing a virus composition (para [0121] "In a specific embodiment, the viral harvest is stabilized by the addition of a concentrated buffer including but not limited to a sucrose phosphate (SP) buffer, which may be added to obtain a final concentration of about 218 mM sucrose, 11 mM phosphate buffer pH 7.0-7.4 in the stabilized viral harvest."; [0186] "In certain embodiments the Benzoinase treated clarified viral harvest is stabilized by the addition of a sucrose phosphate (SP), tris sucrose (TS) or sucrose phosphate glutamate (SPG) buffer. ... In certain embodiments, the stabilization buffer is a SP buffer wherein the buffer comprises sucrose, potassium phosphate (a combination of mono- and dibasic) and potassium chloride.").

Arecor teaches virus vaccine formulations comprising about 0.5-10 mg/ml sodium carboxymethyl cellulose with average molecular weight of about 90,000 and leucine (para [0005] "Recombinant adenovirus vectors are becoming increasingly important for use in vaccine development and gene therapy applications."; para [0073] "The pharmaceutical formulations of the present invention provide long-term storage stability for adenovirus vector compositions at varying degrees of virus concentration"; para [0009] "The present invention is directed to an aqueous formulation comprising an adenovirus vector and at least one anionic polymer.; para [0079] "In some embodiments, the at least one anionic polymer is at a concentration ranging from about 0.5 mg/mL to about 10 mg/mL."; para [0014] "In some embodiments, the at least one anionic polymer is sodium carboxymethyl cellulose. The sodium carboxymethyl cellulose may have an average molecular weight ..., preferably an average molecular weight of about 90000 Da."; para [0056] "Examples of compounds that can be usefully incorporated into the adenovirus formulation as additives and that may, subject to the pH of the formulation, function as displaced buffers are known and include, but are not limited to, ..., leucine").

Takeda teaches a dengue vaccine formulation comprising 25-200 mM sodium chloride (para [0005] "...each of the passages provides an attenuated dengue virus contemplated of use in a pharmaceutically acceptable vaccine composition"; para [000106] "In other embodiments, formulations of use herein stabilize live, attenuated Flaviviruses for commercial use. In some embodiments, the composition further includes a buffer. In accordance with these embodiments, the buffer can include but is not limited to, phosphate buffered saline (PBS). In accordance with these embodiments, the buffer can include at least one of sodium chloride (NaCl), the buffer of the composition can include sodium chloride at a concentration of 25 mM to 200 mM.").

Hananja teaches wherein a vaccine formulation comprises 1-500 mg/ml propylene glycol (para [0054] "The pharmaceutical composition of the invention may comprise one or more therapeutic agents"; para [0056] "In certain embodiments, the therapeutic agent is an analgesic agent, ..., vaccine."; para [0065] "It is understood that the compositions of the invention, in addition to the alkoxy-polyethylene glycol and the therapeutic agent, can comprise a number of other excipients known to those skilled in the art."; para [0068] "Preferred additional solubilizers include ... and propylene glycol. Typically, the solubilizer, if present, is present in an amount of from about 0.1 percent (w/v) to about 50 percent (w/v)").

It would have been obvious to one skilled in the art to have combined, for research or therapeutic purposes, live-attenuated dengue virus, sucrose and trehalose as taught by Panacea, potassium phosphate buffer as taught by Thompson, propylene glycol as taught by Hananja, sodium carboxymethyl cellulose and leucine as taught by Arecor, and sodium chloride as taught by Takeda with the Dengue vaccine formulation made obvious by UT, in order to improve the stability of said formulation. UT, Panacea, Thompson, Hananja, Arecor and Takeda do not specifically teach wherein said formulation comprises 25 mM leucine. However, one of ordinary skill in the art would have conducted routine experimentations to identify the optimal concentration of leucine, and thus would have reached claimed subject wherein the formulation made obvious by UT, Panacea, Thompson, Hananja, Arecor, and Takeda comprises about 25 mM leucine.

Claims 1-3, 8-15, 17-21, 23-27 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.