PATENT COOPERATION TREATY

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THIRD PARTY OBSERVATION

(PCT Administrative Instructions Part 8)

Applicant's or agent's file reference WO181	
International application number PCT/IN2021/050806	International filing date (day/month/year) 21 Aug 2021 (21/08/2021)
Applicant BHARAT BIOTECH INTERNATIONAL LIMITED	

Third party observation submitted by Anonymous	Observation submitted on behalf of
Date of submission(day/month/year) 21 Dec 2022 (21/12/2022)	Language of observation English

Basis and contents of observation

1. The observation is made on the basis of the claims in the international application as filed.
2. The observation comprises:
   References to documents: 5
   Uploaded copies of documents: 6
3. Further explanations:
   Uploaded copies of documents: 1

Citation # 1(Other) (# uploaded documents:3):

Identification of Document: CTRI/2020/07/026300	Publication Date: 01 Jul 2020 (01/07/2020)
Link to document: http://ctri.nic.in/Clinicaltrials/showallp.php?mid1=45184&EncHid&userName=BBV152	
DOI:	
Most relevant passages or drawings: 1-9	Relevant to Claims: 1-18, 20-27

Brief explanation of relevance:

A Phase 1/2 clinical trial, CTRI/2020/07/026300 was registered on 01/07/2020 in India by Bharat Biotech International Limited (BBIL) titled “Whole-virion inactivated SARS-CoV-2 vaccine (BBV152) in healthy volunteers”; hereto annexed as Citation 1, is the pdf print-out from the website of the Clinical Trials Registry–India; the page numbers below refer to the page numbers of the said pdf. The entries for modifications have been collated as “Modifications document” and attached as Citation 1A hereto. This trial appears to be for the vaccine candidate, Covaxin, for which methods of production is claimed by the Applicant of the present Application, WO2022038642 (WO’642), BBIL.

The interventional double blind, multi-centre trial had three arms as described “Whole-Virion Inactivated SARS-CoV-2 vaccine (BBV152) with three formulations, BBV152A, BBV152B and BBV152C. Dose: 0.5 ml, Route of administration: Intramuscular injection, Frequency: Two doses at Day 0 and Day 14” (p.4). The modifications made to the trial do not appear to reflect any modification in the formulations of the vaccine in the three arms of the trial [p.2, Citation 1A].

Though the clinical trial document does not provide the details of the three formulations, the same has been disclosed in the DCGI Permission Letter dated and signed on 29 June 2020, hereto annexed as Citation 1B (last accessed from https://cdsco.gov.in/opencms/resources/UploadCDSCOWeb/2018/UploadCTApprovals/CT-14-2020.pdf), permitting the Applicant (BBIL) to conduct the above clinical trial. It is stated therein that the whole virion, inactivated corona virus antigen (Strain: NIV-2020-770) as the active ingredient is to be administered at 3 mcg or 6 mcg or 6 mcg in 0.5 ml liquid phosphate buffered saline (injection for intramuscular route), with aluminium hydroxide gel in all three formulations, 15 mcg TLR6 agonist in the formulation of 3 mcg & with or without TLR6 agonist in the two formulations of 6 mcg [p.2]. It may be noted that the DCGI Permission Letter also required the Applicant (BBIL) to submit information/documents/certificate of analysis of TLR7 in drug product [p.3, Citation 1B]. However, TLR6 agonist appears to be in the formulation that is provided in the annexure of the DCGI Permission letter [p.2, Citation 1B].

The present Application, WO2022038642 (WO’642), claims a method of preparation of an inactivated, purified SARS-CoV-2 as an active ingredient in a vaccine composition wherein method of preparation of bulk comprises 4 steps: a. growing in Vero, b. scaling up and harvesting, c. inactivating using formalin, BPL, heat, etc., d. purifying (Claims 1–14); method of production of inactivated purified SARS-CoV-2 bulk comprising inactivation followed by a combination of size-exclusion or affinity chromatography and tangential flow filtration (Claim 15); method of preparing an immunogenic composition of inactivated, purified SARS-CoV-2 with formulation having excipients (adjuvant aluminium hydroxide, PBS, stabilizer, preservative) (Claims 16–22), wherein the antigen content and pH are stable (Claim 23), a coronavirus vaccine obtained by claimed method (Claim 24); method of preparation of the bulk (Claims 25–26) & method of treatment and/or prophylaxis of Covid 19 by administration of the immunogenic composition via various routes (intramuscular, etc.) (Claim 27).

However, the Phase 1/2 clinical trial document already discloses the use of the vaccine BBV152 in three formulations, and the corresponding DCGI Permission Letter [Citation 1B] discloses the composition of the three formulations of BBV152 prior to the priority date of the present Application, WO’642, i.e., prior to 21.08.2020. It may be noted that (i) these prior disclosed formulations of BBV152 fall within the scope of the claims of the present Application, WO’642; (ii) the concentrations of the inactive ingredients fall within the ranges claimed for the excipients in WO’642 ; (iii) all modifications to the CTRI [Citation 1A] pertaining to the above disclosures of the CTRI document were made before the priority date of WO’642.

Thus, Claims 1 to 18 & 20 to 27 of the present Application, WO’642, lack novelty (to the extent of overlap) and inventive step.

Citation # 2(Periodical article) (# uploaded documents:1):

Author:	Title of article:	Title of Periodical:	Publication Date:
Zhang, Y., et al.	Immunogenicity and Safety of a SARS-CoV-2 Inactivated Vaccine in Healthy Adults Aged 18-59 years: Report of the Randomized, Double-blind, and Placebo-controlled Phase 2 Clinical Trial	medRxiv	10 Aug 2020 (10/08/2020)

Issue Number of Periodical:	Publisher of Periodical:	Place of publication:
Vol.		
Page range of article within periodical: 1-22	ISBN:	ISSN:

DOI: 10.1101/2020.07.31.20161216

Most relevant passages or drawings:	Relevant to Claims:
Abstract; pp. 5, 8–9, 12, 17–18	1-27

Brief explanation of relevance:

In this preprint version of the publication, Zhang, et al. disclose the details of a phase 2 trial conducted to further evaluate the immunogenicity and safety of inactivated SARS-CoV-2 whole virion vaccine with aluminium hydroxide developed by Sinovac Life Sciences Ltd. (CoronaVac) (Abstract, pp.5, 8). They state that 600 adults were randomly assigned into 3 groups to receive 2 injections of the trial vaccine at a dose of 3 mcg/0.5mL or 6 mcg/0.5mL, or placebo on Day 0,14 schedule or Day 0,28 administered intramuscularly (id, p.8).

They further state that the:
(i) SARS-CoV-2 virus was propagated in Vero cells and harvested.
(ii) Harvested virus was inactivated using beta-propiolactone (BPL) and further purified.
(iii) Bulk vaccine material obtained from this step was then adsorbed onto aluminium hydroxide.
(iv) Inactivated final product formulated with phosphate-buffered saline (PBS) and sodium chloride (p.8).

Zhang, et al. further report that all vaccinated subjects (who received at least one dose) were observed for adverse events, and they did not observe any Grade 3 adverse reaction (pp.9, 12). Further, they state that “the incidence rates of adverse reactions in the 6 mcg and 3 mcg group were comparable, indicating that there was no dose-related aggravating concern on safety” (p.17).

They state that to assess immune response, blood samples were collected from each participant and antibody responses were assessed by checking for neutralizing antibody titres (p.9). The results indicate that “after two-dose vaccination, immune responses induced by Day 0,28 schedule was above the value of Day 0,14 schedule regardless of the dosage of the vaccine” (p.18). Based on their pre-clinical, and phase I and II clinical study results, they highlight the importance of developing an optimum manufacturing process and the integration of multiple-disciplinary techniques, such as genomics and structural biology to support a new era of precision vaccinology (id).

The present Application, WO2022038642 (WO’642), claims a method of preparation of an inactivated, purified SARS-CoV-2 as an active ingredient in a vaccine composition wherein method of preparation of bulk comprises 4 steps: a. growing in Vero, b. scaling up and harvesting, c. inactivating using formalin, BPL, heat, etc., d. purifying (Claims 1–14); method of production of inactivated purified SARS-CoV-2 bulk comprising inactivation followed by a combination of size-exclusion or affinity chromatography and tangential flow filtration (Claim 15); method of preparing an immunogenic composition of inactivated, purified SARS-CoV-2 with formulation having excipients (adjuvant aluminium hydroxide, PBS, stabilizer, preservative) (Claims 16–22), wherein the antigen content and pH are stable (Claim 23) & a coronavirus vaccine obtained by claimed

method (Claim 24); method of preparation of the bulk (Claims 25–26) & method of treatment and/or prophylaxis of Covid 19 by administration of the immunogenic composition via various routes (intramuscular, etc.) (Claim 27). The Applicant of the present Application, WO'642, states that the vaccine composition of the invention is obtained by a process wherein neutralizing antibodies are largely elicited against the spike glycoprotein such as in optimally inactivated virus and refers to optimization of conditions for growth and harvest (pp.13–14, 17).

It appears that the present Application, WO'642, covers the method of producing the marketed product Covaxin (BBV152). It may be noted that the DCGI Permission Letter (Citation 1B) for conducting Phase 1/2 clinical trials for Covaxin (BBV152) discloses that the concentrations of the antigen (a known SARS-CoV-2 strain; see Additional Comments) approved for use within the different formulations are 3 and 6 mcg.

However, Zhang, et al. already disclose an inactivated SARS-CoV-2 whole virion vaccine and its use in Phase 1/2 clinical trials, at 3 and 6 mcg, with promising results at both concentrations. They disclose method of cultivation of virus in Vero cell line followed by its harvesting, inactivation with BPL and purification. They also disclose the use of aluminium hydroxide adjuvant and PBS. Further, WO2017009873 (attached herewith as Citation 3), an earlier application by the Applicant of WO'642 , discloses/claims method of preparation of bulk virus & vaccine including inactivation, purification & formulation for ZIKV, similar to that claimed in WO'642 for SARS-CoV-2. Thus, the Applicant of the present Application, WO'642, has merely applied known technology relating to the production of inactivated viral vaccines to generate an inactivated vaccine for SARS-CoV-2.

Thus, Claims 1 to 27 of the present Application, WO'642, lack novelty (to the extent of overlap) and inventive step.

Citation # 3 (Patent/utility model) (# uploaded documents: 0):

Country code:	Publication number:	Document kind code:
WO	2017/009873	A1

Patent Applicant/Patent Owner:	Title of invention:
Bharat Biotech International Limited	Vaccine compositions

Link to document:
https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2017009873

Publication Date:	Filing Date:	Priority Date:
19 Jan 2017 (19/01/2017)	15 Jul 2016 (15/07/2016)	16 Jul 2015 (16/07/2015)

Source of Abstract:	Accession number:	Publication Date of Abstract:	Retrieval Date of Abstract:

Most relevant passages or drawings:	Relevant to Claims:
Abstract; pp.1–12, 15–17, 36, 52–59, 63–64–23; Claims: 1–2, 5, 7–14, 18–22, 31–34	1-27

Brief explanation of relevance:

WO2017009873 (WO'873), an earlier application by Bharat Biotech International Limited (BBIL), the Applicant of the present Application, WO2022038642 (WO'642), relates to vaccine/immunogenic compositions for prophylaxis & treatment of Zika virus (ZIKV) infections by eliciting immune response (both Th1 & Th2) in mammals; methods of preparation, production & formulations thereof (Abstract; pp.1, 10, 15; Claims 1–2, 52).

WO'873 discloses &/or claims method of preparation of vaccine composition comprising (pp.3–4; Claims 53–58, 63–64):

1. Adaptation & growth of ZIKV using Vero cell line (ATCC No. CCL-81) as substrate for ZIKV culture; the CCL-81 cell line conforms to the Requirements of Biological Substances No.50 recommended by WHO for production of biologicals & qualifies as a host cell for vaccine production (pp.6, 15–16, 21–22; Ex. 1)
2. Scaling up ZIKV culture to 10L harvest volume (pp.4, 22)
3. Inactivating the virus culture (before/after virus purification), using chemical [e.g., formalin, beta

propiolactone (BPL), etc.] or physical inactivating agent or an irradiating agent; in presence/absence of stabilizing agents & amino acids (pp. 5, 7, 16–17), wherein, ZIKV bulk is inactivated by any of the following methods (pp.4, 7, 11, 22–23; Claim 59):
a. Formalin treatment at concentration of 1:500–1:4000 formalin:virus v/v, at (i) 8–37 degree C (degC), preferably 25±3 degC, for at least 1–7 days; or (ii) 2–8 degC for at least 10–30 days;
b. BPL treatment at concentration of 1:500 – 1:4000 BPL:virus v/v, (i) for at least 24–48 hours (h) at 8–30 degC, preferably 25±3 degC, for 48 h; or (ii) 2–8 degC for at least 3–7 days; at concentration of 1:1000 – 1:3500 (BPL:virus, v/v) at 25±5 degC for 24–48 h or 2–8 degC for at least 3–7 days; 1: 2500 BPL: virus, v/v was also tested; at concentration of 1:1000 –1:4000 (BPL: virus, v/v) at 2–37degC for 24h to more than 10 days (pp.17,23; Ex. 3; Fig. 2B)
c. combination of BPL & formalin treatments at any of the aforementioned conditions, preferably 1: 3000 BPL:virus v/v for 24 h followed by 1:3000 formalin:virus v/v for 24–48 h at 15–30 degC, preferably 25±3 degC;
4. Purifying the virus culture by gel filtration (Capto Core 700), affinity matrix chromatography (using cellufine sulfate), etc; other techniques include ultracentrifugation, tangential flow filtration (TFF), etc . (pp.5–6, 16, 22).

WO'873 also claims &/or discloses:
5. A stable vaccine composition comprising (Claims 5, 7–14, 18–22, 31–34):
a. One/more inactivated whole virion arbovirus antigens (p.6)
b. Adjuvant: Aluminium hydroxide, Al(OH)3 (0.1–1.5 mg, preferably 0.25–0.5mg of aluminium (Al) per vaccine dose) (pp.9, 18–19, 21)
Adjuvant confers mucosal & systemic immunity in mammals & may also be selected from Al salts, any TLR ligands (e.g., monophosphoryl lipid A, resiquimod), etc; or combination of any of the aforementioned adjuvants (pp.5, 9, 18–19)
c. Buffer: sodium phosphate buffer containing 5-200 mM phosphate ions & optionally containing NaCl 50-200 mM (pp.7, 18)
d. Stabilizing agent: sorbitol, L-glycine, mannitol, L-glutamic acid, human serum albumin, etc or combinations thereof (pp.5, 7–8, 12, 18–19)
e. Preservative: 2-phenoxy-ethanol (2.5–5 mg/mL) (pp.10, 19)
6. A method of eliciting a protective immune response in mammals (humans) by administering the claimed composition by intramuscular (IM), intradermal (ID), subcutaneous (SC), intravenous, oral, intranasal (IN), etc routes (pp.5, 9, 20; Claim 36)

The present Application WO2022038642 (WO'642) claims:
(a) Method of preparation of Coronavirus (CoV) vaccine comprising inactivated, purified SARS-CoV -2, wherein bulk is prepared by: (i) using Vero cell line, (ii) scaling up to 10L harvest volume, (iii) inactivation (formalin, BPL, etc. using specified concentration & temperature conditions; BPL:virus v/ v = 1:2500 [also Ex. 3.1, p.27] or 1:4000 at 5±3degC for 32 h [p.25], (iv) purification (using size-exclusion-Capto core 700, affinity-Cellufine sulfate, etc; ultracentrifugation; TFF) & obtaining killed-inactivated bulk (Claims 1–15);
(b) Claimed method, wherein killed-inactivated bulk is active ingredient (in 100 mM PBS) in an immunogenic composition (IC) comprising adjuvant [Al(OH)3 (0.1–1.5 mg-preferably 0.25–0.5mg Al per dose)], stabilizing agent, preservative (2-phenoxyethanol 2.5–5 mg/mL); wherein antigen content & pH are stable at -70 degC; & vaccine obtained therefrom;
(c) Method of treatment/prophylaxis &/or eliciting immune response in mammals by administering the IC by IN, IM, oral, SC, etc routes (Claims 16–24, 27);
(d) Method of preparation & inactivation of bulk (Claims 25–26)

However, WO'873 by BBIL, already discloses the preferred use of the validated Vero cell line (CCL-81) for culture, method of preparation of bulk virus (inactivation, purification) & formulation for ZIKV vaccine, similar to that claimed in WO'642 for SARS-CoV-2; thus, Claims 1 to 27 of WO'642 lack inventive step.

Citation # 4 (Patent/utility model) (# uploaded documents: 0):

Country code:	Publication number:	Document kind code:
WO	2020136683	A1

Patent Applicant/Patent Owner:	Title of invention:
Bharat Biotech International Limited	Adaptation of Enterovirus to Vero cells and vaccine formulations thereof

Link to document:
https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2020136683

Publication Date:	Filing Date:	Priority Date:
02 Jul 2020 (02/07/2020)	27 Dec 2019 (27/12/2019)	29 Dec 2018 (29/12/2018)

Source of Abstract:	Accession number:	Publication Date of Abstract:	Retrieval Date of Abstract:

Most relevant passages or drawings:	Relevant to Claims:
Abstract; pp.4–5, 7–12, 15–18; Claims 4, 6–7; Ex., 1, 6; Table 2	1-27

Brief explanation of relevance:

WO2020136683 (WO'683), is an earlier application by Bharat Biotech International Limited (BBIL), the Applicant of present Application, WO2022038642 (WO'642), which shows that BBIL uses the same/similar processes for developing inactivated virus vaccines. WO'683 relates to vaccine composition comprising inactivated Enterovirus D68 (EVD68) antigen & adaptation & propagation of EV in Vero cells; the vaccine capable of inducing specific humoral immune response & neutralizing antibody response (Abstract; p.5).

WO'683 discloses &/or claims:

1. Vero cells, the cell substrate recommended by WHO for vaccine production, is valuable for rapid production of human vaccines due to infinite life span (continuous cell line) with proven safety & suitability for large scale vaccine production.

2. Process of adapting EVD68 to propagate in Vero cells, to high titres at 32-35 degC & harvesting the virus within 6 days (i.e., 144 hours) of infection; & its use as a candidate strain to produce vaccine (pp.4, 7–9, 12; Ex.1; Claim 4)

3. Method of inactivating EVD68 for immunization comprising the steps (pp.4–5, 10–11; Claim 6):

a) Sterile filtration of harvested EVD68

b) Removal of host nucleic acid & concentration by tangential flow filtration (TFF)

c) Inactivating agents for EVD68 include chemical agents (formalin, BPL, etc.) or physical agents & gamma irradiation, wherein chemical inactivation using 1/2000–1/4000 formalin for upto 3 weeks at 25–37 degC or using beta-propiolactone (BPL) at 4–25 degC for upto 120 hours, etc.

d) Purification of viral antigen (before/after inactivation) using gel filtration (Capto Core 700; Ex.6, p .15), Cellufine sulfate (known affinity chromatography resin), etc.

4. Immunogenic composition & formulation comprising inactivated EVD68 antigen (1–20 microg/ dose) in physiologically acceptable vehicle & optionally one or more pharmaceutically acceptable excipients selected from (pp.5, 11, 16–18; Ex.6; Table 2; Claim 7):

a) Buffer: Phosphate buffer

b) Adjuvants: alum (100 microg–1 mg, precisely 200–500 microg; also known as aluminium hydroxide, aluminium phosphate), Toll-like receptor (TLR) ligands like monophosphoryl lipid A, etc.

c) Stabilizers: sorbitol, mannitol (up to 20%); amino acids (up to 2%) like glycine (0.4-1%), glutamic acid; human serum albumin, etc.

d) Preservatives: 2-phenoxyethanol (2.5–20 mg/ml)

4. BPL-inactivated EVD68 antigen adjuvanted with alum elicited even higher IgGl type virus-specific antibody in comparison to IgG2a, indicating further polarization towards Th2-type immune response. Also inactivated antigen adjuvanted with alum showed higher IgG titer compared to the unadjuvanted, on subcutaneous administration in mice (p.20; Ex., 9; Figs. 7, 8)

5. Stability of immunogenic composition at 2–8 degC (6 months) & 37degC (1 month) (Ex., 8; p.19)

The present Application, WO'642 claims:

a) Method of preparation of Coronavirus vaccine, comprising inactivated, purified SARS-CoV-2, wherein bulk is prepared by: (i) using Vero cell line, (ii) scaling up to 10L harvest volume (in 48–80 hours), (iii) inactivation (formalin, BPL, etc using specified concentration & temperature conditions), (iv) purification (size-exclusion-Capto core700, affinity–Cellufine sulfate, etc; ultracentrifugation, TFF) & obtaining killed-inactivated bulk (Claims 1–15);

b) Claimed method, wherein killed-inactivated bulk is used as active ingredient (in 100 mM PBS) in an immunogenic composition (IC) comprising adjuvant Al(OH)3 (0.1–1.5 mg-preferably 0.25–0.5mg Al per dose), stabilizing agent, preservative (2-phenoxyethanol: 2.5–5 mg/mL); antigen content & pH are stable at -70degC; & vaccine obtained therefrom;
c) Method of treatment/prophylaxis &/or eliciting immune response in mammals by administering IC via various routes (Claims 16–24, 27);
d) Method of preparation & inactivation of bulk (Claims 25–26)

However, WO'683 already discloses method of preparing inactivated EVD68 vaccine by virus propagation in Vero cells, inactivation & purification & formulations thereof, similar to that claimed in WO'642 for SARS-CoV-2. Further, another BBIL application, WO2017009873 (Citation 3) also discloses same/similar methods for preparation (including inactivation protocol) & formulation of vaccine comprising inactivated Zika virus & administration thereof.

Thus, it can be seen that BBIL, the Applicant herein, has merely applied its own strategies disclosed for other inactivated vaccines (EVD68 & Zika virus; Citations 4 & 3, respectively) for preparing a similar inactivated SARS-CoV-2 vaccine.

Also, it may be noted that the SARS-CoV-2 strain NIV-2020-770, used by BBIL in the present Application was known & in the public domain prior to the priority date of WO'642 (Singh et al., May 2020; doi: 10.4103/ijmr.IJMR_1253_20) & that its use in the inactivated vaccine was also disclosed in the Government of India Permission Letter (Citation 1B).

Thus, Claims 1 to 27 of WO'642, lack inventive step.

Citation # 5(Periodical article) (# uploaded documents:2):

Author:	Title of article:	Title of Periodical:	Publication Date:
Rhim, J. S., et al.	Biological Characteristics and Viral Susceptibility of an African Green Monkey Kidney Cell Line (Vero)	Proceedings of the Society for Experimental Biology and Medicine	Nov 1969 (11/1969)
Issue Number of Periodical: Volume 132, Issue 2	Publisher of Periodical: Malden, MA [etc.] Blackwell Science [etc.]	Place of publication: United States	
Page range of article within periodical: 670-678	ISBN:	ISSN: 0037-9727	
DOI: 10.3181/00379727-132-34285			
Most relevant passages or drawings: pp. 670–71, 675–77; Fig. 1; Tables III & IV, V		Relevant to Claims: 1-27	

Brief explanation of relevance:

Rhim, et al. state that Vero cell line, a continuous cell line (CCL) derived from kidney tissue of the African green monkey (Cercopithecus aethiops), was found useful for propagation and assay of the Tacaribe group of arboviruses, & was useful in assay of rubella virus, simian virus 5, & certain adenoviruses (pp.670, LHC; 676, RHC). Studies to determine susceptibility of this cell line to additional viruses were carried out. They report that the Vero cell lines grew fast, in a dense, tightly packed sheet, and had higher plating efficiency than BS-C-1 cells, a continuous kidney cell line derived from the African green monkey (p.671, LHC–RHC).

Rhim, et al. note that the highest titers of the T-antigen for certain adenoviruses were previously obtained in Vero cells incubated at 30 degrees Celsius (degC). They examined development of cytopathic effect (CPE) & infectivity titres in Vero cell cultures incubated at 30 & 37 degC (p. 675,

LHC). They reported that there was not only a striking enhancement of CPE in cultures incubated at 30 degC, but significant more virus was obtained (p.675; Fig. 1 & Table III).

They also report that Vero cells were susceptible to infection with various viruses (pp.675–7, Tables IV & V). They state that Vero cells have been shown to be useful in primary isolation, propagation and assay of rubella virus, and they supported the growth of a number of viruses to high titers (pp .676, RHC–677, LHC). They thus conclude that the Vero cell line provides a tool for diagnostic and research work, and offers advantages of large-scale production of viral agents for vaccines (id).

Further, Matsuyama, et al. [2020; doi: 10.1073/pnas.2002589117, attached as Citation 5A] showed that, although SARS-CoV-2 is isolatable using VeroE6, Huh7, or human airway epithelial cells, an engineered cell line, VeroE6/TMPRSS2, is highly susceptible to SARS-CoV-2 infection, thus suggesting the importance of TMPRSS2 in SARS-CoV-2 infection, and its potential utility in isolating & propagating the virus [p.7001, LHC]. They state that “the amount of SARS-CoV-2 RNAs in the culture supernatants of Vero, Calu-3, and A549 cells 48 hp.i. was low and was measurably higher when VeroE6 cells were used” [p.7001, RHC]. They reported that the viral RNA copies in the culture supernatants of the VeroE6/TMPRSS2 cells were >100 times higher than those of Vero E6 cells, suggesting that it would be possible to isolate higher titer virus using TMPRSS2-overexpressing Vero E6 cells [p.7001 RHC]. They state that “VeroE6/TMPRSS2 cells are easily maintained, suitable for large scale propagation, and now available from the Japanese Collection of Research Bioresources Cell Bank” in Japan [p.7002 LHC].

The present Application, WO’642, claims the use of Vero cell line (African Green Monkey Kidney cells) as cell substrates for SARS-CoV-2 virus culture. The Vero cell lines were propagated by incubating at temperatures between 33–37 degC (Claims 1, 3, 4, 25). The Applicant of the present Application, WO’642, also discloses that Vero cells may be Vero or Vero E6 or Vero TMPRSS2 may be as a substrate for the virus culture (p.13 of WO’642). The Applicant of the present Application, WO’642, discloses that the vaccine composition of the invention is obtained by a process wherein neutralizing antibodies are largely elicited against the spike glycoprotein such as in optimally inactivated virus and refers to optimization of conditions for growth and harvest (pp.13–14, 17).

As reported by Rhim, et al., the use of Vero cell lines is obvious to a person skilled in the art, given the biological characteristics and viral susceptibility of African Green Monkey Kidney Cell Line (Vero), for the culturing of various viruses with an incubation temperature range of 30 & 37 degC and specifically at 30 degC. The Applicant of WO’642 discloses that they also performed optimization studies for conditions of growth of SARS-CoV-2, and prefer the incubation temperature at 33-37 degC (slightly above the 30 degC disclosed in Rhim). In any event, WO2017009873 (WO’873) & WO 2020136683 (WO’683) (Citations 3 & 4 resp.) disclose the optimal temperature for incubation, which is within the range now being claimed in WO’642, and disclose the use of parental Vero CCL81 for propagation of the virus.

Further, it may be noted that despite describing use of the VeroE6 and VeroTMPRSS2 cells as alternate substrates, the Applicant of WO’642 has claimed the use only of the Vero cell. This is likely because the Applicant has used the Vero cells earlier, as shown in the Additional Comments, and/ or the fact that the Vero cell conforms to the Requirements of Biological Substances No.50 recommended by WHO for production of biologicals & qualifies as a host cell for vaccine production (also stated in WO’873, Citation 3)

Thus, Claims 1, 3, 4, 25, and when read with WO’873 & WO’683 (Citations 3 & 4), all claims of WO’ 642, lack inventive step.