Appeal decision

Appeal No. 2018-6610

Appellant Novartis AG

Patent Attorney KAWAGUTI & PARTNERS INTELLECTUAL PROPERTY LAW FIRM

The case of appeal against the examiner's decision of refusal of Japanese Patent Application No. 2015-155818, entitled "Decreasing potential iatrogenic risks associated with influenza vaccines" (the application published on November 19, 2015, Japanese Unexamined Patent Application Publication No. 2015-205929) has resulted in the following appeal decision:

Conclusion

The appeal of the case was groundless.

Reasons

[No. 1] History of the procedures

The present application is based on a new application filed on August 6, 2015 based on a part of Japanese Patent Application No. 2013-30624 filed on February 20, 2013, which was a new application based on a part of Japanese Patent Application No. 2011-244904 filed on November 8, 2011, which was a new application based on a part of new Japanese Patent Application No. 2011-158471 filed on July 19, 2011, which was a new application based on a part of Japanese Patent Application No. 2007-530801 whose international filing date is September 9, 2005 (claim of priority under the Paris Convention was received by the foreign receiving office, the European Patent Office, on September 9, 2004,); on May 15, 2018, an appeal against the examiner's decision of refusal was filed and, at the same time, an amendment was made. Later, the body notified reasons for refusal on August 26, 2019, and a written opinion was submitted on February 26, 2020.

[No. 2] The Invention

Inventions according to Claims 1 to 10 of the present application are specified by matters described in Claims 1 to 10 in the scope of claims in the written amendment dated November 29, 2016, and, among the inventions, the invention according to Claim 1 is as shown below (hereinafter, may sometimes be referred to as "the Invention").

"A process for preparing a vaccine antigen from a culture of a Vero cell line, comprising testing for contamination by porcine circovirus wherein the method is carried out with respect to a culture of virus, a substance extracted from the culture of virus, or a substance derived therefrom."

[No. 3] Reasons for refusal indicated in the notice of reasons for refusal by the body

The outline of reason for refusal 1 indicated in the above-mentioned notice of reasons for refusal by the body dated August 26, 2019 is such that inventions according

to Claims 1 to 10 of the present application could have been easily invented by a person skilled in the art based on the descriptions in Documents 1 and 2 which were obviously distributed before the priority date.

1. LEVANDOWSKI R A, DEVELOPMENTS IN BIOLOGICAL STANDARDIZATION, 1999, vol. 98, pp. 171-175
2. Veterinary Immunology and Immunopathology, 1994, vol. 43, no. 4, pp. 357-371

[No. 4] Judgment by the body

1. Described matters in the Cited Documents

Since both Cited Documents 1 and 2 are written in English language, translations prepared by the body are shown below. The underlines were added by the body.

(1) Described matters in Cited Document 1 and Cited Invention

(i) Matters described in Cited Document 1

(A) "Regulatory perspective in the United States on cell cultures for production of inactivated influenza virus vaccines"
(Page 171, Title)

(B) "Abstract: The United States Code of Federal Regulations requires that all influenza virus vaccines produced for use in the United States adhere to specific regulatory standards, including the demonstration of safety and efficacy. For vaccines produced in cell lines, rigorous characterization for manufacturing is particularly important. Influenza vaccines produced by the passage of viruses in mammalian cell lines will require a careful evaluation to ensure the removal or inactivation of potential adventitious agents."
(Page 171, Abstract)

(C) "The characterization of cells used for manufacturing is outlined in 21 CFR 610. 18, ... According to 21 CFR 610. 18(c), characterization of cell lines ... shall include ... . In addition, ... (iii) testing the cell line for potential microbial agents associated with the cell line must be developed. In particular, there is great concern for the potential for cell lines to support or contain known or suspected human pathogen s."
(Page 173, lines 12 to 22).

(D) "The use of mammalian tissue cultures raises somewhat different questions relating to safety. One concern is that influenza virus vaccines produced by mammal cell cultures could harbor agents similar to human pathogen which might be less likely to replicate in eggs. ... Some of the viruses capable of replication in cell lines currently being considered for use in manufacture of influenza virus vaccines are shown in Table 2." ...
...

Table 2: Virus replication in Vero and MCDK

Cell line Virus replication reported
Vero Alphavirus, arenavirus, Bunyavirus, flavivirus, poliovirus, reovirus, rubella virus, measles virus, simian adenovirus, SV-5, SV-40
MDCK ... "
(Page 173, line 5 from the bottom to Page 174, line 4, Page 174, lower column, Table 2 (ruled lines omitted)).

(ii) Cited Invention

According to described matter (A) in Cited Document 1, Cited Document 1 is a document that relates to cell culturing for manufacturing inactivated influenza virus vaccine in the United States. According to described matter (B), influenza vaccines produced by the passage of viruses in mammalian cell lines will require a careful evaluation to ensure the removal or inactivation of potential adventitious agents, and, according to described matter (C), characterization of cell lines must include information on testing cell lines with respect to potential microbial agents associated with the cell line, and, furthermore, it is mentioned that potentiality that the cell line might carry or comprise a known or suspected human pathogen was looked at with great interest. Finally, according to described matter (D), Vero cell line is a cell line that is intended for use in manufacturing influenza virus vaccine, and some of the viruses that can be replicated in this cell is listed.

Then, taking descriptions in Cited Document 1 together, it is acknowledged that Cited Document 1 discloses the following invention (hereinafter, sometimes referred to as "Cited Invention").

"A method for producing influenza virus by the passage of influenza virus using Vero cell line comprising a step to test the vaccine with respect to potential microbial agents associated with Vero cell line."

(2) Described matters in Cited Document 2

(E) "Production, preliminary characterization, and application of a monoclonal antibody to porcine circovirus"
(Page 357, Title)

(F) "Abstract: Preparation of a monoclonal antibody (mAb)to porcine circovirus is described. Preliminary characterization was carried out on 9 types of mAbs obtained by cell fusions carried out twice, including isotype identification, virus neutralization assay, and indirect immunofluorescence staining patterns obtained after immunostaining to both pig kidney (PK/15/W) persistently infected by porcine circovirus (PCV) and Vero (Vero -PCV) cell line. Significant differences between staining patterns were observed in both cells, which seemed to depend on the state of the passage of Vero-PCV cultures. ... "
(Page 357, Abstract, lines. 1 to 7)

(G) "2.1. Virus
Persistent pig kidney cell line (PK/15/W) persistently infected by PCV was used

for preparing a pool of the virus. ... the cellular solubilized substance was centrifuged at 10000 g for 30 minutes to remove supernatant, and again centrifuged at 70000 g for 40 minutes. Obtained viral pellet was resuspended in a small amount of phosphate buffered saline (pH 7.2) (PBS), and the infectious titer was titrated in circovirus-free PK/15/H cells (PK/15/H). The endpoints were read by indirect immunofluorescence (IIF) (Tischer et al., 1982), and the calculated virus titer was $10^{6.0}TCID_{50}0.1ml^{-1}$.

2.2. Antiserum

Hyperimmune rabbit antiserum to PCV ... was provided by Dr. I. Tischer. This serum had an IIF titer of 1:10000 against acetone-fixed PK/15/W/cell culture"
(Page 358, line 15 from the bottom to Page 359, line 6).

* TCID: Tissue culture infectious dose (Page 357, lower column, column for "Abbreviations")

(H) "2.5.3. IIF staining pattern

Acetone-fixed cultures of PK/15/W cells, PK/15/H cells, and Vero cells persistently infected by PCV (Vero-PCV) were subjected to immunostaining for each mAb using the above IIF procedures. This inoculum was allowed to absorb to the cells at 37°C for 1 hour before the addition of 25 ml MEM-E-G and incubation at 37°C for 48 hours. After incubation, supernatant solution was removed and the cells were further subjected to passage for 3 days and, after that, again subjected to passage. This was indefinitely repeated so that cell stocks are stored in liquid nitrogen at the selected passage levels. Cover-glassed specimens were prepared after the first inoculation and 6th, 15th, and 25th passages. However, these preparations, as well as PK/15/W, PK/15/H cultures were subjected to immunostaining for PCV antigen by IIF using polyclonal rabbit anti-PCV antiserum and selected mAbs" (Page 361, lines 1 to 15).

(I) "Table 1
Preliminary characterization of anti-PCV mAb showing indirect immunofluorescence (IIF) and neutralization (SNT) titers against PCV/PK/15/W

MAb	Isotype	IIF	SNT
1H4 (F99)*	IgG3	5120	<10
4B10 (F99)	IgG1	2560	<10
7B4 (F99)	IgG3	5120	40
1C9 (F99)	IgG2b	10240	<10
2E12 (F99)	IgG1	2560	<10
2B7 (F99)	IgG1	5120	<10
2E1 (F99)	IgG2b	>5120	160
6F6 (F93)	IgG3	1280	<10
2F2 (F93)	IgG1	2560	<10

[1]MAb and cell fusion No."
(Page 363, Table 1).

(J) "Table 2

Results of immunostaining of anti- PCV mAb of PK/15/W cell and three different types of Vero -PCV passage cultures

MAb	Cell cultures							
	PK/15/W		Vero-PCV 6th sub		Vero-PCV 15th sub		Vero-PCF 25th sub	
	Nuc.	Cyto	Nuc.	Cyto	Nuc.	Cyto	Nuc.	Cyto
1H4 (F99)	++	+++	++	+++	++	++	-	-
4B10 (F99)	++	++	++	++	++	++	+	-
7B4 (F99)	++	+	++	+	++	+	-	-
1C9 (F99)	++	+	++	+	++	+	++	++
2E12 (F99)	++	-	++	-	++	-	-	-
2B7 (F99)	++	+++	++	+++	++	+++	++	-
2E1 (F99)	+++	+	+++	+	+++	+	++	++
6F6 (F93)	+	-	+	-	+	-	-	+
2F2 (F93)	+	-	+	-	+	-	-	-

+, small number of positive cells; +++, a large number of positive cells; - no positive cell"
(Page 363, Table 2).

(K) "3.3.2. Neutralization titer

Table 1 shows virus neutralization titer of all stable mAb. ... Only 2 types of mAb exhibited neutralization activity (7B4 and 2E1). The neutralization titer (number of positive cells 90% decreased) was low. None of these mAbs perfectly inhibited PCV replication under the dilution degree below the neutralization titer"
(Page 364, lines 11 to 5 from the bottom).

(L) "3.3.3. IIF staining patterns

The results of IIF staining patterns obtained by using stable mAb and rabbit anti-PCV polyclonal antibody are summarized in Table 2. Generally, staining patterns of cells individually infected by PCV could be classified into 8 different types: (a) Cells exhibiting only dark staining of nucleus; (b) cells exhibiting dark staining of nucleus and cytoplasm; (c) cells exhibiting dark staining of nucleus and discrete pin-shaped staining of cytoplasm; (d) cells exhibiting only pin-shaped staining of cytoplasm; (e) cells exhibiting only staining of large inclusions in cytoplasm; (f) cells exhibiting staining of large inclusions in cytoplasm and dark staining of nucleus; (g) cells exhibiting only staining of large inclusions in cytoplasm; and (h) cells exhibiting only discrete staining of inclusions in nucleus. Infected culture contained one or more of the above staining patterns."
(Page 364, lines 4 to 1 from the bottom; Page 366, lines 1 to 8).

(M) "IIF staining in which, after inoculating PCV to Vero cells, rabbit PCV antibody is used proved existence of a large number of cells that exhibit discrete staining of cytoplasm and sparsely scattered cells that exhibit dark staining of inclusions in cytoplasm. No cells whose nucleus were stained were seen on this occasion but staining of nucleus was observed in several cells in 6th passage of this cell line affected by PCV (Vero-PCV). Immunostaining patterns by rabbit serum observed after incubation of cells from 15th and 25th passages of Vero-PCV were the same as those observed after immunostaining of PK/15/W culture and all cells exhibited discrete pin-shaped staining in cytoplasm and a small number of cells exhibited both dark staining of nucleus and staining of cytoplasm. In a small number of cells, many contents in cytoplasm were deeply stained (Figure 1, (a))"
(Page 366, lines 9 to 19).

(N) "IIF staining using mAb of Vero-PCV culture exhibited larger variety in staining patterns. Staining patterns obtained in cells of 6th and 15th passages for which mAbs was used were similar to those obtained in PK/15/W cell culture. Immunostaining in cells from 25th passage culture exhibited a significant change in these staining patterns. In cases in which mAbs 1H4, 7B4 (F99), or 2F2 (F93) was used, no PCV antigen in these cells was proved. MAbs 1C9 and 2E1 exhibited immunostaining of a pattern similar to that of earlier passage culture level of Vero-PCV cells. MAb 4B10 stained only discrete inclusions of nucleus. MAb 2B7 also stained the nuclear antigen with dark staining pattern. In certain cells of this 25th passage culture of Vero-PCV, mAb 2B5 stained only large single inclusions of cytoplasm (Figure 1(b)).

No immunostaining was observed when any of above-mentioned antigens was inoculated to PK/15/H cell, or mAb 2C3 (negative control) is used on PK/15/W cell or Vero -PCV cell"
(Page 367, line 3 from the bottom to Page 368, line 11).

2 Comparison / Judgment

(1) Comparison

The Invention and the Cited Invention are compared.

"A method for producing influenza virus by the passage of influenza virus using Vero cell line" of Cited Invention corresponds to "A process for preparing a vaccine antigen from a culture of a Vero cell line" of the Invention. In addition, since "influenza vaccine" of Cited Invention is derived from "the passage" product obtained "by the passage of influenza virus using Vero cell line," "a step to test the vaccine with respect to potential microbial agents associated with Vero cell line" of Cited Invention corresponds to the method "is carried out with respect to a culture of virus, a substance extracted from the culture of virus, or a substance derived therefrom," which method comprises "testing for contamination by porcine circovirus" of the Invention.

Then, they coincide with each other in that: they relate to

"A process for preparing a vaccine antigen from a culture of a Vero cell line, comprising testing for contamination by potential microbial agents associated with Vero cell line wherein the method is carried out with respect to a culture of virus, a substance extracted from the culture of virus, or a substance derived therefrom" and differ from each other in that, while "potential microbial agents associated with Vero cell line" causing "contamination" are specified to "porcine circovirus" in the Invention, Cited Invention does not have such specification (hereinafter, referred to as the "different feature").

(2) Judgment

(i) Different Feature

The above-mentioned different feature is examined below.

According to described matters (B) and (C) of Cited Document 1, the test in Cited Invention is a step that contributes for detection of microbial agents for ensuring removal or inactivation of potential microbial agent influenza vaccine that might cause

contamination of Vero cell line used for production of antigen, and it can be understood that any microbial agents that might cause contamination can be the object of detection by the test.

Then, Cited Document 1 exemplifies in Table 2 a part of viruses that can be replicated in Vero cell line as the above-mentioned microbial agents (described matter (D)), but it is obvious that it can be deemed that the test of Cited Invention strongly suggests paying appropriate attention to any microbial agent other than exemplified in Table 2 as the object of detection by the test so long as the microbial agent is such that might cause contamination to Vero cell incubation system.

In addition, according to described matter (E) of Cited Document 2, Cited Document 2 is a document relating to monoclonal antibodies against porcine circovirus (PCV); described matter (F) discloses that Vero (Vero-PCV) cell line persistently infected by PCV was used for preliminary characterization of the monoclonal antibody; described matter (G) discloses that persistent pig kidney cell line PK/15/W that had been persistently infected by PCV was used for preparing a pool of viruses; and described matter (H) discloses that persistently infected Vero cell line was obtained after inoculating PCV into Vero, and that cover-glassed specimens were prepared after the first inoculation and 6th, 15th, and 25th passages. According to the result of IIF staining test of described matters (J) to (N) in which mAb of described matters (I) and (K) and rabbit antiserum (rabbit anti-PCV polyclonal antibody) of described matters (G) and (H) were used, it is disclosed that, in Vero after the first inoculation, a large number of cells that exhibit staining of cytoplasm by IIF staining using rabbit PCV antibody were observed and, in Vero after 6th passage, staining of nucleus was observed in several cells, and, in Vero after 15th and 25th passages, staining of cytoplasm of all cells and dark staining of nucleus and staining of cytoplasm in a small number of cells the same as in above-mentioned PK/15/W cell culture was observed (particularly, described matter (M)), staining patterns obtained in 6th and 15th Vero -PCV passage cultures for which mAbs was used were the same as those obtained for PK/15/W culture, but immunostaining pattern of cells from 25th passage culture had been changed (particularly, described matter (M)).

It can be deemed that a person skilled in the art who reads the test results of described matters (I) to (N) based on described matters (E) to (H) can obviously understand at least 1) and 2) below:

1) PCV involves the risk of infection (contamination) of Vero cells and, in fact, at least at the time of 6th, 15th, and 25th passages, it can persistently infect (or, contaminate); namely, get associated with.
2) Immunostaining patterns for which mAb is used are not uniform and vary depending on the type of the used mAb and the state of the passage of Vero-PCV culture. However, taking into consideration the result of immunostaining for which rabbit anti-PCV antibody is used, since PCV was detected by staining of nucleus of Vero-PCV after 6th passage culturing after infection of Vero cells (Vero-PCV), and, in particular, after 15th and 25th passages, exhibits immunostaining pattern similar to that in porcine PK/15/W cell line persistently infected by PCV for preparing a virus pool, it can be deemed that it can be clearly understood that PCV can be replicated and exist in a Vero cell incubation system at least after multiple times (15 and 25 times) of passages the

same as porcine PK/15/W cell lines persistently infected by PCV for preparing PCV virus pool.

Then, it can be deemed that a person skilled in the art who reads these descriptions in Cited Documents 1 and 2 could have easily conceived and carried out, also in Vero cell line used in production of influenza vaccine according to the method of Cited Invention, to recognize PCV as a "potential" microbial agent "associated with" Vero cell line and make it the target of the test for contamination of Vero cell line of Cited Invention.

(ii) Effects of the Invention

(ii-1) With respect to the Invention, the specification of the present application has the following statement (underlines added by the body).

(a) "[0019]
(Infectious agents that do not grow in eggs, but grow in different mammalian cell lines)
The inventor has identified a variety of pathogens that do not grow in hen eggs, do not grow in MDCK cells, but do grow in Vero cells. Testing for contamination by these pathogens was not necessary for vaccines prepared on the traditional egg substrate and is not necessary for vaccines prepared on MDCK cells, but the inventor has realized that quality control of vaccines grown on Vero cells should include tests for one or more of these pathogens in order to ensure the highest safety standards. The pathogens are as follows:
Metapneumoviruses of the Paramyxoviridae family, such as human metapneumovirus (HMPV).
...
· Porcine circoviruses.
... " ([0019]).

(b) "[0026]
(Testing methods)
Methods for detecting the presence of pathogens in cell cultures and in biopharmaceuticals are routinely available. Methods will generally rely on immunochemical detection (immunoassay, western blot, ELISA, etc.) and/or on nucleic acid detection (hybridization methods, such as Southern blots or slot blots, PCR, etc.). As an alternative, it is possible to test for the presence of a pathogen by conventional cell culture inoculation (i.e., test whether the material leads to production of the contaminating pathogen when cultures under suitable conditions).
...
[0028]
General guidance for detecting a pathogen (e.g. virus) of interest can be found in Reference 14. A number of more specific assays are given in the following paragraph, and the skilled person can readily find or prepare an assay for detecting the presence of any chosen pathogen.
[0029]
Reference 15 discloses a multiplex reverse transcription PCR (RT-PCR) assay, ...

for the detection of nine respiratory tract pathogens ... enterovirus ... Detection of porcine circoviruses in human cell lines by PCR and indirect immune fluorescence assays is disclosed in Reference 28. PCR methods for birnavirus detection are disclosed in references 29 & 30.
[0030]

The detection method of the invention may be performed at any stage(s) during vaccine manufacture, starting from the seed virus and/or the cell substrate and/or the culture medium, through the viral infection and growth stages, through viral harvest, through any viral processing (e.g. splitting and/or surface protein extraction), through vaccine formulation and then to vaccine packaging. Thus, the assay used according to the invention can be performed on the materials used to create the viral culture, on the viral culture itself, and on material extracted and derived from the viral culture. ... " ([0026] to [0030]).

(c) "[Description of Embodiments]
[0052]
(MODES FOR CARRYING OUT THE INVENTION)
(MDCK cells)

The inventor has extensive experience of growing influenza viruses on MDCK cells in serum-free culture for the preparation of vaccines. The inventor has realized that the cells are also suitable hosts for other pathogenic agents, and so the ability of various other pathogens to grow in the same condition was tested (specifically, culture of MDCK 33016, deposited as DSMA ACC2219, in serum-free medium, as disclosed in Reference 2).
...
[0056]
(Vero cells)

Following the testing work on MDCK cells, replication of pathogens in Vero cells was investigated. Vero cells support the growth of pathogens such as: pneumoviruses, such as RSV-A and RSV-B; human metapneumoviruses (HMPV), ... porcine circoviruses; canine parvovirus; and Chlamydia" ([0052] to [0056]).

(ii-2) According to these Descriptions, the specification of the present application discloses that the inventor found out that influenza virus PCV is a pathogen that could be a new risk of contamination with respect of preparation of a vaccine antigen against influenza virus using a Vero cell incubation system that differs from a conventional preparation system for which chicken eggs are used from viewpoints of assessment of safeness and quality and took it as the object of the test for contamination (Description (a)), and newly found out as the ground for assumption that PCV can be the risk that PCV replicates (grows) in Vero cells (Descriptions (a), (c), [0056]).

However, Description (c) [0056] does not have any concrete description on the method used for detecting "growth" of PCV in Vero cells. For example, even if a certain method such as "daily available," "immunochemical detection," and/or "nucleic acid detection" for detecting the existence of PCV exemplified in [0026] of Description (b) and, for example, and PCR and indirect immunofluorescence assay of [0029] is used, it is not concretely shown what degree of "growth" of PCV in the above Vero cells

detected with the method is observed under what conditions for Vero cell incubation, etc.

(ii-3) On the other hand, as examined and indicated in (i), it can be understood that although it is not in a vaccine antigen preparation system, Cited Document 2 has descriptions which prove from the result of the immunostaining test in which indirect immunofluorescence (IIF) (that corresponds to immunochemical detection method exemplified in Description (b) [0026] / [0029] in (ii-1)) is used that:
· PCV is a "potential" virulence factor "associated with Vero" cell line that can persistently infect (or contaminate) Vero cells actually at least for 6 to 25 passages; and
· PCV persistently infected the Vero cells can replicate and exist in the nucleus and/or cytoplasm of the Vero cells for 6 to 25 passages.

(ii-4) Then, the result of "growth" of PCV in the Vero cell incubation system in the specification of the present application mentioned in (ii-1) to (ii-2) cannot be deemed to exceed the scope of matters that can be recognized or surmised from the description in Cited Document 2 mentioned in (ii-3).

In addition, if this is true, it cannot be deemed that the specification of the present application made it obvious that, particularly at the time of preparing vaccine antigen of the Invention, unexpected effect that cannot be conceived from Cited Documents 1 and 2 are delivered by specifying PCV as the test object of contamination of a Vero cell incubation system.

(3) The Appellant's allegation in the written opinion dated February 26, 2020 (hereinafter, sometimes, simply referred to as the "written argument")
(i) Outline of the Appellant's allegation

In the written opinion, the Appellant alleges at great length that the Invention does not contain the reason for refusal 1 in [No. 3] and has an inventive step over Cited Documents 1 (1-1 to 1-6), but, in short, the outline of the allegations is as shown in (a) to (d) below.

(a) "Potentiality" of PCV to be "associated with Vero cell line" in the Invention

In culture of Vero cell line used in preparing vaccine antigen in the Invention, unless it is known that the Vero cell line actually carries or comprises PCV, or, even if such fact does not exist, there is a concrete reason that cause to objectively recall that PCV is carried or contained, it cannot be concluded that there is a potentiality that PCV gets associated with Vero cell line in the site of preparation of vaccine antigen.

In this regard, Cited Documents 1 and 2 do not have such disclosure or suggestion.

(b) Risk of "contamination" in the Invention

In a method for preparing vaccine antigen using culture of a certain cell line, any virus that simply infects or contaminates the cell line but does not "replicate" or "reproduce infectious viral particles " in the cell line will never be deemed to be a potential contamination risk to the final vaccine.

Accordingly, it cannot be deemed that there was a motivation to include PCV in the testing for contamination by porcine circovirus PCV in the method for preparing

vaccine antigen according to the Invention just because of Cited Document 2 that discloses that PCV causes persistent infection of Vero cells, but does not disclose that PCV can grow (replicate) in Vero cells (it is written that PCV does not grow (replicate)).

(c) Cited Document 2 and Reference 1

With respect to above (b), as shown in (c1) and (c2) below, from descriptions in Cited Document 2 and the following Reference 1:

Reference 1: XENOTRANSPLANTATION, (2004) 11 pp. 284-294, it was not publicly known to a person skilled in the art that PCV replicates/grows in Vero cells, but, rather, it was believed that PCV does not replicate or grow in Vero cells.

(c1) Regarding Cited Document 2

Since PCV of high concentration as high as 107TCID50 per 1 mL that cannot exist naturally is forced into Vero cells in Cited Document 2, it seemed that what was detected in Table 2 is PCV that was (physically) introduced at such high concentration and invaded into cells and was distributed and existed in daughter cells even after 25 times of passages.

In addition, Table 2 discloses the result of the test for which 9 types of mAbs were used, and, in case in which a larger part thereof (7 types) was used, frequency of detection decreased in inverse ratio of the number of passages.

Namely, in Cited Document 2, PCV forced into Vero cells was diluted and disappeared as replication and passage of the cells were repeated. This fact clearly shows that PCV did not replicate in Vero cells.

Accordingly, it cannot be concluded from the description in Cited Document 2 that PCV replicates in Vero cells, and it cannot be deemed that the description in Cited Document 2 has a motivation to carry out a test for contamination by PCV.

(c2) Reference 1

Reference 1 that was published (later than Cited Document 2) on April 19, 2004 that is close to the priority date of the present application concluded, as a result of a test in which a newly developed reporter gene assay was used, that "Vero did not support PCV replication" (p. 290, left column, lines 3 to 1 from the bottom).

(d) Effects of the Invention

For the first time in the world, the Invention disclosed that PCV replicates (grows) in Vero cells in the situation as mentioned in (c) (specification of the present application, paragraph [0019], etc.), and reported that PCV could actually be a serious risk of contamination in a process for preparing a vaccine antigen from a culture of a Vero cell line. It can be also understood from descriptions in the specification of the present application that such report is correct, and, in addition, it can be confirmed also from Reference 2 (S. M. Gilliland et al., Biologicals (2012): 40: pp. 270 to 277) submitted together with the list of submitted evidences dated August 21, 2017. Reference 2 discloses that a pediatric vaccine (Rotarix) was obliged to temporarily retreat from the market because of contamination by PCV that replicates in cells.

(ii) Judgment on the allegation

Judgment on the above allegation is made below.

· Regarding (a)

(2) As explained in (i), as far as Cited Document 2 shows that PCV can actually cause persistent infection (contamination) of Vero cells up to at least 6 to 25 times of passages and that PCV can replicate and exist in the cells, if any judgement should be made based on such description in Cited Document 2, it cannot be deemed that it requires any special ingenuity of a person skilled in the art to recognize PCV that could persistently infect (contaminate) the Vero cell line incubation system and replicate as "potential microbial agents associated with Vero cell line" and use it as a test object in a manufacturing method for vaccine of Cited Invention in which the same Vero cell line is used.

· Regarding (b)

With respect to allegation (b), the test in the Invention is carried out against "contamination" by PCV in the Vero cell incubation system as defined in Claim 1, but the above-mentioned Appellant's allegation with respect to "replication/growth" of PCV in Vero cells is not any allegation with respect to "contamination" by PCV.

In addition, it can be also understood from the descriptions ((2), (ii), (ii-1), (b)) in [0030] of the specification of the present application that the "final vaccine" called by the Appellant is not specially excluded from "a culture of virus, a substance extracted from the culture of virus, or a substance derived therefrom" from the objects of the "test" for "contamination" in the Invention.

Thus, right from the beginning, in these points, the allegation in (b) cannot be deemed to be based on the definition by the Invention.

In addition, as examined and indicated in (2), (i), Cited Document 2 shows that PCV actually infects (or contaminates) at least Vero cells, and Cited Document 1 states that characterization of cell line must include sufficient information on the fact that the cell line is tested with respect to potential microbial agents associated with the cell line. Furthermore, since it is disclosed that potentiality that the cell line might carry or comprise a known or suspected human pathogen was looked at with great interest, it can be deemed that a person skilled in the art could have arrived at the Invention from Cited Invention as soon as the person skilled in the art learned from Cited Document 2 that PCV can infect (or contaminate) Vero cells.

In addition, for example, even if "contamination" of the Invention requires "growth (replication)" in Vero cell culture as a precondition, since Cited Document 2 shows that PCV can replicate and exist in the cells for at least 6 to 25 passages after infection in the Vero cell incubation system as explained above, this point does not constitute any special different feature.

· Regarding (c)

Since Cited Document 2 is as explained in 1) below, also allegation (c1) cannot be deemed to be cogent.

1) The difference in the result of immunostaining with mAb in Cited Document 2 as seen in Table 2 is a difference due to the type of the used mAb and the condition of passage culture of Vero-PCV (described matters (F), (L), and (N)).

In addition, Cited Document 2 discloses that, in Vero after the first inoculation, by IIF staining using rabbit PCV antibody, many cells that exhibit staining of cytoplasm were observed; in Vero after 6th passage, staining of nucleus was observed in several cells; in Vero after 15th and 25th passages, the same as in above PK/15/W cell culture, staining of cytoplasm in all cells and dark staining in nucleus and staining of cytoplasm in a small number of cells were observed (particularly, described matter (M)), and that the staining patterns obtained in 6th and 15th Vero-PCV passage culture using mAbs were the same as those obtained in PK/15/W culture, but immunostaining pattern of the cell from 25th passage culture showed certain change (especially, described matter (N)). (Furthermore, it is obvious from the statement at the end of described matter (N) that no immunostaining was observed from use of arbitrary antibody for PK/15/H cells (virus-free cells) and use of mAb 2C3 (negative antibody) that all of rabbit PCV antibody and various mAbs specifically recognize PCV).

Namely, as explained in (2), (i), PCV in Vero-PCV passage culture of Cited Document 2 can be recognized or reasonably surmised to be such that can exists in Vero cells persistently repeating replications for at least 6 to 25 passages, and the Appellant's allegation that data in Table 2 shows that " ... PCV forced into Vero cells was diluted and disappeared as replication and passage of the cells were repeated. This fact clearly shows that PCV did not replicate in Vero cells" cannot be deemed to have any basis for reasonable supposition based on the description in Cited Document 2.

In addition, right from the beginning, Reference 1 is not cited in the reason for refusal in [No. 3], and as explained in 2) to 3) below, it does not influence the description in Cited Document 2 or the judgment by the body mentioned in (2) above, and also allegation (c2) cannot be accepted.

2) Reference 1, page 288, left column, lines 3 to 4 and Figure 3B, Panel 4 show that, when Vero cells were infected by PCV and subjected to IFA (indirect fluorescence assay), fluorescence signals were observed in the cells, and a person skilled in the art who read such description can recognize the potentiality of PCV at least getting associated with or contaminating Vero cells.

3) The result of the test disclosed in Reference 1, page 290, left column, lines 2 to 3 from the bottom is the result of an experiment in which specific plasmid (p. 288, right column, line 8 from the bottom to p. 289, right column, last line) that differs from one in 2) above and one from the test disclosed in Cited Document 2, and it does not directly deny the contents of Cited Document 2; it cannot be deemed that a person skilled in the art considered even after reading Cited Document 2 that PCV cannot replicate/grow in Vero cells just because Reference 1 was known to a person skilled in the art.

· Regarding (d)

(2) As explained in (ii), the result of the "growth" of PCV in Vero cell incubation system in the specification of the present application cannot be deemed to have contents exceeding the scope that is understood or surmised from the description in Cited Document 2, and, if it is so, it cannot be deemed that, especially in preparing vaccine antigen of the Invention, specifying PCV as the object of the test for contamination of Vero cell incubation system cannot be deemed to deliver an unexpected effect that cannot be conceived from Cited Documents 1 and 2.

The disclosure in Reference 2 does not influence the judgment by the body based on the described matters of Cited Documents 1 and 2 mentioned in 1 indicated in (2).

(iii) Thus, none of Appellant's allegations in the written opinion including (a) to (d) of (i) can be accepted.

3. Closing

As explained above, since the invention according to Claim 1 of the present application could have easily invented by a person skilled in the art based on the invention disclosed in Cited Document 1 and matters described in Cited Documents 1 and 2, the invention according to Claim 1 of the present application is not patentable under the provisions of Article 29(2) of the Patent Act and, needless to mention other claims, the present application should be refused.

Therefore, the appeal decision shall be made as described in the conclusion.

April 30, 2020

Chief administrative judge: OKAZAKI, Miho
Administrative judge: OKUBO, Motohiro
Administrative judge: MITSUMOTO, Minako