30 September 2010
EMA/11200/2011
Patient Health Protection

Assessment report for Rotarix

Common name: rotavirus vaccine, live

Procedure number: EMEA/H/C/639/A-20/0024

Assessment Report as adopted by the CHMP with all information of a commercially confidential nature deleted.

7 Westferry Circus ● Canary Wharf ● London E14 4HB ● United Kingdom
Telephone +44 (0)20 7418 8400 **Facsimile** +44 (0)20 75 23 70 51
E-mail info@ema.europa.eu **Website** www.ema.europa.eu

1. Background information on the procedure

The European Medicines Agency (EMA) was made aware on 15 March 2010 by the marketing authorisation holder (MAH) of Rotarix, GlaxoSmithKline Biologicals (GSK Biologicals), of new information regarding the unexpected presence of a non-pathogenic viral strain of porcine circovirus 1 (PCV-1) DNA in its rotavirus vaccine. The detection of PCV-1 was not part of the product specifications routine screening and was done using an analytical detection method (metagenomics analysis, with pyrosequencing) which was not part of the current control method used; this was initially reported in a research paper1.

The matter was discussed at the March 2010 Committee for Medicinal Products for Human Use (CHMP) plenary meeting. On 22 March 2010 the Agency informed the European Commission that further in-depth analysis of this issue were appropriate.

In view of the above the European Commission initiated on 23 March 2010 a procedure under Article 20 of Regulation (EC) No 726/2004. The European Commission requested the CHMP to assess the impact of the above findings on the quality of Rotarix, and to give its opinion on measures necessary to ensure the quality of this product, and on whether the marketing authorisation for this product should be maintained, varied, suspended or withdrawn.

The Vaccine and Biologics Working Parties were consulted for the assessment of this procedure, as appropriate.

2. Scientific discussion

Rotarix (rotavirus vaccine, live) is a live attenuated vaccine indicated for the active immunisation of infants from the age of 6 weeks for prevention of gastro-enteritis due to rotavirus infection; Rotarix is administered orally. Rotarix safety profile has been established based on placebo controlled clinical trials data, including monitoring of non serious and serious adverse events. More than 100 000 subjects have been included in clinical trials, of which over 50 000 subjects received Rotarix in over 20 trials. It is estimated that over 68 million infants have been exposed to this vaccine.

The initial evidence indicated that Rotarix vaccine had been tested positive for PCV-1 DNA. The data suggested that the PCV-1 DNA could also be present in the Vero working cell bank (WCB) and the working virus seed (WSV).
PCV-1 is a small (<20 nm), non-enveloped, single-stranded DNA virus of the Circoviridae family. Mammalian circovirus include only two closely related species (PCV-1 and PCV-2), infecting pigs. PCV type 1 is widespread in swine and the virus has not been linked to any animal or human disease. PCV-1 is highly prevalent in healthy pigs, thus human dietary and respiratory exposure to this virus is likely to be common through pork consumption and/or inhalation of particles from pig faeces in the swine industry.

In order to investigate the unexpected presence of PCV in Rotarix, several experiments were initiated. The evaluation focused mainly on investigating the root cause, determining the nature and the content in PCV-1 DNA or viral particles and their infectivity, identifying in which batches PCV-1 had been detected, and if those had been used for vaccination, seroconversion data was to be provided. Efforts also included establishing plans to remove PCV-1 from the vaccine.

2.1. Assessment of the impact of the PCV-1 finding on Rotarix

2.1.1 PCV-1 detection and potential root cause

PCV-1 presence was investigated in several stages of development, from the routine manufacturing process to preparation of current seed materials. Clinical trial and commercial lots were also tested.

[1] Viral Nucleic Acids in Live-Attenuated Vaccines: Detection of Minority Variants and an Adventitious Virus. Victoria JG, Wang C, Jones MS, Jaing C, McLoughlin K, Gardner S and Delwart EL. *J Virol. 2010 Jun;84(12).* http://jvi.asm.org/cgi/content/short/84/12/6033

The results confirmed that HRV (human rotavirus) strain in parent material was negative but at passages upstreamfrom the HRV Master seed, PCV-1 DNA was detected. All starting materials and clinical lots showed the presence of PCV-1 DNA, which indicates that PCV-1 has been present from early stages of the Rotarix production process.

The origin of the confirmed PCV-1 presence in Rotarix was investigated, and it is likely that PCV was introduced into the manufacturing process during HRV strain passages. Rotarix is manufactured using raw materials of animal origin (e.g., trypsin, gelatin derived amino acids). Based on available data, the source of PCV-1 was most probably the porcine-derived trypsin or animal-derived amino acids used to produce starting materials from which Rotarix vaccine is derived, including the Vero Master Cell Bank (Vero MCB). Current testing guidelines have been followed and the quality of porcine trypsin used today by the MAH in commercial vaccine production is guaranteed by strict sourcing conditions (porcine pancreas from USA and Canada) and a testing program for potential adventitious viruses, bacterial or fungal contaminations. Prior to gamma irradiation, all the porcine trypsin batches are submitted to the specific virological testing. At present, a test to screen each new trypsin lot for PCV will be developed by the MAH. However, it cannot be excluded that the (non-irradiated) porcine trypsin used for the establishment of the Vero MCB (in 1983) contained PCV-1. Therefore, the potential root cause of PCV-1 presence is likely related with the development of the vaccine, leading to PCV-1 material to be present in the vaccine lots produced.

To confirm the identity of the circovirus genetic material detected, sequencing analysis were performed. Results were compared with known sequences of PCV-1, PCV-2, DCV (duck circovirus) and BCV (bovine circovirus). Identity with PCV-1 was demonstrated at all tested steps of the rotavirus production process (Vero WCB, HRV seed, harvest, final container). An alignment of the PCV-1 nucleotidic sequences found in the rotavirus vaccine manufacturing process with the consensus wild type PCV-1 sequence was performed. The results confirmed that PCV-1 DNA was present.

2.1.2 Serology and stool samples

Since the probable root cause pre-dates the establishing of the master cell bank, clinical lots used for phase III clinical trials and commercial vaccine lots from both lyophilised and liquid formulation contain PCV-1 material and have been used for vaccination. Therefore, seroconversion data and stool from vaccinated subjects were analysed.

Blinded retrospective laboratory evaluations to assess serologic response to PCV-1 in infants aged 6 to 12 weeks following administration of Rotarix was performed. Serum samples collected at pre-determined time points (pre-vaccination and post dose 2 or 3) from a subset of subjects who participated in clinical studies with Rotarix were tested. Randomised, blinded, placebo controlled clinical trials was selected in order to be able to differentiate between an immune response due to PCV-1 present in the study vaccine (HRV group) or any potential natural PCV-1 exposure (placebo group). The immune response to PCV-1 was assessed using antibodies against PCV-1.
Data from 4 studies was utilised; an overview is presented below:

Study no (No subjects)	Countries	Population (schedule)	Post-vaccination sampling*
Rota 048[2] (N = 20)	Finland	Healthy infants (2 doses)	Stool: Day 7, 15 Serum: post-dose 2
Rota-039[3] (N = 30)	Thailand	Healthy infants (2 doses)	Stool: Day 7, 15 Serum: post-dose 2
Rota-033[4] (N = 10)	Peru	Healthy infants (2 doses)	Stool: Day 3, 7, 10, 15, 30 and 45 Serum: post-dose 2

2 Rota -048: phase II, double-blind, randomised, placebo controlled study with 4 parallel groups. Subjects received 2 doses of either liquid or lyophilised formulation of HRV vaccine, or the respective placebo formulation, according to a 0, 1 month schedule given to healthy infants previously uninfected with HRV and aged 2 months (6 to 12 weeks) at the time of first vaccination. This study had been previously assessed in the context of a Line Extension.

3 Rota-039: evaluated the immunogenicity, reactogenicity and safety of Rotarix when the vaccine was used in conditions different from the recommendations: i.e. when not reconstituted with buffer or when stored for 7 days at 37°C before administration. In addition, the effect of feeding was explored when the vaccine was reconstituted with and without buffer, though stored at recommended temperature. This study had been previously assessed in the context of a follow-up measure.

Study no (No subjects)	Countries	Population (schedule)	Post-vaccination sampling*
Rota-022[5] (N = 20)	South Africa	HIV positive infants (3 doses)	Stool: Day 7, 14/15, 21/22 Serum: post-dose 3

* All stool samples were collected post-dose 1.

Results showed that:

Study Rota-048
The stool from one infant in the HRV group tested positive for PCV DNA at Day 7 post-vaccination, the earliest time point evaluated in the study, and negative at Day 15. The DNA sequence analysis confirmed that the PCV DNA detected had a sequence identical to the PCV-1 sequence present in Rotarix. One infant in Rota-048 had an inconclusive Q-PCR (quantitative polymerase chain reaction) result at Day 7.

Study Rota-039:
The stool from one infant in the HRV group tested positive for PCV DNA at Day 7 post-vaccination, the earliest time point evaluated in the study, and negative at Day 15. The DNA sequence analysis confirmed that the PCV DNA detected had a sequence identical to the PCV-1 sequence present in Rotarix.

Study Rota-033:
The stools from two infants in the HRV group tested positive for PCV DNA at Day 3. In one of these infants, the sample at Day 7 was inconclusive and in the other infant, the sample at Day 7 was negative. DNA sequence analysis at Day 3 confirmed that the DNA detected had a sequence identical to the PCV-1 sequence present in Rotarix. In the placebo group, one infant had PCV DNA detected by Q-PCR at Day 15. However the DNA sequence analysis was negative and did not confirm PCV-1 sequences. Two other subjects in the placebo group had inconclusive Q-PCR results at Day 15 and 30.

Study Rota-022:
No PCV DNA was detected in the stools of any of the HIV-positive infants who received Rotarix.
None of the infants showed evidence of PCV-1 seroconversion.

A summary of stool PCV-1 DNA and sera anti-PCV-1 antibody results (all studies combined) is presented below:

Study arm	Stools analysis by Q-PCR for PCV DNA								Serology by IPMA for anti-PCV-1 antibody	
	Day 0 N=80 (40V, 40P)	Day 3 N=10 (5V, 5P)	Day 7 N=78 (38V, 40P)	Day 10 N=10 (5V, 5P)	Day 15 N=80 (40V, 40P)	Day 22 N=15 (8V, 6P)	Day 30 N=10 (5V, 5P)	Day 45 N=10 (5V, 5P)	Pre-vac N=80 (40V, 40P)	Post-vac N=80 (40V, 40P)
Rotarix	0	2*	2* (2) #	0	0	0	0	0	0	0
Placebo	0	0	0	0	1# (1) #	0	(1) #	0	0	0

* PCV-1 vac sequence identified. () Inconclusive Q-PCR. #No PCV-1 vac sequence identified.
N: Number of tested stool samples
IPMA: immunoperoxidase monolayer assay

The adverse events observed in the infants (all HRV group) whose stools tested positive for PCV-1 DNA (see below) did not raise any safety concerns and were in line with adverse events reported as listed in the current wording of the summary of product characteristics.

4 Rota-033: a lot-to-lot consistency of three lots of the HRV vaccine in terms of immunogenicity two months after dose 2 was studied. This study had been previously assessed in the context of the initial marketing authorisation.

5 Rota 022: phase II, double blind, randomised (1:1), placebo controlled study to assess the safety, reactogenicity and immunogenicity of three doses of GSK Biologicals' oral live attenuated human rotavirus vaccine administered to human immunodeficiency virus (HIV) infected infants at 6, 10 and 14 weeks of age. This study had been previously assessed in the context of a follow-up measure.

Solicited and unsolicited AEs reported in the Q-PCR positive subjects of the HRV group

Study	Post Dose 1 Solicited AEs						Unsolicited AEs
	Fever	Diarrhoea	Vomiting	Loss of appetite	Irritability	Cough	
Rota 033	Day 3 Causality: not related	Not Reported	Not Reported	Not Reported	Day 6-7 Causality: not related	NA	Bronchitis Onset: Day 8 post dose 2 Duration: 16 days Seriousness: no Causality: not related Outcome: recovered
Rota 033	Day 3 Causality: not related	Not Reported	Day 3-5 Causality: not related	Not Reported	Day 0-7 Causality: Related	NA	Rhynopharyngitis Onset: Day 24 post dose 1 Duration: 11 days Seriousness: no Causality: not related Outcome: recovered Perineal dermatitis Onset: Day 26 post dose 1 Duration: 11 days Seriousness: no Causality: not related Outcome: recovered Rhynopharyngitis Onset: Day 16 post dose 2 Duration: 8 days Seriousness: no Causality: not related Outcome: recovered Bronchospasm Onset: Day 22 post dose 2 Duration: 9 days Seriousness: no Causality: not related Outcome: recovered Rhynopharyngitis Onset: Day 26 post dose 2 Duration: 13 days Seriousness: no Causality: not related Outcome: recovered
Rota 039	Not Reported	Not Reported	Not Reported	Not Reported	Not Reported	Not Reported	Neg
Rota 048	Not Reported	Not Reported	Not Reported	Not Reported	Day7 Causality: not related	Neg	Hemorrhage from left mamilla Onset: Day 27 post dose 2 Duration: 3 days Seriousness: no Causality: not related Outcome: recovered

2.1.3 Special patient groups and medication errors

The Committee considered also data from special patient groups, e.g. immunocompromised infants, and data available from medication errors (parental instead or oral administration).

Clinical trial data of the study including HIV-positive infants (study rota 022, see above) were reviewed as immune suppression could favour the development of opportunistic infections. In this trial 50 subjects received HRV and 50 received placebo. Reactogenicity, safety and immunogenicity results were compared and the incidence of fever, vomiting or diarrhoea within the 15-day post-vaccination period was not significantly different between groups. The reactogenicity and safety profile of the HRV group appeared similar to the placebo group. There were 15 fatal cases (6 subjects in the HRV group and 9 subjects in the placebo group) reported. A review of the solicited, unsolicited and serious adverse events reported, did not suggest any association between Rotarix and a hypothetical human PCV infection among these subjects. In fact, no adverse events were found to be significantly more frequently reported among immunocompromised subjects who received Rotarix vaccine compared to the placebo. The only adverse event for which a numerical significant difference was noteworthy was upper respiratory infections occurring at a frequency of 14.2 % in the Rotarix group and 9.9% in the placebo group. This event is not expected to be related to the presence of Rotavirus or PCV-1 in the vaccine.

A review of the available spontaneous adverse event reports received for infants with a medical history of immune-suppression (three cases) was performed. The review of these cases did not reveal any signals suggestive of hypothetical PCV-1 infection or development of opportunistic infections.

Regarding the accidental parental instead of oral administration of Rotarix, 347 spontaneous reports of accidental occurrences of parenteral administration were reviewed. Among these reports, 100 mentioned an associated adverse event. Ten of these events were considered serious. In the remaining ninety non-serious reports, 161 events were reported. The review of these cases of inadequate administration did not reveal any signals suggestive of hypothetical PCV infection.

2.1.4 Infectivity assays

The number of PCV-1 DNA copies per cell were measured using Q-PCR on the master cell bank MCB, on the working cell bank and at different cell passages before inoculation of the rotavirus in order to understand if and when PCV-1 replication occurred. The results showed that Vero cells at production passage contain 3000 times more PCV DNA copies when compared to the WCB. This data showed that PCV-1 is able to replicate in Vero cells. The load of PCV material measured by Q-PCR in the successive manufacturing steps decreased from the HRV working seed to the single harvest. The results suggest that a replicative activity is present, mainly during cell substrate preparation when the cells are in the exponential phase of growth..

Vero and PK15 cell line studies
Cell culture assays were developed in order to evaluate the presence of PCV-1 replication-competent particles in the Rotarix test samples. A step wise approach was followed based on the availability of the required cells and reagents.

Initially tests to ascertain the presence of competent viral particles included reverse transcriptase (RT) PCR, which was performed to detect the presence of circovirus RNA. The experiment was conducted to verify if DNA sequences of PCV-1 detected in Rotarix could be amplified during the vaccine production process. A Q-RT-PCR assay was used to detect mRNAs in nucleic acid material extracted from both the Vero cell bank and from Rotarix harvest. The results indicated that most of the PCV-1 RT-PCR signal was derived from DNA targets rather than RNA targets.
Experiments were then also developed to provide data on possible presence of PCV-1 viral proteins or viral particles in the different stages of the manufacturing process and in different batches of the final vaccines.

Test samples (Vero cells, neutralised HRV harvest and neutralised final container vaccine) were inoculated into PCV-1 free Vero cells, known as a permissive cell line. Neutralisation was achieved using a mixture of two HRV-specific monoclonal antibodies.
The same test samples were also inoculated into PK15 (porcine kidney) cells. This cell line is also recognised to efficiently support the replication of porcine circovirus.
Vero or PK-15 cells were seeded in flasks and inoculated with test samples.. The flasks were incubated with regular cells passages. At each passage, cells were collected and the presence of PCV-1 transcripts was tested by reverse transcriptase RT-Q-PCR assay.
In addition, Vero cells from the first or second passage were seeded into microplates for immunofluorescence readout., in order to detect the presence of PCV-1

The detection of PCV-1 transcriptional activity and immunostained Vero cells during the infectivity assay suggested that replication of PCV-1 on Vero cells is occurring. Results up to the third passage of the infectivity assay using the RT-Q-PCR readouts are presented below for Vero and PK-15 cells. The assay was stopped after the third passage considering that positive results were consistently demonstrated 9 days post-inoculation and after three cell passages.
At Day 0, a few mRNA transcripts were detected in the positive control (cells incubated for 2 hours with 10^3 $CCID_{50}$[6] from a PCV-1 viral stock). The high threshold cycle (Ct) suggests that the amount of mRNA in the positive control is low. This result is most probably due to the presence of residual mRNA in the inoculum. No transcriptional activity was detected in the negative control or in cells inoculated with the test samples.
Up to the third passage, PCV-1 transcriptional activity was detected in the positive controls and in purified bulk test samples. These results confirm the presence of transcriptional activity in HRV purified bulk.

[6] $CCID_{50}$ corresponds to the cell culture infectious dose 50%

Results of the infectivity assay in Vero cells

Sample	Day post-inoculation	Passage	RT-Q-PCR (Ct value)
Culture medium 10^3 $CCID_{50}$ 10^3 $CCID_{50}$ with anti-HRV antibodies HRV Purified Bulk (1500 dose-equivalent)	D0		Negative Positive (37) NT Negative
Culture medium 10^3 $CCID_{50}$ 10^3 $CCID_{50}$ with anti-HRV antibodies HRV Purified Bulk (1500 dose-equivalent)	D3	P1	Negative Positive (31.2) Positive (32.3) Positive (30.6)
Culture medium 10^3 $CCID_{50}$ 10^3 $CCID_{50}$ with anti-HRV antibodies HRV Purified Bulk (1500 dose-equivalent)	D6	P2	Negative Positive (35.8) Positive (37) Positive (35.9)
Culture medium 10^3 $CCID_{50}$ 10^3 $CCID_{50}$ with anti-HRV antibodies HRV Purified Bulk (1500 dose-equivalent)	D9	P3	Negative Positive (32.1) Positive (31.4) Positive (31.7)

NT, not tested

The number of PCR cycles at which the generated fluorescence passes a fixed threshold above the baseline defines a "Threshold Cycle" (Ct value). A Ct value of 40 will indicate that the tested sample is negative, with no fluorescence detected after 40 PCR cycles i.e. no formation PCR product amplified using the specific primers.

Results of the infectivity assay in PK-15 cells

Sample	Day post-inoculation	Passage	RT-Q-PCR (Ct value)
Culture medium	D0		Negative
10^3 $CCID_{50}$			Positive (35.5)
10^3 $CCID_{50}$ with anti-HRV antibodies			NT
HRV Purified Bulk (1500 dose-equivalent)			Negative
Culture medium	D3	P1	Negative
10^3 $CCID_{50}$			Positive (26.8)
10^3 $CCID_{50}$ with anti-HRV antibodies			Positive (26.8)
HRV Purified Bulk (1500 dose-equivalent)			Positive (28.6)
Culture medium	D6	P2	Negative
10^3 $CCID_{50}$			Positive (26.3)
10^3 $CCID_{50}$ with anti-HRV antibodies			Positive (26.9)
HRV Purified Bulk (1500 dose-equivalent)			Positive (34.9)
Culture medium	D9	P3	Negative
10^3 $CCID_{50}$			Positive (22.7)
10^3 $CCID_{50}$ with anti-HRV antibodies			Positive (22.5)
HRV Purified Bulk (1500 dose-equivalent)			Positive (31.8)

NT, not tested
The number of PCR cycles at which the generated fluorescence passes a fixed threshold above the baseline defines a "Threshold Cycle" (Ct value). A Ct value of 40 will indicate that the tested sample is negative, with no fluorescence detected after 40 PCR cycles i.e. no formation PCR product amplified using the specific primers.

A first evaluation of the PCV-1 titre in HRV harvest samples was performed on Vero cell substrate after immunoperoxidase staining using an anti-PCV-1 pig polyclonal antibody as primary antibody. This first evaluation led to conclude to a titre of maximum 3 CCID50 per final container, considering the titre of 102 CCID50/ml obtained on a pool of HRV harvests and considering the dilution factor which should be applied in order to reach the targeted vaccine dose in the final container vaccine. A second estimate of the PCV-1 titre in purified bulks was deduced from the infectivity assay conducted on Vero and PK-15 cells and using RT-PCR as readout. This estimate was based on the comparable results obtained for the HRV purified bulk sample (1500 dose-equivalent) and the positive PCV-1 control titrated at 1000 CCID50. It is recognised that the read-out assay used in this determination does not allow accurate quantification of the PCV-1 transcripts, however the MAH considered that comparison with the positive control allowed to conclude that the PCV-1 load as expressed in CCID50 and present in the HRV purified bulk test sample was at the same level than the PCV-1 positive control, leading to an estimated virus load of maximum 1 CCID50 per vaccine dose.
In addition to these results, two sets of titration results on HRV purified bulks or HRV vaccine in final containers were also generated and are shown below.

The results obtained on the three purified bulks after titration on Vero cell substrate are presented below.

PCV-1 titres as assessed in HRV purified bulks (test on Vero cells)

Purified bulk	PCV-1 titre ($CCID_{50}$/ml)
AROTAVA068	2.5×10^2
AROTAVA074	5.0×10^2
AROTAVA081	7.9×10^2

From these PCV-1 titres as measured at the purified bulk level, the expected titre that should be detected at the final container stage would range from 8 to 26 CCID50 of PCV-1 per Rotavirus vaccine dose, taking into account a 30-fold dilution which is applied on average at vaccine formulation.

The titre obtained on PK-15 cells for the pool of five HRV purifed bulks was 5.0 x 102 CCID50 per ml, which was in line with the titration results obtained on Vero cells (see below).

PCV-1 titres in HRV final containers as compared to PCV-1 DNA copies

Lot number	PCV-1 titre		PCV-1 DNA copies/dose	Ratio DNA copy number /CCID50
	log10CCID50/dose	CCID50/dose		
AROTA202B	< 0.9	< 8	5.99 x 107	> 7.48 x 106
AROTA206A	2.0	100	3.51 x 107	3.51 x 105
AROTA207A	1.4	25	4.66 x 107	1.86 x 106
AROTA209D	< 0.9	< 8	4.44 x 107	> 5.55 x 106
AROTA212A	< 0.9	< 8	2.04 x 107	> 2.55 x 105
AROTA220A	< 0.9	< 8	1.75 x 107	> 2.18 x 105
AROTA221A	1.6	40	1.60 x 107	4.00 x 105
AROTA221B	1.3	20	2.28 x 107	1.14 x 106
AROTA229A	2.1	126	1.45 x 107	1.15 x 105

Consideration was given to spiking Rotarix final container samples with a well characterized PCV-1 viral stock to determine the putative interference effect that the rotavirus vaccine components may have on detection of PCV-1 particles by the different assays, but stocks of PCV-1 virus with high titre are difficult to obtain even from PK-15 cells. The MAH committed to investigate spiking experiments, and to continue to perform infectivity experiments with the titration assay. The availability of anti-PCV-1 antibodies may be a limiting factor and will also be followed up.

Human cell lines studies

Previous studies demonstrated that although PCV gene expression and replication took place in human cells, the infection is non-productive (*Hattermann et al*, 2004). Therefore, in order to clarify the host range, the infectivity of the PCV-1 material detected in rotavirus vaccine was tested on MRC-5 (human diploid cell line), U937 (human monocytic cell line) and Hep2 cells (transformed human cell line) with testing being done mostly at day 0, day 3, day 6 and day 9 post-inoculation. Referring to the infection studies described by *Hattermann et al*., (2004), PCV-1 replication was observed in Hep2 cells. These cells were therefore used as positive control cell line in the infectivity assay on human cells.

Expected results were obtained on Hep2 cell line, and mRNA transcripts were detected after the first and second passages for both PCV-1 positive control and Rotavirus purified bulk. From the third passage, mRNA transcripts were no longer detected. As already observed in previous assays on permissive cells, at day 0, a few mRNA transcripts are detected in the positive control, suggesting the presence of residual mRNA in the inoculum. No transcriptional activity is detected in the negative control or in Hep2 cells inoculated with the test samples.
Similar results were observed for both MRC-5 and U-937 cell lines, i.e. no mRNA transcript detected in cell lysates up to the second or third cell passage.
In addition, further inoculation of day 3 supernatant from all inoculated human cells also gave negative results using rep' RT-Q-PCR readout, including positive Hep2 cells inoculated with the PCV-1 stock. These results showed that if PCV-1 is able to enter human permissive cells, further inoculation of PK-15 permissive cells with the supernatant from PCV-infected cells fails to transmit the infection, clearly indicating that PCV-1 infection of human cells is non-productive (*Hattermann et al*., 2004).

Electron microscopy studies

Electron microscopy experiments were performed on Vero cells, Rotarix bulk and final vaccine but did not reveal the presence of PCV-1 virions. The difficulties and pre-requisites to conduct these analysis were noted, but the MAH committed to continue to investigate detection by electron microscopy and attempt to detect PCV-1 by this method in final container by combining immunoaggregation and on-grid centrifugation.

2.2. Measures to ensure Rotarix quality

PCV-1 free vaccine
A vaccine should not include any other component than necessary for obtaining the vaccination effect, thus the removal of PCV-1 from the vaccine is deemed necessary. The MAH has initiated steps to develop a PCV-1 free vaccine. Proposed actions included the impact of including clearance chromatographic step(s) and other means to remove PCV-1 particles or the development of a new cell bank and a new viral seed. This is however a very complex development, therefore scientific advice has been requested by the MAH and is presently ongoing.
The MAH's proposals and efforts to attempt to eliminate PCV-1 from Rotarix and to develop a PCV-free manufacturing process were endorsed by CHMP. The MAH will provide an implementation plan and continue to update the Committee on this matter on a regular basis.

2.3. Product Information

The MAH initially proposed to update the SPC qualitative and quantitative composition information to reflect that PCV-1 had been detected, but this proposal was not considered appropriate by the CHMP. However, the quality development in light of these findings and the condition to develop a PCV-1 free vaccine was reflected in annex II.

3. Overall discussion and benefit/risk assessment

New information became available regarding the unexpected presence of DNA sequences of a non-pathogenic viral strain of porcine circovirus 1 (PCV-1) in Rotarix. This DNA had been detected using a new non-specific analytical detection method (metagenomics analysis, with pyrosequencing) which was not part of the approved control method used. PCV-1 is a small (<20 nm), non-enveloped, single-stranded DNA virus, that infects many different mammalian cells but does not cause any visible changes. PCV-1 is not known to be pathogenic for humans or animals.

Subsequent analysis performed by the MAH revealed that PCV-1 has been present in the vaccine since early development, thus clinical batches (as used in clinical trials) as well as all commercial batches tested were positive to PCV-1 DNA. At the time of the marketing authorisation application, and despite fulfilment of all the relevant regulatory requirements in force at this time, PCV-1 DNA had not been detected. It is noted that as technology evolves, more relevant and sensitive methods are successively developed and introduced in order to detect viral sequences that had remained undetected before.

Experiments were performed in several stages of Rotarix manufacturing process. It was noted that the total amount of PCV-1 DNA increased during the preparation of the Vero cell substrate. Further downstream (steps that do not involve dividing Vero cells), the PCV-1 DNA titre decreased in accordance with the dilution factor applied during the manufacturing. These data indicate that PCV-1 DNA replicates in the initial phases of the Rotarix manufacturing process (where dividing Vero cells are involved), but no longer in the subsequent steps (downstream from single harvest). Results from infectivity studies indicate that the majority of the PCV-1 DNA in Rotarix does not represent intact PCV-1 virions. These findings show that PCV-1 genome replication (and possibly also expression of viral PCV-1 proteins) occurs in the Vero cells but that few infective PCV-1 virions are produced during the Rotarix manufacturing process. The results in animal cell lines thus confirmed the presence of a very small amount of viral PCV-1 particles. Additional testing also showed that PCV-1 infection of human cells is non-productive.

Seroconversion data and stool from vaccinated subjects were analysed. No seroconversion was observed in all subjects tested and the presence of PCV-1 in a few subjects' stools was transient disappearing one week after vaccination. When considering immunocompromised infants, as immune suppression could favour the development of opportunistic infections, no signals suggestive of PCV-1 infection or development of opportunistic infections were observed.

Rotarix is indicated for the active immunisation of infants from the age of 6 weeks for prevention of gastro-enteritis due to rotavirus infection; the vaccine is effective in preventing rotavirus infections which are responsible for half a million deaths each year, mostly in developing countries. Its safety

profile has been established based on placebo controlled clinical trials data, including monitoring of non serious and serious adverse events. More than 100 000 subjects have been included in clinical trials, of which over 50 000 subjects received Rotarix in over 20 trials. It is estimated that over 68 million infants have been exposed to this vaccine. There are no signals indicating that the presence of PCV-1 has a negative effect on the efficacy or on the safety of the vaccine.

Therefore, the PCV-1 presence does not raise any safety concerns; it is considered an early historical event at the time of establishment of the vaccine, not detected by analytical methods available at that time. This represents a general case for findings following implementation of more sensitive methods as science evolves, which, upon encountering such results, requires a risk-based approach as to what conclusions to draw, as it has been done with Rotarix. Although the PCV-1 findings do not pose a risk to public health, vaccines should not include any other component than necessary for obtaining the vaccination effect; the MAH is committed to eliminate PCV-1 from Rotarix and to develop a PCV-free vaccine. These efforts were endorsed by the Committee as reflected by the condition included in annex II.

Benefit/risk balance
Taken all the above into account, the benefit risk balance for Rotarix is considered favourable.

4. Overall conclusion

Having considered the overall submitted data provided by the MAH in writing on the quality and in relation to the detection of PCV-1 in Rotarix the CHMP concluded that the benefit risk balance of Rotarix remains positive.

The CHMP also concluded that information regarding the development of a PCV- free vaccine should be set out as a condition and recommended the amendment of annex II.

Therefore the CHMP recommended the variation of the marketing authorisation for which the annex II is set out in the annexes of the opinion.

Follow-up measures undertaken by the marketing authorisation holder

The MAH must ensure that the registration dossier is updated to reflect the technical and scientific progress and information which might influence the benefit/risk of the medicinal product.

In relation to this obligation and as requested by the CHMP, the MAH agreed to submit the follow-up measures as listed below :

Area	Description	Due date
Quality	The MAH will perform infectivity experiments with the titration assay. The MAH will also attempt to detect PCV-1 in Rotarix final container and purified bulk by immuno-electron-microscopy. An update, including a plan and timetable should be provided.	31 December 2010
Quality	The MAH will develop and validate trypsin Q-PCR test and to screen each new trypsin lot for PCV-1 and PCV-2. A timetable for implementation of this testing should also be proposed.	31 December 2010

Quality	The following clarifications should be provided: 1. The limited availability of anti-PCV1-antibodies should be clarified. 2. The MAH should comment on the spiking experiments mixing half of the PCV-1 stock with half of samples from final containers of batches that have undetectable PCV-1 infectivity to render information on the possibility that there were inhibitors of the PCV-1 infectivity in the Rotarix final product.	 1. 31 December 2010 2. 29 October 2010

5. Conclusion and grounds for the recommendation

The Committee considered the procedure under Article 20 of Regulation (EC) No 726/2004 for Rotarix initiated by the European Commission.

The Committee considered all available data submitted by the MAH on the quality and in relation to the detection of PCV-1 in Rotarix.

The Committee concluded that the benefit still outweighs the risks in the currently authorised therapeutic indication for Rotarix.

The Committee concluded that a PCV-1 free vaccine should be developed.

The Committee concluded that the marketing authorisation for Rotarix should contain information regarding the development of a PCV-1 free vaccine and therefore recommended the amendment of annex II.