

**BLA STN#: 125259/0**

**Sponsor:** GlaxoSmithKline Biological

**Product:** Cervarix

**BLA:** Original Submission for Approval

**To:** The File – 125259  
Jerry Weir, DVP  
Helen Gemignani, DVRPA

**From:** Robin Levis, DVP

**Subject:** Product review and approval recommendation.

**Memo Date:** Final draft submitted 9/14/09

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**Recommendation:** I have reviewed the quality information related to HPV L1 VLP production and testing. All data reviewed in the submission, during the pre-licensing inspections and as submitted in response to requests for additional information support the manufacturing process and the quality of the product. Based on this review, I recommend approval of the licensure of Cervarix.

**Review Summary:** This review covers the Chemistry, Manufacturing, and Controls sections of the BLA related to HPV16 and HPV18 L1 protein expression, purification and VLP formation. These were detailed in module 3: Quality in the eCTD submission. This review includes details on the manufacturing process for the bulk drug substance and the final container material and validation of each process, and review of the in process and final container quality control testing, including a review of assay validations. In addition, included here is the review of pre-clinical testing on pharmacodynamics and immunogenicity of the product. I did not review quality information related to the AS04 adjuvant. This was reviewed by Dr. Elizabeth Sutkowski, DVRPA.

This review is organized based on the information in the BLA as supplied in eCTD format (for the most part).

Several information requests for data and additional clarifications were submitted to the sponsor during the review cycle. These were:

1. An IR letter was sent to the sponsor on August 7, 2007 requesting information on the scale of manufacturing, additional data on the characterization of the -(b)(4)- ----- cellular feature, the proposed stability study plan and shelf life determination, and more detailed information on the procedures used for the preparation of the baculovirus inocula and on the production of the purified L1 antigen bulks.

- a. The sponsor responded on September 13, 2007 with all of the requested information. Where applicable, this is detailed in the relevant sections of this review.
  - b. With respect to the scale of manufacturing, the sponsor indicated in the BLA that the monovalent adsorbed bulk lots will range in volume from ----(b)(4)---- and that the commercial scale lots will range in size from ----(b)(4)----. I requested a clarification on what would determine the size of the bulks or commercial scale lots and whether or not the respective processes had been validated for the indicated ranges. The response was that the size of the monovalent adsorbed bulk lots were determined by the -(b)(4)- yield from any one production run. In addition, there is a plan for ----- (b)(4) ----- such that monovalent adsorbed bulks up to --- (b)(4) --- could be generated. Final bulks can be used to fill up to -(b)(4)- product lots and the size for the final commercial scale lots will be determined on the size of the final container batch to be filled. This decision will be made based on market need and supply. The range of monovalent bulk sizes and the formulation of commercial scale lots in this range are acceptable based on the explanation provided by the sponsor.
2. An IR letter was sent to the sponsor on October 1, 2007 formally requesting the submission of information that was reviewed during the pre-licensing inspection. This letter requested additional information on process changes related to filter clogging during the filling operation, validation of column and filter re-use, the most recent version of the lot release protocol, removal of production steps detailed in the submission that were not validated (----- (b)(4) -----  
-----), corrections to errors found in the BLA.
  - a. The sponsor responded on October 19, 2007 and submitted the requested information. One critical issue that was identified during the review of this file and during the pre-licensing inspection was a number of errors in the file related to data transcription. Many of the tables in the file had the incorrect data included. The sponsor was asked to fix the errors that I had identified. In addition, the sponsor was asked to review the submission for further errors. The response included the correction of multiple errors in the file.
  - b. The reviews of all other responses, where appropriate, are included in the body of this review.
3. A complete response letter was sent to the sponsor on December 14, 2007. The only CMC request in this letter was for the submission of updated stability data to support product storage and shelf life.
  - a. This data was submitted to the file on January 15, 2008.
4. An email was sent to the sponsor on September 1, 2009 requesting final study data on the storage times for the working cell bank of ---- (b)(4) ---- cells in -(b)(4)-, for the baculovirus working virus seeds and the baculovirus inocula stored at -(b)(4)-, and the most recent stability data for final container product.

- a. The sponsor responded on September 9, 2009 and submitted the final study data for the cell and virus stocks and the details and data of the final container stability studies. See the relevant sections of this review for details.
5. An email was sent to the sponsor on September 8, 2009 requesting information on the specification for -----(b)(4)----- in the purified bulk antigens. The sponsor responded on September 15, 2009. The response included additional information on the establishment of the current specification for -(b)(4)- ----- . The most important point of the response was that they did not consider this in process test as a critical test that required a specification based on process capability. The validation of the process has shown that -(b)(4)- ----- is consistently removed to a level at or near the level of quantification and therefore the specification is acceptable.
6. An email was sent to the sponsor on September 11, 2009 requesting an update to the post marketing commitments related to product stability. Given the time between the BLA submission and final approval we want to ensure that the post marketing commitments for stability are accurately stated in the approval letter.
  - a. It was determined that the sponsor will submit data on the cumulative stability study to CBER as a post marketing commitment. All other stability studies initiated and described in the BLA have been completed. Final data for all of these studies has been submitted to the file. All data support the requested hold time for product intermediates, shelf life for storage of the monovalent adsorbed bulks and final formulated bulk.

***Note: While stability studies to support the cumulative storage of the product are still ongoing, sufficient data has been presented to the file to support the requested expiration dating time of 36 months. Additional stability data will be submitted to the file when available as a post-marketing commitment.***

**Product Summary:** The Cervarix™ vaccine is composed of recombinant C-terminally truncated HPV-16 L1 and HPV-18 L1 proteins, assembled into virus-like particles (VLPs) and adjuvanted with GlaxoSmithKline Biologicals proprietary adjuvant system AS04. The HPV-16 L1 and HPV-18 L1 proteins constitute the active ingredient of the vaccine and are produced with a recombinant Baculovirus expression system. The AS04 adjuvant is composed of an aluminum salt, Al(OH)<sub>3</sub> and 3-*O*-desacyl-4'-monophosphoryl lipid A, (MPL). The MPL immunostimulant is a detoxified derivative of the lipopolysaccharide of the gram negative bacterium *Salmonella minnesota* R595 strain and is manufactured and supplied by Corixa Corporation, doing business as GSK Biologicals North America, Hamilton, Montana, USA.

One dose of Cervarix™ contains 20µg of HPV-16 L1 and 20µg of HPV-18 L1 proteins adjuvanted with AS04 composed, per human dose, of 500µg of aluminum hydroxide and 50µg of MPL. In the Cervarix™ vaccine, the HPV-16 L1 protein, HPV-18 L1 protein and the MPL immunostimulant are separately adsorbed onto aluminum hydroxide after which the three adsorbed bulks are formulated in an isotonic saline solution.

The vaccine is a preservative-free product available as a 0.5 mL single-dose in 3 mL glass vials (fill volume = (b)(4)-) and as a 0.5 mL single-dose in pre-filled, TIP-LOK® disposable 1.25 mL glass syringes (fill volume = (b)(4)-).

The vaccine should be stored at 2° - 8°C. The proposed shelf life is 3 years and the date of manufacturing starts from the filling date of the vaccine.

**Place of Manufacture:** The vaccine is manufactured at two locations:

GlaxoSmithKline Biologicals SA

Rue de l'Institut 89

1330 Rixensart

Belgium

GlaxoSmithKline Biologicals SA

## Parc de la Noire Epine

Rue Fleming, 20

1300 Wavre

Belgium

**Manufacturing Process Development:** (b)(4)-----

[illegible]

**Table 1      Description of the HPV-16 L1 VLP and HPV-18 L1 VLP antigen production processes used during Phase IIb and Phase III development**

Step	HPV-16 L1 and HPV-18 L1 VLPs antigen production processes		Clinical trial study number	Clinical Phase
	Cell substrates	Scale		
1.	(b)(4)		HPV-001/HPV-007	IIb
2.			HPV-008, HPV-012, HPV-013	III
3.			HPV-012, HPV-014, HPV-016	III
4.			HPV-015, HPV-016	III
			Commercial batches	

**Demonstration of process comparability:** The sponsor conducted a series of experiments to demonstrate the comparability of product during late phase manufacturing development, which includes materials used in the phase 2b, 3 clinical studies, and materials manufactured at the final commercial production scale to demonstrate process consistency. The comparability of materials made using the four manufacturing processes; Process 1, -----(b)(4)-----, Process 2, -----(b)(4)-----, Process 3, -(b)(4)----- and Process 4, -----(b)(4)-----, was assessed by comparing the physico-chemical immunological and stability properties of antigen made using each process. To compare physico-chemical properties the following analysis were conducted:

- Comparison of QV data for release of L1 VLP antigen purified bulks, adsorbed monovalent bulks and HPV final bulk/final container vaccine.
- Comparison of the characterization data for each of the materials listed above.
- Comparison of purity profile with respect to process residuals for antigen purified bulks.

The comparison of QC test results for purified antigen bulks for each manufacturing process included testing for identity, purity, ---(b)(4)--- and antigenic activity. HPV16 or 18 type specific antibody reagents were used for all assays. Results included in the BLA show highly comparable results for antigens produced using each of these processes.

Some modifications in test methodologies occurred over process development. These changes were noted in the presentation of the data and did not affect the comparability analysis.

QC test results on adsorbed monovalent bulks: All results presented for the comparison of QC testing done on adsorbed monovalent bulks for each manufacturing process are with in specification.

*Reviewer comment: It is of interest that the HPV16 adsorbed monovalent bulks made during process 3 and 4, the amount of----- (b)(4)-----*

*------. The values are still well within the specification, but should continue to be monitored over time. -----(b)(5)-----.*

Final container results: There is an extensive discussion of product characterization and comparability, see the relevant section of this review. Results from these studies support the comparability of final container products used in all clinical studies and in the final commercial scale materials.

Comparison of characterization data: Extensive characterization studies were performed on the phase 2b and phase 3 materials to better understand the general properties of the vaccine antigens and how this relates to the safety and efficacy of the product. These studies are detailed in section 3.2.S.3.1 – Elucidation of structure and other characteristics of HPV16 and HPV18 – see review of this section for details of the characterization of the L1 antigen.

This section of the BLA on process development looks at the comparability of the characterization data over the different manufacturing process steps.

Purified bulk characterization was conducted on general properties including; (b)(4)-

------. Review of the data shows that L1 VLP antigens made using each manufacturing process have comparable physico-chemical properties. It should be noted that -----(b)(4)----- between the production of phase 2b and phase 3 materials resulted in a (b)(4)- change from -----(b)(4)----- for HPV 16 antigens and from -----(b)(4)----- for HPV18 antigens. This change does not affect antigen quality as shown by QC testing and stability data.

The adsorbed monovalent bulks were characterized for -----(b)(4)-----

------. There was a similar (b)(4)- change for these adsorbed bulks, as described above. Otherwise all characterization data was comparable between all adsorbed monovalent bulks. HPV16 and HPV18 specific antibody reagents were used in the respective tests. Data for all adsorbed monovalent bulks studied as part of the characterization study were comparable for both HPV16 and 18 for each manufacturing process.

Comparability of Immunological Properties: The immunological properties of the phase 2b and phase 3 clinical materials were compared using the following analyses:

- In vitro interactions with antibodies
  - Antigen activity of L1 VLP by interaction with type specific monoclonal antibodies – (b)(4)- for HPV16 VLPs and (b)(4)- for HPV18 VLPs
  - In vitro relative potency by (b)(4)-

- Binding of antibody in human serum samples from trial participants to L1 antigens
- In vivo activity in mice
  - In vivo relative potency of bivalent vaccine in mice
  - Immunogenicity of bivalent vaccine in mice
- Immune response in humans
  - Comparison of the immunogenicity of bivalent vaccines in humans
    - Data for this analysis is from clinical studies HPV012 and HPV016 where different lots, manufactured using different processes were used to immunize trial participants.

*Reviewer comment: Results from all analyses showed that the L1 VLPs produced using the four manufacturing processes induced similar immune responses in vivo in mice and humans and that the antigen/antibody binding properties measured in vitro are comparable.*

Stability profile: Data on the comparability of stability profiles for L1 VLPs manufactured using each of the four manufacturing processes were included in the BLA. Summaries of stability studies conducted on HPV16 and HPV18 adsorbed monovalent bulks and final containers were also included. Data for each of these studies, to support drug substance and drug product stability, storage times and shelf life were reviewed during the pre-licensing inspection. The most current stability data will be submitted to the BLA for review near the action due date.

*Reviewer comment: The data presented in this section support the high level of comparability of product made using each of the four processes. This is critical data as we agreed to allow the sponsor to use stability data for bulks and final drug product from all four processes to support intermediate hold times, bulk and final container storage conditions, and the establishment of shelf life, including a cumulative stability study that encompasses the longest hold times for each stage of manufacturing.*

Comparability of development processes with commercial product manufacturing process: A second comparability assessment was performed on clinical materials manufactured using process 4 -----(b)(4)----- scale and lots manufactured at this scale to demonstrate consistency of commercial product manufacturing. The studies were designed similarly to the previously reviewed studies and included a comparison of physico-chemical and immunological properties of each type of material.

All data submitted to the BLA comparing materials manufactured at the -----(b)(4)----- scale support the manufacturing consistency and product comparability of lots manufactured for use in the clinical trials to support licensure and for lots manufactured for commercial distribution.

**Drug Substance Manufacturing Summary:** The BLA details the commercial scale manufacturing process for the production of HPV L1 antigens, which are assembled into VLPs. There are two main steps to the bulk product manufacturing process. These include -----(b)(4)-----  
14 pages determined to be non-releasable: (b)(4)

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*Reviewer comment: There are no concerns with any of the animal derived materials used in the manufacturing process for the HPV L1 antigens.*

**Drug Substance Characterization:** Studies on the physico-chemical and immunological properties of the recombinant L1 VLP antigens prepared using the final commercial manufacturing process were performed to characterize the HPV antigens present in Cervarix. The tables below summarize the studies performed to complete the product characterization.



Five (5) pages determined to be non-releasable: (b)(4)

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**Validation of Drug Substance Manufacturing Process:** Section 3.2.S.2.5 details the process validation and evaluation. Validation of the VLP antigen production process has been achieved through:

- The demonstration of process consistency for at least 3 consecutive batches showing compliance with the pre-established quality control standards, as detailed in 3.2.S.4.1.
- The identification and validation of the manufacturing process critical parameters.

Consistency of the process is shown by the analysis of quality data collected during the manufacture of the baculovirus inocula (-(b)(4)- scale) and the VLP antigen purified bulks (-(b)(4)- scale) and of quality data for the release of inocula, L1 (-(b)(4)- and VLP purified bulks. These data were collected and submitted in the BLA for three commercial scale batches for both HPV16 and 1 antigen bulks. Included in this review are tables showing the analyses performed and a summary of the results.

Inoculum production: Process data collected to support inoculum production is summarized in 3.2.S.2.5, Table 1.

Three (3) pages determined to be non-releasable: (b)(4)

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*Reviewer comment: While these validation plans are adequate and well described, there was no data to support the performance of the columns and filters used during L1 purification included in the submission. I reviewed the data available during the pre-licensing inspection. There was interim data available for each of the columns and filters used in the process, but not all final validations were completed. I discussed with the firm that available data needed to be submitted to the BLA to support the validation studies detailed in the submission and that final validation data for the lifetime of each filter or column should be submitted to the BLA when available. An information request letter was sent to the sponsor on 10/01/07 requesting complete data on the validations of the columns and filters.*

The firm responded on 10/19/07. The response included updates to the BLA sections related to the validation of column and filter re-use and lifetime studies. Table 2, Validation status of column and filter lifetime summarizes the current status of the lifetime validation studies. In addition, current data for each of these studies has been included in the BLA.

One (1) page determined to be non-releasable: (b)(4)

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**Control of Drug Substance Manufacturing Process:** Section 3.2.S.2.4 details the controls of critical steps and intermediates. These are defined as the steps involved in inoculum production and the production of the -----(b)(4)-----. Process control testing is classified in two categories. The first are process tests that demonstrate control of the process. These tests are validated and have defined specifications. The second type of testing is monitoring tests for process consistency. These tests may or may not have set specification.

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The submission includes the details of the procedures used to measure -----(b)(4)-----, validation of the assays and the establishment of specification for each test. The specification set for each test are summarized in section 3.2.S.2.4 table 2, in process control tests and related specifications during inocula production and L1 antigen production.

Six (6) pages determined to be non-releasable: (b)(4)

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New reference standards will be made following the validated manufacturing process. The new standards will have to comply with all quality specifications for the release of antigen purified bulks. In addition, the (b)(4)- content of the new reference standard material will be tested in the at least (b)(4)- independent assays, with the mean result being used as the (b)(4)- content of the reference standard. To introduce use of the new reference standard, a comparative assessment between the new reference and the current one will be conducted. This assessment will involve (b)(4)- independent assays using the current (b)(4)- method. The assay internal controls and at least ----(b)(4)---- antigen batch will be tested in this comparative assay. The assays must meet all validity criteria



and the results for the new reference standard must be within 10% of the results for the current standard.

*Reviewer comment: The protocol detailed in the submission for the qualification of new reference standards is acceptable.*

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QC data on batches produced during product development and process validation using in clinical trials and on consistency batches for commercial production process validation are inserted below. All data is conforming and support the quality of the antigen purified bulk lots tested. In addition, data support the consistency of manufacturing.

Three (3) pages determined to be non-releasable: (b)(4)

*Reviewer comment: The control tests performed on the final purified bulk antigen are appropriate, have been well validated and will support the quality of the antigen bulks for use in final product formulation.*

**Purified Bulk Stability:** Stability data to support the -(b)(4)- day hold time for purified antigen bulks includes -----(b)(4)----- purified bulk batches. Stability samples from each of these batches were stored -----(b)(4)----- . The tables below show the tests performed for each bulk under the defined storage times and temperatures.

[ --(b)(4)-- ]

While this table specifies testing for -(b)(4)-, identical testing will be performed on -(b)(4)- bulks using the appropriate type-specific reagents.

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**Drug Product Summary:** The final drug product, Cervarix, contains recombinant C-terminally truncated L1 proteins of HPV Type 16 and Type 18 each assembled separately as virus-like particles (VLP) and produced on ----(b)(4)---- cells. The VLPs are formulated with the GlaxoSmithKline Biologicals (GSK) proprietary AS04 adjuvant system composed of aluminum hydroxide and 3-*O*-desacyl-4'-monophosphoryl lipid A (MPL). Table 1 Composition of the HPV Vaccine summarizes the quantity of each ingredient in the vaccine:

**Table 1          Composition of the HPV vaccine**

Ingredients	Quantity (per 0.5ml dose)	Function
<b>Active ingredients</b>		
HPV-16 L1 VLP	20 µg	Antigen
HPV-18 L1 VLP	20 µg	Antigen
<b>Excipients</b>		
3- <i>O</i> -desacyl-4' monophosphoryl lipid A (MPL)	50 µg	Immunostimulant
Aluminium (hydroxide salt)	500 µg	Adjuvant
Sodium Chloride (NaCl)	4.4 mg (150 mM)	Buffer
Sodium dihydrogen phosphate dihydrate (NaH <sub>2</sub> PO <sub>4</sub> ·2H <sub>2</sub> O)	0.624 mg (8 mM)	Buffer
Water for injection	q.s. ad 0.5 ml	Solvent

**Manufacturing process for final container drug product:** The manufacture of the HPV-16/18 L1 VLP/AS04 vaccine consists of the following steps carried out at -(b)(4)-

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- Labeling and packaging operations.

Production of the HPV vaccine is carried out in aseptic conditions (class (b)(4)- - grade (b)(4)-), except for the preparation of -----(b)(4)----- which is performed in a non-aseptic classified room (class (b)(4)-) but with the final sterilizing filtration taking place in an aseptic room (class (b)(4)- – grade (b)(4)-).

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One (1) page determined to be non-releasable: (b)(4)

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The final vaccine formulation is carried out in an aseptic area at ----- (b)(4) ----- . The final bulk is planned to be stored at ---- (b)(4) ---- until filling for ----- (b)(4) ----- in ----- (b)(4) ----- containers or for ----- (b)(4) ----- tanks.

*Reviewer comment: Data to support the storage conditions for final bulk vaccine are finalized and the data has been submitted to the BLA. Please see the discussion of the cumulative stability study to assess the longest possible storage times for final bulk product.*

• **Filling into glass syringes or glass vials:** The HPV vaccine final bulk is aseptically filled (Laminar flow, Class -(b)(4)-) into -(b)(4)- sterile syringes (type -(b)(4)- glass) or into -(b)(4)- ml colorless washed, -(b)(4)-, depyrogenated, sterilized glass vials (type -(b)(4)-) by an automatic filling/stoppering machine. The HPV vaccine final bulk is kept under ----- (b)(4) ----- during the whole filling operation. After filling, the syringes or the vials are automatically closed with grey --- (b)(4) --- stoppers. Vials are then capped with flip-off caps. Filled syringes or filled vials are visually inspected for particulates, and fill volume. The table below presents the maximum batch size and filling volumes/lot per filling line and container:

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The review of filling operations, vial and syringe preparation and qualification for use, visual inspection of final container products and all labeling and packaging operations will be covered in the review submitted by Rebecca Olin, DMPQ.

**Drug Product Process Validation:** The process validation is achieved through:

- The demonstration of process consistency for at least 3 consecutive batches, all of which must show conformance with the pre-established quality standards for the product. Manufacturing of these batches must also show consistency of the unit-step performances, of the residuals clearance profiles and of the manufacturing yields.
- The identification and validation of the manufacturing process critical parameters, or alternatively, monitoring of the unit step in which critical parameters operate.

- **Process consistency:** The consistency of the HPV vaccine production process is demonstrated through analyses of the process data collected during the



manufacture of -----(b)(4)-----  
----- HPV final container vaccine, as well as  
through the analysis of Quality Control data for the release of -----(b)(4)-----  
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final container vaccines.

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- Final container vaccines - Three consecutive HPV vaccine lots were prepared for commercial consistency demonstration. The analysis of the process data, -----(b)(4)-----, collected during the manufacture of HPV vaccines is consistent between the three batches analyzed. In addition, the analysis of the final container quality control data shows the consistency of the HPV vaccine production process.
- **Critical parameter for final container vaccine production:** The critical process parameters for each of the final steps are either validated or, alternatively, the performance of the production step in which these critical parameters operate, are controlled on each batch.
  - AMB and final container vaccine - Critical process parameters of the HPV-16 L1 VLP and HPV-18 L1 VLP AMB and final container product manufacturing process were identified according to the criteria described above and are summarized in the tables below:

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Stability study data submitted in the BLA support the hold time of ---(b)(4)---  
for the -(b)(4)-. Recent data submitted for review, support the requested hold

time of -----(b)(4)----- for the -(b)(4)-. Stability data submitted to the BLA support a final container shelf life of 36 months, when stored at 2 – 8°C. In addition, data on the storage of the formulated final bulk support a hold time of -----(b)(4)----- containers and -----(b)(4)----- containers at -(b)(4)-. See the section in this review on drug product stability studies.

**Control of final product manufacturing process:** Section m3.2.P.3.4 – Control of critical steps and intermediates describes the process control tests conducted during the production of the final container HPV vaccine as well as the Quality Control testing carried out on the following HPV vaccine production intermediates:

- The HPV-16 L1 VLP and HPV-18 L1 VLP adsorbed monovalent bulks (AMBs)
- The MPL ----(b)(4)----
- The MPL adsorbed bulk

This review will cover the in process controls related to the HPV type-specific adsorbed monovalent bulks. MPL -(b)(4)- and adsorbed bulk will be reviewed by Elizabeth Sutkowski, DVRPA.

The tables below summarize the tests, specifications and results for the adsorbed monovalent bulks manufactured using the commercial manufacturing process, process 4.

[ --(b)(4)-- ]

All tests are within specification and support the quality of the adsorbed bulks. It should be noted that the level of -----(b)(4)-----  
-----; however the results are still within specification. This may indicate a difference in the -----(b)(4)----- properties of the different L1 VLPs.

The analytical procedures used for each of the tests specified above are detailed in the submission. In addition, SOPs and validation reports for each assay are included in the BLA. Summaries of validation studies are included in this section of the BLA. Table 7, shown below, summarizes the validation studies and the parameters included.

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**Validation of in process tests:** The identity test and the -(b)(4)- content ---(b)(4)--- test used on adsorbed monovalent bulks are identical to the tests described for purified bulk antigens. Details on the validation of each of these tests performed on the purified antigen bulks are included in the section of this review on the process control for purified antigen bulks. Similar validation studies for each of these tests done on adsorbed monovalent bulks were conducted and detailed in this section of the BLA. Details of these studies and results are similar to those for the validation studies done on the purified bulk antigens and are therefore not detailed in this section of the BLA.

**Characterization of HPV16 and 18 L1 VLP adsorbed monovalent bulks:** -(b)(4)- adsorbed monovalent bulk lots for each HPV type were characterized for content and general physico-chemical properties. The tables below summarize the characterization tests performed and the results for each HPV type.

One (1) page determined to be non-releasable: (b)(4)

(b)(4)

**Final container batch analysis:** The QC test results on the commercial consistency lots are shown in the tables below. All data on the final formulated bulk and on final container material are conforming to the established specifications. In addition to the data shown below, final container test results for all lots made during product development were included in the BLA. These final container development lots were used in the clinical trials. All data for these lots were also conforming.

Two (2) pages determined to be non-releasable: (b)(4)

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**Control of final formulated bulk and final container product:** The tables below summarize the QC testing performed on the final formulated bulks and on final container materials and what the specifications for each of the tests are.





**Table 1 HPV final bulk vaccine specifications**

Tests	Specifications
Sterility test FTM by [REDACTED]	Absence of growth
Sterility test TSB by [REDACTED]	Absence of growth

**Table 2 HPV final container vaccine specifications**

Tests	Specifications
Description	Turbid liquid after shaking. White deposit and colourless supernatant after sedimentation
Identity HPV-16 L1 VLP [REDACTED]	[REDACTED]
Identity HPV-18 L1 VLP [REDACTED]	[REDACTED]
Sterility test FTM [REDACTED] (at 30-35°C)	Absence of growth
Sterility test TSB [REDACTED] (at 20-25°C)	Absence of growth
General safety - Abnormal toxicity on guinea-pigs*	No weight loss , no abnormal reaction
General safety - Abnormal toxicity on mice*	No weight loss , no abnormal reaction
[REDACTED]	[REDACTED]
Volume	
[REDACTED] content by [REDACTED]	
In vitro potency HPV-16 L1 VLP by [REDACTED]	
In vitro potency HPV-18 L1 VLP by [REDACTED]	
Aluminium content by [REDACTED]	
MPL content by [REDACTED]	
[REDACTED]	

\*Performed on first [REDACTED] additional commercial lots

Test procedures and validations for each of the control tests performed on final container materials were detailed in the BLA. All validations were complete and support the specifications set for each test.

*Reviewer comment: The established final container testing profile is adequate and supports the quality of the final container product for release.*

#### **Validation of QC Tests on Final Container Product:**

The HPV final formulated bulk is tested for sterility according the following compendial methods; ---[REDACTED]---, 21 CFR 610.12, and --[REDACTED]--. No additional validation data is included for these tests.

Final Container Testing Validation: The table below lists those final container tests that are compendial methods. Also included are the appropriate test references for each test. No additional validation data is included in the submission for these tests.

Tests		Analytical reference	
Sterility test FTM by	(b)(4)	(at 30-35°C)	21CFR610.12, (b)(4)
Sterility test TSB by	(b)(4)	(at 20-25°C)	21CFR610.12, (b)(4)
General safety - Abnormal toxicity on guinea-pigs		(b)(4)	21CFR610.11
General safety - Abnormal toxicity on mice		(b)(4)	21CFR610.11
(b)(4)			
Volume			

Table 1, Validated QC tests on final container vaccine: test category and validation parameters, lists the remaining final container tests and the validation parameters used to qualify each test.

**Table 1 Validated QC tests on final container vaccine: test category and validation parameters**

In-house tests	Test category	Validation parameters
Identity of the HPV-16 L1 VLP by (b)(4)	Identification test	Specificity
Identity of the HPV-18 L1 VLP antigen by (b)(4)	Identification test	Specificity
(b)(4) content by (b)(4) assay	Quantitative test	Dose response curve, linearity, repeatability, intermediate precision, specificity, accuracy.
<i>In vitro</i> relative potency HPV-16 L1 VLP by (b)(4)	Quantitative test	Accuracy, precision (intermediate and reproducibility), linearity, range, specificity
<i>In vitro</i> relative potency HPV-18 L1 VLP by (b)(4)	Quantitative test	Accuracy, precision (intermediate and reproducibility), linearity, range, specificity
MPL content by (b)(4)	Quantitative test	Linearity, repeatability, intermediate precision, specificity, accuracy
(b)(4)	Quantitative test	Detection limit, quantitation limit
	Quantitative test	Dose response curve, range, intermediate precision, specificity, accuracy.
	Quantitative test	Dose response curve, range, intermediate precision, specificity, accuracy.
	Quantitative test	Linearity, repeatability, intermediate precision, specificity, accuracy.

The following section of this review summarizes each of these tests and the validation studies to qualify them.

Two (2) pages determined to be non-releasable: (b)(4)

-(b)(4)-

Reviewer comment: Samples of purified bulk material, final container product, and reference materials were submitted to CBER for testing in the HPV L1 antigen -(b)(4)-. Dr. Gennady Rezapkin established these assays at CBER and tested all samples. Results from samples tested at CBER were in agreement with the results on the same materials

tested at GSK. There was some difficulty with one set of HPV 16 L1 bulk samples. We contacted GSK on 12/07/07 to discuss these results. It was not possible to determine the problem with those samples that did not yield results, however there was a problem with the labeling of the vials for these samples and it may be that they were not actually type 16. New HPV 16 samples were submitted and these samples were tested and the results were consistent with the other samples tested. The conclusion from Dr. Rezapkin's studies were that the potency assay is well qualified and appropriate for use in determining the antigen content of the -----(b)(4)----- and the final container product. Dr. Rezapkin has written up the results of this study and submitted them to this file.

(b)(4)

One (1) page determined to be non-releasable: (b)(4)

(b)(4)

Reviewer comment: All validation studies for final container QC assays were well designed. Results for all tests were well within the pre-specified performance criteria supporting the validation of the respective assays. These assays, if performed as defined here will provide confidence in the quality of the final container product.

**Reference Standards for Drug Product Control Testing:** The reference standards used in the tests for -----(b)(4)----- are the same standards used in the - (b)(4)- assay for purified bulk antigen. These standards are described in the section of this review on reference standards for antigen bulk testing.

The reference standard used for the testing of the *in vitro* relative potency test of HPV final containers is a final container lot manufactured using process 2. This final container lot met all quality specifications and was used in the clinical trials for this product. To establish a new reference standard for this assay, the new reference material will be assessed in at least -(b)(4)- independent assays. Comparative assessment of the new and current reference materials will be evaluated through the determination of the *in vitro* relative potency of commercial lots. The calculated difference with both current and new reference materials must not exceed the test variability determined through the method validation.

*Reviewer comment: The methodology described for establishment of new reference standards is acceptable.*

**Stability of** -----(b)(4)----- **Bulk Lots:** The stability of -----(b)(4)-----  
----- used in the preparation of Process 2, 3 and 4 vaccine lots has  
been evaluated by -----(b)(4)-----  
according to the stability plan summarized below.

[  
--(b)(4)--  
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Data presented in the BLA show that lots manufactured using the different manufacturing processes are stable at -----(b)(4)----- . The real time studies conducted on materials made with each manufacturing process, stored at -(b)(4)- are at different stages of completeness.

At the time of the BLA submission, data for process 4 commercial lots was out to -(b)(4)- months for -----(b)(4)-----.

*Reviewer comment: A summary of the most recent stability data available for -(b)(4)- included final data at -(b)(4)- for -----(b)(4)----- . The data are all conforming and support the requested -(b)(4)- shelf life for the -(b)(4)-. In a telecon with the sponsor, September 4, 2009, the sponsor asked whether then can submit data to the BLA --(b)(4)----- . CBER stated that it would be best for this request to be made after the licensure of the product. The sponsor agreed.*

**Stability of Final Container Product:** The HPV vaccine has been shown to be a stable vaccine. The currently available data show that after 36 months storage at 2 - 8°C, there is no evidence of any instability of the vaccine lots tested. All stability-indicating parameters remained similar to the initial values. Given that no indication of vaccine stability loss is evidenced in both accelerated stability studies and long-term stability studies up to 36 months, and based on the comparability of Process 2, 3 and 4 vaccine lots a shelf-life of 36 months at 2 - 8°C is proposed for the HPV commercial vaccines. It should be noted that in addition to data to support the final container shelf life, it will be necessary for the sponsor to provide data to support the cumulative shelf life of product over the entire storage times for adsorbed bulks and final formulated product. See the reviewer comment at the end of this section.

Stability study design: The stability studies to support final container storage and shelf life include testing according to the QC testing for the product and in addition, some of the tests for product characterization are included as part of the stability testing profile. The specifications for the testing of stability samples are the same as proposed for routine release of commercial vaccine lots. The submission includes multiple tables detailing the testing to be conducted for all final formulated bulk materials and for final container presentations, included product in vial or syringes. These studies are all appropriately designed and include all appropriate quality indicating tests. As stated above, data currently available support the storage conditions for each intermediate.

To summarize the design of the stability studies detailed in the submission, listed below are the tests to be performed. The main characteristics followed at every time point up to the end of shelf life are:

- Description
- -(b)(4)-
- -(b)(4)-----
- -(b)(4)-----
- -(b)(4)-----
- HPV-16 L1 VLP content by -(b)(4)-
- HPV-18 L1 VLP content by -(b)(4)-
- Potency HPV-16 L1 VLP by -(b)(4)-
- Potency HPV-18 L1 VLP by -(b)(4)-
- Profile by -----(b)(4)-----
- Profile by -----(b)(4)-----.

Other critical parameters -----b(4)-----:

- Sterility
- Potency HPV-16 L1 VLP ---b(4)---
- Potency HPV-18 L1 VLP ---b(4)---



- -----(b)(4)-----  
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In addition, the following parameters are followed at time points 0, 24, and 36:

- Identity
- Volume
- Abnormal toxicity – General safety on mice and guinea-pigs
- -----(b)(4)-----
- Pyrogenicity on rabbits
- ----(b)(4)---
- Aluminium content by -----(b)(4)-----
- MPL content by -(b)(4)-
- Container closure integrity test after storage for 36 months at +2 - 8°C.

*Reviewer comment: Stability data for the storage of each intermediate and final container are supportive of the stability of the product at each stage of development. In discussion with the sponsor, we made them aware that a cumulative stability study was needed to support the shelf life of the product over the entire storage profile of the product. The requirement for this study was established to verify the quality of the antigen stored at -(b)(4)- at each of the intermediate steps, in addition to the final container storage at 2 – 8°C. This study would include data on -(b)(4)- storage of -----(b)(4)----- of final bulk, and three years of final product. This study is underway and final data will be submitted to the file as a post-marketing commitment.*

*Data submitted for stability for lots manufactured using processes 2, 3, and 4 are included in the submission. All data is conforming to specification. The sponsor has made a commitment to submit stability data for final product stored in both syringes and vials and for data on storage of the final bulk for up to ---(b)(4)--- prior to filling. Given the time between the BLA submission and final approval and to ensure that the post marketing commitments for stability are accurately stated in the approval letter we submitted a request on 9/11/09 asking for clarification of the post marketing commitments related to stability. This review will be updated with this information when it is submitted.*

**Lot Release Protocol and CBER lot testing plan:** The lot release protocols that will be submitted with each product lot for distribution were reviewed with Joe Quander, DMPQ and Rajesh Gupta, DPQ. Initially, the sponsor submitted two separate protocols, one for the release of monovalent bulks and the second for the release of final container product. In discussion with the firm it was determined that each monovalent bulk was used for a -----(b)(4)-----  
----- After review of this combined lot release protocol, we requested several changes be made. Critical changes included the addition of data on the quality testing of the aluminum hydroxide and the addition of a test for ----(b)(4)---- that includes the test for -----(b)(4)----- The sponsor accepted all requested changes to

the protocol and submitted the final protocol for review on September 1, 2009. The sponsor will work with the lot release group in DMPQ to set up the protocol and the submission of materials.

The lot release testing plan was finalized in a meeting between DVP and DPQ, held 6/16/09.

The current testing plan, which was agreed upon by everyone on the call, details the quality testing conducted by the sponsor and makes recommendations of testing to be conducted at CBER. The initial plan included testing to be conducted at CBER on the first three lots after licensure for:

1. Test for Aluminum
2. Test for MPL by Gas Chromatography
3. Potency Test

Additional testing would be conducted as deemed necessary. This includes testing up to (b)(4)- of all lots submitted during the first year of licensure and then re-evaluating the needed testing frequency.

Lot release review and product testing will be conducted by DPQ.

**Pre-clinical studies: Summary:** This section of the product review includes the review of the preclinical studies conducted on the pharmacodynamics of the product and the AS04 adjuvant. Ten studies were described in this section of the BLA, each study is summarized here with the major conclusions highlighted.

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Seven (7) pages determined to be non-releasable: (b)(4)

**Review of clinical assays:** This section of the review includes descriptions and validation data for the critical assays used to assess clinical trial materials. These include several PCR assays that were used to measure HPV infection status for HPV16 and 18 and for a variety of non-vaccine HPV types and the assays used to detect antibodies present in serum to determine base line HPV exposure at trial entry and assays to detect antibodies in serum and cervicovaginal samples in response to immunization with Cervarix. A variety of other assays were detailed in the BLA, these assays were used early in clinical development or as secondary assays to assess some parameters of the immune response. They were all reviewed and all data support the use of the assays for the studies they were intended for.

**PCR ASSAYS:**

**Monograph HPVPCRPCV02** - Performance Characteristics and Validation for HPV detection and genotyping by SPF10 PCR DEIA/LiPA assay and Type-specific PCR for HPV-16 and HPV-18 on cervical and cervico-vaginal specimens: This report summarizes the studies performed to validate the assays used to detect HPV types 16 and 18 in cervical and cervico-vaginal specimens collected during the Phase III vaccine efficacy trials HPV-008 and HPV-009. Results from these assays were used to assess vaccine efficacy against persistent HPV infection or HPV infection in the lesional component in women who are diagnosed with cervical intraepithelial neoplasia (CIN2 and CIN1). These assays are performed by -----(b)(4)-----.

Two procedures will be used to detect and identify HPV during the vaccine efficacy trials. These include a broad spectrum HPV PCR (SPF10 PCR) assay coupled with the HPV genotyping Line Probe Assay (LiPA), and HPV-16 and HPV-18 Type-specific PCRs assays based on unique target regions in each of these HPV genomes. These assays are routinely performed at -(b)(4)- and have been used on the following clinical materials:

- Cervical scrapes - General
- Cervical scrapes in Thin Prep
- Paraffin-embedded biopsy specimens
- Micro-dissected biopsy specimens
- Cervical cells on glass slides

The procedure for these assays is summarized below:

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The assays were validated for the following parameters: specificity, limit of detection, precision, accuracy, robustness, interference, and comparison with a reference. Each of the validation studies were conducted with the following controls:

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- Specificity: The specificity of the primers and probes used in each of these assays was assessed by comparison with known HPV sequences for all HPV types submitted to -(b)(4)-. In addition, several experiments using plasmids and clinical samples were conducted to demonstrate reagent specificity. Summaries of the results from each of these studies is included here:

Data for the primers used in the SPF10, DEIA, LiPA, and type specific PCR assays show a high degree of specificity for HPV sequences. This is most critical for the LiPA assay, which is used for identifying what HPV type was amplified in the SPF10 assay. One concern was that some of the LiPA probes also recognized human DNA sequences. This may lead to interference of HPV detection in cervical samples, but only if the human sequences are also amplified by the SPF10 assay.

Specificity of the primers and probes was also demonstrated by testing on plasmids containing the L1 region from -(b)(4)- HPV types. All results were supportive of the specificity of these reagents, including negative results for genotypes for which probes are not present on the LiPA strips.

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For some clinical samples, a positive result was observed by SPF10 PCR/DEIA, whereas a negative result was obtained by LiPA. -(b)(4)- clinical samples were tested; 4580 were positive by SPF10 PCR/DEIA. Of these, 3574 were positive by LiPA. 859 of the LiPA negative samples were sequenced and the data shows that 495 samples matched low risk HPV sequences, 301 samples did not match any known HPV type and 63 samples gave sequences that were not interpretable.

To demonstrate the specificity for HPV vs. other genital pathogens, the assays were performed on cervical specimens (+/- spiking with HPV 16 or 18) known -----(b)(4)-----  
----- . The results show the presence of other  
pathogens does not interfere with detection of HPV.

The final study to demonstrate the specificity of the reagents used in these assays was a -----(b)(4)-----  
----- and the ability of the assays to detect each type was tested. Results from these assays showed a high level of specificity for the reagents.

*Reviewer comment: Review team discussion on clinical data to support the indication of this vaccine for non-vaccine HPV types, especially type 31 reflected the need for this assay to be well qualified for the analysis of non-vaccine types in clinical samples. This data would be supportive of the ability for these samples to be accurately assessed. No specific data is included in this submission for the detection of HPV type 31, however the data to support the specificity of the assay is reasonably strong, based on the qualification of the LiPA assay for the detection of HPV type 31.*

- Limit of Detection: The limit of detection for the SPF10 PCR/DEIA and the type specific PCR/DEIA assays were assessed for HPV types 16 and 18. These limits were established by using -----(b)(4)-----  
16 and 18 as described above. -----(b)(4)-----  
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Based on the results, the sponsor has set the LOD for these assays at -(b)(4)-copies/PCR reaction for HPV-18 and -(b)(4)- copies/PCR reaction for HPV-16.

- Precision: The precision of the assay is linked to the interpretation of LiPA results by different operators performing the assay between different sample runs in different laboratories. To assess these variables and the outcome of

results -(b)(4)- clinical samples were tested on -(b)(4)- different runs by -(b)(4)- different technicians. Data from this analysis showed 100% agreement on all assessments irrespective of the laboratory, the samples or the technician supporting a high level of precision for this assay with respect to operator performance. A second assay for precision was performed to address the impact of batch to batch variation for the nucleic acid isolation kits. -(b)(4)- different lots were used by one technician to extract DNA from a panel of -(b)(4)- clinical samples. Results from this test showed an unacceptable level of variation between kits. It is not clear why this occurred but the decision was made that before use, each kit must be validated using a panel of reference samples.

- Accuracy: A study was conducted as part of a WHO collaboration to assess the accuracy of these methodologies. GSK participated in this study which was based on a panel of -(b)(4)- samples that include various dilutions of HPV plasmid from different HPV types. All samples were prepared in the background of human DNA to mimic a clinical sample. The results showed that the assays were able to detect either HPV 16 or 18 alone or in the presence of other HPV genotypes, if the HPV 16 or 18 DNA were present at a concentration equal to the LOD. No false positives were identified in the negative control.
- Robustness: Robustness was assessed by testing for the affect of temperature variations on the different PCR assays. Results showed that small variations in the annealing and denaturation temperatures did not affect the PCR results. In addition, small variations in the temperatures of the washes used in the LiPA did not affect results.
- Yield of DNA from -----(b)(4)-----: Tests were conducted to assess the yield of DNA from a range of cell numbers to ensure that sufficient DNA could be extracted from clinical specimens of varying sizes. Satisfactory DNA recovery was obtained from samples containing -----(b)(4)-----, with a decrease in yield of samples containing ---(b)(4)---. These results are satisfactory and in line with the level of cells obtained from cervical specimens.
- Assay interference: The effect of repeated freeze thawing and the presence of large amounts of host cell DNA were tested for their impact on these assays. Data show that up to -----(b)(4)----- on samples stored at -(b)(4)- do not impact the results of these assays; however DNA stability may be impacted by storage of samples at -(b)(4)-. Therefore, DNA samples will be stored at -(b)(4)-. The presence of genomic DNA did not impact the results from this assay.

**Monograph HPVPCRBIOPCV02** details the performance characteristics and validation of HPV detection and genotyping on formalin-fixed, paraffin-embedded biopsy



specimens. Critical in this validation report are studies to assess the sample preparation procedure and whether it is possible to obtain enough tissue from these samples to yield enough DNA to perform the PCR assays. Results from these studies show that the technique employed to extract DNA from these samples is appropriate for using the DNAs in the subsequent PCR assays. Because the DNA samples are prepared in a slightly different way, the LOD was re-determined. Values are similar to those obtained from the previous validation study on cervical specimen. Otherwise, data for these validation studies is very similar to the studies described above.

**Monograph HPV TSPCV01** – Performance characteristics and validation of HPV genotyping by -----(b)(4)----- and LiPA on cervical specimens: This monograph summarizes the data generated to validate the -----(b)(4)----- LiPA assay used to detect and to genotype 9 high-risk HPV types (-----(b)(4)-----) purified from cervical swabs/scrapes and biopsy specimens. The algorithms used to perform these assays are similar to the ones described above for the HPV 16 and 18 detection assays.

The multiplex ---(b)(4)--- LiPA assay comprises the following steps;

(b)(4)

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These studies were done in a similar fashion to those studies described in detail for the SPF10 PCR/DEIA LiPA assay. The results of the qualification studies are summarized below:

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Data included in this report demonstrate that the -----(b)(4)----- LiPA assay is suitable for use to detect and genotype HPV-16, -18, -31, -33, -35, -45, -52, -58 and -59 in cervical specimens purified or extracted from swabs, scrapes, or biopsy samples. This assay can be used in place of the previously used -(b)(4)- 16/18 PCR DEIA.

**ANTIBODY DETECTION ASSAYS:**

**Monographs HPV16EIA2PCV01 and HPV18EIA2PCV01 - Performance** characteristics and validation for anti-HPV16 and 18 (L1Protein) ELISA: An assay to measure antibody levels against HPV types 16 and 18 L1 protein was developed at -----(b)(4)----- early in clinical development of this product. That assay was transferred to GSK and was used for the early clinical studies. Changes to the assay were implemented prior to starting the pivotal phase III clinical studies. These changes improved the quality of this assay to measure pre-existing immune responses in trial participants and to measure immune response to immunization. Data on performance characteristics for the original assay developed at -----(b)(4)----- and for the improved assay developed at GSK were included in the BLA as two separate study reports. As there are only minor differences in the two assays and the original assay is no longer being used to evaluate antibody response, this review will only cover the validation of the current assay used to analyze samples from the pivotal phase III studies.

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Determination of important assay characteristics:

- Limit of Detection (LOD): The limit of detection for each assay was determined by assessing the ELISA titer for each HPV type in 299 women who reported as virgins, therefore there should be no previous exposure to any HPV type. Taking into account some values, which indicated previous exposure to either type, LOD values were determined based on the remaining samples. The values calculated for each HPV type were: LOD for type 16 = (b)(4)- and for type 18 = (b)(4)-.
- Limit of Quantification (LOQ): To determine the LOQ for each type, (b)(4)- samples for HPV 16 and (b)(4)- samples for HPV 18 were prepared by making a series of (b)(4)- fold dilutions (High titer samples were pre-diluted to bring the sample into the range of the reference material for each HPV type.). The titer was determined at each dilution and compared, by percentage, with the titer of the first dilution. The last tier which did not differ by more than (b)(4)-fold of the expected titer was considered the LOQ. Using these results, the LOQ for both HPV type 16 and 18 = (b)(4)-. The technical cut off, which allows for results which are reliably quantifiable, to reduce the risk of false positives was set at (b)(4)- for HPV16 and at (b)(4)- for HPV18.
- Specificity: The specificity of the assay was assed in several studies. The first used the samples described above from woman with no supposed exposure to any HPV type. In this assay, 21 of 299 women were positive for HPV 16 and 13 of 299 women were positive for HPV 18. Additional studies were performed by ----- (b)(4) -----  
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----- Taking these cases into account, the specificity for each assay in this population was determined to be (b)(4)- for HPV16 and (b)(4)- for HPV18. A similar study was performed in the general population. This study included sera from 858 women for HPV16 testing and from 851 women for HPV18 testing. A certain number of samples from each population were positive by the type specific test. In addition, there were positive samples in each population that could not be attributed to either HPV16 or 18. These probably reflect the incidence of infection by

other HPV types, which are being recognized in this assay due to a common epitope on the VLP. This was tested by conducting a type specific inhibition test on sera samples by -----(b)(4)----- with VLPs for types -----(b)(4)----- and using an HPV non specific antigen as a negative control. All sera tested were inhibited by each of the HPV types testing strongly suggesting the presence of a common epitope on all VLPs. Taking this information into account, the specificity of the HPV type 16 and 18 assays in the general population were determined to be -(b)(4)- and -(b)(4)-, respectively. These values demonstrate a high level of specificity for this assay to measure existing antibody levels in trial participants.

A study was on the specificity of these assays to measure type specific responses in persons immunized with the bivalent vaccine was conducted to ensure that these assays would measure the relevant antibody response in the presence of antibodies to both HPV types present in the vaccine. This study was conducted by -----(b)(4)-----  
------. The results for both HPV types show that -----(b)(4)-----

------. These results support the specificity of these assays to measure the immune response in trial participant who received the vaccine.

- Sensitivity: The sensitivity of these assays were determined in the general population and in persons immunized with Cervarix. Sensitivity values were determined for the general population by comparing sera test results from trial participants at the start of the trial based on sexual behavior, cytological examination, and HPV test results on cervical swabs. The prevalence for each HPV type was measured for different clinical groups and was shown to be compatible with the published data, suggesting the sensitivity of this assay in the pre-vaccinated subjects is acceptable. The sensitivity of the assay was shown to be 100% for detecting type specific HPV antibodies in the immunized population. This was shown by comparing results from sera samples taken and time 0, prior to immunization and at times 1 month and 12 month post immunization.
- Comparison with previous ELISA assay: A study was done using sera from naturally infected women and immunized women comparing this assay with the previous ELISA assay used to analyze phase II samples. -(b)(4)- sera samples from non-vaccinated subjects, previously tested with the phase II version of this assay were re-tested with this version of the assay. The concordance between the assays for types 16 and 18 was 83.5% and 93.0%, respectively, with the phase III assay having a greater sensitivity. Re-test of phase II samples on immunized trial participants show that the assays performed similarly except that the titers measured for the current assay for type 16 were slightly lower as compared to the phase II assay and were slightly higher at the 12 month time point for the HPV18 assay. These differences were not significant.

- Precision: Precision for this assay was determined by assessing reproducibility and repeatability. The reproducibility was measured by testing -(b)(4)- samples by -(b)(4)- operators on -(b)(4)- different days. One lot of reagent was used for the first two days and a second lot of reagent was used for the other days. To assess repeatability, each of these assays was done in duplicate. Results from this study show that the variability due to the day and the operator is similar. However, there was a high variability observed between reagent lots. Unfortunately, there was a change in supplier for one of the reagents and the new reagent was not validated for use in this assay prior to initiating this study. Routinely, critical assay reagents are validated for their intended use in clinical assays. The effect of using this new reagent is considered the reason for the high degree of variability seen between reagent lots. Taking this into consideration, the precision of the assay is acceptable.
- Robustness: The critical parameter for robustness for this assay was determined to be the time of -(b)(4)-. Testing was done over a range of times. Results show that ------(b)(4)----- affect the assay outcome and therefore this has been set as an upper limit for this assay.
- Linearity: Linearity is defined as the ability of the process to provide a measurement that is directly proportional to the analyte concentration. For the ELISA, linearity was determined by -----(b)(4)-----  
------. The values were corrected based on the dilution factor and the linearity of the results was quantified based on the variation between the successive two-fold dilutions. For HPV16 the CV was -(b)(4)- and for HPV18 the CV was -(b)(4)-. These values support the linearity of this assay of the range of dilutions tested.
- Recovery: Recovery of the assay was determined by the ability of the assay to measure antibodies in samples after -----(b)(4)----- known to contain differing amounts (------(b)(4)-----) of antibody to either HPV16 or 18. Data show that the recovery was close to 100% for both HPV types.
- Interferences: Inference from sample treatment or sample composition was measured for these assays. The effect of sample freeze/thawing was tested by dividing a samples (-(b)(4)- samples for HPV16 and -(b)(4)- samples for HPV18) into -(b)(4)- aliquots. -(b)(4)-----  
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A second test for interference was conducted to look at the affect of -----(b)(4)----- on the assay results. Samples used for the ELISA assay are not -----(b)(4)----- prior to use, but the sera may be used in other assays where this is required. In the case of retesting of samples in the ELISA, it could potentially be required to use serum samples that have been -----(b)(4)-----.

- o One (1) page determined to be non-releasable: (b)(4)

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**Monograph HPV16-18MUCPCQ01** – Performance characteristics and qualification data for the quantification by ELISA of human anti-PV-16 and HPV-18 (L1 protein) IgG in cervicovaginal secretions: This assay was developed to measure the level of anti-HPV IgG in the cervicovaginal secretions following immunization and to see if these levels correlate with serum antibody levels. The assay used for this is identical to the assay described above with the exception of the sample extraction and preparation techniques. This document presents the validation data for the extraction procedure and the qualification data of the ELISA applicable to measurements on samples extracted from cervical specimens.

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Validation of the extraction procedure: A validation study was performed to demonstrate that the anti-HPV antibodies present in the mucosal secretion are recovered using the extraction protocol and that the different reagents, buffers, and material used during the extraction have no impact on the final antibody titer measured in cervical extracted samples. (b)(4)- process steps were identified for the validation of the protocol. These are:

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For each sample, (b)(4)- dilutions at --- (b)(4) --- were tested in both type specific (b)(4)- assays in parallel on the same day. The results from all of these tests support the use of this extraction technique to assess the anti HPV antibodies present in cervicovaginal secretions.

The next set of studies were conducted to qualify the (b)(4)- procedure in terms of performance characteristics, precision, linearity, and accuracy as applied to test samples extracted from cervical specimens. The results from all of these qualification assays were identical to the results for the qualification of this assay on serum samples except for the performance characteristics of the assay with respect the LOD, LOQ and the analytical range. These test results are summarized here:

- LOD: Set at (b)(4)- and (b)(4)- for anti-HPV16 and anti-HPV18 (b)(4)-, respectively.
- LOQ: Set at (b)(4)- and (b)(4)- for anti-HPV16 and anti-HPV18 (b)(4)-, respectively.
- Analytical range: The analytical range for a (b)(4)- pre-diluted mucosal sample for anti-HPV16 is (b)(4)- and for anti-HPV18 is (b)(4)-.

In addition to the assays detailed here, a variety of secondary assays were used during product development and in the assessment of cross protection. They were all reviewed and all data support the use of the assays for the studies they were intended for.