

**Frederick National Laboratory
for Cancer Research**

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HPV Serology Laboratory
Standard Operating Procedure

SOP Title: HPV Neutralization Assay for Titer Determination

Document ID: HSL_LAB_006

Version

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Released by / Effective Date:

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1. PURPOSE

- 1.1. The purpose of this procedure is to describe the method for the Human Papillomavirus (HPV) Pseudovirus (PsV) Neutralization Assay using SEAP Substrate.

2. SCOPE

- 2.1. This procedure applies to the HPV Serology Laboratory located at the Advanced Technology Research Facility (ATRF), room C2007.
- 2.2. The HPV Neutralization Assay is used to determine the reactivity of human serum to HPV Antibodies of the following types: HPV-6, 11, 16, 18, 31, 33, 45, 52, and 58.

3. REFERENCES

- 3.1. HSL_EQ_001: Biosafety Cabinet (BSC) Use and Maintenance
- 3.2. HSL_EQ_002: Operation, Use and Maintenance of C02 Incubators
- 3.3. HSL_EQ_003: Use and Maintenance of the Thermo Fisher Sorvall Legend XTR Centrifuge in the HPV Serology Laboratory
- 3.4. HSL_EQ_005: Use and Maintenance of a Molecular Devices M5 Plate Reader in the HPV Serology Laboratory
- 3.5. HSL_EQ_006: Use and Maintenance of the Cellometer Auto 2000
- 3.6. HSL_EQ_007: Use and Maintenance of a 2-8°C Refrigerator in the HPV Serology Laboratory
- 3.7. HSL_EQ_008: Use and Maintenance of -80°C Freezers in the HPV Serology Laboratory
- 3.8. HSL_EQ_010: Use and Maintenance of the Fisher Scientific Isotemp GDP10 Water Bath
- 3.9. HSL_EQ_012: Use and Maintenance of Pipettes in the HPV Serology Laboratory
- 3.10. HSL_EQ_016: Use and Maintenance of -20°C Freezers in the HPV Serology Laboratory
- 3.11. HSL_EQ_017: Use and Maintenance of a Laboratory Convection Oven
- 3.12. HSL_EQ_018: Use and Maintenance of an Inverted Microscope
- 3.13. HSL_EQ_020: Use and Maintenance of the Eppendorf Centrifuge

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- 3.14. HSL_EQ_023: Use and Maintenance of a Compact Digital Microplate Shaker
- 3.15. HSL_GL_001: Waste Disposal at the Advanced Technology Research Facility
- 3.16. HSL_GL_006: Reagent Preparation for the HPV Serology Laboratory
- 3.17. HSL_LAB_001: 293TT Cell Culturing and Maintenance

4. RESPONSIBILITIES

- 4.1. The Research Associate, hereafter referred to as analyst, is responsible for reviewing and following this procedure.
- 4.2. The Scientific Manager or designee is responsible for training personnel in this procedure and reviewing associated documentation.
- 4.3. The Quality Assurance Specialist is responsible for quality oversight and approval of this procedure.

5. DEFINITIONS

| Term | Definition |
|-------------|--|
| BPV | Bovine Papillomavirus |
| CV | Coefficient of Variance |
| NB | Neutralization Buffer |
| Plate Skirt | Lower protruding outer edge of 96 well plate |
| PBS | Phosphate Buffer Solution |
| RLU | Relative Light Unit |
| TBD | To Be Determined |

6. REAGENTS, MATERIALS AND EQUIPMENT

- 6.1. Equipment
 - 6.1.1. -20°C Freezer
 - 6.1.2. 2-8°C Refrigerator
 - 6.1.3. CO₂ Incubator
 - 6.1.4. -80°C Freezer
 - 6.1.5. Centrifuges (microcentrifuge and bench top)

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- 6.1.6. Class II Biosafety Cabinet (BSC)
- 6.1.7. Convection Oven
- 6.1.8. Inverted Microscope
- 6.1.9. Microplate Reader (Molecular Devices M5 or equivalent)
- 6.1.10. Microplate Shaker
- 6.1.11. Pipettes (Ranging from 2 μ L to 1000 μ L)
- 6.1.12. Serological Pipettor
- 6.1.13. Timer
- 6.2. Reagents
 - 6.2.1. 0.05% Trypsin-EDTA (Life Technologies, Cat# 25300-54)
 - 6.2.2. 293TT cells (HSL_LAB_001)
 - 6.2.3. BPV, HPV-6, HPV-11, HPV-16, HPV-18, HPV-31, HPV-33, HPV-45, HPV-52, and HPV-58 PsV
 - 6.2.4. Cavicide (Warehouse, Cat # 79300360)
 - 6.2.5. Bleach, Concentrated (Warehouse, Cat # 68100251 or equivalent)
 - 6.2.6. Distilled Water (Life Technologies, Cat # 15-230-001)
 - 6.2.7. Dulbecco's PBS (DPBS) (Life Technologies, Cat# 14190-235)
 - 6.2.8. Great EscAPe SEAP Chemiluminescence Kit (Takara, Cat# 631738)
 - 6.2.9. 293TT Pseudovirion Based Neutralization PBNA_M (PBNA_M) (HSL_GL_006, Section 29)
 - 6.2.10. Ster-ahol (VWR, Cat # 14003-358 or equivalent)
 - 6.2.11. ViaStain™ AO/PI Staining Solutions (Nexcelom, Cat # CS2-0106-5mL)

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6.2.12. Negative Control (Anti-HPV-16 (V5) mouse monoclonal antibody (Gift from John Schiller, NCI), Anti-HPV-18 (5074) rabbit polyclonal antibody (Gift from John Schiller, NCI), or HPV Sero Negative Control)

6.2.13. Positive Control (Heparin (Sigma, Cat # H1784-250MG) or Gardasil 9 Serum Control)

6.3. Consumables

6.3.1. 1.5 mL polypropylene tube (VWR, Cat # 87003-294 or equivalent)

6.3.2. 5 mL Serological Pipette (Warehouse, Cat # 66401365 or equivalent)

6.3.3. 10 mL Serological Pipette (Warehouse, Cat # 66401370 or equivalent)

6.3.4. 25 mL Serological Pipette (Warehouse, Cat # 66401361 or equivalent)

6.3.5. 50 mL Serological Pipette (Warehouse, Cat # 66401363 or equivalent)

6.3.6. Alcohol Resistant Lab Marker (Warehouse, Cat # 66400058)

6.3.7. 50 mL Sterile Conical Tubes (Warehouse, Cat # 66401486 or equivalent)

6.3.8. AlumaSeal CS Foil (Thomas Scientific, Cat # 1230Y24)

6.3.9. Plate Sealers (Thomas Scientific, Cat# 6980A01 or equivalent)

6.3.10. 50 mL Reagent Reservoir (Warehouse, Cat # 66401270)

6.3.11. D96, 1.3 mL Deep Well Plate (Thermo Scientific Nunc, Ca t# 260251 or equivalent)

6.3.12. F96, 96-well Flat Bottom Tissue Culture Plate (Corning Costar, Cat # 3596)

6.3.13. O96, OptiPlate (Perkin Elmer, Cat # 6005290)

6.3.14. R96, 96-well Round Bottom Plate (Corning Costar, Cat # 3788)

6.3.15. V96, 96-well V Bottom Plate (Corning Costar, Cat# 3894)

6.3.16. Pipette Tips

6.3.17. Wet Ice and Ice Bucket

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6.3.18. Wypalls Paper Towel (Warehouse, Cat # 79300335 or equivalent)

7. HEALTH AND SAFETY CONSIDERATIONS

- 7.1. Proper safety precautions should be taken while working in a laboratory setting. This includes, but is not limited to, proper protective equipment such as lab coats, safety glasses, closed-toe shoes, and non-latex gloves.
- 7.2. Refer to the respective Safety Data Sheets (SDS) when working with any chemicals.
- 7.3. Refer to "HSL_GL_001: Waste Disposal at the Advanced Technology Research Facility" regarding waste disposal processes at the ATRF.

8. PROCEDURE PRINCIPLES

- 8.1. All processes in the procedure are performed in the BSC.
- 8.2. The Data Reference number is the Logbook number and Page number. For example, Logbook Reference number (*LAB2017003*) and Page number (*001 for page 1*) are combined for final Data Reference number *LAB2017003001*.
- 8.3. Unused plates in a plate sleeve are sealed with tape before removing from the BSC to maintain plate sterility.
- 8.4. Cell culture is prepared per "HSL_LAB_001: 293TT Cell Culturing and Maintenance" prior to start of assay. The assay requires 3.0×10^5 cells/mL.
- 8.5. Cell culture can be prepared exclusively for use in the Neutralization Assay, or provided from an existing culture.
 - 8.5.1. If using cells prepared exclusively for the Neutralization Assay, perform steps 9.2 to 9.10 at cell harvest and document on "HSL_LAB_006.01: HPV Neutralization Assay, Sample Preparation Form."
 - 8.5.2. If using cells from an existing culture, disregard steps 9.2 to 9.10 of this procedure; these steps are performed and documented as part of the ongoing cell culture maintenance in HSL_LAB_001. The required number of cells are provided at passage. N/A cell passage reagents (i.e., DPBS and Trypsin-EDTA).
- 8.6. Do not use cells if they have been passaged greater than 30 passages, unless approved by Scientific Manager. Record comment on HSL_LAB_006.01.
- 8.7. Do not use cells if they appear to be contaminated.

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8.8. Use alcohol resistant lab markers when labeling plates by hand.

9. DAY ONE: NEUTRALIZATION ASSAY

9.1. Prepare F96 plates as follows for cell addition.

9.1.1. Open F96 plates inside a BSC.

9.1.2. Label the skirt of each F96 plate with Plate Number, Data Reference, HPV Type, Analyst Initials and Date. See Attachment 1.

9.2. Examine each flask under an inverted light microscope per "HSL_EQ_018: Use and Maintenance of an Inverted Microscope" and visually inspect confluency. Cells should be 70-95% confluent.

9.3. Discard media from the flask.

9.3.1. Place the flask upright and tilt it so that media collects into the corner of the side of the flask which does not contain cells to minimize the loss of cells.

9.3.2. Aspirate the media in the flask using a sterile serological pipette and discard into a waste container with bleach.

9.4. Using a sterile serological pipette, add 10 mL of sterile DPBS. Do not dispense DPBS directly onto cells, as it may dislodge the cells. Keep flask upright and dispense DPBS onto side of flask that does not contain cells.

9.5. Gently rinse cells with DPBS by slowly rotating the flask so that DPBS washes over the cells. Rotate flask 3-5 times and then stand flask upright.

9.6. Discard DPBS into the waste container.

9.6.1. Tilt flask so the DPBS collects into a corner of the flask where there are no cells adhering to flask surface.

9.6.2. Aspirate DPBS from the flask using a sterile serological pipette and discard into the waste container.

9.7. Repeat steps 9.4 to 9.6 one time.

9.8. Using a sterile serological pipette, add 3 mL of 0.05% Trypsin-EDTA solution, and gently spread over the cells. Incubate for 5 minutes in a 37°C, 5% CO₂ incubator.

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- 9.9. Ensure the cells have detached by examining under an inverted light microscope per HSL_EQ_018. If cells are not completely detached, gently tap the flask to dislodge the cells.
- 9.10. Using a sterile serological pipette, add 10 mL of PBNA_M into the flask to neutralize Trypsin-EDTA.
- 9.11. Transfer cells to a 50 mL conical tube.
- 9.12. Centrifuge cells at 300 x g for 5 minutes at 20-25°C per “HSL_EQ_003: Use and Maintenance of the Thermo Fisher Sorvall Legend XTR Centrifuge in the HPV Serology Laboratory.”
- 9.13. Using a sterile serological pipette, aspirate the supernatant and discard into the waste container.
- 9.14. Tap on the cell pellet on the bottom of the tube with your hand/fingers to loosen the pellet. Add 20 mL of PBNA_M and re-suspend cell pellet to achieve single-cell suspension.
- 9.15. Mix the sample by pipetting up and down 5 times with a serological pipette.
- 9.16. Count cells per “HSL_EQ_008: Use and Maintenance of the Cellometer Auto 2000” or per Attachment 1 of HSL_LAB_001.
- 9.17. Dilute cells in PBNA_M to a final volume of 3.0×10^5 cells/mL. Prepare at least 12 mL of cell suspension for each plate (100 μ L of cells are needed per well) and record calculations in HSL_LAB_006.01.

Example:

$$C_1 = 10 \times 10^6 \text{ cells/mL (Cell stock concentration)}$$

$$C_2 = 3.0 \times 10^5 \text{ cells/mL (Desired cell Concentration)}$$

$$V_2 = 12 \text{ mL (Total Volume required)}$$

$$V_1 = C_2 V_2 / C_1$$

$$V_1 = (3.0 \times 10^5 \times 12) / (10 \times 10^6)$$

$$V_1 = 360 \mu\text{L}$$

360 μ L of the cell stock would be added to 12 mL PBNA_M

- 9.18. Mix cell suspension by gently rotating or inverting the container with cells, then transfer the cells to a reagent reservoir.

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- 9.19. Using a multichannel pipet, dispense 100 μ L of the cells into all the wells of the F96 Plate(s), mixing cell suspension with pipette every few moments between plates to maintain a homogenous mixture of cells during process.
- 9.20. Place lids on F96 Plates, and incubate plates for 120-360 minutes in a 37°C, 5% CO₂ incubator. Record incubation start time on HSL_LAB_006.01.

10. SAMPLE PREPARATION

- 10.1. Remove serum samples and assay controls from -80°C freezer, and thaw on wet ice inside the BSC. Alternately, samples can be placed in a rack and thawed in a refrigerator at 2-8°C.

Note: Samples may not be kept at 4°C for more than 4 hours.

- 10.2. Once the serum samples are thawed, mix by inversion 5 times or vortex at intermediate speed for 10 seconds. Centrifuge the samples at 10,000 x g for 5 minutes at 4°C per "HSL_EQ_020: Use and Maintenance of the Eppendorf Centrifuge."
- 10.3. Prepare serial dilutions in a D96 plate (see Diagram 1). Up to 4 samples can be run on a single plate.
- 10.3.1. Place D96 in the BSC.
- 10.3.2. Add 486 μ L of PBNA_M to Row A, Columns 1-4 of the D96 plate.
- 10.3.3. Add 375 μ L of PBNA_M to Rows B-H, Columns 1-4 of the D96 plate.
- 10.3.4. Add PBNA_M to Row A, Columns 6 and 8 of the D96 plate to dilute the positive (Pos Ct) and negative controls (Neg Ct) being used to recommended dilution per Attachment 2. Record volume of buffer used on HSL_LAB_006.01.

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Diagram 1: D96 Sample Dilution Plate Layout

| | Column 1 | Column 2 | Column 3 | Column 4 | Column 5 | Column 6 | Column 7 | Column 8 | Column 9 | Column 10 | Column 11 | Column 12 |
|---|------------------|------------------|------------------|------------------|----------|----------------|----------|----------------|----------|-----------|-----------|-----------|
| A | Sample 1 1/10 | Sample 2 1/10 | Sample 3 1/10 | Sample 4 1/10 | | Pos Ct 1/10 | | Neg Ct 1/10 | | | | |
| B | 1/40 | 1/40 | 1/40 | 1/40 | | 1/40 | | 1/40 | | | | |
| C | 1/160 | 1/160 | 1/160 | 1/160 | | 1/160 | | 1/160 | | | | |
| D | 1/640 | 1/640 | 1/640 | 1/640 | | 1/640 | | 1/640 | | | | |
| E | 1/2560 | 1/2560 | 1/2560 | 1/2560 | | 1/2560 | | 1/2560 | | | | |
| F | 1/10240 | 1/10240 | 1/10240 | 1/10240 | | 1/10240 | | 1/10240 | | | | |
| G | 1/40960 | 1/40960 | 1/40960 | 1/40960 | | 1/40960 | | 1/40960 | | | | |
| H | 1/163840 | 1/163840 | 1/163840 | 1/163840 | | 1/163840 | | 1/163840 | | | | |

- 10.4. Add serum samples to Row A, Columns 1-4 per Diagram 1. Use volumes listed in Table 1.
- 10.5. Add positive control to Column 6, and negative control to Column 8, per Diagram 1. Use volumes listed in Table 1.
- 10.6. Record volume of serum and controls added to Row A on HSL_LAB_006.01.
- 10.7. Serial Dilute Samples, positive and negative controls by performing a 1:4 dilution from Row A through H, Columns 1-4, 6, and 8 following Table 1. Mix each dilution at least 10 times using a multichannel pipette, and discard tips between each dilution.

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Table 1: Serial Dilutions of Samples and Controls

| Dilution | Sample Volume (µL) | PBNA_M Volume (µL) | Dilution Factor* |
|----------|---|--------------------|------------------|
| Dil 1 | 54 µL from source vial or prediluted controls | 486 | 1:10 |
| Dil 2 | 125 µL from row A | 375 | 1:40 |
| Dil 3 | 125 µL from row B | 375 | 1:160 |
| Dil 4 | 125 µL from row C | 375 | 1:640 |
| Dil 5 | 125 µL from row D | 375 | 1:2560 |
| Dil 6 | 125 µL from row E | 375 | 1:10240 |
| Dil 7 | 125 µL from row F | 375 | 1:40960 |
| Dil 8 | 125 µL from row G | 375 | 1:163840 |

*Note: Dilution factor does not account for any pre-dilution used for serum or controls.

- 10.8. Cover the plates with clear plate sealer and set aside in the hood on wet ice or place in 2-8°C refrigerator while preparing PsV dilutions.

11. PsV PARTICLE PREPARATION

Note: Ensure the proper study-specific PsV is used. Obtain the Working Dilution (see Attachment 2) prior to starting this experiment.

- 11.1. Prepare approximately 12 mL of Working Dilution of PsV per plate with PBNA_M and mix by gently inverting closed tube until mixture is homogeneous (5-10 times). Store on wet ice or in a refrigerator at 2-8°C until use.
- 11.2. Record the PsV dilution preparation on HSL_LAB_006_01.
- 11.3. Label required number of R96 plate(s) according to 9.1.2. These plates will be used to combine diluted samples and PsV. Refer to Diagram 2 unless alternative plate map is used.

Note: For Negative Control, use the corresponding preparation in Column 10.

Note: Document alternate plate map/dilutions in the "Other" plate map section of HSL_LAB_006.01. Follow similar nomenclature as the plate plan in this procedure (e.g., Pos Ct for Positive Control, etc.).

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Diagram 2: Assay Plate Map

| | Columns 1 & 2 | Columns 3 & 4 | Columns 5 & 6 | Columns 7 & 8 | Column 9 | Column 10 | | Columns 11 & 12 |
|---|-------------------------|-------------------------|-------------------------|-------------------------|-----------------------|---|--|--|
| A | Sample 1 1/10 | Sample 2 1/10 | Sample 3 1/10 | Sample 4 1/10 | Pos Ct 1/10 | Neg Ct V5/5074 1/1000 | Neg Ct HPV Sero 1/10 | No PsV/ No Sera (NS/NV) |
| B | 1/40 | 1/40 | 1/40 | 1/40 | 1/40 | 1/4000 | 1/40 | |
| C | 1/160 | 1/160 | 1/160 | 1/160 | 1/160 | 1/16000 | 1/160 | |
| D | 1/640 | 1/640 | 1/640 | 1/640 | 1/640 | 1/64000 | 1/640 | |
| E | 1/2560 | 1/2560 | 1/2560 | 1/2560 | 1/2560 | 1/256000 | 1/2560 | PsV + NB (PsV/NB) |
| F | 1/10240 | 1/10240 | 1/10240 | 1/10240 | 1/10240 | 1/1024000 | 1/10240 | |
| G | 1/40960 | 1/40960 | 1/40960 | 1/40960 | 1/40960 | 1/4096000 | 1/40960 | |
| H | 1/163840 | 1/163840 | 1/163840 | 1/163840 | 1/163840 | 1/16384000 | 1/163840 | |

- 11.4. Using a multichannel pipette, transfer 25 μ L/well of the serially diluted samples from D96 plate into the corresponding columns and rows of the labelled R96 plate.
 - 11.4.1. If using an electronic pipette, use multi-dispense option to dispense in duplicate.
 - 11.4.2. Dispense by columns, one sample at a time.
 - 11.4.3. Change pipette tips for each sample transfer.
- 11.5. Add 25 μ L/well of PBNA_M to Columns 11 and 12, Rows A-H for “No PsV/No Sera” and “PsV + NB” Controls.
- 11.6. Add 100 μ L/well of PBNA_M to Columns 11 and 12, Rows A-D (“No PsV/No Sera Control”).
- 11.7. Pour the Working Dilution of PsV solution from step 11.1 into a reagent reservoir at room temperature.
- 11.8. Using a multichannel pipette, dispense 100 μ L/well of the Working Dilution of PsV into the R96 plate, Column 11 and 12, Rows E-H. Do not touch the tips to the plate/liquid.

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- 11.9. Using a multichannel pipette, dispense 100 μ L/well of the Working Dilution of PsV into the R96 plate, Columns 1 to 10, Rows H-A, starting with H (lowest concentration) then moving to A (highest concentration). DO NOT touch the liquid (samples) in the plate. If pipette tips touch the liquid in the plate, discard tips and subsequently any volume still in tips, and apply new.
- 11.10. Cover the R96 plates with plate lid and incubate for 60 \pm 20 minutes at 2-8°C. Record the start time on HSL_LAB_006.01.

12. SAMPLE ADDITION TO CELLS

- 12.1. After the incubation of the PsV+Samples (step 11.10), take out the F96 plates containing 293TT cells prepared in Section 9 and the R96 plates from Section 11 and place the plates in the BSC.
- 12.2. Using a multichannel pipette, transfer 100 μ L/well of the sample/PsV solutions to the F96 plates that contain 293TT cells gently, to not disturb the cells. Reference plate map diagram (Diagram 2 or alternate) to ensure samples are added to the correct wells.
- Note:** Dispense speed 4 setting on a Rainin electronic pipette is recommended.
- 12.3. Incubate the Assay Plates in a 37°C, 5% CO₂ incubator for 70-74 hours.
- 12.4. Record start date and time of 3-day incubation on HSL_LAB_006.01. The time will start when the final plate is placed into the 37°C, 5% CO₂ incubator.

13. DAY 2: HARVEST

- 13.1. Label one V96 plate and one R96 plate per Assay Plate before starting the harvest with the corresponding unique identifier that is on the Assay Plates, and analyst initials and date.
- 13.2. Remove the Assay Plates from the 37°C, 5% CO₂ incubator. Record incubation end date and time on HSL_LAB_006.01.
- 13.3. Check and record cell confluency on the batch by visually scanning a subsection of each plate on an inverted microscope per HSL_EQ_018. Record results on HSL_LAB_006.01.
- Note:** If the confluency of the Assay Plates on the Harvest Day is less than 75% for about ¼ of the plate or more, consult with Scientific Manager if plate(s) may be harvested or discarded, and record a comment on HSL_LAB_006.01.
- 13.4. Using a multichannel pipette, transfer 100 μ L/well of supernatant from the Assay Plate to corresponding wells of the V96 plate.

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- 13.5. Place the lid from the Assay Plate onto the V96 plate.
- 13.6. Cover Assay Plates and store in the BSC until harvesting is complete. Discard in autoclave bags at end of harvest.
- 13.7. Centrifuge the V96 plates at 300 x g for 5 minutes at 20-25°C per HSL_EQ_003 to pellet down cells that might have carried over from pipetting.
- 13.8. Remove the V96 plates from the centrifuge and place in the BSC. Gently transfer 80 µL/well of the supernatants to the labeled R96 plate from step 13.1.
- 13.9. Transfer the lid from the V96 plate to the R96 plate.
- 13.10. Cover V96 plates and store in the BSC until harvesting is complete. Discard in autoclave bags at end of harvest.
- 13.11. Seal R96 plates with adhesive foil seal and place the lids with the plate map diagram onto the plates. Store in -20°C freezer for at least 12 hours. R96 plates must go through one freeze/thaw cycle prior to use. Record start date and time on HSL_LAB_006.01. The time will start when the final plate is placed into the -20°C freezer.

14. DAY 3: SEAP SUBSTRATE DEVELOPMENT

- 14.1. Turn on the convection oven and set it to 68°C per “HSL_EQ_017: Use and Maintenance of a Laboratory Convection Oven.”
- 14.2. While oven is reaching designated temperature, label one O96 plate per R96 plate (from step 13.11) with the corresponding unique identifier that is on the R96 plate, and analyst initials and date.
- 14.3. Remove bottle(s) of SEAP Substrate from the -20°C freezer and bring to room temperature before using. Approximately 12 mL SEAP Substrate is needed per plate.
- 14.4. Prepare approximately 8 mL 1X Buffer per plate by making a 1:5 dilution using 5X Buffer and Distilled Water. Record dilution on “HSL_LAB_006.02: HPV Neutralization Assay, Substrate Development Form.”
- 14.5. Remove the R96 plates containing supernatants from the -20°C freezer, and thaw at room temperature. Record removal date and time on HSL_LAB_006.02.
- 14.6. Once thawed, mix for 1 minute at room temperature on microplate shaker at 300-400 rpm per “HSL_EQ_023: Use and Maintenance of a Compact Digital Microplate Shaker.”
- 14.7. Centrifuge plates at 1700 x g for 5 minutes at 20-25°C per HSL_EQ_003.

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- 14.8. Remove plates from the centrifuge and place in BSC. Remove foil seal.
- 14.9. Using a multichannel pipette, add 75 μ L/well of 1X Buffer to O96 plate. Using a multichannel pipette, carefully transfer 25 μ L/well of supernatants from R96 plates to appropriate wells in O96 plates per plate map (Diagram 2 or alternate).
- 14.10. Set aside the R96 plate in the BSC in case retesting is needed after completing assay. If retesting is needed, re-seal R96 plate Assay Plates containing supernatant with foil seal, and store at -20°C.
- 14.11. Cover O96 plates with a clear plate sealer. Verify the plate is completely sealed; otherwise, sample may evaporate in oven.
- 14.12. Mix O96 plates for 1 minute at room temperature on microplate shaker at 300-400 rpm per HSL_EQ_023.
- 14.13. Incubate O96 plates in convection oven for 45 ± 2 minutes at 65-70°C per HSL_EQ_017. Record start and end time on HSL_LAB_006.02.
- 14.14. Carefully remove O96 plates from the oven and incubate at 4°C using wet ice or 2-8°C refrigerator. Record start and end time on HSL_LAB_006.02.
- 14.15. Centrifuge plates at 1700 x g for 1 minute at 20-25°C per HSL_EQ_003 to pull down the condensation on the walls of the wells.

Note: Condensation may still be visible on plate sealer.
- 14.16. Place plates into the BSC. Remove the clear plate sealer and dispense the SEAP Substrate from the bottles into a reagent reservoir.
- 14.17. Add 100 μ L of SEAP substrate to each well using a multi-channel pipette, Columns 1-12, starting at Row H and moving to Row A.
- 14.18. Cover O96 plates with new clear plate sealer and mix plate on plate shaker for 1 minute at 300-400 rpm per HSL_EQ_023 while protecting from light (e.g., put a piece of aluminum foil to cover the plates during shaking).
- 14.19. Incubate for 20-25 minutes at room temperature, while protecting plate from light. Record incubation start time on HSL_LAB_006.02.

Note: May place O96 plates in a lab bench drawer during incubation. Be sure to label the drawer so plates are not disrupted during incubation.

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14.20. During incubation, set up Spectramax M5 Microplate Reader using Softmax Pro software. Select the "HPV Neutralization Assay" template. Settings are listed below:

14.20.1. Endpoint

14.20.2. Luminescence

14.20.3. 200 ms Integration Time

14.20.4. Check all wavelengths

14.20.5. Shake 5 seconds before read

14.20.6. Read entire plate

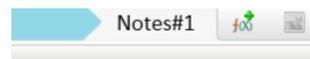
14.20.7. Use 96 well Standard Opaque Plate

14.21. After SEAP Substrate incubation, place individual plate on plate reader. Select "Read" in SoftMax and record the read time/end time on HSL_LAB_006.02.

14.22. Enter the following information into the HPV Neutralization Template.

14.22.1. Notes Section: Logbook Reference with page number, Analyst Initials, PsV Type and Dilution Factor. See Figure 1 below.

Figure 1: Example Notes Section in Template



Logbook Reference:
Analyst:

Plate 1: HPV-16@ 200,000
Plate 2: HPV-18 @ 30,000
Plate 3: HPV-31 @ 200,000
Plate 4: HPV-45 @ 300000
Plate 5: HPV-52 @ 15,000
Plate 6: HPV-58 @ 200,000

14.22.2. Sample Identification (ID): Click the "ID" column and enter the samples being tested. If less than four samples are being tested, enter "No Sample" into the field. When entering fields, use quotation marks around each sample ID as shown in Figure 2 below.

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Figure 2: Example Sample ID Field in Template

Formula Syntax Helper

```
"HPV170620B" & "HPV170620C" & "HPV170706A" & "HPV170710A"
```

SeAP **Sample ID**

| Sample_ID | | | | | |
|-----------|---|-------|------------|------------------|--|
| Sample # | N | Batch | ID | PsV_Manufacturer | |
| 1 | 1 | 1 | HPV170620B | In House | |
| 2 | 2 | 1 | HPV170620C | In House | |
| 3 | 3 | 1 | HPV170706A | In House | |
| 4 | 4 | 1 | HPV170710A | In House | |

14.22.3. Confirm the dilution factors for samples and controls being tested for each plate being analyzed, adjust series if needed. See Figure 3.

Figure 3: Example Dilution Series in Template

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---------|---------|---------|---------|---------|---------|---------|---------|------------|------------|--------------|--------------|
| A | HPV 1.1 | HPV 1.2 | HPV 2.1 | HPV 2.2 | HPV 3.1 | HPV 3.2 | HPV 4.1 | HPV 4.2 | HPV 1...Ct | HPV 1...Ct | HPV 1 NS/NV | HPV 1 NS/NV |
| B | 02 | 02 | 02 | 02 | 02 | 02 | 02 | 02 | 02 | 02 | 01 | 01 |
| C | 03 | 03 | 03 | 03 | 03 | 03 | 03 | 03 | 03 | 03 | 01 | 01 |
| D | 04 | 04 | 04 | 04 | 04 | 04 | 04 | 04 | 04 | 04 | 01 | 01 |
| E | 05 | 05 | 05 | 05 | 05 | 05 | 05 | 05 | 05 | 05 | HPV 1 PsV/NB | HPV 1 PsV/NB |
| F | 06 | 06 | 06 | 06 | 06 | 06 | 06 | 06 | 06 | 06 | 01 | 01 |
| G | 07 | 07 | 07 | 07 | 07 | 07 | 07 | 07 | 07 | 07 | 01 | 01 |
| H | 08 | 08 | 08 | 08 | 08 | 08 | 08 | 08 | 08 | 08 | 01 | 01 |

Assignment Options

Blanks

HPV 1.1

Sample: 01

Dilution Factor: 10.0 titer

14.23. Save file as follows, using the first page of HSL_LAB_006.02 where the experiment is recorded as the logbook number in the Data Reference:

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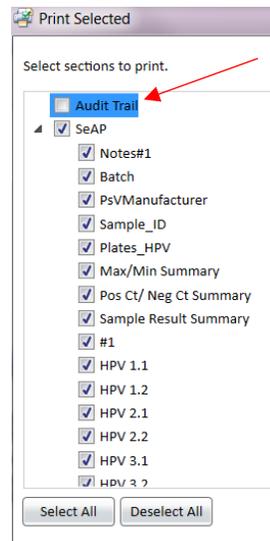
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“Data Reference_HPVNeut_DDMMYYAnalyst Initials”
(LB1234567001_HPVNeut_20May17ABC)

- 14.24. Select “Print Select” option in SoftMax and check all boxes EXCEPT Audit trail then print the document. See Figure 4.

Figure 4: Print Select Options



- 14.25. Sign and date the first page of the raw data file and store in the logbook’s associated Raw Data Binder.

15. ASSAY ACCEPTABILITY GUIDELINES

- 15.1. If the confluency of the Assay Plates on the Harvest Day (day 2) is less than 75% for approximately ¼ of the plate or more, consult with Scientific Manager if plate(s) may be harvested or discarded, and record comment on HSL_LAB_006.02.
- 15.2. If the signal to noise ratio (S:N) result is “flag” then consult with Scientific Manager to determine if any results are valid. Record comment on HSL_LAB_006.02.

16. SYSTEM SUITABILITY

Note: Each plate is assessed individually for system suitability.

Note: Report S:N, percent CV, and RLU to a whole number.

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- 16.1. In the “Max/Min Summary” section, the “PsV + NB” S:N must be ≥ 50 , which is indicated by “PASS”.
- 16.2. The “No PsV/No Sera” (NS/NV) must be ≤ 2000 RLU for the assay to pass.
 - 16.2.1. If the RLU is between 2000-5000 discuss results with Scientific Manager prior to acceptance and assess if wells can be masked. Write comment on HSL_LAB_006.02.
 - 16.2.2. If the RLU is > 5000 , repeat the plate.

17. MASKING OF WELLS

- 17.1. Wells can be masked when the results appear to be erroneous due to pipetting error, well contamination, or other reason as determined by the Scientific Manager.
- 17.2. When masking wells, include two printouts from SoftMax.
 - 17.2.1. The top printout is the reportable/final results where wells have been masked.
 - 17.2.2. The second printout is the original results without any wells masked.
- 17.3. PSV + NB (PSV/NB): No more than two out of eight (2/8) data points are masked per plate.
- 17.4. No PsV/No Sera (NS/NV): No more than two out of eight (2/8) data points are masked per plate.
- 17.5. Samples: If the “Mean Titer” for a sample dilution series has a percent CV $> 20\%$, two wells may be masked even if on the same dilution.

18. ATTACHMENTS

- 18.1. Attachment 1: 96-Well Plate Skirt Label
- 18.2. Attachment 2: HPV PsV Dilutions and Inhibiting Antibody Dilutions
- 18.3. Attachment 3: HSL_LAB_006.01: HPV Neutralization Assay, Sample Preparation Form
- 18.4. Attachment 4: HSL_LAB_006.02: HPV Neutralization Assay, Substrate Development Form

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19. REVISION HISTORY

| Version | Change | Reason |
|---------|---|--|
| 2.0 | Update forms. | Correct missing equipment, added sample results section. |
| | Update Attachment 1. | Include updated dilutions and the dilution schemes for ease of use. |
| | Update reporting results and masking of wells. | Missing from previous version. |
| 3.0 | Added Viastain to Reagents | Used in cell counting procedure. |
| | Step 9.2, added option to vortex. | Reflect current practice. |
| | Updated PsV Recommended Dilutions table: HPV-45 changed from 1:300,000 to 1:400,000, HPV-31 and HPV-16 changed from 1:200,000 to 1:300,000. HPV-11 and HPV-33 added to table (TBD and NA). Note added at bottom of table. | Reflect current practice. |
| | Negative Control Dilutions Table updated: HPV-16 changed to 5074 only. HPV-11 and HPV-33 added. | Reflect current practice. |
| | HSL_LAB_006.01: Positive Control changed to Gardasil 9 Serum Control; reformatted PsV preparation section. | Reflect current practice, ease of use. |
| 4.0 | Changed Version numbers in Revision History (New to 1.0, 1.0 to 2.0). Added 3.0 changes and reasons. | Clarification and ease of tracking. Changes for version 3.0 were not captured prior to release. |
| | Typo and grammar changes throughout document. | Clarification |
| | Updated formatting, forms now separate. | Consistency between procedures. Ease of use. |
| | Added Attachment for 96-Well Plate Skirt Label. | Clarification. |
| | Removed HSL_GL_002, HSL_GL_003, HSL_GL_004, HSL_GL_007, HSL_GL_008, HSL_GL_009, HSL_GL_010 and HSL_EQ_009 from References section. Added HSL_EQ_020 and HSL_LAB_001 to References section. | Removed procedures not referenced in body of procedure. Added procedure referenced in body of procedure. |

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| | | |
|-----|---|--|
| 4.0 | Added centrifuges to Equipment section. | Clarification. |
| | Added 5X Buffer to Reagents. | Used in procedure. |
| | Removed e.g., D96, F96, O96, R96, SDS, V96, SOP, PBNA_M from Definitions list. BPV, CV, NB, Plate skirt, RLU, TBD added to Definitions list. | Removed definitions: acronyms used earlier in procedure, not needed in definitions section. Added definitions: acronyms used later in procedure, needed in definitions section. |
| | Added new Procedural Principles section that includes information about Data Reference, cell culture used, procedures performed in BSC, and handling unused plates. | Clarification. |
| | Added D96 plate diagram. | Clarification. |
| | Updated HSL_LAB_006.01: added plate diagrams, formatting, updated control options, added cell count criteria and pass/fail boxes. | Clarification, ease of use, reflect current practice. |
| | Updated formatting of HSL_LAB_006.02. | Ease of use. |

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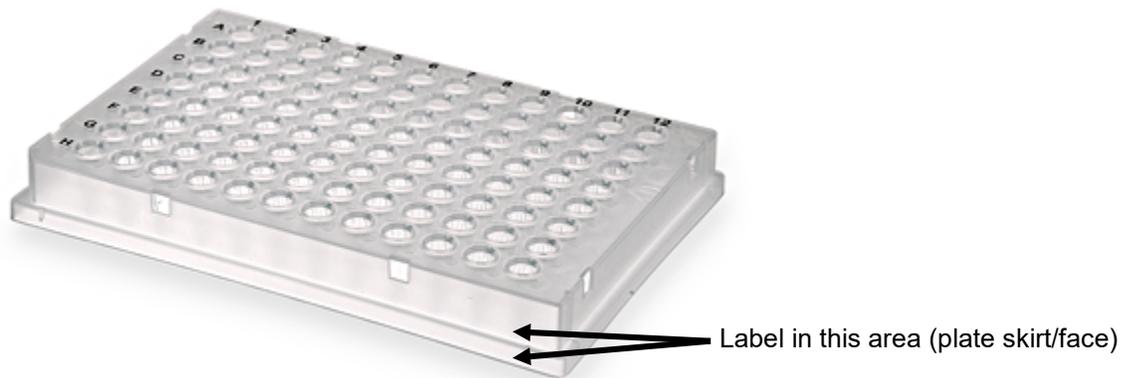
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Attachment 1: 96-Well Plate Skirt Label



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Attachment 2: HPV PsV Dilutions and Inhibiting Antibody Dilutions

PsV Recommended Dilutions

| Pseudovirion (PsV) | Final Dilution for PsV | Recommended Dilution Scheme |
|--------------------|------------------------|--|
| HPV-6 | TBD | N/A |
| HPV-11 | TBD | N/A |
| HPV-16 | 1:300,000 | 1 st Dilution: 10µL of PsV in 990µL of PBNA_M 2 nd Dilution: 40 µL of 1 st Dilution in 11.96 mL of PBNA_M |
| HPV-18 | 1:30,000 | 1 st Dilution: 10µL of PsV in 990µL of PBNA_M 2 nd Dilution: 400 µL of 1 st Dilution in 11.6 mL of PBNA_M |
| HPV-31 | 1:300,000 | 1 st Dilution: 10µL of PsV in 990µL of PBNA_M 2 nd Dilution: 40 µL of 1 st Dilution in 11.96 mL of PBNA_M |
| HPV-33 | TBD | N/A |
| HPV-45 | 1:400,000 | 1 st Dilution: 10µL of PsV in 990µL of PBNA_M 2 nd Dilution: 30 µL of 1 st Dilution in 11.970 mL of PBNA_M |
| HPV-52 | 1:10,000 | 12 µL of PsV in 11.988 mL of PBNA_M |
| HPV-58 | 1:200,000 | 1 st Dilution: 10µL of PsV in 990µL of PBNA_M 2 nd Dilution: 60 µL of 1 st Dilution in 11.94 mL of PBNA_M |

Note: PsVs are stored in the -80°C freezer and are typically pre-diluted 1:10. Confirm any pre-dilution factor prior to following dilution scheme above.

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Negative Control Recommended Dilutions

| Pseudovirion (PsV) | Negative Assay Control (Neg Ct) | Neg Ct Starting Dilution Factor | Recommended Dilution Scheme |
|--------------------|---|---------------------------------|---|
| HPV-6 | 5074 or V5 | 1:1000 | 54µL of 1:100 diluted negative control in 486µL of PBNA_M |
| HPV-11 | 5074 or V5 | 1:1000 | 54µL of 1:100 diluted negative control in 486µL of PBNA_M |
| HPV-16 | 5074 | 1:1000 | 54µL of 1:100 diluted negative control in 486µL of PBNA_M |
| HPV-18 | V5 | 1:1000 | 54µL of 1:100 diluted negative control in 486µL of PBNA_M |
| HPV-31 | 5074 or V5 | 1:1000 | 54µL of 1:100 diluted negative control in 486µL of PBNA_M |
| HPV-33 | 5074 or V5 | 1:1000 | 54µL of 1:100 diluted negative control in 486µL of PBNA_M |
| HPV-45 | 5074 or V5 | 1:1000 | 54µL of 1:100 diluted negative control in 486µL of PBNA_M |
| HPV-52 | 5074 or V5 | 1:1000 | 54µL of 1:100 diluted negative control in 486µL of PBNA_M |
| HPV-58 | 5074 or V5 | 1:1000 | 54µL of 1:100 diluted negative control in 486µL of PBNA_M |
| All Types | Negative response to 9 HPV types included in Gardasil-9 vaccine | 1:10 | 54µL of negative control in 486µL of PBNA_M |

Positive Control Recommended Dilutions

| Pseudovirion (PsV) | Positive Assay Control (Pos Ct) | Pos Ct Starting Dilution Factor | Recommended Dilution Scheme |
|--------------------|---------------------------------|---------------------------------|--|
| All Types | Gardasil-9 Positive Serum | 1:10 | 54µL of positive control in 486µL of PBNA_M |
| All Types | Heparin* | N/A* | 10 µL of pre-diluted heparin in 490 µL of PBNA_M |

*Can vary depending on stock concentration. Final concentration should be ~1 mg/mL for complete inhibition.

Note: If available, use of the serum control is advised over heparin.

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Attachment 3: HSL_LAB_006.01: HPV Neutralization Assay, Sample Preparation Form

| | | | |
|---|------------|--|------------|
| <p>Frederick National Laboratory for Cancer Research <i>sponsored by the National Cancer Institute</i></p> | | <p>HPV Serology Laboratory Standard Operating Procedure Form</p> | |
| <p>Form Title: HPV Neutralization Assay, Sample Preparation Form</p> | | | |
| <p>Document ID: HSL_LAB_006.01</p> | | <p>Version:</p> | <p>4.0</p> |
| <p>Associated SOP: HSL_LAB_006</p> | | <p>Effective Date:</p> | |
| <p>Supersedes Version:</p> | <p>3.0</p> | <p>Page 1 of 6</p> | |

Day 1: HPV Neutralization and Cell Growth

Equipment

| Equipment Description | Equipment ID | Calibration Due Date |
|---------------------------------------|---|----------------------|
| BSC | <input type="checkbox"/> HSL_007 <input type="checkbox"/> HSL_008 <input type="checkbox"/> HSL_009 <input type="checkbox"/> Other: | |
| CO ₂ Incubator | <input type="checkbox"/> HSL_023 <input type="checkbox"/> HSL_024 <input type="checkbox"/> HSL_026 <input type="checkbox"/> HSL_027 | |
| Microscope | <input type="checkbox"/> HSL_020 <input type="checkbox"/> Other: | |
| Microcentrifuge | <input type="checkbox"/> HSL_006 <input type="checkbox"/> Other: | |
| Bench top centrifuge | <input type="checkbox"/> HSL_033 <input type="checkbox"/> Other: | |
| Cellometer Auto 2000 | <input type="checkbox"/> HSL_019 <input type="checkbox"/> Other: | |
| 2-8°C Refrigerator | <input type="checkbox"/> HSL_028 <input type="checkbox"/> Other: | |
| <input type="checkbox"/> N/A Pipette: | µL PIP_ | |
| <input type="checkbox"/> N/A Pipette: | µL PIP_ | |
| <input type="checkbox"/> N/A Pipette: | µL PIP_ | |
| <input type="checkbox"/> N/A Pipette: | µL PIP_ | |
| <input type="checkbox"/> N/A Pipette: | µL PIP_ | |
| <input type="checkbox"/> N/A Pipette: | µL PIP_ | |

Reagents

| Reagent | Lot Number | Expiration Date |
|---|------------------------------|------------------------------|
| DPBS <input type="checkbox"/> N/A | <input type="checkbox"/> N/A | <input type="checkbox"/> N/A |
| 0.05% Trypsin-EDTA <input type="checkbox"/> N/A | <input type="checkbox"/> N/A | <input type="checkbox"/> N/A |
| Vita Stain AOP1 Staining Solution | <input type="checkbox"/> N/A | <input type="checkbox"/> N/A |
| PBNA_M | <input type="checkbox"/> N/A | <input type="checkbox"/> N/A |

Comments:

N/A

| | |
|---------------------------|--|
| Performed by/date: | |
| Reviewed by/date: | |

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| | | | |
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Cell Culture Lot Number: _____ Working Passage #: _____

Cell Count

| Count 1 (Cells/mL) | Viability 1 (%) (≥80%) | Count 2 (Cells/mL) | Viability 2 (%) (≥80%) | Average Count (Cells/mL) | Percent Difference (%) |
|-----------------------|--|-----------------------|--|---|-------------------------------------|
| | <input type="checkbox"/> Pass <input type="checkbox"/> Fail | | <input type="checkbox"/> Pass <input type="checkbox"/> Fail | | |
| Count 3 (Cells/mL) | Viability 3 (%) (≥80%) | Count 4 (Cells/mL) | Viability 4 (%) (≥80%) | Average Count (Cells/mL) (Counts 1-4) | |
| | <input type="checkbox"/> Pass <input type="checkbox"/> Fail | | <input type="checkbox"/> Pass <input type="checkbox"/> Fail | | <input type="checkbox"/> N/A Row |

Cell Dilution to 3.0 x 10⁵ cell/mL

| Average Cell Count (Cells/mL) | Total Volume Required (mL) (~12 mL per plate) | Volume of Cells Used (mL) | Volume of PBNA_M (mL) |
|----------------------------------|--|------------------------------|--------------------------|
| | | | |

F96 Plate Incubation at 37°C, 5% CO₂

| Start Time | End Time | Total Time |
|------------|----------|------------|
| | | |

Comments:

N/A

| | |
|---------------------------|--|
| Performed by/date: | |
| Reviewed by/date: | |

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| Document ID: HSL_LAB_006.01 | | Version: | 4.0 |
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Sample Pre-Dilution

| Sample Number | Identification | Starting Dilution Factor | Sample Volume (µL) | PBNA_M Volume (µL) |
|----------------|----------------|--------------------------|--------------------|--------------------|
| ☐ N/A Sample 1 | | | | |
| ☐ N/A Sample 2 | | | | |
| ☐ N/A Sample 3 | | | | |
| ☐ N/A Sample 4 | | | | |

Assay Control Pre-Dilution

| Plate # | Identification | Lot Number | Starting Dilution Factor | Sample Volume (µL) | PBNA_M Volume (µL) |
|------------|---|------------|--------------------------|--------------------|--------------------|
| 1 ☐ N/A | <input type="checkbox"/> HPV Sero Negative Control <input type="checkbox"/> V5 <input type="checkbox"/> 5074 <input type="checkbox"/> Gardasil 9 Serum Control <input type="checkbox"/> Heparin | | | | |
| 2 ☐ N/A | <input type="checkbox"/> HPV Sero Negative Control <input type="checkbox"/> V5 <input type="checkbox"/> 5074 <input type="checkbox"/> Gardasil 9 Serum Control <input type="checkbox"/> Heparin | | | | |
| 3 ☐ N/A | <input type="checkbox"/> HPV Sero Negative Control <input type="checkbox"/> V5 <input type="checkbox"/> 5074 <input type="checkbox"/> Gardasil 9 Serum Control <input type="checkbox"/> Heparin | | | | |
| 4 ☐ N/A | <input type="checkbox"/> HPV Sero Negative Control <input type="checkbox"/> V5 <input type="checkbox"/> 5074 <input type="checkbox"/> Gardasil 9 Serum Control <input type="checkbox"/> Heparin | | | | |
| 5 ☐ N/A | <input type="checkbox"/> HPV Sero Negative Control <input type="checkbox"/> V5 <input type="checkbox"/> 5074 <input type="checkbox"/> Gardasil 9 Serum Control <input type="checkbox"/> Heparin | | | | |
| 6 ☐ N/A | <input type="checkbox"/> HPV Sero Negative Control <input type="checkbox"/> V5 <input type="checkbox"/> 5074 <input type="checkbox"/> Gardasil 9 Serum Control <input type="checkbox"/> Heparin | | | | |

| | |
|--------------------|--|
| Performed by/date: | |
| Reviewed by/date: | |

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| Document ID: HSL_LAB_006.01 | | Version: | 4.0 |
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PsV Preparation

| Plate # | HPV Type | Pseudovirus Lot Number | Final Dilution Factor | Dilution 1 | | Dilution 2 | |
|-----------|----------|------------------------|-----------------------|---------------|-------------------|----------------------|-------------------|
| | | | | Volume of PsV | Volume of PBNA, M | Volume of Dilution 1 | Volume of PBNA, M |
| 1 □N/A | | | | | | | |
| 2 □N/A | | | | | | | |
| 3 □N/A | | | | | | | |
| 4 □N/A | | | | | | | |
| 5 □N/A | | | | | | | |
| 6 □N/A | | | | | | | |

PsV + Sample Plate Incubation at 2-8°C (40-80 minutes)

| Start Time | End Time | Total Time (mins) |
|------------|----------|-------------------|
| | | |

Comment:

□N/A

| | |
|--------------------|--|
| Performed by/date: | |
| Reviewed by/date: | |

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| | | | |
|---|-----|---|-----|
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Plate Map Used (select one):

Per Procedure

| | Columns 1&2 | Columns 3&4 | Columns 5&6 | Columns 7&8 | Column 9 | Column 10 | | Columns 11&12 |
|---|------------------|------------------|------------------|------------------|----------------|-----------------------------|----------------------------|-----------------------------|
| A | Sample 1 1/10 | Sample 2 1/10 | Sample 3 1/10 | Sample 4 1/10 | Pos Ct 1/10 | Neg Ct V5/6074 1/1000 | Neg Ct HPV Sero 1/10 | No PsV / No Sera (NS/NV) |
| B | 1/40 | 1/40 | 1/40 | 1/40 | 1/40 | 1/4000 | 1/40 | |
| C | 1/160 | 1/160 | 1/160 | 1/160 | 1/160 | 1/16000 | 1/160 | |
| D | 1/640 | 1/640 | 1/640 | 1/640 | 1/640 | 1/64000 | 1/640 | |
| E | 1/2560 | 1/2560 | 1/2560 | 1/2560 | 1/2560 | 1/256000 | 1/2560 | PsV + NB (PsV/NB) |
| F | 1/10240 | 1/10240 | 1/10240 | 1/10240 | 1/10240 | 1/1024000 | 1/10240 | |
| G | 1/40960 | 1/40960 | 1/40960 | 1/40960 | 1/40960 | 1/4096000 | 1/40960 | |
| H | 1/163840 | 1/163840 | 1/163840 | 1/163840 | 1/163840 | 1/16384000 | 1/163840 | |

Other

| | Columns 1&2 | Columns 3&4 | Columns 5&6 | Columns 7&8 | Column 9 | Column 10 | Columns 11&12 |
|---|-------------|-------------|-------------|-------------|----------|-----------|---------------|
| A | | | | | | | |
| B | | | | | | | |
| C | | | | | | | |
| D | | | | | | | |
| E | | | | | | | |
| F | | | | | | | |
| G | | | | | | | |
| H | | | | | | | |

Sample and PsV Incubation at 37°C, 5% CO₂ (70-74 hours)

| | |
|---------------------------|--|
| Start Date / Time: | |
|---------------------------|--|

| | |
|---------------------------|--|
| Performed by/date: | |
| Reviewed by/date: | |

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| | | | |
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Day 2: Harvest

Equipment

| Equipment Description | Equipment ID | Calibration Due Date |
|---|--|----------------------|
| BSC | <input type="checkbox"/> HSL_007 <input type="checkbox"/> HSL_008 <input type="checkbox"/> HSL_009 <input type="checkbox"/> Other: | |
| Microscope | <input type="checkbox"/> HSL_020 <input type="checkbox"/> Other: | |
| Bench top Centrifuge | <input type="checkbox"/> HSL_033 <input type="checkbox"/> Other: | |
| Microcentrifuge | <input type="checkbox"/> HSL_006 <input type="checkbox"/> Other: | |
| -20°C Freezer | <input type="checkbox"/> HSL_034 <input type="checkbox"/> HSL_038 <input type="checkbox"/> Other: | |
| <input type="checkbox"/> N/A Pipette: μL | PIP_ | |
| <input type="checkbox"/> N/A Pipette: μL | PIP_ | |

Sample and PsV Incubation at 37°C, 5% CO₂ (70-74 hours)

| End Date / Time | Total Hours |
|-----------------|-------------|
| | |

| |
|--|
| Plate 1 Cell Confluency >75%? <input type="checkbox"/> Yes <input type="checkbox"/> No: _____ |
| Plate 2 <input type="checkbox"/> N/A Cell Confluency >75%? <input type="checkbox"/> Yes <input type="checkbox"/> No: _____ |
| Plate 3 <input type="checkbox"/> N/A Cell Confluency >75%? <input type="checkbox"/> Yes <input type="checkbox"/> No: _____ |
| Plate 4 <input type="checkbox"/> N/A Cell Confluency >75%? <input type="checkbox"/> Yes <input type="checkbox"/> No: _____ |
| Plate 5 <input type="checkbox"/> N/A Cell Confluency >75%? <input type="checkbox"/> Yes <input type="checkbox"/> No: _____ |
| Plate 6 <input type="checkbox"/> N/A Cell Confluency >75%? <input type="checkbox"/> Yes <input type="checkbox"/> No: _____ |

Sample and PsV Incubation at -20°C

| | |
|---------------------------|--|
| Start Date / Time: | |
|---------------------------|--|

| | |
|---------------------------|--|
| Performed by/date: | |
| Reviewed by/date: | |

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Attachment 4: HSL_LAB_006.02: HPV Neutralization Assay, Substrate Development Form

| Frederick National Laboratory for Cancer Research <small>sponsored by the National Cancer Institute</small> | | HPV Serology Laboratory Standard Operating Procedure Form | |
|---|---|---|-----|
| Form Title: HPV Neutralization Assay, Substrate Development Form | | | |
| Document ID: HSL_LAB_006.02 | | Version: | 4.0 |
| Associated SOP: HSL_LAB_006 | | Effective Date: | |
| Supersedes Version: 3.0 | | Page 1 of 3 | |
| Day 3: Substrate Development | | | |
| Equipment | | | |
| Equipment Description | Equipment ID | Calibration Due Date | |
| Convection Oven | <input type="checkbox"/> HSL_025 <input type="checkbox"/> Other: | | |
| BSC | <input type="checkbox"/> HSL_007 <input type="checkbox"/> HSL_008 <input type="checkbox"/> HSL_009 <input type="checkbox"/> Other: | | |
| 2-8°C Refrigerator | <input type="checkbox"/> HSL_029 <input type="checkbox"/> Other: | | |
| Microcentrifuge | <input type="checkbox"/> HSL_006 <input type="checkbox"/> Other: | | |
| Bench top Centrifuge | <input type="checkbox"/> HSL_033 <input type="checkbox"/> Other: | | |
| Microplate Shaker | <input type="checkbox"/> HSL_030 <input type="checkbox"/> HSL_031 <input type="checkbox"/> HSL_032 <input type="checkbox"/> HSL_054 <input type="checkbox"/> HSL_055 <input type="checkbox"/> Other: | | |
| M5 Microplate Reader | <input type="checkbox"/> HSL_018 <input type="checkbox"/> Other: | | |
| -20°C Freezer | <input type="checkbox"/> HSL_034 <input type="checkbox"/> HSL_038 <input type="checkbox"/> Other: | | |
| <input type="checkbox"/> N/A Pipette: | µL PIP_ | | |
| <input type="checkbox"/> N/A Pipette: | µL PIP_ | | |
| <input type="checkbox"/> N/A Pipette: | µL PIP_ | | |
| Reagents | | | |
| Reagent | Lot Number | Expiration Date | |
| SEAP Substrate | | <input type="checkbox"/> N/A | |
| 5X Buffer | | <input type="checkbox"/> N/A | |
| Distilled Water | | <input type="checkbox"/> N/A | |
| 1X Buffer Preparation | | | |
| Volume of 5X Buffer | Volume of Distilled Water | | |
| | | | |
| Performed by/date: | | | |
| Reviewed by/date: | | | |

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Form Title: HPV Neutralization Assay, Substrate Development Form

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Plate Identification

| Plate # | HPV Type | HSL_LAB_006.01 Data Reference |
|---------|----------|-------------------------------|
| DN/A 1 | | |
| DN/A 2 | | |
| DN/A 3 | | |
| DN/A 4 | | |
| DN/A 5 | | |
| DN/A 6 | | |

Incubations

| Condition | Start Time | End Time | Total Time |
|--|------------|------------|------------|
| Plates at 65-70°C | | | |
| Plates at 4°C | | | |
| Substrate (1 st plate only) | | Read Time: | |

Plate File Name: _____

Comments:

DN/A

Performed by/date:

Reviewed by/date:

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System Suitability

| Plate # | Mean (NB) | Mean (NS/NV) | %CV (NB) | Psv+NB S:N Ratio | Disposition |
|----------|-----------|--|----------|------------------|---|
| | FIO | ≤ 2000 RLU (Pass), 2000-5000 RLU(Check), > 5000 RLU Repeat | FIO | ≥ 50 | Pass / Fail |
| 1 Q/A | | | | | <input type="checkbox"/> Pass <input type="checkbox"/> Fail |
| 2 Q/A | | | | | <input type="checkbox"/> Pass <input type="checkbox"/> Fail |
| 3 Q/A | | | | | <input type="checkbox"/> Pass <input type="checkbox"/> Fail |
| 4 Q/A | | | | | <input type="checkbox"/> Pass <input type="checkbox"/> Fail |
| 5 Q/A | | | | | <input type="checkbox"/> Pass <input type="checkbox"/> Fail |
| 6 Q/A | | | | | <input type="checkbox"/> Pass <input type="checkbox"/> Fail |

Sample Results

| Sample ID: | Q/A Sample 1 | Q/A Sample 2 | Q/A Sample 3 | Q/A Sample 4 |
|-------------------|--------------|--------------|--------------|--------------|
| Q/A Plate 1, HPV- | | | | |
| Q/A Plate 2, HPV- | | | | |
| Q/A Plate 3, HPV- | | | | |
| Q/A Plate 4, HPV- | | | | |
| Q/A Plate 5, HPV- | | | | |
| Q/A Plate 6, HPV- | | | | |

Comments:

Q/A

Performed by/date:

Reviewed by/date:

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