

Frederick National Laboratory for Cancer Research <small>sponsored by the National Cancer Institute</small>	HPV Serology Laboratory Standard Operating Procedure	
ELISA to Determine VLP Specificity		
Document ID: HSL_LAB_015	Version 1.0	Page 1 of 10

Released by/Date Effective:

Author Name	Title	Signature/Date

Approver Name	Title	Signature/Date

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1. PURPOSE

- 1.1. The purpose of this procedure is to describe the ELISA used to detect specificity of VLPs produced by the HPV Serology Laboratory.

2. SCOPE

- 2.1. This procedure applies to the HPV Serology Laboratory located at the Advanced Technology Research Facility, Room C2007.
- 2.2. This procedure will be used to test the specificity of the following HPV types: 6, 11, 16, 18, 31, 33, 45, 52 and 58.

3. REFERENCES

- 3.1. HSL_LAB_015.01: ELISA to Determine VLP Specificity Data Capture Form
- 3.2. HSL_GL_001: Waste Disposal at the Advanced Technology Research Facility
- 3.3. HSL_GL_003: Good Documentation Practices for the HPV Serology Laboratory
- 3.4. HSL_GL_006: Reagent Preparation for the HPV Serology Laboratory
- 3.5. HSL_GL_007: Reagent and Chemical Expiry in the HPV Serology Laboratory
- 3.6. HSL_GL_008: Laboratory Flow and Gowning Procedures for the HPV Serology Laboratory
- 3.7. HSL_GL_009: HPV Serology Laboratory BSL-2 Procedures
- 3.8. HSL_GL_010: Control and Request of Documents in the HPV Serology Laboratory
- 3.9. HSL_EQ_001: Biosafety Cabinet (BSC) Use and Maintenance
- 3.10. HSL_EQ_004: Use and Maintenance of a BioTek Plate Washer in the HPV Serology Laboratory
- 3.11. HSL_EQ_005: Use and Maintenance of a Molecular Devices M5 Plate Reader in the HPV Serology Laboratory
- 3.12. HSL_EQ_007: Use and Maintenance of a 2-8°C Refrigerator the HPV Serology Laboratory
- 3.13. HSL_EQ_008: Use and Maintenance of -80°C Freezers in the HPV Serology Laboratory
- 3.14. HSL_EQ_012: Use and Maintenance of Pipettes in the HPV Serology Laboratory
- 3.15. HSL_EQ_023: Use and Maintenance of a Compact Digital MicroPlate Shaker

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.

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- 4.3. The Quality Assurance Specialist is responsible for quality oversight and approval of this procedure.

5. REAGENTS, CHEMICALS AND EQUIPMENT

5.1. Equipment

- 5.1.1. Pipettes
- 5.1.2. -80°C Freezer
- 5.1.3. 2-8°C Refrigerator
- 5.1.4. Plate Washer
- 5.1.5. Plate shaker
- 5.1.6. M5 Plate Reader
- 5.1.7. Liquid Nitrogen (LN₂) Freezer

5.2. Reagents and Chemicals

- 5.2.1. 1X **Wash Buffer** (HSL_GL_006, Section 11)
- 5.2.2. HPV ELISA **Coating Buffer** (HSL_GL_006, Section 12)
- 5.2.3. Tween-20 (VWR, 500 ml, Cat# EM-PX1296-1)
- 5.2.4. Skim Milk Powder (BD, Cat # 232100)
- 5.2.5. HPV Type-Specific Monoclonal Antibodies
- 5.2.6. Goat anti-Mouse IgG antibody conjugated to peroxidase (GAM) (Sigma Aldrich, Cat# A4416)
- 5.2.7. TMB (KPL, Cat # 50-76-03)
- 5.2.8. 0.36N H₂SO₄ (HSL_GL_006, Section 15)
- 5.2.9. Wet Ice

5.3. Consumables

- 5.3.1. Pipette Tips
- 5.3.2. Maxisorp Plates (Thomas Scientific, Cat # 6925A00)
- 5.3.3. Plate Sealers

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5.3.4. Cluster Tubes

5.3.5. TechniCloth Wipes (VWR, Cat# TWTX1112 or equivalent)

6. HEALTH AND SAFETY CONSIDERATIONS

- 6.1. 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.
- 6.2. Refer to the respective SDS when working with any chemicals.
- 6.3. Refer to "HSL_GL_001: Waste Disposal at the Advanced Technology Research Facility" regarding waste disposal processes at the ATRF.

7. DEFINITIONS

Term	Definition
ELISA	Enzyme Linked Immunosorbent Assay
FME	Facilities, Maintenance and Engineering
GAM	Goat anti-Mouse IgG antibody conjugated to peroxidase
HPV	Human Papillomavirus
HSL	HPV Serology Laboratory
SDS	Safety Data Sheets
SOP	Standard Operating Procedure
VLP	Virus Like Particle

8. REAGENT PREPARATION

Note: Reagents prepared according to this SOP and documented on HSL_LAB_007.01: HPV-16 L1 ELISA Data Capture Form, are for use only with the associated assay where the reagent is documented. Reagents prepared and documented per Reagent Preparation for the HPV Serology Laboratory, HSL_GL_006 may be used for multiple assays.

- 8.1. **Blocking Buffer**, 4% Skim Milk with 0.2% Tween-20 in PBS
 - 8.1.1. Add 8.0±0.4 g of Skim Milk powder to 200 mL of PBS. Mix well to homogenize the solution.
 - 8.1.2. Once solution is homogenous, add 400 µl of Tween-20. Mix gently to avoid producing excessive bubbles in the solution and use buffer within 24 hours of preparation. Store at 2-8°C if not used on same day, label with Reagent Name, Preparation Date, Expiration Date and Logbook Reference.

9. PLATE COATING

- 9.1. Remove VLP aliquot(s) from the -80°C Freezer and thaw on ice prior to use.

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Note: Up to two VLPs can be coated on a single plate at a time (See Attachment 1: Plate Layout). A minimum total volume of 6 mL for each VLP is required to coat ½ a plate.

9.2. After the VLP aliquot(s) is thawed on ice (up to one hour), dilute VLP in HPV ELISA Coating Buffer to a final concentration of 2.7 µg/mL.

9.2.1. For example, to coat ½ a plate.

Dilution 1	
C_1	= 1000 µg/mL (VLP Starting Concentration)
V_1	= Unknown
C_2	= 2.7 µg/mL (Final Coating Concentration)
V_2	= 6000 µL (Total Volume)

$$V_1 = C_2 V_2 / C_1$$

$$V_1 = (2.7 * 6000) / (1000)$$

$$V_1 = 16.2 \mu\text{L}$$

16.2 µL of the VLP would be needed to coat ½ of a single plate.

9.2.2. Label a 96 Well Maxisorp plate with the following information: VLP Lot(s), Logbook Reference, Coating Date/Start Time and Analyst Initials.

Note: Pre-printed labels may be used and filled out with above information and affixed to the plate once coated. See example below.

VLP Lot 1:	VLP Lot 2:	LB Ref:	Coat Date/ Start Time:	Analyst Initials:
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9.2.3. Add 100 µL of coating solution for the first VLP being testing to Rows A-D, Columns 1-12. Add 100 µL of coating solution for the second VLP being testing to Rows E-H, Columns 1-12.

9.2.4. Cover each plate with a clear plate sealer and tap sides gently to ensure all wells are covered. Visually inspect plate for coverage prior to placing it in a 2-8°C refrigerator.

9.2.5. Record incubation start time on Form HSL_LAB_015.01.

9.2.6. Use of the coated plates must occur between 72 and 120 hours.

10. ELISA PROCEDURE

10.1. Remove 1X Wash Buffer, Blocking Buffer and TMB solution from 2-8°C refrigerator and allow to come to room temperature.

10.2. Remove samples and controls from the -80°C Freezer and thaw on ice.

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10.2.1. Once reagents are equilibrated, remove coated plates 2-8°C refrigerator and record incubation end time on Form HSL_LAB_015.01.

- 10.3. Wash plates using the “HPV ELISA” plate washer protocol.
- 10.4. After wash cycle, tap plates gently on an absorbent wipe to remove excess wash buffer.
- 10.5. Add 300 µL of Blocking Buffer to each well using a multichannel pipette.
- 10.6. Cover each plate with a plate sealer and incubate for 90±5 minutes at room temperature (RT) without agitation. Record incubation start and end time on Form HSL_LAB_015.01.
- 10.7. During the Blocking Incubation, prepare the 9 HPV Type-Specific Monoclonal Antibodies in cluster tubes.

Note: If an antibody is not available or needed for a specific run, use Blocking Buffer in place of the reagent.

10.7.1. Prepare at least 300 µL of each initial dilution in Blocking Buffer, according to the recommended dilution factor obtained from the Scientific Manager. Initial dilutions can be made in a cluster tube or micro centrifuge tube.

10.7.2. Document initial dilution preparation on Form HSL_LAB_015.02.

10.7.3. In cluster tubes, prepare four 1:2 serial dilutions in Blocking Buffer per table below. Switch tips between each dilution and either mix ten times with a pipette or gently vortex individual cluster tubes.

	Volume of Blocking Buffer	Volume of Dilution
1:2	400 µL	400 µL of initial dilution
1:4	400 µL	400 µL of 1:2
1:8	400 µL	400 µL of 1:4
1:16	400 µL	400 µL of 1:8

- 10.8. Store prepared antibodies at RT until the blocking incubation is complete.
- 10.9. Wash plates using the “HPV ELISA” plate washer protocol.
- 10.10. After wash cycle, tap plates gently on an absorbent wipe to remove excess wash buffer.
- 10.11. Dispense 100 µL of each antibody according to Attachment 1: Plate Layout.
- 10.12. Seal the plates with plate sealers then incubate at room temperature for 60±5 minutes with gentle shaking at 250 rpm. Record incubation start and end time on Form HSL_LAB_015.01.
- 10.13. Prior to washing plates, dilute conjugate in Blocking Buffer, to the proper final concentration based on qualification of each individual type/lot. Prepare approximately 1 mL of conjugate per column.

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- 10.14. Wash plates using the "HPV ELISA" plate washer protocol.
- 10.15. After wash cycle, tap plates gently on an absorbent wipe to remove excess wash buffer.
- 10.16. Using a multichannel pipette, add 100 µL of diluted conjugate to each well according to Attachment 1: Plate Layout.
- 10.17. Seal the plates with plate sealers then incubate at room temperature for 60±5 minutes with gentle shaking at 250 rpm.
- 10.18. Prepare TMB solution at least 15 minutes prior to use. TMB Reagents A and B should be mixed together in equal volumes and protected from light.
- 10.19. Prepare 12 mL TMB solution per plate by mixing 6 mL of TMB Reagent A with 6 mL of TMB Reagent B. Protect reagent from light.
- 10.20. Wash plates using the "HPV ELISA" plate washer protocol.
- 10.21. After wash cycle, tap plates gently on an absorbent wipe to remove excess wash buffer.
- 10.22. Using a multichannel pipette, add 100 µL of the TMB solution into each well.
- 10.23. Seal the plates with plate sealers then incubate at room temperature for 25±2 minutes protected from light. Record incubation start and end time on Form HSL_LAB_015.01.
- 10.24. During incubation, turn on the Spectramax M5 and open the "Specificity ELISA" template. Enter in assay information such as dilution factors and data references, then save file (O:\HSL\M5 Plate Reader\Raw Data Files\Specificity ELISA) as follows.

"Data Reference_Specificity_DDMMYYAnalyst Initials"
 (LB12345P001_Specificity_20MAY17ABC)

- 10.25. After TMB Incubation, add 100 µL of 0.36N H₂SO₄ to stop reaction. Record stop time on Form HSL_LAB_015.01.
- 10.26. Carefully place plate in M5 Plate Reader, and select "Read" on the computer. Save file after all plates have been read.
- 10.27. Select "Print Select" option in SoftMax and check all boxes EXCEPT Audit trail then print the document.
- 10.28. Sign and date the first page of the raw data file and store in the logbook's associated Raw Data Binder.

11. SYSTEM SUITABILITY CRITERIA

- 11.1. The assay blank (only conjugate antibody) should be <0.05 OD.

12. DATA ACCEPTANCE CRITERIA

- 12.1. The Optical Density (OD) for the HPV L1 or L1L2 antigen and Antibody specific combination should be ≥ 1.0 OD at the initial Dilution Factor (Row "A" and "E").

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- 12.2. All other antibody types (non-specific) should be ≤ 0.05 OD at the initial Dilution Factor (Row "A" and "E").
- 12.3. The Optical Density (OD) for the HPV L1 or L1L2 antigen and Antibody specific combination should demonstrate dilution effect. If a dilution effect is not seen, consult with the Scientific Manager.
- 12.4. If the assay fails the above criteria, consult with Scientific Manager prior to repeating the experiment.

13. ATTACHMENTS

- 13.1. Attachment 1: Plate Layout

Attachment 1: Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12	
	Blocking Buffer	HPV-6 (M48)	Blocking Buffer	HPV-11 (F1)	HPV-16 (V5)	HPV-18 (J4)	HPV-31 (A6)	HPV-33 (B6)	HPV-45 (N5)	HPV-52 (A7)	HPV-58 (D10)	Blocking Buffer	
A		1:10000		1:20000	1:20000	1:10000	1:3000	1:2000	1:3000	1:3000	1:3000		VLP ID 1
B		1:20000		1:40000	1:40000	1:20000	1:6000	1:4000	1:6000	1:6000	1:6000		
C		1:40000		1:80000	1:80000	1:40000	1:12000	1:8000	1:12000	1:12000	1:12000		
D		1:80000		1:160000	1:160000	1:80000	1:24000	1:16000	1:24000	1:24000	1:24000		
E		1:10000		1:20000	1:20000	1:10000	1:3000	1:2000	1:3000	1:3000	1:3000		VLP ID 2
F		1:20000		1:40000	1:40000	1:20000	1:6000	1:4000	1:6000	1:6000	1:6000		
G		1:40000		1:80000	1:80000	1:40000	1:12000	1:8000	1:12000	1:12000	1:12000		
H		1:80000		1:160000	1:160000	1:80000	1:24000	1:16000	1:24000	1:24000	1:24000		
	GAM 1:5000		GAM 1:30000										

Note: This template provides guidance on the plate layout and mouse anti-HPV type specific clones; Dilution factors may vary. Consult with Scientific Manager for current dilution factors.

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14. REVISION HISTORY

Revision Start Date	Version #	Changes	Reasons
01Aug17	New	Create new SOP to confirm the specificity of the VLPs created in HSL against the appropriate HPV Types.	New SOP.

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Plate Coating

	Starting VLP Concentration (µg/mL)	Volume of VLP (µL)	Volume of HPV ELISA Coating Buffer (mL)	Start Time/ Date	Analyst Initials
VLP ID 1					
VLP ID 2					

Blocking Buffer

Amount of Skim Milk (g)	Volume of PBS (mL)	Volume of Tween-20 (µL)	Analyst Initials/ Date

HPV Antibody Types/ Initial Dilution Preparation

HPV Ab Type	Clone Number	Initial Dilution Factor	Volume of mAb (µL)	Volume of Blocking Buffer (µL)
<input type="checkbox"/> N/A HPV-6				
<input type="checkbox"/> N/A HPV-11				
<input type="checkbox"/> N/A HPV-16				
<input type="checkbox"/> N/A HPV-18				
<input type="checkbox"/> N/A HPV-31				
<input type="checkbox"/> N/A HPV-33				
<input type="checkbox"/> N/A HPV-45				
<input type="checkbox"/> N/A HPV-52				
<input type="checkbox"/> N/A HPV-58				

Conjugate

Conjugate Type	Recommended Dilution Factor	Volume of Conjugate/Primary dilution	Volume of Blocking Buffer
GAM 1 (Conjugate Dilution 1)		1° Dil'n:	1° Dil'n:
		2° Dil'n:	2° Dil'n:
GAM 2 (Conjugate Dilution 2)		1° Dil'n:	1° Dil'n:
		2° Dil'n:	2° Dil'n:

TMB Reagent

TMB Reagent A	TMB Reagent B	Analyst Initials/ Date
mL	mL	

Comments:

N/A

Performed By/ Date:	
Reviewed By/ Date:	

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Incubations

	Start Time	End Time	Total Time
Blocking			
Sample			
Conjugate			
TMB		0.36N H ₂ SO ₄ Addition:	

System Suitability

	Highest Blank OD, VLP Lot 1	Highest Blank OD, VLP Lot 2	System Suitability Result
GAM 1 (Conjugate Dilution 1)			<input type="checkbox"/> Pass <input type="checkbox"/> Fail
GAM 2 (Conjugate Dilution 2)			<input type="checkbox"/> Pass <input type="checkbox"/> Fail

Results

VLP ID 1: HPV Type _____

OD Results

HPV-6	HPV-11	HPV-16	HPV-18	HPV-31	HPV-33	HPV-45	HPV-52	HPV-58

Pass Fail

VLP ID 2: HPV Type _____

OD Results

HPV-6	HPV-11	HPV-16	HPV-18	HPV-31	HPV-33	HPV-45	HPV-52	HPV-58

Pass Fail

Comments:

N/A

Performed By/ Date:	
Reviewed By/ Date:	