Released by/Date Effective:

Author Name	Title	Signature/Date

Approver Name	Title	Signature/Date

1. PURPOSE

1.1. The purpose of this procedure is to describe the ELISA used for the detection of HPV antibodies.

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 detect HPV Type specific antibodies in serum.

2.3. This procedure may be used to test for HPV Type specific antibodies in saliva (mouthwash or sponge extracts), however the parameters surrounding those sample types are For Information Only (FIO) and not included in this procedure.

2.3.1. Include appropriate parameters as an attachment to Raw Data when testing non-serum sample types.

3. REFERENCES

3.1. HSL_LAB_007.01: HPV Antibody ELISA Data Capture Form

3.2. HSL_TRN_001: Training Program for the HPV Serology Laboratory

3.3. HSL_GL_001: Waste Disposal at the Advanced Technology Research Facility

3.4. HSL_GL_003: Good Documentation Practices for the HPV Serology Laboratory

3.5. HSL_GL_006: Reagent Preparation for the HPV Serology Laboratory

3.6. HSL_GL_007: Reagent and Chemical Expiry in the HPV Serology Laboratory

3.7. HSL_GL_008: Laboratory Flow and Gowning Procedures for the HPV Serology Laboratory

3.8. HSL_GL_009: HPV Serology Laboratory BSL-2 Procedures

3.9. HSL_GL_010: Control and Request of Documents in the HPV Serology Laboratory

3.10. HSL_EQ_001: Biosafety Cabinet (BSC) Use and Maintenance

3.11. HSL_EQ_004: Use and Maintenance of a BioTek Plate Washer in the HPV Serology Laboratory

3.12. HSL_EQ_005: Use and Maintenance of a Molecular Devices M5 Plate Reader in the HPV Serology Laboratory

3.13. HSL_EQ_007: Use and Maintenance of a 2-8°C Refrigerator in the HPV Serology Laboratory

3.14. HSL_EQ_008: Use and Maintenance of -80°C Freezers in the HPV Serology Laboratory

3.15. HSL_EQ_009: Use and Maintenance of the Liquid Nitrogen Freezer

3.16. HSL_EQ_012: Use and Maintenance of Pipettes in the HPV Serology Laboratory

3.17. HSL_EQ_017: Use and Maintenance of a Laboratory Convection Oven

3.18. HSL_EQ_019: Use and Maintenance of the Milli-Q Integral 3 Water System

3.19. 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.

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 (LN_2) Freezer

5.2. Reagents and Chemicals

5.2.1. HPV VLP Types 6, 11, 16, 18, 31, 33, 45, 52, and 58 (stored in -80°C)

5.2.2. HPV ELISA Coating Buffer (HSL_GL_006, Section 12)

5.2.3. 1X Wash Buffer (HSL_GL_006, Section 11)

5.2.4. DPBS without Calcium and Magnesium (Fisher Scientific, Cat # 14-190-235 or equivalent)

5.2.5. Tween-20 (VWR, 500 ml, Cat# EM-PX1296-1)

5.2.6. Skim Milk Powder (BD, Cat # 232100)

5.2.7. HPV Type Specific Negative Control (CN)

5.2.8. HPV Type Specific Positive Control (CP)

5.2.9. 0.5 mg Goat anti-human IgG antibody conjugated to peroxidase (KPL, Cat # 214-1002)

5.2.10. TMB (KPL, Cat # 50-76-03)

5.2.11. 0.36N H_2SO_4 (HSL_GL_006, Section 15)

5.2.12. Wet Ice

5.3. Consumables

5.3.1. Pipette Tips

5.3.2. Maxisorp Plates (Thomas Scientific, Cat # 6925A00)

5.3.3. Cluster Tubes

5.3.4. Plate Sealers

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
DPBS	Dulbecco's phosphate-buffered saline
ELISA	Enzyme Linked Immunosorbent Assay
FIO	For Information Only
FME	Facilities, Maintenance and Engineering
HPV	Human Papillomavirus
HSL	HPV Serology Laboratory
LLOQ	Lower Limit of Quantitation
LN2	Liquid Nitrogen
SDS	Safety Data Sheets
SOP	Standard Operating Procedure
TMB	3,3',5,5'-Tetramethylbenzidine

8. REAGENT PREPARATION

Note: Reagents prepared according to this SOP and documented on HSL_LAB_007.01: HPV ELISA Data Capture Form, are for use only with the associated assay where the reagent is documented. Reagents prepared and documented on HSL_GL_006.01: Reagent Preparation for the HPV Serology Laboratory, 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 48 hours of preparation.

8.1.2.1. 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

Note: Refer to HPV-Type Specific Attachment to determine the optimal concentrations to use per HPV-Type being tested.

9.1. Document plate coating preparation according to Reagent Preparation for the HPV Serology Laboratory, HSL_GL_006.

9.2. Remove VLP aliquot(s) from the -80°C Freezer prior to use.

9.3. After the VLP aliquot(s) is thawed at room temperature (up to one hour), dilute VLP in coating buffer to a final concentration based on VLP-Type Specific Attachments.

9.3.1. For example, when coating six plates for HPV-16 at a concentration of 2.7 µg/mL, prepare a minimum of 70 mL Plate Coating (6 plates * 96 wells * 100 µL per well = 57.6 mL, round to 60 mL then add at least 10mL for dead volume) as follows.

$\boldsymbol{C_1}$ *= 1000 µg/mL (VLP Starting Concentration)*
$\boldsymbol{C_2}$ *= 2.7 µg/mL (HPV-16 Final Plate Coating Concentration)*
$\boldsymbol{V_2}$ *= 70 mL (Total Volume)*

$V_1 = C_2V_2 / C_1$
$V_1 = (2.7*70) / (1000)$
$\boldsymbol{V_1}$ *= 14.5 µL*

14.5 µL of the HPV-16 VLP would be needed to coat 6 plates.

9.3.2. Label plates with the following information: HPV Type, Coating Date/Time, Reagent Lot Number, and Analyst's Initials. Include Date/Time in the Reagent Logbook in the "Expiration Date" column.

9.3.3. Add 100 µL of coating solution to all wells of a 96-well Maxisorp plate.

9.3.4. Cover each plate coated with plate sealers, and tap sides gently to ensure all wells are covered. Visually inspect plate(s) for coverage prior to placing it in a 2-8°C refrigerator.

9.3.5. Plates must be used between 72 and 120 hours.

10. ELISA PROCEDURE

Note: Record all assay information on HSL_LAB_007.01: HPV Antibody ELISA Data Capture Form on the day of testing.

Note: Refer to HPV-Type Specific Attachment to determine the optimal concentrations to use per HPV-Type being tested.

Note: When performing an assay, the analyst can run up to six plates, for a total of 48 samples.

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

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

10.3. Once reagents are equilibrated, remove coated plates from 2-8°C refrigerator. Remove the adhesive film from the coated plates prior to washing.

10.4. Wash plates using the “HPV ELISA” plate washer protocol (see Attachment 6: BioTek Plate Washer Parameters).

10.5. After wash cycle, tap plates gently on an absorbent wipe to remove excess wash buffer.

10.6. Add 300 µL of Blocking Buffer to all wells using a multichannel pipette.

10.7. Cover each plate with a plate sealer and incubate for 90±10 minutes at room temperature (RT) without agitation.

10.8. Wash plates using the “HPV ELISA” plate washer protocol.

10.9. After wash cycle, tap plates gently on an absorbent wipe to remove excess wash buffer.

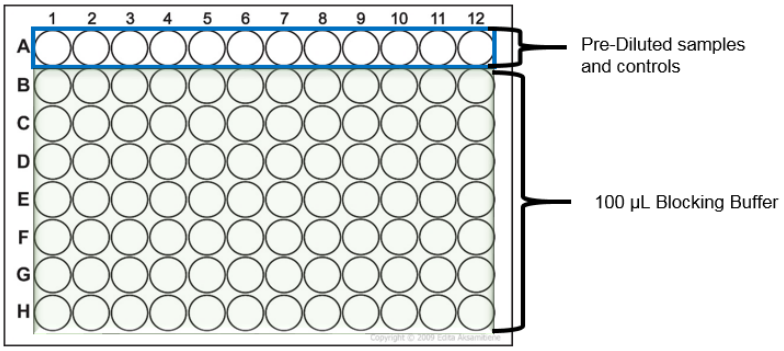
10.10. Determine the starting concentration for standards and samples according to the HPV-Type Specific Attachment.

10.11. Pre-dilute standards, controls and samples in cluster tubes/deep well plate, matching layout according to the plate map.

	1	2	3	4	5	6	7	8	9	10	11	12
	Sample 1	Std	CN	CP1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	CP2
A	100	400	100	300	100	100	100	100	100	100	100	300
B	200	800	200	600	200	200	200	200	200	200	200	600
C	400	1600	400	1200	400	400	400	400	400	400	400	1200
D	800	3200	800	2400	800	800	800	800	800	800	800	2400
E	1600	6400	1600	4800	1600	1600	1600	1600	1600	1600	1600	4800
F	3200	12800	3200	9600	3200	3200	3200	3200	3200	3200	3200	9600
G	6400	25600	6400	19200	6400	6400	6400	6400	6400	6400	6400	19200
H	12800	51200	12800	38400	12800	12800	12800	12800	12800	12800	12800	38400

Note: Concentrations will differ from plate layout above, based on HPV Type being tested.

10.12. Add 100 µL of Blocking Buffer into the plate from Row B to H, Columns 1-12, as seen below.



10.13. Add 200 µL of the pre-diluted standards, controls and samples at the appropriate starting dilutions using a multichannel pipette in Row A using the plate map for standard, control and sample placement.

10.14. Using an electric multichannel pipette, aspirate 100 µL from Row A. Without touching the bottom of the plate, put the tips into the diluent in Row B and mix 10 times. Discard empty tips.

10.15. Continue the serial dilution through to Row H, mixing each dilution ten times and switching tips between each row.

10.16. Discard the extra 100 µL from row H.

10.17. Seal the plate with plate sealer then incubate at room temperature for 60±5 minutes with gentle shaking between 200-300 rpm.

10.18. Prior to washing plates, dilute conjugate in Blocking Buffer, to a final concentration according to the HPV-Type Specific Attachment. Prepare approximately 12 mL of conjugate per plate.

Note: If the conjugate is diluted prior to storage, two dilutions may not be required on the day of the assay. Below is an example for diluting conjugate to achieve a final dilution of 0.0167 µg/mL.

Dilution 1 (1:100)		
C_1	=	0.5 mg/mL
V_1	=	10 µL
C_2	=	5 µg/mL
V_2	=	1000 µL

Dilution 2 (1:300)		
C_1	=	5 µg/mL
V_1	=	40 µL
C_2	=	0.0167 µg/mL
V_2	=	12000 µL

10.19. Wash plates using the “HPV ELISA” plate washer protocol.

10.20. After wash cycle, tap plates gently on an absorbent wipe to remove excess wash buffer.

10.21. Using a multichannel pipette, add 100 µL of diluted conjugate to all wells on the plate.

10.22. Seal the plates with plate sealers then incubate at room temperature for 60±5 minutes with gentle shaking between 200-300 rpm.

10.23. Prepare TMB solution approximately 15 minutes prior to use and protected from light.

10.24. Prepare 12 mL TMB solution per plate by mixing 6 mL of TMB Reagent A with 6 mL of TMB Reagent B.

10.25. Wash plates using the “HPV ELISA” plate washer protocol.

10.26. After wash cycle, tap plates gently on an absorbent wipe to remove excess wash buffer.

10.27. Using a multichannel pipette, add 100 µL of the TMB solution into each well.

Note: It is recommended to start at the lowest concentration and work up (e.g. Row H to A).

10.28. Seal the plates with plate sealers then incubate at room temperature for 25±2 minutes protected from light.

10.29. During the TMB incubation, turn on the Spectramax M5 and open the template (H:\HSL\M5 Plate Reader\SoftMax Templates\HPV ELISA) according to the HPV Type Specific assay being performed.

10.30. Enter in assay information such as dilution factors and data references, then save file as shown below.

"Data Reference_HPVType_DDMMYYAnalyst Initials"
(*LB12345P001_HPV16_20MAY17ABC*)

10.31. Add 100 µL of 0.36N H_2SO_4 to stop reaction.

10.32. Carefully place plate in M5 Plate Reader, and select "Read" on the computer. Save file after all plates have been read. Print file and store in the associated Raw Data Binder.

11. SYSTEM SUITABILITY CRITERIA

11.1. Standard Curve

Parameter	Acceptance Criteria
R^2	≥ 0.990
OD of First Std Dil (A2)	2.0 OD – 4.0 OD
OD of Last Std Dil (H2)	≤ 0.25 OD
ODs for Std Dilutions 1-7	The difference observed between the ODs of the n and the n+1 serial dilutions is ≥ 30% (Delta(OD"Std"))/OD "Std" x 100 >=30%
CV determined on the serial standard values adjusted for dilution	≤ 30%
Back-fit dose, Std Curve Dilution 2	Recalculation must be within 90-110% of the theoretical concentration of the standard

11.2. Controls

Parameter	Acceptance Criteria
Calculated concentration of the negative control	Lower than the cut-off
Calculated concentration of the positive control	Within the range
CV on the serial dilution of the positive control adjusted for dilution	≤ 30%
CV between the 2 replicates of the positive control	≤ 16%
ODn of the Positive Control Dilutions 1-7	The difference observed between the ODs of the n and the n+1 serial dilutions is ≥ 30% (Delta(OD"Std"))/OD "Std" x 100 ≥ 30%
CV calculated on average valid concentration	≤ 30%

12. DATA ACCEPTANCE CRITERIA

Note: Data Acceptance Criteria is for serum samples only. Consult with Scientific Manager to obtain Data Acceptance Criteria for Non-Serum Samples.

12.1. For concentration >20 EU/mL, serum antibody concentration is accepted if the Percent CV is ≤ 30%.

12.2. For concentration 4-20 EU/mL, the serum sample is reanalyzed according to Attachment 5: Repeat Algorithm.

12.3. For concentration <4 EU/mL, the serum concentration is considered negative for the specific HPV type.

13. ATTACHMENTS

13.1. Attachment 1: HPV-16 ELISA Specifications

13.2. Attachment 2: HPV-18 ELISA Specifications

13.3. Attachment 3: HPV-6 ELISA Specifications

13.4. Attachment 4: HPV-11 ELISA Specifications

13.5. Attachment 5: Repeat Algorithm

13.6. Attachment 6: BioTek Plate Washer Parameters

Attachment 1: HPV-16 ELISA Specifications

Parameter	Optimal Value
ELISA Plate **Coating** Concentration	2.7 µg/mL
Initial Dilution Factor of **Standard**	1:800
Initial Dilution Factor for **Serum** Samples	1:100
Negative Control (**CN**) Starting Dilution Factor	1:100
Positive Control (**PC**) Starting Dilution Factor	1:300
Final Optimal Concentration for **Conjugate**	0.0167 µg/mL

Attachment 2: HPV-18 ELISA Specifications

Parameter	Optimal Value
ELISA Plate **Coating** Concentration	2.7 µg/mL
Initial Dilution Factor of **Standard**	1:600
Initial Dilution Factor for **Serum** Samples	1:100
Negative Control (**CN**) Starting Dilution Factor	1:100
Positive Control (**PC**) Starting Dilution Factor	1:300
Final Optimal Concentration for **Conjugate**	0.0333 µg/mL

Attachment 3: HPV-6 ELISA Specifications

Parameter	Optimal Value
ELISA Plate **Coating** Concentration	0.54 µg/mL
Initial Dilution Factor of **Standard**	1:100
Initial Dilution Factor for **Serum** Samples	1:100
Negative Control (**CN**) Starting Dilution Factor	1:100
Positive Control (**PC**) Starting Dilution Factor	1:200
Final Optimal Concentration for **Conjugate**	0.0040 µg/mL

Attachment 4: HPV-11 ELISA Specifications

Parameter	Optimal Value
ELISA Plate **Coating** Concentration	0.54 µg/mL
Initial Dilution Factor of **Standard**	1:200
Initial Dilution Factor for **Serum** Samples	1:100
Negative Control (**CN**) Starting Dilution Factor	1:100
Positive Control (**PC**) Starting Dilution Factor	1:400
Final Optimal Concentration for **Conjugate**	0.0050 µg/mL

Attachment 5: Repeat Algorithm

Test 1	Test 2	Test 3	Final result
T1 <4			FN
FAC-	T2 < cut-off	-	FN
	FAC-	-	FN
	FAC+	T3<4	FN
		FAC-	FN
		FAC+	T2
		T3>20	T2 if T3/T2<=2
			IR if T3/T2 >2
		CV	FIR
		1pt	FIR
		QNS	QNS
	T2>20	T3<4	FN
		FAC-	FN
		FAC+	T2 IF T2/T3 <=2
			FIR if T2/T3 >2
		CV	FIR
		1pt	FIR
		QNS	QNS
	CV	T3 <4	FN
		FAC-	FN
		FAC+	FIR
		T3>20	FIR
		CV	FAP
		1pt	FAP
		QNS	QNS
	1pt	T3 < 4	FN
		FAC-	FN
		FAC+	FIR
		T3 > 20	FIR
		CV	FIR
		1pt	FIR
		QNS	QNS
	QNS	-	QNS

Test1	Test2	Test3	Final results
FAC+	T2 < 4	T3 < 4	FN
		FAC-	FN
		FAC+	T1
		T3 > 20	T2 if T3/T1 <= 2
			FIR if T3/T1 >2
		CV	FIR
		1pt	FIR

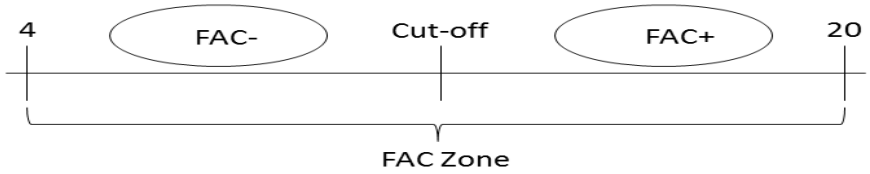
		QNS	QNS
	FAC-	T3 < 4	FN
		FAC-	FN
		FAC+	T1
		T3 > 20	T1 if T3/T1 <=2
			FIR if T3/T1 > 2
		CV	FIR
		1pt	FIR
		QNS	QNS
	FAC+	-	T1
	T2 >20	-	T1 if T2/T1 <=2 To be retested if T2/T1>2
		T3 < 4	FIR
		FAC-	FIR
		FAC+	T1
		T3 > 20	T2 if 0.5 <=T3/T2 <=2 If this is not the case, the final result is IR
		CV	FIR
		1pt	FIR
	CV	T3 < 4	FIR
		FAC-	FIR
		FAC+	T1
		T3 > 20	T1 IF T3/T1 <= 2 FIR if T3/T1 > 2
		CV	FAP
		1pt	FIR
		QNS	QNS
	1pt	T3 < 4	FIR
		FAC-	FIR
		FAC+	T1
		T3 > 20	FIR
		CV	FIR
		1pt	FIR
		QNS	QNS
	QNS	-	QNS

T1 > 20	-	-	T1

CV	T2 < 4	-	FN
	FAC-	T3 < 4	FN
		FAC-	FN
		FAC+	FIR
		T3 > 20	FIR
		CV	FAP

		1pt	FIR
		QNS	QNS
	FAC+	T3 < 4	FIR
		FAC-	FIR
		FAC+	T2
		T3 > 20	T2 IF T3/T2 <= 2 FIR if T3/T2 > 2
		CV	FAP
		1pt	FIR
		QNS	QNS
	T2 > 20	-	T2
	CV	T3 < 4	FN
		FAC-	FAP
		FAC+	FAP
		T3 > 20	T3
		CV	FAP
		1pt	FIR
		QNS	QNS

Terminology:
1. FAC Zone: HPV16 Cut-off value is 8, HPV18 Cut-off value is 7



2. AP: Absence of parallelism
3. CV: A CV result is given if the CV is >= 50% (serum with concentration between the cut-off value and 20 EU/mL) or >=30% (serum with concentration > 20 EU/mL)
4. FAC-: Floating Assay Cut-off negative; Zone with concentrations from 4 to cut-off
5. FAC+: Floating Assay Cut-off positive; Zone with concentrations from cut-off to 20
6. FAP: Final Result “AP”
7. FIR: Final Result “IR”
8. FN: Final Result “Negative”
9. FR: Final Result
10. IR: Invalid Result
11. QNS: Quality Not Sufficient
12. T1, T2 or T3: Test1, Test2 or Test3
13. 1 point: One point available for the calculation

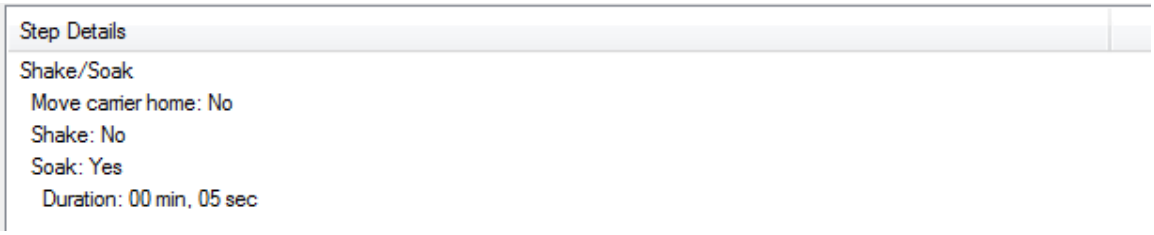
Attachment 6: BioTek Plate Washer Parameters

Protocol Steps

W-Wash 350 µL of buffer B for 3 cycles
Soak for 00:05
W-Aspirate, travel rate 3, delay 0 msec
<end of steps>

Step Details

W-Wash
Pre-dispense before washing: No
Bottom Wash: Yes
Buffer: B
Volume: 300 µL/well
Flow Rate: 7
Z Offset: 121 steps (15.37 mm above carrier)
X Offset: 0 steps (center of well)
Y Offset: 0 steps (center of well)
Pre-dispense: not available
Number of Wash Cycles: 3
Aspirate per cycle
Travel Rate: 3 7.3 & 1.0 mm/sec
Delay: 0 msec
Z Offset: 27 steps (3.43 mm above carrier)
X Offset: 0 steps (center of well)
Y Offset: 38 steps (2.81 mm front of center)
Secondary Aspirate: No
Dispense per cycle
Buffer: B
Volume: 350 µL/well
Flow Rate: 7
Z Offset: 121 steps (15.37 mm above carrier)
X Offset: 0 steps (center of well)
Y Offset: 0 steps (center of well)
Pre-dispense: not available
Delay start of Vacuum until Volume dispensed: 0 µL/well
Shake/Soak after dispense: No
Pre-dispense between cycles: No
Final Aspirate: No


Step Details
Shake/Soak
Move carrier home: No
Shake: No
Soak: Yes
Duration: 00 min, 05 sec


Step Details
W-Aspirate
Travel Rate: 3 7.3 & 1.0 mm/sec
Delay: 0 msec
Z Offset: 27 steps (3.43 mm above carrier)
X Offset: -20 steps (0.91 mm left of center)
Y Offset: 38 steps (2.81 mm front of center)
Secondary Aspirate: No

14. REVISION HISTORY

Revision Start Date	Version #	Changes	Reasons
18Sep17	New	Create new SOP for HPV ELISA Antibody concentrations.	New SOP.

HPV Type: _____

Reagents

Reagent	Identification Number	Expiration Date
Coated Plate (Conc._____µg/mL)		
Standard		
CN control		
CP control		
DPBS		
Tween-20		
Skim Milk		
1X Wash Buffer		
Conjugate (Conc. _____µg/mL)		
TMB Solution		
0.36N H_2SO_4		

Equipment

Equipment Description	Equipment ID	Calibration Due Date
BSC	☐ HSL_007 ☐ HSL_008 ☐ HSL_009 ☐Other:	
2-8°C Refrigerator	☐ HSL_029 ☐Other:	
LN_2 Freezer	☐ HSL_028 ☐Other:	
-80°C Freezer	☐ HSL_022 ☐Other:	
-20°C Freezer	☐ HSL_038 ☐ HSL_034 ☐Other:	☐N/A
Balance	☐ HSL_015 ☐ HSL_016 ☐ Other:	
Plate Shaker	☐HSL_030 ☐HSL_031 ☐HSL_032 ☐Other:	
M5 Plate Reader	☐HSL_018 ☐Other:	
☐N/A Pipette: µL		
☐N/A Pipette: µL		
☐N/A Pipette: µL		
☐N/A Pipette: µL		
☐N/A Pipette: µL		
☐N/A Pipette: µL		
☐N/A Pipette: µL		

Comments:

☐N/A

Performed By/ Date:	
Reviewed By/ Date:	

Reagent Preparation

	Amount of Skim Milk	Volume of PBS	Volume of Tween-20
Blocking Buffer	g	mL	µL

	Starting Concentration	Dilution Factor	Volume of Conjugate	Volume of Blocking Buffer
_____ µg/mL Conjugate				
	□N/A		µL of initial dilution	

	TMB Reagent A	TMB Reagent B
TMB Solution	mL	mL

Standard Preparation

	Dilution Factor	Volume of Standard (µL)	Volume of Blocking Buffer (µL)
Dilution 1			
□N/A Dilution 2		µL of initial dilution	

Control Preparation

	Dilution Factor	Volume of Control (µL)	Volume of Blocking Buffer (µL)
Negative Control (CN)			
Positive Control (CP)			
	□N/A	µL of initial dilution	

Comments

□N/A

Performed By/ Date:	
Reviewed By/ Date:	

Sample Preparation

		Sample ID	Sample Type (*S=Serum, M=Mouthwash, Sp=Sponge*)	Starting Dilution Factor	Volume of Sample (µL)	Volume of Blocking Buffer (µL)
Plate 1 □N/A	1	□N/A				
	2	□N/A				
	3	□N/A				
	4	□N/A				
	5	□N/A				
	6	□N/A				
	7	□N/A				
	8	□N/A				
Plate 2 □N/A	9	□N/A				
	10	□N/A				
	11	□N/A				
	12	□N/A				
	13	□N/A				
	14	□N/A				
	15	□N/A				
	16	□N/A				
Plate 3 □N/A	17	□N/A				
	18	□N/A				
	19	□N/A				
	20	□N/A				
	21	□N/A				
	22	□N/A				
	23	□N/A				
	24	□N/A				

Comments

□N/A

Performed By/ Date:	
Reviewed By/ Date:	

Sample Preparation continued

		Sample ID	Sample Type (*S=Serum, M=Mouthwash, Sp=Sponge*)	Starting Dilution Factor	Volume of Sample (µL)	Volume of Blocking Buffer (µL)
Plate 4 □N/A	25	□N/A				
	26	□N/A				
	27	□N/A				
	28	□N/A				
	29	□N/A				
	30	□N/A				
	31	□N/A				
	32	□N/A				
Plate 5 □N/A	33	□N/A				
	34	□N/A				
	35	□N/A				
	36	□N/A				
	37	□N/A				
	38	□N/A				
	39	□N/A				
	40	□N/A				
Plate 6 □N/A	41	□N/A				
	42	□N/A				
	43	□N/A				
	44	□N/A				
	45	□N/A				
	46	□N/A				
	47	□N/A				
	48	□N/A				

Comments

□N/A

Performed By/ Date:	
Reviewed By/ Date:	

*Incubations

	Start	End	Total Time (mins)
Blocking			
Sample Incubation			
Conjugate			
TMB Solution		Stop:	

*Record Start and End times for first plate only.

Raw Data File References

□N/A Plate 1:
□N/A Plate 2:
□N/A Plate 3:
□N/A Plate 4:
□N/A Plate 5:
□N/A Plate 6:

**Results

	□N/A **Plate 1**	□N/A **Plate 2**	□N/A **Plate 3**	□N/A **Plate 4**	□N/A **Plate 5**	□N/A **Plate 6**
Standard Curve	□Pass □Fail	□Pass □Fail	□Pass □Fail	□Pass □Fail	□Pass □Fail	□Pass □Fail
Negative Control	□Pass □Fail	□Pass □Fail	□Pass □Fail	□Pass □Fail	□Pass □Fail	□Pass □Fail
Positive Controls	□Pass □Fail	□Pass □Fail	□Pass □Fail	□Pass □Fail	□Pass □Fail	□Pass □Fail

**Samples

	□N/A **Plate 1**	□N/A **Plate 2**	□N/A **Plate 3**	□N/A **Plate 4**	□N/A **Plate 5**	□N/A **Plate 6**
Position 1	□Pass □Repeat	□Pass □Repeat	□Pass □Repeat	□Pass □Repeat	□Pass □Repeat	□Pass □Repeat
Position 2	□Pass □Repeat	□Pass □Repeat	□Pass □Repeat	□Pass □Repeat	□Pass □Repeat	□Pass □Repeat
Position 3	□Pass □Repeat	□Pass □Repeat	□Pass □Repeat	□Pass □Repeat	□Pass □Repeat	□Pass □Repeat
Position 4	□Pass □Repeat	□Pass □Repeat	□Pass □Repeat	□Pass □Repeat	□Pass □Repeat	□Pass □Repeat
Position 5	□Pass □Repeat	□Pass □Repeat	□Pass □Repeat	□Pass □Repeat	□Pass □Repeat	□Pass □Repeat
Position 6	□Pass □Repeat	□Pass □Repeat	□Pass □Repeat	□Pass □Repeat	□Pass □Repeat	□Pass □Repeat
Position 7	□Pass □Repeat	□Pass □Repeat	□Pass □Repeat	□Pass □Repeat	□Pass □Repeat	□Pass □Repeat
Position 8	□Pass □Repeat	□Pass □Repeat	□Pass □Repeat	□Pass □Repeat	□Pass □Repeat	□Pass □Repeat

**See Raw Data File for individual results.

Comments

□N/A

Performed By/ Date:	
Reviewed By/ Date:	