

**SOP Title:** Acrylamide Protein Gel Analysis of HPV Virus-Like Particles (VLPs)

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## 1. PURPOSE

- 1.1. The purpose of this procedure is to describe acrylamide gel protein analysis to determine the presence of virus-like particles (VLPs) produced in the Human Papillomavirus (HPV) Serology Laboratory.

## 2. SCOPE

- 2.1. This procedure applies to the HPV Serology Laboratory located at the Advanced Technology Research Facility (ATRF), Room C2007.

## 3. REFERENCES

- 3.1. Mini Gel Tank Manual
- 3.2. HSL\_EQ\_001: Biosafety Cabinet (BSC) Use and Maintenance
- 3.3. HSL\_EQ\_007: Use and Maintenance of a 2-8°C Refrigerator in the HPV Serology Laboratory
- 3.4. HSL\_EQ\_008: Use and Maintenance of -80°C Freezers in the HPV Serology Laboratory
- 3.5. HSL\_EQ\_009: Use and Maintenance of the Liquid Nitrogen Freezer
- 3.6. HSL\_EQ\_012: Use and Maintenance of Pipettes in the HPV Serology Laboratory
- 3.7. HSL\_EQ\_013: Use and Maintenance of the ThermoScientific Thermal Mixer
- 3.8. HSL\_EQ\_016: Use and Maintenance of -20°C Freezers in the HPV Serology Laboratory
- 3.9. HSL\_EQ\_019: Use and Maintenance of the Milli-Q Integral 3 Water System
- 3.10. HSL\_GL\_001: Waste Disposal at the Advanced Technology Research Facility
- 3.11. HSL\_QS\_021: Control, Creation, Issuance and Use of Requested Documents
- 3.12. HSL\_QS\_022: Lot Number Assignment

## 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. DEFINITIONS

Term	Definition
APG	Acrylamide Protein Gel
MW	Molecular Weight
Type I Water	Ultrapure/Reagent Grade/Critical applications
Type II Water	Pure/Analytical Grade, used for standard applications

## 6. REAGENTS, MATERIALS AND EQUIPMENT

### 6.1. Equipment

- 6.1.1. Biosafety Cabinet (BSC)
- 6.1.2. 2-8°C Refrigerator
- 6.1.3. Minifuge
- 6.1.4. Mini Gel Tank
- 6.1.5. Power Ease 90W Power Supply or equivalent
- 6.1.6. ThermoScientific Thermomixer with PCR tube adapter
- 6.1.7. Platform rocker
- 6.1.8. Gel Scanner (Canon Cano or equivalent)
- 6.1.9. Milli-Q System
- 6.1.10. Gel Storage Box
- 6.1.11. Pipettes

### 6.2. Reagents

- 6.2.1. 4X Bolt Lithium Dodecyl Sulfate (LDS) Sample Buffer (Life Technologies, Cat # B0007 or equivalent)
- 6.2.2. 10X Bolt Sample Reducing Agent (Life Technologies, Cat # B0009)
- 6.2.3. 4-12% Bolt Bis-Tris Mini gel (Life Technologies, Cat # NW04122BOX or equivalent)

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- 6.2.4. 20X Bolt 4-Morpholineethanesulfonic acid (MES) Sodium Lauryl Sulfate (SDS) Running Buffer (Life Technologies, Cat # B000202 or equivalent)
- 6.2.5. Novex Sharp Pre-Stained Standard (Life Technologies, Cat # LC5800)
- 6.2.6. Simply Blue Safe Stain (Life Technologies, Cat # LC6065 or equivalent)
- 6.2.7. Dulbecco's Phosphate Buffered Saline (DPBS) (Life Technologies, Cat # 14190-235)
- 6.2.8. Type II water (Milli-Q, HSL\_EQ\_019)
- 6.2.9. Type I water (Milli-Q, HSL\_EQ\_019)

6.3. Consumables

- 6.3.1. 0.2 mL PCR Tubes (Thomas Scientific, Cat # 1194T02 or equivalent)
- 6.3.2. Pipette tips
- 6.3.3. 1L Media Storage Bottle (Thomas Scientific, Cat # 1743D15 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 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. Record experiments on "HSL\_LAB\_010.01: Acrylamide Protein Gel Data Capture Form." Up to four gels may be analyzed per set of forms.
- 8.2. The Assay Tracking Number is assigned per "HSL\_QS\_021: Control, Creation, Issuance and Use of Requested Documents" and the Data Reference is assigned per "HSL\_QS\_022: Lot Number Assignment."

**9. PROCEDURE**

- 9.1. Preheat the Thermomixer to 95°C per "HSL\_EQ\_013: Use and Maintenance of the ThermoScientific Thermal Mixer."

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9.2. Unpack and prepare 4%-12% Bolt Bis-Tris mini-gels by removing the plastic well protector and gently rinsing the gel cartridge and wells with Type II water. Make sure that the acrylamide wells are open, and wells are uninhibited by folded acrylamide after rinsing the gel.

9.3. Remove the tape near the bottom of the gel cartridge. Failure to remove the tape will impede the current from running through the gel correctly.

9.4. Place the precast gels in the Invitrogen Mini Gel Tank per manual.

**Note:** The Bolt gel tank holds two mini gels. Leave one compartment empty when running one gel.

9.5. Prepare 1X MES SDS running buffer from the 20X MES SDS running buffer stock and pour into the gel tank up to the fill line.

9.5.1. For two gels, prepare 1L of 1X MES SDS running buffer. Add 50 mL of 20X MES SDS running buffer to 950 mL Type II water in a media storage bottle.

9.5.2. For one gel, prepare 500 mL of 1X MES SDS running buffer.

**Note:** The tank contains an overflow chamber. If buffer goes past the fill line, it will flow into the chamber on the side of the tank; this will not interfere with the run.

9.6. Label/mark the PCR tubes with the sample ID.

9.7. Thaw samples on wet ice.

9.8. Prepare samples in 4X Bolt LDS sample buffer and 10X Bolt Sample Reducing Agent in the BSC.

9.8.1. To test a sample, add 2.5  $\mu$ L of fraction sample, 7.25  $\mu$ L of DPBS (diluent), 3.75  $\mu$ L of 4X Bolt LDS sample buffer, and 1.5  $\mu$ L of 10X Bolt Sample Reducing Agent.

9.8.2. If testing multiple samples in a gel, perform the following steps.

9.8.2.1. Make a cocktail of DPBS, 4X Bolt LDS sample buffer and 10X Bolt Sample Reducing Agent by adjusting volumes or using amounts described in Table 1.

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Table 1: Cocktail Preparation for Multiple Samples

Reagent/Sample	Amount ( $\mu\text{L}$ ) per tube to test one sample	Amount ( $\mu\text{L}$ ) per tube to test 22 samples	Amount ( $\mu\text{L}$ ) per tube to test 44 samples
DPBS	7.25	183	366
4X Bolt LDS sample buffer	3.75	95	190
10X Bolt Sample Reducing Agent	1.5	38	76

**Note:** When preparing cocktail for more than one sample, volumes must be adjusted up to dispense appropriate amount into sample vials.

9.8.2.2. Add 12.5  $\mu\text{L}$  of prepared cocktail to each tube, then add 2.5  $\mu\text{L}$  of fraction sample to tubes.

- 9.9. Place the lids on the PCR tubes. Gently mix samples by tapping on the PCR tubes.
- 9.10. Spin down in a minifuge for approximately 5 seconds.
- 9.11. Place the tubes in the preheated Thermomixer and heat at  $95 \pm 2^\circ\text{C}$  for 10 minutes.
- 9.12. Remove the tubes from the Thermomixer.
- 9.13. Spin tubes in a minifuge for approximately 10 seconds to bring down the liquid on the wall of the tubes.
- 9.14. Load 8-10  $\mu\text{L}$  of the standard (ladder) and entire volume of each prepared sample into the gel wells. See "Attachment 1: Gel Layout" for an example of sample positioning.
- 9.15. Attach lid to gel apparatus. Refer to Mini Gel Tank manual.
- 9.16. Run gel at max voltage (not greater than 200V) with a constant amp. Set amp to 130 mA and run gel for 1-2 hours. Adjustments to time/speed may be necessary as voltage will vary for each gel run.
 

**Note:** The 15-20 kDa MW marker will be near the bottom of the gel. Refer to "Attachment 2: Protein Markers" to identify band correspondences.
- 9.17. Once the run is complete, turn off the power pack and remove the gels from the gel apparatus.
  - 9.17.1. Remove the gels from the plastic casing by gently cracking the plastic with the gel knife and removing the top of the case.
  - 9.17.2. Unseal the gel from the plate by taking the gel knife and cutting the sides of the gel.

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- 9.17.3. Hold the plate and push the knife into the plug slot at the bottom of the plate to release the gel.
- 9.17.4. The plug and the well dividers can be cut off if desired, though this is only necessary if placing on a membrane to transfer.
- 9.18. Carefully move gels into a plastic container and wash with Type II water three times to remove residual buffer following method 1 or method 2.
- 9.18.1. Method 1:
- 9.18.1.1. Add approximately 100 mL Type II water.
- 9.18.1.2. Gently rock gels for five minutes at room temperature using a platform shaker.
- 9.18.1.3. Discard water into the sink or labeled waste container.
- 9.18.1.4. Repeat steps 9.18.1.1 to 9.18.1.3 two times.
- 9.18.2. Method 2:
- 9.18.2.1. Rinse gel with Type II water.
- 9.18.2.2. Add 100 mL Type II water.
- 9.18.2.3. Microwave up to one minute. Do not allow water to boil or dry out the gel.
- 9.18.2.4. Rock on a platform shaker for one minute.
- 9.18.2.5. Discard water into the sink or labeled waste container.
- 9.18.2.6. Repeat steps 9.18.2.2 to 9.18.2.5 two times.
- 9.19. Add approximately 30 mL of SimplyBlue to adequately cover the gels. Perform Method 1 or Method 2.
- 9.19.1. Method 1: Rock gently for 60 minutes to 120 minutes at room temperature on a platform rocker.
- 9.19.2. Method 2:
- 9.19.2.1. Microwave gel with SimplyBlue up to one minute. Do not allow SimplyBlue to boil or dry out the gel.

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9.19.2.2. Rock gently for 30 minutes to 60 minutes at room temperature on a platform rocker.

9.20. Decant the SimplyBlue and rinse quickly with 100 mL of Type II water. Remove the water and add 100 mL Type II water.

9.21. Using a platform rocker, rock at a medium speed for 30-60 minutes at room temperature. Change the water at least once during the incubation to help remove stain.

9.22. Store gel in Type II water at 2-8°C overnight in Gel Storage box labeled with the data reference, gel number (1 or 2), date and analyst initials.

**Note:** Gels should remain submerged in Type II water at all times. Scanning should occur within one week of preparation; otherwise, make note in logbook.

**Note:** Store one gel per Gel Storage box.

9.23. Name the electronic file/picture as follows: Assay Tracking Number / Data Reference\_APG\_Date&Initials\_Gel Number. Use the first page of HSL\_LAB\_010.01 as the page number in the data reference.

For example:

L0003001\_APG\_01Jan17ABC\_1 for gel 1.

L0003001\_APG\_01Jan17ABC\_2 for gel 2.

9.24. Save an electronic copy as a ".TIFF" file [REDACTED].

9.25. Print gel image with molecular weight label and attach to HSL\_LAB\_010.01.

## 10. SYSTEM SUITABILITY

10.1. Reject a gel if the molecular ladder is not distributed throughout 80% of the full length of the gel.

10.2. Reject a gel if there is a severe wavy appearance throughout the bands in the various lanes of the gel.

For example, if the predominant VLP L1 band at 56 kDa may be associated with a molecular weight higher or lower than the 60 and 50 kDa ladder markers when viewing each sample horizontally, then the samples must be retested.

10.3. Consult with Scientific Manager if there are ambivalent results, or uncertainty with interpretation.

10.4. Record comment on HSL\_LAB\_010.01 if gel does not meet system suitability criteria.

## 11. DATA ANALYSIS

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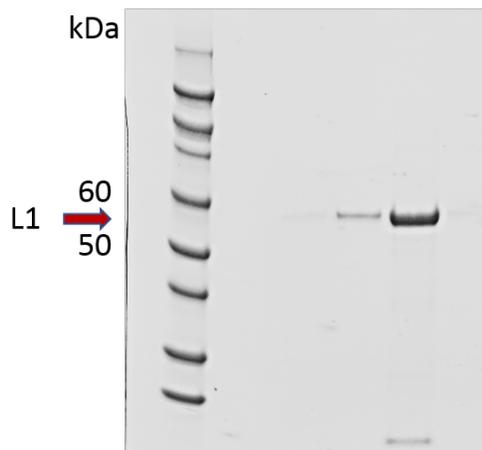
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- 11.1. The molecular weight of HPV L1 is approximately 56kDa. Wells demonstrating a significant band between the 50 and 60kDa markers are considered “Band Observed.” See Figure 1. Record on HSL\_LAB\_010.01.

Figure 1: Example VLP Bands



- 11.2. If a well does not present with a significant band between the 50 and 60 kDa markers it is recorded as “Band not Observed” on HSL\_LAB\_010.01.
- 11.3. Repeat a well (sample) if the correct MW is unable to be determined (for example, there is a significant curvature of the proteins throughout the lane). Select “Repeat” on HSL\_LAB\_010.01.

**Note:** A well may have more than one selection on HSL\_LAB\_010.01. For example, “Band not Observed” and “Repeat” may both be selected for one well.

## 12. ATTACHMENTS

- 12.1. Attachment 1: Gel Layout
- 12.2. Attachment 2: Protein Marker
- 12.3. Attachment 3: HSL\_LAB\_010.01: Acrylamide Protein Gel Data Capture Form

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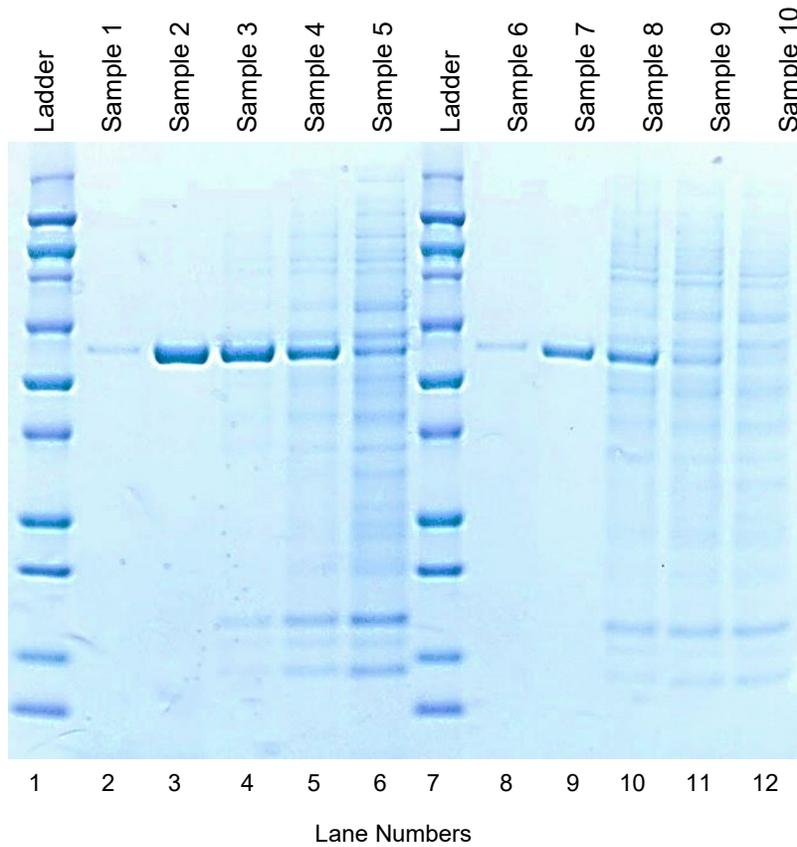
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**Attachment 1: Gel Layout**



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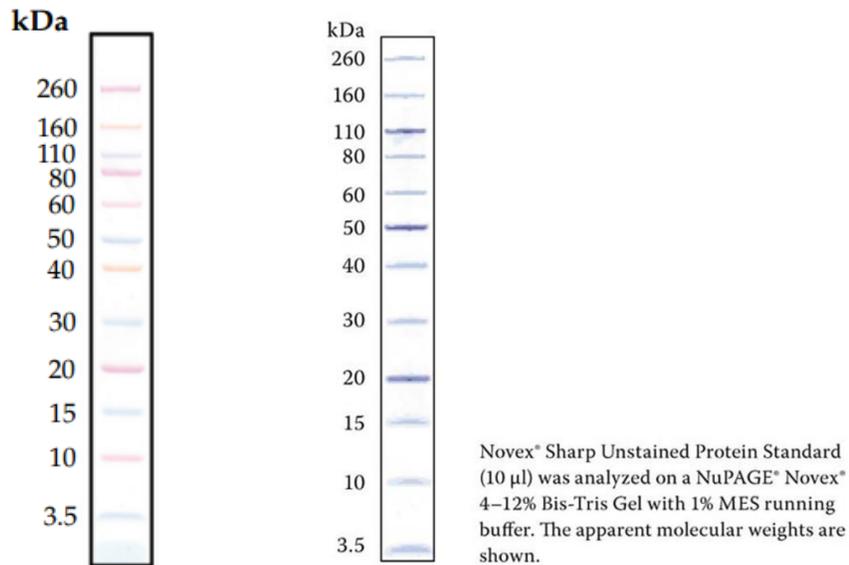
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**Attachment 2: Protein Marker**



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**Attachment 3: HSL\_LAB\_010.01: Acrylamide Protein Gel Data Capture Form**

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<p><b>Form Title:</b> Acrylamide Protein Gel Data Capture Form</p>			
<p><b>Document ID:</b> HSL_LAB_010.01</p>		<p>Version:</p>	<p>4.0</p>
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**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:	
2-8°C Refrigerator	<input type="checkbox"/> HSL_029 <input type="checkbox"/> Other:	<input type="checkbox"/> N/A
Power Supply	<input type="checkbox"/> HSL_013 <input type="checkbox"/> HSL_048 <input type="checkbox"/> Other:	<input type="checkbox"/> N/A
Thermomixer	<input type="checkbox"/> HSL_035 <input type="checkbox"/> Other:	<input type="checkbox"/> N/A
<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
4X Bolt LDS Sample Buffer		
10X Bolt Sample Reducing Agent		
4-12% Bolt Bis-Tris Mini Gel		
20X Bolt MES SDS Running Buffer		
Novex Sharp Pre-Stained Standard (Ladder)		
Simply Blue Safe Stain Solution		
DPBS		
Type II water	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A

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**1X MES SDS Running Buffer Preparation:**

Reagent	Volume Used (mL)
20X MES SDS Running Buffer	
Type II water	

**Cocktail Pool Preparation:**

	Sample Number (X)
Cocktail Pool Calculated based on (X) Samples:	
Reagent	Volume Used (mL)
DPBS (7.25 µL/Sample)	
4X Bolt LDS Sample Buffer (3.75 µL/Sample)	
10X Bolt Sample Reducing Agent (1.5 µL/Sample)	

Comments:

LNA

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Well/Sample Identification

Gel #1			Disposition	Affix Gel Image:
Sample Lot Number(s):				
Gel File Name (.tiff):				
Well 1 <input type="checkbox"/> N/A	<input type="checkbox"/> Ultratube # _____; Fraction # _____ <input type="checkbox"/> Ladder		<input type="checkbox"/> Band Observed <input type="checkbox"/> Band not Observed <input type="checkbox"/> Repeat	Comments:
Well 2 <input type="checkbox"/> N/A	Ultratube # _____; Fraction # _____		<input type="checkbox"/> Band Observed <input type="checkbox"/> Band not Observed <input type="checkbox"/> Repeat	
Well 3 <input type="checkbox"/> N/A	Ultratube # _____; Fraction # _____		<input type="checkbox"/> Band Observed <input type="checkbox"/> Band not Observed <input type="checkbox"/> Repeat	
Well 4 <input type="checkbox"/> N/A	Ultratube # _____; Fraction # _____		<input type="checkbox"/> Band Observed <input type="checkbox"/> Band not Observed <input type="checkbox"/> Repeat	
Well 5 <input type="checkbox"/> N/A	Ultratube # _____; Fraction # _____		<input type="checkbox"/> Band Observed <input type="checkbox"/> Band not Observed <input type="checkbox"/> Repeat	
Well 6 <input type="checkbox"/> N/A	Ultratube # _____; Fraction # _____		<input type="checkbox"/> Band Observed <input type="checkbox"/> Band not Observed <input type="checkbox"/> Repeat	
Well 7 <input type="checkbox"/> N/A	<input type="checkbox"/> Ultratube # _____; Fraction # _____ <input type="checkbox"/> Ladder		<input type="checkbox"/> Band Observed <input type="checkbox"/> Band not Observed <input type="checkbox"/> Repeat	
Well 8 <input type="checkbox"/> N/A	Ultratube # _____; Fraction # _____		<input type="checkbox"/> Band Observed <input type="checkbox"/> Band not Observed <input type="checkbox"/> Repeat	
Well 9 <input type="checkbox"/> N/A	Ultratube # _____; Fraction # _____		<input type="checkbox"/> Band Observed <input type="checkbox"/> Band not Observed <input type="checkbox"/> Repeat	
Well 10 <input type="checkbox"/> N/A	Ultratube # _____; Fraction # _____		<input type="checkbox"/> Band Observed <input type="checkbox"/> Band not Observed <input type="checkbox"/> Repeat	
Well 11 <input type="checkbox"/> N/A	Ultratube # _____; Fraction # _____		<input type="checkbox"/> Band Observed <input type="checkbox"/> Band not Observed <input type="checkbox"/> Repeat	
Well 12 <input type="checkbox"/> N/A	Ultratube # _____; Fraction # _____		<input type="checkbox"/> Band Observed <input type="checkbox"/> Band not Observed <input type="checkbox"/> Repeat	

N/A

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**HPV Serology Laboratory  
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Form**

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**Gel #2**  N/A No Second Gel

Sample Lot Number(s):		
Gel File Name (.tiff):		Disposition
Well 1 <input type="checkbox"/> N/A	<input type="checkbox"/> Ultratube # _____; Fraction # _____ <input type="checkbox"/> Ladder	<input type="checkbox"/> Band Observed <input type="checkbox"/> Band not Observed <input type="checkbox"/> Repeat
Well 2 <input type="checkbox"/> N/A	Ultratube # _____; Fraction # _____	<input type="checkbox"/> Band Observed <input type="checkbox"/> Band not Observed <input type="checkbox"/> Repeat
Well 3 <input type="checkbox"/> N/A	Ultratube # _____; Fraction # _____	<input type="checkbox"/> Band Observed <input type="checkbox"/> Band not Observed <input type="checkbox"/> Repeat
Well 4 <input type="checkbox"/> N/A	Ultratube # _____; Fraction # _____	<input type="checkbox"/> Band Observed <input type="checkbox"/> Band not Observed <input type="checkbox"/> Repeat
Well 5 <input type="checkbox"/> N/A	Ultratube # _____; Fraction # _____	<input type="checkbox"/> Band Observed <input type="checkbox"/> Band not Observed <input type="checkbox"/> Repeat
Well 6 <input type="checkbox"/> N/A	Ultratube # _____; Fraction # _____	<input type="checkbox"/> Band Observed <input type="checkbox"/> Band not Observed <input type="checkbox"/> Repeat
Well 7 <input type="checkbox"/> N/A	<input type="checkbox"/> Ultratube # _____; Fraction # _____ <input type="checkbox"/> Ladder	<input type="checkbox"/> Band Observed <input type="checkbox"/> Band not Observed <input type="checkbox"/> Repeat
Well 8 <input type="checkbox"/> N/A	Ultratube # _____; Fraction # _____	<input type="checkbox"/> Band Observed <input type="checkbox"/> Band not Observed <input type="checkbox"/> Repeat
Well 9 <input type="checkbox"/> N/A	Ultratube # _____; Fraction # _____	<input type="checkbox"/> Band Observed <input type="checkbox"/> Band not Observed <input type="checkbox"/> Repeat
Well 10 <input type="checkbox"/> N/A	Ultratube # _____; Fraction # _____	<input type="checkbox"/> Band Observed <input type="checkbox"/> Band not Observed <input type="checkbox"/> Repeat
Well 11 <input type="checkbox"/> N/A	Ultratube # _____; Fraction # _____	<input type="checkbox"/> Band Observed <input type="checkbox"/> Band not Observed <input type="checkbox"/> Repeat
Well 12 <input type="checkbox"/> N/A	Ultratube # _____; Fraction # _____	<input type="checkbox"/> Band Observed <input type="checkbox"/> Band not Observed <input type="checkbox"/> Repeat

Affix Gel Image:

Comments:

N/A

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**Gel #4**  N/A No Fourth Gel

Sample Lot Number(s):		Disposition	
Gel File Name (.tiff):		Disposition	
Well 1 <input type="checkbox"/> N/A	<input type="checkbox"/> Ultratube # _____; Fraction # _____ <input type="checkbox"/> Ladder	<input type="checkbox"/> Band Observed <input type="checkbox"/> Band not Observed <input type="checkbox"/> Repeat	
Well 2 <input type="checkbox"/> N/A	Ultratube # _____; Fraction # _____	<input type="checkbox"/> Band Observed <input type="checkbox"/> Band not Observed <input type="checkbox"/> Repeat	
Well 3 <input type="checkbox"/> N/A	Ultratube # _____; Fraction # _____	<input type="checkbox"/> Band Observed <input type="checkbox"/> Band not Observed <input type="checkbox"/> Repeat	
Well 4 <input type="checkbox"/> N/A	Ultratube # _____; Fraction # _____	<input type="checkbox"/> Band Observed <input type="checkbox"/> Band not Observed <input type="checkbox"/> Repeat	
Well 5 <input type="checkbox"/> N/A	Ultratube # _____; Fraction # _____	<input type="checkbox"/> Band Observed <input type="checkbox"/> Band not Observed <input type="checkbox"/> Repeat	
Well 6 <input type="checkbox"/> N/A	Ultratube # _____; Fraction # _____	<input type="checkbox"/> Band Observed <input type="checkbox"/> Band not Observed <input type="checkbox"/> Repeat	
Well 7 <input type="checkbox"/> N/A	<input type="checkbox"/> Ultratube # _____; Fraction # _____ <input type="checkbox"/> Ladder	<input type="checkbox"/> Band Observed <input type="checkbox"/> Band not Observed <input type="checkbox"/> Repeat	
Well 8 <input type="checkbox"/> N/A	Ultratube # _____; Fraction # _____	<input type="checkbox"/> Band Observed <input type="checkbox"/> Band not Observed <input type="checkbox"/> Repeat	
Well 9 <input type="checkbox"/> N/A	Ultratube # _____; Fraction # _____	<input type="checkbox"/> Band Observed <input type="checkbox"/> Band not Observed <input type="checkbox"/> Repeat	
Well 10 <input type="checkbox"/> N/A	Ultratube # _____; Fraction # _____	<input type="checkbox"/> Band Observed <input type="checkbox"/> Band not Observed <input type="checkbox"/> Repeat	
Well 11 <input type="checkbox"/> N/A	Ultratube # _____; Fraction # _____	<input type="checkbox"/> Band Observed <input type="checkbox"/> Band not Observed <input type="checkbox"/> Repeat	
Well 12 <input type="checkbox"/> N/A	Ultratube # _____; Fraction # _____	<input type="checkbox"/> Band Observed <input type="checkbox"/> Band not Observed <input type="checkbox"/> Repeat	

Affix Gel Image:

Comments:

N/A

Performed by/date:

Reviewed by/date:

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