

<b>Frederick National Laboratory for Cancer Research</b> <small>sponsored by the National Cancer Institute</small>	HPV Serology Laboratory Standard Operating Procedure	
Protein Analysis of Virus-Like Particles (VLPs) using the Agilent 2100 Bioanalyzer		
<b>Document ID: HSL_ LAB_011</b>	Version 1.0	Page 1 of 10

Released by/Date Effective:

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Author Name	Title	Signature/Date

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**1. PURPOSE**

- 1.1. The purpose of this procedure is to analyze virus-like particles (VLPs) using the Agilent 2100 Bioanalyzer to assess size and estimate protein concentration.

**2. SCOPE**

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

**3. REFERENCES**

- 3.1. HSL\_ LAB\_011.01: Bioanalyzer Data Capture Form
- 3.2. HSL\_ GL\_001: Waste Disposal at the Advanced Technology Research Facility
- 3.3. HSL\_ GL\_002: Equipment Qualification and Calibration in the HPV Serology Laboratory
- 3.4. HSL\_ GL\_003: Good Documentation Practices for the HPV Serology Laboratory
- 3.5. HSL\_ GL\_004: Laboratory Notebook Control and Use for the HPV Serology Laboratory
- 3.6. HSL\_ GL\_005: Signature and Initial Identification System
- 3.7. HSL\_ GL\_006: Reagent Preparation for the HPV Serology Laboratory
- 3.8. HSL\_ GL\_007: Reagent and Chemical Expiry in the HPV Serology Laboratory
- 3.9. HSL\_ GL\_008: Laboratory Flow and Gowning Procedures for the HPV Serology Laboratory
- 3.10. HSL\_ GL\_009: HPV Serology Laboratory BSL-2 Procedures
- 3.11. HSL\_ GL\_010: Control and Request of Documents in the HPV Serology Laboratory
- 3.12. HSL\_ EQ\_007: Use and Maintenance of a 2-8°C Refrigerator in the HPV Serology Laboratory
- 3.13. HSL\_ EQ\_012: Use and Maintenance of Pipettes in the HPV Serology Laboratory
- 3.14. HSL\_ EQ\_013: Use and Maintenance of the ThermoScientific Thermal Mixer
- 3.15. HSL\_ EQ\_016: Use and Maintenance of -20°C Freezers in the HPV Serology Laboratory

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3.16. HSL\_EQ\_027: Use and Maintenance of the Agilent Bioanalyzer

#### **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. -20°C Freezer
- 5.1.3. 2-8°C Refrigerator
- 5.1.4. Thermal Mixer
- 5.1.5. Refrigerated micro centrifuge
- 5.1.6. Agilent Bioanalyzer 2100

##### 5.2. Reagents and Chemicals

- 5.2.1. Agilent Protein 230 Kit (Agilent, Cat # 5067-1517)
- 5.2.2. Dithiothreitol (DTT) Solution 1M (Sigma, Cat # 646563-10X.5ML)
- 5.2.3. Type I Water
- 5.2.4. Wet Ice

##### 5.3. Consumables

- 5.3.1. Pipette Tips
- 5.3.2. PCR Tubes (0.2 mL) (VWR, Cat # 75796-164)

#### **6. HEALTH AND SAFETY CONSIDERATIONS**

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- 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
ATRF	Advanced Technology Research Faculty
FME	Facilities, Maintenance and Engineering
HPV	Human Papillomavirus
HSL	HPV Serology Laboratory
SDS	Safety Data Sheets
SOP	Standard Operating Procedure
Type I Water	Ultrapure/Reagent Grade/Critical applications

## 8. SUMMARY OF TEST METHODS AND PROCEDURE

- 8.1. Chip Priming Station
  - 8.1.1. Replace the syringe after every ten uses, or if there is no longer any “bounce-back” after releasing from the clip post-addition of the gel-dye mix and subsequent one minute incubation.
    - 8.1.1.1. Unscrew the syringe from the lid of the chip priming station and release it by popping out the syringe.
    - 8.1.1.2. Remove the syringe cap of the new syringe and insert it into the clip by sliding it into the hole of the luer-lock adapter and screwing it tightly into the priming station.
  - 8.1.2. Ensure the base plate is inserted into position “A” (you may need to use a small Phillips’ Head screwdriver to move/tighten the base plate).
  - 8.1.3. The syringe clip needs to be in the middle position of the clip (this can be achieved by releasing the lever of the clip and sliding it into the correct position).
- 8.2. Reagent Preparation
  - 8.2.1. Gel-Dye Mix
    - 8.2.1.1. Add 25 µL of the protein 230 dye concentration (blue vial) to the protein 230 gel matrix (red vial).

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8.2.1.2. Vortex well to mix the dye with the gel matrix.

8.2.1.3. Spin down for 15 seconds using a mini centrifuge.

8.2.1.4. Transfer the gel-dye mix to a spin filter and centrifuge using a microcentrifuge at 2500 x g for 15 minutes

#### 8.2.2. Destaining Solution

8.2.2.1. Pipette 650  $\mu$ L of the gel matrix (red vial) into a new spin filter.

8.2.2.2. Centrifuge using a microcentrifuge at 2500 x g for 15 minutes at room temperature.

8.2.2.3. One vial can be used for 25 chips.

#### 8.2.3. Denaturing Solution

8.2.3.1. Add 7  $\mu$ L 1M DTT (Dithiothreitol) to the sample buffer vial (white tube).

8.2.3.2. Vortex for five seconds.

#### 8.3. Sample Preparation and Denaturing

8.3.1. Add 2  $\mu$ L of the prepared denaturing solution to 4  $\mu$ L of each protein sample in 0.2 $\mu$ L PCR tubes with lids.

8.3.2. Add 6  $\mu$ L of the 230 Ladder (yellow tube) to a 0.2  $\mu$ L PCR tube with lid.

8.3.3. Boil the samples at 95°C for five minutes using a heat block or thermocycler. Allow the tubes to cool.

8.3.4. Spin the tubes using a mini centrifuge for 15 seconds.

8.3.5. Add 84  $\mu$ L Type I Water to each sample and ladder and vortex briefly.

#### 8.4. Preparing the Agilent 2300 Protein Chip

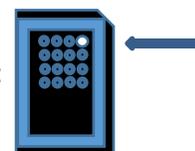
8.4.1. Place a new protein chip on the chip priming station.

8.4.2. Pipette 12  $\mu$ L of the gel-dye mix into the well labeled "G":

8.4.3. Pull the syringe plunger all the way to the 1 mL mark.

8.4.4. Close the priming station (make sure you hear the latch "click").

8.4.5. Push the plunger down until it is held by the clip and wait for 60 seconds.



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8.4.6. Release the clip and allow the plunger to push back up for five seconds before pulling it completely up to the 1 mL mark on the syringe.

8.4.7. Remove any solution left over in the well.



8.4.8. Pipette 12 µL of the gel-dye mix into all wells labeled “G.”

8.4.9. Pipette 12 µL of the destaining solution into the well labeled “DS.”

8.4.10. Pipette 6µL of sample into wells 1-10. If wells are not to be used, add 6µL deionized water to the well.



8.4.11. Pipette 6µL of the ladder in the well marked “”.

8.4.12. Place the chip in the bioanalyzer and start immediately.

#### 8.5. Use of the Agilent Software

8.5.1. Users of the software must be set up through Windows in order to have an account to use the Agilent program.

8.5.2. The “2100 Expert Logon” window will pop up and the following fields must be filled out:

User Name  
Password  
Role (Advanced or Standard Operator can run the program)

8.5.3. Select “Okay” on the screen.

#### 8.6. Running the Software

8.6.1. Make sure that the “Method” is set for running the protein 230 series II program.

8.6.2. Fill out samples with specific information on what is being analyzed.

8.6.3. Fill out chip lot number and kit lot number information.

8.6.4. There is room for any additional comments that should be entered concerning the run.

8.6.5. Once the chip is loaded, hit the “Start” button.

#### 8.7. User Confirmation

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8.7.1. Prior to starting the run, an additional screen will pop up asking for log on information and additional comments. This needs to be filled out before the run will begin.

## 8.8. Results and Analyses

8.8.1. Once the program has started, do not open the lid to the Bioanalyzer or the program will be voided.

8.8.2. The marker will be analyzed first and results can be shown in either an electrocardiogram or column format (user can toggle between the two options).

8.8.3. Samples will then be analyzed in numerical order from one to ten and results will be displayed as they are analyzed.

8.8.4. Data can be presented in table format, as well as by putting the mouse over the band in question to determine estimated kDa and protein concentration.

8.8.5. Results will be automatically saved in the secure folder at the location of the computer and Bioanalyzer however files can also be saved as PDF's and placed in the following folder: O:\HSL\HSL\_Agilent\Bioanalyzer Raw Data Files.

8.8.6. Save file as:

LB Reference with Page Number\_BIOA\_Date&Analyst Initials  
(For example, LAB2017099P001\_BIOA\_18Jul17ABC)

## 9. SYSTEM SUITABILITY CRITERIA

9.1. The run is considered acceptable if the markers in Lane 1 are distinguishable. See "Attachment 1: Bioanalyzer Example" for details.

## 10. DATA ACCEPTANCE CRITERIA

10.1. Sample results will vary based on the purity of the VLPs being analyzed. Sample integrity will be determined by Research Associate and/or the Scientific Manager.

10.2. A sample will be considered as either FIO (For Information Only), Data Suitable or Repeat.

10.2.1. A sample will be considered **FIO** when the data is not required based on another assay's result, due to the sample type or when directed by the Scientific Manager.

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10.2.2. A sample will be considered **Data Suitable** when a clear band is seen and the data extracted from the results matches expectations.

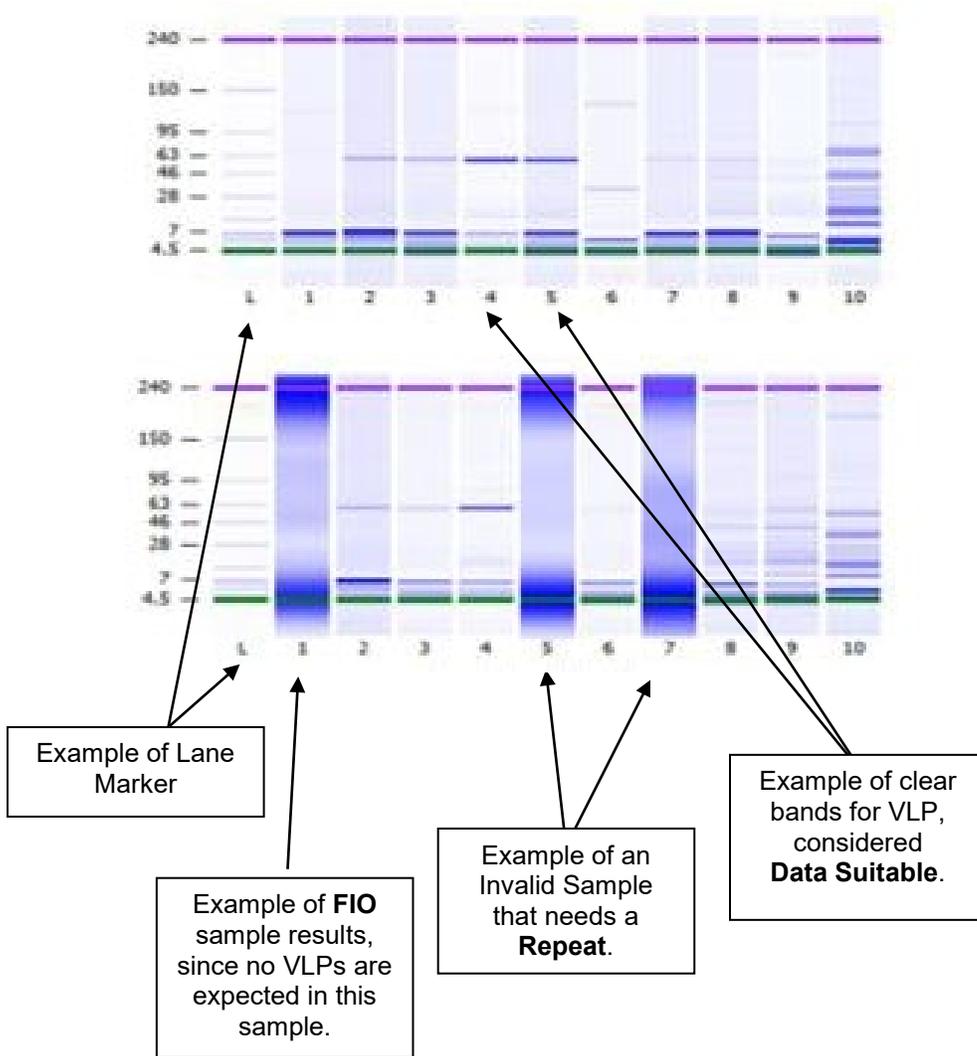
10.2.3. A sample will need a **Repeat** when an invalid result occurs, based on experience, expectations related to other assays, or if determined by the Scientific Manager.

10.3. See "Attachment 1: Bioanalyzer Example" for details.

## 11. ATTACHMENTS

11.1. Attachment 1: Bioanalyzer Example

Attachment 1: Bioanalyzer Example



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**12. REVISION HISTORY**

Revision Start Date	Version #	Changes	Reasons
18Jul17	New	Create new SOP for the Agilent 2100 Bioanalyzer.	New SOP.

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**Reagents**

	Identification Number	Expiration Date
Protein Chip		
Protein Dye		
Protein Matrix		
Denaturing Solution		
Ladder Mix		
1M DTT (Dithiothreitol)		

**Equipment**

	Identification Number	Calibration Due Date
Agilent Bioanalyzer	<input type="checkbox"/> HSL_040 <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:	
Thermomixer	<input type="checkbox"/> HSL_035 <input type="checkbox"/> Other:	<input type="checkbox"/> N/A
-20°C Freezer	<input type="checkbox"/> HSL_034 <input type="checkbox"/> Other:	<input type="checkbox"/> N/A
Micro Centrifuge	<input type="checkbox"/> HSL_006 <input type="checkbox"/> Other:	<input type="checkbox"/> N/A
<input type="checkbox"/> N/A Pipette:                      μL		
<input type="checkbox"/> N/A Pipette:                      μL		
<input type="checkbox"/> N/A Pipette:                      μL		

**Sample Information**

1:	6:
2:	7:
3:	8:
4:	9:
5:	10:

Comments:	<input type="checkbox"/> N/A
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Performed By/ Date:	
Reviewed By/ Date:	

