

<b>Frederick National Laboratory for Cancer Research</b> <small>sponsored by the National Cancer Institute</small>	HPV Serology Laboratory Standard Operating Procedure	
DNA Quantitation using the NanoDrop		
<b>Document ID: HSL_ LAB_016</b>	Version 1.0	Page 1 of 6

Released by/Date Effective:

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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 quantitation of DNA in a sample using the NanoDrop 1000 spectrophotometer.

**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. NanoDrop 1000 Spectrophotometer user manual
- 3.2. HSL\_LAB\_016.01: NanoDrop Data Capture Form
- 3.3. HSL\_EQ\_021.01: NanoDrop 1000 Use and Maintenance Form
- 3.4. HSL\_EQ\_019: Use and Maintenance of the Milli-Q Integral 3 Water System
- 3.5. HSL\_GL\_001: Waste Disposal at the Advanced Technology Research Facility
- 3.6. HSL\_GL\_002: Equipment Qualification and Calibration in the HPV Serology Laboratory
- 3.7. HSL\_GL\_003: Good Documentation Practices for the HPV Serology Laboratory
- 3.8. HSL\_GL\_004: Laboratory Notebook Control and Use for the HPV Serology Laboratory
- 3.9. HSL\_GL\_006: Reagent Preparation for the HPV Serology Laboratory
- 3.10. HSL\_GL\_007: Reagent and Chemical Expiry in the HPV Serology Laboratory
- 3.11. HSL\_GL\_008: Laboratory Flow and Gowning Procedures for the HPV Serology Laboratory
- 3.12. HSL\_GL\_009: HPV Serology Laboratory BSL-2 Procedures
- 3.13. HSL\_GL\_010: Control and Request of Documents in the HPV Serology Laboratory

**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. NanoDrop 1000 Spectrophotometer
- 5.2. Type I Water
- 5.3. Kimwipes (VWR, Cat # 21905-026 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
ATRF	Advanced Technology Research Facility
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. ASSAY PROCEDURE

- 8.1. Open program ND-1000 Version 3.8. A window message will pop-up asking to initialize the instrument. Click OK.
- 8.2. A log-in window will pop-up. Choose user from the drop-down menu and enter user password. Click OK.
- 8.3. With the sample arm open, wipe both the upper and lower pedestals with kimwipe, damp with nuclease free water (Type I water).
- 8.4. Load 2 µL of Type I water onto the lower measurement pedestal and close the sampling arm then click OK.
- 8.5. The message "Initializing Spectrometer- please wait" will appear. When this message disappears, the instrument will be ready for use.

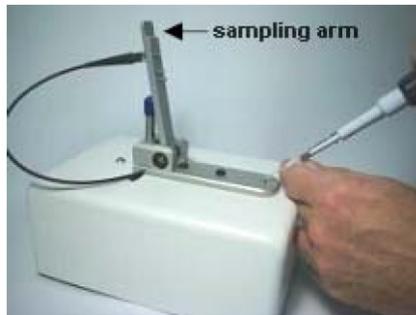
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8.6. A window menu with the following option is available:



- 8.7. Click on **"Nucleic Acid"** from the main menu.
- 8.8. Click on which sample type (DNA) is being measured from the main menu. Add a sample ID for each reading (example: HPV16 pSheLL)
- 8.9. With the sample arm open, wipe both the upper and lower pedestals with kimwipe, damp with Type I water.
- 8.10. With the sample arm open, pipette 2  $\mu$ L of the sample onto the lower measurement pedestal (see picture below).

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- 8.11. Close the sampling arm and initiate a spectral measurement by pressing the F1 button.
- 8.12. When the measurement is complete, open the sampling arm and wipe the sample from both the upper and lower pedestals with kimwipe.
- 8.13. Wipe both the upper and lower pedestals with a kimwipe that is damp with Type I water then close the sampling arm.
- 8.14. Perform at least two measures per sample and the average concentration will be defined as the final result.
- 8.15. Name the data file as follows:
 

"Data Reference\_NanoDrop\_DDMMYYAnalyst Initials"  
(LB12345P001\_NanoDrop\_20MAY17ABC)
- 8.16. Save the data file in the following location:
 

O:\HSL\HSL\_Nanodrop\Analyst Initials\Nucleic Acid
- 8.17. Print data file, initial and date the first page, and store in the Raw Data binder.
- 8.18. Complete "HSL\_LAB\_016.01: NanoDrop Data Capture Form" to capture the data.

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**9. REVISION HISTORY**

<b>Revision Start Date</b>	<b>Version #</b>	<b>Changes</b>	<b>Reasons</b>
14Aug17	New	Create new SOP for DNA quantitation using the NanoDrop	New SOP.

NanoDrop Data Capture Form

**Form ID: HSL\_LAB\_016.01**  
Document ID: HSL\_LAB\_016

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**Reagents**

	Lot Number	Expiration Date
<input type="checkbox"/> N/A Distilled Water		
<input type="checkbox"/> N/A Blank Diluent		

**Note:** Record lot number if bottled water is used.

**Equipment**

	Equipment ID	Calibration Due Date
NanoDrop		
Pipette: <span style="margin-left: 150px;">µL</span>		

**Sample Information**

Sample Identifier (HPV Plasmid ID)	HPV Plasmid Isolation Lot #	Result 1	Result 2	Final Result Average
<input type="checkbox"/> N/A				
<input type="checkbox"/> N/A				
<input type="checkbox"/> N/A				
<input type="checkbox"/> N/A				
<input type="checkbox"/> N/A				
<input type="checkbox"/> N/A				

Data File: \_\_\_\_\_

Comments:

N/A

Analyst/Date	
Review By/ Date	