

# Facilitating the implementation of NGS-based Diagnostic Testing in Infectious Disease Laboratories

Atis Muehlenbachs, MD PhD FCAP

Laboratory Medical Director, CDC Atlanta

CLIA Compliance Program, Office of Infectious Diseases



# CDC's QMS Risk-Based Approach to Next Generation Sequencing

CAP  
Guidelines

FDA  
Regulations

CLIA  
Regulations

ISO  
Standards

**Quality Management System –  
provides the foundation to build upon**

# Challenges of NGS to Regulatory Compliance and Patient Safety on a PHL CLIA Certificate

- Use of non-validated, uncontrolled technologies.
- Experts in use and development of NGS technologies often less versed in clinical laboratory standards or regulations.
- Ever expanding laboratory activities can potentially impact patient care (and directly impact CLIA certification):
  - Patient identifiers de-coded offsite
  - Outbreak investigations and “research use only” testing
  - “Behind the scenes” testing
- Challenges compounded by complexity of novel technology and difficulty in interpreting specific CLIA regulations.

# Diagnostic NGS at CDC Infectious Disease Laboratories

- Two diagnostic tests using NGS on the Roybal campus CLIA menu:
  - FVIII Gene Sequencing
  - Enteric Bacterial Identification
- Other NGS activities (unable to report at a patient level):
  - Pathogen characterization\*
  - Phylogenetic analysis
  - Hospital infection control
  - Antimicrobial resistance/susceptibility\*
  - Metagenomics/pathogen discovery\*

*\*in pipeline towards CLIA activity*
- CDC reference labs are often “end of the line” for diagnostic testing: arguable need to provide this specialized testing to PHL partners and US population.

# OID/CDC Efforts to Support Diagnostic NGS Implementation

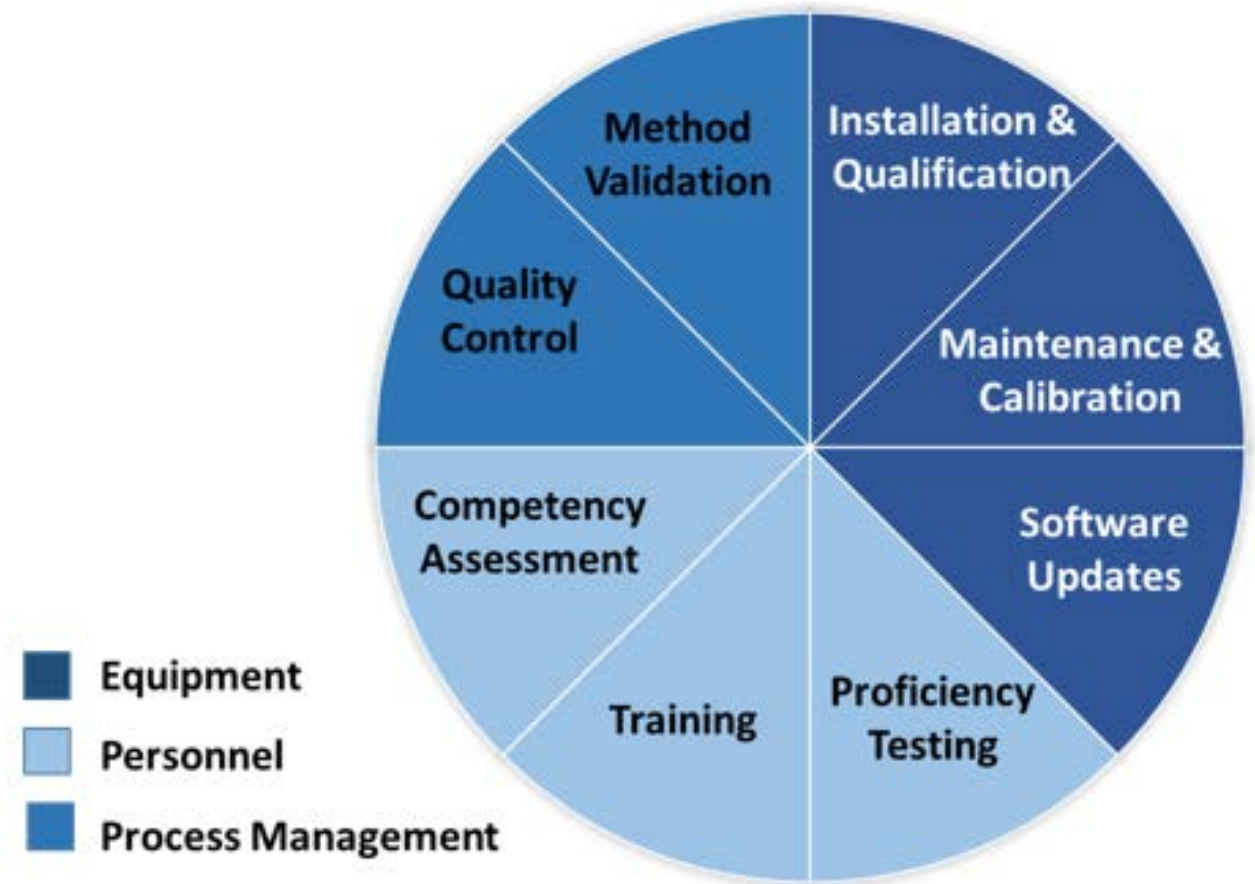
- Challenge: Multiple, specialized laboratories. Re-inventing the wheel is impractical.
  - Solution: Generate ready-to-implement SOPs and forms, each made flexible for customization to individual laboratory needs.
- Resources available: Scientific, technologic, quality systems and bioinformatic expertise throughout organization.
  - Engagement: Provide a venue to communicate and define best practices.
- Desire to work with external partners.

# CDC NGS Quality Workgroup: Description

- The NGS Quality Workgroup meets monthly to identify challenges and gaps in laboratories performing NGS for both research and diagnostics.
  - Lead: Rebecca Hutchins, started in 2015.
  - Participation from multiple Centers: NCEZID, NCIRD, NCHHSTP, CSELS, NCEH.
- The workgroup develops SOPs, forms, guidance, and tools to address the gaps.
- Key success factors:
  - Inclusion of laboratorians, bioinformaticians, and quality managers (NGS users).
  - Interactive and inclusive discussions.
  - Systematic approach.
  - Surveyed NGS users to determine areas of greatest need from their perspective.

# Alignment to Quality System 12 Essential Elements

- Workgroup output aligned to the 12 QSEs (Clinical and Laboratory Standards Institute).
- In 2015, a survey to NGS-using laboratories, identified the QSEs of **Equipment, Personnel** and **Process Management** to have the largest gaps and posed the greatest **risk**.
- These were prioritized to address.



# CDC NGS Quality Workgroup: Output

#	Document Title	Document Type	QSE
1	Ion PGM Sequencer Competency Assessment Form	Form	Personnel
2	Ion PGM Sequencer Competency Assessment SOP	SOP	Personnel
3	MiSeq Competency Assessment Form	Form	Personnel
4	MiSeq Competency Assessment SOP	SOP	Personnel
5	Ion PGM Sequencer Training Form	Form	Personnel
6	Ion PGM Sequencer Trainer Designation Form	Form	Personnel
7	Ion PGM Sequencer Training SOP	SOP	Personnel
8	MiSeq Employee Training Form	Form	Personnel
9	MiSeq Trainer Designation Form	Form	Personnel
10	MiSeq Training SOP	SOP	Personnel
11	Ion Chef Preventive Maintenance Log	Log	Equipment
12	Ion Chef Preventive Maintenance SOP	SOP	Equipment
13	Ion OneTouch 2 Preventive Maintenance Log	Log	Equipment
14	Ion OneTouch 2 Preventive Maintenance SOP	SOP	Equipment
15	Ion OneTouch ES Preventive Maintenance Log	Log	Equipment
16	Ion OneTouch ES Preventive Maintenance SOP	SOP	Equipment
17	Ion PGM Equipment Error Log	Log	Equipment
18	Ion PGM In-Use Equipment Daily Maintenance Log	Log	Equipment
19	Ion PGM In-Use Equipment Weekly Maintenance Log	Log	Equipment
20	Ion PGM Power Off Equipment Maintenance Log	Log	Equipment
21	Ion PGM Preventive Maintenance Wash Flowchart	Job Aid	Equipment
22	Ion PGM Sequencer Preventive Maintenance SOP	SOP	Equipment
23	MiSeq Equipment Error Log	Log	Equipment
24	MiSeq In-Use Equipment Maintenance Log	Log	Equipment
25	MiSeq Preventive Maintenance SOP	SOP	Equipment
26	MiSeq Preventive Maintenance Wash Flowchart	Job Aid	Equipment
27	MiSeq Standby Equipment Maintenance Log	Log	Equipment
28	Ion PGM System Equipment Pre-Installation Checklist	Job Aid	Equipment
29	MiSeq Equipment Pre-Installation Checklist	Job Aid	Equipment
30	Vendor-Performed IQ/OQ Coversheet	Form	Equipment
31	Ion PGM Sequencer Software Update Evaluation SOP	SOP	Equipment
32	Ion PGM Sequencer Software Update Form	Form	Equipment
33	MiSeq Software Update Evaluation SOP	SOP	Equipment
34	MiSeq Software Update Form	Form	Equipment
35	NGS QC Guidance for Illumina Workflows	SOP	Process Management
36	Bioinformatics QC Workflows	SOP	Process Management
37	Sequencing QC SOP	SOP	Process Management
38	Pre-Analysis QC SOP	SOP	Process Management
39	Assembly QC SOP	SOP	Process Management

- The Workgroup collaborated to develop guidance, SOPs and Forms for QSE’s **Equipment, Personnel and Process Management.**
- A total of 39 documents have been reviewed for external release.
- Multiple additional documents that have not been reviewed for external release (e.g. analytical SOPs) or are in “working drafts”.

*Hutchins, R. Manuscript in preparation*






# Example Forms: Personnel Training & Equipment Pre-Installation

MiSeq Employee Training Form			
Doc. No.	Rev. No.	Effective Date:	Page 1 of 3
Employee Name		Training Start Date	
Section I – Base Knowledge (Video and Reading Requirements) <i>[select videos and documents relevant to your lab processes; add other videos and documents as appropriate]</i>			
Video Title	Trainee Initials	Date Watched	
MiSeq: Sequencing Chemistry			
MiSeq: Introduction to the MiSeq System			
MiSeq: How to Start a Run			
MiSeq: Instrument Washes			
TruSeq: Best Practices			
TruSeq: Controls			
TruSeq: Sample Purification Bead Size Selection and Best Practices			
Nextera DNA Sample Prep			
Nextera Sample Prep: Best Practices			
Illumina Experiment Manager			
MiSeq: Does My Run Look Good?			

CDC Infectious Diseases Laboratories			
Equipment Pre-Installation Checklist			
Doc. No.	Rev. No.	Effective Date:	Page 1 of 3
Equipment Name: Illumina MiSeq			
Before purchasing equipment, verify that the following requirements are, or can be, met:			
Requirement	Requirement Met?	Comments	
<b>a. Electrical (two options):</b> <input type="checkbox"/> 100-110 V AC with 10-amp grounded dedicated line <input type="checkbox"/> 220-240 V AC with 6-amp grounded line	Yes <input type="checkbox"/> No <input type="checkbox"/>		
<b>b. Wattage:</b> <b>400 Watts</b>	Yes <input type="checkbox"/> No <input type="checkbox"/>		
<b>c. Power Protection:</b> <b>Uninterrupted Power Supply</b>	Yes <input type="checkbox"/> No <input type="checkbox"/>		
<b>d. Water:</b> Access to one of the following types of laboratory grade water <input type="checkbox"/> Illumina PW1 <input type="checkbox"/> 18 Megohm water	Yes <input type="checkbox"/> No <input type="checkbox"/>		

These relatively simple forms are ready to implement and customizable. Such forms can save laboratories the work of creating *de novo*.

# Example Procedure and Form: Software Updates

 <b>CDC Infectious Diseases Laboratories</b>			
<b>MiSeq Software Update Evaluation</b>			
Doc. No.	Rev. No.	Effective Date:	Page 1 of 3

**5.3.7** Complete a verification run as described below prior to releasing the equipment back into service.


- Using a standard, well-characterized sample previously ran in the laboratory, perform a sequencing run.
- If the sequencing data obtained with the new software versions are comparable to the data obtained with the prior software versions, no further action is needed.
- If the sequencing data obtained with the new software versions are not comparable to the data obtained with the prior software versions, **conduct a revalidation of the assay.**

**5.3.8** Attach additional information as needed (e.g. Release Notes documentation, Verification / Validation data) to the MiSeq Software Update Form.

**5.3.9** Sign, date, and obtain applicable reviews and approvals.

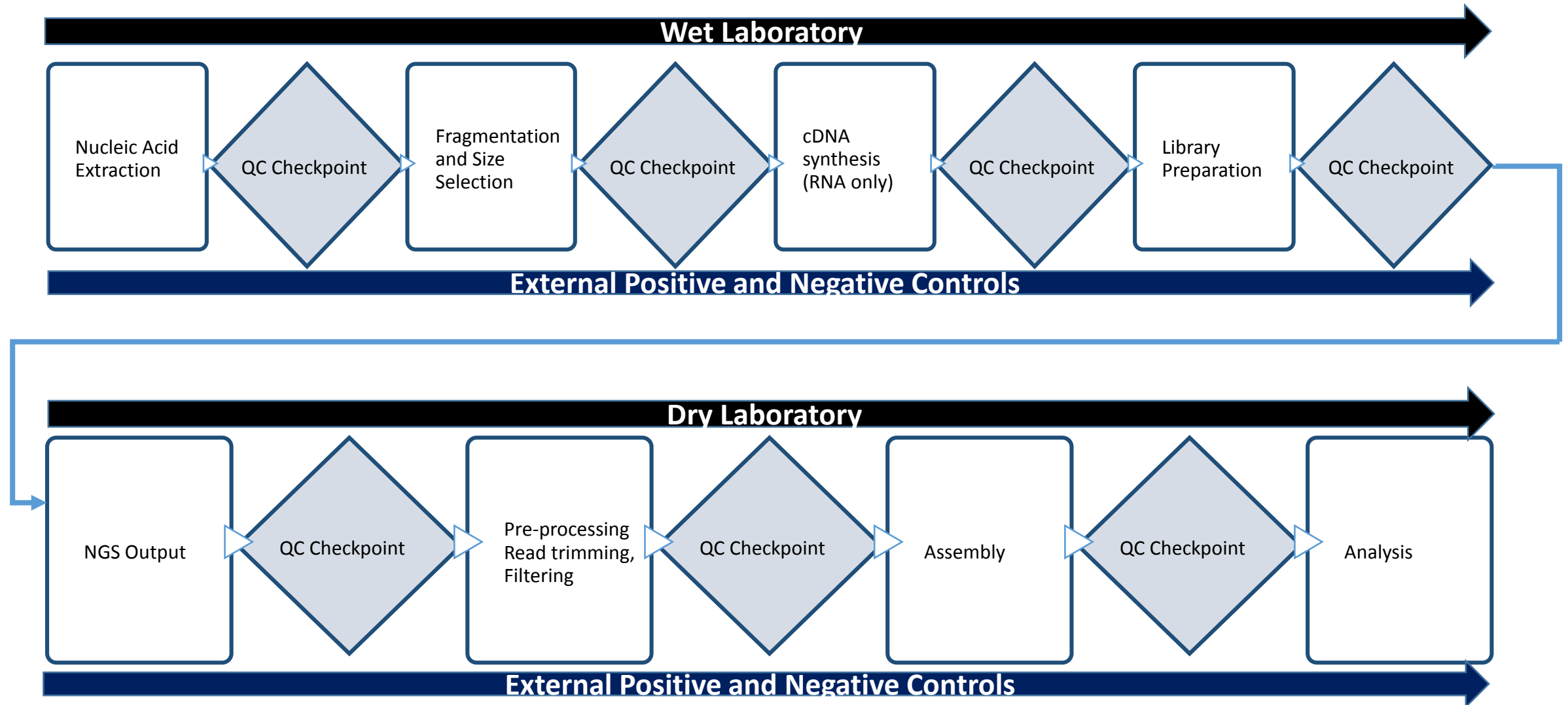
**6.0** Revision History

Rev #	DCR #	Change Summary	Date
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 <b>CDC Infectious Disease Laboratories</b>			
<b>MiSeq Software Update Form</b>			
Doc. No.	Rev. No.	Effective Date:	Page 1 of 1
<b>Lab:</b> Equipment: MiSeq Manufacturer: Illumina Serial #: Log Start Date:		<b>Building #:</b> Equipment ID: Model #: ESO/CDC Barcode #: Log End Date:	
<b>Current Software Versions:</b>			
<b>New Software Versions:</b>			
<b>Illumina Sequencing Workflow(s) currently used in the laboratory:</b>			
<b>Release Notes Reviewed?</b> <input type="checkbox"/> Yes <input type="checkbox"/> No	<b>Do the updates affect the sequencing workflow used in the laboratory?</b> <input type="checkbox"/> Yes <input type="checkbox"/> No	<b>Do the updates potentially affect the sequencing data output results?</b> <input type="checkbox"/> Yes <input type="checkbox"/> No	
<b>Required Action:</b>		<input type="checkbox"/> Verification <input type="checkbox"/> None	
<b>Results of Verification (and Validation, if applicable)</b>			

This procedure and form has been adapted by non-NGS laboratories, highlighting the strength of the quality systems approach to identify needs.

# Process Control for the Wet and Dry NGS laboratories





# *Ad hoc* Discussions to Determine Best Practices

- Consideration of external sequence data as a clinical sample.
  - Acceptance criteria driven by meeting defined QC checkpoints.
- Internal/external controls and individualized quality control plans (ongoing).
- Venue to communicate reagent recalls and identify reagent quality issues.

# Work Group Expertise Provided Input to the CLIAC Federal Advisory Committee, April 2018

- Provided the public health voice at this session.
- Identified specific CLIA regulatory challenges and described CDC best practices to address:
  - Personnel.
  - Process control, including distributive testing.
  - System validation and re-validation.
  - Analysis (including record retention) and reporting.
- CLIAC recommended formation an NGS workgroup.

Hutchins, R: “Diagnostic NGS Challenges: CDC PHL Perspective”

[http://ftp.cdc.gov/pub/CLIAC\\_meeting\\_presentations/pdf/Addenda/cliac0418/7\\_Hutchins\\_NextGen\\_Sequencing\\_Public\\_Health.pdf](http://ftp.cdc.gov/pub/CLIAC_meeting_presentations/pdf/Addenda/cliac0418/7_Hutchins_NextGen_Sequencing_Public_Health.pdf)



# CDC NGS Quality Workgroup: Future Direction

- The Workgroup is tackling the QSEs of **Process Management, Organization, Information Management** and **Assessments**:
  - NGS Method Validation: Guidance, Procedures and forms.
  - Individualized Quality Control Plan.
  - Quality Assurance planning.
  - Information Management Guidance (data file retention).
  - Proficiency testing



12 QSEs, CLSI

# Next Steps

- Manuscript in preparation (including 39 documents and forms) on personnel, equipment and process control.
  - Will be publically available, but a “snapshot” as field rapidly evolves.
- Plan to strengthen collaboration with CDC’s Division of Laboratory Systems (CSELS) and APHL
  - Engagement and interaction.
  - Development of resources to support public health quality management of NGS-based testing.



# Acknowledgements

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  - **Collette Fitzgerald**
  - **Adeeba Saboor**

Contact: [vkd6@cdc.gov](mailto:vkd6@cdc.gov)

Disclaimer: The findings and conclusions in this presentation are those of the author and do not necessarily represent the views of Centers for Disease Control and Prevention.



# CDC NGS Quality Workgroup: Approach

- Systematic approach to improve quality management systems for labs that perform NGS testing

