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

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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.

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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.

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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".

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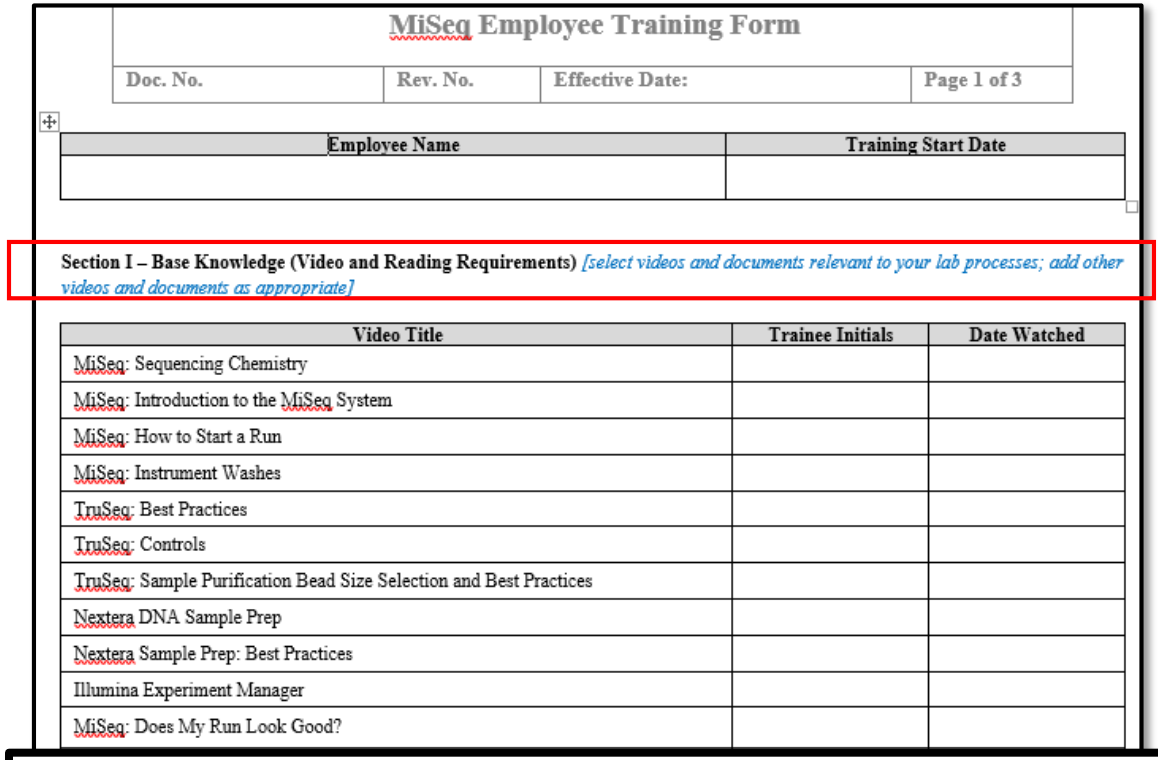
Example Forms: Personnel Training & Equipment Pre-Installation



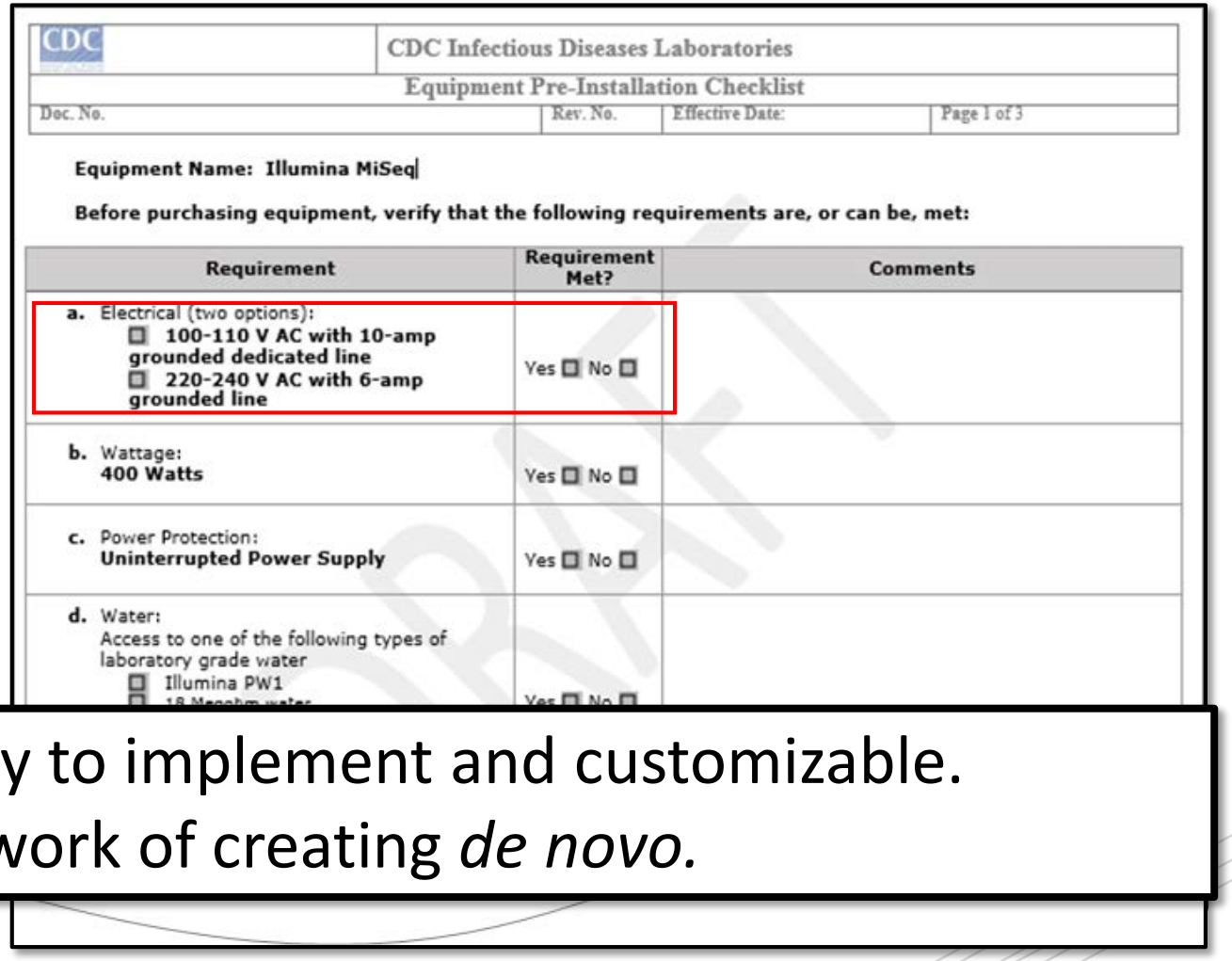
MiSeq Employee Training Form

Doc. No.	Rev. No.	Effective Date:	Page 1 of 3

Employee Name	Training Start Date

Section I – Base Knowledge (Video and Reading Requirements) *[select videos and documents relevant to your lab processes; add other videos and documents as appropriate]*

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

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Equipment Name: Illumina MiSeq

Before purchasing equipment, verify that the following requirements are, or can be, met:

Requirement	Requirement Met?	Comments
a. Electrical (two options): ☐ **100-110 V AC with 10-amp grounded dedicated line** ☐ **220-240 V AC with 6-amp grounded line**	Yes ☐ No ☐	
b. Wattage: **400 Watts**	Yes ☐ No ☐	
c. Power Protection: **Uninterrupted Power Supply**	Yes ☐ No ☐	
d. Water: Access to one of the following types of laboratory grade water ☐ Illumina PW1 ☐ 18 Megohm water	Yes ☐ No ☐	

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

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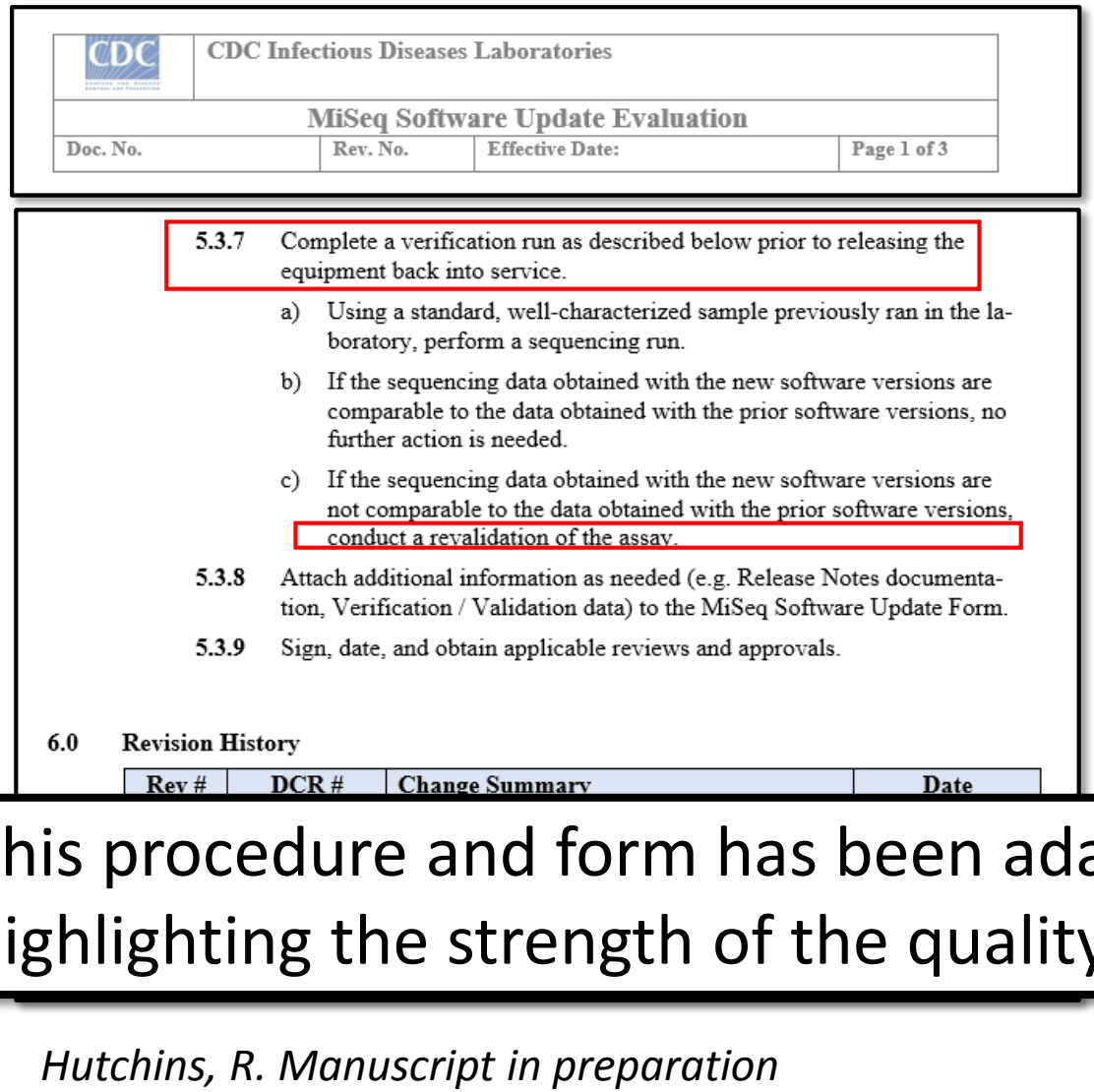
Example Procedure and Form: Software Updates



CDC Infectious Diseases Laboratories

MiSeq Software Update Evaluation

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

a) Using a standard, well-characterized sample previously ran in the laboratory, perform a sequencing run.

b) 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.

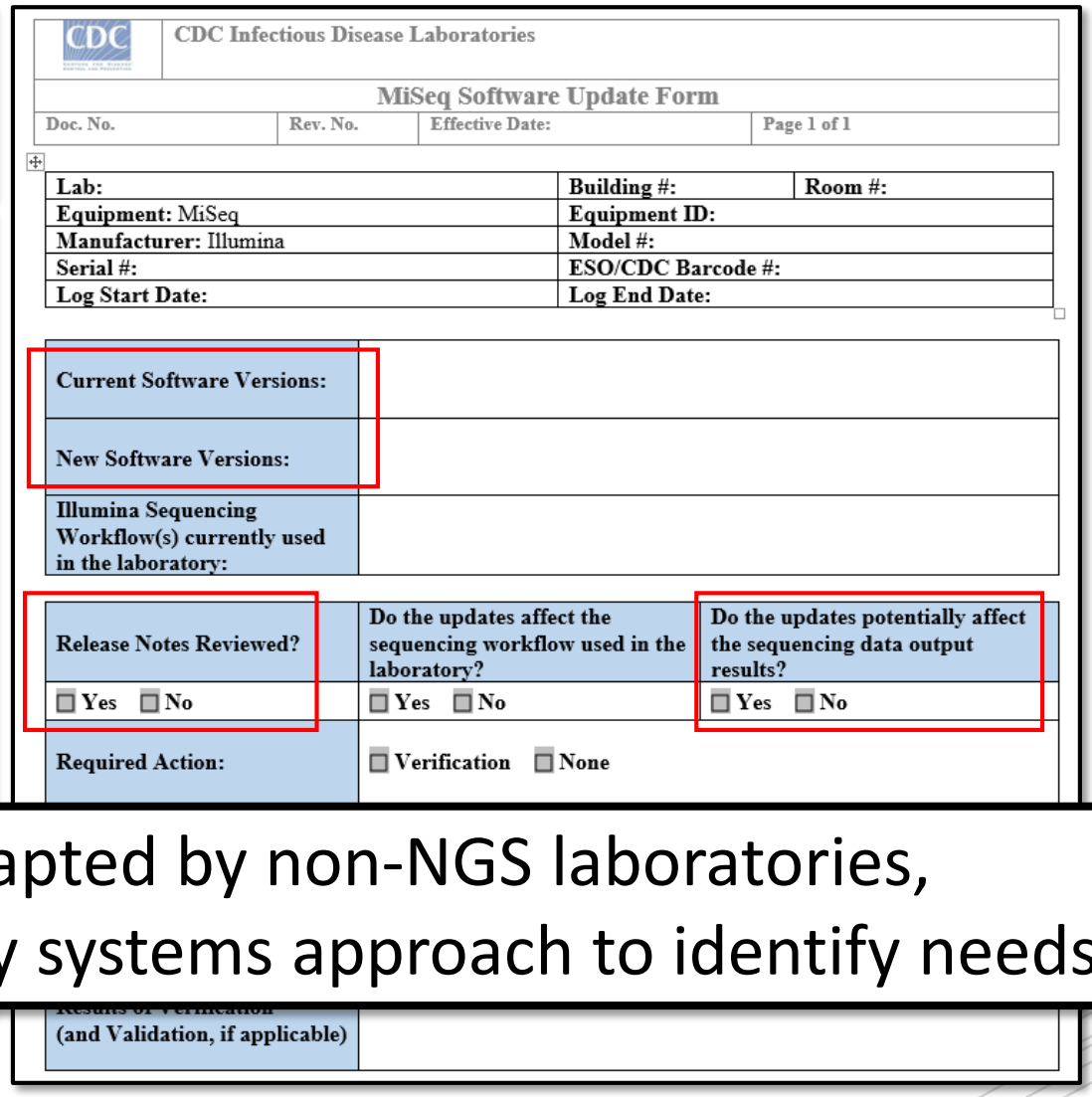
c) If the sequencing data obtained with the new software versions are not comparable to the data obtained with the prior software versions, conduct a revalidation of the assay.

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



CDC Infectious Disease Laboratories

MiSeq Software Update Form

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Lab:	**Building #:**	**Room #:**
Equipment: MiSeq	**Equipment ID:**	
Manufacturer: Illumina	**Model #:**	
Serial #:	**ESO/CDC Barcode #:**	
Log Start Date:	**Log End Date:**	

Current Software Versions:	
New Software Versions:	
Illumina Sequencing Workflow(s) currently used in the laboratory:	

Release Notes Reviewed?	**Do the updates affect the sequencing workflow used in the laboratory?**	**Do the updates potentially affect the sequencing data output results?**
☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
Required Action:	☐ Verification ☐ None	
(and Validation, if applicable)		

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

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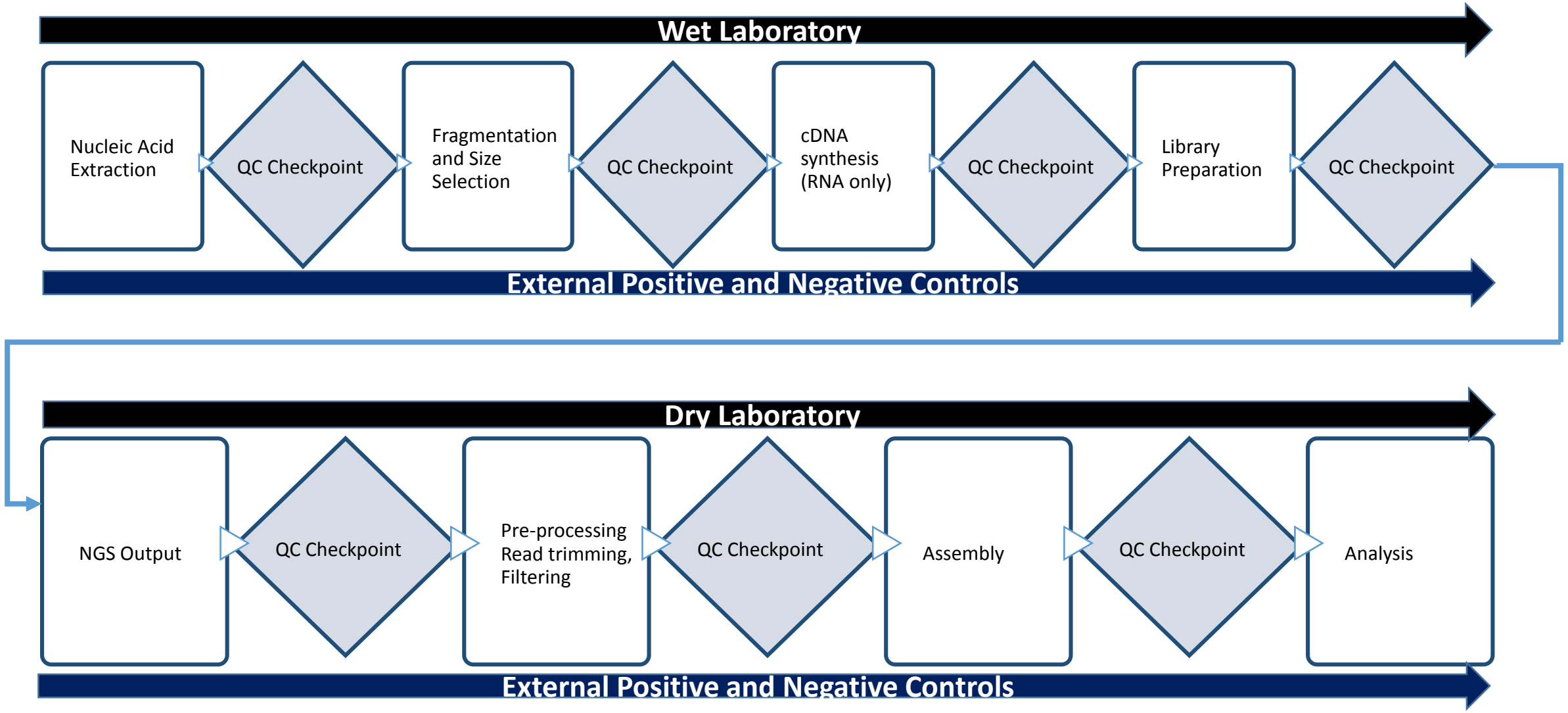
Process Control for the Wet and Dry NGS laboratories



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Example QC Checkpoint Checklist

CDC	CDC Infectious Diseases Laboratories		
NGS QC Guidance for Illumina Workflows			
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QC Checkpoint (Process Step)	Method (SOPs)	Expected Results*
cDNA Synthesis* (2.6) • **Quantitate purity and concentration** **for RNA sample only*	Purity (**choose one**): ☐ NanoDrop Nucleic Acid Quantitation Assay ☐ Other _____ *AND* Concentration (**choose one**): ☐ Qubit dsDNA or RNA Quantitation Assay ☐ Quant-iT Assay ☐ Other _____ *OR* Electrophoresis Instrument for NGS (**choose one**): ☐ TapeStation Assay ☐ Bioanalyzer Assay ☐ BluePippin DNA Size Selection Assay ☐ Other	**Purity:** A_{260}/A_{280} = 1.8-2.0 **Concentration:** > 500 ng in a 20-100 µL sample
Library Preparation (2.8) • **Quantitate concentration and confirm size selection**	Concentration (**choose one**): ☐ Qubit dsDNA or RNA Quantitation Assay ☐ Quant-iT Assay ☐ KAPA qPCR ☐ Other _____ *AND* Electrophoresis Instrument for NGS (**choose one**): ☐ TapeStation Assay ☐ Bioanalyzer Assay ☐ BluePippin DNA Size Selection Assay ☐ Other _____	**Concentration:** > 1 mM **Electropherogram results:** Single peak of desired size with no tailing and excessive broadening per lab specifications

*The expected results included are based on standard NGS methods in use at the time of document development. The advancement of new methods and technologies may allow for successful sequencing with QC results differing from those listed in this document.

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

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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- Office of Infectious Diseases/Office of the Director:
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- Division of Laboratory Systems/Center for Surveillance, Epidemiology, and Laboratory Services:
  - **Collette Fitzgerald**
  - **Adeeba Saboor**

Contact: vkd6@cdc.gov

CDC NGS Quality Workgroup: Approach

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

Identify Critical Risk Areas

- Survey NGS labs
- Form risk mitigation strategies
- Develop QMS implementation plan

Develop Quality Documentation

- Develop consensus on best practices
- Develop SOPs and Forms to assure quality
- Publish documents for NGS lab use

Evaluate for Effectiveness

- Review and revise documentation as needed
- Engage with core groups to understand ongoing needs
- Address challenges via NGS workgroup input