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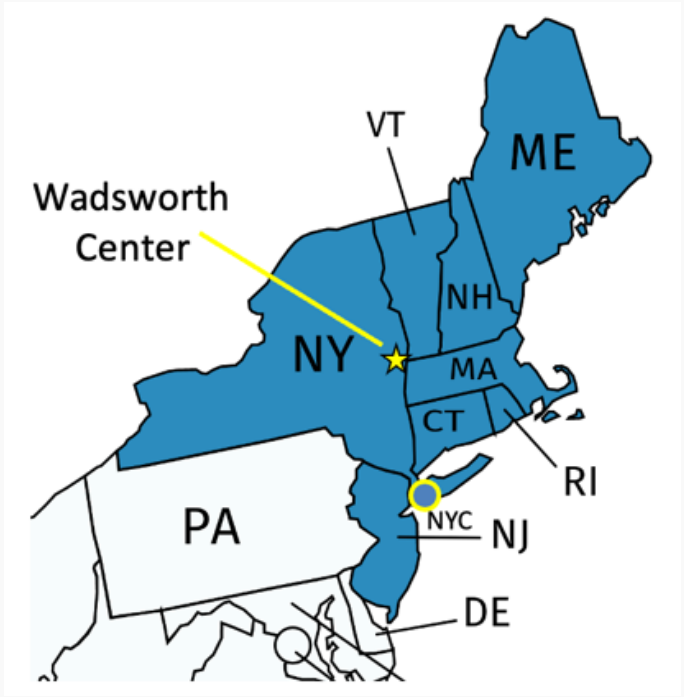
LDT Validation at the Wadsworth Center

Kimberlee Musser, PhD
Wadsworth Center

OVERVIEW

- Wadsworth Center Overview
- NYS Clinical Laboratory Evaluation Program (CLEP)
- Laboratory Developed Test Submission
  - Risk Assessment
  - Checklist
  - SOP
  - Validation Study
  - Submission Form, Report

WADSWORTH CENTER- SCIENCE IN THE PURSUIT OF HEALTH®



Large state public health laboratory

750 staff, 5 sites

Notable features

Regulatory role- Clinical Laboratory Evaluation Program (CLEP)

Utilize hundreds of lab developed tests (LDTs)

In-house developed LIMS (CLIMS)

Mission - Wadsworth Center is a science-based community committed to protecting and improving the health of New Yorkers through laboratory analysis, investigations and research, as well as laboratory certification and educational programs.

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Wadsworth Center

History and Vision

1901 Antitoxin Laboratory established
1914 Designated the Division of Laboratories and Research

Dr. Wadsworth (Director 1914-1945):

- Urged staff to pursue original investigations
- Understood that research, public health testing and science education were complementary
- Created a synergy that continues to inform the vibrant scientific community that now bears his name

https://www.wadsworth.org/about/history

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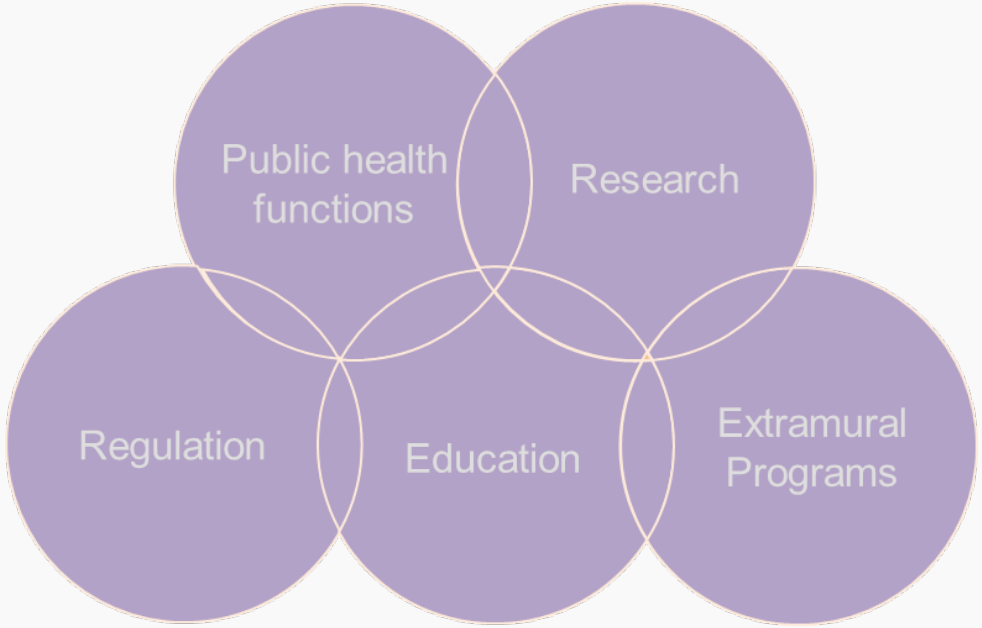
WADSWORTH CENTER- MODEL



- Science-based and data-driven organization
- Committed to protecting and improving the health of New Yorkers through laboratory analysis, investigations and research
- Laboratory certification and educational programs.

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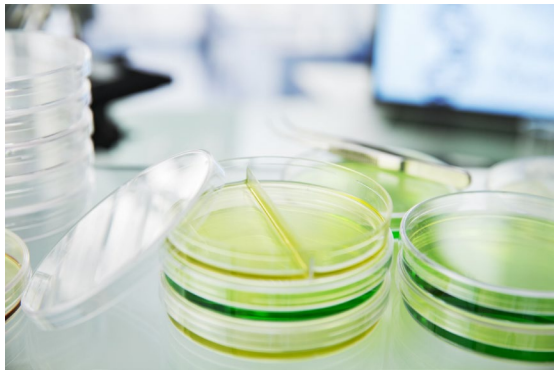
Regulatory History in NY

On July 1, 1965, New York became the first state in the nation to initiate a certification and licensing program for a limited number of clinical laboratories operating within the state in selected areas of patient testing.



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Wadsworth Center Regulatory Programs
Division of Laboratory Quality Certification (DLQC)

Consists of seven programs that provide regulatory oversight in several areas.

- Clinical Laboratory Evaluation Program (CLEP)
- Physicians Office Laboratory Evaluation Program (POLEP)
- Tissue Resources Program (TRP)
- Regulated Medical Waste Program (RMW)
- Environmental Laboratory Approval Program (ELAP)
- Regulated Medical Waste Program (RMW)
- Laboratory Investigative Unit (LIU)

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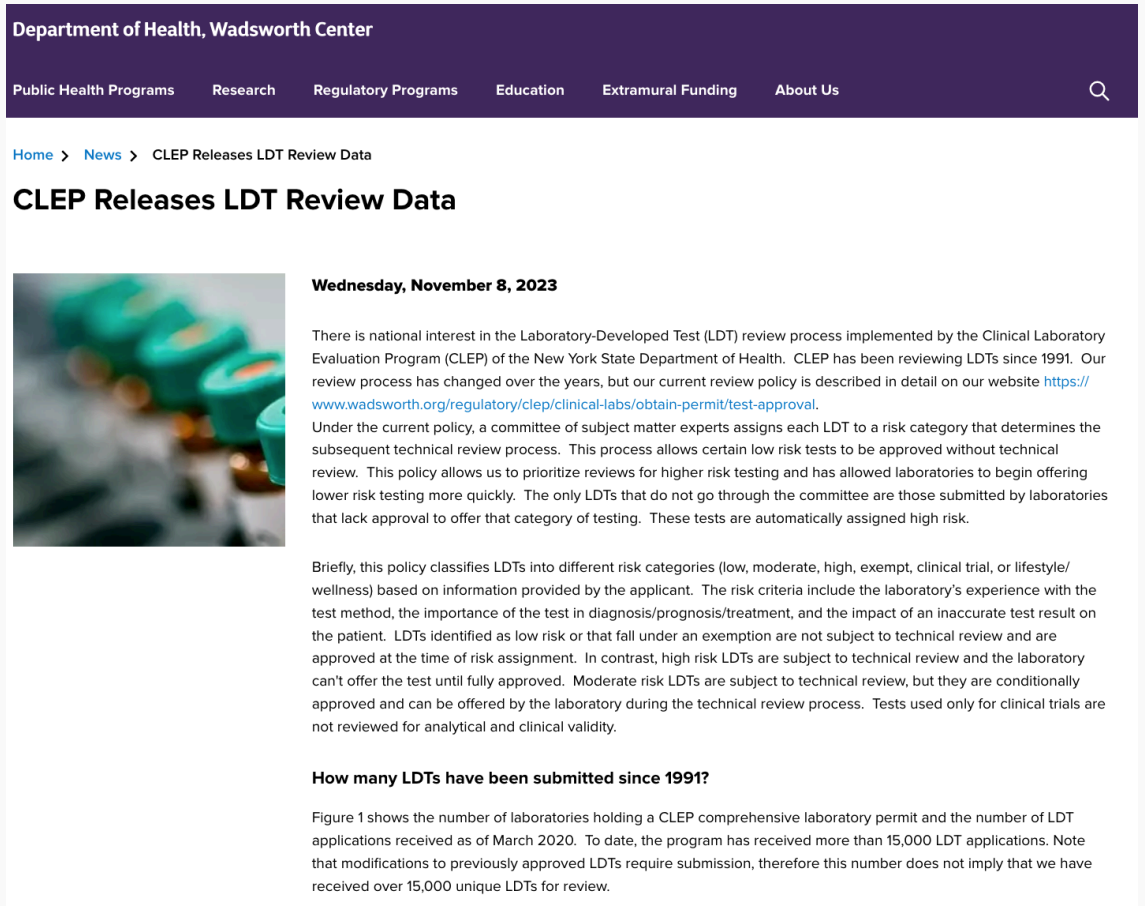
CLEP by the numbers…

- 1150 permitted labs (high complexity testing) (34 categories)
- 11125 limited-service laboratories (waived testing)
- 3572 Certificate of Qualifications (CQs)
- **>15,000 unique laboratory developed tests received for review since 1991**
  - 54% require >1 review
  - 77% are from labs outside of NY



Department of Health, Wadsworth Center

Public Health Programs | Research | Regulatory Programs | Education | Extramural Funding | About Us

Home > News > CLEP Releases LDT Review Data

CLEP Releases LDT Review Data

Wednesday, November 8, 2023

There is national interest in the Laboratory-Developed Test (LDT) review process implemented by the Clinical Laboratory Evaluation Program (CLEP) of the New York State Department of Health. CLEP has been reviewing LDTs since 1991. Our review process has changed over the years, but our current review policy is described in detail on our website https://www.wadsworth.org/regulatory/clep/clinical-labs/obtain-permit/test-approval.
Under the current policy, a committee of subject matter experts assigns each LDT to a risk category that determines the subsequent technical review process. This process allows certain low risk tests to be approved without technical review. This policy allows us to prioritize reviews for higher risk testing and has allowed laboratories to begin offering lower risk testing more quickly. The only LDTs that do not go through the committee are those submitted by laboratories that lack approval to offer that category of testing. These tests are automatically assigned high risk.

Briefly, this policy classifies LDTs into different risk categories (low, moderate, high, exempt, clinical trial, or lifestyle/wellness) based on information provided by the applicant. The risk criteria include the laboratory's experience with the test method, the importance of the test in diagnosis/prognosis/treatment, and the impact of an inaccurate test result on the patient. LDTs identified as low risk or that fall under an exemption are not subject to technical review and are approved at the time of risk assignment. In contrast, high risk LDTs are subject to technical review and the laboratory can't offer the test until fully approved. Moderate risk LDTs are subject to technical review, but they are conditionally approved and can be offered by the laboratory during the technical review process. Tests used only for clinical trials are not reviewed for analytical and clinical validity.

How many LDTs have been submitted since 1991?

Figure 1 shows the number of laboratories holding a CLEP comprehensive laboratory permit and the number of LDT applications received as of March 2020. To date, the program has received more than 15,000 LDT applications. Note that modifications to previously approved LDTs require submission, therefore this number does not imply that we have received over 15,000 unique LDTs for review.

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https://www.wadsworth.org/news/clep-releases-ldt-review-data

23 years later…Oversight of Clinical Laboratory Testing at the Federal Level

In 1988, Congress passed the Clinical Laboratory Improvement Amendments of 1988 (CLIA 88) establishing quality standards for all clinical laboratory testing to ensure the accuracy, reliability and timeliness of patient test results regardless of where the test was performed. The final CLIA regulations were published in the Federal Register on February 28, 1992.

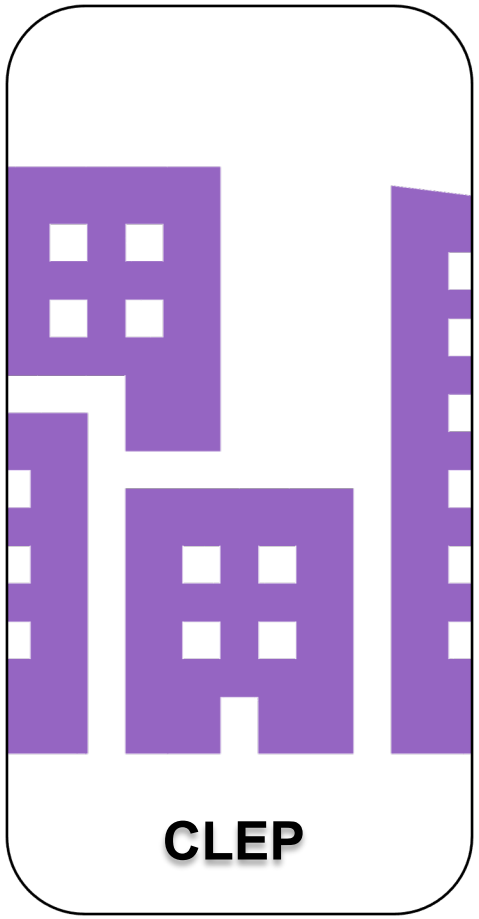
The Centers for Medicare and Medicaid Services (CMS) has the primary responsibility for the operation of the CLIA Program.

Oversight of Clinical Laboratory Testing at the Federal and State Level

CMS performed an exhaustive review of our laws, regulations, and the CLEP program. CMS determined that New York State's laws and regulations for the oversight of clinical labs are equal to or more stringent than CLIA 88.

Because of this, NYS was first granted exempt status in 1995 and more recently in 2021. As a result of this exemption, we are responsible for providing oversight of NYS clinical labs. If a lab in NYS has a NYS permit, they will automatically meet the Federal requirements to carry out testing.

Managing Oversight of Permitted Clinical Laboratories



- Routes information to other Wadsworth divisions
- Evaluation of proficiency testing results
- Processing of fees
- Contact point for the labs we regulate
- Handles applications
- Reviews applications and issues permits, CQs
- Performs on-site inspections (surveys)

Subject Matter Experts in Divisions:

- Review non-FDA approved methods and LDTs
- Assist in evaluation of survey results
- Review non-FDA approved methods and LDTs
- Assist in the evaluation of laboratory director qualifications
- Evaluate proficiency test samples
- Participate in surveys
- Provide training and education

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WADSWORTH CENTER

CLINICAL LABORATORY EVALUATION PROGRAM (CLEP) OVERVIEW

Oversight of clinical laboratories performing testing on specimens originating from New York State.

Elements of oversight:

- On-site survey
- Monitor proficiency testing performance
- Review of laboratory developed tests (LDT)
- Director Certificate of Qualification (CQ) approval

Lab Directors and Research Scientists are Subject Matter Experts on externally submitted CLEP LDT reviews- we are also submitters to this LDT review process

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OVERSIGHT OF CLINICAL LABORATORIES

NYS Public Health Law and NYS Regulations

- Provides DOH the authority to permit clinical laboratories located in or accepting specimens from New York State.
- Describes requirements that need to be met to obtain or maintain a permit to operate a clinical laboratory.
- The Centers for Medicare and Medicaid Services (CMS) has determined that New York State's laws and regulations for the oversight of clinical labs are equal or more stringent than Federal requirements (i.e.,CLIA88).
- Because of this, NYS has been granted exempt status, and we act as an agent of CMS to provide oversight of NYS clinical labs. If a lab is issued a NYS permit, they will automatically meet the Federal requirements to carry out testing in NYS.

NYS CLEP LDT SUBMISSIONS

CLINICAL LABORATORY EVALUATION PROGRAM
BIGGS LABORATORY, WADSWORTH CENTER
NEW YORK STATE DEPARTMENT OF HEALTH
EMPIRE STATE PLAZA
ALBANY, NY 12237

RISK ATTESTATION FORM
For Laboratory Developed Tests

PFI: Office Use Only: Project ID
LABORATORYNAME:
LDT TITLE:

Provide a summary (not more than 500 words) of the proposed test including:
- Intended use to include target population if applicable
- Methodology and technology (e.g., sequencing by next generation sequencing)
- Specimen type(s)

Respond to the following questions:

1. Does the intended use of the assay make clinical claims or direct reference to recognized diseases/ condition(s)? Note that any materials submitted to the Department may be shared with federal Centers for Medicare and Medicaid Services (CMS) CLIA Program. The intended use must be clearly stated above.
   Yes No

Enter relevant literature references here; full citations required. References must be available, upon request.

2. Is this test used only in a clinical trial?
   No Yes

2a. If yes, does the LDT as a device in the clinical trial have IRB APPROVAL?
   Yes - Submit the following: No
   - validation summary
   - sample reports for all possible outcomes

And submit at least one of the following:
- IRB Approval letter
- clinicaltrials.gov (NTC) identifier
- FDA letter stating LDT meets requirements as NSR device

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APPROVAL OF MICROBIOLOGY NUCLEIC ACID AMPLIFICATION ASSAYS

Please submit all information as outlined below. Submit one hard copy of the entire package and one electronic copy (as a PDF file on a CD or flash drive) to:
US Postal Service: Clinical Laboratory Evaluation Program, Biggs Laboratory, Wadsworth Center, New York State Department of Health, Empire State Plaza, Albany, NY 12237; Attn: Assay Validation Review
UPS, FedEx, Courier: Clinical Laboratory Evaluation Program, Biggs Laboratory, Wadsworth Center, New York State Department of Health, Dock J - P1 Level, Empire State Plaza, Albany, NY 12237; Attn: Assay Validation Review
Materials submitted, including related data packages, will not be returned to the laboratory. All materials are maintained under strict confidentiality. As relates to New York State's Freedom of Information Law (commonly called FOIL): The Department's Records Access Officer has advised Wadsworth Center that if documents are marked "proprietary"; "confidential"; or with any labeling indicative of the submitter's desire for an increased level of protection based on the submission content, such protection from immediate release based on a FOIL request is justified. Laboratories will be given an opportunity to block information release if a request for the material is filed under the FOIL, by presenting evidence that the materials contain trade secrets. Marking should minimally appear on the cover page of each unit of material. Documents not marked with such terms will not block release of the submission through a FOIL request.

SECTION 1: GENERAL INFORMATION

Laboratory Name:_____ NYS PFI: _____
Contact Person: _____
Phone:_____ Fax:_____ Contact E-mail: _____
Assay (Test) Name (e.g., detection of *E. coli*): _____
Target Population (if applicable):_____
Methodology (e.g., PCR; Sequencing, RFLP): _____
Analyte(s) included: _____
Validated Specimen Type(s) (e.g., blood, urine) _____
Clinical Purpose (e.g., detection, quantification): _____

Laboratory Director/Assistant Director (NYS Certificate of Qualification Holder for applicable Permit category)

CQ Code_____ Signature _____

Laboratory Director (if not the responsible CQ Holder for applicable Permit category)

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https://www.wadsworth.org/regulatory/clep/clinical-labs/obtain-permit/test-approval

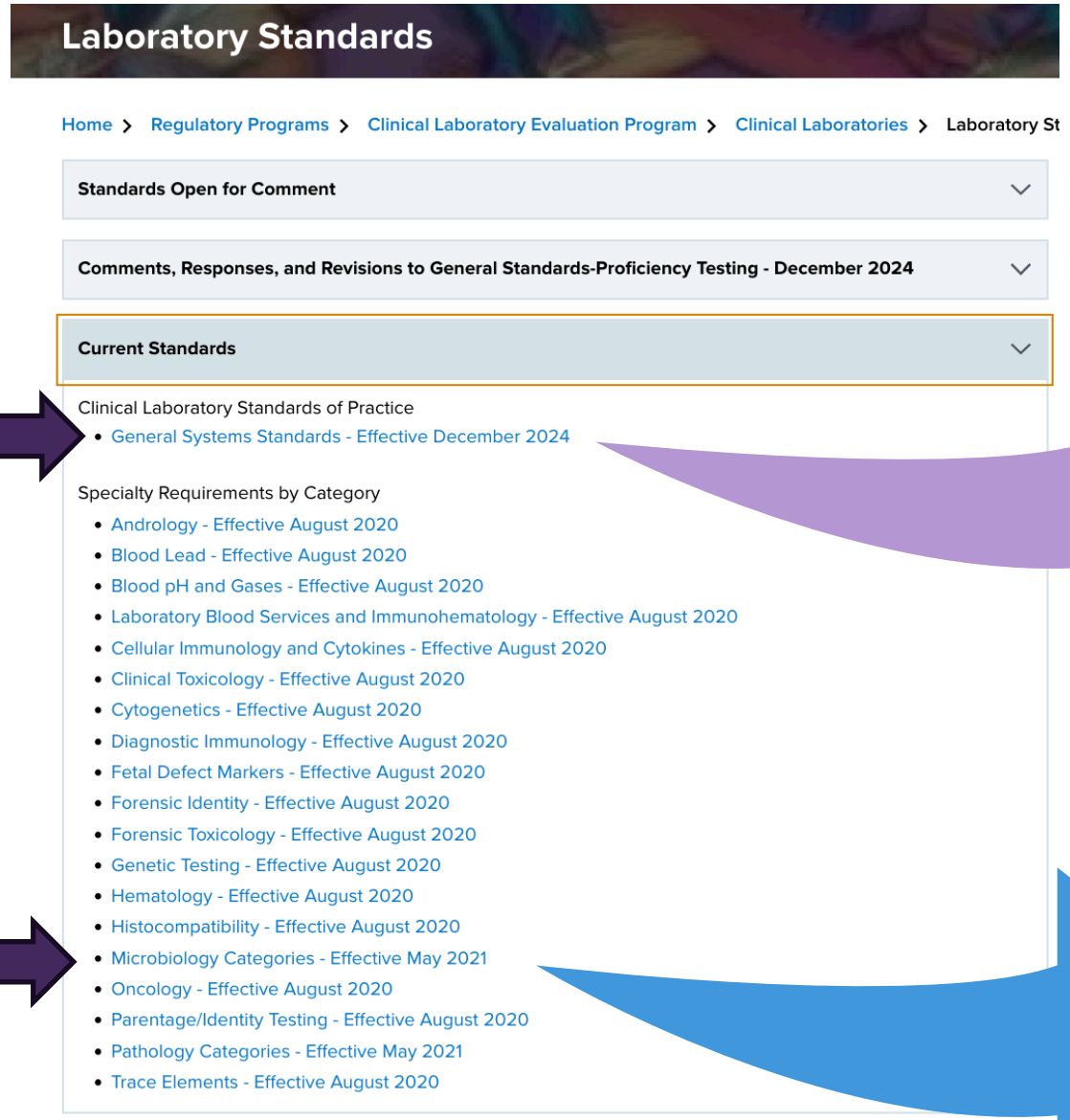
OVERSIGHT OF LABORATORY DEVELOPED TESTS

WHAT ARE LABORATORY DEVELOPED TESTS (LDTS)?

Non-FDA cleared or approved tests that are developed and validated by the laboratory offering the assay.

Tests that include a combination of reagents and/or kits prepared by the laboratory, labeled as Analyte Specific Reagents (ASR), Research Use Only (RUO), or Investigational Use Only (IUO).

Modified FDA-approved tests where there has been a change in intended use. For example, a change in the specimen type; the type of analysis (e.g., qualitative vs. quantitative); the purpose of the assay (e.g., screening, diagnosis, prognosis, monitoring, and /or confirmation); or the target population(s).



Quality Management System
Director Responsibilities
Human Resources
Facility Design
Laboratory Safety
Laboratory Information Systems
Resource Management
Document Control
Pre-Analytic Systems
Analytic Systems
Post-Analytic Systems
Document and Specimen Retention
Proficiency Testing
Investigation and Corrective Action

Microbiology
Nucleic Acid (MNA) Amplification Assay
Bacteriology
Mycobacteriology
Mycology
Parasitology
Virology

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NYS CLEP Laboratory Standards

Test Performance Specification Standard of Practice 2 (TPS S2): Laboratory Developed Tests The laboratory must establish method performance specifications before a test method is used to report specimen results. For laboratory developed tests (LDTs), modified FDA cleared, approved, or exempted tests, and modifications to standard methods (e.g., textbook methods), the laboratory must:	Information on Departmental approval of a laboratory developed test (LDT) is available at: https://www.wadsworth.org/regulatory/clep/clinical-labs/obtain-permit/test-approval.

Analytic Systems	
Standard	***Guidance***
a) **establish** performance specifications for accuracy, precision, reportable range, reference range(s), analytical sensitivity and specificity (to include interfering substances); clinical sensitivity and specificity; and other applicable performance characteristics; b) determine the acceptability of the established performance specification; and c) adhere to LDT guidelines established by the Department and submit required documents to the Department for approval. ***Regulatory authority: 10 NYCRR subdivision 58-1.10(g)***	

Example Standard

Microbiology	
Microbiology Nucleic Acid (MNA) Amplification Assay	
Standard	***Guidance***
Microbiology Nucleic Acid Amplification Assay Standard of Practice 4 (MNA S4): Task Separation for FDA-Approved Closed System Amplification Test For a closed system amplification test (CSAT), the laboratory must: a) handle, process, and store clinical specimens, reagents and supplies in a manner that prevents exposure to amplicon, plasmids, and culture-amplified materials; and	A CSAT refers to an assay in which all steps, including post-amplification steps, are performed and contained within a closed system. A closed system is defined as an instrument in which the patient specimen is directly added to the test unit, device, or cartridge, and then the testing process is initiated with no additional external manipulation or addition of reagents unless approval is received by the Department. CSAT instrumentation should be segregated from areas in which specimens are routinely processed in order to avoid cross-contamination.

Submission Checklist

Check One	Assay Type	Necessary Forms for Package Submission
	Laboratory developed test that does not utilize Analyte Specific Reagents (ASRs)	Full validation package-See Section 2
	Laboratory developed test that utilizes Analyte Specific Reagents	Full validation package-See Section 2
	Kit labeled for Investigational Use Only (IUO) or Research Use Only (RUO)	Full validation package-See Section 2
	FDA-cleared/approved assay	DOH 3519f "Notification to Add/Delete FDA-Approved Tests" Form
	Modification of a FDA or NYS-approved assay	Major modification- Full validation package-See Section 2 Minor modification- See Section 3
	Addition of an assay under an approved exemption	Provide the Project ID from your original exemption approval letter, a description of the original exemption, the name of the assay to be added, a summary of the validation performed, and sample reports for all possible outcomes. Attach additional materials as necessary.

	Guidance: The following controls are necessary for infectious agent nucleic acid amplification tests. The procedure should contain details on how each of the following functions is controlled in the assay, including the composition of the control and the step in which the control is included in the assay. Additional guidance on acceptable control materials and procedures is provided below: • **Positive Lysis/Extraction Control** The positive lysis/extraction control will monitor the performance of the entire assay, including the lysis/extraction process, to ensure that it is performing as expected. This control should contain whole organism (bacterial cells, viruses, parasites, fungi) or, if these are unavailable, nucleic acid. This positive control should be included at a low but easily detectable concentration, and should be run through the entire assay. • **Negative Lysis/Extraction Control** A negative lysis/extraction control will monitor potential contamination that could occur during the entire assay, including the lysis/extraction process. This control should be run through the entire assay (lysis, extraction, and amplification). Ideally, this control should consist of a known negative specimen in the same matrix that is being tested. The use of carriers such as tRNA, glycogen, or DNA can be used to detect low level nucleic acid contamination. • **Inhibition Control** An inhibition control ensures that patient samples are free of amplification inhibitors, which could lead to false negative results. In some cases, the inhibition control may also serve as an extraction control. In all cases, an expected value or range should be developed for the inhibition control, and results should be interpreted accordingly. If inhibition is tested using exogenous spiked nucleic acid, controls must be included to monitor for contamination with this material. Inhibition controls are not required if the run includes isolates only and not primary patient specimens. Examples of inhibition controls include, but are not limited to: -specimens spiked with an exogenous nucleic acid control that is detected in a separate assay -specimens spiked with a control nucleic acid containing primer-binding sites identical to the target, but with a heterologous probe-binding internal sequence -specimens dispensed into at least two aliquots tested in parallel; one aliquot is spiked with a low level of a positive or inhibition control • Alternatively, data must be provided that demonstrate the absence (or only rare occurrence) of inhibition, in each specimen type. If the extraction method is well established, with supportive evidence in the peer-reviewed literature, these references can be submitted together with the laboratory's own validation data. For each specimen type, the data set should include a total of 500 hundred drawn from the literature, plus 100 samples directly tested in the submitting laboratory that demonstrate that the method is performing according to expectations. The specimens tested in the submitting laboratory should contain low but easily detectable amounts of target. Specimens should be extracted and assayed, with results provided to show no loss in detection. • If independent supportive evidence is not available, the laboratory needs to provide more extensive in-house data. Typically, 500 samples are adequate to verify that inhibition is occurring in less than 1% of the samples. It should be noted that it is acceptable to obtain data assayed from the same matrix containing different but similar analytes if it is needed to attain the required number of samples. • **Reagent Contamination Control** A reagent contamination control (i.e. no-template control) ensures that the amplification reagents are not contaminated. This control should consist of water or buffer in a reaction tube that contains all of the reagents for amplification, but does not contain nucleic acid template. • The inclusion of additional negative controls is strongly recommended whenever the prevalence of the target agent is routinely expected to be high, e.g., in HCV screening of intravenous drug users, or patients with suspected hepatitis.

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https://www.wadsworth.org/sites/default/files/WebDoc/Microbiology_NAAT_Checklist_0211.pdf

Submission Checklist

Section 2.2: Test Requisition And Reports	
Page/Tab	
_____	A sample requisition form containing all the required elements in **Requisition Sustaining Standard of Practice 4 (Requisition S4): Request Form**.
_____	Examples of test reports containing all findings (e.g. positive (quantitative or qualitative), negative, indeterminate, inconclusive, etc.) with interpretive text, assay limitations and any disclaimers required by the federal government for tests utilizing Analyte-Specific Reagents (ASRs) and in compliance with **Reporting Sustaining Standard of Practice 1 (Reporting S1): Report Content)**. If preliminary or presumptive positive results will be reported without confirmation, include examples of these reports containing the appropriate statements explaining the presumptive/preliminary nature of the results.

Section 2.3: References	
Page/Tab	
_____	A list of relevant literature references that describe the scientific basis and clinical validity of the assay. Provide copies of only primary references.
_____	Applicable package inserts

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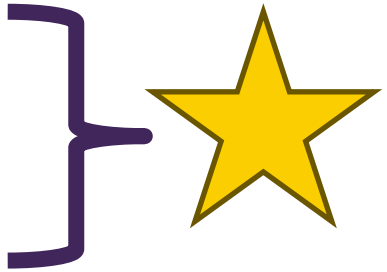
Submission Validation

Section 2.4: Validation	
Page/Tab	
_____	**Validation Data Summary**: Succinctly summarize why this new assay is needed, the means by which the new assay was validated, and the results. This should include a description of testing (authentic patient or spiked samples and total number), comparison method (gold standard or other FDA or NYS-approved assay) and overall results. Authentic clinical specimens are preferred, but spiked clinical samples are acceptable.
_____	**Specificity:** • Provide a list of all organisms tested in the specificity study and the verification run, including the source and concentration of each organism or nucleic acid target if whole organism is not available. • Provide the results of the specificity study. If there is any cross-reactivity, provide additional information on how the results will be resolved or interpreted.
	Guidance: • Specificity of the assay should be demonstrated using genetically-related organisms, organisms that can produce similar symptoms or illness, and other organisms that can be present in the specimen matrix/matrices to which the assay will be applied. If any cross-reacting organisms are noted, they should be clearly specified in the application, as well as on the patient report of test findings. In addition, assay a minimum of 5 different strains/isolates of the intended target organism/virus if available. If there are additional genotypes/serotypes/subtypes/variants, these should be included as well. • Molecular amplification assays for microbial detection that are not probe-based, such as SYBR® Green or real-time PCR with melting curve analysis, typically require the use of a secondary method in order to report a result as confirmatory. These assays can be used as screening assays but must be confirmed with an alternative method, i.e., a probe-based, hybridization-based, or sequence-based method. In the absence of such confirmation, positive results are considered presumptive, and this should be clearly indicated on the report (Also see Accuracy Verification section below).



Submission Validation

	Sensitivity: • Provide a brief description of experiments used to demonstrate the limit of detection (LOD) of the assay. • Provide the results of the LOD experiments for each specimen matrix.
	Guidance: **There are various methods for the determination of the LOD. Methods that statistically determine the LOD with 95% confidence are acceptable. Listed below are the minimum accepted requirements. All LODs determined from primary matrices must be performed from extraction.**

Microbiology Molecular Checklist 02/2011 Page 4 of 11

	• **Primary Matrix** Provide data from a dilution series of a known concentration of the target (preferably whole organism) that is spiked into negative clinical samples for each matrix being validated. Perform 3 separate extractions of this dilution series and test in duplicate. The dilution series must encompass six dilutions including one below the limit of detection of the assay. If the LOD is determined to be > 100 gene copies, linearity data must be provided. • **Multiple Specimen Matrices** When an assay is validated in multiple sample types (matrices), a separate dilution series should be performed for each matrix, although some sample types may be grouped. If it is unclear whether you need to independently spike different matrices (for example, different types of body fluids) please contact CLEP for advice. • **Microbial or Viral Isolates** Provide data as stated above for primary matrix except isolates may be diluted in water or buffer. • **LOD Requirements for Virology Applications** For virology applications, the LOD in genomic copy number is required. For quantitative viral assays, the limit of quantitation (LOQ) should be determined. Quantitation based on plaque titrations of viruses is not acceptable. It is acceptable to perform 1:10, 1:100, or 1:1000 dilutions of specimens that exceed the validated upper limit of quantification provided that the diluted specimen falls within the acceptable reportable range and internal controls are used to ensure that the diluent does not introduce inhibitors.

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Submission Validation

_____	**Inter-assay Reproducibility:** • Provide a brief experimental description and results demonstrating inter-assay reproducibility.
	Guidance: At least 3 authentic clinical samples or spiked clinical samples should be run on three different days. If spiked, the three samples should include one concentration at or near the limit of detection. Alternatively, a single positive control run repeatedly over many days (15 or more) may be used.
_____	**Intra-assay Reproducibility:** • Provide a brief experimental description and data demonstrating intra-assay reproducibility.
	Guidance: At least 3 authentic clinical samples or spiked clinical samples should be run in triplicate. If spiked, the three samples should include at least one concentration at or near the limit of detection. Also, if different instruments, platforms, models or technicians will be used to perform the assay, demonstrate the assay's consistency across these variables.

https://www.wadsworth.org/sites/default/files/WebDoc/Microbiology_NAAT_Checklist_0211.pdf

Submission Validation

Numbers provided
Data of interest to have submitted
Test run- 1 needed
Summary data

Accuracy Verification:

- Provide a description of the validation study design. See guidance below for specific details.
- Provide data from *at least* 30 positive samples and 10 negative samples for each specimen type together with controls used in the assay. Preferably present the results in tables (2 x 2), showing the qualitative results compared to results obtained by the comparison method.
- For each assay, provide the number and type of specimens tested, including information on the subtypes, genotypes, etc tested in the assay. If multiple clinically relevant sub-types or genotypes are available for the organism being assayed, please include an example of each available type, or a representative range of subtypes.
- Provide a brief summary of the results, including an explanation of any discrepant results and how the discrepancy was resolved.
- Submit one representative example of test results (one high-quality original printout of an actual test run), a condensed summary of the raw data (such as Ct values), and a complete description of how all results were interpreted.
- Molecular amplification assays for microbial detection that are not probe-based, such as SYBR Green or real-time PCR with melting curve analysis, data must be submitted from a secondary confirmatory method. These assays can be used as screening assays but must be confirmed with an alternative method, i.e., a probe-based, hybridization-based, or sequence-based method. In the absence of such confirmation, positive results are considered presumptive, and this should be clearly indicated on the report. (Also see Specificity validation guidance above).

Guidance: Specimen characteristics for accuracy verification:

- Accuracy should be verified by conducting a randomized, blinded validation study where the assay results are compared to those of a gold standard or FDA or CLEP approved assay, or results of spiked clinical samples that are compared to predicted results based on the spiking values.
- If multiple sub-types or genotypes are available for the organism being assayed, please include an example of each type, or a representative range of subtypes. As many different specimens and different strains, types, or clinical isolates as possible should be used in spiking studies (especially for specimen matrices that are routinely tested and pathogens that are routinely isolated in the laboratory). If your laboratory has difficulty in obtaining sufficient samples of a particular specimen type, or any other problems in fulfilling the validation requirements, please contact CLEP for guidance.
- The samples should be authentic clinical specimens, but spiked samples are acceptable when clinical specimens are not available. If spiked samples are used, at least 10 samples should be close to the LOD (maximum, 10-fold above that level). If authentic clinical samples are used, at least 10 of the 30 positives should be weak positives, if the methods used allow for this determination. Include multiple different strains and specimens.
- For quantitative assays, please provide data across the full range of concentrations likely to be encountered in clinical samples.
- Any discrepant results need to be explained.
- Multiplex assays (especially those with multiple sample types) can present a difficult situation for the design of a validation study, since it can be a large and expensive endeavor to assay 40 samples (30 positive and 10 negative) for each analyte in each specimen type. Samples can be spiked with multiple organisms, so as to reduce the number of samples required. CLEP may be contacted for guidance prior to the submission of a package with multiplex analysis having four or more simultaneous targets.

https://www.wadsworth.org/sites/default/files/WebDoc/Microbiology_NAAT_Checklist_0211.pdf

Submission Checklist

Section 2.5: Quality Assurance	
Page/Tab	
_____	A plan for proficiency testing including criteria for passing, the number of samples included in the proficiency panel, and the corrective actions that would be taken in the event of inadequate verification of the assay and individual analyst performance, compliant with **Quality Assessment Sustaining Standard of Practice 3 (QA S3): Ongoing Verification of Examination Accuracy**

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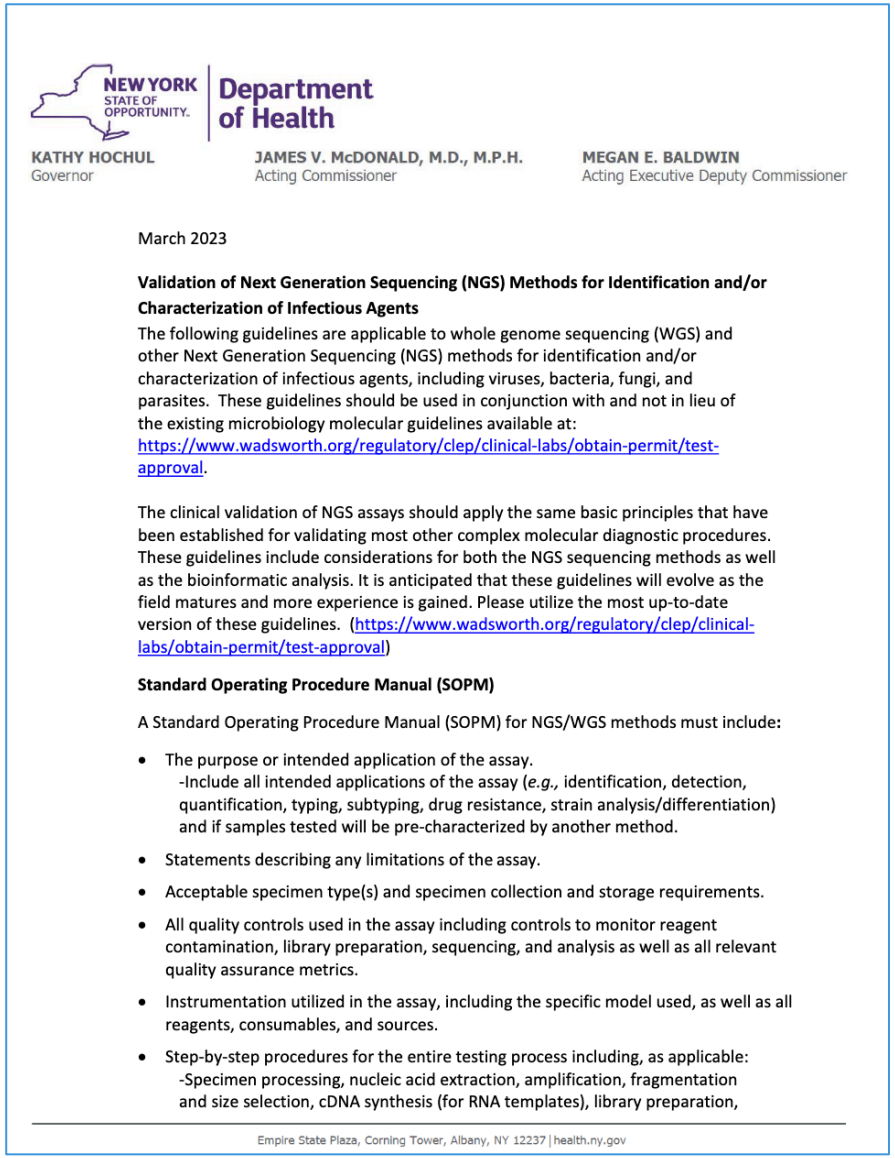
https://www.wadsworth.org/sites/default/files/WebDoc/Microbiology_NAAT_Checklist_0211.pdf

Making a Submission

If a full method validation submission is required as described above, please select the appropriate checklist for your category from the following:

- Cellular Immunology Checklist
- Cytogenetics FISH Checklist
- Diagnostic Immunology Checklist
- Forensic Identity Checklist
- Genetic Testing Molecular Checklist
  - Guidelines for Germline Variant Detection Using Next Generation Sequencing (NGS)
- Microbiology Molecular Checklist
  - MALDI-TOF Assays in Microbiology Checklist
  - Validation of Next Generation Sequencing (NGS) Methods for Identification and/or Characterization of Infectious Agents



NEW YORK STATE OF OPPORTUNITY. Department of Health

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March 2023

Validation of Next Generation Sequencing (NGS) Methods for Identification and/or Characterization of Infectious Agents

The following guidelines are applicable to whole genome sequencing (WGS) and other Next Generation Sequencing (NGS) methods for identification and/or characterization of infectious agents, including viruses, bacteria, fungi, and parasites. These guidelines should be used in conjunction with and not in lieu of the existing microbiology molecular guidelines available at: https://www.wadsworth.org/regulatory/clep/clinical-labs/obtain-permit/test-approval.

The clinical validation of NGS assays should apply the same basic principles that have been established for validating most other complex molecular diagnostic procedures. These guidelines include considerations for both the NGS sequencing methods as well as the bioinformatic analysis. It is anticipated that these guidelines will evolve as the field matures and more experience is gained. Please utilize the most up-to-date version of these guidelines. (https://www.wadsworth.org/regulatory/clep/clinical-labs/obtain-permit/test-approval)

Standard Operating Procedure Manual (SOPM)

A Standard Operating Procedure Manual (SOPM) for NGS/WGS methods must include:

- The purpose or intended application of the assay.
  -Include all intended applications of the assay (*e.g.*, identification, detection, quantification, typing, subtyping, drug resistance, strain analysis/differentiation) and if samples tested will be pre-characterized by another method.
- Statements describing any limitations of the assay.
- Acceptable specimen type(s) and specimen collection and storage requirements.
- All quality controls used in the assay including controls to monitor reagent contamination, library preparation, sequencing, and analysis as well as all relevant quality assurance metrics.
- Instrumentation utilized in the assay, including the specific model used, as well as all reagents, consumables, and sources.
- Step-by-step procedures for the entire testing process including, as applicable:
  -Specimen processing, nucleic acid extraction, amplification, fragmentation and size selection, cDNA synthesis (for RNA templates), library preparation,

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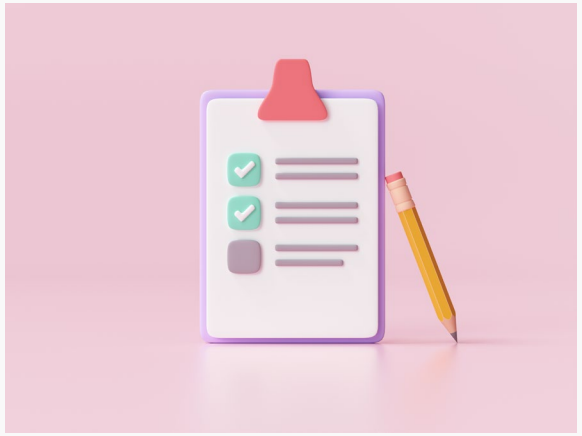
Examples: AR Pipeline for AR Gene and MLST



- **7/18/2022**
  NYS Clinical Laboratory Evaluation Program (CLEP) Approval

- **Wadsworth Center CLIA Director Approval**

NEW YORK STATE OF OPPORTUNITY. Department of Health

KATHY HOCHUL
Governor

MARY T. BASSETT, M.D., M.P.H.
Commissioner

KRISTIN M. PROUD
Acting Executive Deputy Commissioner

VALIDATION SUBMISSION RECEIPT - CONDITIONAL ASSAY APPROVAL FOR MODERATE RISK TEST

July 18, 2022

Kimberlee A. Musser, Ph.D.
Wadsworth Center - David Axelrod Institute
120 New Scotland Ave
Albany, NY 12208

PFI: 8523
Project ID: 93979

Dear Dr. Musser:

Thank you for submitting portions of your standard operating procedure manual (SOPM) and validation summary/data needed to evaluate your next generation sequencing method for whole genome sequencing for identification of antimicrobial resistance genes in cultured bacterial isolates. After conducting a risk assessment based on the information provided with the materials, the Clinical Laboratory Reference System (CLRS) Laboratory Developed Test Evaluation Panel has determined the testing to be Moderate Risk. According to the established policy, the SOPM and validation materials have been routed for review by the Wadsworth Center's Clinical Laboratory Reference System (CLRS) Reviewers. While under CLRS initial review, you may offer this assay under your current permit in the category of Bacteriology. Upon completion of the initial review, you will be notified that the testing has been fully approved or that additional information is required in order to achieve final approval. **If additional information is requested, the laboratory must provide a complete response within 60 business days in order to continue making the test available pending final approval.** This letter may be shared with New York clients.

Please refer to your laboratory's PFI number and the Project ID should you have questions. Note the revised *Test Approval Status Key Definitions* in the LDT Approval tab on the eCLEP page of the Department's Health Commerce System (HCS) website.

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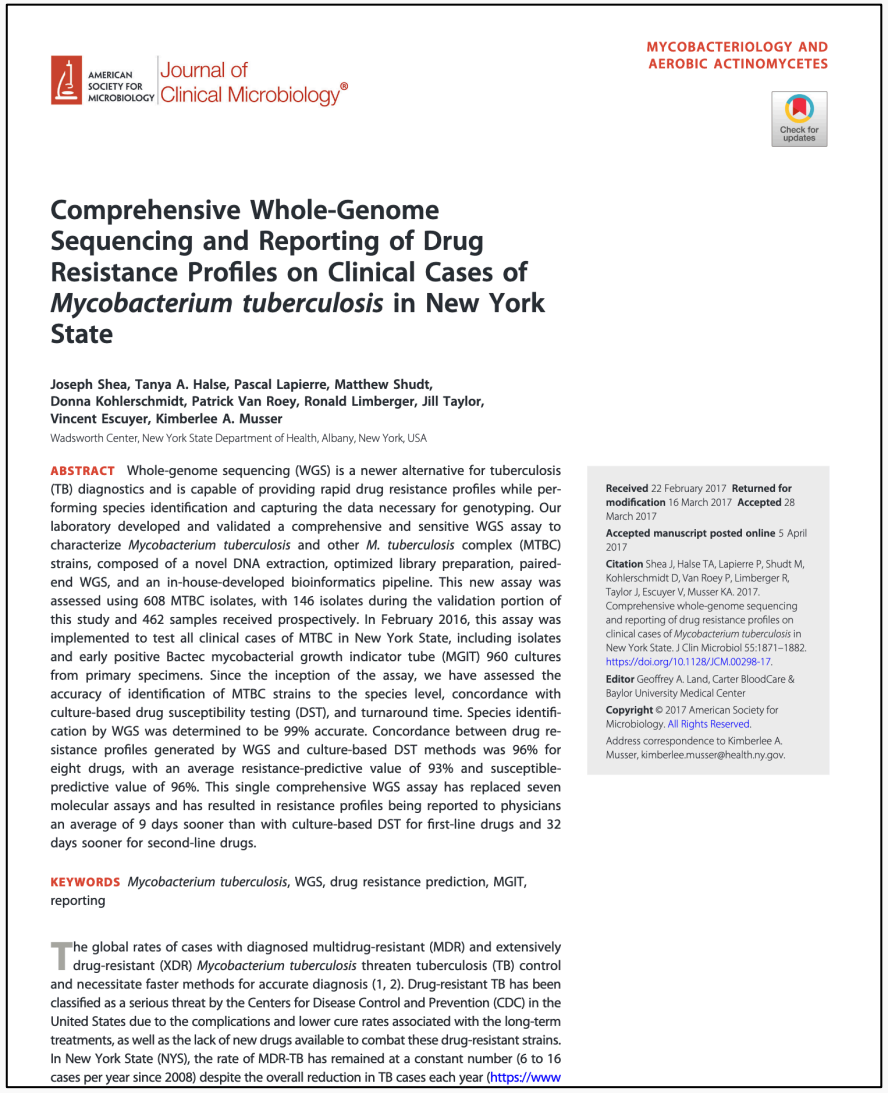
Examples: WGS MTBC Drug Resistance

- SOP
- Quality Control Metrics- monitoring entire assay
- Reagent control and testing
- Software updates
- Revalidation plan
- Controls (Neg, Pos, Inhibition)
- Reporting
- Validation
  - Specificity
  - Limit of Detection (specimens)
  - Reproducibility (Inter- and Intra-)
  - Accuracy



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Check for updates

Comprehensive Whole-Genome Sequencing and Reporting of Drug Resistance Profiles on Clinical Cases of *Mycobacterium tuberculosis* in New York State

Joseph Shea, Tanya A. Halse, Pascal Lapierre, Matthew Shudt, Donna Kohlerschmidt, Patrick Van Roey, Ronald Limberger, Jill Taylor, Vincent Escuyer, Kimberlee A. Musser

Wadsworth Center, New York State Department of Health, Albany, New York, USA

ABSTRACT Whole-genome sequencing (WGS) is a newer alternative for tuberculosis (TB) diagnostics and is capable of providing rapid drug resistance profiles while performing species identification and capturing the data necessary for genotyping. Our laboratory developed and validated a comprehensive and sensitive WGS assay to characterize *Mycobacterium tuberculosis* and other *M. tuberculosis* complex (MTBC) strains, composed of a novel DNA extraction, optimized library preparation, paired-end WGS, and an in-house-developed bioinformatics pipeline. This new assay was assessed using 608 MTBC isolates, with 146 isolates during the validation portion of this study and 462 samples received prospectively. In February 2016, this assay was implemented to test all clinical cases of MTBC in New York State, including isolates and early positive Bactec mycobacterial growth indicator tube (MGIT) 960 cultures from primary specimens. Since the inception of the assay, we have assessed the accuracy of identification of MTBC strains to the species level, concordance with culture-based drug susceptibility testing (DST), and turnaround time. Species identification by WGS was determined to be 99% accurate. Concordance between drug resistance profiles generated by WGS and culture-based DST methods was 96% for eight drugs, with an average resistance-predictive value of 93% and susceptible-predictive value of 96%. This single comprehensive WGS assay has replaced seven molecular assays and has resulted in resistance profiles being reported to physicians an average of 9 days sooner than with culture-based DST for first-line drugs and 32 days sooner for second-line drugs.

KEYWORDS *Mycobacterium tuberculosis*, WGS, drug resistance prediction, MGIT, reporting

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Editor Geoffrey A. Land, Carter BloodCare & Baylor University Medical Center

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The global rates of cases with diagnosed multidrug-resistant (MDR) and extensively drug-resistant (XDR) *Mycobacterium tuberculosis* threaten tuberculosis (TB) control and necessitate faster methods for accurate diagnosis (1, 2). Drug-resistant TB has been classified as a serious threat by the Centers for Disease Control and Prevention (CDC) in the United States due to the complications and lower cure rates associated with the long-term treatments, as well as the lack of new drugs available to combat these drug-resistant strains. In New York State (NYS), the rate of MDR-TB has remained at a constant number (6 to 16 cases per year since 2008) despite the overall reduction in TB cases each year (https://www

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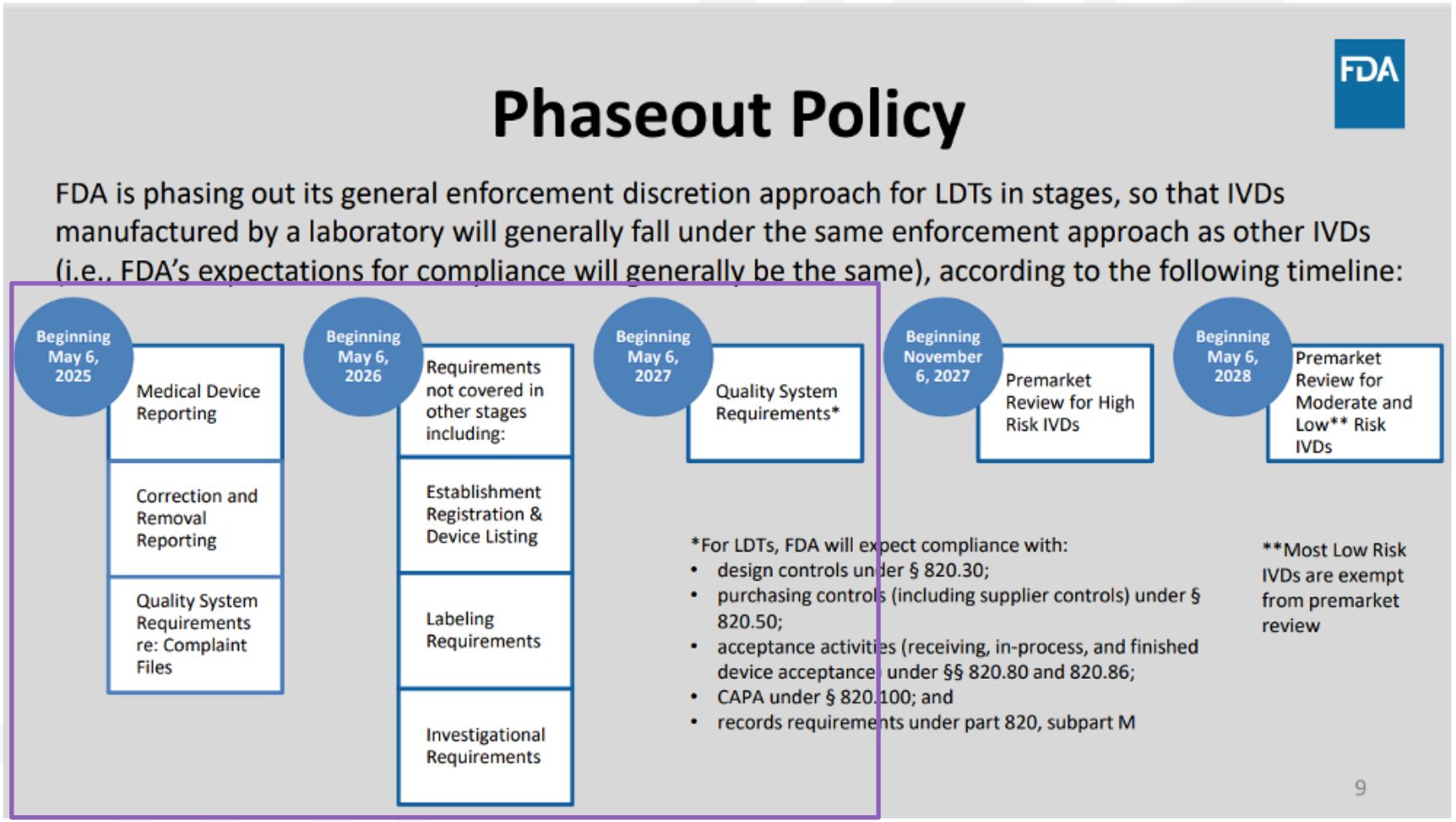
WADSWORTH CENTER AND THE FDA RULE

NYS CLEP permitted labs need to comply with Phase 1, 2 and 3



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IVD=In vitro diagnostic

APHL LDT Guidebook 2024

QUESTIONS?

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