Preparing for and implementing a laboratory-developed test

Learning objectives

- Describe how a laboratory can prepare to design and implement a laboratory-developed test (LDT)
- Outline the typical steps of LDT implementation and identify key questions that must be addressed at each stage of the process

Introduction

Research and development (R&D) tests comprise a unique subcategory of tests in a clinical laboratory. R&D testing should be segregated from clinical testing operations, conducted using different platforms, and preferably be performed in separate laboratory spaces.

Clinical and R&D samples should not be included in the same run, and instrumentation used for an R&D assay that is located within the footprint of a clinical laboratory should be clearly labeled “For Research Use Only” and “Not for clinical testing.” It is preferable to avoid sharing equipment; but if sharing is necessary, the equipment should be clearly identified as being for either clinical testing or research use on specific days or at specific times of operation.

If an R&D test has clinical utility, a laboratory may elect to implement it as an LDT. An LDT is a diagnostic test designed, validated, and performed by an individual laboratory. If an LDT is not transferred, licensed, or sold to other laboratories, it is not considered an *in vitro* diagnostic (IVD) test by the U.S. Food and Drug Administration (FDA). The decision to develop an LDT is based largely on the patient population, requests from healthcare providers, and the technical expertise in the laboratory.

Although LDTs can only be used by the laboratories that develop them, a laboratory may consider an LDT the best option when a diagnostic test is needed to meet specific clinical requirements. It is often the case that no FDA-approved IVD assay is available to test for a rare disease or condition. A laboratory may also opt for an LDT when there is a newly identified or newly reported marker that is not included in the panel of an existing FDA-approved IVD assay.

An LDT must be verified by demonstrating that it has clinical utility for the intended patient population, but it can be implemented quickly for emergency use. This is an important advantage when a test must be developed and deployed in response to an emergency like an epidemic or pandemic.

How are LDTs developed?

The need for an LDT is justified based on clinical input to improve patient care. Input from a clinical advocate is thus key in the assay development process. The laboratory that develops the assay must be knowledgeable about how the test will be used and understand the specimen type, range of detection, turnaround requirements, and potential complementary tests.

It is at this stage when the laboratory decides on the technology, specimen type, and test methodology for the LDT. Test methodology might be as simple as adding a new analyte to an established test or using a different platform in the laboratory. LDT methodology could require more intensive development if it involves a new specimen type, new instrumentation, or new biomarkers. It may also require optimization to improve sensitivity, reduce turnaround time, or benefit a new patient demographic.

A clinical laboratory can develop an LDT for a new analyte or biomarker(s) *de novo* and take it through the necessary analytical, performance, and clinical validation studies as defined in 42 CFR §493.1443[1] to satisfy the criteria of the laboratory director and regulatory officers at the state level.

Deploying an LDT requires a research and development process that documents standard operating procedures (SOPs) for the assay and includes basic analytical validation studies to show that the assay satisfies clinical requirements. It may also include pilot clinical studies to document the range of values that can be expected in the target patient population. Handoff to a clinical testing laboratory should include a validation report, which provides direction for the laboratory to verify the SOPs or validate modified SOPs.

Licensure and certification

While any laboratory can develop an LDT, a testing laboratory must be certified under the Clinical Laboratory Improvement Amendments (CLIA) and licensed in the state in which it operates. A laboratory must also be licensed in other states if an assay it develops will be used to test samples from patients who are residents of those states.

The approval criteria for LDTs in the United States vary from state to state. New York state performs the most stringent review of LDTs prior to their approval for use on patient samples. Several other states, including Washington, have specific requirements for LDT approval.

A laboratory must satisfy both CLIA and state requirements if it plans to provide test results for clinical patient management and receive payment from the Centers for Medicare and Medicaid Services (CMS) or private insurance.

Quality management and documentation

A CLIA-certified laboratory must have a quality management system (QMS) that specifies which documentation is required and how it is archived. This applies to all tests, including IVD tests and LDTs. The QMS encompasses all SOPs; quality control (QC) monitoring; temperature records; personnel training and competency records; minutes from quality management meetings with the laboratory director; communications from vendors regarding recalls or changes in performance; and corrective and preventative action (CAPA) documentation.

Personnel who perform test development do not need to be licensed technologists, but those who validate or verify LDTs should be trained and qualified to perform testing on patient specimens and have state or national licensure. The qualifications for medical technologists, supervisors, and directors can be found in 24 CFR §493.1449[2] and on state health department websites[3].

Once a test has been validated and the validation report is signed by the laboratory director, all technologists who may perform testing must be trained. Competency must be assessed and documented before the technologists are qualified to perform the new test on patient specimens.

All equipment, testing instrumentation, and reagents must be specified by vendor, model, serial number, lot number, or other applicable category in laboratory records. These specifications should not be modified after IVD verification or validation of an LDT without performing additional studies. Since IVD reagents are packaged in kits, reagents from different lots and non-kit reagents should not be mixed or used for testing.

LDT reagents can be analyte-specific reagents (ASRs), reagents for research use only (RUO), or reagents for investigational use only (IUO). All reagent specifications and documentation should be provided by the vendor and retained as laboratory records. A reagent from a new lot must be tested in parallel with the equivalent reagent from the old lot for both IVD tests and LDTs, and documentation of acceptable performance must be signed by the laboratory director.

If a different testing platform is selected for an existing LDT, a parallel study must be performed to compare performance on the old and new platforms. For example, a laboratory may develop an LDT using a technology platform that is readily available in the laboratory, such as conventional PCR. The laboratory might later switch to a newer or different platform like digital PCR. To remain compliant, the laboratory must demonstrate that the performance of the assay is comparable on the two platforms. Quantitative and qualitative comparisons must be performed and documented to ensure that the reported results are clinically equivalent.

If a different LDT reagent is used, a verification study must be performed to demonstrate that the test results are consistent with clinical interpretation in the original validation. If the expected patient ranges need to be adjusted, these changes must be documented in the QMS, updated in the laboratory information system (LIS) or hospital information system (HIS), and appropriately noted on each report form.

When LDTs are designed and validated, a variety of quality and process controls should be in place to verify that all steps of the assay are working appropriately. Depending on the technology behind the test, appropriate controls might include extraction controls, controls to assess clinical accuracy, hybridization controls, external analyte controls, or internal controls for analytes that are expected to be present in each test sample. An example of an internal control is human DNA on a nasopharyngeal swab used to test for SARS-CoV-2. A human DNA concentration above a predetermined threshold value would confirm that enough sample was collected to detect the virus in asymptomatic individuals.

Proficiency requirements

All laboratories that perform LDTs or FDA-cleared or FDA-approved IVD clinical assays must conduct routine proficiency testing or regularly exchange samples with other laboratories that perform similar tests. All proficiency training must be performed by qualified technical staff, and samples must be treated exactly as patient samples would be treated. If an appropriate proficiency testing survey is not available, it is acceptable to archive and store patient samples with appropriate consent after reporting.

Remnant samples should be added blindly to the laboratory testing queue at least twice per year, and they must have proven stability at the archiving temperature. Patient consent is not required to use remnant samples for test validation and parallel studies if the samples have previously been reported and could have been discarded.

Personnel training documentation

IVD tests and LDTs share the same regulatory requirements for documentation of results. SOPs must be reviewed annually or every two years depending on state requirements, and the review must be signed by the laboratory director. Any changes or updates can be reviewed off cycle or during the required review timeframe. Changes and updates may necessitate retraining and documentation of retraining as well as competency assessments for all testing personnel.

The QMS will require that all previous versions be archived and available for retrieval when needed. All software integrated into the LIS must safeguard protected health information (PHI) and ensure patient confidentiality under the Health Insurance Portability and Accountability Act (HIPAA). Most LISs are designed to trace access by everyone who logs into a test workflow and provide an audit trail. Security levels are determined before the software is implemented in the laboratory and maintained to prevent unauthorized access.

Implementing an LDT

How does a CLIA-certified laboratory implement an R&D assay as an LDT? There are multiple steps in the decision-making process leading to the launch of an LDT. Technical and legal feasibility must be evaluated first. The laboratory director and clinical consultant should draft a development plan with key clinicians to determine the assay's design along with its intended use and performance specifications. IVD product specifications for assays that have similar clinical applications should be compared to the expected performance of the LDT. A freedom to operate analysis should be conducted to assess the patent landscape for the contemplated LDT and any potential patent infringement issues. The LDT testing technology and platform should be suitable for the assay as designed. It is equally important to determine whether the platform is already available in the clinical laboratory.

Feasibility and design assessment

Figure 1 illustrates the steps of LDT implementation. Key parameters include the reason for developing the test, how it is performed, and how it fits into laboratory operations. Implementation in a clinical laboratory must be feasible in terms of the space, airflow, and technological training it will require. Once the testing procedure is optimized, the test must be analytically validated according to state requirements and/or Clinical Laboratory Standards Institute (CLSI) guidelines. These include requirements for accuracy, sensitivity, specificity, reproducibility, and other parameters that will depend on the sample source and technology involved. CLIA-approved tests do not have to have clinical utility, but the results should be verified by another laboratory or with another method to ensure accuracy.

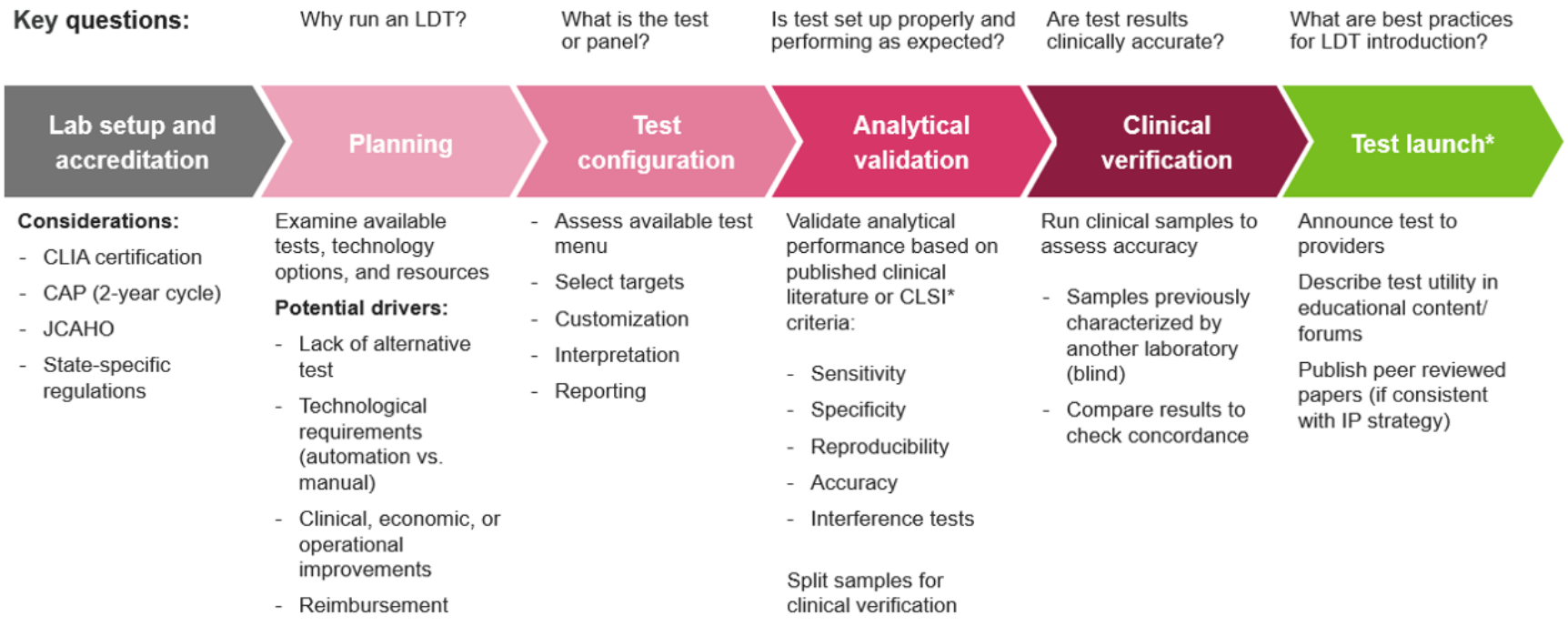

Figure 1. Typical LDT implementation process from planning to launch.

The path from development to launch

How long does it take to develop and validate an LDT for clinical use? Depending on the technology, it can take up to 12 months to complete all phases. Once the technology has been established in the project planning phase, the test platform must be qualified for use and available to the development team. The testing procedure must be developed and optimized, and reagents, calibrators, controls, and consumables must be identified, ordered, and placed in inventory.

Depending on the technology, calibrators and controls for molecular LDTs may be purchased from IVD or RUO vendors or prepared in-house from purified reagents spiked into appropriate matrices. Primers and probes selected for genetic sequence analysis must be tested for specificity and confirmed by BLAST analysis. DNA and RNA extraction reagents can be purchased and optimized for a given sample matrix if necessary. PCR master mixes may be commercially available or developed in-house.

Reagents used for an LDT must meet the quality requirements of the laboratory if they are purchased from vendors who also provide IVD kits, even though they would be considered LDT reagents rather than IVD reagents. When the laboratory uses the reagents, the LDT must also meet the specified accuracy requirements.

After the project planning phase, the assay must be optimized and analytically validated for accuracy, sensitivity, and specificity just as it would in an IVD analytical validation study. The CLIA Program requires that all testing procedures be well-documented. SOPs for an LDT must be specific and updated regularly, and documentation must show that the SOPs have been followed appropriately. An LDT laboratory must document analytical validation studies, but the CLIA Program does not require clinical trials. Clinical utility can be assessed by comparing test results from a subset of samples to results from another CLIA-certified laboratory, so it is not necessary to report outcomes in patient cohorts.

Although IVD assay and LDT development both require project planning, instrument identification, qualification, staff training, and pre-validation planning, the analytical validation period is quite variable. Analytical validation of an LDT often takes longer due to limitations in technical resources. IVD tests are clinically validated by the vendor using samples from a wide range of patients. Clinical validation of an IVD test can overlap with analytical validation, but specific clinical specimens must be analyzed to verify the usefulness of an LDT for patients. Clinical verification of an LDT is less stringent than IVD validation, although this will depend on the availability of specimens from patients with and without the disease of interest and the presence or absence of the target analyte in patient samples.

Conclusion

LDTs have a specific role in clinical laboratories in the U.S. As a single site test, an LDT is owned by the laboratory that develops it. For legal reasons, intellectual property and the freedom to operate should be established prior to LDT development. A laboratory that designs an LDT must consider which testing platforms are available as well as the clinical need the test will fulfill. For example, an LDT for a highly sensitive chemical analysis might require a new platform. A blood test for molecular identification of a new parasite might be adapted from a urinalysis assay.

Since all clinical laboratories must be CLIA-certified, LDTs must satisfy laboratory QMS requirements. CLIA-certified laboratories must also participate in proficiency testing programs as well as maintain training and competency documentation for all employees. All LDT analytical validation studies must document the accuracy, sensitivity, specificity, and reproducibility of the tests. Clinical verification studies are also required for LDTs to show that the tests have clinical utility for the target populations defined by the testing laboratories.

References

1. Standard; Laboratory Director Qualifications, 42 CFR §493.1443 (2003).
2. Standard; Technical supervisor qualifications., 24 CFR §493.1449 (1993).
3. Washington State Office of Laboratory Quality Assurance, https://www.doh.wa.gov/LicensesPermitsandCertificates/FacilitiesNewReneworUpdate/LaboratoryQualityAssurance.

About Arizona State University College of Health Solutions

Recognized by U.S. News & World Report as the country's most innovative school, Arizona State University (ASU) is where students and faculty work together to advance research, strategic partnerships, entrepreneurship and economic development.

ASU's College of Health Solutions is dedicated to translating scientific health research and discovery into practical interventions. The college's Master's Degree in Biomedical Diagnostics is designed to address the role of diagnostics in clinical and research decision making. The only program of its kind in the world, ASU is dedicated to continuing to be the academic and intellectual center of the diagnostics industry.