An introduction to diagnostic testing in laboratories

Learning objectives

- Describe different types of *in vitro* diagnostic (IVD) tests
- Identify differences between a laboratory developed test (LDT) and an IVD test
- Understand the benefits of running LDTs and IVD tests
- View examples of LDTs and different applications for them

In this overview, we describe different diagnostic assays and explain why they are important for laboratories in clinical, hospital, academic, and specialized settings. We discuss potential areas for growth in the use of LDTs as well as what a laboratory must do to implement one. We also describe more traditional IVD tests that are approved by the U.S. Food and Drug Administration (FDA), the role of IVD tests in the U.S. healthcare system, and some of the potential benefits associated with them. Finally, we review regulations that must be understood by IVD manufacturers and laboratories that offer LDTs.

IVD tests and LDTs can be used for patient testing in clinical laboratories. IVD tests are regulated by the FDA and validated by manufacturers prior to approval for marketing. In contrast, an LDT is designed and validated by a laboratory that wishes to expand its services and client base, deliver personalized medicine, or provide support to clinicians in a particular specialty. LDTs are not approved by the FDA and can only be used by the laboratories that develop them.

What is an LDT?

An LDT is a diagnostic test for clinical use that is designed, manufactured, and performed by an individual laboratory. If a clinical laboratory develops its own assay and uses it for health screening or diagnostic purposes, the FDA considers the test an LDT as long as it is not transferred, licensed, or sold to other laboratories. FDA oversight of LDTs is based on risk. In contrast, the FDA considers diagnostic tests that are performed on human specimens to be IVD tests. IVD tests are categorized as medical devices if they are marketed and sold to laboratories, health systems, or individual consumers. The FDA requires premarket approval or premarket clearance for IVD assays, and it regulates their manufacture and use. LDTs and IVD tests are compared in Table 1.

Table 1. Differences between IVD tests registered with the U.S. FDA and LDTs.

IVD test with FDA approval	LDT
Developed for sale to diagnostic laboratories, health clinics, or consumers	Developed by individual laboratories; not transferred, licensed, or sold
Standardized instrument qualification procedures and training required	Instrument qualification and training requirements established by individual laboratories
Must be pre-validated with a data analysis and bioinformatics report	Often developed in-house by necessity—no standard assay available
Must be clinically validated	Must be clinically verified and can be implemented quickly for emergency use*

* Must comply with the Clinical Laboratory Improvement Amendments (CLIA) of the U.S. Centers for Medicare and Medicaid Services.

LDTs include a wide variety of assays developed for different applications (Figure 1). For example, large hospital-based laboratories and academic reference centers have developed LDTs to identify pathogens in blood or sputum during outbreaks and to detect and quantify antibodies after infection or vaccination. Reference laboratories and clinical laboratories in academic hospitals are often asked to perform tests for rare diseases that are not generally available as IVD tests. For example, an assay may be developed to detect proteins or genes associated with a rare condition like Tay-Sachs disease [1].

Some laboratories develop assays to monitor disease progression or treatment response when no standardized assay exists. A hospital or specialty laboratory may develop an LDT for a complex multigenic disease like cancer by creating its own panel for genetic polymorphisms that can be used to classify a disease or determine whether a particular therapy is appropriate. An LDT can also be used to identify novel biomarkers for a specific disease.

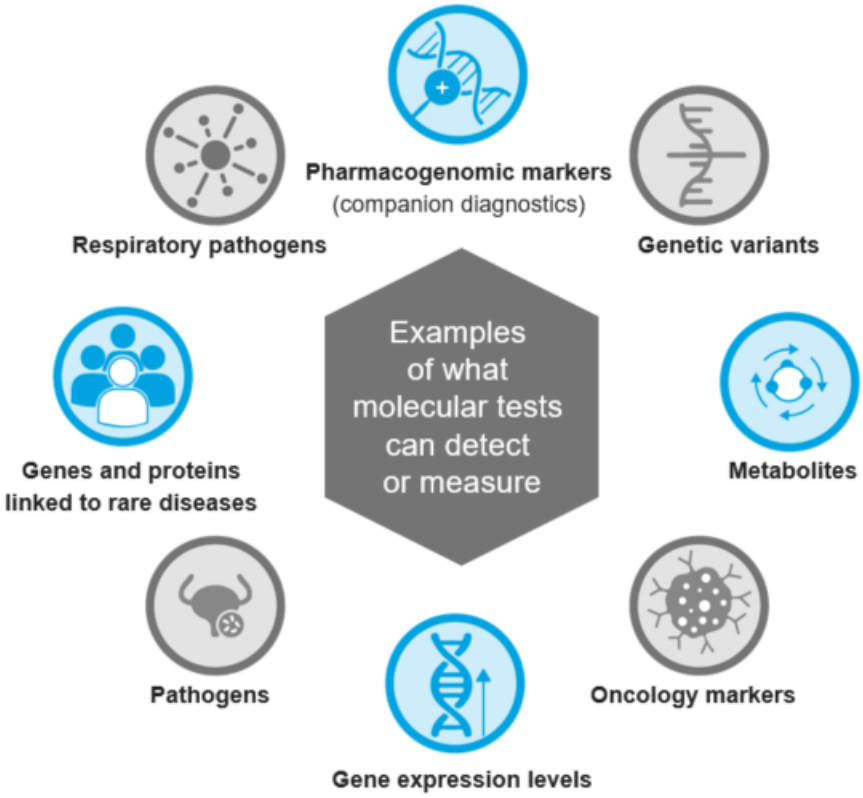

Figure 1. Molecular LDT and IVD tests include a wide variety of assays for different applications.

LDTs have been developed for a broad array of targets, including small molecules, proteins, RNA, DNA, cells, and pathogens. LDT technologies range from molecular diagnostics to immunoassays and mass spectrometry. An LDT or IVD test can be a simple assay developed to measure a particular biomarker, or it may require a highly complex algorithm to assess multiple analytes and biomarkers. The Clinical Laboratory Improvement Amendments (CLIA) Program, administered by the U.S. Centers for Medicare and Medicaid Services, recognizes three levels of IVD test complexity. A simple waived test requires little technical training. Moderately complex tests are usually performed with automated clinical laboratory equipment, while highly complex tests require a high level of expertise (Table 2). In contrast to IVD tests, LDTs are always considered highly complex tests.

Table 2. Clinical laboratory tests fall into three main categories. The U.S. FDA uses a patient and public health risk–based classification system for IVD tests that are registered for clinical use. The scoring system considers test complexity, the stability of calibrators and controls, any pre-analytical steps required, and the level of expertise needed to interpret test results.

CLIA category	**Waived tests**	**Moderately complex tests**	**Highly complex tests**
Description	Simple to perform with a low risk of interpretation error; require little technical training; many sold over the counter (OTC) for consumer use	Usually performed with automated clinical laboratory equipment	Require clinical laboratory expertise beyond automation; may require additional data analysis expertise
Examples	• Pregnancy tests • Tests for drugs of abuse • Strep tests • Dipsticks • Glucometers and other simple devices • Lateral flow SARS-CoV-2 antigen tests	• Electrolyte profiles • Chemistry profiles • Complete blood count • Urinalysis • Urine drug screen • Automated immunoassays	• Cytology • Immunohistochemistry assays • Peripheral smears • Flow cytometry • Gel electrophoresis • Most molecular diagnostic tests like RT-PCR, gene chip arrays, multiplexed analyses, dot blots, viral loads, expression arrays and CGH arrays

The business case for molecular diagnostics

IVDs

Human samples are often analyzed with IVD tests to measure the concentrations of specific analytes, such as sodium or cholesterol. IVD tests can also be performed to confirm the presence or absence of a particular marker or set of markers, such as a genetic mutation or an immune response to infection. Healthcare providers regularly conduct IVD testing to diagnose conditions, guide treatment decisions, and mitigate or prevent future disease. For example, a screening test may be performed to estimate a patient's risk of developing a given condition in the future.

The FDA has regulated medical devices since the Medical Device Amendments were passed in 1976. Medical devices include products that are intended for use in the diagnosis of diseases or other conditions. The FDA also has the authority to regulate the components of a diagnostic test, such as reagents, that are used to detect or measure other substances. In the current regulatory framework, IVD tests developed for the commercial market are subject to FDA regulatory requirements to ensure their safety and effectiveness.

IVD tests have significant advantages over LDTs (Table 3). First and foremost, a manufacturer can sell an IVD test to other laboratories, healthcare systems, or point-of-care locations after obtaining FDA approval or clearance as long as its use falls within the scope stated on the approval or clearance label. Marketing IVD tests enables sponsors to generate a broad customer base, because many clinical laboratories can perform IVD assays. Revenue from the sale of an IVD test can then be used to offset any regulatory costs associated with the IVD pathway.

Healthcare providers may also benefit by purchasing IVD tests. Laboratories, healthcare systems, and point-of-care facilities may choose to perform IVD testing themselves rather than obtain LDT services from other laboratories. This is because the FDA requires the design and manufacture of IVD tests to be extensively controlled (Figure 2). The FDA also requires post-approval surveillance, such as monitoring and reporting of adverse events. These FDA-mandated protection mechanisms can thus make an IVD test more attractive to a clinical laboratory than LDT-based services.

Table 3. Advantages of IVD tests for individual laboratories.

Advantage	Example
Quality system	Tests are subject to various requirements, including design controls, manufacturing controls, and monitoring complaints.
Simplified inventory control	Users only need to order manufactured tests for anticipated use, which reduces the amount of documentation required. Use of an LDT requires an inventory of the actual test and all of the components needed to perform it. It requires less time and effort to maintain in-house inventories for IVD tests.
Technical support	Customer can contact supplier technical support to troubleshoot and replace faulty products.
Clinical validity	Clinical validation of the test ensures that it detects or measures the specified target analyte, or that it is useful for determining the presence or absence of a clinical condition or predisposition to the condition prior to marketing.
Broad distribution	Many laboratories utilizing the test provide data that can increase or potentially reduce confidence in it. Laboratories performing the same IVD test can report proficiency results to confirm the accuracy of the test.

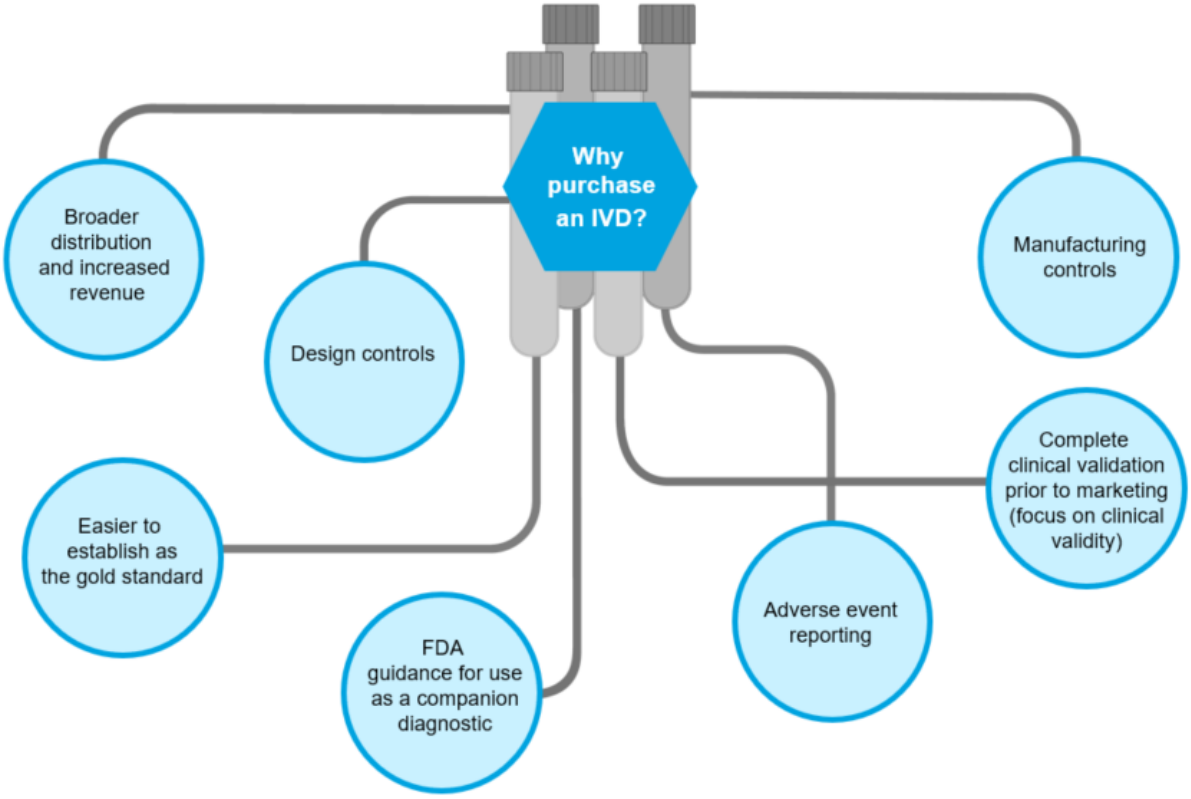

Figure 2. Reasons a laboratory would choose to purchase an IVD test.

LDTs

Over the past decades, independent laboratories, university laboratories, laboratories associated with hospitals, and reference laboratories have developed thousands of LDTs. These tests are often developed in-house out of necessity. A comparable commercial test may not be available if the market for such an assay is too small, which is frequently the case for laboratories investigating rare diseases. When there is an immediate need for a unique assay, an LDT is often the faster path for *de novo* development and biomarker panel selection. In other cases, LDTs are developed because applying for authorization would be cost-prohibitive for the laboratory or sponsoring agency.

Jonathan Genzen, an associate professor of clinical pathology at the University of Utah, addressed this topic in a 2019 paper published in the American Journal of Clinical Pathology [2]. “Given the high costs of obtaining premarket approval, as well as the limited financial incentive for IVD manufacturers to develop esoteric tests or tests for rare diseases, [these] laboratories address unmet clinical needs through the development of LDTs that are performed in a single laboratory location.” Table 4 lists some of the advantages LDTs have for individual laboratories, and reasons for choosing to develop an LDT are summarized in Figure 3.

Table 4. Advantages of LDTs for individual laboratories.

Advantage	Description
Control over content	Laboratories can select specific and relevant target(s) and applications.
Rapid adaptation	LDTs can be developed and modified relatively quickly to respond to market needs.
Lower cost per test	Technological advances and the availability off-the-shelf bulk reagents have made complex analyses faster and more affordable.
Consolidation into a single test	Testing for multiple analytes provides more data per sample and may enable faster diagnosis.
Laboratory qualification	The laboratory quality management system (QMS) covers all tests, including LDTs, which is not the case for IVD kit production.

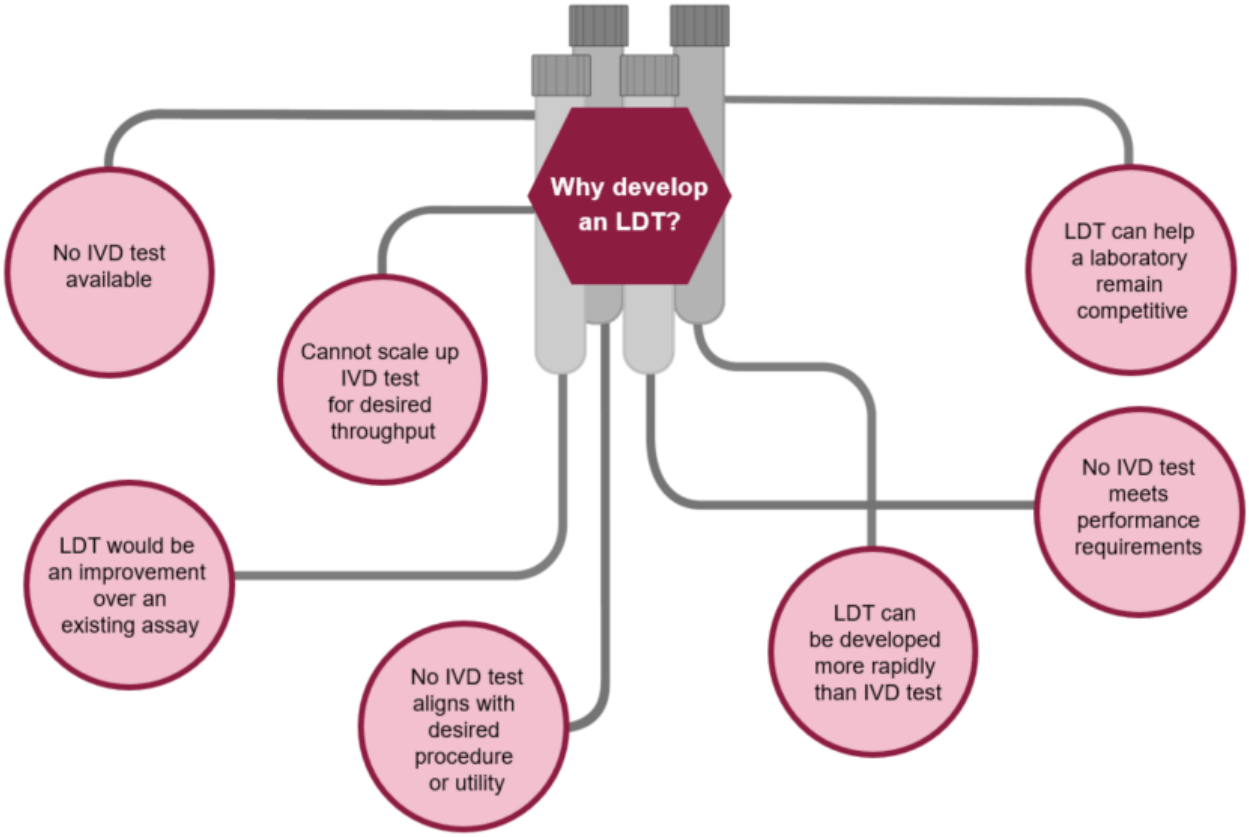

Figure 3. Reasons a laboratory would choose to develop an LDT.

Rapid modification of LDTs for complex diseases

Novel LDTs can be rapidly developed and launched, because LDT deployment does not require prior approval or authorization by a government agency or third party. This makes LDTs particularly useful when rapid adoption is necessary to respond to changing circumstances, or when the biomarkers requiring measurement have not been defined or standardized. One such example is the Genomic Health™ Oncotype™ Dx test, which is used to estimate the likelihood that a woman will experience a recurrence of breast cancer within ten years of diagnosis [3]. The original assay was used to query the activity of 21 genes, and patients were stratified according to metastasis risk [4].

A modified test has since been developed that queries 12 genes associated with ductal carcinoma *in situ* (DCIS) breast cancer. The modified test helps oncologists determine whether radiation therapy will benefit women with DCIS breast cancer. LDTs are especially useful for stratification and the diagnosis of complex diseases, because the biomarker panels of these assays can be modified quickly. However, if a CLIA-certified laboratory changes an LDT protocol, the changes must be documented. The test must also be validated per the requirements of the quality management system.

LDTs as companion diagnostics for personalized medicine

Companion diagnostics belong to a special class of LDTs that are developed for specific therapies. For example, Herceptin™ (trastuzumab) was developed by Genentech™ as an immunotherapeutic drug for breast and gastrointestinal cancer. However, the test can only be used if malignant cells express the HER2 receptor. Companion diagnostics can guide clinicians in tailoring treatment regimens for individual patients. Many LDT-based companion diagnostic assays may be initially evaluated in National Cancer Institute (NCI) trials by regional cancer research networks, such as the Eastern Cooperative Oncology Group (ECOG) or the SWOG Cancer Research Network. These tests are ultimately submitted to the FDA for approval as IVD tests. Creating an LDT can thus be a foundational step in developing a companion IVD test.

LDTs for clinicians exploring new MIS-C biomarkers

LDTs can help clinicians explore new biomarkers as indicators of disease or treatment response. A condition for which an LDT could be useful is multisystem inflammatory syndrome in children (MIS-C), one of the long-term SARS-CoV-2 sequelae. Researchers are still trying to identify SARS-CoV-2 biomarkers, determine how many biomarkers should be evaluated, and decide when samples should be collected [5].

Circulating tumor DNA (ctDNA) is a biomarker that has recently been validated as an indicator of certain types of cancer. MicroRNAs (miRNAs) are increasingly being used as biomarkers for the diagnosis and treatment of large B cell lymphoma, renal fibrosis, and breast carcinomas [6]. However, standardized protocols for the collection, transport, and storage of samples and data analysis still need to be developed for these LDTs.

Improving existing assays and testing platforms

Laboratories that develop LDTs can improve existing assays and testing platforms. For example, a laboratory can try to increase throughput or efficiency by automating or scaling sections of a workflow. Modifying an existing LDT is permissible as long as any changes are documented appropriately and comply with the laboratory's quality systems. LDTs are important vehicles for diagnostic innovation, because they permit laboratories to experiment and improve novel and highly complex tests. Laboratories can and should conduct their own internal studies to fully characterize an assay's performance, establish its analytical limitations, and identify potential interferents. The laboratory should also determine which specimen types are appropriate for a given LDT, establish specimen stability limits, and identify appropriate reference intervals for test samples. Unlike LDT modification, modification of an FDA-cleared or FDA-approved IVD test must be done by the manufacturer and may require regulatory notification or resubmission to the FDA. An IVD test that is modified by a testing laboratory becomes an LDT that will require additional validation and documentation by the laboratory.

Rapid response to health crises

LDTs are critical for mounting a rapid response to a health crisis. Early in the SARS-CoV-2 pandemic, scientists at the U.S. Centers for Disease Control and Prevention were able to develop a reverse-transcription PCR test for SARS-CoV-2. The test was developed within ten days of the release of the SARS-CoV-2 genetic sequence, which was before community spread was first reported in the U.S. Almost simultaneously, virologists at the Charité hospital in Germany developed a test that was adopted for the protocol of the World Health Organization. Many laboratories were eager to contribute their services in response to the emerging outbreak. The University of Washington Virology Laboratory developed their own SARS-CoV-2 LDT by the end of January 2020. At the same time, geneticists at the Broad Institute of MIT and Harvard began supplying SARS-CoV-2 diagnostic kits to hospitals in Africa.

It was recognized early in the pandemic that SARS-CoV-2 antibody tests would be important for determining the total number of SARS-CoV-2 cases in the population and identifying individuals who had acquired immunity and could safely return to public life [7]. Responding to this perceived need, laboratories rapidly developed a variety of antibody tests that included ELISAs, neutralization assays, and chemiluminescent immunoassays. Unfortunately, the FDA had no standardized means by which to evaluate claims for these serological LDTs.

Laboratories were forbidden from offering coronavirus antibody tests until they were granted emergency use authorization at the end of February 2020. This impeded scale-up of diagnostic capacity and delayed efforts to control the spread of SARS-CoV-2 [8,9]. The global crisis brought about by SARS-CoV-2 clearly illustrates how important diagnostics can be when testing is urgently needed.

Laboratories must be prepared to develop and deploy necessary tests during a health emergency, and they must be sufficiently staffed to conduct testing. Some contend that LDTs are too complex for use in clinical laboratories. It should be noted that validation of an LDT or IVD test generally involves the same steps (Table 5). Validation of an LDT test may require fewer samples and less rigorous review, while IVD assays may receive less scrutiny from inspectors of the CLIA Program or the College of American Pathologists (CAP). However, the validation processes are quite similar overall, and accreditation of laboratories that develop LDTs and IVD assays tends to follow the same trajectory (Table 6).

Table 5. LDT and IVD test validation requirements.

	IVD FDA test validation*	LDT validation*
Utility	Per product labeling	Determined by lab, as demonstrated in validation studies
Reproducibility (CV)	High and low controls: • Intra-run precision (10 or more samples) • Inter-run precision (10 days)	High and low controls: • Intra-run precision (10 or more samples) • Inter-run precision (10 days)
Analytical sensitivity	Determine LOD with serial low-end dilutions	Determine LOD with serial low-end dilutions
Analytical specificity	Identify interferents (mucus, normal flora, etc.)	Varies with sample type
Analytical range	Validate established package insert cutoff with 10 or more samples	Establish normal range using samples from a mixed male and female cohort
Clinical sensitivity	Verify performance per package insert with samples from patients with and without disease	Verify performance with samples from patients with and without disease
Clinical specificity	Verify performance per package insert with samples from patients with and without disease	Verify performance with samples from patients with and without disease
Method correlation study (R^2, slope)	Not usually applicable (refer to IVD label)	Comparison with a different platform
Interpretation	IVD label	Criteria established by laboratory
Documentation for inspector	• QC • Calibration • PT • Reviewed, updated, and approved procedures • Training records • Personnel qualifications	• QC • Calibration • PT • Reviewed, updated, and approved procedures • Training records • Personnel qualifications defined by laboratory

* IVD tests are regulated by the FDA and must be registered with the agency. LDT validation procedures and laboratory requirements are determined by the laboratory based on the criteria of the accrediting body or the policies and regulations of state and/or local agencies.

Table 6. Accreditation and validation parameters for LDTs and IVD tests.

	IVD FDA test validation*	LDT validation*
Accreditation	CLIA + CAP* or JCAHO*	CLIA + CAP or JCAHO
Assay reagents	IVD Kit	LDT kit determined by lab
Controls	Provided	Determined by lab
Calibrators	Provided	Determined by lab
Calibration verification (linearity)	Third party† every 6 months	Third party† every 6 months, depending on technology
Proficiency Testing	Third party††, 2–3 tests per year	Third party 2–3 times per year or in-house testing at various concentrations using previously reported blind samples

* CAP: College of American Pathologists
** JCAHO: Joint Commission on Accreditation of Healthcare Organizations
† Third party calibration (CAP, American Petroleum Institute, Maine Standards, American Association of Bioanalysts)
†† CLIA-approved third party (Accutest Laboratories, AAFP Foundation Proficiency Testing Program, American Association of Bioanalysts, American Proficiency Institute, College of American Pathologists, Medical Laboratory Evaluation Proficiency Testing Program, Pennsylvania Department of Health, Wisconsin State Laboratory of Hygiene, American Society of Clinical Pathology)

Conclusion

A particularly relevant feature of LDTs is that they enable laboratories to be agile, so they can adapt rapidly to changing circumstances. LDTs can also be cost-effective and give researchers and clinicians the flexibility to search for new biomarkers and address unmet clinical needs. LDTs will continue to have a vital role in personalized medicine, emergency response, and the diagnosis and treatment of rare diseases. Although IVD tests cannot be as quickly developed and deployed as LDTs, they do offer benefits. IVD tests allow healthcare stakeholders like laboratories, healthcare systems, and POC facilities to choose diagnostic assays that the FDA has either approved or cleared for emergency use authorization. Faith in the FDA's process provides some measure of certainty, and stakeholders may not have as much confidence in services tied to LDTs.

In addition, reimbursement for LDTs can be complex and require multiple inputs over time.

Clinical and medical laboratories have provided critical infrastructure and technical expertise to quickly respond to emergencies and scale up testing capacity during past and present epidemics. There will undoubtedly be other situations for which the development of a diagnostic assay is necessary to quickly address an immediate need. To address clinical needs and improve patient care with LDTs, it is essential that laboratories have the flexibility to develop unique assays or modify existing IVD tests.

Glossary

Term	Description
CLIA	Clinical Laboratory Improvement Act of 1988. All laboratories performing clinical tests with human specimens must have a valid CLIA certificate. The CLIA Program is focused on the quality and competence of laboratories, whereas the FDA focuses solely on IVD tests.
De novo	Latin for "from the new." In the context of LDTs, a laboratory may develop a new or *de novo* test rather than modifying an existing test.
EUA	Emergency Use Authorization (EUA). EUAs are issued during public health emergencies for unapproved medical products or unapproved use of FDA-regulated products. EUAs provide an expedited path to market for new drugs and devices by compressing the development timeline for products and mitigating risk for sponsors.
FDA	Food and Drug Administration, a federal agency that oversees clinical laboratories in the United States.
Genetic polymorphism	A variation of a DNA sequence. The most common type of polymorphism involves a single base pair (https://www.genome.gov/genetics-glossary/Polymorphism).
Immunoassay	A procedure for detecting or measuring specific proteins or other molecules based on their properties as antigens or antibodies.
IVD	Refers to *in vitro* diagnostic products, reagents, instruments, and systems used to diagnose disease and other conditions with human specimens. The FDA regulates IVD products according to the rules set forth in 21 CFR Part 809.
Laboratory-developed test (LDT)	An *in vitro* diagnostic test for clinical use that is designed, manufactured, and performed by a single laboratory. LDTs are not currently regulated by the FDA.
Mass spectrometry (MS)	An analytical technique used to identify unknown compounds by molecular weight determination, to quantify known compounds, and to determine the structures and chemical properties of molecules (Broad Institute).
Molecular diagnostics	Molecular diagnostic tests are used to detect specific biological molecules, or biomarkers, in patient tissue and fluid samples. Molecular diagnostic tests can be used to identify an appropriate cancer therapy and/or to monitor the effects of treatment based on the characteristics of a biomarker or biomarker changes (Molecular diagnostics - National Cancer Institute).
Multigenic disease	A genetic disorder caused by abnormalities in two or more genes or chromosomes. Examples include Tay-Sachs disease and Alport's Syndrome.
Quality management system (QMS)	A formalized system to document processes, procedures, and responsibilities in a laboratory to ensure that accurate, precise, and timely results are obtained with the overall goal of patient satisfaction.
RT-PCR	Reverse-transcription polymerase chain reaction. RT-PCR is used to detect specific genetic sequences.
Validation report	A summary of findings and results used to assess the quality of a product or service.

References

1. ARUP Laboratories (2018) A rare niche: testing for hard-to-diagnose disorders (https://www.aruplab.com/magnify/2018/rare).
2. Genzen JR (2019) Regulation of laboratory-developed tests: a clinical laboratory perspective. *Am J Clin Pathol* 152(2):122–131 (https://doi.org/10.1093/ajcp/aqz096).
3. https://www.breastcancer.org/symptoms/testing/types/oncotype_dx.
4. Fowble B, Bevan A, Alvarado M, Melisko M (2010) Cancer of the Breast. In: Hoppe RT, Phillips TL, Roach M (editors), Leibel and Phillips Textbook of Radiation Oncology, Third Edition. Philadelphia: Elsevier Saunders. pp 1215–1323 (https://www.sciencedirect.com/topics/medicine-and-dentistry/oncotype-dx).
5. Samprathi M and Jayashree M (2021) Biomarkers in COVID-19: an up-to-date review. *Front Pediatr* 8:972 (https://www.frontiersin.org/articles/10.3389/fped.2020.607647/full).
6. Condrat CE, Thompson DC, Barbu MG et al. (2020) miRNAs as biomarkers in disease: latest findings regarding their role in diagnosis and prognosis. *Cells* 9(2):276 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7072450/).
7. Brennan Z and Lim D (2020) FDA pushed through scores of inaccurate antibody tests without agency review. *Politico* (https://www.politico.com/news/2020/04/27/reliable-antibody-tests-coronavirus-207589).
8. Baird R. What went wrong with coronavirus testing in the U.S. *The New Yorker* (https://www.newyorker.com/news/news-desk/what-went-wrong-with-coronavirus-testing-in-the-us).
9. Yong E (2020) How the pandemic defeated America. *The Atlantic* (https://www.theatlantic.com/magazine/archive/2020/09/coronavirus-american-failure/614191/).

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 The 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.