Lab testing 101:

An introduction to using labs in clinical practice

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Preface

Fullscript's Integrative Medical Advisory team (IMAT) has developed this guide using a variety of resources including government documentation, peer-reviewed articles, and information from key players in the laboratory (lab) testing industry. This guide provides an introductory overview of the lab testing industry, the regulatory environment in the United States, and other practical information providing practitioners a baseline understanding of lab testing.

Disclaimer
Practitioners should be aware of the potential risks of lab testing. Risks to patient health can include lack of diagnosis or misdiagnosis that can alter the course of a patient's treatment plan or cause undue mental harm. No test is 100% accurate. Decisions to use tests to inform treatment should always evaluate the potential benefits versus the risks to patient safety, and how testing will ultimately influence the treatment plan. This guide is not meant to provide recommendations for the use of specific tests. Practitioners should use their clinical discretion and expertise when evaluating the appropriateness of testing for their patients.

Introduction

This guide is meant to provide an introduction to lab testing for health practitioners in the United States. With the number of lab tests rapidly growing, it is important to have a basic understanding of some of the fundamentals of lab testing.

Use of lab tests

Lab testing is an essential resource in an integrative medical practitioner's toolkit. Being able to provide objective data, along with a patient's relevant history and/or physical examination, can be instrumental in making a confident diagnosis. Lab testing is used to inform approximately 70 to 80% of all clinical decisions. (Katayev 2010)(Rohr 2016)(St. John 2020) Many integrative practitioners use lab testing as a strategy to get patients invested in their health plan and boost treatment adherence by taking the time to adequately explain results and show progress in outcomes over time. (Bailey 2021)

Beyond providing a diagnosis, lab work can also be very useful for monitoring a patient's condition. Decision making on the modification, maintenance, or discontinuation of treatment can be greatly aided by repeat lab testing at certain intervals after treatment has begun.

Industry growth and value

Given that lab tests benefit both practitioners and patients, it is no surprise that both the lab testing and integrative medicine industries have grown rapidly. Many research firms cite that the acceleration of the North American and global diagnostics market is regularly attributed to factors such as the increased demand for point-of-care and at-home diagnostic products, a greater focus on preventative medicine, the growing rates of chronic disease, and the current global pandemic. (BioSpace 2021) (Precedence Research 2021)

This rapid growth is exemplified by the North American in vitro diagnostics market projected to grow from US$29B in 2020 to US$40B in 2027. (Precedence Research 2021) Globally, this market was valued at US$85B in 2020 and is expected to grow to US$118B by 2028. (BioSpace 2021)

Test availability

As lab tests are so widely used, it is reasonable for practitioners to wonder how tests become available and to have questions about which tests should be used from an evidence-based perspective. Though the lab testing regulatory environment and evidence requirements are complicated subjects to approach, this section provides a high-level overview of these topics.

U.S. regulatory environment

In partnership, the Food and Drug Association (FDA) and the Centers for Medicare and Medicaid Services (CMS) handle the regulatory oversight of lab tests and the laboratories that conduct and analyze tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA'88). The FDA primarily oversees the manufacturing, clearance or approval, marketing, and post-market surveillance of tests intended for commercial distribution. In contrast, the CMS has duties related to the quality assurance of laboratory practices. (FDA 2021a)

The labs that provide tests must also demonstrate the use of quality assurance practices related to sample collection, analysis, and interpretation. (FDA 2021a) Most facilities performing even one test require certification from the CMS or state agency. (CMS 2021) New York State and Washington State have their own stricter regulatory programs. (CMS ND) A summary of the requirements needed for certification will be provided in the "Lab certification" section of this guide.

The steps needed to bring a lab test to the market for medical use are highlighted in Diagram 1. Please note that the terms found in this diagram are explained throughout the following sections.

View Diagram 1. Development, distribution, and use of lab tests

In vitro diagnostics versus laboratory-developed tests

From a regulatory standpoint, all diagnostic tests are either considered in vitro diagnostics (IVDs) or laboratory-developed tests (LDTs). Under the CLIA'88, the FDA primarily regulates IVDs but reserves the right to oversee LDTs. However, the CMS primarily manages LDTs. (Genzen 2019)(Graden 2021)

IVDs are defined by the FDA as "reagents, instruments, and systems intended for use in the diagnosis of disease or other conditions, including a determination of the state of health, in order to cure, mitigate, treat, or prevent disease or its sequelae. Such products are intended for use in the collection, preparation, and examination of specimens taken from the human body." (FDA-21CFR809.3, 2022)

LDTs are sometimes referred to as "homebrew" tests. (CMS 2013)(Genzen 2019) Legally, an LDT is a type of IVD, but it (1) has been developed entirely from scratch and analyzed by a single lab in one location, (2) was an existing IVD that has been modified by a lab,

or (3) was an existing IVD, but the lab does not use the FDA-cleared/approved protocols when running the test (e.g., running an IVD using a nasal swab instead of saliva when the test was cleared or approved for analysis using saliva only). (Genzen 2019)

Often, the development of LDTs occurs when there is an unmet clinical need for innovation in an area of medicine and patient care. Though the classification as an IVD or LDT does not necessarily mean that one test type will be of higher quality or more accurate than another, (Kim 2018) there are differences in how these test types can be made available to patients.

If either type of test was being developed to be offered as a completely new product, manufacturers would initiate **pre-clinical development** (or the laboratory phase) to establish the design, performance, and usability of the test (e.g., **analytical accuracy**). At this stage, tests would be labeled for research use only and would not be available for commercial distribution. **Research use exemptions** and **investigational device exemptions** can be granted to allow manufacturers to provide tests for pre-clinical and clinical research development, respectively. (FDA 2013) These steps are typically required for IVDs (but not LDTs) needing FDA approval or clearance. (FDA 2019a)(Sarata 2014)

From here, depending on whether the test is legally considered an LDT or IVD, or if the manufacturer purposely wants to have the test cleared or approved by the FDA, the product will undertake different paths to market.

The steps to bring an IVD to the market include:

1. Determining the IVD classification
2. Submitting pre-market documentation based on the classification for FDA review
3. Following regulatory rules based on the test classification, such as adverse event reporting (FDA 2020c)

Depending on the lab test's risk classification, manufacturers may be preemptively required to conduct studies that demonstrate the benefits of the test in comparison to other products or procedures (more detail on clinical accuracy studies is provided in the "Evidence requirements" section). (FDA 2013)

Classification based on risk assessment is one of the critical steps in the IVD regulatory process as it determines the extent of requirements that the test will be required to meet prior to and after marketing (i.e., higher-risk tests have more requirements). (FDA 2021b)

Manufacturers will need to **register** and **list** their test with the FDA as one of three classes outlined in Table 1.

Table 1. Classification of tests (Lorick N.D.)(Piermatteo 2019)

Classification	Risk	Examples
Class I	Lowest risk of harm	Lactic acid, ESR, non-specific pathology stains
Class II	Moderate risk of harm	TSH tests, allergen tests
Class III	Highest risk of harm	Hepatitis B, C, or HPV tests, total PSA for cancer screening

Classification is done by comparing the new test to the FDA's classification database to determine if the new test is similar to a legally marketed **predicate test**. (FDA 2018)

The FDA compares:

- Whether the new test has the same intended use and technical characteristics; and
- The available safety and effectiveness data compared to the predicate test (FDA 2022a) (FDA 2020e)

Risk and safety assessments are based on the health decisions that patients may make as a result of the test and potential for improved or worsened health outcomes. Diagnostic errors can lead to health safety issues, including misdiagnosis, undiagnosed conditions, or delays in diagnosis. Ultimately, these errors can cause scenarios in which improper treatments are used, treatments are not undertaken, or treatment delays may risk worsening of health. (Balogh 2015)

In the event that no predicate test exists, the new test is automatically classified as Class III (regardless of the true risk of the test), leading to the highest level of requirements. However, manufacturers can submit **De Novo Requests** to apply to reclassify the test as Class I or II by showing that the test has reasonable assurance of safety and effectiveness through the provision of non-clinical or clinical data. (FDA 2017b)

Class I tests are simple and safe enough that they are not required to be cleared or approved by the FDA and are therefore **exempt** from most controls. This applies to most Class I IVDs and some Class II IVDs, listed in the Medical Device Exemptions 510(k) and GMP Requirements database. (FDA 2020e) (FDA 2022a)

Most Class II tests require a **510(k) premarket notification application**, which is the process through which the FDA reviews and ensures that the test follows special controls befitting the moderate risk of these products.

Information required in this application can include, but is not limited to, the proposed labeling, marketing, and messaging for the test's intended use(s); comparing and contrasting data and technical characteristics to existing tests (pre-clinical data); clinical data (as necessary); and the general details on the condition that the test is explicitly intended to diagnose, treat, prevent, cure, or mitigate in a specific population. (FDA 2022a) The process can take around 90 days, leading to the test being labeled as **FDA-cleared**, but not **FDA-approved**. (FDA 2022a)(Kessler 2010)

Class III tests (and some Class II) require the submission of a **pre-market approval (PMA)** application, leading to a test being considered as FDA-approved. The process for gaining FDA approval is more rigorous and comprehensive, time consuming (~180 days), and expensive, and it requires continuous **post-market reporting/surveillance**. (Kessler 2010) In contrast to FDA-cleared tests, FDA-approved tests are required to have submitted clinical study information detailing the **clinical accuracy**. (FDA 2020d)(FDA 2019b)(FDA 2010)

Unless exempt, all IVDs are additionally reviewed for their compliance with Good Manufacturing Practice (GLPs) regulations (also known as Quality System regulations). (FDA 2022d) GMPs are different from Good Laboratory Practices (GLP) as they provide guidelines for the production of tests, whereas GLPs provide standards for evaluating the test's safety in non-clinical studies. (FDA 2022d)

Similarly to GMPs, GLPs set criteria such as for personnel qualifications, quality assurance of the facilities in which studies are performed, equipment maintenance and calibration, and detailed documentation of operating procedures, materials, study protocols, and results. (FDA 2022b)

With a few exceptions, LDTs are not required to go through any of the pre-market approval processes to which IVDs are subject. A summary of the difference between LDTs and IVDs are provided in Table 2.

Table 2. Summarized differences between regulatory environments for IVDs and LDTs (FDA 2014)

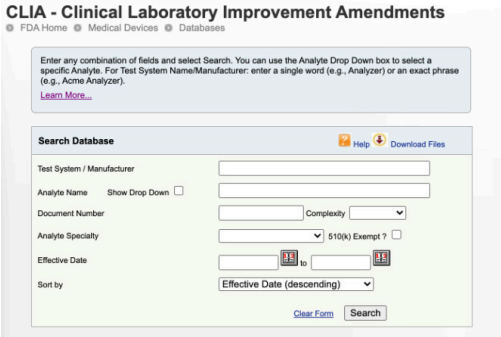
Classification	IVD	LDT
Intended for commercial distribution	✓	✗*
Risk-based classification	✓	✗
FDA pre-market review	✓	✗
Test registration	✓	✗
Labeling review	✓	✗
Evidence for marketing claims	✓	✗
Lab quality and personnel assessments	✓	✓
Manufacturing quality assessments	✓	✗
Analytical testing validity	✓	✓
Clinical testing validity	✓	✗
Pre-market testing review	✓	✗
Regulatory review results publicly available	✓	✗
Mandatory adverse event reporting	✓	✗
Mandatory recalls can be issued	✓	✗

*LDTs are now widely accessible making this a grey zone.

For those interested in determining if an available test is an IVD or an LDT, here are a couple of factors to consider.

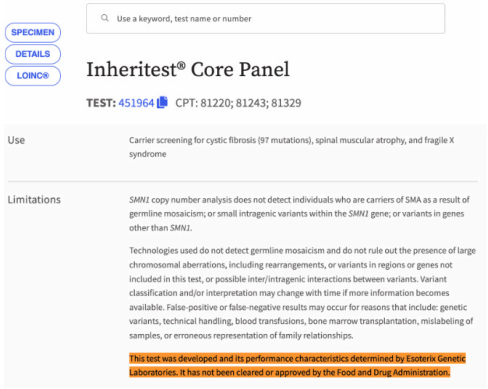
The CLIA test database (Image 1) shows all tests that have been cleared or approved by the FDA or that were exempt. The database can be searched using the test name or more broadly for specific markers (i.e., individual markers, tests, or analytes). Clicking on each test will provide a summary of information for how that test has been classified, the regulatory control pathway that it needed to take during the FDA's review, and a summary document that may also contain performance information (e.g., analytical validity of the test).

Image 1. CLIA test database



Additionally, LDTs may be subject to including the following statement on test results provided to patients: "This test was developed and its performance characteristics determined by (Laboratory Name). It has not been cleared or approved by the U.S. Food and Drug Administration." (FDA 2022c) These statements can also sometimes be found in the test's product descriptions (Image 2).

Image 2. FDA disclaimers in LDT product descriptions



Lab certification

Throughout the review process, the FDA also categorizes IVDs based on their complexity. (FDA 2021a)(FDA 2020a) This step determines how and where lab tests can be made available on the market. The tests are scored and categorized as:

- Waived tests
- Moderate-complexity tests
- High-complexity tests (CDC 2018) (FDA 2020a)

Depending on the level of complexity of the test available, the lab will be required to acquire the corresponding lab certification through the CMS in order to legally run the test and analyze results for patients.

Waived tests are home-based tests with an extremely low risk of harm if the performance of the test is incorrect. They are accurate and straightforward enough to make the likelihood of an inaccurate result unimportant or scarce. (FDA 2020b)

If granted a waiver, the FDA adds the test to the waived test database (FDA 2017a) found in the CLIA waived tests database. All over-the-counter tests that have been cleared or approved by the FDA can be found in the Over The Counter database.

In contrast, **moderate- and high-complexity tests** typically require that samples being taken are analyzed directly in the lab or sent back to the lab after collection. (FDA 2020b) All LDTs are automatically classified as high-complexity tests. (AACC 2020)

As previously mentioned, the CMS is responsible for monitoring compliance to lab quality practices in the collection, analysis, and interpretation of tests. (FDA 2021a) The type of certification depends on the complexity of the test's performance in the laboratory.

The types of **CMS lab certifications** are provided in Table 3. These certificates are valid for two years. (CMS 2021) The requirements to achieve each certification are extensively detailed in the Laboratory Requirement regulations under the CLIA'88.

Table 3. The five types of lab certifications provided by the CMS (CMS N.D.)(CMS N.D.) (CMS 2021)(CMS 2019)

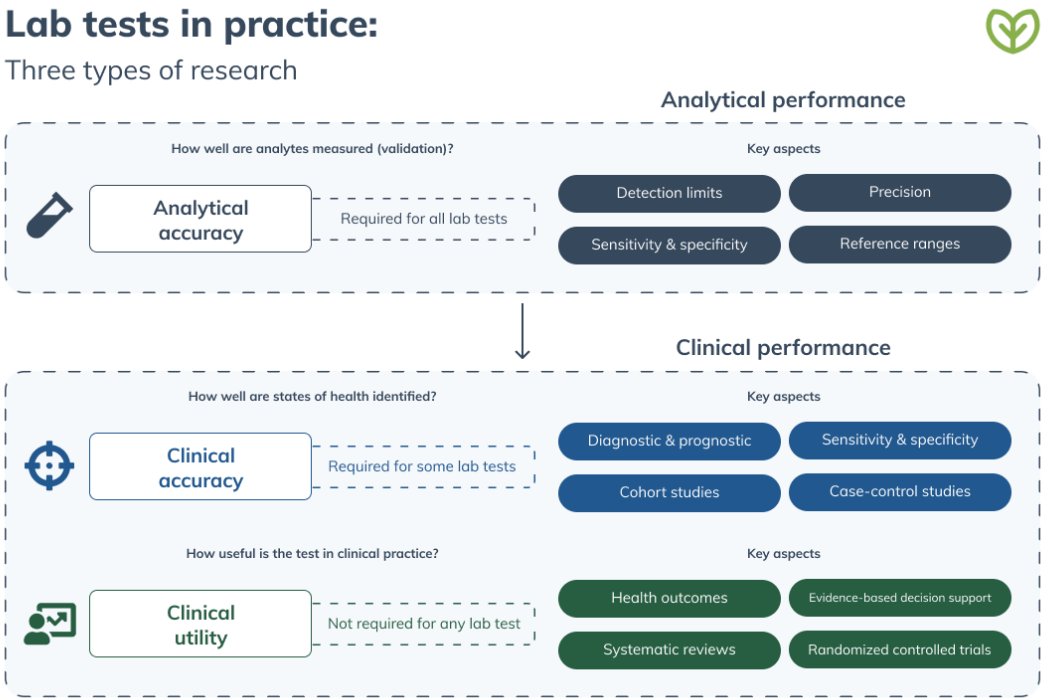
Certification	Description
Waived	Low complexity and risk; cleared or approved for over-the-counter and home use
Provider-performed microscopy procedures (PPMP)	Limited to a few types of microscopic tests (e.g., microscopic urinalysis, nasal smears for eosinophils, etc.) of moderate complexity during a patient's visit with their physician, mid-level practitioner, or dentist
Registration	Temporary certification provided until CMS inspection of compliance/accreditation to CLIA regulations occurs
Compliance	Received after passing CMS/state inspection of lab performing moderate-high complexity tests
Accreditation	Received after passing inspection performed by CMS-recognized accreditation body

Evidence requirements

For a test to be available in the U.S. market, the required scientific evidence is dependent on whether the test is an IVD or LDT. LDTs only require that labs demonstrate analytical validity, which is the same for Class I and most Class II IVDs. Class III IVDs (and some Class II) do require the submission of clinical data showing their safety and effectiveness for use in a particular population and indication. (FDA 2022a)(FDA 2019b)(GovInfo 2011) (Kessler 2010)

Typically, lab test studies are broken down into two types with three phases (Diagram 2): analytical validation (analytical accuracy phase) and clinical validation (diagnostic accuracy and clinical utility phases). (Flatland 2014)

Diagram 2. Lab tests in practice: Three types of research



Analytical performance

When tests have been analytically verified, this means that the lab has demonstrated that it and its personnel are deemed capable of accurately measuring a marker using a specific test according to a manufacturer's specifications. (FDA 2020f) This is a requirement to receive lab certification when offering IVDs that have been cleared or approved by the FDA. (CMS ND)

In contrast, when tests have been analytically validated, the lab has compared the performance indicators of a new test (IVD or LDT) to those of an established reference standard that may be currently used to measure a particular marker. (FDA 2020f) Validation is the demonstration of how well the technical aspects of the test work. (Flatland 2014)

Several performance measures that are often encountered include:

- Accuracy: how close the test results are to a gold-standard value
- Detection limits: the lowest amount of the marker that can be detected
- Precision: agreement between replicated measures over time and how reproducible this level of precision is between laboratories
- Range: upper and lower concentrations of the marker
- Ruggedness/robustness: how unaffected the test remains in the face of introduced variations as would be expected under real world circumstances
- Sensitivity: how much the test responds to a change in the marker's concentration
- Specificity: how well the test measures the marker and not others (FDA 2020f)

This is the stage at which a test's reference ranges may also be set using samples that match specific population characteristics for the test's intended use. (Burd 2010) A minimum of 120 samples is recommended to establish "normal ranges." (CSLI 2010)(FDA 2021b)

Clinical performance

Validation of clinical performance is the indication for how well the test can differentiate between individuals with or without the specified condition (i.e., how well it can screen for or diagnose), how well it can differentiate between two stages of a particular condition, or the prognostic/predictive accuracy of the test. (Mathes 2019)(Simundic 2009) This is referred to as **clinical accuracy**.

The most commonly referred to metrics of accuracy are the test's **specificity** and **sensitivity**. In combination, they indicate how well the test identifies patients with or without a condition (diagnostic value), or how well it identifies the progression (or lack thereof) of outcomes related to a condition (prognostic value), in comparison to a reference standard. (Chikere 2019)(Mathes 2019)(Simundic 2009)

Specificity:

- *Correctly* identifying patients as being *free* from the condition or that had no progression in outcome
- Highly specific tests will rarely falsely include patients as having a condition or developing an outcome when in reality, they did not.
- Also known as the "True Negative Rate"

Sensitivity:

- *Correctly* identifying patients as *having* the condition or identifying the risk of progression of an outcome (e.g., progression of cognitive impairment to dementia)
- Highly sensitive tests will rarely omit patients as not having a condition or developing an outcome when they truly do/did.
- Also known as the "True Positive Rate"

When specificity and sensitivity are calculated, many practitioners may refer to the **SPIN** (Specific test when Positive rules IN the condition) and **SNOUT** (Sensitive test when Negative rules OUT the condition) rules. (Sackett 1998) The SPIN rule might be used to rule in the presence of a condition when a positive result appears on a highly specific test because there is a low chance of a false positive result. The SNOUT rule might be used to rule out the presence of a condition when a negative result appears on a highly sensitive test because there is a low chance of a false negative result.

Diagram 3. Interpreting sensitive and specific test results

However, SPIN and SNOUT rules answer the question of "the chance of a positive or negative test result in the presence or absence of a condition" instead of the more clinically relevant question of "the chance of the presence or absence of the condition when receiving a positive or negative test result." (Baeyens 2019) Thus, a more useful clinical interpretation of the chance of the condition being present or absent in the context of a positive or negative test result relates to the test's predictive ability.

A test's predictive ability is often described in terms of positive and negative predictive values (**PPV** and **NPV**). (Baeyens 2019)(Simundic 2009) The PPV describes the proportion of patients with the disease and a positive test result out of all of the positive results (including false positives) in a population. The NPV

describes the proportion of patients without the condition and a negative test result out of all of the negative results (including the false negatives) in a population. It should be noted that PPV and NPV are highly dependent on and differentially affected by the prevalence of the condition in a population, limiting these measures' transferability to distinct populations. (Baeyens 2019)(Simundic 2009)

Hence, positive and negative likelihood ratios have been described as a potentially more clinically useful metric of a test's accuracy. The positive likelihood ratio (**LR+**) describes the ratio of the proportion of patients with the condition as well as a positive test result to the proportion of patients without the condition but who have a positive result. It indicates how much more likely a positive test result is in patients with the condition compared to patients without the condition. In contrast, the negative likelihood ratio (**LR-**) describes how much more likely a negative test result is in patients with the condition than in patients without. It is therefore the ratio of patients with the condition with a negative test result to patients without the condition with a negative result. (Baeyens 2019)(Simundic 2009)

Interpretation of a LR+ or LR- value of 1 indicates that a test's results are not useful (i.e., there is a 50:50 chance of getting a positive or negative result). The accuracy values of a test for LR+ ranges from one to infinity, whereas the accuracy values of a LR- ranges from zero to one. A summary of the interpretation of values is provided in Table 4.

Table 4. Interpretation of likelihood ratio calculations for diagnostic accuracy (Baeyens 2019) (Fagan 1975)(McGee 2002)

Likelihood ratio	Calculated result	Interpretation
LR+	>10	Strong likelihood of the condition's presence
	5-10	Moderate likelihood of the condition's presence
	2-5	Weak likelihood of the condition's presence
LR-	0.2-0.5	Weak likelihood of the condition's absence
	0.1-0.2	Moderate likelihood of the condition's absence
	<0.1	Strong likelihood of the condition's absence

Studies of the test's diagnostic accuracy may use any of these aforementioned types of measures to define performance. During the FDA's review of tests seeking to be cleared or approved, submission of valid scientific evidence (FDA 2020d) with "well-controlled" design criteria is required. (FDA 2020d)(FDA 2019b) The design of studies is typically dependent on whether the clinical accuracy study is classified as diagnostic or prognostic. (Mathes 2019)

Diagnostic accuracy studies examine the likelihood that a condition or state of health is present or absent at a particular point in time (i.e., use cross-sectional designs). Prognostic accuracy studies examine the risk of future outcomes that are not present when the test was used and observe the natural development of a condition over time (i..e, use longitudinal designs). (Mathes 2019)

Cohort and case-control studies are the study types most frequently used to assess clinical accuracy. In cohort designs, a single group of patients suspected of having the condition but without a confirmed diagnosis use the reference test and the test of interest. (Chasse 2019)(Colli 2014)(Mathes 2019) In case-control studies (also known as two-gate design), (Rutjes 2005) patients are selected from two known and distinct groups: patients with the condition and healthy controls. Both sets of patients then use the test of interest for comparison with a reference standard. (Chasse 2019) Case-control studies may be advantageous in their convenience and feasibility compared with cohort studies, but they may be less generalizable and can lead to overestimations in test accuracy. (Chasse 2019)(Colli 2014)

Though less common, (Rodger 2012) clinical accuracy studies can also use comparative designs (non-randomized or randomized), which provide the added benefit of being able to compare the accuracy of two different index tests in addition to the reference standard. (Chasse 2019) Their controlled nature can help to further reduce the likelihood of study bias, but are more resource intensive.

Clinical utility

Clinical utility studies assess whether the use of the test leads to improved health outcomes. (Bossuyt 2012)(Burke 2014) They determine whether patients "fare better" than others who had either not been tested or were evaluated using a different test in areas such as risk of the condition or death, quality of life, or cost effectiveness. (Leeflang 2019)

For example, one study compared the clinical utility of using a combination of a specific antibody test kit (Pierce™ Direct IP Kit) using filamin-A to using prostate-specific antigen (PSA) screening alone to distinguish patients with benign prostatic hyperplasia (BPH) from patients with prostate cancer. (Kiebish 2021) The panel was both shown to be more effective at distinguishing BPH from prostate cancer, but also led to a 43% reduction in patients without prostate cancer receiving an unneccessary biopsy referral. (Kiebish 2021) This may be particularly clinically useful given that up to

three quarters of patients may be unnecessarily recommended biopsies (Kim 2021) due to a high number of false positives created from low positive predictive values (25%) from using PSA tests alone. (Mistry 2003)

However, clinical utility studies are not required for IVDs to be cleared or approved by the FDA. Only clinical accuracy studies are needed. (FDA 2007) Test accuracy does little to inform clinicians about the degree of clinical importance or even whether the differences between accuracies of tests are clinically important at all. (Rodger 2012)

As clinical utility studies are meant to capture health outcomes of implementing a test as a form of intervention, practitioners are likely to be most interested in this form of evidence.

Evaluating the evidence

To help practitioners distinguish between the quality of studies across study types that they may encounter in the literature, a summary of the accepted hierarchies is shown in Table 5.

Table 5. A summary of the hierarchy of research designs across test study types (Shekelle 2013)(Centre for Evidence-based Medicine 2011)

Levels	Diagnostic accuracy	Prognostic accuracy	Clinical utility (outcomes)
Level 1 ★★★★★	Systematic review/meta-analysis of cross-sectional studies	Systematic review/meta-analysis of longitudinal studies	Systematic review/meta-analysis of randomized controlled trials (RCTs)
Level 2 ★★★★	Cross-sectional cohort	Prospective cohort	RCTs
Level 3 ★★★	Cross-sectional case-control	All or none study	Non-RCTs
Level 4 ★★	Diagnostic yield (no reference standard)	Retrospective cohort, single-ar arm RCTs	Observational (cohort, case-control, case-series)
Level 5 ★	Background, mechanistic, or expert opinion	Longitudinal case-control or case-series	Background, mechanistic, or expert opinion

Types of lab tests

Lab testing plays a crucial role in health assessment and diagnosis, encompassing a range of tests—from basic tests to advanced and specialty evaluations. This section highlights some of the lab test types available to practitioners and patients.

Basic labs

Basic lab testing includes routine tests like the complete metabolic panel (CMP), complete blood count (CBC), iron panels, lipid panels, and thyroid function tests. It's used for initial screening and general health assessment, helping to evaluate overall health, identify potential issues early, and monitor existing conditions. These labs are familiar and cost-effective, providing clinicians with a reliable starting point in their assessment process.

Advanced labs

Advanced lab testing offers more comprehensive and detailed insights to aid in understanding the physiologic dysfunctions contributing to patient symptoms and health challenges. An example would be adding inflammation markers and lipid measurements that assess for lipid size and density to a standard cholesterol panel. Other examples would be comprehensive nutrition assessments like a complete iron panel, vitamin and mineral levels, and organic and fatty acids. These tests provide additional depth to clinical evaluations to guide clinicians' assessment.

Specialty labs

Specialty lab testing, often utilized in functional and integrative medicine, includes specialized tests like stool testing for gut health, saliva and dried urine hormone testing, genetic panels, and labs to assess environmental and toxin exposure. Specialty labs often utilize innovative technology and capture patterns that help clinicians uncover the underlying causes of chronic conditions and offer a holistic view of a patient's health.

Diagnostic versus functional tests

There are a great number of different lab tests available to practitioners. They differ across the kind of material sample medium, with considerable overlap. For example, certain hormones may be tested via blood, saliva, or urine.

Many integrative and functional practitioners frequently refer to certain saliva or urine tests as functional medicine tests. The difference between these tests and conventional tests is not typically described in the research literature. However, many functional tests are considered direct-to-consumer tests because they can be accessed without a practitioner's prescription in some states. (Galior 2020) Though not an exhaustive list, some common functional medicine tests include:

- Comprehensive stool analyses for gut bacteria, yeast, parasites, pathogens, and various metabolic markers
- Genetic testing via saliva (legality varies by state)
- Heavy metals and neurotransmitters via urine
- Hormone levels via saliva and urine
- IgG food sensitivity testing via blood

Diagnosis and monitoring of many conditions tend to be based on evidence from blood testing, (Clarke 2016) with some notable exceptions such as routine urinalysis for urinary tract infections or stool testing for parasites, for example.

Single marker

With **single-marker** testing, a practitioner will select one or often several individual markers of health. For example, a practitioner may choose to test only thyroid stimulating hormone (TSH) or they may choose to test two single markers, TSH and free T3.

This may be done when a practitioner only wants information on specific markers. It may also be done for financial considerations. For example, testing single markers is typically less expensive than ordering an entire panel.

Panels

Practitioners may also choose to requisition a collection of single markers, referred to as a **panel**. For example, a practitioner may requisition a thyroid panel, which may include TSH, free T3, and free T4 together, among others. There may or may not be a "bundling" cost advantage to requisitioning a panel depending on the fee structure set by the lab.

Panels are more often run by practitioners requisitioning more information (i.e., a higher volume of markers) at one time or when a more comprehensive assessment (compared to single-marker screening) is indicated.

Kits

Kits contain a collection of materials including both the instructions and the tools required to take a particular sample. Kits may be contained within a box, envelope, or other medium.

For example, a fecal occult blood stool sample kit may contain paper instructions, a requisition featuring patient information, tools to aid in stool collection, and a card, envelope, and/or test tube in which to place the stool.

Kits are most often used for at home collection of samples. They are often given to the patient by a clinic, or sent directly from a lab to the patient's residence. They may be dropped off or even picked up by a courier for delivery back to the lab.

Test mediums

There are several different mediums available for lab testing that may require the collection of samples, including blood, saliva, stool, and urine. Genetic tests are also common and may require the collection of samples.

Blood tests

Blood is a very common test medium. Depending on the test, samples are added to color-coded tubes, which may contain different compounds (e.g., ethylenediamine tetraacetic acid (EDTA) for anticoagulation, sodium citrate for anticoagulation) or no compounds at all. Samples may or may not be centrifuged in order to separate out the serum from the plasma. (Bayot 2021) Plasma contains red blood cells, white blood cells, platelets, and other clotting factors. (Mathew 2021)

The serum contains most other markers in which a practitioner may be interested. For example, a practitioner may assess nutrient status from vitamin B12 levels, (Ankar 2021) liver health from alanine aminotransferase (ALT), (Moriles 2021) and/or a marker of cardiovascular health from triglycerides (TG). (Boullart 2012)

Other tests may require dried blood spot samples. For these, patients will often be provided with instructions and disposable equipment that pricks the finger in order to elicit a drop or two of blood onto a test card. This is somewhat similar to how a person with diabetes may test their blood glucose at home.

Saliva tests

Saliva, while less commonly tested by conventional practitioners, may be more sensitive in detecting changes in sex hormones, compared to serum, (O'Leary 2000) which may lend itself well to monitoring patients prescribed hormone-replacement therapy. From cortisol and DHEA-S to sex and thyroid hormones, saliva may be a useful testing medium, especially for circumstances in which access to labs for blood draws is more difficult. (Gröschl 2008)

Stool tests

Stool is a medium commonly used to investigate digestive issues, but may also offer insight into atopy-related conditions. (Joseph 2022) Common stool tests are fecal occult blood and parasitology-related tests. However, fecal testing may also include tests such as fecal calprotectin and lactoferrin for inflammatory bowel disorders. (Mosli 2015)

Urine tests

Urine is another medium through which hormones may be tested via their metabolites. Other than routine urinalysis, urine testing tends to be utilized more by functional medicine practitioners. It may test for additional hormones (e.g., melatonin, estrogen metabolites) or other compounds such as neurotransmitters that may be otherwise difficult to test. (Gröschl 2008) Urine may also be useful for heavy metal testing, such as mercury testing. (Fields 2017)

Genetic tests

Genetic testing is usually performed on saliva or buccal (cheek) swab mediums. (Galior 2020) It examines the DNA present in such samples in order to help in genealogical pursuits to see if there is any biological relation between certain people. Genetic testing can also offer potential insights into genetic predispositions with regard to health, such as information on predisposition to certain diseases.

However, due to the multifactorial etiology of health conditions, interpretation of genetic tests results should be done with caution. By 2017, there were 3,200 genome-wide association studies that analyzed over 55,000 single nucleotide polymorphisms (SNPs) for associations with over 3,000 diseases. (Malgorzata 2022) Testing for this many associations can yield spurious associations (i.e., statistically but possibly not clinically significant associations). (Austin 2006)

However, together with relevant history, other labs, and overall clinical picture, single genetic markers can help to inform a practitioner's assessment and treatment plan. For example, if a patient has a certain variation in their methylenetetrahydrofolate reductase (MTHFR) gene, this may indicate that their vitamin B12 levels are low. (Surendran 2018) In this case, it would be very useful to run blood work for vitamin B12 and homocysteine levels, as well as inquire about any overt symptoms of deficiency. With this information, a practitioner would be able to assess if a B complex, or B12 supplement alone, would be indicated.

Notable exceptions in poor correlation would be the APOE4 gene for Alzheimer's disease risk, as having one of the copies of this gene can carry stronger correlation to risk for Alzheimer's (OR=6.96) (Reiman 2020) or heart disease (OR=1.68). (Malgorzata 2022) (Newman 2001)

Test settings

Blood draws are often carried out in a laboratory setting, though some clinics may have qualified staff performing the blood draws within the clinic. Urine samples may be collected in the clinic or in a lab. Samples that are collected in a clinic will often be processed, refrigerated, and sent out to the lab for analysis. This has the advantage of convenience for the patient, but can offer logistical challenges for the clinic.

Home testing may be used whenever the medium allows; stool, urine, saliva, and even dried blood spot samples may be taken at home.

Commonly offered tests

Table 6 was developed by searching the more commonly used labs in the United States and populating the table with the various "popular" lab test markers and panels offered on their websites until redundancy was achieved. Data was aggregated together roughly based on clinically relevant groupings.

Table 6. Popular markers and panels across different labs in the United States (Labcorp 2022)(Quest Diagnostics 2022)

Marker grouping	Popular (blood-based) markers
Cardiovascular	Fasting blood glucose Hemoglobin A1C (HbA1C) High-density lipoprotein cholesterol (HDL-C) Low-density lipoprotein cholesterol (LDL-C) Non-HDL-C Total cholesterol Triglycerides (TG)
Electrolytes	Bicarbonate Calcium Chloride Magnesium Phosphate Potassium Sodium
Hematology	Complete blood count (CBC) Ferritin

Marker grouping	Popular (blood-based) markers
Inflammation	C-reactive protein (CRP) Erythrocyte sedimentation rate (ESR) Fibrinogen Highly-sensitive C-reactive protein (hs-CRP)
Kidneys	Creatinine Estimated glomerular filtration rate (eGFR)
Liver	Alanine aminotransferase (ALT) Alkaline phosphatase (ALP) Aspartate aminotransferase (AST) Bilirubin Gamma-glutamyl transferase (GGT)
Men's health	Follicle stimulating hormone (FSH) Free testosterone Luteinizing hormone Total testosterone
Nutritional	25-Hydroxyvitamin D (Vitamin D3) Vitamin B12
Thyroid	Free T3 (fT3) Free T4 (fT4) Thyroid stimulating hormone (TSH)
Urinalysis	Bilirubin Blood Glucose Ketones Leukocytes pH Protein Nitrites Specific gravity
Women's health	Beta-human chorionic gonadotropin (b-hCG) Estradiol FSH LH Progesterone

Test analysis

Once a sample is taken, it is analyzed and results are obtained. The results of a test are often compared to a reference range.

Types of results

The way results are reported varies based on the type of test. Some may be compared to reference ranges, while others may yield a binary or a graded result (see Diagram 4). An example of the latter would be a fecal occult blood test, which may be interpreted as positive or negative for containing blood in the stool. Some results may have graded or categorical results, such as a stool microbiology test for yeast, which may be reported as "None," "Trace," "Mild," "Moderate," or "Many" in reference to the quantity of yeast in the sample. These may still feature a reference range, such as "None-Trace" in this example, as it may be normal to have none or trace amounts of a given marker.

Diagram 4. Binary and graded test results

Binary results example: Testing for the presence of H. pylori

Antibiotic resistance genes, phenotypes					
Helicobacter		**Result**			**Expected result**
Clarithromycin		**Positive**			Absent
A2142C	Absent	A2142G	Absent	A2143G	**Present**
Fluoroquinolones		**Negative**			Absent
gyrA N87K	Absent	gyrA D91N	Absent	gyrA D91G	Absent
gyrB S479N	Absent	gyrB R484K	Absent		

Graded results example: Thyroid hormone levels

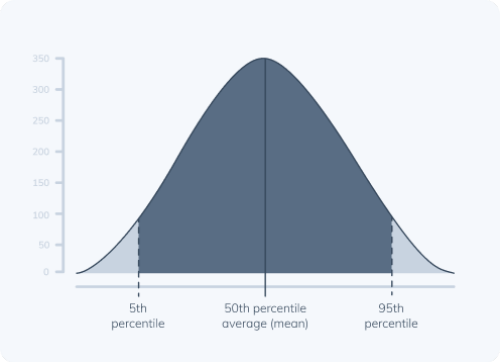
More commonly, tests will come back with a number, a unit of measurement, and a corresponding reference range to indicate what a "normal" range should be for this result. For example, hemoglobin levels, which may be measured in grams per deciliter (g/dL), has a reference range for males of 14 to 17 g/dL. (American College of Physicians 2022) If a male had a result of 15 g/dL, they would be within the reference range, whereas 10 g/dL would be considered outside of the range and would warrant attention from the practitioner.

Units of measurement differ across countries, with Standard International (SI) units tending to be the norm outside of the United States. An example would be millimoles per liter (mmol/L) for glucose in Canada versus milligrams per deciliter (mg/dL) for glucose in the United States.

Diagnostic versus functional intervals

Reference ranges are typically developed from percentiles of a surveyed population. For example, when attempting to set a reference range, a population of people classified as healthy (i.e., people with no known health conditions, especially in regards to the particular marker) may all have their blood collected and the levels of a particular marker analyzed. The results may come back on a bell curve, as shown in Diagram 5.

Diagram 5. Reference range percentiles



Depending on the marker and the entity setting the reference range (e.g., professional/medical association, lab, research group, etc.), different cutoffs may be used. Typically, the bottom 2.5% (i.e., 2.5th percentile) and top 2.5% (i.e., 97.5th percentile) of the population are excluded as statistical outliers. (Jones 2008) Such people would be the highest and lowest in the range and may not be representative of where an average person's marker "ought" to be for normal or better health.

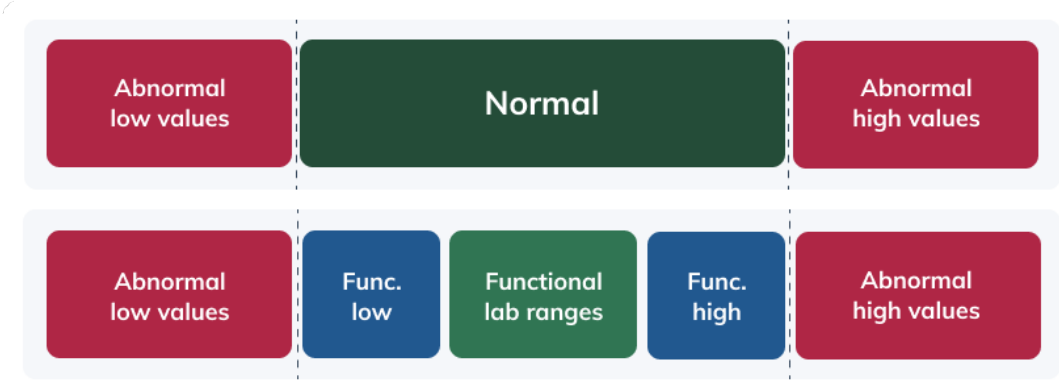
Some reference ranges are set to the first and 99th percentiles, specifically cardiac troponins, blood glucose for diabetes and macrovascular risk, and therapeutic intervals for drug monitoring. (Garber 2012) Furthermore, some reference ranges have no lower limit, such as C-reactive protein. (Jones 2008)

Of course, debate may exist over where a reference range should be set for a particular marker. For example, the reference range cutoff

for high TSH, which was once 9 mIU/L, was dropped to 4.5 mIU/L and is now recommended at 3 mIU/L by some, while others argue it should be 2 mIU/L or less. (Garber 2012)

Some practitioners will use more narrow reference ranges. These may be termed "functional (medicine) reference ranges," as opposed to the more traditional "diagnostic reference ranges" (see Diagram 6). These ranges may be based on large research studies, as noted in the next section will regard to cholesterol, or they may be based on anecdotal evidence, clinical practice experience, or other information.

Diagram 6. Diagnostic and functional range results



As mentioned in the example above for TSH, the definition of "healthy" intervals for populations may change over time, leading to questions of where a "true" reference range should lie. A patient may question if their health is "okay" if they are right on the borderline of the normal range, for example. These issues and questions frequently lead to practitioners formulating their own opinion on reference ranges. Therefore, an important question is: who are the entities that set reference ranges?

Setting reference ranges and their challenges

Each laboratory is ultimately responsible for setting the reference ranges that accompany a patient's lab results. (Katayev 2010) Finding the original source for each reference range can be very challenging. The source may be professional organizations, certain large studies, a labs' own data, other labs' data, or the manufacturers of the lab equipment.

Examples of professional organizations that set reference ranges include the American College of Physicians and the American Board of Internal Medicine. An example of a governmental association that sets reference ranges is Statistics Canada, which sets Canadian ranges based off of the Canadian Health Measures survey. (Clarke 2016) Certain sets of markers, for example cardiovascular markers, are set by bodies or associations such as the American Heart Association. (Jones 2008)

Often, these associations set their ranges based off of large research studies. For example, the target reference range for non-high-density lipoprotein cholesterol (non-HDL-C) of less than 2.6 mmol/L (100 mg/dL) originates from a study by Brunner et al., 2019, in which researchers examined cardiac markers from 524,444 individuals from 19 countries and found that when stratified into five categories of non-HDL-C status, the risk of 30-year CVD events was lowest in the non-HDL-C category of less than 2.6 mmol/L. (Brunner 2019)

Due to the difficulties carrying out and interpreting the results of such large, rigorous trials, clinicians and researchers may often disagree on exact reference ranges across the many different markers possible. Therefore, published clinical practice guidelines or position statements may vary.

Evidence may not always exist for setting reference ranges for newer and/or less commonly known or used markers, such as with functional medicine markers based on urine, stool, bloodspot, or saliva testing. Such markers require reference ranges of their own to be developed, even if the same marker exists in blood (serum) testing. To this end, labs may establish their own reference range for that marker by examining the breadth of data taken from their own patients and setting percentile-based cutoffs, such as 5th and 95th percentile cutoffs. (Groves 2015)

Labs may perform their own reference range study; however, they can be difficult and cost-prohibitive in order to meet adequate standards and represent different age, sex, and ethnicity population groups. Such studies will often need 120 participants per group (e.g., male, female, etc.) in order to develop a statistically sound 90% confidence interval. (Jones 2008)(Solberg 1998)

It can be difficult for researchers to know what groups in which to partition participants, however. Partitioning by sex is simple, but what ages should groups be split into? For example, if groups were to be split by age, what cutoffs should be set (e.g., 0 to 19 versus 0 to 13)? Such sociodemographic categories may be arbitrarily set. Ideally, they are based on sound new data; (Clarke 2016) however, the data may be old, such as the American Heart

Association's data that stratifies cholesterol levels by age in childhood. (NCEP 1992) Addressing these research questions, along with trying to exclude subjects with subclinical disease, are difficult tasks in reference range studies. (Jones 2008)

For these reasons, labs may just "grandfather in" older reference ranges. Labs may also refer to ranges established by other labs.

In addition, ranges may be given to a lab by a manufacturer of the lab equipment used to do the testing. (Jones 2008) A difficulty with these reference ranges is that the equipment used to establish these older reference ranges may be significantly different from current equipment. (Jones 2008) The ultimate origin of each of these sources of reference ranges can be very difficult to track down.

Conclusion

Lab testing has become a core tool at the integrative practitioner's disposal for supporting patients' health and wellness journeys. It has a high level of utility throughout the patient journey, including helping with treatment adherence.

The ways through which tests are made available on the market depend on factors such as risks to patient health, the complexity of running tests, the type of test, and more.

All available tests are required to demonstrate analytical validity, while those that are reviewed by the FDA can also require validation of the test's clinical accuracy. Though this type of data does not necessarily indicate a test's clinical utility, referring to tests with an evidence base for improving health outcomes is encouraged.

Lab testing is a rapidly growing industry both in North America and internationally. Many factors, such as comparing conventional and functional tests, choosing between test types and mediums, and understanding how reference ranges are established, can make applying and analyzing lab testing difficult. These challenges make easier access to the wide variety of labs and lab tests important for today's practice.

By providing educational information on the basics of lab tests in the current regulatory lab testing landscape in the United States, the authors of this guide hope that integrative practitioners and their patients feel more empowered to incorporate lab testing in their health journeys.

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