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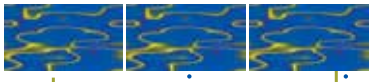
AN OVERVIEW OF DIAGNOSTIC TEST TECHNOLOGIES

ABOUT THIS DOCUMENT

This document is partly a primer on diagnostic technology and partly a status report on trends in the field of diagnostic tests. It is intended for those who may have some background in health care and who want a deeper understanding of the different technologies and trends that are driving the use of diagnostics. This field is highly complex, however, and this document is only intended to provide an overview.

For the purposes of this document, the term "diagnostic tests" refers only to those tests that analyze body fluids such as blood and urine for clues regarding the prevention, diagnosis, and treatment of diseases and conditions using the tools and expertise found in chemistry, hematology, microbiology, and molecular pathology. Anatomic pathology (the examination of body organs and tissues) and imaging, or radiology, tests such as MRI and X-ray are not addressed here.

Prepared by 
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Executive Summary

Diagnostic tests[1] are an essential component of the health care system, providing vital information that impacts provider decisions regarding the prevention, diagnosis, treatment, and management of disease. With advances in technology over the last several decades, diagnostics have become even more integral to the practice of medicine today, enabling, for example, more personalized and more productive treatments. While testing capabilities continue to evolve rapidly through development of new technologies, there are certain concepts of measurement used to evaluate their effectiveness that are fundamental and unchanging.

This document describes the fundamentals of diagnostic testing and examines the complexities involved in diagnostic technologies today and the trends that are redefining diagnostics at a rapid pace. Whereas most diagnostic tests are currently performed on fluid and tissue samples that are first removed from the body (*in vitro*), research is underway that will lead to a new wave of diagnostic testing that enables clinicians to take their measurements directly from within the body (*in vivo*). This shift will mark a dramatic change in care, as it will eliminate the major source of errors in diagnostic testing and open up opportunities for new applications of the technologies used to test and monitor human health.

DIAGNOSTICS TODAY

EXPANDING THE ROLE OF DIAGNOSTIC TESTS Viewed narrowly, diagnostic tests are those that aid in identifying illness in a person who presents with symptoms by confirming or ruling out the presence of a specific disease or infection. As our knowledge of human biology has grown, so too has the definition of "diagnostic tests." This term is regularly used as a reference to tests that contribute to preventive care and, increasingly, to identifying and managing treatment. Today, advances in molecular testing have opened up a new area of diagnostics that enables providers to evaluate the likelihood that someone will develop an inheritable disease potentially many years in the future.

UNDERSTANDING THE FOUNDATION OF TESTING All diagnostic tests, regardless of what role they play in care or what technology is used to perform them, require some minimum level of accuracy and precision, and sensitivity and specificity in order to demonstrate they are effective. These areas of measurement represent the foundation of diagnostic testing, and those tests that achieve the greatest results among these measurements are often considered the "gold standard." While gold standards may provide the best understanding of what is happening to a patient, sometimes the time, complexity, and cost of running a gold standard test routinely is not in the best interest of patient care. This is particularly true when there are alternative tests available that, while falling short of the gold standard in one or more of the key areas of measurement, provide sufficiently credible information in less time or with less labor required.

1. The term "diagnostic tests," (also referred to as "*in vitro* diagnostics") as used in this document, refers only to those tests that analyze body fluids such as blood and urine for clues regarding the prevention, diagnosis, and treatment of diseases and conditions using the tools and expertise found in chemistry, hematology, microbiology, and molecular pathology. Anatomic pathology or imaging tests are not addressed.

ACKNOWLEDGING THE COMPLEX PROCESS OF *IN VITRO* TESTING What the patient sees of the testing process, and often, what the health care provider who prescribes the test sees, is only a small part of a complex process that involves many opportunities for error. Testing-related errors typically are not due to the quality of the test itself, but arise during pre- and post-analysis. The vast majority of testing-related errors occur in the pre-analytical phase[2] in which a fluid or tissue sample is collected from the patient, processed through a test tracking system, stored temporarily for transport, and then transported to a lab (in most cases). Diagnostic error also may arise when tests results are not appropriately communicated or used appropriately to inform health care decisions.[3]

DIAGNOSTICS TOMORROW

EVENTUAL ELIMINATION OF PRE-ANALYTICAL ERRORS A great deal of research is underway today into *in vivo* testing, which would enable analysis of the same fluids through devices that are worn on the skin or implanted in the body. As such tests are developed and approved for use, the gradual elimination of the need to collect, process, transport, and store patient test samples will be seen.

POTENTIAL BENEFITS OF REAL-TIME ANALYSIS Wearable and implantable test technologies currently in development will allow for constant monitoring of body chemistry and collection of previously inaccessible data. As the anticipated massive volume of data is analyzed for the individual and in aggregate, a seemingly boundless range of possible benefits to care emerge: earliest possible identification of cancer-causing mutations and infections; new treatments for chronic conditions; and detection of early signs of fatigue or stress that could be used to avoid accidents in high-risk activities. It will likely take several more years before the first wave of such technologies receives approval for use.

2. Hammerling, J. (2012). "A Review of Medical Errors in Laboratory Diagnostics and Where We Are Today." *Medscape News & Perspective*. Lab Med. 2012;43(2):41–44.
3. National Academies of Sciences, Engineering, and Medicine, 2015, *Improving diagnosis in health care*, Washington, DC, The National Academies Press.

Introduction

As technologies advance, clinical diagnostic tests have become an ever more essential part of health care. Particularly in the area of *in vitro* diagnostics, breakthroughs in molecular diagnostics have opened up entirely new opportunities for diagnostics to drive medical decision-making and care.

In vitro diagnostics (IVD) refers to tests for disease or infection on samples that are removed from the body for analysis.[4] In clinical diagnostics, these samples are typically fluids such as blood, urine, saliva, and sometimes, cerebrospinal fluid, or secretions and cells from the nose, throat, vagina, or an open wound. Various technologies are used to test for infections and analyze the proteins, genes, enzymes, and other analytes that are indications to one degree or another of a health problem.

The vast majority of diagnostic testing is conducted using diagnostic technologies in laboratory settings. By one estimate, more than seven billion diagnostic tests are performed each year. Results from these tests allow health care providers and their patients to better understand a person's health status, and to make informed decisions. Through reporting and monitoring programs, many of these results will also impact public health at the local and national levels.

The complex nature of *in vitro* testing can make it challenging to understand and interpret a test result without a deep knowledge of the technologies involved. Even many health care providers are unfamiliar with the nuances of testing and may rely on the interpretations provided by laboratory professionals. However, a shift is underway in which testing is increasingly done at the point of care using portable *in vitro* technologies that can be used by health care providers with varying degrees of technical expertise, and even by patients in some cases, with wearable and implantable *in vivo* testing technologies on the horizon. This shift will eliminate some of the variables in testing and place more of the responsibility on users outside of the traditional laboratory.

4. In this document, all IVD tests will be referred to as "diagnostic tests".

THE EVOLVING ROLE OF DIAGNOSTIC TESTS

Over the years, the use of diagnostics tests has grown beyond its original role as a tool for making or confirming a diagnosis. Today, health care providers are able to catch disease early and so prevent more dire health consequences; to better manage treatment; and thanks to recent advances in molecular testing, to predict the likelihood of future health problems. See Table 1 for a description of the different roles of *in vitro* testing.

TABLE 1. ROLES OF DIAGNOSTIC TESTING

	WHAT THE TESTS DO	WHY THEY MATTER
SCREENING	**RISK ASSESSMENT**	
	These tests go beyond family and medical history to evaluate the likelihood of an individual developing a particular condition	Lifestyle changes can sometimes be made or treatment done to minimize risk or the impact of the condition should it develop
	EARLY DETECTION	
	Routine and at-risk screening tests that may catch disease in its early stages	Disease impacts can be minimized, and sometimes prevented, if caught early enough for treatment
ASSESSMENT	**DIAGNOSIS**	
	Tests that confirm or rule out specific diagnoses	Needed to understand next steps in care
	STAGING AND PROGNOSIS	
	Tests used to determine how advanced or severe a condition might be or its predicted course. May also be used to assess risk of recurrence and to inform adjuvant therapy decisions	Determines whether and what kind of treatment is necessary
MANAGEMENT	**THERAPY SELECTION**	
	Tests that predict the effectiveness and potential side effects of specific treatments	Avoids suffering and wasted time from, and cost of, unproductive treatments
	MONITORING/TREATMENT ASSESSMENT	
	Tests that ensure ongoing safety and effectiveness of prescribed treatments or course of care	Enables timely intervention to adjust or change treatment as necessary

SOURCE: Adapted from DxInsights White Paper, January 2012, as used in *The Essentials of Diagnostics series: Molecular Diagnostics*, AdvaMedDx and DxInsights, 2013.

TEST CATEGORIES

The thousands of *in vitro* tests depend on telltale indicators captured in samples taken from the body to communicate to health care providers what may be amiss with the patient. These indicators and how they are detected and measured define the four disciplines into which the tests are organized:

CHEMISTRY Chemistry tests measure or detect specific substances in the body to determine if they are present or present in "normal" amounts. The human body maintains a relatively predictable level of a wide range of chemicals or other substances when it is healthy. Determining whether a substance — more commonly known in this context as an "analyte" — is present or present in too high or too low amounts can be an indication that something is wrong and may help identify a specific disease or condition. Today, sophisticated equipment is typically used to analyze the patient's samples, usually a fluid sample like blood, urine, or saliva.

HEMATOLOGY Hematology tests focus on blood and the components of blood. White blood cells, red blood cells, and platelets are created in the bone marrow and are present in a healthy human's blood in predictable numbers, with identifiable characteristics. Proteins in the fluid portion of the blood that help regulate appropriate blood clot formation (coagulation) are present in predictable amounts and level of activity. Automated counting and assessment of blood cells and evaluation of coagulation proteins are typically used to determine specific blood-related diseases or conditions. Bone marrow is evaluated to help diagnose blood cell cancers such as leukemia.

MICROBIOLOGY Microbiology tests look for agents of infectious disease, including bacteria, viruses, parasites, mycobacteria, and fungi, or the body's immune response (typically antibodies) to these microbes. These may be found in patient samples taken from noses, throats, open wounds, other body sites, as well as blood and other body fluids. Samples may be cultured in Petri dishes, examined using a microscope, or analyzed with automated instruments to determine the presence of a disease agent or antibody produced in response to it. Additional testing is frequently performed to determine the susceptibility of an infectious agent to antimicrobial therapies.

MOLECULAR Molecular tests analyze DNA, RNA, or the expression of proteins. They look for abnormalities or variations in the genetic code, or identify the presence of specific genes in order to determine predisposition or presence of disease, presence of an infectious agent, or what particular treatment options are likely to be most effective. Samples are typically prepared so that specific genes on tiny fragments of DNA or RNA can be detected or evaluated for mutations. Some tests evaluate these fragments directly using nucleic acid "probes" but most tests use amplification, a process that produces many copies of the DNA in the fragment in order to allow detection and quantifying of the genes of interest. Following amplification, the presence of the genes of interest is determined and, when required, the genes are counted to provide very specific information to the health care provider for risk assessment, diagnosis, prognosis, and treatment.

TABLE 2. EXAMPLES OF DIAGNOSTIC TESTS BY CATEGORY

	CHEMISTRY	HEMATOLOGY	MICROBIOLOGY	MOLECULAR
SCREENING	**RISK ASSESSMENT**			
	Cholesterol (cardiovascular disease)	Platelet count (risk of bleeding) Factor V Leiden and PT 20210 (risk of blood clots)	Rubella antibody (determines immunity in pregnant women, risk of infection if exposed to virus)	BRCA1 BRCA 2 Cystic Fibrosis (CF) Mutation Panel (carrier status in prospective parents–risk of passing on the disease)
	EARLY DETECTION			
	BUN, creatinine (kidney damage or disease)	Hemoglobin (anemia)	Hepatitis C antibody test (hep C infection) PCR or culture screening (determine bacterial presence in pregnant woman that may be harmful to newborn if passed during birth)	CfDNA (screen for DS in pregnant woman at risk of having baby with DS) PCR screening (determine bacterial presence in pregnant woman that may be harmful to newborn if passed during birth)
ASSESSMENT	**DIAGNOSIS**			
	Hemoglobin A1c (diabetes)	CBC (Anemia) Immunophenotyping (leukemia)	Hepatitis C RNA test (distinguish between current and past Hep C infection) Blood culture (septicemia) Mycobacterial culture (tuberculosis)	Hepatitis C RNA test (distinguish between current and past Hep C infection) CF Mutation Panel (cytic fibrosis) PML-RARA (leukemia) BCR-ABL (leukemia)
	STAGING AND PROGNOSIS			
	CEA (cancer) eGFR (kidney disease) CCP antibody (RA)	Bone marrow aspiration, biopsy (leukemia)	HIV viral load (HIV infection)	KRAS mutation (lung, colon cancer)
MANAGEMENT	**THERAPY SELECTION**			
	TPMT (who can safely receive thiopurines)	PML-RARA (likely benefit from treatment with all-trans retinoic acid)	Antimicrobial susceptibility testing (many infections)	HER2/neu (breast cancer)
	MONITORING/TREATMENT ASSESSMENT			
	CEA (cancer) Hemoglobin A1c (diabetes)	PT/INR (Warfarin therapy)	Hepatitis C viral load (hep C infection) HIV viral load (HIV infection) Repeat mycobacterial cultures (assess drug response and clearance of tuberculosis)	Hepatitis C viral load (hep C infection) HIV viral load (HIV infection) PML-RARA (quantitative) BCR-ABL (quantitative)

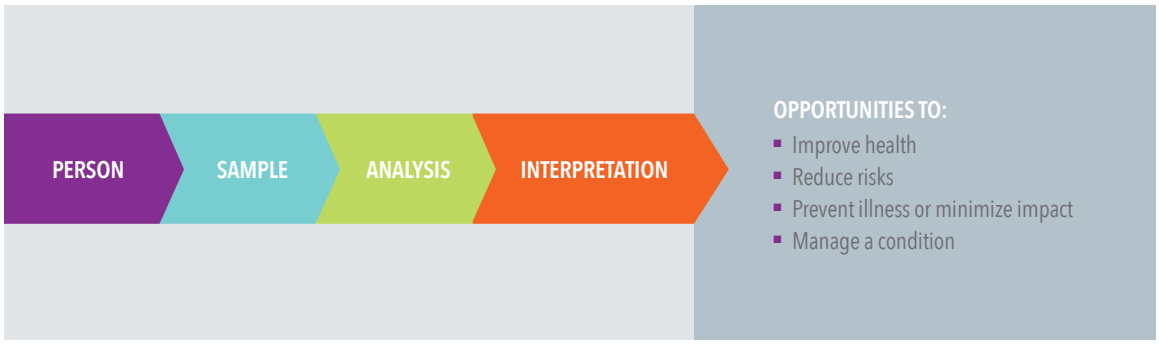
Often-Unseen Complexities of Diagnostic Testing

The human body works as a series of interconnected systems with extensive feedback loops, error notifications, damage control, self-correction, and repair. In a world of perfect health, all of the body's systems would work in constant harmony and balance. Daily living and lifestyle choices, however, put that balance under constant strain. Infectious diseases, elements in our environment, and injuries are just a few of the factors that can weaken our systems. Sometimes, the genes that are responsible for creating our harmonious metabolic systems actually make it more difficult for us to maintain or achieve a healthy balance.

In this continuing state of flux, diagnostic tests provide information on what is happening, has happened, or might happen in the body. Are the kidneys and liver functioning well? Is there an elevated risk of heart disease that might lead to a heart attack? Does a sick child have a cold or strep throat? What are the chances a woman will develop breast cancer?

Diagnostic tests allow health care providers to do the detective work that they need, giving them test results that provide answers or clues to further investigate and determine solutions that will help restore a person to good health.

On the surface, the diagnostic testing process appears simple. The health care provider prescribes a test, the patient provides a sample, and the lab performs the analysis and returns the result to the provider. The provider discusses it with the patient, and together, they determine a treatment plan.



This description, however, is only the tip of the iceberg when it comes to obtaining a useful result that can impact a person's health. Underlying each of these steps are numerous factors that can potentially affect test results. These factors are largely invisible to the patient, and many are invisible to the health care provider.

The complexities of testing derive from a variety of methodologies, each requiring different technology and equipment that, like different computer operating systems, perform the same tasks in different ways. Reagents (solutions), controls, and standards designed for compatibility with specific instruments create another layer of sophistication. In the end, the many variables inherent in *in vitro* testing produce results that can only be adequately interpreted in the context of the technology used to produce them.

EXAMPLE OF THE OFTEN UNSEEN COMPLEXITIES IN TESTING

- Physician writes Rx for test
- Collects sample or sends patient to lab for sample collection
- Lab uses appropriate methodology for analysis
- Receives report from lab with test results

Test Method (Type of Technology)

Analytical Instrument (by Company A):

- Type, model, brand of equipment that will perform test using test method
- Technology "under the hood" (hardware and software) establishes a test platform (way of working)
- Will vary by lab

Test 1 (by Company A)
Reagents (solutions), controls, and standards created to perform a specific test on this instrument. May also work on other Company A models.

Test 2 (by Company B)
Reagents (solutions), controls, and standards created to be compatible with this instrument in order to perform a specific test. May also work with some other instrument brands/models that have the same/similar test platform.

FOUNDATION FOR *IN VITRO* TESTING

Despite these complexities, there are two pairs of important characteristics that form the foundation for all *in vitro* testing:

- Accuracy and Precision
- Sensitivity and Specificity

These four key characteristics must be statistically reliable, regardless of methodology, in order for a particular test to be validated and for the health care provider to have confidence in the test results.

ACCURACY AND PRECISION Accuracy and precision communicate how effectively a test is able to measure the amount of a specific analyte that is present in the test sample. While many diagnostic tests produce quantitative results, some, such as a routine pregnancy test, produce a qualitative result, either positive or negative. Even qualitative tests, however, are based on an inherent capacity for measurement that can be assessed for its accuracy and precision.

Accuracy is a measure of how close reported test results are to the "true" value, which is typically a target defined by a "gold standard" test (see sidebar). A diagnostic test is useful and its results considered accurate when it repeatedly is able to come close to or hit the pre-determined target. If the "true" value is considered to be the bullseye on a target, then optimally, an accurate test will hit the bullseye every time.

Precision is a measure of how close test results are to each other when they are repeated multiple times on the same sample. Referring again to the target analogy, precision measures how tightly clustered the arrows are, regardless of whether they hit the bullseye. A precise test, then, is one that consistently and reliably produces the same results (on an identical sample), whether or not they are accurate.

Naturally, the most effective tests are those that have a high degree of both accuracy and precision. If a test does not meet minimum standards for accuracy and precision, it is unlikely it will be approved for use in a clinical setting.

SENSITIVITY AND SPECIFICITY Sensitivity and specificity[5] are used to communicate the probability that an individual does or does not have a particular condition.

Sensitivity, sometimes referenced as clinical sensitivity, measures the probability that a person has the disease or condition suspected by the health care provider. For example, if a test has 95% sensitivity, it means that for every 100 people who have the disease, the test will positively identify 95 of them. Five of the 100 will be incorrectly identified by a negative test result as not having the disease (false-negative).

Specificity, also known as clinical specificity, measures the probability that a person does not have the suspected disease or condition. In this case, assume that 100 healthy people are evaluated for a disease using a test that is 95% specific. Five of those people will be incorrectly identified as positive for the disease, creating a 5% false-positive rate.

While test developers strive to attain the optimal balance between sensitivity and specificity, the technical challenges involved may lead to a trade-off between the two. In general, tests with high sensitivity may be useful for screening (catching a high number of true positives while minimizing false negatives), and highly specific tests may be more useful as diagnostic, confirmatory tests by minimizing the number of false-positives. The sometimes-difficult balance between sensitivity and specificity is often why two or more different tests (screening and confirmatory) are performed before a diagnosis is made.

5. All references are to *clinical* sensitivity and *clinical* specificity, which are distinct from *analytical* sensitivity and *analytical* specificity, both of which are defined in the glossary.

THE GOLD STANDARD

In general, the four cornerstones of *in vitro* testing are measured against a "gold standard," a test that is considered the "most true or best available true." It is likely to be the most sensitive and/or specific. Gold standard tests may be technically challenging, labor intensive, costly, invasive, slow, and/or be more of a hazard to perform — such as a radioactive test, or one that requires patient medical monitoring while it is performed.

During diagnostic test development, test kits and test methods are compared to the gold standard to ensure that their results are acceptably close to it, or better. This includes the ability of other test kits to arrive at the same value (true result = 20, test result = 20), and/or the ability of another test method to accurately identify the same condition (positive = pregnant). For instance, the gold standard test for infection of the throat caused by group A *streptococcus*, commonly known as strep throat, is a culture, but that test may take 24–48 hours for results. For this reason, experts recommend that a rapid antigen strep test be performed. Though it is not as sensitive as a culture, it is sufficiently specific, and results are available in 10–20 minutes. The patient is likely to be treated sooner and does not have to return for a second office visit.

UNDERLYING TEST COMPONENTS

Accuracy, precision, sensitivity, and specificity are the four cornerstones of diagnostic testing technologies. As such, they are the criteria against which the effectiveness of a test's underlying component parts is measured. These components include:

- *Test Method:* The method defines the procedural steps necessary to perform the test. It is "how" the test is done.
- *Test Materials:* The test materials may or may not be provided as a "kit" and include the reagents (solutions), standards, controls, and instructions. These are needed to perform the test using a specified instrument.
- *Instrument:* This is the equipment used to perform test. It houses the test method and uses the test materials or kit to execute the analysis defined by the method.
- *Patient Test Sample:* This is the body fluid or other sample provided by the patient, processed and prepared or "as-is," depending on the testing protocols.
- *Connectivity:* The four items above are the essential components for performing a test. This fifth component is equally critical to testing effectiveness, though it is not directly related to the test analysis. An effective communications network is needed in order for the test results to have meaning.

The test method, test kit, and analytical instrument can all differ by manufacturer, and require standardization (calibration) and quality assurance procedures (quality control) to ensure the test performs as intended. These factors can affect the test's accuracy, precision, sensitivity, and specificity, and so, the reported test results. Comparing results across instruments from different manufacturers (usually from different labs) can also challenge the health care provider to make sense of the data. A result of 5.0 reported from one lab may be "normal," but a 5.0 from a second lab may be "high."

TEST APPROVAL, VALIDATION, AND QUALITY CONTROL

Many diagnostic tests are manufactured and sold as packaged kits to multiple laboratories. Such tests require the approval of the US Food and Drug Administration (FDA). The premarket review process is designed to ensure the safety of the test and its effectiveness for the patient.

Individual laboratories also may develop their own tests, so-called laboratory developed tests, or LDTs. Currently, FDA has not yet exercised its enforcement discretion to regulate LDTs, although the agency has announced intentions to subject LDTs to the same regulatory requirements as those that apply to manufacturer developed tests.

Laboratories, whether or not they produce LDTs, are subject to oversight by the Centers for Medicare and Medicaid Services (CMS) to ensure good laboratory practices and also may be subject to third-party accreditation processes.

TESTING PHASES

As discussed, test technology is a complex process with many variables that depend on strict adherence to protocols designed to minimize testing errors and maximize test reliability. These protocols are defined in the following three phases of testing: Preanalytical, Analytical, and Post-analytical.

PREANALYTICAL The preanalytic phase has the largest number of variables of the three phases and, not surprisingly, has been shown to be the source of the vast majority of laboratory errors. In this testing phase, many of the variables are related to the communication between the patient, the health care provider, and laboratory personnel. The following list covers many pre-testing variables but is not all-inclusive.

Test Ordering and Communication

- Right test is selected by the doctor and/or communicated correctly when ordered.
- Right data, including the test name and patient name, are entered into health care electronic information system.

Patient Status and Preparation (relevance depends upon the tests ordered)

- Stable health is desired, as emotional or physical stress or acute illness are among several factors that can affect some test results.
- Fasting is sometimes required.
- Dietary or drug restrictions prior to testing can be simple or extensive.

Sample Collection

- Collecting samples from the right person and properly labeling the sample containers with patient identifiers, such as name, date of birth, patient ID number, etc.
- Right type and number of samples, matched to the test performed (blood, urine, sputum, cerebrospinal fluid, etc.) and collected in proper containers.
- Good quality and quantity of sample must be provided, e.g. a sputum sample must be fluid (mucus) from the respiratory tract (lungs), not saliva from the mouth.
- Mixing the appropriate additive with the blood immediately after the blood is drawn is important, as is adding the right sample preservative when appropriate.
- Some substances in the sample can interfere with testing, e.g. difficult blood draws and small needles can cause red blood cells to break (hemolysis).
- Prolonged tourniquet use on the arm can alter some test results.

Sample Transport, Processing, and Storage

- Prolonged time at room temperature or exposure to light can affect some substances and so, alter the test results. Immediate cold storage and/or prompt testing are sometimes necessary for certain samples.
- Excess heat, freezing, and rough handling can damage blood samples.
- Sample should be stored and stability maintained for further testing, should it be requested.

ANALYTICAL The quality of test results relies on the analytical instrument to perform the test correctly and on the laboratory staff to identify and resolve any quality issues that arise. Typical areas of concern that the staff must be aware of include:

- Interference (due to sample condition or other problems). The laboratory staff must have a good understanding of any limitations associated with their test methods.
- Analytical instrument status, including equipment calibration and maintenance as well as the potential for the failure of a component. An example of this might be an instrument lamp that has exceeded its useful life.
- Reagents and test kit controls and standards are acceptable and stable (not contaminated or outdated).
- Sample accurately identified by the instrument, such as through the use of a bar code.
- The right test request data is communicated to the analytical instrument to ensure that the right test is performed.
- Test performed correctly, including the right series of steps, completed in the proper order, with appropriate timing and measurement.
- If a sample is diluted, it is done correctly.
- If calculations are performed, they are performed correctly.

When any of these factors are not properly controlled, the assay may not reflect the accuracy, precision, sensitivity, or specificity required for correct results and an accurate interpretation.

POST-ANALYTICAL After testing, the results are sent to the health care provider, who interprets them in the context of the patient's history and presentation of symptoms to devise a plan for a positive health outcome. Post-testing variables that can impact this process include:

- Right test results communicated from the analytical instrument or input into the electronic information system.
- Communication of results or interpretation of results to the health care provider occurs in a timely manner, especially for critical values (those requiring prompt attention and immediate medical intervention).
- Right report with correct test name, results, reference ranges to aid in interpretation, and with high, low, and critical values properly flagged.
- Interpretation of the test by the doctor (or by the laboratory scientist and communicated to the doctor), including an understanding of test limitations to help determine appropriate next steps.
- Results and their impacts communicated to the patient in a timely manner.
- Additional testing ordered as warranted for confirmation or further investigation. Sometimes this is automatically done by the laboratory and sometimes the same sample may be used for additional testing.
- Patient follows up with additional testing and/or recommended treatment or lifestyle changes.

EVALUATING SAMPLES TO OBTAIN A USABLE RESULT

As noted in the preceding section, all diagnostic testing follows a similar set of general steps regardless of the test ordered. The central step — evaluation of the test sample — is accomplished through one of a handful of standard, routine mechanisms.

ANALYTICAL METHODS The method of detecting and measuring an analyte, the materials used, and the mechanism used to make measurement possible are among the variables that generate a usable result.

Examples of methods of detection and measurement include:

- How much light is absorbed, scattered, transmitted, or reflected when a light source is applied to a sample. The amount of change detected reflects the concentration of the analyte and/or evaluates some aspect of one of its physical characteristics (such as cell volume).
- The amount of color change or fluorescence indicates the amount of analyte present.
- Electrical impedance (resistance) or detection of electrical charge can be used to count cells as they pass a detector and relate the voltage "pulse" size to the volume of each cell.

- A biosensor recognizes a biologic activity or biochemical reaction, and includes an electrochemical or optical detector. Point-of-care instruments, which are used to perform testing at the patient's side, may use this method.
- Migration rate measures how fast an analyte travels through or across a medium, helping to identify what it is and its concentration.

Electrophoresis testing best illustrates the variability of test materials and the mechanism used to perform the test. Electrophoresis involves the movement of particles within a medium under the influence of an electrical field. The rate of movement depends upon the size and shape and electrical charge of molecules as well as the type of medium.

Medium options for electrophoresis each have a different purpose. Common options include:

- Agarose — a gel that separates proteins based on their charge-to-mass ratio.
- Polyacrylamide — a polymer gel that separates proteins and nucleic acids based upon both charge-to-mass ratio and molecular size.

Examples of mechanisms used to perform electrophoresis:

- Isoelectric focusing electrophoresis — uses a pH gradient to increase resolution of protein separation.
- Capillary electrophoresis — performs electrophoresis in thin tubes at high voltage, rapidly.

AUTOMATED ANALYTICAL INSTRUMENTS AND DIAGNOSTIC TEST KITS For many tests today, there are two essential components for conducting an analysis: an instrument and a test kit. Manufacturers develop analytical instruments with their own versions of technology and different models will likely share proprietary technologies "under the hood." The internal workings of the equipment, such as detection methods, hardware, software, the way that samples are handled, and the sequences of operations will make up a fixed instrument/test platform. The instrument(s) will each have an

inherent set of capabilities, ranging from a potentially broad test menu to a small test menu and down to a single test, such as a point-of-care glucose test.

These models may be considered to have the same instrument platform and test platform, or they may encompass a couple of different ones.

Diagnostic test kits may be designed to work with specific, proprietary test platforms, or they may be more generic. Proprietary platforms are typically unique to a manufacturer's specific instrument or product line and usually require use of test kits made by the same manufacturer. Alternatively, some test kits are designed to work with instruments from several different manufacturers and are typically made by a third party.

A manufacturer may develop and offer a specific test in several configurations, with each configuration intended to work with a different test platform. There may be one specialized test kit that has been developed to run on a specific model of an analytical instrument, or there may be many different choices.

INSTRUMENTS MAY USE ONE OR MORE ANALYTICAL METHODS OR TECHNIQUES Instruments may use a single analytical method or technique or a combination of them. A single method typically still has at least two parts to it, such as a component that causes a change and a component that detects the change, or a component that detects a change and then a component that turns that change into an electrical signal. An example of an instrument with a single method is a spectrophotometer that has a light source at a specific wavelength and a detector that measures changes in the light that are caused by the analyte in the test sample.

Combining methods in a mix-and-match fashion is a manufacturer's choice. In some cases, combining methods is done to get a usable result. In others situations, it enables additional capabilities, and/or it may make the overall instrument platform perform better (more sensitive, better separation of analytes, etc.).

One example of a multiple methods instrument is a hematology analyzer that may utilize electrical impedance (resistance) and then either flow cytometry and optical scatter, or fluorescent flow cytometry and a fluorescence detector, to count, distinguish, and evaluate red blood cells (RBCs), white blood cells (WBCs), and platelets. In addition, a method for measuring reticulocytes (immature RBCs) may also be a component part of the hematology analyzer in order to add this testing option to the instrument.

DIFFERENT METHODS CAN BE USED TO EVALUATE AN ANALYTE Tests may be run using different methods, but these methods will not necessarily produce interchangeable results and may be used for different purposes.

- In most cases, test methods are invisible to the health care provider, who would order a Complete Blood Count (CBC) with no awareness of what test methodology is used. Methods used reflect the choice of whoever purchased the hematology analyzer.

- Laboratories select different test methods based upon a needs evaluation. What tests does their laboratory want to offer routinely and at what volume? With what turnaround time? What are the needs of the health care providers that the lab serves? What are their anticipated needs in the next few years?
- Selections are also made based upon the capabilities and limitations and cost of the test method and instrumentation. A central laboratory with high volume may choose a highly automated hematology analyzer with an on-board reticulocyte analyzer. A doctor's clinic, which typically would have much lower volume, may only need a basic hematology analyzer that performs only the most routine tests.
- In some cases, a specific test method may be selected by a specialist, such as a pediatrician or an oncologist, because of identified advantages to her population of patients, or it may be selected in order to perform the same test repeatedly on a person, where comparison of results is important. Monitoring for cancer recurrence is one example where repeat testing using the same test method would be valuable.
- Methods may also be selected based on the level of expertise of the laboratory or the facility performing the testing. Some laboratories may not be equipped to perform highly complex tests, and a physician's office, for example, may only perform relatively simple tests that do not require laboratory expertise.
- Some methods are more closely identified with specific tests.
- Some instruments and test methods are dedicated to a single purpose because of the functions that are built into them. They are intended to do one thing well.

EXAMPLE 1

A blood culture system (right) automates blood culture management, making the process less labor intensive and speeding up the process of detecting a systemic infection. This system manages the blood culture sample (agitation and incubation), continuously monitors it, and rapidly identifies the presence of microorganisms.

EXAMPLE 2

A point-of-care glucometer (left) is used to measure blood glucose. Since glucose is by far the most frequently monitored analyte, being performed daily or multiple times a day by diabetics, a dedicated piece of equipment that is small and portable enough to use at the point of care has great value.

Test Categories, Methodologies, and Uses

Methodologies used to perform the sample analysis differ by test category. Chemistry, hematology, and microbiology testing have long been the core disciplines in the medical laboratory. Molecular testing, once found only in reference laboratories and research settings, is now rapidly making its way into routine clinical use and is, in some cases, providing an alternative to or supplementing more traditional chemistry, hematology, and microbiology testing.

CHEMISTRY Chemistry has by far the largest menu of tests and test methods. Laboratories typically have multiple types of instruments for different purposes, and most tests performed in chemistry are automated. The automated instrument that is the workhorse in this area is usually simply referred to as a "Chemistry Analyzer" regardless of manufacturer.

HEMATOLOGY Hematology's workhorse is the "Hematology Analyzer." Most testing of blood cells that is performed in hematology utilizes this analyzer. Traditionally, a microscope was used to perform some tests, such as the white blood cell differential, but the more sophisticated hematology analyzers in use today perform that task. Other automated instruments are used for coagulation testing, such as platelet function or coagulation factor activity.

MICROBIOLOGY Microbiology is still relatively "hands-on" compared to other areas of the lab. Traditionally, many microbiology tests involved culturing samples and incubating them to grow and identify microbes and to determine what drugs they are susceptible to. Stained slides are evaluated under a microscope to help quickly identify microorganisms. As testing technology has evolved in this area, some culturing processes have been automated or semi-automated, e.g. blood culture systems. Many microorganisms are identified using molecular methods that are automated, and some are identified through kit-based testing — such as Giardia antigen. One important advance is the development and use of MALDI-TOF MS which stands for "Matrix Assisted Laser Desorption/Ionization" and "Time of Flight Mass Spectrometry" for identification of microbes.

MOLECULAR Molecular testing uses a wide variety of techniques to evaluate tiny fragments of DNA or RNA. Testing falls into two broad categories: direct nucleic acid testing or amplified nucleic acid testing. With direct testing, the sample is prepared, hybridized (attached) to a nucleic acid probe that seeks out the genetic material being tested for if it is present (like a key fitting into a lock), then the product is detected. With amplification, millions of copies of the DNA or RNA fragment are created, then identified and measured using a detector. Test results are compared to a known reference. When looking for genetic variations, a test will only look for the most common ones. In some cases results will be compared to a database of known genetic variants.

For more information and examples of specific diagnostic technologies, see **Supplement: Select Test Methodologies by Category** at the end of this document.

Trends in Test Technology

Analytical instruments shape the use and availability of diagnostic tests. Two important forces are driving recent trends in test technology:

- *Location* — placing more diagnostic tests and test results in locations where they are as close to the patient as possible, referred to as point-of-care testing.
- *Automation* — for efficiency in testing and reporting, decreased labor, cost savings, and to allow sophisticated testing, such as molecular diagnostics, to become more widely adopted into routine use and in a variety of settings.
- *Personalized Medicine* — enables therapies to be targeted to a subpopulation most likely to benefit from it and/or least likely to experience serious side effects from it, also enables dosage to be tailored to the individual.

POINT OF CARE TESTING (POCT)

Instrument and diagnostic test manufacturers continue to innovate and produce a range of instruments and diagnostic tests with varying capabilities that are robust enough to be reliably used by people with varying degrees of training, education, and resources. Point of care testing (POCT) brings the test to the health care provider and patient, and usually provides faster results. POCT includes tests performed at:

- The hospital bedside, intensive care unit, emergency room, diagnostic treatment center, outpatient clinic, emergency responders/ambulance, and field hospitals
- The doctor's office or clinic, urgent care and walk-in clinics, nursing homes, and health fairs
- A local pharmacy or retail clinic, workplace clinic, and a person's home

VALUE: These tests can allow for more immediate feedback to patients and providers. They can make health care more convenient for the patient, provide results and diagnosis faster, allow for a single doctor visit, expedite therapy implementation (if needed), and faciilitate monitoring. All of these attributes increase the likelihood of treatment success.

EXAMPLES	USES
HIV-1/HIV-2 Rapid Test	Screen for HIV
Rapid influenza test	Diagnose flu infection
Glucose	Monitor diabetes and adjust treatment
Blood gas analyzer	Measure oxygen (pO_2), carbon dioxide (pCO_2), and acidity (pH) Some also offer other tests, such as electrolytes
Troponin	Help diagnose heart attack
D-dimer	Rule out a blood clot

HOME TESTING

Home testing brings POC testing to the patient outside of a health care setting. For some types, the tests are performed at home, while for others, the sample is collected at home and then sent to a laboratory. For those tests that actually are performed at home, they are designed to be easy for a person to understand, perform, and interpret results.

VALUE: These tests bring health care to the patient at home, often provide rapid test results, help determine whether a visit to the doctor may be required, and help monitor existing conditions such as diabetes. They provide convenience and privacy that may encourage some people to get tested who might not otherwise.

EXAMPLE TESTS FOR RESULTS AT HOME	USES
Glucose	Monitor diabetes
PT/INR	Monitor warfarin (Coumadin®) therapy
HIV oral antibody test	Screen for and diagnose HIV infections
Cholesterol	Determine the risk of developing heart disease
Luteinizing Hormone (LH)	Help determine timing of ovulation
Pregnancy test	Determine if a woman is pregnant

EXAMPLES FOR HOME COLLECTION KITS	USES
Hepatitis C screening test	Screen for and diagnose Hepatitis C infection
HIV antibody screening test	Screen for and diagnose HIV infections
Fecal occult blood test	Detect blood in the stool that can be associated with colon cancer

AUTOMATING MOLECULAR TESTING AND NEXT GENERATION SEQUENCING

At one time molecular testing was only performed by reference laboratories and large hospitals, but the technology has become more automated, and the testing is now being adopted into laboratories of all sizes. The use of molecular testing is growing rapidly, in part because of continuing trends towards personalized medicine and the use of companion diagnostics.

Most molecular tests detect a single gene or handful of common gene mutations by looking in a single location — like looking for a red, blue, or green bead at a position 3 inches down a chain. Sequencing reads all of the "beads" on a section of the chain and then evaluates what is there, typically by comparing it to known reference data. In the past, sequencing was used sparingly as it was expensive and reference data was limited. This is starting to change with next generation sequencing (NGS).

NGS encompasses several methods of sequencing a greater number of genes — more accurately, much faster, and at a lower cost. Instead of targeting a single or just a few genes at a time, NGS allows the potential evaluation of thousands of genes or an entire genome.

VALUE: Molecular diagnostics expands information available to the provider for use in tailoring therapies to an individual person based upon their own genetics or the genetics of their condition or disease; understanding the risks of inheriting and developing specific diseases; and identifying infectious diseases and microorganisms more rapidly than traditional methods. NGS applications may include a more in-depth evaluation of cancers (tumor tissue), non-invasive prenatal testing (fetal DNA in maternal blood), and gene-related disease identification. NGS generates large amounts of data that must be compared to reference data. This requires adequate bioinformatics capabilities as well as existing reference data reflecting the current state of scientific knowledge.

COMPANION DIAGNOSTICS

Companion diagnostics are tests that are used to provide a health care practitioner with information that is essential for the safe and effective use of a therapeutic product.

VALUE: The test identifies people who are likely to benefit from and/or those who should not receive the treatment, those who are likely to be at an increased risk for serious side effects, and people who may require careful monitoring in order to establish a tailored dose of medication (i.e., they may need much less or more than the standard drug dose for their treatment to be effective).

EXAMPLE TESTS	THERAPY	USES
HER2 gene amplification, protein overexpression	Herceptin (trastuzumab)	Breast cancer, gastric cancer
BRAF V600E gene mutation	Vemurafenib	Metastatic melanoma
KRAS gene mutation	Cetuximab, panitumumab	Colorectal cancer
ALK gene rearrangements	Crizotinib	Non-small cell lung cancer

Conclusion: Where the Trends Lead

One vision of the future of diagnostic testing is reflected in the Qualcomm Tricorder XPRIZE, a $10 million global competition inspired by the tricorder, a fictional medical scanning device first introduced in 1966 on the Star Trek television series. In this make-believe world, a handheld scanner is waved slowly over a crewman or alien creature to provide the doctor with a plethora of biological and medical information within seconds. The tricorder epitomizes the trends being seen in diagnostics today to "take the test to the patient." It is small, lightweight, and mobile; non-invasive; fast and accurate for a wide range of conditions at the point-of-care; and able to transmit data to a provider who may or may not be physically present. Most importantly, such technology performs the diagnostics *in vivo*, thereby doing away with the need for sample collection, processing, transport, and storage, and *eliminating the source of most diagnostic errors*.

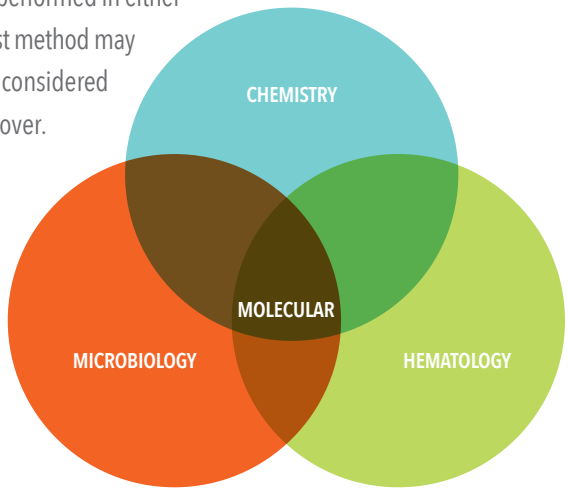
While Star Trek's tricorder is still well in the future, developing trends towards wearable and implantable devices and increased connectivity are pointing us in this direction. The Qualcomm XPRIZE and other research efforts are the beginning of a long transition that likely will see many tests shift from *in vitro* to *in vivo* analysis. Just in the last few years, there has been substantial progress in the development of wearable and implantable "instruments" capable of analyzing concentrations of specific analytes such as glucose and cholesterol. Such devices are able to transmit results to a patient's handheld device from which they can be sent to the health care provider.

The so-called "lab-on-a-chip" is a device small enough to be implanted under the skin and capable of performing multiple diagnostic tests *in vivo*. Such a device has been tested successfully on mice. Other research is focusing on technologies that are worn on the skin and even worn on the eye like a contact lens. At this time, these wearable devices tend to be tailored to sensing and analyzing a single chemical in the blood, sweat, or tears.

Apart from reducing diagnostic errors by eliminating pre-analytical sampling, handling, and preparation, these new technologies are likely to have significant health impacts in specific areas, further expanding the role of diagnostics as nearly instantaneous test results allow for faster data-gathering and better-informed decision-making. For example, some researchers suggest that such new devices could aid in monitoring heat stress in firefighters or fatigue in first responders, determining a pilot's stress and alertness, or even quickly diagnosing a concussion in a high school athlete. The additional data from continuous *in vivo* monitoring that these devices would produce may also open doors to new knowledge and treatments and, some believe, could eventually become a breakthrough technology in the management of chronic conditions.

Supplement: Select Test Methodologies by Category

Many different analytical methods are used in different test categories. The "methodology briefs" that follow offer examples of analytical methods that are used in different test categories. Some of the equipment in these areas is dedicated to its category. However, there is some overlap or cross-over between categories. For example, testing that detects antibodies produced in response to infections may be performed in either a chemistry lab or microbiology lab. Or the principles of one test method may be applied in more than one area of the laboratory. These are considered "Category Cross-overs." Molecular testing has the most cross-over.



ANALYTICAL INSTRUMENT: Chemistry Analyzer | CHEMISTRY

Analytical Methods: Photometric, reflectance photometers, fluorometers, spectrophotometers, luminometers, colorimetric, ion-selective electrode

Category Cross-Over: Typically dedicated to Chemistry; but may be combined with an immunoassay analyzer module for microbiology tests

How It Works: Samples are loaded into the analyzer and tests are ordered. A test sample is mixed with a reagent. This causes a change — such as a color change — that is measured. The intensity of the change is related to the concentration of the analyte of interest. Results are sent to a printer or computer.

Photometry: A common analytical method which measures the intensity of light absorbed by a test sample. With reflectance photometry, reflected light is measured.

Spectrophotometry: Measurement of the intensity of light at selected wavelengths.

Fluorometry: Measurement of emitted fluorescent light.

Ion-selective Electrode: Measure the concentration of ions by measuring electric current flow.

How It Is Used: Broad menu of routine tests measure analytes in body fluids to evaluate the health of body organs and systems. Comprehensive Metabolic Panel (CMP), glucose, calcium, total protein, albumin, sodium, potassium, chloride, CO_2, BUN, creatinine, alkaline phosphatase, alanine amino transferase, aspartate amino transferase, bilirubin; lipids, and many other tests.

ANALYTICAL INSTRUMENT: Immunoassay Analyzer, Immunoassay System | CHEMISTRY

Analytical Methods: Fluorescence/Fluorometry, Electrochemiluminescence, chemiluminescene, fluorescence polarization, microparticle enzyme, ion-capture. May use multiple technologies.

Category Cross-Over: Microbiology

How It Works: Uses antibodies or antigens to detect and measure analytes using a variety of different technologies. These instruments are sometimes combined with chemistry analyzers to provide a large number of tests on the menu.

How It Is Used: Measure and/or detect drugs of abuse, therapeutic drug monitoring, autoantibodies, enzymes, cardiac markers, cancer markers, thyroid tests, hormones, allergy testing, antibodies to bacteria, and viruses.

ANALYTICAL INSTRUMENT: Blood Gas Analyzer | CHEMISTRY

Analytical Methods: Ion selective electrodes, optical fluorescence, optical sensor, pulse oximeters, electrochemical sensors

Category Cross-over: Hematology

How It Works: Use ion selective membranes and optical sensors to measure arterial oxygen tension (pO_2), carbon dioxide tension (pCO_2), and acidity (pH) in an arterial blood sample. Additional analytes may include electrolytes, calcium, glucose, and lactate, and in some cases hemoglobin, hematocrit, bilirubin, creatinine, coagulation parameters, and cardiac markers. Most manufacturers produce point-of-care instruments as well as small, portable, stand-alone instruments. Pulse oximeters are a way to noninvasively and continuously monitor SO_2Hb.

How It Is Used: Measure and monitor oxygen and pH levels and to resolve imbalances. Manage patients in acute care settings.

ANALYTICAL INSTRUMENT: Hematology Analyzer, Automated Blood Cell Analysis | HEMATOLOGY

Analytical Method: Flow cytometry and optical scatter, fluorescent flow cytometry and fluorescence detector and/or "Coulter principle" electronic impedance (resistance)

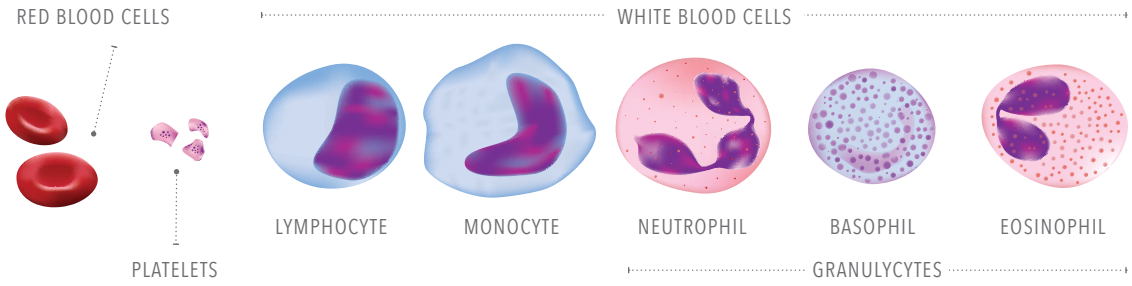
Category Cross-Over: An instrument using this test method would be dedicated for use in hematology. However flow cytometry and fluorescent flow cytometry are also used in Chemistry.

How It Works: Hematology analyzers vary in complexity, testing specifics, and automation. Computer software is used to analyze and graph data, and to report test results.

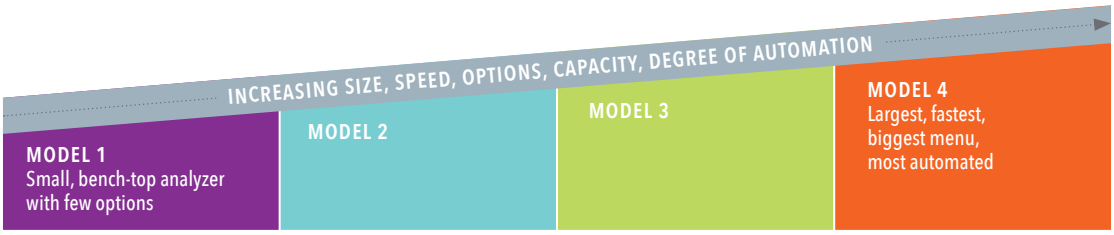
Flow cytometry used for hematology sends red blood cells (RBCs), white blood cells (WBCs), and platelets past a laser beam one cell at a time. Light that is absorbed and scattered (optical scatter) is measured from different angles to evaluate each cell's size and internal complexity and granularity. Flow cytometry and fluorescent dyes can be used with a fluorescence detector to determine additional information about specific cell populations.

There are 5 types of WBCs and these methods can produce a 5-part WBC differential that counts and identifies all 5 different types of WBCs. They can also count reticulocytes (immature RBCs).

Coulter principle: Red blood cells (RBCs), white blood cells (WBCs), and platelets cells are evaluated as they pass one at a time between two electrodes and through a tiny aperture. Changes in electrical resistance from the cells cause voltage pulses that are measured and analyzed to count cells and measure their volume. The rate of counting can be up to 10,000 cells a second, so thousands of cells are evaluated during testing. This method groups WBC into 3 types for a 3-part WBC differential (granulocytes, lymphocytes, and monocytes).



Manufacturers of hematology analyzers may combine technologies to develop proprietary combinations. Each manufacturer would typically have a line of instruments to accommodate different places where testing is performed. Similar to an automobile manufacturer's line-up of trucks, minivans, and large to small cars.



How It Is Used: Determine if quantities of WBC, RBC, and platelets and proportions of WBC types are normal in quantity, size, type, and shape. Screens for and helps diagnose conditions such as anemia, infections, bleeding disorders, inflammation, and cancer.

ANALYTICAL INSTRUMENT: Coagulation Analyzer | HEMATOLOGY

Analytical Methods: Measures endpoint which is the number of seconds until detection of a clot: increased impedance or turbidly, or decreased optical clarity

Category Cross-Over: Typically dedicated to Hematology

How It Works: Coagulation factors are proteins within the fluid portion of blood that are activated in a sequence of steps to help form a clot and stop bleeding. Automated systems are used to evaluate the function of these coagulation factors. They typically examine the rate of clot formation in a sample. Reagent(s) is added that activate the sequence of reactions that ultimately lead to clot formation and precipitation of proteins. Depending on the instrument used, this is detected by measuring a decrease in optical clarity or increase in impedance or turbidity. Coagulation analyzers are usually "stand-alone" instruments.

How It Is Used: Evaluates the function of the coagulation system (hemostasis) to screen for or help diagnose bleeding or excessive clotting disorders. Tests such as the prothrombin time and partial thromboplastin time provide overall evaluation of several coagulation factors that are part of the different sequence of steps that form clots (intrinsic, extrinsic and common coagulation pathways).

ANALYTICAL INSTRUMENT: Hematocrit Determination | HEMATOLOGY

Analytical Method: Microhematocrit

Category Cross-Over: Dedicated for hematocrit testing

How It Works: This is a simple test that has been in use for many years. A small volume of blood is collected into thin capillary tubes, one end is plugged with clay, and the tubes are spun in a specialized centrifuge to separate the cells from the liquid portion of the blood. A physical Microhematocrit reader is used to look at the proportion of packed red blood cells to liquid.

How It Is Used: May be performed at the point of care to screen for anemia.

ANALYTICAL INSTRUMENT: Mass Spectrometer | MICROBIOLOGY

Analytical Method: Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF)

Category Cross-Over: Chemistry

How It Works: Uses excitation by a laser to ionize chemicals in the proteins of a microorganism. Organism from a pure culture is put onto a plate and mixed with a chemical matrix. Then a laser is applied, heating the matrix and creating ions in the organism sample. Ions enter a flight tube. Lighter ions will travel faster down the tube. They are measured using a detector and their mass-to-charge ratio and signal intensity is evaluated. The characteristics are compared to those of known organisms in a computerized database.

How It Is Used: Identify microorganisms including bacteria, viruses, and fungi but limited to what is in the database.

ANALYTICAL TECHNIQUE: Nucleic Acid Amplification | MICROBIOLOGY

Analytical Method: PCR (Polymerase Chain Reaction) and Product Detection

Category Cross-Over: Molecular

How It Works: See Molecular category. Detects DNA/RNA from bacteria when present in samples.

How It Is Used: Detect the mecA gene which indicates methicillin-resistant *Staphylococcus aureus*. Detect or measure several different microbes including viruses such as influenza virus, hepatitis B, HIV and bacteria such as *C. difficile*.

ANALYTICAL INSTRUMENT: Instrument-Based Blood Culture System | MICROBIOLOGY

Analytical Method: Automation and Continuous monitoring

Category Cross-Over: Dedicated to Microbiology and blood cultures

How It Works: Different manufacturers use different methods to automate blood cultures and allow for rapid detection of microorganisms.

Examples include:

- Automated incubation and agitation of blood culture bottles
- Use of fluorescence to measure CO_2 released by microorganisms
- Continuous monitoring

Or the measure CO_2-derived pH changes with a colorimetric sensor in bottle. Sensor changes color.

How It Is Used: Rapidly detect microorganism growth in blood cultures.

ANALYTICAL TECHNIQUE: Nucleic Acid Amplification | MOLECULAR

Analytical Method: PCR (Polymerase Chain Reaction) and Product Detection

Category Cross-Over: Chemistry, Hematology, Microbiology

How It Works: DNA exists as a double-helix and contains each person's inherited genes. These genes govern protein production, determine a person's physical characteristics (such as eye color), and have a significant influence on body functions.

PCR (polymerase chain reaction) takes a small portion of DNA and replicates a fragment of interest, making many copies of it through amplification so that it can be measured and evaluated.

Repeated cycles of heat and cold (thermocycling) are used to perform amplification. This process is done in specialized equipment, called a thermocycler.

Heat is used to denature (melt) the DNA fragment and separate it into two strands. It is then cooled and interacts with primers and DNA polymerases (enzymes that assemble DNA molecules) to make copies of the portion of interest. Repeating the process of heating, cooling, and copying over and over again allows up to billions of copies of the original genetic material to be generated.

PCR is the most widely known amplification method, but there are many other methods such as branched DNA (bDNA), ligase chain reaction (LCR), and loop mediated (LAMP).

Many different analytical methods can be used to detect PCR products (amplified genetic material). Examples include:

- Electrophoresis
- Real-time PCR — adding a fluorescent dye or probe before amplifying so that the genetic copies can be analyzed during or after amplification
- Discrimination by mass, such as by using mass spectrometry (MS)

How It Is Used:

- Detect genes associated with increased risks of disease and guide treatment
- Evaluate the genetic makeup of cancer tissue to guide treatment
- Diagnose and monitor infectious diseases such as HIV/AIDS

ANALYTICAL TECHNIQUE: Direct Nucleic Acid Test | MOLECULAR

Analytical Method: Hybridization and Probes

Category Cross-Over: Chemistry, Microbiology

How It Works: During hybridization, double-stranded hybrid DNA is created from single-stranded DNA and then detected using probes.

A probe is a set of known nucleic acids whose identity and genetic sequence is known. It is used to hybridize to a target nucleic acid in order to identify it and measure it. If the nucleic acid of interest is present, the probe nucleic acid will hybridize with it.

The probes or targets may be labeled or unlabeled depending on the detection method. Hybridization categories include solid-phase and solution-phase.

Examples of Solid-Phase (probe or target attached to a surface):

- Medium-density arrays: Analyze 20 to 500 spots
- Microarrays (DNA chips): Analyze thousands to millions of spots

Examples of Solution-Phase (probe and target in solution):

- Single-Copy Visualization: Probe labeled with multi-color fluorescent molecules

How It Is Used:

- Medium-density arrays: Useful for testing for multiple mutations in specimens. Can be used for cancers, genetic diseases, and pharmacogenetics.
- Microarrays (DNA chips): Used for gene expression and detecting variations in nucleotides. Potential uses in many areas.
- Single-Copy Visualization: Targets identified by color and counted, can measure mRNA in tissue specimen.

ANALYTICAL TECHNIQUE: Direct Nucleic Acid Test | MOLECULAR

Analytical Method: Fluorescence *in situ* hybridization

Category Cross-Over: Hematology

How It Works: Fluorescent probes bind to target portions of chromosomes that have been fixed to slides. Detection of fluorescence using a special microscope indicates the presence of target DNA sequences on chromosomes.

How It Is Used: Can show gene deletions, duplications, amplifications, and translocations by evaluating chromosomes. Multiple target areas can be evaluated using multi-color probes.

- Breast Cancer: HER-2/neu for drug therapy
- Leukemia: BCR-ABL fusion confirms a diagnosis of CML and guides therapy
- Down syndrome (trisomy 21). Extra (third) copy of chromosome 21

Glossary of Commonly Used Diagnostic Terms

AMPLIFICATION Amplification of DNA is a process in which the polymerase chain reaction (PCR) is repeated for a number of cycles to exponentially increase the number of copies of a specific target region of a gene to a level at which they are detectable by a sensor component, enabling a test result.

ANALYTE A substance measured by a diagnostic test, for instance, a specific blood chemistry component such as sodium.

ANALYTIC TECHNIQUE See **TEST METHOD**

ANALYTICAL INSTRUMENT Piece of equipment used to perform *in vitro* diagnostic testing.

ANALYTICAL SENSITIVITY The smallest quantity of a substance in a sample that can accurately be measured by a test.

ANALYTICAL SPECIFICITY The ability of a test to measure one specific substance in a sample, while not measuring other interfering or cross-reacting compounds.

ANALYTICAL VALIDITY A determination of whether a test reliably and correctly detects what it says it does. This includes accuracy, precision, analytical sensitivity, and analytical specificity.

ANTIBODY (AB) A substance produced by the body in response to an antigen that specifically reacts with the antigen to destroy, inhibit, or neutralize it. The body produces antibodies as a defense against foreign substances. Antibodies may be identified and measured to determine whether an individual has been infected by a pathogen.

ANTIGEN Any substance foreign to the body that causes a person's immune system to produce antibodies against it. Antigens can be microorganisms (bacteria, viruses, mold, fungus), chemicals, toxins, pollens, or any other material that triggers a response. If the immune system targets part of the body's cells or tissue (self-antigen), then the antibody produced is an autoantibody (against-self) which is seen with autoimmune conditions.

CHROMOSOME Long, thin structures within the nucleus of most cells of the body that contain inherited genes in the form of DNA. Humans normally have 46 chromosomes: 22 pairs and then 1 pair of sex chromosomes (XX for a female or XY for a male).

CLINICAL CHEMISTRY Also known as clinical biochemistry or clinical pathology, clinical chemistry is the area of science that is generally concerned with analysis of bodily fluids.

COMPANION DIAGNOSTIC A diagnostic test that provides information that is essential for the safe and effective use of a therapeutic product. A test that is used as a "companion" to inform prescription or dosing of the drug based on test results (i.e., the patient would receive the test prior to a treatment decision to ensure it is appropriate for that particular patient, or the test could be used to monitor drug response and alter dose).

DIAGNOSTICS The use of clinical tests to inform clinical decision-making for the purpose of disease prediction, screening, diagnosis, treatment selection, prognosis, and monitoring.

DNA Deoxyribonucleic acid (DNA) is the molecule that encodes genetic material and is used within cells to form proteins. In cells, DNA usually exists as two long intertwined chains twisted into a double helix and held together by weak bonds between base pairs of nucleotides.

DNA SEQUENCING DNA sequencing is the determination of the precise sequence of nucleotides in a sample of DNA.

FLOW CYTOMETRY A method of measuring physical and chemical attributes of cells or other biological particles by sensors, as they move by, one after the other, homogeneously suspended in fluid. This rapid (about 10,000 particles per second) analytic method allows identification of important cell types, such as malignant cells, T cells and B cells.

FLUORESCENCE *IN SITU* HYBRIDIZATION (FISH) FISH is a test that "maps" the genetic material in a person's cells. This test can be used to visualize specific genes or portions of genes.

GENE A hereditary unit consisting of a sequence of DNA that occupies a specific location on a chromosome and determines a particular characteristic or biological process in an organism. Genes carry information for making all the proteins required by an organism. These proteins determine, for example, how the organism looks, how well its body metabolizes food, or how effectively it fights infection. Genes undergo mutation when their DNA sequence changes during cell division.

GENE SEQUENCING Determining the order of DNA nucleotides or bases in a gene. Gene sequencing tests are typically used to identify genetic changes or mutations in DNA that can influence or cause disease. The technology to sequence the entire genome of a patient exists but it has not yet entered routine clinical practice.

GENETIC TESTING A direct analysis of genetic information (DNA, RNA, genes, chromosomes) to determine the presence or risk of developing a particular disease(s) or condition.

GENOME All of the DNA in an organism or a cell, including the DNA in the nucleus of a cell and the DNA in the cell mitochondria.

HEMATOLOGY The scientific study of blood and blood-forming tissues.

IMMUNOASSAY Tests used to detect or quantify a specific substance, the analyte, in a blood or body fluid sample, using an immunological reaction. Immunoassays are highly sensitive and specific. Their high specificity results from the use of antibodies and purified antigens as reagents. High sensitivity is achieved by using an indicator system (e.g., enzyme label) that results in amplification of the measured product.

IMMUNOCHEMISTRY The study of the chemical properties of antigens and antibodies, complement, and T cell receptors.

IMMUNODIAGNOSTICS The use of specific antibodies to measure a substance. This tool is useful in diagnosing infectious diseases and the presence of foreign substances in a variety of human and animal fluids (e.g., blood, urine, etc).

INSTRUMENT PLATFORM See **PLATFORM**

***IN VITRO* DIAGNOSTIC (IVD)** *In vitro* diagnostic (IVD) products are those reagents, instruments, and systems intended for use in 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 complications. IVDs are medical devices.

MASS SPECTROMETRY Mass spectrometry (MS) is an analytical technique that measures the mass-to-charge ratio of charged particles. It is used for determining masses of particles, for determining the elemental composition of a sample or molecule, and for elucidating the chemical structures of molecules, such as peptides and other chemical compounds. The MS principle consists of ionizing chemical compounds to generate charged molecules or molecule fragments and measuring their mass-to-charge ratios.

MICROARRAY A multiplex microarray is a lab-on-a-chip that enables a single instrument to simultaneously perform multiple diagnostic tests.

MICROBIOLOGY The branch of biology that is concerned with the study of microorganisms, including bacteria, archaea, viruses, algae, protozoa, and fungi, and their effect on humans.

MOLECULAR DIAGNOSTICS Diagnostic tests that identify a disease, predisposition for a disease, or progress in treating a disease by detecting specific molecules such as DNA, antibodies, and proteins.

MUTATION In molecular biology and genetics, mutations are changes in a genomic sequence: the DNA sequence of a cell's genome or the DNA or RNA sequence of a virus. Mutations are caused by radiation, viruses, and mutagenic chemicals as well as errors that occur during DNA replication. Mutation can result in several different types of change in DNA sequences; these can have either no effect, alter the product of a gene, or prevent the gene from functioning properly or completely. Due to the damaging effects that mutations can have on genes, organisms have mechanisms such as DNA repair to remove mutations.

NUCLEIC ACID A polymer (large molecule) made up of nucleotide monomers (smaller molecules). Examples of nucleic acids are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).

NUCLEOTIDES / NUCLEOBASES Nucleotides and nucleobases are the building blocks for DNA and RNA. They include A (adenine), C (cytosine), G (guanine), T (thymine) and U (uracil). DNA is made up of long chains of base pairs using C–G and A–T in specific sequences (order). RNA substitutes U for T, to make C–G and A–U base pairs.

PANEL TESTING A laboratory procedure in which a series of tests is performed on one specimen, usually related to a single condition or disease, or for differential diagnosis.

PLATFORM — INSTRUMENT PLATFORM AND TEST PLATFORM An instrument platform is characterized by *how* a piece of laboratory equipment performs testing. Test platforms are linked to instrument platforms. It is the component parts (hardware and software) and how they are configured by a manufacturer. It is also the sequence of steps that occur from the time a test sample is identified to the time that a test result is reported. In most cases much or all of the internal workings of the equipment make up a fixed instrument platform. In some cases, some components or modules may be able to be exchanged for a more flexible instrument platform. Manufacturers may have a line of chemical analyzers, for instance, that range from a large, sophisticated model to a simple bench-top model. They may share technologies "under the hood" and be considered to have the same instrument platform (depending on how defined) and test platform. Test kits are designed and developed to work with specific instrument platforms and in this context are referred to as a specific test platform. This is similar to ink cartridges that are created to work with specific models of ink-jet printers.

POINT OF CARE TESTING (POCT) Point of care testing (POCT) occurs where the patient is located or in the immediate location of the patient's health care provider (e.g., the hospital bedside, home, or physician office).

POLYMERASE CHAIN REACTION (PCR) The polymerase chain reaction (PCR) is a scientific technique in molecular biology to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence in order to enable the sequence's automated detection. PCR is a foundational methodology in molecular diagnostics and is important in biotechnology, forensics, medicine, and genetic research.

PRIMER A segment of DNA or RNA that is complementary to a given DNA sequence and that is needed to initiate replication by DNA polymerase for a polymerase chain reaction (PCR) to occur.

REAGENT A substance used to produce a specific chemical reaction. It may be used to detect, measure, or prepare other substances.

RNA Ribonucleic acid (RNA) is a chemical found in the nucleus and cytoplasm of cells. It plays an important role in protein synthesis and other chemical activities of the cell. The structure of RNA is similar to that of DNA, but it is usually single stranded. There are several classes of RNA molecules, including messenger RNA, transfer RNA, ribosomal RNA, and other small RNAs, each serving a different purpose.

SCREENING TEST A test used to determine whether an *asymptomatic* patient has a particular disease.

SINGLE NUCLEOTIDE POLYMORPHISM (SNP) DNA sequence variations that occur when a single nucleotide (A, T, C, or G) in the genome sequence is altered. Although the majority of human DNA sequences are the same, variations in DNA sequence can have a major impact on how humans respond to disease; environmental factors such as bacteria, viruses, toxins, and chemicals; and drugs and other therapies. This makes the identification of SNPs and their effects valuable for biomedical research and for developing medical diagnostics.

TEST METHOD, ANALYTICAL METHOD, ANALYTICAL TECHNIQUE These terms are used somewhat interchangeably in several ways: A procedure that is used to measure an analyte in a test sample, or evaluate a physical characteristic (size, volume, granularity). The method uses specific materials (reagents, controls, standards) and may use a specific type of equipment (analytical instrument) in order to carry out a series of tasks in sequence and end up with a usable test result. Also used as more of an "umbrella" term to refer to a type of testing, such as electrophoresis, that may involve variations in materials, equipment, and procedural steps used but that has the same testing foundation — the movement and separation of particles across a medium based upon their response to an electric field. The term "analytical technique" can also be used more narrowly to describe the specifications for the performance of a specific task, such as creating sequential/serial sample dilutions, piercing the cap of a test sample tube with a hollow needle then aspirating a sample, pipetting a specific volume of reagent, etc.

TEST PLATFORM *See* **PLATFORM**

Additional Resources

The following resources offer a variety of perspective on diagnostics and diagnostics technologies that may supplement the information contained here. In some cases, these resources served as sources that informed the content of this document, and so, may offer more detail on specific topics covered.

A BRIEF HISTORY OF MEDICAL DIAGNOSIS AND THE BIRTH OF THE CLINICAL LABORATORY

- Part 1: Ancient times through the 19th century
  http://www.academia.dk/Blog/wp-content/uploads/KlinLab-Hist/LabHistory1.pdf
- Part 2: Laboratory science and professional certification in the 20th century
  http://www.academia.dk/Blog/wp-content/uploads/KlinLab-Hist/LabHistory2.pdf
- Part 3: Medicare, government regulation, and competency certification
  http://www.academia.dk/Blog/wp-content/uploads/KlinLab-Hist/LabHistory3.pdf
- Part 4: Fraud and abuse, managed care, and lab consolidation
  http://www.academia.dk/Blog/wp-content/uploads/KlinLab-Hist/LabHistory4.pdf

ADVAMEDDX/DX INSIGHTS: INTRODUCTION TO MOLECULAR DIAGNOSTICS

http://advameddx.org/download/files/AdvaMedDx_DxInsights_FINAL(2).pdf

BIOCHIMICA ET BIOPHYSICA ACTA 1842 (2014) 1932–1941

Next generation sequencing technology: Advances and applications
http://www.sciencedirect.com/science/article/pii/S092544391400180X

CLINICAL LABORATORY IMPROVEMENT AMENDMENTS (CLIA)

https://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/index.html?redirect=/clia/

DARK DAILY: LABORATORY INSTRUMENTS AND LABORATORY EQUIPMENT

http://www.darkdaily.com/category/laboratory-pathology/laboratory-instruments-laboratory-equipment#axzz3gEzkyEFD

FDA: OFFICE OF *IN VITRO* DIAGNOSTICS AND RADIOLOGICAL HEALTH

http://www.fda.gov/AboutFDA/CentersOffices/OfficeofMedicalProductsandTobacco/CDRH/CDRHOffices/ucm115904.htm

FDA VOICE: FDA TAKING GENOMIC TESTING TO THE NEXT LEVEL

http://blogs.fda.gov/fdavoice/index.php/tag/next-generation-sequencing-ngs/

FUTURE MEDICINE: PERSONALIZED MEDICINE

http://www.futuremedicine.com/loi/pme

IOM: IMPROVING DIAGNOSIS IN HEALTH CARE
http://iom.nationalacademies.org/reports/2015/improving-diagnosis-in-healthcare.aspx

LAB TESTS ONLINE
http://labtestsonline.org

LABCOMPARE: THE BUYER'S GUIDE FOR LABORATORY EQUIPMENT
http://www.labcompare.com/

LABORATORY MEDICINE | JOURNAL OF ASCP
http://labmed.oxfordjournals.org

MEDICAL DAILY: INNOVATION
http://www.medicaldaily.com/innovation

MDDI: MEDICAL DEVICE AND DIAGNOSTIC INDUSTRY
http://www.mddionline.com

MEDICAL LABORATORY OBSERVER
http://www.mlo-online.com/

MEDICAL NEWS TODAY: MEDICAL DEVICES / DIAGNOSTICS
http://www.medicalnewstoday.com/categories/medical_devices

NATIONAL HEART LUNG AND BLOOD INSTITUTE: WHAT ARE BLOOD TESTS?
http://www.nhlbi.nih.gov/health/health-topics/topics/bdt

NATIONAL LIBRARY OF MEDICINE, MEDLINEPLUS: LABORATORY TESTS
https://www.nlm.nih.gov/medlineplus/laboratorytests.html

QUALCOMM TRICORDER XPRIZE
http://tricorder.xprize.org

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