“Sensitivity” and “Specificity” Reconsidered: The Meaning of These Terms in Analytical and Diagnostic Settings

Alfred J. Saah, MD, MPH; and Donald R. Hoover, PhD, MPH

Imprecise usage of the terms “sensitivity” and “specificity” produces confusion in the diagnostic use of sophisticated laboratory test results. “Analytical sensitivity” represents the smallest amount of substance in a sample that can accurately be measured by an assay. “Analytical specificity” refers to the ability of an assay to measure one particular organism or substance, rather than others, in a sample. An assay's clinical diagnostic sensitivity and diagnostic specificity are distinct from that assay's clinical diagnostic sensitivity and diagnostic specificity. “Diagnostic sensitivity” is the percentage of persons who have a given disorder who are identified by the assay as positive for the disorder. High analytical sensitivity does not guarantee acceptable diagnostic sensitivity. “Diagnostic specificity” is the percentage of persons who do not have a given condition who are identified by the assay as negative for the condition. False-positive reactions occur because of sample contamination and diminish the diagnostic specificity of the assay. The terms “sensitivity” and “specificity” should be used with the requisite adjectives because the “diagnostic” and the “analytical” meanings of these terms are very different.

The terms “sensitive” and “specific” are used in many different contexts. In fact, in a discussion of molecular assays [1], the term “ultrasensitive” was introduced to describe an assay that offered “more sensitivity” than its predecessor. Most health care professionals have at least an intuitive notion of these concepts, but the vernacular of test sensitivity and specificity has become more complex and seemingly more contradictory as the use of molecular assays has become more widespread [2]. Thus, difficulties arise when the clinical performance of an assay does not seem to meet expectations. For instance, as many as 2% to 15% of persons who are documented as being seropositive for human immunodeficiency virus type 1 (HIV-1) infection have negative results on the exquisitely sensitive polymerase chain reaction (PCR) assay [3–5]. Understandably, confusion arises when a “highly sensitive” [6] assay often misses the diagnosis or when a “highly specific” test gives a false-positive result [7, 8].

The problem is that any given laboratory test has not one but two kinds of sensitivity and specificity: analytical and diagnostic. Despite important differences between analytical sensitivity and diagnostic sensitivity, these terms are used interchangeably in the clinical setting without being distinguished by their respective adjectives. The same is true for analytical specificity and diagnostic specificity. Understanding the different meanings of these terms is key to properly requesting and interpreting diagnostic test results.

Analytical and Diagnostic Sensitivity

Any assay that purports to measure a given substance has inherent characteristics that are described as “analytical” to distinguish them from the assay’s “diagnostic” characteristics [9]. The analytical sensitivity of an assay is that assay's ability to detect a low concentration of a given substance in a biological sample, whether that substance is blood glucose or HIV–1 proviral DNA. This type of sensitivity is expressed as a concentration (for example, in mg/dL or in gene copies/50 million cells) [10]. The lower the detectable concentration, the greater the analytical sensitivity. Synonyms for analytical sensitivity include “limit of detection” and “minimal detectable concentration.” Analytical sensitivity may also be expressed in terms of an assay's ability to detect a change in concentration. The smaller the detectable change, the greater the analytical sensitivity.

Analytical sensitivity is determined in one of two ways: empirically, by testing serial dilutions of specimens with a known concentration of the target substance; or statistically, by testing multiple negative specimens and using 2 or 3 standard
deviations above the mean as the lower limit of detection. With either method, the goal is to determine the detection limit of the assay [11]. For molecular assays, it is logical to use the empirical method to determine the detection limit.

When an assay is applied to a population to detect a condition or disease, diagnostic sensitivity becomes relevant. The diagnostic sensitivity of a test is the test's ability to detect persons with the condition of interest in a population or group and is expressed as a proportion or percentage: the number of persons who have both the condition and a positive test result divided by the number of persons who have the condition. Diagnostic sensitivity often has more to do with the ability to obtain the target substance in a processed sample from a person who has the condition than with the ability to detect very low concentrations of a substance. If the target substance is not in the processed sample because of vagaries of sampling or processing, an assay with perfect analytical sensitivity still fails to give a positive result.

Polymerase chain reaction is a technologically advanced assay in which a small amount of DNA (very low numbers of copies of a gene or gene fragment) is amplified to make its detection feasible. Since it was invented 11 years ago, PCR has gained wide applicability because the amplification process has been automated [12-14]. It has been used to identify Borrelia burgdorferi [15] sequences in synovial fluid and Treponema pallidum [16] in brain tissue. Additionally, by altering methods in assay performance, quantitation of an infectious agent (such as HIV-1 DNA or viral RNA) has become routine. In some cases, PCR has greater potential usefulness than do the previous serologic, antigen-detection, or culture-based assays designed to detect various agents or conditions.

However, sizable proportions of persons with documented diseases or infections have negative results on PCR when this assay is used for diagnosis [4, 6, 17-20]. This occurs because the target DNA may be missing from a sample obtained from an infected person. Even with infinite analytical sensitivity, PCR cannot detect the target DNA if that DNA is absent. A similar problem arises when blood culture is used to diagnose bacterial endocarditis. Only one pathogenic organism needs to be in the culture bottle that is obtained and processed from the patient in order for a diagnosis to be yielded. However, capturing and successfully processing that organism may be challenging because its presence in any given sample is unlikely.

The frequent subordination of diagnostic sensitivity to analytical sensitivity is exemplified by the dissonant message conveyed in the following sentence [21], which was written to explain why false-negative results are seen on PCR assays for HIV: “Absence of virus rather than false negativity may explain some of these results.” If the patient is truly infected with HIV, as manifested by serologic reactivity, then a negative result on PCR is falsely negative if it is used to diagnose HIV infection. From a diagnostic point of view, it does not matter that the absence of virus in the reaction vessel explains the negative results: The results remain falsely negative and frustrate the clinician's attempt at diagnosis.

False-negative results on PCR or other amplification assays also occur when primer sequences are not properly complementary to the target molecules or when primers fail to bind and amplification fails despite the presence of HIV DNA [3, 22]. This phenomenon also occurs in certain viral load assays for HIV RNA and is due to genetic intraspecies variation in HIV-1 [23]. Amplification failure may also result from the inhibition of Taq polymerase [24].

Analytical and Diagnostic Specificity

Analytical specificity is the ability of an assay to exclusively identify a target substance or organism rather than similar but different substances (insulin rather than proinsulin; HIV-1 rather than HIV-2) in a sample or specimen. In PCR, the primers for the HIV-1 proviral genome are highly "specific"; that is, they do not measure the proviral DNA of retroviruses other than HIV-1. When an assay is analytically nonspecific, it often produces a positive result when the specimen is truly negative for the exact agent being sought. This problem also diminishes diagnostic specificity, which is the ability of an assay to correctly identify a person who does not have the disease in question.

However, because an assay such as PCR is extraordinarily sensitive analytically, the slightest exogenous contamination with previously amplified HIV-1 DNA (carryover) causes a false-positive test result in an assay that is "very highly
specific" (analytically). The result, therefore, may be that analytical and diagnostic specificity diverge because the assay maintains its very high analytical specificity but becomes diagnostically misleading because of external contamination.

Given the potential for contamination due to carryover, a clinical dilemma may arise when the only indication of HIV-1 infection is a positive result on PCR. There is no standard by which to measure the true state of infection, except for the passage of time and the emergence of HIV-specific antibodies. The problem of carryover is one issue that may limit the general applicability of PCR results for HIV-1 in the diagnostic and screening setting ([25]; U.S. Food and Drug Administration Conference on the Feasibility of Genetic Technology to Close the HIV Window in Donor Screening, Silver Spring, MD, 1994).

Yet another issue affects the diagnostic specificity of PCR for detecting true as opposed to “perceived” infection. The PCR assay may detect DNA fragments that do not represent intact organisms capable of reproducing or causing disease [15, 26]. Here again, the test has not lost analytical specificity but gives diagnostically incorrect results.

Diagnostic Predictive Value and Clinical Relevance

Sensitivity and specificity define the operating characteristics of an assay, but it is the predictive value (positive or negative) of the assay that is generally of diagnostic importance to clinician and patient. Positive predictive value is the probability that a person whose test result is positive truly has the disease or condition of interest (that is, of every 100 patients who have positive test results, the number of patients who have the disease). Negative predictive value is the probability that a person whose test result is negative does not have the disease of interest (that is, of every 100 patients who have negative test results, the number of patients who do not have the disease). Strong diagnostic sensitivity improves negative predictive values, and strong diagnostic specificity improves positive predictive values (regardless of analytical sensitivity and analytical specificity). For example, if a test has perfect diagnostic sensitivity and perfect diagnostic specificity, then all persons who have positive test results have the disease and all persons who have negative test results do not. Accordingly, assays that have very high analytical sensitivity and specificity but have low diagnostic sensitivity and specificity have a poor diagnostic predictive value. For example, if an "ultrasensitive" assay (one with high analytical sensitivity) often gives positive results because of contaminated samples, then a positive test result may not strongly suggest disease. On the other hand, if an "ultrasensitive" assay fails to give a positive result because the patient samples do not contain the target molecules or if heterologous molecules are improperly measured because of very high analytical specificity, then a negative test result does not guarantee the absence of disease.

Summary

Imprecise usage of the terms “sensitivity” and “specificity” produces confusion in the diagnostic use of laboratory tests, particularly certain molecular assays, such as PCR. Important distinctions exist between the analytical and diagnostic use of these terms. The analytical sensitivity of an assay represents the smallest amount of a substance that can be accurately measured in a biological sample; analytical specificity is the assay's ability to measure a particular organism or substance, rather than another, in a sample. These characteristics are distinct from diagnostic sensitivity and specificity. In the clinical setting, diagnostic sensitivity is defined by the percentage of persons who have the disorder of interest who have positive results on the assay. Although one might expect that an analytically sensitive assay should more readily identify those persons, the ability to measure a very small quantity of a substance does not always translate into high diagnostic sensitivity. This apparent contradiction results from the shortcomings of sampling a very small volume, variations in the clinical spectrum of disease, and possible difficulties with specimen preparation and technical performance of the assay. Diagnostic specificity is defined by the percentage of persons who do not have the condition of interest who have negative results on the assay. False-positive reactions diminish the diagnostic specificity; these reactions may be particularly likely to occur in molecular assays as a result of contamination with amplified material from other reactions (carryover).

Assays with extraordinarily high analytical sensitivity and specificity will almost certainly not perform at these very high levels diagnostically. Thus, great care is required when test results are being interpreted for patient management.
Clinicians must be cautious when the terms "sensitivity" and "specificity" are used without the requisite adjectives by laboratory-based colleagues or in advertisements for laboratory services or test kits, because the "diagnostic" and "analytical" meanings of these terms are not the same.

Dr. Hoover: Johns Hopkins University, School of Public Health, 615 North Wolf Street, Room E6014, Baltimore, MD 21205.

* Copyright ©2004 by the American College of Physicians

References


20. Sanchez P, Wendel JD Jr, Grimmel E, Goldberg M, Hall M, Arencibia-


Articles citing this article

Breathing New Life into Pneumonia Diagnostics

Full Text Full Text (PDF)

Novel Diagnosis of Lyme Disease: Potential for CAM Intervention
A. Vojdani, F. Hebroni, Y. Raphael, J. Erde, and B. Raxlen
Evid Based Complement Alternat Med September 1, 2009 6:283-295

Abstract Full Text Full Text (PDF)

PCR-Based Methods for Detecting Single-Locus DNA Methylation Biomarkers in Cancer Diagnostics, Prognostics, and Response to Treatment
L. S. Kristensen and L. L. Hansen
Clin. Chem. August 1, 2009 55:1471-1483

Abstract Full Text Full Text (PDF)

Novel Diagnosis of Lyme Disease: Potential for CAM Intervention
A. Vojdani, F. Hebroni, Y. Raphael, J. Erde, and B. Raxlen
Evid Based Complement Alternat Med October 15, 2007 0:nem138v1-nem138

Abstract Full Text Full Text (PDF)

Misdiagnosis of HIV Infection
J. D. Rich, E. Mylonakis, and T. P. Flanigan
ANN INTERN MED October 5, 1999 131:547-548

Full Text Full Text (PDF)

Outcome of Diagnostic Tests Using Samples from Patients with Culture-Proven Human Monocytic Ehrlichiosis: Implications for Surveillance

"Sensitivity" and "Specificity" Reconsidered: The Meaning of These Term... http://www.annals.org/content/126/1/91.full
Misdiagnosis of HIV Infection by HIV-1 Plasma Viral Load Testing: A Case Series
J. D. Rich, N. A. Merriman, E. Mylonakis, T. C. Greenough, T. P. Flanigan, B. J. Mady, and C. C.J. Carpenter
ANN INTERN MED January 5, 1999 130:37–39