

Educational Case: Point-of-Care Testing

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The following fictional case is intended as a learning tool within the Pathology Competencies for Medical Education (PCME), a set of national standards for teaching pathology. These are divided into three basic competencies: Disease Mechanisms and Processes, Organ System Pathology, and Diagnostic Medicine and Therapeutic Pathology. For additional information, and a full list of learning objectives for all three competencies, see <http://journals.sagepub.com/doi/10.1177/2374289517715040>.¹

Keywords

pathology competencies, diagnostic medicine, general principles, point of care testing, sensitivity, specificity, preanalytical errors, postanalytical errors

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Primary Objective

Objective GP1.8: Point-of-Care Testing. Explain how “point-of-care” (POC) testing in the physician office, multispecialty clinic, and hospital can enable better patient and population management of acute and chronic disease and why values generated using POC methods could differ from values generated in a high-throughput laboratory.

Competency 3: Diagnostic Medicine and Therapeutic Pathology; Topic GP: General Principles; Learning Goal 1: Laboratory Tests.

Secondary Objectives

Objective GP1.1: Pre- and Postanalytical Errors. Give examples of common sources of preanalytical and postanalytical errors and categorize errors when the following procedures are not properly followed: pairing patient/specimen identification with the requisition forms, using correct specimen containers/tubes for specific tests, and timing of collection, transport, and storage.

Competency 3: Diagnostic Medicine and Therapeutic Pathology; Topic GP: General Principles; Learning Goal 1: Laboratory Tests.

Objective GP1.2: Sensitivity and Specificity. Evaluate the quality of an assay in differentiating disease versus nondisease states, including graphically presenting and interpreting the data. Determine the relationship between sensitivity and specificity for this assay.

Competency 3: Diagnostic Medicine and Therapeutic Pathology; Topic GP: General Principles; Learning Goal 1: Laboratory Tests.

Patient Presentation

In spite of receiving their annual influenza vaccines in November from local pharmacy, 2 adolescent children are taken to their local outpatient clinic by their parents in February with

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Table 1. Diagnostic Laboratory Testing Information.

	Point-of-care test	Reference laboratory test
Name	Kit* Influenza A+B Rapid Influenza Diagnostic Test	Analyzer* Influenza A+B Assay
Method	Influenza viral nucleoprotein antigen detection via lateral flow immunoassay	Qualitative multiplex real-time reverse transcription polymerase chain reaction
CLIA† complexity	Waived	High
Sample type	Nasopharyngeal swab	Nasopharyngeal swab
Turnaround time	15 minutes	75 minutes + specimen packing, courier pickup, transport, and specimen receipt at facility, processing and storage, once daily batch analyzing, result certification, and result transmittal to requesting medical treatment facility
Cost	\$22.25	\$273.81

*Names are arbitrarily assigned and do not represent actual testing platforms.

†CLIA = Clinical Laboratory Improvement Amendments of 1988.

flu-like symptoms. Patient A is a 14-year-old Hispanic male complaining of 3 days of fever, chills, nonbloody diarrhea, splitting headache, fatigue, and body aches. Patient A's temperature taken in the clinic was 101.2 °F (38.4 °C). The patient was given 325 mg of oral acetaminophen, which dropped his temperature to 99.5 °F/37.4 °C. Two nasopharyngeal (NP) swabs were collected. The first NP swab was tested in the clinic and yielded a negative result for influenza A or B. The second NP swab was sent to a reference laboratory for further characterization. Ultimately, patient A was discharged with instructions for supportive care of hydration and acetaminophen as needed for fever and aches. Four days later, after the holiday weekend, Patient A's physician calls his parents to notify them that patient A's reference laboratory result was positive for influenza A.

Patient B is a 16-year-old Native American female with past medical history significant for asthma complaining of one-and-a-half days of fever, sore throat, headache, muscle aches, dehydration, dizziness upon standing, and nausea with 3 episodes of nonbloody vomiting earlier this morning. Patient B's temperature at the clinic was 100.6 °F (38.1 °C), she was hypertensive for her age at 132/83 mm Hg, tachypneic at 18 breaths per minute, and tachycardic at 128 bpm. Patient B was given 650 mg of oral acetaminophen, 8 mg of oral dissolving tablet ondansetron, and 1 L of intravenous normal saline; repeated vital signs 1 hour later were temperature of 98.9 °F (37.2 °C), blood pressure of 116/73 mm Hg, respiratory rate of 14 breaths per minute, and heart rate of 86 bpm. Two NP swabs were collected; using a point-of-care rapid antigen detection test, the first NP swab produced a positive result for influenza A; the second NP swab was shipped to a reference laboratory for testing. Patient B was given a 5-day course of oseltamivir 75 mg taken orally twice daily, supportive care instructions, and was sent home. Four days later, after the President's Day Holiday weekend, Patient B's family nurse practitioner calls her parents to follow up and to notify her that the reference laboratory test result was positive for both influenza A and influenza B.

Table 2. Nasopharyngeal Swab Sensitivity and Specificity Comparisons by Methodology.

	Immunoassay	>/=/ <	Real-time reverse transcription polymerase chain reaction
Influenza A sensitivity	83%	<	100%
Influenza A specificity	89%	<	98.7%
Influenza B sensitivity	62%	<	98.4%
Influenza B specificity	98%	>	95.5%

Diagnostic Findings

Diagnostic laboratory testing information is given in Table 1.²⁻⁴

Comparison of testing sensitivities and specificities for NP swab samples is given in Table 2.²⁻⁴

Questions/Discussion Points

What Type of Virus Is the Influenza Virus, and How Does Infection Usually Present?

Influenza is a viral disease caused by a highly contagious, single-stranded, negative sense RNA virus enveloped in a lipid bilayer. Of the 3 types (A, B, and C), influenza A is responsible for seasonal antigenic drift-related epidemics (viral protein antigen changes from spontaneous mutations) and antigenic shift-related pandemics (recombination of viral RNA due to multiple flu types infecting the same cell) and is capable of infecting humans, pigs, horses, and birds.⁵

Common presenting symptoms include abrupt headache, myalgia, malaise, and fever 1 to 4 days after infection. The disease course is usually benign, requiring only supportive care, but can cause severe or even fatal complications in immunocompromised, elderly, and young patients; for patients with certain risk factors (eg, diabetes mellitus, Native American ancestry, lung disease, etc), antiviral drugs are often prescribed early in the disease course (within 48 hours).⁵

What Is Point-of-Care Testing and What Are the Advantages of This Type of Testing?

Point-of-care testing (POCT) is a descriptive category for tests able to be performed and resulted outside of a laboratory setting, like at a patient’s bedside/in a patient treatment room/clinic setting. The test complexity will be low/waived (low risk of obtaining an incorrect result) by CLIA (Clinical Laboratory Improvement Amendments of 1988) standards, meaning that personnel other than laboratory technicians can be trained to effectively run the test and interpret results.⁶ With advances in technology, more complex testing platforms are becoming available to nonlaboratorians, like the popular iSTAT analyzer, for example. Some common POCT analytes include glucose, blood gases, rapid strep, and influenza.

Point-of-care testing is advantageous in that nonlaboratory personnel (such as medical technicians working in the clinic) can be trained to run the diagnostic laboratory testing on-site, even at the bedside/in the patient treatment room, reducing time required to obtain a reliable result and factor it into medical decision-making. This equates to the patient receiving specific care while in the clinic versus possibly having to be called to come back in due to important disease findings noted by diagnostic laboratory testing.

Why Didn’t the Point-of-Care Test Result Match the Result From the Reference Laboratory?

The reference laboratory platform and the POCT use different methods to analyze patient samples. The POCT kit uses immunoassay, a technique common in POCT, like in pregnancy tests. A lawn of sensitive monoclonal antibodies specific to viral nucleoproteins are fixed onto the test strip—they bind to any present viral influenza nucleoproteins. Reagents react with the bound viral fragments, producing a visual color change which indicates a positive result.⁴

The reference laboratory, on the other hand, uses a much more complex method: real-time reverse transcription polymerase chain reaction. Through several complex reactions in different temperature ranges, viral nucleic acids are extracted, targeted, bound by oligonucleotide primers, and complementary DNA (cDNA) is created by reverse transcriptase. Said cDNA is amplified exponentially and probes which bind to the cDNA are cleaved, generating fluorescent signal when excited by light transmitted at a certain wavelength, generating a signal and a positive result.^{2,3,7,8}

Upon comparison, the 2 methodologies have their own respective sensitivities and specificities, as seen in Table 2.⁹ Thus, the distinct test analyzing methodologies are a likely source of the different results. Other possible reasons why the test results don’t match include pre- or postanalytical error.

What Are Preanalytical Error and Postanalytical Error?

Preanalytical and postanalytical errors are sources of error either before or after running a test that lead to spurious results

Table 3. Determining Sensitivity and Specificity.

Test result	Predetermined diagnosis	
	Disease	No disease
Positive	True positive (TP)	False positive (FP)
Negative	False negative (FN) (Sensitivity) TP TP + FN	True negative (TN) (Specificity) TN TN + FP

(falsely positive, falsely negative, erroneously increased, or decreased values). Preanalytical errors could be from interfering substances—for example, if blood from a sample is grossly hemolyzed (the red blood cells are lysed, spilling their contents into plasma/serum), certain tests will not be accurate, such as potassium, for example, and will be increased significantly, while other analytes, such as cardiac troponin T, will be decreased significantly. Other possible preanalytical errors may include using the wrong collection method, such as a cotton swab instead of a Dacron swab when collecting samples for influenza testing, mislabeling the sample another patient’s name, or using kits past their expiration date.⁹

Postanalytical errors occur after analysis of a sample. Clerical errors may occur if the results are manually transcribed; the testing operator could annotate the wrong result or list the result under the wrong patient, for example.⁹

What Are Sensitivity and Specificity?

The probability that a diagnostic test will give a positive value from a patient with the disease is known as sensitivity. Mathematically, sensitivity is the percentage of true positive values when compared to all tested patients with the disease (see Table 3).⁹ Inversely, the higher the sensitivity, the more accurate a negative value is. Thus, a higher sensitivity test is more effective in ruling out a disease.

Specificity, on the other hand, is the percentage of true negative values compared to all tested patients without the disease in question (Table 3). In other words, the chance that a test will yield a negative value from a patient without the disease is the specificity. The more specific a test is, the more reliable a positive value is; a test with a high specificity is better at ruling in a disease. A positive value is more likely a true positive. This is the basis for the mnemonic SPIN/SNOUT (SPecificity rules IN, SeNsitivity rules OUT).

These values are characteristic of the test in question, not the population; a population’s disease prevalence will not alter the test’s ability to accurately elicit a positive or negative value.⁹ Upon closer review of patient B’s results, the POC test gave her a negative value for influenza B, but the sensitivity of the “kit” POCT for NP swabs is only 62%, meaning that the test has a higher potential for giving a false-negative value when compared to tests with higher sensitivities, like the “analyzer” assay (see Table 2). Patient B’s influenza B infection was not picked

Table 4. Kit Rapid Test Sensitivities and Specificities for Influenza A and Influenza B by Sample Type.

Specimen type	Influenza A sensitivity	Influenza A specificity	Influenza B sensitivity	Influenza B specificity
Nasal swab	94%	90%	70%	97%
Nasopharyngeal swab	83%	89%	62%	98%
Nasal aspirate/nasal wash	77%	99%	82%	99%
Frozen nasal wash	86%	95%	Not evaluated	Not evaluated

up by the POCT, making it a false negative, but was identified by the reference laboratory test.

To generate the sensitivity or specificity for a test, the manufacturer must compare their test's results with a proven method for obtaining a "true" diagnosis (eg, an expensive, invasive procedure such as a bronchoscopy with immunohistochemical-stained lung biopsy, or even a current, Food and Drug Administration–approved test for the same disease). This method is usually the current "gold standard" for ruling in/ruling out the disease in question.

Keep in mind, different sample collection methods can have different sensitivity and specificity profiles (see Table 4).⁴ Upon review of Table 4 for the POCT, the nasal aspirate/wash has a higher sensitivity and specificity for evaluating influenza B. Correlate with clinical findings though influenza A is responsible for more devastating pandemics and epidemics than influenza B and test sensitivity and specificity are worse for nasal washes/aspirates! A better decision might be to update standard operating procedures to collect nasal swabs instead of NP swabs. Looking at Table 4, nasal swabs boast significantly more effective sensitivities for both influenza A and B (94% vs 83% and 70% vs 62%), with a slightly better specificity for influenza A (90% vs. 89%) and slightly worse specificity for influenza B (97% vs 98%).

What Are the Benefits of Point-of-Care Testing Versus Diagnostic Laboratory Testing?

Point-of-care testing is able to give providers a relatively reliable result in minutes at a fraction of the cost (see Table 1). Even so, diagnostic laboratory testing assays from reference laboratories tend to have better sensitivities and specificities when compared to POCT.

Reference laboratory testing can take from days to weeks to even months to obtain a result. One must factor in time for proper specimen labeling, temperature packaging, and storage while waiting for a courier—some may only come once a day, meaning the sample could be delayed in shipment until the following day or even until after a holiday weekend. When the courier brings the sample to a reference laboratory, the sample must be checked by the receiving laboratory for accurate labeling and that it was received within specimen processing requirements (see Table 5).^{2,3,10} If these criteria are not met, the receiving laboratory will reject the sample!

If the sample is correctly labeled, processed, and within proper temperature requirements, the sample will be transferred to the proper section for analysis (for an influenza

Table 5. Reference Laboratory Website Information.

Test: Influenza type A and B RNA, qualitative, real-time reverse transcriptase polymerase chain reaction (PCR)
Preferred specimen(s): Throat swab or nasal/nasopharyngeal swab in 3.5 mL viral transport medium
Collection instructions: Use sterile vials containing 3.5 mL of sterile media. Use only sterile Dacron or Rayon swabs with plastic or wire shafts. Do not use calcium alginate swabs, as they may contain substances that inhibit PCR testing. Break applicator sticks off near the tip to permit tightening of the cap
Transport temperature: Refrigerated (1-8 °C; use cold packs)
Specimen stability: Room temperature (20 °C-25 °C): 48 hours; refrigerated (1 °C -8 °C): 7 days; frozen (below 0 °C): 30 days
Methodology: Real-time reverse transcriptase PCR
Setup schedule: Monday to Friday; report(s) available: next business day

sample, it could be sent to either an immunology/serology section or virology section within the reference laboratory's microbiology department). To maximize efficiency and reduce costs (eg, the cost of reagents needed by the analyzer to run testing), certain uncommon, esoteric laboratory tests are batched (see Table 5 setup schedule). This means that the test may be run only once a day, or even once a week, adding more delays into the test turnaround time.

Once the test is run, the results have to be reviewed and certified by a medical laboratory technician with an appropriate level of medical knowledge/competency. Depending on the test complexity, certification may require an additional reviewer, like the section technical supervisor, a position held by a medical laboratory technologist. Once certified, the test results need to be sent back to the requesting medical treatment facility—depending on laboratory interoperability, the results may be transferred digitally (within minutes), called telephonically (like for critical values that need to be addressed for the sake of life, limb, or eyesight), or faxed.

Can Hospital Laboratories Run More Sensitive/Specific Testing Platforms Than POCT?

Yes, hospital laboratories do run testing platforms that are more sensitive/specific than offered POCT assays. Rapid molecular

assays, for example, have made their way into hospital laboratories and are generally performed when patients meet clinical criteria (usually set forth by the infectious disease service to avoid overuse or inappropriate use of these assays). For example, within 47 to 67 minutes, a 300 μ L (0.3 mL) sample can be analyzed and reliably detect the presence of 17 to 18 different viral pathogens (including influenza A) and 3 to 4 different bacterial pathogens concurrently.¹¹ These molecular assays detect viral RNA/nucleic acids with excellent sensitivity and specificity, but they do not distinguish viable or replicating virus from the presence of viral RNA/nucleic acids.⁷

Teaching Points

- Point-of-care testing is an invaluable tool utilized outside of the laboratory in a patient care environment by nonlaboratory personnel to provide health care providers with testing results in a timely manner to help aid in medical decision-making.
- Reference laboratory testing can give more specific testing results and may need to be ordered even when POCT is performed.
- Preanalytical errors, along with postanalytic errors, can both significantly alter testing results. When in doubt, testing can be repeated with the same sample or a redrawn sample.
- Common preanalytical errors include hemolyzed specimen, wrong collection method, mislabeled specimens, or using expired testing kits.
- Common postanalytical errors include annotating the wrong result or listing the result under the wrong patient.
- Sensitivity of a test refers to the probability that a diagnostic test will give a positive value from a patient with the disease, while specificity of a test refers to the chance that a test will yield a negative value from a patient without the disease.
- Virtually no testing platform is 100% accurate. Sensitivities and specificities calculated for a testing platform using a certain sample type can aid providers in determining which method is best for utilization in their care setting.
- Both POCT and conventional laboratory testing have their advantages and disadvantages, in accuracy, timing, and cost, which must be factored in before ordering and utilizing them.
- Don't treat the laboratory value, treat the patient. Diagnostic laboratory testing is a tool, not an absolute. Treat patients based on all information—history, physical examination, imaging, and laboratory findings, all factor into making an educated decision for the best plan of care.

Author's Note

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