TABLE 87-1 FDA-CLEARED METHODS OF DIRECT TESTING FROM SPECIMENS

TEST METHODS*	DIAGNOSTIC METHOD	ANALYTE DETECTED
Smear stain preparations	Gram stain	Bacteria, yeast
	Fluorescence	DFA: *Pneumocystis jirovecii*, viruses†
		Auramine: mycobacteria
		Calcofluor: fungi
	Special: acid-fast (Kinyoun), partial acid-fast (PAF), India Ink‡	Smear use determined by laboratory and based on primary stained specimen§
	Wright stain	Leukocyte differentiation and count
Antigen-antibody	Latex agglutination	*Legionella* or *Streptococcus pneumoniae* urinary antigen
		Cryptococcal antigen in serum and CSF
	Lateral flow antibody/antigen	GAS, RSV, influenza A or B
	Serology for IgG, IgM, Western blot	Multiple analytes; detection and/or confirmation of immune status and acute disease
	Biomarker	Procalcitonin,‖ C-reactive protein
Molecular¶	Hybridization and signal amplification	HPV, bacterial vaginosis or vaginitis, GAS
	Amplification of RNA or DNA or both	Small bundle with ≤5 targets: sexually transmitted pathogens (GC, CT, TV)
		Multiplex amplification with ≥10 targets: blood sepsis, respiratory, gastrointestinal pathogens
		Chip array: multiple targets, HCV genotyping
	Amplification with quantification of nucleic acids	HIV, HCV, HBV

CSF, Cerebrospinal fluid; CT, *Chlamydia trachomatis*; DFA, direct fluorescent antibody; DNA, deoxyribonucleic acid; FDA, U.S. Food and Drug Administration; GAS, group A streptococci; GC, *Neisseria gonorrhoeae*; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HPV, human papillomavirus; Ig, immunoglobulin; RNA, ribonucleic acid; RSV, respiratory syncytial virus; TV, *Trichomonas vaginalis*

*One-hour, same-day testing.

†Because the direct fluorescent antibody (DFA) is organism specific (e.g., *P. jirovecii*, varicella zoster, herpes simplex 1 or 2, cytomegalovirus), these smears are better than histologic stains (e.g., silver stain) and Tzanck preparations (e.g., nucleated giant cells), which can yield similar appearances for many infectious causes.

‡Cryptococcal antigen from cerebrospinal fluid (CSF) or serum is the recommended test. India ink often yields false-positive results and is used by the laboratory for confirmation of suspected yeast in a Gram stain of CSF.

§For example; partial acid fast testing is performed if the Gram stain shows branching of gram-positive rods and *Nocardia* is suspected; acid fast testing is performed if the auramine-stained sample is positive.

‖Procalcitonin is a better marker for identification of bacterial sepsis than C-reactive protein.

¶Common examples of pathogens are listed for each group, but many more analytes are available.

preparations, which may take days. However, because adequate specimens are often difficult to obtain, sensitivity may be lacking with smear and culture, and alternative methods such as molecular and empirical therapy are still warranted in specific cases.

POINT-OF-CARE OR NEAR-PATIENT TESTING

POC or near-patient testing offers rapid results, typically while the patient is still in the clinical care setting, and it allows directed treatment. However, most tests done in practitioners' offices or on-site laboratories are rapid antigen tests. The predictive values

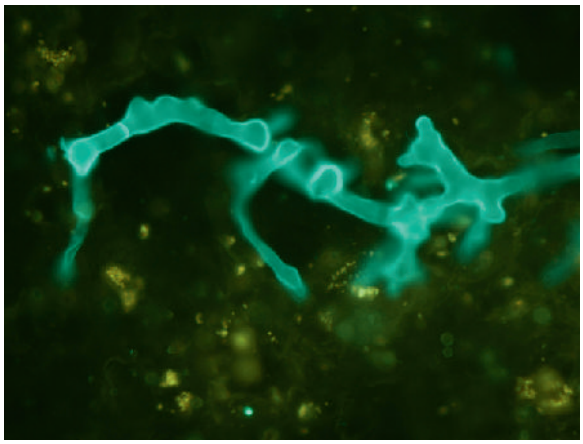

FIGURE 87-1 Calcofluor fluorescent stain depicts fungal hyphae from a wound specimen.

of these tests depend on the specimen type collected (e.g., nasopharyngeal swab is better than a throat swab for influenza A or B testing), the test analyte (e.g., group A streptococci performance is more reliable than influenza A or B), and the prevalence of disease at the time of testing.

False-positive results for rapid antigen tests are unusual, and the patient can be treated based on a positive result. In contrast, a negative test result can be quite misleading. For example, data from the novel H1N1 influenza outbreak demonstrated very poor sensitivity for rapid antigen tests (about 50%) compared with molecular tests. When a multiplex viral panel was used, other viral pathogens were identified as the cause of influenza-like illness in more than 50% of patients admitted to hospitals (Fig. 87-2). Molecular detection of influenza is recommended for hospitalized patients with influenza-like illness if a rapid antigen test result is negative. Many manufacturers have submitted amplified testing assays for influenza, seeking Clinical Laboratory Improvement Amendment (CLIA)–waived status for rapid influenza detection tests that can be performed in the laboratories of physicians' offices or urgent care centers.

Molecular Assays

Molecular identification of infectious diseases has intensified in the past 10 years. The basic categories are shown in Table 87-1.

FDA-cleared direct molecular tests include hybridization and amplification methods. The main difference between these methods is that with hybridization methods, the nucleic acid is not multiplied beyond what is already in the sample. For assays that target DNA, the sensitivity is limited because DNA exists as a single copy. For assays that target proteins or RNA, detection sensitivity is somewhat increased because these components are naturally amplified in the microbe. Familiar hybridization assays include fluorescent in situ hybridization (FISH) for targets in tissue and the protein nucleic acid (PNA) smear (Fig. 87-3). Hybridization assay systems can increase their sensitivity by pairing with signal amplification, such as for human papillomavirus (i.e., Qiagen/Digene HPV test).

In contrast, amplification assays increase the original nucleic acid copy number through a variety of processes, including PCR, transcription-mediated amplification (TMA), and isothermal loop amplification (LAMP). Real-time PCR refers to