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Review

Molecular Methods and Platforms for Infectious Diseases Testing

A Review of FDA-Approved and Cleared Assays

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The superior sensitivity and specificity associated with the use of molecular assays has greatly improved the field of infectious disease diagnostics by providing clinicians with results that are both accurate and rapidly obtained. Herein, we review molecularly based infectious disease diagnostic tests that are Food and Drug Administration approved or cleared and commercially available in the United States as of December 31, 2010. We describe specific assays and their performance, as stated in the Food and Drug Administration's Summary of Safety and Effectiveness Data or the Office of In Vitro Diagnostic Device Evaluation and Safety's decision summaries, product inserts, or peer-reviewed literature. We summarize indications for testing, limitations, and challenges related to implementation in a clinical laboratory setting for a wide variety of common pathogens. The information presented in this review will be particularly useful for laboratories that plan to implement or expand their molecular offerings in the near term. ***(J Mol Diagn 2011, 13:583–604; DOI: 10.1016/j.jmoldx.2011.05.011)***

In 1986, the Food and Drug Administration (FDA) approved the first nucleic acid test, the DNA probe for identification of Legionnaires' disease from bacterial culture, marketed by Gen-Probe Inc. (San Diego, CA).[1] Seven years later, the FDA cleared the AMPLICOR CT test (Roche Molecular Systems, Branchburg, NJ), the first DNA amplification-based test for detection of *Chlamydia trachomatis* (CT) directly from a clinical sample.[2] Since then, the field of clinical molecular testing in infectious diseases has grown enormously; it represents approximately 70% of the global molecular testing market.[3]

The FDA regulates *in vitro* diagnostic devices (IVDs), which include the reagents, systems, and products used in the molecular diagnostic assays as class I, II, or III medical devices, with increasing regulatory oversight, to ensure safety and effectiveness according to the risk

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Disclaimer: The findings and conclusions herein are those of the author (L.W.) and do not necessarily represent the official position of the CDC.

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posed to the patient if the results are incorrect. Several specific guidance documents regarding the classification and review criteria of these tests are available from the FDA Medical Devices website (*http://www.fda.gov/MedicalDevices/default.htm*, last accessed December 31, 2010). The FDA also determines test complexity as high, moderate, or waived, with most molecular IVDs being designated as high-complexity tests. The term FDA cleared is used for assays that are routed by a 510(k), submission showing substantial equivalence to any assay already cleared by the FDA or marketed before 1976. The term FDA approved is used when an assay is routed by a premarket approval application to demonstrate its efficacy and safety. A searchable database of FDA-approved or FDA-cleared assays can be accessed (*http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMN/pmn.cfm*, last accessed December 31, 2010), and an updated list of FDA-cleared assays is available at the Association for Molecular Pathology website (*http://www.amp.org/FDATable/FDATable.doc*, last accessed December 31, 2010).

Molecular infectious disease (MID) testing offers several advantages, including rapid test results facilitating detection of outbreaks and, in some cases, newly emerging strains; and sensitivity, specificity, identification of resistant organisms, and quantifiable correlation to disease severity, all of which contribute to timely therapeutic clinical decisions and early infection control interventions.[4] Multiplex methods can simultaneously detect multiple infectious agents in a single clinical specimen. In addition, these methods are able to identify organisms that may be difficult to isolate or have not been cultured by traditional methods. Assays that provide sequence or genotype can trigger collection of epidemiological information, track disease outbreaks, provide strain resistance data and/or treatment prognosis, and determine the method or source and means of spread of infection.

This review complements a new Clinical and Laboratory Standards Institute guideline, MM19: Establishing Molecular Testing in Clinical Laboratory Environments, under review at the time of this article's submission (Leslie Hall, personal communication in June 2011). The assays described were selected by reviews of several databases, including the FDA database, the Association for Molecular Pathology website, and the PubMed database for publications related to MIDs. Although we have attempted to provide a comprehensive review of commercially available FDA-cleared or FDA-approved IVDs and platforms, we do not endorse or promote any one of these over the other. Our review is limited to FDA-cleared or FDA-approved assays available in the US market as of December 31, 2010. Esoteric assays, such as those for bioterrorism agents and emergency use, are beyond the scope of this review. However, additional information can be obtained at the CDC and FDA websites (*http://www.selectagents.gov/index.html* and *http://www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm*, respectively; last accessed December 31, 2010).

Specific Considerations for Commercially Available MID Assays

We focus on commonly used assays and relevant information to assist the molecular laboratory director in assay selection and implementation. Assays available for diagnosis and treatment are presented herein in four groups: sexually transmitted diseases (STDs) (Table 1), health care–associated infections (HAIs) and surveillance (Table 2), respiratory tract and central nervous system (CNS, Washington, DC) infections (Table 3), and other infections and culture confirmations [eg, hepatitis B virus (HBV), various cultures, and ancillary assays for HAI and surveillance] (Table 4). For each area, we highlight several MID assays; and reference to additional FDA-cleared assays is found in Tables 1–4.

During the FDA IVD review process, each MID assay described in the specific product insert is approved or cleared for a specific patient population, specimen type, and extraction method. If a laboratory chooses to deviate from the product insert (eg, to offer a specimen type that is not described in the product insert), the modified assay is considered off-label use and the laboratory is obligated to perform a thorough validation to ensure that the modification does not alter performance claims. We present assay sensitivity, specificity, limit of detection, dynamic range, and percentage positive or negative agreement with culture results, as applicable.

It is out of the scope of this article to describe the actual process of implementation of MID testing; however, there are several excellent recent references[5,6] to assist the laboratory.

STD Data

HPV Data

More than 100 genotypes of human papilloma virus (HPV) (Table 1) have been identified based on DNA sequence heterogeneity, and >40 of these infect anogenital or ororespiratory tracts. HPV genotypes have been divided into four oncogenic risk classes: low, intermediate/high, high, and unknown. The World Health Organization's International Agency for Research on Cancer (IARC), in 2009, classified HPV-16, HPV-18, HPV-31, HPV-33, HPV-35, HPV-39, HPV-45, HPV-51, HPV-52, HPV-56, HPV-58, and HPV-59 as high-risk (hr) HPV types with sufficient evidence for causing cervical cancer, with special emphasis on HPV-16 as being the most aggressive type.[7] Periodically, the IARC has revised its listing of hr-HPV, and the components of the list have varied.

One prevailing viewpoint finds HPV testing differs from other molecular assays in that analytic sensitivity for detection of HPV is not the prime driver of assay performance. A high analytic sensitivity can decrease the clinical specificity, resulting in more false referrals for colposcopy and biopsy, decreased correlation with histological presence of disease, and a consequent distrust of a positive result by the treating physician.[8] This approach was further emphasized by Meijer et al,[9] who set

forth requirements for a candidate HPV screening molecular assay. A historical review of the use of HPV testing in the screening and management of abnormal cervical screening results is provided in a recent article by Cox,[10] and we recommend reading this as part of a molecular laboratory director's preparation for the introduction of HPV testing. Although some commercially available HPV assays target both low-risk and hr-HPV types, we limit our discussion to the application of assays detecting hr-HPV (Table 1 contains a complete listing).

The Digene Hybrid Capture 2 (HC2) HPV DNA Test (Qiagen, Gaithersburg, MD) has been the most widely used molecular HPV assay in most clinical trials and has been extensively reviewed.[11] This assay is FDA approved for triage in cases of equivocal cytology results in the presence of atypical squamous cells of undetermined significance to determine which patients should be referred for a colposcopy and also as a screening test for use in addition to cytology in women ≥30 years. The National Cancer Institute–sponsored clinical trial of Atypical Squamous Cells of Undetermined Significance and Low-Grade Squamous Intraepithelial Lesion Triage Study and the Canadian Cervical Cancer Screening Trial demonstrated the assay's greater accuracy compared with cytology alone in detecting histologically confirmed cervical intraepithelial neoplasia 2/3 lesions of the cervix.[12] However, Kitchener et al[13] found that liquid-based cytology appears to be closing this gap and may have an improved sensitivity over conventional cytology.

The HC2 assay uses unlabeled single-stranded full-genomic-length RNA probes specific for all of the types recommended by IARC-2009 with the addition of HPV-68. The RNA:DNA hybrids are detected by a microplate chemiluminescent signal amplification method. The use of full-genome probes prevents false negatives resulting from gene deletions. The HC2 assay lacks an internal control to evaluate sample adequacy or the presence of interfering substances. This assay has a false-positive rate of 7.8% for detection of hr-HPV because of the cross-reactivity with many untargeted low-risk HPV types.[14] In an attempt to demarcate these cross-reactive false positives, which usually show a weak reaction/signal, several groups recommended a readjustment of the cutoff value or retesting of initial borderline samples. In 2006, the concept of an HC2 gray zone was introduced with the recommendation of retesting repeatedly borderline samples (relative light unit per cutoff of 1.0 to 2.5) by a different HPV assay having a high analytic specificity. The manufacturer subsequently changed (only slightly) the criteria for interpretation of positive results, restricted to samples collected in ThinPrep PreservCyt solution (Hologic Inc., Bedford, MA) and not applicable to those collected in the Digene Specimen Transport Medium (Qiagen Inc., Valencia, CA). The Digene-recommended algorithm for low-positive HPV results (gray or retest zone) required a retest followed by a second retest if the result of the first retest was <1.0 relative light unit per cutoff. This algorithm was evaluated by Muldrew et al,[15] who showed that although retesting of an initial gray zone sample was necessary, a second retest did not offer advantages over the first retest. Another group[16] showed that increasing the HC2 positive cutoff value to 2.0 relative light unit would improve clinical specificity, with only a minimal reduction in clinical sensitivity. However, these recommendations are not part of the FDA approval of this assay.

The Cervista HPV HR (Hologic, Inc., Bedford, MA) assay uses manual extraction with a single-well Invader Biplex technology format that simultaneously detects HPV and a Histone H2be DNA internal control in the same reaction. The assay is an isothermal signal amplification method using Invader chemistry. The probe pools detect 14 HPV types (IARC-2009 12 hr-HPV plus HPV-66 and HPV-68) and identify type-specific single-nucleotide polymorphisms, effectively decreasing cross-reactivity with low-risk types and false-positive results. The assay only requires 2.0 mL of sample (half the requirement of Digene HC2). The inclusion of an internal control is a quality control measure that differentiates between a true negative and a sample with insufficient DNA present and is also a verification of the procedure. The same sample specimen used in the Cervista HPV HR may then be reflexed to the Cervista HPV 16/18 genotyping assay, which specifically identifies the presence of HPV types 16 and 18, now implicated in approximately 70% of cases progressing to cancer.[17,18]

In a postapproval clinical study (SHENCCAST II, conducted in China) comparing the HC2 with the Cervista hr-HPV assays, the HC2 showed better sensitivity (95.6% versus 92.9%), whereas the Cervista assay demonstrated a statistically significantly higher specificity (91.1% versus 88.6%; $P < 0.05$).[19]

HPV testing is performed predominantly on liquid-based cytology samples, and sample collection is determined by the method in use. The HC2 assay has been validated for use with the Digene Specimen Transport Medium and the ThinPrep PreservCyt solution. Use of other collection media (eg, SurePath liquid cytology medium) is considered unapproved off-label use. The Cervista assay has been validated for use with the PreservCyt solution. The typical turnaround time is 1 to 3 days, depending on the platform and availability of automation.

In addition to molecular assays for the detection of HPV, the FDA has also approved the Cervista HPV 16/18 genotyping assay, briefly mentioned earlier (Hologic, Inc.). This assay is based on the same Invader technology as the Cervista hr-HPV detection test and, as indicated by its name, specifically detects and distinguishes HPV types 16 and 18. For cytology-negative, hr-HPV–positive women, HPV 16/18 genotyping can be used to determine who should be referred for immediate colposcopy. If the HPV 16/18 genotyping test result is negative, then cytology and hr-HPV testing are recommended to be repeated in 12 months. The American Society for Colposcopy and Cervical Pathology Consensus Conference Recommendations for HPV 16/18 detection do not recommend the use of HPV genotyping in women with atypical squamous cells of undetermined significance who test positive for hr-HPV. Alternatively, the American Society for Colposcopy and Cervical Pathology recommends that these women are referred to colposcopy (American Society for Colposcopy and Cervical Pathol-

Table 1. STD Assays

Organism	Specimen/sample type	Assay/platform (FDA no.)
HPV	Cervical cytology specimen in ThinPrep PreservCyt and cervical cytology specimen in Specimen Transport Medium	Digene HC2 HPV DNA Test (P890064/S006)†
	Cervical cytology specimen in ThinPrep PreservCyt and cervical cytology specimen in Specimen Transport Medium	Digene HC2 High-Risk HPV DNA typing kit (P890064/S009)†
	Cervical cytology specimen in ThinPrep PreservCyt	Cervista HPV HR (P080014)¶
	Cervical cytology specimen in ThinPrep PreservCyt	Cervista HPV 16/18 (P080015)¶
HSV	Vaginal lesion swab only (not for prenatal screening or in females <18 years); collected in Copan Universal Transport Medium or identical Copan- manufactured media formulation; an extractable sample processing control target is added to the specimen before lysis	MultiCode RTx HSV 1&2 Kit (K100336)††
		MagNA Pure LC Total NA Kit and Magna Pure Instrument LightCycler 1.2‡‡
CT	Endocervical (female) and urethral (male) swabs; urine specimens (male and female); gynecological specimens collected in BD SurePath preservative fluid	BD ProbeTec CT Q^x Amplified DNA Assay (K090824, K091724, K081824)§§
	Endocervical and vaginal (female) and urethral (male) swabs; urine specimens (male and female); patient-collected vaginal swab specimen is also accepted	APTIMA Assay for *Chlamydia trachomatis* (K043072, K053446, K061413, K063451)¶¶
	Specimen that has been processed and tested positive by PACE 2 system for CT	PACE 2 CT (K920302)¶¶
	Digene Cervical Sampler or Dacron swab placed in Digene Specimen Transport Medium	Digene HC2 CT-ID DNA Test (K990023, K010892)†
	Endocervical (female) and urethral (male) swabs; urine specimens (male and female)	COBAS AMPLICOR (CT/NG) Test (K973707, K973718, K070174, K053287)‡‡
NG	Cervical and vaginal (female) and urethral (male) swabs; urine (male and female); PreservCyt specimens; Surepath specimens	BD ProbeTec GC Q^x Amplified DNA Assay (K081825, K090971)§§
NG	Vaginal and cervical swabs; urethral swabs (male); urine (male and female); PreservCyt specimen	APTIMA Assay for NG (K043144, K061509, K062440, K063664)¶¶
	Specimen that has been processed and tested positive by PACE 2 system for NG	PACE 2 NG (K920301)¶¶
	Cervical specimens collected using Digene Cervical Sampler and Digene Swab Specimen Collection kit	Digene HC2 GC-ID DNA Test (K981485, K010893)†
	Cervical (female) and urethral (male) swabs; urine (male)	AMPLICOR CT-NG for NG (K974503, K974342, K070172, K053289)‡‡
CT-NG combined	Vaginal (female) and urethral (male) swabs; urine (male and female); patient-collected vaginal swab specimen	RealTi*me* CT-NG assay (K092704, K080739)***
	Endocervical (female) and urethral (male) swabs; urine specimens (male and female)	BD ProbeTec ET CT-GC (K012351)§§
CT-NG combined	Endocervical (female) and urethral (male) swabs; urine specimens (male and female)	APTIMA COMBO 2 (K003395, K022874, K032554, K043224, K060652)¶¶
	Endocervical (female) and urethral (male) swab specimens collected with the PACE Specimen Collection kits	PACE 2C CT-NG (K940979)¶¶
	Cervical specimens collected using *Digene* HC2 DNA Collection Device or *Digene* Female Swab Specimen Collection Kit	Digene HC2 CT/GC Dual DNA Test (K981567, K010891)†,†††
HIV-1	Human plasma specimens (ACD-A and EDTA)	RealTi*me* HIV-1 assay (BP060002)*** and m2000 (m2000sp + 2000rt)

(*table continues*)

Table 1. *Continued*

Method	Target	Linear range (%)*	
		Sensitivity	Specificity
Hybridization protection assay with signal amplification using microplate chemiluminescence hybrid capture of RNA-DNA hybrid	RNA probe cocktail for 13 hr types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) and 5 low-risk types (6, 11, 42, 43, and 44)	93.0‡	61.1
Hybridization protection assay with signal amplification using microplate chemiluminescence hybrid capture of RNA-DNA hybrid	RNA probe cocktail to detect 13 hr-HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68)§	93.0‡	61.1
Invader technology	E6/E7/L1; cocktail of 14 hr-HPV DNA probes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68)‖; human histone 2 gene (internal control)	92.8	67.2 (aged ≥30 years)
Invader technology	Cocktail of two types of sequence-specific DNA probes**; human histone 2 gene (internal control)	PPA, 85.7 (65.4–95.0)	NPA, 95.9 (94.9–96.7)
Real-time PCR using isoC:isoG synthetic DNA bp technology	*Glycoprotein* gene segment of HSV-1 and HSV-2	HSV-1, 92.4	HSV-1, 98.3
		HSV-2, 95.2	HSV-2, 93.6
Strand displacement amplification	Alternate region of the cryptic plasmid	94.5	98.9
Transcription-mediated amplification	23S rRNA	95.6	98.8
Probe competition assay; CT confirmation test	23S rRNA	91.7	98.5
Hybrid capture	RNA probe cocktail complementary to CT genomic DNA; cryptic plasmid	92.3–97.7‖‖	98.2–98.6‖‖
PCR	Cryptic plasmid	92.9–94.1	94.7–98.4
Strand displacement amplification	*Pilin* gene	99.3	99.4
Transcription-mediated amplification	16S rRNA	92.3	99.8
Probe competition assay; GC confirmation test	rRNA	95.1	98.7
Hybrid capture	RNA probe cocktail complementary 0.5% of the NG genome, cryptic plasmid	92.6–95.2‖‖	98.5–98.9‖‖
PCR	*M.NgoPII* putative methyl transferase gene of NG	95.9–96.5	98.7–97.3
Real-time PCR	CT, cryptic plasmid NG, *Opa* gene	CT, 92.5–97.8 NG, 87.0–100	CT, 98.3–99.8 NG, 99.3–100
Strand displacement amplification	CT, cryptic plasmid GC, *Pilin* gene	CT, 92.0 GC, 96.1	CT, 94.9 GC, 98.2
Transcription-mediated amplification	CT, 23S rRNA NG, 16S rRNA	CT, 95.2–96.5 NG, 96.5–99.1	CT, 97.6–98.7 NG, 98.4–99.4
Probe competition assay	rRNA	CT-NG, 96.8	CT-NG, 93.6
Hybrid capture	CT-GC RNA probe cocktail	CT, 96.1 GC, 93.0	CT, 98.7 GC, 99.1
Real-time RT-PCR	Integrase region in *pol* gene	40–10,000,000 copies/mL (HIV-1 groups M, N, and O)	

(*table continues*)

Table 1. *Continued*

Organism	Specimen/sample type	Assay/platform (FDA no.)
	Plasma extracted from blood collected using EDTA or ACD‡‡‡	AMPLICOR HIV-1 MONITOR test, version 1.5 (BP950005/4)‡‡ and COBAS AMPLICOR HIV-1 MONITOR test, version 1.5 (BP950005)‡‡
	Plasma separated from blood collected using EDTA	COBAS AmpliPrep/COBAS TaqMan HIV-1 test (BP050069/0)‡‡ and COBAS AmpliPrep/COBAS TaqMan‡‡
HIV-1	Plasma extracted from blood collected using EDTA or ACD‡‡‡	VERSANT HIV-1 RNA 3.0 Assay (bDNA) (BP000028/0)§§§ and VERSANT Molecular System§§§
	Plasma or serum	APTIMA HIV RNA Qualitative Assay (BL103966/5040)¶¶
	Plasma or serum	Procleix ULTRIO Assay¶¶ Procleix HIV-1, HCV, and/or HBV Discriminatory Assays (BL 125113/33)¶¶
	Plasma or serum	COBAS AmpliScreen HIV-1 Test (BL 125059/37)‡‡
HIV-1 drug resistance¶¶¶	Plasma samples from blood collected in EDTA	ViroSeq HIV-1 Genotyping System (BK030033)‖‖‖ and ABI 3100/3130 capillary electrophoresis platform****
	Plasma samples from blood collected using ACD or EDTA anticoagulants	TRUGENE *HIV-1* Genotyping Kit (BK090077)§§§ and OpenGene DNA Sequencing System§§§

(*table continues*)

*Sensitivity, specificity, linear range, and percentage positive or negative agreement with culture data are sourced from FDA submission material or product inserts.
†Obtained from Qiagen, Gaithersburg, MD.
‡Atypical squamous cells of undetermined significance referral Papanicolaou stain population, Kaiser Study, PreservCyt solution specimens.
§Cross-reacts with HPV types 40, 53, and 66.
¶Obtained from Hologic, Madison, WI.
‖Cross-reacts with HPV types 67 and 70.
**Cross-reacts with high levels of HPV type 31.
††Obtained from EraGen Biosciences, Madison, WI.
‡‡Obtained from Roche Molecular Diagnostics, Pleasanton, CA.
§§Obtained from BD Diagnostics, Sparks, MD.
¶¶Obtained from Gen-Probe, Inc., San Diego, CA.
‖‖‖Depending on brush or swab specimens.
***Obtained from Abbott Molecular, Inc., Des Plaines, IL.
†††This assay is indicated for use as an initial test and requires confirmation with the individual MID assays.
‡‡‡ACD specimens will yield approximately 15% lower test results because of the dilution effect of 1.5 mL ACD in the collection tube.
§§§Obtained from Siemens Healthcare Diagnostics, Deerfield, IL.
¶¶¶Provides information on resistance to nucleoside and nonnucleoside reverse transcriptase and protease inhibitors.
‖‖‖‖Obtained from Celera Diagnostics, Alameda, CA.
****Obtained from Applied Biosystems, Foster City, CA.
ACD-A, anticoagulant citrate dextrose solution A; bDNA, branched DNA; NPA, negative predictive accuracy; PPA, positive predictive accuracy.

ogy, HPV Genotyping Clinical Update, *http://www.asccp.org/pdfs/consensus/clinical_update_20090408.pdf*, last accessed December 31, 2010).

CT and NG Data

CT and *Neisseria gonorrhoeae* (NG) are the most common cause of bacterial STDs, and both can cause urogenital tract infections ranging from acute to asymptomatic disease. CT is an obligate intracellular bacterium comprising 15 serovars, whereas NG is a fastidious intracellular diplococcus. Significant underreporting of disease can occur as the result of silent infections affecting the reproductive age group. Identification and treatment is important to prevent the sequelae of infection, such as infertility, chronic pain, and pelvic inflammatory disease.

Urogenital specimens commonly exhibit amplification inhibition. The inhibitory substances can be removed by including nucleic acid purification steps in the sample preparation. The sample preparation protocols vary among the commercially available assays, ranging from the use of crude lysates (AMPLICOR) to purified nucleic acids. The Roche AMPLICOR assay uses an amplification control in the sample that allows for detection of inhibitory substances. This control consists of a plasmid-containing CT primer binding sites and a randomized internal sequence. The BD ProbeTec (BD Diagnostics, Sparks, MD) uses 1000 copies of a linearized NG DNA containing plasmid as the internal amplification control.

Commercially available assays for CT and NG (Table 1) use target amplification methods, with the one exception being the Digene HC2 assay, which uses a signal amplification method with an RNA probe cocktail complementary to approximately 39,300 bp (4%) of the *Chlamydia* genomic DNA and one probe complementary to 100% of the cryptic plasmid. Nucleic acid amplification

Table 1. *Continued*

Method	Target	Linear range (%)*	
		Sensitivity	Specificity
End point RT-PCR	142 bp in highly conserved region of *Gag* gene	Standard, 400–750,0000 copies/mL; ultrasensitive, 50–100,000 copies/mL (HIV-1 group M)	
Real-time RT-PCR	*Gag* gene	48–10,000,000 copies/mL (HIV-1 group M)	
bDNA technology	*Pol* gene	75–500,000 copies/mL (HIV-1 groups M and O)	
Transcription-mediated amplification	Highly conserved regions of HIV-1 RNA	100	99.83
Transcription-mediated amplification	Highly conserved regions of HIV-1 RNA, HCV RNA, and HBV DNA	100 (ULTRIO) 100 (HIV-1 discriminatory)	99.5 (ULTRIO) 99.7–100 (HIV-1 discriminatory)
RT-PCR	*Gag* gene	96.5–98	98.9–99.7
RT-PCR, population sequence analysis	HIV-1 subtype B *protease* gene and partial sequence of the reverse transcriptase regions of the *pol* gene	Validated for detection of drug-resistance mutations in 40 of 60 mutant/wild-type mixture samples with a viral load range of 2000–750,000 copies/mL	
RT-PCR, population sequence analysis	*protease* gene and part of the reverse transcriptase regions	Requires samples with viral loads ≥1000 copies/mL	

testing assays typically increase sensitivity by targeting multiple copy genes or plasmids. For CT, the targets include cryptic plasmid DNA present in nearly all serovars (5 to 10 copies), genes such as *omp1*, and ribosomal RNA (rRNA; 16S and 23S). For NG, targets include the cytosine methyl transferase gene (*M.NgoPII),* the *Opa* gene, *Piv-1* genes, and 16S and 23S rRNA. The specimen type approved for CT and NG testing is assay specific (Table 1) and includes urethral swab and urine for males and endocervical/cervical samples, vaginal swabs, urine, and PreservCyt (Hologic, Inc.) specimens for females. A male first-void urine specimen and vaginal swabs are considered optimal specimens, according to the Association of Public Health Laboratories (*http://www.aphl.org/aphlprograms/infectious/std/documents/ctgclabguidelinesmeetingreport.pdf*, last accessed December 31, 2010).

The AMPLICOR NG detection kit (Roche Molecular Diagnostics, Pleasanton, CA) targets the *M.NgoPII* gene, whereas the BD ProbeTec ET CT/GC targets the cryptic plasmid of CT and the *Piv-1* gene of NG. Both of these assays have cross-reactivity with some *Neisseria* species. Confirmatory testing using a different gene target is an option in such instances. Cross-reactivity has not been reported for the Real Time CT/NG (Abbott Laboratories, Des Plaines, IL), APTIMA COMBO 2 (Gen-Probe Inc., San Diego, CA), and PACE 2 (Gen-Probe Inc.) assays.[20]

Coinfection with CT and NG occurs in many patients. Simultaneous detection of both organisms in a single test is achieved by several assays (Table 1). The APTIMA COMBO 2 is a second-generation assay that uses target capture with transcription-mediated amplification and chemiluminescent hybridization protection. In contrast to PCR and strand displacement assays, which amplify

Table 2. HAI Assays

Organism	Specimen/sample type	Assay/platform (FDA no.)	Method	Target	Sensitivity (%)*	Specificity (%)*
MRSA/SA (screening, surveillance)	Lesion (skin/soft tissue) double swab	Xpert MRSA/SA SSTI (K080837)† and GeneXpert System†	Real-time PCR	Staphylococcal protein A *(spa),* the gene for *MecA*-mediated oxacillin resistance (*mecA*), and SCC*mec* inserted in the SA chromosomal *attB* site	MRSA % positive agreement with culture, 93.8; SA % positive agreement with culture, 95.7	MRSA % negative agreement with culture, 97.3; SA % negative agreement with culture, 89.5
	Nasal swabs (surveillance)	Xpert MRSA (K070462)† and GeneXpert System†	Real-time PCR	SCC inserted into the SA chromosomal *attB* site	% positive agreement, 80.9–90.6	% negative agreement, 92.3–96.3
	Nasal swabs (surveillance)	BD GeneOhm MRSA ACP (K093346)‡ and SmartCycler†	Real-time PCR	SCC*mec-orfX*junction area of the SCC*mec*adjacent to the integration site MREJ	92.00	94.6
	Nasal swabs (presurgical screen)	Xpert SA Nasal Complete (K100822)† and GeneXpert System†	Real-time PCR	SCC inserted into the SA chromosomal *attB* site	% positive agreement, 80.9–90.6	% negative agreement, 92.3–96.3
	Nasal swabs (surveillance)	LightCycler MRSA Advanced test (K091409)§ and LightCycler 2.0	Real-time PCR	Sequence incorporating the insertion site of the SCC*mec* in the SA *orfX* gene	% positive agreement with direct chromogenic culture, 95.2	% negative agreement with direct chromogenic culture, 96.4
Enterococcus	Rectal swab (surveillance)	Xpert *vanA* (K092953)† and GeneXpert System†	Real-time PCR	Gene sequences for *VanA*-encoded resistance to vancomycin-teicoplanin	98–99	81–90
C. difficile	Unformed (liquid or soft) stools	Xpert *C. difficile* (K091109)† and GeneXpert System†	Real-time PCR	*C. difficile tcd*B	93.50	94.0
		Prodesse ProGastro Cd (K090239)¶	Multiplex real-time PCR	*C. difficile tcd*B found only in toxigenic strains	91.7	94.7
		BD GeneOhm Cdiff Assay (K081920)‡	Real-time PCR	*C. difficile tcd*B	93.8	95.5
		Illumigene *C. difficile* (K091109)‖	LAMP technology	*C. difficile tcd*A of the PaLoc	95.2	95.3

LAMP, loop-mediated amplification; PaLoc, pathogenicity locus; SCC, staphylococcal cassette chromosome.
*Sensitivity, specificity, and percentage positive or negative agreement with culture data are sourced from FDA submission material or product inserts.
†Obtained from Cepheid, Sunnyvale, CA.
‡Obtained from BD Diagnostics-Infectious Diseases, LaJolla, CA.
§Obtained from Roche Molecular Diagnostics, Pleasanton, CA.
¶Obtained from Gen-Probe Prodesse, Inc., Waukesha, WI.
‖Obtained from Meridian Bioscience, Inc., Cincinnati, OH.

bacterial DNA, transcription-mediated amplification amplifies specific regions of the 23S rRNA/16S rRNA. The APTIMA COMBO 2 assay does not have an internal control; however, it uses target capture technology, which removes inhibitors. The assay is a dual kinetic assay, with one signal scoring for CT and the second signal scoring for NG. Assays that target bacterial rRNA rather than plasmid DNA have a greater ability to detect lower concentrations of organisms because of the presence of up to a 1000-fold greater amount of RNA than plasmid DNA in the infected cell.

Cryptic plasmid–based detection assays could yield false-negative results because CT strains without the plasmid or with deletions in the plasmid have been described (the Swedish variant, with a 377-bp fragment deletion). False-negative results have been reported for

the Roche AMPLICOR and the Abbott RealTi*m*e CT/NG assays that target the deleted region, whereas those assays that target outside of this region or the chromosomal regions detect the mutant strain.[21] The BD ProbeTec assay is able to detect the CT Swedish variant because the cryptic plasmid target is outside the area of deletion. The newer Abbott RealTi*m*e CT/NG assay, FDA cleared in June 2010, includes an additional 140-bp cryptic plasmid target outside of the 377-bp deletion area. The Abbott RealTi*m*e CT/NG assay also contains a small fragment of noninfectious linearized DNA plasmid for use as an internal control throughout the sample preparation process.

A low prevalence of STDs in a specific population may reduce the positive predictive value of the molecular result. However, the test can be repeated with a separate aliquot of the same specimen or a second specimen and a different test method and/or a different target to confirm the positive result.[20] The efficacy of this strategy is debatable.[22]

HIV-1 Assay

Qualitative assays, such as the Procleix ULTRIO and Discriminatory HIV-1/HCV/HBV assays (Gen-Probe Inc.) and the COBAS AmpliScreen HIV-1 test (Roche Molecular Systems, Pleasanton), are available for donor screening applications. The transcription-mediated amplification–based APTIMA HIV-1 RNA qualitative assay (Gen-Probe Inc.) can be used for diagnosing acute and primary infections and can detect infection before seroconversion and confirm infections in individuals when antibody test results are positive.[23,24] However, in this segment, we will focus on the quantitative and genotyping assays that are the main HIV-1–related assays performed in the Molecular Diagnostics Laboratory.

HIV-1 viral load assays are important for monitoring HIV-1–infected individuals, predicting the progression of HIV disease, and monitoring antiretroviral treatment.[25,26] HIV-1 is classified into three major groups (ie, M, N, and O). Group M is the most prevalent and is sub-classified into seven subtypes (ie, A–D and F–H) that are geographically distinct. Several commercial kits are available for quantitative determination of HIV viral load to assess patient prognosis during antiretroviral therapy (Table 1). Ascertaining the viral load is a prerequisite to initiating drug therapy in adherence to FDA guidelines and serves to evaluate the efficacy of antiretroviral therapy (*http://www.aidsinfo.nih.gov/guidelines*, last accessed December 31, 2010). In 1996, the AMPLICOR HIV-1 MONITOR test (Roche Molecular Systems) was the first FDA-approved quantitative HIV end point–based RT-PCR assay. Quantification of HIV-1 RNA copy number is determined by comparing optical density readings of the HIV-1 signal with an external quantitation standard signal, which has a known copy number input. However, a small dynamic range of 400 to 750,000 copies/mL (or 50 to 100,000 copies/mL for the ultrasensitive method) limits the assay. Recently, real-time RT-PCR HIV assays with options of automation, closed-system platform characteristics, broad dynamic range, and good specificity have become commercially available.[27] By using the COBAS AmpliPrep/COBAS TaqMan HIV-1 Test (Roche Molecular Systems), patient specimens are extracted on the COBAS AmpliPrep instrument and amplification/detection occurs on the COBAS TaqMan Analyzer. The assay targets the conserved region in the *gag* gene, which is prone to a high level of mutation and is only intended for the detection of group M subtypes of HIV-1. Calibration is not required because specific calibration values and predefined assay control ranges are included with each kit and because uracil-*N*-glycosylase reduces the risk of carryover contamination. Initial underestimations regarding quantification of HIV-1 group M non-B subtypes, when compared with the COBAS AMPLICOR HIV-1 MONITOR Version 1.5 and the Abbott RealTi*m*e HIV-1 assay, have been reported.[28–32] A second version of the COBAS AmpliPrep/COBAS TaqMan HIV-1 Test version 2.0 (Roche Molecular Systems) was recently approved. This assay improves the underquantification and subtype inclusivity issues present with the original assay. The version 2.0 assay uses a two-target approach with the combination of the new *ltr* primer-probe set with the original *gag* primer-probe set to detect the various group M HIV-1 subtypes A–D and F–H and group O. The assay has a quantitation range of 20 to 10^7 RNA copies/mL.[33,34] With the Abbott RealTi*m*e HIV-1 kit (Abbott Molecular, Inc., Abbott Park, IL), patient specimens are extracted on the m2000sp instrument and detected on the m2000rt instrument. The assay allows for flexibility in the sample input volumes and detects both group M and group O HIV-1 subtypes. In contrast to these real-time PCR-based assays, the VERSANT HIV-1 RNA 3.0 Assay (Siemens Health Care Diagnostics, Deerfield, IL) uses branched DNA chemistry, which relies on signal amplification. The assay has a lower risk of contamination because of the lack of amplicon production, and the VERSANT HIV-1 RNA 3.0 Assay has been validated for samples containing group M subtypes A–G (in 2002, subtype E was still believed to be a true subtype); however, it has decreased sensitivity compared with target amplification assays.[35,36]

During treatment of HIV infections, mutant HIV-1 strains emerge that are resistant to one or more drugs.[37] The identification of viral resistance genotypes allows treatment strategies to be modified.[38] Retrospective and prospective intervention-based studies[38–41] have provided evidence supporting the clinical utility of genotype testing for resistance, and this is recommended by the International AIDS Society–USA panel for selecting new regimens after treatment failure and monitoring therapy for pregnant women. Genotype testing should also be considered before initiation of therapy for acute infections and for treatment-naïve patients with established infection.[38]

Two genotyping systems are commercially available: TRUGENE HIV-1 Genotyping Kit (Siemens Diagnostics, Tarrytown, NY) and ViroSeq HIV-1 Genotyping Systems (Celera Diagnostics, Alameda, CA). The ViroSeq kit provides reagents for viral RNA isolation from plasma, and both kits provide reagents for RT-PCR and sequencing.[39,40,42] Typically, the entire protease and the 5′ reverse transcription coding regions of the *pol* gene are

Table 3. Respiratory Tract and CNS Infection Assays

Organism	Specimen/sample type	Platform/assay (FDA no.)
Respiratory virus panel: influenza A/H1 and A/H3; influenza B; adenovirus; RSV; metapneumovirus (hMPV); parainfluenza (hPIV) 1, 2, and 3; and rhinovirus	Nasopharyngeal swab	*xTAG* Respiratory Viral Panel (K063765)†
Respiratory virus panel: influenza A and B and RSV	Nasopharyngeal swab, cultured clinical specimens	Verigene Respiratory Virus Nucleic Acid Test (K083088)‡ and Verigene Respiratory Virus Nucleic Acid Test on the Verigene SP system (K092566)‡
	Nasopharyngeal swab	ProFlu+ (K073029, K081030, K092500)§ and SmartCycler¶
Respiratory virus panel: influenza A and B and RSV	Nasopharyngeal swab	Simplexa Flu A/B and RSV (K102170)‖; MagNA Pure LC Instrument and the MagNA Pure Total Nucleic Acid Isolation Kit** or a Qiagen QIAamp Viral RNA Mini Kit†† and 3M Integrated Cycler‡‡
Respiratory virus: influenza A/H1N1-2009	Nasopharyngeal swabs, nasal swabs, NPAs	Simplexa Influenza A H1N1 (2009) (K100148)‖; MagNA Pure LC Instrument and the MagNA Pure Total Nucleic Acid Isolation Kit** or a Qiagen QIAamp Viral RNA Mini Kit†† and 3M Integrated Cycler‡‡
Respiratory virus: influenza A/H1N1-2009	Nasopharyngeal swabs and nasal swabs	CDC Human Influenza Virus Real-Time RT-PCR Detection and Characterization Panel (K101564)§§ and Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument¶¶
Respiratory virus panel: seasonal influenza A/H1, A/H3, and A/H1N1-2009	Nasopharyngeal swab	ProFAST+ (K101855)§ and SmartCycler¶
Respiratory virus: influenza A/H5 (Asian lineage)	Nasopharyngeal and throat swabs	JBAIDS Influenza A subtype A/H5 (Asian lineage) (K100287)‖‖ and JBAIDS Instrument
Respiratory virus: hPIV	Nasopharyngeal swab	ProParaFlu+ (K091053)§ and SmartCycler¶
Respiratory virus: hMPV	Nasopharyngeal swab	Pro hMPV+ (K082688)§ and SmartCycler¶
Respiratory virus: adenovirus	Nasopharyngeal swab	ProAdeno+ (K102952)§ and SmartCycler¶
Enterovirus	CSF	Xpert EV (K061062) and GeneXpert System¶
	CSF	NucliSENS EasyQ Enterovirus (K063261)*** and NucliSENS EasyQ System
MTB	Sputum and bronchial specimens	AMPLIFIED MTD (Mycobacterium Tuberculosis Direct) Test (P940034/S008)†††

(*table continues*)

*Sensitivity, specificity, and percentage positive or negative agreement with culture data are sourced from FDA submission material or product inserts.
†Obtained from Luminex Corporation, Austin, TX.
‡Obtained from Nanosphere, Inc., Northbrook, IL.
§Obtained from Gen-Probe Prodesse, Inc., Waukesha, WI.
¶Obtained from Cepheid, Sunnyvale, CA.
‖Obtained from Focus Diagnostics, Inc., Cypress, CA.
**Obtained from Roche Molecular Diagnostics, Pleasanton, CA.
††Obtained from Qiagen, Gaithersburg, MD.
‡‡3M, St Paul, MN.
§§Obtained from the CDC, Atlanta, GA.
¶¶Obtained from Life Technologies Inc., Carlsbad, CA.
‖‖Obtained from the Department of Defense.
***Obtained from bioMérieux Inc., Durham, NC.
†††Obtained from Gen-Probe, Inc., San Diego, CA.
hMPV, human metapneumovirus; hPIV, human parainfluenza virus; NASBA, nucleic acid sequence-based amplification; NPA, nasopharyngeal aspirate.

Table 3. *Continued*

Method	Target	Sensitivity (%)*	Specificity (%)*
RT-PCR, allele-specific primer extension, tag sorting	*Matrix* gene of influenza A, *Hemagglutinin* gene of influenza A/H1 and A/H3	Influenza A, 96.4	Influenza A, 95.9
	Influenza B, adenovirus, RSV A/B metapneumovirus	Influenza A/H1, 100	Influenza A/H1, 100
	Parainfluenza 1, 2, and 3; and rhinovirus	Influenza A/H3, 91.7 Influenza B, 91.5 RSV A, 100 RSV B, 100 Adenovirus, 78.3 hMPV, 96 hPIV 1, 100 hPIV 2, 100 hPIV 3, 84.2 Rhinovirus, 100	Influenza A/H3, 98.7 Influenza B, 96.7 RSV A, 98.4 RSV B, 97.4 Adenovirus, 100 hMPV, 98.8 hPIV 1, 99.8 hPIV 2, 99.8 hPIV 3, 99.6 Rhinovirus, 91.3
Multiplex RT-PCR multiplex gold nanoparticle hybridization technology Verigene System‡	Influenza A *matrix* gene Influenza B *NS* and *matrix* genes *L* and *F* genes of RSV	Influenza A, 99.2 Influenza B, 96.8 RSV, 89.8	Influenza A, 90.1 Influenza B, 98.5 RSV, 91.5
Multiplex real-time RT-PCR TaqMan chemistry	Influenza A *matrix* gene Influenza B non-structural *NS1* and *NS2* genes RSV *polymerase* gene	Influenza A, 100 Influenza B, 97.8 RSV, 89.5	Influenza A, 92.6 Influenza B, 98.6 RSV, 94.9
Real-time RT-PCR	Target RNA of highly conserved region of *matrix* protein genes of influenza A and B and RSV	Influenza A, 100 Influenza B, 100 RSV, 98	Influenza A, 99.3 Influenza B, 99.8 RSV, 96.9
Real-time PCR	Influenza A *matrix* gene and unique region in *Hemagglutinin* gene of 2009 H1N1 influenza virus	% positive agreement for swabs, 100; NPA, 100	% negative agreement for swabs, 92.5; NPA, 96.1
Real-time PCR	Influenza A *matrix* gene and *Nucleoprotein* gene specific for 2009 H1N1 and *Hemagglutinin* gene specific for 2009 H1N1	96	96
Real-time RT-PCR	Target RNA of conserved region of *Hemagglutinin* gene	% positive agreement for A/H1, 100; A/H3, 100; A/H1N1-2009, 95.4	% negative agreement for A/H1, 99.0; A/H3, 99.0; A/H1N1-2009, 100
Real-time RT-PCR	Two target RNA sequences 5′ and 3′ of the *Hemagglutinin* precursor cleavage site within the conserved regions of the *Hemagglutinin* gene of influenza A/H5 (Asian lineage) virus	96.9–100	95.3–97.1
Multiplex real-time RT-PCR TaqMan chemistry	Conserved regions of *Hemagglutinin-Neuraminidase* gene of hPIV 1, 2, and 3	hPIV-1, 88.9 hPIV-2, 96.3 hPIV-3, 97.3	hPIV-1, 99.9 hPIV-2, 99.8 hPIV-3, 99.2
Real-time RT-PCR TaqMan chemistry	hMPV: *Nucleocapsid* gene	% positive agreement, 94.1	% negative agreement, 99.3
Multiplex real-time PCR TaqMan chemistry	Adenovirus serotypes 1–51, *Hexon* gene	97.5	95.6
Real-time PCR	Consensus region of enterovirus, 5′ UTR between nucleotides 452 and 596	96.3–100	97.0–97.2
NASBA	Enterovirus RNA	70.9–100	99.3–100
Transcription- mediated amplification, hybridization protection assay	Mycobacterial 16S rRNA	96.9	100

amplified to generate a large amplicon that is then used as a sequencing template for multiple primers that generate a consensus sequence. Software is available for comparing the consensus with a known reference, to determine any mutations present, based on which treatment options are made available. These are kept current by a panel of HIV experts and recommendations of the International AIDS Society–USA panel.[37,43,44] Reportedly, genotyping assays may have limitations of sensitivity in detecting a minority variant species in a patient.[45] In addition, potential mutations may be missed at positions not previously characterized as resistance mutants.

HSV Data

Herpes simplex virus (HSV) is one of the most common STDs in the United States. Genital herpes is a chronic life-long infection caused primarily by HSV-2, although the role of HSV-1 is increasing.[46,47] Most patients infected with genital herpes are asymptomatic, and the clinical presentation is diverse. Because of the availability of effective antiviral therapy, there is an increased demand for rapid accurate laboratory diagnosis of HSV. HSV genotyping may aid in tracing of contacts and in case evaluation.[48,49] The MultiCode-RTx HSV-1&2 Kit (EraGen Biosciences, Madison, WI) is a PCR-based qualitative IVD HSV typing assay, using vaginal swab specimens from symptomatic female patients. The assay is not approved for cerebrospinal fluid (CSF) or any other genital or oral lesion specimens. The assay uses fluorophore-labeled HSV-1 and HSV-2 primers that target the glycoprotein B gene. The extraction methods cleared for the test include the MagNA Pure LC Instrument and the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche Molecular Diagnostics). The PCR amplification is performed using the LightCycler 1.2 instrument (Roche Molecular Diagnostics), after which the HSV genotypes are discriminated by melt curve analysis. Evaluation of the appropriate specimen/lesion and specimen collection procedures is essential because lesion type and location may affect the sample quality and the assay performance.

HAI Data

Different HAI assays have specific intended uses, such as surveillance, presurgical, and diagnostic testing; and the product insert should be reviewed carefully before implementation.

MRSA Data

Methicillin resistance is associated with increased mortality in patients with staphylococcal bacteremia.[50] Approximately half of all *Staphylococcus aureus* pneumonias in the United States are due to methicillin-resistant *S. aureus* (MRSA).[51] MRSA ventilator-associated pneumonias also appear to be associated with a higher mortality rate compared with methicillin-sensitive *S. aureus* ventilator-associated pneumonias.[52] Patients colonized with MRSA (nasal carriers) are also at increased risk of developing MRSA disease and can spread the infection as well.

For direct MRSA detection from patient samples, the Xpert MRSA, Xpert SA Nasal Complete assays for the GeneXpert System (Cepheid, Sunnyvale, CA), the BD GeneOhm MRSA ACP (BD Diagnostics, La Jolla, CA), and the LightCycler MRSA Advanced test (Roche Molecular Systems) can be used. The GeneXpert System is a fully integrated and automated nucleic acid preparation, amplification, and real-time detection system. The Xpert SA Nasal Complete (Cepheid) assay can be used for surveillance of both *S. aureus* and MRSA carrier status, which can aid in reducing the risk of HAIs. In a recent multicenter clinical evaluation, no statistically significant performance differences were observed between the Xpert MRSA and MRSA ACP assays compared with culture.[53,54] The recently approved LightCycler MRSA Advanced test was similarly evaluated compared with the BD GeneOhmMRSA ACP and had a similar sensitivity (92.2% and 93.2%, respectively) but a significantly better specificity (98.9% and 94.2%, respectively).[55] All of these assays target the *S. aureus orfX* gene sequence incorporating the insertion site (*attBssc*) of the staphylococcal cassette chromosome *mec* (SCC*mec*) for the detection of MRSA. Targeting the *mecA* alone could result in false positivity because a large percentage of coagulase-negative staphylococcus species would also test positive. False-negative results can occur as the result of novel SCC*mec* elements and variants resulting from recombination. Targeting the *orfX* region alone can give false-negative results in those instances of *mec*A insertion into other sites (although this is rare).[56]

Both the BD GeneOhm MRSA ACP and the BD GeneOhm StaphSR assays target the MREJ (types i to vii) of the SCC*mec* insertion into the *orfX* gene and the *S. aureus* species-specific *nuc* gene, which is distinct from the SCC*mec* cassette. Therefore, false-positive results are reduced. However, false-positive results may still occur because of SCC*mec* variants with missing or nonfunctional *mecA* genes (empty cassette variants) and false-negative results from MREJ variants other than types i to vii.[57]

The LightCycler MRSA Advanced assay targets the sequence incorporating the insertion site of the SCC*mec* in the *S. aureus orfX* gene. Specifically, it targets types 2, 3, and 7 of the right extremity of the SCC*mec-orfX* junction. Thus, it may give false-negative results if the targeted right extremity types are not present. This assay uses the uracil-*N*-glycosylase enzyme before amplification to eliminate any amplicon contamination. The Xpert MRSA and Xpert SA Nasal Complete assays offer ease of use, minimal hands-on time, and a closed-tube method of testing. The assay can also be run either as a single on-demand assay or in batch mode. In addition, the Xpert SA Nasal Complete assay has high specificity because of inclusion of three targets (*spa*, *mecA*, and SCC*mec*), reducing false-positive results due to empty cassette variants. In this assay, all three targets must be detected for the assay to give a positive MRSA result. Therefore, coagulase-negative staphylococcus species, which may contain the *mecA* gene but not the *S. aureus*–specific *spa*

gene, will not render a positive MRSA result. Assays may still test positive if there is mixed flora of both methicillin-sensitive *S. aureus* and coagulase-negative staphylococcus species in the testing sample; however, the reported incidence of such cocolonization is low.[58]

Cultured material can also be tested for the presence of methicillin-susceptible or resistant *S. aureus* causing sepsis (Table 4). Positive blood culture material may be analyzed by the nonamplified peptide nucleic acid (PNA) fluorescence *in situ* hybridization (FISH) method (AdvanDx, Woburn, MA). These pathogen-specific assays are based on positive blood culture and Gram stain results for detecting *S. aureus* and MRSA as soon as the instrument signal on continuously monitoring blood culture systems is positive.[59–61] Small quantities of positive blood culture material can also be analyzed using the multiplex real-time PCR assays [BD GeneOhm StaphSR (BD Molecular Diagnostics) and Xpert MRSA/SA BC (Cepheid)]. The latter assay operates under a revised Corrective Action Letter from Cepheid that instructs laboratories not to report an MRSA-negative result when an MRSA-negative/SA-positive result is generated on the Cepheid MRSA/SA Blood Culture Assay. Instead, MRSA-indeterminate/SA-positive antimicrobial susceptibility testing pending is the recommendation, with further antimicrobial susceptibility testing performed to determine the MRSA status. The reporting of MRSA-positive/SA-positive results generated on the Cepheid MRSA/SA Blood Culture Assay is not affected.

Results can be achieved by real-time PCR, closed, walk-away systems more rapidly than by more traditional PCR assays. The commercial assays have excellent sensitivity and specificities when compared with culture. In particular, genetic excisions within the SCC*mec* region of MRSA strains may also yield positive PCR results in the absence of a functional *mecA* gene and may cause PCR-positive but phenotypically methicillin-susceptible *S. aureus* results (empty cassette). This prevalence has differed by geographical region and appears to be more common outside the United States.[62,63] Decisions on which assay to implement will depend on laboratory capabilities, the urgency for the result, and the impact on patient care. It is recommended that the laboratory routinely perform clinical correlation of the assay results, both positive and negative, to keep abreast of diverse and evolving MRSA strains.

VRE Data

Screening for vancomycin-resistant enterococcus (VRE) directly from perianal, perirectal, rectal, or stool specimens has been recommended by the CDC, Health Care Infection Control Practices Advisory Committee (*http://www.cdc.gov/hicpac/mdro/mdro_0.html*, last accessed December 31, 2010) to limit the spread of antimicrobial resistance within certain high-risk populations. For surveillance of VRE, the Xpert*vanA* (Cepheid) assay can be performed directly on rectal swab specimens from patients (Table 2). Gram-positive cocci in pairs and chains–*Enterococcus faecalis*/other enterococci PNA FISH (AdvanDx) is also available to identify VRE from positive blood culture results. Testing for VRE helps identify patients colonized with resistant enterococci in approximately 1 to 2 hours. Positive test results indicate the presence of either the *vanA* or *vanB* gene, or *vanA* alone, which confers vancomycin resistance in *E. faecalis*, *Enterococcus faecium*, and other bacteria that may colonize the human intestine. In general, although a positive result does not imply disease caused by VRE, the presence of *vanA* or *vanB* genes correlates with colonization and clinical correlation is required to determine active VRE disease. PCR testing should decrease the spread of VRE by rapid identification and isolation of colonized patients; however, conventional bacterial cultures may still be required to isolate VRE from clinical specimens (eg, blood) for the diagnosis of VRE infection. This approach allows antimicrobial susceptibility testing for selection of appropriate antimicrobial treatment and strain typing of isolates in outbreak situations.

Clostridium difficile Infection

C. difficile infection is an important cause of diarrhea in patients who are hospitalized, in long-term care facilities and receiving antibiotics, and in community settings.[64] Four assays are available for the detection of toxigenic strains of *C. difficile* (Table 2). The Illumigene *C. difficile* assay (Meridian Bioscience Inc., Cincinnati, OH) uses loop-mediated isothermal amplification technology to detect the pathogenicity toxin A gene (*tcd*A) in the pathogenicity locus of toxigenic *C. difficile*. The *C. difficile* pathogenicity locus is a gene segment present in several known toxigenic *C. difficile* strains. It codes for both *tcd*A and the toxin B gene (*tcd*B). The test includes a manual extraction step but does not require costly capital equipment, and results are available in approximately 1 hour. The Xpert *C. difficile* (Cepheid), BD GeneOhm Cdiff (BD Diagnostics), and proGastro Cd (Gen-Probe Prodesse, Inc., Waukesha, WI) assays are based on real-time PCR and target *tcd*B of *C. difficile*. A positive test result does not necessarily indicate the presence of viable *C. difficile* organisms, but it does indicate the presence of *tcd*B. Specimen extraction and amplification for the Xpert *C. difficile* test is self-contained and automated, and the results are available in approximately 45 minutes. The BD GeneOhm Cdiff assay results are available in <2 hours. Mutations or polymorphisms in primer- or probe-binding regions may affect detection of *C. difficile tcd*A or *tcd*B variants, resulting in false-negative results; however, variant toxigenic *C. difficile* without *tcd*B or with a nonfunctional toxin B protein is rare. An assay may be positive for *tcd*B without TcdB toxin production (noncytotoxic, IX subtype), as reported in community-associated cases in Canada.[65] Because of the enhanced sensitivity of these amplification methods, testing for *C. difficile* should be limited to patients with clinical symptoms of *C. difficile* infection. Testing should be limited to diarrheal or loose stools (ie, those that take the shape of the container), and the assays should also not be used for test of cure. Assay performance is unknown for asymptomatic patients.[66] Tables 2 and 4 list details of assays for HAIs.

Table 4. Other Organisms and Culture Confirmations

Organism	Specimen/sample type	Platform/assay (FDA no.)
HCV quantitative	EDTA plasma or serum	COBAS AmpliPrep/COBAS TaqMan HCV Test (P060030)†
	Plasma or serum extracted from blood collected using EDTA or ACD	VERSANT HCV RNA 3.0 Assay (P020022)‡
HCV qualitative	Plasma or serum	APTIMA HCV RNA Qualitative Assay (P020011)§
	EDTA plasma or serum	AMPLICOR HCV test, version 2.0 (P000010)†
	EDTA plasma or serum	COBAS AMPLICOR HCV test, version 2.0 (P000012)†
HBV quantitative	EDTA plasma or serum	COBAS TaqMan HBV Test (P050028)†
	EDTA plasma or serum	RealTi*me* HBV Assay (P080026)¶
GBS	Vaginal and rectal swabs incubated in Lim broth overnight	BD MAX GBS assay and BD MAX System (K090191)‖
	Vaginorectal swab	*IDI-Strep B* (K022504)‖
	Vaginorectal swab	Xpert GBS (K060540)**
	Directly from vaginal and rectal swabs or from LIM broth culture	Smart GBS test (K062948)**
	Vaginal and rectal swabs incubated in Lim broth overnight	GBS PNA FISH (K082612)††
	Vaginal and anorectal swabs in Lim broth culture	AccuProbe Group B Streptococcus Culture Identification Kit (K974572)§
CA, GV, and TV	Vaginal sample	BD Affirm VPIII Microbial Identification Tests (K931374, K923133, K931151)‖
Group A *Streptococcus*	Throat swab	GASDirect Test (K924715)§
Bacterial identification from culture	Cultures	AccuProbe *Neisseria gonorrhoeae* (K895583)§
		AccuProbe *Listeria monocytogenes* (K901397)§
Organisms causing sepsis: GPCC	GPCC-positive cultures	BD GeneOhm StaphSR (K071026)‖
	GPCC-positive cultures	Xpert MRSA/SA BC (K082140)**
	GPCC-positive cultures	*S. aureus* PNA FISH (K060099)††
		S. aureus/CNS PNA FISH (K092166)††
	GPCC-positive cultures	AccuProbe *S. aureus* (K902213)§
Organisms causing sepsis: GPCPC	GPCPC-positive cultures	AccuProbe *Streptococcus pneumoniae* (K902908)§
Organisms causing sepsis: GPCPC	GPCPC-positive cultures	*E. faecalis*/OE PNA FISH (K083074)††
Organisms causing sepsis: smears from GNR	GNR-positive cultures	EC, PA PNA FISH (K081309, K092236)††
	GNR-positive cultures	EC, KP, PA PNA FISH (K101558)††
Organisms causing sepsis: yeast	Smears made directly from yeast-positive blood cultures	*Candida albicans* PNA FISH (K062461)††
		Yeast Traffic Light PNA FISH (K080719)††
		C. albicans/C. glabrata PNA FISH (K092784)††
	Yeast-positive blood cultures	AccuProbe *Blastomyces dermatitidis* (K903201)§
		AccuProbe *Coccidiodes immitis* (K904047)§
		AccuProbe *Histoplasma capsulatum* (K896859)§
Mycobacterial identification from cultures	Growth from appropriate solid media or broth	AccuProbe *M. avium* (K896494)§
		AccuProbe *M. avium* complex (K897078)§
		AccuProbe *Mycobacterium gordonae* (K896492)§
		AccuProbe *Mycobacterium intracellulare* (K897077)§
		AccuProbe *Mycobacterium kansasii* (K904463)§
		AccuProbe MTB complex (K896493)§

(*table continues*)

Table 4. *Continued*

Method	Target	Linear range (%)* Sensitivity	Specificity
Real-time RT-PCR	Transcript of a 244-base sequence in the highly conserved 5′ untranslated region of HCV; genotypes 1–6	43–69,000,000 IU/mL	
bDNA	5′UTR and core regions of the HCV genome	3200–40,000,000 IU/mL	
Transcription-mediated amplification	5′UTR of the HCV genome	91.8–100	97.8–98.5
RT-PCR	5′UTR of the HCV genome	92–94	96–97
RT-PCR	5′UTR of the HCV genome	92–94	96–97
Real-time PCR	Core-precore region of the HBV genome; primer pairs to genotypes A–G of HBV and the precore mutation	20–170,000,000 IU/mL	
Real-time PCR	Surface (*S*) gene of the HBV genome; primer pairs to genotypes A–G of HBV	10–1,000,000,000 IU/mL	
Real-time PCR	124-bp region of the *cfb* gene	95	96.7
Real-time PCR SmartCycler system**	GBS *cfb* gene	94	95.9
GeneXpert System (real-time PCR)**	3′ region adjacent to the *cfb* gene	91.1	96.0
Real-time PCR SmartCycler system**	DNA 3′ region adjacent to the *cfb* gene	Direct from swabs, 81.6 From Lim broth, 98.7	Direct from swabs, 96.4 From Lim broth, 90.4
PNA-FISH	16S rRNA of *Streptococcus agalactiae*	89.2–100	86.8–100
Culture confirmation; hybridization protection assay	DNA probe that detects rRNA sequences unique to *S. agalactiae*	97.5	99.8
Single-stranded DNA probes, hybridize with complementary rRNA target sequences to form hybrids	rRNA	CA, 80.6 GV, 83.8 TV, 92.8	CA, 98.2 GV, 99.1 TV, 99.9
Hybridization protection assay	rRNA	91.7	99.3
Hybridization protection assay	rRNA AccuProbe *Listeria monocytogenes* (K901397)§	100 100	100 99.7
Real-time PCR	SCC*mec* and *mecA* gene; *nucA* gene	MRSA % positive agreement, 100 SA % positive agreement, 98.8–100	MRSA % negative agreement, 98.2–100 SA % negative agreement, 96.5
GeneXpert System (real-time PCR)**	*Spa* and *mecA* genes and SCC*mec* inserted into *S. aureus* chromosomal *attB* insertion site	MRSA, 98.3 SA, 100	MRSA, 99.4 SA, 98.6
FISH assay	Species-specific 16S rRNA of *S. aureus*‡‡	100	100‡‡
Hybridization protection assay	rRNA	100	100
Hybridization protection assay	rRNA	100	100
PNA; FISH assay	Species-specific 16S rRNA in *E. faecalis* and other enterococci§§	% positive agreement with culture, 100	% negative agreement with culture, 100
PNA; FISH assay	Species-specific rRNA of EC and PA	EC, 100 PA, 97.5	100¶¶ 100
PNA; FISH assay	Species-specific rRNA of EC, KP, and PA	EC, 100 KP, 98.7 PA, 96.9	EC, 97.5 KP, 97.5 PA, 97.5
PNA FISH species-specific probes	16S rRNA of *C. albicans*	100	100
Hybridization protection assay	rRNA	98.1 98.8 100	99.7 100 100
Hybridization protection assay	rRNA	99.3 99.9 98.8 100 98.0 99.2	100 100 99.7 100 96.8 99.9

(*table continues*)

Table 4. *Continued*

Organism	Specimen/sample type	Platform/assay (FDA no.)
Mycoplasma species	Tissue culture	*Mycoplasma* Tissue Culture NI (MTC-NI) Rapid Detection System (K860574)[§]

(table continues)

*Sensitivity, specificity, linearity range, and percentage positive or negative agreement with culture data are sourced from FDA submission material or product inserts.
†Obtained from Roche Molecular Diagnostics, Pleasanton, CA.
‡Obtained from Siemens Healthcare Diagnostics, Deerfield, IL.
§Obtained from Gen-Probe, Inc., San Diego, CA.
¶Obtained from Abbott Molecular, Inc., Des Plaines, IL.
‖Obtained from BD Diagnostics, Sparks, MD.
**Obtained from Cepheid, Sunnyvale, CA.
††Obtained from AdvanDx, Inc., Woburn, MA.
‡‡False-positive results with Staphylococcus schleiferi may occur because of a single-base mismatch.
§§*Enterococcus moraviensis* is identified as *E. faecalis* because of sequence identity.
¶¶False-positive results may occur with *Shigella* species (serogroups A, B, C, or D), *Escherichia albertii*, and *Escherichia fergusonii* because of sequence similarity.
ACD, anticoagulant citrate dextrose; bDNA, branched DNA; CA, *Candida* species; EC, *Escherichia coli*; GNR, Gram-negative rod; GPCC, Gram-positive cocci in clusters; GPCPC, Gram-positive cocci in pairs and chains; GV, *Gardnerella vaginalis*; KP, *Klebsiella pneumonia*; PA, *Pseudomonas aeruginosa*; TV, *Trichomonas vaginalis*.

Respiratory Tract and CNS Infections

Detection of MTB Complex from Clinical Specimens

Data for the United States describe 11,181 cases of tuberculosis infections in 2010. Approximately one third of the 40 million people living with HIV/AIDS worldwide are coinfected with tuberculosis. People with HIV are up to 50 times more likely to develop tuberculosis in a given year than HIV-negative individuals (World Health Organization, *http://www.who.int/tb/challenges/hiv/en/index.html*, last accessed December 31, 2010). Several strains of *Mycobacterium tuberculosis* (MTB) are resistant to multiple antibiotics, and detection of these strains is critical for patient treatment and public health concerns.

The AMPLIFIED MTD (Mycobacterium Tuberculosis Direct) Test (Gen-Probe Inc.) (Table 3) is the only FDA-approved test available for the qualitative detection of MTB. The assay detects MTB complex rRNA directly from smear-positive and smear-negative sputum, bronchial specimens, and tracheal aspirates, with results available in <4 hours. The sensitivity and specificity of the MTD assay are 72% and 99.3%, respectively, for smear-negative patients and 96.9% and 100%, respectively, for smear-positive patients (package insert, IN0014 revision L, dated August 2001). Other specimen types (eg, CSF, blood, and lymph node tissue) are not FDA approved for use with this assay. Culture of the specimen is still required given the imperfect sensitivity of the MTD assay for smear-negative specimens and for susceptibility testing. Non-specific inhibition was reported in 3% to 7% of sputum specimens. Pollock et al[67] have shown that dilution of the processed sputum sediment by 1:10 using an MTD reaction buffer overcomes non-specific inhibition and improves sensitivity of the MTD assay. However, this dilution technique is not part of the FDA-approved assay. A positive MTD result in a smear-positive patient helps to initiate antimycobacterial drug therapy much earlier than awaiting culture results. The decision to remove patients from isolation should not be based solely on a negative MTD test result because of imperfect sensitivity, especially in smear-negative patients. The limit of detection of the AMPLIFIED MTD assay is one colony-forming unit per test. Because of the global importance of tuberculosis, nucleic acid tests are recommended by the CDC and the American Thoracic Society to improve detection and treatment of this infection (*http://www.cdcnpin.org/scripts/tb/cdc.asp*, last accessed December 31, 2010).

Respiratory Tract Viral Infections

Acute respiratory tract infections are the most common infections in humans, and respiratory tract viruses cause 80% of these infections. Respiratory tract virus infections range from mild self-limiting upper respiratory tract infections to severe lower respiratory tract infections. Influenza causes 200,000 hospitalizations and 36,000 deaths in the United States annually, and respiratory syncytial virus (RSV) is the most common cause of severe lower respiratory tract disease in infants and young children worldwide (World Health Organization, *http://www.who.int/vaccine_research/diseases/ari/en*, last accessed December 31, 2010). Influenza A and B and RSV account for the most serious respiratory tract diseases, with antiviral therapy available for treatment.

The commercially available IVDs for respiratory viral agents include single or multiple pathogen (multiplex panel) detection and devices for the identification or typing of these causative agents (Table 3). Because most of the respiratory viral agents cause similar symptoms, the multiplex assays provide the added value of enabling the simultaneous detection of multiple agents with a single test. Several multiplex panels are available, and their applications will be laboratory and patient population dependent.

Several closed-tube real-time PCR systems are available for the detection of respiratory tract viruses. These include the proFlu+ (Gen-Probe Prodesse, Inc.) test, which simultaneously detects influenza A and B and RSV, with a platform designed for a high-throughput laboratory. The Verigene System (Nanosphere, Inc., Northbrook, IL) and the Simplexa Flu A/B and RSV (Focus Diagnostics, Inc., Cypress, CA) detect influenza A and B

Table 4. *Continued*

Method	Target	Linear range (%)*	
		Sensitivity	Specificity
DNA probe	rRNA of *Mycoplasma* and *Acholeplasma* species	Not applicable	Not applicable

and RSV. The ProParaFlu+ (Gen-Probe Prodesse, Inc.) detects parainfluenza 1, 2, and 3; and the Pro hMPV+ (Gen-Probe Prodesse, Inc.) detects human metapneumovirus (Table 3). The pro hMPV+ assay shows 94.1% sensitivity and 99.3% specificity against a composite reference method of RT-PCR targeting the nucleocapsid and fusion genes of hMPV. The ProAdeno+ (Gen-Probe Prodesse, Inc.) assay is approved for qualitative detection of human adenovirus serotypes 1 to 51 in nasopharyngeal specimens. Compared with shell vial culture, the ProAdeno+ assay is 98% sensitive and 96% specific. The ProParaflu+ assay is approved for qualitative detection of parainfluenza 1, 2, and 3 from nasopharyngeal swab specimens. The ability of the ProParaflu+ assay to detect parainfluenza virus 1, 2, and 3 ranges from a sensitivity of 89% to 97% and from a specificity of 99% to 100% when compared with culture. The ProFAST+ assay is designed to detect and differentiate influenza A/H1, influenza A/H3, and the 2009 H1N1 influenza from nasopharyngeal specimens. Additional IVDs for influenza A and H1N1 influenza are the Simplexa Influenza A H1N1 (2009) (Focus Diagnostics, Inc.) and the CDC influenza assay. However, the CDC assay is not commercially available. The FDA also cleared the Department of Defense biological warfare agent detection device, the JBAIDS diagnostic system for Influenza A/H5 (avian influenza) diagnosis. The JBAIDS assay is not available for general commercial use.

A moderately multiplexed assay, such as the xTAG Respiratory Viral Panel (Luminex Corp, Austin, TX), detects a panel of 12 viruses and subtypes influenza seasonal H1 and H3 viruses. By using decision tree modeling, Mahony et al[68] demonstrated that the least costly strategy to diagnose respiratory tract virus infection was the xTAG RVP (Luminex Corporation, Austin, TX) test alone when the prevalence of infection was ≥11% and DFA (Direct Fluorescent Antibody) alone when the prevalence was <11%. The xTAG assay is relatively complex, and the assay's open-tube format has the potential for contamination. The assay is approved for nasopharyngeal swab specimens. The assay cannot adequately detect adenovirus species C or serotypes 7a and 41, and rhinovirus is not differentiated from enterovirus (EV). A nonsubtypable influenza A result must also be carefully evaluated because this may be the first indication of an epidemic caused by a new influenza strain. In addition to the potential of detecting more viral coinfections, the multiplex assays, although not FDA cleared for this population, may prove useful in the evaluation of immunosuppressed patients and in older patients in whom viral titers may typically be lower.[69]

CNS Viral Infections

Viral CNS infections usually manifest as meningitis and encephalitis. Although several microorganisms are associated with CNS infections, the only MID IVD available is for EV, the leading cause of seasonal meningitis. Nucleic acid testing aids in rapid diagnosis of viral meningitis and prevents unnecessary antibiotic use and potential repeat spinal taps, especially in children.[70,71] More recently, rapid nucleic acid amplification tests, including real-time RT-PCR, nucleic acid sequence-based amplification, and fully automated systems capable of extraction, amplification, and detection, have replaced conventional RT-PCR methods.[72–75] CSF is the diagnostic specimen for detection of EV in patients with aseptic meningitis. Pleocytosis and elevation of protein level in CSF are good markers for CSF infection. The absence of pleocytosis in CSF may be a good predictor of a negative EV RT-PCR result in children >2 months. However, elevation of the CSF protein level is not a good predictor of RT-PCR positivity for EV.[76]

Two qualitative IVDs are available for testing CSF specimens for EV: Xpert EV (Cepheid) and NucliSENS EasyQ Enterovirus (bioMérieux, l'Etoile, France). The Xpert EV assay is performed on a GeneXpert System (Cepheid). In a multicenter evaluation of 102 CSF specimens, the assay had a sensitivity of 97.1% (95% CI, 84.7% to 99.9%) and a specificity of 100% (95% CI, 94.6% to 100%) when compared with culture.[74] Although this initial study demonstrated that moderate amounts of blood did not interfere with assay performance, a subsequent study[77] indicated that the presence of red blood

cells could produce an invalid result in up to 8.2% of CSF specimens. It also showed that xanthochromia and specimen clotting did not affect results. Specimens with any invalid results could be diluted at 1:5 or repeat tested after a freeze-thaw cycle to overcome inhibition, with a minimal reduction in sensitivity (<3.6%). This is an easy-to-use assay format that affords random access capability and minimal hands-on time, with results available in <2.5 hours.

The NucliSENS EasyQ Enterovirus assay (bioMérieux), a nucleic acid sequence-based amplification, involves nucleic acid extraction, amplification, and real-time detection of an internal control and EV RNA by Molecular Beacons that are labeled with two different dyes: 6-FAM for EV and 6-ROX for the internal control. In a study of 449 prospectively collected CSF specimens tested by cell culture and NucliSENS EasyQ Enterovirus, method agreement was 86.4% (95% CI, 79.3% to 91.2%; FDA-K063261 submission data). In a premarket evaluation study, [73] this assay was more sensitive than culture (97.9% versus 65.6%; $P < 0.001$) and inhibition was noted in only 0.5% of CSF specimens. The GeneXpert System and nucleic acid sequence-based amplification assays were compared in a recent multicenter trial for detection of EV from CSF and had a sensitivity of 100% and 87.5%, respectively.[75] Table 3 lists assays for respiratory tract and CNS infections.

Other Infections

HBV Data

HBV infection is a global public health problem, with 400 million worldwide long-term carriers and up to 25% mortality.[78,79] HBV is classified with A–H genotypes, and prevalence varies greatly by geography and population subgroups. Because there are significant differences in genotype-dependent hepatocellular carcinoma, it is essential to detect and quantitate all genotypes. Typically, the conserved region in the precore/core or N-terminal portion of the *S* gene is targeted for target amplification assays; and multiple probes are used to detect all genotypes. There is no role for molecular testing in the diagnosis of acute HBV other than in the detection of asymptomatic patients during pretransfusion screening of blood products.

There are two commercial FDA-approved assays for HBV quantification (Table 4). The COBAS TaqMan HBV Test for use with the manual High Pure System Viral Nucleic Acid Kit (Roche Molecular Diagnostics) is approved for serum and plasma specimen types. Quantification of HBV viral DNA is performed using the quantitation standard that is incorporated in each sample. The dynamic range of the test is 29 to 110,000,000 IU/mL, with a limit of detection of 10 IU/mL. The RealTi*m*e HBV assay on the m2000 system (Abbott Molecular) has recently been approved to quantify HBV viral load in plasma and serum. This test has a detection capability that ranges from 10 to 1 billion IU/mL and spans all known HBV genotypes (A–H). The same quantitative test should be used throughout a patient's treatment course.

HCV Data

Hepatitis C virus (HCV) infection is the most common cause of chronic viral hepatitis. Nearly 20% of the 4 million carriers develop liver cirrhosis. Viral RNA can be detected in HCV-infected individuals as early as 1 to 4 weeks before the increase of liver enzymes, and it peaks in the first 8 to 12 weeks after infection.[80] HCV infections are typically diagnosed by the detection of antibodies directed against specific HCV antigens. However, HCV serological tests demonstrate low specificity and may require confirmation of positive results. Qualitative PCR assays, such as the FDA-cleared Cobas AMPLICOR HCV Test, version 2.0 (Roche Molecular Diagnostics), the APTIMA HCV RNA qualitative assay (Gen-Probe Inc.), or the VERSANT HCV RNA Qualitative assay (Siemens Diagnostics), can be used to confirm serological findings.

Treatment for chronic HCV typically consists of a combination of pegylated interferon-α and ribavirin, and response to therapy is genotype dependent. Quantitative viral load assays are used to establish viral load at baseline, monitor viral load during therapy, and determine response to treatment. Both the rapid and complete early virologic responses have been used to predict if a patient will achieve a sustained virologic response. The early virologic response is defined as a $\geq$2-$\log_{10}$ reduction in HCV RNA levels during the first 12 weeks of therapy. The rapid and complete early virologic responses are defined as no virus detected at 4 and 12 weeks after initiation of antiviral agent, respectively.[81] A completely negative test result for HCV RNA at week 12 (complete early virologic response) is a better predictor of a sustained virologic response than a 2-$\log_{10}$ reduction in HCV RNA.[82] A sustained virologic response is achieved when <5 IU/mL HCV RNA is detected after 24-week treatment. After this, the treatment is discontinued for genotypes 2 and 3 and continued for an additional 24 weeks for genotypes 1 and 4. Currently, there are no FDA-approved HCV genotyping tests. Therefore, the quantitative assays used to measure HCV viral load need to be sensitive and need to generate accurate results for all genotypes and subtypes. The same quantitative test should be used throughout a patient's treatment course. The American Association for the Study of Liver Diseases has published guidelines for the use of qualitative and quantitative molecular assays for detection and quantification of HCV RNA in serum and plasma.[83]

There are two commercial FDA-approved assays for HCV quantitation. The COBAS AmpliPrep/COBAS TaqMan System (Roche Molecular Diagnostics) is an automated real-time RT-PCR that targets a highly conserved sequence in the HCV 5′ untranslated region.[84,85] The VERSANT HCV RNA 3.0 Assay (branched DNA) (Siemens Healthcare Diagnostics) is a branched DNA signal amplification method targeting highly conserved sequences in both the 5′UTR and the core gene.[86] The former method has a dynamic range of detection from 25 to 3.9×10^8 IU of HCV RNA/mL of plasma or serum. The

range of the latter assay is from 615 to 7.69 × 10^6 IU of HCV RNA/mL of plasma or serum.

Group B Streptococcus

Streptococcus agalactiae [group B *Streptococcus* (GBS)] is a leading cause of sepsis, meningitis, and death among newborn infants in the Western world. Between 10% and 40% of healthy adult women are colonized by GBS in the genital and gastrointestinal tracts; although not associated with disease in healthy women, GBS can cause disease during pregnancy and delivery.[87] The CDC, in collaboration with the American College of Obstetrics and Gynecology and the American Academy of Pediatrics, issued revised guidelines that included recommending universal prenatal screening of all pregnant women between 35 and 37 weeks of gestation to determine their vaginal/rectal GBS colonization status. The CDC recommends universal antepartum GBS screening in all pregnant women, primarily with culture from vaginal-rectal swabs, to identify candidates for intrapartum prophylaxis to decrease early-onset GBS-related complications in the newborn.[87] Use of vaginal-rectal swabs improves GBS isolation by 40%, compared with use of vaginal specimens alone.[88] Culture requires up to 36 hours of incubation and, therefore, rapid screening using molecular test methods may alleviate unnecessary antibiotic treatment in patients with preterm labor. A rapid and sensitive intrapartum real-time PCR assay offers the advantage of ascertaining the colonization status before delivery.

Six assays are commercially available for GBS testing (Table 4), using either direct vaginal-rectal swabs or these swabs incubated in Lim broth (culture confirmation): AccuProbe Group B Streptococcus culture confirmation test (Gen-Probe Inc.), IDI-Strep B (GeneOhm Sciences, San Diego), BD MAX GBS assay (BD Diagnostics), Smart GBS (Cepheid), Xpert GBS (Cepheid), and the GBS PNA FISH (AdvanDx) assay. The BD MAX System (BD Diagnostics) is an integrated system that uses probes (Molecular Beacons), whereas the GeneXpert (Cepheid) system uses TaqMan probes. The Xpert GBS assay is the only FDA-cleared assay for use on both intrapartum and antepartum specimens. FDA-cleared molecular methods allow for increased sensitivity and a rapid turnaround time. The GeneXpert system facilitates near-point-of-care analysis in an intrapartum scenario. The BD GeneOhm StrepB and the Smart GBS PCR assays are not yet automated and require varying degrees of hands-on specimen manipulation and sample preparation. In a high-volume laboratory setting, this may not be practical. The BD MAX system allows automation of a PCR assay that is run in a batch mode in the laboratory for antepartum testing. Although PCR tests have initially been adopted selectively in facilities with sufficient demand and resources, a general recommendation for their use by the CDC and other agencies would require the capacity for effective implementation in a wide range of hospital settings. Some disadvantages of intrapartum PCR testing include delays in the administration of antibiotics, pending test results, and lack of an isolate for susceptibility testing. Susceptibility testing is of particular concern for women who are allergic to penicillin.

Fungal or Bacterial Identification from Culture

Pathogen-specific rRNA PNA FISH probes can be selected based on a Gram stain result and are available for bacteria and yeast. These probes have a high impact on antimicrobial stewardship and HAI.[59–61,89–92] *Candida* species are a leading cause of both community- and hospital-associated fungemia. The identification of *Candida* species in blood cultures is routinely based on presumptive identification by Gram stain as yeast, followed by final identification after subculture and biochemical analysis. There are several PNA FISH assays available from AdvanDx. The Yeast Traffic Light PNA FISH identifies *Candida albicans*/*Candida parapsilosis*, *Candida tropicalis*, and *Candida glabrata*/*Candida krusei* and aids in appropriate drug therapy because of decreased susceptibility (*C. glabrata*) and inherent resistance (*C. krusei*) to fluconazole. Because these MID IVDs do not provide antimicrobial susceptibility profiles, culture should also be performed.

AccuProbe kits (Gen-Probe Inc.) performed on culture specimens can identify several fungi (*Coccidioides immitis*, *Histoplasma capsulatum*, and *Blastomyces dermatitidis)* that require special handling in biological safety cabinets, thereby decreasing reporting times considerably. Probes for bacteria include the following: GBS, *Streptococcus pneumoniae*, *S. aureus*, *Listeria monocytogenes*, and NG. *Mycobacterium* kits are also available, including probes to identify MTB complex, *Mycobacterium avium* complex, and species-specific *M. avium*, *Mycobacterium intracellulare*, *Mycobacterium gordonae*, and *Mycobacterium kansasii*. In contrast to molecular methods, traditional biochemical methods to speciate *Mycobacterium* may take as long as 2 months, whereas these probes can complete identification in <1 hour. Table 4 lists culture confirmation and other assays.

Summary and Conclusions

This review is intended to provide the current state-of-the-art information on FDA- cleared/approved molecular assays to diagnose infectious diseases. The technology has evolved rapidly over the past 25 years from a chemiluminescent probe performed on the routine bacteriology bench top to sophisticated and automated platforms where a patient specimen is extracted and molecular targets are detected within short turnaround times. Although the application of MID tests in the diagnosis and management of infectious diseases has greatly improved health care, it is limited to institutions with certain capacities. The availability of well-characterized FDA-cleared or FDA-approved MID tests reduces the burden on laboratories in developing or validating laboratory-developed tests, facilitates implementation of molecular testing in more laboratories, and allows for the standardization and comparability of tests, thus greatly improving health care outcomes. Because of the impact on patient care and the

evolving technology, we can expect major changes in molecular testing in the next few years.

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