Identification of Microorganisms Using Nucleic Acid Probes

Description

Nucleic acid probes can identify microorganisms more rapidly than traditional culture. Direct probes identify organisms that are present using immunoassays or fluorescence in situ hybridization (FISH). Polymerase chain reaction (PCR) can be used to amplify probe signals to increase the sensitivity of detection. Quantitative probes are also available for some organisms, with which an estimate of the number of organisms present is made.

Background

Until recently, identification of microorganisms depended either on culture of body fluids or tissues or identification of antigens, using a variety of techniques including direct fluorescent antibody technique and qualitative or quantitative immunoassays. These techniques are problematic when the microorganism exists in very small numbers or is technically difficult to culture. Indirect identification of microorganisms by immunoassays for specific antibodies reactive with the microorganism is limited by difficulties in distinguishing between past exposure and current infection; although to some extent IgM versus IgG antibodies can be helpful. Response to treatment is typically assessed according to the patient’s clinical response or by rising titers of specific antibodies and falling antigen titers.

The availability of nucleic acid probes has permitted the rapid direct identification of microorganisms’ DNA or RNA. Amplification techniques, including but not limited to the polymerase chain reaction (PCR), results in exponential increases in copy numbers of a targeted strand of microorganism-specific DNA. The most commonly used amplification technique is the polymerase chain reaction (PCR) or reverse transcriptase PCR. In addition to PCR, other nucleic acid amplification techniques have been developed such as transcription-mediated amplification (TMA), loop-mediated isothermal DNA amplification (LAMP), strand displacement amplification, nucleic acid sequence-based amplification and branched chain DNA signal amplification. After amplification, target DNA can be readily detected using a variety of techniques. The amplified product can also be quantified to give an assessment of how many microorganisms are present. Quantification of the amount of nucleic acids permits serial assessments of response to treatment; the most common clinical application of
quantification is the serial measurement of human immunodeficiency virus (HIV) RNA (called viral load), which serves as a prognostic factor.

A number of different microorganisms are reviewed as follows:

*Bartonella henselae or quintana*: *Bartonella henselae or quintana* is thought to be responsible for cat scratch disease, which is characterized by chronic regional lymphadenopathy developing about 2 weeks after contact with a cat. A cat scratch skin antigen test is positive in the majority of patients with cat scratch disease, but this test cannot distinguish between active and remote infection.

Bartonella may also cause an opportunistic infection in HIV-infected patients, in whom it is characterized by an acute febrile bacteremic illness, evolving to an asymptomatic bacteremia and finally indolent vascular skin lesions. The organism is typically detected using culture techniques, although an incubation period of 5 to more than 30 days is required. DNA probe technology has been investigated as a diagnostic technique.

*Borrelia burgdorferi*: *Borrelia burgdorferi* is responsible for Lyme disease. Antibody assays are typically the first diagnostic laboratory test performed; but these assays may be negative during early disease, and, in the later course of the disease, immunologic assays cannot distinguish between past and present infections, a severe limiting factor in areas of high prevalence. The spirochete is also difficult to culture, in part because the number of organisms in clinical specimens is extremely low. Therefore, in some instances, PCR amplification has been used to confirm the diagnosis of active Lyme disease. High sensitivities have been reported from synovial fluid samples; sensitivities of the PCR technique for cerebrospinal fluid, blood, and urine have been disappointing (low and/or variable).

*Candida species*: A commonly occurring yeast, *Candida species* normally can be found on diseased skin, throughout the entire gastrointestinal tract, expectorated sputum, the female genitalia, and in urine of patients with indwelling Foley catheters. Clinically significant *Candida* infections are typically diagnosed by clinical observation or by identification of the yeast forms on biopsy specimens. *Candida species* are a common cause of vaginitis.

*Chlamydia pneumoniae*: *Chlamydia pneumoniae* is an important cause of pneumonia, bronchitis, and sinusitis. Culture and isolation of the microorganism is difficult; a microimmunofluorescence serum test may be used. The use of PCR amplification now offers a rapid diagnosis.

*Chlamydia trachomatis*: *Chlamydia trachomatis* is a significant intracellular pathogen causing, most prominently, urogenital disease (including pelvic inflammatory disease) and perinatal infections. *Chlamydia trachomatis* is also responsible for lymphogranuloma venereum (LGV). Due to its prevalence and association with pelvic inflammatory disease and perinatal disease, widespread testing of chlamydia is recommended; routine chlamydia testing has been adopted as a quality measure by Healthcare Effectiveness Data and Information Set (HEDIS). This microorganism can be diagnosed by: 1) identifying the typical intracytoplasmic inclusions in cytology specimens; 2) isolation in tissue culture; 3) demonstration of chlamydial antigen by enzyme-linked immunosorbent assay or by immunofluorescent staining; or 4) demonstration of DNA using a direct probe or amplification technique.
**Cytomegalovirus:** Cytomegalovirus (CMV) is a common virus that infects many, but rarely causes clinical disease in healthy individuals. However, this virus can cause protean disease syndromes, most prominently in immunosuppressed patients, including transplant recipients or those infected with the HIV virus. CMV can also remain latent in tissues after recovery of the host from an acute infection. Diagnosis depends on demonstration of the virus or viral components or demonstration of a serologic rise. DNA probe techniques, including amplification, have also been used to identify patients at risk for developing CMV disease as a technique to triage antiviral therapy.

**Clostridium difficile:** *Clostridium difficile* is an anaerobic, toxin-producing bacteria present in the intestinal tract. It causes clinical colitis when the normal intestinal flora is altered and overgrowth of *C. difficile* occurs. The common precipitant that disrupts the normal intestinal flora is previous treatment with antibiotics. The disorder has varying severity but can be severe and in extreme cases, life-threatening. *C. difficile* is easily spread from person-to-person contact and is a common cause of hospital-acquired outbreaks. Hospital infection control measures, such as wearing gloves and handwashing with soap and water, are effective methods of reducing the spread of *C. difficile*. The standard diagnosis is made by an assay for the *C. difficile* cytotoxin or by routine culture methods.

**Enterovirus:** Enteroviruses are single-stranded ribonucleic acid (RNA) viruses. This group of viruses includes the polioviruses, coxsackieviruses, echoviruses, and other enteroviruses. In addition to 3 polioviruses, there are more than 60 types of non-polio enteroviruses that can cause disease in humans. Most people who are infected with a non-polio enterovirus have no disease symptoms at all. Infected persons who develop illness usually develop either mild upper respiratory symptoms, flu-like symptoms with fever and muscle aches, or an illness with rash. Less commonly, some persons have "aseptic" or viral meningitis. The use of amplified probe DNA test(s) can be used to detect enteroviruses.

**Gardnerella vaginalis:** A common microorganism, *Gardnerella vaginalis* is typically found in the human vagina and is usually asymptomatic. However, *G. vaginalis* is found in virtually all women with bacterial vaginosis and is characterized by inflammation and perivaginal irritation. The microorganism is typically identified by culture. The role of *G. vaginalis* in premature rupture of membranes and preterm labor is also under investigation.

**Hepatitis B, C, and G:** Hepatitis is typically diagnosed by a pattern of antigen and antibody positivity. However, the use of probe technology permits the direct identification of hepatitis DNA or RNA, which may also provide prognostic information. Quantification techniques are used as a technique for monitoring the response to interferon and/or ribavrin therapy in patients with hepatitis C.

**Herpes simplex virus (HSV):** Herpes simplex infection of the skin and mucous membranes is characterized by a thin-walled vesicle on an inflammatory base typically in the perioral, ocular, or genital area, although any skin site may be involved. The diagnosis may depend on pathologic examination of cells scraped from a vesicle base or by tissue culture techniques. Herpes simplex encephalitis is one of the most common and serious sporadic encephalitides in immunocompetent adults. The PCR technique to detect HSV in the cerebrospinal fluid has been used to provide a rapid diagnosis of herpes virus encephalitis.
**Herpes virus-6**: Human herpes virus-6 (HHV-6) is widespread in the general population and is also responsible for roseola, a benign rash and fever occurring in young children. HHV-6 may also cause meningitis, encephalitis, pneumonitis, and hepatitis in children and adults. Diagnosis is typically based on rising serologic titers.

**HIV-1, HIV-2**: DNA probe technology for HIV-1 is widely disseminated, and HIV-1 quantification has become a standard laboratory test in HIV-1 infected patients. HIV-2 can result in severe immunosuppression and the development of serious opportunistic diseases. Although HIV-2 has been reported in the United States, it is most commonly found in Western Africa. Blood donations are routinely tested for HIV-2, but due to its rarity in this country, clinical testing for HIV-2 is typically limited to those in contact with persons in a country where HIV-2 is endemic or when the clinical picture suggests HIV infection, but testing for HIV-1 is negative.

**Influenza virus**: Influenza virus is a very common pathogen that accounts for a high burden of morbidity and mortality, especially in elderly and immunocompromised patients. The most common means of identifying influenza is by viral culture, which takes 48-72 hours to complete. Influenza is highly contagious and has been the etiology of numerous epidemics and pandemics. Identification of outbreaks is important so that isolation measures may be undertaken to control the spread of disease. Anti-viral treatment can be effective if instituted early in the course of disease. Therefore, rapid identification of influenza virus is important in making treatment decisions for high-risk patients and in instituting infection control practices.

**Legionella pneumophila**: Legionella pneumophila is among the most common microbial etiologies of community acquired pneumonia. Laboratory diagnosis depends on culture, direct fluorescent antibody tests, urinary antigens, or DNA probe. DNA probe techniques have also been used in epidemiologic investigations to identify the source of a Legionella outbreak.

**Mycobacteria species**: Although mycobacterium can be directly identified in sputum samples (i.e., acid fast bacilli), these organisms may take 9 to 16 days to culture. DNA probes have also been used to identify specific mycobacterial groups (i.e., mycobacterial tuberculosis, avian complex, or intracellulare) after culture. In addition, amplification techniques for mycobacterium tuberculosis may be used in patients who have a positive smear. The rapid identification of *Mycobacteria tuberculosis* permits prompt isolation of the patient and identification of the patient’s contacts for further testing.

**Mycoplasma pneumoniae**: Mycoplasma pneumoniae is an atypical bacterium that is a common cause of pneumonia. It is most prevalent in younger patients, younger than age 40 years and in individuals who live or work in crowded areas such as schools or medical facilities. The infection is generally responsive to antibiotics of the macrolide or quinolone class. Most patients with mycoplasma pneumonia recover completely, although the course is sometimes prolonged for up to 4 weeks or more. Extra-pulmonary complications of mycoplasma pneumonia occur uncommonly, including hemolytic anemia and the rash of erythema multiforme.

**Neisseria gonorrhoeae**: Isolation by culture is the conventional form of diagnosis for this common pathogen. Direct DNA probes and amplification techniques have also been used. Neisseria is often tested for at the same time as Chlamydia.
Papillomavirus: Papillomavirus species are common pathogens that produce epithelial tumors of the skin and mucous membranes, most prominently the genital tract. Physical examination is the first diagnostic technique. Direct probe and amplification procedures have been actively investigated in the setting of cervical lesions. The ViraPap test is an example of a commercially available direct probe technique for identifying papillomavirus. There has also been interest in evaluating the use of viral load tests of papilloma virus to identify patients at highest risk of progressing to invasive cervical carcinoma.

Streptococcus, group A: Also referred to as Streptococcus pyogenes, this pathogen is the most frequent cause of acute bacterial pharyngitis. It can also give rise to a variety of cutaneous and systemic conditions, including rheumatic fever and poststreptococcal glomerulonephritis. Throat culture is the preferred method for diagnosing S pharyngitis. In addition, a variety of commercial kits are now available that use antibodies for the rapid detection of group A carbohydrate antigen directly from throat swabs. While very specific, these kits are less sensitive than throat cultures, so a negative test may require confirmation from a subsequent throat culture. DNA probes have also been used for direct identification of streptococcus and can be used as an alternative to a throat culture as a back-up test to a rapid, office-based strep test.

Streptococcus, group B (GBS): Also referred to as Streptococcus agalactiae, GBS is the most common cause of sepsis, meningitis, or death among newborns. Early-onset disease, within 7 days of birth, is related to exposure to GBS colonizing the mother’s anogenital tract during birth. The Centers for Disease Control and Prevention (CDC), the American College of Obstetrics and Gynecology (ACOG), and the American Academy of Pediatricians (AAP) recommend either maternal risk assessment or screening for GBS in the perinatal period. Screening consists of obtaining vaginal and anal specimens for culture at 35 to 37 weeks’ gestation. The conventional culture and identification process requires 48 hours. Therefore there has been great interest in developing rapid assays using DNA probes to shorten the screening process, so that screening could be performed in the intrapartum period with institution of antibiotics during labor.

Trichomonas Vaginalis: Trichomonas is a single-cell protozoan that is a common cause of vaginitis. The organism is sexually transmitted and can infect the urethra or vagina. The most common way of diagnosing trichomonas is by clinical signs and by directly visualizing the organism by microscopy in a wet prep vaginal smear. Culture of trichomonas is limited by poor sensitivity. Treatment with metronidazole results in a high rate of eradication. The disease is usually self-limited without sequelae, although infection has been associated with premature birth and higher rates of HIV transmission, cervical cancer, and prostate cancer.

A list of current U.S. Food and Drug Administration (FDA)-approved or cleared nucleic acid-based microbial tests is available at: http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm330711.htm

The Association of Molecular Pathology (AMP) website also provides a list of current U.S. FDA approved tests for diagnosis of infectious diseases (available online at: http://www.amp.org/FDATable/FDATable.pdf).
### FDA Approved Diagnostic Test

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Test Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus anthracis</td>
<td>Real-time PCR</td>
</tr>
<tr>
<td>Coxiella burnetii (Q fever)</td>
<td>Real-time PCR</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>PNA (Peptide nucleic acid) FISH</td>
</tr>
<tr>
<td>Escherichia coli and Pseudomonas aeruginosa</td>
<td>PNA FISH</td>
</tr>
<tr>
<td>Escherichia coli and/or Klebsiella pneumoniae</td>
<td>PNA FISH</td>
</tr>
<tr>
<td>Escherichia coli, Klebsiella pneumoniae and</td>
<td>PNA FISH</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
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<tr>
<td>Francisella tularensis</td>
<td>Real-time PCR</td>
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<tr>
<td>Leishmania</td>
<td>Real-time PCR</td>
</tr>
<tr>
<td>Yersinia pestis</td>
<td>Real-time PCR</td>
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<tr>
<td>Adenovirus</td>
<td>Multiplex Real-time RT-PCR</td>
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<tr>
<td>Avian Flu</td>
<td>Real-time RT-PCR</td>
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<td>Human metapneumovirus</td>
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<tr>
<td>Influenza virus A/H5</td>
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<tr>
<td>Influenza virus H1N1</td>
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<td>Respiratory virus panel</td>
<td>RT-PCR, ASPE, Tag sorting</td>
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### Policy

*This policy statement applies to clinical review performed for pre-service (Prior Approval, Precertification, Advanced Benefit Determination, etc.) and/or post-service claims.*

The status of nucleic acid identification using direct probe, amplified probe, or quantification for 30 microorganisms are summarized in the following table:

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Direct Probe</th>
<th>Amplified Probe</th>
<th>Quantification</th>
</tr>
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<tbody>
<tr>
<td>Bartonella henselae or quintana</td>
<td>inv</td>
<td>inv</td>
<td>inv</td>
</tr>
<tr>
<td>Borrelia burgdorferi</td>
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<td>Inv</td>
<td>Inv</td>
</tr>
<tr>
<td>Candida species</td>
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<td>Med nec</td>
<td>inv</td>
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<tr>
<td>Chlamydia pneumoniae</td>
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<td>Inv</td>
<td>Inv</td>
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<tr>
<td>Chlamydia trachomatis</td>
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<td>med nec</td>
<td>inv</td>
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<td>Clostridium difficile</td>
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<td>Mednec</td>
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<td>Cytomegalovirus</td>
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<td>med nec</td>
<td>Mednec</td>
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<td>Enterovirus</td>
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<tr>
<td>Vancomycin resistance (e.g., enterococcus vanA, vanB)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Influenza virus</td>
<td>med nec</td>
<td>med nec</td>
<td>med nec</td>
</tr>
</tbody>
</table>
Gardnerella vaginalis  med nec  inv  inv
Hepatitis B  med nec  med nec  med nec
Hepatitis C  med nec  med nec  med nec
Hepatitis G  inv  inv  inv
Herpes simplex virus  med nec  med nec  inv
Herpes virus-6  inv  inv  inv
HIV-1  med nec  med nec  med nec
HIV-2  med nec  med nec  med nec
Legionella pneumophila  inv  inv  inv
Mycobacterium species  med nec  inv  inv
Mycobacterium tuberculosis  med nec  med nec  inv
Mycobacterium avium intracellulare  med nec  inv  inv
Mycoplasma pneumoniae  inv  inv  inv
Neisseria gonorrhoeae  med nec  med nec  inv
Papillomavirus  med nec  med nec  inv
Staphylococcus aureus  med nec  med nec  med nec
Staphylococcus aureus, methicillin resistant  med nec  med nec  med nec
Streptococcus group A*  med nec  inv  inv
Streptococcus group B  med nec  med nec
Trichomonas vaginalis  med nec  Med nec

*The direct DNA probe test for streptococcus A is designed to be an alternative to a confirmatory culture. Therefore, the simultaneous use of confirmatory culture and DNA probe test is considered **not medically necessary**. Antibiotic sensitivity of streptococcus A cultures is frequently not performed for throat cultures. However, if an antibiotic sensitivity is considered, then the most efficient method of diagnosis would be a combined culture and antibiotic sensitivity.

**Policy Guidelines**

It should be noted that the technique for quantification includes both amplification and direct probes; therefore, simultaneous coding for both quantification with either amplification or direct probes, is not warranted.

In the evaluation of Group B streptococcus, the primary advantage of a DNA probe technique compared to traditional culture techniques is the rapidity of results. This advantage suggests that the most appropriate use of the DNA probe technique is in the setting of impending labor, for which prompt results could permit the initiation of intrapartum antibiotic therapy.
Many probes have been combined into panels of tests. For the purposes of this policy, other than the respiratory virus panel, only individual probes are reviewed.

Rationale

Although nucleic acid probe technologies offer the potential for rapid, sensitive detection for a variety of microorganisms, there are many technologic limitations, and the clinical application of these techniques is still developing. This technology requires the identification and manufacture of nucleic acid probes, i.e., short strands of either DNA or RNA, which are specific to the target microorganism. Amplification requires the use of specific short segments of complementary DNA, called primers, to initiate the repetitive rounds of DNA duplication. For many of the microorganisms, these probes or primers are not commercially available, and different reference laboratories may use different products. Amplification techniques raise considerable concerns regarding contamination from one specimen to another, creating the potential for false positive results. Nonspecific amplification is also a concern related in part to the specificity of the probes used. The clinical interpretation of results may also be challenging. Amplification of organisms representing latent infection or colonization cannot be distinguished from active, clinically significant infections. In addition, amplification techniques may amplify fragments of nucleic acids, representing dead microorganisms, thus further clouding the clinical interpretation. Finally, specificities, sensitivities, and positive and negative predictive values have not been reported in large groups of patients for many of the microorganisms. Newer nucleic acid amplification techniques have been developed to reduce concerns regarding possible laboratory cross contamination and improve the clinical relevance of test results with higher sensitivity rates.

In general, nucleic acid probe techniques are used when traditional culture is difficult due to the low numbers of the organisms (i.e., HIV), fastidious or lengthy culture requirements (i.e., mycobacterium, chlamydia, or neisseriae), or difficulty in collecting an appropriate sample (herpes simplex encephalitis). (1-4) Quantification is a useful clinical tool when the viral load can be used as a prognostic indicator or to follow the patient’s response to therapy; this is an established practice in patients with HIV or hepatitis C. The clinical utility, and medical necessity, of these probes will be determined in part by the accuracy of the test (sensitivity, specificity and predictive value) compared to standard identification techniques. The rapidity of results will also be considered, with the clinical utility of early identification considered in the context of each clinical situation to determine medical necessity.

Bartonella henselae or quintana. Microbiologic detection of Bartonella henselae or quintana is difficult, and molecular testing is not readily available. However, a monoclonal antibody (mAB) to B henselae has become commercially available. A 2009 study (5) evaluated the usefulness of immunohistochemical analysis (IHC) for diagnosing B henselae on surgical specimens and compared these results with polymerase chain reaction (PCR) detection and serologic testing. The study included 24 formalin-fixed, paraffin-embedded (FFPE) cases of lymphadenitis with histologic and/or clinical suspicion of B henselae. Control cases included 14 cases of lymphadenopathy. FFPE tissue sections were evaluated with a mAB to B henselae, Steiner silver stain (SSS), and PCR that targeted B henselae and B quintana. Positive cases were as follows: SSS, 11 (46%); PCR, 9 (38%); and IHC, 6
(25%). Only 2 cases (8%) were positive for all 3 techniques. All control cases were negative for IHC and PCR. The diagnostic sensitivity of these 3 tests is low for Bartonella. SSS seems to be the most sensitive test but is the least specific. PCR is more sensitive than IHC and may, therefore, serve as a helpful second-line test on all IHC negative cases.

*Borrelia burgdorferi.* DNA probes are available to aid in diagnosis of Lyme disease caused by *Borrelia burgdorferi.* A recent study (6) evaluated the sensitivity of 5 direct diagnostic methods (culture and nested PCR of a 2-mm skin biopsy specimen, nested PCR, and quantitative PCR [qPCR] performed on the same 1-mL aliquot of plasma and a novel qPCR-blood culture method) in 66 untreated adult patients with erythema migrans, the most common clinical manifestation. The results found one or more of these tests were positive in 93.9% of the patients. Culture was more sensitive than PCR for both skin and blood, but the difference was only statistically significant for blood samples (p<0.005). Blood culture was significantly more likely to be positive in patients with multiple erythema migrans skin lesions compared to those with a single lesion (p=0.001). Positive test results among the 48 patients for whom all 5 assays were performed invariably included either a positive blood or a skin culture. Results of this study demonstrated that direct detection methods such as PCR and culture are highly sensitive in untreated adult patients with erythema migrans. Erythema migrans eventually resolves even without antibiotic treatment. However, the infecting pathogen can spread to other tissues and organs, causing more severe manifestations that can involve a patient's skin, nervous system, joints, or heart. Diagnosed cases are usually treated with antibiotics for 2-4 weeks, and most patients make an uneventful recovery. (7) Therefore, laboratory evidence of infection is essential for diagnosis, except in the case of typical erythema migrans.

*Candida species.* DNA probes are available to aid in diagnosis of possible *Candida* species infections. Amplified peptide nucleic acid tests have demonstrated high sensitivity and specificity levels of up to 100%. (8, 9) Some tests have been able to detect up to 6 *Candida* species. (10) A real-time quantitative PCR assay, developed for the detection of the most common pathogenic *Candida* species using a single-reaction PCR assay targets a selected region of the 28S subunit of the fungal rDNA gene. In a 2012 study, the sensitivity and specificity of an assay based on quantitative real-time assay using duplex mutation primers were 100 and 97.4%, respectively (9). The data suggest that this assay may be appropriate for use in clinical laboratories as a simple, low-cost, and rapid screening test for the most frequently encountered *Candida* species.

*Chlamydia pneumoniae or trachomatis.* Probes are commercially available for the detection of *Chlamydia pneumonia* or *trachomatis.* A 2011 study (7) demonstrated a Chlamydia-specific real-time PCR which targeted the conserved 16S rRNA gene. The test can detect at least 5 DNA copies and shows very high specificity without cross-amplification from other bacterial DNA. The PCR was validated with 128 clinical samples positive or negative for *Chlamydia trachomatis* or *C. pneumoniae.* Of 65 positive samples, 61 (93.8%) were found to be positive with the new PCR. Another study (11) demonstrated the VERSANT® CT/GC DNA 1.0 Assay performed with 99.2% specificity for *Chlamydia trachomatis* detection and sensitivity of 100%. As a clinical consideration, patients with suspected *C trachomatis* accept antibiotic treatment before their infection status had been confirmed. Treatment of individuals with *C trachomatis* genital infection prevents sexual transmission and complications, including pelvic inflammatory disease. Treatment of pregnant women will prevent the transmission of infection to infants during delivery. The benefits of treatment of respiratory infections due to *C*
*pneumoniae* are more difficult to assess, primarily because of the lack of U.S. Food and Drug Administration (FDA)-approved, specific diagnostic tests for detection of the organism in clinical samples. (12)

**Clostridium difficile.** DNA probes for *Clostridium difficile* using PCR have been commercially available since 2009. (13-16) Eastwood et al. (14) compared the performance characteristics of numerous DNA probes with cytotoxic assays and cultures. The results demonstrated a mean sensitivity of 82.8% (range 66.7-91.7%) and a mean specificity of 95.4% (range 90.9-98.8%). Rapid identification of *C difficile* allows for early treatment of the disease and timely institution of isolation measures to reduce transmission. Because of the advantages of early identification of *C difficile*, these probes may be considered medically necessary.

**Cytomegalovirus (CMV).** There is interest in using viral load tests for cytomegalovirus (CMV), specifically to identify asymptomatic immunosuppressed patients (i.e., transplant recipients) who would be candidates for preemptive antiviral therapy. For example, among transplant recipients, CMV infections account for about two thirds of deaths in the immediate post-transplant period (i.e., up to 50 days post-transplant), and thus a variety of preventive therapies have been investigated. One strategy proposes that all at-risk patients (i.e., seropositive patients or seronegative patients receiving a seropositive organ) be treated prophylactically with antiviral therapy during the first 100 days after transplantation. While this strategy has been shown to be effective in reducing the risk of CMV disease, it results in a large number of patients being treated unnecessarily. Therefore, preemptive therapy has become an accepted option, in which antiviral therapy is initiated when a laboratory technique identifies an increasing viral load. Late CMV disease, defined as occurring after 100 days, is also a concern, and viral loads can also be monitored to prompt antiviral therapy. A variety of laboratory techniques are available to evaluate viral loads. For example, pp65 antigenemia refers to a fluorescent antigen detection technique that identifies an antigen specific to CMV. However, this test is described as labor intensive and requiring specialized personnel for interpretation, and thus a variety of tests to detect CMV DNA have been developed, including but not limited to Hybrid Capture (Digene Corporation), Amplicor CMV Monitor Tests (Roche Molecular System), and TaqMan. The specific techniques used may vary by local availability, but studies have suggested that all provide complementary information. (17-21)

**Enterovirus.** Amplified DNA probes are available for detecting this group of viruses including the polioviruses, coxsackieviruses, echoviruses, and other enteroviruses. In addition to 3 polioviruses, there are more than 60 types of non-polio enteroviruses that can cause disease in humans. Several FDA-approved test kits are available including the GeneXpert Enterovirus Assay (GXEA), with a sensitivity, specificity, positive predictive value and negative predictive value of 82.1%, 100%, 100% and 96.2%, respectively. In this study, molecular assays were superior to viral culture for detecting Enterovirus RNA in CSF. GXEA showed a high specificity but a lower sensitivity for the detection of Enterovirus RNA compared to the RT-qPCR assay. (22) Management is supportive and addresses symptoms. No antiviral medications are currently approved for the treatment of Enterovirus infections. These amplified probes can be part of a panel that includes other respiratory viruses. (See “respiratory panels.”)
Vancomycin-resistant Enterococcus. Probes are available for detecting vancomycin resistance of organisms; e.g., for Enterococcus. These probes are able to detect vancomycin resistance in a rapid and accurate manner so that appropriate antibiotic selection can be made and infectious precautions, such as isolation, can be instituted. (23, 24)

Gardnerella vaginalis. A 2006 study (25) evaluated vaginal specimens, from 321 symptomatic women, that were analyzed for bacterial vaginosis by both Gram stain using Nugent criteria and a DNA hybridization test (Affirm VPIII hybridization test). Of the 321 patients, 115 (35.8%) were Gram-positive for bacterial vaginosis and 126 (39.2%) were negative. 80 patients (25.0%) demonstrated intermediate Gram staining that was also considered negative. The DNA hybridization test detected Gardnerella vaginalis in 107 (93.0%) of 115 vaginal specimens positive for bacterial vaginosis diagnosed by Gram stain. Compared to the Gram stain, the DNA hybridization test had a sensitivity of 87.7% and a specificity of 96.0%. Positive and negative predictive values of the DNA hybridization test were 93.0% and 92.7%, respectively. The study concluded the Affirm VPIII hybridization test correlated well with Gram stain and may be used as a rapid diagnostic tool to exclude bacterial vaginosis in women with genital complaints.

Hepatitis B. Viral load has also been investigated in patients with hepatitis B receiving the antiviral therapy lamivudine. Research interest has focused on assessing response to therapy and identifying the emergence of resistant strains of hepatitis B. (26-29) Although clearly many aspects of viral load measurements in hepatitis B are primarily research tools, it does appear that measurements of viral load may be used to determine when to initiate therapy. For example, treatment may not be required in asymptomatic Hbe-Ag-negative patients with normal liver enzymes who have a viral load below 10^5 genomes per milliliter. In contrast, there are questions about how viral load measurements should be used to monitor the response to therapy. (30)

Hepatitis C. Diagnostic tests for hepatitis C can be divided into 2 general categories: 1) serological assays that detect antibody to hepatitis C virus (anti-HCV); and 2) molecular assays that detect, quantify, and/or characterize HCV RNA genomes within an infected patient. Detection of HCV RNA in patient specimens by polymerase chain reaction (PCR) provides evidence of active HCV infection and is potentially useful for confirming the diagnosis and monitoring the antiviral response to therapy. Two main technologies exist for assessing HCV RNA levels or viral load. Quantitative PCR is the most sensitive test for determining hepatitis C viral load. Molecular tests have also been developed to classify HCV into distinct genotypes; the clinical importance of HCV genotype is related directly to treatment options. After the introduction of the HCV RNA PCR test, it became clear that interferon therapy can cure hepatitis C infections in a certain number of patients. Widespread therapy was introduced after a co-drug ribavirin was found to reduce relapse rates, and 2 pivotal trials with recombinant interferon showed sustained virological responses in about 50% of patients, with much higher positive outcomes in genotype 2 and 3. (31) Therapy-induced sustained virological remission has been shown to reduce liver-related death, liver failure, and to a lesser extent hepatocellular carcinoma.

Hepatitis G. It is possible that hepatitis C is part of a group of GB viruses, rather than just a single virus. It is unclear if hepatitis G causes a type of acute or chronic illness. When diagnosed, acute hepatitis G infection has usually been mild and brief and there is no evidence of serious complications,
but it is possible that, like other hepatitis viruses, it can cause severe liver damage resulting in liver failure. The only method of detecting hepatitis G is by reverse transcriptase-polymerase chain reaction (RT-PCR) and direct sequencing for 4 randomly selected samples followed by phylogenetic analysis.

**Herpes simplex virus.** Typing of Herpes simplex virus (HSV) isolates is required to identify the virus isolated in culture. The methods available for this include antigen detection by immunofluorescence (IF) assays and polymerase chain reaction (PCR). A 2009 cross-sectional study (32) utilized 4 reference strains and 42 HSV isolates obtained from patients between September 1998 and September 2004. These were subjected to testing using a MAb-based IF test and a PCR that detects the polymerase (pol) gene of HSV isolates. The observed agreement of the MAb IF assay with the pol PCR was 95.7%. A total of 54.8% (23/42) of isolates tested by IF typing were found to be HSV-1, 40.5% (17/42) were HSV-2, and 2 (4.8%) were un typable using the MAb IF assay. The 2 untappable isolates were found to be HSV-2 using the pol PCR. According to the American Academy of Family Physicians, antiviral medications have expanded treatment options for the 2 most common cutaneous manifestations, HSV-1 and HSV-2. Acyclovir therapy remains an effective option; however, famciclovir and valacyclovir offer improved oral bioavailability and convenient oral dosing schedules but at a higher cost. Patients who have 6 or more recurrences of genital herpes per year can be treated with daily regimens which are effective in suppressing 70 to 80% of symptomatic recurrences.

**Herpes virus-6.** Herpes virus-6 is the common collective name for Human herpesvirus 6A (HHV-6A) and Human herpesvirus 6B (HHV-6B). These closely related viruses are 2 of the 9 herpesviruses known to have humans as their primary host. HHV-6A has been described as more neurovirulent (33) and as such, is more frequently found in patients with neuroinflammatory diseases, such as multiple sclerosis. (34) HHV-6B primary infection is the cause of the common childhood illness exanthema subitum. Additionally, HHV-6B reactivation is common in transplant recipients, which can cause several clinical manifestations such as encephalitis, bone marrow suppression and pneumonitis. (35)

**Human Immunodeficiency Virus 1 (HIV-1).** Validated DNA probes are widely available for diagnosis and quantification. Quantification is regularly done to determine viral load in infected patients to monitor response to anti-retroviral therapies.

**Human Immunodeficiency Virus (HIV-2).** DNA probes are available for diagnosis and quantification of HIV-2. HIV-2 is most commonly found in Western Africa, although it has been reported in the United States. Blood donations are routinely tested for HIV-2, but clinical testing for HIV-2 is typically limited to those in contact with persons in a country where HIV-2 is endemic or when clinical evaluation suggests HIV infection, but testing for HIV-1 is negative. HIV-2 quantification is regularly done to determine viral load in infected patients to monitor response to anti-retroviral therapies.

**Human papillomavirus (HPV).** There has also been research interest in exploring the relationship of human papilloma viral load and progression of low-grade cervical lesions to cervical cancer. While studies have reported that high-grade lesions are associated with higher viral loads (36-37), clinical utility is based on whether or not the presence of increasing viral loads associated with low-grade lesions is associated with disease progression. For example, current management of cervical smears with “atypical cells of uncertain significance” suggests testing with HPV, and then, if positive, followed by colposcopy. It is hypothesized that colposcopy might be deferred if a low viral load were associated
Influenza virus. Numerous different strains of influenza virus can be identified by DNA probes. Published studies indicate improved sensitivity of PCR for identifying influenza and distinguishing influenza from related viruses. Lassauniere et al. (41) used a multiplex real-time PCR probe to identify 13 respiratory viruses, including influenza A and B. Screening of 270 samples that were negative on immunofluorescence assays revealed the presence of a respiratory virus in 44.1%. Probes have also been developed to identify specific strains of influenza associated with epidemics, such as the H1N1 influenza virus. (42) Because of the importance of early identification of outbreaks for infection control purposes, use of this test may be considered medically necessary.

Legionella pneumophila. DNA probes for Legionella pneumophila have been developed. A recent study (43) compared the usefulness of 2 quantitative real-time PCR assays (qrt-PCRmip targeting L pneumophila, and qrt-PCR16S targeting all Legionella species) performed on lower respiratory tract (LRT) samples for diagnostic and prognostic purposes in 311 patients hospitalized for community-acquired pneumonia (CAP). The New Legionella urinary antigen test from Binax (Portland, ME, USA) was used as a reference test. One subset of 255 CAP patients admitted to Chambéry hospital in 2005 and 2006 was evaluated and the sensitivities, specificities, positive predictive and negative predictive values for both qrt-PCR tests were 63.6, 98.7, 77.7 and 97.4%, respectively. High bacterial loads in LRT samples at hospital admission were significantly associated with the need for hospitalization in an intensive care unit and for prolonged hospitalization.

Mycobacterium species. DNA probes are available to distinguish between Mycobacterium species. In a recent study, (44) the extracted DNA specimens from Mycobacterium species and non-Mycobacterial species were tested using peptide nucleic acid (PNA) probe-based real-time PCR assay to evaluate potential cross-reactivity. A total of 531 respiratory specimens (482 sputum specimens and 49 bronchoalveolar washing fluid specimens) were collected from 230 patients in July and August, 2011. All specimens were analyzed for the detection of Mycobacteria by direct smear examination, Mycobacterial culture, and PNA probe-based real-time PCR assay. In cross-reactivity tests, no false-positive or false-negative results were evident. When the culture method was used as the gold standard test for comparison, PNA probe-based real-time PCR assay for detection of Mycobacterium tuberculosis complex (MTBC) had a sensitivity and specificity of 96.7% (58/60) and 99.6% (469/471), respectively. Assuming the combination of culture and clinical diagnosis as the standard, the sensitivity and specificity of the real-time PCR assay for detection of MTBC were 90.6% (58/64) and 99.6% (465/467), respectively. The new real-time PCR for the detection of non-tuberculous mycobacteria had a sensitivity and specificity of 69.0% (29/42) and 100% (489/489), respectively.

Mycobacterium tuberculosis. DNA probes are available to diagnose Mycobacterium tuberculosis infection. In a recent study, (45) an in house IS6110 real-time PCR (IH IS6110), MTB Q-PCR Alert (Q-PCR) and GenoType® MTBDRplus (MTBDR) were compared for the direct detection of M tuberculosis complex (MTBC) in 87 specimens. This included 82 first smear-positive specimens and three smear-negative specimens. The sensitivities of IH IS6110, Q-PCR, MTBDR, and IH ITS for MTBC detection were 100%, 92%, 87%, and 87% respectively, compared to culture. Both IS6110-based real-time PCRs (in-house and Q-PCR) were similar in performance with 91.2% concordant results for MTBC detection.
However, none of the real-time PCR assays tested provide drug resistance data. Detection and drug resistance profiling are necessary for successful treatment of infection.

**Mycobacterium avium and Mycobacterium intracellulare.** DNA probes are available to diagnose *Mycobacterium avium* and *Mycobacterium intracellulare* infection. A recent study (46) evaluated the performance of the GenoType Mycobacteria Direct (GTMD) test for rapid molecular detection and identification of the MTBC and 4 clinically important non-tuberculous mycobacteria (*M avium*, *M intracellulare*, *M kansasii*, and *M malmoense*) in smear-negative samples. A total of 1,570 samples (1,103 bronchial aspiration, 127 sputum, and 340 extrapulmonary samples) were analyzed. When evaluated, the performance criteria in combination with a positive culture result and/or the clinical outcome of the patients, the overall sensitivity, specificity, and positive and negative predictive values were found to be 62.4, 99.5, 95.9, and 93.9%, respectively, whereas they were 63.2, 99.4, 95.7, and 92.8%, respectively, for pulmonary samples and 52.9, 100, 100, and 97.6%, respectively, for extrapulmonary samples. Among the culture-positive samples which had *Mycobacterium* species detectable by the GTMD test, 3 samples were identified to be *M intracellulare* and one sample was identified to be *M avium*. However, 5 *M intracellulare* samples and an *Mycobacterium kansasii* sample could not be identified by the molecular test and were found to be negative. The GTMD test is a reliable, practical, and easy tool for rapid diagnosis of smear-negative pulmonary and extrapulmonary tuberculosis so that effective precautions may be taken and appropriate treatment may be initiated.

**Mycoplasma pneumonia.** Probes for mycoplasma pneumonia have been developed. (47, 48) Chalker et al. (47) tested 3,987 nose and throat swabs from patients presenting with symptoms of a respiratory tract infection. Mycoplasma DNA was present in 1.7% of patients overall and was more common in children aged 5-14 years, in whom 6.0% of samples were positive. Probes have also been developed to test for mycoplasma strains with macrolide resistance. Peuchant et al. (48) found that 9.8% (5/51) of mycoplasma strains were macrolide resistant. However, the clinical utility of this probe is uncertain given that the disease is usually self-limited. It is unclear whether early identification of mycoplasm, and/or identification of resistance, leads to improved outcomes.

**Neisseria gonorrhoeae.** Probes for *Neisseria gonorrhoeae* have been developed for commercial use. These probes are often a combination test with *C trachomatis*. A recent study (49) demonstrated the positive predictive value of the screening PCR (cobas 4800 CT/NG PCR screening assay) in urine specimens remained high (98.75%) even though the prevalence of *gonorrhoeae* was low. Another study (11) demonstrated the VERSANT® CT/GC DNA 1.0 assay performed with 99.4% and 99.2% of specificity for *N gonorrhoeae* and *C trachomatis* detection, respectively, whereas sensitivity was 100% both for *C trachomatis* and *N gonorrhoeae*. As a comparator, culture methods were 100% specific, but far less sensitive. As a clinical consideration, patients accept antibiotic treatment before their infection status has been confirmed.

**Respiratory viral panel.** A broad spectrum of pathogens is causative for respiratory tract infections, but symptoms are mostly similar. The identification of the causative viruses is only feasible using multiplex PCR or several monoplex PCR tests in parallel. Several studies of various respiratory viral panels, (50-52) demonstrate the multiplex assay detected clinically important viral infections in a single genomic test and thus, may be useful for detecting causative agents for respiratory tract disorders. A 2011 study by Brittain-Long (53) on a randomized population of 406 patients with access to a rapid- multiplex-PCR
assay used to detect 13 viruses had lower antibiotic prescription rates (4.5% vs. 12.3%, respectively) versus delayed identification with no significant difference in outcome at follow-up (p=0.359). Access to a rapid method for etiologic diagnosis of respiratory tract infections may reduce antibiotic prescription rates at the initial visit in an outpatient setting. Rapid identification of influenza may also lead to more effective early treatment with antivirals and more effective infection control measures.

**Staphylococcus aureus and Methicillin-resistant Staphylococcus aureus.** Probes are available for the detection of *Staphylococcus aureus*. (54, 55) These probes are able to not only distinguish between coagulase-negative Staphylococcus and *S aureus*; they can also detect methicillin-resistant species (MRSA) with high accuracy. (24, 25) Given the importance of establishing an early and accurate diagnosis in clinical situations in which an *S aureus* infection is likely and there is substantial likelihood of MRSA, testing may be considered medically necessary in these situations. Probes are also available for the detection of enterovirus, although the clinical applicability of these probes has not been demonstrated.

**Streptococcus, Group A.** Confirmation of the diagnosis of *streptococcus A* is typically based on culture. However a direct DNA probe test for *streptococcus A*, using a throat swab, has been used as an alternative to culture, with the advantage of a 45-minute turnaround, compared to several days for culture. The summary of clinical studies included in the product label indicates a 97.4% agreement with confirmatory culture. (56) Furthermore, a recent study (57) of a laboratory-developed internally-controlled rapid Group A streptococcus (GAS) PCR assay using flocked swab throat specimens compared the GAS PCR assay to GAS culture results using a collection of archived throat swab samples obtained during a study comparing the performance of conventional and flocked throat swabs. The sensitivity of the GAS PCR assay as compared to the reference standard was 96.0% (95% confidence interval [CI]: 90.1% to 98.4%), specificity 98.6% (95% CI: 95.8% to 99.5%), positive predictive value (PPV) 96.9% (95% CI: 91.4% to 99.0%) and negative predictive value (NPV) of 98.1% (95% CI: 95.2% to 99.3%). The GAS PCR assay appeared to perform as well as conventional throat swab culture, the current standard of practice. Since the GAS PCR assay, including DNA extraction, can be performed in approximately 1 hour, prospective studies of this assay are warranted to evaluate the clinical impact of the assay on management of patients with pharyngitis.

**Group B Streptococcus.** Several different rapid polymerase chain reaction (PCR)-based tests for Group B streptococcus (GBS) have been developed, with reported sensitivities and specificities similar to that of conventional culture. DNA probes have also been developed to identify GBS from cultured specimens. (58, 59)

**Trichomonas vaginalis.** Nye et al. (60) compared the performance characteristics of PCR testing for trichomonas with wet prep microscopy and culture in 296 female and 298 male subjects. In both women and men, DNA probe testing of vaginal swabs was more sensitive than culture. However, in men wet prep testing was more sensitive than DNA probe testing. Munson et al. (61) compared DNA probe testing and culture in 255 vaginal saline preparations. The DNA probe identified trichomonas in 9.4% (24/255) of specimens that were negative on culture. This probe offers the ability to better distinguish between causes of vaginitis, which can be difficult clinically and using standard culture
Nucleic acid amplification tests have demonstrated higher clinical sensitivity than culture and wet mount microscopy (60), as well as single-probe nonamplified testing in general. A 2011 prospective multicenter study of 1,025 asymptomatic and symptomatic women found nucleic acid amplification testing had clinical sensitivity of 100% for both vaginal and endocervical swabs while urine specimen sensitivity was 95.2%. (62) Specificity levels ranged from 98.9% to 99.6%. Other studies have also reported similar results. (63) PCR amplification tests have higher clinical sensitivity and are considered the standard of care for diagnosing Trichomonas vaginalis when culturing is not an option.

**Summary**

Nucleic acid probes are available for identification of a wide variety of microorganisms, offering more rapid identification compared to standard cultures. Nucleic acid probes can also be used to quantitate the number of microorganisms present. This technology offers advantages over standard techniques when rapid identification is clinically important and/or when treatment decisions are based on quantitative results. Using these criteria, nucleic acid probes for numerous microorganisms can be considered **medically necessary**, as delineated in the policy statement.

**References**

<table>
<thead>
<tr>
<th>Subject:</th>
<th>Identification of Microorganisms Using Nucleic Acid Probes</th>
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56. Package Insert, GenProbe. Group A Streptococcus Direct Test.


Policy History

<table>
<thead>
<tr>
<th>Date</th>
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<tr>
<td>March 2012</td>
<td>New Policy</td>
<td>Policy updated with literature search, references updated, new information added to rationale for numerous probes. New medically necessary indication added for respiratory virus panel amplified probes.</td>
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<tr>
<td>December 2013</td>
<td>Update Policy</td>
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HIV-2, Diagnosis  
Influenza, Diagnosis  
Legionella, Diagnosis  
Mycobacteria, Diagnosis  
Mycoplasma, Diagnosis  
Neisseria Gonorrhoeae, Diagnosis  
Nucleic Acid Test (NAT)  
Papillomavirus, Diagnosis  
Streptococcus, Diagnosis  
Trichomonas vaginalis, Diagnosis  

This policy was approved by the FEP Pharmacy and Medical Policy Committee on December 6, 2013 and is effective January 15, 2014.

Signature on File  
Deborah M. Smith, MD, MPH