BlueCross BlueShield Federal Employee Program.

FEP 2.04.10 Identification of Microorganisms Using Nucleic Acid Probes

Effective Date: April 15, 2018

Related Policies:
5.01.15 IV Antibiotics Lyme Disease
2.04.127 Multitarget Polymerase Chain Reaction Testing for Diagnosis of Bacterial Vaginosis

Identification of Microorganisms Using Nucleic Acid Probes

Description

Nucleic acid probes are available for the identification of a wide variety of microorganisms. 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 when microbial identification using standard culture is difficult or impossible, and/or when treatment decisions are based on quantitative results.

FDA REGULATORY STATUS

A list of current U.S. Food and Drug Administration–approved or cleared nucleic acid–based microbial tests is available online.[3] Table 1 lists tests approved or cleared by the Food and Drug Administration that do not have specific CPT codes.

Table 1. FDA-Approved/Cleared Tests Without CPT Codes

FDA-Approved/Cleared Diagnostic Test	Test Type
Bacillus anthracis	Real-time PCR
Central Nervous System Panel (FilmArray Meningitis/Encephalitis- FDA 510k)	PCR of cerebrospinal fluid
Coxiella burnetii (Q fever)	Real-time PCR
Enterococcus faecalis	PNA FISH
Escherichia coli and *Pseudomonas aeruginosa*	PNA FISH
Escherichia coli and/or *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*	PNA FISH
Escherichia coli, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*	PNA FISH
Francisella tularensis	Real-time PCR
Leishmania	Real-time PCR
Yersinia pestis	Real-time PCR
Adenovirus	Multiplex real-time RT-PCR
Avian flu	Real-time RT-PCR
Human *metapneumovirus*	Multiplex real-time RT-PCR
Influenza virus A/H5	Real-time RT-PCR
Influenza virus H1N1	Real-time RT-PCR
Dengue virus	Real-time RT-PCR
Gram-positive/gram-negative bacteria panel	Multiplex nucleic acid amplification

FDA: U.S. Food and Drug Administration; FISH: fluorescence in situ hybridization; PCR: polymerase chain reaction; PNA: peptide nucleic acid; RT: reverse transcriptase.

FEP 2.04.10 Identification of Microorganisms Using Nucleic Acid Probes

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

POLICY STATEMENT

The use of nucleic acid testing using a direct or amplified probe technique (*without* quantification of viral load) may be considered **medically necessary** for the following microorganisms (see Policy Guidelines):

- *Bartonella henselae* or *quintana*
- *Candida* species
- *Chlamydia trachomatis*
- *Clostridium difficile*
- *Enterococcus*, vancomycin-resistant (eg, enterococcus vanA, vanB)
- *Enterovirus*
- *Gardnerella vaginalis*
- Herpes simplex virus
- Human papillomavirus
- *Legionella pneumophila*
- *Mycobacterium* species
- *Mycobacterium tuberculosis*
- *Mycobacterium avium-intracellulare*
- *Mycoplasma pneumoniae*
- *Neisseria gonorrhoeae*
- Respiratory virus panel
- *Staphylococcus aureus*
- *Staphylococcus aureus*, methicillin-resistant
- *Streptococcus*, group A
- *Streptococcus*, group B
- *Trichomonas vaginalis*

The use of nucleic acid testing using a direct or amplified probe technique (*with or without* quantification of viral load) may be considered **medically necessary** for the following microorganisms:

- Cytomegalovirus
- Hepatitis B virus
- Hepatitis C virus
- HIV-1
- HIV-2
- Human herpesvirus 6
- Influenza virus

The use of nucleic acid testing with quantification of viral load is considered **investigational** for microorganisms that are not included in the list of microorganisms for which probes with or without quantification are considered medically necessary.

The use of nucleic acid testing using a direct or amplified probe technique with or without quantification of viral load is considered **investigational** for the following microorganisms:

- *Chlamydophila pneumoniae*
- Hepatitis G virus
- Gastrointestinal pathogen panel

- Central nervous system pathogen panel

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.

Antibiotic sensitivity of streptococcus A cultures is generally 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.

For uncomplicated infections, testing for only 1 candida species, *Candida albicans*, may be considered medically necessary. For complicated infections, testing for multiple candida subspecies may be considered medically necessary. The Centers for Disease Control and Prevention (2010) classifies uncomplicated vulvovaginal candidiasis as being sporadic or infrequent; or mild to moderate; or likely to be *C. albicans*; or in nonimmunocompromised women. Complicated vulvovaginal candidiasis is classified as being recurrent or severe; or not a *C. albicans* species; or in women with uncontrolled diabetes, debilitation, or immunosuppression.

In the evaluation of group B streptococcus, the primary advantage of a DNA probe technique compared with 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 gastrointestinal pathogen panel, central nervous system panel, and the respiratory virus panel, only individual probes are reviewed.

BENEFIT APPLICATION

Screening (other than the preventive services listed in the brochure) is not covered. Please see Section 6 General exclusions.

Benefits are available for specialized diagnostic genetic testing when it is medically necessary to diagnose and/or manage a patient's existing medical condition. Benefits are not provided for genetic panels when some or all of the tests included in the panel are not covered, are experimental or investigational, or are not medically necessary.

Experimental or investigational procedures, treatments, drugs, or devices are not covered (See General Exclusion Section of brochure).

RATIONALE

Summary of Evidence

For individuals who have suspected *C. pneumoniae* who receive a nucleic acid probe for *C. pneumoniae*, the evidence includes prospective and retrospective evaluations of the tests' sensitivity and specificity. Relevant outcomes are test accuracy and validity, other test performance measures, symptoms, and change in disease status. The body of evidence is limited. One study was identified that reported relatively high sensitivity and specificity for a polymerase chain reaction–based test. However, the total number of patients in this study was small (N=56), and most other studies were conducted in the investigational setting. In addition to the limitations in the evidence base on test characteristics, the clinical implications of these tests are unclear. The evidence is insufficient to determine the effects of the technology on health outcomes.

For individuals who have hepatitis who receive a nucleic acid probe for hepatitis G, the evidence is lacking. Relevant outcomes are test accuracy and validity, other test performance measures, symptoms, and change in disease status. The clinical implications of this test are unclear. The evidence is insufficient to determine the effects of the technology on health outcomes.

For individuals who have signs and/or symptoms of gastroenteritis who receive nucleic acid–based gastrointestinal pathogen panel, the evidence includes prospective and retrospective evaluations of the tests' sensitivity and specificity. Relevant outcomes include test accuracy and validity, other test performance measures, symptoms, and change in disease status. The evidence suggests that gastrointestinal pathogen panels are likely to identify both bacterial and viral pathogens with high sensitivity, compared with standard methods. Access to a rapid method for etiologic diagnosis of gastrointestinal infections may lead to more effective early treatment and infection-control measures. However, in most instances, when a specific pathogen is suspected, individual tests could be ordered. There may be a subset of patients with an unusual presentation who would warrant testing for a panel of pathogens at once, but that subset has not been well defined. The evidence is insufficient to determine the effects of the technology on health outcomes.

For individuals who have signs and/or symptoms of meningitis and/or encephalitis who receive a nucleic acid–based central nervous system pathogen panel, the evidence includes retrospective evaluations of the tests' sensitivity and specificity. Relevant outcomes include test accuracy and validity, other test performance measures, symptoms, and change in disease status. Access to a rapid method that can simultaneously test for multiple pathogens may lead to the faster initiation of more effective treatment and conservation of cerebrospinal fluid. The available central nervous system panel is highly specific for the included organisms, but the sensitivity for each pathogen is not well-characterized. More than 15% of positives in the largest clinical validity study were false-positives. A negative panel result does not exclude infection due to pathogens not included in the panel. The evidence is insufficient to determine the effects of the technology on health outcomes.

SUPPLEMENTAL INFORMATION

Practice Guidelines and Position Statements

No guidelines or statements were identified.

U.S. Preventive Services Task Force Recommendations

Not applicable.

Medicare National Coverage

There is no national coverage determination (NCD). In the absence of an NCD, coverage decisions are left to the discretion of local Medicare carriers.

REFERENCES

1. Yoshikawa T. Human herpesvirus 6 infection in hematopoietic stem cell transplant patients. *Br J Haematol.* Feb 2004;124(4):421-432. PMID 14984492
2. Verani JR, McGee L, Schrag SJ, et al. Prevention of perinatal group B streptococcal disease--revised guidelines from CDC, 2010. *MMWR Recomm Rep.* Nov 19 2010;59(RR-10):1-36. PMID 21088663
3. U.S. Food and Drug Administration. Nucleic Acid Based Tests. 2017; http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm330711.htm. Accessed November 27, 2017.
4. Jensen WA, Fall MZ, Rooney J, et al. Rapid identification and differentiation of Bartonella species using a single-step PCR assay. *J Clin Microbiol.* May 2000;38(5):1717-1722. PMID 10790087
5. Johnson G, Ayers M, McClure SC, et al. Detection and identification of Bartonella species pathogenic for humans by PCR amplification targeting the riboflavin synthase gene (ribC). *J Clin Microbiol.* Mar 2003;41(3):1069-1072. PMID 12624031

6. Sander A, Penno S. Semiquantitative species-specific detection of Bartonella henselae and Bartonella quintana by PCR-enzyme immunoassay. *J Clin Microbiol.* Oct 1999;37(10):3097-3101. PMID 10488160
7. Hansmann Y, DeMartino S, Piemont Y, et al. Diagnosis of cat scratch disease with detection of Bartonella henselae by PCR: a study of patients with lymph node enlargement. *J Clin Microbiol.* Aug 2005;43(8):3800-3806. PMID 16081914
8. Caponetti GC, Pantanowitz L, Marconi S, et al. Evaluation of immunohistochemistry in identifying Bartonella henselae in cat-scratch disease. *Am J Clin Pathol.* Feb 2009;131(2):250-256. PMID 19141385
9. Flahaut M, Sanglard D, Monod M, et al. Rapid detection of Candida albicans in clinical samples by DNA amplification of common regions from C. albicans-secreted aspartic proteinase genes. *J Clin Microbiol.* Feb 1998;36(2):395-401. PMID 9466748
10. Xia QF, Liu JB, Liu P, et al. Development of a novel quantitative real-time assay using duplex mutation primers for rapid detection of Candida species. *Mol Med Rep.* Jan 2012;5(1):207-210. PMID 21964617
11. Das S, Brown TM, Kellar KL, et al. DNA probes for the rapid identification of medically important Candida species using a multianalyte profiling system. *FEMS Immunol Med Microbiol.* Mar 2006;46(2):244-250. PMID 16487306
12. Workowski KA, Berman S, Centers for Disease Control and Prevention (CDC), et al. Sexually transmitted diseases treatment guidelines, 2010. *MMWR Recomm Rep.* Dec 17 2010;59(RR-12):1-110. PMID 21160459
13. Stanek G, Wormser GP, Gray J, et al. Lyme borreliosis. *Lancet.* Feb 4 2012;379(9814):461-473. PMID 21903253
14. Marangoni A, Foschi C, Nardini P, et al. Evaluation of the new test VERSANT CT/GC DNA 1.0 assay for the detection of Chlamydia trachomatis and Neisseria gonorrhoeae in urine specimens. *J Clin Lab Anal.* Feb 2012;26(2):70-72. PMID 22467321
15. Centers for Disease Control and Prevention (CDC). Recommendations for the laboratory-based detection of Chlamydia trachomatis and Neisseria gonorrhoeae--2014. *MMWR Recomm Rep.* Mar 14 2014;63(RR-02):1-19. PMID 24622331
16. Tondella ML, Talkington DF, Holloway BP, et al. Development and evaluation of real-time PCR-based fluorescence assays for detection of Chlamydia pneumoniae. *J Clin Microbiol.* Feb 2002;40(2):575-583. PMID 11825973
17. Gaydos CA, Roblin PM, Hammerschlag MR, et al. Diagnostic utility of PCR-enzyme immunoassay, culture, and serology for detection of Chlamydia pneumoniae in symptomatic and asymptomatic patients. *J Clin Microbiol.* Apr 1994;32(4):903-905. PMID 8027341
18. Barbut F, Monot M, Rousseau A, et al. Rapid diagnosis of Clostridium difficile infection by multiplex real-time PCR. *Eur J Clin Microbiol Infect Dis.* Apr 13 2011;30(10):1279-1285. PMID 21487764
19. Eastwood K, Else P, Charlett A, et al. Comparison of nine commercially available Clostridium difficile toxin detection assays, a real-time PCR assay for C. difficile tcdB, and a glutamate dehydrogenase detection assay to cytotoxin testing and cytotoxigenic culture methods. *J Clin Microbiol.* Oct 2009;47(10):3211-3217. PMID 19710274
20. Huang H, Weintraub A, Fang H, et al. Comparison of a commercial multiplex real-time PCR to the cell cytotoxicity neutralization assay for diagnosis of clostridium difficile infections. *J Clin Microbiol.* Nov 2009;47(11):3729-3731. PMID 19741082
21. Knetsch CW, Bakker D, de Boer RF, et al. Comparison of real-time PCR techniques to cytotoxigenic culture methods for diagnosing Clostridium difficile infection. *J Clin Microbiol.* Jan 2011;49(1):227-231. PMID 20980562
22. Wootton SH, Aguilera E, Salazar L, et al. Enhancing pathogen identification in patients with meningitis and a negative Gram stain using the BioFire FilmArray((R)) Meningitis/Encephalitis panel. *Ann Clin Microbiol Antimicrob.* Apr 21 2016;15:26. PMID 27101869
23. Launes C, Casas-Alba D, Fortuny C, et al. Utility of FilmArray meningitis/encephalitis panel during outbreak of brainstem encephalitis caused by enterovirus in Catalonia in 2016. *J Clin Microbiol.* Jan 2017;55(1):336-338. PMID 27795349
24. Messacar K, Breazeale G, Robinson CC, et al. Potential clinical impact of the film array meningitis encephalitis panel in children with suspected central nervous system infections. *Diagn Microbiol Infect Dis.* Sep 2016;86(1):118-120. PMID 27342782
25. Rhein J, Bahr NC, Hemmert AC, et al. Diagnostic performance of a multiplex PCR assay for meningitis in an HIV-infected population in Uganda. *Diagn Microbiol Infect Dis.* Mar 2016;84(3):268-273. PMID 26711635
26. Arora HS, Asmar BI, Salimnia H, et al. Enhanced identification of group B Streptococcus and Escherichia Coli in young infants with meningitis using the Biofire Filmarray Meningitis/Encephalitis Panel. *Pediatr Infect Dis J.* Jul 2017;36(7):685-687. PMID 28114152

27. Lee CK, Chiu L, Yan G, et al. False negative results caused by erroneous automated result interpretation algorithm on the FilmArray 2.0 instrument. *Clin Chem Lab Med.* Aug 01 2017. PMID 28763299
28. Leber AL, Everhart K, Balada-Llasat JM, et al. Multicenter evaluation of biofire filmarray meningitis/encephalitis panel for detection of bacteria, viruses, and yeast in cerebrospinal fluid specimens. *J Clin Microbiol.* Sep 2016;54(9):2251-2261. PMID 27335149
29. Hanson KE, Slechta ES, Killpack JA, et al. Preclinical Assessment of a fully automated multiplex PCR panel for detection of central nervous system pathogens. *J Clin Microbiol.* Mar 2016;54(3):785-787. PMID 26719436
30. Graf EH, Farquharson MV, Cardenas AM. Comparative evaluation of the FilmArray meningitis/encephalitis molecular panel in a pediatric population. *Diagn Microbiol Infect Dis.* Jan 2017;87(1):92-94. PMID 27771208
31. Boivin G, Belanger R, Delage R, et al. Quantitative analysis of cytomegalovirus (CMV) viremia using the pp65 antigenemia assay and the COBAS AMPLICOR CMV MONITOR PCR test after blood and marrow allogeneic transplantation. *J Clin Microbiol.* Dec 2000;38(12):4356-4360. PMID 11101564
32. Humar A, Gregson D, Caliendo AM, et al. Clinical utility of quantitative cytomegalovirus viral load determination for predicting cytomegalovirus disease in liver transplant recipients. *Transplantation.* Nov 15 1999;68(9):1305-1311. PMID 10573068
33. Li H, Dummer JS, Estes WR, et al. Measurement of human cytomegalovirus loads by quantitative real-time PCR for monitoring clinical intervention in transplant recipients. *J Clin Microbiol.* Jan 2003;41(1):187-191. PMID 12517846
34. Razonable RR, van Cruijsen H, Brown RA, et al. Dynamics of cytomegalovirus replication during preemptive therapy with oral ganciclovir. *J Infect Dis.* Jun 1 2003;187(11):1801-1808. PMID 12751039
35. Weinberg A, Hodges TN, Li S, et al. Comparison of PCR, antigenemia assay, and rapid blood culture for detection and prevention of cytomegalovirus disease after lung transplantation. *J Clin Microbiol.* Feb 2000;38(2):768-772. PMID 10655383
36. de Crom SC, Obihara CC, van Loon AM, et al. Detection of enterovirus RNA in cerebrospinal fluid: comparison of two molecular assays. *J Virol Methods.* Jan 2012;179(1):104-107. PMID 22024398
37. Tsai JD, Tsai HJ, Lin TH, et al. Comparison of the detection rates of RT-PCR and virus culture using a combination of specimens from multiple sites for enterovirus-associated encephalomyelitis during enterovirus 71 epidemic. *Jpn J Infect Dis.* 2014;67(5):333-338. PMID 25241681
38. Dewan M, Zorc JJ, Hodinka RL, et al. Cerebrospinal fluid enterovirus testing in infants 56 days or younger. *Arch Pediatr Adolesc Med.* Sep 2010;164(9):824-830. PMID 20819964
39. Giulieri SG, Chapuis-Taillard C, Manuel O, et al. Rapid detection of enterovirus in cerebrospinal fluid by a fully-automated PCR assay is associated with improved management of aseptic meningitis in adult patients. *J Clin Virol.* Jan 2015;62:58-62. PMID 25542472
40. Appleman MD, Citron DM, Kwok R. Evaluation of the Velogene genomic assay for detection of vanA and vanB genes in vancomycin-resistant Enterococcus species. *J Clin Microbiol.* Apr 2004;42(4):1751-1752. PMID 15071039
41. Patel R, Uhl JR, Kohner P, et al. Multiplex PCR detection of vanA, vanB, vanC-1, and vanC-2/3 genes in enterococci. *J Clin Microbiol.* Mar 1997;35(3):703-707. PMID 9041416
42. Gazi H, Degerli K, Kurt O, et al. Use of DNA hybridization test for diagnosing bacterial vaginosis in women with symptoms suggestive of infection. *APMIS.* Nov 2006;114(11):784-787. PMID 17078859
43. Claas EC, Burnham CA, Mazzulli T, et al. Performance of the xTAG(R) gastrointestinal pathogen panel, a multiplex molecular assay for simultaneous detection of bacterial, viral, and parasitic causes of infectious gastroenteritis. *J Microbiol Biotechnol.* May 2013;23(7):1041-1045. PMID 23711521
44. Khare R, Espy MJ, Cebelinski E, et al. Comparative evaluation of two commercial multiplex panels for detection of gastrointestinal pathogens by use of clinical stool specimens. *J Clin Microbiol.* Oct 2014;52(10):3667-3673. PMID 25100818
45. Onori M, Coltella L, Mancinelli L, et al. Evaluation of a multiplex PCR assay for simultaneous detection of bacterial and viral enteropathogens in stool samples of paediatric patients. *Diagn Microbiol Infect Dis.* Jun 2014;79(2):149-154. PMID 24656922
46. Biswas JS, Al-Ali A, Rajput P, et al. A parallel diagnostic accuracy study of three molecular panels for the detection of bacterial gastroenteritis. *Eur J Clin Microbiol Infect Dis.* Nov 2014;33(11):2075-2081. PMID 24935616
47. Beckmann C, Heininger U, Marti H, et al. Gastrointestinal pathogens detected by multiplex nucleic acid amplification testing in stools of pediatric patients and patients returning from the tropics. *Infection.* Dec 2014;42(6):961-970. PMID 25015433

48. Al-Talib H, Latif B, Mohd-Zain Z. Pentaplex PCR assay for detection of hemorrhagic bacteria from stool samples. *J Clin Microbiol.* Sep 2014;52(9):3244-3249. PMID 24958797
49. Jiang Y, Fang L, Shi X, et al. Simultaneous detection of five enteric viruses associated with gastroenteritis by use of a PCR assay: a single real-time multiplex reaction and its clinical application. *J Clin Microbiol.* Apr 2014;52(4):1266-1268. PMID 24478418
50. Sorrell MF, Belongia EA, Costa J, et al. National Institutes of Health Consensus Development Conference Statement: management of hepatitis B. *Ann Intern Med.* Jan 20 2009;150(2):104-110. PMID 19124811
51. Lok AS, McMahon BJ. Chronic Hepatitis B: Update 2009. *Hepatology.* 2009;50(3):661-662. PMID 19714720
52. Abraham AM, Babu M, Kavitha S, et al. A molecular method for typing Herpes simplex virus isolates as an alternative to immunofluorescence methods. *Indian J Med Microbiol.* Jan-Mar 2009;27(1):22-26. PMID 19172054
53. Bressollette-Bodin C, Nguyen TV, Illiaquer M, et al. Quantification of two viral transcripts by real time PCR to investigate human herpesvirus type 6 active infection. *J Clin Virol.* Feb 2014;59(2):94-99. PMID 24380721
54. Agut H, Bonnafous P, Gautheret-Dejean A. Laboratory and clinical aspects of human herpesvirus 6 infections. *Clin Microbiol Rev.* Apr 2015;28(2):313-335. PMID 25762531
55. Abba MC, Mouron SA, Gomez MA, et al. Association of human papillomavirus viral load with HPV16 and high-grade intraepithelial lesion. *Int J Gynecol Cancer.* Mar-Apr 2003;13(2):154-158. PMID 12657116
56. Lorincz AT, Castle PE, Sherman ME, et al. Viral load of human papillomavirus and risk of CIN3 or cervical cancer. *Lancet.* Jul 20 2002;360(9328):228-229. PMID 12133661
57. Josefsson AM, Magnusson PK, Ylitalo N, et al. Viral load of human papilloma virus 16 as a determinant for development of cervical carcinoma in situ: a nested case-control study. *Lancet.* Jun 24 2000;355(9222):2189-2193. PMID 10881891
58. Schlecht NF, Trevisan A, Duarte-Franco E, et al. Viral load as a predictor of the risk of cervical intraepithelial neoplasia. *Int J Cancer.* Feb 10 2003;103(4):519-524. PMID 12478669
59. Ylitalo N, Sorensen P, Josefsson AM, et al. Consistent high viral load of human papillomavirus 16 and risk of cervical carcinoma in situ: a nested case-control study. *Lancet.* Jun 24 2000;355(9222):2194-2198. PMID 10881892
60. Saslow D, Solomon D, Lawson HW, et al. American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology screening guidelines for the prevention and early detection of cervical cancer. *Am J Clin Pathol.* Apr 2012;137(4):516-542. PMID 22431528
61. Wheeler CM, Hunt WC, Cuzick J, et al. The influence of type-specific human papillomavirus infections on the detection of cervical precancer and cancer: A population-based study of opportunistic cervical screening in the United States. *Int J Cancer.* Aug 1 2014;135(3):624-634. PMID 24226935
62. Lassauniere R, Kresfelder T, Venter M. A novel multiplex real-time RT-PCR assay with FRET hybridization probes for the detection and quantitation of 13 respiratory viruses. *J Virol Methods.* May 2010;165(2):254-260. PMID 20153377
63. Wenzel JJ, Walch H, Bollwein M, et al. Library of prefabricated locked nucleic acid hydrolysis probes facilitates rapid development of reverse-transcription quantitative real-time PCR assays for detection of novel influenza A/H1N1/09 virus. *Clin Chem.* Dec 2009;55(12):2218-2222. PMID 19797710
64. Maurin M, Hammer L, Gestin B, et al. Quantitative real-time PCR tests for diagnostic and prognostic purposes in cases of legionellosis. *Clin Microbiol Infect.* Apr 2010;16(4):379-384. PMID 19519843
65. Diederen BM, Kluytmans JA, Vandenbroucke-Grauls CM, et al. Utility of real-time PCR for diagnosis of Legionnaires' disease in routine clinical practice. *J Clin Microbiol.* Feb 2008;46(2):671-677. PMID 18094136
66. Mentasti M, Fry NK, Afshar B, et al. Application of Legionella pneumophila-specific quantitative real-time PCR combined with direct amplification and sequence-based typing in the diagnosis and epidemiological investigation of Legionnaires' disease. *Eur J Clin Microbiol Infect Dis.* Aug 2012;31(8):2017-2028. PMID 22278293
67. Choi YJ, Kim HJ, Shin HB, et al. Evaluation of peptide nucleic acid probe-based real-time PCR for detection of Mycobacterium tuberculosis complex and nontuberculous mycobacteria in respiratory specimens. *Ann Lab Med.* Jul 2012;32(4):257-263. PMID 22779066
68. Seagar AL, Neish B, Laurenson IF. Comparison of two in-house real-time PCR assays with MTB Q-PCR Alert and GenoType MTBDRplus for the rapid detection of mycobacteria in clinical specimens. *J Med Microbiol.* Oct 2012;61(Pt 10):1459-1464. PMID 22790204
69. Bicmen C, Gunduz AT, Coskun M, et al. Molecular detection and identification of mycobacterium tuberculosis complex and four clinically important nontuberculous mycobacterial species in smear-negative clinical samples by the genotype mycobacteria direct test. *J Clin Microbiol.* Aug 2011;49(8):2874-2878. PMID 21653780
70. Chalker VJ, Stocki T, Mentasti M, et al. Mycoplasma pneumoniae infection in primary care investigated by real-time PCR in England and Wales. *Eur J Clin Microbiol Infect Dis.* Jul 2011;30(7):915-921. PMID 21311941

71. Peuchant O, Menard A, Renaudin H, et al. Increased macrolide resistance of Mycoplasma pneumoniae in France directly detected in clinical specimens by real-time PCR and melting curve analysis. *J Antimicrob Chemother.* Jul 2009;64(1):52-58. PMID 19429926
72. Ishiguro N, Koseki N, Kaiho M, et al. Sensitivity and specificity of a loop-mediated isothermal amplification assay for the detection of mycoplasma pneumonia from nasopharyngeal swab samples compared with those of real-time PCR. *Clin Lab.* Jun 2015;61(5-6):603-606. PMID 26118195
73. Olson D, Watkins LK, Demirjian A, et al. Outbreak of Mycoplasma pneumoniae-associated Stevens-Johnson syndrome. *Pediatrics.* Aug 2015;136(2):e386-394. PMID 26216320
74. Miyashita N, Kawai Y, Inamura N, et al. Setting a standard for the initiation of steroid therapy in refractory or severe Mycoplasma pneumoniae pneumonia in adolescents and adults. *J Infect Chemother.* Mar 2015;21(3):153-160. PMID 25533771
75. Izumikawa K, Izumikawa K, Takazono T, et al. Clinical features, risk factors and treatment of fulminant Mycoplasma pneumoniae pneumonia: a review of the Japanese literature. *J Infect Chemother.* Mar 2014;20(3):181-185. PMID 24462437
76. Hopkins MJ, Smith G, Hart IJ, et al. Screening tests for Chlamydia trachomatis or Neisseria gonorrhoeae using the cobas 4800 PCR system do not require a second test to confirm: an audit of patients issued with equivocal results at a sexual health clinic in the Northwest of England, U.K. *Sex Transm Infect.* Nov 2012;88(7):495-497. PMID 22661631
77. Mansuy JM, Mengelle C, Da Silva I, et al. Performance of a rapid molecular multiplex assay for the detection of influenza and picornaviruses. *Scand J Infect Dis.* Dec 2012;44(12):963-968. PMID 22830610
78. Dabisch-Ruthe M, Vollmer T, Adams O, et al. Comparison of three multiplex PCR assays for the detection of respiratory viral infections: evaluation of xTAG respiratory virus panel fast assay, RespiFinder 19 assay and RespiFinder SMART 22 assay. *BMC Infect Dis.* Jul 24 2012;12:163. PMID 22828244
79. Pierce VM, Hodinka RL. Comparison of the GenMark Diagnostics eSensor respiratory viral panel to real-time PCR for detection of respiratory viruses in children. *J Clin Microbiol.* Nov 2012;50(11):3458-3465. PMID 22875893
80. Brittain-Long R, Westin J, Olofsson S, et al. Access to a polymerase chain reaction assay method targeting 13 respiratory viruses can reduce antibiotics: a randomised, controlled trial. *BMC Med.* Apr 26 2011;9:44. PMID 21521505
81. Kaplan S, Marlowe EM, Hogan JJ, et al. Sensitivity and specificity of a rapid rRNA gene probe assay for simultaneous identification of Staphylococcus aureus and detection of mecA. *J Clin Microbiol.* Jul 2005;43(7):3438-3442. PMID 16000472
82. Zhang K, Sparling J, Chow BL, et al. New quadriplex PCR assay for detection of methicillin and mupirocin resistance and simultaneous discrimination of Staphylococcus aureus from coagulase-negative staphylococci. *J Clin Microbiol.* Nov 2004;42(11):4947-4955. PMID 15528678
83. Shulman ST, Bisno AL, Clegg HW, et al. Clinical practice guideline for the diagnosis and management of group A streptococcal pharyngitis: 2012 update by the Infectious Diseases Society of America. *Clin Infect Dis.* Nov 15 2012;55(10):e86-102. PMID 22965026
84. Hologic. Group A Streptococcal Direct Test (103887 Rev. 001). 2016; http://www.hologic.com/sites/default/files/package%20inserts/103887-IFU-PI_001_01.pdf. Accessed December 13, 2017.
85. Quidel. LyraDirect Strep Assay. n.d.; https://www.quidel.com/sites/default/files/product/documents/PIM112001EN00.pdf. Accessed November 17, 2017.
86. Meridien Bioscience. Package Insert: Illumigene Group A Streptococcus DNA Amplification Assay. 2015; http://www.meridianbioscience.com/Content/Assets/PackInsert/8.5%20x%2011_5%20lang_CLEAN_SN11187_280150_illumigene%20Group%20A%20Strep%20PI_REV.%2004-15.pdf. Accessed November 17, 2017.
87. Slinger R, Goldfarb D, Rajakumar D, et al. Rapid PCR detection of group A Streptococcus from flocked throat swabs: a retrospective clinical study. *Ann Clin Microbiol Antimicrob.* Sep 02 2011;10:33. PMID 21888649
88. Anderson NW, Buchan BW, Mayne D, et al. Multicenter clinical evaluation of the illumigene group A Streptococcus DNA amplification assay for detection of group A Streptococcus from pharyngeal swabs. *J Clin Microbiol.* May 2013;51(5):1474-1477. PMID 23447639
89. Henson AM, Carter D, Todd K, et al. Detection of Streptococcus pyogenes by use of Illumigene group A Streptococcus assay. *J Clin Microbiol.* Dec 2013;51(12):4207-4209. PMID 24048538

90. Upton A, Bissessor L, Farrell E, et al. Comparison of illumigene Group A Streptococcus assay with culture of throat swabs from children with sore throats in the New Zealand school-based rheumatic fever prevention program. *J Clin Microbiol.* Jan 2016;54(1):153-156. PMID 26560542
91. Cohen DM, Russo ME, Jaggi P, et al. Multicenter Clinical Evaluation of the Novel Alere i Strep A Isothermal Nucleic Acid Amplification Test. *J Clin Microbiol.* Jul 2015;53(7):2258-2261. PMID 25972418
92. Faron ML, Ledeboer NA, Granato P, et al. Detection of Group A Streptococcus in pharyngeal swab specimens using the AmpliVue GAS Isothermal helicase-dependent amplification assay. *J Clin Microbiol.* Jul 2015;53(7):2365-2367. PMID 25972419
93. Boyanton BL, Jr., Darnell EM, Prada AE, et al. Evaluation of the Lyra Direct Strep Assay to detect Group A Streptococcus and beta-hemolytic groups C/G Streptococcus from pharyngeal specimens. *J Clin Microbiol.* Jan 2016;54(1):175-177. PMID 26491174
94. Bergeron MG, Ke D. New DNA-based PCR approaches for rapid real-time detection and prevention of group B streptococcal infections in newborns and pregnant women. *Expert Rev Mol Med.* Nov 2001;3(27):1-14. PMID 14585149
95. Bergeron MG, Ke D, Menard C, et al. Rapid detection of group B streptococci in pregnant women at delivery. *N Engl J Med.* Jul 20 2000;343(3):175-179. PMID 10900276
96. Nye MB, Schwebke JR, Body BA. Comparison of APTIMA Trichomonas vaginalis transcription-mediated amplification to wet mount microscopy, culture, and polymerase chain reaction for diagnosis of trichomoniasis in men and women. *Am J Obstet Gynecol.* Feb 2009;200(2):188 e181-187. PMID 19185101
97. Munson E, Napierala M, Basile J, et al. Trichomonas vaginalis transcription-mediated amplification-based analyte-specific reagent and alternative target testing of primary clinical vaginal saline suspensions. *Diagn Microbiol Infect Dis.* Sep 2010;68(1):66-72. PMID 20727473
98. Schwebke JR, Hobbs MM, Taylor SN, et al. Molecular testing for Trichomonas vaginalis in women: results from a prospective U.S. clinical trial. *J Clin Microbiol.* Dec 2011;49(12):4106-4111. PMID 21940475
99. Andrea SB, Chapin KC. Comparison of Aptima Trichomonas vaginalis transcription-mediated amplification assay and BD affirm VPIII for detection of T. vaginalis in symptomatic women: performance parameters and epidemiological implications. *J Clin Microbiol.* Mar 2011;49(3):866-869. PMID 21248097

POLICY HISTORY

Date	Action	Description
March 2012	New Policy	
December 2012	Update Policy	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.
December 2013	Update Policy	Policy updated with literature search. Candida species amplified probe changed from investigational to medically necessary. Medically necessary indication for Trichomonas vaginalis amplified probe added. References 8, 10, and 62-63 added.
December 2014	Update Policy	Policy updated with literature review. Added gastrointestinal pathogen panel as investigational to the policy statement. References 1, 27-28, 44-45 and 63-64 added.
March 2018	Update Policy	Policy updated with literature review through October 16, 2017; references updated/added. Medically necessary statement added for nonquantified nucleic acid-based testing for enterovirus, *Legionella pneumophila*, *Mycoplasma pneumoniae*, and *Bartonella spp*, and for quantified testing for human herpesvirus 6. *Borrelia* testing removed from policy.Investigational policy statement added for probes with quantification of viral load that do not meet criteria for quantification. Investigational statement added for central nervous system pathogen panel.