Medical Policy

Blue Cross Blue Shield Blue Care Network of Michigan

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(See policy history boxes for previous effective dates)

Title: Identification of Microorganisms Using Nucleic Acid Probes

Description/Background

This policy does NOT discuss nucleic acid amplification testing or polymerase chain reaction testing related to the diagnosis of vaginitis (bacterial vaginosis, candidiasis, trichomonas). See related policies.

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, microbial identification using standard culture is difficult or impossible, and/or treatment decisions are based on quantitative results.

Nucleic Acid Probes

A nucleic acid probe is used to detect and identify species or subspecies of organisms by identifying nucleic acid sequences in a sample. Nucleic acid probes detect genetic materials, such as RNA or DNA, unlike other tests, which use antigens or antibodies to diagnose organisms.

The availability of nucleic acid probes has permitted the rapid direct identification of microorganism DNA or RNA. Amplification techniques result in exponential increases in copy numbers of a targeted strand of microorganism-specific DNA. The most used amplification technique is 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, loop-mediated isothermal DNA amplification, 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 assess how many microorganisms are present. Quantification of the number 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 RNA (called viral load).

The direct probe technique, amplified probe technique, and probe with quantification methods vary based on the degree to which the nucleic acid is amplified and the method for measurement of the signal. The direct probe technique refers to detection methods in which nucleic acids are detected without an initial amplification step. The amplified probe technique refers to detection methods in which either target, probe, or signal amplification is used to improve the sensitivity of the assay over direct probe techniques, without quantification of nucleic acid amounts.

- Target amplification methods include PCR (including PCR using specific probes, nested or multiplex PCR), nucleic acid-based sequence amplification, transcription-mediated amplification, and strand displacement amplification. Nucleic acid-based sequence amplification and transcription-mediated amplification involve amplification of an RNA (rather than a DNA) target.
- Probe amplification methods include ligase chain reaction.
- Signal amplification methods include branched DNA (bDNA) probes and hybrid capture methods using an anti-DNA/RNA hybrid antibody.

The probe with quantification techniques refers to quantitative PCR or real-time PCR methods that use a reporter at each stage of the PCR to generate absolute or relative amounts of a known nucleic acid sequence in the original sample. These methods may use DNA-specific dyes (ethidium bromide or SYBR green), hybridization probes (cleavage-based[TaqMan] or displaceable), or primer incorporated probes.

Direct assays will generally have lower sensitivity than amplified probes. In practice, most commercially available probes are amplified, with a few exceptions. For this evidence review, indications for direct and/or amplified probes without quantification are considered together, while indications for a probe with quantification are considered separately.

Classically, identification of microorganisms relies either on the 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.

Potential reasons for a nucleic acid probe to be associated with improved clinical outcomes compared with standard detection techniques include the following (note: in all cases, for there to be clinical utility, making a diagnosis should be associated with changes in clinical management, which could include initiation of effective treatment, discontinuation of other therapies, or avoidance of invasive testing):

- Significantly improved speed and/or efficiency in making a diagnosis.
- Improved likelihood of obtaining any diagnosis in cases where standard culture is difficult. Potential reasons for difficulty in obtaining standard culture include low numbers of the organisms (e.g., HIV), fastidious or lengthy culture requirements (e.g., *Mycobacteria, Chlamydia*, *Neisseria* species), or difficulty in collecting an appropriate sample(e.g., herpes simplex encephalitis).
- There is no way to definitively make a diagnosis without nucleic acid testing.

- The use of nucleic acid probe testing provides qualitatively different information than that available from standard cultures, such as information regarding disease prognosis or response to treatment. These include cases where quantification of viral load provides prognostic information or is used to measure response to therapy.

The risks of nucleic acid testing include false-positive and false-negative results; inaccurate identification of pathogens by the device, inaccurate interpretation of test results, or incorrect operation of the instrument.

- False-positive results can lead to unnecessary treatment, with its associated toxicities and side effects, including allergic reaction. In addition, true diagnosis and treatment could be delayed or missed altogether.
- False-negative results could delay diagnosis and initiation of proper treatment.
- It is possible that these risks can be mitigated by the use of a panel of selected pathogens indicated by the clinical differential diagnosis while definitive culture results are pending.

Regulatory Status

The U.S. Food and Drug Administration maintains a list of nucleic acid amplification tests (NAATs) that have been cleared by the Center for Devices and Radiological Health. NAATs have been cleared for many of the microorganisms discussed in this review and may be reviewed on this site.

Table 1 summarizes the NAATs cleared for central nervous system panels when diagnosing meningitis and/or encephalitis, for gastrointestinal panels when diagnosing gastroenteritis, for respiratory panels and for urogenital Infections .

Table 1. FDA Cleared NAATs for CNS, GI, and Respiratory Panels

NAAT	Manufacturer	510(k) Number	Product Code
Meningitis/Encephalitis (CNS) Pathogen Panels			
FilmArray Meningitis/Encephalitis Panel	BioFire Diagnostics, LLC (Salt Lake City, UT)	DEN150013, K160462	PLO
Gastroenteritis Pathogen Panels			
xTAG Gastrointestinal Pathogen Panel (GPP)	Luminex Molecular Diagnostics, Inc (Toronto, Ontario, CA)	DEN130003, K121454	PCH
PANNAT STEC Test	Micronics, Inc. (Redmond, WA)	K173330	PCH
Progastro SSCS Assay	Gen-Probe Prodesse, Inc (Waukesha, WI)	K123274	PCH
Biocode Gastrointestinal Pathogen Panel (GPP)	Applied Biocode (Santa Fe Springs, CA)	K180041	PCH
Biocode Gastrointestinal Pathogen Panel	Applied Biocode (Santa Fe Springs, CA)	K190585	PCH
EntericBio Dx Assay	Serosep, Ltd (Annacotty, IE)	K182703	PCH
Filmarray Gastrointestinal Panel	BioFire Diagnostics, LLC (Salt Lake City, UT)	K140407, K160459	PCH
ProGastro SSCS	Hologic/Genprobe (Waukesha, WA)	K123274	PCH
BD MAX Enteric Bacterial Panel (EBP)	BD Diagnostics (Sparks, MD)	K170308	PCH
Verigene Enteric Pathogen Panel (EP)	Nanosphere, Inc (Northbrook, IL)	K142033K140083	PCH

xTAG Gastroenterology Pathogen Panel (GPP) Multiplex Nucleic Acid-Based Assay System	Luminex Molecular Diagnostics, Inc (Toronto, Ontario, CA)	K121894	PCH
FilmArray GI Panel	BioFire Diagnostics, Inc (Salt Lake City, UT)	K140407	PCH
Great Basin Stool Bacterial Pathogens Panel	Great Basin Scientific, Inc. (Salt Lake City, UT)	K163571	PCH
Respiratory Viral Panels			
Curetis Unyvero Lower Respiratory Panel	Opgen		
BIOFIRE SPOTFIRE Respiratory (R) Panel	BioFire Diagnostics, Inc (Salt Lake City, UT)	K230719	QOF
BIOFIRE SPOTFIRE Respiratory (R) Panel Mini	BioFire Diagnostics, Inc (Salt Lake City, UT)	K230719	QOF
QIAstat-Dx Respiratory Panel; QIAstat-Dx Analyzer	QIAGEN GmbH (Germantown, MD)	K183597	OCC
ID-TAG Respiratory Viral Panel Nucleic Assay System	Luminex Molecular Diagnostics, Inc (Toronto, Ontario, CA)	DEN070013, K063765	OCC
Biocode Respiratory Pathogen Panel	Applied BioCode, Inc. (Santa Fe Springs, CA)	K192485	OCC
Nxtag Respiratory Pathogen Panel	Luminex Molecular Diagnostics, Inc (Toronto, Ontario, CA)	K193167	OCC
NxTAG Respiratory Pathogen Panel v2 (NxTAG RPP v2)	Luminex Molecular Diagnostics, Inc(Toronto, Ontario, CA)	K231758	QOF
xTAG Respiratory Virus Panel (RVP)	Luminex Molecular Diagnostics, Inc (Toronto, Ontario, CA)	K081483	OCC
Qiastat-Dx Respiratory Panel	QIAGEN GmbH (Germantown, MD)	K183597	OCC
xTAG Respiratory Virus Panel FAST	Luminex Molecular Diagnostics, Inc (Toronto, Ontario, CA)	K103776	OCC
eSensor® Respiratory Virus Panel (RVP)	Clinical Micro Sensors, Inc (Carlsbad, CA)	K113731	JJH
Verigene Respiratory Pathogens Plus Nucleic Acid Test	Nanosphere, Inc (Northbrook, IL)	K103209	OCC
BioFire FilmArray Respiratory Panel (RP)	BioFire Diagnostics, Inc (Salt Lake City, UT)	K123620	OCC
Urogenital Infections			
Mycoplasma genitalium	Hologic, Inc.	DEN180047	QEP

CDC: Centers for Disease Control and Prevention; CNS: central nervous system; DEN: de novo; GI: gastrointestinal; NAAT: nucleic acid amplification test; FDA: Food and Drug Administration.

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments (CLIA).Laboratories that offer laboratory-developed tests must be licensed by the CLIA for high-complexity testing.

Medical Policy Statement

The use of nucleic acid testing using either single pathogens or panel testing is established in specified situations. It may be considered a useful diagnostic tool when indicated.

Inclusionary and Exclusionary Guidelines

The status of nucleic acid identification (using either direct probe, amplified probe, or quantification) for certain microorganisms are summarized in Table 2 by CPT code (if applies) and status of the procedure (established versus investigational):

Table 2. Determination Table for Microorganism by Test, CPT Code (If Applies), and Efficacy

Microorganism	*Direct Probe*	*Amplified Probe*	*Quantification*	*Other Techniques*
Bartonella henselae or quintana	EST	87471 - EST	87472 - INV	N/A
Candida species – non-vaginal	87480 – EST	87481 - EST	87482 - INV	N/A
Central nervous system pathogen panel[a]	EST	87483 - EST	EST	N/A
Chlamydia pneumoniae	87485 - EST	87486 - EST	87487 - INV	N/A
Clostridium difficile	EST	87493 - EST	INV	N/A
Cytomegalovirus	87495 - EST	87496 - EST	87497 - EST	N/A
Enterococcus, vancomycin-resistant	EST	87500 - EST	INV	N/A
Enterovirus	EST	87498 - EST	INV	N/A
Gastrointestinal pathogen panel	INV	87505, 87506 EST 87507 - INV	INV	N/A
Hepatitis B	EST	87516 - EST	87517 - EST	N/A
Hepatitis C	87520 - EST	87521 - EST	87522 - EST	N/A
Hepatitis D	NA	N/A	87523 – EST	N/A
Hepatitis G	87525 - INV	87526 - INV	87527 - INV	N/A
Herpes virus 6	87531 - EST	87532 - EST	87533 - EST	N/A
Human papillomavirus	EST	EST	INV	87623-87625 EST
Influenza virus	EST	87501-87503 - EST	EST	N/A
Legionella pneumophila	87540 - EST	87541 - EST	87542 - INV	N/A
Mycobacterium species	87550 - EST	87551 - EST	87552 - INV	N/A
Mycobacterium tuberculosis	87555 - EST	87556 - EST	87557 - INV	N/A
Mycobacterium avium intracellulare	87560 - EST	87561 - EST	87562 - INV	N/A
Mycoplasma pneumoniae	87580 - EST	87581 - EST	87582 - INV	N/A
Papillomavirus	87623-87625 - EST	87623-87625 - EST	INV	N/A
Respiratory syncytial virus (RSV)	N/A	87634 - EST	N/A	N/A
Respiratory virus panel	EST	87631-87633 - EST	INV	N/A
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[b]	N/A	87635 - EST	N/A	N/A
Sexually Transmitted Diseases				
• Chlamydia trachomatis	87490 – EST	87491 - EST	87492 – INV	N/A
• Herpes Simplex Virus	87528 - EST	87529 – EST	87530 – INV	N/A
• HIV 1	87534 - EST	87535 - EST	87536 - EST	N/A
• HIV 2	87537 - EST	87538 - EST	87539 - EST	N/A
• Mycoplasma Genitalium	N/A	87563 - EST	N/A	N/A
• Neisseria gonorrhoeae	87590 - EST	87591 - EST	87592 - INV	N/A
Staphylococcus aureus	EST	87640 - EST	INV	N/A
Staphylococcus aureus, methicillin-resistant	EST	87641 - EST	INV	N/A
Streptococcus, group A	87650 - EST	87651 - EST	87652 - INV	N/A
Streptococcus, group B	EST	87653 - EST	INV	N/A
Urinary tract infections[a]	EST	EST	INV	N/A
Zika virus	EST	87662 - EST	INV	N/A

[a] Considered established when criteria below are met.
[b] This medical policy does not address antibody testing (serological IgG assays).
EST – established; INV - investigational.

Panel testing using nucleic acid probes for **central nervous system** pathogens are considered established when **ONE** of the following criteria are met:

- As an adjunct to standard work-up with cerebral spinal fluid (CSF) culture and sensitivity and other epidemiologic and laboratory data
- The individual has clinical findings consistent with a central nervous system infection (e.g., meningitis, encephalitis)
- Viral etiology is suspected or CSF culture is inconclusive for a pathogen (i.e., viral, bacterial fungal, yeast)

Repeat panel testing for the same clinical indication will only be covered when **ALL** of the following are met:

- First panel yielded a negative result.
- There is a high index of suspicion for a pathogen as the cause of symptoms.
- The individuals clinical condition is not improving or is deteriorating after a clinically appropriate length of time.

Single nucleic acid probe testing for **urinary tract pathogens** to diagnose an infection (i.e., prostatitis, urinary tract infection) is considered established when **ALL** the following are met:

- Documentation includes proper technique for urine specimen collection (i.e., clean catch, straight catheter)
- Urinary tract symptoms (dysuria, frequency, urgency) remain after treatment with two courses of antibiotics based on results of urine culture & sensitivity.
- The individual has a current immunocompromised[a] condition or has post-surgical abnormal genital urinary tract anatomy.

Note: Criteria for single nucleic acid probe testing for urinary tract pathogens is established for covered organisms without a specific CPT code and up to 10 units/pathogens in infection diagnoses (i.e., prostatitis, UTI)

Polymerase chain reaction (PCR) testing for the following microorganisms that do not have specific CPT codes are considered established:

- Actinomyces, for identification of actinomyces species in tissue specimens
- Adenovirus, to diagnose any of the following:
  - Adenovirus myocarditis
  - Adenovirus infection in immunocompromised[a] hosts, including transplant recipients.
- Avian influenza A virus, for diagnosis of avian influenza A (H5N1) in persons with both:
  - Symptoms consistent with Avian influenza A virus
  - A history of travel to or contact with persons or birds from a country with documented H5N1 avian influenza infections within 10 days of symptoms onset.
- Bacillus anthracis
- BK polymavirus in transplant recipients receiving immunosuppressive therapies and persons with immunosuppressive disease[a.]
- Bordetella pertussis and B. parapertussis, for diagnosis of whooping cough in individuals with coughing
- Brucella spp., for members with signs and symptoms of Brucellosis, and history of direct contact with infected animals and their carcasses or secretions or by ingesting unpasteurized milk or milk products.
- Burkholderia infections (including B. cepacian, B. gladioli)
- Chancroid (Haemophilus ducreyi), for diagnosis of persons with genital ulcer disease

- Clostridium difficile
- Coxiella burnetiid (Q fever)
- Dengue virus
- Epidemic typhus (Rickettsia prowazekii)
- Epstein Barr Virus (EBV): for detection of EBV in post-transplant lymphoproliferative disorder; or for testing for EBV in persons with lymphoma; or for those who are immunocompromised[a] for other reasons.
- Francisella tularensis, for presumptive diagnosis of tularemia
- Hantavirus, diagnosis
- Hemorrhagic fevers and related syndromes caused by viruses of the family Bunyaviridae (Rift Valley fever, Crimean-Congo hemorrhagic fever, hemorrhagic fever with renal syndromes), for diagnosis in acute phase in persons with clinical presentation suggestive of these conditions.
- Hepatitis E virus (HEV), for definitive diagnosis in persons with anti-HEV antibodies
- Human metapneumovirus
- Human T Lymphotropic Virus type 1 and type 2 (HTLV-1 and HTLV-2), to confirm the presence of HTLV-1 and HTLV-2 in the cerebrospinal fluid of persons with signs or symptoms of HTLV-1/HTLV-2
- JC polyomavirus, in transplant recipients receiving immunosuppressive therapies, in persons with immunosuppressive diseases[a], and for diagnosing progressive multifocal leukoencephalopathy in persons with multiple sclerosis or Crohn's disease receiving natalizumab (Tysabri)
- Leishmania
- Measles virus (Morbilliviruses; Rubeola), for diagnosis of measles
- Mumps
- Neisseria meningitis, to establish diagnosis where antibiotics have been started before cultures have been obtained.
- Parvovirus, for detecting chronic infection in immunocompromised[a] persons.
- Psittacosis, for diagnosis of Chlamydophila (Chlamydia) psittaci infection
- Rubella, diagnosis
- Severe acute respiratory syndrome (SARS), for detection of SARS coronavirus RNA in persons with signs or symptoms of SARS who have traveled to endemic areas or have been exposed to persons with SARS.
- Toxoplasma gondii, for detection of T. gondii infection in immunocompromised[a] persons with signs and symptoms of toxoplasmosis, and for detection of congenital Toxoplasmagondii infection (including testing of amniotic fluid for toxoplasma infection)
- Varicella-Zoster infections
- Whipple's disease (T. whippeli), biopsy tissue from small bowel, abdominal or peripheral lymph nodes, or other organs of persons with signs and symptoms, to establish the diagnosis.
- Yersinia pestis

[a] Immunocompromised individuals consist of those with weakened immune systems including human immunodeficiency virus or acquired immunodeficiency syndrome, individuals who are taking immunosuppressive medications (i.e., chemotherapy, biologics, transplant-related immunosuppressive drugs, high-dose systemic corticosteroids) and those with inherited diseases that affect the immune system (i.e., congenital immunoglobulin deficiencies).

Exclusions:

- The use of nucleic acid testing with direct or amplified probes for the following microorganisms:
  - Hepatitis G
- Direct probe, amplified probe, or panel testing of pathogens used for the diagnosis of an uncomplicated urinary tract infection.
- Any nucleic acid panel or single pathogen testing for any of the following conditions:
  - Wound infection
  - Blood stream infection/sepsis
    - Exception: when criteria above are met for meningitis
- Testing that is performed as a test of cure.
- Molecular-based panel testing for general screening of microorganisms (e.g., MicroGenDX qPCR+ NGS)
- Any nucleic acid or PCR testing that is not FDA approved or identified above as established.

CPT/HCPCS Level II Codes *(Note: The inclusion of a code in this list is not a guarantee of coverage. Please refer to the medical policy statement to determine the status of a given procedure.)*

Established codes:

86794	87154	87471	87480	87481	87483
87485	87486	87490	87491	87493	87495
87496	87497	87498	87500	87501	87502
87503	87505	87506	87516	87517	87520
87521	87522	87523	87528	87529	87531
87532	87533	87534	87535	87536	87537
87538	87539	87540	87541	87550	87551
87555	87556	87560	87561	87563	87580
87581	87590	87591	87623	87624	87625
87631	87632	87633	87634	87635	87640
87641	87650	87651	87653	87662	87797
87798	0202U	0223U			

Other codes (investigational, not medically necessary, etc.):

87472	87482	87487	87492	87507	87525
87526	87527	87530	87542	87552	87557
87562	87582	87592	87652	87799	0115U
0225U	0321U	0455U	0480U	0483U	0484U
0505U					

Other various codes may apply.

**Established for covered organisms without a specific CPT code and up to 10 units/pathogens in infection diagnoses (i.e., prostatitis, UTI).*

Notes:
A panel cannot be unbundled and billed as individual components regardless of the fact that the test reports multiple individual pathogens and/or targets. The panel is a closed system performed on a single platform, and as such, is a single test panel with multiple components. Specific single organisms (not included in panel testing) may be ordered in addition to panel testing if the policy criteria determines that the specific single organism is established.

Code(s) may not be covered by all contracts or certificates. Please consult customer or provider inquiry resources at BCBSM or BCN to verify coverage. Established codes may be considered investigational for the purpose of this policy.

Rationale

Evidence reviews assess whether a medical test is clinically useful. A useful test provides information to make a clinical management decision that improves the net health outcome. That is, the balance of benefits and harms is better when the test is used to manage the condition than when another test or no test is used to manage the condition.

The evidence review section of this policy update focuses on pathogen panels. The supplemental information section contains supporting information for the medical necessity of the use of the organism-specific nucleic acid amplification tests (NAATs) which have guideline support. Guidelines from the Centers for Disease Control and Prevention, National Institute of Health, Infectious Disease Society of America or America Academy of Pediatrics were used to evaluate appropriate indications for a wide variety of microorganisms and to inform treatment decisions.

The purpose of nucleic acid-based specified pathogen panels is to provide a diagnostic option that is an alternative to or an improvement on existing tests for a wide variety of microorganisms in patients with signs and/or symptoms of disease and to inform treatment decisions.

True-positive and true-negative results lead to faster diagnosis and correct treatment, or no unnecessary treatment, as well as fewer repeated tests.

False-positive and false-negative results, inaccurate identification of a pathogen by the testing device, failure to correctly interpret test results, or failure to correctly operate the instrument may lead to misdiagnosis resulting in inappropriate treatment while postponing treatment for the true condition. Such a situation could lead to incorrect, unnecessary, or no treatment, necessity for additional testing, and delay of correct diagnosis and treatment.(12)

Review of Evidence

CENTRAL NERVOUS SYSTEM BACTERIAL AND VIRAL PANEL

Individuals with signs and/or symptoms of meningitis and/or encephalitis are managed by infectious disease specialists and emergency medicine professionals in an emergency or inpatient clinical setting. Testing with a CNS pathogen panel leads to reduced time to diagnosis compared with standard laboratory techniques (approximately 1-8 hours).(1)

The FilmArray Meningitis/Encephalitis (ME) Panel (BioFire Diagnostics, Salt Lake City, UT) is a nucleic acid-based test that simultaneously detects multiple bacterial, viral, and yeast nucleic acids from cerebrospinal fluid (CSF) specimens obtained via lumbar puncture from patients with signs and/or symptoms of meningitis and/or encephalitis. The test has been cleared for marketing through the FDA 510(k) process. The test identifies 14 common organisms responsible for community-acquired meningitis or encephalitis:

Bacteria: *Escherichia coli K1; Haemophilus influenzae; Listeria monocytogenes; Neisseria meningitidis; Streptococcus agalactiae; Streptococcus pneumoniae.*

Viruses: Cytomegalovirus; enterovirus; herpes simplex virus 1; herpes simplex virus 2; human herpes virus 6; human parechovirus; varicella-zoster virus.

Yeast: *Cryptococcus neoformans/gattii*

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Run-time is approximately 1 hour per specimen.

The standard approach to the diagnosis of meningitis and encephalitis is culture and pathogen-specific polymerase chain reaction (PCR) testing of CSF based on clinical characteristics. These techniques have a slow turnaround time, which can delay administration of effective therapies and lead to unnecessary empirical administration of broad-spectrum antimicrobials.

The systematic review and meta-analysis by Tansarli and Chapin (2020) examined the diagnostic accuracy of the BioFireFilmArray meningitis/encephalitis (ME) panel.(2) Thirteen studies (n=3764) were included in the review and 8 of them (n=3059) were pooled in a meta-analysis. The summary estimates of sensitivity and specificity with 95% confidence intervals (CI) was 90% (95% CI 86e93%) and 97% (95% CI 94e99%), respectively. When we looked specifically at studies that assessed further the false positive and false negative results, false positive detections were 11.4% and 4% before and after adjudication, respectively. The highest proportion of false positive was observed for *Streptococcus pneumoniae* followed by *Streptococcus agalactiae*. False negative isolates were 2.2% and 1.5% before and after adjudication, respectively. Herpes simplex virus 1 and 2, enterovirus and *Cryptococcus neoformans/gattii* had the highest proportions of false negative determinations. False negative *C. neoformans/gattii* were mostly patients with positive antigen titres, on treatment or cleared disease. Evidence suggested that the ME panel is a highly specific method with a high sensitivity. Concerns about false negative results with HSV-1/2 lead to recommendations for singleplex PCR and evaluation of other sources (i.e., blood, lesions) if the panel is negative and there is high clinical suspicion for HSV-1/2. The authors concluded that the ME panel can significantly improve the clinical and diagnostic algorithm used for CNS infections. However, the decision for implementation should be individualized based on the needs of the patient population, the capabilities of the laboratory, and the knowledge of the healthcare providers who are utilizing the test.

The study by Leber et al (2016), was a multicenter evaluation of BioFire FilmArray meningitis/encephalitis panel for the detection of bacteria, viruses, and yeast in cerebrospinal fluid specimens.(3) One thousand five hundred and sixty cerebral spinal fluid samples were evaluated for the 14 most frequent pathogens that cause meningitis/encephalitis (*Escherichia coli K1, Haemophilus influenzae, Listeria monocytogenes, Neisseria meningitidis, Streptococcus pneumoniae, Streptococcus agalactiae*, cytomegalovirus, enterovirus, herpes

simplex virus 1 and 2, human herpesvirus 6, human parechovirus, varicella-zoster virus, and *Cryptococcus neoformans/Cryptococcus gattii)* and compared them to culture and PCR. The FilmArray ME Panel demonstrated a sensitivity or positive percentage of agreement of 100% for 9 of 14 analytes. Enterovirus and human herpesvirus type 6 had agreements of 95.7% and 85.7%, and *L. monocytogenes* and *N. meningitidis* were not observed in the study. For *S. agalactiae*, there was a single false-positive and false-negative result each, for a sensitivity and specificity of 0 and 99.9%, respectively. The specificity or negative percentage of agreement was 99.2% or greater for all other analytes. Authors concluded that the FilmArray ME Panel is able to detect a broad range of pathogens directly in the CSF with good performance relative to culture and molecular reference methods. The panel may have significant utility in several patient populations (i.e., young infants, immunocompromised) and the simplicity of the testing process means it can be offered in a variety of care settings.

Cuesta et al (2024) prospectively evaluated the performance of a multiplex PCR assay (QIAstat-Dx ME panel) compared to conventional diagnostic methods and the Biofire FilmArray ME Panel for diagnosing meningoencephalitis in 50 CSF samples.(4)Conventional methods identified a pathogen in 29 CSF samples (58%), with 41% bacterial and 59% viral etiologies. The QIAstat-Dx ME panel demonstrated a sensitivity of 96.5% (95% CI, 79.8% to 99.8%) and specificity of 95.2% (95% CI, 75.2% to 99.7%),with high positive and negative predictive values (96.4% and 95.2%) and complete agreement (91.8%) with conventional methods based on Cohen's kappa. In contrast, the FilmArray ME panel had a lower sensitivity (85.1%; 95% CI, 55.9% to 90.2%),specificity (57.1%; 95 %CI, 29.6% to 70.3%), positive and negative predictive values and only moderate agreement (43.5%) with conventional methods. The FilmArray ME panel reported 7 single-pathogen and 5 polymicrobial false positive results, most commonly for HSV-1, while the QIAstat-Dx ME panel had only one false positive (VZV) and one false negative (HSV-1) result. Limitations include the enrichment of positive samples in the QIAstat-Dx ME analysis and the inability to evaluate all panel targets due to a lack of some positive CSF samples.

López et al (2024) retrospectively reviewed the performance of the Biofire FilmArray ME panel compared to conventional diagnostic methods in 313 patients with suspected ME seen at a single-center from 2018 to 2022.(5) FilmArray was positive in 84 cases (26.8%) (HSV-1 [10.9%], VZV [5.1%], Enterovirus [2.6%], and S. pneumonia [1.9%]). In the 136 cases where both FilmArray and routine methods were performed, there was a 25.7% lack of agreement. In the overall tested population, the sensitivity was estimated to be 81% (95% CI, 70.6% to 89%) with a specificity of 89% (95% CI, 85.4% to 93.4%). The authors reported a high NPV (93.4%; 95 %CI, 89.9% to 95.7%) and modest PPV (73%; 95 %CI, 64.6% to 80.1%). While FilmArray had a low false negative rate of 6.6%, it reported a high false positive rate of 28.6%, mainly due to HSV-1. The authors observed that the positive predictive value dropped to 36.9% in cases without pleocytosis and 70.2% in those lacking high CSF protein levels; other test characteristics were less impacted by individual CSF characteristics. Limitations include the retrospective single-center design and that conventional testing could not be performed on all samples due to insufficient volume.

In 2015, the FDA issued a *de novo* classification for BioFire Diagnostics FilmArray Meningitis/Encephalitis (ME) Panel.(56) The FilmArray Meningitis/Encephalitis (ME) Panel is a qualitative multiplexed nucleic acid based in vitro diagnostic test intended for use with FilmArray and FilmArray 2.0 systems. The FilmArray ME Panel is capable of simultaneous detection and identification of multiple bacterial, viral, and yeast nucleic acids directly from cerebrospinal fluid (CSF) specimens obtained via lumbar puncture from individuals with signs

and/or symptoms of meningitis and/or encephalitis. The Center for Devices and Radiological Health of the Food and Drug Administration determined that the FilmArray ME Panel is indicated as an aid in the diagnosis of specific agents of meningitis and/or encephalitis and results are meant to be used in conjunction with other clinical, epidemiological, and laboratory data. Results from the FilmArray ME Panel are not intended to be used as the sole basis for diagnosis, treatment, or other patient management decisions.

Section Summary: Central Nervous System Bacterial and Viral Panel
The FilmArray ME Panel provides fast diagnoses compared with standard culture and pathogen-specific PCR and, because it combines multiple individual nucleic acid tests, clinicians can test for several potential pathogens simultaneously. The test uses only a small amount of CSF, leaving remaining fluid for additional testing if needed. The test is highly specific for the included organisms. D*e novo* classification was granted by the FDA (2015) as adjunct testing to aid in the diagnosis of meningitis and/or encephalitis.

GASTROINTESTINAL PATHOGEN PANEL
The most common 2 types of GI pathogens are either bacterial or viral, including but not limited to the following:(6,7,8)

- Bacterial (common to U.S. and may be foodborne): *Bacillus* cereus, *Campylobacter, Clostridioides (Clostridium) difficile, Clostridium botulinum, Clostridium perfringens, Cronobacter sakazakii, Esherichia coli, Listeria monocytogenes, Salmonella* spp., *Shigella spp., Staphylococcus* aureus, *Yersinia enterocolitica*
- Viral: norovirus, rotavirus, adenovirus, astrovirus, sapovirus

Norovirus is the most common cause of foodborne illness in the U.S.(9)

These panels are capable of qualitatively detecting the DNA or RNA of multiple pathogens, including but not limited to *Campylobacter*, *Clostridioides (Clostridium) difficile, Plesiomonas shigelloides, Salmonella* spp., *Yersinia* spp., enteroaggregative *Escherichia coli*, enteropathogenic *E coli*, enterotoxigenic *E coli*, Shiga toxin-producing *E coli, E coli* O157, Shigella/enteroinvasive *E coli,* adenovirus F 40/41, astrovirus, norovirus, rotavirus, and sapovirus.

For community-acquired diarrheal illness, extensive GI panels for parasites and viruses may be unnecessary because these illnesses are usually self-limited and, as viruses, are treated with supportive care and hydration.(10) In situations in which the GI condition is likely foodborne based on patient history, GI pathogen panels may be limited to the most common pathogens typically found with foodborne illness. For patients who are immune competent, such a panel could include *Salmonella, Campylobacter, Shigella, Cryptosporidium* (parasite), Shiga toxin-producing *E. coli* (STEC), and STEC O157. More pathogen targets may be included if testing for *C. difficile* or testing patients who are critically ill or immunocompromised.(10)

Individuals with signs and/or symptoms of gastroenteritis and GI conditions are managed by primary care clinicians, infectious disease specialists, and emergency medicine professionals in an emergency or inpatient clinical setting. Time to a result of testing with a gastrointestinal pathogen panel is reduced compared with standard laboratory techniques (< 6 hours).(11)

Infectious gastroenteritis may be caused by a broad spectrum of pathogens resulting in the primary symptom of diarrhea. Panels for gastrointestinal pathogens use multiplex amplified

probe techniques and multiplex reverse transcription for the simultaneous detection of many gastrointestinal pathogens such as *C. difficile, Escherichia coli, Salmonella, Shigella, norovirus, rotavirus*, and *Giardia.* The performance study of the first FDA-cleared GI panel (xTAG Pathogen Panel [GPP], Luminex Molecular Diagnostics, Inc, Toronto, Ontario, CA), showed high sensitivity and specificity and overall strong positive percent agreement for the organisms on the panel (Table 3).(13)

Table 3. Prospective Performance Data by Organism

Organism	Sensitivity, %	95% CI, %	Specificity, %	95% CI, %
Campylobacter	100	43.8–100	98.2	97.3–98.8
Cryptosporidium	9.23	66.7–98.6	95.5	94.2–96.6
E. coli O157	100	34.2–100	99.2	98.5–99.6
Giardia	100	51.0–100	96.7	95.5–97.6
Salmonella	100	72.2–100	98.4	97.6–99.0
STEC	100	20.7–100	98.6	97.8–99.2
Shigella	100	34.2–100	98.5	97.7–99.1
Organism	**Positive Percent Agreement**	**95% CI, %**	**Negative Percent Agreement**	**95% CI, %**
C. difficile Toxin A/B	93.9	87.9–97.0	89.8	87.8–91.5
ETEC	25.0	7.1–59.1	99.7	99.1–99.9
Norovirus GI/GII	94.9	87.5–98.0	91.4	89.6–92.9
Rotavirus A	100	34.2–100	99.8	99.4–100

Source: FDA Decision Summary.
CI: Confidence Interval; ETEC: enterotoxigenic *Escherichia coli;* GI: gastrointestinal; STEC: Shiga toxin–producing *E. coli.*

Several studies of GI pathogen panels have demonstrated overall high sensitivities and specificities and indicated the panels might be useful for detecting causative agents for GI infections, including both foodborne and infectious pathogens. Claas et al (2013) assessed the performance characteristics of the xTAG Gastrointestinal Pathogen Panel (GPP; Luminex, Toronto, ON, Canada) compared with traditional diagnostic methods (i.e., culture, microscopy, enzyme immunoassay/direct fluorescent antibody, real-time PCR (rtPCR), or sequencing) using 901 stool samples from multiple sites.(14) The sensitivity of GPP against rtPCR was > 90% for nearly all pathogens tested by rtPCR; the one exception was adenovirus at 20%, but sensitivity could be higher because rtPCR did not distinguish between adenovirus species. Kahre et al (2014) found similar results when they compared the FilmArray GI panel (BioFire Diagnostics, Salt Lake City, UT, USA) with the xTag GPP.(15) Both panels detected more pathogens than routine testing. Of 230 prospectively collected samples, routine testing identified 1 or more GI pathogens in 19 (8.3%) samples; FilmArray detected 76 (33.0%), and xTag detected 69 (30.3%). Two of the most commonly detected pathogens in both assays were *C. difficile* (12.6%–13.9% prevalence) and norovirus (5.7%–13.9% prevalence). Both panels showed > 90% sensitivity for the majority of targets.

Using the xTAG GPP, Beckmann et al (2014) evaluated 296 patients who were either children with gastroenteritis (n = 120) or patients who had been to the tropics and had suspected parasite infestation (adults, n = 151; children, n = 25).(11) Compared with conventional diagnostics, the GPP showed 100% sensitivity for rotavirus, adenovirus, norovirus, *C. difficile, Salmonella* species, *Cryptosporidium,* and *Giardia lamblia.* Specificity was >90% for all but norovirus (42%) and *G. lamblia* (56%), which both also had lower positive predictive value (PPV) at 46% and 33%, respectively. Salmonella species also had low PPV at 43%; all others had 100% PPV. Negative predictive value was 100% for all pathogens.

Buchan et al (2013) evaluated a multiplex rtPCR assay (ProGastro SSCS, Gen-Probe Prodesse, San Diego, CA) limited to *Campylobacter* spp., *Salmonella* spp., and *Shigella* spp.

against culture; and they tested for Shiga toxin-producing Escherichia coli (STEC) against broth enrichment followed by enzyme immunoassay.(16) A total of 1244 specimens from four U.S. clinical laboratories were tested. Bidirectional sequencing was used to resolve discrepancies between ProGastro and culture or enzyme immunoassay. The overall prevalence of pathogens detected by culture was 5.6%, whereas the ProGastro assay and bidirectional sequencing showed an overall prevalence of 8.3%. The ProGastro SSCS assay showed a sensitivity of 100% and a specificity of 99.4% to 100% for all pathogens. This is compared with a sensitivity of 52.9% to 76.9% and a specificity of 99.9% to 100% for culture compared with ProGastro SSCS assay.

Al-Talib et al (2014) assessed the diagnostic accuracy of a pentaplex PCR assay with specific primers to detect hemorrhagic bacteria from stool samples.(17) The primers, which were mixed in a single reaction tube, were designed to detect *Salmonella* spp., *Shigella* spp., enterohemorrhagic *E. coli* , and *Campylobacter* spp., all of which are a particular danger to children in developing countries. The investigators used 223 stool specimens from healthy children and spiked them with hemorrhagic bacteria. All primers designed had 100% sensitivity, specificity, PPV, and negative predictive value.

Jiang et al (2014) developed a reverse transcription and multiple xrtPCR assay to identify 5 viruses in a single reaction.(18) The viruses included norovirus genogroups I and II; sapovirus genogroups I, I, IV, and V; human rotavirus A; adenovirus serotypes 40 and 41; and human astrovirus. Compared with monoplex rtPCR, multiplex rtPCR assay had sensitivity ranging from 75% to 100%; specificity ranged from 99% to 100%.

The health technology assessment and systematic review by Freeman et al (2017) evaluated multiplex texts to identify GI pathogens in people suspected of having infectious gastroenteritis.(19) Tests in the assessment were xTAG® GPP and FilmArray GI Panel. Eligible study included patients with acute diarrhea, compared multiplex GI pathogen panels tests with standard microbiology tests, and assessed patient, management, and/or test accuracy outcomes. Of the 23 identified studies, none provided an adequate reference standard for comparing the accuracy of GI panels with standard tests, so sensitivity and specificity analyses were not performed. Positive and negative test agreement were analyzed for individual pathogens for the separate panel products and are not detailed in this review. The meta-analysis of 10 studies found high heterogeneity in participants, country of origin, conventional methods used, and pathogens considered. Using conventional methods as the determinant of clinically important disease, the meta-analysis results suggested GI panel testing is reliable and could supplant current microbiological methods. An increase in false positives would result, along with the potential for overdiagnosis and incorrect treatment. However, if GI panel testing is identifying important pathology being missed with conventional methods, the result could be more appropriate treatments. The clinical importance of these findings is unclear, and assessment of GI panel testing effect on patient management and outcomes, compared with conventional testing, is needed.

Kosai et al (2021) evaluated the Verigene Pathogens Nucleic Acid Test (Luminex Corporation), testing 268 clinical stool samples for bacteria and toxins and 167 samples for viruses.(20) Of these samples, 256 and 160 samples, respectively,(95.5% and 95.8%) had fully concordant results between the Verigene EP test and the reference methods (which were culture for bacteria and toxins and xTAG GPP for viral detection). Overall sensitivity and specificity were 97.0% and99.3%, respectively. Sensitivity for individual pathogens ranged from 87.5% to

100%, and specificity ranged from 98.7%to 100%. A total of 13 false-positive and 6 false-negative results were reported.

Ahmed et al (2024) evaluated the performance of the BioFire FilmArray GI Panel for diagnosing infectious diarrhea caused by parasitic and bacterial infections in intensive care unit patients in Egypt.(21) The study included 50 stool samples subjected to conventional identification (microscopic examination, stool culture, and bacterial identification) and molecular diagnosis by the FilmArray Panel. For parasitic infections, the sensitivity and specificity of the panel compared to microscopy were 83.3% and100% for Cryptosporidium oocysts and 100% and 92.5% for Giardia lamblia cysts, respectively. For bacterial infections, the BioFire FilmArray GI Panel demonstrated 100% sensitivity and specificity for both Escherichia coli and Salmonella compared to stool culture. The overall agreement between the BioFire FilmArray GI Panel and conventional methods was 98% for Cryptosporidium, 94% for G. lamblia, and 100% for both E. coli and Salmonella.

Meltzer et al (2022) conducted a single-center RCT investigating antibiotic use in patients with moderate to severe suspected infectious diarrhea presenting to the emergency department.(22) Patients were randomized to receive multiplex PCR testing with the BioFire FilmArray GI panel (n=38) or standard care (usual testing or no testing; n=36). In the PCR arm, subjects received antibiotics in 87% of bacterial or protozoal diarrheal infections (13/15) compared to 46% (6/13) in the control arm (p=.042). No significant differences were found between groups in follow-up symptoms as assessed on days 2, 7, and 30, or emergency department length of stay. The study was terminated early due to the COVID-19 pandemic and thus was underpowered. Additional limitations include potential antibiotic prescribing at subsequent healthcare visits that was not captured and lack of a standardized reference test for the control arm.

A 9-month, prospective, multi-center study by Cybulski et al (2018) assessed the effect of the BioFire FilmArray GI PCR panel on clinical diagnosis and decision-making. It also compared the diagnostic accuracy for patients with positive results obtained exclusively using the GI panel with results obtained using conventional stool culture.(23) (Study characteristics in Table 3.) Testing on 1887 consecutive fecal samples was performed in parallel using the GI panel and stool culture. The GI panel detected pathogens in significantly more samples than culture; median time from collection to results and collection to initiation of treatment was also significantly less. The use of a GI panel also led to a significant trend toward targeted therapy rather than empirical (r^2=0.65; *p*=0.009 by linear regression). Results of the GI panels resulted in discontinuation of antimicrobials in 8 of 9 Shiga toxin-producing *E. coli* (STEC), with just 1 example of GI panel results affecting clinical decision-making. (Other results summarized in Table 10.) Limitations of the study include the limit to 2 hospitals within a single healthcare system and certain subgroups that were too small for analysis. In addition, it was unclear how the historic controls were used since the current samples tested were both tested with GI panel and culture.

The prospective study by Beal et al (2017) also aimed to assess the clinical impact of the BioFire FilmArry GI panel.(24) (Table 4) Stool samples from 241 patients (180 adults and 61 children) were tested with the GI panel and compared with 594 control patients from the previous year who were tested via culture. The most common pathogens detected by the GI panel were enteropathogenic *E. coli* (n=21), norovirus (n=21), rotavirus (n=15), sapovirus (n=9), and *Salmonella* (n=9). GI panel patients had significantly fewer subsequent infectious stool tests compared with the control group. GI panel patients also had 0.18 imaging studies

per patient compared with 0.39 (p=.0002) in the control group. The GI panel group spent fewer days on antibiotic(s) per patient: 1.73 versus 2.12 in the control group. In addition, average length of time from stool culture collection to discharge was 3.4 days for the GI panel group and 3.9 days for the controls (p=.04). (Other results summarized in Table 9.) The GI panel improved patient care in several ways: (1) it identified a range of pathogens that might not have been detected by culture, (2) it reduced the need for other diagnostic tests, (3) it resulted in less unnecessary use of antibiotics, and (4) it led to shorter length of hospital stay. Some limitation of the study include not confirming the results in which the GI panel did not agree with standard testing, and the study used a historical cohort as a control group.

Table 4. Summary of Key Observational Comparative Study Characteristics

Study	Study Type	Country	Dates	Participants	Test 1	Test 2
Cybulski (2018)	Prospective multi-center, parallel design	U.S.	Jan-Sep 2017 (controls from 2016)	Newly admitted inpatients (<3 d) and outpatients aged 0-91 y; historical control group was patients with positive stool samples from same laboratory during the same period the previous year. (N=1887 specimens)	BioFire FilmArray GI panel (n=1887 specimens)	Stool culture (n=1887)
Beal (2017)	Prospective single-center	U.S.	Jun 2016-Jun 2017 (controls from Jun-Dec 2015)	ED or admitted patients with stool samples submitted with an order for culture; historical controls were from a previous period.(N=835)	BioFire FilmArray GI Panel (n=241)	Stool culture (n=594)

ED: emergency department; GI: gastrointestinal.

Table 5. Summary of Key Observational Comparative Study Results

Study	Pathogens Detected, % of specimens	Time to Results	Time From Collection to Treatment	Empirical Initiation of Antimicrobial, %	Overall Positivity Rate, %	No. of Additional Stool Tests
Cybulski et al (2018)		Median	Median			
GI panel	35.3	18 h	26 h	23.5	NR	NR
Culture	6.0	47 h	72 h	40.0	NR	NR
p-value	NA	<.0001	<.0001	.015	NR	NR
Beal et al (2017)		Mean				
GI panel	NR	8.94 h	NR	NR	32.8	0.58
Culture	NR	54.75 h	NR	NR	6.7	3.02
95% CI	NA	1.44 to 82.8	NR	NR	NR	2.89 to 3.14
p-value	NA	<.0001	NR	NR	NR	.0001

CI: confidence interval; GI: gastrointestinal; NA: not applicable; NR: not reported.

Section Summary: Gastrointestinal Pathogen Panel

Most GI panels combining multiple individual nucleic acid tests provide faster results compared to standard stool culture. Sensitivity and specificity are generally high, but the yield of testing may be affected by the panel composition. Results of comparisons of conventional methods for ova and parasites to nucleic acid tests are limited. Prospective observational studies were available to evaluate the clinical utility of a GI panel, which was shown in faster turnaround times leading to quicker treatment and a trend away from empirical treatment toward targeted

therapy. Access to a rapid method for etiologic diagnosis of GI infections may lead to more effective early treatment and infection-control measures. However, in most instances, when there is suspicion for a specific pathogen, individual tests could be ordered, or a limited pathogen panel could be used. There may be a subset of patients with an unusual presentation who would warrant testing for a panel of pathogens.

RESPIRATORY PATHOGEN PANEL

The available evidence notes that respiratory pathogen panels are particularly effective for high-risk individuals.

High-risk individuals can include:

- Immunocompromised individuals, such as
  - Hematopoietic stem cell or solid organ transplant recipients
  - Individuals receiving high-dose chemotherapy and/or steroids.
  - These individuals can be adult or pediatric patients.
- Adults who appear acutely ill with respiratory conditions—particularly in certain settings such as influenza outbreaks
- Critically ill adult individuals—particularly ICU patients

The respiratory pathogens panel is used to diagnosis respiratory infection due to bacteria or viruses and to help guide management of the infection. This panel is performed primarily when a patient is seriously ill, hospitalized, and/or at an increased risk for severe infection with complications or multiple infections. Not everyone with symptoms is tested (e.g., fever, aches, sore throat, and cough). Samples are collected by nasopharyngeal swab in universal transport medium or respiratory wash (i.e., nasal wash, nasal aspirate, or bronchoalveolar lavage wash). Examples of these pathogens include adenovirus, coronavirus (HKU1, NL63, 229E, OC43), human metapneumovirus, human rhinovirus/enterovirus, influenza A (H1, H1-2009, H3), influenza B, parainfluenza (1, 2, 3, 4), respiratory syncytial virus, *Bordetella pertussis, Chlamydophila pneumoniae*, and *Mycoplasma pneumoniae*.

Clark et al (2023) conducted a systematic review and meta-analysis of the impact of multiplex PCR testing among individuals with a suspected acute respiratory tract infection in the hospital setting.(25) Twenty-seven studies representing 17,321 patients were identified for analysis. Multiplex testing was associated with a reduction in both time to results (-24.22 h; 95% CI, -28.70 to -19.74 h) and hospital length of stay (-0.82 days; 95% CI, -1.52 to -0.11). Antivirals were more likely to be prescribed among influenza positive individuals (RR, 1.25; 95% CI, 1.06 to 1.48) as was use of an appropriate infection control facility (RR, 1.55; 95% CI, 1.16 to 2.07).

Huang et al (2018) published a systematic review and meta-analysis of a multiplex PCR system for the rapid diagnosis of respiratory virus infections.(26) Authors summarized diagnostic accuracy evidence on the detection of viral respiratory infections for BioFire FilmArray RP (Film Array), Nanosphere Verigene RV+ test, and Hologic Gen-Probe Prodesse assays. The study reviewed 20 studies with 5510 patient samples. Multiplex PCRs were found to have high diagnostic accuracy with AUROC $\geq$ 0.98 for all reviewed viruses expected adenovirus (AUROC 0.89). All three reviewed multiplex PCR systems were shown to be highly accurate.

Several studies of various respiratory viral panels have demonstrated 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.(27-29)

Cartuliares et al (2023) conducted a prospective, multicenter, randomized controlled trial to evaluate the impact of point-of-care multiplex PCR on antibiotic prescribing for patients admitted with suspected community-acquired pneumonia in Denmark.(30) Lower respiratory tract samples were collected from 294 patients randomized to either the PCR group (Biofire FilmArray Pneumonia Panel plus added to standard care) or the standard care only group. The primary outcome, prescription of no or narrow-spectrum antibiotics at 4 hours, did not differ significantly between the PCR (62.8%) and standard of care (59.6%) groups(OR 1.13; 95% CI 0.96 to 1.34; p=.134). However, the PCR group had significantly more targeted antibiotic prescriptions at 4hours (OR 5.68; 95% CI 2.49 to 12.94; p<.001) and 48 hours (OR 4.20; 95% CI 1.87 to 9.40; p<.001), and more adequate prescriptions at 48 hours (OR 2.11; 95% CI 1.23 to 3.61; p=.006) and day 5 (OR 1.40; 95% CI 1.18 to 1.66; p<.001). There were no significant differences in ICU admissions, 30-day readmissions, length of stay, 30-day mortality, or in-hospital mortality.

Randomized Controlled Trials

Andrews et al (2017) published a quasi-randomized study assessing the impact of multiplex PCR on length of stay and turnaround time compared with routine, laboratory-based testing in the treatment of patients aged ≥ 16 years presenting with influenza-like illness or upper or lower respiratory tract infection (Table 6).(31) Patients were selected at inpatient and outpatient clinics in 3 areas of a hospital. FilmArray RP PCR systems were used. Of eligible patients (N=606), 545 (89.9%) were divided into a control arm (n=211) and an intervention arm (n=334). While PCR testing was not associated with a reduction in length of stay, turnaround time was reduced. (See Table 11 for detailed results.) Limitations of the study included design and patient allocation (patients were allocated to the intervention arm on even days). Additionally, the patients considered in the study were not noted to be high-risk individuals as defined above, only those with pertinent symptoms.

The parallel-group, open-label RCT by Brendish et al (2017) evaluated the routine use of molecular point-of-care testing (POCT) for respiratory viruses in adults presenting to a hospital with acute respiratory illness.(32) (Table 6) In a large U.K. hospital, over 2 winter seasons, investigators enrolled adults within 24 hours of presenting to the emergency department or acute medical unit with acute respiratory illness or fever > 37.5°C, or both. A total of 720 patients were randomized (1:1) to either molecular POCT for respiratory viruses (FilmArray Respiratory Panel; n = 362) or routine care (n = 358), which included diagnosis based on clinical judgment and testing by laboratory PCR at the clinical team's discretion. All patients in the POCT group were tested for respiratory viruses; 158 (45%) of 354 patients in the control group were tested. Because patients presenting with symptoms are often put on antibiotics before tests can be run, the results of the POCTs were unable to influence the outcome in many patients; therefore, a subgroup analysis was necessary for those who were only given antibiotics after test results were available. The results of the analysis showed antibiotics were prescribed for 61 (51%) of 120 patients in the POCT group and for 107 (64%) of 167 in the control group (difference = -13.2%; 95% CI, -24.8% to -1.7%; *p* =.0289). Mean test turnaround time for POCT was 2.3 hours (SD = 1.4) versus 37.1 hours (SD = 21.5) in the control group. The percentage of patients prescribed a neuraminidase inhibitor who tested positive for influenza was significantly higher for the POCT group than the control group (82% vs. 47%), and it was significantly lower for the percentage who tested negative for influenza (18% vs. 53%). In addition, the time to first dose was 8.8 hours (SD = 15.3) for POCT and 21.0 hours (SD = 28.7) for the control group. (See Table 11 for more results.) Blinding of the clinical teams to which group a patient had been randomized to was not possible because the purpose of the

study was to inform the clinical team of POCT results. In addition, the limit of the study to the winter months means the findings cannot be extrapolated to the rest of the year.

Table 6. Summary of Key RCT Characteristics

Study; Trial	Countries	Sites	Dates	Participants	Interventions	
					Active	***Comparator***
Andrews et al (2017)[a]	United Kingdom	1	Jan-Jul 2015	Patients with influenza-like illness/upper RTI +/- lower RTI N = 454	FilmArray POC testing (even days of month) n = 334	Routine, laboratory-based RP PCR testing +/- atypical serology (odd days)n = 211
Brendish et al (2017)	United Kingdom	1	Jan 2015-Apr 2016 and Oct 2015-Apr, 2016[b]	Adults who could be recruited within 24 h of triage in ED or arrival at acute medical unit with acute respiratory illness or fever >37.5°C for ≤7 d N = 720	POCT n = 362	Diagnosis based on clinical judgment and PCR testing at clinical team's discretion = 358

ED: emergency department; PCR: polymerase chain reaction; POCT: point of care testing (using FilmArray Respiratory Panel); RCT: randomized controlled trial; RTI: respiratory tract infection

[a] Quasi-randomized study

[b] The dates do not make sense because they overlap, likely due to an error in the article. Another place in the article says the "winter seasons in 2014-15 and 2015-16."

Table 7. Summary of Key RCT Results

Study	Test Efficacy	Length of Stay	Antimicrobic Use Duration	All-Cause Mortality[a]	Readmission[b]
Andrews et al (2017)		Median (IQR)	Median (IQR)		
Active	24%	98.6 h (48.1–218.4)	6.0 d (4.0–7.0)	4%	19%
Comparator	20%	79.6 h (41.9–188.9)	6.8 d (5.0–7.3)	4%	20%
Estimated intervention effect	NR	NR	Absolute difference in natural logarithm of duration: -0.08 (95% CI: -0.22–0.054)	[a]OR: 0.9 (95% CI: 0.3–2.2)	OR: 0.9 (95% CI: 0.6–1.4)
Adjusted p-value	NR	NR	0.23	0.79	0.70
Brendish et al (2017)		Mean (SD)	Mean (SD)		
Active	NR	5.7 d (6.3)	7.2 d (5.1)	3%	13%
Comparator	NR	6.8 d (7.7)	7.7 d (4.9)	5%	16%
Difference (95% CI)	NR	-1.1 d (-2.2 to -0.3)	-0.4 (-1.2–0.4)[c]	-2.0% (-4.7%–0.6%)	-3.0% (-8.3%–2.0%)
OR (95% CI)	NR	NR	0.95 (0.85–1.05)[d]	0.54 (0.3–1.2)	0.78 (0.5–1.2)
p-value	NR	0.04	0.32	0.15	0.28

CI: confidence interval; IQR: interquartile range; NR: not reported; OR: odds ratio; RCT: randomized controlled trial; SD: standard deviation.

[a] 30 days post-enrollment.

[b] Within 30 days of study participation.

[c] Mean risk difference.

[d] Unadjusted odds ratio.

Tables 12 and 13 display notable limitations identified in each study.

Table 8. Study Design and Conduct Limitations

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]

Andrews et al (2017)	2. Patients allocated to study arms based on even vs. odd days of the week; patient groups unbalanced in favor of FilmArray group	
Brendish et al (2017)		1. Patients and data collectors not blinded

The study limitations stated in this table are those notable in the current review; this is not a comprehensive limitations assessment.
[a] Selection key: 1. Selection not described; 2. Selection not random or consecutive (i.e., convenience).
[b] Blinding key: 1. Not blinded to results of reference or other comparator tests.
[c] Test Delivery key: 1. Timing of delivery of index or reference test not described; 2. Timing of index and comparator tests not same; 3. Procedure for interpreting tests not described; 4. Expertise of evaluators not described.
[d] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.
[e] Data Completeness key: 1. Inadequate description of indeterminate and missing samples; 2. High number of samples excluded; 3. High loss to follow-up or missing data.
[f] Statistical key: 1. Confidence intervals and/or p values not reported; 2. Comparison with other tests not reported.

Table 9. Study Relevance Limitations

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
Andrews et al (2017)	4. Patients were not noted to be high-risk				
Brendish et al (2017)				3. Sensitivity and specificity not reported (study was on clinical utility)	

The study limitations stated in this table are those notable in the current review; this is not a comprehensive limitations assessment.
[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.
[b] Intervention key: 1. Classification thresholds not defined; 2. Version used unclear; 3. Not intervention of interest.
[c] Comparator key: 1. Classification thresholds not defined; 2. Not compared to credible reference standard; 3. Not compared to other tests in use for same purpose.
[d] Outcomes key: 1. Study does not directly assess a key health outcome; 2. Evidence chain or decision model not explicated; 3. Key clinical validity outcomes not reported (sensitivity, specificity and predictive values); 4. Reclassification of diagnostic or risk categories not reported; 5. Adverse events of the test not described (excluding minor discomforts and inconvenience of venipuncture or noninvasive tests).
[e] Follow-Up key: 1. Follow-up duration not sufficient with respect to natural history of disease (true-positives, true-negatives, false-positives, false-negatives cannot be determined).

Section Summary: Respiratory Pathogen Panels

The evidence for the clinical validity or clinical utility of respiratory pathogen panels in diagnosing respiratory infections includes a systematic review and 2 RCTs. The systematic review reported that all 3 reviewed multiplex PCR systems were highly accurate. The clinical utility demonstrated by the RCTs showed benefits to the respiratory panel in test results turnaround time, time to receive treatment, and length of hospital stay. Significant differences were not seen in antibiotic prescription, readmission, or mortality.

SEXUALLY TRANSMITTED PATHOGENS

Van De Pol et al (2020) conducted a multicenter study to assess the performance of the cobas Trichomonas vaginalis (TV)/MG assay (cobas) for the detection of *M. genitalium*.(57) Two thousand, one hundred and fifty urogenital specimens were collected from symptomatic and nonsymptomatic men and women at diverse geographical sites across the United States. Women provided specimens in the following order: first-catch urine (FCU), vaginal swabs, an

endocervical swab in cobas PCR media, and a cervical specimen in PreservCyt solution obtained with a spatula, cytobrush, or broom. Men first provided meatal swabs for use with the cobas test, followed by an FCU sample. Participants were randomized to either self-obtained or clinician obtained for collection of swabs used in the cobas assay. In total, 59 women and 60 men were considered infected. Of these infected participants, 67.8% of women and 51.7% of men reported symptoms. The overall sensitivity of the cobas test for the detection of *M. genitalium* in women was highest in vaginal swab samples (96.6% [95% CI, 88.5 to 99.1]; clinician and self-collected combined). The overall sensitivity of the test for female urine, PreservCyt samples, and endocervical samples ranged from 83.1% to 86.4%. The overall sensitivity of cobas for *M. genitalium* in male urine samples and meatal swab samples was 100% (95% CI, 94.0 to 100%) and 85.0% (95% CI, 73.9 to 91.9%), respectively. There were no statistically significant sensitivity differences between the clinician- and self-collected vaginal swabs (96.3% versus 96.9%, respectively; P 0.99) and meatal swabs (83.9% versus 86.2%, respectively; P 0.99) as determined by the Z-test analyses. Additional Z-test analyses similarly showed no statistically significant specificity differences between the clinician- and self-collected vaginal swabs (96.8% versus 97.3%, respectively; P 0.63) and meatal swabs (97.5% versus 98.2%, respectively; P 0.74). The specificity of the cobas assay for *M. genitalium* ranged from 96.0 to 99.8% across male and female symptomatic and asymptomatic samples. *M. genitalium* prevalence was higher in symptomatic than asymptomatic patients, and the overall prevalence ranged from 5.4% to 5.8% across male and female specimens. The PPV of the cobas for detection of *M. genitalium* was 58.6 to 94.7%, and the NPV was 98.7 to 100% across all specimen types evaluated. Authors concluded that the cobas assay had high sensitivity and specificity for the detection of *M. genitalium* in both male and female sample types, regardless of symptom status. This study provides evidence of a fully validated, high-throughput PCR assay for the detection of *M. genitalium*.

Hu et al (2019) obtained 1,328 clinical specimens from 3 hospitals to detect 9 sexually transmitted disease (STD) pathogens using multiplex real-time PCR melting curve and Sanger sequencing, to evaluate the sensitivity, specificity, and consistency of the technology.(58) Testing included evaluation for *chlamydia trachomatis, Neisseria gonorrhoeae, Mycoplasma genitalium, Mycoplasma hominis, Ureaplasma urealyticum, Ureaplasma parvum*, and *herpes simplex virus*. The consistency between the results of the novel assay method and those of Sanger sequencing was more that 0.85 (Kappa test, P < 0.001), and the assay was highly sensitive and specific.

URINARY PATHOGENS

The gold standard for the diagnosis of a urinary tract infection is the detection of the pathogen in the presences of clinical symptoms via a urine culture using a mid-stream urine catch.(59) There is little to no support for use of PCR based molecular testing to detect urinary tract infection organisms as a routine screening modality. Small scale and industry sponsored trials suggest that NAP testing for urinary pathogens is effective. Although the American Urological Association discusses PCR testing in their guideline, concern is raised that use of the technology to evaluate lower urinary tract symptoms may lead to over treatment with antibiotics. No guidelines have been identified which recommend the use of PCR based molecular testing to detect urinary tract infections. Price et al reported that compared to expanded-spectrum enhanced quantitative urine culture, standard urine culture missed 67% of uropathogens overall and 50% in participants with severe urinary symptoms (n=75). In addition, approximately 40% percent of individuals with missed uropathogens reported no symptom resolution after treatment based on standard urine culture results.(60) Results from molecular panel tests must be interpreted with caution as they detect significantly more

pathogens than conventional methods of testing. Not all positive results indicate an active infection as these tests detect microbial nucleic acid and they do not require live, actively replicating organisms.(61,62) Determination of whether these additional detected organisms are pathogens or colonizers is important to prevent overuse of antibiotics. In the event of complicated urinary tract infections which may put the individual at higher risk for decreased efficacy of therapy (i.e., anatomic or functional abnormality of the urinary tract, immunocompromised host) (63) NAP testing may improve health outcomes.

Summary of Evidence

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 a systematic review and a pivotal prospective study. Relevant outcomes include test accuracy and validity, other test performance measures, medication use, 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 and was granted FDA *de novo* classification in 2015. The evidence is sufficient to determine that the technology results in an improvement in the net 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 and prospective studies on utility. Relevant outcomes include test accuracy and validity, other test performance measures, medication use, 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. The evidence is sufficient to determine that the technology results in an improvement in the net health outcomes.

For individuals who have signs and/or symptoms of respiratory infection who receive a nucleic acid-based respiratory pathogen panel, the evidence includes a systematic review and 2 randomized controlled trials (RCTs). Relevant outcomes include test accuracy and validity, other test performance measures, medication use, symptoms, and change in disease status. The systematic review reported that all 3 reviewed multiplex polymerase chain reaction systems were highly accurate. One RCT and 1 quasi-RCT evaluated utility of a respiratory panel and found benefits in time-to-treat and length of hospital stay; in addition, 1 sub analysis found fewer antibiotics being prescribed for patients diagnosed with the panel. The panel did not significantly affect duration of antibiotic use, readmission, or mortality rates. The evidence is sufficient to determine that the technology results in an improvement in the net health outcomes.

For individuals who have signs and/or symptoms of sexually transmitted infections, the evidence suggests that nucleic acid amplification test (NAAT) technologies are effective. Assays were found to be highly sensitive and specific in symptomatic individuals and one study indicated that there were no statistically significant sensitivity differences between the clinician- and self-collected swabs. Multiple societies recommend that symptomatic patients should be tested using NAAT technologies.

For individuals with or without signs and/or symptoms of urinary tract infection, the evidence is lacking regarding standard use. Guideline statements regarding diagnosis of urinary tract infections continues to rely on culture and sensitivity specimens as the gold standard. Although PCR based molecular testing to detect urinary tract infection organisms was discussed in the American Urology Association guidelines, more information is needed before recommendations can be placed based on clinical utility for use as standard care. In complicated urinary tract infections, PCR testing may improve health outcomes.

Supplemental Information

PRACTICE GUIDELINES AND POSITION STATEMENTS

Numerous guidelines have been identified concerning the use of nucleic acid amplification tests (NAATs) for the diagnosis of the pathogens discussed in this review. Table 10 provides an index of NAAT recommendation by Virus/Infection.

Table 10. Index of NAAT Recommendations by Virus/Infection

Microorganism	Guidelines Recommending the Use of NAATs (Location)	Guidelines Not Recommending the Use of NAATs[a] (Location)
Bartonella hensalae	NIH (2.1.1), IDSA (3.1), AAP (5.1)	NA
CNS Pathogen Panel	IDSA (3.2, 3.3)	NA
Chlamydia pneumonia	CDC (1.5.3), IDSA (3.1[c])	AAP (5.1)
Chlamydia trachomatis	CDC (1.5.2,[c] 1.6[c]), IDSA (3.1), AAP (5.1)	NA
Clostridium difficile	NIH (2.1.2), AAP (5.1)	IDSA (3.1, 3.4)
Cytomegalovirus	CDC (1.1), NIH (2.1.3), IDSA (3.1,[c] 3.3)	AAP (5.1)
Enterovirus	IDSA (3.1), AAP (5.1)	NA
GI Pathogen Panel	CDC (1.4[c]), IDSA (3.5), ACG (6.1)	NA
Hepatitis B	NIH (2.1.4), IDSA (3.1), AAP (5.1)	NA
Hepatitis C	CDC (1.5.5[c]), NIH (2.1.5), IDSA (3.1), AAP (5.1)	NA
Herpes Simplex Virus	CDC (1.5.6[c]), NIH (2.1.6), IDSA (3.1,[c] 3.3), AAP (5.1)	NA
Human Herpesvirus 6	IDSA (3.1,[c] 3.3)	AAP (5.1)
Human Papillomavirus	CDC (1.5.8[c]), AAP (5.1)	NA
HIV 1	CDC (1.5.7[c]), IDSA (3.1), AAP (5.1)	NA
Influenza virus	IDSA (3.1[c]), AAP (5.1)	NA
Legionella pneumophila	IDSA (3.1), AAP (5.1)	NA
Meningitis	NA	IDSA (3.6)
Mycobacteria Species	CDC (1.8), NIH (2.1.7), IDSA (3.1, 3.3)	AAP (5.1)
Mycoplasma pneumoniae	CDC (1.2[c]), IDSA (3.3), AAP (5.1)	NA
Neisseria gonorrhoeae	CDC (1.6[c]), IDSA (3.1), AAP (5.1)	NA
Respiratory Panel	None Identified	NA
Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)	IDSA (3.8)	NA
Staphylococcus aureus	IDSA (3.1), AAP (5.1)	NA
Streptococcus, Group A	IDSA (3.1)	AAP (5.1)
Streptococcus, Group B	CDC (1.7), AAP (5.2)	IDSA (3.1), AAP (5.1)
Vancomycin-resistant enterococcus	AST (4.1)	IDSA (3.1), AAP (5.1)
Zika	CDC (1.3), IDSA (3.1), AAP (5.1)	NA

AAP: American Academy of Pediatrics; ACG: American College of Gastroenterology; AST: American Society of Transplantation; CDC: Centers for Disease Control and Prevention; IDSA: Infectious Disease Society of America; NA: not applicable (none found);
NAAT: nucleic acid amplification test; NIH: National Institutes of Health.
[a] Guidelines Not Recommending includes not only guidelines that recommend again NAATs but also those that were neutral.

on the use of NAATs.

[b] CDC recommends culture for first-line identification of Candida species; it recommends NAAT for complicated infections and for second-line diagnosis.

[c] Indicates guidelines in which the issuing body specifically recommends that U.S. Food and Drug Administration (FDA)-cleared NAATs be used.

Centers for Disease Control and Prevention

The Centers for Disease Control and Prevention (CDC) has published multiple recommendations and statements regarding the use of NAATs to diagnose the viruses and infections discussed in this evidence review since 2009.

The CDC published guidance for laboratory testing for Cytomegalovirus (CMV), the guideline stated that the standard laboratory test for congenital CMV is polymerase chain reaction (PCR) on saliva, with confirmation via urine test to avoid false-positive results from ingesting breast milk from CMV seropositive mothers. Serologic tests were recommended for persons > 12 months of age.(33)

The CDC published diagnostic methods for mycoplasma pneumoniae.(34) They cited NAAT as a method of diagnosis, along with culture or serology.

The CDC published updated guidance on Zika virus testing.(35) Routine testing for Zika virus in asymptomatic pregnant patients is not recommended, but NAAT testing may still be considered for asymptomatic pregnant women with recent travel to an area with risk of Zika outside the U.S. and its territories. Symptomatic pregnant patients should receive NAAT testing if they have recently traveled to areas with a risk of Zika virus or if they have had sex with someone who lives in or recently traveled to areas with risk of Zika virus. If a pregnant woman (with risk of Zika virus exposure) has a fetus with prenatal ultrasound findings consistent with congenital Zika virus infection, Zika virus NAAT and IgM testing should be performed on maternal serum and NAAT on maternal urine. If amniocentesis is being performed as part of clinical care, Zika virus NAAT testing of amniocentesis specimens should also be performed.

In 2017, the CDC updated its guidelines on norovirus gastroenteritis outbreak management and disease prevention.(36,37) Real-time reverse transcription-PCR assays, specifically, TaqMan-based real-time assays, which can contain multiple probes, is considered the effective laboratory diagnostic protocol for testing suspected cases of viral gastroenteritis.

In 2015, the CDC made recommendations for the use in NAATs in diagnosing numerous sexually transmitted infections.(38) These recommendations were most recently updated in 2021, with the publication of new guidelines and the following recommendation:(39)

- For Gonococcal Infections:
  - "Culture, NAAT, and POC [point of care] NAAT, such as GeneXpert (Cepheid), are available for detecting genitourinary infection with N. gonorrhoeae."
  - "NAATs and POC NAATs allow for the widest variety of FDA-cleared specimen types, including endocervical and vaginal swabs and urine for women, urethral swabs and urine for men, and rectal swabs and pharyngeal swabs for men and women. However, product inserts for each NAAT manufacturer should be consulted carefully because collection methods and specimen types vary."
- For Chlamydia Infection:
  - NAATs are the most sensitive tests for these specimens and are the recommended test for detecting *C. trachomatis* infection. NAATs that are FDA cleared for use with

vaginal swab specimens can be collected by a clinician or patient in a clinical setting. Patient collected vaginal swab specimens are equivalent in sensitivity and specificity to those collected by a clinician using NAATs, and this screening strategy is highly acceptable among women. Optimal urogenital specimen types for chlamydia screening by using NAAT include first catch urine (for men) and vaginal swabs (for women). Recent studies have demonstrated that among men, NAAT performance on self-collected meatal swabs is comparable to patient-collected urine or provider-collected urethral swabs."

- For Hepatitis C infection (HCV):
  - In addition, “testing for HCV infection should include use of an FDA-cleared test for antibody to HCV…followed by NAAT to detect HCV RNA for those with a positive antibody result.” Persons with HIV infection with low CD4+ T-cell count might require further testing by NAAT because of the potential for a false-negative antibody assay.”
- For diseases characterized by genital, anal, or perianal ulcers (e.g., herpes simplex virus [HSV], syphilis):
  - "Specific evaluation of genital, anal, or perianal ulcers includes syphilis serology tests and dark field examination from lesion exudate or tissue, or NAAT if available; NAAT or culture for genital herpes type 1 or 2; and serologic testing for type-specific HSV antibody. In settings where chancroid is prevalent, a NAAT or culture for Haemophilus ducreyi should be performed;"
  - "PCR is also the test of choice for diagnosing HSV infections affecting the central nervous system(CNS) and systemic infections (e.g., meningitis, encephalitis, and neonatal herpes). HSV PCR of the blood should not be performed to diagnose genital herpes infection, except in cases in which concern exists for disseminated infection (e.g., hepatitis)."
- For Human immunodeficiency virus (HIV-1):
  - The use of NAAT is not mentioned; serologic tests are recommended for detecting antibodies against HIV-1 and by virologic tests that detect HIV antigens or RNA.
- For Human Papillomavirus (HPV):
  - There are several FDA-cleared HPV tests that detect viral nucleic acid or messenger RNA; however, there are currently no algorithms for HPV 16/18/45 testing in the clinical guidelines;
  - Testing for non-oncogenic HPV (types 6 and 11) is not recommended; and
  - “HPV assays should be FDA-cleared and used only for the appropriate indications” and should not be performed if the patient is “deciding whether to vaccinate against HPV;” when “providing care to persons with genital warts or their partners;” when “testing persons aged <25 years as part of routine cervical cancer screening;” or when “testing oral or anal specimens.”
- In 2014, the CDC published recommendations regarding the laboratory-based detection of *C.* trachomatis and N. *gonorrhoeae* infections.(40) It stated:
  - NAATs are superior other available diagnostic tests in “overall sensitivity, specificity, and ease of specimen transport;”
  - The use of “NAAT to detect chlamydia and gonorrhea except in cases of child sexual assault involving boys and rectal and oropharyngeal infections in prepubescent girls” is supported by evidence; and
  - Only NAATs that have been cleared by the FDA for detection of C. trachomatis and N. gonorrhoeae should be used “as screening or diagnostic tests because they have been evaluated in patients with and without symptoms”.

- In 2009, the CDC published updated guidelines for the use of NAATs in diagnosing Mycobacterium tuberculosis bacteria.(41) The CDC recommended that “NAA testing be performed on at least one respiratory specimen from each patient with signs and symptoms of pulmonary TB [tuberculosis] for whom a diagnosis of TB is being considered but has not yet been established, and for whom the test result would alter case management or TB control activities.” Although it noted that “culture remains the gold standard for laboratory confirmation of TB and is required for isolating bacteria for drug-susceptibility testing and genotyping,” the guideline stated that “NAA testing should become standard practice for patients suspected to have TB, and all clinicians and public health TB programs should have access to NAA testing for TB to shorten the time needed to diagnose TB from 1–2 weeks to 1–2 days.”
- The CDC (2021) released the following statement:(38)
    - M. genitalium is a slow-growing organism. Culture can take up to 6 months, and technical laboratory capacity is limited to research settings. NAAT for M. genitalium is FDA cleared for use with urine and urethral, penile meatal, endocervical, and vaginal swab samples. Molecular tests for macrolide (i.e., azithromycin) or quinolone (i.e., moxifloxacin) resistance markers are not commercially available in the United States. However, molecular assays that incorporate detection of mutations associated with macrolide resistance are under evaluation.
    - Men with recurrent NGU should be tested for M. genitalium using an FDA-cleared NAAT. If resistance testing is available, it should be performed and the results used to guide therapy. Women with recurrent cervicitis should be tested for M. genitalium, and testing should be considered among women with PID. Testing should be accompanied with resistance testing, if available. Screening of asymptomatic M. genitalium infection among women and men or extragenital testing for M. genitalium is not recommended. In clinical practice, if testing is unavailable, M. genitalium should be suspected in cases of persistent or recurrent urethritis or cervicitis and considered for PID.

National Institute of Health et al

The NIH, CDC, and HIV Medicine Association of the IDSA published guidelines for the prevention and treatment of opportunistic infections in adults and adolescents with HIV.(42) the most recent update took place in 2024. In these guidelines, NAATs are discussed in the following situations:

- Bartonella species
    - For patients with suspected bacillary angiomatosis, serologic tests are the standard of care and the most accessible test for diagnosing Bartonella infection. There are PCR “methods that have been developed for identification and speciation of Bartonella are becoming increasingly available through private laboratories, as well as the CDC and may aid in diagnosis of Bartonella in freshly biopsied tissue samples or whole blood.”
- *Clostridioides (Clostridium) difficile*
    - Detection of either the *C. difficile* toxin B gene, using NAAT, or the *C. difficile* toxin B protein, using an enzyme immunoassay, is required for diagnosis. PCR assays have high sensitivity and can detect asymptomatic carriers.
- Cytomegalovirus
    - For patients with suspected cytomegalovirus disease, diagnosis is based on clinical symptoms and the presence of CMV in cerebral spinal fluid (CSF) or brain tissue. “In rare cases, the diagnosis may be unclear, and PCR of aqueous or vitreous humor specimens for CMV and other pathogens—especially herpes simplex virus, varicella zoster virus, and Toxoplasma gondii—can be useful for establishing the diagnosis."

- Hepatitis B
  - The CDC, the United States Preventive Services Task Force, and the AASLD recommend that patients with HIV infection should be tested for hepatitis B; however, NAATs are not recommended for initial testing in patients with HIV.
- Hepatitis C
  - Patients with HIV are recommended to undergo routine hepatitis C screening, initially "performed using the most sensitive immunoassays licensed for detection of antibody to HCV in blood." The use of NAATs are not mentioned for initial testing in patients with HIV.
- Herpes Simplex Virus
  - "HSV DNA PCR… is the preferred method for diagnosis of mucocutaneous HSV lesions caused by HSV."
- *Mycobacterium tuberculosis* Infection and Disease
  - "NAA tests provide rapid diagnosis of TB, and some assays also provide rapid detection of drug resistance."
  - "NAA assays, if positive, are highly predictive of TB disease when performed on Acid-Fast Bacillus(AFB) smear-positive specimens. However, because nontuberculous mycobacterial infections (NTM) may occur in people with HIV with advanced immunodeficiency, negative NAA results in the setting of smear-positive specimens may indicate NTM infection and can be used to direct therapy and make decisions about the need for respiratory isolation."
  - "NAA tests are more sensitive than AFB smear, being positive in 50% to 80% of smear negative, culture-positive specimens and up to 90% when three NAA tests are performed. Therefore, it is recommended that for all patients with suspected pulmonary TB, a NAA test be performed on at least one specimen. NAA tests also can be used on extrapulmonary specimens with the caveat that the sensitivity is often lower than with sputum specimens."

Infectious Disease Society of America et al

Since 2008, the IDSA has partnered with various societies to publish 9 recommendations regarding the use of NAATs to diagnose the viruses and infections discussed in this evidence review.

In 2024, the IDSA and the American Society for Microbiology published a guide on the diagnosis of infectious diseases.(43) NAATs were recommended diagnostic procedures for Enterovirus, Hepatitis C, Hepatitis B, Cytomegalovirus, bacterial vaginosis, Herpes Simplex Virus, Human Herpesvirus 6, HIV, Influenza Virus, and Zika Virus. In addition to providing guidance on diagnosing these diseases, the guidelines also provided recommendations on testing for other conditions by testing for common etiologic agents. Table 11 describes selected conditions for which IDSA recommends NAATs for diagnosing etiologic agents.

Table 11. IDSA Recommended Conditions for Use of NAATs in Identifying Etiologic Agents of Other Conditions*

Etiologic Agents	Recommended Conditions for Use of NAATs in Diagnosis when Specific Etiologic Agents is Suspected
Bartonella spp	Bloodstream infections, encephalitis
Chlamydia pneumoniae	Bronchiolitis, Bronchitis, and Pertussis; Community-Acquired Pneumonia
Chlamydia trachomatis	Pre-septal and orbital cellulitis, lacrimal and eyelid infections, and conjunctivitis; pharyngitis; Periocular structure infections/ Conjunctivitis, Orbital and Periorbital Cellulitis, and Lacrimal and Eyelid Infections; Proctitis; Epididymitis and Orchitis; Pathogens Associated with Cervicitis/Urethritis; Pathogens Associated with Pelvic Inflammatory Disease and Endometritis

Clostridioides (Clostridium) difficile	Gastroenteritis, Infectious, and Toxin-Induced Diarrhea
Cytomegalovirus	Pericarditis and Myocarditis[a]; Encephalitis; Pneumonia in the Immunocompromised Host; Esophagitis; Gastroenteritis, Infectious, and Toxin-Induced Diarrhea; Burn Wound Infections[b]
Enterovirus	Meningitis; Encephalitis; Brochiolitis, Bronchitis, and Pertussis; Community-Acquired Pneumonia; Gastroenteritis, Infectious, and Toxin-Induced Diarrhea; pre-septal and orbital cellulitis, lacrimal and eyelid infections, and conjunctivitis; infectious keratitis; endophthalmitis, panophtalmitis, uveitis, and retinitis
Herpes Simplex Virus	Meningitis; Encephalitis; Esophagitis; Proctitis; Pathogens Associated with Cervicitis/Urethritis; Burn Wound Infection[b]; Periocular structure infections/ Conjunctivitis, Orbital and Periorbital Cellulitis, and Lacrimal and Eyelid Infections; Periocular Structure Infections/Keratitis; Pharyngitis; Genital Lesions; endophthalmitis, panophthalmitis, uveitis, and retinitis; pneumonia in the immunocompromised host
HIV	Pericarditis and Myocarditis; Meningitis[c]; Pharyngitis[c]
Human Herpesvirus 6	Encephalitis
Influenza	Encephalitis; Bronchiolitis, Bronchitis, and Pertussis; Community-Acquired Pneumonia; Hospital-Acquired Pneumonia and Ventilator-Associated Pneumonia; Pulmonary Infections in Cystic Fibrosis;
Legionella spp	Community-Acquired Pneumonia; Hospital-Acquired Pneumonia and Ventilator-Associated Pneumonia; Surgical Site Infections
Mycobacteria species-both Tuberculosis and NTM	Community-Acquired Pneumonia; Infections of the Pleural Space; meningitis; osteomyelitis; encephalitis
Neisseria gonorrhoeae	Joint infection; Pharyngitis; Proctitis; Native Joint Infection and Bursitis; Epididymitis and Orchitis; Pathogens Associated with Cervicitis/Urethritis; Pathogens Associated with Pelvic Inflammatory Disease and Endometritis
Staphylococcus aureus	Joint infection; trauma-associated cutaneous infection; surgical site infections; osteomyelitis
Streptococcus, Group A	Pharyngitis; periprosthetic joint infection

* The IDSA provided recommendations for many situations in which NAATs are recommended for diagnosing certain etiologic agents commonly seen with the listed conditions noted under the Recommended Conditions for Use of NAATs in Diagnosis Column.

HIV: human immunodeficiency virus; IDSA: Infectious Disease Society of America; MSRA: methicillin-resistant Staphylococcus aureus; NAAT: nucleic acid amplification test: NTM: nontuberculous mycobacteria.

[a] Recommended as first choice if available;

[b] Where applicable and laboratory-validated;

[c] The guidelines caution that NAAT is not 100% sensitive in individuals with established HIV infection due to viral suppression; therefore, if NAAT is used, subsequent serologic testing is recommended.

Use of NAATs for diagnosing *Streptococcus* Group B, and Vancomycin-resistant enterococcus as etiologic agents was not recommended.

In 2017, the IDSA published clinical practice guidelines for the management of healthcare-associated ventriculitis and meningitis.(44) When making diagnostic recommendations, the IDSA notes cultures as the standard of care in diagnosing healthcare-associated ventriculitis and meningitis, but that "nucleic acid amplification tests, such as PCR, on CSF may both increase the ability to identify a pathogen and decrease the time to making a specific diagnosis (weak, low)." (Strength of recommendation and quality of evidence established using the GRADE [Grading of Recommendations Assessment, Development and Evaluation] methodology).

In 2008, the IDSA published clinical practice guidelines for the management of encephalitis.(45) The following recommendations were made:

- "Biopsy of specific tissues for culture, antigen detection, nucleic acid amplification tests (such as PCR), and histopathologic examination should be performed in an attempt to establish an etiologic diagnosis of encephalitis (A-III)." (Strength of recommendation level "A indicates good evidence to support recommendation for use." Quality of

evidence level III indicates “evidence from opinions of respected authorities based on clinical experience, descriptive studies, or reports of expert committees.”).(46)
- “Nucleic acid amplification tests (such as PCR) of body fluids outside of the CNS may be helpful in establishing the etiology in some patients with encephalitis (B-III).” (Strength of recommendation level B indicates “moderate evidence to support recommendation.” Quality of evidence level III indicates “evidence from opinions of respected authorities based on clinical experience, descriptive studies, or reports of expert committees.”).(46)
- “Nucleic acid amplification tests (such as PCR) should be performed on CSF specimens to identify certain etiologic agents in patients with encephalitis (A-III). Although a positive test result is helpful in diagnosing infection caused by a specific pathogen, a negative result cannot be used as definitive evidence against the diagnosis.”
- The use of NAATs was recommended for diagnosing CMV, HSV-1 and -2, Human herpesvirus 6, Bartonella henselae, Mycoplasma pneumoniae, and Mycobacterium tuberculosis.

In 2018, the IDSA and the Society for Healthcare Epidemiology of America (SHEA) published weak recommendations with low quality evidence for the use of NAATs to diagnose Clostridium difficile.(47)
- “The best-performing method (i.e., in use positive and negative predictive value) for detecting patients at increased risk for clinically significant C. difficile [CDI] infection” is use of a “stool toxin test as part of a multistep algorithm…rather than NAAT along for all specimens received in the clinical laboratory when there are no pre-agreed institutional criteria for patient stool submission.”
- “The most sensitive method of diagnosis of CDI in stool specimens from patients likely to have CDI based on clinical symptoms” is use of “a NAAT alone or a multistep algorithm for testing…rather than a toxin test alone when there are pre-agreed institutional criteria for patient stool submission.”

In 2017, the IDSA published clinical practice guidelines for the diagnosis and management of infectious diarrhea.(48) The following recommendations were made:
- In situations where enteric fever or bacteremia is suspected, “culture-independent, including panel-based multiplex molecular diagnostics from stool and blood specimens, and when indicated, culture-dependent diagnostic testing should be performed” (GRADE: strong, moderate).
- In testing for Clostridium difficile in patients >2 years of age, “a single diarrheal stool specimen is recommended for detection of toxin or toxigenic C. difficile strain (e.g., nucleic acid amplification testing)” (GRADE: strong, low).
- NAATs are not recommended for diagnosing Cytomegalovirus.
- It was also noted that “clinical consideration should be included in the interpretation of results of multiple-pathogen nucleic acid amplification tests because these assays detect DNA and not necessarily viable organisms” (GRADE: strong, low).

In 2016, the IDSA published updated clinical practice guidelines for managing candidiasis.(49) The guideline noted many limitations of PCR testing. No formal recommendation was made, but the guidelines did state that “the role of PCR in testing samples other than blood is not established.”

In 2020, the IDSA established a panel composed of 8 members including frontline clinicians, infectious diseases specialists and clinical microbiologists who were members of the IDSA,

American Society for Microbiology (ASM), Society for Healthcare Epidemiology of America (SHEA), and the Pediatric Infectious Diseases Society (PIDS). Panel members represented the disciplines of adult and pediatric infectious diseases, medical microbiology, as well as nephrology and gastroenterology. The panel created a COVID-19 Diagnosis guideline using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach for evidence assessment; and, given the need for rapid response to an urgent public health crisis, the methodological approach was modified according to the GIN/McMaster checklist for development of rapid recommendations. The panel published recommendations for COVID-19 Diagnosis in an online format, as when substantive new information becomes available the recommendations will require frequent updating.(50) The current recommendations (published December 23, 2020) support SARS-CoV-2 nucleic acid testing for the following groups:

- all symptomatic individuals suspected of having COVID-19;
- asymptomatic individuals with known or suspected contact with a COVID-19 case;
- asymptomatic individuals with no known contact with COVID-19 who are being hospitalized in areas with a high prevalence of COVID-19 in the community;
- asymptomatic individuals who are immunocompromised and being admitted to the hospital, regardless ofCOVID-19 exposure;
- asymptomatic individuals prior to hematopoietic stem cell transplant or solid organ transplantation, regardless of COVID-19 exposure;
- asymptomatic individuals without known exposure to COVID-19 undergoing major time-sensitive surgeries;
- asymptomatic individuals without a known exposure to COVID-19 who are undergoing a time-sensitive aerosol generating procedure (e.g., bronchoscopy) when personal protective equipment (PPE) is limited, and testing is available;
- asymptomatic individuals without known exposure when the results will impact isolation/quarantine/ PPE usage decisions, dictate eligibility for surgery, or inform administration of immunosuppressive therapy.

The IDSA panel further recommends the following:

- collecting nasopharyngeal, or mid-turbinate or nasal swabs rather than oropharyngeal swabs or saliva alone for SARS-CoV-2 RNA testing in symptomatic individuals with upper respiratory tract infection (URTI) or influenza like illness (ILI) suspected of having COVID-19 (conditional recommendation, very low certainty of evidence).
- nasal and mid-turbinate (MT) swab specimens may be collected for SARS-CoV-2 RNA testing by either patients or healthcare providers, in symptomatic individuals with upper respiratory tract infection (URTI) or influenza like illness (ILI) suspected of having COVID-19 (conditional recommendation, low certainty of evidence).
- a strategy of initially obtaining an upper respiratory tract sample (e.g., nasopharyngeal swab) rather than a lower respiratory sample for SARS-CoV-2 RNA testing in hospitalized patients with suspected COVID-19 lower respiratory tract infection. If the initial upper respiratory sample result is negative, and the suspicion for disease remains high, the IDSA panel suggests collecting a lower respiratory tract sample (e.g., sputum, bronchoalveolar lavage fluid, tracheal aspirate) rather than collecting another upper respiratory sample (conditional recommendations, very low certainty of evidence)
- performing a single viral RNA test and not repeating testing in symptomatic individuals with a low clinical suspicion of COVID-19 (conditional recommendation, low certainty of evidence).

- repeating viral RNA testing when the initial test is negative (*versus* performing a single test) in symptomatic individuals with an intermediate or high clinical suspicion of COVID-19 (conditional recommendation, low certainty of evidence).
- using either rapid reverse-transcriptase (RT)-PCR or standard laboratory-based NAATs over rapid isothermal NAATs in symptomatic individuals suspected of having COVID-19 (conditional recommendation, low certainty of evidence).

American Society of Transplantation

In 2019, the American Society of Transplantation Infectious Diseases Community of Practice published guidelines which addressed vancomycin-resistant enterococci (VRE) infections in solid organ transplant patients.(51) The guidelines noted the cost-effectiveness and accuracy of "emerging molecular diagnostics for VRE colonization, including multiplexed PCR performed after culture on selective media," compared with culture alone.

American Academy of Pediatrics

The current edition of the AAP Red Book describes the diagnostic and treatment options of many infectious diseases in the pediatric population.(52) Their recommendations for appropriate diagnostic tests for the viruses and infections discussed in this policy are detailed in Table 12.

Table 12. Redbook Diagnostic Test Recommendations for the Pediatric Population

Infection	Diagnostic Test Recommendation
Bartonella henselae	EIA IFA NAAT (PCR)
Candida Species	Clinical Evaluation Microscopy PNA FISH probes and PCR assays developed for rapid detection directly from positive blood cultures.
Chlamydia pneumoniae	NAATs (PCR) are the preferred method for diagnosis of acute infection. Serologic antigen test is an option, but is technically complex and interpretation is subjective
Chlamydia trachomatis	NAATs are recommended for C trachomatis urogenital infections and in postpubescent individuals. They are not recommended for diagnosis C trachomatis conjunctivitis or pneumonia or in the evaluation of prepubescent children for possible sexual assault.
Clostridioides (Clostridium) difficile	NAATs detect genes responsible for the production of toxins A and B, rather than free toxins A and B in the stool, which are detected by EIA. NAAT could be considered alone if a policy in place to screen symptoms; if no policy in place, multi-step algorithms involving EIA, GDH, NAAT plus toxin is recommended
Cytomegalovirus	Saliva PCR is the preferred diagnostic tool for screening.
Enterovirus	RT-PCR and culture from a variety of specimens
Gardnerella vaginalis	Microscopy Numerous NAATs have been recommended when microscopy is unavailable
Hepatitis B	Serologic antigen tests NAATs
Hepatitis C	IgG antibody enzyme immunoassays NAATs
Herpes Simplex Virus	Cell culture NAATs- diagnostic method of choice for neonates with CNS infections, older children, and adults with HSE
Human Herpesvirus 6	Few developed assays are available commercially and do not differentiate between new, past, and reactivated infection. Therefore, these tests "have limited utility in clinical practice:" Serologic tests; PCR- the assays are not sensitive in younger children.

HIV 1	HIV DNA PCR or RNA PCR - preferred test to diagnose HIV-1 infection in infants and children younger than 18mo; highly sensitive and specific by 2weeks of age and available
Human Papillomavirus	“Detection of HPV infection is based on detection of viral nucleic acid”
Influenza Virus	“RT-PCR, viral culture tests, and rapid influenza molecular assays are available options for testing; optimal choice of influenza test depends on the clinical setting.”
Legionella pneumophila	BCYE Media Legionella antigen in urine Direct IFA Genus-specific PCR reaction-based assays
Meningitis	Cultures of blood and CSF NAATs- “useful in patients who receive antimicrobial therapy before cultures are obtained.”
Mycobacteria Species	M tuberculosis disease: Chest radiography and physical examination Several NAATs are cleared for rapid detection of M tuberculosis, but expert consultation is recommended for interpretation of results Nontuberculous Mycobacteria: “definite diagnosis of NTM disease requires isolation of the organism.”
Mycoplasma pneumonia	“PCR tests for M pneumoniae are available commercially and increasing replacing other tests, because PCR tests performed on respiratory tract specimens have sensitivity and specifically between 80% and 100%, yield positive results earlier in the course of illness than serologic tests, and are rapid.”
Neisseria gonorrhoeae	“NAATs are far superior in overall performance compared with other N gonorrhoeae culture and nonculture diagnostic methods to test genital and nongenital specimens, but performance varies by NAAT type.”
Staphylococcus aureus	“NAATS are approved for detection and identification of S aureus, including MRSA, in positive blood cultures.”
Streptococcus, Group A	“Children with pharyngitis and obvious viral symptoms should not be tested or treated for GAS infection.. Laboratory confirmation before initiation of antimicrobial treatment is required for cases in children without viral symptoms… culture on sheep blood agar can confirm GAS infection.”
Streptococcus, Group B	“Gram-positive cocci in pairs or short chains from a normally sterile body fluid provides presumptive evidence of infection.”
Trichomonas vaginalis	Microscopy NAATs are “the most sensitive mean of diagnosing T vaginalis infection and is encouraged for detection in females and males.”
Vancomycin-resistant enterococcus	“Selective agars are available for screening of vancomycin-resistant enterococcus from stool specimens. Molecular assays are available for direct detection of vanA and vanB genes from rectal and blood specimens to identify vancomycin-resistant enterocci"
Zika	NAATs Trioplex real-time PCR assay Serologic testing

BCYE: buffered charcoal yeast extract; CNS: central nervous system; CSF: cerebrospinal fluid; DNA: deoxyribonucleic acid; EIA: enzyme immunoassay; FDA: Food and Drug Administration; GDH: glutamate dehydrogenase; HIV: human immunodeficiency virus; HPV: human papillomavirus; HSE: herpes simplex encephalitis;
IFA: indirect fluorescent antibody; MSRA: methicillin-resistant Staphylococcus aureus; NAAT: nucleic acid amplification test; NTM: nontuberculous mycobacteria; PCR: polymerase chain reaction; PNA FISH: peptide nucleic acid fluorescent in situ hybridization; RNA: ribonucleic acid; RT: reverse transcriptase; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2.

In 2019, the AAP published guidelines on managing infants at risk for GBS.(53) It recommends antenatal vaginal-rectal culture performed by using a broth enrichment “followed by GBS identification by using traditional microbiologic methods or by NAAT-based methods.” However, point-of-care NAAT-based screening should not be the primary method of determining maternal colonization status due to reported variable sensitivity as compared with traditional culture, as well as “because most NAAT-based testing cannot be used to determine the antibiotic susceptibility of colonizing GBS isolates among women with a penicillin allergy.”

American College of Gastroenterology
In 2016, the American College of Gastroenterology published clinical guidelines on the diagnosis, treatment, and prevention of acute diarrheal infections in adults.(54) It recommended that, given that “traditional methods of diagnosis (bacterial culture, microscopy with and without special stains and immunofluorescence, and antigen testing) fail to reveal the etiology of the majority of cases of acute diarrheal infection,… the use of FDA-approved culture-independent methods of diagnosis can be recommended at least as an adjunct to traditional methods. (Strong recommendation, low level of evidence).” These are described in the rationale as multiplex molecular testing.

American Society for Microbiology
In 2020, the American Society for Microbiology updated the 2010 guidelines on detecting and identifying GBS that were originally published by the CDC, with plans to continue updating regularly.(55) The most recent update took place July 2021. The guidelines state that "intrapartum NAAT without enrichment has an unacceptably high false negative rate...As such we do not recommend the use of intrapartum NAAT without enrichment to rule out the need for prophylaxis." All GBS screening specimens should be incubated in selective enrichment broth prior to agar media plating or NAAT. "Nucleic acid amplification-based identification of GBS from enrichment broth is acceptable" for GBS screening, "but not sufficient for all patients" due to high false-negative rates.

European Guideline on the Management of Non-gonococcal Urethritis
The International Union against Sexually Transmitted Infections recommends confirmation of urethritis in symptomatic men before starting treatment. It does not recommend testing asymptomatic men for the presence of urethritis. All men with urethritis should be tested for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* and ideally *M. genitalium* using a NAAT as this is highly likely to improve clinical outcomes.(64)

British Association for Sexual Health
The British Association for Sexual Health and HIV National Guideline indicated *M. genitalium* infection is unequivocally and strongly associated with non-gonococcal urethritis (NGU). Typically, the prevalence of *M. genitalium* in men with NGU is 10-20% and in male patients with non-chlamydial nongonococcal urethritis is 10-35%, as compared to 1-2% in the general population. Several studies support an association of *M. genitalium* infection in cisgender women with post coital bleeding and cervicitis, endometritis and pelvic inflammatory disease (PID). A recent meta-analysis has demonstrated significant associations between *M. genitalium* and cervicitis (pooled OR 1.66) and PID (pooled OR 2.14), in addition to pre-term birth and spontaneous abortion (pooled ORs 1.89 and 1.82 respectively). The evidence suggests that the majority of people infected with *M. genitalium* in the genital tract do not develop disease. Current treatments are imperfect and associated with development of antimicrobial resistance. There is no evidence that screening asymptomatic individuals will be of benefit, and indeed is likely to do harm at a population level. Grading recommendations are as follows:(63)

1B - Test for *M. genitalium* infection in people with non-gonococcal urethritis
1B - Test for *M. genitalium* infection in all individuals with signs and symptoms suggestive of pelvic inflammatory disease
1D - Test current sexual partners of persons infected with *M. genitalium.*
2B - Consider testing for *M. genitalium* infection in people with signs or symptoms of mucopurulent cervicitis, particularly post-coital bleeding
2D - Consider testing for *M. genitalium* infection in people with epididymitis.

2D - Consider testing for *M. genitalium* infection in people with sexually-acquired proctitis.

American Urological Association
The American Urological Association updated their guideline (2022) for recurrent uncomplicated urinary tract infections in women. Testing recommendations in symptomatic women included urinalysis and urine cultures with sensitivity. Discussion included the PCR technology, however the committee indicated that more evidence is needed before these technologies become incorporated into the guideline. There is concern is that adoption of this technology in the evaluation of lower urinary tract symptoms may lead to over treatment with antibiotics.(65)

U.S. PREVENTIVE SERVICES TASK FORCE RECOMMENDATIONS
Not applicable.

ONGOING AND UNPUBLISHED CLINICAL TRIALS
A search of ClinicalTrials.gov did not identify any ongoing or unpublished trials that would likely influence this review.

Government Regulations
National:
There is no national coverage determination. In the absence of a national coverage determination, coverage decisions are left to the discretion of local Medicare carriers.

During the Public Health Emergency for the COVID-19 pandemic, a number of Medicare exceptions and waivers have been implemented. For further information on testing, see the CMS website regarding Coronavirus Disease.(49)

Local:
Local Coverage Determination: **MolDX: Molecular Syndromic Panels for Infectious Disease Pathogen Identification Testing** L39044; Original Effective Date: 4/17/22; Revised 6/9/22.(66)

Coverage Indications, Limitations, and/or Medical Necessity
This policy provides limited coverage for outpatient testing with panels using molecular syndromic panels for infectious disease pathogen identification testing. This policy does NOT address coverage for the inpatient setting.

This policy defines a panel as a test that detects > 1 pathogen. This policy also differentiates (where appropriate) between small, targeted panels (up to 5 pathogens) and larger, expanded panels (≥6 pathogens). This distinction is primarily applied to the Respiratory and Gastrointestinal Panels. A 'syndromic panel' is further defined as one that simultaneously detects multiple different pathogens associated with similar and overlapping clinical symptomatology.

General Criteria For Coverage For A Molecular Syndromic Infectious Disease Pathogen Identification Panel Test
This Medicare Contractor will cover molecular syndromic infectious disease pathogen identification panel tests when ALL of the following criteria are met:
- The patient has a clinical indication for infectious disease testing:

- For immunocompetent patients, the clinical indication includes a presumption of active infection OR infection-associated complications (which may include exacerbation of underlying disease) *that require the identification of a causative organism for appropriate management*. Atypical clinical presentations of disease are considered appropriate indications for special populations who may not present with classic symptoms of infection (i.e., the elderly).
- For immunocompromised patients (i.e., those with weakened immune systems including those with human immunodeficiency virus (HIV) or acquired immunodeficiency syndrome (AIDS), patients who are taking immunosuppressive medications (i.e., chemotherapy, biologics, transplant-related immunosuppressive drugs, high-dose systemic corticosteroids) and those with inherited diseases that affect the immune system (i.e., congenital immunoglobulin deficiencies), atypical clinical presentations of disease are considered appropriate indications for testing. In this patient population, testing may be performed ONCE as part of a pre-transplant evaluation, regardless of the presence of symptoms.
- **Note:** For certain panels, such as the Urogenital/Anogenital Panel, epidemiologic indication or potential exposure to pathogens as a result of a high-risk experience is considered a covered clinical indication, even in the absence of clinical symptoms. These are specifically noted below in **LIMITED COVERAGE FOR *EXPANDED* (>5 Pathogens) PANEL TESTING**.

- The results of testing will impact clinical management in a manner already demonstrated in the peer-reviewed published literature to improve patient outcomes.
- Testing is performed according to the intended use of the test in the intended patient population for which the test was developed and validated.
  - This includes performing the test using the intended sample types along with parallel testing that must accompany the test (i.e., the meningoencephalitis and bloodstream pathogen tests include requirements for parallel testing using conventional Gram stain and culture-based detection for correlation of results).
  - This also includes the provision - by the laboratory to ordering providers - of the major limitations of a given panel test.
- An evaluation for more than 1 pathogen by molecular testing is necessary for patient management (testing for a single pathogen is NOT reasonable and necessary for the specific infection, patient, or indication). The panel performed includes *at least* the minimum pathogens required for clinical decision making for its intended use that can be reasonably detected by the test.
- Expanded panel testing is only indicated when targeted panel testing is not appropriate (i.e., will not provide sufficient information for the appropriate clinical management of the patient). See **LIMITED COVERAGE FOR *EXPANDED* (>5 Pathogens) PANEL TESTING** below.
- Services that do not have Food and Drug Administration (FDA)-cleared/approved indicated uses, as well as FDA-approved tests performed in ways not consistent with their intended-use labeling directions, will require registration with Molecular Diagnostic Services Program (MolDX®) and a Technical Assessment (TA) to demonstrate compliance of the service with this policy. Similarly, tests (and CPT codes) for which there are no accompanying ICD-10 codes in the associated Billing and Coding Article will require registration with MolDX® and a TA to demonstrate compliance of the service with this policy.
- Registered tests must demonstrate equivalent or superior test performance characteristics - analytical validity (AV) and clinical validity (CV) - to established standard-of-care (SOC)

methods (i.e., culture, pathogen-specific polymerase chain reaction [PCR]) *for the majority of targets included on the panel*.
    - CV of any new organisms and analytes that are not already established as SOC or that do not have a predicate test that is covered by this contractor must be established through a study published in the peer-reviewed literature for the intended use of the test in the intended population.
- Documentation of the following is clearly stated in the medical record:
    - Specific clinical indications for testing (i.e., clinical suspicion of a pathogen as the cause of the patient's condition)
    - Specific reasons for performing panel testing.
    - Provider type/specialty and Place of Service
- Testing must be performed according to Clinical Laboratory Improvement Amendments (CLIA) and/or FDA regulations. For example, CLIA-non-waived tests may only be performed in certified laboratories and according to CLIA regulations. CLIA-waived tests may be performed in healthcare settings that operate under a CLIA Certificate of Waiver or Certificate of Compliance/Certificate of Accreditation. Panels intended for home use (including those that have been FDA approved or cleared) do NOT meet the coverage criteria of this policy.

Non-Coverage Criteria

Molecular Syndromic Panel Tests will NOT be covered in the following circumstances:

- If the test is performed as a test of cure.
- If the patient has been previously tested by molecular diagnostic methods for the same pathogens within 14 days for the same clinical indication.
    - If a previous panel test was performed with a similar/duplicative intended use, a subsequent test is only reasonable and necessary if the non-duplicative content of the second test is reasonable and necessary.
    - Exception: Repeat panel testing for the same clinical indication will only be covered if first panel yielded a negative result AND there is a high index of suspicion for a pathogen as the cause of symptoms AND the patient's clinical condition is not improving or is deteriorating after a clinically appropriate length of time. In such cases, 1 additional panel test may be covered between 1 and 14 days after the initial panel test, so long as the test fulfills the criteria for coverage as set forth in this policy.

LIMITED COVERAGE FOR *EXPANDED* (>5 Pathogens) PANEL TESTING FOR THE SPECIFIC PANEL TYPES LISTED BELOW, ALL OF THE FOLLOWING ADDITIONAL CRITERIA MUST BE MET:

- **Respiratory (RP) and Pneumonia (PNP) Panels** will only be covered when targeted testing is not appropriate AND according to the following additional criteria:
    - For immune-competent patients, at least 1 of the following must apply:
        - Testing is ordered by a clinician specialist in Infectious Diseases or Pulmonology for a patient with severe and established underlying respiratory pathology (i.e., severe asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, pulmonary fibrosis, radiation therapy to the lung) AND treatment with antibiotics may be indicated according to established guidelines. Specific examples that do NOT meet coverage criteria according to established guidelines include the following:
            - Asthma exacerbations without the additional presence of either fever and purulent sputum or radiographic evidence of pneumonia
            - Uncomplicated community acquired pneumonia (CAP)

        - The patient is seriously or critically ill or at imminent risk of becoming seriously or critically ill (as defined by the American Hospital Association's "General Guide for the Release of Information on the Condition of Patients") as a result of a presumed respiratory infection AND the patient is being treated in an appropriate critical care facility.
    - For immune-suppressed patients: Testing is ordered by a clinician specialist in 1 of the following: Infectious Diseases, Pulmonology, Oncology, Transplant OR the patient is being managed in an appropriate critical care facility.
    - For ALL patients: Only 1 of the following panels - RP OR PNP- will be covered for a given patient for the same clinical indication. The PNP should be prioritized in the evaluation of pneumonia from lower respiratory tract specimens (i.e., bronchoalveolar lavage samples [BALs]). For the purposes of repeat panel testing for the same clinical indication, RP and PNP will be considered as equivalent tests, such that if criteria for repeat testing are met (as defined above), a clinician may choose to perform the repeat test using the PNP, even if the original test was performed using the RP.
    - For ALL patients, exceptions to the limitation on medical specialists who can order expanded panel tests are provided in the accompanying Billing and Coding Article, such that patient geography and access to care do not preclude the receipt of appropriate diagnostic testing when indicated.
- **Gastrointestinal (GI) Panels** will <u>only</u> be covered when targeted testing is not appropriate AND according to the following additional criteria:
    - For immune-competent patients, at least 1 of the following must apply:
        - Testing is ordered by a clinician specialist in Infectious Diseases or Gastroenterology for a patient with severe and established underlying GI pathology (i.e., inflammatory bowel disease (IBD), paralytic ileus, radiation therapy to the intestine) AND identification of an infectious cause is necessary to determine next steps in patient management.
        - The patient is <u>seriously or critically ill or at imminent risk of becoming seriously or critically ill</u> (as defined by the American Hospital Association's "General Guide for the Release of Information on the Condition of Patients") as a result of a presumed GI infection AND the patient is being treated in an appropriate critical care facility.
        - The patient's clinical indication for GI panel testing is diarrhea, and ALL of the following apply:
            - The diarrheal illness MUST be acute or persistent with signs or risk factors for severe disease (i.e., fever, bloody diarrhea, dysentery, dehydration, severe abdominal pain) that may warrant hospitalization AND/OR
            - The diarrheal illness is not resolving after 7 days AND the patient has NOT taken laxatives within 24 hours of the test.
    - For immune-suppressed patients:
        - Testing is ordered by a clinician specialist in 1 of the following: Infectious Diseases, Gastroenterology, Oncology, Transplant OR the patient is being managed in an appropriate critical care facility.
    - For ALL patients, exceptions to the limitation on medical specialists who can order expanded panel tests are provided in the accompanying Billing and Coding Article, such that patient geography and access to care do not preclude the receipt of appropriate diagnostic testing when indicated.
- **Urogenital/Anogenital (UG/AG) Panels**
    - For the UG/AG panels, epidemiologic indication or potential exposure to sexually transmitted pathogens (i.e., in the case of clinical concern for multiple sexually

transmitted infections (STIs) due to a high-risk experience) is considered a covered clinical indication, even in the absence of clinical symptoms. Documentation of the high-risk reason for panel testing is clearly stated in the medical record.
    - In the absence of a high-risk experience, if the primary clinical concern is for a few specific pathogens due to specific signs and symptoms (i.e., lesions suggestive of herpes simplex virus [HSV]), then it is expected that only a small, targeted panel (i.e., including HSV-1 and HSV-2) will be performed. In such cases, expanded panels are NOT considered reasonable and necessary and will NOT be covered.
    - For the diagnosis of infectious vaginosis/vaginitis, it is reasonable to perform a (targeted or expanded) panel that includes a combination of at least 2 of the following: Gardnerella vaginalis, other BV-associated bacteria (BVAB) (such as Atopobium vaginae and/or Megasphaera types), Trichomonas vaginalis, and Candida species.
- **Meningoencephalitis (ME) Panels** will be covered according to the following additional criteria:
    - For immune-competent patients: the patient has at least 2 of the following indicators of central nervous system (CNS) infection: cerebrospinal fluid (CSF) markers, radiology, clinical signs and symptoms consistent with meningitis or encephalitis, epidemiologic indication or exposure. For immune-compromised patients, at least 1 of these indicators is required.
    - For all patients: Testing is from a sample collected via lumbar puncture, and NOT an indwelling medical device (i.e., CSF shunts).
- **Bloodstream Infection (BSI) Panels** will be covered according to the following additional criteria:
    - There is clinical concern for bacteremia or sepsis AND microbe(s) were seen on a Gram stain from the patient's blood AND the patient is being managed in an appropriate critical care facility (this includes the Emergency Room), AND
    - Personnel (i.e., an antimicrobial stewardship team [ASP]) are equipped for rapid (within 24 hours) tailoring of antimicrobial therapy as a result of rapid testing.
- **Urinary Tract Infection (UTI) Panels** will be covered according to the following additional criteria:
    - The patient is symptomatic AND at higher risk for UTI complications (i.e., the elderly, patients with recurrent symptomatic UTIs and/or complicated urinary tract anatomy) AND/OR is seen in urogynecology or urology specialty care settings.

Article: **Billing and Coding: MolDX: Molecular Syndromic Panels for Infectious Disease Pathogen Identification Testing**. A58761; Original Effective Date: 4/17/22; Revised 7/1/24. Additional information:(67) *Bullet points below represent the highlights of the article. Refer to actual article for further information.*

- Any diagnosis submitted must have documentation in the patient's record to support coverage and medical necessity.
- Panels intended for home use (including those that have been FDA approved or cleared) do NOT meet the coverage criteria of the policy.
- This contractor expects that critically ill patients will be tested and managed in the appropriate critical care facility.
- The test panel is a single test with multiple components and is characterized by a single unit of service. A panel cannot be unbundled and billed as individual components regardless of the fact that the test reports multiple individual pathogens and/or targets. If additional organisms are not included in a panel, testing for those organisms separately

may be reasonable and necessary when ordered in addition to the panel and supported by documentation in the medical record.

- Repeat panel tests for the same clinical indication will NOT be reimbursed, except according to the criteria outlined in the related LCD (i.e.,1 additional panel test may be performed between 1 and 14 days after the initial panel test, so long as the test fulfills the criteria for coverage as set forth in the policy).
- It is understood that in certain instances in which only targeted testing is appropriate, institutions may not have access to small panels and may have to perform larger panels for technical reasons. In such cases, Palmetto will pay only for components of a service that are reasonable and necessary.
- For Expanded (>5 pathogens) RP, PNP, and GI Panels the following additional conditions apply:
    - For immune-competent beneficiaries, the test must be ordered by an Infectious Disease Specialist or 1 of the following: Pulmonologist (for the RP and PNP panels) or Gastroenterologist (for the GI panels) who is diagnosing and treating the beneficiary.
    - For immune-compromised beneficiaries, the test must be ordered by a clinician specialist in 1 of the following: Infectious Diseases, Oncology, Transplant (for any panel), Pulmonologist (for the RP and PNP panels), or Gastroenterologist (for the GI panels) who is diagnosing and treating the beneficiary.
    - Regarding above 2 hollow bullets, an exception may be made in geographic locations where the specialist(s) cannot be reasonably reached by the beneficiary, and the ordering provider is located closer to the beneficiary's place of residence than the nearest specialist. We would generally expect that beneficiaries for whom the test is ordered under this exception to be living in rural locations, islands, or some other location where access to care is limited.
- The expanded/targeted panel distinction is not applicable to all panels, except as otherwise indicated in the related policy.

See article for coding information.

(The above Medicare information is current as of the review date for this policy. However, the coverage issues and policies maintained by the Centers for Medicare & Medicare Services [CMS, formerly HCFA] are updated and/or revised periodically. Therefore, the most current CMS information may not be contained in this document. For the most current information, the reader should contact an official Medicare source.)

Related Policies

- Diagnosis of Vaginitis (Including Bacterial Vaginosis, Trichomonas and Candidiasis) Using Multi-Target PCR Testing
- Polymerase Chain Reaction (PCR) Testing in the Diagnosis of Onychomycosis

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The articles reviewed in this research include those obtained in an Internet based literature search for relevant medical references through 8/23/24, the date the research was completed.

Joint BCBSM/BCN Medical Policy History

Policy Effective Date	BCBSM Signature Date	BCN Signature Date	Comments
7/1/18	4/17/18	4/17/18	Joint policy established
7/1/21			Routine maintenance – Tabled
1/1/23	10/18/22		• Reformatted to cover past and present BCBSA coverage, FDA approved tests and align with Avalon (slp) • Added as EST: M. Gen, SARS-CoV-2, Dengue virus, CNS panel, chlamydia pneumoniae, Zika virus. • PCR use in UTIs is covered with criteria for small population per discussion with SME. • Criteria added for CNS pathogen panels. • Added exclusions for wound infection, sepsis and nail infection and testing that is performed as a test of cure. • Inclusions added for UTI and CNS pathogens and repeat panel testing. • Note added to coding r/t unbundling. • Codes used by Medicare added as EI (0115U, 0225U) • 0321U (EI) per code update • Codes added as EST: 0202U and 0223U per workgroup • Changed definition of immunocompromised to align with Medicare. • Defined abnormal urinary tract to mean post-surgical
1/1/24	10/25/23		• Vendor managed: Avalon (slp) • Vaginitis pathogens and codes removed – transferred to "Diagnosis of Vaginitis (Including Bacterial Vaginosis, Trichomonas and Candidiasis) Using Multi-Target PCR Testing". Includes

			87482, 87510, 87511, 87512, 87660, 87661. • Non vaginal candida species remains on this policy per vendor. • Panel testing for CNS – only one bullet needs to be met (was originally “all”) • Exclusion for blood stream infections clarified to have exception for meningitis.
1/1/25	10/15/24		• Vendor managed: Avalon (slp) • RSV moved to table and code added (EST; 87634) • Hep D moved to table and code added (EST; 87523) • Rubeola added to measles information for clarity • Nail infections removed from policy – addressed by related policy • Language removed from coding section regarding proprietary lab codes • Exclusion added for MicroGenDX qPCR+NGS • Coding changes include: o Added as EI – 87482, 0445U, 0480U, 0483U, 0484U, 0505U o Added as EST – 87523 o Moved to EST - 87797

Next Review Date: 4th Qtr, 2025

BLUE CARE NETWORK BENEFIT COVERAGE
POLICY: IDENTIFICATION OF MICROORGANISMS USING NUCLEIC ACID PROBES

I. Coverage Determination:

Commercial HMO (includes Self-Funded groups unless otherwise specified)	Covered; criteria apply
BCNA (Medicare Advantage)	Refer to Medicare section under Government Regulations
BCN65 (Medicare Complementary)	Coinsurance covered if primary Medicare covers the service.

II. Administrative Guidelines:

- The member's contract must be active at the time the service is rendered.
- Coverage is based on each member's certificate and is not guaranteed. Please consult the individual member's certificate for details. Additional information regarding coverage or benefits may also be obtained through customer or provider inquiry services at BCN.
- The service must be authorized by the member's PCP except for Self-Referral Option (SRO) members seeking Tier 2 coverage.
- Services must be performed by a BCN-contracted provider, if available, except for Self-Referral Option (SRO) members seeking Tier 2 coverage.
- Payment is based on BCN payment rules, individual certificate and certificate riders.
- Appropriate copayments will apply. Refer to certificate and applicable riders for detailed information.
- CPT - HCPCS codes are used for descriptive purposes only and are not a guarantee of coverage.
- Duplicate (back-up) equipment is not a covered benefit.