

## Identification of Microorganisms using Nucleic Acid Probes

Policy Number: AHS – M2097 – Identification of Microorganisms Using Nucleic Acid Probes	Prior Policy Name and Number, as applicable:
Effective Date: 02/01/2025	

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### I. Policy Description

Nucleic acid hybridization technologies utilize complementary properties of the DNA double-helix structures to anneal together DNA fragments from different sources. These techniques are utilized in polymerase chain reaction (PCR) and fluorescent resonance energy transfer (FRET) techniques to identify microorganisms (Khan, 2014).

A discussion of every infectious agent that might be detected with a probe technique is beyond the scope of this policy. Many probes have been combined into panels of tests. For the purposes of this policy, only individual probes are reviewed.

For guidance on nucleic acid identification of *Candida* in vaginitis, please refer to AHS-M2057-Diagnosis of Vaginitis Including Multi-Target PCR Testing.

### II. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual’s benefit coverage at the time of the request.

- 1) The coverage status of nucleic acid identification using direct probe, amplified probe, or quantification for the microorganism's procedure codes is summarized in Table 1 below. "MCC" in the table below indicates that the test **MEETS COVERAGE CRITERIA**; while "DNMCC" tests indicates that the test **DOES NOT MEET COVERAGE CRITERIA**.

<b>Microorganism</b>	<b>Direct Probe</b>	<b>Amplified Probe</b>	<b>Quantification</b>
<i>Bartonella henselae</i> or <i>quintana</i>		87471 (MCC)	87472 (DNMCC)
Non-vaginal <i>Candida</i> species	87480 (DNMCC)	87481 (DNMCC)	87482 (DNMCC)
<i>Chlamydia pneumoniae</i>	87485 (MCC)	87486 (MCC)	87487 (DNMCC)
<i>Clostridium difficile</i>		87493 (MCC)	
<i>Cytomegalovirus</i>	87495 (MCC)	87496 (MCC)	87497 (MCC)
<i>Enterococcus</i> , Vancomycin-resistant (e.g., <i>enterococcus vanA</i> , <i>vanB</i> )		87500 (MCC)	
<i>Enterovirus</i>		87498 (MCC)	
Hepatitis G	87525 (DNMCC)	87526 (DNMCC)	87527 (DNMCC)
Herpes virus-6	87531 (DNMCC)	87532 (DNMCC)	87533 (MCC)
<i>Legionella pneumophila</i>	87540 (MCC)	87541 (MCC)	87542 (DNMCC)
Orthopoxvirus		87593 (MCC)	
<i>Mycoplasma pneumoniae</i>	87580 (MCC)	87581 (MCC)	87582 (DNMCC)
Respiratory syncytial virus		87634 (MCC)	
<i>Staphylococcus aureus</i>		87640 (MCC)	
<i>Staphylococcus aureus</i> , methicillin resistant		87641 (MCC)	

- 2) Simultaneous ordering of any combination of direct probe, amplified probe, and/or quantification for the same organism in a single encounter **DOES NOT MEET COVERAGE CRITERIA**.

### III. Table of Terminology

<b>Term</b>	<b>Definition</b>
CDC	Centers for Disease Control and Prevention
CDI	<i>Clostridioides difficile</i> infection
CIDT	Culture-independent diagnostic test
CMV	Cytomegalovirus
CPT	Current procedural terminology
DFA	Direct fluorescent antibody testing

DNA	Deoxyribonucleic acid
EVD	Ebola virus disease
FDA	Food and Drug Administration
FRET	Fluorescent resonance energy transfer
HHV-6	Human herpesvirus 6
IDSA	Infectious Diseases Society of America
ITS	Internal transcribed region
Mpox	Monkeypox
MRSA	Methicillin-Resistant Staphylococcus Aureus
NAATs	Nucleic acid amplification tests
NGU	Nongonococcal urethritis
PCR	Polymerase chain reaction
PID	Pelvic inflammatory disease
qPCR	Quantitative polymerase chain reaction
rDNA	Recombinant deoxyribonucleic acid
RNA	Ribonucleic acid
rRT-PCR	Real-time reverse transcriptase-polymerase chain reaction
RSV	Respiratory syncytial virus infection
RT-PCR	Reverse transcriptase-polymerase chain reaction
SARS	Severe acute respiratory syndrome

#### IV. Scientific Background

Nucleic acid hybridization technologies, including polymerase chain reaction (PCR), ligase- or helicase-dependent amplification, and transcription-mediated amplification, are beneficial tools for pathogen detection in blood culture and other clinical specimens due to high specificity and sensitivity (Khan, 2014). The use of nucleic acid-based methods to detect bacterial pathogens in a clinical laboratory setting offers “increased sensitivity and specificity over traditional microbiological techniques” due to its specificity, sensitivity, reduction in time, and high-throughput capability; however, “contamination potential, lack of standardization or validation for some assays, complex interpretation of results, and increased cost are possible limitations of these tests” (Mothershed & Whitney, 2006).

#### V. Guidelines and Recommendations

##### World Health Organization (WHO)

For detection of mpox, the WHO recommends “detection of viral DNA by polymerase chain reaction (PCR)” as the preferred laboratory test and recommends that any individual with a suspected case should be offered testing. They note that the best specimens for diagnosis are taken directly from the rash. Antigen and antibody detection may not be able to distinguish between orthopoxviruses (WHO, 2022).

## **2018 Infectious Diseases Society of America (IDSA)**

Specific guidelines for testing of many organisms listed within the policy coverage criteria is found in the updated 2018 Infectious Diseases Society of America (IDSA) guidelines and recommendations titled, “A Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases: 2018 Update by the Infectious Diseases Society of America and the American Society for Microbiology” (Miller et al., 2018). “This document is organized by body system, although many organisms are capable of causing disease in >1 body system. There may be a redundant mention of some organisms because of their propensity to infect multiple sites. One of the unique features of this document is its ability to assist clinicians who have specific suspicions regarding possible etiologic agents causing a specific type of disease. When the term “clinician” is used throughout the document, it also includes other licensed, advanced practice providers. Another unique feature is that in most chapters, there are targeted recommendations and precautions regarding selecting and collecting specimens for analysis for a disease process. It is very easy to access critical information about a specific body site just by consulting the table of contents. Within each chapter, there is a table describing the specimen needs regarding a variety of etiologic agents that one may suspect as causing the illness. The test methods in the tables are listed in priority order according to the recommendations of the authors and reviewers” (Miller et al., 2018).

## **Centers of Disease Control and Prevention (CDC)**

### *Candida Auris (C. auris)*

The CDC writes that “Molecular methods based on sequencing the D1-D2 region of the 28S rDNA or the Internal Transcribed Region (ITS) of rDNA can identify *C. auris*.” The CDC further notes that various PCR methods have been developed for identifying *C. auris* (CDC, 2024d).

### *Chlamydia Pneumoniae (C. pneumoniae)*

The CDC writes that RT-PCR is the “preferred” method of detecting an acute *C. pneumoniae* infection (CDC, 2024e).

### *Clostridioides difficile (C. diff)*

The CDC states that there are four laboratory tests that can be used to diagnose *Clostridioides difficile* infection (CDI). “FDA-approved PCR assays are same-day tests that are highly sensitive and specific for the presence of a toxin-producing *C. diff* organism.” The CDC does note that “molecular assays can be positive for *C. diff* in asymptomatic individuals and those who do not have an infection” and “when using multi-pathogen (multiplex) molecular methods, read the results with caution as the pre-test probability of *C. diff* infection might be less” (CDC, 2024b).

### *Cytomegalovirus (CMV)*

The CDC states that “The enzyme-linked immunosorbent assay is the most common serologic test for measuring antibody to CMV.” The CDC also notes that “congenital CMV infection cannot be diagnosed with antibody testing (IgG and IgM)” and recommends “the standard

laboratory test for diagnosing congenital CMV infection is a PCR on saliva, with a confirmatory test on urine.” (CDC, 2024f).

### *Mpox Virus*

The CDC defines a suspect case of Mpox as a “new characteristic rash or meets one of the epidemiologic criteria and has a high clinical suspicion for mpox.” A probable case is defined as “no suspicion of other recent *Orthopoxvirus* exposure (e.g., Vaccinia virus in ACAM2000 vaccination) AND demonstration of the presence of *Orthopoxvirus* DNA by polymerase chain reaction of a clinical specimen OR *Orthopoxvirus* using immunohistochemical or electron microscopy testing methods OR Demonstration of detectable levels of anti-orthopoxvirus IgM antibody during the period of 4 to 56 days after rash onset.” A confirmed case of Mpox is defined as “demonstration of the presence of Mpox virus DNA by polymerase chain reaction testing or Next-Generation sequencing of a clinical specimen OR isolation of Mpox virus in culture from a clinical specimen” (CDC, 2024k).

The CDC states that “Mpox is diagnosed using real time PCR tests” and further notes “clinicians should collect two swabs from each lesion (generally from 2-3 lesions) in case additional testing, such as clade-specific testing, is needed for these patients” (CDC, 2024l).

### *MRSA*

The CDC remarks that “Providers can test some patients to see if they carry MRSA in their nose or on their skin. This test involves rubbing a cotton-tipped swab in the patient’s nostrils or on the skin. The only way to know if MRSA is the cause of an infection is to test for the bacteria in a laboratory.” The CDC further states “There are many methods laboratorians can use to test for MRSA” and lists that “Phenotypic methods recommended for the detection of MRSA include: cefoxitin broth microdilution, oxacillin broth microdilution, and cefoxitin disk diffusion testing.” The CDC includes additional methods including “Nucleic acid amplification tests, such as the polymerase chain reaction (PCR), to detect the *mecA* gene, which mediates oxacillin resistance in staphylococci” but notes “*mecA* PCR tests will not detect novel resistance mechanisms or uncommon phenotypes (e.g., *mecC* or borderline-resistant oxacillin resistance)” (CDC, 2024h).

### *Non-Polio Enterovirus*

The CDC remarks that their laboratories “routinely” perform qualitative testing for enteroviruses, parechoviruses, and uncommon picornaviruses and states that “CDC and some health departments test with molecular sequencing methods, or a real-time reverse transcription polymerase chain reaction (rRT-PCR) lab test” (CDC, 2024j).

### *Respiratory Syncytial Virus (RSV)*

The CDC writes that “PCR tests can be used to diagnose anyone for RSV. Antigen tests are only effective when testing infants and young children” (CDC, 2024c).

### *Miscellaneous*

The CDC does not mention the need to quantify [through PCR] *Bartonella*, *Legionella pneumophila*, or *Mycoplasma pneumoniae*. However, PCR can be performed for both *Bartonella*,

*Legionella pneumophila*, and *Mycoplasma pneumoniae* specimen (CDC, 2024a, 2024g, 2024i). “Nucleic Acid Amplification Tests (NAATs) are the preferred method of diagnostic testing for *M. pneumoniae* infections” (CDC, 2024i). No guidance was found on Hepatitis G.

### **Committee on Infectious Diseases, American Academy of Pediatrics, 31st Edition (2018-2021, Red Book)**

The Committee on Infectious Diseases released joint guidelines with the American Academy of Pediatrics. In it, they note that “the presumptive diagnosis of mucocutaneous candidiasis or thrush usually can be made clinically.” They also state that FISH probes may rapidly detect *Candida* species from positive blood culture samples, although PCR assays have also been developed for this purpose (AAP Committee on Infectious Diseases, 2018).

### **European Centre for Disease Prevention and Control (ECDC)**

On May 23, 2022, the ECDC released a rapid risk assessment of the Mpox multi-country outbreak. They recommend that patients with probable cases should be tested with a “Mpox virus specific PCR or an orthopoxvirus specific PCR assay which is then confirmed through sequencing” (ECDC, 2022b).

On June 2, 2022, ECDC released interim advice on risk communication and community engagement during the 2022 Mpox outbreak in Europe. This is a joint report with the WHO regional office for Europe. They recommend speaking to your doctor about getting tested for Mpox if you develop a rash with a fever or feeling of discomfort or illness (ECDC, 2022a).

### **United Kingdom Health Security Agency (UKHSA)**

The UKHSA states that “Mpox is diagnosed by PCR test for the Mpox virus (MPXV) on a viral swab taken from one or more vesicles or ulcers.” Specifically, it is recommended that healthcare workers “Take a viral swab in viral culture medium or viral transport medium (for example Virocult®) from an open sore or from the surface of a vesicle. If other wounds are present, ensure that the sample is definitely taken from a vesicle, an ulcer or a crusted vesicle. Rub the swab over the lesion and place the swab in the collection tube. If there are pharyngeal lesions, a throat swab should also be taken” (UKHSA, 2024). UKHSA also suggests that “A viral throat swab can be taken for high-risk contacts of a confirmed or highly probable case who have developed systemic symptoms but do not have a rash or lesions that can be sampled. Please note that even if the throat swab is negative, the individual must continue with monitoring and isolation as instructed by their local health protection team, and should be reassessed and sampled if further symptoms develop.” Lastly, “If follow-up testing is required from a confirmed or highly probable case, either because of clinical deterioration or to inform discharge from isolation to an inpatient setting, additional samples should be taken and should include the following:

- a lesion swab and throat swab in viral transport medium
- a blood sample in an EDTA tube
- a urine sample in a universal sterile container” (UKHSA, 2024).

The UKHSA states that “Following the identification of a cluster of sexually transmitted HCID Clade I mpox in 2023, there is an increased risk of mpox HCID infection circulating

unrecognized on the background of Clade II infections.” They therefore recommend “All diagnostic samples from all individuals testing positive for mpox should now be subject to clade confirmation. Positive mpox samples should be sent to RIPL for clade specific testing if clade differentiation is not available through local mpox testing services” (UKHSA, 2024).

The UKHSA states that mpox DNA viruses can be detected in semen up to 11 days after acute infection, and recommends that: “Following the initial 12 weeks and up to 6 months after recovery from infection, UKHSA recommends performing MPXV PCR on semen samples (and where necessary, oropharyngeal and/or rectal swabs) if the patient:

- is undergoing fertility treatment or planning pregnancy
- is undergoing planned semen storage (for example prior to chemotherapy)
- has an immunocompromised sexual partner (including a pregnant partner)
- is concerned about transmission to sexual partner or partners for any other reason and requests a test from their clinician” (UKHSA, 2024).

### **HHV-6 Foundation**

The human herpesvirus 6 (HHV-6) foundation also states that “a negative finding in the plasma does not rule out a localized active infection in an organ (e.g. uterus, brain, thyroid, liver). Persistent HHV-6 infections have been found in the liver, brain, lungs, heart tissue and uterus, with no trace of HHV-6 DNA in the plasma. Quantitative testing on blood and tissues is preferred because it can differentiate between the very low levels occasionally found in healthy controls and high levels found in diseased tissues” (HHV-6 Foundation, 2024).

The HHV-6 foundation states that *qualitative* PCR DNA tests on whole blood are “useless for differentiating active from latent infection” but notes that the test may be useful for differentiating between herpes virus-6A and herpes virus-6B. The HHV-6 foundation states that *quantitative* PCR DNA tests on whole blood can differentiate active from latent infection “If the viral load is >200 copies per ml or 20 copies per microgram of DNA then this is an active infection.”

## **VI. Applicable State and Federal Regulations**

### **Food and Drug Administration (FDA)**

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

A list of current U.S. Food and Drug Administration (FDA, 2022) approved or cleared nucleic acid-based microbial tests is available at: <https://www.fda.gov/medical-devices/vitro-diagnostics/nucleic-acid-based-tests>.

## VII. Applicable CPT/HCPCS Procedure Codes

Procedure codes appearing in medical policy documents are only included as a general reference. This list may not be all inclusive and is subject to updates. In addition, codes listed are not a guarantee of payment.

CPT	Code Description
87471	Infectious agent detection by nucleic acid (DNA or RNA); Bartonella henselae and Bartonella quintana, amplified probe technique
87472	Infectious agent detection by nucleic acid (DNA or RNA); Bartonella henselae and Bartonella quintana, quantification
87480	Infectious agent detection by nucleic acid (DNA or RNA); Candida species, direct probe technique
87481	Infectious agent detection by nucleic acid (DNA or RNA); Candida species, amplified probe technique
87482	Infectious agent detection by nucleic acid (DNA or RNA); Candida species, quantification
87485	Infectious agent detection by nucleic acid (DNA or RNA); Chlamydia pneumoniae, direct probe technique
87486	Infectious agent detection by nucleic acid (DNA or RNA); Chlamydia pneumoniae, amplified probe technique
87487	Infectious agent detection by nucleic acid (DNA or RNA); Chlamydia pneumoniae, quantification
87493	Infectious agent detection by nucleic acid (DNA or RNA); Clostridium difficile, toxin gene(s), amplified probe technique
87495	Infectious agent detection by nucleic acid (DNA or RNA); cytomegalovirus, direct probe technique
87496	Infectious agent detection by nucleic acid (DNA or RNA); cytomegalovirus, amplified probe technique
87497	Infectious agent detection by nucleic acid (DNA or RNA); cytomegalovirus, quantification
87498	Infectious agent detection by nucleic acid (DNA or RNA); enterovirus, amplified probe technique, includes reverse transcription when performed
87500	Infectious agent detection by nucleic acid (DNA or RNA); vancomycin resistance (eg, enterococcus species van A, van B), amplified probe technique
87525	Infectious agent detection by nucleic acid (DNA or RNA); hepatitis G, direct probe technique
87526	Infectious agent detection by nucleic acid (DNA or RNA); hepatitis G, amplified probe technique
87527	Infectious agent detection by nucleic acid (DNA or RNA); hepatitis G, quantification
87531	Infectious agent detection by nucleic acid (DNA or RNA); Herpes virus-6, direct probe technique
87532	Infectious agent detection by nucleic acid (DNA or RNA); Herpes virus-6, amplified probe technique
87533	Infectious agent detection by nucleic acid (DNA or RNA); Herpes virus-6, quantification



CPT	Code Description
87540	Infectious agent detection by nucleic acid (DNA or RNA); Legionella pneumophila, direct probe technique
87541	Infectious agent detection by nucleic acid (DNA or RNA); Legionella pneumophila, amplified probe technique
87542	Infectious agent detection by nucleic acid (DNA or RNA); Legionella pneumophila, quantification
87580	Infectious agent detection by nucleic acid (DNA or RNA); Mycoplasma pneumoniae, direct probe technique
87581	Infectious agent detection by nucleic acid (DNA or RNA); Mycoplasma pneumoniae, amplified probe technique
87582	Infectious agent detection by nucleic acid (DNA or RNA); Mycoplasma pneumoniae, quantification
87593	Infectious agent detection by nucleic acid (DNA or RNA); orthopoxvirus (eg, monkeypox virus, cowpox virus, vaccinia virus), amplified probe technique, each
87634	Infectious agent detection by nucleic acid (DNA or RNA); respiratory syncytial virus, amplified probe technique
87640	Infectious agent detection by nucleic acid (DNA or RNA); Staphylococcus aureus, amplified probe technique
87641	Infectious agent detection by nucleic acid (DNA or RNA); Staphylococcus aureus, methicillin resistant, amplified probe technique

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## VIII. Evidence-based Scientific References

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## IX. Revision History

Revision Date	Summary of Changes
01/01/2022	Initial Effective Date
04/12/2022	Updated background, guidelines, and evidence-based scientific references. Literature review did not necessitate any modifications to the coverage criteria
09/14/2022	Updated background, guidelines, and evidence-based scientific references. Literature review did not necessitate any modification to the coverage criteria. Edits made for clarity: Removed “*DNMCC= Does Not Meet Coverage Criteria; MCC = Meets coverage criteria.” from beneath the table in CC1, as the definition was redundant with what was already provided in CC1. Revised code disclaimer statement

04/04/2023	<p>Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the following changes in coverage criteria:</p> <p>Addition of “Orthopoxvirus” to the table in CC1. Code 87593 added in the “Amplified Probe” column in its row as “MCC” to allow for coverage of the amplified probe test for orthopoxvirus (monkeypox is an orthopoxvirus)</p> <p>CC2 edited for clarity, now reads: “Simultaneous ordering of any combination of direct probe, amplified probe, and/or quantification for the same organism in a single encounter DOES NOT MEET COVERAGE CRITERIA.”</p> <p>CC3 edited to remove specific list of organisms, as it was not all inclusive. Now reads: “For any other microorganism without a specific CPT code, PCR testing MEETS COVERAGE CRITERIA.”</p> <p>Added CPT code 87593 (new orthopoxvirus CPT code effective 7/26/2022)</p> <p>Committee approved 4/4/2023</p>
08/15/2023	<p>Reviewed and Updated: Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the following changes in coverage criteria:</p> <p>In the table within CC1, references to Candida testing for vaginitis were removed, row now specifies “non-vaginal Candida”. Directive to see M2057 for vaginal Candida moved into policy description.</p> <p>87493 for C. diff moved from “Direct Probe” column to “Amplified Probe” column.</p> <p>Heptatis B removed from the table in CC1 due to the expansion of G2036 to include Hepatitis B testing.</p> <p>Removed former CC3: “3) For any other microorganism without a specific CPT code, PCR testing MEETS COVERAGE CRITERIA.”</p> <p>Former “Policy Guideline” was moved into the Policy Description.</p> <p>Removed CPT codes 87516, 87517, 87797, 87798, and 87799.</p> <p>Committee approved: 08/15/2023</p>
10/21/2024	<p>Reviewed and Updated: Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the following changes in coverage criteria:</p> <p>Removed Mycoplasma genitalium from this table, as management of testing for M. genitalium is now contained within G2157- Diagnostic Testing of Common Sexually Transmitted Infections</p> <p>Changed direct probe for HHV6 (87531) from MCC to DNMCC, as quantitative testing for HHV6 can differentiate between active and latent infection, whereas qualitative does not and direct and amplified probe coverage should match for HHV6.</p> <p>Removed CPT code 87563</p> <p>Committee approved: 10/21/2024</p>