



Identification of Microorganisms using Nucleic Acid Probes

Policy Number: AHS – M2097 – Identification of Microorganisms Using Nucleic Acid Probes	
Effective Date: 12/01/2023	

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I. Policy Description

Nucleic acid hybridization technologies utilize complementary properties of the DNA doublehelix 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. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

 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.

Microorganism	Direct Probe	Amplified Probe	Quantification
Bartonella henselae or		87471 (MCC)	87472
quintana			(DNMCC)
Non-vaginal Candida species	87480 (DNMCC)	87481 (DNMCC)	87482
			(DNMCC)
Chlamydia pneumoniae	87485 (MCC)	87486 (MCC)	87487
			(DNMCC)
Clostridium difficile		87493 (MCC)	





Microorganism	Direct Probe	Amplified Probe	Quantification
Cytomegalovirus	87495 (MCC)	87496 (MCC)	87497 (MCC)
Enterococcus, Vancomycin-		87500 (MCC)	
resistant (e.g., enterococcus			
vanA, vanB)			
Enterovirus		87498 (MCC)	
Hepatitis G	87525 (DNMCC)	87526 (DNMCC)	87527
			(DNMCC)
Herpes virus-6	87531 (MCC)	87532 (DNMCC)	87533 (MCC)
Legionella pneumophila	87540 (MCC)	87541 (MCC)	87542
			(DNMCC)
Orthopoxvirus		87593 (MCC)	
Mycoplasma pneumoniae	87580 (MCC)	87581 (MCC)	87582
			(DNMCC)
Mycoplasma genitalium		87563 (MCC)	
Respiratory syncytial virus		87634 (MCC)	
Staphylococcus aureus		87640 (MCC)	
Staphylococcus aureus,		87641 (MCC)	
methicillin resistant			

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

Term	Definition
CDC	Centers for Disease Control and Prevention
CIDT	Culture-independent diagnostic test
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
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





Term	Definition
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, 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 monkeypox, 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 also can identify *C. auris*." The CDC further notes that various PCR methods have been developed for identifying *C. auris* (CDC, 2020a).

Chlamydia Pneumoniae (C. pneumoniae)

The CDC writes that RT-PCR is the "preferred" method of detecting an acute *C. pneumoniae* infection. The CDC further notes that a positive culture should be confirmed by a second test, such as PCR (CDC, 2021a).

Ebola

The CDC states that for diagnosis of Ebola, "there must be a combination of symptoms suggestive of EVD **AND** a possible exposure to EVD within 21 days before the onset of symptoms." Such exposures include

- blood or body fluids from a person sick with or who died from EVD,
- objects contaminated with blood or body fluids of a person sick with or who died from EVD,
- infected fruit bats and nonhuman primates (apes or monkeys), or
- semen from an individual who has recovered from EVD.

The CDC notes that PCR is one of the most common diagnostic methods, but also cautions that "When the virus is no longer present in great enough numbers in a patient's blood, PCR methods will no longer be effective. Other methods, based on the detection of antibodies an EVD case produces to an infection, can then be used to confirm a patient's exposure and infection by Ebola virus" (CDC, 2022c).

Giardia

The CDC states that microscopy with direct fluorescent antibody testing (DFA) is considered the test of choice for diagnosing giardiasis, but rapid immunochromatographic cartridge assays, enzyme immunoassay kits, microscopy with trichrome staining, and molecular assays may be alternatively used as well. To obtain more accurate test results, the CDC recommends collecting three stool specimens from patients over the course of a few days. But, only molecular testing (e.g., DNA sequencing) can identify *Giardia* strains (CDC, 2021b).

Monkeypox Virus





The CDC defines a <u>suspect case</u> of monkeypox as a "new characteristic rash, or meets one of the epidemiologic criteria and has a high clinical suspicion for monkeypox." A <u>probable case</u> 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 monkeypox is defined as "demonstration of the presence of Monkeypox virus DNA by polymerase chain reaction testing or Next-Generation sequencing of a clinical specimen OR isolation of Monkeypox virus in culture from a clinical specimen" (CDC, 2022b).

MRSA

The CDC remarks that nucleic acid amplification tests (NAATs, such as PCR) "can be used for direct detection of mecA, the most common gene mediating oxacillin resistance in staphylococci," but will not detect novel resistance mechanisms or uncommon phenotypes (CDC, 2019a).

Mycoplasma genitalium

For individuals with a penis who are experiencing recurrent nongonococcal urethritis (NGU), the CDC recommends FDA-cleared NAAT, with resistance testing done when available. The CDC also recommends that individuals with recurrent cervicitis should be tested for *M. genitalium*. Testing should be considered for individuals with pelvic inflammatory disease (PID). Testing should be accompanied with resistance testing, if available. The CDC does not recommend screening for *M. genitalium* for asymptomatic individuals, nor do they recommend extragenital testing for *M. genitalium*. "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" (CDC, 2021d).

Non-Polio Enterovirus

The CDC remarks that their laboratories "routinely" perform qualitative testing for enteroviruses, parechoviruses, and uncommon picornaviruses (CDC, 2018).

Respiratory Syncytial Virus (RSV)

The CDC writes that real-time reverse transcriptase-polymerase chain reaction (rRT-PCR) and antigen detection tests are the most commonly used diagnostic tests and are effective in infants and young children. However, the highly sensitive rRT-PCR is recommended to be used when testing older children and adults with RSV (CDC, 2022d).

Salmonella

The CDC writes that diagnosis requires detection of the *Salmonella* bacteria, be it through culture or a "culture-independent diagnostic test (CIDT)" (CDC, 2019b).

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 *Legionella pneumophila* and *Mycoplasma pneumoniae* specimen (CDC, 2020b, 2021c, 2022a). 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 monkeypox multi-country outbreak. They recommend that patients with probable cases should be tested with a "monkeypox 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 monkeypox 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 monkeypox if you develop a rash with a fever or feeling of discomfort or illness (ECDC, 2022a).

United Kingdom Heath Security Agency (UKHSA)

The UKHSA states that "Mpox is diagnosed by PCR test for the monkeypox 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, 2023). 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, 2023).





VI. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: <u>https://www.cms.gov/medicare-coverage-database/search.aspx</u>. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

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: <u>https://www.fda.gov/medical-devices/vitro-diagnostics/nucleic-acid-based-tests</u>.

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.

СРТ	Code Description
	Infectious agent detection by nucleic acid (DNA or RNA); Bartonella
87471	henselae and Bartonella quintana, amplified probe technique
	Infectious agent detection by nucleic acid (DNA or RNA); Bartonella
87472	henselae and Bartonella quintana, quantification
	Infectious agent detection by nucleic acid (DNA or RNA); Candida species,
87480	direct probe technique
	Infectious agent detection by nucleic acid (DNA or RNA); Candida species,
87481	amplified probe technique
	Infectious agent detection by nucleic acid (DNA or RNA); Candida species,
87482	quantification
	Infectious agent detection by nucleic acid (DNA or RNA); Chlamydia
87485	pneumoniae, direct probe technique
	Infectious agent detection by nucleic acid (DNA or RNA); Chlamydia
87486	pneumoniae, amplified probe technique
	Infectious agent detection by nucleic acid (DNA or RNA); Chlamydia
87487	pneumoniae, quantification
	Infectious agent detection by nucleic acid (DNA or RNA); Clostridium
87493	difficile, toxin gene(s), amplified probe technique





СРТ	Code Description
	Infectious agent detection by nucleic acid (DNA or RNA); cytomegalovirus,
87495	direct probe technique
	Infectious agent detection by nucleic acid (DNA or RNA); cytomegalovirus,
87496	amplified probe technique
	Infectious agent detection by nucleic acid (DNA or RNA); cytomegalovirus,
87497	quantification
	Infectious agent detection by nucleic acid (DNA or RNA); enterovirus,
87498	amplified probe technique, includes reverse transcription when performed
	Infectious agent detection by nucleic acid (DNA or RNA); vancomycin
	resistance (eg, enterococcus species van A, van B), amplified probe
87500	technique
	Infectious agent detection by nucleic acid (DNA or RNA); hepatitis G, direct
87525	probe technique
	Infectious agent detection by nucleic acid (DNA or RNA); hepatitis G,
87526	amplified probe technique
07507	Infectious agent detection by nucleic acid (DNA or RNA); hepatitis G,
87527	quantification
07521	Infectious agent detection by nucleic acid (DNA or RNA); Herpes virus-6,
87531	direct probe technique
97522	Infectious agent detection by nucleic acid (DNA or RNA); Herpes virus-6,
87532	amplified probe technique
07522	Infectious agent detection by nucleic acid (DNA or RNA); Herpes virus-6, quantification
87533	Infectious agent detection by nucleic acid (DNA or RNA); Legionella
87540	pneumophila, direct probe technique
0/340	Infectious agent detection by nucleic acid (DNA or RNA); Legionella
87541	pneumophila, amplified probe technique
07541	Infectious agent detection by nucleic acid (DNA or RNA); Legionella
87542	pneumophila, quantification
07512	Infectious agent detection by nucleic acid (DNA or RNA); Mycoplasma
87563	genitalium, amplified probe technique
0,000	Infectious agent detection by nucleic acid (DNA or RNA); Mycoplasma
87580	pneumoniae, direct probe technique
	Infectious agent detection by nucleic acid (DNA or RNA); Mycoplasma
87581	pneumoniae, amplified probe technique
	Infectious agent detection by nucleic acid (DNA or RNA); Mycoplasma
87582	pneumoniae, quantification
	Infectious agent detection by nucleic acid (DNA or RNA); orthopoxvirus
	(eg, monkeypox virus, cowpox virus, vaccinia virus), amplified probe
87593	technique, each
	Infectious agent detection by nucleic acid (DNA or RNA); respiratory
87634	syncytial virus, amplified probe technique
	Infectious agent detection by nucleic acid (DNA or RNA); Staphylococcus
87640	aureus, amplified probe technique





СРТ	Code Description
	Infectious agent detection by nucleic acid (DNA or RNA); Staphylococcus
87641	aureus, methicillin resistant, amplified probe technique

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VIII. Evidence-based Scientific References

AAP Committee on Infectious Diseases. (2018). Red Book® 2018.

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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	Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the following changes in coverage criteria:
	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)
	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."
	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."





	Added CPT code 87593 (new orthopoxvirus CPT code effective
	7/26/2022)
	Committee approved 4/4/2023
08/15/2023	Reviewed and Updated: Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the following changes in coverage criteria:
	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.
	87493 for C. diff moved from "Direct Probe" column to "Amplified Probe" column.
	Heptatis B removed from the table in CC1 due to the expansion of G2036 to include Hepatitis B testing.
	Removed former CC3: "3) For any other microorganism without a specific CPT code, PCR testing MEETS COVERAGE CRITERIA."
	Former "Policy Guideline" was moved into the Policy Description.
	Removed CPT codes 87516, 87517, 87797, 87798, and 87799.
	Committee Approved: 08/15/2023