Clinical Guidance on the Use of Next Generation Sequencing (NGS) Tests for Infectious Diseases

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EXECUTIVE SUMMARY:

Next-Generation Sequencing tests should ONLY be considered when the following criteria are met:

1. **Clearly identifiable focus on infection** (do not use in undifferentiated clinical conditions)
2. **Anticipated prolonged course of antimicrobial therapy** (i.e. weeks to months; avoid NGS if anticipating short [*≤14 day*] course of empiric therapy or no plans to treat)
3. **Negative conventional workup at >48 hours** (or longer, depending on the clinical scenario, suspected pathogen, and type of conventional testing sent)

An Infectious Diseases consult is **required** to order these tests.

Next-generation sequencing (NGS) is a culture-free method of analyzing the microbes within a sample. These tests sequence all or part of the microbial genes in a patient specimen, such as serum, tissue, or CSF. This review will focus on the clinical use of NGS tests, including the **Karius Test, University of Washington Broad-Range PCR**, and **Delve Bio (previously UCSF Center for Next-Gen precision diagnostics) CSF cfDNA**. Separate guidelines regarding the clinical use of multiplex molecular panels are available on the UNMC Clinical Microbiology website: https://www.unmc.edu/intmed/divisions/id/asp/clinicalmicro.html

An Infectious Disease consult is required to obtain next-generation sequencing. Only ID clinicians are able to order these tests in One Chart so they assist with interpretation of results and management decisions.

Abbreviations:

CAP = College of American Pathologists
CLIA = Clinical Laboratory Improvement Amendments of 1988
FDA = Food and Drug Administration
HSCT = hematopoietic stem cell transplant
IFI = Invasive Fungal Infection
LDT = Laboratory Developed Test
LRTI = lower respiratory tract infection
MRSA = methicillin-resistant staphylococcus aureus
MSSA = methicillin-sensitive staphylococcus aureus
NGS = next generation sequencing
SOTR = solid organ transplant recipient
URTI = upper respiratory tract infection

Table 1: Next-generation sequencing technology[1]

Type of next-generation sequencing (NGS)	Mechanism	Examples
Whole genome sequencing (WGS)	Sequencing of the entire microbial genome from isolated colonies	Identification of microbial pathogens for hospital and public health epidemiological studies
Metagenomic NGS (mNGS)	Sequencing of all the nucleic acids directly from patient specimens (including pathogen and human DNA) without culture	Karius Test and Delve Bio CSF test
Targeted NGS (tNGS)	Sequencing 16s rDNA (bacterial) or internal transcribed spacer (ITS; fungal) regions following amplification directly from patient specimens	University of Washington Broad-Range PCR

Table 2: Available molecular diagnostic assays

Clinical specimen	Available tests	Turnaround time	Estimated cost
Multiplex molecular panels (please see separate guidance documents for use and interpretation)			
CSF	BioFire Meningoencephalitis Panel	60 min	$$
Respiratory	BioFire Respiratory Pathogen Panel (for URTI)	45 min	$$
	BioFire Pneumonia Pathogen Panel (for LRTI)	60 min	$$
	Fungal plus PCR profile 1 and 2 (for LRTI)	24 hours from receipt of specimen (send-out)	$$$
Stool	BioFire Gastrointestinal Pathogen Panel	60 min	$$
Joint	BioFire Joint Pathogen Panel	60 min	$$
Next generation sequencing assays			
Plasma*	Karius Test (cell free DNA)	2-4 business days from receipt of specimen (send-out)	$$$$ *List price (2024): $2,000*
Tissue	University of Washington Broad-Range PCR (can include bacterial, fungal, nontuberculous mycobacterial, and/or MTB complex targets all of which must be ordered seperately)	5-7 business days from receipt of specimen (send-out)	$$ - $$$$ (depending on how many target groups are selected for testing, i.e. bacterial only, bacterial + fungal, etc) *List prices (2024):* - *Bacterial: $300* - *Fungal: $490* - *MTB: $220* - *Non-MTB: $330*
CSF	Delve Bio cell free DNA (previously UCSF Center for Next-Gen precision diagnostics)	1-2 weeks from receipt of specimen (send-out)	$$$$ *List price (2024): $3,100*

**Plasma specimens can be stored at ambient temperature for 96 hours and at -20°C for up to 6 months. Karius testing can be performed on specimens that are >96 hours old, if they were appropriately frozen.[2]*

Clinical utility and limitations of NGS tests

Patient outcomes are typically not included in studies of NGS tests, making it difficult to determine the clinical utility of these tests. Real-world studies have demonstrated limited meaningful clinical impact, even when these tests are restricted to Infectious Diseases specialists.[3,4,5] NGS testing may be advantageous for detecting obscure or rare pathogens, pathogens that are difficult to grow using conventional methods, or in patients previously treated with antimicrobials (where culture-based testing may be falsely negative[6]). Some NGS tests (i.e. Karius Test) also offer a relatively rapid turnaround time, which can be advantageous for a limited number of clinical syndromes described later in this document; however, the majority of NGS tests take several weeks to result. The numerous NGS testing limitations must be taken into account including:

Potential Limitations of NGS:

1. While additional pathogens may be detected via NGS, false negative results do occur when compared to conventional testing. Thus a negative result does not rule out infection and must be put in context of the pre-test probability of disease.
2. NGS tests do not distinguish commensal or colonizing organisms from pathogenic organisms, so results must be interpreted based on the clinical picture.
3. NGS tests do not usually provide antimicrobial susceptibility data, although the Karius Test now incorporates some common antimicrobial resistance markers (e.g. *mecA*, CTX-M, etc.). As with all genotypic resistance testing, detection of the resistance markers does not always correlate with phenotypic resistance, nor does the abscence of the markers guarantee susceptbility. As such, NGS-identified resistance markers must be interpreted with caution and should be confirmed with standard cultures as much as possible.
4. The Karius Test only detects pathogens that have DNA (e.g. bacteria, fungi, DNA viruses). Therefore it is not able to detect RNA viruses such as HIV, hepatitis C, Zika, or coronaviruses.

All currently available NGS tests have not been approved by the FDA, although the FDA is actively exploring regulatory approaches to this technology to ensure that NGS tests have adequate analytical and clinical performance.[7] Rather, these tests are developed by their respective parent laboratories as Laboratory Developed Tests (LDT). As a result, each test is only commercially available from its own CLIA-certified, CAP-accredited laboratory, leading to longer turnaround times due to shipping of the specimen.

Additional details regarding how to order and interpret each test are provided at the end of this document.

Recommended clinical use of NGS tests:

Table 3a: Focal site(s) of infection

Table 3(a-e) is loosely adapted from the American Society of Transplantation (AST) consensus conference's statements on best practice use of next generation sequencing assays in SOT recipients.[8]

Clinical syndrome	Clinical scenario	Testing order of preference	Guidance on Use of NGS
Focal site(s) of infection (Figure 1)	Suspected infectious focus on radiographic evaluation that cannot be sampled due to anatomical site or procedure risk (ex: deep brain abscess) or has negative conventional testing (ex: spinal osteomyelitis)	(1) Tissue cultures and histopathology (2) Broad-Range PCR if tissue is available* (ID consult required) (3) Karius Test if tissue not available (ID consult required)	**Conventional testing is the preferred initial step** (i.e. biopsy with cultures and histopathology, blood cultures, antigen testing, and serology as appropriate). If conventional testing is negative, NGS testing from an infected sample source may be considered. ● NGS testing of tissue specimens (Broad-Range PCR) is preferred over Karius Test. Karius Test can be considered if samples from infected sites are negative by standard testing or cannot be obtained. ● Negative NGS testing does not rule out infection.

** Note: The yield of Broad-Range PCR can vary, depending on the quality of the tissue specimen.*

Figure 1: Algorithm for considering NGS test for patients with focal sites of infection
Algorithms modeled after University of Washington / Seattle Children's infectious encephalitis algorithm.[9]

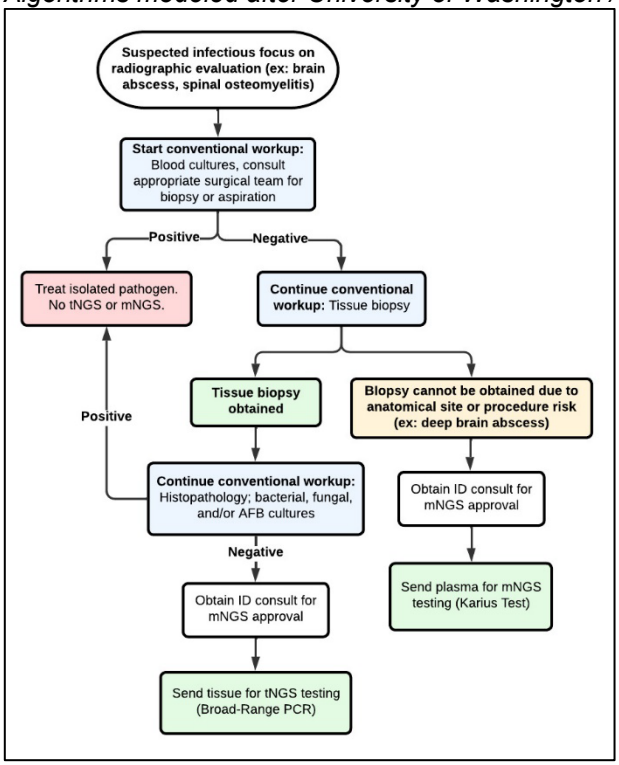


Table 3b: Endovascular Infection

Clinical syndrome	Clinical scenario	Testing order of preference	Guidance on Use of NGS
Endovascular Infection (Figure 2)	Culture-negative endocarditis Mycotic aneurysm or vascular graft infection	(1) Blood cultures (2) Tissue cultures and histopathology (3) If blood cultures are negative at 48 hours, Infectious Disease should be consulted and send second line of testing (i.e. Q fever, *Bartonella quintana* and *henselae*, Brucella Antibodies, and *Histoplasma* serum and urine Ag). Simultaneously, save a plasma sample for possible Karius Test. (4) Broad-Range PCR if tissue is available (ID consult required) (5) Karius Test if tissue is not available (ID consult required)	**Conventional testing is the preferred initial step** (i.e. biopsy with cultures and histopathology, blood cultures, antigen testing, and serology as appropriate). Select populations (SOTR with severe illness, HSCT) may benefit from plasma cfDNA testing earlier in the disease course due to the diversity of pathogens encountered. ● Conventional testing (including testing for infections associated with culture-negative endocarditis such as Q fever, *Bartonella*, Brucella, and *Histoplasma*) should precede NGS testing. ● Other causes of culture-negative endocarditis including Legionella, Mycoplasma and *Tropheryma whipplei* should be considered before or in paralell with Karius testing ● Plasma samples obtained early in the course of the infection can be saved and frozen for later NGS testing in case conventional testing is unrevealing. ● NGS testing of plasma cfDNA after broad antimicrobial use may provide a retrospective diagnosis, limiting prolonged or unnecessary broad-spectrum antimicrobial therapy, although the likelihood of a positive test decreases with each day of therapy. Karius Test (either by stored plasma or as initial testing) can be preferred if bacterial endocarditis is suspected but patient recieved antibiotic therapy prior to blood culture collection. **NOTE**: Although the usefulness of NGS for blood culture-negative endocarditis (BCNE) is still being evaluated, the ISCVID Working Group, in their updated 2023 Duke Criteria for Infectious Endocarditis (IE), has proposed that a positive result for *C. burnetti, Bartonella* species, or *T. whipplei* from a NGS platform should constitue a Major Criterion for IE. Positive NGS testing for any other organisms should be considered as a Minor Criterion, although keeping in mind that data is limited regarding differentiation of "true positive" from "contamination" in NGS tests as well as that these tests can have limited sensitivity.[10]

Figure 2: Algorithm for considering NGS test in patients with endovascular infections

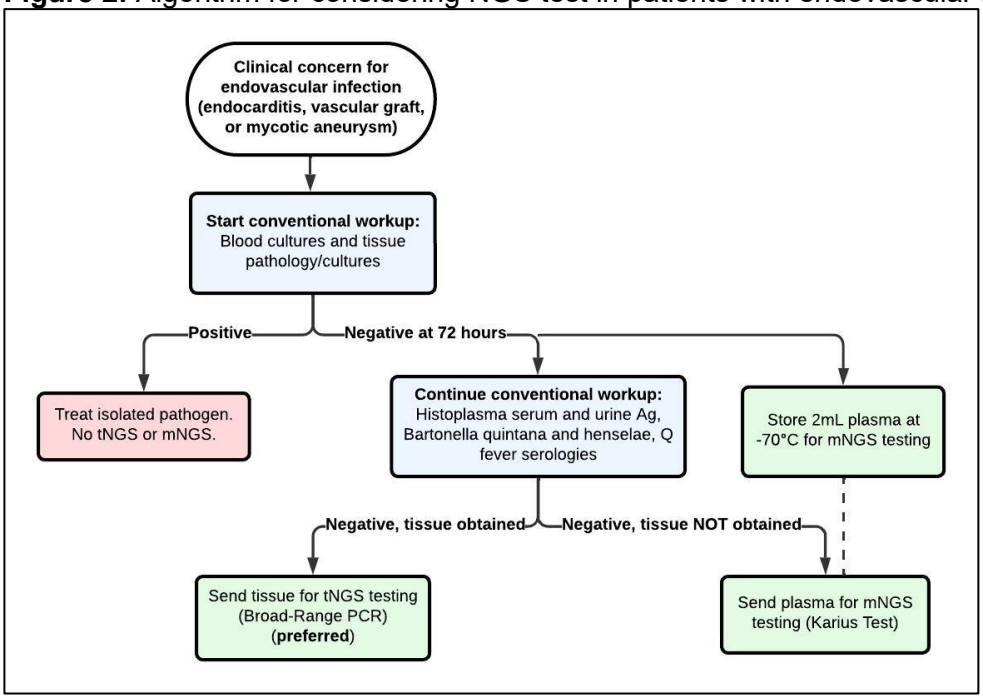


Table 3c: Respiratory Infection

Clinical syndrome	Clinical scenario	Testing order of preference	Guidance on Use of NGS
Respiratory Infection (Figure 3)	Radiographic findings (interstitial infiltrates, tree-in-bud opacities, consolidation, nodules, cavities) and negative/ inconclusive conventional testing Culture-negative severe community-acquired pneumonia Immuno-compromised host with concern for lower respiratory opportunistic infection	(1) If sputum available, obtain pneumonia pathogen panel and sputum Gram stain and culture (2) If sputum not available, obtain respiratory viral panel, urine streptococcal Ag, urine *Legionella* Ag (3) Serum Beta-D-Glucan if suspicion for *Pneumocystis* pneumonia (PCP) (4) Bronchoscopy specimens for Gram stain, fungal stain, aerobic bacterial culture, nocardia culture, fungal culture and pneumonia PCR panel if not previously obtained. Consider fungal studies if concern for invasive fungal infection (see IFI below), or silver stain if concern for PCP; Infectious Disease should be consulted in these cases. (5) Broad-Range PCR if tissue is available (i.e. lung biopsy) (ID consult required). This should <u>not</u> be sent from BAL fluid. (6) If all standard cultures/ serologies are negative, infection is still suspected, and Karius Test is being considered, Infectious Disease <u>must</u> be consulted for input.	**Conventional testing is the preferred initial step** (i.e. sputum cultures, pneumonia pathogen panel, bronchoscopy as appropriate). If conventional testing is negative, NGS testing may be considered. ● <u>If a tissue specimen is available NGS testing of infected sites (Broad-Range PCR) is preferred over Karius Test.</u> We recommend sending Broad-Range PCR from tissue specimens only; it should *not* be sent directly from BAL fluid. ● <u>If a respiratory specimen is available (i.e. BAL) and invasive fungal infection is suspected, consider Fungal Plus PCR 1 or 2 prior to Karius Test</u> ● Non-pathogenic members of the respiratory microbiome must be clinically differentiated from pathogens. **We do not recommend NGS testing if planning to treat with short empiric course of therapy (<14 days).**

Figure 3: Algorithm for considering NGS test in patients with respiratory infections

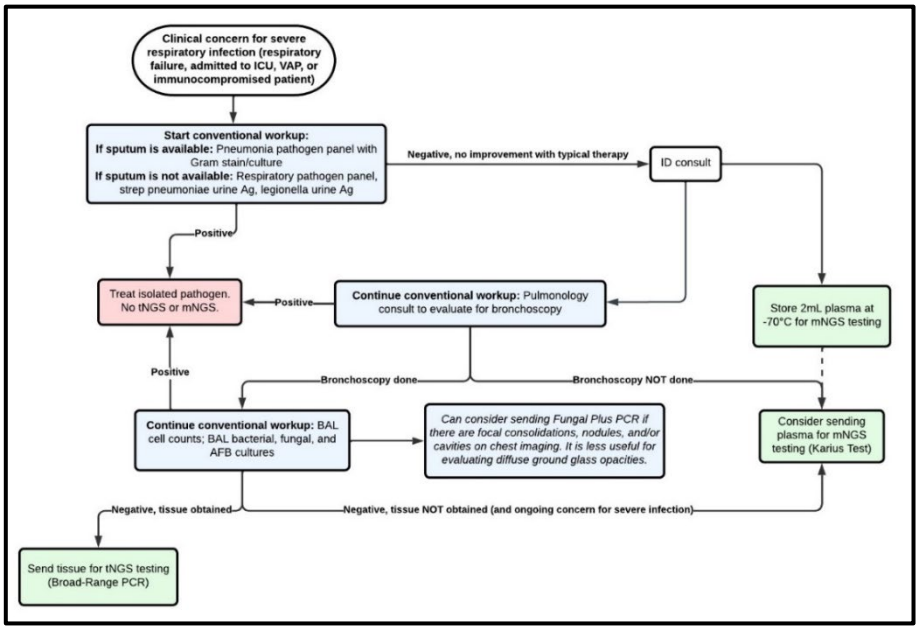


Table 3d: Meningoencephalitis

Clinical syndrome	Clinical scenario	Testing order of preference	Guidance on Use of NGS
Meningo-encephalitis (Figure 4)	Clinical syndrome of meningo-encephalitis, CSF pleocytosis, or other abnormal parameters suggestive of infection, but negative conventional testing	(1) CSF cell count, protein, glucose (2) Meningoencephalitis panel and CSF cultures (3) Consider studies for West Nile virus, toxoplasmosis, syphilis, endemic fungal infection, and tuberculosis as appropriate. (4) If standard cultures are negative, CSF indices are suggestive of infection, and Delve Bio CSF mNGS is being considered, consult Infectious Disease for input.	**Conventional testing is the preferred initial step** (i.e. CSF cultures, meningoencephalitis panel). If conventional testing is negative, NGS testing may be considered although the incremental diagnostic yield is low and it may miss organisms detected using traditional testing. ● NGS testing of CSF (Delve Bio) is preferred over Karius Test due to the proximity of CSF to the source of infection. ● CSF samples obtained early in the course of the infection can be saved and frozen for later NGS testing in case conventional testing is unrevealing.* ● In cases where conventional testing is positive for a likely pathogen, do not use NGS to confirm a diagnosis.

**Samples can be stored <6 hours at room temperature, <7 days at 2-8°C, and up to 90 days at -70°C. If the CSF specimen is frozen, it is acceptable for testing provided it has been stored at -70°C, and has undergone no more than 2 freeze/thaw cycles. We would recommend discussing with Delve Bio / UCSF prior to sending frozen specimens, to ensure that the specimen will be accepted.*

Figure 4: Algorithm for considering NGS test in patients with meningoencephalitis

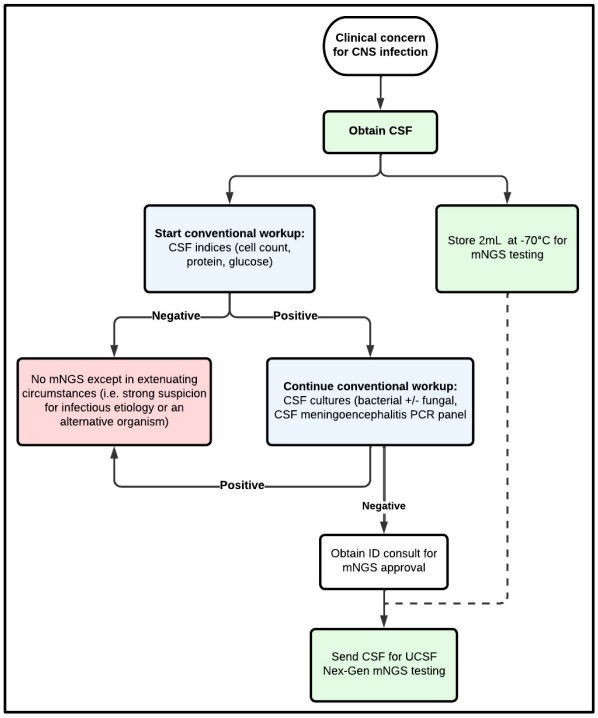


Table 3e: Invasive Fungal Infection, Febrile Neutropenia, and Fever of Unknown Origin

Syndrome	Clinical scenario	Testing order of preference	Guidance on Use of NGS
Invasive fungal infection (IFI)	Clinical concern for invasive fungal infection based on clinical, laboratory, and radiographic evaluation (particularly lung infiltrates or sinusitis) but negative conventional workup Biopsy specimen with fungal forms on pathologic evaluation but negative conventional workup	**The menu of diagnostic testing is extensive, and should be tailored to the individual patient based on risk factors, patient history, clinical presentation, epidemiology, and radiologic appearance.** (1) See Table 3c regarding initial workup for suspected lower respiratory infection. This should include consideration of Pulmonology consult and bronchoscopy. (2) See Table 3a and Table 3d regarding workup for CNS lesions (either focal site of infection or meningoencephalitis). **(3)** If there is concern for fungal infection, Infectious Diseases should be consulted to help guide workup based on the patient's risk factors and the radiographic appearance. **The following diagnostic tests can be considered but will vary according to patient's immunocompromised status:** - **Blood fungal diagnostic tests:** (1,3)-β-d-glucan (Fungitell), cryptococcal Ag, Aspergillus galactomannan, and endemic fungi serologies (i.e. Histoplasma, Coccidioides, Blastomyces depending on geographic location or exposures) - **Urine fungal diagnostic tests:** Histoplasma Ag - **Bronchoscopy fungal studies:** Fungal stains, fungal cultures, Aspergillus galactomannan, nocardia culture. Consider *Fungal Plus PCR 1 or 2* panel if there are focal consolidations, nodules, and/or cavities on chest imaging; it is less useful for evaluating diffuse ground glass opacities. (4) Biopsy of involved organ (lung, sinus, skin, GI tract, etc.) for fungal culture and histopathology with GMS stains - Consider adding Fungal Broad-Range PCR if biopsy is done (5) If biopsy is not done, IFI is strongly suspected, and Karius Test is being considered, Infectious Disease must be consulted for input	**Conventional testing is the preferred initial step** (i.e. galactomannan, fungal antigens/serology, etc.). NGS testing has not consistently exceeded detection of invasive fungal infections by conventional testing and clinical criteria even in high-risk populations such as SOT or HSCT recipients. ● Note that NGS demonstrated decreased sensitivity in detecting infection with *Aspergillus* species as compared to other molds.
Febrile neutropenia (Fevers with ANC <500 or	**LOW RISK:** MASCC Risk-Index Score of ≥21 or CISNE score of <3	(1) Blood cultures; additional testing based on symptoms and physical exam findings (CXR, urinalysis, GI pathogen panel or *C. difficile* assay, viral	**Conventional testing is the preferred initial step** (i.e. blood cultures, other cultures, antigen testing, and serology as

<1000 with predicted decline to <500 over the next 48 hours)	**HIGH RISK:** MASCC Risk-Index Score of <21 or CISNE score of ≥3	diagnostics, etc.) (2) Consider additional diagnostics based on initial clinical presentation (i.e. abdominal CT, sinus CT, etc.), particularly if neutropenic fevers last >3-5 days. Early ID consultation of is recommended. (3) If lung infiltrates or sinusitis are present, consider workup for IFI evaluation (see above). (4) If patient is high-risk (see criteria to the left), there is no obvious focal site of infection, all standard cultures/ serologies are negative, fevers persist, an infectious cause is still suspected, and Karius Test is being considered, Infectious Disease must be consulted for input.	appropriate). If conventional testing is negative, NGS testing from an infected sample source in a high-risk patient may be considered. ● NGS testing of affected sites/tissues (Broad-Range PCR) is preferred over Karius Test. ● Karius Test could be considered if samples from infected sites are negative by standard testing or cannot be obtained (ex: patient with severe thrombocytopenia). ● Negative NGS testing does not rule out infection.
Fever of unknown origin	Patient with temperature >101F for >5 days inpatient or >10 days outpatient but negative conventional workup	**We recommend a tiered evaluation based on clinical history and exam.** Some considerations, which should be tailored to the individual patient, include: (1) Blood cultures (2) Imaging (i.e. CT chest / abdomen / pelvis, transthoracic echocardiogram, CT/PET scan) (3) Simultaneous evaluation for malignancy and rheumatologic conditions as appropriate (4) Infectious Disease consult and consideration of targeted infectious workup based on exposure history and clinical findings (i.e. endemic fungi, TB, rickettsial, etc). (5) If all standard cultures/ serologies are negative, fevers persist, infection is still suspected, and Karius Test is being considered, Infectious Disease must be consulted for input.	**Conventional testing is the preferred initial step** (i.e. blood cultures, antigen testing, and serology as appropriate). If conventional testing is negative, NGS testing from an infected sample source may be considered. ● NGS testing of infected sites/tissues (Broad-Range PCR) is preferred over Karius Test. However Karius Test could be considered if samples from infected sites are negative by standard testing or cannot be obtained. ● Saving of plasma specimen for Karius Test is not needed as patients with FUO should have persistant fevers and not be on antimicrobials. ● Negative NGS testing does not rule out infection.

Additional Clinical Guidance on the Use of Karius Testing

Karius testing has the ability to detect approximately 1,250 different pathogens (including bacteria, fungi, viruses, and parasites). The full pathogen list is available here: https://kariusdx.com/karius-test/pathogens. As noted at the top of this document, it only detects DNA-based organisms, so it is unable to detect RNA-based pathogens such as HIV, hepatitis C, Zika, or coronaviruses. It also does not distinguish commensal organisms from pathogenic organisms, so test results must be interpreted with care. The sensitivity of the test is undefined and a negative test result does not necessarily rule out infection unless the pre-test probably is already low. Although some antimicrobial resistance markers can be reported, the detection of these markers does not always correlate with phenotype resistance and we generally recommend correlating these results with standard cultures. The typical turnaround time for this test is 2-4 business days (includes shipping time). Karius is expensive and often not covered by insurance, although it can be billed under diagnosis-related group codes. Finally, the clinical actions which should occur based on test results are currently unclear. For these reasons we recommend judicious use of this test and provide guidance regarding appropriate clinical scenarios below.

Clinical scenarios in which Karius Testing may be considered	
Culture-negative endovascular infections (endocarditis, mycotic aneurysm, vascular graft infections)	**Tiered evaluation:** ● **Perform standard evaluation (see Table 3b)** ● **Day 3+**: Karius Test (if no surgical intervention) or ● If valve surgery performed consider Broad-Range tissue PCR (University of Washington; send out test)
Deep seated infection with contraindication to or desire to avoid an invasive procedure	See Table 3a. Examples: - Deep brain abscesses with no plan for aspiration or surgery - Spinal osteomyelitis/discitis with negative blood and bone/disc cultures (if biopsy was done, would recommend Broad-Range tissue PCR prior to Karius Test)
Deep seated infection with nondiagnostic invasive workup, but imaging or pathology remain highly suspicious for infection	If standard microbiological workup (including tissue cultures and pathology) negative but infection is still highly suspected, consider Broad-Range PCR prior to Karius testing.

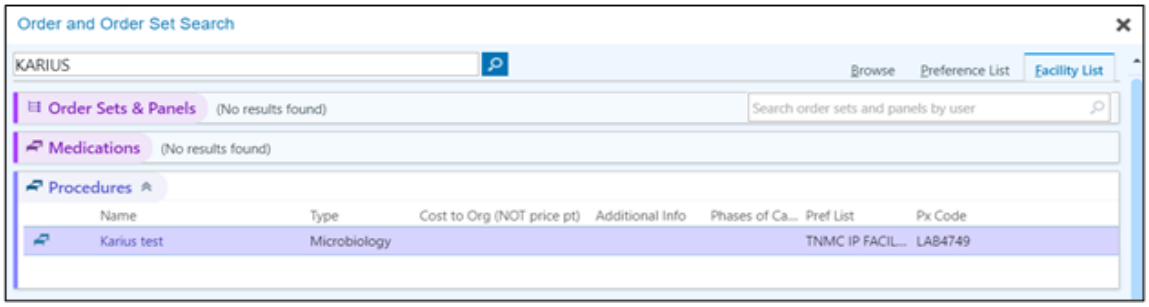
Clinical scenarios in which Karius Testing should usually be avoided	
Severe pneumonia*	**Tiered evaluation:** For high risk patients (patients with severe CAP, patients with CAP on expanded-spectrum therapy such as vancomycin/cefepime, patients not responding to typical therapy, or patients with hospital-acquired or ventilator-associated pneumonia), we recommend: (1) Sputum culture, pneumonia pathogen panel, etc. (see Table 3c) (2) Bronchoscopy (with Pulmonology input) (3) Karius Test (with ID input) only if all testing is unrevealing and infectious cause is still strongly suspected and anticipating prolonged (>14 days) of antimicrobial therapy
Fever of unknown origin (i.e. temperature >101°F for >5 days inpatient or >10 days outpatient, with negative workup)	See Table 3e. Limited data (case reports/series only) suggests that Karius testing is unlikely to be beneficial in FUO cases. Could consider if microbiological workup is negative and infectious etiology remains high on the differential.

**Data is strongest for pediatric patients with complicated pneumonia, suggesting that Karius has a much higher yield than standard testing and can significantly impact antimicrobial management.[11,12] A prospective study among mechanically ventilated adult patients demonstrated only marginally better yield than standard antimicrobial evaluation (60% vs 52%), and it was unclear if this led to any meaningful outcomes.[13] Karius testing may be useful in adult patients with complicated pneumonia if standard microbiological workup is unrevealing, particularly in deescalating/targeting antibiotics.*

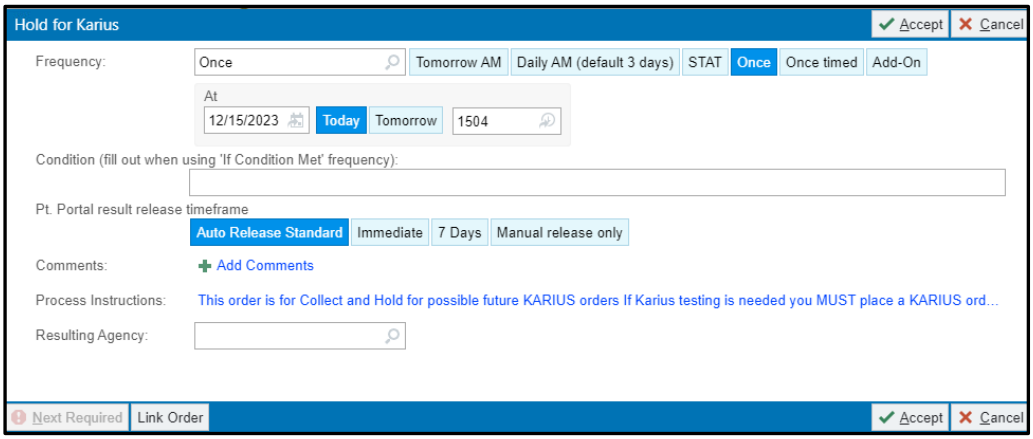
Clinical scenarios in which Karius Testing should NOT be used	
Sepsis or suspected bacteremia	There is no data regarding Karius testing's impact on meaningful outcomes compared to standard testing (i.e. duration of hospital stay, mortality, etc.).
Febrile neutropenia	See Table 3e. There is no data regarding Karius testing's impact on clinical outcomes compared to standard testing (i.e. duration of hospital stay, mortality, etc.)
Advanced HIV/AIDS (CD4 <200 cell/mm^3) with fevers	There is no evidence that Karius provides utility over standard microbiologic testing, and many fevers may resolve with appropriate treatment of the HIV
Polyarthritis	There is no data regarding Karius testing's impact on diagnostic yield or meaningful outcomes in patients with polyarthritis.
Serial monitoring in asymptomatic transplant recipients	Prospective studies suggest that Karius testing has some ability to predict fungal infections and bacterial bloodstream infections, however there is no data yet regarding the test's impact on meaningful clinical outcomes and cost justification in order to rationalize serial testing.
Outbreak investigation for rare or difficult-to-isolate pathogens	Karius testing has been used as a diagnostic tool for outbreaks caused by very difficult-to-grow pathogens (i.e. NTMs), but in general we would not recommend its use.

How to order a Karius Test

Karius testing can be ordered in Epic under "**Karius Test**":



Specimens can also be requested to be held for possible future testing. This can decrease the effect of empiric antimicrobial therapy on Karius Test results while awaiting standard microbiological workup. This can be ordered in Epic under "**Hold for Karius**":

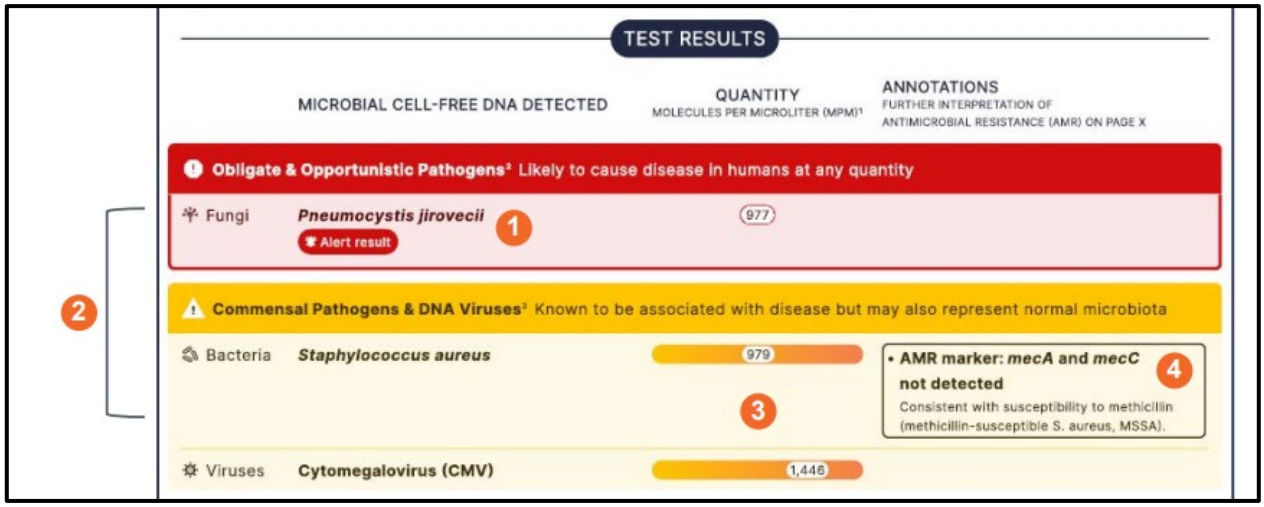


If Karius testing is needed you must place a Karius order and notify the Sendout Dept to let them know a sample is being held and now needs to be sent out. **Samples will only be held for one week then disposed.**

Sendout Lab can be notified via email (SendoutLab@nebraskamed.com) or phone call (**402.559.9353**); email would be the preferred method for documentation purposes.

Specific information about the tube top, serum volume, storage, etc. can be found here: https://kariusdx.com/karius-test/karius-test-process. Plasma specimens can be stored at ambient temperature for up to 96 hours and at -20°C for up to 6 months.[1]

How to read the Karius report



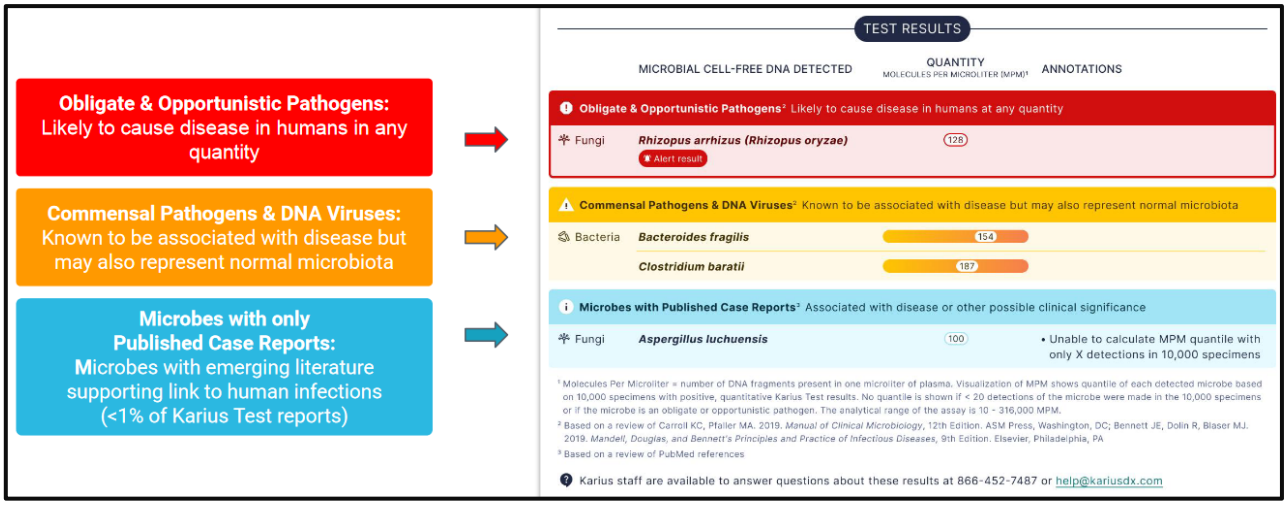
(1) **Microbe(s) detected**: Must be part of Karius test's database (see website for full list), and must have cell-free DNA detected in statistically significant amounts

(2) **Classification of microbes**: Microbes will be classified by Karius into one of three categories: (1) Obligate & Opportunistic Pathogens, (2) Commensal Pathogens & DNA Viruses, or (3) Microbes with Published Case Reports.

(3) **Quantification:** The concentration of microbial DNA is measured in molecules of DNA fragments per microliter of plasma (molecules per microliter, **MPM**). Many variables can affect the MPM value, including the turnover rate of the organism and the genome size of the organism, so **the MPM value should not be compared across different microorganisms**. The gradient visualizes the MPM based on 10,000 specimens with positive Karius Test results.

(4) **Antimicrobial resistance:** The presence or absence of 7 AMR markers (SCC*mec*, *mecA, mecC, vanA, vanB,* CTX-M, and KPC) are reported across 18 bacterial pathogens. These results are typically reported as an amended report the following day.

Karius Test classification of microorganisms



Karius Test results: Example scenarios

What is detected	Example of microorganism(s) detected	Example of clinical scenario	Interpretation
No organisms detected	N/A	Adult with valvular vegetation and does not meet any other Duke Criteria for infective endocarditis	Typically indicates no infection or source has been controlled, however it should be noted that negative NGS testing does not completely rule out infection. Test results can be affected by location of the infection and antimicrobial therapy.
Microorganism with very high quantification	>316,000 MPM *Toxoplasma gondii*	Immunocompromised patient with fevers after stem cell transplant engraftment	Typically associated with advanced or disseminated disease
Obligate pathogen with low quantification	87 MPM *MTB complex*	Patient post-liver transplant with nodular pneumonia	Typically indicates infection
Microorganism with very low quantification	20 MPM *Candida albicans*	Immunocompromised patient with febrile neutropenia	Clinical context required - could represent early infection, end of infection, pre-treated infection, or commensal/colonizer/ contaminant
Polymicrobial, with one significant microorganism and multiple non-specific microorganisms	4,764 MPM *Histoplasma capsulatum* 1,470 MPM *Veillonella parvula* 408 MPM *E Coli* 322 MPM *Neisseria bacilliformis* ***NOTE: Cannot compare MPMs across different microbes!***	Adult with pneumonia and multiple pulmonary nodules	One organism (*Histoplasma* in this case) is the possible causative pathogen, and the other organisms are consistent with GI commensals (suggestive of mucosal barrier disruption)
Polymicrobial, with multiple non-specific microorganisms	979 MPM *Fusobacterium nucleatum* 852 MPM *Actinomyces oris* 580 MPM *Streptococcus salivarius* 512 MPM *Rothia dentocariosa*	Adult with prolonged hospitalization in ICU and on pressor support for 3 weeks for septic shock	Multiple commensal organisms consistent with mucosal barrier injury and GI translocation Can be seen in critically ill patients and immunocompromised patients
Polymicrobial, with multiple pathogenic microorganisms	4,764 MPM *Histoplasma capsulatum* 2,480 *Trichosporon faecale* 648 *Candida albicans*	Immunocompromised patient with known candidemia and disseminated *Histoplasmosis*, now with febrile neutropenia	Clinical context required - all organisms could potentially be pathogenic

Factors to consider when interpreting the Karius Test:	Frequent incidental findings:
- Location of infection - Recent infections - Latent DNA viruses - Antimicrobial pre-treatment - Mucosal barrier injury - GVHD - Poor dentition - Aspiration	- *H. pylori* - Low level DNA viruses - Commensal microbes (i.e. *staphylococcus epidermidis, corynebacterium, streptocci, actinomyces*, etc.)

How to order University of Washington Broad-Range PCR

Broad-Range PCR testing can be ordered in Epic as discussed below. See screenshot from Epic for ordering details. You must be logged into an Infectious Disease context and have saved these labs as "preferences" to be able to sign the order.

To order PCR testing for individual bacterial targets, fungal targets, or mycobacterial targets (non-TB mycobacteria and MTB complex), order the desired individual test as follows:

- **Bacterial Detect PCR** (Code: LAB4531)
- **Fungal Detection PCR** (Code: LAB4532)
- **Non TB Mycobact PCR** (Code: LAB4533)
- ***M. tuberculosis* PCR** (Code: LAB4534)

To order PCR testing for both mycobacterial targets (non-TB and mycobacteria), use the following test order:

- **AFB PCR** (Code LAB4538)

To order PCR testing for all four targets (bacterial, fungal, NTM, and MTB) for a specimen, use the following test order:

- **Broadrange PCR** (Code: LAB 4537)

Locating the specimen:

(1) If you place the order before the sample is collected, the sample for broad-range PCR generally gets split in Specimen Receiving. Send-outs will receive a portion without further intervention. You can confirm that send-outs has received the specimen by calling 402-559-9353.

   (a) Note: If the patient discharges before Specimen Receiving receives the sample, the broad-range PCR order will auto cancel. If you are requesting broad-range PCR on a sample obtained through an outpatient procedure or on a patient that will be discharging, please contact send-outs to confirm that the specimen has been received.

(2) If you are adding broad-range PCR on after cultures in micro have been set, please call the micro lab at 402-552-2090 and request that tissue be sent to send-outs for broad-range PCR. If multiple samples were collected for the patient, please indicate if you would like the specimens pooled. Tissue samples are held for 7 days. Please note that the micro lab will not always have remaining tissue, depending on the quantity of specimen received and the orders placed.

(3) If you are adding broad-range PCR on to a sample in pathology (such as a paraffin block), please call send-outs at 402-559-9353 to coordinate with Pathology. Note that formalin-fixed specimens will have lower sensitivity than fresh specimens, due to degradation of the DNA.

How to read the Broad-Range report

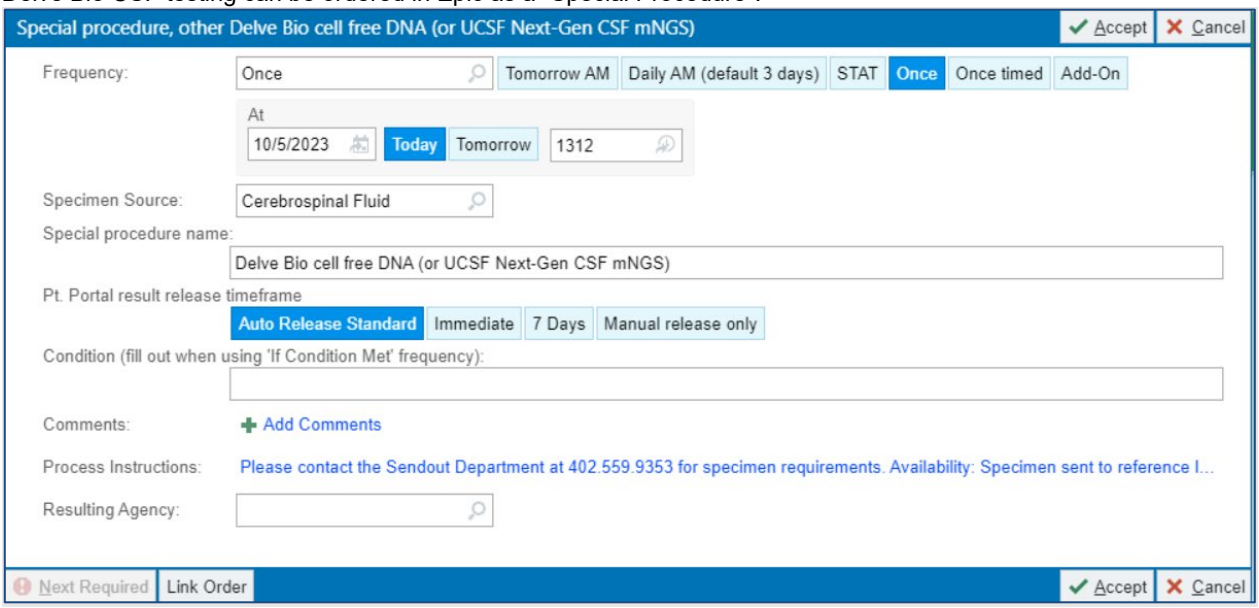
```
Bacterial Detection by PCR

Detection, 16S rDNA: **Detected**
Identification: Streptococcus pneumoniae

Specimen Description: Aorta

Specimen comment: None

Specimen DNA Extraction: Confirmed

Reviewed by Pathologist

Method Note: Presence of inhibitors excluded unless otherwise
specified. Diagnostic yield of organisms detected may be
influenced by: diagnostics target copy number, DNA extraction
efficiency, PCR efficiency, and the amount of microbial DNA
present in the sample received.

Fresh tissue is the optimal specimen of choice, as it reduces
the chance of introducing exogenous DNA templates or
microorganisms during formalin fixation and paraffin embedding.

Formalin, staining, or acid decalcification may dramatically
reduce the sensitivity of the assays due to decreased template
yield and quality.
```

Each PCR category (Bacterial, Fungal, NTM, and MTB complex) will list if any pathogens were ****Detected****, followed by the name of the pathogen that was identified.

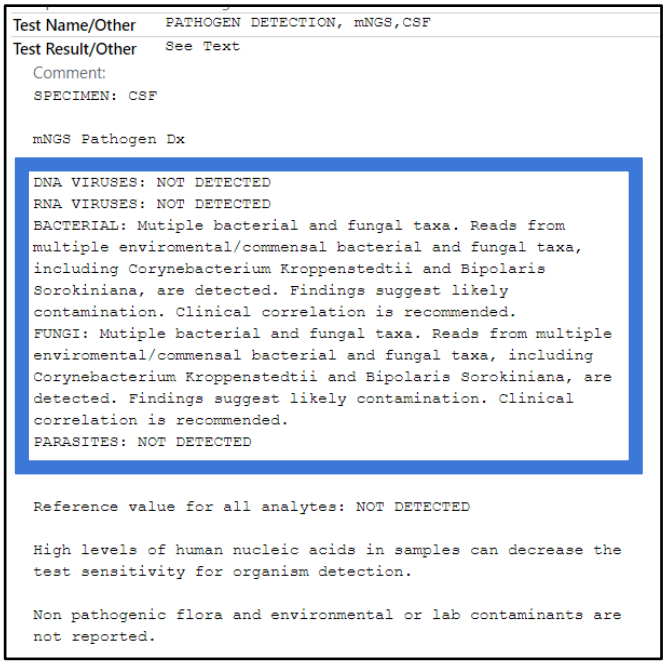
If a pathogen is detected in one section, then one or more of the other sections might be labeled as ****Indeterminate**** due to testing interference.

How to order Delve Bio cell free DNA (previously University of California San Francisco Center for Next-Gen precision diagnostics CSF cfDNA)

Delve Bio CSF testing can be ordered in Epic as a “Special Procedure”:



How to read the Delve Bio report



Test Name/Other PATHOGEN DETECTION, mNGS,CSF
Test Result/Other See Text
Comment:
SPECIMEN: CSF

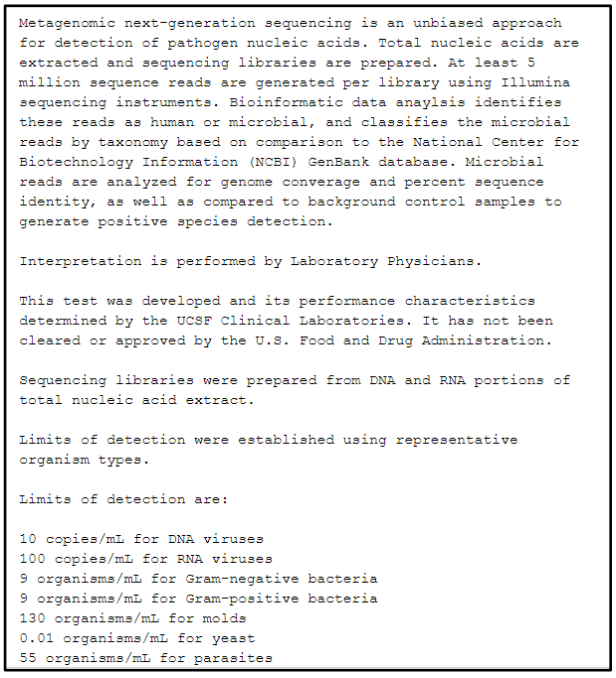
mNGS Pathogen Dx

DNA VIRUSES: NOT DETECTED
RNA VIRUSES: NOT DETECTED
BACTERIAL: Mutiple bacterial and fungal taxa. Reads from multiple enviromental/commensal bacterial and fungal taxa, including Corynebacterium Kroppenstedtii and Bipolaris Sorokiniana, are detected. Findings suggest likely contamination. Clinical correlation is recommended.
FUNGI: Mutiple bacterial and fungal taxa. Reads from multiple enviromental/commensal bacterial and fungal taxa, including Corynebacterium Kroppenstedtii and Bipolaris Sorokiniana, are detected. Findings suggest likely contamination. Clinical correlation is recommended.
PARASITES: NOT DETECTED

Reference value for all analytes: NOT DETECTED

High levels of human nucleic acids in samples can decrease the test sensitivity for organism detection.

Non pathogenic flora and environmental or lab contaminants are not reported.



Metagenomic next-generation sequencing is an unbiased approach for detection of pathogen nucleic acids. Total nucleic acids are extracted and sequencing libraries are prepared. At least 5 million sequence reads are generated per library using Illumina sequencing instruments. Bioinformatic data anaylsis identifies these reads as human or microbial, and classifies the microbial reads by taxonomy based on comparison to the National Center for Biotechnology Information (NCBI) GenBank database. Microbial reads are analyzed for genome converage and percent sequence identity, as well as compared to background control samples to generate positive species detection.

Interpretation is performed by Laboratory Physicians.

This test was developed and its performance characteristics determined by the UCSF Clinical Laboratories. It has not been cleared or approved by the U.S. Food and Drug Administration.

Sequencing libraries were prepared from DNA and RNA portions of total nucleic acid extract.

Limits of detection were established using representative organism types.

Limits of detection are:

10 copies/mL for DNA viruses
100 copies/mL for RNA viruses
9 organisms/mL for Gram-negative bacteria
9 organisms/mL for Gram-positive bacteria
130 organisms/mL for molds
0.01 organisms/mL for yeast
55 organisms/mL for parasites

Each section (DNA viruses, RNA viruses, Bacterial, Fungi, and Parasites) will list if any pathogens were **Detected**, followed by the name of the pathogen. Results are interpreted by laboratory physicians prior to release, so organisms that are considered commensal or environmental are excluded from the report.

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