Overview

Useful For
Rapid detection of meningitis and encephalitis caused by:
- *Escherichia coli* K1 (K1 serotype only)
- *Haemophilus influenzae*
- *Listeria monocytogenes*
- *Neisseria meningitidis* (encapsulated strains only)
- *Streptococcus agalactiae* (Group B Strep)
- *Streptococcus pneumoniae*
- Cytomegalovirus (CMV)
- Enterovirus
- Herpes simplex virus 1 (HSV-1)
- Herpes simplex virus 2 (HSV-2)
- Herpes simplex virus 6 (HHV-6)
- Human parechovirus
- Varicella zoster virus (VZV)
- *Cryptococcus neoformans/gattii*

This test is **not intended for use** with CSF collected from indwelling medical devices (e.g., CSF shunts).
This test is **not recommended** as a test of cure.

Testing Algorithm
See [Meningitis/Encephalitis Panel Algorithm](#) in Special Instructions.

Special Instructions
- [Meningitis/Encephalitis Panel Algorithm](#)

Highlights
This test is used to rapidly detect the nucleic acid of 14 of the most common pathogens that cause encephalitis or meningitis.

The FilmArray Meningitis/Encephalitis panel is a multiplex PCR test capable of qualitatively detecting DNA or RNA of 14 pathogens (bacteria, viruses, and yeast) in approximately 1 hour from spinal fluid.

This test is used to diagnose infection caused by *Escherichia coli* K1, *Haemophilus influenzae*, *Listeria monocytogenes*, *Neisseria meningitidis*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, cytomegalovirus, enterovirus, herpes simplex virus 1 and 2, human herpesvirus 6, human parechovirus, varicella zoster virus, and *Cryptococcus neoformans/gattii*.

Method Name
Multiplex Polymerase Chain Reaction (PCR)

NY State Available
Yes
Specimen

Specimen Type
CSF

Ordering Guidance
It is not usually recommended that the following tests be concomitantly ordered if this test is ordered:
- LCMV / Cytomegalovirus (CMV), Molecular Detection, PCR, Varies
- LENT / Enterovirus, Molecular Detection, PCR, Varies
- HSVC / Herpes Simplex Virus (HSV), Molecular Detection, PCR, Spinal Fluid
- HHV6V / Human Herpesvirus-6, Molecular Detection, PCR, Spinal Fluid
- LVZV / Varicella-Zoster Virus, Molecular Detection, PCR, Varies

Additional Testing Requirements
1. In some cases, there may be local public health requirements that impact Mayo Clinic Laboratories’ (MCL) clients and require additional testing on specimens with positive results from this panel. Clients should familiarize themselves with local requirements. MCL recommends that clients retain an aliquot of each specimen submitted for this test to perform additional testing, as needed.
2. It is recommended that the following testing be ordered with this test:
   - CCCF / Cell Count and Differential, Spinal Fluid
   - TPSF / Protein, Total, Spinal Fluid
   - GLSF / Glucose, Spinal Fluid
   - GEN / Bacterial Culture, Aerobic, Varies
   - GRAM / Gram Stain, Varies
3. If clinically indicated, the following testing should also be ordered with this test:
   - FS / Fungal Smear, Varies
   - FGEN / Fungal Culture, Routine
   - LFACX / Cryptococcus Antigen with Reflex, Spinal Fluid

Shipping Instructions
Specimen must arrive at refrigerated temperature within 7 days of collection.

Necessary Information
Specimen source is required.

Specimen Required
Specimen Type: Spinal fluid
Container/Tube: Sterile vial
Specimen Volume: 1 mL
Collection Instructions: Frozen specimens are not acceptable.

Forms
If not ordering electronically, complete, print, and send 1 of the following forms with the specimen:
- Neurology Specialty Testing Client Test Request (T732)
- Microbiology Test Request (T244)
Reject Due To

- Any specimen in transport media
- Any specimen that has been centrifuged
- Shunt fluid

Specimen Minimum Volume
0.5 mL

Specimen Stability Information

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Clinical & Interpretive

Clinical Information

Bacteria:

*Escherichia coli* K1 strains account for nearly 80% of *E coli* isolated from cerebrospinal fluid (CSF). While most *E coli* are harmless enteric organisms residing in the intestines of humans and animals, some cause gastrointestinal illness and extraintestinal infections (eg, urinary tract infections, bacteremia, and meningitis). *E coli* associated with meningitis contain virulence factors that contribute to their pathogenesis by allowing them to spread through the blood, hijack normal host cell functions, infiltrate endothelial cells, and gain access to the tissues of the central nervous system (CNS). The K1 antigen is a capsule that protects the bacteria from the immune system. These infections are of particular concern for preterm babies and neonates and are responsible for nearly 45% and 30% of meningitis cases in these age groups with a mortality rate of 13% and 25%, respectively. Infections in adults are less common and generally opportunistic in nature following exposure of sterile organs to contents of the gastrointestinal tract following trauma or surgical procedures; the mortality rate for adults is reported to be 28% to 36%.

*Haemophilus influenzae* is a gram-negative coccobacillus that is isolated exclusively from humans. Strains of *H influenzae* are divided into 2 groups based on the presence or absence of a polysaccharide capsule. Encapsulated strains are further divided into 6 serotypes (a through f). Prior to widespread use of the *H influenzae* type b (Hib) conjugate vaccines, Hib caused more than 80% of invasive *H influenzae* infections, predominantly in children under the age of 5, with a mortality rate of 3% to 6% and a further 20% to 30% developing permanent sequelae ranging from mild hearing loss to mental retardation. In areas with routine vaccination, the majority of invasive *H influenzae* infections are caused by nontypeable strains and remain an important cause of meningitis, particularly for persons with predisposing conditions such as otitis or sinusitis, diabetes, immune deficiency, or head trauma with CSF leakage. Meningitis due to *H influenzae* occurs at an estimated rate of 0.08 cases per 100,000 in the United States, and it has been reported as the etiologic agent of bacterial meningitis in 20% to 50% of cases worldwide over the last several decades.

*Listeria monocytogenes*, the causative agent of listeriosis, is a gram-positive bacillus that is ubiquitous in soil and water and can be found in the gastrointestinal tract of up to 5% of healthy human adults. Listeriosis is considered one of the most severe bacterial foodborne infections due to its high mortality rate even with early antibiotic treatment (11%-60%). Invasive listeriosis can result in abortion, sepsis, meningitis, and meningoencephalitis. Populations at risk for developing invasive listeriosis include the immunosuppressed, pregnant women, neonates, fetuses, and the elderly. Meningitis due to *L monocytogenes* is reported to be approximately 0.05 cases per 100,000 persons in the United States per year, and causes from 0.5% to 2.0% of bacterial meningitis cases in non-US countries.(1)

*Neisseria meningitidis* (encapsulated) is a fastidious, aerobic, gram-negative diplococcus that is transmitted by contact with mucus or respiratory droplets, often from asymptomatic carriers. There are at least 12 different serogroups of *N*
**meningitidis**, 6 of which are associated with epidemics (groups A, B, C, W135, X, and Y). The serogroup refers to types of capsular antigens, generally only encapsulated *N meningitidis* are considered pathogenic. Meningococcal disease (meningitis and meningococcemia) is rare in developed countries, but can occur in outbreaks and is still a public health issue in developing countries. It is most common in infants, children, and young adults, and appears in places with crowded living conditions (e.g., college dormitories and military barracks). Seasonal incidence peaks in late winter and early spring with an annual incidence of about 0.2 cases per 100,000 in the United States. The disease can progress extremely quickly (<24 hours) with hypotension, multiorgan dysfunction, shock, peripheral ischemia, and limb loss, and has a mortality rate of approximately 5% to 10%. There are licensed meningococcal vaccines available in United States that may be used in persons of all ages, depending on the vaccine. Despite extensive vaccination efforts worldwide, several serogroups of *N meningitidis* still cause seasonal outbreaks, particularly in sub-Saharan Africa. Extreme reductions in serogroup C meningococcal meningitis have been observed in countries where vaccines providing protection for this serogroup have been introduced.

*Streptococcus agalactiae* (group B *Streptococcus* or GBS) is an important cause of meningitis in neonates, particularly those that are preterm, and is often coincident with neonatal sepsis. The most important risk factor for neonatal disease is maternal colonization with GBS. Since 1996, the CDC guidelines (updated in 2010) have called for prophylactic antibiotic treatment several hours before delivery in at-risk deliveries, resulting in declining rates of neonatal GBS. In adult patients, GBS is associated with advanced age or severe underlying health conditions. Overall incidence in the United States is estimated to be 0.25 infections per 100,000 and neonatal GBS disease has ranged from 0.2 to 2.4 per 1,000 births in Europe over the last few decades. Mortality rates range from 10% for neonates to 25% to 30% in adults. *Streptococcus pneumoniae* colonizes the upper respiratory tract and is the most frequently isolated respiratory pathogen in community-acquired pneumonia. It is also a major cause of meningitis, particularly in pediatric and older adult patients, and especially in those with underlying medical conditions, with an incidence rate of approximately 0.8 infections per 100,000 in the United States, and causes 20% to 31% of bacterial meningitis cases in non-US countries. The mortality rate is also high: 8% to 15% for children and 20% to 37% for adults. Mortality approaches 50% in resource-poor countries, especially where HIV coinfection is a factor. Neurological sequelae (cognitive impairment, deafness, epilepsy) are reported in up to 40% of survivors. Vaccines have helped reduce the risk of both invasive disease and pneumococcal pneumonia by 50% to 80%.

**Viruses:**

Human cytomegalovirus (CMV) is a double-stranded DNA virus of the Herpesviridae family. Seroprevalence data show that infection is nearly ubiquitous in the population worldwide, with rates approaching 100% in developing countries and 36% to 90% in the United States depending on age and race/ethnicity. While severe illness in immunocompetent patients is rare, CMV is an opportunistic pathogen in immunocompromised or immunosuppressed individuals, either as an initial infection or activation of a latent infection. In some patients (e.g., transplant recipients), CMV may infect the central nervous system and cause meningoencephalitis.

Enteroviruses (EV) are small RNA viruses that are members of the Picornaviridae family and are associated with human illnesses ranging from asymptomatic or mild infections to serious CNS illnesses requiring hospitalization. Infection rates are highest in children, with the majority of infections occurring during summer months. The most common EV serotypes are coxsackie viruses A9 and B1, and echoviruses 6, 9, and 18, which account for over 50% of serotyped detections. Infections are spread via fecal-oral and respiratory routes and can spread quickly in community settings, particularly in areas with poor sanitation. EV is one of the commonly identified causes of infectious encephalitis/meningitis, with prevalence rates reported between 5.5% and 30% depending on location and patient demographics.

Herpes simplex viruses 1 and 2 (HSV-1 and HSV-2) are DNA viruses of the Herpesviridae family named for the spreading skin ulcerations caused by infection with these viruses. HSV-1 infections usually occur early in childhood and manifest primarily as oral lesions, whereas HSV-2 is primarily associated with genital lesions and infections are acquired later in
life and are associated with sexual activity. HSV establishes residency in nerve cells following initial infection (which is asymptomatic in most cases). Viral activation resulting in lesions or other severe disease outcomes (such as CNS infection), may occur throughout life, and are associated with fever, injury, exposure to ultraviolet irradiation (sunlight), emotional stress, hormone irregularities, and changes in immune status. In the United States, overall seroprevalence for HSV-1 is around 60%. The overall seroprevalence for HSV-2 is around 16%, but varies with age, sex, and ethnicity. Worldwide, it is estimated that approximately 90% of people are infected with HSV-1; HSV-2 is less common with 15% to 80% of people infected. HSV is one of the most common causes of viral encephalitis and is a significant cause of meningitis. In a large study of over 1,600 CSF specimens in the United Kingdom, HSV-1 was found in 25 (1.5%) patients (almost all of whom had encephalitis) and HSV-2 was found in 33 (1.9%) patients (almost all of whom had meningitis). This overall prevalence of approximately 3% in CSF is similar to that seen in a recent study of CSF patients in New York State. This study also saw a similar distribution of HSV-1 and HSV-2 in encephalitis versus meningitis.

Human herpesvirus 6 (HHV-6) was discovered in the mid-1980s, when the rise of immunocompromised patients led to an increase in the population susceptible to severe disease outcome. There are 2 species: HHV-6A and HHV-6B. Studies have shown that over 95% of persons over the age of 2 are positive for 1 or both variants and the infection establishes latency due to viral integration into host cells. While primary infection with HHV-6B causes roseola in infants, the clinical manifestations of primary infection with HHV-6A remain somewhat undefined; however, some studies have suggested that HHV-6A infection may be linked to inflammatory or neurological disease, and that HHV-6A may have an increased neurotropism compared to HHV-6B. This hypothesis is supported by the finding that HHV-6 inhabits CNS tissues, including the brain, where it may cause tissue damage leading to encephalitis or meningitis. Furthermore, HHV-6 was identified in CSF of 1.8% of patients with encephalitis or meningitis in a recent study. CNS disease associated with HHV-6 is found in both children and adults, suggesting CNS invasion during primary infection is possible. While immunocompetent patients may experience CNS infection, it is much more common in severely immunosuppressed individuals. However, HHV-6 is known to reactivate in asymptomatic patients and can be detected by PCR in otherwise healthy individuals without signs of active HHV-6 infection. Studies of HHV-6 in normal brain tissue have also identified HHV-6 DNA via PCR in up to 85% of patients without signs of active infection and HHV-6 DNA may persist in the CSF after acute infection. In a study of 56 allogeneic stem cell transplant patients, HHV-6 DNA was detected in the CSF of 14 (27%) patients without CNS symptoms. Given the prevalence of latent infection and potential for asymptomatic reactivation, positive HHV-6 results should be carefully interpreted in association with clinical symptoms and supplemental laboratory testing.

Human parechoviruses (HPeV) comprise another genus of the Picornaviridae family. HPeV were originally classified as enterovirus upon their discovery in the 1950s and at least a dozen serotypes have been identified. Seroprevalence for HPeV-1 approaches 100% in adult populations, with most infections occurring during early childhood. As with EV, infections are spread via fecal-oral and respiratory routes; with the most common symptoms being mild respiratory or gastrointestinal illness. CNS disease from HPeV-1 is rare, but HPeV-3 is associated with severe disease outcomes such as sepsis, encephalitis, meningitis, and hepatitis in children younger than 3 months of age. Recent studies of CSF from infants with suspected CNS illness or sepsis have demonstrated HPeV at a prevalence of 3% to 17%, nearly all of which were HPeV-3. Magnetic resonance imaging studies of infants who survive HPeV CNS disease show damage to the white matter of the brain and developmental disabilities later in life.

Varicella zoster virus (VZV) is a double-stranded DNA virus of the Herpesviridae family that usually causes infections in childhood (chicken pox) and establishes latent presence in cells that can reactivate later in life (zoster or shingles). VZV is primarily spread through respiratory secretions or direct contact with lesions of an infected individual, and infection of new hosts begins within the epithelial cells of the respiratory tract. Following primary infection (fever and malaise accompanied with a maculopapular rash), VZV establishes itself in the sensory ganglia of the nervous system where it remains latent. In the United States, nearly 90% of the population had been infected with VZV before the advent of vaccines. Similar rates have been reported in European countries. Of those infected, between 10% and 30% develop
zoster (a painful rash along the dorsal ganglia), primarily later in life. It is estimated that the median global incidence of zoster is 4.0 to 4.5 per 1,000 person-years, which highlights the frequency of VZV reactivation worldwide. Studies have shown that VZV is transiently detectable by PCR in the blood of older, asymptomatic individuals (both immunocompetent and immunocompromised), suggesting reactivation occurs throughout life but is usually managed by the immune system. Encephalitis and meningitis are complications of both varicella and zoster infections. In 1 study, VZV was the third most detected virus among patients with signs and symptoms of encephalitis or meningitis, with a reported prevalence of 1.9% in the study population. There are live, attenuated VZV vaccines licensed for use in the United States for the vaccination of children against varicella and adults against zoster.

Yeast:

_Cryptococcus neoformans_ and _Cryptococcus gattii_ are pathogenic fungi that are acquired by inhalation and can spread to other organ systems (particularly the brain and meninges). _C neoformans_ is considered an opportunistic pathogen of immunocompromised individuals. It is the AIDS-defining illness in up to 50% of AIDS patients. _C gattii_ infections are relatively rare but appear to be increasing. While typically associated with tropical and subtropical climates, since the 1990s, _C gattii_ infections have been reported in British Columbia, Canada, the US Pacific Northwest region, the Northeastern US, and in Europe. In addition to those with reduced immune function, _C gattii_ can also cause disease in the immunocompetent, particularly in persons with underlying health conditions. Mortality from cryptococcal meningitis is high, ranging from 10% to nearly 50% in immunocompromised patients.(1)

Reference Values
Negative (for all targets)

Interpretation
A positive result for 1 or more of the organisms suggests that nucleic acid from the organism was present in the sample. A negative result suggests that the nucleic acid of 14 common pathogens of the central nervous system was not present in the sample. A negative result should not rule-out central nervous system (CNS) infection in patients with a high pretest probability for meningitis or encephalitis. The assay does not test for all potential infectious agents of CNS disease. Negative results should be considered in the context of a patient’s clinical course and treatment history, if applicable. False-negative results may occur when the concentration of nucleic acid in the specimen is below the limit of detection for the test. Detection of multiple viruses or bacteria or viruses and bacteria may be observed with this test. In these situations, the clinical history and presentation should be reviewed thoroughly to determine the clinical significance of multiple pathogens in the same specimen.

Results are intended to aid in the diagnosis of illness and are meant to be used in conjunction with other clinical and epidemiological findings.

Cautions
The detection of microbial DNA or RNA is dependent upon proper sample collection, handling, transportation, storage, and preparation. There is a risk of false-negative results due to the presence of strains with sequence variability or genetic rearrangements in the target regions of the assays. False-negative results for herpes simplex virus (HSV) may occur; therefore, consider HSV testing by another nucleic acid amplification test (eg, HSVC / Herpes Simplex Virus (HSV), Molecular Detection, PCR, Spinal Fluid) if the meningitis encephalitis panel result is negative and clinical suspicion is high.

The meningitis encephalitis panel may be negative for _Cryptococcus neoformans/gattii_ in patients with cryptococcal meningitis, especially those who are receiving treatment; therefore, cryptococcal antigen testing on CSF (CLFAT / Cryptococcus Antigen Titer, Lateral Flow Assay, Spinal Fluid) should be considered if results of the meningitis encephalitis panel are negative, and clinical suspicion is high. Serum (SLFA / Cryptococcus Antigen Screen with Titer, Serum) may also
be indicated in such a situation. The meningitis encephalitis panel should not be used to monitor response to treatment for cryptococcal meningitis.

Repeat testing should not be performed on samples collected less than 7 days apart.

The following information is provided by the test manufacturer:

-The presence of excessively elevated protein (albumin) levels in the sample may interfere with testing.

-The performance of this test has not been specifically evaluated for CSF specimens from immunocompromised individuals.

-The effect of antibiotic treatment on test performance has not been evaluated. Test results may be affected by concurrent antimicrobial therapy or levels of organism in the sample that are below the limit of detection.

*Escherichia coli* K1: Strains possessing the K1 capsular antigen will be detected. All other *E coli* strains and serotypes will not be detected.

*Haemophilus influenzae*: Assay may cross react with *Haemophilus haemolyticus*. *H haemolyticus* is found in the respiratory tract, but are rarely isolated from CSF. *H influenzae* can be shed from the respiratory tract of healthy individuals. Caution should be exercised during specimen collection and testing to prevent contamination leading to false-positive results.

*Neisseria meningitidis*: Only encapsulated strains of *N meningitidis* will be detected. Unencapsulated *N meningitidis* will not be detected.

*Streptococcus pneumoniae*: *S pneumoniae* can be shed from the respiratory tract of healthy individuals. Caution should be exercised during specimen collection and testing to prevent contamination leading to false-positive results.

Human herpesvirus 6 (HHV-6) or cytomegalovirus (CMV): Can exist in a latent form that is reactivated during infection due to other pathogens, including agents not detected by the FilmArray ME panel that may cause meningitis or encephalitis (eg, *Mycobacterium tuberculosis* or HIV). Chromosomally integrated HHV-6 may result in a positive result. When detected by the FilmArray ME, HHV-6 or CMV should be considered as the likely cause of meningitis or encephalitis only in appropriate clinical settings and following expert consultation.

*Varicella zoster virus* (VZV): Viral shedding into the CSF often occurs in cases of zoster (shingles). VZV may not be the cause of CNS disease in these cases.

*Cryptococcus neoformans/gattii*: Assay may cross-react with *Cryptococcus amylolentus*, a near-neighbor of *C neoformans* that does not infect humans.(1)

**Supportive Data**

The Meningitis/Encephalitis Multiplex PCR Panel detects 14 pathogens (bacterial n=6, viral n=7, and fungal n=1) from spinal fluid (CSF). The performance of the panel was evaluated using clinical, residual CSF samples (n=291) that had tested positive by a routine methods (eg, bacterial culture, individual real-time PCR assay) for a pathogen represented on the panel. Of note, a subset (n=76) of the CSF specimens were collected during the prevaccine era, and had been characterized as positive for a bacterial pathogen. The panel demonstrated an overall percent positive agreement (PPA) of 97.5% (78/80) for bacterial pathogens, 90.1% (145/161) for viruses, and 52% (26/50) for *Cryptococcus neoformans/gattii*. Despite the low overall agreement (52%) between the panel and antigen testing for detection of *C neoformans/gattii*, the percent positive agreement of the panel *C neoformans/gattii* assay was 92.3% (12/13) when compared directly to the results of routine fungal smear or culture. Refer to Clinical Reference 1 for additional information.
Clinical Reference
1. FilmArray Meningitis/Encephalitis [ME] Panel CE IVD Instruction Booklet, BioFire Diagnostics, LLC, Salt Lake City, Utah, RFIT-PRT-0276-03 June 2017

Performance

Method Description
The FilmArray Meningitis/Encephalitis panel is a closed system that performs the chemistry required to isolate, amplify, and qualitatively detect nucleic acid from multiple bacterial, viral, and yeast pathogens from a spinal fluid specimen of patients suspected to have meningitis or encephalitis. A panel contains reagents in freeze-dried form and is divided into discrete segments where the required chemical processes are carried out. Patient sample and hydration fluid are drawn by vacuum into the panel and then placed into the FilmArray instrument. The detection process operations are automated (nucleic acid purification, first-stage PCR, second-stage PCR, and melt analysis) and complete in about an hour in this closed system:

-Nucleic Acid Purification:
The sample is lysed by a combination of chemical and mechanical mechanisms and the liberated nucleic acid is captured, washed and eluted using magnetic bead technology.

-First-Stage PCR:
A reverse transcription step is performed to convert viral RNA into cDNA prior to amplification. The purified nucleic acid solution is combined with a preheated master mix to initiate the reverse transcription step and subsequent thermocycling for multiplex PCR.

-Second-Stage PCR:
Products of first-stage PCR are diluted and mixed with fresh PCR reagents containing an intercalating fluorescent DNA dye (LCGreen Plus, BioFire Diagnostics), which is distributed over the second-stage PCR array. The individual wells of the array contain primers for different assays (in triplicate) that target specific nucleic acid sequences from each of the pathogens detected, as well as control template material.

-DNA Melting Analysis:
The temperature is slowly increased and fluorescence in each well of the array is monitored and analyzed to generate a melt curve.

-Analysis of Melt Curves:
The software evaluates the DNA melt curve for each well to determine if a PCR product was present in that well. If the melt profile indicates the presence of a PCR product, then the analysis software calculates the melting temperature of
the curve, which is then compared against the expected range for the assay. When the software determines that the melt curve is positive and in range, it is called positive. When it determines that the melt curve is negative or is not in the appropriate range, it is called negative.

-Analysis of Replicates:
Melt curves of each of the 3 replicates for each assay are evaluated to determine the assay result. For an assay to be called positive, at least 2 of the 3 associated melt curves must be called positive, and the temperature for at least 2 of the 3 positive melt curves must be similar (within 1 degree C). Assays that do not meet these criteria are called negative. (Instruction manual: FilmArray Meningitis/Encephalitis Panel CE IVD Instruction Booklet, BioFire Diagnostics, LLC, Salt Lake City, Utah, RFIT-PRT-0276-03 June 2017)

PDF Report
No

Performing Laboratory Location
Rochester

Fees & Codes

Test Classification
This test has been cleared, approved, or is exempt by the US Food and Drug Administration and is used per manufacturer's instructions. Performance characteristics were verified by Mayo Clinic in a manner consistent with CLIA requirements.

CPT Code Information
87483

LOINC® Information

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