Targeted Molecular Testing for Meningitis/Encephalitis and its Impact on Patient Management and Healthcare

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Background
Meningitis and encephalitis are often some of the most terrifying diseases in medicine mainly because of the high mortality rates if left untreated or if treated incorrectly. Meningitis refers to inflammation of the meninges, or the membranous layers that surround the brain and spinal cord, while encephalitis refers to inflammation of the brain itself. Inflammation of the meninges along with the surrounding cerebral tissue is referred to as meningoencephalitis. These diseases along with other central nervous system (CNS) infections can have a wide range of symptoms including fever, headache, seizures, and confusion. They are also generally characterized by altered consciousness that lasts over 24 hours.

In developed countries, meningitis affects between 4 to 30 people per 100,000 each year while encephalitis affects between 3 to 7 people per 100,000 each year. In the United States, there are approximately 20,000 encephalitis-related hospitalizations per year with an average of 1,400 deaths each year. There are over 70,000 meningitis-related hospitalizations in the US each year with a mortality rate as high as 11.4%. Overall, these conditions result in over $2 billion in hospitalization and healthcare costs in the US each year.

The most common causes of meningitis are viral infections, followed by bacterial and, rarely, fungal or parasitic infections. Viral, or aseptic, meningitis is usually mild and often clears on its own. Viruses that can cause this infection include enteroviruses, herpes simplex virus (HSV 1 & 2), and varicella-zoster virus (VZV). Several strains of bacteria can cause acute bacterial meningitis, most commonly Streptococcus pneumoniae (pneumococcal meningitis), Neisseria meningitides (meningococcal meningitis), Haemophilus influenza, Escherichia coli, Listeria monocytogenes, and Mycobacterium tuberculosis. Parasitic or fungal meningitis is relatively uncommon. Parasitic Cryptococcus neoformans, cysticercosis (a tapeworm), as well as cerebral malaria are also causes.

While the majority of all reported cases stem from viral etiologies, up to 60% of cases remain undiagnosed. The most common causes are HSV 1, HSV 2, VZV, enterovirus, and arboviruses (such as West Nile virus). Arbovirus refers to any virus that is transmitted to humans and other vertebrates by an arthropod vector, such as ticks or mosquitoes.

About 30% of herpes simplex encephalitis cases result from primary HSV 1 or 2 infections while the majority of cases are from viral reactivation. HSV 1 is typically acquired during childhood, however, HSV 2 is often transmitted through sexual contact. Over 90% of the global population has HSV, with each individual at risk of developing herpes simplex encephalitis.

VZV is also recognized as one of the leading causes of adult encephalitis. Studies have shown that VZV is the second most common etiology identified, second only to HSV. Before the availability of a vaccine, it was estimated that greater than 90% of the population would acquire VZV by the age of 15 and each of these persons would be at risk for developing acute VZV encephalitis.

Patient management is highly dependent on pathogen identification, with unfavorable outcomes directly correlated to delays of correct treatments and therapies. Delayed or incorrect treatments stemming from poor diagnosis can lead to severe neurological disabilities and ultimately death.

Testing Methods
Proper diagnosis and treatment can be difficult because of the complicated nature of identifying the pathological cause. Current guidelines recommend collecting cerebrospinal fluid (CSF) for use in distinguishing between a bacterial or viral infection by testing the leukocyte count with differential, erythrocyte count, protein level, and glucose levels. Culture, serology, and molecular tests may also be used to determine specific etiologies. These may be used alongside neuroimaging, EEG, and other tests to determine a diagnosis, treatment course, and evaluations.

Traditionally, culture and serology tests have been used to determine specific etiologies. For bacterial infection,
Gram staining and culture are part of the diagnostic algorithm and guidelines for determining an exact etiology of meningitis or encephalitis. A concentrated CSF Gram stain is a rapid test, but sensitivity can depend on the microorganism and bacterial load. For instance, sensitivity can be high for *S. pneumoniae* and Group B *Streptococcus* meningitis (60-90%) but is very low for *Listeria monocytogenes* meningitis (10-35%). CSF culture and blood culture are also traditionally utilized tests for bacterial meningitis, but suffer from poor sensitivity if the patient has had antibiotics up to 4 hours before sample collection. For viral infection, culture is no longer recommended for clinical diagnosis, although it is sometimes used for antiviral resistance testing or typing. Serology tests may be used in conjunction with other tests for both bacterial and viral infection to aid in diagnosis of the exact disease-causing organism.

Nucleic acid amplification tests (NAATs), including single or multiple target assays and panel approaches, have been on the rise for etiological identification and have the potential to improve the speed of diagnosis. If a virus is suspected, PCR testing is recommended in all cases to determine if the infectious agent is HSV 1, HSV 2, VZV, or enterovirus. Data suggests that NAATs perform similarly or better than traditional testing methods for bacteria and are capable of identifying tough to culture bacterial organisms.

DiaSorin Molecular provides the first and only FDA cleared stand alone molecular assays for HSV 1 & 2 and VZV for CSF samples using the LIAISON® MDX. The Simplexa™ Direct kits allow for the direct detection and differentiation of HSV 1/2 and VZV in about an hour. The assays are CLIA moderate complexity requiring 50 μl of CSF for HSV and 50 μl for VZV testing. Clinical studies of the Simplexa™ HSV 1 & 2 Direct assay and Simplexa™ VZV Direct assay were performed on all age groups, from birth to over 60 years old.

The FDA has also cleared a PCR-based panel test, which detects 14 targets including various bacterial, viral, and fungal targets. There have been many studies surrounding the usefulness and clinical utility of these types of panel tests. Although there are clear advantages to syndromic based panel testing for meningitis and encephalitis, including the quick identification of a wide variety of CNS infectious pathologies, studies are showing that currently available panels fail to reveal the etiology of the disease. Several studies have reported both false positive and false negatives that lead to costly and ineffective treatments, which ultimately lead to poor patient outcomes.

In one case, a false-positive HSV 1 result caused a delay in treatment of an immunocompromised patient ultimately leading to his poor neurologic recovery and long-term disability. In another case, a kidney transplant patient developed cryptococcal meningitis that was reported as negative during two tests causing needless treatment. There are also concerns about contamination, which can lead to false positives, especially when testing for organisms that can be found as normal oral flora in laboratory staff. Healthcare providers must be familiar with the limitations of this type of test, especially false positive, which can lead to needless treatments and delay life-saving therapy.

Extended hospital stays also increase the cost for the hospital. It is beneficial to both hospital and patient to decrease their length of stay through proper diagnosis and treatment. In one older 2002 study, direct variable costs were estimated at $450 per day for low-intensity neonatal intensive care, $502 per day for pediatric patients, and $609 per day for adult non-day of admission or non-day of discharge. Today, neonatal intensive care unit stays can cost up to $2,500 per day. More recent studies show that the mean hospital stay for a meningitis or encephalitis patient can range from over $15,000 per patient to over $40,000 depending on their length of stay and severity of disease. It is critical for hospitals to utilize the right tests to ensure proper diagnosis and patient management to improve patient care and reduce costs.

### Results Using Targeted PCR

In a study of 363 patients, the Simplexa™ HSV 1 & 2 Direct resulted in faster acyclovir discontinuation compared to a laboratory developed test (29.2 hours versus 14.3). The mean turnaround time also decreased by 14.5 hours and the median hospital length of stay was reduced by 20% after implementing the Simplexa™ test (Table 1).

Access to the test also increased as the laboratory was able to offer the Simplexa™ test 24 hours a day every day rather than only 9am-5pm for 6 days of the week. This is important because 45.3% of results using the Simplexa™ test were reported during the night shift, indicating a large patient population would have had to wait before receiving possible life-saving results. Lasty, STAT testing was able to be offered as tests could be run direct from sample immediately upon receipt, using the Simplexa™ procedure (Table 1).

Another study demonstrated that 6 months after implementing a targeted PCR assay they noticed a reduction in the length of stay for 12 pediatric patients. They projected a total decrease of about 55 hospital days for the first year of implementation, indicating up to $43,000 worth of savings (Table 1).
### Table 1

Results of studies conducted using targeted PCR assays.

<table>
<thead>
<tr>
<th>Metric</th>
<th>Result</th>
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<tbody>
<tr>
<td>Mean Turnaround Time&lt;sup&gt;18&lt;/sup&gt;</td>
<td>Decreased by 14.5 hours</td>
</tr>
<tr>
<td>Mean Time from Sample Collection to Acyclovir Discontinuation&lt;sup&gt;18&lt;/sup&gt;</td>
<td>Decreased by 17.1 hours</td>
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<tr>
<td>Median Hospital Length of Stay&lt;sup&gt;18&lt;/sup&gt;</td>
<td>Decreased by 20%</td>
</tr>
<tr>
<td>Test Availability&lt;sup&gt;18&lt;/sup&gt;</td>
<td>Able to offer the test 24 hours per day, 7 days a week</td>
</tr>
<tr>
<td>Test Procedure&lt;sup&gt;18&lt;/sup&gt;</td>
<td>Direct from sample, immediately upon receipt of sample</td>
</tr>
<tr>
<td>Projected Hospital Day Savings&lt;sup&gt;18&lt;/sup&gt;</td>
<td>−55.2 days</td>
</tr>
</tbody>
</table>

### Conclusions

Implementing targeted PCR for meningitis and encephalitis testing allows for significant gains clinically, operationally, and financially. Using the Simplexa™ HSV 1 & 2 Direct assay, turnaround time and time to acyclovir discontinuation significantly decreased. The easy workflow also allowed for the laboratory to offer the test 24 hours a day every day of the week and the ability to run STAT samples. This ultimately lead to better patient management and more effective treatment. Median hospital length of stay was also shown to decrease using targeted PCR methods, which leads to a reduction in healthcare cost. These results show that targeted PCR and the Simplexa™ kits offer an effective and efficient solution for labs to provide a rapid and accurate diagnosis, which is necessary for successful treatment of CNS infections.

### References


11. DiaSorin Molecular. HSV and VZV clinical study validations (data on file).


Your solution for meningitis and encephalitis testing

Ordering information – Simplexa™ Direct Kits

<table>
<thead>
<tr>
<th>CATALOG NUMBER</th>
<th>DESCRIPTION</th>
<th>REACTIONS/KIT</th>
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<tbody>
<tr>
<td>MOL2150</td>
<td>Simplexa™ HSV 1 &amp; 2 Direct Kit*</td>
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<tr>
<td>MOL2160</td>
<td>Simplexa™ HSV 1 &amp; 2 Positive Control Pack</td>
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<td>MOL3650</td>
<td>Simplexa™ VZV Direct Kit*</td>
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<tr>
<td>MOL3660</td>
<td>Simplexa™ VZV Positive Control Pack</td>
<td>10</td>
</tr>
</tbody>
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*Direct Amplification Discs are included with kit.

Contact DiaSorin Molecular today!
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