

VIASURE MULTIPLEX

H. influenzae, *N. meningitidis* & *S. pneumoniae*
Real Time PCR Detection Kit

Pathogen and product description

The most common causes of bacterial meningitis in adults are *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Haemophilus influenzae*. These organisms are spread from person to person by close contact with respiratory secretions. Once acquired, each species can colonize the mucosa of the nasopharynx and oropharynx, which is known as pharyngeal carriage. From there, they may cross the mucosa and enter the blood. Once in the blood, they can reach the meninges, causing meningitis, or other body sites causing other syndromes.

Haemophilus influenzae, a pleomorphic gram-negative coccobacillus. Is a common commensal microorganism of the upper respiratory tract. It is a human-only pathogen that can cause severe invasive disease, including meningitis, pneumonia, and septicemia. *H. influenzae* strains are divided based on the presence or absence of a polysaccharide capsule; there are 6 encapsulated serotypes (Hia-HIif) and nonencapsulated, nontypeable *H. influenzae* (NTHi) strains. Among them, Hib strains are considered the most prevalent.

Neisseria meningitidis may either be encapsulated or unencapsulated. However, nearly all invasive *N. meningitidis* organisms are encapsulated, or surrounded by a polysaccharide capsule. This capsular polysaccharide is used to classify *N. meningitidis* into 12 serogroups. Six of these serogroups cause the great majority of infections in people: A, B, C, W135, X, and Y. Meningitis epidemics are generally caused by serogroup A, although outbreaks have also been caused by serogroups C, W135, and X.

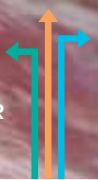
Streptococcus pneumoniae cause severe infections like meningitis, community-acquired pneumonia (CAP), bacteremia, bronchitis, sinusitis, and otitis media. More than 90 different serotypes of *S. pneumoniae* have been identified to date on the basis of the biochemical structure of the capsular polysaccharide that is a major virulence factor. The distribution of serotypes can vary with age, geography and time.

Cerebrospinal fluid culture is considered the diagnostic reference standard for bacterial meningitis, and bacterial isolation is important for antimicrobial susceptibility testing and molecular epidemiology. However, CSF culture requires at least a day or more, and has limited sensitivity. Real-Time Polymerase Chain Reaction (qPCR) of CSF and blood allows a rapid diagnostic test for bacterial meningitis, and amplification of DNA from non-viable bacteria could potentially facilitate diagnosis in culture negative cases. Using multiple analytic approaches, we found that qPCR assays on CSF and blood specimens were highly accurate for diagnosis of *S. pneumoniae*, *N. meningitidis*, and *H. influenzae* meningitis.

VIASURE *H. influenzae*, *N. meningitidis* & *S. pneumoniae* Real Time PCR Detection Kit is designed for the diagnosis of *H. influenzae*, *N. meningitidis* and/or *S. pneumoniae* in clinical samples. After DNA isolation, the identification of *H. influenzae*, *N. meningitidis* and *S. pneumoniae* is performed by the amplification of a conserved region of the *hdp* gene for *Haemophilus influenzae*, *lytA* and *piaA* genes for *Streptococcus pneumoniae* and *ctrA* gene for *Neisseria meningitidis*, using specific primers and a fluorescent-labelled probe.



THE REAL ONE STEP qPCR



Analytical sensitivity

VIASURE *H. influenzae, N. meningitidis & S. pneumoniae* Real Time PCR Detection Kit has a detection limit of ≥ 10 DNA copies per reaction for *H. influenzae, N. meningitidis* and *S. pneumoniae*. (figures 1, 2 and 3).

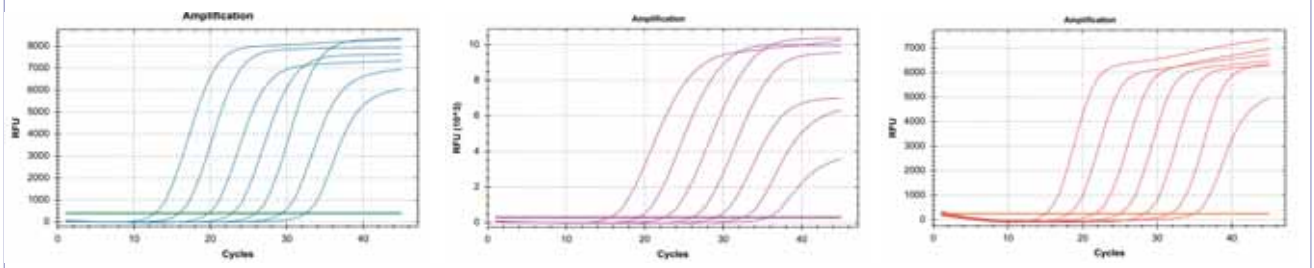


Figure 1. Dilution series of *H. influenzae* (10^7 – 10^1 copies/rxn) template run on the Bio-Rad CFX96™ Real-Time PCR Detection System (channel FAM).

Figure 2. Dilution series of *N. meningitidis* (10^7 – 10^1 copies/rxn) template run on the Bio-Rad CFX96™ Real-Time PCR Detection System (channel Cy5).

Figure 3. Dilution series of *S. pneumoniae* (10^7 – 10^1 copies/rxn) template run on the Bio-Rad CFX96™ Real-Time PCR Detection System (channel ROX).

Components

Reagent/Material	Description	Colour	Quantity
<i>H. influenzae, N. meningitidis & S. pneumoniae</i> 8-well strips	A mix of enzymes, primers-probes, buffer, dNTPs, stabilizers and Internal control in stabilized format	White	6/12 x 8-well strip
Rehydration Buffer	Solution to reconstitute the stabilized product	Blue	1 vial x 1,8 mL
<i>H. influenzae, N. meningitidis & S. pneumoniae</i> Positive Control	Non-infectious synthetic lyophilized cDNA	Red	1 vial
Negative Control	Non template control	Violet	1 vial x 1 mL
Water RNase/DNase free	RNase/DNase free water	White	1 vial x 1 mL
Tear-off 8-cap strips	Optical caps for sealing wells during thermal cycling	Transparent	6/12 x 8-cap strip

Kit References

Reference	Description
VS-HNS106L	VIASURE <i>H. influenzae, N. meningitidis & S. pneumoniae</i> Real Time PCR Detection Kit 6 x 8-well strips, low profile
VS-HNS106H	VIASURE <i>H. influenzae, N. meningitidis & S. pneumoniae</i> Real Time PCR Detection Kit 6 x 8-well strips, high profile
VS-HNS112L	VIASURE <i>H. influenzae, N. meningitidis & S. pneumoniae</i> Real Time PCR Detection Kit 12 x 8-well strips, low profile
VS-HNS112H	VIASURE <i>H. influenzae, N. meningitidis & S. pneumoniae</i> Real Time PCR Detection Kit 12 x 8-well strips, high profile
VS-HNS113L	VIASURE <i>H. influenzae, N. meningitidis & S. pneumoniae</i> Real Time PCR Detection Kit 96-well plate, low profile
VS-HNS113H	VIASURE <i>H. influenzae, N. meningitidis & S. pneumoniae</i> Real Time PCR Detection Kit 96-well plate, high profile

Work Flow

