# Syndromic Meningoencephalitis Panels – Multiplex PCR : the Limits of an Innovative Tool

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## Keywords
Meningitis ; meningoencephalitis ; multiplex PCR ; syndromic meningoencephalitis panel ; Haemophilus influenzae ; false positive.

## Abstract

The main challenge in a clinical presentation of meningoencephalitis lies in the early detection of pathogens in the cerebrospinal fluid. Owing to their rapid results and imperviousness to prior antibiotic use, syndromic panels using multiplex PCR have emerged as an innovative tool for the etiological diagnosis of meningoencephalitis.

However, this test presents limitations, as illustrated by two patient cases: one with questionable results and another strongly suggestive of likely false-positive result for *Haemophilus influenzae* on multiplex PCR.

This case report highlights the importance of interpreting the results provided by this technique by integrating the patient's clinical and biological data.

## Introduction

Central nervous system infections, although rare and of diminishing prevalence for bacterial etiologies, thanks to the introduction of conjugate vaccines into vaccination programs, remain a significant cause of morbidity and mortality in the pediatric population (1).

In high-income countries, the main responsible germs in the neonatal period are *Escherichia coli*, *Streptococcus agalactiae*, and, more rarely, *Listeria monocytogenes*. In older children, *Streptococcus pneumoniae* and *Neisseria meningitidis* remain the most frequently encountered pathogens (2). Finally, although historically responsible for a significant number of bacterial meningitis cases, *Haemophilus influenzae* type B has been rarely observed in Belgium since the introduction of vaccination against this strain in 1993 (3).

The recent advent of new diagnostic techniques using molecular biology, such as multiplex PCR syndromic panels for meningoencephalitis, has enabled significant progress in the management of patients suspected of bacterial meningitis.

Easier to use and significantly faster than conventional methods, with an average turnaround time of less than 2 hours (compared to 1 to 5 days for cultures), these tools simultaneously detect 90% of pathogens (bacteria, viruses, and fungi) involved in meningoencephalitis using only 200 µL of cerebrospinal fluid (CSF). This allows early treatment adaptations and helps to reduce the duration of antibiotic therapy when found negative for bacterial etiologies, compared to relying solely on conventional diagnostic methods (4,5). Moreover, prior use of antibiotics does not affect the panel's performance (unlike cultures), as it detects bacterial DNA rather than live bacteria (5).

Currently, two meningoencephalitis panels are available: BioFire® FilmArray® (FA) as from 2015 and, more recently, QIAstat-Dx® (QIA) as from 2022. The former detects 14 targets, including 6 bacterial (*E. coli K1, S. agalactiae, L. monocytogenes, S. pneumoniae, N. meningitidis,* and *H. influenzae*), 7 viral, and 1 fungal pathogen. The latter additionally detects *Mycoplasma pneumoniae* and *Streptococcus pyogenes* while omitting one viral target, totaling 15 targets.

While providing undeniable advantages, these new diagnostic tools demonstrated certain limitations in clinical practice, particularly in terms of diagnostic accuracy. Indeed, false-negative results have been reported, particularly for herpes simplex virus, as multiplex panels tend to be less sensitive than most singleplex PCR assays. In case of strong clinical suspicion of herpetic encephalitis, diagnostic sensitivity can be enhanced by employing singleplex assays and by repeating the PCR later in the disease course (4,6).

Moreover, false-positive results have also been observed. Through two clinical cases, we highlight the importance of interpreting positive multiplex PCR findings with caution.

## Case report

### Case 1

A 3-year-old boy, presented to the emergency department with headache, fever, vomiting, anorexia, rhinorrhea, apathy, irritability, and left otorrhea. History taking revealed prior hospitalizations for enteroviral meningitis at 3 months and severe rotavirus gastroenteritis at 18 months. He had received all recommended vaccinations. His vital signs were normal, and clinical examination identified a decline in general condition, neck stiffness, and bilateral acute otitis media, for which oral amoxicillin 80 mg/kg/day was initiated 24 hours prior.

Given this clinical picture, meningitis was suspected. Bloodwork revealed a severe inflammatory syndrome with neutrophilic leukocytosis: CRP 290 mg/L [reference: <5 mg/L], 16.75x10³/mm³ white blood cells [reference: 4-12x10³/mm³], including 14.9x10³/mm³ neutrophils [reference: 1.5-8.5x10³/mm³]. A small amount of CSF was collected via traumatic lumbar puncture, revealing 1654/mm³ red blood cells (with 4.18x106/mm³ in peripheral blood), 12 nucleated cells (reported to 5.37 after correction factor for contaminated CSF) [reference: <5], with negative direct examination and culture. CSF protein, glucose, and multiplex PCR could not be performed due to insufficient sample volume.

In the context of an unidentified etiology and persistent lack of clinical improvement, a second lumbar puncture was performed after three days of intravenous cefotaxime therapy. The multiplex PCR (FA panel) returned positive for *H. influenzae*. Cytology and chemistry results were normal, and direct examination was negative. Blood and CSF cultures returned negative. A diagnosis of *H. influenzae* meningitis was made in a fully vaccinated child. The patient had a favorable outcome with intravenous cefotaxime for 10 days. Subsequent immune testing was normal.

### Case 2

A 17-day-old female newborn, born at term with no notable history, presented with fever, nasal congestion, and blood-streaked diarrhea. Vital signs were normal except for T° 38.8°C [reference: 36.5-37.5°C]. Clinical examination was unremarkable. A complete infectious workup was performed due to her age. Blood analysis revealed a mild inflammatory syndrome (CRP 29 mg/L) and normal leukocytes with mixed formula. Nasopharyngeal aspiration was positive for SARS-CoV-2 and catheterized urine showed pyuria (336 white blood cells/µL, [reference: <13]), along with a positive culture for *Klebsiella pneumoniae* (> 100,000 CFU/mL). Blood cultures and stool analysis (culture and rotavirus/adenovirus antigens) were negative.

Lumbar puncture showed no pleocytosis, with normal glucose, protein, and lactate levels, along with negative direct examination and culture results (15 days of incubation). Surprisingly, the multiplex PCR (QIA panel) was positive for *H. influenzae*. A subsequent analysis on the same sample using the FA panel returned negative, suggesting a false positive for *H. influenzae* on the QIA panel.

## Discussion

While interpretation is challenging due to prior antibiotic use, our first case demonstrated discordant microbiological results warranting further analysis. This raised doubts about the positive result for *H. influenzae* on the FA panel.

If a false positive is considered, it's not an isolated case. Despite the FA panel's good sensitivity and specificity (90% and 97% respectively, per Tansarli and Chapin), false positives — mainly for *S. pneumoniae* and *S. agalactiae*, but also some for *H. influenzae* — have been reported (4). Zanella et al. identified an alarming likely false-positive rate for *H. influenzae* on FA (78% of tested cases). Most of positive cases for *H. influenzae* had alternative diagnoses, and only a third were treated as bacterial meningitis, reflecting discordance between positive panels and non-suggestive clinical presentations. Given careful sample handling and low false positives for *S. pneumoniae* (which in theory, could be a contaminant as often as *H. influenzae*), it likely stems from pre-analytical issues or analytical artifacts rather than contamination (7).

Additionally, FA assays target multiple genetic sequences per pathogen; for *H. influenzae*, two specific gene targets are typically analyzed. Concurrent positivity for both targets significantly increases the likelihood of a true positive result, given the low probability of simultaneous false positives. Conversely, detection of only one target should be interpreted with caution, as it may reflect a low-level or non-specific signal. Our patient's PCR was retrospectively found to be positive for both targets, suggesting a true-positive result nonetheless.

As mentioned, the child received appropriate antibiotic therapy despite initial diagnostic uncertainty. Indeed, he exhibited clinical signs consistent with meningitis, and inflammatory markers suggested a bacterial origin. Correction factors for CSF white blood cell counts are not sufficiently reliable to definitively exclude pleocytosis, and mild or even absent pleocytosis can occasionally be found in cases of bacterial meningitis (8). Considering this, and more broadly, even when the likelihood is low, the threshold for initiating appropriate treatment should remain low, as the consequences of untreated disease are more serious than the potential adverse effects of overtreatment.

Our second case leaves less room for doubt. Although clinical assessment may be more challenging due to the patient's young age, samples collected prior to any antibiotic administration showed no evidence of CSF infection. Moreover, two other infectious foci — SARS-CoV-2 (respiratory) and *K. pneumoniae* (urinary) — were identified. The QIA panel's result, discordant with other findings, was quickly considered as likely false positive by the negative FA panel's result. This limited the potential consequences of a false positive, such as unnecessary additional tests, prolonged antibiotic therapy, extended hospital stays, epidemiological overestimation, and undue concern.

Beyond PCR amplification curves, QIA offers enhanced analytical detail compared to FA, including cycle threshold (Ct) values, which provide useful insight when assessing whether a positive result reflects a true infection or a potential false positive. Retrospective analysis of our case revealed a normal amplification curve, but the elevated Ct value (36.2, i.e. very little target genetic material detected) suggested a likely false positive.

Also, we could have decided not to perform the panel test immediately, given its cost and the lack of supporting evidence for meningoencephalitis following the initial laboratory results. It could instead be carried out at a later stage and in a more targeted manner in cases where meningoencephalitis is genuinely suspected.

Finally, false positivity could have been confirmed through specific (singleplex) PCR, a test requiring technical expertise, but capable of accurately detecting a single bacterial pathogen (e.g., *H. influenzae*) and useful in discordant cases like ours.

## Conclusion

With the roll-out of syndromic panels as an additional tool for diagnosing bacterial meningitis, these cases highlight their limitations, particularly pertaining to false positives.

To minimize their impact, clinicians must carefully select candidates for multiplex PCR as well as understand and interpret the results it provides, including PCR amplification curves and cycle threshold (Ct) values if available, while integrating clinical context and other laboratory findings.

Consulting with experts in microbiology and infectious diseases is recommended for discordant or complex cases to ensure proper follow-up testing, including specific PCR, and to determine the best course of care for patients while balancing the potential consequences of misinterpreting a result as false.

The authors have no conflicts of interest to declare about the topic discussed in this manuscript.

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