Detection of Neisseria meningitidis From Negative Blood Cultures and Cerebrospinal Fluid

With the FilmArray Blood Culture Identification Panel

Joe Pardo,a Kenneth P. Klinker,a Samuel J. Borgert,a Brittany M. Butler,b Kenneth H. Rand,b
Nicole M. Iovineb#

Department of Pharmacy, UF Health Shands Hospital, Gainesville, FL, USAa;
Department of Pathology, University of Florida, Gainesville, FL, USAb

Running head: Molecular detection of N. meningitidis

#Corresponding authors:
Submission-related issues: Joe Pardo, PharmD, BCPS, pardoj@shands.ufl.edu, 352-231-3485
For the published article: Nicole M. Iovine, MD, iovinnm@medicine.ufl.edu
Abstract

The FilmArray® Blood Culture Identification (BCID) panel is a rapid molecular diagnostic test approved for use with positive blood culture material. We report a fatal case of meningococcemia with CNS involvement detected with the BCID from culture-negative blood and cerebrospinal fluid.
CASE REPORT

A 5-month-old previously healthy female infant was taken to the emergency department for evaluation of rash and fever. The patient was in her usual state of health until early morning the day of admission when she developed fever. The patient tolerated oral intake of milk and temporarily defervesced after one dose of acetaminophen. Within a few hours she awoke again with fever, but continued to tolerate oral intake and a second dose of acetaminophen.

She defervesced and went back to sleep. At approximately 07:00, the patient had a high fever and was irritable, prompting the parents to bring the patient to their pediatrician. At approximately 08:00, the pediatrician observed two small petechial lesions on the patient’s chest. The parents were instructed by the pediatrician to bring the child immediately to the emergency department.

Upon arrival to the emergency department, the petechial rash had spread over her trunk, and purpuric lesions developed on the patient’s back, trunk and extremities. There was strong suspicion for purpura fulminans secondary to meningococcemia. Ceftriaxone was administered intramuscularly (100 mg/kg) in the emergency department, approximately 1 hour prior to the collection of two sets of blood cultures and a urine specimen. Due to worsening hemodynamic instability, it was not possible to perform a lumbar tap. The patient was intubated and transferred to the pediatric intensive care unit with septic shock, metabolic acidosis, and disseminated intravascular coagulation (blood pressure = 83/46 mmHg, arterial pH = 7.24, serum lactate = 9.3 mmol/L, international normalized ratio = 3.4, fibrinogen = 55 mg/dL, d-dimer = 16.3 mg/L). Despite massive volume resuscitation, vasopressor and ventilator support, initiation of additional antimicrobial therapy (doxycycline, gentamicin, vancomycin and
a second dose of ceftriaxone), and extracorporeal membrane oxygenation, the patient
developed progressive hypotension and expired at 18:00 on the day of admission.

**Diagnostic Work-Up.** The blood specimens obtained in the emergency department
were collected in Bactec Peds Plus Medium bottles and sent for incubation in a Bactec 9240
continuously monitoring blood culture instrument (Becton Dickinson DIS, Sparks, MD). Blood
cultures remained negative after 6 days of incubation. On day 6, the bottles were removed
from the incubation system and sub-cultured onto chocolate and blood agar plates. No
bacterial growth was observed after 3 days.

Also on day 6, an aliquot of the negative blood culture fluid was tested on the
FilmArray® Blood Culture Identification (BCID) Panel (BioFire Diagnostics, Inc., Salt Lake City,
UT). The sample was processed in accordance with the BCID Instruction Booklet (1). Each BCID
test uses a closed-system disposable pouch that contains all reagents necessary to amplify and
detect nucleic acids from common bacterial and fungal pathogens. First, the freeze-dried
reagents were hydrated with sterile water. Next, 0.1mL of the blood culture medium was
mixed with extraction buffer provided by the manufacturer and approximately 0.3mL of
sample/buffer mixture was added to the BCID pouch and loaded into the FilmArray® platform
for processing. The BCID test indicated the presence of *N. meningitidis*, consistent with the
peripheral blood smear at autopsy which showed clusters of organisms with diplococcal
morphology under Wright-Giemsa stain.

A lumbar puncture was not performed before the patient expired. However, lumbar
and cisternal cerebrospinal fluid (CSF) specimens were collected during autopsy. Both
specimens were cultured but did not yield bacteria. Additionally, a sample of cisternal CSF was stored in an EDTA-containing tube in refrigerated conditions until the day the negative blood cultures were tested on the BCID. The CSF specimen was also tested on the BCID, with an identical procedure (except that 0.1mL of CSF was used in place of blood culture fluid). *N. meningitidis* was also detected in the patient’s CSF. CSF was also sent to the CDC, where *N. meningitidis* type B was confirmed by real-time polymerase chain reaction assays for *sodC* and serogroup-specific capsule genes (2, 3).

Pathogen identification is critical for the optimal management of infectious diseases. Traditional biochemical tests and cultures have progressed significantly over the past several decades and remain the standard of care for many diagnoses (4). However, rapid molecular tests are changing the landscape of diagnostic microbiology. These systems provide fast and accurate results, bridging some of the gaps that exist in traditional pathogen identification schemes. The FilmArray® BCID is a multiplexed polymerase chain reaction-based diagnostic test approved for use with positive blood culture material. From positive cultures, the BCID can detect the presence of 24 pathogens and 3 genes associated with antimicrobial resistance (Figure 1), in roughly 1 hour. Our case describes the off-label use of the BCID to provide diagnostic information that was not obtained through standard laboratory procedures.

Several factors are known to impair blood culture yield (4). For our patient, we suspect low blood volume collection (actual amount is unknown) and administration of antibiotics prior to blood collection contributed to blood culture negativity (4, 5, 6). Intramuscular ceftriaxone
reaches peak plasma concentrations in as little as 2 hours (7). Our patient likely had a significant concentration of ceftriaxone in their blood at the time samples were obtained, rendering the collected bacteria non-viable. This theory is supported by the fact that diplococcal organisms were visualized in a peripheral blood smear but did not grow on chocolate or blood agar plates following several days of incubation.

CSF cultures are also vulnerable to yield-blunting effects of antibiotic administration. The chance of CSF culture positivity drops significantly when lumbar puncture occurs greater than four hours after antibiotic administration and is highly unlikely when lumbar puncture occurs more than eight hours after antibiotic administration (8). Due to issues surrounding CSF culture, PCR-based methods have been evaluated for the diagnosis of meningitis. PCR amplification of the crgA gene from CSF specimens facilitated identification of \textit{N. meningitidis} in 66/81 (81%) meningitis cases when initial laboratory culture remained negative (9). The BCID has not been approved for use with CSF or other non-blood specimens. Our report suggests use of the BCID warrants further study in the scenario when antibiotics negate CSF culture positivity.

To our knowledge there are no clinical reports of off-label BCID use with negative blood culture material or non-blood specimens (i.e., CSF). Prior to Food and Drug Administration approval, the clinical performance of the BCID was evaluated in a multi-center clinical study that included 2207 positive blood cultures (1). The BCID detected \textit{N. meningitidis} with 100% sensitivity and specificity (36/36 isolates detected; 2171/2171 negatives called negative). In this study, the average concentration of \textit{N. meningitidis} in blood bottles at the time of positivity was $2.5 \times 10^8$ CFU/mL. Patients with meningococcemia often present with an extremely high
bacterial burden, which has been measured as high as $10^8$ CFU/mL in fulminant disease (10). The high organism load associated with severe meningococcemia may account for the ability of the BCID to detect *N. meningitidis* in our case. It is important to point out that most cases of meningococcemia have bacterial counts in the range of $10^4$ – $10^8$ CFU/mL (10) and with dilution into the 30 – 40 ml volume of Bactec blood culture bottles may not have high enough levels of bacterial DNA for detection. Furthermore, we deliberately waited until more than the standard 5 days of incubation had passed before entering the blood culture bottle and testing with the BCID. We strongly recommend against entering blood culture bottles before the end of their prescribed incubation time because of the risk of contamination. Finally, we do not recommend routinely testing negative blood cultures or CSF with the BCID unless further research is conducted.

Advances in molecular methods continue to change the landscape of diagnostic microbiology and challenge the practice of clinical microbiology. This report illustrates a case where off-label use of a multiplex PCR test, the FilmArray® BCID, aided in the diagnosis of meningococcal septicaemia and meningitis.
References


http://dx.doi.org/10.1093/cid/cit278


Figure 1. FilmArray BCID results report for the patient’s blood and CSF specimens.
### FilmArray BCID Panel

**Run Summary**

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Run Date</th>
<th>Controls</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. meningitides</td>
<td>Passed</td>
<td>Passed</td>
<td>Passed</td>
</tr>
</tbody>
</table>

**Antimicrobial Resistance Genes**

- **Gram Positive Bacteria**
  - Not Detected: *S. pneumoniae*, *Staphylococcus aureus*, *S. pyogenes*, *Group B Streptococcus*, *Streptococcus pneumoniae*
  - Detected: *Enterococcus faecalis*, *Enterococcus faecium*

- **Gram Negative Bacteria**
  - Not Detected: *Acinetobacter baumannii*, *Citrobacter freundii*
  - Detected: *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella flexneri*, *Salmonella enterica*, *Escherichia coli*

- **Yeasts**
  - Detected: *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Candida auris*, *Cryptococcus neoformans*