Case Report
Successful off-label use of the Cepheid Xpert GBS in a late-onset neonatal meningitis by Streptococcus agalactiae

Vincenzo Savini¹, Roberta Marrollo¹, Eleonora Coclite², Paola Fusilli², Carmine D’Incecco², Paolo Fazii¹

¹Clinical Microbiology and Virology, Spirito Santo Hospital, Pescara (Pe), Italy; ²Neonatal Intensive Care Unit and Neonatal Pathology, Spirito Santo Hospital, Pescara (PE), Italy

Received June 4, 2014; Accepted July 24, 2014; Epub July 15, 2014; Published August 1, 2014

Abstract: We report the case of a late-onset neonatal meningitis by Streptococcus agalactiae (group B Streptococcus - GBS) that was diagnosed with a latex agglutination assay (on cerebrospinal fluid, CSF), as well as by using, for the first time, Xpert GBS (Cepheid, US) on CSF. Due to empirical antibiotics given before sampling, both CSF and blood culture were negative, so the abovementioned diagnostics was crucial. Moreover, the Xpert GBS assay, performed according to an off-label, modified protocol (the system is designed for GBS-carriage intrapartum screening, based on a completely automated real time-Polymerase Chain Reaction) quickly detected the organism’s genome target. Although further investigation on this test’s performance on CSF is required, then, we trust it may be a promising, quick and precise diagnostic method for infections in newborns.

Keywords: Streptococcus agalactiae, GBS, Lancefield group B, pregnancy, gestation, meningitis, Cepheid, Xpert GBS

Short communication
A 45-day old male patient was taken to the Civic Hospital of Pescara, Italy, due to paracetamol-refractory fever (stably 39.5°C) and deep malaise that had begun 1 day before, at home. Negative urine dipstick, neutrophilia, along with elevated procalcitonin and C-reactive protein (PCR) suggested infectious meningitis. Although the infant had received intramuscular ceftriaxone at the time of fever onset, rachicentesis was performed, and two blood aliquots were introduced into BacT/Alert bottles (bio-Mérieux, France) for culture. Hypoglycorrachia, along with polymorphonuclear cells (> 5 cells/mm³) and Gram positive cocci in pairs and chains were observed at cerebrospinal fluid (CSF) analysis, suggesting a central nervous system (CNS) infection.

A CSF aliquot was suddenly inoculated and incubated aerobically onto Trypticase soy and Sabouraud agar (Liofilchem®, Italy), as well as Chocolate agar (Liofilchem®), under microaerophilic conditions. Given the abovementioned results and the patient’s age, Streptococcus agalactiae (Group B Streptococcus, GBS) disease was suspected; therefore, to improve diagnostic accuracy, we screened a CSF aliquot with the Xpert GBS system (Cepheid, US). This is a completely automated genome-based methodology that is designed for intrapartum GBS screening. For this purpose, rectovaginal swabs are directly introduced into specific cartridges that are placed into the instrument for processing. We made therefore an off-label use of the system, based on a modified procedure, by collecting CSF with a sterile swab, then placing the latter into the Xpert GBS cartridge. A further aliquot was screened with the Pastorex Meningitis latex assay (Bio-Rad) for detection of antigens of common CSF pathogens (Neisseria meningitidis A-C, Y and W135; Escherichia coli K1; Hemophilus influenzae type b; Streptococcus pneumoniae; GBS).

Interestingly, Xpert GBS detected GBS target by the 39th minute from starting test (Figure 1). Accordingly, the latex assay provided agglutination with GBS-specific antiserum. Unfortunately,
after 24-h incubation (and still after 4-day incubation), both CSF and blood cultures were negative, reasonably due to the antibiotic therapy that preceded sampling. Treatment was shifted to combined intravenous ceftriaxone and gentamicin that led to a slow clinical resolution.

During gestation, the infant’s mother underwent negative antepartum screening for GBS, although this was performed more than five weeks far from delivery. She received no intrapartum prophylaxis and, during a 48-h period observation after birth (that was full term), child clinical examination and PCR were normal, so he was dismissed.

Source for the infection remained therefore unclear, although breast milk might be indicted [1]. The latter may contain a number of bacteria, viruses and parasites (i.e. GBS, Listeria monocytogenes, Cytomegalovirus, Toxoplasma gondii) [2]. Nevertheless, GBS shows the ability to adhere to the newborn’s buccal epithelium, with adherence increasing with the decrease of the gestational age [3]; also, amniotic fluid has been known not to inhibit the abovementioned phenomenon that may therefore in part explain susceptibility of premature infants to GBS disease [3]. Consequently, it is nowadays debatable as to what came first, between the chicken and the egg, as source for GBS late-onset infections has not been fully explored yet [1] and children might indeed acquire the organism through the birth canal, get colonized in the oral cavity, then transmit the pathogen to the mother’s nipples via breast feeding, rather than vice versa [4].

Clinical picture of GBS meningitis is potentially serious, then timely diagnosis and prompt antibiotic approach are of paramount importance pending culture-based microbiology results [5]. CSF glucose evaluation and observation under a microscope may provide preliminary data, whereas organisms’ (if any) cultivation require 24-h incubation at least. Therefore, latex-based search for antigens of most commonly encountered CSF pathogens is a relatively quick and reliable test providing a first indication.

Nonetheless, agglutination tests may be laborious; for instance, the one we used requires that CSF be warmed up to 100°C, cooled at room temperature, centrifugated for 5 minutes to obtain surnatant; finally, ten minute agitation and observation are needed to detect agglutination, if any. A sufficient CSF quantity should be collected, moreover, as 40 µL surnatant must be dispensed in each of the nine reaction areas where sample and antisera are mixed.

Figure 1. Raising of the GBS curve, indicating test positivity, after about 25 RT-PCR cycles.
Xpert GBS is instead a genome-based methodology, that integrates sample lysis, nucleic acid purification and amplification, and final detection of the target sequence using real-time Polymerase Chain Reaction (RT-PCR); of interest, the whole procedure is completely automated, so neither manual DNA extraction nor, consequently, technically skilled personnel are required. The system consists of an instrument, a personal computer, and a preloaded software for running tests on collected samples and viewing results, and the only technical step needed is the insertion of the collected sample into a specific cartridge [5]. Single-use disposable Xpert cartridges are in fact used, that hold the PCR reagents and host the PCR process. Notably, as cartridges are self-contained, cross-contamination concerns are minimized.

Xpert GBS includes reagents for concomitant detection of the target GBS DNA, a sample-processing control (SPC) aiming to monitor processing conditions, and an internal control (IC) to monitor PCR conditions as well as the absence of reaction inhibition. The probe check feature checks reagent rehydration, PCR-tube filling in the cartridge, probe integrity, and dye stability. GBS primers and probe detect a target within a 3’ DNA region adjacent to the GBS cfb gene.

The system prepares the sample by eluting the material from the swab, mixing the sample reagent with the SPC (Bacillus globigii in the form of a bead within the cartridge) and the treatment reagent, capturing cell material on a filter, lysing cells, and eluting DNA. The DNA solution is then mixed with dry PCR reagents and transferred into the integrated reaction tube for RT-PCR and detection. Results are interpolated from measured fluorescent signals and embedded calculation algorithms, taking the whole process approximately 50 minutes or less.

We first used, based on published literature, Xpert GBS with CSF. Further studies are therefore needed to define performance of the methodology on this clinical fluid, but we trust it may represent a valid aid for neonatal diagnostics. Particularly, sensitivity and specificity in intrapartum screening have reached 98.5% and 99.6%, respectively, in part due to the complete automatization [5], so we might expect that future observations will confirm similar accuracy with CSF, as well.

Potential application of a quick, easy-to-perform, automated RT-PCR to CSF is intriguing, as CNS disease diagnostics must be fast and precise. It is finally remarkable that, in the described experience, Xpert GBS required a truly scant sample quantity to be used. The CSF aliquot processed, in fact, was simply taken with a sterile swab, and the latter introduced into the cartridge, without any previous supernatant collection.

As a main take-home message from this case, therefore, we would like to suggest that fast and reliable diagnostics of newborn CNS infections be supported by combined methods; particularly, observation under the microscope, CSF glucose evaluation, and latex assays targeting most common pathogens do provide preliminary, fundamental indications. Nonetheless, we feel Xpert GBS could be taken into account as a timely and reliable, RT-PCR-based diagnostic system in newborn diseases and, even if quite expensive; its accuracy might be life-saving for children, mostly when an empiric treatment may result in negative cultures. Particularly, we believe that, even in infants’ meningitis, Xpert GBS represents a quick, easy-to-perform assay providing accurate GBS detection and of course deserves, therefore, further trust in the next future as to its suitability for neonatal pathology.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Vincenzo Savini, Clinical Microbiology and Virology, Spirito Santo Hospital, Via Fonte Romana 8, CAP 65124, Pescara (Pe), Italy. Tel: 0039 340-7379737; E-mail: vincenzo_savini@libero.it

References

Cepheid Xpert GBS and neonatal meningitis

