

## Brief Communication

# Liofilchem® O.A. *Listeria* agar and direct CAMP test provided sooner *Listeria monocytogenes* identification from neonatal bacteremia

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**Abstract:** *Listeria monocytogenes* infection in pregnant women and newborns is a cause for serious concern, and invasive disease outcome strongly depends on prompt antibiotic therapy. To provide sooner identification from neonatal bacteremia we performed a CAMP test directly on positive blood aliquots and inoculated the Liofilchem® O.A. *Listeria* chromogenic agar as well, thus providing a 24-h turn-around time for response.

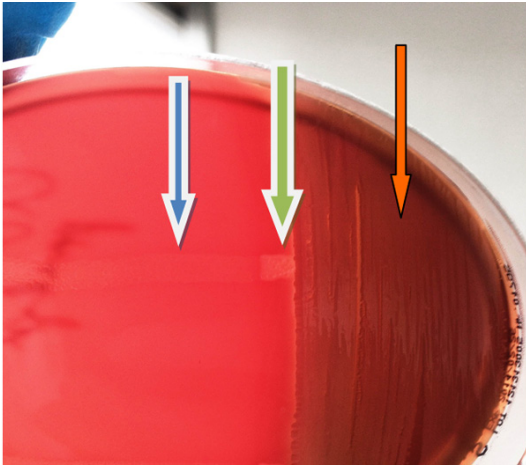
**Keywords:** *Listeria monocytogenes*, neonate, newborn, pregnancy, CAMP test, Liofilchem® O.A. *Listeria* agar

*Listeria monocytogenes* is a gram positive, facultatively aerobic, facultatively intracellular bacterium that is responsible for human and veterinary listeriosis [1, 3]. It is almost ubiquitous in soil, vegetables, silage and water, and inhabits the enteric tract of humans and animals [1]. The organism is therefore a foodborne pathogen [1] and, due to its ability to survive hostile conditions (wide pH range, elevated salt concentration, refrigeration), it represents a cause for concern in the food industry [3]. Disease includes cases and outbreaks of febrile enteritis in otherwise healthy subjects as well as invasive disease, mostly affecting pregnant women, neonates, the elderly, and patients with underlying degenerative disorders and/or T-cell-related immune system impairment [1, 3]. Clinically, meningitis and septicemia are mostly reported [1, 3]. Mortality rate is high (20-30% of cases), and neurological and psychiatric sequelae may be observed in survivors, that is up to 11% of neonates and 30% of adults who survive central nervous system infection [1, 3]. During pregnancy, particularly, cellular immunity is minimal owing to the increased progesterone levels, which make women significantly susceptible to intracellular

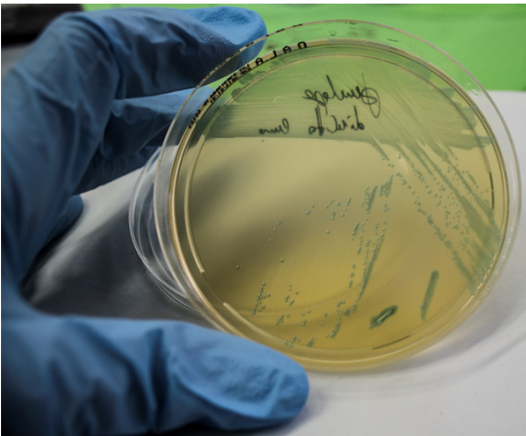
microorganisms [3]. Susceptibility to *L. monocytogenes* is particularly high in late gestation; listeriosis, in fact, mostly occurs in the third trimester, rarely in the second, exceptionally in the first, although early infection usually has a poorer prognosis for fetuses and generally results in miscarriage or stillbirth; vertical transmission, again, is frequent as the organism shows uterus and placenta tropism [3]. Among the 13 serotypes of *L. monocytogenes* described thus far, 1/2a, 1/2b and 4b are responsible for most of the human disease cases [3].

Affected women may be asymptomatic or suffer from a nonspecific syndrome (flu-like symptoms, headache, backache, muscular pain, vomiting and diarrhea, sore throat), so listeriosis is often ignored, thus resulting in chorioamnionitis, miscarriage, stillbirth, preterm delivery, maternal and newborn septicemia [3].

Early-onset neonatal listeriosis (called *granulomatosis infantiseptica*) is acquired *in utero* via transplacental transmission, it develops at a mean of 36h after birth and presents (probably owing to aspiration of infected amniotic fluid) with sepsis (81-88%), pneumonia or respiratory distress (38%), meningitis (24%); formation and



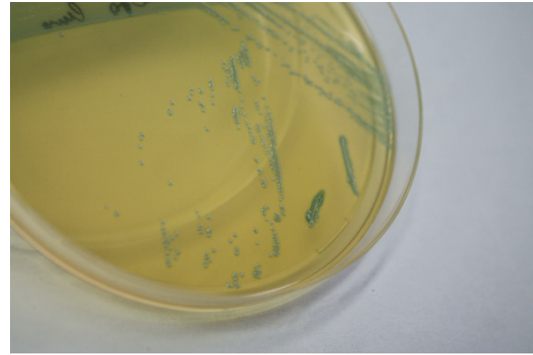
**Figure 1.** Direct CAMP test: orange arrow,  $\beta$ -hemolysin-producing *Staphylococcus pseudintermedius* isolate (tester strain); green arrow, zone of CAMP reaction (indicating test positivity); blue arrow, *L. monocytogenes*-containing blood inoculum (mild *L. monocytogenes* hemolysis is visible).



**Figure 2.** *Listeria monocytogenes* on the Liofilchem® O.A. *Listeria* agar (24h incubation).

dissemination of abscesses and granulomas in multiple organs may also occur and the mortality rate for infants born alive is around 20% (the frequency of abortion and stillbirth increases the overall mortality to more than 50%) [3].

The late-onset disease develops instead 2-3 weeks after delivery; infection, in this case, occurs by contamination through the birth canal, and presents as sepsis, meningitis or meningoencephalitis [3]. Mortality rate is about 10% and numerous surviving babies develop serious and chronic neurological sequelae, such as blindness and delayed mental development [3].



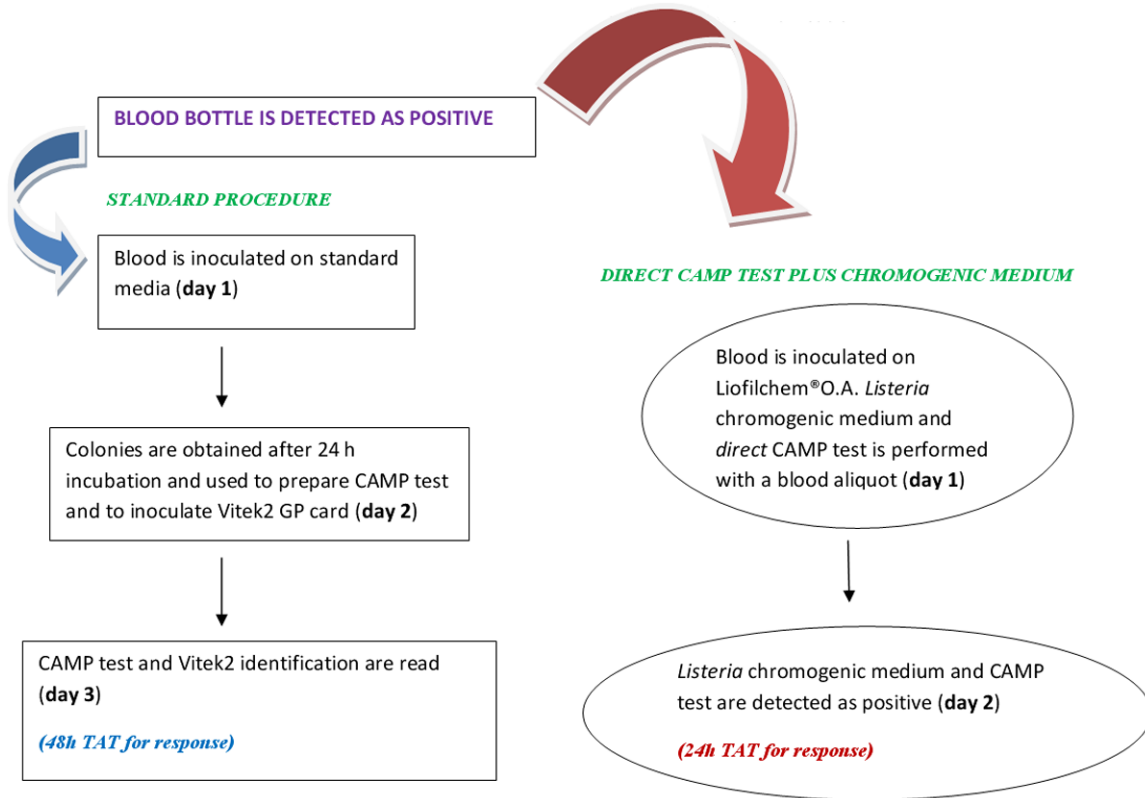
**Figure 3.** Particular from Figure 1.

Microscopic examination may fail in presumptively identifying *L. monocytogenes* from positive blood samples, as the pathogen may appear as Gram variable and pleomorphic, thus mimicking Gram negative bacteria as well as streptococci, enterococci, *Nocardia* and coryneforms [8]; again, expression of flagella is temperature-dependent in this species and, at mammalian host physiologic temperature (37°C), *L. monocytogenes* cannot produce them and it is therefore nonmotile, while at  $\leq 30^\circ\text{C}$  motility is observed, as flagellar gene transcription occurs [2].

Hence, usually, as soon as blood bottles positivity is detected, cultures on agar media are performed, and 24h-incubation colonies are used for biochemical identification (i.e. by the Vitek2 GP card, BioMérieux, France) and CAMP test [6]. Both assays are read after further 24 hours, then clinicians receive response with a 48-turn around time (TAT) from detection of blood positivity.

When managing two neonatal bacteremias occurred in the Pescara Civic Hospital, during 2013, we tried to shorten TAT for response by providing clinicians with a soon identification of *L. monocytogenes*, if present. Then, on the same day that blood samples were detected as positive (through the BacT/Alert system, BioMérieux) CAMP test was performed directly with blood aliquots; concomitantly, Trypticase Soy, Mac Conkey, Mannitol Salt and Sabouraud agar (plates by Liofilchem®, Italy) were inoculated as well as, additionally, the Liofilchem® O.A. *Listeria*. The latter is a chromogenic medium designed to detect the organism from food specimens. After 24h incubation, both CAMP tests were positive [6] (**Figure 1**),

## *Listeria monocytogenes* neonatal bacteremia



**Figure 4.** Left: example of standard diagnostic algorithm for neonatal bacteremia; right, alternative algorithm to shorten TAT.

and light-blue colonies (indicating *L. monocytogenes*, according to the manufacturer) were grown on the chromogenic medium (Figures 2, 3). Clinicians then received response with a 24h-TAT from blood bottle positivization, instead of the above 48h (Figure 4).

Identification was finally confirmed through Vitek2, along with sequencing of 16S rRNA and *hlyA* gene, using *L. monocytogenes* ATCC 7644 as reference strain. The isolates were stored into the laboratory bacterial collection under the accession numbers Lm317 and Lm338, and were found to belong to 1/2a and 1/2b serotypes, respectively (molecular characterization and serotyping were carried out at the Faculty of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, Italy).

First described several years ago, CAMP test still remains a reliable tool to identify *L. monocytogenes* [6, 7]. The assay, nevertheless, is time-consuming, as it is performed on 24h-incubation colonies and requires further 24h to be

read (total 48h from blood bottle positivization) [6]. Based on our experience, conversely, we emphasize that, to shorten TAT, CAMP test may be performed directly by inoculating the positive blood sample, instead of waiting for colony growth (occurring 24 hours later); a *Listeria* bacteremia, so, is diagnosed 1 day earlier (Figure 4).

Additionally, we made an *off-label* use of the O.A. *Listeria* medium. The latter, and *Listeria* chromogenic media in general, are in fact designed to be used with food samples [5, 9]; we got, nonetheless, a fast (24h TAT) and reliable result by using it with neonatal blood (Figure 4).

Our will to anticipate TAT for response (Figure 4) is based on the evidence that the outcome of listeriosis in pregnancy and newborns strongly depends on early administration of drugs with rapid and bactericidal activity (ampicillin alone, or combined to gentamicin, is the first choice treatment for neonates; second-line antibiotics are erythromycin, cotrimoxazole, vancomycin,

linezolid, fluoroquinolones, rifampin, chloramphenicol and carbapenems, resistance to which is low in clinical isolates) [3, 4].

As presentation of listeriosis in pregnant women may be aspecific, each symptom, even mild, and all febrile episodes during gestation should be referred and investigated through blood cultures [3]. It is then crucial that *Listeria* be promptly recognized, both from the pregnant woman and the neonate blood. For this purpose, CAMP test may be performed directly on positive blood samples, to promptly exclude or confirm *Listeria*. Additionally, using *Listeria* chromogenic media might not be limited to food microbiology only, but implemented in the clinical practice, as they are easy-to-interpret, cost-effective tools that may provide microbiologists and clinicians with a sooner response.

#### Disclosure of conflict of interest

None.

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#### References

- [1] Dos Reis CMF, Barbosa AV, Rusak LA, Vallim DC, Hofer E. Antimicrobial susceptibilities of *Listeria monocytogenes* human strains isolated from 1970 to 2008 in Brazil. *Rev Soc Bras Med Trop* 2011; 44: 173-176.
- [2] Lemon KP, Higgins DE, Kolter R. Flagellar motility is critical for *Listeria monocytogenes* biofilm formation. *J Bacteriol* 2007; 189: 4418-4424.
- [3] Mateus T, Silva J, Maia RL, Teixeira P. Listeriosis during pregnancy: a public health concern. *ISRN Obstet Gynecol* 2013; 2013: 851712.
- [4] Morvan A, Moubareck C, Leclercq A, Hervé-Bazin M, Bremont S, Lecuit M, Courvalin P, Le Monnier A. Antimicrobial resistance of *Listeria monocytogenes* strains isolated from humans in France. *Antimicrob Agents Chemother* 2010; 54: 2728-2731.
- [5] Park SH, Chang PS, Ryu S, Kang DH. Development of a novel selective and differential medium for the isolation of *Listeria monocytogenes*. *Appl Environ Microbiol* 2014; 80: 1020-5.
- [6] Savini V, Kosecka M, Marrolo R, Carretto E, Międzobrodzki J. CAMP-test detected *Staphylococcus delphini* ATCC 49172  $\beta$ -haemolysin production. *Pol J Microbiol* 2013; 62: 465-466.
- [7] Savini V, Marrolo R, D'Antonio M, D'Amario C, Fazii P, D'Antonio D. *Streptococcus agalactiae* vaginitis: nonhemolytic variant on the Liofilchem® Chromatic StreptoB. *Int J Clin Exp Pathol* 2013; 6: 1693-1695.
- [8] Stefanovic A, Reid J, Nadon AC, Grant J. Potential nosocomial acquisition of epidemic *Listeria monocytogenes* presenting as multiple brain abscesses resembling nocardiosis. *Can J Infect Dis Med Microbiol* 2010; 21: 57-60.
- [9] Stessl B, Luf W, Wagner M, Schoder D. Performance testing of six chromogenic ALOA-type media for the detection of *Listeria monocytogenes*. *J Appl Microbiol* 2009; 106: 651-659.