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## Isolation of *Leptospira* from blood culture bottles

Dominique Girault<sup>1</sup>, Marie-Estelle Soupé-Gilbert<sup>1</sup>, Sophie Geroult<sup>1</sup>, Julien Colot<sup>2</sup>, Cyrille Goarant<sup>1\*</sup>

<sup>1</sup> Institut Pasteur in New Caledonia, Institut Pasteur International Network, Leptospirosis Research and Expertise Unit, Noumea, New Caledonia

<sup>2</sup> Institut Pasteur in New Caledonia, Institut Pasteur International Network, Medical Microbiology Unit, Noumea, New Caledonia

\* Corresponding author

Cyrille Goarant.

Email: [cgoarant@pasteur.nc](mailto:cgoarant@pasteur.nc)

Tel: +687 277531

Institut Pasteur International Network, Institut Pasteur in New Caledonia, Leptospirosis Research and Expertise Unit, 9–11 Avenue Paul Doumer, BP 61, 98 845 Noumea, New Caledonia

### Abstract

With the increasing use of real time PCR techniques, *Leptospira* isolation has mostly been abandoned for the diagnosis of human leptospirosis. However, there is a great value of collecting *Leptospira* isolates to better understand the epidemiology of this complex zoonosis and to provide the researchers with different isolates. In this study, we have successfully isolated different *Leptospira* strains from BacT/Alert aerobic blood culture bottles and suggest that this privileged biological material offers an opportunity to isolate leptospires.

### Highlights

- *Leptospira* isolation is rare, yet isolates are needed for epidemiology and research
- Blood culture bottles provide a useful source for *Leptospira* isolation and culture
- BacT/Alert bottles allowed isolation of *Leptospira* over than 2 days from collection

### Note

Leptospirosis is a bacterial zoonosis increasingly recognized as emerging or re-emerging. Because of its multiple and non-specific clinical presentation, its medical diagnosis is especially challenging and

clinical suspicions warrant systematic biological confirmation (Goarant, 2016; World Health Organization & International Leptospirosis Society, 2003). This biological confirmation in the acute stage involves the evidence of pathogenic leptospires (or their products, most frequently DNA) in biological fluids like blood, cerebrospinal fluid or urine or the rise of a specific immune response at the convalescent (immune) stage (Levett, 2001). In acute specimens, *Leptospira* isolation from biological fluids has been considered the gold standard for decades. However, leptospires are fastidious and slow-growing organisms and cultures must be kept and checked for up to 14 weeks (Faine, Adler, Bolin, & Perolat, 1999), resulting in low sensitivity (Limmathurotsakul, et al., 2012) so that isolation is of limited use for medical diagnosis and treatment. Direct observation of spirochetes using dark field microscopy was used but is insensitive and possibly poorly specific, because of false positive results, so that this technique is not recommended (World Health Organization & International Leptospirosis Society, 2003). In contrast, the advent of molecular amplification techniques has revolutionized the biological diagnosis of acute leptospirosis; the use of conventional PCR (Merien, Amouriaux, Perolat, Baranton, & Saint Girons, 1992), then more recently real time PCR notably allows evidencing minute amounts of leptospiral DNA in a matter of hours. As a result, most clinicians do not request isolation anymore and most medical laboratories have more or less abandoned *Leptospira* culture and isolation. And yet, there is a great value of gaining access to bacterial isolates: they provide biological material for epidemiology (serological and/or molecular) as well as research (e.g. virulence, comparative genomics). As a paradigm example, they are currently the only biological material allowing serological typing at the serovar level.

Many severe leptospirosis cases present as septic shock and the differential diagnosis often includes bacterial septicemia, leading clinicians to collect blood cultures bottles. These bottles are sampled as early as possible, before the onset of antibiotics when possible, and special attention is paid to disinfection at the collection site. These culture bottles are then rapidly sent without refrigeration to the microbiology lab. In leptospirosis cases, they may provide a unique high quality biological sample.

In New Caledonia, leptospirosis is endemic with a high incidence; the medical community has a high level of awareness and leptospirosis is usually considered early in the differential diagnosis of acute fevers (Goarant, et al., 2009). In this study, we attempted to isolate pathogenic leptospires from blood culture bottles incubated at 37°C in a BacT/Alert® Microbial detection system, targeting aerobic bottles from patients with leptospirosis confirmed by a positive specific real time PCR. From 39 patients with a positive *Leptospira* real time PCR (Stoddard, Gee, Wilkins, McCaustland, & Hoffmaster, 2009), we collected 500µL from an aerobic blood culture bottle (BacT/Alert®SA for adults or BacT/Alert®PF with activated charcoal for children, BioMérieux, France) to inoculate a 5-mL R1 dilution EMJH supplemented with 5% decomplexed Fetal Bovine Serum (FBS); an additional ten-fold R2 dilution was made in a second tube. Both were incubated at 30°C with constant shaking. The blood bottles had been collected from less than one to almost 2.5 days (mean 1.4 days) before sub-sampling for *Leptospira* isolation. For 3 patients, whole blood from a total blood count was also used in parallel to attempt *Leptospira* isolation. We obtained 13 isolates after 4 to 30 days culture (mean 17.4 days) belonging to the two species (*Leptospira interrogans* and *Leptospira borgpetersenii*) involved in human cases in New Caledonia (Perez & Goarant, 2010). These isolates represented at least three different strains based on the identification of serogroups Icterohaemorrhagiae and Pyrogenes in *L. interrogans* isolates and Ballum in the *L. borgpetersenii* isolate. The results are summarized in Table 1.

We successfully isolated pathogenic *Leptospira* from aerobic blood culture bottles in one third of the patients investigated. *Leptospira* isolation is recognized to be insensitive when compared to molecular and other diagnostic methods (Balassiano, Vital-Brazil, & Pereira, 2012; de Abreu Fonseca,

et al., 2006; Limmathurotsakul, et al., 2012; Truccolo, Serais, Merien, & Perolat, 2001), with sensitivity as low as 3% being reported in some studies (Merien, Baranton, & Perolat, 1995). With our small study sample, we had a 33% isolation rate from blood culture bottles, in agreement with more traditional blood samples. The selection of samples from leptospirosis cases confirmed by real-time PCR as well as the rapid turnaround time probably account for this relatively high isolation success. Because of the sample used (blood culture bottles), it can be assumed that the clinical presentation suggested a possible bacterial septicemia and/or sepsis. Whether a similarly high isolation rate could be obtained from other clinical presentations remains to be determined.

Beside anecdotal evidence (Gelman, et al., 2002), there have been studies considering the use of blood culture bottles for the isolation or diagnostic of leptospirosis. Palmer et al. (Palmer & Zochowski, 2000) used *in vitro* cultures to evaluate the survival of various leptospires in different blood culture bottles and demonstrated viability up to 48 hours at 37°C. In another study, Griffith et al. (Griffith, et al., 2006) demonstrated the viability of leptospires in specialized mycobacterial blood culture bottles for as long as 14 days. This very interesting finding hardly applies to clinical settings, because mycobacterial infections are not a frequent differential diagnosis of leptospirosis. However, these studies used quite large inocula with  $10^5$  (Palmer & Zochowski, 2000) or  $10^3$  to  $10^7$  (Griffith, et al., 2006) *in vitro*-cultured leptospires / mL, which hardly reflects the *in vivo* condition in patients. In contrast in our study, the bacterial load estimated by quantitative PCR suggests that the blood culture bottles allowing *Leptospira* isolation contained  $10 - 10^5$  leptospires / mL. More recently, Dittrich and colleagues evaluated the use of blood cultures bottles for the molecular diagnosis of leptospirosis and concluded to a limited utility, mostly because of low sensitivity (Dittrich, et al., 2016). In our study, a limited number of patients could be evaluated but isolation was successful in some patients in spite of a low leptospiraemia.

Our series extended over more than 18 months, so that different batches of the culture bottles were used and supported *Leptospira* survival. However, there might be batch to batch differences in efficiencies. In addition, we have not been able to test other brands of blood culture systems, which might give different results, so that our results would not apply to all blood culture bottles from different brands.

We advocate that *Leptospira* isolation might be attempted from blood culture bottles at least until 2.5 days after collection. The specific attention paid to blood cultures (most frequently collected before antibiotic therapy, with a good disinfection at the collection site, sent at ambient temperature and as rapidly as possible to the microbiology laboratory) make these biological specimens a unique opportunity to successfully gain access to live leptospires for isolation. The use of this technique in other settings with a wider diversity of *Leptospira* strains would help evaluate this strategy for different *Leptospira* strains and serovars.

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Table 1: Successful *Leptospira* isolation from blood culture bottles

Leptospiraemia from blood qPCR (day since symptom onset)	Blood bottle (time since blood collection)	Days to positive EMJH culture	<i>Leptospira</i> isolate
5490 / mL (day 1)	BacT/Alert®SA (2.1 days)	16 (R1 first)	<i>L. interrogans</i> serogroup Icterohaemorrhagiae
601 / mL (day 1)	BacT/Alert®SA (2.3 days)	22 (R1 first) (60 days from whole blood)	<i>L. interrogans</i> serogroup Pyrogenes
965 / mL (day 3)	BacT/Alert®SA (2.1 days)	18 (R1 first) (negative from whole blood)	<i>L. interrogans</i> serogroup Icterohaemorrhagiae
613 / mL (day 3)	BacT/Alert®SA (2 days)	28 (R1 first)	<i>L. interrogans</i> serogroup Icterohaemorrhagiae
1360 / mL (day 3)	BacT/Alert®SA (0.7 day)	30 (R1 first)	<i>L. borgpetersenii</i> serogroup Ballum
2880 / mL (day 3)	BacT/Alert®SA (1.9 days)	10 (R1 & R2)	<i>L. interrogans</i> serogroup Icterohaemorrhagiae
9520 / mL (day 2)	BacT/Alert®SA (0.8 day)	9 (R1 first)	<i>L. interrogans</i> serogroup Pyrogenes
14 / mL (day 4)	BacT/Alert®PF (0.9 day)	18 (R1 & R2)	<i>L. interrogans</i> serogroup Pyrogenes
1133 / mL (day 2)	BacT/Alert®PF (1.6 days)	10 (R2 first)	<i>L. interrogans</i> serogroup Icterohaemorrhagiae
49100 / mL (day 3)	BacT/Alert®SA (1.2 day)	4 (R1 & R2)	<i>L. interrogans</i> serogroup Icterohaemorrhagiae
472 / mL (unknown)	BacT/Alert®SA (0.3 day)	21 (R1 & R2)	<i>L. interrogans</i> serogroup Icterohaemorrhagiae
51900 / mL (unknown)	BacT/Alert®SA (0.7 day)	28 (R2 only)	<i>L. interrogans</i> serogroup Icterohaemorrhagiae
243 / mL (day 5)	BacT/Alert®SA (1.6 days)	12 (R1 & R2)	<i>L. interrogans</i> serogroup Icterohaemorrhagiae

## References:

- Balassiano, IT, Vital-Brazil, JM, & Pereira, MM (2012) Leptospirosis diagnosis by immunocapture polymerase chain reaction: a new tool for early diagnosis and epidemiologic surveillance. *Diagn Microbiol Infect Dis*, 74: 11-15.
- de Abreu Fonseca, C, Teixeira de Freitas, VL, Caló Romero, E, Spinosa, C, Arroyo Sanches, MC, da Silva, MV, & Shikanai-Yasuda, MA (2006) Polymerase chain reaction in comparison with serological tests for early diagnosis of human leptospirosis. *Trop Med Int Health*, 11: 1699-1707.
- Dittrich, S, Rudgard, WE, Woods, KL, Silisouk, J, Phuklia, W, Davong, V, Vongsouvath, M, Phommason, K, Rattanavong, S, Knappik, M, Craig, SB, Weier, SL, Tulsiani, SM, Dance, DA, & Newton, PN (2016) The Utility of Blood Culture Fluid for the Molecular Diagnosis of *Leptospira*: A Prospective Evaluation. *The American journal of tropical medicine and hygiene*, 94: 736-740.

- Faine, S, Adler, B, Bolin, C, & Perolat, P (1999) *Leptospira and Leptospirosis, Second Edition*. Melbourne, Australia: MedSci, Melbourne, Vic. Australia.
- Gelman, SS, Gundlapalli, AV, Hale, D, Croft, A, Hindiyyeh, M, & Carroll, KC (2002) Spotting the spirochete: rapid diagnosis of leptospirosis in two returned travelers. *Journal of travel medicine*, 9: 165-167.
- Goarant, C (2016) Leptospirosis: risk factors and management challenges in developing countries. *Res Rep Trop Med*, 7: 49-62.
- Goarant, C, Laumond-Barney, S, Perez, J, Vernel-Pauillac, F, Chanteau, S, & Guigon, A (2009) Outbreak of leptospirosis in New Caledonia: diagnosis issues and burden of disease. *Trop Med Int Health*, 14: 926-929.
- Griffith, ME, Horvath, LL, Mika, WV, Hawley, JS, Moon, JE, Hospenthal, DR, & Murray, CK (2006) Viability of *Leptospira* in BacT/ALERT MB media. *Diagn Microbiol Infect Dis*, 54: 263-266.
- Levett, PN (2001) Leptospirosis. *Clin Microbiol Rev*, 14: 296-326.
- Limmathurotsakul, D, Turner, EL, Wuthiekanun, V, Thaipadungpanit, J, Suputtamongkol, Y, Chierakul, W, Smythe, LD, Day, NP, Cooper, B, & Peacock, SJ (2012) Fool's gold: why imperfect reference tests are undermining the evaluation of novel diagnostics. A re-evaluation of five diagnostic tests for leptospirosis. *Clin Infect Dis*, 55: 322-331.
- Merien, F, Amouriaux, P, Perolat, P, Baranton, G, & Saint Girons, I (1992) Polymerase chain reaction for detection of *Leptospira* spp. in clinical samples. *J Clin Microbiol*, 30: 2219-2224.
- Merien, F, Baranton, G, & Perolat, P (1995) Comparison of Polymerase Chain Reaction with Microagglutination Test and culture for diagnosis of Leptospirosis. *J Infect Dis*, 172: 281-285.
- Palmer, MF, & Zochowski, WJ (2000) Survival of leptospires in commercial blood culture systems revisited. *Journal of clinical pathology*, 53: 713-714.
- Perez, J, & Goarant, C (2010) Rapid *Leptospira* identification by direct sequencing of the diagnostic PCR products in New Caledonia. *BMC microbiology*, 10: 325.
- Stoddard, RA, Gee, JE, Wilkins, PP, McCaustland, K, & Hoffmaster, AR (2009) Detection of pathogenic *Leptospira* spp. through TaqMan polymerase chain reaction targeting the LipL32 gene. *Diagn Microbiol Infect Dis*, 64: 247-255.
- Truccolo, J, Serais, O, Merien, F, & Perolat, P (2001) Following the course of human leptospirosis: evidence of a critical threshold for the vital prognosis using a quantitative PCR assay. *FEMS Microbiol Lett*, 204: 317-321.
- World Health Organization, WHO, & International Leptospirosis Society, ILS (2003) *Human Leptospirosis: guidance for diagnosis, surveillance and control*: World Health Organization.