

Survival of leptospire in commercial blood culture systems revisited

M F Palmer, W J Zochowski

Abstract

Aim—To assess the ability of commercial blood culture systems to maintain leptospire.

Methods—Nine different commercial blood culture bottles were compared for their ability to maintain four leptospiral strains at two temperatures, 30°C and 37°C. Bottles were subcultured at 48 hours, and one, two, three, and four week intervals and examined microscopically for the presence of viable leptospire.

Results—The results were comparable with those of an earlier study, which showed that different commercial blood culture systems varied in their ability to maintain leptospire.

Conclusions—No single factor appears to influence the viability of leptospire in blood culture systems. In general, the combination of an aerobic blood culture and an incubation temperature of 30°C enhances the viability of leptospire, and hence would increase the chances of their subsequent isolation from suspected cases of leptospiraemia.

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In human and animal infections leptospiraemia is considered to be of short duration. Normally organisms are only found in the blood during the first eight days of illness.¹ Therefore, the time that a blood culture is taken after the development of symptoms in a suspected case of leptospirosis is an important factor in the subsequent isolation of that organism. Similarly, the initiation of appropriate chemotherapy will also influence the isolation rate. In addition, a study in 1984 revealed large differences in the ability of commercial blood culture systems to maintain various strains of leptospire.² It is important to determine the suitability of these commercial blood culture systems to maintaining leptospire because no other maintenance or culture media are

available in routine laboratories in the UK for the isolation of the organism from suspected cases of leptospiraemia. Therefore, all suspected cases of leptospirosis where culture is attempted are referred to the reference facility. The most readily available clinical sample is usually a blood culture; therefore, we need to know whether the blood culture sent to the reference laboratory is able to maintain leptospire to enhance the chances of their isolation using conventional culture methods. The introduction of automated blood culture systems into most microbiology laboratories has had a major influence on the investigation of suspected cases of bacteraemia and septicaemia. Therefore, we thought it appropriate to investigate the survival times of leptospiral strains in some of these "newer" blood culture systems, and compare them with some of those examined in the previous study.

Methods

Four different leptospiral strains were incorporated into the study: *Leptospira interrogans* serovars: *icterohaemorrhagiae*, *hardjo*, *bataviae* and *grippityphosa*. Serovars *icterohaemorrhagiae* and *hardjo* were chosen because they are the most common serovars causing human infection in the UK. Serovar *bataviae* is found mainly in Asia and serovar *grippityphosa* in mainland Europe.³

Each serovar was inoculated into Johnson and Harris's modification of Ellinghausen and McCullough's medium (EMJH)¹ and incubated at 30°C for seven days. Each culture was checked for purity by culturing on blood agar at 37°C and 30°C, and the numbers of leptospire/ml of culture medium were estimated using darkground microscopy and a Thoma counting chamber.

Blood from healthy seronegative adults was added aseptically to each blood culture bottle according to the manufacturer's recommendation (table 1). Each blood culture bottle was aseptically inoculated with the appropriate leptospiral strain, giving a final concentration of approximately 10⁵ leptospire/ml of blood culture. Appropriate controls were included to monitor the viability of each strain throughout our study.

Blood cultures were incubated at 37°C, simulating the conditions found in most routine microbiology laboratories, and also at 30°C, the optimum temperature for the growth of pathogenic leptospire.

One drop (50 µl) of blood from each blood culture system was aseptically removed and inoculated in duplicate into 3 ml of leptospira isolation media (EMJH plus 2% rabbit serum

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Table 1 Blood culture media examined

Manufacturer	Medium	Volume	Code
Southern Group Laboratories	Glucose broth	50 ml	1505C
	"Signal" blood culture system	80 ml	BC102
BioMerieux*	"Vital" aerobic	50 ml	52511
	"Vital" anaerobic	50 ml	52512
Becton and Dickinson	BACTEC aerobic/F	40 ml	4402190
	BACTEC anaerobic/F	40 ml	4402191
Organon Teknika*	BacT/Alert aerobic	40 ml	52968
	BacT/Alert anaerobic	40 ml	59269
	Pedi-Bact aerobic	20 ml	59502

*Newer blood culture systems.

Table 2 Duration of viability of *Leptospira interrogans* strains in aerobic blood culture systems

Serovar	Vital		Signal		Southern Group		BacT/Alert		Pedi-Bact		BACTEC	
	30°	37°	30°	37°	30°	37°	30°	37°	30°	37°	30°	37°
<i>L. icterohaemorrhagiae</i>	4 wk	48 hr	48 hr	48 hr	7 wk	48 hr	1 wk	48 hr	1 wk	48 hr	3 wk	1 wk
<i>L. hardjo</i>	4 wk	48 hr	48 hr	48 hr	7 wk	48 hr	1 wk	–	1 wk	–	3 wk	1 wk
<i>L. bataviae</i>	3 wk	48 hr	48 hr	–	6 wk	48 hr	–	–	48 hr	48 hr	3 wk	48 hr
<i>L. grippityphosa</i>	4 wk	48 hr	48 hr	–	7 wk	48 hr	1 wk	–	1 wk	48 hr	3 wk	1 wk

48 hr refers to ≥ 2 days but < 7 days.

–, No growth at 48 hr; hr, hours; wk, weeks.

and 0.1% agar).⁵ A further four serial dilutions were made from each of the original dilutions in isolation medium and all the bottles were incubated at 30°C. These were then examined microscopically under dark ground illumination at weekly intervals for six weeks for the presence of viable leptospires.

The process of subculturing from the original blood culture systems was performed at 48 hours, and one, two, three, and four week intervals. Any blood culture still positive after four weeks incubation were incubated further and subcultured until becoming negative.

Results

Our results indicate that there is still a variation between different commercial blood culture systems in their ability to maintain leptospires. Of the “newer” systems, the Vital aerobic and anaerobic systems compared favourably against some of the other media, particularly when the bottles were incubated at 30°C (tables 2 and 3). The Southern Group glucose broth also gave good viability at 30°C, confirming the results of the 1983 study.² Overall, most systems, particularly the aerobic bottles, gave enhanced viability times at 30°C compared with identical bottles that were incubated at 37°C.

Discussion

The variation in the ability of commercial blood systems to maintain leptospires is likely to be the result of several factors.

Leptospires are chemo-organotrophic, using long chain fatty acids as their main source of carbon, and inorganic ammonium salts as their

major source of nitrogen. Therefore, the exacting chemical metabolic requirements of leptospires might account for the failure of some systems to maintain them. Variation in chemical formulation between some of the systems might affect their ability to maintain leptospires. As suggested in previous studies, even when systems have similar components there may be variations in some of the commercial peptones that make up these formulations.

It has been shown that the temperature of incubation is an important factor in the successful isolation of leptospires from blood culture systems. Those bottles incubated at 30°C clearly showed improved maintenance times compared with those incubated at 37°C.

Another factor may be that, with the exception of the BacT/Alert aerobic and Pedi-Bact bottles, the viability studies were carried out on unvented preflushed bottles. Previous investigations have shown that venting of blood culture bottles can improve the isolation of some organisms.⁶ In addition, the preflushing of bottles, such as anaerobic blood culture bottles, with carbon dioxide and nitrogen appeared to be detrimental to the viability of leptospires in most blood culture systems tested in our study.

Conclusion

In general, the use of an aerobic blood culture bottle and an incubation temperature of 30°C significantly increases the chances of successful isolation from cases of suspected leptospiroemia.

Table 3 Duration of viability of *Leptospira interrogans* strains in anaerobic blood culture systems

Serovar	Vital		BacT/Alert		BACTEC	
	30°	37°	30°	37°	30°	37°
<i>L. icterohaemorrhagiae</i>	4 wk	48 hr	–	–	–	–
<i>L. hardjo</i>	4 wk	48 hr	–	–	–	–
<i>L. bataviae</i>	4 wk	48 hr	–	–	–	–
<i>L. grippityphosa</i>	4 wk	48 hr	–	–	–	–

–, No growth at 48 hr; hr, hours; wk, weeks.

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