JOURNAL OF CLINICAL MICROBIOLOGY, July 1979, p. 114-115
0095-1137/79/07-0114/02$02.00/0

Vol. 10, No. 1

Rapid Isolation of *Legionella pneumophila* from Seeded Donor Blood

GORDON L. DORN* AND WAYNE R. BARNES

Wadley Institutes of Molecular Medicine, Dallas, Texas 75235

Received for publication 24 April 1979

The laboratory isolation of *Legionella pneumophila* from seeded donor blood, using the lysis-centrifugation technique, is described. Time to pure culture isolate was 3 to 4 days.

The clinical significance of *Legionella pneumophila* as it relates to documented outbreaks of various forms of pneumonic illness was recently described by several researchers (4). This unusual microorganism has also been suspected as the etiological agent of a number of unresolved cases of respiratory illness (6). Although some cases resolve uneventfully, a 15 to 20% mortality is not uncommon (1). The diagnosis and subsequent therapy of Legionnaires disease are presently made on the basis of indirect fluorescent antibody tests and rising serological titers, since the microorganism cannot be isolated by conventional laboratory procedures. Positive blood cultures have not been reported, and, in addition, it is not known at this time if the microorganism disseminates into the blood stream during a progressive infection.

It has recently been shown that this microorganism can be successfully grown on primary isolation medium, namely, Mueller-Hinton agar supplemented with 1% IsoVitaleX and 1% hemoglobin (MH-IH) (1). Furthermore, it has been suggested that an effort be made to develop a blood culture medium for this organism (5). In the present communication, we report the successful application of a lysis-centrifugation blood culture technique (3) in combination with MH-IH for the rapid detection of *L. pneumophila* strain Philadelphia-1 from freshly drawn donor blood.

It has previously been reported that between 25 and 35% of all positive blood specimens contain $\geq$10 microorganisms per ml of sample (2). To date there is no information on the number of *L. pneumophila* present in the blood of patients with Legionnaires disease. Since this disease is associated with pneumonia, it is anticipated that moderate numbers of the bacteria could be present during the acute phase of the disease. To more closely simulate a moderate in vivo septicemia and also to provide reliable quantitative results, approximately 130 colony-forming units per 7 ml (19 colony-forming units per ml) of donor blood were introduced into each of six centrifugation vehicles per experiment. Concomitantly, approximately 130 colony-forming units per 10 ml of blood was equally divided into two 50-ml vented Becton-Dickinson blood culture tubes containing 0.03% sodium polyanethanolsulfonate and supplemented peptone broth II. The Becton-Dickinson tube was selected as the broth control because, according to the manufacturer's specification, it contains an enriched peptone source and an adequate level of L-cysteine, an essential amino acid for the growth of *L. pneumophila* (5). Blood culture bottles were incubated at 35°C, examined daily for turbidity, and subcultured every 48 h through day 10 onto MH-IH media (5). MH-IH agar was chosen to grow the microorganism because individual colonies appeared sooner and were easier to count than on the recently described F-G agar (5). All subculture plates were incubated at 35°C in a candle extinction jar for a total of 6 days.

Laboratory-simulated septicemia employing the centrifugation tube was performed as previously described (3). After the seeded blood was aseptically injected into the tube, the tube was placed in the centrifuge, angle stopper down with the angular plane facing toward the center of the rotor. The centrifugation vessels were spun for 30 min at 3,000 $\times$ *g* in a Beckman TJ6 centrifuge (TA-10 rotor). After centrifugation, a sterile cotton-plugged vent needle was inserted in the top stopper. A 10-ml syringe was fitted with a 21 gauge, 1.5-inch (ca. 38.1-mm) needle. This needle was inserted through the bottom of the angle stopper to its upper limit, and the majority of the supernatant (~7 ml) was slowly removed and discarded. The remaining 1.5 ml of solution was vigorously mixed and removed with a 3-ml syringe. The contents within this syringe were equally dispensed onto five plates of MH-IH and incubated as described above. Plates were examined daily for growth, and colonies were counted when they reached approximately

1 to 2 mm in diameter. For both the broth and centrifugation processes, the *L. pneumophila* was in contact with the blood for a maximum of 20 min prior to introduction into the respective containers. All experiments were performed in accordance with strict safety protocols advised by the Center for Disease Control, Atlanta, Ga.

Table 1 shows that the bacterium could be efficiently recovered with the lysis-centrifugation technique using freshly drawn blood (average recovery of 75.5 ± 14.5%). An overall survival index (*K*) of 0.79 ± 0.17 reflects that little toxicity was observed with the present vehicle and process for the microorganism tested (3). With this technique, the time to positive was approximately 3 days, although accurate enumeration was not possible until day 4. The microorganism could not be isolated from the blood culture bottles containing the same inoculum load as that used in the reconstruction experiments. During the routine examination of the blood culture bottles, no turbidity was observed, even after a 10-day incubation.

If *L. pneumophila* disseminates into the blood stream during an active infection, the lysis-centrifugation technique offers the clinical laboratory a potentially simple and efficient way in which to isolate the organism from a blood sample. Efforts are currently being made to obtain blood samples from patients suspected of having Legionnaires disease.

TABLE 1. *Reconstruction data using L. pneumophila Philadelphia-1*

No. of tubes	Blood	Avg. no. of colony-forming units per tube	% Recovery	*K* factor	Day positive
6	Fresh	156	71.6 ± 9.4	0.75 ± 0.10	3.0
6	Fresh	120	76.8 ± 16.3	0.83 ± 0.27	3.0
6	Fresh	98	78.1 ± 17.4	0.79 ± 0.18	2.5

This research was supported by the Wadley Guild Research Fund, Leukemia Association of North Central Texas, Inc., and by a donation from Olive Ruth Jester.

We thank the Center for Disease Control, Atlanta, Ga., for contributing *L. pneumophila* Philadelphia-1 for this study.

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