Modified cold Z-N staining for presumptive identification of \textit{Brucella}

Pankaj A. Joshi, R.D. Kulkarni & R.M. Powar*

\textit{Department of Microbiology, Government Medical College, Miraj, India}

Received April 13, 2004

\textbf{Brucellosis} is one of the most common zoonotic diseases in India. Growth of \textit{Brucella} is slow and needs meticulous biochemical tests and antisera for confirmation of the isolates. In the present study modified cold ZN staining was tried on the broth cultures for early presumptive identification of \textit{Brucella} growth. Blood cultures were attempted in 22 seropositive patients. In the five blood cultures positive for \textit{Brucella}, acid-fast coccobacilli were seen in broth smears stained with modified cold ZN stain, thus providing presumptive identification of \textit{Brucella} growth. Acid-fast bacteria were not seen in the broth smears of the remaining 17 broths negative for \textit{Brucella} growth. The method is simple, reliable and reproducible and needs to be evaluated further on a larger sample.

\textbf{Key words} Blood culture - \textit{Brucella} - ZN staining

\textbf{Brucellosis} is an important zoonotic disease in India. Although it is not considered to be so common, but in fact should be given due consideration in an agrarian country like India. The incidence reported in different studies varies from 1 to 20 per cent in patients with pyrexia of unknown origin (PUO)\textsuperscript{1-5}.

The disease is often missed in its early stages due to its protean manifestations, lack of suspicion and lack of facilities for establishing the diagnosis in most laboratories. An absolutely certain diagnosis is made only when the causative organism is isolated on culture. But culture is not routinely sought by clinicians mainly due to the time required to obtain the culture report which varies from one to four weeks as \textit{Brucella} grows very slowly \textit{in vitro}. One has, therefore, to depend on serology for the diagnosis of the disease. However, serological diagnosis has certain limitations that should be kept in mind while interpreting the results like presence of \textit{Brucella} antibodies in normal individuals and the cross-reactivity with other organisms. Though significantly high titres indicate an acute infection, rise in titres of antibodies in serum samples collected one week apart is diagnostic. This takes almost the same time as required for culture, which in most cases can be

*Present address: Professor & Head, Department of Microbiology, Govt. Medical College, Nagpur
obtained within a week. So culture remains the Gold standard for the diagnosis. Although many new diagnostic tests like automated blood culture systems and molecular methods like polymerase chain reaction (PCR), nucleic acid probes etc., have been developed for the diagnosis of brucellosis, they are quite expensive and need sophisticated equipment and skilled expertise. As this is not possible for all the laboratories, simple, cost effective, reliable and reproducible methods need to be developed and tested.

The major drawback of culture is that it is time consuming. We have tried to overcome this drawback by using a simple and inexpensive method i.e., a modified cold ZN staining for detecting the growth of Brucella in blood culture broths. This method has been used for demonstrating Brucella in certain animal tissues especially aborted materials. This method provides a presumptive indication of Brucella growth at least four days earlier than the conventional identification method.

A total of 325 serum samples from PUO cases received in the laboratory of Department of Microbiology, Government Medical College, Miraj, from January to December 2001 for Widal test were screened simultaneously for presence of Brucella antibodies by slide agglutination test. The samples showing presence of Brucella antibodies by slide test were further tested by tube agglutination test to determine the titres. The antigens used in both the tests were obtained from Indian Veterinary Research Institute (IVRI), Izatnagar, Uttar Pradesh. A total of 22 seropositive persons were subjected to blood culture. Ten ml of blood was collected aseptically from each patient and five ml each was inoculated directly into the bottles containing sterile brain heart infusion (BHI) broths. Bottles were incubated at 37°C, one in aerobic atmosphere and another in atmosphere of 5 per cent CO₂ in a candle jar.

Subcultures were made on solid BHI agar plates every fourth day. During each subculture two smears were made from the broths and stained by modified cold ZN stain and Gram stain. Presence of red acid-fast coccobacilli against blue background was considered to be indicative of Brucella growth in broths. Cultures were considered negative for Brucella only after four weeks of incubation.

All the isolates were sent to IVRI, Izatnagar and Bijapur Libral District Education Association's (BLDEA’s) Shri B.M. Patil Medical College Hospital and Research Centre, Bijapur and HiTech Laboratories Belgaum for confirmation and typing.

Of the 22 cultures, Brucella was isolated from five. In all these five cultures acid-fast coccobacilli were seen in the broth smears made during the subculture and were subsequently confirmed as Br. melitensis. None of the 17 broths negative for Brucella showed presence of acid-fast coccobacilli. All the broth positive for Brucella also showed the presence of Gram-negative coccobacilli.

Twenty consecutive routine blood culture broths, which showed turbidity on incubation, were also smeared and stained by modified cold ZN stain. None of the broths showed acid-fast coccobacilli and none yielded the growth of Brucella.

The presumptive indication of Brucella growth was available on 4th day in the 5 culture positive cases by modified cold ZN staining. It took another four days for bacteriological identification by different biochemical tests. Thus, modified cold ZN stain can be used as a useful adjunct in presumptive identification of Brucella growth in blood culture broths. The agreement between modified ZN stain results and culture confirmation was 100 per cent.

Though the number of cultures performed and isolates obtained is not large, considering profound agreement between the modified cold ZN stain and culture we feel that this method should be further evaluated. An advantage of presumptive diagnosis by culture four days in advance deserves attention. Because of simplicity, reliability and reproducibility this test can be routinely incorporated in the laboratory for diagnosis of brucellosis.
References


Reprint requests: Dr R.D. Kulkarni, Professor & Head, Department of Microbiology Govt. Medical College, Miraj 416410, Maharashtra, India e-mail: san_katul@sancharnet.in