The results are shown in the Table. The exponential character of turbidity increase was less sensitive yet a more specific screening criterion, thus it is suitable for preliminary confirmation of significant bacteriuria. The non-specified turbidity increase estimation has a high negative predictive value and is suitable (just like the biochemical screening) for preliminary exclusion of significant bacteriuria. Using a microwell plate and a photometer with a kinetic programme, the photometric method can be used for the reliable, rapid and inexpensive screening of bacteriuria.

References

*M Bednar, V Nemeckova*
Department of Medical Microbiology (MB), Charles University, 3rd Faculty of Medicine, Prague; and Teaching Hospital Royal Vineyards (VN), Prague, Czech Republic

Dear Editor,

Human brucellosis varies from an acute febrile illness to a chronic, low-grade ill defined disease. It is a systemic disease, characterised by paucity of signs but accompanied with a myriad of non-specific symptoms such as fever, nocturnal sweating, malaise, fatigue, myalgia and backache. Patients with brucellosis may have been ill for many weeks or months before a diagnosis of brucellosis is considered. Many times Widal false positive reactions occur in patients with brucellosis giving a wrong diagnosis of typhoid fever. The disease has a worldwide distribution with higher prevalence in Middle East, Mexico, Central and South America and the Indian subcontinent. There is a paucity of literature on human brucellosis in India due to the highly infectious nature of the organism. In the absence of an isolate, serological investigation of the patient is of paramount importance for diagnosis of the disease and the future management of the patient. But before we do so, it is important to know the background prevalence of *Brucella* agglutinins. This study was carried out to investigate the background prevalence of *Brucella* agglutinins in blood donor population in and around Chandigarh.

A total of 292 serum samples obtained from blood donors attending the Blood Transfusion Medicine of Postgraduate Institute of Medical Research, Chandigarh were investigated for *Brucella* antibodies. The age range of the subjects was 18 to 55 years with a male:female ratio of 1.8:1.

*Brucella* antigen along with control positive and negative sera were procured from Indian Veterinary Research Institute (IVRI), Izatnagar, Uttar Pradesh. The sera were checked for *Brucella* agglutinins both by the slide and the serum
agglutination test (SAT) methods by the protocols provided by them. Briefly 40 µL and 80 µL of each serum sample was put on a glass slide to each of which a drop of the Brucella abortus coloured antigen was added and rocked gently from side to side for 4 to 5 minutes. Macroscopic agglutination occurring instantaneously was considered as strongly indicative of a positive reaction (80 IU/mL upwards). Those occurring after 2-3 minutes with medium particulation were considered to be doubtful (40 IU/mL approx) and those occurring after 4-5 minutes with powdery particulation as negative (10 - 20 IU/mL).

The serum agglutination test using the tube method was carried out in 273 of the above samples. Serial dilutions of the serum samples were made and standardised Brucella antigen for SAT was added to each tube and incubated in a water bath for 20h. A set of control tubes with different quantities of the Brucella antigen without the serum samples was also included. After incubation the tubes were kept at room temperature for 30 min and the degree of agglutination recorded. The total number of international units /mL of the serum was calculated. 80 IU/mL was considered as positive, 40 as doubtful and 20 as negative for human brucellosis.

Of 292 samples checked by the slide method, one was positive (80 IU/mL) and another doubtful (40 IU/mL). Of the above 292 samples, 273 were also investigated for agglutinins by SAT. A lone sample was found to be positive at 160 IU/mL and 46 (16.8%) others had insignificant levels. Table shows the distribution of Brucella agglutinins by slide and tube methods.

Brucellosis is a zoonotic disease transmitted by cattle to man and therefore of great economic importance. It is endemic in most of the developing countries manifesting as acute, subacute and chronic disease. Man is usually infected with Brucella organisms either by direct contact with infected animals or indirectly by infected dairy products or by accidental exposure to animal vaccines or laboratory isolates. The variable symptoms and occurrence of sub clinical and atypical infections makes the clinical diagnosis of human brucellosis very difficult and the laboratory aid is therefore essential. Isolation of Brucella organisms is not only cumbersome and expensive, but also quite hazardous to the workers. The organisms must be handled with particular care and under appropriate containment conditions in the laboratory. Alternatively, serological investigation provides a very safe method of diagnosing brucellosis in human beings. The combination of potential exposure, consistent clinical features and raised levels of Brucella antibodies with or without positive cultures confirms the diagnosis of brucellosis.

Due to its variable clinical manifestations the disease is only identified if a high index of suspicion is maintained. In India the disease has been found wherever it has been looked for and even in patients infected with human immunodeficiency virus. A seroprevalence of 0.9% in man has been reported by Sharma et al., whereas Panjarathinam et al. reported 6.5% seropositivity in women with spontaneous abortion. Patil et al. found a male/female ratio of 3:2 in Brucella serology. Bhat et al. reported a seroprevalence of 8.5% in the general population of Belgaum. Kadri et al. reported a seroprevalence of 0.8% having a male preponderance among hospitalised patients. However, no study on the prevalence of brucellosis among blood donors is available.

In our blood donor population, a very low level of Brucella agglutinins was present in 46 (16.8%) of the samples from healthy subjects. This was most likely due to the endemicity of the disease. However, only one sample (0.36%) was found to be truly positive for Brucella agglutinins at 160 IU/mL. This sample came from an 18-year-old female student who originated from Dharampur, a hilly terrain in Northern India. The blood was obtained during a voluntary blood donation camp.

The incidence of brucellosis is higher in rural areas where agriculture is the main occupation. Occupational infections occur to butchers, milkmen, laboratory staff, veterinarians, farmers, cattle-breeders etc. Non-occupational exposure may occur due to consumption of raw milk or milk products or handling of contaminated meat. Bhat et al. reported a seroprevalence of 5.8 to 14.3% in veterinary workers. To the best of our knowledge, this is the first study investigating the prevalence of brucellosis in blood donors. We do not know the reason for Brucella agglutinin positivity in the young female in our study, but it could be envisaged to be due to the consumption of raw milk or exposure to an agricultural background.

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A Novel Method for Differentiation of *Candida dubliniensis* from other *Candida* Species

Dear Editor,

*Candida dubliniensis* is being increasingly reported as an opportunistic infection in patients with human immunodeficiency virus (HIV) infection. It has an ability to rapidly develop fluconazole resistance *in vitro*; therefore, it is important to identify it. *C. dubliniensis* and *C. albicans* have a close genotypic relationship resulting in sharing a broad range of phenotypic characteristics. This hampers the accurate and rapid differentiation of the two species. We utilized Staib agar media for differentiating the isolates of *C. dubliniensis* from other *Candida* spp.

The present study comprised 63 clinical isolates of *C. albicans*, *C. dubliniensis*, *C. parapsilosis*, and *C. tropicalis* including two reference strains of *C. albicans* (CA132A) and *C. dubliniensis* (CD36). As described by Staib et al., colonial morphology of *C. dubliniensis* is observed on Staib agar along with the characteristics of chlamydospores produced by these *Candida* spp. *C. dubliniensis* and *C. parapsilosis* showed rough colonies with fine fringe on this media. However, *C. parapsilosis* could be easily differentiated as no chlamydospores were produced by this species, while *C. dubliniensis* produced chlamydospores in characteristic doublets and triplets. *C. albicans* and *C. tropicalis* produced mucoid colonies without fine edges and no chlamydospore production on this media (Figure).

The currently used important tests to differentiate *C. dubliniensis* from *C. albicans* are colony color on CHROM agar *Candida* medium, lack of growth at 45°C, PCR, immunofluorescence, and co-aggregation with *Fusobacterium nucleatum* tests.

![Figure](image)

**Figure:** Colonies of *C. albicans* on Staib agar (left). Colonies of *C. dubliniensis* on Staib agar (right).

Staib et al. reported that growth on Staib agar was an efficient means of differentiating *C. dubliniensis* and *C. albicans*. However, Mosaid et al. reported that colony morphology rather than chlamydospore production was more accurate for species identification. Our results suggest that colony morphology along with chlamydospore production on Staib agar when used in combination easily differentiates *C. dubliniensis* from other *Candida* isolates in clinical samples. The proposed method of phenotypic differentiation on Staib agar has a number of advantages over carbohydrate profile analysis as well as over commercially available yeast identification systems. It is rapid, reliable, easy to perform, inexpensive, readily available, and amenable to the analysis of large number of isolates. Thus, Staib agar provides a simple test for accurate identification of *C. dubliniensis* from clinical samples.

**Acknowledgement**

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**References**


