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Combining Vital Staining with Fast Plaque: TB Assay

Dear Editor,

The conventional culture technique for the diagnosis of *Mycobacterium tuberculosis* takes a minimum of 3-4 weeks. Results using automated systems also take an average of 10-21 days. This leads to a significant delay in confirmation of the diagnosis of tuberculosis.

The fast plaque TB (Biotech Labs Ltd., Ipswich, UK) is a rapid manual test for the detection of *M. tuberculosis* from clinical specimens within 48 h. This test utilizes specific mycobacteriophages (Actiphage™) to reflect the presence of viable *M. tuberculosis*. Mycobacteriophages are added to a clinical specimen and allowed to incubate for one hour to allow phage infection of target tubercle bacilli. After the incubation period, a virucidal solution (Virusol™) is added, which destroys all phages that have not infected the bacilli. The remaining phages replicate in the infected bacilli until new progeny phages are released as the cells lysis. The progeny phages are amplified by the addition of a non-pathogenic rapidly growing mycobacterial host *M smegmatis* (Sensor™ cell), which is also able to support phage replication. This is visualized as plaques, which are clear areas in a lawn of Sensor™ cell growth. The number of plaques visualized is directly related to the number of viable tubercle bacilli in the original sample.

This is one of the most critical steps in the procedure of fast plaque assay. The reading of plaques is dependent on the number, size and depression on the agar surface. This observation has been reiterated in our experience where reading of the plates was hindered when the number of plaques was very few. An attempt was made to modify the fast plaque technique with an addition of vital staining to the final steps.

Vital staining is a technique that has been propounded for elucidation of growing viable organisms. The various stains used are bismarck brown, trypan blue, neutral red, alamar blue, etc. Bismarck brown is considered to be a very effective vital stain with minimal toxicity to the bacilli.

We utilized the principle of vital staining of viable bacteria to increase the contrast provided by the formation of plaques, for 25 samples. After performing the original method of fast plaque, bismarck brown was added to the plate and it was incubated overnight. The viable bacterial lawn stained yellow to golden brown, as against the plaques, which retained the colour of the medium, i.e. a pale cream colour. This colour contrast greatly aided in the reading and counting of the plaques (Figure).

This concept has been utilized in the past in tests such as microwell alamar blue assay (MABA) and tetrazolium redox dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay where oxidation-reduction dyes have been used to obtain drug susceptibility to bacteria including *Mycobacterium tuberculosis*. Studies comparing the fast plaque assay to culture have found it to have high specificity but low sensitivity. Inability to discern the plaques may be one of the causes of low sensitivity, and the modification applied may improve results. A larger series of samples will aid in the statistical significance of this modified application.

References

Disseminated Histoplasmosis

Comment 1

I read with interest the case report on Disseminated Histoplasmosis by Joshi et al.\textsuperscript{1} The case is not the first culturally confirmed case of disseminated histoplasmosis in AIDS patients in India. Although the disease is under-diagnosed and under-reported from India, such a case was reported\textsuperscript{2} from Calcutta School of Tropical Medicine, along with four other chronic disseminated histoplasmosis in non-AIDS patients. Those were detected between February 1996 and September 1997 and all were culturally confirmed.

In the past, maximum number of this rare fungal disease were reported from this centre, including the first reporting of an Indian case of histoplasmosis in 1954 and the only report of isolation of causal fungus from Indian soil\textsuperscript{3} in 1975. In spite of under-reporting, at least 38 cases have been documented from India up to 1992. In view of the rising incidence of AIDS in India, the alarm of appearance of histoplasmosis from India up to 1992. In view of the rising incidence of AIDS in India, the alarm of appearance of histoplasmosis as an emerging opportunistic infection in eastern India was given with first reported histoplasmosis as an emerging case infected with HIV. The apprehension has now come to a reality after detecting nine more cases of disseminated histoplasmosis in HIV-infected cases who attended Calcutta School of Tropical Medicine from 2000 to 2006 (S. Basak, Personal communication). Thus, the Indian scenario of histoplasmosis in HIV-infected cases who attended Calcutta School of Tropical Medicine from 2000 to 2006 (S. Basak, Personal communication). Thus, the Indian scenario of histoplasmosis as a common co-morbid condition. This is the single largest series on disseminated histoplasmosis from India including culture-proven disseminated histoplasmosis.

The figures published have been transposed and do not correspond to the text. The yeast cell in the picture is not clear.