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PARAFFIN SLIDE CULTURE TECHNIQUE FOR "BAITING" NON-TUBERCULOUS MYCOBACTERIA

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Summary: The ability of Nocardia to utilise paraffin as the sole source of carbon has been used for its isolation from clinical specimens. Some mycobacteria also possess the same property, which is not so well known. The paraffinophilic nature of non-tuberculous mycobacteria as well as *M.tuberculosis* using commercially prepared Infectech Identikit™ (USA) was investigated.

Fifteen known species of non-tuberculous mycobacteria (NTM) and one strain each of *M.tuberculosis* $H_{37}R_V$ and *Nocardia asteroides* were inoculated into tubes containing paraffin coated slides. Visible growth was seen for all NTM within 7-14 days (and compared with *Nocardia asteroides* which acted as growth control) while *M.tuberculosis* strain did not grow even after 8 weeks of incubation. Thus, the paraffin slide culture (PSC) technique proved to be useful in distinguishing between NTM and *M.tuberculosis*.

Further tests like nitrate, urease, Tween 80 hydrolysis and tellurite reduction could also be performed in PSC system to distinguish species such as *M.kansasii, M.avium-intracellulare* and *M.fortuitum*. The colonies could also be subcultured on fresh Lowenstein Jensen medium for further characterisation. The kit can also be modified for drug susceptibility tests by incorporating drugs in Czapek broth.

Key words: Paraffin Slide Culture (PSC), Non-tuberculous mycobacteria (NTM)

INTRODUCTION

Paraffin baiting was developed for isolating organisms like Nocardia and Mycobacteria from soil[1,2] because of their ability to utilize paraffin wax as the sole source of carbon. The baiting was accomplished by growing the organisms in a medium lacking any carbon source into which were dipped glass rods coated with paraffin wax. The technique was applied for the isolation of *Nocardia asteroides* from clinical specimens[3].

The ability of non-tuberculous mycobacteria (NTM) and the inability of *M.tuberculosis* complex to utilize carbon present in paraffin wax for their growth has been shown by Ollar et al[4]. This technique is therefore useful for isolating NTM from clinical specimens since this unique property of paraffin metabolism is not commonly found amongst other human pathogens. The use of a paraffin coated glass slide instead of a glass rod allows the use of *in situ* staining procedures and finding out the acid-fast nature of the organism growing on the slide. The technique can also be used for speciation and antibiotic susceptibility testing of NTM[4,5].

The present study was, therefore, undertaken to assess the ability of a commercially available paraffin slide culture sysem (INFECTECH IDENTIKIT, Sharon, Pennsylvania, USA) for supporting the growth of known strains of NTM, *M.tuberculosis* and *N.asteroides* and to judge if the system could also be used for performing the biochemical tests used for speciation of NTM[4].

MATERIAL AND METHODS

Fifteen known strains of NTM *viz M.avium, M.kansasii, M.fortuitum, M.thamnopheos, M.terrae, M.diernhoferi, M.chelonae, M.asiaticum, M.szulgai,*

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M.phlei, M.flavescene, M.gadium, M.abscessus, M.gordonae, and *M.marinum* as well as *M.tuberculosis* $H_{37}R_V$ and one strain of *Nocardia asteroides,* used as the positive control, were grown in 5ml each of Middlebrook 7H9 medium with OADC (oleic acid, albumin, dextrose and catalase) supplement and incubated at 37° C for 7 days or till the turbidity matched that of MacFarlands' No.1 standard. Then, 0.5ml of the stock broth culture was inoculated into 4.5ml of sterile Czapek broth into which were dipped sterile paraffin coated glass slides, incubated at 37°C and checked daily for growth.

As soon as growth was observed on the slide, either in the form of discrete colonies or confluent growth (Fig.1), the slide was taken out and stained by Kinyoun's method by placing it into a tube containing Kinyoun carbol fuchsin for 15min, immersing it several times in distilled water; placing it in another tube containing 3% acid alcohol for 5 min; immersing it again in a tube containing distilled water and finally immersing it in a test tube containing 1% aqueous methylene blue counter stain for 1min. The slide was then gently blotted dry and examined under 10x, 40x, & oil immersion lens for presence of acid fast colonies and bacillary forms.

In order to judge whether the system could be used also for speciation of NTM, *M.kansasii, M.avium - intracellulare* and *M.fortuitum* growths were tested for reduction of nitrate and tellurite and hydrolysis of urea and Tween-80, using separate slides with growth of the test organism for each test. Uninoculated media, with paraffin slide without growth were used as reagent control.

Fig.1 Paraffin slide cultures showing growth of NTM (*M.abscessus-2, M.thamnopheos-3* & *N.asteroides* -4 but no growth of *M.tuberculosis*-1)

RESULTS

After inoculation, growth was visible within 1-2 weeks on the paraffin coated slides for *Nocardia asteroides* and all the NTM but *M.tuberculosis* $H_{37}R_V$ did not grow even after 8 weeks of incubation. (Fig.1). All the slides with growth showed the presence of acid fast colonies (Fig.2) and acid fast bacillary forms were seen under the oil immersion lens. All the three NTM species specially tested gave the positive and negative reactions as expected.

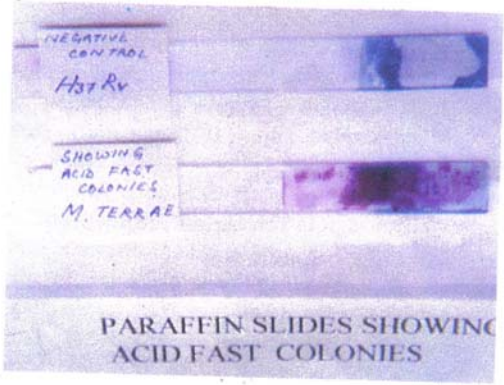

Fig. 2 Growth of *M:terrae* on paraffin slide stained with Kinyoun acid alcohol fast staining

DISCUSSION

The paraffin slide culture (PSC) technique (Infectech, USA) was found to be useful for baiting and supporting the growth of the NTM strains tested. It also served as a useful means for distinguishing NTM from *M.tuberculosis,* since the latter did not grow because of its inability to utilise wax as the sole source of carbon for growth. Further, the *in situ* Kinyoun's staining with 3% acid alcohol technique enabled differentiation to be made between NTM and Nocardioform organisms which

are only 1% acid fast. The PSC system was also sucessfully used to carry out the important biochemical reactions to speciate most of the NTM. In this study, all the tested NTM i.e. *M.kansasii, M.avium-intracullare* and *M.fortuitum* produced the relevant positive and negative results, thereby showing that this system could be successfully used for the purpose.

This method can prove to be a major breakthrough in the cultivation of non-tuberculous mycobacteria. It has been used for isolating *M.avium-intracellulare* from blood cultures[4]. According to the present authors, while isolating NTM from clinical specimens, this system has an added advantage of reducing the risk of contamination as very few human pathogens are able to grow on paraffin wax. The system can be made even more specific by adding a cocktail of antibiotics like Polymyxin B, Amphotericin B, Naladixic acid, Trimethoprim and Azlocillin to the medium in order to prevent the growth of Pseudomonas and *C.tropicalis* which can also grow on these slides. PSC has also been used for drug susceptibility testing of *M.avium-intracellulare* by a modified broth dilution assay[5]. The growth on the slides can even be used for DNA extraction[4] for molecular studies. **This paraffin slide culture technique provides an inexpensive and simple alternative for isolation, speciation and drug susceptibility testing of NTM.**

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