

Why Not Use a Low Tech, Low Cost, Highly Specific and Sensitive Paraffin Slide Culture System to Detect the Presence of Non Tuberculous *Mycobacteria* in Countries of the Developing World When More Advanced Technologies are Not Available

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Received: April 15, 2019; **Published:** July 31, 2019

"Paraffin slide culture Technique" is being utilized to easily distinguish between TB complex and Non-Tuberculous Mycobacteria".

The presence of Non-Tuberculous *Mycobacteria* (NTM) *Mycobacteria* have been reported in many countries Developed World as opportunistic pathogens in AIDS patients [1]. By contrast, however, the presence of Non Tuberculous *Mycobacteria* in patients with AIDS or Non-AIDS Patients have been rarely reported in Developing World Countries [1,3].

This is probably due to the fact that many of the labs in these Developing World Countries (especially those labs located in small rural communities) "are not able to" diagnose the presence of Non Tuberculous *Mycobacteria* based solely upon the stained slide analyses using traditional Acid-Fast Microscopic Staining, or the more advanced slide analyses based upon the Auramine-O- Fluorochrome staining protocol. It is well known that both Tuberculous and Non-Tuberculous *Mycobacteria* are both acid-fast whether one uses either of the two aforementioned methods cited above to detect acid-fastness!!

The antibiotics often used to treat TB are not effective against Non-Tuberculous *Mycobacterium* such as *Mycobacterium avium* complex and other Non-Tuberculous *Mycobacteria* [5]. Can this be one of the reasons why patients treated for TB based solely upon microscopy are victims of failed antibiotic regimens? This why it is so important that labs whether they are located in countries of the Developed or Developing World have the ability to diagnose the presence of these NTM *Mycobacteria*!! Sadly, the myth that often exists in these countries of the Developing World is that "NTM *Mycobacteria* are not a problem" [1]. In the twenty years that I have worked with my fellow Mycobacteriologists in India (the years 1999-2019), at the Mahatma Gandhi Institute of Medical Sciences located in Sevagram in rural India, we have debunked this myth, and proved that NTM *Mycobacteria* are an important group of pathogens in a country of the "Developing World" (7,8). These studies in India revealed that NTM *Mycobacteria* are present and must be identified, and treated in AIDS-Patients. These NTM *Mycobacteria* are quite frequently found as a co-infecting agent in patients who have been also found to already have *Mycobacterium tuberculosis* in countries in the Developing World like India [7,8].

India is indeed a leader in the countries of the Developing World in the routine Identification, and treatment of NTM Mycobacterial Diseases.

The ability to detect NTM Mycobacterial presence has been made possible because in even in poorly equipped labs located in rural India, a low cost and low technology system called the "paraffin slide culture Technique" is currently available and can be utilized to easily distinguish between TB complex and Non-Tuberculous *Mycobacteria* [4-8].

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Paraffin wax utilization or baiting of *Mycobacterium avium* organisms and other 'NTM Mycobacteria' and the inability *Mycobacterium tuberculosis* and TB complex organisms to utilize paraffin wax as a carbon source in a basal salt media where the paraffin wax is a sole carbon makes the distinction possible [4]. This is a known if not forgotten fact [4]. In fact, very few organisms have this ability to utilize paraffin wax as a sole carbon source. To further enhance the system a cocktail of antibiotics (which can be prepared in house) are added to the system prior to inoculation with a patient specimen [4-8]. Thus, the system has a low risk of contamination.

The basic system consists of a sterile paraffin wax coated slide (which is the solid phase and sole carbon source) that is introduced into a Basal Salt Liquid Media called Czapek Broth (liquid phase) which is lacking other forms of carbon [4]. A variety of specimen types both patient (sputum, stool, etc.) and environmental (water, soil) have been successfully utilized with this system [8,9]. One usually inoculates several tubes with patient or environmental specimens.

This low cost biphasic system enables a small rural lab and field station to perform the following analyses: a) staining of one the slides (showing "in situ growth") via acid-fast staining directly on the slide [4-8]. A positive acid-fast staining reaction of the paraffin slide informs the user that this "is not a TB Complex Organism" because "TB complex organism cannot grow in this basal salt media as they cannot utilize paraffin wax as a sole carbon source". A second tube containing positive "in situ growth" can have the growth scrapped off the paraffin surface with a flamed sterilized inoculating loop for subculture upon Middlebrook Media Agar Slant Cultures or traditional Egg Yolk Media like Lowenstein Jensen Media. The additional inoculated slides can be placed into speciation chemical solutions. We have used this low tech speciation testing of 'in situ" growth" to accurately detect the presence of *Mycobacterium Avium* Complex organisms. In addition, this simple system has been even utilized to perform "in vitro" antibiotic sensitivity assays from the samples which had been previously subcultured upon media like Middlebrook 7H9 Agar Slants or Egg Yolk Media [4,5].

This system has even been utilized in connection with advanced molecular biology based technologies. The "in situ growth" can be directly scrapped off the paraffin wax coated slide so that a DNA extraction protocol can be achieved. The DNA derived from the extraction off of the slide can then be analyzed by Horizontal Agarose Gel Electrophoresis, as well as, with Polymerase Chain Reaction Protocols using a variety of primers. In addition, it even has been utilized with Southern Blotting Systems and in RFLP Analysis [6].

Thus this robust yet flexible system can even be utilized in studies in countries of the Developed World involving NTM *Mycobacterial* presence in in Chronic Obstructive Pulmonary Disease COPD Patient related studies involving: a) Isolation of NTM Mycobacterial presence in water supplies, b) NTM Mycobacterial growth as biofilms on plumbing, c) The artificial "in situ" generation of biofilm growth on the paraffin wax slide culture surface [2,9].

Thus, in conclusion the existence of a simple low cost and low tech paraffin slide culture provides countries of the Developing World with a practical methodology for the detection of NTM Mycobacterial Presence even in a scenario where there is the existence of co-infecting TB Complex organisms.

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Volume 8 Issue 8 August 2019

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