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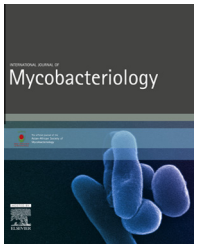

Review

Microscopy as a diagnostic tool in pulmonary tuberculosis

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ARTICLE INFO

Article history:
Received 26 November 2014
Received in revised form
17 December 2014
Accepted 20 December 2014
Available online 2 February 2015

Keywords:
Microscopy
Tuberculosis
Ziehl–Neelsen
Mycobacteria
Fluorescence

ABSTRACT

Tuberculosis continues to cast a huge impact on humanity with its high incidence and mortality, especially in developing countries. For tuberculosis case detection, microscopy continues to be indispensible, given its low cost, rapidity, simplicity of procedure and high specificity. Modifications have attempted to improve the sensitivity of microscopy which include: concentration methods such as centrifugation, N-acetyl cysteine–sodium hydroxide, bleach, ammonium sulfate or chitin. Furthermore, classical Ziehl–Neelsen (ZN) staining has been subjected to varying carbol fuchsin concentrations or replaced by Kinyoun staining, fluorescent microscopy or immune-fluorescence. Currently, light emitting diode fluorescence is recognizably the most plausible method as an alternative to ZN staining.

Contents

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Peer review under responsibility of Asian African Society for Mycobacteriology.
http://dx.doi.org/10.1016/j.ijmyco.2014.12.006

Introduction

Tuberculosis (TB) continues to intimidate the human race since time immemorial as a severely debilitating disease. The socio-economic burden of TB has been the subject of much concern, and major efforts are under way to try to achieve its control. In 2012, an estimated 8.6 million cases developed TB and 1.3 million died from the disease [1]. Directly Observed Treatment Strategy (DOTS) was formally introduced in 1997 and involved documentation and surveillance of TB and brought about a degree of control [2]. Here, emphasis is given to TB diagnosis by identification of acid-fast bacilli (AFB) on un-concentrated sputum (direct smears) with Ziehl–Neelsen (ZN) staining [3].

The historical perspective

In the 19th century in Eastern Germany, physician and scientist Robert Koch (1843–1910) established bacterial techniques to diagnose bacterial infections. On the evening of March 24, 1882, Robert Koch presented his landmark lecture with a statement on tuberculosis: "If the importance of a disease for mankind is measured by the number of fatalities it causes, then tuberculosis must be considered much more important than those most feared infectious diseases, plagues, cholera and the like. One in seven of all human beings dies from tuberculosis. If one only considers the productive middle-age groups, tuberculosis carries away one-third, and often more". He demonstrated the presence of the rod-shaped bacterium, Mycobacterium tuberculosis (MTB), by the staining methods invented by him. He used various adaptations of the staining methods of Carl Weigert in smear microscopy. Subsequent to Robert Koch's discovery, several other researchers (Ehrlich, Ziehl, Rindfleisch, and Neelsen), intending to improve on Koch's method, introduced modifications to the reagents and used carbolic acid (phenol) as the mordant. Paul Ehrlich developed the alum hematoxylin stain and demonstrated the tubercle bacillus in 1886. Ehrlich's method was further modified first by German bacteriologist Franz Ziehl (1859–1926) who modified the procedure by using carbolic acid (phenol) as the mordant. Subsequently, pathologist Friedrich Neelsen (1854–1898) kept Ziehl's mordant, but changed the primary stain to the basic fuchsin (first used by Ehrlich in 1882). This method became known as the Ziehl–Neelsen method in the early to mid-1890s and is a special bacteriological stain used to identify acid-fast organisms, mainly Mycobacteria [4]. In this method, heat is used to help drive the primary stain into the waxy cell walls of these difficult-to-stain cells. The use of heat in this method has been the reason that this technique is called the "hot staining" method. The Ziehl–Neelsen method has endured as a reliable and effective way to demonstrate the acid-fast bacteria [4]. Simultaneously, in Denmark, Hans Christian Gram developed a method for broadly distinguishing bacteria into two groups on the basis of a particular staining characteristic. However, Mycobacteria are gram positive, but many species stain poorly even after the prolonged heating. In 1915, Kinyoun published a method that has become known as the "cold staining" method because the heating step was removed in favor of using a higher concentration of the carbol-fuchsin primary stain [4].

Utility with the road blocks

Most National TB control programs in developing countries are implementing direct sputum microscopy primarily for tuberculosis case detection [1]. Though culture is more sensitive than microscopy, in developing countries, diagnosis is primarily based on AFB microscopy owing to its simplicity, less cost and rapidity. It is highly specific for MTB, which appear as long, curved and beaded. The Non-Tuberculous Mycobacteria (NTM) may appear as short, straight bacilli with no specific morphology [2].

The MTB forms tight ropes called cords in liquid media which can be identified on AFB smear. Cord formation has been used for presumptive identification of MTB as compared with the MOTT as it is rapid, sensitive and low-cost compared with the conventional identification system [5].

ZN staining has a low sensitivity of 22–43% for a single smear. Maximum sensitivity has been found to be up to 60% under optimal conditions when compared with that of cultures [6,7].

The threshold of detection of AFB in sputum samples under optimal conditions is found to be between 10^4 and 10^5 bacilli per ml. The yield is often decreased further under program conditions due to technical and operational constraints [8]. The sensitivity is even lower in pediatric and human immunodeficiency virus (HIV)/AIDS patients who usually present a pauci-bacillary picture [9,10]. Children under 12 years of age with pulmonary TB rarely produce sputum and are usually unable to expectorate voluntarily. When sputum samples cannot be obtained, gastric aspirate samples are used for detection and isolation of MTB. Even though AFB stain of sputum is positive in up to 75% of adults with pulmonary TB, fewer than 20% of children with TB have a positive AFB smear of sputum or gastric aspirate [10]. A total of 412 adults with culture-proven pulmonary tuberculosis were studied, of whom 185 (44.9%) were HIV sero-positive and had a significantly lower sputum smear positivity than HIV sero-negatives (68% versus 79%, $p < 0.05$) [9].

The collected sample

If pulmonary TB is suspected, specimens originating from the respiratory tract should be collected, i.e., sputum, induced sputum, broncho-alveolar lavage or a lung biopsy. Earlier, for the diagnosis of pulmonary TB, three first-morning spu-