

Short Communication

A faster and safer staining technique for acid fast bacilli in resource-poor setting

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Accepted 17 April, 2011

The traditional Ziehl-Neelsen (ZN) method used for the staining of acid fast bacilli contains phenol and because of the hazardous nature and inherent danger of phenol, this study was carried out to develop a faster and safer method in our environment that will exclude toxic phenol from the staining solution. "Morning fresh", a commercial liquid dish washing solution with super grease cutting power and citrus extract distributed by PZ Industry PLC, Nigeria was substituted for phenol in preparing carbol fuchsin. This study was carried out between September 2006 – May 2007 at the Public Health Laboratory, Department of Community Medicine, Ahmadu Bello University and Histopathology Department, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. Departmental slides known to contain acid fast bacilli were stained in duplicates using both the traditional carbol fuchsin and the staining solution containing "Morning fresh" following the standard procedures. The modified and the traditional ZN stained red for acid fast bacilli, nuclei, cytoplasm and cytoplasmic element stained blue on a clear background. The results were compared with departmental control slides for acid fast bacilli stained by traditional ZN method. The liquid dish washing solution "Morning fresh" with super grease cutting power and citrus extract was found to be very efficient because of its affinity for lipids, its ease to handle and safety when compared to phenol. This study method stains acid fast bacilli more efficiently than the traditional carbol fuchsin method using phenol and it is also cheaper and readily available for use in our environment, and in the light of this finding, this may be a more suitable alternative in the detection of acid fast bacilli especially in resource poor and constrained countries where mycobacterial infections are a major public health problem.

Key words: Tuberculosis, Morning fresh, PZ, traditional ZN stain, Nigeria.

INTRODUCTION

In 1882, Koch was the first person to describe the stained appearance of acid fast bacilli using alkaline methylene blue to demonstrate the organism (Koch, 1882). In the same year, the Ziehl-Neelsen (ZN) method was also developed by Ehrlich and described the properties of acid fast bacilli organism (Ehrlich, 1882). But he was unable to remove the stain from mycobacteria with dilute acids after staining with a fuchsin-aniline mixture. In 1882 and 1883, modifications were made to the method by using basic fuchsin with carbolic acid (phenol C₆H₅OH) (Ziehl et al., 1882; Ziehl et al., 1883). Neelsen in 1883 used the same staining solution as Ziehl but destained tissue elements with 25% sulphuric acid (Neelsen et al., 1883). Victoria

Blue, Night blue, triphenyl and diphenyl naphthylamine dyes and new fuchsin have been substituted for basic fuchsin in the original method. Fluorescence methods using Auramine O⁸ as well as recent years immunopathological methods have been recommended and developed using monoclonal antibodies (Barbolini et al., 1989) and mycobacteria have been detected using polymerase chain reaction (Eisenach et al., 1991). Despite all these alternative methods for demonstration and detection of mycobacteria, Ziehl- Neelsen is the commonest method requested and used to detect its presence both in rural and urban areas.

The cell wall of mycobacterial organisms contains complex lipids known as -hydroxy carboxylic acids, phitocerols and polymethyl acids. Other complex lipids and glycolipids have been isolated. The -hydroxy carboxylic acid which has the property of acid fastness and other

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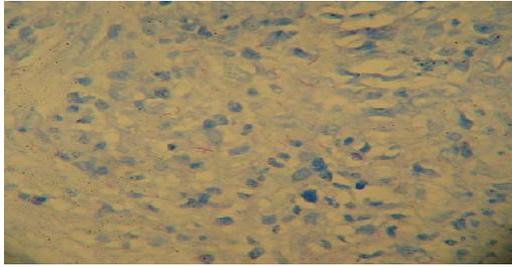


Figure 1. A slide using traditional carbol fuchsin stain.

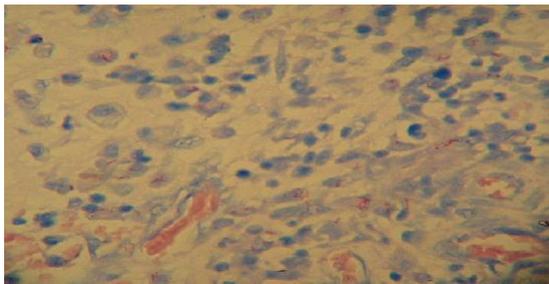


Figure 2. A slide showing citrus extract super grease cutting power of morning fresh. Acid fast organisms with a better intensity to the carbol fuchsin traditional

cell wall lipids present a barrier to dye entry as well as elution. The original Ziehl-Neelsen method over this is by adding phenol, a lipophilic agent to an aqueous solution of basic fuchsin by heating and prolonging the staining time.

Phenol's function is to increase lipophilia and acid passage of the dye through protective lipid wall (Prento et al., 1991), enabling the dye to attach with negatively charged ions present in the bacterial structure. After staining the lipid coating is reconstituted by removing the lipophilic agent. An acid/alcohol solution from subsequent differentiation removes the dye from the tissue structures but leaves dye in the organism with lipid wall acting as a barrier to the removal of the dye. Due to the hazardous exposure of phenol with inherent danger to staff of histopathology that have to handle the concentrated solution, the study was carried out with an alternative agent "Morning fresh" to ascertain the faster and safer suitability in the staining of acid fast bacilli in Ahmadu Bello University Teaching Hospital, Zaria, Nigeria.

MATERIALS AND METHODS

The study was conducted and carried out between September 2006 – May 2007 at the Public Health Laboratory, Department of Community Medicine, Ahmadu Bello University and the Modern Histopathology Department, Ahmadu Bello University Teaching Hospital, Zaria Kaduna, Nigeria that was recently commissioned. Ethical permission was obtained.

Reagents that were used are staining solution constituting; Basic

fuchsin 1 g; Absolute ethyl alcohol 10 ml; 1 ml Morning fresh (super grease cutting power with citrus extract); distilled water 100 ml.

The constituents of the "Morning fresh" are water, anionic surfactants, hydrotropes, magnesium salt, perfume, preservatives and colour.

The staining solution keeps well and can be used repeatedly for staining until stock solution is exhausted. Others are 1 ml hydrochloric acid in 99 mls of 70% methylated spirit and 0.20% aqueous methylene blue.

All the tissue was fixed in 10% neutral buffered formalin. Sections were routinely cut at 5 µm from tissue embedded in paraffin wax. Departmental control slides with *Mycobacterium tuberculosis* were used as control during the process of this method.

The study was done using the following procedure:

1. Dewaxing and hydration was done.
2. The slides were flooded with the staining solution and allowed to steam, staining for 5 min.
3. The slides were then washed in running water for 1 min.
4. Differentiating in 1% acid alcohol was done until a pale pink colour was obtained.
5. Washing was done briefly in water.
6. Counter stain with 0.20% methylene blue 30 s.
7. Wash in water, dehydrate in alcohol, clear in xylene and mount in DPX.

RESULT

The modified basic fuchsin solution stained acid fast bacilli with a similar intensity of colour to that of the carbol fuchsin. Acid fast bacilli stained red, nuclei stained blue, other tissue constituents stained blue. The background staining was clearer with the new staining solution than with the regular traditional ZN stain, allowing the organisms to be identified more readily.

Below are two slides, one stained by carbol fuchsin (traditional ZN method), (Figure 1) and another stained by our study method using "Morning fresh", extract (Figure 2). They were examined by medical laboratory scientists and our pathologists along with the control slides stained by each method. Different medical laboratory scientists and pathologists examined the slides without being able to distinguish the conventional carbol fuchsin stain from the stain using "morning fresh".

DISCUSSION

This faster, cheaper and safer modified basic carbol fuchsin staining solution stains acid fast organisms with a similar intensity to the traditional method of ZN because the standard staining solution does contain phenol that is expensive, toxic and difficult to handle. "Morning fresh", a commercial liquid dish washing solution with super grease cutting power and citrus extract has a power that actually breaks the lipophilic cell wall of the *M. tuberculosis* to give access to basic fuchsin into the bacilli thereby producing a deeper intensity of the colour. This agent is very efficient and it is not toxic. Apart from non-toxicity, it is cheap and readily available and it can be used in areas where phenol is not available. This staining

technique does not need special storage facilities and is safer to handle, and the "Morning fresh" can be used for other purposes, such as washing of glass wares.

In the light of this finding, this may be a more suitable alternative in the detection of acid fast bacilli especially in resource poor and constrained countries where Mycobacterial infections are a major public health problem in order to improve diagnostic services for case detection rate.

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