Rev. Inst. Med. trop. São Paulo
38 (4): 299-302, julho/agosto, 1996

A SINGLE METHOD TO STAIN *Malassezia furfur* AND *Corynebacterium minutissimum* IN SCALES

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SUMMARY

A single and practical method to stain *Malassezia furfur* and *Corynebacterium minutissimum* in lesions' scales is described.

The scales are collected by pressing small pieces of scotch tape (about 4 cm lenght and 2 cm width) onto the lesions and following withdrawl the furfuraceous scales will remain on the glue side. These pieces are then immersed for some minutes in lactophenol-cotton blue stain. Following absorption of the stain the scales are washed in current water to remove the excess of blue stain, dried with filter paper, dehydrated via passage in two bottles containing absolute alcohol and then placed in xylene in a centrifugation tube. The xylene dissolves the scotch tape glue and the scales fall free in the tube. After centrifugation and decantation the scales concentrated on the bottom of the tube are collected with a platinum-loop, placed in Canada balsam on a microscopy slide and closed with a cover slip. The preparations are then ready to be submitted to microscopic examination. Other stains may also be used instead of lactophenol-cotton blue. This method is simple, easily performed, and offers good conditions to study these fungi as well as being useful for the diagnosis of the diseases that they cause.

KEYWORDS: *Malassezia furfur*; *Corynebacterium minutissimum*; Pityriasis versicolor; Erythrasma; Staining method.

INTRODUCTION

The staining of *Malassezia furfur* and *Corynebacterium minutissimum*, etiologic agents of pityriasis versicolor and erythrasma respectively, in the lesions' scales is hard, time consuming and usually difficult to perform. Fresh direct examination easily shows *M. furfur* mycelia if is performed by the Jarbas Porto procedure[1] to collect the scales by pressing scotch tape onto the lesions. When the scotch tape is withdrawn it will retain the infected scales within the glue. The pieces of scotch tape with the adherent scales are placed with the glue face down on a slide and examined using 20 or 40 microscope objectives. If present, the round and filamentous mycelia of *M. furfur* will be immediately found. However it is not possible to detect the filamentous and coccoides forms of *C. minutissimum* using this procedure and in addition the morphologic details of *M. furfur* cannot be appropriately studied. On the other hand to stain these scales in the fresh preparations clarified with potassium or sodium hydroxide, in slide and cover slip, to be examined with immersion objective, is extremely time

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consuming and requires a special ability seldom rewarded by the results obtained.

A new method to simplify and facilitate the detection and study of *M. furfur* and *C. minutissimum* will be described to obviate the above mentioned difficulties.

METHOD

1. To collect the material to be stained, two or three pieces of scotch tape of about 4 cm lenght and 2 cm width are pressed, with the glue face down, on pityriasis versicolor or erythrasma lesions. When taken off they will carry the furfuraceous scales of the lesions on the glutinous side.

2. The scotch tape pieces containing the scales are immersed for a few minutes in a Borrel bottle containing lactophenol-cotton blue stain (crystals of phenol-20 gm, lactic acid-20 ml, glycerol-40 ml, distilled water-20 ml, cotton blue-0.10 gm). Other stains may also be used, for example changing the lactophenol-cotton blue for 2% methylene blue in distilled water whilst keeping the other steps identical.

3. The scotch tape pieces with adherent scales are washed in current water to remove excess stain until only the scales remain blue.

4. The remaining water on the scotch tape pieces is blotted gently with a filter paper.

5. Dehydration of the stained scales is achieved by passing the scotch tape pieces sucessively in 2 Borrel bottles each one containing absolute alcohol and then in another one with xylene. This latter stage is carried out preferably in a centrifuge tube.

6. The xylene dissolves the scotch-tape glue and the stained scales floating in the liquid will drop to the bottom of the tube after centrifugation for 10 minutes at 1,500 r.p.m. Then the xylene is decanted and the stained scales remaining on the bottom are easily distinguished.

7. The stained scales are collected with a loop, placed on a microscope slide in a drop of Canada balsam and covered with a cover slip. They are then ready for microscopic examination using the 20, 40, and immersion objectives.

The morphologic elements of the *M. furfur* (Fig. 1) and of the *C. minutissimum* (Fig. 2) stained a distinct blue, can be examined in detail and are distinct from the scales shown in a much lighter blue.

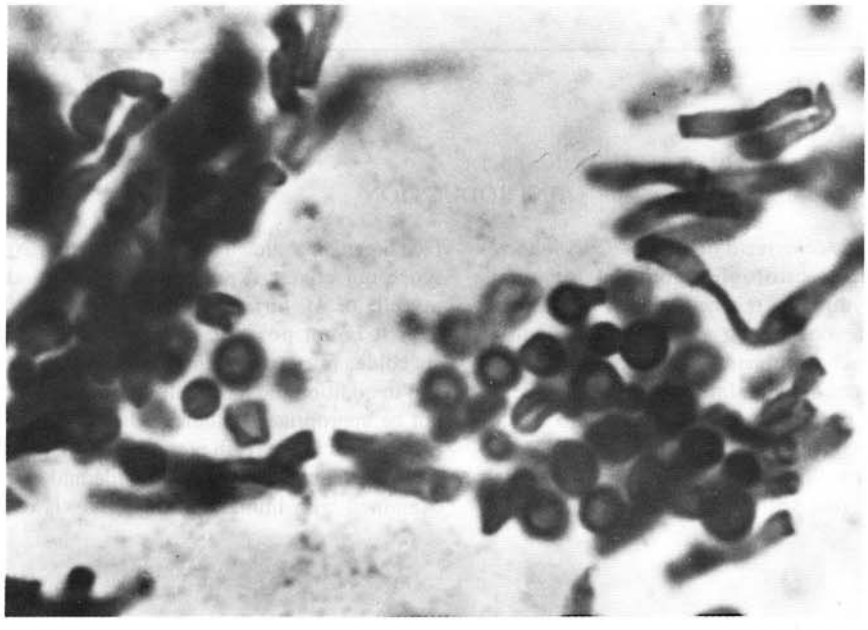

Fig. 1 - *Malassezia furfur* in pityriasis versicolor scales stained with lactophenol-cotton blue by the new method (X 1.000).

In addition to the lactophenol-cotton blue or the methylene blue which are simpler and easier to perform, other staining methods such as periodic acid-Schiff (PAS), Gram, Giemsa and Ziehl-Neelsen can be successfully used.

To perform the PAS staining the steps of the lactophenol-cotton blue staining are substituted as follows. The scotch tape pieces carrying the scales are placed in 95% alcohol for 2 minutes, then in 5% periodic acid for 7 minutes followed by washing in tap water for 2 minutes and then in Schiff reagent for 7 minutes. They are then washed for 2 minutes with tap water. Dehydration is carried out as previously described and the scales are closed on a slide and cover slip with Canada balsam. Both microorganisms are stained bordeaux and the scales very light bordeaux.

COMMENTS

The new procedure described to stain *M. furfur* and *C. minutissimum* in the lesions' scales collected with the aid of scotch tape pieces has proven easy to perform and has shortened the time needed to produce a good preparation adequate for microscopic diagnosis and morphologic study of these two microorganisms. The lactophenol-cotton blue was the staining technique mostly used in this study because it is simpler and easier to perform. The method described can be also used to perform other staining techniques aiding a better knowledge of the microscopic aspects of *M. furfur* and *C. minutissimum*.

RESUMO

Um método simples para corar *Malassezia furfur* e *Corynebacterium minutissimum* nas escamas

É descrito um método simples e prático para corar *Malassezia furfur* e *Corynebacterium minutissimum* nas escamas das lesões. O material é colhido com o auxílio de fita durex que será usada na maior parte das etapas do método para ajudar a fácil execução do processo de coloração. Para colher as escamas, pequenos pedaços de fita durex com cerca de 4 cm de comprimento por 2 cm de largura são colocados e pressionados sobre as lesões, e quando retirados trazem aderidas as escamas furfuráceas na face com goma. Esses pedaços de fita durex são imersos por alguns minutos no corante lactofenol-azul cotton e logo que as escamas estiverem coradas em azul são lavadas em água corrente para remover o excesso de corante azul, secos com papel de filtro, desidratados pela passagem em dois frascos com álcool absoluto e colocados em frascos de centrifugação com xilol. O xilol dissolve a goma da fita durex e as escamas caem soltas no tubo. Após centrifugação e decantação as escamas concentradas no fundo do tubo

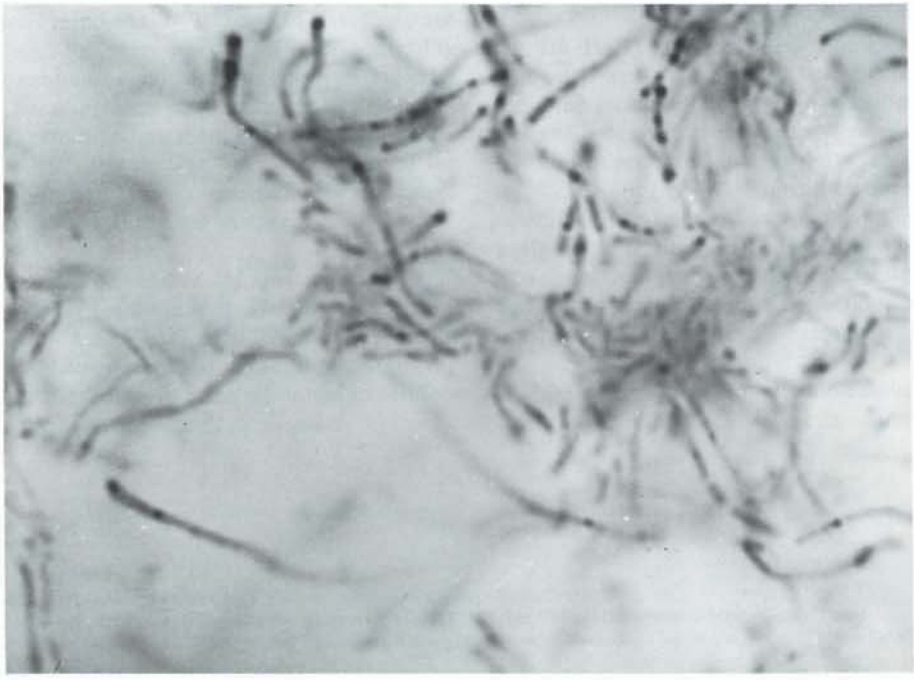

Fig. 2 - *Corynebacterium minutissimum* in erythrasma scales stained with lactophenol-cotton blue by the new method (X 1.000).

são colhidas com alça de platina, colocadas em bálsamo de Canadá sobre uma lâmina de microscopia e fechadas com uma lamínula. A preparação está assim pronta para exame microscópico. Outros processos de coloração em lugar do lactofenol-azul cotton podem ser usados. Este método é entretanto simples, fácil de ser processado e proporciona boas condições para o estudo destes fungos e para o diagnóstico das doenças por eles causadas.

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Recebido para publicação em 26/12/1995
Aceito para publicação em 28/06/1996