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Comparing the Effect of Different Concentration of Sulfuric Acid Differentiation Solution on Acid Fast Dyeing

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Abstract

Objective: In the acid-fast staining experiment of pathological tissues, sulfuric acid and hydrochloric acid were used to evaluate the staining results, so as to get the best staining method. **Methods:** Using sulfuric acid differentiation solution and hydrochloric acid differentiation solution, the paraffin blocks of pathological tissues known to contain Mycobacterium tuberculosis were compared to evaluate the staining effect. **Results:** When 0.5% hydrochloric acid differentiation solution is used and the differentiation time is 6 s, the dyeing effect is better than that of sulfuric acid differentiation solution.

Keywords

Acid-Fast Dyeing, Sulfuric Acid, Differentiation Solution, Dyeing Effect, Hydrochloric Acid

1. Introduction

Tuberculosis (TB) is a systemic chronic infectious disease. In recent years, its incidence is on the rise all over the world, which has attracted the attention of the World Health Organization [1] [2] [3]. Mycobacterium tuberculosis (TB) is the pathogen causing this disease, which mainly invades the lungs, lymph nodes and kidneys. Under the microscope, most of them are slender and curved rods, with uneven lengths, scattered single rods, or parallel clusters, sometimes branching [4] [5] [6]. At present, commonly used TB detection methods include Ziehl-Neelsen acid-fast staining, real-time fluorescence quantitative PCR and ISH, etc. Although histopathology has developed to molecular pathology at present, many diseases can be diagnosed by immunohistochemistry and molecular biology

techniques, acid-fast staining still occupies an important position in clinical pathological diagnosis, and its advantages are high efficiency and economy [7]. It is shown that the traditional staining method of acid-fast bacilli is Ziehl-Neelsen method, that is, basic fuchsin and phenol are used for staining, and then Wade-Fite has made an improvement on this basis. In this paper, sulfuric acid solution and hydrochloric acid solution are used to differentiate acid-fast bacilli, so that the staining results are clearer and the contrast is sharper.

2. Experimental Methods

Experimental materials: Collect the cases from December 2018 to November 2020 in the Department of Pathology, the Seventh Affiliated Hospital of Sun Yat-sen University, select 10 cases of pathological tissues known to contain Mycobacterium tuberculosis, and make them into tissue wax blocks by fixed dehydration.

Main instruments and equipment: Dehydrator (HP300, Dakota, China), medical refrigerator (HYC-356, Qingdao Haier), microtomes (RM2235, Leica, Germany), oven (BPG-9070A, Shanghai Heng Science Instrument Co., Ltd.), wet box.

Experimental reagents: TO biological transparent agent, phenol basic fuchsin, 0.5% sulfuric acid solution, 0.5% hydrochloric acid solution, Mayer's hematoxylin, xylene, neutral gum.

Experimental methods: 10 cases of wax blocks were sliced into 2 pieces in succession, and randomly divided into group A and group C, with 0.5% hydrochloric acid solution and 0.5% sulfuric acid solution corresponding to Group A and Group C respectively.

Experimental steps of acid-fast dyeing

1) Slice pathological paraffin blocks with a thickness of 5 um, and bake them in the oven for 60 min;

2) Put the slices in two cylinders of TO biological transparent agent for dewaxing for 20 min each cylinder;

3) Gently absorb the active agent on the glass slide with absorbent paper, place it in hot water at 80˚C and lift it up and down for more than 10 times;

4) Rinse with running water for 10 min;

5) Add phenol basic fuchsin for 20 min, put it in the incubator, be careful not to dry it;

6) Drop 0.5% hydrochloric acid solution and 0.5% sulfuric acid solution into Group A and Group C respectively for 6 s;

7) Rinse with running water for 10 min;

8) Mayer' s hematoxylin staining for 1 min; Pet-name ruby water flushing for 10 min; Attending oven drying for 20 min, transparent xylene, and sealing with neutral gum.

3. Scoring Standard

Double-blind scoring, with a score of 10 points; Acid-fast bacilli have strong

positive staining degree, clear structure, sharp contrast with surrounding tissues, and clean background, with 10 points for each index; If the staining effect of section tissue is average, but it does not affect the diagnosis, 1 - 3 points will be deducted; if the staining effect of section tissue is poor and the structure is fuzzy, which has certain influence on diagnosis, 4 - 7 points will be deducted; if the staining effect is very poor, and the tissue structure cannot be identified, which seriously affects the diagnosis result, 8 - 10 points will be deducted.

4. Statistical Processing

All statistical processing was done in SPSS 13.0 software. The scores of two groups A and C were analyzed by variance homogeneity, and two independent samples were selected for comparison by T-test. The inspection level is $\alpha = 0.05$, and $P < 0.05$ is regarded as a statistically significant criterion.

5. Result

Group A scored (9.20 + 0.79); Group C scored (0.50 + 0.53); the acid-fast dyeing effect of 0.5% hydrochloric acid solution is obviously better than that of 0.5% sulfuric acid solution, with strong positive degree, clear structure and clean background, and the difference is statistically significant ($t = -10.077$, $P < 0.005$) (Figure 1 & Figure 2).

6. Discussion

Acid-fast bacilli contain lipid in the cell wall, and a waxy shell is formed by glycolipid, which combines with phenol basic fuchsin to form a compound. This

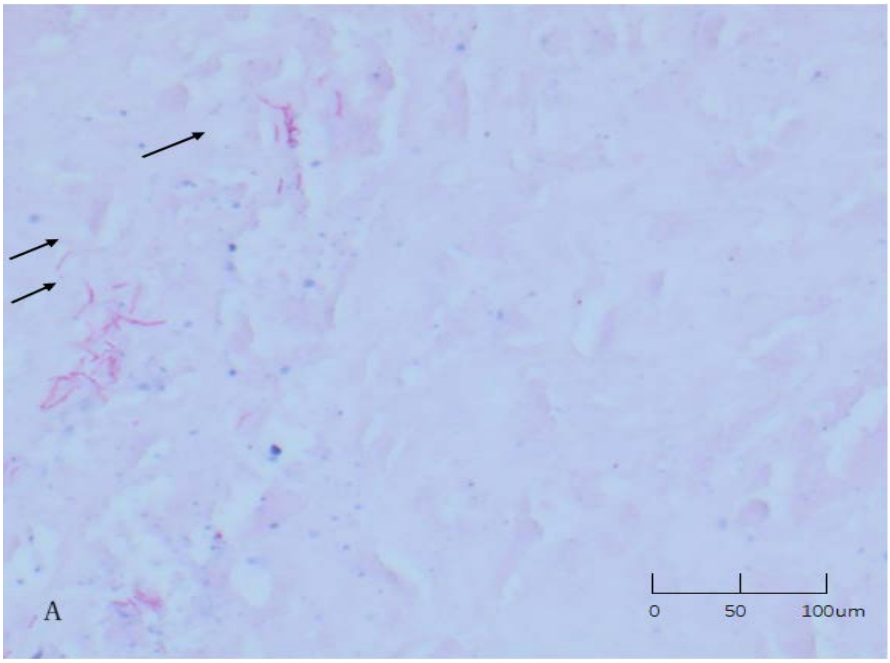

Figure 1. A 0.5% hydrochloric acid differentiation solution differentiated for 6 s in group A, and the arrow is Mycobacterium tuberculosis (scale 100 μm).

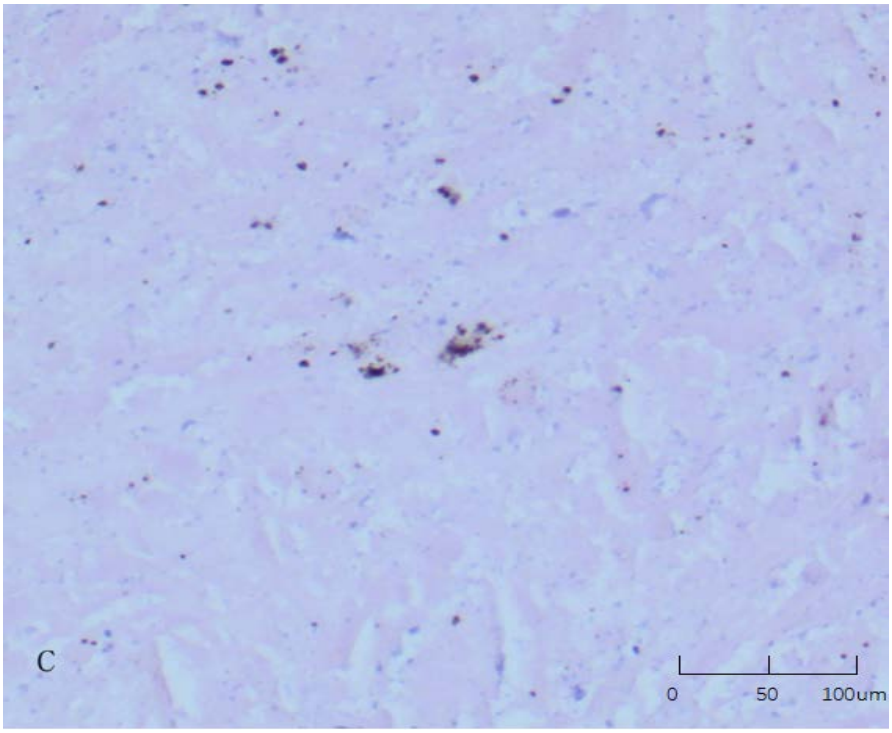

Figure 2. Group C: 0.5% sulfuric acid differentiation solution differentiated for 6 s (scale 100 μm).

compound can resist the decolorization of acids after being combined with bacteria, thus making acid-fast bacilli red. As a mordant, phenol can promote the combination of bacteria and basic fuchsin, and help the dissolution of basic fuchsin. Hydrochloric acid and sulfuric acid are differentiation solutions, which can decolorize other tissues without decolorizing acid-fast bacteria after dyeing [8] [9]. Tuberculosis is small rod-shaped in acid-fast staining, and it needs to be observed under 100× high-power microscope. Sometimes, tuberculosis lesions are rare. Therefore, the positive degree of staining and clear background contrast are crucial to clinical diagnosis. In this experiment, the author made the following improvements:

1) The conventional paraffin section is 3um, but the thickness is 5um in the staining of Mycobacterium tuberculosis. Because the section is too thin, the Mycobacterium tuberculosis will be chopped up, and it will become crumb under the microscope, which is not conducive to observation and judgment;

2) In this experiment, the biological agent turpentine was dewaxed, followed by washing with warm water. Compared with the traditional xylene dewaxing method, it can better preserve Mycobacterium tuberculosis. Acid-resistance of acid-fast bacilli comes from their lipid cell walls. Xylene and ethanol can dissolve the lipid and destroy the cell walls, resulting in the decrease or even disappearance of acid-fast bacilli.

3) Select appropriate differentiation solution and strictly control differentiation time. In traditional operation, sulfuric acid solution is often used as differentiation agent in acid-fast bacteria staining kit. However, in actual operation, it

is found that the differentiation time is not easy to control, and the whole pathological section has turned white after only a few seconds of differentiation, and the positive degree of acid-fast bacteria observed under the microscope is extremely low. Therefore, this experiment uses 0.5% hydrochloric acid differentiation solution, and the time is controlled at 6 s, so that the differentiation state can be observed under the microscope and the best degree of acid-fast bacteria staining can be obtained.

7. Conclusion

Compared with the experimental results of Group A and Group C, it was found that the time of differentiating tissue slices with 0.5% sulfuric acid solution was too fast, and it was difficult to master the operation, which often led to excessive differentiation, weakened positive degree, and even affected the diagnosis results. Using 0.5% hydrochloric acid solution for differentiation, the time is easy to control, and there is sufficient time to observe the differentiation degree under the microscope and stop it at the right time, so that the staining positive degree is stronger, the tissue structure contrast is clear, and it is more conducive to microscopic diagnosis.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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