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Ability of Ziehl-Neelsen and Fite Faraco Staining for Detection of Mycobacterium Sp. In Tissue Samples: A Literature Review

Riska Dwi Putri[1], Hermin Aminah Usman[2], Hasrayati Agustina[3]
[1]Ilmu Kedokteran Dasar, Universitas Padjadjaran / BioMedical Science, Padjadjaran University
[2]Patologi Anatomi, Universitas Padjadjaran/ Anatomic Pathology, Padjadjaran University
[3]Patologi Anatomi, Universitas Padjadjaran/ Anatomic Pathology, Padjadjaran University

Corresponding author: Dr. dr. Hermin Aminah Usman, Sp.PA(K).,
Kompleks Unpad II Cigadung Jln. Delta No. 3 Kav 8 Bandung
Phone number: 08112204910
Email: hermin@unpad.ac.id

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ABSTRACT

Histopathology is an essential method for disease diagnosis. It is crucial for clinicians to have an ideal diagnostic method that is simple, specific, and highly sensitive. The sensitivity and specificity of a test can be determined by comparing it with other tests. Tuberculosis is an infectious disease caused by Mycobacterium tuberculosis. Mycobacterium tuberculosis is diagnosed using Ziehl-Neelsen staining, which differentiates acid-fast bacilli from non-acid-fast bacilli. The Fite Faraco staining technique is used to detect Mycobacterium sp in tissue specimens. All reviewed articles show that Ziehl-Neelsen staining has a sensitivity between 21%-97.6%, specificity between 85.7%-92%, NPV between 34.3%-75%, PPV between 30.9%-100% in detecting Mycobacterium sp in tissue samples. Fite Faraco staining shows a sensitivity between 50%-74.6%, specificity between 84%-100%, NPV between 33.6%-56.7%, PPV 38.1% in detecting Mycobacterium sp in tissue samples.It is detected that Ziehl-Neelsen and Fite Faraco can be used to detect bacteria, Mycobacterium sp especially bacteria Mycobacterium tuberculosis and Mycobacterium leprae. However, Ziehl-Neelsen staining has better ability in terms of sensitivity, PPV, and NPV than Fite Faraco in detecting bacteria Mycobacterium sp, especially Mycobacterium tuberculosis. As Fite-Faraco staining is superior in terms of specificity. Other things that must be Considered in carrying out Ziehl-Neelsen and Fite Faraco staining are specific types of samples, making modifications such as modifying microwave heating on the Ziehl-Neelsen staining method and combining examination with H&E staining and multiplex PCR to increase the validity of the two staining methods.

Keywords: Ziehl-Neelsen, Fite Faraco, Mycobacterium Sp, Specificity, Sensititivity, Tissue

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INTRODUCTION

Histopathological examination in infectious diseases involves direct microscopic visualization of tissue samples to identify the infectious agent, particularly useful when culture cannot be performed or when the infectious agent grows slowly or requires special handling.[1,2] Histopathology is an essential method in the diagnosis of diseases. Although microbiological culture remains the gold standard for Mycobacterium infections, it is time-consuming and has limited sensitivity and specificity.[3] Clinicians must have an ideal, simple, precise, sensitive diagnostic method. The sensitivity and specificity of a test can be determined by comparing it with other tests.[1]

The validity of an examination technique requires sensitivity and specificity in its assessment to determine whether a test can be used or not in detecting a disease.[4,5] PPV (positive predictive value) is the proportion of patients who test positive and genuinely have the disease. In contrast, NPV (negative predictive value) is the proportion of patients who test negative and do not have the disease.[5]

Tuberculosis is an infectious disease caused by Mycobacterium tuberculosis. This bacterium commonly infects lung tissue but can also infect outside the lungs.[6] Mycobacterium tuberculosis is diagnosed using Ziehl-Neelsen staining, which differentiates acid-fast bacilli from non-acid-fast bacilli.[3] The concentration of the primary stain (carbol fuchsin) and counterstain (methylene blue) is essential for detecting Mycobacterium tuberculosis. The World Health Organization (WHO) recommends using 0.3% carbol fuchsin and 0.3% methylene blue. In clinical settings, staining with 1% carbol fuchsin for 10 minutes and counterstaining with 0.1% methylene blue for 1 minute provides better results.[7]

The Fite Faraco staining technique is used to detect Mycobacterium leprae in tissue specimens.[3] Confirming the diagnosis of leprosy is an essential indication for histopathological examination. The parameters used for histopathological classification are well-defined and accurate and also take into account the immunological manifestations.[8] The Fite Faraco staining method uses Xylene-peanut oil for deparaffinization and is stained with the Ziehl-Neelsen primary stain.[9] Xylene-peanut oil is used to protect the wax coating from acid-fast bacteria which will prevent shrinkage and loss of bacteria during the coloring process.[3]

Ziehl-Neelsen stain and Fite Faraco stain use the same main dye, namely carbol-fuchsin. The main dye will give a red color for acid fast bacteria Mycobacterium Sp.[3] It is known that histopathological examination using Ziehl-Neelsen and Fite Faraco stains can reveal acid-fast bacilli in tissue sections. Ziehl-Neelsen stain is used more frequently because it is easy to obtain and low cost.[10] In a study also showed good results on Ziehl-Neelsen staining visualize mycobacterium tuberculosis in tissue samples.[11] Both of these colorings have been effectively shown to visualize mycobacterium sp.[12,13]

METHODS

Search Strategy

The search was conducted using two search engines: PUBMED and Google Scholar. The keywords used were "Ziehl-Neelsen, Fite Faraco, Mycobacterium Sp, specificity, sensitivity, and Tissue," with a range of years from 2000 to 2022 without any date limitations.

Selection Criteria

All literature was assessed for eligibility by the authors. All authors evaluated each article's title, abstract, and full text identified in the search engines. All literature was assessed for eligibility by the authors. All authors evaluated the title, abstract, and full text. Studies were deemed eligible based on the inclusion and exclusion criteria. The inclusion criteria for this study were articles containing the exact keywords as the research topic, such as specificity, sensitivity, NPV%, PP%, and histopathological features, the article is a full paper, the article was published from 2000 to 2022, the article must be a research result, and the article must be in English. The exclusion criteria for this study were duplicated articles and inconsistencies in the title and abstract of the article. All articles were published in all countries worldwide. Any dif erences of opinion arising during the selection assessment were resolved through discussion.

Data Extraction

Data were extracted based on the results of Ziehl-Neelsen staining or Fite Faraco staining in Mycobacterium sp studies that include sensitivity, specificity, NPV%, PPV%, and the Mycobacterium species tested. If data were unavailable, a dash (-) was recorded in the data collection.

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RESULT AND DISCUSSION

The analysis flow of the study is shown in Figure 1. After conducting the search, 40 articles were obtained, 21 from PubMed and 19 from Google Scholar. 30 articles were excluded, with 27 excluded because they did not present the desired data and 3 excluded because they were found to be the same or duplicates. Then, the identified journals were reviewed, and 10 were selected for inclusion and will be discussed in this article.

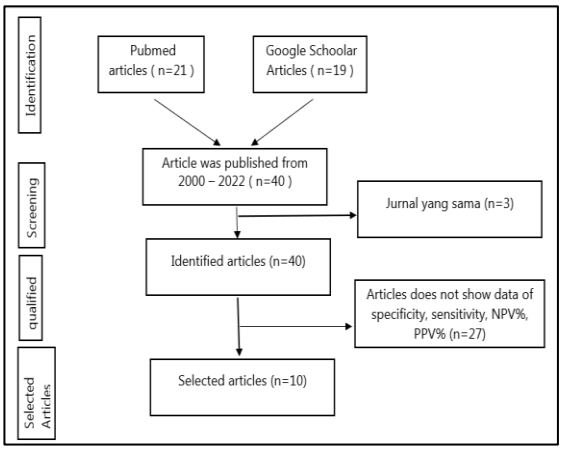

Figure.1 The analysis flow of the study

Table 1. Sensitivity, specificity, NPV, PPV of Ziehl-Neelsen and Fite faraco staining in detecting mycobacterium sp in tissue samples.

Researcher	Tissue Sample Type	Bacteria	Ziehl-Neelsen				Fite-Faraco			
			Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)
Kelly Atherton BS et al	Skin	Mycobacterium tuberculosis and mycobacterium leprae					57.1			
	Lung FNA/ lymph		43				58.8 <50			
Kamle et al	Skin scrapings	Mycobacterium Leprae			64.2	30.9			54	38.1
	Biopsy				52.6	45.2			33.6	61.9
Abu Hena Hasanoor Reja et al	Biopsy	Mycobacterium Leprae	56.9		45.7	69	74.6		56.7	85.9
Sunil V. et al	Biopsy	Mycobacterium Leprae								57.7
Crothers et al	Paraffin embedded tissue	Mycobacterium tuberculosis and Mycobacterium avium	21	92			61	84		
Priyanka Agarwala et al	Biopsy	Mycobacterium Leprae	40							
Pooja Prapanna et al	Fine-needle aspiration	Mycobacterium Tuberculosis	56.9							
Gehan Mohammed Ahmed et al	Excision biopsy	Mycobacterium Tuberculosis	97.6	85.7	75	98.8				
Selfu Girma et al	Skin scrapings	Mycobacterium Leprae	59.3		34.3	100				
	Biopsy						77	100	51.8	100
Wilda Mahdani et al	Paraffin embedded tissue	Mycobacterium Tuberculosis	81	90	64	96				

Specificity and sensitivity

In this review article, there are 2 studies that have the highest sensitivity and specificity in the Ziehl-Neelsen staining technique, 97.6%, 85.7%[19] and 81%,90%[21]. Both of these studies have their own strategy in increasing the sensitivity and specificity of Ziehl-Neelsen staining in detecting mycobacterium sp. On the article, Gehan mohammed Ahmed et al explained that they modified the Ziehl-Neelsen staining technique by using a microwave oven heater in the carbol fuchsin staining process with the best time and temperature obtained, namely level 1 (60w) for 1.5 minutes, this proved that heating could help open the mycolic acid layer on bacteria mycobacterium sp so that the main carbol fuchsin dye can enter and color the bacteria.[19] Another strategy used to increase the sensitivity and specificity of Ziehl-Neelsen staining was carried out by Wilda Mahdani et al, which uses a combination of HE staining techniques to pre-detect specific granulomas caused by Mycobacterium tuberculosis.The specific granulomas referred to are epitheloid cells, lymphocytes, fibroblasts and Langhans giant cells. Samples that had been

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stained with HE and identified the presence of specific granulomas,were stained with Ziehl-Neelsen so that bacteria Mycobacterium tuberculosis detect ability and increase sensitivity and specificity.[21]

The other articles have a fairly good sensitivity to the Ziehl-Neelsen technique staining is 56.9%[1,18] and 59.3%[20]. Article Pooja Prapanna et al and Abu Hena Hasanoor Reja et al showed the same sensitivity to Ziehl-Neelsen staining of 56.9% even though the bacteria detected were from different mycobacterial species, Mycobacterium Tuberculosis and Mycobacterium Leprae.[1,18] These results can prove that Ziehl-Neelsen staining can detect Mycobacterium Tuberculosis and Mycobacterium Leprae with good sensitivity. In another study the resulting sensitivity for the Ziehl-Neelsen color in detecting Mycobacterium Leprae was 59.3%, this indicated an increase.[20]

Priyanka Agarwala et al concluded that the results of the sensitivity of Ziehl-Neelsen staining were 40% in detecting Mycobacterium Tuberculosis and Mycobacterium Leprae, in his study Ziehl-Neelsen staining was compared to fluorescent staining using samples suspected of leprosy andcutaneous tuberculosis which resulted in a sensitivity that was not too significant, 49.2%.[17] Thus, Ziehl-Neelsen staining and fluorescent staining have almost the same sensitivity values in detecting Mycobacterium Tuberculosis and Mycobacterium Leprae bacteria.

Other articles have a low sensitivity to Ziehl-Neelsen staining 33%[14] and 21.[16] Research conducted by Kelly Atherton BS et al, stated that Ziehl-Neelsen staining has a sensitivity of 33% in detecting Mycobacterium tuberculosis and Mycobacterium Leprae. This study compared various staining techniques for detecting acid-fast bacteria, namely Ziehl-Neelsen, auramine-rhodamine, Fite Faraco and Kinyoun staining with samples and objectives. which is to determine which stain is better in detecting acid-fast bacteria.[14] The lowest sensitivity in this review article was 21% for the Ziehl-Neelsen stain.[16] Crothers et al compared the conventional staining examination of Ziehl-Neelsen, Fite Faraco with a method that uses specific antibodies, namely immunohistochemistry which uses samples of biological material stored for 2 years and all samples identified as mycobacterium. Although the sensitivity obtained for Ziehl-Neelsen staining is 21%, the specificity obtained is 91%[16], it can be concluded that in this study, Ziehl-Neelsen staining was able to avoid the number of false positives well because of its high specificity.

Other staining methods commonly used in detection Mycobacterium sp was Fite Faraco staining, not inferior to Ziehl-Neelsen staining. Fite Faraco staining also showed good sensitivity and specificity. Like the research done Selfu Germa et al and Abu Hena Hasanoor Reja et al, these two studies concluded that Fite Faraco staining had a sensitivity of 77% and 74.6% in detecting Mycobacterium Leprae. Good sensitivity is obtained with proper sample selection. To detect Mycobacterium Leprae this study used samples of various types of leprosy, such as lepramatous leprosy, borderline lepramatous, borderline tuberculoid, tuberculoid and indeterminate leprosy.[20] By selecting a specific sample, the Fite Faraco stain has good sensitivity. To increase the percentage value of the sensitivity of Fite Faraco staining, a combination of examinations can also be carried out, as was done by Abu Hena Hasanoor Reja et al, in his research combined Fite Faraco staining with Multiplex-PCR so that the resulting sensitivity was 74.6% in detecting Mycobacterium Leprae.[1]

This review article can prove that Faraco's staining is also quite good at detectingMycobacterium tuberculosis. This is shown in the results of research conducted by Kelly Atherton BS et al that the sensitivity of the Fite-Faraco stain obtained was 57%[1], 58.8%, and <50%. Variations in sensitivity werw obtained because this study used different tissue samples, skin,lung and FNA/Lymph.[14] Other study have demonstrated the ability of Fite Faraco staining to detect bacteria Mycobacterium Tuberculosis reaserch conducted by Crothers et al which resulted in a sensitivity percentage 61% and specificity of 81% this study used stored biological samples.[16]

PPV and NPV

Likewise, the sensitivity and specificity varies in article reviews This,positive predictive value (PPV) and Negative predictive value (NPV) also varied due to modifications to the staining technique, the number and type of samples used in each study. As is research Gehan Mohammed Ahmed et al, which stated that the PPV and NPV obtained by heating modification in the Ziehl-Neelsen staining procedure were 98.8% and 75% using 90 samples with the category of clinically suspect tuberculous lymphadenitis, of the total samples 82 samples tested positive and 8 others were negative in detecting bacteria Mycobacterium tuberculosis.[19]

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Wilda Mahdani et al also obtained good results for PPV and NPV, namely 96% and 64%, using 37 block samples of lung tissue, lymph nodes, skin and bones that had been clinically diagnosed as granulomatus inflammation caused by Mycobacterium tuberculosis carrying out H&E staining, then 23 samples showed positive results and 14 showed negative results after Ziehl-Neelsen staining.[21]

Other articles show quite good PPV and NPV namely research Abu Hena Hasanoor Reja et al and Kamle et al the two studies both compared Ziehl-Neelsen staining and Fite Faraco staining by producing PPV and NPV in detecting bacteria Mycobacterium leprae.

Abu Hena Hasanoor Reja et al has a PPV percentage of 69%, NPV 45.7% for Ziehl-Neelsen staining and a PPV percentage of 85.9%, NPV 56.7% for Fite Faraco staining. This study used 165 punch biopsy samples taken from patients with spots or nodules on the skin who had been clinically diagnosed with leprosy with leprosy type, 10 patients with indeterminate type, 27 patients with tuberculoid type, 71 patients with borderline tuberculoid type, 38 patients with borderline type lepramotous, and 19 patients with lepramotous type Ziehl-Neelsen staining has 84 positive samples with 81 negative samples. While Fite Faraco staining 99 samples were positive and 66 samples were negative.[1]

Kamle et al conducted his research using 2 types of samples so that the PPV and NPV obtained were divided into 2, with a total sample of 42 samples that had been clinically diagnosed with leprosy with Ziehl-Neelsen staining and Fite Faraco staining. For skin smear samples it had a PPV of 30.9% and NPV of 64.2%, and for skin biopsy samples it had a PPV of 45.2% and NPV of 52.6% for Ziehl-Neelsen staining. PPV and NPV produced by Fite Faraco staining in this study were PPV 38.1% and NPV 54% in skin smear samples and PPV 61.9%, NPV 33.6% in skin biopsy samples. This means that on Fite Faraco staining the skin biopsy samples had more positive results than the skin smear samples.[2] Study Sunil V et al detect bacteria Mycobacterium Lepraewith 56 samples of skin biopsies taken from leprosy patients with 25 positive samples using Fite Faraco staining which resulted in a PPV of 57.7%.[15]

Although using different samples in this study showed the same results, namely lower PPV than NPV, which means that Ziehl-Neelsen staining and Fite Faraco staining gave more negative results than positive results in detecting Mycobacterium Leprae. As well as Ziehl-Neelsen staining and Fite Faraco staining of skin biopsy samples had results more positive than skin smear samples. This proves that sample selection affects the results on staining.

The impressive thing from the other articles included in this review article is that the PPV results reached a percentage of 100%, this research was conducted by Selfu Girma et alwhich detects bacteria Mycobacterium Lepraeusing Ziehl-Neelsen staining and Fite Faraco staining with 137 samples of skin smears and biopsies of patients who had been clinically diagnosed with leprosy with the PPV and NPV percentages being 100% and 34.3% for Ziehl-Neelsen staining and 100% and 51.8% for Ziehl-Neelsen staining, respectively Fite Faraco.[21]

All reviewed articles show the validity of Ziehl-Neelsen staining with sensitivity between 21%-97.6%, specificity 85.7%, NPV 34.3%-75%, PPV 30.9%-100% in detecting Mycobacterium sp, especially Mycobacterium Tuberculosis and Mycobacterium Leprae tissue samples. Fite Faraco staining showed sensitivity 50%-74.6%, specificity 84% - 100%, NPV 33.6%-56.7%, PPV 38.1% in detecting Mycobacterium sp, especially Mycobacterium Tuberculosis and Mycobacterium Leprae on tissue samples.

In comparison that these two staining can detect Mycobacterium Sp especially bacteria Mycobacterium Tuberculosis and Mycobacterium Leprae in tissue samples, Ziehl-Neelsen staining has a sensitivity of 33%-59.3%, NPV 34.3%, and PPV 100% in detecting bacteria Mycobacterium Leprae. In contrast, Fite Faraco stain has a sensitivity of 50%-61% and a specificity of 84% in detecting bacteria Mycobacterium Tuberculosis.

Both of these stains are good diagnostic tests and screening tests because of the speed in the examination and the cheap price of the examination. In several reviewed articles, it is shown that these two methods also require things that must be considered, such as the research carried out Gehan Mohammed Ahmed et al where heating also affects the staining results and can increase the validity of the coloring method. The validity of this method can also be increased by combining this staining method with other methods such as the IHC disclosed by Crothers et al[(16)] and in combination with PCR as disclosed by Abu Hena Hasanoor Reja et al.[1] Another thing that must be considered in carrying out Ziehl-Neelsen and Fite Faraco staining is the specific type of sample.[1,14,21] which will help detect

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bacteria Mycobacterium Sp especially bacteria Mycobacterium tuberculosis and Mycobacterium Leprae by paying attention to these things we will get good validity results in using the coloring method.

CONCLUSIONS AND SUGGESTIONS

In this review we get the fact that Ziehl-Neelsen stain and Fite Faraco stain can be used to detect bacteria Mycobacterium Sp especially especially bacteria Mycobacterium tuberculosis and Mycobacterium Leprae. However, Ziehl-Neelsen staining has better ability in terms of sensitivity, PPV and NPV than Fite Faraco staining in detecting bacteria Mycobacterium sp especially Mycobacterium Tuberculosis. As well as Fite Faraco staining is superior in terms of specificity. By comparing the results of the sensitivity and specificity of several studies that have been done it is detected that Ziehl-Neelsen staining has better ability than Fite Faraco staining in detecting bacteria Mycobacterium spespecially myco-bacterium tuberculosis. Another thing to watch out for in carrying out Ziehl-Neelsen and Fite Faraco staining is to use a specific type of sample that will help detect bacteria Myco-bacterium Sp especially bacteria Myco-bacterium tuberculosis and Mycobacterium Leprae. Some of the research results reviewed in this article also provide suggestions for modifications such as modification of microwave heating on the Ziehl-Neelsen staining method and combining examinations with H&E staining and Multiplex PCR to increase the validity of the two staining methods which can also improve diagnostic test results and a screening test of this method when used.

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