TECHNOLOGY REVIEW (MINI-HTA)

LIGHT EMITTING DIODE (LED) FLUORESCENT MICROSCOPE IN DETECTING MYCOBACTERIUM LEPRAE

Malaysian Health Technology Assessment Section (MaHTAS)
Medical Development Division
Ministry of Health Malaysia
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Please contact htamalaysia@moh.gov.my if further information is required.

Malaysian Health Technology Assessment Section (MaHTAS)
Medical Development Division
Ministry of Health Malaysia
Level 4, Block E1, Precinct 1
Government Office Complex
62590, Putrajaya
Tel: 603 8883 1229

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AUTHOR

Fatin Nabila Mokhtar
Research Officer
Assistant Director
Malaysian Health Technology Assessment Section (MaHTAS)
Medical Development Division
Ministry of Health Malaysia

REVIEWER

Dr. Izzuna Mudla Mohamed Ghazali
Public Health Physician
Deputy Director
Malaysian Health Technology Assessment Section (MaHTAS)
Medical Development Division
Ministry of Health Malaysia

EXTERNAL REVIEWERS (alphabetical order)

Mr. Amrish Shah Osman
Science Officer (Microbiology)
Leprosy Unit
National Public Health Laboratory
Ministry of Health Malaysia

EXECUTIVE SUMMARY

Introduction

Leprosy (also known as Hansen's disease) is an age-old disease affecting mankind with myriad clinicopathological forms. It is a chronic infectious disease caused by *Mycobacterium leprae*. It manifests in various forms based on the immunological profiles and bacterial load in patients. Leprosy is classified as indeterminate, tuberculoid, borderline tuberculoid, borderline, borderline lepromatous and lepromatous leprosy. More recently for therapy purposes, the World Health Organization (WHO) implemented another classification depending on the number of lesions. Patients with five or less skin lesions are considered as paucibacillary cases whereas those with six or more lesions are regarded as multibacillary.

The earliest symptoms are usually skin lesions that are typically flat, pale (hypopigmented) or reddish (erythematous) spots in the skin with slightly decreased sensitivity to touch or pain. These lesions typically do not present with other symptoms, such as burning or pain. There may be some hair loss in the affected area. As the skin lesions progress, they may become raised and, in some cases, nodules may form. The symptoms of nerve involvement include diminished sensation or feeling in the affected areas (anaesthesia) and, sometimes, burning and tingling sensations (paraesthesias). In more advanced cases, there may be weakness, paralysis, and atrophy of muscle in the hands or feet.

According to WHO Weekly Epidemiological Record on global leprosy update in 2020, the registered prevalence of leprosy (the number of cases on treatment at the end of 2020) was 129192, with a rate of 16.6 per million populations. Globally, 127396 new cases were reported for a case detection rate of 16.4 per million populations. Both figures were much lower than in the previous years, with a 27.7% reduction in registered prevalence and a 37.1% reduction in new cases. This change is probably due to less detection and reporting during the COVID-19 pandemic. The highest proportions of both cases registered for treatment (61.1%) and new cases detected (66.6%) were in South-East Asia Region.

In terms of diagnostic, since *Mycobacterium leprae* cannot be cultivated *in vitro*, clinical signs such as presence of lesions, sensory loss and thickened peripheral nerves serve as the primary tool of leprosy diagnosis. However, the disease can easily be confused with other skin pathologies especially by less experienced physicians. Even though the most popular tools like Ziehl-Neelsen and Fite-Faraco staining are available at lower level health institutions of resources-limited countries, their performance in detecting *Mycobacterium leprae* bacilli is low, particularly in paucibacillary patients. Therefore, for these problematic cases, this highlights the need for more sensitive techniques to support clinical diagnosis. Auramine O staining is a fluorescence-based method widely used to detect mycobacterial species such as *Mycobacterium tuberculosis* and *Mycobacterium leprae* and has been previously evaluated to be more sensitive for detection in tissue sections compared to Fite-Faraco and is less time consuming.

Auramine O staining works along with a light emitting diode (LED) fluorescence microscope. A fluorescence microscope is much the same as a conventional light microscope with added features to enhance its capabilities. The conventional

microscope uses visible light (400-700 nanometers) to illuminate and produce a magnified image of a sample. A fluorescence microscope, on the other hand, uses a much higher intensity light source which excites a fluorescent species in a sample of interest. This fluorescent species in turn emits a lower energy light of a longer wavelength that produces the magnified image instead of the original light source.

This technology review was requested by the National Leprosy Control Programme, Disease Control Division, Ministry of Health Malaysia, to evaluate the efficacy, safety, cost-effectiveness and organisational issues related to the LED fluorescence microscope in detecting *Mycobacterium leprae*.

Objective/aim

To evaluate the efficacy, safety, cost-effectiveness and organisational issues related to the light emitting diode fluorescence microscope in detecting *Mycobacterium leprae*.

Results and conclusions

A total of 1552 titles were retrieved. After removing duplicates, applying inclusion and exclusion criteria, there were five studies reported on LED fluorescence microscope in detecting *Mycobacterium leprae* included in this review; one case-control study and four diagnostic studies conducted in Ethiopia and India.

Based on the review, the outcomes of the LED fluorescence microscope varied depending on the different skin section taken.

The retrievable evidence showed that, the LED fluorescence microscope works along with Auramine O stain, able to visualise more bacillary load and had higher sensitivity rates compared to Fite-Faraco and Ziehl-Neelsen methods.

There was no retrievable study on the safety of LED fluorescence microscope in detecting *Mycobacterium leprae*. According to the Medical Device Authority Malaysia, there were one fluorescent microscope device (Nova View 2.0 Automated Fluorescent Microscope) and four Auramine O stain registered. The devices had also received 510(k) from United States Food and Drug Administration.

There was no study retrieved on cost-effectiveness of LED fluorescence microscope in detecting *Mycobacterium leprae.* The price range of the technologies depended on types, brands and specifications. Meanwhile, the service charge varied depending on their membership or ward status which was provided by the local provider.

Methods

Electronic databases were searched through the Ovid interface; Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R) 1946 to 13 March 2022, Ovid MEDLINE(R) and Epub Ahead of Print, In-Process, In-Data-Review & Other Non-Indexed Citations and Daily 1946 to April 7, 2022, Ovid MEDLINE(R) and In-Process, In-Data-Review & Other Non-Indexed Citations 1946 to April 7, 2022, Ovid MEDLINE(R) and Epub Ahead of Print, In-Process, In-Data-Review & Other Non-Indexed Citations and Daily 2017 to April 7, 2022, Ovid MEDLINE(R) 1946 to March Week 3 2022, Ovid MEDLINE(R) 1996 to March Week 3 2022, Ovid MEDLINE(R) Epub Ahead of Print April 7, 2022, Ovid MEDLINE(R) Daily Update April 7, 2022 and Ovid MEDLINE(R) 2017 to April Week 2 2022. Searches were also run in PubMed,

INAHTA, Cochrane Library and US Food and Drug Administration. Google was used to search for additional web-based materials and information. Additional articles were identified from reviewing the references of retrieved articles. Last search was conducted on 14 April 2022.

TABLE OF CONTENTS

ABBREVIATIONS

CASP	**Critical Appraisal Skills Programme**
COVID-19	**Coronavirus Disease 2019**
LED	**Light Emitting Diode**
USFDA	**United States Food and Drug Administration**
WHO	**World Health Organization**

1.0 BACKGROUND

Leprosy (also known as Hansen's disease) is an age-old disease affecting mankind with myriad clinicopathological forms. It is a chronic infectious disease caused by *Mycobacterium leprae*.[1] It manifests in various forms based on the immunological profiles and bacterial load in patients. Leprosy is classified as indeterminate, tuberculoid, borderline tuberculoid, borderline, borderline lepromatous and lepromatous leprosy.[2] More recently for therapy purposes, the World Health Organization (WHO) implemented another classification depending on the number of lesions. Patients with five or less skin lesions are considered as paucibacillary cases whereas those with six or more lesions are regarded as multibacillary.[3]

The earliest symptoms are usually skin lesions that are typically flat, pale (hypopigmented) or reddish (erythematous) spots in the skin with slightly decreased sensitivity to touch or pain **(see Figure 1)**. These lesions typically do not present with other symptoms, such as burning or pain. There may be some hair loss in the affected area. As the skin lesions progress, they may become raised and, in some cases, nodules may form. The symptoms of nerve involvement include diminished sensation or feeling in the affected areas (anaesthesia) and, sometimes, burning and tingling sensations (paraesthesias). In more advanced cases, there may be weakness, paralysis, and atrophy of muscle in the hands or feet.[4]

According to WHO Weekly Epidemiological Record on global leprosy update in 2020, the registered prevalence of leprosy (the number of cases on treatment at the end of 2020) was 129 192, with a rate of 16.6 per million populations. Globally, 127 396 new cases were reported for a case detection rate of 16.4 per million populations. Both figures were much lower than in the previous years, with a 27.7% reduction in registered prevalence and a 37.1% reduction in new cases. This change is probably due to less detection and reporting during the COVID-19 pandemic. The highest proportions of both cases registered for treatment (61.1%) and new cases detected (66.6%) were in South-East Asia Region.[5]

In terms of diagnostic, since *Mycobacterium leprae* cannot be cultivated *in vitro*, clinical signs such as presence of lesions, sensory loss and thickened peripheral nerves serve as the primary tool of leprosy diagnosis. However, the disease can easily be confused with other skin pathologies especially by less experienced physicians.[3,6,7] Even though the most popular tools like Ziehl-Neelsen and Fite-Faraco staining are available at lower level health institutions of resources-limited countries, their performance in detecting *Mycobacterium leprae* bacilli is low, particularly in paucibacillary patients.[8] Therefore, for these problematic cases, this highlights the need for more sensitive techniques to support clinical diagnosis. Auramine O staining is a fluorescence-based method widely used to detect mycobacterial species such as *Mycobacterium tuberculosis* and *Mycobacterium leprae* and has been previously evaluated to be more sensitive for detection in tissue sections compared to Fite-Faraco and is less time consuming.[9-12] **Figure 2** shows the different appearances of acid fast bacilli that confirm the presence of *Mycobacterium leprae*.

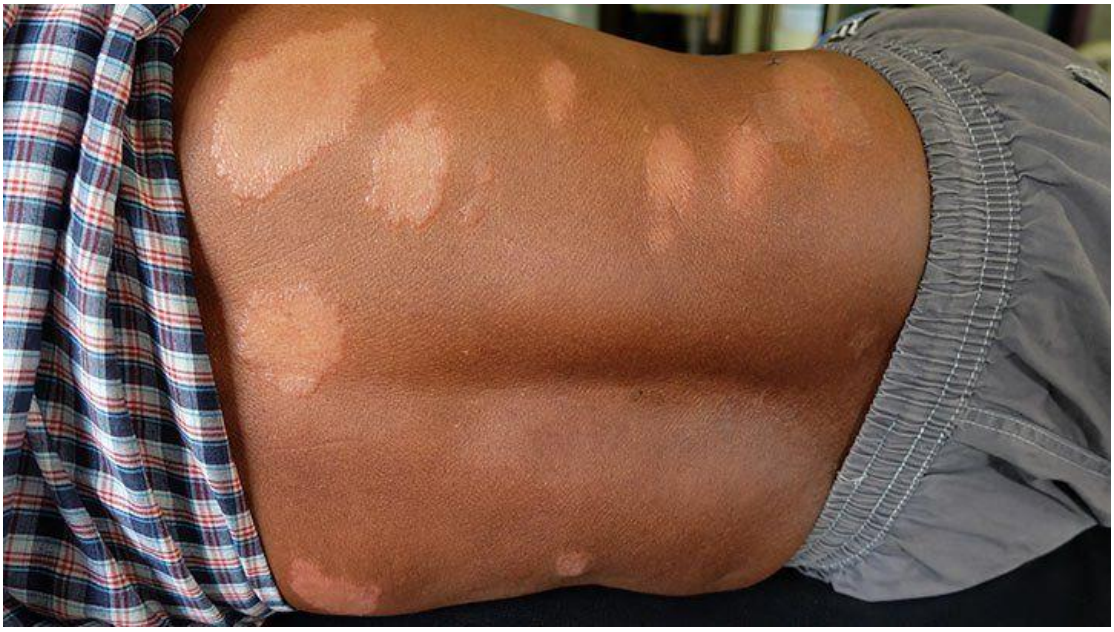
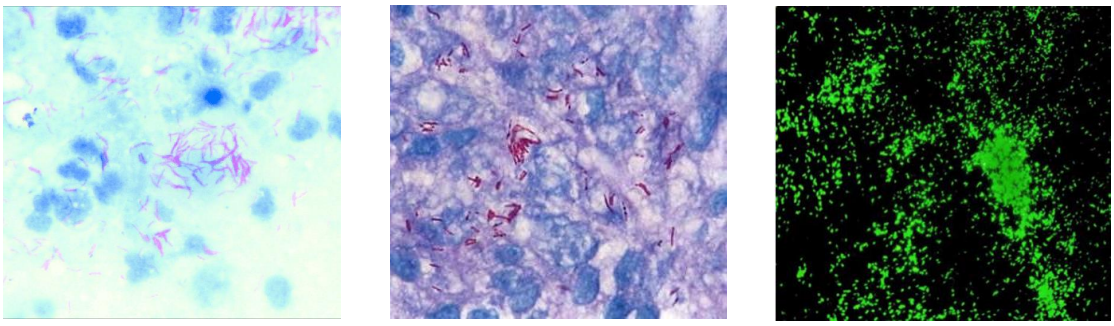


Figure 1: Light or dark spots on the back, arms or legs is a common symptom of leprosy.[13]



(a) (b) (c)

Figure 2: a) Ziehl-Neelsen stained and b) Fite-Faraco stained (acid-fast bacilli stain red/pink, background blue/purple), c) Auromine O stained of a light-emitting diode fluorescence microscope.[14-16]

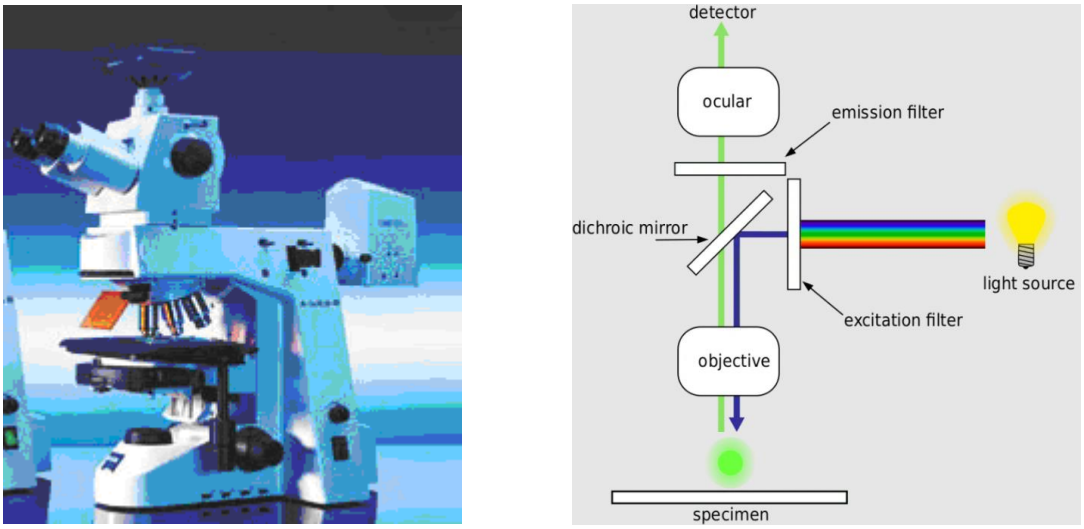
Auramine O staining works along with a light emitting diode (LED) fluorescence microscope. A fluorescence microscope is much the same as a conventional light microscope with added features to enhance its capabilities. The conventional microscope uses visible light (400-700 nanometers) to illuminate and produce a magnified image of a sample. A fluorescence microscope, on the other hand, uses a much higher intensity light source which excites a fluorescent species in a sample of interest. This fluorescent species in turn emits a lower energy light of a longer wavelength that produces the magnified image instead of the original light source.[17]

This technology review was requested by the National Leprosy Control Programme, Disease Control Division, Ministry of Health Malaysia, to evaluate the efficacy, safety, cost-effectiveness and organisational issues related to the LED fluorescence microscope in detecting *Mycobacterium leprae*.

2.0 OBJECTIVE/ AIM

To evaluate the efficacy, safety, cost-effectiveness and organisational issues related to the light emitting diode fluorescence microscope in detecting *Mycobacterium leprae*.

3.0 TECHNICAL FEATURES



(a) (b)

Figure 3: a) A light emitting diode (LED) fluorescence microscope[18], b) working mechanism of an LED fluorescence microscope.[18]

Components of an LED fluorescence microscope **(see Figure 3)**[18]

1. Light source: Xenon arc lamp or mercury-vapor lamp are common; power LED and lasers are used in more advanced forms.
2. A set of optical filters: Optical filters include a set of a compatible excitation filter, emission filter, and dichroic beam splitter;
   a. An excitation filter selects the wavelengths to excite a particular dye within the specimen.
   b. A dichroic beam splitter or dichroic mirror reflects light in the excitation band and transmit light in the emission band, enabling the classic epifluorescence incident light illumination.
   c. An emission filter serves as a kind of quality control by letting only the wavelengths of interest emitted by the fluorophore pass through.
3. Darkfield condenser: It provides a black background against which the fluorescent objects glow.

The filters are often plugged in together in a filter cube (compound microscopes) or in a flat holder (mainly stereo microscopes).

Principle of the working mechanism[18]

The sample should be first labelled with a fluorescent dyes or substance known as a fluorophore (e.g. Auramine O staining) before observing it through a fluorescence

microscope. Higher energy light shorter wavelength of lights (ultraviolet rays or blue light) generated from mercury vapor arc lamp passes through the excitation filter which allows only the short wavelength of light to pass through and removes all other non-specific wavelengths of light. The filtered light is reflected by the dichroic filter and falls on the sample (i.e. fluorophore-labeled). The fluorochrome absorbs shorter wavelength rays and emits rays of longer wavelength (lower energy) that passes through the emission filter. The emission filter blocks (suppresses) any residual excitation light and passes the desired longer emission wavelengths to the detector. Thus the microscope forms glowing images of the fluorochrome-labeled microorganisms against a dark background. From the perspective of an observer, the background is dark as there is no visible light and only the labelled specimen appear bright (fluoresce).

Limitations of fluorescence microscope[18]

Fluorophores gradually lose their ability to fluoresce as they are illuminated in a process called **photobleaching**. Photobleaching can severely limit the time over which a sample can be observed by fluorescence microscopy. However, several techniques exist to reduce photobleaching such as the use of more robust fluorophores, by minimising illumination, or by using photoreactive scavenger chemicals. Even though fluorescence microscopy has enabled analysis of live cells, fluorescent molecules generate reactive chemical species under illumination that enhances the **phototoxic effect**, to which live cells are susceptible. In addition, fluorescence microscopy **only allows observation of the specific structures** which have been labelled for fluorescence. For example, observing a tissue sample prepared with a fluorescent DNA stain by fluorescence microscopy only reveals the organisation of the DNA within the cells and reveals nothing else about the cell morphologies.

4.0 METHODS

4.1 Searching

Electronic databases were searched through the Ovid interface:

- Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R) 1946 to 13 March 2022
- Ovid MEDLINE(R) and Epub Ahead of Print, In-Process, In-Data-Review & Other Non-Indexed Citations and Daily 1946 to April 7, 2021
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Searches were also run in PubMed, INAHTA, Cochrane Library and US Food and Drug Administration. Google was used to search for additional web-based materials

and information. Additional articles were identified from reviewing the references of retrieved articles. Last search was conducted on 14 April 2022.

Appendix 1 shows the detailed search strategies.

4.2 Selection

A reviewer screened the titles and abstracts against the inclusion and exclusion criteria and then evaluated the selected full text articles for final article selection. The inclusion and exclusion criteria were:

Inclusion criteria

Population	Diagnosis of *Mycobacterium leprae*
Interventions	Light emitting diode fluorescence microscope
Comparators	Conventional microscope
Outcomes	**Efficacy:** Sensitivity rate **Cost-analysis**
Study design	Health Technology Assessment (HTA) reports, Systematic Review (SR) and Meta-Analysis, Randomised Control Trial (RCT), Non-randomised Control Trial (RCT), cohort studies, cross-sectional studies, case studies
Type of publication	English, full text articles

Exclusion criteria

Study design	Studies conducted in animals, narrative reviews
Type of publication	Non-English full text articles

Relevant articles were critically appraised using Critical Appraisal Skills Programme (CASP) checklist and evidence graded according to the US/Canadian Preventive Services Task Force (See **Appendix 2**). Data were extracted from included studies using a pre-designed data extraction form (evidence table as shown in **Appendix 3**) and presented in tabulated format with narrative summaries. No meta-analysis was conducted for this review.

5.0 RESULTS

5.1 Selection of the included studies

A total of 1552 titles were retrieved. After removing duplicates, applying inclusion and exclusion criteria, there were five studies reported on LED fluorescence microscope in detecting *Mycobacterium leprae* included in this review; one case-control study and four diagnostic studies as shown in **Figure 4**. The studies were conducted in Ethiopia and India.

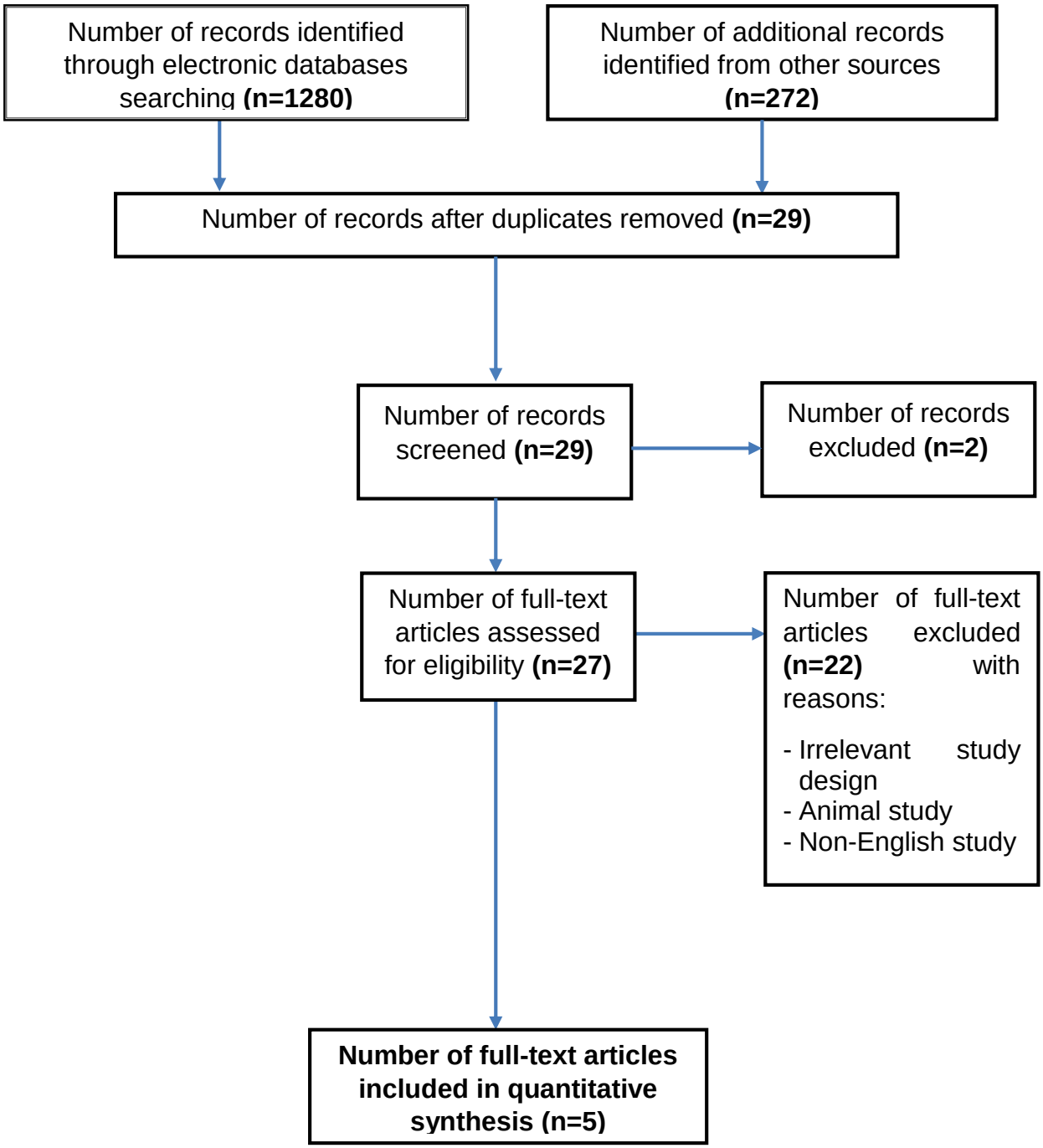

Figure 4: Flow chart of study selection

5.2 Critical appraisal of the included studies

The studies were appraised using the Critical Appraisal Skills Programme (CASP). For the case control study, the yellow judgement showed that there was insufficient number of controls selected compared to the number of cases (case=141, control=28), no potential confounding factors had been taken into account in sensitivity analysis, and no available evidence from randomised controlled trial, systematic review, cohort study and other case-control study to be compared. However, the appraisal of the literature indicated that the literature in this review was of acceptable relevance **(see Figure 5)**.

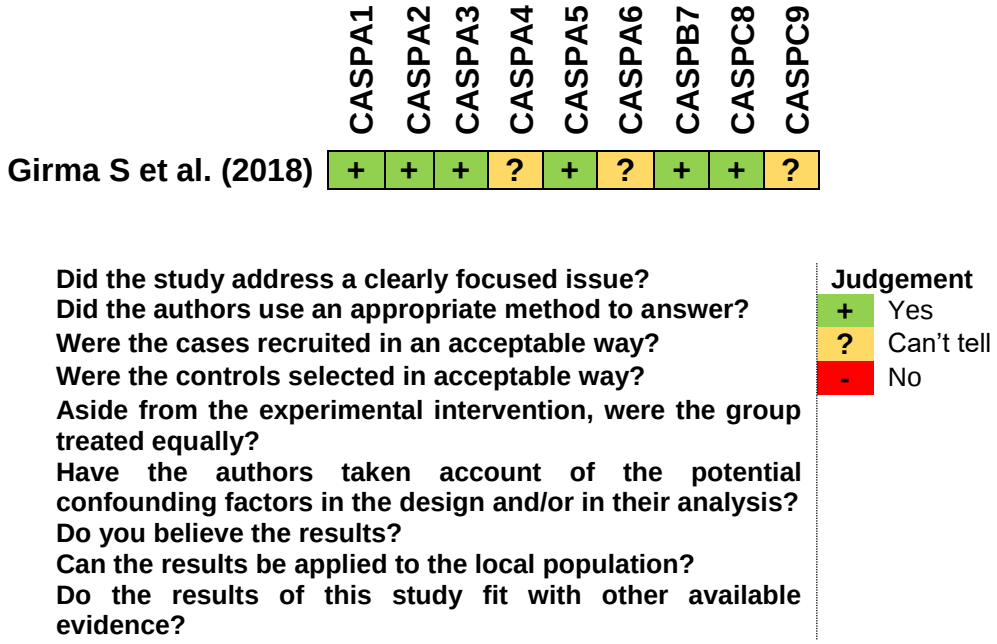

Figure 5: Assessment of risk of bias of case control

Meanwhile for the diagnostic studies, the results of the appraisal were outlined in **Figure 6**. From the CASP appraisal, three literature reviews shared the same score. The red judgement (CASPA4) indicated there was blinding process in the studies and the tests were performed independently. Therefore, it showed that the results had not been influenced by the results of the reference standard. In contrast, one study appeared there was no blinding and no independent test, thus the results may have high risk of bias.

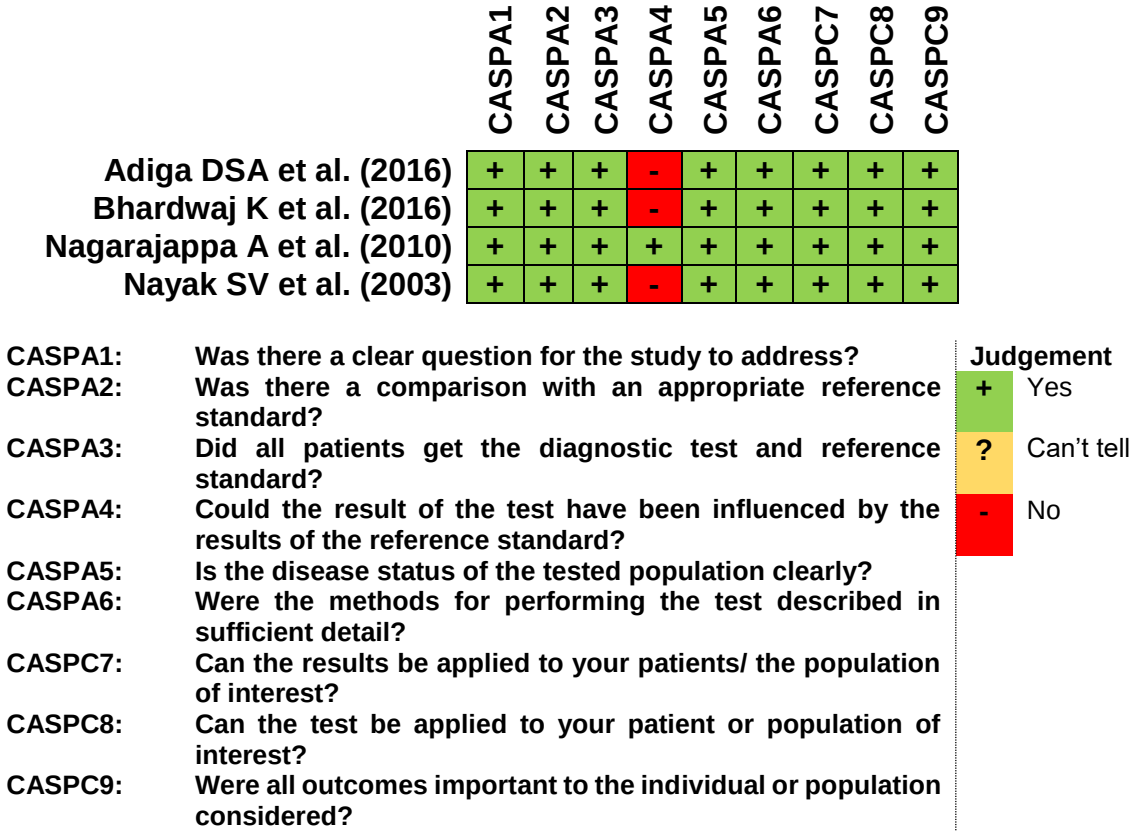

Figure 6: Assessment of risk of bias of diagnostic studies

5.3 Efficacy/ Effectiveness

There were one case-control study and four diagnostic studies on the efficacy of LED fluorescence microscope in detecting *Mycobacterium leprae*.

A case control study by Girma et al. (2018) was conducted to assess the performance of the fluorescent Auramine O staining and polymerase chain reaction (PCR) with different skin samples using combination of Ziehl-Neelsen, Fite-Faraco and Haematoxylin & Eosin staining as the gold standard. A total of 141 leprosy cases comprising 136 newly diagnosed treatment and five relapse leprosy patients with any form of the disease were enrolled at the ALERT center from January 2015 to April 2016. All cases were clinically diagnosed and confirmed by a dermatologist. Non-leprosy patients (n=28) visiting the minor surgery department of the ALERT hospital were enrolled in the study as a control group. These patients did not present signs of leprosy.[16, level II-2]

The study reported that **the sensitivity of Auramine O in slit skin smear (45.4%) was slightly higher than Ziehl-Neelsen (32.7%, p<0.05)** whereas the sensitivity of Auramine O in tissue (60%) was similar to Fite-Faraco (61.8%, p<0.05) but statistically lower than PCR (83.6%, p<0.05).[16, level II-2]

Bhardwaj et al. (2016) conducted a diagnostic study to compare conventional modified Fite-Faraco based detection of lepra bacilli detection with Auramine stain-based

fluorescence microscopy. One hundred eighteen skin biopsies were obtained from patients clinically diagnosed as leprosy. Disease was classified into indeterminate (n=28), tuberculoid (n=2), borderline tuberculoid (n=67), mid borderline (n=2), borderline lepromatous (n=13) and lepromatous (n=6). Each biopsy was stained by Auramine and modified Fite-Faraco. The sections were screened for the detection of lepra bacilli. Sections stained by Auramine was seen under fluorescent microscope which showed bright yellow rods against dark background.[10, level II-3]

The result showed, **out of 112 biopsies, 73 (65.2%) were positive by Auramine while only 39 (34.8%) by modified Fite-Faraco (see Table 1)**.[10, level II-3]

Table 1: Positivity rates according to histopathological diagnosis.

Histopathological Diagnosis	Total no. of samples	Auramine (per total no. of samples)	Modified Fite-Faraco (per total no. of samples)
Indeterminate	28	12 (42.9%)	2 (7.1%)
Borderline tuberculoid	67	42 (62.7%)	20 (29.9%)
Borderline lepromatous	13	13 (100%)	11 (84.6%)
Lepromatous	6	6 (100%)	6 (100%)

Bhardwaj K, Ghate S, Dhurat R. *Detection of Mycobacterium Leprae in Tissue Sections Using Auramine O Fluorescent Stain versus Modified Fite-Faraco: a Comparative Study.* Internal Journal of Infectious Diseases. 2016; 1-477. Doi: http://dx.doi.org/10.1016/j.ijid.2016.02.830

Nagarajappa et al. (2010) in another diagnostic study compared modified Fite-Faraco method with fluorescent dye (Auramine-Rhodamine) method, to detect *Mycobacterium leprae* bacilli in tissue sections. Seventy patients clinically suspected of leprosy were studied for a period of two-years duration. The disease was classified based on clinical features, histopathological findings, slit skin smears and modified Fite-Faraco method. Two sections were taken for routine Haematoxylin and Eosin staining and five each for fluorescent and Fite-Faraco stain. For fluorescent staining, sections were taken on clean scratch free glass slides without egg albumin or any other adhesive. These tissue sections were stained with fluorescent dye (Auramine-Rhodamine) with minor alterations in deparaffinisation. The study reported that **positivity rate of fluorescent stain was superior to modified Fite-Faraco**. Moreover, the sensitivity of fluorescent stain in indeterminate leprosy, tuberculoid leprosy, borderline tuberculoid leprosy and mid borderline leprosy was 100%.[12, level II-3]

Another diagnostic study conducted by Nayak et al. (2003), also compared modified Fite-Faraco method with fluorescent dye method, to detect *Mycobacterium leprae* bacilli in tissue sections. In this study, fifty-six patients from the outpatient department of Victoria Hospital and Bowring and Lady Curzon Hospital (Bangalore, India), clinically suspected of having leprosy (fresh cases) from April 2001 to March 2002 were the subjects of this study. Sterile disposable 5 mm punches were used to take punch biopsies from the active lesion. The procedure on fluorescent stain was done as Nagarajappa et al. (2010). The study showed, **39 (69.6%) biopsies were positive by the fluorescent method and 25 (44.6%) were positive by the modified Fite-Faraco method**. The bacillary positivity rates in each type of leprosy by both methods are also depicted in **Table 2**.[11, level II-3]

Table 2: Comparison of fluorescent method and modified Fite-Faraco method in detecting *Mycobacterium leprae* bacilli in tissue sections.

		Modified Fite-Faraco Method			Fluorescent Method		
Leprosy Type	No. of Cases	Positive	Positivity Rate	Negative	Positive	Positivity Rate	Negative
Indeterminate	25	5	20%	20	13	52%	12
Tuberculoid	9	4	44.4%	5	7	77.7%	2
Borderline tuberculoid	18	12	66.6%	6	15	83.3%	3
Lepromatous	4	4	100%	0	4	100%	0
	56	25		31	39		17

Note: There were no borderline lepromatous leprosy and mid borderline leprosy cases in the study.

Nayak SV, Shivarudrappa AS, Mukkamil AS. *Role of Fluorescent Microscopy in Detecting Mycobacterium leprae in Tissue Sections.* Annals of diagnostic pathology. 2003; 7: 78–81. https://doi.org/10.1053/adpa.2003.50012 PMID: 12715331

Adiga et al. (2016) conducted a diagnostic study to compare the efficacy of Auramine-Rhodamine stain with Ziehl-Neelsen and modified Fite-Faraco staining in diagnosing *Mycobacterium leprae* in tissue sections. The study was retrospective, spanning four years, from July 2006 to June 2010 at Shri BM Patil Medical College, Bijapur including a total of sixty skin biopsies from patients clinically diagnosed as leprosy. For fluorescent staining ribbons containing four to five serial sections were taken on clean scratch free slides. No adhesives like egg albumin were used. For each batch of sections that were stained, sections from a skin biopsy of a typical lepromatous leprosy patient and a skin biopsy from a normal individual were used as controls.[8]

The study showed the fluorescent stain in indeterminate leprosy cases was significantly more positive than that with Ziehl-Neelsen or Fite-Faraco stain **(see Table 3)**. Furthermore, fluorescent method retained good (r=0.73) and statistically significant correlation (p<0.0001) even at low bacillary loads. Thus, **fluorescent method was more sensitive in detecting lepra bacilli in cases with low bacillary load** (BI<3). Fluorescent stain also showed 100% sensitivity as against Ziehl-Neelsen which showed only 75% sensitivity compared to Fite-Faraco method. Among paucibacillary and multibacillary cases, fluorescent stain showed a higher bacteriological index compared to Fite-Faraco.[8]

Table 3: Comparison of positivity rates of Ziehl-Neelsen, modified Fite-Faraco and fluorescent stains. IL (indeterminate leprosy), TT (tuberculoid leprosy), BT (borderline tuberculoid leprosy), BB (mid borderline leprosy), BL (borderline lepromatous leprosy), LL (lepromatous leprosy).[8]

Histopathological Diagnosis	Total No. of Patients	ZN Stain	Modified Fite-Faraco Stain	Fluorescent Stain
		Positivity Rate n (%)	Positivity Rate n (%)	Positivity Rate n (%)
IL	30	1 (3.3)	1 (3.3)	8 (26.7)
TT	2	0	0	0
BT	14	2 (14.3)	4 (28.6)	4 (28.6)
BB	0	0	0	0
BL	2	1 (50)	2 (100)	2 (100)
LL	12	12 (100)	12 (100)	12 (100)
Total	60	16 (26.7)	19 (31.7)	26 (43.3)

Adiga DSA, Hippargi SB, Rao G et al. *Evaluation of Fluorescent Staining for Diagnosis of Leprosy and its Impact on Grading of the Disease: Comparison with Conventional Staining.* Journal of Clinical and Diagnostic Research. 2016; 10(10): 23-26. Doi: 0.7860/JCDR/2016/22470.8739.

The efficacy was summarised as follow:

Table 4: The sensitivity rate of LED fluorescence microscope in detecting *Mycobacterium leprae*

Outcome	Section	Remarks
Higher rate compared to conventional microscope	Slit skin smear	Auramine O (45.4%) versus Ziehl-Neelsen (32.7%), p<0.05[16, level II-2]
	Tissue	Auramine O (73;62.5%) versus Fite-Faraco (39;34.8%)[10, level II-3]
	Tissue	Auramine O (39;69.6%) versus Fite-Faraco (25;44.6%)[11, level II-3]
	Tissue	With low bacillary load (Bacteriological Index<3), p<0.0001[8]
100% sensitivity in LED fluorescent microscope	Tissue	In indeterminate leprosy, tuberculoid leprosy, borderline tuberculoid leprosy and mid borderline leprosy[12, level II-3]
	Tissue	Against Ziehl-Neelsen[8]

5.4 Safety

There was no retrievable study on the safety of LED fluorescence microscope in detecting *Mycobacterium leprae*. According to the Medical Device Authority Malaysia, there were one fluorescent microscope device (Nova View 2.0 Automated Fluorescent

Microscope) and four Auramine O stain devices registered.[19] The devices had also received 510(k) from United States Food and Drug Administration.[20]

5.5 Cost-analysis/ Cost-effectiveness

There was no study retrieved on cost-effectiveness of LED fluorescence microscope in detecting *Mycobacterium leprae*. However, there were price range of the LED fluorescent microscope and Auramine O stains, and service charge by the facilities that provide services related to this technology **(see Table 5)**.

Table 5: Equipment rental rate and service charges.[21-26]

Price of LED Fluorescent Microscope	$2,400* (RM11,316) to $21,000* (RM99,015) • depend on the specifications and customisations
Price of Auramine O Stains	$10* (RM47.15) to $1433.60* (RM6759.42) • depend on the manufacturers • sold by per mass value: eg. µg/ g/ Kg
Service Charge	RM6 to RM60 • depend on membership status and ward status
Local Provider	Hospital Tengku Ampuan Rahimah (HTAR), Hospital Universiti Sains Malaysia (HUSM), Hospital Pengajar Universiti Putra Malaysia (HPUPM), Universiti Kebangsaan Malaysia (UKM)

*United States Dollar

5.6 Organisational Issue

There was no retrievable study on the organisational issue of LED fluorescence microscope in detecting *Mycobacterium leprae*.

5.7 Limitations

This technology review has a limitation. Although there was no restriction in language during the search but only English full text articles were included in this review.

6.0 CONCLUSION

Based on the review, the outcomes of the LED fluorescence microscope varied depending on the different skin section taken.

The retrievable evidence showed that, the LED fluorescence microscope works along with Auramine O stain, able to visualise more bacillary load and had higher sensitivity rates compared to Fite-Faraco and Ziehl-Neelsen methods.

There was no retrievable study on the safety of LED fluorescence microscope in detecting *Mycobacterium leprae*. According to the Medical Device Authority Malaysia, there were one fluorescent microscope device (Nova View 2.0 Automated Fluorescent Microscope) and four Auramine O stain registered. The devices had also received 510(k) from United States Food and Drug Administration.

There was no study retrieved on cost-effectiveness of LED fluorescence microscope in detecting *Mycobacterium leprae.* The price range of the technologies depended on types, brands and specifications. Meanwhile, the service charge varied depending on their membership or ward status which was provided by the local provider.

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8.0 APPENDICES

8.1 Appendix 1: Search strategy

Ovid MEDLINE® In-Process & Other Non-Indexed Citations and Ovid MEDLINE® 1946 to 13 March 2022

1 MYCOBACTERIUM LEPRAE/ (5821)
2 mycobacterium leprae.tw. (4406)
3 txid1769.tw. (0)
4 LEPROSY/ (20441)
5 hansen disease.tw. (106)
6 hansen's disease.tw. (1045)
7 leprosy.tw. (21193)
8 1 or 2 or 3 or 4 or 5 or 6 or 7 (27814)
9 MICROSCOPY, FLUORESCENCE/ (80255)
10 fluorescence microscop*.tw. (37214)
11 immunofluorescence microscop*.tw. (10505)
12 STAINING.mp. and LABELING/ [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms] (0)
13 histological labeling*.tw. (11)
14 labeling.mp. and staining.tw. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms] (39696)
15 staining.mp. and labeling.tw. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms] (18053)
16 staining*.tw. (333624)
17 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 (439175)
18 8 and 17 (449)

OTHER DATABASES	
Ovid MEDLINE(R) and Epub Ahead of Print, In-Process, In-Data-Review & Other Non-Indexed Citations and Daily 1946 to April 7, 2022	Same MeSH, keywords, limits used as per MEDLINE search
Ovid MEDLINE(R) and In-Process, In-Data-Review & Other Non-Indexed Citations 1946 to April 7, 2022	
Ovid MEDLINE(R) and Epub Ahead of Print, In-Process, In-Data-Review & Other Non-Indexed Citations and Daily 2017 to April 7, 2022	
Ovid MEDLINE(R) 1946 to March Week 3 2022	
Ovid MEDLINE(R) 1996 to March Week 3 2022	
Ovid MEDLINE(R) Epub Ahead of Print April 7, 2022	
Ovid MEDLINE(R) Daily Update April 7, 2022	
Ovid MEDLINE(R) 2017 to April Week 2 2022	
Cochrane Library	

PubMeD

(((((((MYCOBACTERIUM LEPRAE[MeSH Terms]) OR (LEPROSY[MeSH Terms])) OR (mycobacterium leprae[Text Word])) OR (txid1769[Text Word])) OR (hansen disease[Text Word])) OR (hansen's

disease[Text Word])) OR (leprosy[Text Word])) AND (((((((MICROSCOPY, FLUORESCENCE[MeSH Terms]) OR (STAINING AND LABELING[MeSH Terms])) OR (fluorescence microscop[Text Word])) OR (immunofluorescence microscop[Text Word])) OR (histological labeling[Text Word])) OR (labeling[Text Word] AND staining[Text Word])) OR (staining[Text Word] AND labeling[Text Word])) OR (staining[Text Word]))

8.2 Appendix 2: Hierarchy of evidence for effectiveness/ diagnostic

I Evidence obtained from at least one properly designed randomised controlled trial.

II-I Evidence obtained from well-designed controlled trials without randomization.

II-2 Evidence obtained from well-designed cohort or case-control analytic studies, preferably from more than one centre or research group.

II-3 Evidence obtained from multiple time series with or without the intervention. Dramatic results in uncontrolled experiments (such as the results of the introduction of penicillin treatment in the 1940s) could also be regarded as this type of evidence.

III Opinions or respected authorities, based on clinical experience; descriptive studies and case reports; or reports of expert committees.

SOURCE: US/CANADIAN PREVENTIVE SERVICES TASK FORCE (Harris 2001)

8.3 Appendix 3: Evidence tables

Evidence Table : Efficacy
Question : What is the effectiveness of Light Emitting Diode (LED) Fluorescent Microscope in Detecting Mycobacterium Leprae?

Bibliographic citation	Study Type / Methodology	LE	Number of patients and patient characteristics	Intervention	Comparison	Length of follow up (if applicable)	Outcome measures/ Effect size	General comments
1. Girma S, Avanzi C, Bobosha K et al. Evaluation of Auramine O Staining and Conventional PCR for Leprosy Diagnosis: a Comparative Cross-sectional Study from Ethiopia. PLoS Negl Trop Dis. 2018; 12(9): e0006706. Doi: https://doi.org/10.1371/journal.pntd.0006706 ETHIOPIA	**Case control study** **Objective:** To assess the performance of the fluorescent Auramine O staining and polymerase chain reaction (PCR) with different skin samples using combination of Ziehl Neelsen, Fite-Faraco and Haematoxylin & Eosin staining as the gold standard. **Method:** A total of 141 leprosy cases comprising 136 newly diagnosed treatment naïve and five relapse leprosy patients with any form of the disease were enrolled in this prospective comparative cross-sectional study at the ALERT center from January 2015 to April 2016. All cases were clinically diagnosed and confirmed by a dermatologist. Non-	II-3	141 leprosy cases 28 non-leprosy case (control)	Fluorescent Auramine O staining	Ziehl Neelsen, Fite-Faraco and Haematoxylin & Eosin staining	-	**Auramine O staining:** On analyses of the 137 slit skin smear, the sensitivity of Auramine O in skin smear examination (65.5%) was slightly higher ($p<0.05$) than Ziehl Neelsen (59.3%) while specificity was 100% for both tests. The sensitivity and specificity of 137 tissue sections stained with Fite-Faraco staining were 77% and 100%, respectively, while other statistical parameters, positive predictive value and negative predictive value were 100% and 51.8%, respectively. Sensitivity and specificity of Auramine O-tissue staining were similar ($p<0.05$) to Fite-Faraco with 77.9% and 100%, respectively, using the established gold standard method. The overall sensitivity of both Auramine O in tissue and Fite-Faraco was significantly higher ($p<0.05$) than Auramine O in slit skin smear and Ziehl Neelsen. In addition, sensitivity of the different tests was higher in the form lepromatous leprosy, borderline lepromatous and borderline than tuberculoid, borderline tuberculoid and indeterminate leprosy ($p<0.05$) as expected since the number of bacilli was higher in the first three forms. The sensitivity of Auramine O in slit skin smear (45.4%) was slightly higher than Ziehl Neelsen (32.7%, $p<0.05$) whereas the sensitivity of Auramine O in tissue (60%)	

Evidence Table : Efficacy
Question : What is the effectiveness of Light Emitting Diode (LED) Fluorescent Microscope in Detecting Mycobacterium Leprae?

Bibliographic citation	Study Type / Methodology	LE	Number of patients and patient characteristics	Intervention	Comparison	Length of follow up (if applicable)	Outcome measures/ Effect size	General comments
	leprosy patients (n = 28) visiting the minor surgery department of the ALERT hospital were enrolled in the study as a control group. These patients did not present signs of leprosy.						was similar to Fite-Faraco (61.8%, $p<0.05$) but statistically lower than PCR (83.6%, $p<0.05$).	

Evidence Table : Efficacy
Question : What is the effectiveness of Light Emitting Diode (LED) Fluorescent Microscope in Detecting Mycobacterium Leprae?

Bibliographic citation	Study Type / Methodology	LE	Number of patients and patient characteristics	Intervention	Comparison	Length of follow up (if applicable)	Outcome measures/ Effect size	General comments
2. Bhardwaj K, Ghate S, Dhurat R. Detection of Mycobacterium Leprae in Tissue Sections Using Auramine O Fluorescent Stain versus Modified Fite-Faraco: a Comparative Study. Internal Journal of Infectious Diseases. 2016; 1-477. Doi: http://dx.doi.org/10.1016/j.ijid.2016.02.830 INDIA	**Diagnostic study** **Objective:** To compare conventional Modified Fite-Faraco based detection of lepra bacilli detection with Auramine stain-based fluorescence microscopy. **Method:** One hundred eighteen skin biopsies was obtained from patients clinically diagnosed as leprosy. Disease was classified into Indeterminate (I)(n=28), Tuberculoid (TT=2), Borderline tuberculoid (BTH,n=67), Borderline borderline (BB,n=2), Borderline Lepromatous (BL)(n=13) and Lepromatous (LL,n=6). Each biopsy was stained by Auramine and Modified Fite-Faraco. The sections were screened for the detection of lepra bacilli. Sections stained by Auramine was seen under Fluorescent microscope	II-3	118 leprosy cases	Fluorescent Auramine staining	Modified Fite-Faraco	-	Out of 112 biopsies, 73 were positive by Auramine while only 39 by Modified Fite- faraco. Out of 28 Indeterminate cases 12(42.9%) showed bacilli by Auramine and 2(7.1%) by Modified Fite-Faraco. 42(62.7%) out of 67, and 20(29.9%) out of 67 BTH patients were positive by Auramine and Modified Fite-Faraco respectively. Biopsies from BB were positive by both the methods. In BL/LL Modified Fite-Faraco detected bacilli in 11 out of 13(84.6%) and all 6(100%) respectively while Auramine detected bacilli in all 19(100%)BL/LL biopsies.	

Evidence Table : Efficacy
Question : What is the effectiveness of Light Emitting Diode (LED) Fluorescent Microscope in Detecting Mycobacterium Leprae?

Bibliographic citation	Study Type / Methodology	LE	Number of patients and patient characteristics	Intervention	Comparison	Length of follow up (if applicable)	Outcome measures/ Effect size	General comments
	which showed bright yellow rods against dark background.							

Evidence Table : Efficacy
Question : What is the effectiveness of Light Emitting Diode (LED) Fluorescent Microscope in Detecting Mycobacterium Leprae?

Bibliographic citation	Study Type / Methodology	LE	Number of patients and patient characteristics	Intervention	Comparison	Length of follow up (if applicable)	Outcome measures/ Effect size	General comments
3. Nagarajappa A, Prabhu D. Sensitivity of Fluorescent Microscopy in Detecting Mycobacterium Leprae in Tissue Sections. The Internet Journal of Pathology. 2010; 11(2). INDIA	**Diagnostic study** **Objective:** To compare modified Fite-Faraco method with fluorescent dye (Auramine-Rhodamine) method, to detect mycobacterium leprae bacilli in tissue sections. **Method:** Seventy patients clinically suspected of leprosy were studied for a period of two-years duration. The disease was classified based on clinical features, histopathological findings, slit skin smears and modified Fite-faraco method. Two sections were taken for routine haematoxylin and eosin staining and five each for fluorescent and Fite-faraco stain. For fluorescent staining, sections were taken on clean scratch free glass slides without egg albumin or any other adhesive. These tissue sections were stained with fluorescent dye	II-3	70 leprosy cases	Fluorescent Auramine-Rhodamine	Modified Fite-Faraco	-	Comparative analysis of positivity rates of modified Fite-faraco stain and fluorescent stain on histological diagnosis showed that positivity rate of fluorescent stain was superior to modified Fite-faraco. Sensitivity of fluorescent stain in indeterminate leprosy, tuberculoid leprosy, borderline tuberculoid leprosy and mid borderline leprosy was 100%.	

Evidence Table : Efficacy
Question : What is the effectiveness of Light Emitting Diode (LED) Fluorescent Microscope in Detecting Mycobacterium Leprae?

Bibliographic citation	Study Type / Methodology	LE	Number of patients and patient characteristics	Intervention	Comparison	Length of follow up (if applicable)	Outcome measures/ Effect size	General comments
	(Auramine-Rhodamine) with minor alterations in deparaffinization							

Evidence Table : Efficacy
Question : What is the effectiveness of Light Emitting Diode (LED) Fluorescent Microscope in Detecting Mycobacterium Leprae?

Bibliographic citation	Study Type / Methodology	LE	Number of patients and patient characteristics	Intervention	Comparison	Length of follow up (if applicable)	Outcome measures/ Effect size	General comments
4. Nayak SV, Shivarudrappa AS, Mukkamil AS. Role of Fluorescent Microscopy in Detecting Mycobacerium Leprae in Tissue Sections. Annals of Diagnostic Pathology. 2003; 7(2): 78-81. INDIA	**Diagnostic study** **Objective:** To compare modified Fite-Faraco method with fluorescent dye method, to detect mycobacterium leprae bacilli in tissue sections. **Method:** Fifty-six patients from the outpatient department of Victoria Hospital and Bowring and Lady Curzon Hospital (Bangalore, India), clinically suspected of having leprosy (fresh cases) from April 2001 to March 2002 were the subjects of this study. Sterile disposable 5 mm punches were used to take punch biopsies from the active lesion. For fluorescent staining ribbons containing five serial sections were taken on clean, scratch-free glass slides without egg albumin or any other adhesive. Rhodamine-Auramine fluorescent stain was used.	II-3	56 leprosy cases	Fluorescent Auramine-Rhodamine	Modified Fite-Faraco	-	Thirty-nine biopsies were positive by the fluorescent method and 25 were positive by the modified FiteFaraco method. The bacillary positivity rates in each type of leprosy by both methods are also depicted in Table 1.	

Table 1. Comparison of Fluorescent Method and Modified Fite-Faraco Method in Detecting *Mycobacterium leprae* Bacilli in Tissue Sections

		Modified Fite-Faraco Method			Fluorescent Method		
Leprosy Type	No. of Cases	Positive	Positivity Rate	Negative	Positive	Positivity Rate	Negative
Indeterminate	25	5	20%	20	13	52%	12
Tuberculoid	9	4	44.4%	5	7	77.7%	2
Borderline tuberculoid	18	12	66.6%	6	15	83.3%	3
Lepromatous	4	4	100%	0	4	100%	0
	56	25		31	39		17

NOTE. There were no borderline lepromatous leprosy and midborderline leprosy cases in our study.

Evidence Table : Efficacy
Question : What is the effectiveness of Light Emitting Diode (LED) Fluorescent Microscope in Detecting Mycobacterium Leprae?

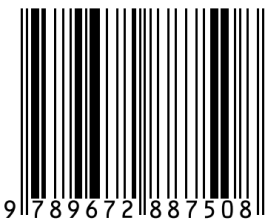
Bibliographic citation	Study Type / Methodology	LE	Number of patients and patient characteristics	Intervention	Comparison	Length of follow up (if applicable)	Outcome measures/ Effect size	General comments
5. Adiga DSA, Hippargi SB, Rao G et al. Evaluation of Fluorescent Staining for Diagnosis of Leprosy and its Impact on Grading of the Disease: Comparison with Conventional Staining. Journal of Clinical and Diagnostic Research. 2016; 10(10): 23-26. Doi: 0.7860/JCDR/ 2016/22470.8739. INDIA	**Diagnostic study** **Objective:** To compare the efficacy of Auramine-Rhodamine stain with Ziehl Neelsen and modified Fite-Faraco staining in diagnosing mycobacterium leprae in tissue sections. **Method:** The current study was retrospective one, spanning four years, from July 2006 to June 2010 at Shri BM Patil Medical College, Bijapur including a total of sixty skin biopsies from patients clinically diagnosed as leprosy. For fluorescent staining ribbons containing four to five serial sections were taken on clean scratch free slides. No adhesives like egg albumin were used. For each batch of sections that were stained, sections from a skin biopsy of a typical lepromatous leprosy patient and a skin biopsy	II-3	60 leprosy cases	Fluorescent Auramine-Rhodamine	Modified Fite-Faraco, Ziehl Neelsen	-	Fluorescent stain in indeterminate leprosy cases was significantly more positive than that with Ziehl Neelsen or Fite-Faraco stain (Table/ Fig-2). **[Table/Fig-2]:** Comparison of positivity rates of ZN (Ziehl Neelsen), Modified Fite-Faraco and fluorescent stains. IL- Indeterminate Leprosy, TT- Tuberculoid Leprosy, BT- Borderline Tuberculoid Leprosy, BB- Mid Borderline Leprosy, BL- Borderline Lperomatous Leprosy, LL- Lepromatous Leprosy. Ziehl-Neelsen method correlated well (r=0.89) with Fite-Faraco method at higher bacteriological index (>3) but poorly and insignificantly (p=0.81) with lower bacteriological index (<3). However, fluorescent method retains good (r=0.73) and statistically significant correlation (p<0.0001) even at low bacillary loads. Thus, fluorescent method is more sensitive in detecting lepra bacilli in cases with low bacillary load (BI<3). Fluorescent stain showed 100% sensitivity as against Ziehl Neelsen which showed only 75% sensitivity compared to Fite-Faraco method. Among paucibacillary cases, fluorescent stain showed a higher bacteriological index compared to Fite-Faraci	

Histopathological Diagnosis	Total No. of Patients	ZN Stain	Modified Fite-Faraco Stain	Fluorescent Stain
		Positivity Rate n (%)	Positivity Rate n (%)	Positivity Rate n (%)
IL	30	1 (3.3)	1 (3.3)	8 (26.7)
TT	2	0	0	0
BT	14	2 (14.3)	4 (28.6)	4 (28.6)
BB	0	0	0	0
BL	2	1 (50)	2 (100)	2 (100)
LL	12	12 (100)	12 (100)	12 (100)
Total	60	16 (26.7)	19 (31.7)	26 (43.3)

Evidence Table : Efficacy
Question : What is the effectiveness of Light Emitting Diode (LED) Fluorescent Microscope in Detecting Mycobacterium Leprae?

Bibliographic citation	Study Type / Methodology	LE	Number of patients and patient characteristics	Intervention	Comparison	Length of follow up (if applicable)	Outcome measures/ Effect size	General comments
	from a normal individual were used as controls.						in nine cases, while among multibacillary cases, only one additional case had a higher bacteriological index compared to Fite-Faraco. No net additional case could be detected by Ziehl Neelsen stain compared to Fite-Faraco. Ziehl Neelsen stain showed a lesser bacteriological index compared to Fite-Faraco among seven multibacillary cases.	

LIGHT EMITTING DIODE (LED) FLUORESCENT MICROSCOPE INDETECTING MYCOBACTERIUM LEPRAE



CAWANGAN PENILAIAN TEKNOLOGI KESIHATAN (MAHTAS)