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CAT
Critically Appraised Topic

Sputum smear microscopy in the diagnosis of pulmonary tuberculosis: What are the options anno 2014

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CLINICAL/DIAGNOSTIC SCENARIO

Tuberculosis was a disease known to the ancients, and has plagued humankind throughout known history and human prehistory. Formerly known as 'phthysis', Schönlein introduced 'tuberculosis' in 1839 where he indicated the disease resulting from the infection with the tubercle, which was described earlier by Sylvius, in 1650. The first major break-through however, was by Jean-Antoine Villemin (1827-1892), who showed in 1865 by animal experiments that tuberculosis was a contagious disease (1,2). Almost two decades later, on 24 March 1882 Robert Koch announced the discovery of the responsible microorganism: the tubercle bacillus. The weeks following that moment, the news spread around the world and Koch became, almost overnight, a household name, and 'Koch's bacillus' and 'Koch's disease' entered medical jargon (3).

Anno 2014, tuberculosis continues to be the world's most important infectious cause of mortality and morbidity among adults. More than 2 billion people (about one-third of the world population) are estimated to be infected with Mycobacterium *tuberculosis* and yearly about 1.6 million people die from it (5). Despite this enormous global burden, case detections are low, posing an enormous hurdle for tuberculosis control (5). Although much work is currently being conducted in order to develop new diagnostics, the diagnosis of pulmonary tuberculosis relies -especially in low-resource countries- primary on sputum smear microscopy. This century-old technique has evolved and been adjusted from its first introduction by Koch in 1882, until now. This has left us nowadays with several options and methods for smear-staining, each with its own benefits and disadvantages.
The first part of this CAT will guide you through the most recent literature in order to provide an up-to-date recommendation concerning which staining method should be used in which setting for the diagnosis of pulmonary tuberculosis anno 2014. The second part describes the results of our field study, where three relevant staining techniques (Kinyoun, auramine O and acridine orange staining) were compared to each other in a prospective setting.

QUESTION(S)

1) *What are the current available and recommended staining methods for sputum smear microscopic examination in the diagnosis of tuberculosis?*
2) *What are the results of our own study, were three well-established staining methods are compared to each other?*

SEARCH TERMS

1) *MeSH Database (PubMed): MeSH term: "tuberculosis diagnosis, sputum smear staining, Ziehl-Neelsen, Kinyoun, fluorescence staining, acridine orange, auramine O, auramine rhodamine"*
2) *PubMed Clinical Queries (from 1966; http://www.ncbi.nlm.nih.gov/entrez/query.fcgi): Systematic Reviews; Clinical Queries using Research Methodology Filters (diagnosis + specific, diagnosis + sensitive, prognosis + specific)*
3) *Pubmed (Medline; from 1966), SUMSearch (http://sumsearch.uthscsa.edu/), National Guideline Clearinghouse (http://www.ngc.org/), Institute for Clinical Systems Improvement (http://www.icsi.org), The National Institute for Clinical Excellence (http://www.nice.org.uk/), Cochrane (http://www.update-software.com/cochrane, Health Technology Assessment Database (http://www.york.ac.uk/inst/crd/htahp.htm)*
4) *National Committee for Clinical Laboratory Standards (NCCLS; http://www.nccls.org/), International Federation of Clinical Chemistry (IFCC; http://www.ifcc.org/ifcc.asp), American Diabetes Association (ADA; http://www.diabetes.org/home.jsp), National Diabetes Information Clearinghouse (NDIC; http://diabetes.niddk.nih.gov/), Westgard QC (http://www.westgard.com), Clinical Laboratory Improvement Amendments (CLIA; http://www.cms.hhs.gov/clia/)*
5) *UpToDate Online version 12.2 (2004)*

RELEVANT EVIDENCE/REFERENCES

1. *Daniel TM. The history of tuberculosis. Resp medicine 2006; 100:1862-1870*
2. *Ehrlich P. A method for staining the tubercle bacillus. 1882*
3. *Sakula A. Robert Koch: centenary of the discovery of the tubercle bacillus, 1882. Thorax 1982; 37:246-251*
4. *Kaufman S. Robert Koch's highs and lows in the search for a remedy for tuberculosis. Nature Medecine Special Web Focus 2000*
5. *Minion J. Light-emitting diode technologies for TB diagnosis: what is on the market? Expert Rev Med Devices 2009; 6:341-345*
6. *World Health Organisation 2011. Fluorescent light-emitting diode (LED) microscopy for diagnosis of tuberculosis: Policy Statement.*
7. *Harries A. An approach to the problems of diagnosing and treating adult smear-negative pulmonary tuberculosis in hogh-HIV-prevalence seetings in seub-Saharian Africa. Bull World Health Organ 1998; 76: 651-662*
8. *Foulds J. New tools for the diagnosis of tuberculosis: the perspective of deverloping countries. Int J Tuberc Lung Dis 1998; 2:778-83*
9. *Tuberculosis Division International Union Against Tuberculosis and Lung Disease. Tuberculosis bacteriology – priorities and indications in high prevalence countries: position of the technical staff of the tuberculosis division of the international union against tuberculosis and lung disease. Int J Tuberc Lung Dis 2005; 9: 355-61*
10. *Corbet E. The growing burden of tuberculosis: global trends and interactions with the HIV epidemic. Arch Intern Med 2003; 163:1009-21*
11. *Dye C. Evolution of tuberculosis control and prospects for reducing tuberculosis incidence, prevalence, and deaths globally. JAMA 2005; 293:2767-75*
12. *Luelmo F. What is the role of sputum microscopy in patients attending health facilities? Geneva: World Health Organisation, 2004:7-13*
13. *Perkins M. New diagnostic tools for tuberculosis. Int J Tuberc Lung Dis 2000; 4:S182-88*
14. *Ramsay A. Front-loading sputum microscopy services: an opportunity to optimize smear-based case detection of tuberculosis in high prevalence countries. J Trop Med 2009; 2009:1-6*
15. *Cattamanchi A. Integrated strategies to optimize smear microscopy: a prospective observational study. Am J Respir Crit Care Med 2011; 183: 547-551*
16. *Cuevas L. A multi-country non-inferiority cluster randomized trial of frontloaded smear microscopy for the diagnosis of pulmonary tuberculosis. PLoS Med 2011; 8:e1000443*
17. *Cuevas L. LED fluorescence microscopy for the diagnosis of pulmonary tuberculosis: a multi-country cross sectional evaluation. PLoS Med 2011; 8:e1001057*
18. *Myneedu V. A pilot study of same day sputum smear examination, its feasibility and usefulness in diagnosis of pulmonary TB. Indian J Tuberc 2011; 58:160-167*
19. *Steingart K. Fluorescene versus conventional sputum smear microscopy for tuberculosis: a systematic review. Lancet Infect Dis 2006; 6: 570-581*
20. *Iadememarco M. Evaluation of laboratory methods used to examine sputum specimens for Mycobacterium tuberculosis. Abstracts of the 30th World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease; Madrid, Spain. 1999. Abstract No. 216-PD*
21. *Somoskövi A. Lessons From a Proficiency Testing Event for Acid-Fast Microscopy. Chest 2001; 120: 250-257*
22. *Foulds J. New tools for the diagnosis of tuberculosis: the perspective of deverloping countries. Int J Tuberc Lung Dis 1998; 2:778-83*
23. *Isenberg H. Clinical Microbiology Procedures Handbook. 2004*
24. *Collins F. Microscopic counts carries out on M. leprae and M. tuberculosis suspension: a comparison of three staining procedures. Int J Lepr Other Mycobact Dis 1980; 48: 402-407*
25. *Allen J. A modified Ziehl-Neelsen stain for mycobacteria. Med Lab Sci 1992; 49: 99-102*
26. *Slosarek M. Comparison of microscopic positivity in smears from sputa stained according to Ziehl-Neelsen in different modifications. J Hyg Epidemiol Micrbiol Immunol 1977; 21: 7-15*
27. *Slosarek M. Cold staining methods for mycobacteria. J Hyg Epidemiol Microbiol Immunol 1974; 18:22-26*
28. *Gruft H. Evaluation of Mycobacteriology Laboratories: The Acid-Fast Smear (1978) Health Lab Sci. 1978 Oct;15(4):215-20.*
29. *Mathew S. Evaluation of a cold staining method for acid-fast bacilli in sputum. Indian J Chest Dis Allied Sci. 1994 Jul-Sep;36(3):125-31.*
30. *Perkins M. New diagnostic tools for tuberculosis. Int J Tuberc Lung Dis 2000; 4:S182-88*
31. *Katila M. Acridine orange staining of smears for demonstration of Mycobacterium tuberculosis. 1982. Eur J Clin Microbiol. 1:351-353*
32. *Smithwick R. Phenolic Acridine Orange Fluorescent Stain for Mycobacteria. J Clin Microbiol 1995; 2763-2764*
33. *Ba F. A comparison of fluorescence microscopy with the Ziehl-Neelsen technique in the examination of sputum for acid-fast bacilli (1999) Int J Tuberc Lung Dis 3:1101-110551*
34. *Collins FM, Morrison NE, Dhople AM, Watson SR. Microscopic carried out on Mycobacterium leprae and M. tuberculosis suspension: a comparison of three staining procedures.(1980) Int J Lepr Other Mycobact Dis. 48: 402-407.*
35. *Kent, P.T., and G. P. Kubica. 1985. Public Health Mycobacteriology. A Guide for the Levele III Laboratory, p. 57-68. U.S. Department of Health and Human Services, Centers for Disease Control, Atlanta, Ga.*

36. *Salfinger, M. and G. E. Pfyffer. 1994. The new diagnostic mycobacteriology laboratory. Eur. J. Clin. Microbiol. Infect. Dis. 13:961-979.*
37. *Laifangbam S. A comparative study of fluorescent microscopy with Ziehl-Neelsen staining and culture for the diagnosis of pulmonary tuberculosis. Kathmandu University medical journal (KUMJ) 7:27 pg 226-30*
38. *Ulukanlgil M. A comparative study on the different staining methods and number of specimens for the detection of acid-fast bacilli. Mem Inst Oswaldo Cruz 2000; 95:855-8*
39. *Habtamu M. Light-emitting diode with various sputum smear preparation techniques to diagnose tuberculosis. Int J Tuberc Lung Dis 2012; 16: 402-7*
40. *den Hertog A. No added value of performing Ziehl-Neelsen on auramine-positive samples for tuberculosis diagnostics. Int Tuberc Lung Dis 20103; 17:1094-1099*
41. *World Health Organisation 2011. Fluorescent light-emitting diode (LED) microscopy for diagnosis of tuberculosis: Policy Statement.*
42. *Bennedsen J. Examination for tubercle bacilli by fluorescence microscopy. Scan J Respir Dis 1966; 47: 114-120*
43. *Toman K. What are the advantages and disadvantages of fluorescence microscopy? Geneva World Health Organisation 2004; 31-34*
44. *Marzouk M. Comparison of LED and conventional fluorescence microscopy for detection of acid-fast bacilli in an area with high tuberculosis incidence. Diag Microbiol and Inf Dis 2013; 76: 306-308*
45. *Holst E. Examination of smears for tubercle bacilli by fluorescence microscopy. Indian J Med Res 1959; 47: 495-499*
46. *Weiser O. Fluorochrome staining of mycobacteria. Tech Bull Regist Med Technol 1966; 36:257-58*
47. *Kivihya-Ndugga L. A comprehensive comparison of Ziehl-Neelsen and fluorescence microscopy for the diagnosis of tuberculosis in a resource-poor urban setting. Int J Tuberc Lung Dis 2003; 7:1163-1171*
48. *Sohn H. Fluorescence microscopy is less expensive than Ziehl-Neelsen microscopy in Thailand. Int J Tuberc Lund Dis 2009; 13:266-268*
49. *Rieder H. Priorities for Tuberculosis Bacteriology Services in Low-Income Countries (2nd Edition). International Union Against Tuberculosis and Lung Disease, paris, France 2007*
50. *Kalich R. Comparison of auramine-rhodamine B and acridine orange for staining of acid-fast bacteria. J Hyg Epidemiol Immunol 1979; 23:307-17*
51. *Narayan S. A comparison of three different staining methods for the detection of acid-fast bacilli (Mcobacterium tuberculosis) in sputum samples. JPBMS, 2012; 14*
52. *Sunil S. Evaluation of acridine orange staining for rapid detection of mycobacteria in primary specimen. Ind J Pathol and Microbiol 2010*
53. *Gottfried M. Light emitting diode microscope illumination for green fluorescent protein of fluorescein isothiocyanate epifluorescence. BioTechniques 2005; 38:204-206*
54. *Hanscheid T. The future looks bright: low-cost fluorescent microscopes for detection of Mycobacterium tuberculosis and Coccidiae. Trans R Soc Trop Med Hyg 2008; 102:502-21*
55. *Minion J. Comparison of LED and conventional fluorescencemicroscopy for detection of acid-fast bacilli in a low incidence setting. PLoS One 2011; 6:e22495*
56. *Nwofor A. Optimising Mycobacterium tuberculosis detection in resource limited settings. BMJ Open 2014; 4:e004093*
57. *Manpreet B. Performance of light-emitting diode fluorescence microscope for diagnosis of tuberculosis. Int J Mycobaceriol 2 2013; 2:174-178*
58. *Xia H. Multicentre evaluation of Ziehl-Neelsen and light-emitting diode fluorescen microscopy in China. Int J Tuberc Lung Dis 2013; 17: 107-112*
59. *Khatun Z. Usefulness of Light Emitting Diode (LED) fluorescent microscopy as a tool for rapid and effective method for the diagnosis of pulmonary tuberculosis. Bangladesh Med Res Counc Bull 2011; 37:7-10*

PART 1: LITERATURE

1.1 CONVENTIONAL LIGHT MICROSCOPY: Ziehl-Neelsen and Kinyoun

Other scientists had perhaps have seen the bacillus in tuberculous material around the same time as Koch, but they were unable to stain and demonstrate it as Koch had done (3). Koch's initial staining method consisted of staining the dried preparations in a weakly alkaline solution of methylene blue. After 24 hours they were then treated with a solution of vesuvin (Bismarck's brown). Then the preparations became brown, and under the microscope all of the substances were strongly brown, while the bacillus remained an intense blue (2,4).

Not much later Paul Ehrlich, who had been present at Koch's lecture in 1882, introduced a new more accurate and less-time consuming staining method. He used for staining aniline instead of methylene blue, and used a shorter staining time (15 to 30 minutes instead of Koch's 24 hours). He also applied 30% nitric acid and alcohol for a few seconds in order to decolorize the surrounding tissues, while the tubercle bacillus remained red. On counterstaining with a yellow or blue dye, the red tubercle bacilli showed up more clearly than by Koch's method. It was also Ehrlich who introduced heat-fixation of the preparations. This was done by keeping the preparations for one hour at 100-110°C or taking the dried preparations with forceps and passing them three times through the flame of a Bunsen burner (2,3). Later Ziehl introduced carbolic instead of aniline, while Neelsen advocated the use of sulphuric instead of nitric acid. In this way the "Ziehl-Neelsen" and the ''acid-alcohol fast bacillus" were born.

Because of the advantages of other staining techniques (*see below*), conventional light microscopy has become more and more abandoned as reference staining method for the diagnosis of tuberculosis in high-income countries. However, because of the simplicity, inexpensiveness and predictive power of the Ziehl-Neelsen/Kinyoun sputum smear microscopy, it has kept its role as the most applicable (and available) diagnostic tool of choice in developing countries (6), where more of 90% of tuberculosis cases occur (7,8,9,10,11). Classical microscopy is indeed rapid, inexpensive, and highly specific in areas where there is a high prevalence of tuberculosis (12,13). Most studies report excellent specificities, ranging from 96% to 100%. Sensitivity-reports however are more variable, with reported values varying from 20% to 86% (14,15,16,17,18,19). Sensitivity is not only influenced by the staining technique, but also by numerous other factors, such as the prevalence and severity of disease, the type of specimen, the method of processing (direct or concentrated), the method of centrifugation, and the quality of examination. A study of Iadermarco et al showed that the ZN method can be similary or even significantly more sensitive than the fluorochrome methods if the slides are prepared and examined according to the standard recommendations (20). However, Somoskövi et al found out in his large proficiency test for acid-fast microscopy in 167 laboratories in the state New York, that even though 91% of the participants used commercial staining kits, a lot of unexpected errors occurred concerning concentration of carbol fuchsin, time of staining and counterstaining, and the concentration of acid alcohol for decolorization. These errors, together with the factors described above, influence significantly the sensitivity (21).

In 1914 Joseph Kinyoun described a new staining method, without the necessity of a heating step. Kinyoun's carbol fushsin was used for staining, 3% acid-alcohol for decolorizing and methylene blue or brilliant green for counterstaining (22,23).

Anno 2014, the classical ZN method has been grossly replaced by the less toxic Kinyoun staining. Somoskövi *et al*. however, showed a significantly lesser performance of the KI staining compared with ZN, and these results are in accordance with several other studies, which indicate a significantly lower sensitivity of Kinyoun compared to Ziehl-Neelsen (21,24,25,26,27,28,29). Despite this inferior diagnostic performance, the Kinyoun staining method is the most frequently used classical light microscopy technique in many laboratories, mainly because of its ease in use and its lesser toxicity (no need for sophisticated suction-systems).

GUIDELINES Overall, most guidelines do not recommend conventional light microscopy for the diagnosis of tuberculosis. If used, which is so in most developing countries, Ziehl-Neelsen should be chosen over Kinyoun.

1.2 FLUORESCENCE MICROSCOPY: Auramine O, auramine-rhodamine and acridine

The spontaneous fluorescence of M. tuberculosis under kristal-violet was first observed by Kaiserling in 1917, although the use of fluorochrome staining was introduced by Hageman in 1938. Hageman used auramine O or auramine-rhodamine as acid-fast fluorescent dye with an intense light source such as a halogen of high-pressure mercury vapour lamp (19,30).

More recently, Katila and Mantyjarvi evaluated in 1982 a fluorescence acid-fast staining method that used acridin orange as the specific dye. In 1995 then, the group of Smithwick introduced the use of phenol to accelerate dye penetration through mycobacterial cell walls and this layed the foundation for the phenolic acridine orange staining (31,32).

Fluorescence microscopy has several advantages over classical light microscopy using Ziehl-Neelsen of Kinyoun staining and is frequently used in high-income countries. In Belgium, most laboratories use auramine fluorescence smear microscopy as a diagnostic tool for pulmonary tuberculosis (*own questionary*).
The first advantage is an overall better performance. Several studies report a superior performance of fluorescence microscopy over the conventional Ziehl-Neelsen/Kinyoun technique for the detection of acid-fast bacilli (33,34,35,36,37). These findings were confirmed in a recent systematic review of 45 relevant studies, were Steingart et al concluded a 8-10% greater sensitivity of fluorescence microscopy compared with conventional light microscopy (19). There is a general concern though that the specificity could be lower than conventional smear microscopy (38,39). Therefore, in some countries national guidelines for tuberculosis diagnostics continue to recommend the confirmation of acid-fast bacilli detected by auramine using Ziehl-Neelsen. However, Steingart et al showed in her systematic review no decrease in specificity of auramine compared to conventional microscopy (19). Moreover, recently den Hertog et al concluded in a retrospective study of 10276 samples that there is no added value of confirming auramine-positive direct smears of respiratory samples with Ziehl-Neelsen, and that reanalysis of these samples will have no impact on patient management and is thus a waste of resources (40). However, some authors occasionally do report a lower specificity of fluorescence compared to conventional light microscopy (17). One possible explanation could be that a higher proportion of non-tuberculous mycobacteria are detected by this method which are missed by Ziehl-Neelsen, as others have suggested (17), but this remains to be demonstrated. Anyhow, WHO guidelines recommend staining tuberculosis suspected smears with one stain only, preferably auramine (41).
A second and equally important advantage of fluorescence microscopy is that it uses a lower power objective lens (typically 25x) than conventional microscopy (typically 100x), enabling the microscopist to assess the same area of slide more quickly and efficiently (42,43). It has been estimated that using fluorescence microscopy may take up to 75% less time than conventional light microscopy (19). For illustration: an average of 15 minutes reading time is required for Ziehl-Neelsen/Kinyoun stained slides VS approximately 2.6 minutes per slide when fluorescent microscopy is used (44).
Other advantages includes the ease and simplicity of recognizing the acid-fast bacilli by using fluorescence microscopy as with conventional light microscopy interpretation is a lot more difficult (45,46).
So as described, fluorescence microscopy has taken a primary place in the diagnosis of tuberculosis in high-income countries. This implementation however has been more difficult in developing countries (41). Most cited reason for this is the high capital cost for conventional mercury vapor fluorescent microscopes (5,41,44). However, two groups demonstrated the cost effectivity of fluorescence microscopy, even in low-income countries because of the high sensitivity and greater time efficiency (47,48). Nevertheless, the requirement of significance maintenance of the microscopes, the limited lifespan of the bulbs, the need for a dark room away from dusty environments and the toxic exposure when broken are other road blocks to global implementation of fluorescence microscopy, especially in low-income countries (49).

The phenolic acridine orange staining is much less known (and used) that the popular auramine staining. Therefore only a few studies have been conducted in order to evaluate the performance of this staining technique in the detection of acid fast bacilli in sputum smears.
Katila and Smithwick showed in their studies mentioned above that the results of the phenolic arcidine orange staining were comparable to those of the auramine staining (31,32). A study across six laboratories in Europe found that acridine orange even outperformed auramine staining (50). A recent Indian study also showed a superior sensitivity of acridine orange staining compared with auramine in the diagnosis of tuberculosis in a setting of a developing country (51).
Anyhow, the literature on this staining technique is scarce, and its performance has to be further evaluated. Overall, in the available studies, the differentiation of the bacilli was said to be better if stained with acridine orange, as the dull green background enabled easy visualization. This in contrary to the yellowish green fluorescence of the bacilli against an also pale green background, which is seen in auramine stained preparations. Another general conclusion is that with the auramine staining, more than with acridine orange, fluorescent debris can be mistaken for acid-fast bacilli and thus could have an influence on specificity (51,52). These conclusions, suggest that the acridine orange staining can be seen, next to auramine, as a comparable fluorescence staining technique in the diagnoses of tuberculosis.

GUIDELINES Most guidelines recommend fluorescence microscopy for the diagnosis of tuberculosis. WHO, CDC, ATS and IDSA2013 state that fluorescence microscopy is a better technique than conventional light microscopy. Ziehl-Neelsen are good alternatives (especially in developing countries), but results in lower sensitivities. No guidelines make a distinguish between the different fluorescent staining methods. If mentioned, auramine staining is most noted.

1.3 NEW-GENERATION MICROSCOPY: LED

In 2005 Martin et al. described a new-generation light-emitted-diodes (LED's- microscopes being used as excitatory light sources for diagnostic fluorescence stains, where it was demonstrated that a LED could replace a mercury arc lamp and produce light of sufficient intensity for use with fluorescence microscopy (53).

LED microscopes were developed mainly to give resource-limited countries access to the benefits of fluorescence microscopy (41). Moreover, LED fluorescence microscopy have many practical advantages over conventional mercury vapour fluorescence microscopes. The spectrum of light produced by LED devices is narrower than that provided by mercury vapour conventional fluorescence microscopes, and its wavelength is produced to match specifically the peak absorbance of auramine strains (54). This explains why they can be used without a dark room. This could significantly improves the workflow and maximizes space utilization in the lab (55).

In 2009, a meta-analysis found out a significantly greater sensitivity by 6% (95% CI, 0.1-13%), with no appreciable lost in specificity, when compared with Ziehl-Neelsen (41). More recent studies confirm the superior performance of LED-microscopy over Ziehl-Neelsen for the detection of acid-fast bacilli (41,54,58,59). The systematic review mentioned above showed significant gains in time for reading as conventional light microscopy, with about half of the time for smear examination compared with Ziehl-Neelsen. And finally the same authors also found out a better cost-effectiveness with LED than with Ziehl-Neelsen, with improved efficiency. Recently, Xia confirmed this cost-effectiveness: he became in his study as average cost unit for Ziehl-Neelsen 2.20USdollar (+/-0.58) versus 1.97USdollar (+/-0.71) for LED-fluorescence microscopy (P<0.05) (58). In 2011 Whitelaw found in his cost-analysis also an less average cost per slide for LED-microscopy (1.63USdollar) compared with Ziehl-Neelsen staining (2.10USdollar).

When compared to classical fluorescence microscopy the WHO expert group performed a meta-analysis which showed that LED microscopy was 5% (95% CI, 0-11%) more sensitive and 1% (95% CI, -0.7-3%) more specific. More recent studies confirm these findings: LED-microscopy shows a better sensitivity than classical fluorescence microscopy (54) and implicates a substantial increase in smear positive detection (4,41). Some studies report comparable diagnostic performance compared with classical fluorescence, but none show LED as an inferior test. Most studies report similar specificities (44,55,57) and identical time required to examine slides with LED-microscopy and conventional fluorescence microscopy respectively (44,55,56,57,58). No studies, to our knowledge, have been conducted to compare the costs per slide of fluorescence microscopy versus LED-microscopy.

Overall, general user acceptability in all field studies was reported as excellent and most studies confirm many anticipated advantages, as described above, including use of the devices without a dark room, durability and portability.

GUIDELINES

The WHO recommends that conventional fluorescence microscopy be replaced by LED microscopy in all settings where fluorescence microscopy is currently used and that LED microscopy be phased in as an alternative to conventional Ziehl-Neelsen light microscopy in both high- and low-volume laboratories.

PART 2: FIELD – WORK

In our hospital (AZ Sint-Jan campus Bruges) the microscopic method for diagnosing pulmonary tuberculosis, is the acridine orange fluorescence staining technique. Because of the scarce amount of literature concerning the performance of this staining method, we evaluated in cooperation with UMC Saint-Pierre Brussels and AZ ZENO Knokke-Heist/Blankenberge the acridine orange staining in comparison with two established staining methods, the auramine O fluorescence and Kinyoun staining.

Culture served as the reference method to assess diagnostic performance of Ziehl-Neelsen, acridine orange and auramine O staining. In all, 186 consecutive routine samples were included in the study protocol. After decontamination and preparation of the sample, three slides were prepared by one technician on the exact same manner. The slides were stained with auramine O, acridine orange and Kinyoun staining. All slides were examined triple blinded by experienced technicians or clinical biologists following standard principles. With 10.3% positive samples, we became a sensitivity of 68.4% for both the Kinyoun as the auramine O staining method, and 63.2% for the acridine orange staining. For specificity we became 96.4%, 99.4% and 98.8% respectively. With respect to positive predictive value, the results were 68.6%, 92.9% and 85.7% for Kinyoun, auramine O and acridine orange staining respectively. The negative predictive value was a comparable 96.4%; 95.5% and 96.0% respectively.

PART 3: GENERAL CONCLUSIONS

Despite several other emerging rapid diagnostic techniques for diagnosing pulmonary tuberculosis, sputum smear microscopy stays the most important tool. According to guidelines, and other published data, LED-microscopy gains importance in the microscopic smear diagnosis of tuberculosis. Although not yet globally implemented, this technique implicates a lot of advantages compared with conventional techniques as well concerning cost-efficacy, ease in use and interpretation as in its diagnostic performance. LED-microscopy could be of great value in developing countries, where the practical disadvantages of conventional fluorescence microscopy can be overruled by LED-microscopy.

Fluorescence microscopy is in high-income countries the most frequently used staining method for diagnosing pulmonary tuberculosis anno 2014. Most studies show a good diagnostic performance, but the costs and practical disadvantages are road blocks for global implementation. Auramine O staining method is the most known, described and thus frequently used fluorescence technique. Acridine orange seems a valuable alternative, although literature concerning the latter method is scarce.

Ziehl-Neelsen or Kinyoun remains the most important staining method in developing countries. It is a century-old, relatively cheap technique, which is globally implemented. Most guidelines disadvise the use of classical light microscopy for the diagnosis of pulmonary tuberculosis because of a lesser diagnostic performance compared to newer techniques, such as fluorescence staining methods or LED-microscopy.

In our prospective trial we compared three established staining methods for the detection of acid-fast bacilli.

The results of our study could not confirm the superior performance of fluorescence staining microscopy compared to classical Kinyoun staining for the diagnosis of tuberculosis. However, this could confirm the findings of Iademarco, who stated that the sensitivity of conventional light microscopy (Ziehl-Neelsen or Kinyoun) could be comparable to fluorescence microscopy if used and interpreted in a perfect standardized setting (20).

The results neither could confirm a superior or comparable diagnostic performance of acridine orange staining compared to auramine O staining for the diagnosis of tuberculosis.

Anyhow, the performance of the three staining methods separately is in line with previous findings, with good sensitivities en negative predictive values.