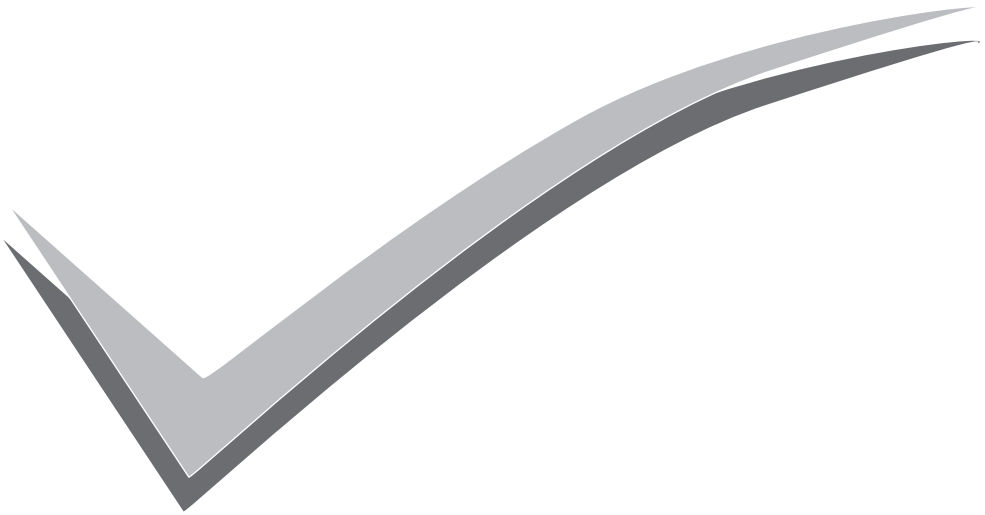
CE

ISO 9001: 2008
EN ISO 13485: 2012

Performance Evaluations



MYCOSTAIN

AFB stain set for screening of *M. tuberculosis* and *M. leprae*

Tulip Group

Performance Evaluations

CE

ISO 9001: 2008
EN ISO 13485: 2012

SCIENTIFIC REPORT

INDEX	
S. No.	**Name**
1.	Performance evaluation of Mycostain against certified ZN Stain from Sigma Aldrich, U.S.A from reputed labs and hospitals across India.

MYCOSTAIN

AFB stain set for screening of *M. tuberculosis* and *M. leprae*

Tulip Group

Scientific Report

Performance evaluation of Mycostain against certified ZN Stain from Sigma Aldrich, U.S.A. from reputed labs and hospitals across India.

Dr. R. J. Sood, MD (Path.)
Sr. Visiting Consultant, Manipal Health Systems
email: spedilab@sify.com

ABSTRACT

Objective - To evaluate performance and technical parameters of acid-fast staining kit for *Mycobacterium sp.* - Mycostain, from MICROXPRESS, A Division of Tulip Group of Companies against a certified ZN Stain kit, from Sigma Aldrich U.S.A based on Ziehl-Neelsen hot method for AFB staining.

Study Design and Methods

Samples from 96 patients tested randomly at selected laboratories & hospitals were used in this study. The specimens were analyzed using Ziehl-Neelsen Stains from MICROXPRESS and Sigma Aldrich in all the laboratories and hospitals. The results of both the stains were compared for their accuracy in identification of tuberculous bacilli, differentiation between acid fast and non acid fast organism, clarity of the smear & visibility if the organism against the background.

Results

Data from all the laboratories and hospitals were analyzed in the present study on the basis of the stain quality. Since Carbol Fuchsin and Methylene Blue stain from Sigma Aldrich is a certified stain, the study revealed that Carbol Fuchsin and Methylene Blue stain provided in Mycostain kit from MICROXPRESS was correlating well with the used reference stain. Both the stains showed excellent performance when compared for the characteristics like – smear clarity, visibility of organism, differentiation between tuberculous & non-tuberculous bacilli & background distinction against red color bacilli.

Conclusions

The study shows that the stain quality and purity play a significant part to make microscopy an excellent tool in diagnosis. For exact analysis of the organism morphology and their distinction from one another based on cell structure, the stain used for microscopy should be certified. The result comparison done by different laboratories and hospitals using Mycostain, MICROXPRESS and ZN Stain from Sigma Aldrich, U.S.A. for the same samples following similar acid fast techniques revealed that the quality of Mycostain is well comparable with certified reference stain.

It was also observed that the performance of Mycostain in differentiating tuberculous red-rods from non-tuberculous blue bacilli/cocci, clear background distinction, visibility of organism morphology as well as smear clarity was equivalent to standardized ZN Stain kit from Sigma, Aldrich,U.S.A.

Background

Tuberculosis is a common and often deadly infectious disease caused by *Mycobacterium tuberculosis* in humans. A third of the world's population is thought to be infected with *M. tuberculosis* and new infections occur at a rate of about one per second. The proportion of pulmonary tuberculosis is approximately 75% among all TB cases and it should be diagnosed at the earliest to prevent the spread. Pulmonary TB diagnosis can be done effectively at the initial stage by examination of sputum with the aid of acid fast microscopy. The recommendations of the International Union Against Tuberculosis and Lung Disease (IUATLD) and World Health Organization (WHO) are that patients with suspected pulmonary tuberculosis should have three sputum smears, stained by Ziahl-Neelsen method, examined by microscopy for *Mycobacterium tuberculosis.* Since it is more than 100 years old and still remains the initial and most rapid step in the diagnosis of TB. The specificity of acid-fast microscopy is excellent for mycobacterium species, whereas the sensitivity of microscopy is influenced by numerous factors, such as quality of the stain used, concentration of the stain and various other parameters of the stain - namely its pH, ionic strength, conductivity and optical density. Mainly the concentration of the dyes used in Acid -fast staining method (i.e. Carbol Fuchsin and Methylene Blue) play a critical role. General commercially available dyes can undoubtedly help in mere staining the cells but the performance of the certified dyes when used is outstanding when it comes to the level of morphology of cells, their distinction and correct differentiation based on the cell wall structure.

It is essential for a stain used in AFB microscopy to fulfill the following criteria in order to produce accurate results: (1) Visibility of the organisms (2) Clarity of the smear. (3) Differentiation between tuberculous and non-tuberculous bacilli. (4) Distinction between background and bacilli. (5) Any particles if found in the smear. And these mentioned criterion's are only fulfilled when a standardized stain is made use of.

Materials And Methods

Stains used in Hot Ziehl-Neelsen method are Carbol Fuchsin Reagent (a reagent containing basic fuchsin and phenol), Decolorization Agent (a reagent containing ethanol and hydrochloric acid) and Methylene Blue Reagent.

In This study Carbol Fuchsin Reagent and Methylene Blue Reagent provided in Mycostain Kit from MICROXPRESS (CAT NO.: 20307100, Lot No.: ZN-B-901T/ZN-B-902T, Mfg.:Nov'09/Dec'09, Exp.:Oct'12/Dec'12) is compared with certified ZN Stain Kit from Sigma, Aldrich,U.S.A (Lot No.:ZN-A-901T/ZN-A-902T,Mfg.:Nov'09/Dec'09, Exp.:Oct'12/Dec'12) on its performance based on above mentioned criteria.

Preparation of Smear - The smear was prepared by standard protocol as mentioned in Clinical Diagnosis & Management by Laboratory Methods, Todd Stanford.17th Edition 1988 and Practical Medical Microbiology, Mackie & Mc Cartney, 13th Edition.

Mycostain kit and Sigma Aldrich Kit were provided to all the laboratories and hospitals. They were asked to evaluate both the kits by using same samples collected by them from TB patients and TB negative healthy volunteers.

Analysis of Results

AFB microscopy results with X1000 immersion magnification were interpreted and reported by the labs and hospitals according to standard recommendations.

Data from all reputed laboratories and hospitals were analyzed in the present study on the basis of the quality of the dyes present in MYCOSTAIN & Sigma, Aldrich ZN-Stain.

Since Carbol Fuchsin and Methylene Blue stain by sigma are certified stains, the study revealed that Carbol Fuchsin and Methylene Blue stain provided in Mycostain was well comparable with the known reference certified stain. (Table: 1)

Labs & Hospitals	Number of POSITIVE SAMPLES		Number of NEGATIVE SAMPLES		**Number of TOTAL SAMPLES**
	Brand A Sigma Aldrich,U.S.A.	**Brand B** Mycostain, MXP	**Brand A** Sigma Aldrich,U.S.A.	**Brand B** Mycostain, MXP	
LAB 1	6	6	29	29	35
HOSP 1	10	10	2	2	12
HOSP 2	1	1	23	23	24
HOSP 3	7	7	18	18	25

Table: 1 – Evaluation response Data*

* Data on MICROXPRESS file

The Kits were also compared on the following criteria's in order to evaluate the performance of the stain (Table: 2)

Sr. No.	**CHARACTERISTICS OBSERVED**	**REMARKS for ZN Stain Kit**	
		Sigma Aldrich, U.S.A. (poor/good/ excellent)	Mycostain, MICROXPRESS *(poor/good/ excellent)*
1.	Clarity of the smear	Excellent	Excellent
2.	Visibility of the organism	Excellent	Excellent
3.	Differentiation between tuberculous bacilli & non-tuberculous bacilli.	Excellent	Excellent
4.	Distinction of background against red color bacilli	Excellent	Excellent
5.	Any partials if found in the smear.	Good	Good

Labs & Hospitals	**RESULTS OBSERVED**				
	Clarity of smear *(poor /good/ excellent)*	**Visibility of the organism** *(poor /good/ excellent)*	**Differentiation b/w Gram negative & Gram positive organisms** *(poor/ good/ excellent)*	**Distinction b/w back-ground** *(poor/ good/ excellent)*	**Any particles if found any** *(poor /good/ excellent)*
LAB 1	Excellent	Excellent	Excellent	Excellent	Excellent
HOSP 1	Excellent	Excellent	Excellent	Excellent	Excellent
HOSP 2	Excellent	Excellent	Excellent	Excellent	Excellent
HOSP 3	Excellent	Excellent	Excellent	Excellent	Excellent

Table: 2 – It depicts the important criterion's that play a critical role in microscopy.*

From the analysis it is clear that - the quality and other parameters of a particular stain plays a significant part to make microscopy an excellent tool in diagnosis. For exact analysis of the morphology of organism and distinction of one organism from another, the stains have to be of certain Standard.

Comparison of the results obtained by using ZN Staining Kits - Mycostain from MICROXPRESS and ZN Stain Kit from Sigma Aldrich, U.S.A. following similar technique revealed that the quality of ZN reagents Carbol Fuchsin & Methylene Blue in Mycostain kit from MICROXPRESS correlates well with ZN Stain reagents Carbol Fuchsin & Methylene Blue provided in certified ZN Stain kit from Sigma Aldrich, U.S.A.

It was also observed that the performance of Mycostain, MICROXPRESS in differentiating pink colored tuberculous bacilli from purple colored non tuberculous, clear distinction of background against organisms,

visibility of (cell morphology) organisms, no observation of particles in the smear as well as clarity of the smear was equivalent to standardized ZN Stain kit from Sigma Aldrich, U.S.A.

Discussion

For exact microscopic identification and result interpretation it is very critical to analyze the cell morphology of the organism. There are various criteria on which the interpretation depends like -Visibility of the organisms, Clarity of the smear, Differentiation between tuberculous and non- tuberculous bacilli, Distinction between background and bacilli, and any particles if found in the smear. And these mentioned criterion's are only fulfilled when a standardized stain is made use of.

A standardized stain is a stain in which the concentration of the dye is optimal in order to fulfill all the above characteristics. And when these standardized dyes are certified by a globally known body, it is than called as a certified stain. The reference stain used in this study (ZN Stain, from Sigma Aldrich, U.S.A.) is a certified by Biological Stain Commission and is ISO 9001 certified. Since the study depicts that the performance of Mycostain, from MICROXPRESS showed excellent correlation with Zn Stain, from Sigma Aldrich, U.S.A., we can therefore conclude that Mycostain, from MICROXPRESS is equivalent to a certified stain.

References

1. Kumar, Vinay ; Abbas, Abul K ; Fausto, Nelson & Mitchell, Richard N. (2007). Robbins Basic Pathology(8th Edition). Saunders Elsevier,pp;516-522. ISBN 978-1-4160-2973-1.
2. Jasmer RM, Nahid P, Hopewell
3. PC (December 2002). “Clinical practice. Latent tuberculosis infection.” N. Engl. J. Med. 347(23): 1860-6.
4. “Tuberculosis”, World Health Organization.2007. Retrieved 12 November 2009. Fact Sheet No. 104.
5. Laboratory service in tuberculosis control: Part 2. Microscopy. Geneva. Switzerland: World Health Organization, 1998: 258.
6. Salfinger M, Pfyffer GE. The new diagnostic mycobacteriology laboratory. Euc. J. Clin Microbio Infect Disease Control, 1985.
7. Kent PT, Kubica GP. Public Health Mycobacteriology : a guide for a level III laboratory. Atlanta, GA; Center for Disease Control, 1985.
8. Smithwick RW. Laboratory manual for acid fast microscopy. Atlanta, GA; Center for Disease Control, 1976.
9. Lipsky BA, Gates J, Tenover FC, et al. Factors affecting the clinical value of microscopy for acid-fast bacilli. Rev Infect Dis 1984; 6:214-222.
10. Mc Carter YS, Robinson A. Quality evaluation of sputum specimens for mycobacterial culture. Am J clin Pathol 1996; 105:7
11. .Rickman TW, Moyer NP. Increased sensitivity of acid-fast smears. J Clin Microbio 1980; II: 618-620.
12. Peterson GM, Nakasone A, Platon-De Leon JM, et al. Comparison of direct & Concentrated acid fast smears to Identify specimens culture positive for Mycobacterium spp. J Clin Microbio 1999; 37 : 3564-3568.
13 Clinical Diagnosis and Management by Laboratory methods, Todd Standford, 17th editided by John Bernard Henry.
14. Practical Medical Microbiology, Mackie & McCartney, 13th Edition 1989, Edited by J.G. Collee, J.P. Daguid
.15 Reider HL, Choude TM, Myking H, et al. The Public Health service national reference laboratory and the national laboratory network : minimum requirenments, role and operation in low income country. Paris, France: International Union against Tuberculosis and Lung Disease,1998.
16 Laboratory Services in tuberculosis control: Part 2. Microscopy.Geneva, Switzerland:World Health Organization, 1998;258.
17. Enarson DA, Rieder HL, Arnadottir T. Tuberculosis guide for low income countries;Paris : International Union Against Tuberculosis & Lung Disease, 3rd edition 1994.
18 World Health Organization, Framework for effective tuberculosis control, Geneva: WHO 1994.

For further information contact:

TULIP GROUP OF COMPANIES

Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex Post Office, Goa - 403202, INDIA.

Tel.: +91 832 2458546-50 **Fax :** +91 832 2458544 **E-mail :** sales@tulipgroup.com **Website :** www.tulipgroup.com