ULTRACARE DIAGNOSTICS

Mycostain®

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

Summary
Mycostain® is provided as a ready to use stain set. It is a standard Ziehl Neelsen Hot Stain Set for the screening of Mycobacterium tuberculosis from biological specimens. Infection with Mycobacterium tuberculosis remains a major public health problem. The epidemic of tuberculosis and Multi-Drug Resistance (MDR) reflects the failure of public health and social programs towards prompt treatment of infected cases and screening of high-risk population. While culture, isolation and sensitivity of Mycobacterium tuberculosis from patient groups using standard methods remain the gold standard for Mycobacterium tuberculosis detection and effective and swift treatment worldwide, Acid Fast Bacilli staining is the first line microscopic procedure performed towards this goal.

Principle
Carbol Fuchsin forms acid insoluble complex with the Mycolic acid present on the Acid-Fast Bacilli and renders red / pinkish red color to Mycobacterium tuberculosis or Mycobacterium leprae. Mycobacterium species being acid fast in nature, and resist decolorization by strong acid. Other elements present in the smear are decolorized and hence take up the counter stain (methylene blue) and are stained blue.

Reagent / Contents
Mycostain® comprises of:
1. **Mycostain® (A)** – Ready to use Carbol Fuchsin Strong (1%).
2. **Mycostain® (B)** – Ready to use Acid Fast Decolorizer.
3. **Mycostain® (C)** –Ready to use Loeffler's Methylene Blue.

Note: The ready-to-use Loeffler's Methylene Blue included with the **Mycostain®** staining solutions, should not be used with **Novachrom®.**

Accessory: Plug-in dispenser - 2 nos.; Plastic dropper - 1 no.

Appearance
Mycostain® Stain (A) – Dark pink coloured liquid
Mycostain® Stain (B) – Clear colourless liquid
Mycostain® Stain (C) – Dark Blue coloured liquid

Storage and Stability
Store the **Mycostain®** solutions at room temperature 15°C-25°C, away from light. The stability of the **Mycostain®**
solutions is as per the expiry date mentioned on the label.

Materials required but not provided
Glass slides, sterile plating loops (10 uL), biosafety hood with Bunsen burner, activated 2% Glutaraldehyde solution, distilled water, microscope with oil immersion lens , filter paper, cedar wood oil.

Type of Specimen
Biological specimens such as sputum, CSF, urine and isolated culture. It is also used in the identification of Mycobacterium *leprae* from ulcerated nodules on skin and ear lobe, scrapings and secretions from nasal mucosa and sputum specimens.

Specimen Collection and Preparation
Collect specimen prior to using an antimicrobial agent. Wherever possible, indicate clearly that patient is on antimicrobial drugs.

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CSF: Collect as much as possible in a syringe, clean skin with alcohol before aspirating specimen.
Body fluids: Disinfect the site and collect specimen with aseptic precautions.
Sputum: Collect 5 to 10 mL in a sterile container from an early morning specimen of deep productive cough. For induced specimen use sterile saline. Have patients rinse mouth with water to minimize specimen contamination with food particles, mouthwash, or oral drugs.
Urine: As organisms accumulate in the bladder overnight, first morning void provides best yield. Collect midstream clean catch urine, first morning catheterization/suprapubic taps in sterile containers.
Ulcerated nodules: Split an un-ulcerated nodule on the skin or from the ear lobe with a sterile scalpel and squeeze to exude the fluid on to the slide.
Nasal mucosa: Aseptically collect scrapings or secretions from the nasal mucosa and transfer onto the glass slide.

Specimen Preparation
Proper decontamination and concentration of specimen containing normal microbial flora are crucial for the detection of Acid-Fast Bacilli. Specimen obtained from sterile sites such as CSF, peritoneal or pleural fluids do not need decontamination. However, since most specimens for Acid-Fast Bacilli smear are from respiratory tract and the mucous traps Acid-Fast Bacilli and protect other organisms from effective decontamination, their concentration, decontamination, and liquefaction are a must. Most satisfactory for this purpose is a combination of N-acetyl-L- cysteine (mucolytic agent) and 2% NaOH (decontaminant) – (available as **LYFECTOL®** from **UltraCare Diagnostics**). Petroffs method of decontamination can also be used.

Test Procedure
Preparing the Smear: For direct sputum screening macroscopic examination is made and small portions of the specimen are sampled by wire loop, care should be taken to ensure that purulent material, if any, is included in the sample. Alternately, use small portions of decontaminated and concentrated specimens.
(1) Place the specimen under test on a clean, scratch-free, preferably new glass slide using a sterile plating loop.
(2) Homogenize and evenly spread the sample with the loop by tracing concentric circles well separated to cover approximately 1/3rd of the whole area of the glass slide to form a fairly thick uniform smear.
(3) When the smear is completed, plunge the inoculating loop into liquid disinfectant (2% Glutaraldehyde) and shake to remove any sputum, then flame sterilize the loop.
(4) Air-dry the smear.
(5) Flame the edges of the slides and place it on a drying rack.
(6) The slide is then air dried and heat fixed by passing three times through a flame.
(NOTE: While passing the smear slide through the flame, ensure that the side opposite the smear is facing the flame).

Staining Procedure
(1) Replace the caps of the reagent bottles and fit them with provided Plug-in dispenser.
(2) Add **Mycostain® (A)** Carbol fuchsin strong (1%) stain to cover the smear.
(3) Heat (till the steam rises from the stain) every 1 minute for 5 minutes. (Do not allow the stain to boil and dry. If dried immediately add the stain to cover it).
(4) Allow the stain to cool for 2 minutes.
(5) Wash the slide under running tap water.
(6) Tilt the slide to drain.
(7) Cover the smear with **Mycostain® (B)** Acid Fast Decolorizer.
(8) Allow it to stand initially for 1 minute.
(9) Discard and repeat step 7.
(10) Allow to stand for 2 minutes.
(11) Discard and repeat step 7 and 10.
(12) Discard and repeat step 5 and 6.
(13) Cover the smear with **Mycostain® (C)** Loeffler's Methylene blue stain.
(14) Allow to stand for 25-30 seconds and repeat step 5 and 6.
(15) Air dry the slide and observe under oil immersion lens.

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Note: 1.The above method can be employed for staining of Mycobacterium leprae.
2. Do not repeat the steps-9,10,11 for M.leprae.

Interpretation of Results

1. Presence of pink to red coloured slender Bacilli – Smear is Acid Fast Bacilli positive.
2. Absence of pink to red coloured slender Bacilli – Smear is Acid Fast Bacilli negative.
3. Pus cells and other bacteria stain purple to blue colour.

Grading of results

After 5 minutes of examination covering about 100 fields, report the results as follows:

No. of Acid Fast Bacilli	Report as	Or Report as Found at 1000x
0	Negative for AFB Negative for AFB	
1-2 per 300 fields	Number Seen (Order repeat specimen)	Number Seen (Order repeat specimen)
1-9 per 100 fields	1+	Number seen per 100 fields
1-9 per 10 fields	2+	Number seen per 10 fields
1-9 per field	3+	Number seen per field
>9 per field	+	>Number seen per field

Remarks

(1) As with all diagnostic tests, the test result must always be correlated with clinical findings.
(2) Improper decontamination and concentration of sputum specimens will yield erroneous results.
(3) Treat the specimens and used slides by immersing in 2% activated Glutaraldehyde for at least two hours before incineration and disposal.
(4) Good laboratory practices and hazard precautions must be observed at all times.
(5) Do not allow **Mycostain® (A)** to boil or dry during heating.
(6) Observe stain timings which are essential to obtain correct staining results.
(7) Under stain or over decolorization may give false results.
(8) Decolorization of the smear by **Mycostain® (B)** Acid fast decolorizer depends on the thickness of the smear.
(9) Artefacts could be mistaken for Acid Fast Bacilli.
(10) Do not intermix the stain droppers provided in the kit.
(11) Mycostain® (A), **Mycostain® (B)** and **Mycostain® (C)** of different lots must not be mixed and used.
(12) Any modifications to the above procedure and / or use of other reagents will invalidate the test procedure.

Warranty

UltraCare Diagnostics Myostatin® is a reagent for laboratory use only.
This product is designed to perform as described on the label and pack insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

References

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Disclaimer
Information provided is based on our inhouse technical data on file, it is recommended that user should validate at his end for suitable use of the product.

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