

Intended use

Rapid two step cold AFB stain for screening of *Mycobacterium tuberculosis* and *Mycobacterium leprae*.

Summary

Novachrom® staining system is simple to perform and very reproducible. It has a sensitivity comparable to the traditional Ziehl Neelsen hot staining method and as compared to Acid Fast Bacilli culture results. It is clinically proven, easy to use, time, labor, and cost saving. This simplicity of staining makes **Novachrom®** an ideal screening tool for *Mycobacterium tuberculosis*.

Infection with *Mycobacterium tuberculosis* remains a major public health problem. The epidemic of Tuberculosis and Multi Drug Resistant Tuberculosis 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 an acid insoluble complex with Mycolic acid present on the Acid-Fast Bacilli and renders red/ pinkish red color to *Mycobacterium tuberculosis*. Other elements present in the smear take up counter stain (Methylene blue) and are stained bluish.

Novachrom® Rapid Two Step Cold AFB Stain avoids the extra decolorization step associated with traditional staining techniques as the decolorizing component is incorporated within the counter stain.

Reagent / Contents

Novachrom® comprises of:

a) **Novachrom® AFB stain (A)** - Ready to use Carbol Fuchsin Strong (1%)

b) **Novachrom® AFB stain (B)** - Ready to use Loeffler's Methylene blue

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

Appearance

Novachrom® Stain (A) - Dark pink coloured liquid

Novachrom® Stain (B) - Dark blue coloured liquid

Storage and Stability

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

Materials required but not provided

Sterile plating loops (10 µL), biosafety hood with Bunsen burner, activated 2% Glutaraldehyde solution, distilled water, microscope with oil immersion lens , 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 anti- tubercular drugs.

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, the first morning void provides best yield. Collect midstream clean catch urine, first morning catheterization/suprapubic taps in sterile containers.

Specimen Preparation

Proper decontamination and concentration of specimens containing normal microbial flora are crucial for the detection of *Mycobacterium tuberculosis*. 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). Petroff's method of decontamination can also be used.

Test Procedure

For direct sputum screening use 10 µL of purulent sputum or use 10 µL of decontaminated, concentrated specimen.

1. Place the specimen under the test on a clean, scratch-less glass slide using a sterile plating loop.
2. Spread by tracing concentric circles well separated over an area of 200 mm square (20 mm x 10 mm), take care as to not to reach the edge of the slide. Alternatively, for dense mucoid specimen press the specimen between two glass slides and pull apart gently to form a thin film of mucous.
3. When the smear is completed, plunge the inoculating loop into liquid disinfectant (2% Glutaraldehyde) and shake to remove any sputum, then flame sterilize loop.
4. Air-dry the smear.
5. Fix the smear by passing the slide approximately three times on the flame.
(**Note:** While passing the smear slide on the flame see that the side opposite the smear is facing the flame).
6. Add **Novachrom® AFB stain (A)** over the smear to cover it completely (4-5 drops ≈ 0.2 mL, may be required).
7. Keep for 6 minutes, then rinse with plenty of distilled water slowly to remove excess of **Novachrom® AFB stain (A)**.
8. Tilt slide to drain and add **Novachrom® AFB stain (B)** over the smear to cover it completely (4-5 drops ≈ 0.2 mL).
9. Keep for 6 minutes, then rinse the smear with distilled water to remove excess of **Novachrom® AFB stain (B)**.
10. Air-dry and observe under oil immersion (magnification 100X).

Interpretation of Results

1. Presence of pink to red colored slender Bacilli - Smear Acid Fast Bacilli positive.
2. Absence of pink to red colored slender Bacilli - Smear Acid Fast Bacilli negative.
3. Pus cells and other bacteria stain purple to blue color.

Grading of results: After 5 minutes of examination covering about 100 fields.

No. of Acid-Fast Bacilli

Observed

No Acid-Fast Bacilli
1-10 Acid Fast Bacilli
> 10 Acid Fast Bacilli

Report

Negative
Actual
Number
+

Masses of Acid-Fast Bacilli in Several Fields ++

Warranty

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

Reference

Data on file: UltraCare Diagnostics .

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.



Manufacturer
CAT NO.
Number Lot number
Date of manufacture
Use by (Expiration date)



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