**HiCold Stain TB- Kit**

**Intended Use:**
HiCold Stain TB- Kit is recommended for microscopic investigation of Mycobacterium by Cold Acid-Fast staining method (Kinyoun method).

**Composition**

**Ingredients**

**Carbol fuchsin solution (S080)**
- Basic Fuchsin: 4.0 gm
- Ethyl alcohol, 95%: 25.0 ml
- Phenol: 10.0 ml
- Distilled Water: 876.0 ml

**Decolourizer (S099)**
- Hydrochloric acid, concentrated: 3.0 ml
- Ethyl alcohol, 95%: 97.0 ml

**Counter stain (Loeffler’s methylene blue) (S081)**
- Methylene blue chloride: 0.3 gm
- Ethyl alcohol, 95%: 30.0 ml
- KOH solution (0.1% aqueous): 100.0 ml

**Directions**

**A. Smear Staining:**
1. Flood the fixed smear with the carbol fuchsin solution (S080) and wait for 15 min without heating.
2. Wash the smear with running tap water until no further colour is given off.
3. Pour decolourizing reagent (S099) on the slide and allow to stand for 30 sec for thin smear and upto 2 min for thick smear.
4. Wash immediately with tap water.
5. Counter stain for 1-2 min with Loeffler’s methylene blue (S081).
6. Rinse well with tap water and dry.
7. Observe under low power objective and examine under oil immersion objective.

**B. Tissue (histological section) staining:**
1. Deparaffinize sections in 2 changes of Xylene, and absolute alcohol. Air dry slides.
2. Flood the slide with carbol fuchsin (S080), and allow standing at 37°C for 1 hour or at 56°C for 30 min.
3. Rinse in tap water until no colour is given off. (1 min)
4. Pour decolourizing reagent (S099) on the slide and allow to stand for 30 sec–1 min.
5. Wash immediately with tap water.
6. Counter stain for 5 min with Loeffler’s methylene blue (S081).
7. Rinse well with tap water.
8. Dehydrate clear in Xylene and observed under oil immersion objective.

**Principle And Interpretation**

Mycobacteria (AFB/Acid Fast Bacteria) are difficult to stain due to high lipid and wax content in their cell walls. HiCold TB stain kit is modified Ziehl-Neelsen staining method which does not require heating (Kinyoun method). The omission of heating step is made possible by increasing the concentration of basic fuchsin in carbol fuchsin solution.

When stained with strong stains (carbol fuchsin solution), acid acid-fast bacilli retain their colour even after treatment with strong decolourizing solutions. They remain red after counterstaining with Loeffler’s methylene blue, whereas the microorganisms susceptible to acid take on the blue colour.
**Type of specimen**

Any isolated colony on primary or subculture plates can be isolated from following specimens. Clinical specimen: Blood, urine, CSF, pus, wounds, lesions, body tissues, sputum etc.

**Specimen Collection and Handling**

All testing for acid-fast bacilli is sent to the reference laboratory in an effort to meet the 24 hr TAT time for smear results. Use sterile, leak proof disposable plastic containers for collection. Do not use wax containers as these can cause false positive smear results. Do not use any fixative or preservatives. Swabs are not recommended as a collection device for the isolation of mycobacteria. They are acceptable only if the specimen can not be obtained by any other means. A negative result from a swab specimen is not reliable. In general, the number of acid fast bacilli in a specimen is small. Early morning specimens are the specimens of choice for urine and sputum because the mycobacteria have had a chance to pool and concentrate, and so increase the chances of recovery. Always collect and submit the maximum volume possible of specimens normally considered sterile. Do not submit 24-hour collections, as they are likely to be diluted and contaminated.

Collect specimens before antimicrobial therapy is started. Even a few days of therapy may kill or inhibit sufficient numbers of mycobacteria to prevent recovery on culture and so leave confirmation of disease in doubt. If a specimen is submitted after therapy has been initiated, note on the request. Avoid contamination of the specimen with tap water, as environmental mycobacteria exist and their recovery by smear or culture can cause confusion for the patient diagnosis.

**Warning and Precautions**

In Vitro diagnostic use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

**Limitations**

1) Smears that are too thick may flake during staining and may be difficult to decolorize.
2) Excessive washing following the carbol fuchsin may cause a heightens decolourization effect.
3) Excessive washing after the counterstain lightens the blue colour of the non acid fast material.
4) Absence of heating,may lead to less penetration of the stain in to the cells thus the number of acid fast bacilli detected may reduce.

**Quality Control**

**Microscopic examination**

Cold acid fast staining is carried out and staining characteristics is observed under microscope using oil immersion lense.

**Results**

- Acid Fact Bacteria: Bright Red bacilli
- Other types of bacteria: Blue
- Other tissue element (Macrophage cells): Blue

**Storage and Shelf Life**

Store between 10 - 30°C in tightly closed container and away from bright light. Use before expiry date on label. On opening, product should be properly stored in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use.

**Disposal**

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,2).
Reference

5. George Clark; Staining procedures, fourth edition. Published by Williams and Wilkins Baltimore. 1981.
7. Clinical Microbiology Procedures Handbook, 1992, Isenberg, American Society for Microbiology. LabCorp Specimen Collection Instructions

Disclaimer:
User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.