HiCold Stain TB- Kit

K062S

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	0.3gm
Ethyl alcohol,95%	30.0ml
KOH solution (0.1% aqueous)	100.0ml

**Formula adjusted, standardized to suit performance parameters

Directions

A. Smear Staining:

1. Flood the fixed smear with the carbol fuchsin solution (S080) and wait for 15min 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 2min for thick smear.
4. Wash immediately with tap water.
5. Counter stain for 1-2min 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 37ºC for 1hour or at 56ºC for 30 min.
3. Rinse in tap water until no colour is given off. (1min)
4. Pour decolourizing reagent (S099) on the slide and allow to stand for 30 sec– 1min.
5. Wash immediately with tap water.
6. Counter stain for 5min 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 o bserved 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^{0}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

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2. Thorpe,J.E., R.P.Banghman,P.T. Frame,T.A. Wessler,and J.L. Staneck.1987.Bronchoalveolar lavage for diagnosing acute bacterial pneumoniae .J.Infect.Dis.155:855-861
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
4. Godkar, P. B; Textbook of Medical Laboratory Technology. Bhalani Publishing House, Bombay.1996.
5. George Clark; Staining procedures, fourth edition. Published by Williams and Wilkins Baltimore. 1981.
6. Kinyoun, J.J. A note on uhlenhuths method for sputum examination for tubercle bacilli.American Journal of Public Health, 5, 867-70.
7. Clinical Microbiology Procedures Handbook, 1992, Isenberg, American Society for Microbiology. LabCorp Specimen Collection Instructions
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IVD	In vitro diagnostic medical device
CE	CE Marking
30°C 10°C	Storage temperature
	Do not use if package is damaged
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EC REP	CE Partner 4U ,Esdoornlaan 13, 3951 DB Maarn The Netherlands, www.cepartner 4u.eu

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