HiCold Stain TB Kit- Kit for Mycobacteria

K062

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

Decolorizer(S099)

Conc. HCl	3.0ml
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

Pretreatment of sputum:

Aseptically mix 0.1 gm of N-Acetyl-L-Cysteine (R034) in 20 ml of Sodium citrate-hydroxide buffer (R032). Use immediately and within 24 hours only. Transfer a maximum volume of 10 ml of sputum/ clinical sample to a sterile graduated 50 ml plastic centrifuge tube having a leak proof cap. Add an equal volume of the above solution. Mix the contents thoroughly by inverting with the cap tightened. Mix on vortex mixer for approximately 20s until the contents are liquefied. Allow the mixture to stand for 15 minutes at 20 to 25°C with occasional gentle shaking by hand. Do not overprocess as this will reduce the recovery of mycobacteria. Add phosphate buffer (R033) up to the 50 ml mark on the tube. Recap the tube and swirl it by hand to mix the contents well. Centrifuge the solution for at least 15 minutes at >= 3000 rpm. Preferably use a refrigerated centrifuge. Carefully decant the supernatant fluid into a splash-proof discard container containing suitable disinfectant. Smear out the sediment and allow to dry.

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 30sec 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 solution(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 to 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), 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.

Quality Control

Microscopic examination

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

Results

Acid fast Bacteria : Bright Red bacilli
Other type Bacteria : Blue
Other tissue element : Blue

Storage and Shelf Life

Store below 30°C in tightly closed container and away from bright light. Use before expiry date on label.

Reference

1.Godkar, P. B; Textbook of Medical Laboratory Technology. Bhalani Publishing House, Bombay.1996.

2. George Clark; Staining procedures, fourth edition. Published by Williams and Wilkins, Baltimore. 1981.

3. Kinyoun, J.J. A note on uhlenhuths method for sputum examination for tubercle bacilli. American Journal of Public Health, 5, 867-70.

4. Valle, Sylvia; Journal of Histology. Vol. 9, No. 4, December 1986.

Important Note:

1. This kit is for 'in-vitro' diagnostic use only.

2. Upon completion of work keep the all the reagent bottles tightly closed, away from bright light, under recommended storage conditions.

3. Staining must be carried out by qualified personnel. National guidelines for work and safety must be followed.

4. The clinical interpretation of result (positive or negative), should be carried out by a qualified pathologist. The interpretation should be complemented by morphological studies and appropriate controls. It is further advised to evaluate the staining results with patient's clinical history, symptoms and parallel diagnostic test, for confirmation.

5. Used and expired solutions must be disposed as special waste in accordance with local guide lines.

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