



Modified Neisser's Metachromatic Stain-Kit

K063

Modified Neisser's Metachromatic Stain-Kit is a modification of Neisser's metachromatic stain. It not only detects metachromatic granules present in *Corynebacterium diphtheriae*, but also differentiates between *Nostocodia limicola*, *Microthrix parvicella* and phosphate solubilising bacteria (Bio-P bacteria).

Composition**

Ingredients

- 1.Modified Neissers methylene blue(S065) - Proprietary
- 2.Neissers Crystal Violet (S060) - Proprietary
- 3.Chrysoidin Y(S061) - Proprietary

Directions

Working Methylene blue-crystal violet solution: Prior to staining, mix 2 parts of Modified Neisser's methylene blue (S065) and 1 part Neisser's Crystal Violet (S060).

A. Filamentous bacteria staining:

- 1.Prepare smear of the specimen sample on grease free clean slide and gently heat fix.
- 2.Stain the slide with freshly made working methylene blue-crystal violet solution (S065 + S060; 2:1) for 10-15seconds.
- 3.Drain off the excess stain.
- 4.Add solution Chrysoidin Y solution (S061) to the slide and stain for 45 seconds.
- 5.Rinse the slide with tap water (with the flow against the back of the slide).
- 6.Air dry the slide (Drying can be speeded up by removing most of the water carefully with filter paper).
- 7.Allow the slide to dry and then view under 100x bright field objective.

B. Diphtheria Staining procedure:

1. Prepare a thin smear of the specimen sample on grease free, clean slide.
2. Allow it to air dry and gently heat fix.
- 3.Stain the smear with freshly made working methylene blue-crystal violet solution (S065 + S060, 2:1)for 10-15 seconds.
4. Drain off the excess stain. (Do not wash)
5. Counter stain with Y solution (S061) and stain for 45 seconds.
6. Rinse the slide with tap water.
7. Blot dry the slide and examine under oil immersion objective.

Principle And Interpretation

Modified Neisser's metachromatic stain kit is a modification of routine neisser's staining method. The modified solution used in this kit helps in rapid detection, within 1 min, of metachromatic granules (Volutin bodies) present in the cell. The kit provides an indispensable aid for the identification of *Corynebacterium diphtheriae* and certain strains of filamentous bacteria. Furthermore, biological phosphate removal bacteria (Bio-P bacteria),responsible for biological phosphate removal, can also be detected.

Metachromatic granules tend to stain more strongly with basic dye (methylene blue and crystal violet) while the bacterium cell takes up the contrasting counter stain (Chrysoidine Y).The *Corynebacterium diphtheriae* gives its characteristic volutin-staining reactions best in a young culture (18-24 hours) cultivated on a blood or serum medium.

Quality Control

Microscopic examination

Metachromatic staining is carried and the stained slides are observed under oil immersion objective

Results

Four main groups of Neisser positive bacteria can be distinguished:

1. Filamentous bacteria (*Nostocoida limicola*) : Grey-violet
2. Filamentous bacteria (*Microthrix parvicella*): Show blue-black coloured polyphosphate globules (in pair) .
3. Biological phosphate removal bacteria : Colonies of blue-black coloured cells. (Bio-P bacteria)
4. Diphtheria bacteria : Yellowish brown slender rods cells with dark brown to black polar bodies

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]. Alfred C. Coles (1899). A modification of Neisser's diagnostic stain for Diphtheria Bacillus. The British Medical Journal, 1 (2003), 1213.

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