Modified Neisser's Metachromatic Stain-Kit

**Intended Use:**
Modified Neisser's Metachromatic Stain-Kit is used to detect metachromatic granules present in *Corynebacterium diphtheriae*. It is a modification of Neisser's metachromatic stain.

**Composition**

**Ingredients**

**Modified Neisser's methylene blue (S065)**
- Neisser's methylene blue: 1.0 gm
- HCl: 30.0 ml
- Methanol: 970.0 ml

**Neisser's Crystal Violet (S060)**
- Crystal violet: 3.3 gm
- Ethyl alcohol: 67.0 ml
- Distilled water: 930.0 ml

**Chrysoidine Y (S061)**
- Chrysoidine Y: 10.0 gm
- Distilled water: 990.0 ml

**Directions**

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 10-15 seconds.
3. Drain off the excess stain.
4. Add solution Chrysoidine 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.

**A. Filamentous bacteria staining:**
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.

**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.

**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. From environment: Air, water, soil, sludge, waste water, food, dairy samples etc.

**Specimen Collection and Handling**
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4, 5).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1, 3).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards. (2)
Generally the smear is made in laboratory; however, when there is a concern that transport will be delayed or that the preservation for culture will alter the specimen, prepare smear and submit slides to the laboratory.

**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. Overstaining may reduce the contrast between the bacteria and background, or between the cytoplasm and granules (6).
2. Some strains of *Propionibacterium*, *Actinomyces*, and pleomorphic forms of streptococci may mimic the characteristic stained appearance of *C. diphtheriae*. (6)

**Quality Control**

**Microscopic examination**
Metachromatic staining was carried and the stained slides were observed under oil immersion objective lens.

**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. Diptheria bacteria: Yellowish brown slender rods cells with dark brown to balck polar bodies

**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**

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<tr>
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