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ZIEHL-NEELSEN STAIN

Used in the differentiation of acid-fast bacteria

REF: BS.1/ZS03.100.0300 3 X 100 ml

CLINICAL SIGNIFICANCE

The acid-fast stain is performed on samples to demonstrate the characteristic of acid fastness in certain bacteria and the cysts of Cryptosporidium and Isospora. Clinically, the most important application is to detect Mycobacterium tuberculosis in sputum samples to confirm or rule out a diagnosis of tuberculosis in patients.

METHOD PRINCIPLE

The lipoid capsule of the acid-fast organism takes up carbolfuchsin and resists decolorization with a dilute acid rinse. The lipoid capsule of the mycobacteria is of such high molecular weight that it is waxy at room temperature and successful penetration by the aqueousbased staining solutions (such as Gram's) is prevented.

REAGENT COMPOSITION

Item	Formula per liter of medium
Solution 1	Carbol fuchsin stain
Solution 2	Decolorizing solvent
Solution 3	Counterstain

PRECAUTIONS AND WARNINGS

Media to be handled by entitled and professionally educated person. Do not ingest or inhale.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.
S57: use appropriate container to avoid environmental Contamination.
S61: avoid release in environment.
For further information, refer to Ziehl - Neelsen Stain material safety data sheet.

STORAGE AND STABILITY

BioScien Ziehl - Neelsen Stain are stable until expiration date stated on label when properly stored 10-25°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle.

PROCEDURE

1. Heat fix an air dried smear at 80o C for at least 15 minutes or for 2 hours on an electric hot plate at 65o C – 70o C
2. Place a slide with an air-dried and heat-fixed smear on suitable staining device. Cut a piece of absorbent paper to fit the slide and saturate the paper with the carbolfuchsin stain.
3. Carefully heat the underside of the slide by passing a flame under the rack or by placing the slide on a hot plate until steam rises (without boiling!). Keep the preparation moist with stain and steaming for 5 minutes, repeating the heating as needed. (CAUTION: overheating causes spattering of the stain and may crack the slide).
4. Wash the film in a gentle and indirect stream of tap water* until no color appears in the effluent.
5. Holding the slide with forceps, wash the slide with the decolorizing solvent. Immediately wash with tap water*, as above. Repeat the decolorizing and the washing until the stained smear appears faintly pink and the fluid washing off the slide runs clear.
6. Flood the smear with the methylene blue counterstain for 20 to 30 seconds, and wash with tap water*, as above.
7. Gently blot, or air dry the smear.
8. Examine under oil immersion.

** Acid-fast bacteria appear red, and non-acid-fast bacteria (and other organisms and cellular materials) appear blue.

SPECIMEN COLLECTION AND PRESERVATION

Clinical samples: Blood

PERFORMANCE CHARACTERISTICS

The appearance of bright red stained bodies that are slender, slightly curved, long or short rods, and sometimes granular or beaded are typically acid-fast bacteria (AFB). Some atypical forms appear thick or diptheriod, sometimes coccoid, or are very long.

Other non-acid-fast organisms will stain blue, as will background material.

QUALITY CONTROL

To ensure adequate quality control, it is recommended that positive and negative control included in each run. If control values are found outside the defined range, check the system performance. If control still out of range please contact ***BioScien*** technical support.

REFERENCES

1. Anderson, N.L., et al. Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.
2. Jorgensen., et al. Manual of Clinical Microbiology, American Society for Microbiology, Washington, D.C.
3. Tille, P., et al. Bailey and Scott's Diagnostic Microbiology, C.V. Mosby Company, St. Louis, MO.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook, Vol. I, II & III. American Society for Microbiology, Washington, D.C.

SYMBOLS IN PRODUCT LABELLING			
IVD	For in-vitro diagnostic use	Σ	Number of <n> test in the pack
LOT	Batch Code/Lot number	⚠	Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		