ZN Acid Fast Stains-Kit (contains S033, S005 and S022)

Intended Use:
ZN Acid Fast Stains-Kit is used for staining of acid fast bacteria.

Composition**

Ingredients
- Carbol Fuchsin (ZN, Strong) (S005)
  - Basic Fuchsin: 0.300 gm
  - Ethyl alcohol, 95%: 10.000 ml
  - Phenol: 5.000 ml
  - Distilled Water: 95.000 ml

- Acid Fast Decolourizer (S033)
  - Hydrochloric acid, concentrated: 3.0 ml
  - Ethyl alcohol, 95%: 97.0 ml

- Methylene Blue (Loeffler's) (S022)
  - Methylene Blue: 0.300 gm
  - Ethyl alcohol, 95%: 30.000 ml
  - Distilled Water: 100.00 ml

**Formula adjusted, standardized to suit performance parameters

Directions
1) Prepare a smear on a clear, dry glass slide.
2) Allow it to air dry and fix with gentle heat.
3) Flood the smear with Carbol Fuchsin stain (S005). Heat to steaming for 5 minutes with a low flame; do not boil the stain and do not permit drying of the smear.
4) Allow it to stand for 5 minutes without further heating.
5) Wash in running tap water.
6) Decolourize with Acid Fast Decolourizer (S033) for 2 minutes or until no more stain comes off in the washings. (If washing is not thorough, you may get false positive results).
7) Wash with tap water.
8) Counterstain for 30 seconds with Methylene Blue (S022).
9) Wash with tap water, dry in air, then examine 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. This differential staining technique is useful for identification of the tubercle bacillus, other Mycobacteria, and Nocardia, which depends on the chemical composition of the bacterial cell wall. Because of the difficulty in staining these organisms with ordinary dyes, basic dyes in the presence of controlled amounts of acid are used. Generally, heat must be applied during the staining procedure, or wetting agents must be used, to aid dye penetration. Organisms exhibiting the property of acid fastness, once stained, are not easily decolourized by alcohol. Non-acid fast organisms are decolourized by acid fast decolourizer and take up the counter stain.

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 heightened decolourization effect.
3) Excessive washing after the counterstain lightens the blue colour of the non acid fast material.

Quality Control
Microscopic examination
Acid fast staining is carried and staining characteristics of organisms is observed under microscope by using oil immersion lens

Results
Bright red - Acid fast organism
Blue - Other organisms and cellular material

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 (6,7).

Reference
8. Clinical Microbiology Procedures Handbook, 1992, Isenberg, American Society for Microbiology. LabCorp Specimen Collection Instructions