ACID FAST STAIN FOR MYCOBACTERIA (KINYOUN)

Principle

To stain Mycobacteria present in specimens and cultures.

Mycobacteria are different to stain with common aniline dyes. However, they will stain with basic fuschsin. Once stained, they retain the dye despite treatment with mineral acids i.e. HCl H_2SO_4. This property of acid fastness may be due to a lipd fraction called mycolic acid. Mycobacteria also exhibit degrees of resistance to decolourization with alcohol.

Materials

Kinyoun Carbol fuchsin
3% HCl in 95% ethanol
Brilliant green

Procedure

1. Prepare smear over an area of 2-3 sq. cm.

2. Heat fix smear on heating block (56^0C/1 hr). Then hold to incinerator for 10 secs.

3. Place slide on stain rack and allow to cool. Flood with Kinyoun Carbol fuschsin for 5 min.

4. Rinse off stain with water.

5. Decolourize with 3% acid alcohol for 3 mins.

6. Rinse with water.

7. Repeat decolourization for 1-2 mins. or until no red appears.

8. Rinse with water.

9. Flood slide 3-4 mins. with Brilliant green.

10. Rinse with water.

11. Air dry. DO NOT BLOT.

Microscopy

Place a drop of oil between the specimen and coverslip and oil again on top. Smears are examined with oil immersion lens. The coverslip prevents cross contamination. An average of 15 mins. is spent on each slide. The total area of the specimen must be examined.

References

1. Baker, F.J., Breach, M.R. 1980. Medical Microbiological Technique, p. 15