

TB-Ziehl-Neelsen

Intended use

Tuberculosis stains (TB) are used to perform the staining of cultures or samples suspected of containing mycobacteria, in order to perform the early diagnosis of mycobacterial infection and its characterisation.

For *in vitro* diagnostic use only.

For professional use only.

Clinical significance

The use of TB stains is fully accepted as procedure for early diagnosis of infection by mycobacteria, as well as to provide information about the bacterial load in the sample.

Even though most acid-fast microorganisms are mycobacteria, this feature is also present in other bacteria like *Nocardia* and some parasites like *Cryptosporidium*.

Principle

Tuberculosis (TB) Stains are used to differentiate acid-fast microorganisms. These microorganisms have a special lipid cell wall containing mycolic acid. This enables the wall to resist discoloration with acid-alcohol after staining with basic dyes such as carbol fuchsin. The cell wall of these microorganisms appears thus reddish-pink, while the rest are stained by the counterstain. It is assumed that permeability through intact membranes plays an essential role in the mechanism of acid-alcohol resistance.

Kinyoun staining is a variant of the classic Ziehl-Neelsen staining procedure which uses fuchsin with high phenol concentrations as primary stain, which allows cold staining. The Ziehl-Neelsen procedure, on the other hand, required heat during the primary staining phase.

Reagent composition

A: Carbol Fuchsin (1 x 250 ml):

Basic Fuchsin	0,2%
Phenol	4,6%

B: TB Decolorizer (1 x 250 ml):

Ethanol	97%
HCl	3%

C: Kühne's Methylene Blue (1 x 250 ml):

Methylene blue	0,5%
Phenol	1%
Ethanol	30%

Caution

Carbol fuchsin: Danger. H226 – Flammable liquid and vapour. H314 – Causes severe skin burns and eye damage. H341 – Suspected of causing genetic defects. P210 – Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P260 – Do not breathe dust/fume/gas/mist/vapours/spray. P264 – Wash thoroughly after handling. P280 – Wear protective gloves/protective clothing/eye protection/face protection. P310 – Immediately call a POISON CENTER/doctor. P305+P351+P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P370+P378 – In case of fire: Use the means described in point 5 of the Safety Data Sheet.

TB Decolorizer: Danger. H225 – Highly flammable liquid and vapour. P210 – Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P233 – Keep container tightly closed. P280 – Wear protective gloves/protective clothing/eye protection/face protection. P303+P361+P353 – IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower. P370+P378 – In case of fire: Use the means described in point 5 of the Safety Data Sheet. P403+P235 – Store in a well-ventilated place. Keep cool. P501 – Dispose of contents/container according to point 13 of the Safety Data Sheet.

Kühne's methylene blue: Warning. H226 – Flammable liquid and vapour. H315 – Causes skin irritation. H319 – Causes serious eye irritation. H341 – Suspected of causing genetic defects. P201 – Obtain special instruction before use. P210 – Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P280 – Wear protective gloves/protective clothing/eye protection/face protection. P362+P364 – Take off contaminated clothing and wash it before reuse. P370+P378 – In case of fire: Use the means described in point 5 of the Safety Data Sheet. P403+P235 – Store in a well-ventilated place. Keep cool. P501 – Dispose of contents/container according to point 13 of the Safety Data Sheet.

All waste should be properly disposed of in accordance with the applicable local regulations.

Preparation

All reagents are ready for use.

Storage and stability

The reagents will remain stable until the expiration date stated on the label when stored at 15-30°C protected from light.

Containers must always be kept tightly closed.

A light precipitate may form for some reagents over time. This, nevertheless, does not affect their functionality.

Additional equipment required but not provided

- Standard microbiology lab equipment and supplies like microscopy slides, Bunsen burner, inoculation loop and filter paper
- Device for staining (manual or automated)
- Microscope with immersion lens

Samples

Smears of bacterial cultures. Samples of various body fluids: sputum, lung fluid, urine sediment, cerebrospinal fluid, tissue, etc.

Spread the sample with an inoculation loop onto a slide to obtain a uniform and thin smear. Air dry and heat-fix by passing the slide through a low flame 2 or 3 times. Leave to cool before performing the staining.

Handle the samples with care due to their potentially infectious nature.

Test procedure

1. Place the smear on a staining bridge and cover it with a piece of filter paper. Soak with **Carbol Fuchsin**. Let stand for 5-10 min. During this period, and from time to time, heat until the stain emits vapours, but do not boil and prevent the preparation from drying out.
2. Rinse softly under tap water and decant excess water.
3. Take the paper off, cover with **TB Decolorizer** and gently swing the smear. Rinse with water. If red staining is still present in the sample, repeat the decolouration step.
4. Rinse softly under tap water and decant excess water.
5. Cover the slide with **Kühne's Methylene Blue** for approximately 1 min.
6. Rinse with water and air dry.
7. Examine under the microscope with an immersion lens.

Interpretation of the results

Alcohol-acid resistant bacteria: Dark red to pink.

Non-alcohol-acid resistant bacteria: Blue.

Notes

The result of the TB staining must be treated as a guide. Positive staining provides presumptive evidence of the presence of mycobacteria in the sample and should be confirmed with additional tests (culture, molecular tests etc). Negative staining does not necessarily indicate that the sample is negative for mycobacteria.

The technique outlined above may be modified in accordance to the technician's preferences in order to obtain variations in the staining intensity. This entails modifications in the staining, de-staining and rinsing times. If running tap water is used for rinsing, please be aware that strongly chlorinated water may weaken the contrast staining. Excessive rinsing after adding the fuchsin may produce false negative results. Excessive rinsing after counterstaining may reduce the staining of the non-acid-fast microorganisms.

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

Quality Control

The use of QC samples is recommended in order to assess the appropriate staining of the sample components. Each laboratory should establish its own QC scheme and corrective actions if the controls do not fulfil the established criteria.

We recommend following the QC practices defined by the CLSI. For this purpose, carry out controls with ATCC microorganisms or other previously characterized control strains.

Bibliography

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2. Clark, G. (1981) "Staining Procedures", pp.380-382, 4th ed. Williams & Wilkins.
3. CLSI Guidelines and Standards, CLSI, Wayne, P.A.
4. Young D.S., Effect of drugs on Clinical Lab. Test, 5th Ed. AAC Press (2000).

