

CLIN-TECH

LIMITED

GRAM/TWORT STAINING KIT

IVD

Catalogue number
631250



INTENDED USE

For the demonstration of Gram positive and Gram negative bacteria in tissues sections.

This method utilises acid alcohol rather than acetone to decolourize, and Twort's stain as a counterstain leading the nuclei of Gram negative organisms to take up the neutral red component, and the background cytoplasm and collagen to take up the fast green component.

KIT PRESENTATION

Reagents

<input type="checkbox"/> Gram's Crystal Violet	500mL
<input type="checkbox"/> Grams Iodine	500mL
<input type="checkbox"/> Twort's Stain	500mL

Also required but not supplied:-

- 2% acetic acid in alcohol
- 100% Ethyl Alcohol (Dehydrate)
- Xylene (Clear)

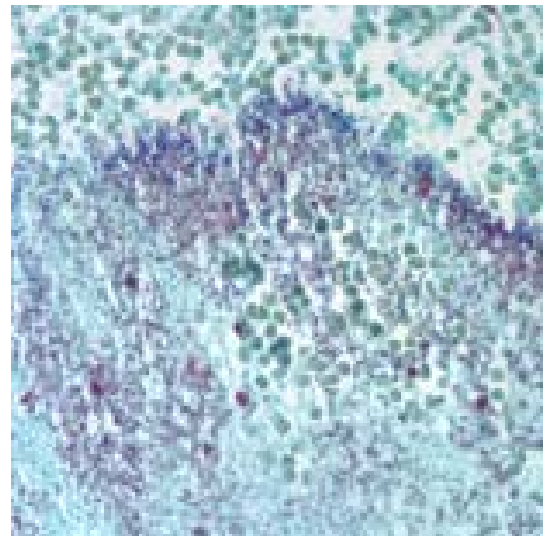
Do not use beyond the expiry date printed on the reagent labels.

TEST PROTOCOL

1. Dewax and rehydrate paraffin sections. (Remove mercury deposits if necessary.)
2. Stain with Crystal Violet for approximately 3 minutes
3. Rinse in gently running tap water.
4. Treat with Gram's Iodine for 3 minutes.
5. Rinse with tap water and blot dry, dry in a warm place.
6. Differentiate with 2% acetic acid alcohol, section should appear light brown or straw coloured.
7. Rinse with water
8. Stain with Twort's for 5 minutes.
9. Wash in distilled water.
10. Immerse slides in 2% acetic acid alcohol for about 5 seconds with agitation
11. Dehydrate and clear
12. Mount.

13. Examine under the microscope, using the oil immersion lens.

RESULTS



Placenta infected with *E. coli* and *Streptococcus* stained with Gram Twort's stain.

GRAM Negative:	pink / red.
GRAM Positive:	blue / black
Nuclei	red
Cytoplasm, Collagen	green
Elastic fibres	black

PROCEDURAL NOTES

The preferred fixative for tissue sections is neutral buffered formalin

Sections should be in paraffin embedded

In step 10, rinse until no more red leaves the section.

REFERENCES

1. Bancroft J.D.& Gamble M. (2008) Theory and Practice of Histological Techniques 6th Ed, Churchill Livingstone, pg 313
2. Twort F. W. (1924) J. State Med. **32** pg 351
3. Ollett W.S. (1947) J. Pathol & Bacteriol. **59** pg 357

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Instructions for use edition C22-53