



Borax Carmine (Grenacher's), Alcoholic

S003

Intended use

Borax carmine is used as staining solution for nuclei and cytoplasmic organelles in whole organisms.

Composition**

Ingredients

Borax Carmine	70.0 gm
Methanol	300.0 ml
Distilled water	700.0 ml

**Formula adjusted, standardized to suit performance parameters

Directions

1. Transfer material to 35 or 50% Borax Carmine Staining Solution to stain for 3-24 hours.
2. Add concentrated hydrochloric acid drop wise, agitating container vigorously until all the carmine is precipitated as a brick red floc. Let it stand for 6 hours to overnight.

(NOTE: With the small volume of material usually stained in protozoal work, it is easily possible to pass from basic to a strongly acid solution with the dye again soluble, the floc being dissolved before one is aware that the process is well under way. In such very acid solutions, the protozoans may be consumed. After each drop, the container should be shaken or tipped until no more action (precipitation) is apparent. End point is reached when there is little or no more of the original deep red translucent solution. If, with a drop of concentrated HCl, the floc begins to dissolve again, add a small drop of borax carmine staining solution).

3. Add an equal volume of 3% alcoholic hydrochloric acid (either in 50% or 70% alcohol) and agitate gently to mix thoroughly. Let it stand until the stained material settles. Decant or pipette off stain suspension, repeating the process several times, as needed to remove most of the stain.

(NOTE: It is this stage which limits the convenience of this stain for protozoans. Individuals smaller than large Stentor, if they are not attached to tissues (as lichenophora on respiratory tree wall) or in smears (as termite flagellates or blood parasites) should be affixed to coverslips.

4. Cover the material about 3 mm deep in fresh 3% HCl in 70% alcohol in a petri plate and observe under microscope until nuclei, zones of membranellles and other organelles retaining stain are deep pink.

(NOTE: If decolourization appears to be happening in a few minutes, put material in 70% alcohol until the process is stopped; examine some in glycerin under the microscope. If the general cytoplasm is still stained, continue the differentiation in acid-alcohol, but with more dilute, 1% or even 0.5% HCl-alcohol).

5. When cytoplasm is transparent (nuclei and fibrillar structures should still be deep pink), remove acid alcohol.
6. Wash with two 5 minutes changes of 80% alcohol, hold in a third change for 60 minutes.
7. Dehydrate, clear, mount in resinous medium.

(NOTE: Lynchs Carmine gives much more transparent stains than haematoxylin on the same subjects; it gives useful stains of Opalina and Nyctotherus, or of small flagellates and trichonymphas in the same termite gut smear or small and large rumen ciliates in the same batch; this is not usually possible with haematoxylin)

Principle And Interpretation

Borax carmine is a biological stain prepared by dissolving the carmine lake powder in water with sodium borate (borax). Borax carmine is a red dye, used in optical microscopy, that stains nuclei and cytoplasm pink. It is frequently used to stain large pieces of animal tissue

Type of specimen

Clinical samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines. After use contaminated materials must be sterilized by autoclaving before discarding.

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. Results may differ due to human error during preparation of slides and processing of staining.
2. If decolourization not done properly, may affect the results.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature

Quality Control

- **Appearance** : Violet coloured solution.
- **Clarity** : Clear without any particles.
- **Microscopic Examination** : Nuclear staining is carried out where Borax Carmine (Grenacher's) Alcoholic stain is used as one of the stain.
- **Results** : Nuclei, zone of membranelles: Deep pink
Other organelles: Deep pink
Cytoplasm: Transparent

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

Reference

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
2. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
3. Downes F. P. and Ito K. (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th ed., APHA, Washington, D.C.



Storage temperature



Do not use if package is damaged



In vitro diagnostic medical device



CE Marking



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