Borax Carmine (Grenacher's)Aqueous Stain

S004

Composition**

Ingredients

Borax carmine	70.000 gms
Distilled water	1000.000 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 dropwise, 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 Stentors, if they are not attached to tissues (as licnophora 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 membranelles 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 haematoxylins 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 haematoxylins).

Principle And Interpretation

Borax carmine test method is designed to see nuclei and cytoplasmic organelles in whole organisms.

Quality Control

Appearance

Violet coloured solution.

Clarity

Clear without any particles.

Microscopic Examination

Nuclear staining is carried out where Borax Carmine (Grenacher's) Aqueous stain is used as one of the stains and staining characteristics is observed under microscope by using oil immersion lens.

Results

Nuclei, zone of membranells -Deep pink
Other organelles - Deep pink
Cytoplasm - Transparent

Storage and Shelf Life

Store below 30°C in tightly closed container and away from bright light. Use before expiry date on label.

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