



## Neutral Red Solution

S037

### Intended use

Neutral Red solution is used for Neisser's staining method of metachromatic granules.

### Composition\*\*

#### Ingredients

Neutral red	1.0 gm
Acetic acid	2.0 ml
Distilled water	1,000.0 ml

\*\*Formula adjusted, standardized to suit performance parameters

### Directions

1. Prepare a smear on clean, dry glass slide.
2. Allow it to air dry and fix with gentle heat.
3. Stain with Neisser's Methylene Blue (S023) for 3 minutes.
4. Wash off with dilute (1:10 in distilled water) Gram's Iodine Solution (S013) and leave some of this solution on the slide for 1 minute.
5. Wash with water.
6. Counterstain with Neutral Red Solution (S037) for 3minutes.
7. Wash with water and air dry it and observe under oil immersion objective.

### Principle And Interpretation

Well developed granules of volutin (polyphosphate) may be seen in unstained wet preparations as round refractile bodies within the bacterial cytoplasm. With basic dye, they tend to stain more strongly than the rest of the bacterium, and with toluidine blue or methylene blue they stain metachromatically, a reddish purple colour. They are demonstrated most clearly by special methods, such as Albert's and Neisser's, which stain them dark purple but the remainder of the bacterium with a contrasting counterstain. The diphtheria bacillus gives its characteristic volutin-staining reactions best in a young culture (18-24 hours) on a blood or serum medium.

### Type of specimen

Any isolated colony on primary or subculture plates can be isolated from following specimens. Clinical specimen: Blood, urine, CSF, pus, wounds, lesions, body tissues, sputum etc. From environment: Air, water, soil, sludge, waste water, food, dairy samples etc.

### Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines.

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines.

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.

Generally, the smear is made in laboratory; however, when there is a concern that transport will be delayed or that the preservation for culture will alter the specimen, prepare smear and submit slides to the laboratory.

## 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. Overstaining may reduce the contrast between the bacteria and background, or between the cytoplasm and granules.
2. Some strains of Propionibacterium, Actinomyces, and pleomorphic forms of streptococci may mimic the characteristic stained appearance of C. diphtheriae.

## 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** : Red coloured solution.
- **Clarity** : Clear without any particles.
- **Microscopic Examination** : Metachromatic staining is carried out where Neutral red is used as counter stain and staining characteristic of organism is observed under microscope by using oil immersion lens.
- **Results** : Metachromatic granules : deep blue  
The Organisms : pink coloured.

## 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. Downes F. P. and Ito K. (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th ed., APHA, Washington, D.C.
2. Rice E.W., Baird, R.B., Eaton A. D., Clesceri L. S. (Eds.), 2012, Standard Methods for the Examination of Water and Wastewater, 22nd ed., APHA, Washington, D.C.
3. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Tenover, G., Tenover, G., Landry, M.L., Richter, S.S and Warnock, D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

6. Shanholtzer, C.J., P. Schaper, and L.R. Peterson 1982. Concentrated Gram stain smear prepared with a cytospin centrifuge. J. clin. Microbiol.16:1052-1056
7. Thorpe,J.E., R.P.Banghman,P.T. Frame,T.A. Wessler,and J.L. Staneck.1987.Bronchoalveolar lavage for diagnosing acute bacterial pneumoniae .J.Infect.Dis.155:855-861.



Storage temperature



Do not use if package is damaged



In vitro diagnostic medical device



CE Marking



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