remel

5% ALPHA NAPHTHOL (VOGES-PROSKAUER A)

INTENDED USE
Remel 5% Alpha Naphthol (VP A) is a reagent recommended for use in qualitative procedures to determine the ability of gram-negative bacilli to produce acetylmethylcarbinol (acetoin) from glucose fermentation.

SUMMARY AND EXPLANATION
Voges and Proskauer reported certain bacteria, when grown in an appropriate medium such as MR-VP Broth, produce acetylmethylcarbinol from glucose fermentation.[1,2] Barritt recommended using 5% Alpha Naphthol (VP A) and 40% Potassium Hydroxide (VP B) for detection of acetylmethyl-carbinol production.[3]

PRINCIPLE
The Voges-Proskauer test is based on the detection of acetoin (acetylmethylcarbinol) from glucose metabolism.[4] Glucose is metabolized to pyruvic acid from which the neutral products, acetoin and 2,3-butanediol, are formed. In the presence of oxygen and alkali, acetoin and 2,3-butanediol are oxidized to diacetyl, the reactant for the pink color produced in the Voges-Proskauer test. VP A is a catalyst which serves to intensify the color and increase the sensitivity of the reaction. VP B is an oxidizing agent which hastens oxidation of acetoin to diacetyl.

REAGENTS (CLASSICAL FORMULA)*
Alpha Naphthol (CAS 90-15-3) ..... 50.0 g
Ethyl Alcohol (CAS 64-17-5) ..... 1000.0 ml

*Adjusted as required to meet performance standards.

PRECAUTIONS
DANGER! POISON, may be fatal or cause blindness if swallowed. Cannot be made nonpoisonous. Vapor harmful**. FLAMMABLE,** keep away from heat, sparks and flame. Avoid breathing vapor and eye/skin contact.

This product is for *In Vitro* diagnostic use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after use. Directions should be read and followed carefully. Refer to Material Safety Data Sheet for additional information**.**

STORAGE
Store product in its original container at 2-8°C until used. Allow product to equilibrate to room temperature before use. Protect product from light.

PRODUCT DETERIORATION
This product should not be used if (1) the color has changed, (2) the expiration date has passed, or (3) there are other signs of deterioration.

SPECIMEN COLLECTION, STORAGE, TRANSPORT
Specimens should be collected and handled following recommended guidelines**.**[5,6]

MATERIALS REQUIRED BUT NOT SUPPLIED
(1) Loop sterilization device, (2) Inoculating loop, swabs, collection containers, (3) Incubators, alternative environmental systems, (4) Supplemental media, (5) Quality control organisms, (6) MR-VP Broth (REF R061430), (7) 40% Potassium Hydroxide (VP B) (REF R21281), (8) Ethanol (95%) (REF R40132), (9) Pipettes, (10) Sterile screw cap test tubes.

PROCEDURE
Reconstitute Alpha Naphthol powder by adding 12 ml of 95% ethanol to the vial and mix well. The reconstituted product has a shelf life of 2-weeks when stored at 2-8°C.

1. Inoculate a tube of MR-VP Broth with a pure culture of the isolate to be tested and incubate at 35-37°C for 24-72 hours.
2. Following incubation, transfer 1 ml of inoculated MR-VP Broth to a separate tube.
3. Add 0.6 ml of 5% Alpha Naphthol (VP A).
4. Add 0.2 ml of 40% Potassium Hydroxide (VP B) and shake well.
5. Allow mixture to stand up to 15 minutes at room temperature and observe for the development of a pink color.

INTERPRETATION
Positive Test - Pink color development within 15 minutes

Negative Test - No color development within 15 minutes

QUALITY CONTROL
All lot numbers of 5% Alpha Naphthol (VP A) have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
Enterobacter cloacae ATCC®13047	Aerobic, 24 h @ 35-37°C	Positive
Escherichia coli ATCC®25922	Aerobic, 24 h @ 35-37°C	Negative

LIMITATIONS

1. An organism may fail to produce a positive result due to destruction of acetoin. To verify a negative result, gently heat the suspension after adding the reagents to effect development of a pink color.[7]
2. The methyl red and Voges-Proskauer tests are only part of the overall scheme for identification of *Enterobacteriaceae.* Further testing is required for definitive identification. Consult appropriate references for further instructions.[5,6]
3. The order of adding reagents must be followed exactly; reversal of the order may cause a weak positive or false-negative result.[4]
4. The exact amount of VP B must be added, as an excess of this reagent may mask a weak positive Voges-Proskauer reaction by development of a copper color, which is not considered a positive reaction.[4]

BIBLIOGRAPHY

1. Voges, O. and B. Proskauer. 1898. Zeit. Hyg. 28:20-32.
2. Clark, W.M. and H.A. Lubs. 1915. J. Infect. Dis. 17:160-173.
3. Barritt, M.M. 1936. J. Pathol. Bacteriol. 42:441-454.
4. MacFaddin, J.F. 2000. Biochemical Tests for Identification of Medical Bacteria. 3rd ed. Lippincott Williams & Wilkins, Philadelphia, PA.
5. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.L. Landry, and M.A. Pfaller. 2007. Manual of Clinical Microbiology. 9th ed. ASM Press, Washington, D.C.
6. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2007. Bailey and Scott's Diagnostic Microbiology. 12th ed. Mosby Elsevier, St. Louis, MO.
7. MacFaddin, J.F. 1985. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Vol. 1. Williams & Wilkins, Baltimore, MD

PACKAGING

5% Alpha Naphthol (VP A):
REF R21200 ..... 12 ml/Btl

Symbol Legend

REF	Catalog Number
IVD	In Vitro Diagnostic Medical Device
LAB	For Laboratory Use
	Consult Instructions for Use (IFU)
	Temperature Limitation (Storage Temp.)
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)

CAS (Chemical Abstracts Service Registry No.)

IFU 21200, Revised December 17, 2008 Printed in the U.S.A

12076 Santa Fe Drive, Lenexa, KS 66215, USA
General Information: (800) 255-6730 Technical Services: (800) 447-3641 Order Entry: (800) 447-3635
Local/International Phone: (913) 888-0939 International Fax: (913) 895-4128
Website: www.remel.com Email: remel@remel.com