IFU

Instructions for Use

# RAPID ANGINOSUS ID KIT

| | | |
|---|---|---|
| Cat. no. Z14 | Rapid Anginosus ID Kit | 10 tests/kit |
| | Each kit contains:<br>Z14A - Rapid Arginine, 13x100mm Tube, 0.5ml<br>Z14B - Rapid VP, 13x100mm Tube, 0.5ml | <br>10 tubes<br>10 tubes |

## INTENDED USE

Hardy Diagnostics Rapid Anginosus ID Kit is used to detect arginine decarboxylase activity and perform the Voges-Proskauer test, in as little as four hours, to assist in the identification of the *Streptococcus anginosus* group (formerly *S. milleri*).

## SUMMARY

Hardy Diagnostics Rapid Anginosus ID Kit can be used to identify streptococcal isolates suspected of belonging to the anginosus group (*S. anginosus, S. constellatus, S. intermedius*).

Infection by *Streptococcus anginosus* group (formerly known as *Streptococcus milleri*) can cause endocarditis, CNS infections and abscesses, bacteremia, oral infections, neonatal sepsis, deep-seated sepsis, intra-abdominal infections, pyogenic liver abscesses, deep-tissue abscesses, appendicitis, and pulmonary infections.[1-3] *S. anginosus* can also cause soft tissue infections, which become severe in patients that are intravenous-drug users or have uncontrolled diabetes.[3] Early detection and treatment of *S. anginosus* group is essential, and with Hardy Diagnostics Rapid Anginosus ID Kit, results can be obtained in as little as four hours.

Conventional methods for detecting arginine hydrolysis require an extended period of incubation. The tests generally involve the degradation of arginine, resulting in an increase in pH and indicated by the development of a purple color. Tests are usually incubated for a period of up to seven days. An overlay of mineral oil acts as a barrier to oxygen and prevents alkalinization of the surface of the medium.

In 1898, Voges and Proskauer, first observed the production of a red color after the addition of potassium hydroxide to cultures grown on specific media.[6] Harden later revealed that the development of the red color was a result of acetyl-methyl carbinol production.[7] In 1936 Barrit made the test more sensitive by adding alpha-naphthol to the medium before adding potassium hydroxide.[8]

The Voges-Proskauer test identifies bacteria that are able to metabolize pyruvic acid to form acetyl-methyl carbinol (acetoin). This end product, in the presence of atmospheric oxygen and 40% potassium hydroxide, is converted to diacetyl. Diacetyl, under the catalytic action of alpha-naphthol and creatine, is converted into a pink-red complex.[3] This is a positive Voges-Proskauer (VP) test reaction. The conventional Voges-Proskauer test tube method requires an incubation of up to 72 hours.

The Voges-Proskauer test is also useful for differentiating coagulase-positive species *S. aureus* (positive VP) from *S. hyicus* and *S. intermedius* (negative VP).[4]

Rapid Arginine consists of peptones and yeast extract which supply nitrogenous and other nutrients necessary for bacterial growth. Bromcresol purple is a pH indicator. If the amino acid arginine is hydrolyzed, the pH of the medium shifts upward resulting in a color change. This reaction requires an anaerobic environment, which can be created by a mineral oil overlay.

Rapid VP consists of peptones and glucose (which supply nitrogenous and other nutrients necessary for bacterial growth) in a phosphate-buffered solution. A color change results from the above described reaction between acetoin and diacetyl.

## FORMULA

Ingredients per liter of deionized water:*

| **Rapid Arginine (Z14A):** | |
|---|---|
| L-Arginine | 10.0gm |
| Meat Peptone | 5.0gm |
| Yeast Extract | 3.0gm |
| Bromcresol Purple | 0.01gm |

Final pH 5.5 +/- 0.2 at 25ºC.

| **Rapid VP (Z14B):** | |
|---|---|
| Dipeptone | 7.0gm |
| Dextrose | 5.0gm |
| Potassium Phosphate | 5.0gm |

Final pH 6.9 +/- 0.2 at 25ºC.

* Adjusted and/or supplemented as required to meet performance criteria.

## STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8ºC. Media should not be used if there are any signs of deterioration, discoloration, contamination, or if the expiration date has passed. Product is temperature sensitive; protect from excessive heat and moisture.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

## PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory

personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

## PROCEDURE

Specimen Collection: This product is not intended for primary isolation of patient specimens. It should be used only with cultures of isolated organisms. This product is used in conjunction with other biochemical tests to identify cultures of isolated organisms.

Method of use:

1. A suspect colony should be grown on a non-selective media, such as Chocolate Agar, Cat. no. E14 or Blood Agar, Cat. no. A10, however Chocolate Agar will yield higher growth of *Streptococcus* spp. and is recommended.

2. From this 18-24 hour old pure culture plate, heavily inoculate (greater than a McFarland Latex #4, Cat. no. ML4) one tube of Rapid Arginine and one tube of Rapid VP.

3. Overlay the inoculated Rapid Arginine broth with at least 0.5ml of Sterile Mineral Oil (Cat. no. Z80).

4. Incubate aerobically at 35-37 degrees C. for four hours.

**VOGES-PROSKAUER TEST performed AFTER incubation**

5. Add three drops of 5% alpha-naphthol (Voges-Proskauer Reagent A, Cat. no. Z91) to the Rapid VP tube and agitate tube to mix.

6. Add one drop of 40% KOH (Voges-Proskauer Reagent B, Cat. no. Z92) to the Rapid VP tube and agitate tube to mix.

7. Allow tube to remain undisturbed at room temperature for 15-30 minutes.

## INTERPRETATION OF RESULTS

**ARGININE HYDROLYSIS**

For reference, compare an uninoculated reference tube with an inoculated tube.

Positive reaction: development of a purple color. Must be darker than the uninoculated reference tube.

Negative reaction: no development of color (remains pale yellow to light gray).

**VOGES-PROSKAUER TEST**

A positive VP test is demonstrated by the development of a pink-red color on the surface of the medium 15-30 minutes after the addition of the reagents.

A negative VP test is demonstrated by the appearance of a yellow color on the surface of the medium. Development of a copper-like color is also interpreted as negative.

**PRESUMPTIVE IDENTIFICATION BASED ON RESULTS OBTAINED WITH RAPID ANGINOSUS ID KIT**

| Viridans Streptococcal Group | Arginine | VP |
|---|---|---|
| Anginosus group | +<br>purple | +<br>pink-red |
| Mitis group | V<br>purple/yellow | -<br>yellow |
| Mutans, Salivarius, and Bovis groups | -<br>yellow | +<br>pink-red |

## LIMITATIONS

In addition to the *Streptococcus anginosus* group, *S. porcinus* (rarely isolated from humans), and *S. ratti* are also positive for both arginine hydrolysis and the Voges-Proskauer test, thus it is recommended that further biochemical tests be performed on pure cultures for complete identification. For more information, see appropriate references.[3-6] Acidification of fermentation broth with sorbitol (Cat. no. Y93 or Y114) is useful as *S. porcinus* and *S. ratti* are positive while the *S. anginosus* group is negative.

Some organisms that are capable of producing acetyl methyl carbinol (acetoin) produce false-negative VP reactions when incubated for 48-72 hours. Rapid VP should not exhibit false-negative VP reactions because of the heavy suspension and short incubation time (as little as four hours).

When adding the VP reagents to the suspension, it is important that the alpha-naphthol be added first and the KOH added second. A change in the order may produce invalid test results.

False-positive VP results may occur if VP tests are read beyond one hour following the addition of reagents.

Refer to the document "Limitations of Procedures and Warranty" for more information.

## MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, Blood Agar (Cat. no. A10), other culture media, swabs, applicator sticks, incinerators, Sterile Mineral Oil (Cat. no. Z80), Saline, 0.85% (Cat. no. K248 or R45), Voges-Proskauer Reagent A (Cat. no. Z91), Voges-Proskauer Reagent B (Cat. no. Z92), McFarland Latex #4 (Cat. no. ML4), pipets, and incubators, etc. are not provided.

## QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

| Test Organisms | Inoculation Method* | Incubation | | | Reaction | |
|---|---|---|---|---|---|---|
| | | Time | Temperature | Atmosphere | Arginine | Voges-Proskauer |
| *Streptococcus agalactiae* ATCC® 12386** | E | 4hr | 35°C | Aerobic | Positive (purple) | Negative (yellow) |
| *Streptococcus salivarius* ATCC® 13419** | E | 4hr | 35°C | Aerobic | Negative (yellow) | Positive (pink-red) |
| *Streptococcus anginosus* ATCC® 33397** | E | 4hr | 35°C | Aerobic | Positive (purple) | Positive (pink-red) |

* Refer to the document "Inoculation Procedures for Media QC" for more information.

** Recommended QC strains for User Quality Control according to the CLSI document M22 when applicable.

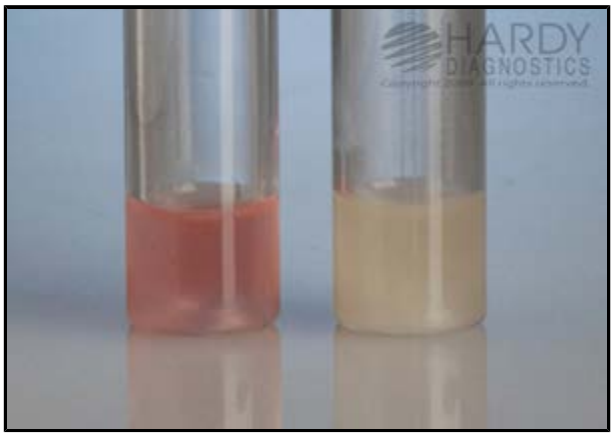
### USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

## PHYSICAL APPEARANCE

Rapid Arginine should appear clear, and pale yellow.
Rapid VP should appear clear, and light amber in color.


**Z14A - Rapid Arginine tube**
**LEFT:** *Streptococcus anginosus* (ATCC® 33397)
Positive reaction at four hours.
**RIGHT:** *Streptococcus salivarius* (ATCC® 13419)
Negative reaction at four hours.




**Z14B - Rapid VP tube**
**LEFT:** *Streptococcus anginosus* (ATCC® 33397)
Positive reaction at four hours.
**RIGHT:** *Streptococcus agalactiae* (ATCC® 12386)
Negative reaction at four hours.

## REFERENCES

1. Ruoff, K.L., 1988. *Streptococcus anginosus* ("*Streptococcus milleri*"): The Unrecognized Pathogen. *Clin. Microbiol. Rev.*; Vol. 1, p. 102-108.

2. Whitworth, J.M., 1991. Use of Rapid Carbohydrate Utilisation Test for Identifying " *Streptococcus milleri* Group". *J. Clin. Pathol.*; Vol. 44, p. 329-333.

3. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology.* J.B. Lippincott Company, Philadelphia, PA.

4. Versalovic, J., et al. *Manual of Clinical Microbiology.* American Society for Microbiology, Washington, D.C.

5. Tille, P.M., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.

6. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.

7. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

8. Voges, O. and B. Proskauer. 1898. *Zeit. Hyg.*; 28:20-32.

9. Harden, A. 1906. *Proc. Roy. Soc.*, (London); 77:424-425.

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