IFU

Instructions for Use

TRYPTONE BROTH, 2%

Cat. no. R40	Tryptone Broth, 2%, 13x100mm Tube, 2ml	20 or 100 tubes/box

INTENDED USE

Hardy Diagnostics Tryptone Broth, 2% is recommended for use in the detection of indole production by microorganisms, especially the Enterobacteriaceae.

SUMMARY

Detection of indole in a tryptophan enriched medium was first described by Kovacs in 1928.[5] Organisms possessing the enzyme tryptophanase degrade tryptophan to produce indole and other metabolic products.

Indole production can be determined by the production of a red-violet color complex upon application of Kovacs (Cat. no. Z67) or Ehrlichs Reagent. If present, indole reacts with the aldehyde group of p-dimethylaminobenzaldehyde, the active chemical in either reagent. Microorganisms that do not possess tryptophanase will not produce a red-violet color change upon application of the reagent.

FORMULA

Ingredients per liter of deionized water:*

Pancreatic Digest of Casein	20.0gm
Sodium Chloride	5.0gm

Final pH of 6.8 +/- 0.3 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-30ºC. Media should not be used if there are any signs of deterioration, discoloration, contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat and freezing.

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

Method of use with Kovacs Reagent:

1. Prior to inoculation, allow the medium to equilibrate to room temperature.

2. Obtain organisms from an 18-24 hour pure culture from an appropriate plate medium and inoculate to the broth tube.

3. Incubate the inoculated broth aerobically at 35ºC. for 24-48 hours; incubate with loosened caps.

4. Following incubation, add 4-5 drops of Kovacs Reagent (Cat. no. Z67) to the tube, shake gently.

5. Allow the tube to stand approximately 10 minutes.

6. Observe for the production of a dark red color in the top alcohol layer.

Method of use with Ehrlichs Reagent:[6]

1. Prior to inoculation, allow the medium to equilibrate to room temperature.

2. Obtain organisms from an 18-24 hour pure culture from an appropriate plate medium and inoculate to the broth tube.

3. Incubate the inoculated broth aerobically at 35ºC. for 24-48 hours; incubate with loosened caps.

4. Following incubation, add 1ml xylene to the broth. Shake the tube well and allow it to stand for a few minutes until the solvent rises to the surface.

5. Add approximately 0.5ml of Ehrlichs Reagent down the sides of the tube; a ring will form between the medium and the solvents.

INTERPRETATION OF RESULTS

Kovacs Reagent: A positive indole test reaction is indicated by the appearance of a dark red color in the top alcohol layer.

Ehrlichs Reagent: A positive indole test reaction is indicated by the development of a brilliant red ring just below the solvent layer.

A negative reaction is indicated by the medium remaining colorless or light yellow due to the color of the reagent.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

Either Kovacs or Ehrlichs Reagent may be used to detect the production of indole in broth media; of the two reagents, Ehrlichs is the more sensitive and is best suited for detecting indole production in non-fermenters or anaerobes. Ehrlichs Reagent is not suitable for use in agar media. If using Ehrlichs Reagent, an extraction with xylene is necessary. Kovacs Reagent does not require the extraction step.

Prior to the addition of either reagent, it is recommended that a 2ml portion of the inoculated broth be aseptically removed and transferred to a separate sterile tube. If the test is negative, the remaining broth from the original tube should be reincubated for an additional 24 hours and then retested.

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, slides, staining supplies, Kovacs or Ehrlichs Reagent, other culture media, microscopes, incinerators, incubators, etc., as well as serological and biochemical reagents, 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			Results
		Time	Temperature	Atmosphere	
Escherichia coli ATCC® 25922	E	18-24hr	35°C	Aerobic	Growth; turns pink to red after adding 4-5 drops of Kovacs Reagent and agitation
Staphylococcus aureus ATCC® 25923	E	18-24hr	35°C	Aerobic	Growth; no pink color after adding Kovacs Reagent

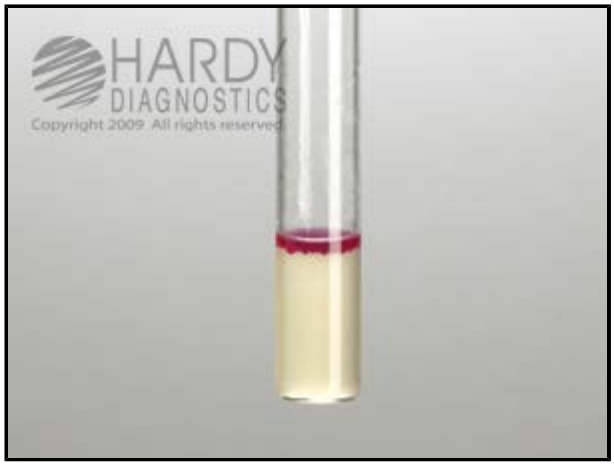
* Refer to the document "Inoculation Procedures for Media QC" for more information.

USER QUALITY CONTROL

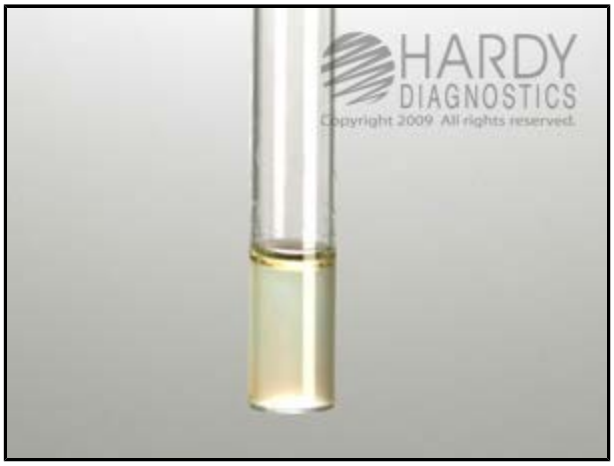
End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory 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

Tryptone Broth, 2% should appear clear, and light amber in color.



Showing positive indole reaction.
Escherichia coli (ATCC® 25922) was incubated in Tryptone Broth under aerobic conditions for 24 hours at 35ºC. Five drops of Indole Kovac's Reagent (Cat. no. Z67) was added directly to the broth and the tube was gently shaken. The top alcohol layer shows a positive reaction.



Showing negative indole reaction. *Staphylococcus aureus* (ATCC® 25923) was incubated in Tryptone Broth (Cat. no. R40) under aerobic conditions for 24 hours at 35ºC. Five drops of Indole Kovac's Reagent (Cat. no. Z67) was added directly to the broth and the tube was gently shaken. The top alcohol layer shows a negative reaction.

Uninoculated tube of Tryptone Broth, 2% (Cat. no. R40).

REFERENCES

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

2. 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.

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

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

5. Kovacs, N. 1928. *Zeit. Immunitest, Exper. Therap.*; 53:311.

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

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

HDQA 2207F [D]