

Instructions for Use

INDOLE TEST REAGENTS

Cat. no. Z65	Indole Spot Reagent	15ml
Cat. no. Z67	Indole Kovacs Reagent	15ml

INTENDED USE

Hardy Diagnostics Indole Spot Reagent and Indole Kovacs Reagent are recommended for use in determining the indole reaction of bacteria.

SUMMARY

The indole test is a qualitative procedure for determining the ability of bacteria to produce indole by deamination of tryptophan.

Using Kovacs tube method, indole combines, in the presence of a tryptophan rich medium, with p-Dimethylaminobenzaldehyde at an acid pH in alcohol to produce a red-violet compound.

In the spot test, indole combines, in the filter paper matrix, at an acid pH with p-Dimethylaminocinnamaldehyde (DMACA) to produce a blue to blue-green compound. Indole Spot Reagent (DMACA) has been reported to be useful in detecting indole production by members of the family *Enterobacteriaceae* and certain anaerobic species.

REAGENT FORMULA

Ingredients per liter:*

Indole Spot Reagent:	
p-Dimethylaminocinnamaldehyde (DMACA)	10.0gm
Hydrochloric Acid, 37%	100.0ml
Deionized Water	900.0ml

Indole Kovacs Reagent:	
p-Dimethylaminobenzaldehyde	50.0gm
Hydrochloric Acid, 37%	250.0ml
Amyl Alcohol	750.0ml

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-30°C. Products should not be used if there are any signs of deterioration or if the expiration date has passed.

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. This product is used in conjunction with other biochemical tests to identify cultures of isolated organisms.

Indole Spot Reagent (DMACA): Place several drops of Indole Spot Reagent on a piece of filter paper. With an inoculating loop or wooden applicator stick, pick a portion of an 18-24 hour isolated colony from a non-selective media and rub it onto the reagent saturated area of the filter paper.

Interpretation of Results: A positive reaction is denoted by the appearance of a blue to blue-green color change on the bacterial smear, or red-violet in the case of *Providencia alcalifaciens*, within 10 seconds. Negative reactions remain colorless or light pink.

The filter paper may appear pink to purple after the reagent is applied, but only the color change of the bacterial smear itself should be indicative of a positive reaction.

Indole Kovacs Reagent: Lightly inoculate Tryptone Broth (Cat. no. R40) or Peptone Broth with the test organism. Incubate 24-48 hours at 35°C. Add 4-5 drops of Kovacs Reagent to the tube, shake gently.

Interpretation of Results: A positive Kovacs tube test reaction is denoted by the appearance of a pink to red color in the top alcohol layer. Negative reactions remain colorless or light yellow.

If Kovacs Indole is to be used with a commercial identification test strip, such as API® or EnteroPluri, or Microgen, consult the manufacturer's literature.

LIMITATIONS

Indole tests may be used as an aid in the identification and differentiation of gram-positive and gram-negative organisms. Additional biochemical testing using pure cultures is recommended for complete identification.

The tube test is a more sensitive method of detecting indole than the spot test.

When performing a spot test, Kovacs Indole Reagent may be used as a substitute for the spot test reagent. However, Kovacs Indole Reagent, when used as the spot test reagent, is less sensitive in detecting indole than the Indole Spot Reagent (DMACA).⁽⁶⁾

Kovacs Indole Reagent is not recommended for use with anaerobic bacteria. The Indole Spot Reagent (DMACA) is suitable for anaerobe use.

Since peptones have been shown to vary with regard to their suitability for use with indole testing, media selected for indole determination should be tested with known positive and negative organisms to insure suitability.

Media containing glucose should not be used for indole testing due to the formation of acid end products which have been shown to reduce indole production. Mueller Hinton Agar should also not be used for this test because tryptophan is destroyed during acid hydrolysis of casein.

Media containing dye, such as MacConkey and EMB, are unsuitable sources of inoculum due to possible carryover of dye and subsequent interference of indole color interpretation.

Indole-positive colonies have been reported to cause adjacent indole-negative colonies to appear false-positive due to diffusion of indole into the media. To avoid false-positives, select colonies of different morphologies that are separated by at least 5mm for indole testing.⁽⁶⁾

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, Tryptone Broth (Cat. no. R40), Peptone Broth, needles, incubators, and incinerators, etc., as well as biochemical and serological 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	Reaction
Indole Spot	
<i>Escherichia coli</i> ATCC® 25922	Positive; blue to blue-green color change
<i>Pseudomonas aeruginosa</i> ATCC® 27853	Negative; no color change or pink
Indole Kovacs	
<i>Escherichia coli</i> ATCC® 25922	Positive; red color change
<i>Pseudomonas aeruginosa</i> ATCC® 27853	Negative; no color change or light yellow

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

- Indole Spot Reagent should appear clear, and yellow to orange in color.
- Indole Kovacs Reagent should appear clear, and light yellow in color.



Showing positive indole reaction.

Filter paper was saturated with Indole Spot Reagent (Cat. no. Z65) and *Escherichia coli* (ATCC® 25922) growth was applied subsequently. *E. coli* was incubated aerobically for 24 hours at 35°C. on a TSA plate (Cat. no. G60).



Showing negative indole reaction.

Filter paper was saturated with Indole Spot Reagent (Cat. no. Z65) and *Pseudomonas aeruginosa* (ATCC® 27853) growth was applied subsequently. *P. aeruginosa* was incubated aerobically for 24 hours at 35°C. on a TSA plate (Cat. no. G60).



Showing positive indole reaction.

Escherichia coli (ATCC® 25922) was incubated in Tryptone Broth under aerobic conditions for 24 hours at 35°C. 5 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.

Pseudomonas aeruginosa (ATCC® 27853) was incubated in Tryptone Broth (Cat. no. R40) under aerobic conditions for 24 hours at 35°C. 5 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.

REFERENCES

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5. MacFaddin, J.F. *Biochemical Tests for Identification of Medical Bacteria*, Lipincott Williams & Wilkins, Philadelphia, PA.
6. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II, & III. American Society for Microbiology, Washington, D.C.
7. Centers for Medicare and Medicaid, *Appendix C, Survey Procedures and Interpretive Guidelines for Laboratories and Laboratory Services*. Subpart K - Quality System for Non-Waived Testing. 493;1200-1265.
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API is a registered trademark of bioMeriuex, France.

ATCC is a registered trademark of the American Type Culture Collection.

IFU-10494[B]



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