

---

**remel**

## **BILE SOLUBILITY REAGENT**

### **2% and 10% (SODIUM DESOXYCHOLATE)**

#### **INTENDED USE**

Remel Bile Solubility Reagent, 2% and 10%, are recommended for use in qualitative procedures for presumptive identification and differentiation of *Streptococcus pneumoniae* from other alpha-hemolytic streptococci.

#### **SUMMARY AND EXPLANATION**

*S. pneumoniae* possesses an autolytic enzyme that lyses the organism's own cell wall during cell division. In 1900, Neufeld used sodium desoxycholate, a bile salt, to detect autolysis which differentiated bile-soluble *S. pneumoniae* from bile-insoluble, alpha-hemolytic streptococci.<sup>1</sup> Downie et al. employed 10% bile salt solutions to lyse broth cultures of pneumococci.<sup>2</sup> Greey later reported the effectiveness of applying bile directly to a blood agar plate culture of pneumococci.<sup>3</sup> In 1965, Hawn and Beebe demonstrated a rapid method of bile solubility on blood agar using 2% sodium desoxycholate.<sup>4</sup> The 10% reagent may provide more rapid reactions; however, both concentrations have been reported to provide reliable results with both the tube and spot test methods.<sup>5-7</sup>

#### **PRINCIPLE**

The bile solubility test is based on the observation that *S. pneumoniae* cells visibly lyse when 2% or 10% sodium desoxycholate is applied, while other alpha-hemolytic streptococci do not. *S. pneumoniae* produces an autolytic, intracellular enzyme that causes the organism to undergo rapid autolysis when cultivated on artificial medium.<sup>8</sup> The actual mechanism for bile solubility is not fully understood, but it is speculated that bile salts lower surface tension at the medium-membrane interface and under these conditions, *S. pneumoniae* is particularly susceptible to disruption by enzyme action or by chemical agents. Goebel and Avery concluded that the action of bile salts is independent of the autolytic enzyme, whereas Dubos and Hirsch reported that bile salts may activate the enzyme by either alteration or removal from the cells of an autolysis inhibitor.<sup>9,10</sup>

#### **REAGENTS (CLASSICAL FORMULA)\***

##### **Bile Solubility Reagent, 2% and 10%:**

Sodium Desoxycholate .....	20.0 or 100.0	g
Tincture Thimerosal .....	0.15	ml
Ethyl Alcohol .....	200.0	ml
Demineralized Water .....	800.0	ml

\*Adjusted as required to meet performance standards.

#### **PRECAUTIONS**

**DANGER! COMBUSTIBLE**, keep away from heat and flame. May cause irritation to skin, eyes, and respiratory tract. Avoid breathing vapor and eye/skin contact. Refer to Material Safety Data Sheet for additional information on reagent chemicals.

These products are 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.

#### **STORAGE**

This product is ready for use and no further preparation is necessary. Store product in its original container at room temperature until used. Some flocculation may occur if product is stored below 20°C. This will not reduce the effectiveness of the reagent. Protect product from light.

#### **PRODUCT DETERIORATION**

These products 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, AND TRANSPORT**

Specimens should be collected and handled following recommended guidelines.<sup>5</sup>

#### **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) Test tubes, 13 x 100 mm (Tube Test only), (7) McFarland 0.5 and 1.0 turbidity standards (R20410 and R20411) or equivalents, (8) Saline (pH 7.0), (9) Pipettes.

#### **PROCEDURE**

Perform preliminary tests, such as Gram stain and catalase, to verify that test isolate is a *Streptococcus*.

##### **Tube Test:**

1. Prepare a saline suspension of the test isolate from an 18-24 hour, pure culture.
2. Adjust turbidity to that of a 0.5 to 1.0 McFarland standard or equivalent.
3. Aliquot 0.5 ml of the suspension into each of 2 tubes. Label one tube as Test, the other as Control.
4. Add 0.5 ml of 2% or 10% Bile Solubility Reagent to the tube marked Test, and 0.5 ml of saline (pH 7.0) to the tube marked Control. Gently agitate tubes to suspend bacteria.
5. Incubate tubes at 35-37°C and examine periodically for up to 3 hours.
6. Observe for clearing in the Test suspension. The Control suspension should remain turbid.

##### **Spot Test:**

1. Add 1 drop of 2% or 10% Bile Solubility Reagent to a well-isolated, 18-24 hour colony of the test isolate growing on sheep blood agar.
2. Incubate the plate aerobically in an upright position at 35-37°C and examine periodically for up to 30 minutes. Leave lid slightly ajar to enhance evaporation of the reagent.
3. Observe the colony for disintegration or solubility.

#### **INTERPRETATION**

##### **Tube Test:**

- |                 |   |
|-----------------|---|
| Positive Test - | Clearing of the test suspension within 3 hours; control suspension remains turbid |
| Negative Test - | Test and control suspensions remain turbid within 3 hours                         |

##### **Spot Test:**

- |                 |   |
|-----------------|---|
| Positive Test - | Colony disintegration within 30 minutes |
| Negative Test - | Colony remains intact within 30 minutes |

## QUALITY CONTROL

All lot numbers of Bile Solubility Reagent 2% and 10% 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
<b>TubeTest:</b>		
<i>Streptococcus pneumoniae</i> ATCC® 6305	Ambient, 3 h @ 35-37°C	Positive
<i>Streptococcus sanguinis</i> ATCC® 10556	Ambient, 3 h @ 35-37°C	Negative
<b>Spot Test:</b>		
<i>Streptococcus pneumoniae</i> ATCC® 6305	Ambient, 30 min. @ 35-37°C	Positive
<i>Streptococcus sanguinis</i> ATCC® 10556	Ambient, 30 min. @ 35-37°C	Negative

## LIMITATIONS

1. Test only alpha-hemolytic streptococci.
2. When performing the spot test, keep the plate level to prevent reagent from running, as some nonpneumococcal colonies may wash away and falsely appear as being disintegrated.<sup>7</sup>
3. Colonies that are older than 24 hours may have lost their active enzyme, resulting in a false-negative test.<sup>7</sup>

## BIBLIOGRAPHY




1. Neufeld, F. 1900. Z. Hyg. Infektionskr. 34:454-464.
2. Downie, A.W., L. Stent, and S.M. White. 1913. Br. J. Exp. Pathol. 12:1-9.
3. Greey, P.H. 1939. J. Infect. Dis. 64:206-207.
4. Hawn, C.V.Z. and E. Beebe. 1965. J. Bacteriol. 90:549.
5. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller, and R.H. Tenen. 2003. Manual of Clinical Microbiology. 8<sup>th</sup> ed., Vol. 1. ASM, Washington, D.C.

6. Clinical and Laboratory Standards Institute (CLSI). 2002. Abbreviated Identification of Bacteria and Yeast; Approved Guideline, M35-A. CLSI, Wayne, PA.
7. MacFaddin, J.F. 2000. Biochemical Tests for Identification of Medical Bacteria. 3<sup>rd</sup> ed. Lippincott Williams & Wilkins, Philadelphia, PA.
8. Cowan, S.T. and K.J. Steel. 1970. Manual for the Identification of Medical Bacteria. Cambridge University Press, Cambridge, England.
9. Goebel, W.F. and O.T. Avery. 1929. J. Exp. Med. 49:267-286.
10. Dubos, R.J. and J.G. Hirsch. 1965. Bacterial and Mycotic Infections of Man. 4<sup>th</sup> ed. J.B. Lippincott Co., Philadelphia, PA.

## PACKAGING

REF R21206, Bile Solubility Reagent, 10%.....25 ml/Btl  
REF R21209, Bile Solubility Reagent, 2%.....25 ml/Btl

## Symbol Legend

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

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

IFU 21206, Revised July 17, 2012

Printed in the U.S.A.