

broth culture to a sheep blood agar plate; otherwise, incubate an additional 24 hours before discarding.<sup>9</sup>

### Expected Results

Growth in broth medium is indicated by the presence of turbidity compared to an uninoculated control.

Subculture to a **Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)** plate and incubate for 18-24 hours, or up to 48 hours if necessary. Identify organisms suggestive of group B streptococci ( $\beta$ - or non-hemolytic, gram-positive and catalase negative). Specific identification may be performed; e.g., using streptococcal grouping sera, the CAMP test or other procedures.

### References

1. Todd and Hewitt. 1932. *J. Pathol. Bacteriol.* 35:973.
2. Updyke and Nickle. 1954. *Appl. Microbiol.* 2:117.
3. Jones, Hebert and Cherry. 1978. Fluorescent antibody techniques and bacterial applications, HEW Publication (CDC) No. 78-8364. Center for Disease Control, Atlanta, Ga.
4. MacFaddin. 1985. *Media for isolation-cultivation-identification-maintenance of medical bacteria*, vol. 1. Williams & Wilkins, Baltimore, Md.
5. Federal Register. 1994. Prevention of group B streptococcal disease: a public health perspective. *Fed. Regist.* 59:64764.
6. Centers for Disease Control and Prevention. 2002. *Morbidity and Mortality Weekly Report*. 51(No. RR-11): 1.
7. Forbes, Salm and Weissfeld. 1998. *Bailey & Scott's diagnostic microbiology*, 10th ed. Mosby, Inc., St. Louis, Mo.
8. Facklam and Washington II. 1991. *In* Balows, Hausler, Hermann, Isenberg and Shadomy (ed.), *Manual of clinical microbiology*, 5th ed. American Society for Microbiology, Washington, D.C.
9. Isenberg (ed.). 1992. *Clinical microbiology procedures handbook*, vol. 1. American Society for Microbiology, Washington, D.C.

### Availability

#### Bacto™ Todd Hewitt Broth

##### SMWVW

Cat No.	249230	Dehydrated – 100 g
	249240	Dehydrated – 500 g
	249210	Dehydrated – 2 kg
	249220	Dehydrated – 10 kg

#### BBL™ Todd Hewitt Broth

##### SMWVW

Cat. No.	211735	Dehydrated – 100 g
	211736	Dehydrated – 500 g
	297778	Prepared Tubes (K Tubes), 0.5 mL – Pkg. of 10
	221713	Prepared Tubes (K Tubes), 5 mL – Pkg. of 10
	221714	Prepared Tubes (K Tubes), 5 mL – Ctn. of 100

#### BBL™ Todd Hewitt Broth with Gentamicin and Nalidixic Acid

Cat No.	299486	Prepared Tubes (K Tubes) – Ctn. of 100*
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\*Store at 2-8°C.

## Tomato Juice Media

### Tomato Juice Agar • Tomato Juice Agar Special

### Tomato Juice Broth

#### Intended Use

Tomato Juice Agar is used for cultivating and enumerating *Lactobacillus* species.

Tomato Juice Agar Special is used for cultivating and enumerating lactobacilli and other acidophilic microorganisms from saliva and other specimens.

Tomato Juice Broth is used for cultivating yeasts and other aciduric microorganisms.

#### Summary and Explanation

In 1925, Mickle and Breed<sup>1</sup> reported the use of tomato juice in culture media used for cultivating lactobacilli. Kulp<sup>2</sup> investigated the use of tomato juice on bacterial development and found that the growth of *L. acidophilus* was enhanced. Tomato Juice Agar, prepared according to Kulp and White's<sup>3</sup> modification, is especially useful in cultivating *L. acidophilus* from clinical specimens and foodstuffs.<sup>4</sup>

Tomato Juice Agar Special is recommended for the direct plate count of lactobacilli from saliva and for cultivation of other acidophilic microorganisms. The acidic pH of Tomato Juice Agar Special encourages growth of lactobacilli while inhibiting growth of accompanying bacteria. The number of lactobacilli in saliva is an index of a predisposition to dental caries as described by Jay.<sup>5, 6</sup> Many dentists use the direct count of lactobacilli for

the diagnosis of caries. This medium is more selective for lactobacilli than Tomato Juice Agar.

Tomato Juice Broth is recommended for use in cultivating and isolating yeasts, lactobacilli and other aciduric microorganisms from clinical specimens and foods.

#### Principles of the Procedure

##### Tomato Juice Agar and Tomato Juice Agar Special

Tomato juice is a source of carbon, protein and nutrients. Peptone provides a source of nitrogen, amino acids and carbon. Peptonized milk contains lactose as an energy source. Agar is the solidifying agent.

##### Tomato Juice Broth

Tomato juice is a source of carbon, protein and nutrients. Yeast extract is a source of trace elements, vitamins and amino acids. Dipotassium phosphate and monopotassium phosphate provide buffering capability. Magnesium sulfate, ferrous sulfate and manganese sulfate provide inorganic ions. Sodium chloride is a source of essential ions.

## User Quality Control

### Identity Specifications

#### Difco™ Tomato Juice Agar

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	5.1% solution, soluble in purified water upon boiling. Solution is medium to dark amber, very slightly opalescent.
Prepared Appearance:	Medium to dark amber, very slightly opalescent.

Reaction of 5.1% Solution at 25°C:	pH 6.1 ± 0.2
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#### Difco™ Tomato Juice Agar Special

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	6.0% solution, soluble in purified water upon boiling. Solution is medium to dark amber, slightly opalescent.
Prepared Appearance:	Medium to dark amber, slightly opalescent.

Reaction of 6.0% Solution at 25°C:	pH 5.0 ± 0.2
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#### Difco™ Tomato Juice Broth

Dehydrated Appearance:	Tan, free-flowing, homogeneous and may contain dark particles.
Solution:	4.1% solution, soluble in purified water upon boiling. Solution is dark amber, clear.
Prepared Appearance:	Dark amber, clear.
Reaction of 4.1% Solution at 25°C:	pH 6.7 ± 0.2

### Cultural Response

#### Difco™ Tomato Juice Agar

Prepare the medium per label directions. Inoculate using the pour plate technique and incubate at 35 ± 2°C for 40-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Lactobacillus acidophilus</i>	4356	10 <sup>2</sup> -10 <sup>3</sup>	Good
<i>Lactobacillus rhamnosus</i>	9595	10 <sup>2</sup> -10 <sup>3</sup>	Good
<i>Lactobacillus delbrueckii</i> subsp. <i>lactis</i>	4797	10 <sup>2</sup> -10 <sup>3</sup>	Good

#### Difco™ Tomato Juice Agar Special

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours (72 hours if necessary).

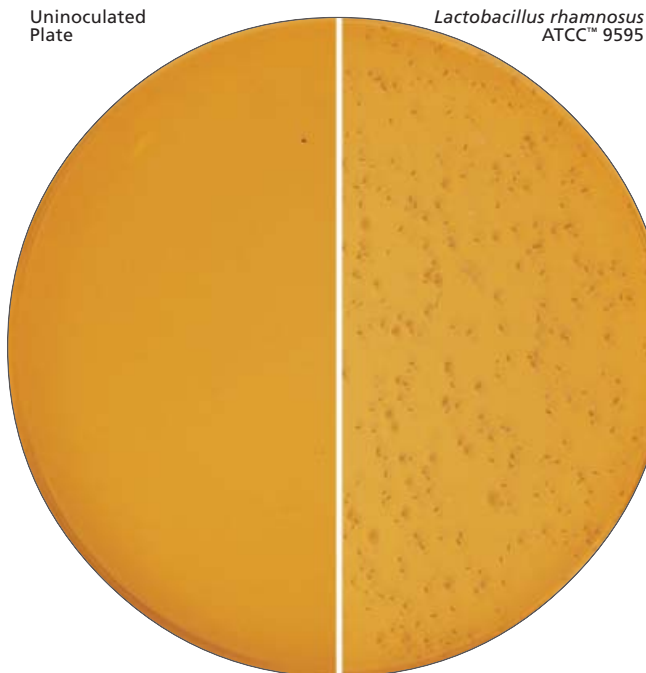
ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Lactobacillus acidophilus</i>	4356	10 <sup>2</sup> -10 <sup>3</sup>	Good
<i>Lactobacillus rhamnosus</i>	9595	10 <sup>2</sup> -10 <sup>3</sup>	Good
<i>Lactobacillus delbrueckii</i> subsp. <i>lactis</i>	4797	10 <sup>2</sup> -10 <sup>3</sup>	Good

#### Difco™ Tomato Juice Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Lactobacillus rhamnosus</i>	9595	10 <sup>2</sup> -10 <sup>3</sup>	Good
<i>Lactobacillus delbrueckii</i> subsp. <i>lactis</i>	4797	10 <sup>2</sup> -10 <sup>3</sup>	Good
<i>Saccharomyces cerevisiae</i>	9080	10 <sup>2</sup> -10 <sup>3</sup>	Good
<i>Saccharomyces cerevisiae</i>	9763	10 <sup>2</sup> -10 <sup>3</sup>	Good

Uninoculated Plate



## Formulae

### Difco™ Tomato Juice Agar

Approximate Formula* Per Liter	
Tomato Juice (from 400 mL)	20.0 g
Peptone	10.0 g
Peptonized Milk	10.0 g
Agar	11.0 g

### Difco™ Tomato Juice Agar Special

Approximate Formula* Per Liter	
Tomato Juice (from 400 mL)	20.0 g
Peptone	10.0 g
Peptonized Milk	10.0 g
Agar	20.0 g

### Difco™ Tomato Juice Broth

Approximate Formula* Per Liter	
Tomato Juice (from 400 mL)	20.0 g
Yeast Extract	10.0 g
Dextrose	10.0 g
Dipotassium Phosphate	0.5 g
Monopotassium Phosphate	0.5 g
Magnesium Sulfate	0.1 g
Sodium Chloride	0.01 g
Ferrous Sulfate	0.01 g
Manganese Sulfate	0.01 g

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

## Directions for Preparation from Dehydrated Product

Equilibrate the medium to room temperature before opening.

1. Suspend the powder in 1 L of purified water:  
 Difco™ Tomato Juice Agar – 51 g;  
 Difco™ Tomato Juice Agar Special – 60 g;  
 Difco™ Tomato Juice Broth – 41 g.  
 Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Avoid overheating Tomato Juice Agar Special, which could cause a softer medium.
4. Test samples of the finished product for performance using stable, typical control cultures.

## Procedure

See appropriate references for specific procedures.

## Expected Results

Refer to appropriate references and procedures for results.

## References

1. Mickle and Breed. 1925. Technical Bulletin 110. N.Y. State Agriculture Exp. Station, Geneva, N.Y.
2. Kulp. 1927. Science 66:512.
3. Kulp and White. 1932. Science 76:17.
4. MacFaddin. 1985. Media for isolation-cultivation-identification- maintenance of medical bacteria, vol 1. Williams & Wilkins, Baltimore, Md.
5. Jay and Gordon (ed). 1938. Bacteriology and immunology of dental caries and dental science and dental art. Lea and Febiger, Philadelphia, Pa.
6. Jay, Pelton and Wisan. 1949. Dentistry in public health. W. B. Saunders Company, Philadelphia, Pa.

## Availability

### Difco™ Tomato Juice Agar

Cat. No. 211794 Dehydrated – 500 g\*

### Difco™ Tomato Juice Agar Special

Cat. No. 238910 Dehydrated – 500 g\*

### Difco™ Tomato Juice Broth

Cat. No. 251720 Dehydrated – 500 g\*

251710 Dehydrated – 10 kg\*

\*Store at 2-8°C.

# Transport Media

## Transport Medium Amies • Transport Medium Amies without Charcoal • Transport Medium (Stuart, Toshach and Patsula) • Cary and Blair Transport Medium

### Intended Use

Transport Medium Amies, Transport Medium Amies without Charcoal and Transport Medium (Stuart, Toshach and Patsula) are used for collecting, transporting and preserving microbiological specimens.

Cary and Blair Transport Medium is used for collecting, transporting and preserving microbiological specimens, particularly those containing *Vibrio cholerae*.

### Summary and Explanation

Transport media are chemically defined, semisolid, nonnutritive, phosphate buffered media that provide a reduced environment. Transport media are formulated to maintain the viability of microorganisms without significant increase in growth.

In 1948, Moffett, Young and Stuart described a medium for transporting gonococcal specimens to the laboratory.<sup>1</sup> Stuart, Toshach and Patsula improved this formulation, introducing what is now known as Stuart's Transport Medium.<sup>2</sup> The ability of Stuart's medium to maintain the viability of gonococci during transport<sup>3,4</sup> led other researchers to explore its use with a variety of specimens. This medium is currently recommended for throat, vaginal and wound samples.

In 1964, Cary and Blair modified Stuart's medium by substituting inorganic phosphates for glycerophosphate and raising the pH to 8.4.<sup>5</sup> The modified medium was effective in maintaining the viability of *Salmonella* and *Shigella*<sup>6,7</sup> in fecal samples.

Due to its high pH, Cary and Blair Transport Medium is also effective in maintaining the viability of *Vibrio* cultures for up to four weeks.<sup>8</sup> Cary and Blair Transport Medium is currently recommended for fecal and rectal samples.

Amies<sup>9</sup> confirmed Cary and Blair's observations that an inorganic salt buffer was superior to the glycerophosphate. He further modified the formulation by using a balanced salt solution containing inorganic phosphate buffer, omitting the methylene blue and adding charcoal. This modified medium yielded a higher percentage of positive cultures than the transport medium of Stuart. Transport Medium Amies, available with and without charcoal, is recommended for throat, vaginal and wound samples. Amies media are especially suited for specimens containing *Neisseria gonorrhoeae*.

### Principles of the Procedure

In the formulations, potassium chloride, calcium chloride, magnesium chloride and sodium chloride provide essential ions that help maintain osmotic balance while controlling permeability of bacterial cells. Monopotassium phosphate and disodium phosphate provide buffering capabilities. Sodium thioglycollate suppresses oxidative changes and provides a reduced environment. Sodium glycerophosphate is a buffer for use with calcium chloride. Methylene blue is a colorimetric pH indicator of the oxidation-reduction state. Charcoal neutralizes fatty acids that are toxic to microorganisms. Agar makes the media semi-solid.