

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.¹ Stuart, Toshach and Patsula improved this formulation, introducing what is now known as Stuart's Transport Medium.² The ability of Stuart's medium to maintain the viability of gonococci during transport^{3,4} 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 8.4.⁵ The modified medium was effective in maintaining the viability of *Salmonella* and *Shigella*^{6,7} 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.⁸ Cary and Blair Transport Medium is currently recommended for fecal and rectal samples.

Amies⁹ 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.

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco™** and **BBL™** brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Transport Medium Amies

Dehydrated Appearance:	Black, free-flowing, homogeneous.
Solution:	2.0% solution, soluble in purified water upon boiling. Solution is black, opaque.
Prepared Appearance:	Black, opaque, semi-solid.
Reaction of 2.0% Solution at 25°C:	pH 7.3 ± 0.2

Difco™ Transport Medium Amies without Charcoal

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	1.0% solution, soluble in purified water upon boiling. Solution is colorless to very light amber, opalescent with precipitate.
Prepared Appearance:	Colorless to very light amber, opalescent with precipitate, semi-solid.
Reaction of 1.0% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Difco™ Transport Medium Amies or Transport Medium Amies without Charcoal

Prepare the medium per label directions. Inoculate sterile swabs with suspensions of test organisms containing 10³-10⁴ CFU/0.1 mL. Place swabs in the medium and incubate at room temperature for 18-24 hours. Remove swabs, streak on prepared chocolate agar plates and incubate appropriately. All cultures should be viable.

ORGANISM	ATCC™
<i>Bacteroides fragilis</i>	25285
<i>Haemophilus influenzae</i> Type b	10211
<i>Neisseria gonorrhoeae</i>	43069
<i>Neisseria meningitidis</i> Group B	13090
<i>Streptococcus pneumoniae</i>	6305
<i>Streptococcus pyogenes</i> Group A	19615

Formulae

Difco™ Transport Medium Amies

Approximate Formula* Per Liter	
Sodium Chloride	3.0 g
Potassium Chloride	0.2 g
Calcium Chloride	0.1 g
Magnesium Chloride	0.1 g
Monopotassium Phosphate	0.2 g
Disodium Phosphate	1.15 g
Sodium Thioglycollate	1.0 g
Charcoal	10.0 g
Agar	4.0 g

Difco™ Transport Medium Amies without Charcoal

Consists of the same ingredients without the charcoal.

BBL™ Transport Medium (Stuart, Toshach and Patsula)

Approximate Formula* Per Liter	
Sodium Thioglycollate	1.0 g
Sodium Glycerophosphate	10.0 g
Calcium Chloride	0.1 g
Methylene Blue	2.0 mg
Agar	3.0 g

Identity Specifications

BBL™ Transport Medium (Stuart, Toshach and Patsula)

Dehydrated Appearance:	Slightly moist, granular, softly clumped, free of extraneous material, may contain minute to small white particles.
Solution:	1.41% solution, soluble in purified water upon boiling. Solution is pale, yellow with light blue-green top, clear to slightly hazy.
Prepared Appearance:	Pale, yellow with light blue-green top, clear to slightly hazy.
Reaction of 1.41% Solution at 25°C:	pH 7.3 ± 0.2

BBL™ Cary and Blair Transport Medium

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	12.6 g/991 mL, soluble in purified water upon boiling. Solution is light to medium, gray, hazy to cloudy.
Prepared Appearance:	Light to medium, gray, hazy to cloudy.
Reaction of 12.6 g/991 mL Solution at 25°C:	pH 8.0 ± 0.5

Cultural Response

BBL™ Transport Medium (Stuart, Toshach and Patsula)

Prepare the medium per label directions. Inoculate charcoal impregnated sterile swabs with heavy suspensions of the test organisms. Place in the medium and incubate at 25 ± 2°C for 66-72 hours. Remove swabs, streak on TSA with 5% sheep blood plates or Chocolate II agar plates for (*) organisms and incubate plates at 35 ± 2°C for 18-24 hours under appropriate atmospheric conditions.

ORGANISM	ATCC™	RECOVERY
<i>Haemophilus influenzae</i> *	10211	Good
<i>Neisseria gonorrhoeae</i> *	19424	Good
<i>Streptococcus pneumoniae</i>	6305	Good

BBL™ Cary and Blair Transport Medium

Prepare the medium per label directions. Inoculate sterile swabs with heavy suspensions of the test organisms. Place in the medium and incubate at 25 ± 2°C for 18-24 hours. Remove swabs, streak on TSA with 5% sheep blood plates or Chocolate II agar plates for (*) organisms and incubate plates at 35 ± 2°C for 18-24 hours under appropriate atmospheric conditions.

ORGANISM	ATCC™	RECOVERY
<i>Haemophilus influenzae</i> *	10211	Good
<i>Neisseria gonorrhoeae</i> *	19424	Good
<i>Shigella flexneri</i>	9199	Good
<i>Streptococcus pneumoniae</i>	6305	Good

BBL™ Cary and Blair Transport Medium

Approximate Formula* Per Liter	
Sodium Thioglycollate	1.5 g
Disodium Phosphate	1.1 g
Sodium Chloride	5.0 g
Agar	5.0 g

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

Directions for Preparation from Dehydrated Product

Difco™ Transport Medium Amies or Transport Medium Amies without Charcoal

1. Suspend the powder in 1 L of purified water:
Difco™ Transport Medium Amies – 20 g;
Difco™ Transport Medium Amies w/o Charcoal – 9.8 g.
Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Dispense into 6-8 mL screw-cap vials to within 5 mm of the top. Cap tightly.
4. Autoclave at 121°C for 15 minutes.
5. Retighten caps, if necessary. For Transport Medium Amies, invert vials just prior to solidification to uniformly distribute the charcoal.
6. Test samples of the finished product for performance using stable, typical control cultures.

BBL™ Transport Medium (Stuart, Toshach and Patsula)

1. Suspend 14.1 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Dispense in small screw-capped bottles or vials, filling them almost to capacity. Leave only enough space to permit acceptance of a small swab without overflow when in use.
6. Autoclave at 121°C for 10 minutes or steam for 1 hour. After autoclaving, tighten caps immediately.
7. Test samples of the finished product for performance using stable, typical control cultures.

BBL™ Cary and Blair Transport Medium

1. Suspend 12.6 g of the powder in 991 mL of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Cool to 50°C and add 9 mL of 1% aqueous calcium chloride.
4. Adjust the pH to approximately 8.4, if necessary.
5. Dispense in 7 mL amounts in 9 mL screw-capped test tubes.
6. Steam for 15 minutes. Cool. Tighten caps.
7. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

1. Obtain specimen with sterile swab. Insert specimen swab(s) into the upper third of the medium in the transport container.
2. Cut with sterile scissors or break-off the protruding portion of the swab stick. Tightly screw the lid on the bottle or vial.
3. Label the bottle or vial and send to the laboratory with minimum delay. Specimens may be refrigerated until ready for shipment.
4. Submit to laboratory within 24 hours for culture and analysis.

Expected Results

Survival of bacteria in a transport medium depends on many factors including the type and concentration of bacteria in the specimen, the formulation of the transport medium, the temperature and duration of transport and inoculation to appropriate culture media within 24 hours.

Optimal growth and typical morphology can only be expected following direct inoculation and appropriate cultivation.

Limitations of the Procedure

1. Specimens taken from transport media will not exhibit the optimal or comparative growth as expected from direct inoculation and cultivation. These media do, however, provide an adequate degree of preservation for those specimens which cannot be forwarded immediately to the laboratory for prompt evaluation.
2. Viability of cells will diminish over time and some degree of multiplication or growth of contaminants can occur during prolonged periods of transit. This is particularly true of fecal specimens that contain substantial numbers of coliform organisms.
3. The condition of the specimen received by the laboratory for culture is a significant variable in recovery and final identification of the suspect pathogen. An unsatisfactory specimen (overgrown by contaminants, containing nonviable organisms, or having the number of pathogens greatly diminished) can lead to erroneous or inconclusive results.
4. For transport of specimens that may contain *N. gonorrhoeae*, the use of a selective medium, such as JEMBEC™ or Gono-Pak systems, should also be considered.

References

1. Moffett, Young and Stuart. 1948. Br. Med. J. 2:421.
2. Stuart, Toshach and Patsula. 1954. Can. J. Public Health 45:73.
3. Stuart. 1946. Glasgow M. J. 27:131.
4. Stuart. 1959. Public Health Reports 74:431.
5. Cary and Blair. 1964. J. Bacteriol. 88:96.
6. Cary, Matthew, Fusillo and Harkins. 1965. Am. J. Clin. Path. 43:294.
7. Neuman, Benenson, Hubster and Thi Nhu Tuan. 1971. N. Am. J. Clin. Path. 57:33.
8. Kelly, Hickman-Brenner and Farmer. 1991. In Balows, Hausler, Herrmann, Isenberg and Shadomy (ed.), Manual of clinical microbiology, 5th ed.. American Society for Microbiology, Washington D.C.
9. Amies. 1967. Can. J. Public Health 58:296.

Availability

Difco™ Transport Medium Amies

Cat. No. 212225 Dehydrated – 500 g

Difco™ Transport Medium Amies without Charcoal

Cat. No. 283210 Dehydrated – 500 g

BBL™ Amies Transport Medium without Charcoal

Cat. No. 297643 Prepared Tubes, 9 mL (K Tubes) – Ctn. of 100

BBL™ Transport Medium (Stuart, Toshach and Patsula)

Cat. No. 211743 Dehydrated – 500 g*

BBL™ Cary and Blair Transport Medium

Cat. No. 211102 Dehydrated – 500 g*
297611 Prepared Tubed Deeps, 10 mL (D Tubes) –
Ctn. of 100

*Store at 2-8°C.