

Precautions⁵

1. Biosafety Level 2 practices, containment equipment and facilities are recommended for activities with clinical specimens of human or animal origin containing or potentially containing pathogenic *Brucella* spp.
2. Biosafety Level 3 practices, containment equipment and facilities are recommended for all manipulations of cultures of the pathogenic *Brucella* spp. and for experimental animal studies.

Directions for Preparation from Dehydrated Product

Refer to the final concentration of Biosate Peptone in the formula of the medium being prepared. Add product as required.

Procedure

See appropriate references for specific procedures using Biosate Peptone.

Expected Results

Refer to appropriate references and procedures for results.

References

1. Artemenko, Ivanova, Nenashev, Kuznetsova and Ochkina. 1985. Zhurnal Mikrobiologii, Epidemiologii, i Immunobiologii. 11:37.
2. Siegel and Metzger. 1980 Appl. Environ. Microbiol. 40:1023.
3. Ponce, Martínez and Alvarez. 1989. Archivos de Investigacion Medica. 20:123.
4. Dorsman and Bijl. 1985. J. Parasitol. 71:200.
5. U.S. Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. 1999. Biosafety in microbiological and biomedical laboratories, 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Government Printing Office, Washington, D.C.

Availability

BBL™ Biosate™ Peptone

Cat. No. 211862 Dehydrated – 454 g
294312 Dehydrated – 25 lb (11.3 kg)

User Quality Control

Identity Specifications

BBL™ Biosate™ Peptone

Dehydrated Appearance: Yellow-tan powder, fine, homogeneous, free of extraneous material.

Solution: 2.0% solution, soluble in purified water. Solution is light to medium, yellow to tan, clear to slightly hazy.

Reaction of 2.0% Solution at 25°C: pH 6.3-7.5

Cultural Response

BBL™ Biosate™ Peptone

Prepare a sterile solution of peptone agar using 10 g of Biosate Peptone, 2.5 g of sodium chloride and 6.5 g of agar in 500 mL of purified water. Adjust final pH to 7.2-7.4. Inoculate and incubate plates at 35 ± 2°C for 2-3 days.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Brucella abortus</i>	11192*	10 ³ -10 ⁴	Good
<i>Escherichia coli</i>	25922	10 ³ -10 ⁴	Good
<i>Staphylococcus aureus</i>	6538P	10 ³ -10 ⁴	Good
<i>Streptococcus pyogenes</i>	49117	10 ⁴ -10 ⁵	Good

*If this strain is not available, verify performance with a known isolate.

Biotin Assay Medium

Intended Use

Biotin Assay Medium is used for determining biotin concentration by the microbiological assay technique.

Summary and Explanation

Vitamin assay media are used in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;
3. Assay Media: To permit quantitation of the vitamin under test. They contain all the factors necessary for optimal growth of the test organism except the single essential vitamin to be determined.

Biotin Assay Medium is prepared for use in the microbiological assay of biotin using *Lactobacillus plantarum* ATCC™ 8014 as the test organism.

User Quality Control

Identity Specifications

Difco™ Biotin Assay Medium

Dehydrated Appearance: Light beige, homogeneous with a tendency to clump.

Solution: 3.75% (single strength) solution, soluble in purified water upon boiling 2-3 minutes. Solution is light amber, clear, may have a slight precipitate.

Prepared Appearance: Light amber, clear, may have a slight precipitate.

Reaction of 3.75% Solution at 25°C: pH 6.8 ± 0.2

Cultural Response

Difco™ Biotin Assay Medium

Prepare the medium per label directions. The medium supports the growth of *Lactobacillus plantarum* ATCC™ 8014 when prepared in single strength and supplemented with biotin. The medium should produce a standard curve when tested with a biotin reference standard at 0.0 to 1.0 ng per 10 mL. Incubate tubes with caps loosened at 35-37°C for 16-20 hours. Read the percent transmittance using a spectrophotometer at 660 nm.

Principles of the Procedure

Biotin Assay Medium is a biotin-free dehydrated medium containing all other nutrients and vitamins essential for the cultivation of *L. plantarum* ATCC 8014. The addition of a biotin standard in specified increasing concentrations gives a growth response by this organism that can be measured titrimetrically or turbidimetrically.

Formula

Difco™ Biotin Assay Medium

Approximate Formula* Per Liter

Vitamin Assay Casamino Acids	12.0	g
Dextrose	40.0	g
Sodium Acetate	20.0	g
L-Cystine	0.2	g
DL-Tryptophan	0.2	g
Adenine Sulfate	20.0	mg
Guanine Hydrochloride	20.0	mg
Uracil	20.0	mg
Thiamine Hydrochloride	2.0	mg
Riboflavin	2.0	mg
Niacin	2.0	mg
Calcium Pantothenate	2.0	mg
Pyridoxine Hydrochloride	4.0	mg
<i>p</i> -Aminobenzoic Acid	200.0	µg
Dipotassium Phosphate	1.0	g
Monopotassium Phosphate	1.0	g
Magnesium Sulfate	0.4	g
Sodium Chloride	20.0	mg
Ferrous Sulfate	20.0	mg
Manganese Sulfate	20.0	mg

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

Precautions

Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present. Take precautions to keep sterilization and cooling conditions uniform throughout the assay.

Directions for Preparation from Dehydrated Product

1. Suspend 7.5 g of the powder in 100 mL of purified water.
2. Heat with frequent agitation and boil for 2-3 minutes to completely dissolve the powder.
3. Dispense 5 mL amounts into tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust tube volume to 10 mL with purified water.
6. Autoclave at 121°C for 5 minutes.

Procedure

Stock Cultures

Stock cultures of the test organism, *L. plantarum* ATCC 8014, are prepared by stab inoculation of Lactobacilli Agar AOAC. After 16-24 hours incubation at 35-37°C, the tubes are stored in the refrigerator. Transfers are made weekly.

Inoculum

Inoculum for assay is prepared by subculturing from a stock culture of *L. plantarum* ATCC 8014 to 10 mL of single-strength Biotin Assay Medium supplemented with 0.5 ng biotin. After 16-24 hours incubation at 35-37°C, the cells are centrifuged under aseptic conditions and the supernatant liquid decanted. The cells are washed three times with 10 mL sterile 0.85% saline. After the third wash, the cells are resuspended in 10 mL sterile 0.85% saline and finally diluted 1:100 with sterile 0.85% saline. One drop of this suspension is used to inoculate each 10 mL assay tube.

Standard Curve

It is essential that a standard curve be constructed each time an assay is run. Autoclave and incubation conditions can influence the standard curve reading and cannot always be duplicated. The standard curve is obtained by using biotin at levels of 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8 and 1 ng per assay tube (10 mL).

The concentration of biotin required for the preparation of the standard curve may be prepared by dissolving 0.1 gram of d-Biotin or equivalent in 1,000 mL of 25% alcohol solution (100 µg per mL). Dilute the stock solution by adding 2 mL to 98 mL of purified water. This solution is diluted by adding 1 mL to 999 mL purified water, giving a solution of 2 ng of biotin per mL. This solution is further diluted by adding 10 mL to 90 mL purified water, giving a final solution of 0.2 ng of biotin per mL. Use 0.0, 0.5, 1, 1.5, 2, 2.5, 3, 4 and 5 mL of this final solution. Prepare the stock solution fresh daily.

Biotin Assay Medium may be used for both turbidimetric and titrimetric analysis. Before reading, the tubes are refrigerated for 15-30 minutes to stop growth. Turbidimetric readings should be made after 16-20 hours at 35-37°C. Titrimetric determinations are made after 72 hours incubation at 35-37°C. The most effective assay range, using Biotin Assay Medium, has been found to be between 0.1 ng and 1 ng biotin.

For a complete discussion of vitamin assay methodology, refer to appropriate procedures outlined in the reference.¹

Expected Results

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.
2. Determine the amount of vitamin at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of vitamin in the sample from the average of these volumes. Use only those values that do not vary more than ±10% from the average. Use the results only if two-thirds of the values do not vary by more than ±10%.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.

2. Aseptic technique should be used throughout the assay procedure.
3. The use of altered or deficient media may cause mutants having different nutritional requirements that will not give a satisfactory response.
4. For successful results to these procedures, all conditions of the assay must be followed precisely.

Bird Seed Agar

Intended Use

Bird Seed Agar is a selective and differential medium used in the identification of *Cryptococcus neoformans*.

Summary and Explanation

Bird Seed Agar was initially described by Staib.¹ He found that *Cryptococcus neoformans* produced characteristic brown colonies when cultivated on a growth medium containing an extract prepared from the seeds of the Indian thistle plant *Guizotia abyssinica*. *C. neoformans* is the only yeast known to produce this pigmentation.²

Shields and Ajello later modified the original formulation by the addition of the antimicrobial agent chloramphenicol.³ The concentration of chloramphenicol has been doubled in this medium to improve the inhibition of bacteria.

Principles of the Procedure

The seed extract contains caffeic acid, which serves as a substrate for phenol oxidase, an enzyme present in the cell wall of *C. neoformans*. The subsequent enzymatic reaction produces the brown pigment melanin, resulting in tan to brown pigmentation of the yeast colonies. *C. neoformans* is the only species known to produce this enzyme, although with occasional isolates (particularly serotype C) the production of phenol oxidase may have to be induced.²

Intended Use

This medium conforms with specifications of *The United States Pharmacopeia (USP)*.

Bismuth Sulfite Agar is a highly selective medium used for isolating *Salmonella* spp., particularly *Salmonella typhi*, from food and clinical specimens.

Summary and Explanation

Salmonellosis continues to be an important public health problem worldwide, despite efforts to control the prevalence of *Salmonella* in domesticated animals. Infection with nontyphi *Salmonella* often causes mild, self-limiting illness.¹ Typhoid fever, caused by *S. typhi*, is characterized by fever, headache, diarrhea and abdominal pain, and can produce fatal respira-

Reference

1. United States Pharmacopeial Convention, Inc. 2001. The United States pharmacopeia 25/The national formulary 20 – 2002. United States Pharmacopeial Convention, Inc., Rockville, Md.

Availability

Difco™ Biotin Assay Medium

Cat. No. 241910 Dehydrated – 100 g*

*Store at 2-8°C.

The addition of the antimicrobial agent chloramphenicol improves the recovery of *Cryptococcus* from specimens containing mixed flora by suppressing bacterial growth.

Procedure

To prepare plates from agar deeps, liquefy medium in boiling water bath and pour molten medium into a sterile Petri dish; allow medium to solidify and dry before use.

Using a sterile inoculating loop or needle, pick two or three isolated colonies from the subculture medium and streak over slant or plate surface. Incubate media at 25-30°C for up to 7 days.

Expected Results

Yeast-like organisms that produce tan to brown colonies on this medium within 4-7 days may be presumptively identified as *C. neoformans*.

References

1. Staib. 1962. Z. Hyg. Infekt. Med. Mikrobiol. Immunol. 148:466.
2. Warren and Hazen. 1999. In Murray, Baron, Pfaller, Tenover and Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
3. Shields and Ajello. 1966. Science 151:208.

Availability

BBL™ Bird Seed Agar

Cat. No. 297875 Prepared Plates – Pkg. of 10*
297096 Prepared Pour Tubes (20 mL) – Pkg. of 10

*Store at 2-8°C.

Bismuth Sulfite Agar

Intended Use

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Summary and Explanation

Salmonellosis continues to be an important public health problem worldwide, despite efforts to control the prevalence of *Salmonella* in domesticated animals. Infection with nontyphi *Salmonella* often causes mild, self-limiting illness.¹ Typhoid fever, caused by *S. typhi*, is characterized by fever, headache, diarrhea and abdominal pain, and can produce fatal respira-

tory, hepatic, splenic and/or neurological damage. These illnesses result from consumption of raw, undercooked or improperly processed foods contaminated with *Salmonella*. Many cases of *Salmonella*-related gastroenteritis are due to improper handling of poultry products. United States federal guidelines require various poultry products to be routinely monitored before distribution for human consumption but contaminated food samples often elude monitoring.

Bismuth Sulfite Agar is a modification of the Wilson and Blair²⁻⁴ formula. Wilson^{5,6} and Wilson and Blair²⁻⁴ clearly showed the superiority of Bismuth Sulfite medium for isolation of *S. typhi*. Cope and Kasper⁷ increased their positive findings of typhoid from 1.2 to 16.8% among food handlers and from 8.4 to 17.5% among contacts with Bismuth Sulfite Agar.