TRANSPORT MEDIA

PRODUCT:

Tube Media:[a]

Amies Transport Medium With Charcoal, item no. T6120, T6123
Amies Transport Medium Without Charcoal, item no. T6130
Cary-Blair Transport Medium, item no. T6262, T6264
Enteric Pathogen Transport, item no. E1000, E1020, E1021
Stuart Transport Medium, item no. T7522

[a]see catalog for ordering options.

INTENDED USE:

Transport media preserve the viability of microbes and help maintain the original ratio of microbes in the specimen during its transportation over a 24- to 48-hour time period.[13]

PRINCIPLE:

A buffered medium containing no carbohydrates, peptones, or other nutrients and containing sodium thioglycollate and methylene blue indicator was developed by Stuart in 1946[12] for the preservation of *Neisseria gonorrhoeae* during transport. Other investigators, including Moffitt, Young, LeMinor, and Coombes, found Stuart's transport effective in the recovery of other microbes.[13] By not providing any nutrients, the microbes are maintained in the ratio present in the specimen; sodium thioglycollate serves as a reducing agent and prevents oxidation which causes bacterial death during transport. The addition of agar also prevents oxygenation as well as preventing spillage and dehydration.

Amies et al.,[1,4] further modified the medium by replacing glycerophosphate with a buffered salt solution containing inorganic phosphate and calcium and magnesium salts. They discovered that glycerophosphate could be hydrolyzed by glycerophosphotase-producing bacteria resulting in the release of by-products which provide a source of energy for growth. The substitution of inorganic phosphates and calcium and magnesium salts for glycerophosphate proved to continue to control the permeability of the bacterial cell and aid in the survival of the microbes. Amies also incorporated charcoal directly into the medium, reduced the agar concentration to counteract the adverse effects on the agar by charcoal, and eliminated the methylene blue indicator. By adding finely powdered neutral charcoal of pharmacological quality, the settling of the charcoal to the bottom of the container was prevented and eliminated the time-consuming preparation and unsightly appearance of the charcoal-prepared swabs as recommended by Stuart et al.[11] The incorporation of charcoal helped to preserve nonfastidious microorganisms and neutralized the toxic ions produced by irradiation of the medium and the toxic properties of wood sticks, cotton wool, agar, and the by-products of bacterial metabolism.[7]

Cary and Blair[2] made further modifications after discovering that increasing the pH of the transport medium lead to greater survival rate of fecal pathogens, e.g., *Salmonella* sp., *Shigella* sp., *Vibrio* sp., and *Yersinia* species. Preliminary studies by various investigators[3,9] revealed that salmonellas and shigellas could be recovered from the transport medium after 45 days, *Vibrio comma* after 22 days, and *Yersinia pestis* after 75 days. Further modification of Cary-Blair, with the reduction of the amount of agar, improved the recovery of *Campylobacter fetus* and *Campylobacter jejuni*, and other enteric pathogens were recovered as well with the modification.[9]

Enteric Pathogen Transport (EPT) is a buffered modified Cary-Blair medium with the addition of phenol red. The buffer is added to protect enteric pathogens that are sensitive to changes in pH. Phenol red acts as an indicator to detect the buffering capability of the medium. If the medium becomes acidic, which could be toxic to some enteric pathogens, the medium becomes yellow and makes the medium unsuitable for use.

FORMULAS:

Approximate, per liter of deionized filtered water.

(1) Amies Transport Medium With Charcoal:

Sodium Chloride	3.00 g
Potassium Chloride	0.20
Calcium Chloride	0.10
Magnesium Chloride	0.10
Disodium Phosphate	1.15
Monopotassium Phosphate	0.20
Sodium Thioglycollate	1.00
Charcoal	10.00
Agar	4.00

Final pH 7.3 ± 0.2 at 25°C

(2) Amies Transport Medium Without Charcoal:
Same as (1) above except Charcoal is not added.

(3) Cary-Blair Transport Medium:

Sodium Thioglycollate	1.50 g
Disodium Phosphate	1.10
Sodium Chloride	5.00
Calcium Chloride	0.09
Agar	5.00

Final pH 8.0 ± 0.5 at 25°C

(4) Enteric Pathogen Transport (EPT):
Same as (3) above except it also contains 3.0 mg of Phenol Red Indicator, 0.2 g of Monopotassium Phosphate, and Agar is decreased to 1.6 g.

(5) Stuart Transport Medium:

Sodium Thioglycollate	1.0 g
Sodium Glycerophosphate	10.0
Calcium Chloride	0.1
Agar	3.0
Methylene Blue	2.0 mg

Final pH 7.3 ± 0.2 at 25°C

PRECAUTIONS:*

For in vitro diagnostic use. Observe approved biohazard precautions.

Storage: Upon receipt store at 2-30°C away from direct light (except EPT). Upon receipt store EPT at 10-30°C. Media should not be used if there are signs of contamination, deterioration (cracking, shrinking, or discoloration), or if the expiration date has passed.

Limitations: The glycerophosphate present in Stuart Transport Medium may permit the multiplication of certain microbes and this overgrowth may mask significant pathogens that are present in low numbers.

Stuart and Amies Without Charcoal Transport Media are no longer recommended for the transport of *Neisseria gonorrhoeae*.

When Gram staining smears made from specimens held in Amies Transport Medium With Charcoal, small particles of charcoal may be confused with gram-positive cocci and large aggregates of charcoal may obscure cells.

Some microbes that are held in Amies and Stuart Transport Media increase in number when maintained at 25°C.

Methylene blue inhibits some microorganisms, especially anaerobes.

Enteric Transport Medium (EPT) may affect the recovery of some extremely fastidious organisms such as *Campylobacter jejuni*, especially with extended transport periods and/or elevated temperatures.

PROCEDURE:*

Material Required but Not Provided: Standard microbiological supplies and reagents such as loops, needles, incubators, incinerators, and staining reagents are not provided.

Specimen Collection: Information on specimen collection is found in standard reference material. In general, specimens held in transport media should be protected from extremes of heat, cold, or desiccation and should be delivered expediently to the microbiology laboratory. Specimens need to be collected before initiation of antimicrobial therapy.

Method of Use for Amies, Stuart, and Cary-Blair Transport Media: Obtain the specimen and immediately place it in the transport medium, making sure it is well submerged. If using a swab, make sure the tip is well submerged and then break off the protruding portion of the stick.

Upon arrival at the laboratory, inoculate the specimen onto appropriate primary isolation media using sterile technique. Using standard microbiological procedure, steak the inoculum so as to obtain isolated colonies. Incubate at appropriate temperature and environment so as to enhance the growth of pathogens expected from the site of collection. Examine after the recommended incubation period and perform biochemical and/or serological tests to identify the microorganisms.

Method of Use for Enteric Pathogen Transport (EPT): Collect the stool in a clean, dry, waterproof container making sure the stool is not contaminated with urine. Place formed stool about the size of a walnut into the vial or fill liquid stool to the line on the vial label. Mix thoroughly and transport to the laboratory.

Upon receipt in the laboratory, inoculate the specimen in the EPT onto appropriate primary isolation media and streak the inoculum so as to obtain isolated colonies. Incubate appropriately under the temperature and environment suitable for the isolation of enteric pathogens. Examine after the recommended incubation period and perform biochemical and/or serological tests to identify the microorganisms.

Interpretation: These media are transport media and culture analysis is made from the media to which the specimen is cultured. Refer to the specific medium for culture interpretation and any further information.

QUALITY CONTROL:*

Transport Media Used:	Microorganisms Used (ATCC #):	Expected Subculture Results:
Amies Transport, w/ Charcoal	*Streptococcus pyogenes* (19615)	Growth
	Neisseria gonorrhoeae (43069)	Growth
	Bacteroides fragilis (25285)	Growth
Enteric Pathogen Transport	*Campylobacter jejuni* (33291)	Growth
	Shigella flexneri (12022)	Growth
	Yersinia enterocolitica	Growth
Amies, Stuart, Cary-Blair Transport	*Streptococcus pyogenes* (19615)	Growth
	Haemophilus influenzae (10211)	Growth
	Bacteroides fragilis (25285)	Growth

User Quality Control: Check for signs of contamination and deterioration.

SPECIFIC PERFORMANCE CHARACTERISTICS:

Number of *Campylobacter* strains showing 100% and 50% recovery of the initial inoculum after 24, 48, 72, and 96 hours, respectively, in EPT transport media at 2-8°C and 24-26°C and non-*Campylobacter* strains showing 100% recovery in 96 hours at 2-8°C and 24-26°C.

ORGANISM	NO. TESTED	INOCULUM CONCENTRATION	TEMP	EPT 24HRS 100%	24HRS 50%	48HRS 100%	48HRS 50%	72HRS 100%	72HRS 50%	97HRS 100%
Campylobacter jejuni	9	10^3 CFU/ML	2-8°C	6/9	9/9	6/9	7/9	4/9	4/9	4/9
			24-26°C	5/9	9/9	4/9	5/9	4/9	4/9	4/9
Salmonella spp.	1	10^3 CFU/ML	2-8°C	1/1		1/1		1/1		1/1
			24-26°C	1/1		1/1		1/1		1/1
Shigella spp.	2	10^3 CFU/ML	2-8°C	2/2		2/2		2/2		2/2
			24-26°C	2/2		2/2		2/2		2/2
V. parahaemolyticus	1	10^3 CFU/ML	2-8°C	1/1		1/1		1/1		1/1
			24-26°C	1/1		1/1		1/1		1/1
Y. enterocolitica	1	10^3 CFU/ML	2-8°C	1/1		1/1		1/1		1/1
			24-26°C	1/1		1/1		1/1		1/1

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*For more detailed information, consult appropriate references and/or details in the preface of the PML Technical Manual.

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FIXATIVES FOR SPECIMENS CONTAINING INTESTINAL PARASITES

PRODUCT:

Vial:[a]

Formalin, 10%, item no. E2000, E2020
Polyvinyl Alcohol,item no. E9000, E9020
Polyvinyl Alcohol, Modified, item no. E8000, E8020
Polyvinyl Alcohol, Zinc Sulfate, item no. E9906, E9907
Sodium Acetate, Acetic Acid, Formalin,item no. E3000, E3010
Empty Clean Vial, item no. E1050, E1060

[a]see catalog for ordering options

PURPOSE:

Each of these fixatives, when properly mixed with a clinical specimen, will preserve diagnostic stages of intestinal parasites for later examination. The empty vial is used to transport unpreserved stool specimens for parasite examination (within 30 minutes of collection), occult blood, and fat determination.

PRINCIPLES:

Specimens for parasitic examination should be properly collected and then examined within 30 minutes of collection before some of the diagnostic stages are lost. It is not always possible to examine specimens within the first hour, and fixative reagents were developed to overcome this limitation. These fixatives, when properly used, preserve diagnostic stages and characteristics for some time without adversely affecting them.[5,6]

Each fixative has its limitations.[5,6] Formalin, which preserves cysts, eggs, and larvae, can be used in examinations of wet mounts or concentration procedures but not for permanent smear preparations. Polyvinyl Alcohol (PVA) and Polyvinyl Alcohol, Modified (m-PVA), and Zinc Sulfate Polyvinyl Alcohol (Zinc-PVA), which preserve trophozoites, cysts, and eggs, are used for making permanent smears for staining procedures but are not routinely used for examinations of wet mounts and are not applicable to concentration procedures.[1,2,3,4]

For many years Schaudinn and Polyvinyl Alcohol (PVA) fixatives with mercuric chloride ($HgCl_2$) base have been used to preserve stool specimens for the recovery and identification of intestinal parasites.[1] During the past 10 years the question of mercury disposal has been raised by clinical laboratories. In the m-PVA formulation, the $HgCl_2$ has been replaced with copper sulfate, eliminating the mercury hazard. A study[3] of $ZnSo_4$ found this mercury substitute to be an acceptable alternative, and can also be used with trichrome stain. Sodium Acetate, Acetic Acid, Formalin (SAF), which preserves trophozoites, cysts, eggs, and larvae, can be used for direct examinations, concentration procedures, and smears for permanent staining procedures.[5] With the substitute fixatives and methods available at this time, Garcia, et al, is recommending the use of Zinc-PVA stained with trichrome as a replacement for the current use of mercury-containing PVA stained with trichrome with a second, equally acceptable, choice being the SAF fixative coupled with iron hematoxylin stain.

The Empty Clean Vial can be used to send unpreserved stool specimens to the lab for several determinations. If the specimen will be examined within 30 minutes of collection, parasite examination and bacterial cultures can be performed. The specimen can also be used for occult blood and fat determination.

FORMULAS:

Approximate ingredients.

(1) Formalin, 10%:

Ingredient	Amount
Disodium Phosphate	760.0 mg
Monosodium Phosphate	15.0
Formaldehyde, 40%	100.0 ml
Water, Deionized	900.0

(2) PVA:

Polyvinyl Alcohol .... 50.0 g
Mercuric Chloride, Sat. Sol. 45.0
Ethanol, Absolute .... 310.0 ml
Glycerol 15.0
Glacial Acetic Acid 50.0
Water, Deionized 625.0

(3) m-PVA:

Polyvinyl Alcohol .... 53.0 g
Copper Sulfate 16.0
Ethanol, Absolute .... 317.0 ml
Glycerol 16.0
Glacial Acetic Acid 53.0
Water, Deionized 633.0

(4) Zinc-PVA:

Polyvinyl Alcohol .... 53.0 g
Zinc Sulfate 89.0
Ethanol, Absolute .... 317.0 ml
Glycerol 16.0
Glacial Acetic Acid 53.0
Water, Deionized 614.0

(5) SAF:

Sodium Acetate .... 15.0 g
Glacial Acetate Acid .... 20.0 ml
Formaldehyde, 40% 40.0
Water, Deionized 925.0

PRECAUTIONS: *

For in vitro diagnostic use only. Observe all safety precautions consistent with the hazard(s) stated on the product label and/or Material Safety Data Sheet. Fixatives should not be used if there are signs of deterioration or if the expiration date has passed. Avoid contact with eyes, skin, and mucous membranes. If contact occurs, flush area with running water. If irritation continues, contact a physician. These formulations are poisonous. **DO NOT TAKE INTERNALLY**. If swallowed, contact a physician or poison control center immediately. Do not breathe the vapors directly from an opened PVA vial.

Storage: Upon receipt store at 10-30°C away from direct light.

Limitations: A small amount of sediment may form on the vial bottom. It will not interfere with the examination. Avoid cold storage temperatures. PVA will show some sediment, varying from lot to lot, but will become excessively sedimented if exposed to cold. Warm excessively sedimented PVA prior to use. Sediment does not affect the fixation properties.

Inadequate fixation or use of fixatives beyond their expiration date will cause significant changes in the resulting stained smear. False-negative examinations may occur if too much or too little specimen is used in the concentration procedure.

Entamoeba coli cysts may not fix well in the SAF procedure, making it difficult to see them on the stained smear. Doubling the fixing time will improve on their appearance.

Although PVA, m-PVA, and Zinc-PVA fixative can be used for sputum, abscess aspirates, sigmoidoscopic materials, and urogenital materials, it is best to prepare direct smears from those specimens and then place them into Schaudinn or some other fixative for transporting to the laboratory. This fixative is primarily used for protozoan cysts and helminth eggs.

Iospora cysts do not survive fixation in PVA.

PROCEDURE: *

Specimen Collection: Collect the stool specimen in a clean, dry, and waterproof container. Do not contaminate it with urine. Immediately fill the vial(s) of choice to the fill line on the label (1 part of the specimen with 3 parts of the fixative) and mix thoroughly.

If the specimen can be taken to the laboratory immediately upon collection and processed, a preservative is not needed. Information on specimen collection can be found in standard reference material on the subject.[1,4] The unpreserved specimen should be examined within 30 minutes of collection.

Methods of Use: Depending on the fixative used, some or all of the following procedures can be used for examining clinical specimens. For complete descriptions and related information of the following procedures, refer to reference materials in clinical parasitology.[5,6]

Wet Mount Examinations (Formalin, SAF):

1. Saline mount. Place a drop of physiological saline on a glass microscope slide and emulsify a small portion of the preserved specimen in it. Overlay the suspension with a cover slip. One should be able to read newspaper print through the slide and suspension. Microscopically examine smear for the presence of parasites.
2. Iodine mount. Same procedure as for saline, except a drop of a one percent solution of iodine is used instead of the saline. The iodine will highlight internal structures of parasites that may be present in the specimen making them more recognizable.

Concentration Procedure (Formalin, SAF):

1. Stir the preserved specimen to resuspend it.
2. Strain the preserved specimen through 1 or 2 layers of narrow mesh gauze into a centrifuge tube (10 ml).
3. Centrifuge 1-2 minutes at 2000-2500 rpm.
4. Decant supernatant. Repeat steps 1-3 if necessary until 0.5-1.0 ml of sediment is remaining, resuspending sediment with saline.
5. Mix 10 ml of fresh water or saline with the sample and shake vigorously.
6. Centrifuge the water or saline suspension for 1 minute at 1,500 rpm. Decant the supernatant.
7. Repeat steps 7 and 8 until the supernatant is clear.
8. Add 9 ml of 10% neutral formalin to the sediment.
9. Add 4 ml of diethyl ether to the sediment/formalin suspension. Stopper the tube and shake vigorously for 30 seconds in an inverted position.
10. Centrifuge the suspension for 1 minute at 1800 rpm. Four layers should result:
    a. Ether layer at the top.
    b. Plug of debris.
    c. Formalin solution.
    d. Sediment.
11. Ring the plug (second layer) with an applicator stick and decant all but the sediment and a small amount of liquid. Remove debris from the side of the tube using a cotton-tipped applicator.
12. Mix the sediment with the remaining liquid and add several drops of neutral formalin to neutralize the ether.
13. Make a wet mount as described above, using the sediment, and examine microscopically for parasites. Sediment may be stored several days by adding 1-2 ml of formalin.

NOTE: Comparable results have been reported substituting ethyl acetate for diethyl ether. Ethyl acetate is less flammable and, therefore, less dangerous.

Permanent Smear Procedures (PVA, m-PVA, Zinc-PVA, SAF):
PVA, m-PVA, Zinc-PVA
1. To collect some of the specimen, dip an applicator stick into the preserved specimen.
2. Place a drop or two of specimen on a glass slide and then spread (do not smear) a thin film over one third of the glass surface. Make sure the smear is not too thick and that it extends to the sides of the slide to reduce peeling.
3. Allow the smear to air dry thoroughly, preferably overnight at 37°C, before staining.
4. Dried films can be held for several weeks before being stained.
5. Prior to staining, the dried PVA films are placed in 70% alcohol and iodine to remove the mercuric bichloride. m-PVA and Zinc-PVA smears to not require this step.
6. Stain the smear with the appropriate parasitological stain procedure. Staining techniques can be found in standard texts on clinical parasitology. See Parasitology Stains, Data #915.
7. Microscopically examine smears for the presence of parasites.

SAF
1. Place a drop of Mayer's Albumin on a clean glass slide.
2. Mix a small portion of the sediment from the centrifugation button or directly from the SAF-preserved specimen with the drop of albumin.
3. Spread the mixture over the slide to form a thin film. If the film is too thick it may have a reddish hue to it.
4. Allow the smear to dry at room temperature until it becomes tacky, usually from 5-10 minutes.
5. Immerse the slide in 70% ethyl alcohol until the albumin coagulates, usually 25-35 minutes.
6. Stain the smear using the procedure of choice. Treatment of these smears with an iodine/alcohol solution prior to staining is not necessary because there is no mercuric chloride present.
7. Microscopically examine the stained smear for the presence of parasites.

Interpretation: Refer to standard reference material and to Parasitology Stains, Data #915.

Other Materials Required but Not Supplied: Standard microbiological supplies and equipment such as centrifuge, microscope, slides, and stains are not provided.

QUALITY CONTROL: *

Stock cultures of parasites are used to test the effectiveness of the fixative. Permanent smears or wet mounts are examined for the preservation of the parasites used and for appropriate staining characteristics.

The morphological characteristics of parasites should appear as defined for the staining procedures used. See standard references.

User Quality Control: Check for signs of deterioration.

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*For more detailed information, consult appropriate references and/or details in the preface of the PML Technical Manual.

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