Thermo
SCIENTIFIC

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Consult Instructions For Use

**Protocol™ Cary Blair Medium, 30 mL Vials**
**23-005-47**

REF IVD Rx 20°C 30°C

## INTENDED USE

Protocol™ Cary Blair Medium provides a method for collecting and preserving fecal specimens for the culture of intestinal enteric bacteria. Because the medium is capable of maintaining the bacteria for 96 hours, immediate transportation and processing of the specimen is not necessary.

## SUMMARY AND EXPLANATION

The diagnosis of intestinal disease caused by bacterial infection is confirmed by the isolation and identification of the pathogenic agent from a fresh stool specimen. The ability to recover and identify these bacteria depends on the immediate collection, transportation and culture of the specimen. A transport medium should be used if a delay of more than two hours is anticipated. The best recovery of *Shigella* species is obtained by inoculating the media directly at the bedside. Buffered glycerol saline may provide the best results if this is not possible. Cary Blair transport medium is the method of choice for maintaining the viability of intestinal pathogens such as *Campylobacter* and *Vibrio* species. Buffered glycerol saline is not recommended for these bacteria. Cary Blair medium will maintain the pathogenic bacteria for up to 96 hours while limiting the overgrowth of normal flora.

## CONTENTS

Protocol™ Cary Blair is a non-nutritive, buffered, isotonic solution with a pH indicator added. The phenol red indicator will turn yellow when the solution is acidic and the conditions are not optimal for recovery of the intended organisms. Each vial contains 15 ml of solution and a built in sample collection spoon. The kit is available with or without a multilingual instruction sheet and resealable bag.

## COLLECTION, STORAGE and TRANSPORT

1. Collection of fecal specimens for the recovery of intestinal bacteria should always be performed prior to the use of antacids, barium, bismuth, antidiarrheal medication, antibiotics, antimalarials or oily laxatives.
2. A minimum of three specimens, obtained during the acute stage of diarrheal disease, is recommended to insure the recovery of enteric pathogens.
3. Fecal specimens should be collected in a clean, dry, wide-mouthed container. A bedpan is ideal. However, a waxed half-pint container with a tight fitting lid, or a clean dry milk carton with the top two thirds removed is acceptable.
4. Using the spoon built into the cap, small samples should be added to the vial. Pay particular attention to areas that appear bloody or watery. Add sufficient sample to raise the liquid level to the red fill line on the label.
5. Use the spoon to mix the sample. Recap the vial, making sure that the lid is securely fastened. Firmly shake the vial until the contents appear homogeneous.
6. Fill out the patient information on the side of the vial. Return the vial to the physician or laboratory.
7. Proper specimen collection from the patient is critical for successful isolation and identification of enteric organisms. If immediate transportation to a laboratory is not possible refrigerate at 2-8°C for up to 96 hours.

## LABORATORY EXAMINATION

Caution: Because of the variety of bacteria that may be encountered, the use of disposable gloves is recommended when examining the contents of the vial. Subculture the bacteria in the following manner:

1. Line a shallow tray with a paper towel and wet the towel with an appropriate disinfectant.
2. Gently mix the Cary Blair vial to resuspend the stool sample.

3. Tap the bottom of the vial on the counter to remove any liquid remaining from the cap.
4. Place vial upright on the wet towel and remove the cap.
5. Inoculate the appropriate media using the appropriate receptacle.

The type of media used for identification of organisms transported in Cary Blair specimen is arbitrary and based on the particular requirements of the laboratory. Refer to one or more of the references in the bibliography for a more complete discussion of the various regimens available. In general, fecal specimens should be inoculated to several media including an enrichment broth and several selective and/or differential plated media.

**PRECAUTIONS**

For *in vitro* diagnostic use

1. The collected specimen is potentially infectious. Always practice good laboratory hygiene when handling.
2. Disinfect or sterilize the vial prior to disposal according to regulations.
3. If the solution in the vial is beyond its expiration date, appears yellow or cloudy prior to use, it should be discarded.
4. If the collected specimen exceeds the buffering capacity of the solution (indicated by a yellow color) conditions are no longer optimal for recovery of enteric bacteria.
5. Do not refrigerate the vials prior to, collection.
6. Cary Blair solution is a mild irritant. In case of contact, flush thoroughly with water. If irritation persists contact a physician immediately.
7. Protocol™ Cary Blair Medium is for use by trained and qualified personnel.
8. For single use only
9. For prescription use only

**MATERIALS NOT PROVIDED**

Materials for cultivation, isolation, identification and other microbiological procedures of bacteria from clinical specimens are not provided. Fisher swab (23-400-122) used for pre-market test is not included. Refer to referenced laboratory standards for the cultivation, isolation and identification of bacteria from clinical specimens

**STABILITY and STORAGE**

Protocol™ Cary Blair Medium is stable for at least eighteen months from the date of manufacture when stored at room temperature. The user should conduct a visual inspection prior to use. If the solution has turned yellow it should not be used

**CAS NUMBERS**

| | |
|---|---|
| Agar | 9002-18-0 |
| Reagent Water | 7732-18-5 |
| Sodium Thioglycolate | 367-51-1 |
| $KH_2PO_4$ | 7778-77-0 |
| $Na_2HPO_4$ | 7558-79-4 |
| Sodium Chloride | 7647-14-5 |
| Phenol Red | 143-74-8 |

**VIAL INTEGRITY**

Before use inspect each vial for leakage, cracks, or other defects. Use only intact undamaged vials for specimen collection.

**QUALITY CONTROL**

Protocol™ Cary Blair Medium is provided as a non-sterile product. Each lot is tested for pH and bio-burden to assure that the pH and level of viable organisms is within specifications.

All bacterial test isolates and testing procedures were established using criteria outlined in the Clinical and Laboratory Standards Institute's M40-A2 document, where applicable.

**LIMITATIONS**

1. Protocol™ Cary Blair Medium is intended for the transport of enteric pathogens listed in the Performance Characteristics section and has not been validated for other types of organisms.
2. Product performance has been tested out to 96 hours at 2-8°C and 20-25°C.
3. Extreme temperature should be avoided during transportation of Protocol™ Cary Blair Medium

4. Protocol™ Cary Blair Medium is recommended for the collection and transport of enteric bacteriological samples only. Anaerobic bacteria, viruses, *Chlamydia*, *Mycoplasma*, and *Ureaplasma* require a transport medium formulated specifically for use with these organisms.
5. Reliable specimen collection and transport depends on many factors, including collection and handling techniques, specimen condition, volume, and timing. Best results are achieved when specimens are processed shortly after the time of collection.

**CAUTION**

Federal law restricts this device to sale by or on the order of a medical, clinical or hospital professional.

**BIBLIOGRAPHY**


1. Edwards, P.R. and W.H. Ewing. 1972. Identification of Enterbacteriaceae, 3rd ed. Burgess Publishing Co.: Minneapolis. pp. 28, 337-338.

2. Ewing, W.H. 1971. Transport methods for enterobacteriacae and allied bacteria. Public Health Lab 29: 8-23.

3. Finegold, S.M. and E.J. Baron. 1986. Diagnostic Microbiology, 7th ed. C.V. Mosby Co.: St. Louis. pp. 260-278.

4 Lenette, E.H. et. Al. Manual of Clinical Microbiology, 3rd ed. ASM.

5. Sonnenwirth, A.C. 1970, "Collection and culture of specimens and guidelines for bacterial identification," in Gradwohl's Clinical Laboratory Methods and Diagnosis, 7th ed. C.V. Mosby Co.: St Louis. pp. 1149-1152.

6. Data on file, Medical Chemical Corp., Torrance, California.

7. M40-A2 Quality Control of Microbiological Transport Systems; Approved Standard-Second Edition.


**Protocol™ Cary Blair Vials**

| | Item# |
|---|---|
| Protocol™ Cary Blair Vials, 20 vl/pk | 23-005-47 |

**Other Protocol™ Parasitology Products**

| | Item# |
|---|---|
| Protocol SAF Vials, 20 vl/pk | 23-005-41 |
| Protocol Clean Vials, 20 vl/pk | 23-005-31 |
| Protocol 10% Buffered Formalin Vials, 20 vl/pk | 23-005-46 |
| Protocol C&S Vials, 20 vl/pk | 23-005-43 |
| Protocol Modified (Cu) PVA Vials, 20 vl/pk | 23-005-33 |
| Protocol Zn-PVA Vials, 20 vl/pk | 23-005-37 |
| Protocol SAF/Clean Kit, 2x10 vl/pk | 23-005-44 |
| Protocol Modified (Cu) PVA/Formalin Kit, 2x10 vl/pk | 23-005-27 |
| Protocol Zn-PVA/10% Formalin Kit, 2x10 vl/pk | 23-005-45 |
| Protocol Zn-PVA/Formalin/Clean Kit, 3x10 vl/pk | 23-005-28 |
| Protocol Modified (Cu) PVA/Formalin/Clean Kit, 3x10 vl/pk | 23-005-30 |
| Protocol Trichrome, 500 mL | 23-005-38 |
| Protocol Zn-PVA Bulk, 500 mL | 23-005-40 |
| Protocol 50 mL Concentration System, 120 ea | 23-005-50 |
| Protocol 15 mL Concentration System 50 ea | 23-005-51 |
| Protocol Ethyl Acetate | 23-005-68 |

**PERFORMANCE CHARACTERISTICS**

Protocol™ Cary Blair Medium was tested for its ability to maintain pathogenic enteric organisms. Organisms in a human fecal matrix and pure culture were tested using the CLSI M40-A2 method. Clinically negative human fecal matrix was added to Cary Blair vials that were then seeded with suspensions of representative enteric organisms, all other enteric organisms were taken from pure culture for testing. Vials were held at 2-8°C and 20-25°C and serial sampled at 0, 72, 96 and 120 hours for representative organisms and tested at 0 and 96 hours for all other enteric organisms. Recovery of viable organisms was tested with Swab Elution and Roll-Plate method. A polyester spun swab (Fisher 23-400-122) was used in the roll plate method only. Representative enteric organisms were cultured on selective media to assure accurate recovery from fecal matrix. All other enteric organisms were cultured on non-selective media for recovery.

**Representative Enteric Organisms evaluated:**

| | |
|---|---|
| *Salmonella enterica* | ATCC 10708 |
| *Vibrio parahaemolyticus* | ATCC 17802 |
| *Escherichia coli* O157 | ATCC 43894 |

**Other Enteric Organisms**

| | | | |
|---|---|---|---|
| *Escherichia coli* | ATCC 8739 | *Enterococcus faecalis* | ATCSC 29212 |
| *Staphylococcus aureus* | ATCC 6538 | *Shigella dysenteriae* | ATCC 9361 |
| *Pseudomonas aeruginosa* | ATCC 9027 | *Vibrio parahaemolyticus* | ATCC 17802 |
| *Salmonella enterica* | ATCC 10708 | *Bacillus subtilis* | ATCC 6633 |
| *Clostridium difficile* | ATCC 9689 | *Campylobacter jejuni* | ATCC 33201 |

Acceptance criteria for recovery of bacteria as recommended in the CLSI document M40-A2 were followed. For the Roll-Plate Method, for the viability to be considered acceptable, there shall be ≥5 CFU following the specified holding time from the specific dilution that yielded zero-time plate counts closest to 300 CFU. For viability in the Swab Elution Method to be considered acceptable there shall be no more than a 3 log10 (1 x 103 +/- 10%) decline in CFU between the zero-time CFU count and the CFU count for the predetermined end point.

The results of the Roll-Plate Method study and the Swab Elution Method studies are presented in Tables 1, 2, and 3 respectively. The results demonstrate the ability of Protocol™ Cary Blair Medium to sustain the viability and recovery of test bacteria within acceptance criteria for at least 96 hrs refrigerated at (2-8°C) and room temperature (20-25°C).

Viability performance studies also included an assessment of bacterial overgrowth at the refrigerated temperature only. Overgrowth assessment as defined in CLSI M40-A2 guideline is greater than 1 log10 increase in CFU between zero-time and the holding time point at 2-8°C. There was no increase in bacterial count when the samples were stored at 2-8°C for 96 hrs and analyzed by the Roll-Plate Method (Table 1.) and the Swab Elution Method (Table 2. and 3.). No overgrowth limit is defined by CLSI M40-A2 at room temperature (20-25°C) because most commercial transport media cannot control for it. Protocol™ Cary Blair is no different; overgrowth at room temperature (20-25°C) was recorded as Too Numerous To Count (TNTC).

The performance criterion was no more than a 2 log increase or decrease in viable enteric organisms at 2-8°C. Protocol™ Cary Blair Medium maintained counts of seeded organisms, with and without fecal matrix, within the specified limits at 2-8°C, whether evaluated by Swab Elution or Roll-Plate method.

**PERFORMANCE CHARACTERISTICS (continued)**

**Table 1. Representative Enteric organism recovery results for Protocol™ Cary Blair Medium using Roll-Plate Method.**

| Organism | Hold Temperature | Average CFU's Recovered: Time 0 hrs | Average CFU's Recovered: Time 72 hrs | Average CFU's Recovered: Time 96 hrs | Average CFU's Recovered: Time 120 hrs | | T=120 hrs Log reduction/ Log increase |
|---|---|---|---|---|---|---|---|
| *Salmonella enterica* | 2-8°C | 220 | 140 | 160 | 130 | | -0.23 |
| | 20-25°*C | 39 | ** | ** | 250 | | +0.81 |
| *Vibrio parahaemolyticus* | 2-8°C | 52 | 160 | 190 | 100 | | +0.28 |
| | 20-25°C | 110 | ** | ** | ** | | N/A |
| *Escherichia coli* | 2-8°C | 79 | 48 | 55 | 64 | | -0.09 |
| | 20-25°C | 75 | ** | ** | 150 | | +0.30 |

0.5 McFarland microorganism suspension diluted with fecal matrix and Cary Blair medium to $2.0 \times 10^4$ unless noted

* diluted $2.0 \times 10^5$

** Too numerous to count

**Table 2. Representative Enteric organism recovery results for Protocol™ Cary Blair Medium using Swab Elution Method.**

| Organism | Hold Temperature | Average CFU's Recovered: Time 0 hrs | Average CFU's Recovered: Time 72 hrs | Average CFU's Recovered: Time 96 hrs | Average CFU's Recovered: Time 120 hrs | | T=120 hrs Log reduction/ Log increase |
|---|---|---|---|---|---|---|---|
| *Salmonella enterica* | 2-8°C | $2.4 \times 10^2$ | $2.5 \times 10^2$ | $2.8 \times 10^2$ | $2.1 \times 10^2$ | | -0.06 |
| | 20-25°C | $8.0 \times 10^2$ | ** | ** | ** | | N/A |
| *Vibrio parahaemolyticus* | 2-8°C | $2.9 \times 10^2$ | $1.6 \times 10^2$ | $8.3 \times 10^2$ | $1.4 \times 10^2$ | | -0.32 |
| | 20-25°C | $2.1 \times 10^2$ | ** | ** | ** | | N/A |
| *Escherichia coli* | 2-8°C | $2.9 \times 10^2$ | $1.6 \times 10^2$ | $5.2 \times 10^2$ | $6.4 \times 10^1$ | | -0.66 |
| | 20-25°C | $1.2 \times 10^2$ | $7.0 \times 10^2$ | $5.2 \times 10^2$ | $2.3 \times 10^2$ | | +0.28 |

0.5 McFarland microorganism suspension diluted with fecal matrix and Cary Blair medium at 1:2000

** Too numerous to count

**PERFORMANCE CHARACTERISTICS (continued)**

**Table 3. All Other Enteric Organism recovery results for Protocol™ Cary Blair Medium using Swab Elution Method.**

| Organism | Hold Temperature | Average CFU's Recovered: Time 0 hrs | Average CFU's Recovered: Time 96 hrs | T=96 hrs Log reduction/ Log increase |
|---|---|---|---|---|
| *Escherichia coli* | 2-8°C | $2.2x10^7$ | $2.7x10^7$ | +0.09 |
| | 20-25°C | $2.1x10^7$ | $2.8x10^8$ | +1.12 |
| *Staphylococcus aureus* | 2-8°C | $1.6x10^7$ | $1.3 x10^7$ | -0.09 |
| | 20-25°C | $1.5 x10^7$ | $1.6 x10^7$ | +0.03 |
| *Pseudomonas aeruginosa* | 2-8°C | $7.6x10^6$ | $8.2x10^6$ | +0.03 |
| | 20-25°C | $8.1x10^6$ | $2.9x10^8$ | +1.55 |
| *Salmonella enterica* | 2-8°C | $4.2x10^7$ | $4.5x10^7$ | +0.03 |
| | 20-25°C | $5.8x10^7$ | $2.8x10^8$ | +0.68 |
| *Bacillus subtilis* | 2-8°C | $4.0x10^6$ | $4.4x10^6$ | +0.04 |
| | 20-25°C | $4.7x10^6$ | $1.5x10^7$ | +0.50 |
| *Vibrio parahaemolyticus* | 2-8°C | $9.9x10^5$ | $9.1x10^5$ | -0.04 |
| | 20-25°C | $1.1x10^6$ | $9.8x10^5$ | -0.05 |
| *Clostridium difficile* | 2-8°C | $1.4x10^6$ | $1.2x10^6$ | -0.07 |
| | 20-25°C | $1.4x10^6$ | $1.2x10^6$ | -0.07 |
| *Campylobacter jejuni* | 2-8°C | $4.1x10^6$ | $3.7x10^6$ | -0.04 |
| | 20-25°C | $5.4x10^6$ | $4.8x10^6$ | -0.05 |
| *Enterococcus faecalis* | 2-8°C | $2.1x10^7$ | $1.9x10^7$ | -0.04 |
| | 20-25°C | $1.8x10^7$ | $1.6x10^7$ | -0.05 |
| *Shigella dysenteriae* | 2-8°C | $2.4x10^7$ | $2.2x10^7$ | -0.04 |
| | 20-25°C | $2.1x10^7$ | $1.4x10^7$ | -0.18 |
| 0.5 McFarland microorganism suspension diluted with Cary Blair medium at 1:15 | | | | |

**Symbols Glossary:**

| | | | |
|---|---|---|---|
| EC REP | Authorized Representative in the European Community | 20°C 30°C | Temperature Limitation |
| CE | CE Marking of Conformity | | Consult Instructions For Use |
| REF | Catalog Number | Rx | Prescription Use |
| IVD | In Vitro Diagnostic Medical Device | | Manufacturer |

EC REP
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