

INSTRUCTIONS FOR USE – READY-TO-USE BOTTLED MEDIA

BA-257179.00

# **BD™ Kirchner Medium with PACT**

# **INTENDED USE**

**BD Kirchner Medium with PACT** is a liquid selective medium for the isolation of mycobacteria, especially *Mycobacterium tuberculosis*, from clinical specimens.

# PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

**BD Kirchner Medium with PACT** is a modification of the liquid enrichment medium developed by Kirchner. Mitchison and coworkers modified the medium by adding a small amount of casein and calf serum.<sup>1</sup> The Mitchison supplement, consisting of polymyxin B, amphotericin, carbenicillin, and trimethoprim (= PACT) is added to render the medium selective.<sup>2</sup> Kirchner medium, both with and without selective agents, is mentioned in the DIN Standard 58943-3 and in the MiQ Quality Standard for the primary isolation of mycobacteria from clinical specimens.<sup>3,4</sup>

In **BD Kirchner Medium with PACT**, Casitone and asparagine are nitrogen sources, and magnesium is a growth factor. Glycerol is a preferred energy source of most mycobacteria. Phosphates are included to maintain the pH stable. Citrate, together with the antimicrobials polymyxin B, amphotericin B, carbenicillin, and trimethoprim (=PACT) and phenol red are inhibitors of the accompanying fungal and bacterial flora. Calf serum is a source of complex nutrients.

**BD Kirchner Medium with PACT** is used for the isolation of mycobacteria from clinical specimens. Specimens containing normal flora (e.g., sputa) must be decontaminated before inoculation of the medium. Specimens from sterile body sites (e.g., cerebrospinal fluid) must not be pretreated before inoculation of the medium.

# REAGENTS

#### **BD Kirchner Medium with PACT**

Formula\* Per Liter Purified Water

Bacto™ Casitone	0.5 g
L-Asparagine	5.0
Magnesium Sulfate	0.6
Glycerol	20.0 ml
Potassium Dihydrogen Phosphate	2.0 g
Disodium Hydrogen Phosphate	7.5
Sodium Citrate	2.5
Phenol Red	0.1
Polymyxin B	40000 Units
Amphotericin B	0.01
Carbenicillin	0.02
Trimethoprim	0.002 g
Calf Serum	100.0 ml

pH 7.4 +/- 0.2

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

# PRECAUTIONS

IVD . For professional use only.

Do not use vials if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

Laboratory procedures involving tuberculous mycobacteria require special equipment and techniques to minimize biohazards.<sup>5-7</sup> Biosafety Level 3 is required for handling of specimens and cultures.

Consult **GENERAL INSTRUCTIONS FOR USE** document for aseptic handling procedures, biohazards, and disposal of used product.

# STORAGE AND SHELF LIFE

On receipt, store vials in the dark at 2 to 8° C until just prior to use. Avoid freezing and overheating. The vials may be inoculated up to the expiration date (see container or package label) and incubated for the recommended incubation times.

Vials from opened packages can be used up to the expiration date. Opened vials must be used immediately.

# USER QUALITY CONTROL

Inoculate representative samples of the medium with 0.01 ml of McFarland 0.5 suspensions of the strains mentioned below. For further details, consult **GENERAL INSTRUCTIONS FOR USE** document. Incubate *M. tuberculosis* for 2 to 3 weeks, and the remaining strains for 2 weeks at 35 to 37° C.

Strain	Result
Mycobacterium tuberculosis H37Ra ATCC™ 25177	Growth
Mycobacterium fortuitum DSM 46621	Growth
Mycobacterium smegmatis DSM 43061	Growth
Staphylococcus aureus ATCC 25923	Inhibition (complete)
Escherichia coli ATCC 25922	Inhibition (complete)

# PROCEDURE

#### Materials Provided

**BD Kirchner Medium with PACT**. Microbiologically controlled.

# Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

#### Specimen Types

**BD Kirchner Medium with PACT** can be used for the isolation of mycobacteria (including *Mycobacterium tuberculosis*) from all types of clinical specimens. Consult the references for appropriate collection techniques.<sup>6,7</sup>

#### **Reagent Preparation**

Principally, this medium can be used without further supplementation. However, if *Mycobacterium haemophilum* is suspected to be contained in a specimen, the medium must be supplemented with ferric ammonium citrate or hemin. It has been shown that *M. genavense* requires mycobactin J for growth. Consult the reference for special nutritional requirements of *Mycobacterium* species.<sup>7</sup>

#### **Test Procedure**

Prior to the inoculation of the medium, specimens containing normal flora must be pretreated (digested and decontaminated) according to reference procedures. The N-acetyl-L-cystein (NALC) procedure is recommended. The SDS (Lauryl Sulfate) procedure may also be used. Specimens from normally sterile body sites may be inoculated without digestion and decontamination. Consult the appropriate references.<sup>3,4,7</sup>

Inoculate each vial of **BD Kirchner Medium with PACT** with 0.2 to 0.5 ml of the specimen concentrated by centrifugation. Larger volumes may be used for liquid specimens with a low expected organism density such as urines.<sup>4,7</sup>

For optimal recovery of mycobacteria, a combination of solid and liquid media should be used. Incubate the inoculated medium at 35 to 37° C for up to 8 weeks. Tubes are read once weekly.

Note that *Mycobacterium haemophilum*, *M. marinum*, *M. ulcerans*, and *M. chelonae* require incubation at 28 to 30° C.<sup>4,7</sup>

### Results

In liquid media, many mycobacteria tend to produce granular growth rather than a homogenous turbidity. Growth should be subjected to a differential stain for mycobacteria and microscopy.<sup>4,7</sup> Subcultures onto appropriate solid media must be made to examine the purity of the culture and to obtain growth for further differential tests.

Further tests are needed for the differentiation and identification of the isolated organisms. Consult the references.<sup>4,8,9</sup>

# PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

**BD Kirchner Medium with PACT** is a liquid selective medium for the isolation of mycobacteria, including *Mycobacterium tuberculosis,* from clinical specimens.<sup>3,4</sup>

Specimens containing normal flora must be digested and contaminated before inoculation of this medium (see **Test Procedure**).

State-of-the-art diagnosis of tuberculosis requires that several media formulations are used simultaneously.<sup>3,4,8,9</sup>

Certain mycobacteria require supplementation of this medium and incubation at 28 to 30° C. Consult **Reagent Preparation**, **Test Procedure**, and the references.<sup>4,7</sup>

# REFERENCES

- 1. Mitchison, D.A. et al. 1983. Selective Kirchner medium in the culture of specimens other than sputum for mycobacteria. J. Clin. Pathol. 36: 1357-1361.
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- 3. DIN 58943-3. 1996. Diagnosis of tuberculosis part 3: detection of mycobacteria by culture methods. Edited by DIN Deutsches Institut für Normung, Berlin. Beuth Verlag, Berlin, Germany.
- Küchler, R., et al. 1998. Tuberkulose Mykobakteriose. *In:* Mauch, H., Lüttiken, R., and S. Gatermann (eds.): MiQ Qualitätsstandards in der mikrobiologisch-infektiologischen Diagnostik, vol. 5. G. Fischer, Munich, Germany.
- 5. Kent, P.T., and G.P. Kubica. 1985. Public health mycobacteriology: a guide for the level III laboratory. USDHHS. Centers for Disease Control, Atlanta.
- 6. Sommers, H.M., and J.K. McClatchy. 1983. Cumitech 16, Laboratory diagnosis of the mycobacterioses. Coordinating ed., J.A. Morello. American Society for Microbiology, Washington, D.C.
- Pfyffer, G.E., B.A. Brown-Elliot, and R.J. Wallace jr. 2003. *Mycobacterium:* general characteristics, isolation, and staining procedures. *In:* Murray, P.R., E. J. Baron, J.H. Jorgensen, M.A. Pfaller, and R.H. Yolken (ed.). Manual of clinical microbiology, 8<sup>th</sup> ed. American Society for Microbiology, Washington, D.C.
- Vincent, V., et al. 2003. *Mycobacterium:* phenotypic and genotypic identification. *In:* Murray, P.R., E. J. Baron, J.H. Jorgensen, M.A. Pfaller, and R.H. Yolken (ed.). Manual of clinical microbiology, 8<sup>th</sup> ed. American Society for Microbiology, Washington, D.C.
- 9. DIN 58943-9. 1993. Diagnosis of tuberculosis part 9: Minimum requirements for the identification of tubercle bacilli. Edited by DIN Deutsches Institut für Normung, Berlin. Beuth Verlag, Berlin, Germany.

# PACKAGING/AVAILABILITY

# **BD Kirchner Medium with PACT**

Cat. No. 257179 Ready-to-use Bottled Medium, cpu 50: 10 ml in 28 ml screw-cap vial

# FURTHER INFORMATION

For further information please contact your local BD representative.

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