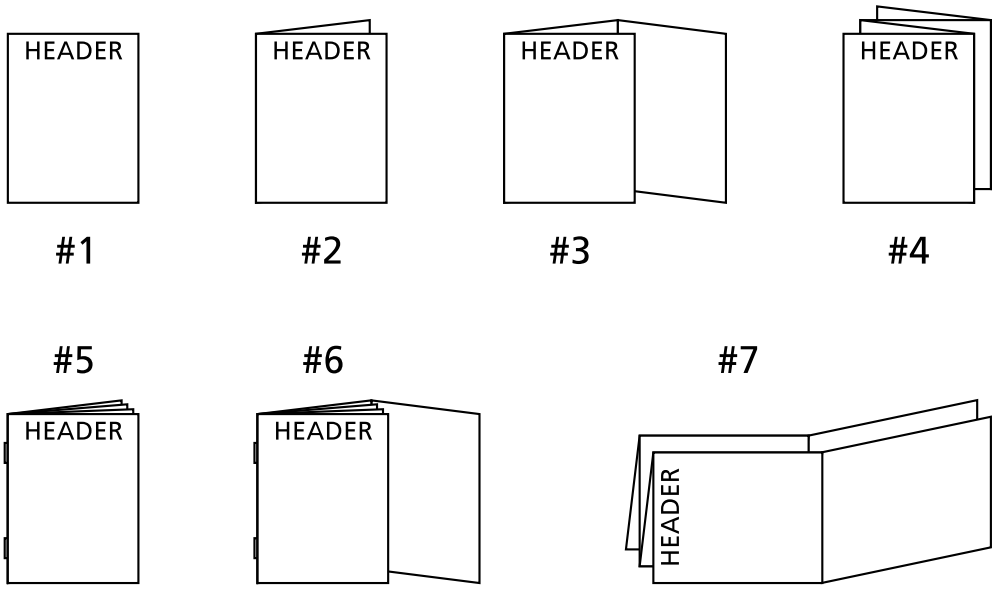
Revisions				SO 0046-2
Rev From	Rev To	ECO #	Date	Appr.
0999	1103	2389-03		

Notes:

1. BD Cat. No. 233901
2. Blank (Sheet) Size : Length: 11" Width: 5.5"
   Number of Pages: 4 Number of Sheets: 1
   Page Size: Length 11" Width 2.75" Final Folded Size: 2.75" x 2.75"
3. Style (see illustrations below): #2



4. See Specification Control No. Difco for Material Information
5. Ink Colors: Printed two sides [X] Yes [ ] No
   No. of Colors: 1 PMS #2755 Blue
6. Graphics are approved by Becton, Dickinson and Company. Supplier has the responsibility for using the most current approved revision level.

Label Design Date Proofer Date Checked By Date Part Number: S1010JAA		BD Becton, Dickinson and Company 250 Schilling Circle Cockeysville, MD. 21030-0243 USA	
	Category and Description Package Insert Difco Legionella	Sheet: 1 of 5 Scale: 1 : 1	A

BD Difco™ Legionella Agar Enrichment

S1010JAA
2003/11

See symbol glossary at end of insert. / Se symbolglossaret i slutningen af indlægssedlen. / Voir le glossaire des symboles à la fin de la notice. / Siehe Symbol-Erklärungen am Ende der Packungsbeilage. / Δείτε το γλωσσάριο των συμβόλων στο τέλος του ένθετου. / Vedere il glossario dei simboli alla fine del foglio illustrativo. / Consulte o glossário de símbolos no fim do folheto informativo. / Consulte el glosario de símbolos al final del prospecto. / Se symbolförteckningen vid slutet av bipacksedeln.

Contact your local BD representative for instructions. / Kontakt den lokale BD repræsentant for at få instruktioner. / Veuillez contacter le Service d'Assistance Technique de BD pour toute instruction. / Um Anleitungen zu erhalten, wenden Sie sich bitte an Ihren BD-Kundendienst. / Επικοινωνήστε με τον τοπικό αντιπρόσωπο της BD για οδηγίες. / Contattare il rappresentante BD di zona per ottenere il foglietto illustrativo. / Contacte o seu representante local da BD para obter instruções. / Para obtener el prospecto del producto, comuníquese con el representante de BD. / Kontakta lokal Becton Dickinson-representant för anvisningar.

INTENDED USE

Legionella Agar Enrichment is intended for use in the the preparation of Legionella Agar. The complete medium is based on the Charcoal Yeast Extract (CYE) Agar formula of Feeley et al.,[1] as modified by Edelstein,[2] and is recommended for use in the isolation and cultivation of *Legionella* from clinical and non-clinical materials.

SUMMARY AND EXPLANATION

Legionella pneumophila was first demonstrated as a causative agent of pneumonia by McDade et al., who isolated this organism from lung tissue of patients who died of Legionnaires Disease.[3] Since that time, six additional species of *Legionella* have been described and have been shown to be either causative agents of pneumonia,[4-7] or to be associated with pneumonia patients by serological procedures.[8,9]

Legionellae have been frequently isolated from environmental aquatic sources including air conditioning evaporation condensers,[10] cooling towers[11] and potable water sources such as shower heads[12] or water fixtures.[13] Presumably, these and other aquatic sources serve as reservoirs for this group of organisms and represent the means by which legionellosis is acquired.

Initial isolation of *L. pneumophila* was performed by McDade et al., using guinea pigs and embryonated chicken eggs.[3] In 1978, Weaver reported that Mueller Hinton Chocolate Agar would support good growth of this organism.[14] Feeley et al., subsequently determined that ferric pyrophosphate and L-Cysteine would respectively replace the hemoglobin and enrichment components of Mueller Hinton Chocolate Agar.[14] Feeley et al., also showed that growth of *L. pneumophila* was optimal at pH 6.9. These observations were incorporated into an improved legionella agar, which they called F-G Agar.

In 1979, Feeley et al., described a modification of F-G Agar.[1] This medium, which they called Charcoal Yeast Extract (CYE) Agar was found to provide better growth of *Legionella* than obtained on F-G Agar. Subsequently, Pasculle et al., reported that the performance of CYE Agar could be further improved by the addition of ACES buffer.[15] Edelstein described a further modification of CYE Agar in which he incorporated both ACES buffer and alpha ketoglutarate.[2] Edelstein reported that this further modification, which he referred to as BCYEα Agar, improved the growth and recovery of *Legionella*. Legionella Agar is a modification of the BCYEα Agar formula of Edelstein. In the formula, the concentration of ACES buffer was reduced from 10.0 g/L to 6.0 g/L.

PRINCIPLES OF THE PROCEDURE

Legionella Agar Enrichment contains L-Cysteine and ferric pyrophosphate. L-Cysteine and ferric pyrophosphate were included in the medium as sources of nutrients or growth factors.[1,2,16]

REAGENTS

Legionella Agar Enrichment

Approximate Formula* per vial.

L-Cysteine HCl . . . . . .0.35 g

Ferric Pyrophosphate . . . . . .0.14 g

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

Warnings and Precautions:

For Laboratory Use.

Use aseptic technique in rehydrating Legionella Agar Enrichment and adding the supplement to the base. Follow proper, established laboratory procedures in handling and disposing of infectious materials. SInce legionellosis is apparently acquired by the airborne route, care should be taken to minimize aerosis. Use of a biological safety cabinet in the handling and inoculation of specimens suspected to contain *Legionella* organisms and in the handling and examination of inoculated plates is recommended. Work surfaces should be disinfected with 5% phenol or 5% hypochlorite solutions.

Storage Instructions: On receipt, store at 2-8ºC in the dark. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light.

Product Deterioration: Do not use if product shows evidence of microbial contamination, evaporation, or other signs of deterioration. Do not use the rehydrated enrichment if it is contaminated, partially or completely evaporated, or shows other signs of deterioration.

PROCEDURE

Materials Provided: Legionella Agar Enrichment.

Materials Required But Not Provided: Legionella Agar Base, other ancillary culture media, reagents, quality control organisms and laboratory equipment as required for this procedure.

Preparation of Legionella Agar Enrichment:

Aseptically add 5 mL sterile purified water. Invert gently several times to resuspend the powder.

Preparation of Legionella Agar:

1. To rehydrate the base, suspend 18.5 g of Legionella Agar Base in 500 mL purified water. Adjust pH to 7.1 - 7.2 with 1 N KOH. Do not heat prior to sterilization.
2. Autoclave for 15 min at 121ºC.
3. Cool to 45 - 50ºC.
4. Aseptically add 5 mL of rehydrated Legionella Agar Enrichment to the molten agar.
5. Mix thoroughly.
6. Check pH. It should be 6.85 to 7.0. Adjust if necessary with 1N HCl or 1N KOH.
7. Dispense into sterile 90 - 100 mm Petri Dishes, approximately 20 mL per dish. Maintain agitation during dispensing to prevent setting of the charcoal particles.

Inoculation and Incubation:

Process specimens as appropriate for that specimen and inoculate directly onto the surface of a Legionella Agar plate. Streak for isolation.

For information on processing of specimens intended for *Legionella* cultures, consult appropriate references.[13,17-19]

Incubate plates aerobically in a humidified atmosphere containing 2.5% CO_2 at 35ºC for a minimum of 4 days. Examine daily for evidence of growth.

RESULTS

Colonies of *Legionella* should be visible after 2 to 5 days incubation and appear light blue to blue-gray in color. Upon longer incubation, colonies become larger, smoother and gray-white to white in appearance. Colonies suspected of being *Legionella* should be Gram-stained and subcultured to a fresh Legionella Agar plate and to a Blood Agar plate not containing L-Cysteine. Gram-negative organisms that grow on Legionella Agar but fail to grow on Blood Agar (without L-Cysteine) may be presumptively identified as Legionella species.[17,18-21] Definitive identification is performed on the basis of growth, morphology, and biochemical and immunological reactions. Appropriate references should be consulted for further information on identification procedures.[17,19-20]

USER QUALITY CONTROL

1. Examine the lyophilized and rehydrated supplement for evidence of deterioration as described under "Product Deterioration".
2. Check the performance of the enrichment by testing in the complete medium. Plates should be inoculated with approximately 100 - 200 colony-forming units of the test cultures and incubated aerobically under humidified conditions at 35ºC. Examine plates for growth after 48 and 72 h incubation. Results should be as stated below:

	Expected Results	
Organism	**Growth**	**Colonial Morphology**
Legionella pneumophila ATCC™ 33153	Good	Light-blue to blue-gray colonies becoming gray-white to white in appearance.
Legionella pneumophila ATCC 33155	Good	Light-blue to blue-gray colonies becoming gray-white to white in appearance.
Legionella dumoffii ATCC 33343	Good	Light-blue to blue-gray colonies becoming gray-white to white in appearance.

LIMITATIONS OF THE PROCEDURE

1. Legionella Agar Base and Legionella Agar Enrichment are intended for use in the preparation of Legionella Agar. Although this medium is recommended for use in the isolation and cultivation of *Legionella* species, organisms other than *Legionella* will grow on this medium and must be differentiated from Legionella by appropriate testing.
2. Due to variations in the nutritional requirements of this group of organisms, some strains of *Legionella* may be encountered that fail to grow or grow poorly on this medium.
3. Growth of *Legionella* has been shown to be significantly affected by pH.[14] Care should be taken in adjusting the pH of the medium in order to obtain optimal performance.

AVAILABILITY

Cat. No.	**Description**
218301	Legionella Agar Base, 500 g.
233901	Legionella Agar Enrichment, 6 x 5 mL.

REFERENCES

1. Feeley, J.C., R.J. Gibson, G.W. Gorman, N.L. Langford, J.K. Rasheed, D.C. Mackel, and W.B. Baine. 1979. Charcoal yeast extract agar: primary isolation medium for *Legionella pneumophila*. J. Clin. Microbiol. *10*:437-441.
2. Edelstein, P.H. 1981. Improved semiselective medium for isolation of *Legionella pneumophila* from contaminated clinical and environmental specimens. J. Clin. Microbiol. *14*:298-303.
3. McDade, J.E., C.C. Shepard, D.W. Fraser, T.S. Tsai, M.A. Redus, W.R. Dowdle, and the field investigation team. 1977. Legionnaires' disease: isolation of a bacterium and demonstration of its role in other respiratory disease. N. Engl. J. Med. *297*:1197-1203.
4. Pasculle, A.W., R.L. Myerowitz, and C.R. Rinaldo. 1979. New bacterial agent of pneumonia isolated from renal transplant recipients. Lancet. *2*:58-61.
5. Bozeman, F.M., J.W. Humphries, and J.M. Campbell. A new group of rickettsialike agents recovered from guinea pigs. Acta. Virol. *12*:87-93.
6. Lewallen, K.R., R.M. McKinney, D.J. Brenner, C.W. Moss, D.H. Dail, B.M. Thomason, and R.A. Bright. 1979. A newly identified bacterium phenotypically resembling but genetically distinct from *Legionella pneumophila*: an isolate in a case of pneumonia. Ann. Intern. Med. *91*:831-834.
7. McKinney, R.M., R.K. Porschen, P.H. Edelstein, M.L. Bissett, P.P. Harris, S.P. Bondell, A.G. Steigerwalt, R.E. Weaver, M.E. Ein, D.S. Linquist, R.S. Kops, and D.J. Brenner. 1981. *Legionella longbeachae* species nova, another etiologic agent of human pneumonia. Ann. Intern. Med. *94*:739-743.
8. Cherry, W.B., G.W. Gorman, L.H. Orrison, C.W. Moss, A.G. Steigerwalt, H.W. Wilkinson, S.E. Johnson, R.M. McKinney, and D.J. Brenner. 1982. *Legionella jordanis*: a new species of *Legionella* isolated from water and sewage. J. Clin. Microbiol. *15*:290-297.
9. Morris, G.K., A. Steigerwalt, J.C. Feeley, E.S. Wong, W.T. Martin, C.M. Patton, and D.J. Brenner. 1980. *Legionella gormanii* sp. nov. J. Clin. Microbiol. *12*:718-721.
10. Cordes, L.G., D.W. Fraser, P. Skaliy, C.A. Perlino, W.R. Elsea, G.F. Mallison, and P.S. Hayes. 1980. Legionnaires disease outbreak at an Atlanta, Georgia country club: evidence for spread from an evaporative condenser. Am. J. Epidemiol. *111*:425-431.

11. Dordero, T.J., R.C. Randtorff, G.W. Mallison, R.M. Weeks, J.S. Levy, W.E. Wong, and W. Schaffner. 1980. An outbreak of Legionnaires disease associated with a contaminated air conditioning cooling tower. N. Engl. J. Med. *302*:365-370.
12. Cordes, L.G., A.M. Wiesenthal, G.W. Gorman, J.T. Phair, H.M. Sommers, A. Brown, V.L. Yu, M.H. Magrussen, R.D. Meyer, J.S. Wolf, K.N. Shands, and D.W. Fraser. 1981. Are showers a source of nosocomial Legionnaires' disease? Ann. Intern. Med. *94*:195-197.
13. Edelstein, P.H. 1982. Comparative study of selective media for isolation of *Legionella pneumophila* from potable water. J. Clin. Microbiol. *16*:697-699.
14. Feeley, J.C., G.W. Gorman, R.E. Weaver, D.C. Mackel, and W.H. Smith. 1978. Primary isolation media for Legionnaires disease bacterium. J. Clin. Microbiol. *8*:320-325.
15. Pasculle, A.W., J.C. Feeley, R.J. Gibson, L.G. Cordes, R.L. Myerowitz, C.M. Patton, G.W. Gorman, L.L. Carmack, J.W. Ezzell, and J.N. Dowling. 1980. Pittsburgh pneumonia agent: direct isolation from human lung tissue. J. Infect. Dis. *141*:727-732.
16. Pine, L., J.R. George, M.W. Reeves, and W.K. Harrel. 1979. Development of a chemically defined liquid medium for growth of *Legionella pneumophila*. J. Clin. Microbiol. *9*:615-626.
17. Winn, W.C. Jr. 1999. *Legionella*, p. 572-585. *In* P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
18. Morris, G.K., P. Skaliy, C.M. Patton, and J.C. Feeley. 1979. Method for isolating Legionnaires' disease bacterium from soil and water samples, p. 85-90. *In* G.L. Jones and G.A. Hibert (ed.), "Legionnaires", the disease, the bacterium and methodology. Center for Disease Control, Atlanta, GA.
19. Feeley, J.C., G.W. Gorman, and R.J. Gibson. 1979. Primary isolation media and methods, p. 77-84. *In* G.L. Jones and G.A. Hibert (ed.), "Legionnaires", the disease, the bacterium and methodology. Center for Disease Control, Atlanta, GA.
20. Weaver, R.E., and J.C. Feeley. 1979. Cultural and biochemical characterization of Legionnaires disease bacterium, p. 19-25. *In* G.L. Jones and G.A. Hibert (ed.), "Legionnaires", the disease, the bacterium and methodology. Center for Disease Control, Atlanta, GA.
21. Myerowitz, R.L. 1982. *Legionella pneumophila* and other *Legionella* species: an update. Lab. Med. *13*:611-617.

REF Catalog number / Katalognummer / Catalogusnummer / Tuotenumero / Numéro catalogue / Bestellnummer / Αριθμός καταλόγου / Numero di catalogo / Katalognummer / Número do catálogo / Número de catálogo / Katalognummer

Temperature limitation / Temperaturbegrænsning / Temperatuurlimiet / Lämpötilarajoitus / Température limite / Zulässiger Temperaturenbereich / Όριο θερμοκρασίας / Temperatura limite / Temperaturbegrensning / Limitação da temperatura / Limitación de temperatura / emperaturbegränsning

Use by / Anvendes før / Houdbaar tot / Viimeinkäyttöpäivä / A utiliser avant / Verwendbar bis / Ημερομηία λήξης / Usare entro / Brukes før / Utilizar em / Usar antes de / Använd före / YYYY-MM-DD / YYYY-MM (MM = end of month) / ÅÅÅÅ-MM-DD / ÅÅÅÅ-MM (MM = slutning af måned) / JJJJ-MM-DD / JJJJ-MM (MM = einde maand) VVVV-KK-PP / VVVV-KK (kuukauden loppuun mennessä) AAAA-MM-JJ / AAAA-MM (MM = fin du mois) / JJJJ-MM-TT / JJJJ-MM (MM = Monatsende) / EEEE-MM-HH / EEEE-MM (MM = τέλος του μήνα) / AAAA-MM-GG / AAAA-MM (MM = fine mese) / ÅÅÅÅ-MM-DD / ÅÅÅÅ-MM (MM = slutten av måneden) AAAA-MM-DD / AAAA-MM (MM = fim do mês) / aaaa-mm-dd / aaaa-mm (mm = fin del mes) / ÅÅÅÅ-MM-DD / ÅÅÅÅ-MM (MM = slutet på månaden)

LOT Batch Code (Lot) / Batch kode (Lot) / Chargenummer (lot) / Eräkoodi (LOT) / Code de lot (Lot) / Chargencode (Chargenbezeichnung) / Κωδικός παρτίδας (Παρτίδα) / Codice del lotto (partita) / Batch-kode (Serie) / Código do lote (Lote) / Código de lote (Lote) / Satskod (parti)

Becton, Dickinson and Company
7 Loveton Circle
Sparks, Maryland 21152 USA
800-638-8663

Becton, Dickinson France S.A.
11 rue Aristide Bergès
38800 Le Pont de Claix, France