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# Difco Agar Manual

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All plated agar require refrigeration on arrival for long term storage. Sterile Packs, Serum Bottles, Boston Rounds and Wide Mouth Jars of BD BBL Agar and Broth ready to use. Difco BBL supplier distributor dealer. MICROBIOLOGICAL and CLINICAL Printed in U. S. A. Acknowledgment is also made of the use of Foreword. This edition of the Difco Manual, the ninth published since 1927, has The Ninth Edition is the first to When we first introduced prepared dehydrated culture media to the Today, Difco Difco products are not only being used by a continually increasing number Research laboratories maintained by the Federal Government are also Recommendation and approval have been extended to our products by American Public Health Association, the American Dairy Science Associa Bacto Dehydrated Culture Media and laboratory reagents are prepared Each ingredient is The new section The dehydrated media are stable and resist deterioration over long Culture Media are truly economical when actual cost comparisons are Grateful acknowledgment is made of the support we have received It is the desire of our organization to Difco Laboratories. Table of Contents Introduction 11. Origin of Dehydrated Culture Media 15. General Conditions Pertaining to the Cultivation of Microorganisms.. 16. Preparation of Media from Dehydrated Culture Media, Difco. 21. Dehydrated Culture Media 23. Guides for the Selection of Culture Media 23. Media for Examination of Water and Sewage 29. Supplementary Media for Water and Sewage Examination. 41. Media for Examination of Dairy and Other Food Products. 57. Supplementary Media for Dairy and Other Food Products. 68. Media for Lactobacilli 72. Media for Cultivation of Pathogenic Microorganisms 76.

Infusion Media 76. Peptone Media without infusions 99. Differential Media 130. Schema for Examination of Stools 130. Primary Plating Media 131. Differential Liquid Enrichments 156. Differential Tube Media 159. Sterility Test Media 195. <http://eaupureinternational.com/userfiles/brother-tape-printer-manual.xml>

Media for Microbiological Assays 203. Media for the Assay of Antibiotics 203. Media for the Assay of Vitamins and Amino Acids 212. Media for Mycology 237. Ingredients of Culture Media 255. Peptones, Difco 255. Enzymatic Hydrolysates 255. Hydrolysates, Acid 265. Amino Acids 268. Extracts 269. Enrichments 271. Enzymes 278. Bile Products 286. Dehydrated Meats for Infusions 288. Solidifying Agents 290. Carbohydrates, Polyhydric Alcohols and Glucosides 291. Carbohydrate Solutions in Ampuls 292 Biochemicals 296. Miscellaneous Ingredients 299. Tissue Culture Media Reagents, Difco 301. Methods of Tissue Culture 307. Serological Reagents for Diagnosis of Syphilis 309. Reagents for Complement Fixation Tests 309. Reagents for Precipitation Tests 311. Cardiolipin Antigens 313. Reagents for Preparation of Antigens 315. Diagnostic Reagents 317. Miscellaneous Products 338. Dehydrated Media in Special Packages 338. Prepared Media in Tubes and Bottles 338. Index 343. Introduction. Bacteriology emerged as a definite branch of science as a result of the monu When in 1876 Robert.

Koch, for the first time in history, propagated a pathogenic bacterium in pure The decade immedi The fundamental principles elaborated at that time, of which the most im Nevertheless, With a suitable culture The bacteriologist of The chemical analyses of bacteria indicate that they are essentially water plants, Almost without exception wherever bacteria occur in nature, and this is particu So complex is the structure of many of these substances, Such alterations are effected by processes of hydrolysis, These changes are ascribed to the activities Abstract as such studies of bacterial metabolism may seem, their practical From these studies has come a better understanding Naegelis report covered the use of a large variety of sub The first reference to the use of peptone for the cultivation of microorganisms Because of its content of amino acids and other In the light of our present knowledge, proteins are believed to be complex com When subjected to hydrolysis proteins yield metaproteins, proteoses, peptones, The intermediate products should be considered as classes of compounds, rather The relation of amino acids to bacterial metabolism, and the ability of bacteria Many other Indispensable as amino acids are to From the data thus far summarized, it is apparent that the problem of bacterial It is not improbable While the importance of nitrogenous substances for bacterial growth was It is highly desirable, in fact essential, to Many laboratory methods, such as The use of protein hydrolysates, particularly gelatin and casein, has stimu The importance of these new paths of investigation Closely associated with research of this nature are such In this brief discussion of certain phases of bacterial nutrition we have at Difco Laboratories has been en Difco Dehydrated Culture Media, and Muenchen, 102771880. Exp. Med., 133651911. Infectious Diseases, 154551914. It is a pleasure to include, as a part of this book, the abstract given below It is to be noted that Dr.

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Frosts University of Wisconsin. Abstract of Paper at Boston 1909 Meeting of the Society of American Bacteri In order to overcome the generally recognized faults of bacterial culture media, The authors work on this problem, covering nearly a decade of time, is con There is apparently no reason why the different culture media cannot be put Not only the ordinary, but probably most of the It is interesting to note that Doerr, in Kraus and Uhlenhut Handbuch der. Mikrobiologischen Technik, states he also prepared powdered culture media by The practical application of the dehydration of culture media was initiated and The development of microorganisms upon culture media is dependent upon a A satisfactory microbiological culture medium must contain available sources These were originally supplied in the form of the meat in Beef extract frequently replaces meat infusions, but the preparation of this sub The addition of peptone provides a readily available

Peptone is used in culture media to supply an available form of nitrogen since Most organisms are capable Certain bacteria require additions of other food substances such as serum, Carbohydrates may also be desirable at times, and certain salts such as those of Dyes may be added to media as indicators of metabolic activity or because of their Growth promoting substances of a vitaminlike nature The consistency of a liquid medium may be modified by the addition of agar, One of the principal landmarks in bacteriology was the preparation of a Until the intro Most bacteria are capable of growth under ordinary conditions of oxygen Between these Eh range.

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Anaerobic conditions for growth of microorganisms are obtained in a number Methods of readily obtaining anaerobic conditions in the laboratory are dis For anaerobic conditions a cotton plug is placed just A solution of The tube is then ready for Remove the percussion tip from the Seal the culture tube with a rubber stopper Proper moisture conditions must prevail in the culture media employed for the The pH or reaction of the culture medium, expressing its hydrogen ion concen The pH or reaction of the It should be noted that additions of acid or alkali The usual range of temperature suitable for the growth of microorganisms lies All organisms exhibit three cardinal points in their thermic relations In addition to a suitable temperature for growth of microorganisms it is Some organisms re For example, media in plates inoculated with Incubators should have open containers filled with The media upon which microorganisms are grown must be sterile or free from The usual method for immediate sterilization of culture If larger volumes are to The medium is Tubes should be placed in racks or packed loosely in baskets. Flasks should never In the operation of the autoclave, all the enclosed air must be allowed to Pressuretemperature relations PressureTemperature Relations in Autoclave Pressure in Pounds Temperature Through the courtesy of. Dr. F. W. Tanner 2 we are able to reproduce the following chart which plainly Effect of Entrapped Air on Temperature of Autoclave. PER CENT AIR IN SitAM. When the operator is assured that all the air is replaced by steam, which is best When the ther.

<http://idc504.com/images/Dell-2135Cn-Manual-Cz.pdf>

A maximum of 15 minutes is recommended for the sterilization of carbohydrate After the sterilization period Pressure should not drop too rapidly or the Pressure should, Ordinarily about 8, and not more than 1 2, minutes The media should be For the sterilization of coagulable material such as serum, see the method given Oversterilization or prolonged heating will change the composition of the The same lot of medium sterilized for This demonstrates that oversterilization resulted in a break Agar media on prolonged sterilization or heating are apt to show a precipitate. Media contain This flocculent agar Excessive heating of media also results in an increase in acidity. The reaction Some media which It is possible to destroy completely the jellifying properties Culture media which may be injured by autoclaving are sometimes sterilized This procedure consists of heating Body fluids and sera are sometimes sterilized External contamination of culture media is prevented by plugging the tubes or Plugs should fit neither too Tubes of Kligler Iron Agar The same medium with tubes loosely Media should always be stored in a cool moist atmosphere to prevent evapora Agar tubes should be Blood or other body fluids to be cultured should always be taken prior to the If drugs have been administered their The addition of j!amino BactoPenase, a concen BactoBrain Heart. Infusion with P.A.B. and Agar with added BactoPenase is an ideal medium for The advantages of dehydrated media and their efficiency for the cultivation of a Public Health Association. The preparation of media from Bacto Dehydrated Culture Media is a time The composition of each medium is stated on the label of the bottle with the For ease of preparation and for best results with Bacto. Dehydrated Media, a discussion of these methods is given in detail. Distilled water should be used in the rehydration of dehydrated culture media.

Distilled water that has been stored at room temperature for any length of It is recommended that

freshly distilled water or boiled. The quantity of dehydrated culture medium to use per liter is specified on each. Best results will be obtained by adding the powder to a stirring rod may be used to. The entire amount of distilled water is added when all broth or liquid media are readily soluble in. Agar media must be heated to the boiling point for. For small quantities another satisfactory suspension is then finally added to the boiling. Gelatin media are best. Care should be exercised to avoid contamination of media during the rehydration. It has been shown that detergent-free highly clean glassware. For example, the importance of clean glassware in the assay. Following rehydration, Difco culture media require no filtration. In some Levine E.M.B. Agar and Bacto-Niacin Assay Medium. Removal of these precipitates. The filtration of any medium. They were able to. The addition of starch to the medium. The same observation was also made with centrifuge. Adjustment of the reaction of the medium is not required. The final reaction. The temperature of the. The ionization constant increases with a rise in. Uniform standardized media are readily prepared in large or small amounts. By the use of these products microbiology. Dehydrated Culture Media. The tables on the following pages will assist in the selection of media for various. For the reason that media other than those listed may be preferred in some laboratory tests for Coliform Organisms. Plate Counts. Presumptive. Confirmed. Completed. Nutrient. Gelatin. Tryptone Glucose Extract. Agar. Lactose Broth. Lauryl Tryptose. Broth. Endo Agar. Levine E.M.B. Agar. Brilliant Green. Bile 2%. Formate Ricinoleate. Nutrient Agar. Control of Water Filtration Plant Operation. Selective Broths. Selective Agars. Differential Test Media. Fuchsin Lactose Broth. Brilliant Green Bile 2%. M.B.B.C.P. Medium. Formate Ricinoleate Broth. Crystal Violet Broth. Eijkman Lactose Medium. E C Medium.

MacConkey Broth. MacConkey Agar. Violet Red Bile Agar. Desoxycholate Lactose. Brilliant Green Bile Agar. Bacto-Tryptone. M.R.V.P. Medium. Koser Citrate Medium. Culture Media for the Examination of Dairy and Other Food Products. Plate Counts. Tryptone Glucose Extract Agar. Proteose Tryptone Agar. Beef Lactose Agar. Nutritive Caseinate Agar. Heart Infusion Agar. Brucella. Tryptose Agar. Lactobacilli. Tomato Juice Agar. Trypsin Digest Agar. Peptonized Milk. Skim Milk. Micro Assay Culture Agar. Micro Inoculum Broth. Snyder Test Agar. Hemolytic Streptococci. Coliform Organisms. Desoxycholate Agar. Desoxycholate Lactose Agar. Thermophiles. Dextrose Tryptone Agar. Thermoacidurans Agar. Molds and Yeasts. Potato Dextrose Agar. Malt Agar Phosphate. Starch Agar. Agar CO Ph 3 4J. Sr oH. QCoffi 6 CO i G c3 Is Striated Jg C d cj Id M bo Proteose Peptone, No. 3, Difco. 10 g. Sodium Chloride 5 g. Bacto-Agar 15 g. Bacto-Veal Infusion Agar is prepared from select lean veal and is recommended. It is also a suitable basal medium for enrichment by the addition of blood. To rehydrate the medium suspend 40 grams Bacto-Veal Infusion Agar in. Since most organisms prefer a fresh medium with a moist surface, it is suggested. One pound of Bacto-Veal Infusion Agar will make 11.3 liters of medium. Infusion from 50 g. Bacto Peptone 10 g. Bacto-Agar 14 g. Tryptose and Bacto-Yeast Extract were satisfactory peptones in a medium for Bacto-PPLO Agar. Bacto-PPLO Agar, enriched with Bacto-Ascitic Fluid or Bacto-PPLO Serum. Fraction will permit the development of colonies of PPLO visible microscopically. PPLO colonies are round. They vary from 10 to 500 microns in diameter 0.010.5 mm. and grow into the vacuoles. Vacuoles are seen in the periphery of. They are the large bodies characteristic of the pleuropneumonia. To rehydrate the medium, suspend 34 grams of Bacto-PPLO Agar in 1000 ml. Distribute in flasks and sterilize in the autoclave for 15 minutes at 15 pounds pressure. Bacto-PPLO Serum Fraction or 25 per cent Bacto-Ascitic Fluid.

Mix thoroughly. The reaction of the unenriched medium will. One pound of Bacto-PPLO Agar will make 13.3 liters unenriched medium. Beef Heart, Infusion from 250 g. Proteose Peptone, Difco 10 g. Bacto-Dextrose 2 g. Disodium Phosphate 2.5 g. Bacto-Brain Heart Infusion Agar is recommended as a solid medium for the. A selective medium for. The liquid medium, Bacto-Brain Heart Infusion, has for many years been. This medium, solidified with agar to. Heart Infusion with 2 per cent agar was more satisfactory than 1 per cent. Dex. Incubation in an atmosphere of 5 per cent carbon dioxide was required for best. Howel used Bacto-Brain Heart Infusion to which was added 2 per cent Bacto-Agar and 10 per cent sterile defibrinated horse blood for the cultivation of. *Histoplasma capsulatum*. A

selective medium for the isolation of this organism Potato Dextrose Agar, the Brain Heart Infusion Agar gave a greater number of *H. capsulatum* from tissues of experimentally infected mice. Their results showed *H. capsulatum* from the tissues of the infected mice. BactoBrain Heart Infusion Agar contains 0.2 per cent BactoDextrose, mak Base or BactoBlood Agar Base, as discussed on pages 87, 115 and 88, should To rehydrate the medium, suspend 52 grams BactoBrain Heart Infusion Agar Heat to boiling to dissolve the medium com A selective medium for fungi is prepared by adding 20 units penicillin and One pound of BactoBrain Heart Infusion Agar will make 8.7 liters of Edition452 1950. Proteose Peptone, Difco 10 g. BactoDextrose 10 g. BactoCystine Heart Agar, enriched with BactoHemoglobin, is recommended The use of this medium is suggested BactoCystine Heart Agar without enrichment sup A large number of the media first em Blood Dextrose Cystine Agar in his later investigation as being a satisfactory Cystine Heart Agar proved to be entirely satisfactory for the cultivation of *P. tularensis*.

In three or four days the growth is sufficient for the preparation of BactoCystine Heart Agar was originally developed in collaboration with. Rhamy. As mentioned in the paper by Rhamy, referred to above, W. M. Simp Also cooperating in these preliminary trial Cystine Heart Agar and BactoHemoglobin entirely satisfactory for growing *P. tularensis*. When used with BactoHemoglobin, the medium is prepared for use as A. Suspend 10.2 grams BactoCystine Heart Agar in 100 ml. Sterilize in the. B. Place 2 grams BactoHemoglobin in a dry flask and add 100 ml. cold The hemoglobin When a plain Cystine Dextrose Agar, without hemoglobin, is desired, the One pound of BactoCystine Heart Agar will make 8.9 liters of the enriched, BactoGasamino Acids, Technical.. 17.5 g. Starch 1.5 g. Bacto Agar 1 7 g. BactoMueller Hinton Medium duplicates the formula recommended by In an attempt to develop a simple transparent medium containing no heat The medium chosen for study As a result of the fractionation In addition, they found Technical. Growth of the gonococcus and meningococcus on the developed For the cultivation of the gonococcus, it is imperative to have the incubation Satisfactory conditions can be obtained if A can with a suitable About 200 ml. of water added in Plates incu If the culture requires carbon dioxide for growth this To rehydrate the medium, suspend 38 grams of BactoMueller Hinton. Medium in 1000 ml. of cold distilled water and heat to boiling to dissolve the It is recommended that the dissolved medium be distributed into test tubes One pound of BactoMueller Hinton Medium is sufficient to prepare 11.9 Veal, Infusion from 375 g. Monosodium Phosphate 1.25 g. Sodium Chloride 3.75 g. BactoBrain Veal Agar is a medium containing extractives of fresh calf brain BactoBrain Veal Agar is prepared according to their BactoBrain Veal Agar is a satisfactory medium for the preparation of bacterial Brain Veal Agar for the isolation of the gonococcus.

Their medium was pre Although BactoBrain Veal Agar will support good growth of the gonococcus, BactoHemoglobin, enriched with BactoSupplement A or BactoSupplement B, The procedure is given in de For cultivation of the gonococcus in pure culture we To rehydrate the medium, suspend 53 grams of BactoBrain Veal Agar in Veal Agar should be used the same day it is prepared or, if not used at once, the A moist surface is particu One pound of BactoBrain Veal Agar will make 8.5 liters of medium. BactoBeef Extract 5 g. BactoPotato Infusion Agar is prepared according to the formula used by. Stockman and MacFadyean for the isolation of *Brucella abortus*. This medium BactoTryptose Agar, To rehydrate the medium suspend 49 grams of BactoPotato Infusion Agar Distribute in tubes or flasks and The presence of this precipitate in no way interferes with Best results are obtained on freshly prepared media with a moist surface. It One pound of BactoPotato Infusion Agar will make 6 liters of medium. Sodium Chloride 5.5 g. BactoAgar 20 g. BactoBordet Gengou Agar Base, enriched with 15 to 20 per cent blood, is rec This is a modification The addition of 1 per cent Proteose. Peptone to the medium is suggested if employed for mass culture of *H. pertussis* This method Medium for the isolation and propagation of *H. pertussis*. Eldering and Ken With this modification of the Bordet Gengou Medium, enriched with 15 to 20 The plates are then examined twice daily, Three standard tuberculosis media were They recommended the addition of

25 per cent These media They were also satis To rehydrate the medium, suspend 3 grams of BactoBordet Gengou Agar. Base in 100 ml. of a 1 per cent solution of glycerol in distilled water and heat Distribute in flasks and sterilize in. The blood should be For mass cultivation of *H. pertussis* as One pound of BactoBordet Gengou Agar Base is sufficient for 15 liters of Proteose Peptone, Difco 5.5 g. Disodium Phosphate 3 g. Sodium Chloride 2.7 g. BactoAgar 11 g.

BactoEndamoeba Medium is a Liver Infusion Agar prepared especially for The formula cor. Collier.2. Cleveland and his associates made a comprehensive study of the cultivation of *E. histolytica*. They used egg, serum and various other materials for cultivating BactoEndamoeba Medium Cleveland and his coworkers. This medium, furthermore, is reported by them to be almost specific for *E. histolytica*, as far as the intestinal amoebae of man are concerned. They This report also mentions that other in To rehydrate the medium, suspend 33 grams of BactoEndamoeba Medium in For the cultivation of *E. histo* BactoRice Powder is sterilized in a One pound of BactoEndamoeba Medium will make 13.7 liters of medium. Proteose Peptone, Difco 10 g. BactoGelatin 10 g. BactoDextrose 0.5 g. BactoIsoelectric Casein 5 g. Disodium Phosphate 4 g. Sodium Citrate 3 g. BactoAgar 7.5 g. BactoStock Culture Agar is recommended for the maintenance of cultures of Their medium was developed from a formula originally The success of their medium probably lies in the fact BactoStock Culture Agar is prepared to duplicate the medium described by. Ayers and Johnson. This medium, likewise, will support luxuriant growth of BactoCooked Meat Medium, as discussed on page 85, is also recommended For carrying cultures of gonococcus, To rehydrate the medium, suspend 50 grams of BactoStock Culture Agar in One pound of BactoStock Culture Agar will make 9 liters of medium. Veal, Infusion from 500 g. Proteose Peptone, Difco 20 g. Neopeptone, Difco 1.3 g. BactoTryptone 1.3 g. BactoDextrose 5 g. BactoIsoelectric Casein 2 g. Sodium Nitrate 2 g. BactoGelatin 20 g. BactoAgar 15 g. The use of the anaerobic BactoLiver Veal Agar gives excellent growth of the sporulating anaerobes. In Gas production is With proper dilution giv To rehydrate the medium, suspend 97 grams of BactoLiver Veal Agar in After solidification, 5 ml.

Agar is poured over the medium as a cover layer to prevent the spreading of One pound of BactoLiver Veal Agar will make 4.6 liters of medium. Lab. Clin. Med., 162031930. Personal Communication. Extractives from fresh meat have been considered Media containing peptone without meat The opportunities for variation in media prepared from fresh meat are ob The age of the animal, the cut of the Likewise, the infusion of meat is These conditions were recognized by the A standard medium must be uniform in composition and For isolation or cultivation of strains of many highly pathogenic organisms Difco Laboratories that make the addition of infusions of fresh meat unnecessary *Brucella* and others. For example. BactoTryptose Agar, developed and prepared in 1938, proved more satisfactory BactoProteose No. 3 Agar and Bacto. Dextrose Starch Agar also developed in 1938 for the isolation and cultivation of These media contain specially The simplicity of preparation and uniformity of composition, combined with It is a well recognized fact that infusion media contain varying amounts of A small quantity BactoDextrose 2 g. Disodium Phosphate 2.5 g. A procedure Erlenmeyer When growth occurs in the flasks, transfers are The addition of 0.10.2 per cent BactoAgar to Tryptose Phosphate Broth The advantages of Heart Infusion, page 77. Phosphate Broth. An incubation tem. BactoTryptose Phosphate Broth Tryptose Phosphate Broth, BactoDextrose Broth or BactoNutrient Broth were To rehydrate the medium, dissolve 29.5 grams of BactoTryptose Phosphate. Broth in 1000 ml. distilled water. Distribute in tubes or flasks. For blood culture Erlenmeyer flasks or bottles. The If the medium is not used immediately after preparation, it should be heated One pound of BactoTryptose Phosphate Broth will make 15.3 liters of BactoTryptose 10 g. BactoDextrose Broth is recommended as a liquid enrichment medium for the It is a superior medium for the cultivation of organ The fact that dextrose ptose 10 g.

Proteose Peptone No. 3, Difco. 5 g. Proteose Peptone, Difco 5 g. BactoDextrose 1 g. Saccharose, Difco 50 g. Dipotassium Phosphate 4 g. Trypan Blue 0.075 g. BactoCrystal Violet 0.0008 g.

BactoAgar 15g. BactoMitis Salivarius Agar is prepared according to the formula described by Tryptose Blood Agar Base. The final medium, containing BactoChapman Tel Different methods have been employed for the isolation of streptococci and Enterococci form colonies dark blue or black in color, shiny, slightly raised, 12 Erysipelothrix rhusiopathiae produce colorless circular convex colonies. He also Using this medium. Chapman was able to demonstrate pathogenic streptococci in about 95 per cent Pathogenicity of these streptococci was BactoMitis Salivarius Agar, to which BactoChapman Tellurite Solution has To rehydrate the medium, suspend 90 grams of BactoMitis Salivarius Agar BactoChapman Tellurite Solution discussed in detail on page 277. Prepare Final reaction of the medium will One pound of BactoMitis Salivarius Agar will make 5 liters of medium. BactoBeef Extract 3 g. Sodium Azide 0.2 g. BactoAzide Blood Agar Base is a selective medium for the isolation of strepto It is also suggested as a selective The medium may be employed with The addition of sodium azide to culture media as a selective agent has been Bryan, Devereux, Hirschey BactoTryptose for the isolation of pathogenic bacteria from cheese. Sodium azide has also been used in liquid media for the detection of fecal BactoS F Medium, page 46, BactoB A G G Broth, page 47, and BactoAzide. Dextrose Broth, page 48 for the discussion of these media. To rehydrate the medium, suspend 33 grams of BactoAzide Blood Agar Base Distribute in tubes or flasks. One pound of BactoAzide Blood Agar Base will make 13.7 liters of medium. Differential Liquid Enrichments. The use of selective enrichment media is a recommended procedure for aiding Preliminary inoculation of the suspected sample Salmonella. BactoBile Salts 1 g. Calcium Carbonate 10 g.

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