GENERAL INSTRUCTIONS FOR USE
Ready-to-use and partially completed media

This document provides information on the structure of the **Instructions for Use** documents and contains additional information on the use of **BD™** ready-to-use and partially completed media.

> **Additional information not provided in the individual Instructions for Use documents is given here in the text boxes.**

The **header** on page 1 of all documents contains the **CE mark**, if the product is an **IVD** according to the European IVD Directive[1] Also, the **document number and *version*** (e.g., PA-**123456**.***01*** for plated media, and BA-**123456**.***01*** for bottled media), and **the revision date** (month and year) are given. The document number and version is repeated in the **footer** on each page.

Next, the **product name** is given. Some **Instructions for Use** documents contain the descriptions of several media with similar formulations and applications.

INTENDED USE
The area of application is indicated. If used for other applications, the medium or procedure must be validated by the user.

> **BD** Diagnostic Systems does not assume responsibility if the product is used for applications, microorganisms, or procedures not recommended in the **Instructions for Use**.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE
In this section, the principle of the method ("Microbiological method") is indicated. Furthermore, information on the history and development of the medium, the principles of the procedure and the function of the ingredients as listed under **REAGENTS** are explained.

REAGENTS
This section contains the formula and the pH of the product.

> In the formula Table, the amounts of the ingredients [g, mg, µg, ml, IU, U, etc] are mentioned only <u>once</u> and further on only if they are changed. An **example** is given below:
>
> **BD CHROMagar™ O157**
> Formula* Per Liter Purified Water

Chromopeptone	22.0 g	
Sodium Chloride	5.0	←***amount in [g]***
Potassium Tellurite	2.5 mg	
Cefixime	0.05	← ***amount in [mg]***
Cefsulodin	4.0	← ***amount in [mg]***
Special Chromogenic and Selective Mix	1.0 g	
Agar	12.0	← ***amount in [g]***

> pH: 7,1 +/- 0,2

PRECAUTIONS

If labelled IVD, **BD** ready-to-use or partially completed media are for in vitro diagnostic use, as defined in the European In Vitro Diagnostic Device Directive.[1] They may also be used in other fields of microbiology, as indicated for the respective product.
These products are for professional use only and must only be used by educated or trained personnel. They must not be used for self testing by patients.
If labelled „For Laboratory Use", these products are for use in industrial or general microbiology, biotechnology, or general hygiene only and must not be used for processing human clinical specimens.

Do not use product that shows evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

Observe aseptic handling of specimens and the product before, during and after use.

Biological and chemical safety of the product
This section may also contain information on specific biological and/or chemical hazards, indicated by the appropriate symbols, together with the appropriate R (risk)- and S (safety)-phrases.[2]

Concerning risks originating from animal material used, **BD** is committed to source such materials primarily from Australia, New Zealand, and the United States. None of these countries have reported cases of BSE (bovine spongiform encephalopathy) in native cattle. **BD** sources animal origin materials in other countries [e.g., Argentina, Brazil, Columbia, Mexico, South Africa and Uruguay] as well. None of these other countries have reported cases of BSE in native cattle.

Biohazard originating from specimens and microorganisms cultivated on microbiological media
Observe established precautions against microbiological hazards. Specimens and cultures of microorganisms must be handled according to local biohazard guidelines and legislation. According to the European Directive 2000/54/EC, most bacterial and fungal pathogens are included in risk group 2. Risk group 3** has been created to include *Salmonella* Typhi, enterohemorrhagic *Escherichia coli* (EHEC; also referred to as STEC = Shiga toxin-producing *E. coli*), *Shigella dysenteriae* (type 1) and a several other bacteria and fungi. Among several other bacterial and fungal pathogens, all *Brucella* spp., *Mycobacterium tuberculosis, M. bovis, M. africanum, M. ulcerans,* and *Histoplasma capsulatum* are included in risk group 3. For details, consult Annex III of Directive 2000/54/EC.[3] This and other European Directives can be directly assessed through **http://europa.eu.int/eur-lex**

Product Disposal
After use and prior to discarding, specimen containers and all contaminated material, including the used culture media and contaminated culture containers, must be autoclaved for 20 to 30 min at 121° C or higher (if large volumes of disposed materials must be sterilized), or incinerated by validated procedures.

STORAGE AND SHELF LIFE

BD ready-to-use plated media must be stored at +2 to +8° C. Media in bottles and dipslides must be stored at the same or at different storage temperatures.

In addition to the specific **Instructions for Use** document, the storage temperature is indicated on the package label or box of the product.

Avoid freezing and overheating.

Freezing may result in complete deterioration of agar gels or in precipitations in liquid media. Temperatures exceeding the indicated storage temperature for a longer time may lead to deterioration of media ingredients. This holds especially true for selective agents, such as antimicrobials.
Excessive moisture due to condensation water may develop after extreme subsequent temperature changes (e.g. from 2° C to 25° C and back to 2° C) on all solid media.
Plated media with excessive moisture must be dried before inoculation, e.g. by placing them with lids ajar into a clean incubator at 30 to 37° C for not longer than one hour. Do not desiccate the media! The exact exposure time depends on the air humidity in the incubator.

The **storage of the opened package** is also indicated. The storage of opened stacks of plated media indicated refers to refrigerated storage in a clean place.

Contamination during storage must be avoided by the user, e.g., by packing the plates in clean plastic bags.

For the storage of Bottled Media from opened packaging units, the shelf life given on the package label and on the bottles or vials applies, as long as they have not been opened.

All ready-to-use or partially completed media must be stored in the dark.

If exposed to artificial light, sunlight, or UV light for a longer time, the performance of all media may be reduced. Several media, such as chromogenic media, Endo Agar, and others are especially sensitive to strong illumination before and during incubation.

All **BD** ready-to-use or partially completed media may be used up to the **expiration** date and incubated for the recommended incubation times.

As an example, this includes inoculation of a mycobacterial medium on its day of expiry, followed by four or six weeks of incubation. Avoid desiccation of the medium during incubation.
The expiry date (hour-glass symbol) is indicated on the individual containers and on the package label. The Year-Month-Day format is used on all products, e.g., 2004-06-09 means June 9, 2004.

USER QUALITY CONTROL

The quality control procedures indicated in the **Instructions for Use** should be performed by the user. The test strains mentioned there usually, but not always, correspond to the strains used in quality control procedures for the release test of the manufacturer which may include additional challenge strains. Most often, strains from the American Type Culture Collection (= ATCC™; **www.atcc.org**) are used, but strains from European collections, e.g., the Deutsche Sammlung von Mikroorganismen und Zellkulturen (= DSM; **www.dsmz.de**), or the National Collection of Type Cultures (= NCTC; **www.hpacultures.org.uk**) are also used.

Please refer to the **Certificate of Analysis** of the respective product lot which states the actual test strain battery and results used for lot release.

The procedures for conducting a microbiological performance test may be dependent on the type of medium and method followed.

Always use fresh test strain suspensions, prepared from overnight cultures in appropriate liquid media (e.g., Tryptic or **Trypticase**™ Soy Broth for aerobes, and Schaedler Broth with hemin and vitamin K for anaerobes). Alternatively, fresh suspensions prepared from overnight cultures on plated media can be used. Incubation times of precultures must be extended if the test strain grows slowly.

For **testing the nutritive capacity of a plated medium** according to the CLSI standard M22, dilute the inoculum suspension to provide 1 to 2 x 10^4 CFU per plate.[4] A tenfold lighter inoculum should be used if this does not provide isolated colonies. According to DIN EN 12322, the growth-promoting properties are tested with 100 to 1000 CFU or a sufficient amount of CFU to provide isolated colonies by an appropriate streaking plate technique.[5] If the strains are inoculated by a quantitative plating technique, 50 to 500 CFU per plate are usually appropriate to obtain a countable number of colonies. Following the guidelines of the USP and EP, 10 to 100 CFU per plate (or container) must be used.[6,7]

For **testing the inhibitory capacity of a selective plated medium**, according to CLSI M22, 1 to 2 x 10^5 CFU per plate must be used for inoculation, and about 10^4 or more CFU according to DIN EN 12322.[4,5] Very high inocula of unwanted strains may "overload" the medium, leading to "breakthrough" growth.

For comparison, always include a growth reference medium which should be a nonselective medium that provides optimal growth of all test strains. For aerobic strains, Columbia Agar with 5% Sheep Blood, for fastidious strains (like *Neisseria gonorrhoeae*) Chocolate Agar, for anaerobes Schaedler Agar with Vitamin K and 5% Sheep Blood, and for fungi Sabouraud Glucose Agar are suitable for this purpose. If tested quantitatively, growth of "desired" strains on the test medium should be at least 70% of that on the reference medium. On selective media, growth of "undesired" strains must be partially to completely inhibited. The degree of inhibition depends on the medium and the strains, but growth is usually reduced by a factor of 10^3 to 10^4 (or more) as compared to the growth on the nonselective growth reference medium.

For testing the **growth performance of media in vials or bottles**, comparable methods are used. Smaller tubes and vials should be inoculated with 10^5 cfu according to the CLSI M22-A2 standard.[4] The EP, USP, and DIN EN 12322 procedures require the same inocula as listed above for the plated media.[5-7] Vials or bottles with fill volumes above 10 ml should first be aliquoted in 5 or 10 ml amounts in sterile tubes and tested in the same way.

Incubate and inspect the inoculated media as described for the respective product.

Determine the **pH** potentiometrically at room temperature (25° C) for adherence to the values specified for the product. The pH range given in the **Instructions for Use** document is the one determined after manufacture of the medium. It may vary slightly over the shelf life and may be dependent on the electrode system used.

The **sterility of the product** may be tested by the user by incubating several plates (or containers, e.g. bottles) at a suitable incubation temperature (e.g. 28 to 35° C) for 5 to 7 days or as appropriate for the procedures to be followed.[6,7]

Liquid media with a natural turbidity should be subcultured onto a suitable solid medium (e.g. **Trypticase™** Soy Agar plates) after the incubation to determine sterility. Also, Gram stains and microscopy from the media may be valuable in case of question.

PROCEDURE

In **Materials Provided**, the media and the type of container are indicated. Most **BD** ready-to-use plated media are provided in **BD Stacker™** 90 mm dishes, designed to interlock to minimize the hazard of sliding stacks. Certain **BD** ready-to-use plated media are provided in different types of dishes, such as divided dishes (=biplates), contact dishes, 150 mm dishes or square dishes, depending on the application.
BD ready-to-use or partially completed media in bottles or vials are available in many different sizes, fill volumes, and with different closures, depending on their use and application.
Here, information on the microbiological state of the product is given:

Plated media, dipslides, and certain media in bottles or vials are aseptically filled; they are “microbiologically controlled”. For these media, the DIN EN 12322 standard allows a contamination rate of ≤ 5% is allowed.[5] However, internal release criteria are more stringent.
Bottled media that are sterilized in their final container are labelled with the “sterile” symbol, together with the “thermometer” symbol (for “sterilized by autoclaving”). For these media, the criteria of sterile products according to the EP apply.[6]

In **Materials Not Provided**, special equipment necessary to perform the test are given. Regular materials such as inoculating loops, spreaders, pipettors, incubators etc. are not indicated here since they are universally needed in a microbiological laboratory.

In **Specimen Types,** the specimen(s) to be used with the specific medium are indicated. if necessary, special requirements for the collection and transport of the specimen are given in **Collection and Transport.**

Specimens must be appropriately collected and transported, using suitable transport media to avoid desiccation, excessive oxygen exposure, or overgrowth of commensal organisms. Also, the time needed from collection to processing of the specimens in the laboratory is important and must be as short as possible. For details on the collection and transport methods for specific pathogens, appropriate references should be consulted.[8]

In **Test Procedure**, general and specific information for the inoculation of the specific medium, and, if necessary, the use of additional media is described.

Media Inoculation
It is good laboratory practice in microbiology that inoculation of media with the specimen is performed by streaking for isolation, applying three streaking steps on each plate with a resterilized loop before each streaking step. If presterilized loops are used, a separate loop must be used for each streaking step. If material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak for isolation from this inoculated area with loops, applying two streaking steps.
This technique allows the isolation of single colonies from mixed cultures. Pure cultures, obtained from single colonies, are mandatory for identification and susceptibility testing of the isolates.

Also, the incubation temperature and time is mentioned here.

It is good laboratory practice to regularly inspect the actual **temperature of incubators.** Temperatures below and above the given range may lead to a loss of viability of the organisms in the cultures, to reduced growth, or to irreproducible results.
During incubation of ready-to-use plated media, provide adequate **humidity**, especially if they are incubated for an extended period of time. In this case, incubators with air circulation should not be used since media may desiccate significantly, especially when the air humidity in the laboratory is low.. Plate cultures of microorganisms that are adapted to moist environments, e.g. *Legionella,* must be incubated in humidified chambers or jars. Plates may also be sealed with adhesive tape to reduce evaporation. However, an minimal gas exchange must still be possible.
This also applies to tubed media: screw caps should always be slightly loosened during incubation.

In the **Results** section, information on the appearance of the organisms on the specific medium is provided. For general purpose isolation and growth media, this specific information cannot be given for all organisms able to grow on the medium. Appropriate references should be consulted.[9]
Eventually, specific information is provided in **Calculation and Interpretation of Results**. This section is found if a diagnosis depends on the amount of the pathogens in a specimen. As an example, this holds true for media such as CLED Agar, used for counting bacteria in urine.

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

The performance of the medium is discussed, and, if applicable, the types of organisms that can be isolated with the medium is given, together with proof sources. For newly or recently introduced media, results from performance evaluations may be included here.

It must be stressed that a single medium is only rarely adequate to detect all organisms of potential significance in a specimen or sample. Also, there may exist single strains within a microbial population that do not grow properly on a certain medium even though the medium is suitable for the detection of most other strains of this species. Therefore, for most specimens and samples, two or three different media are inoculated simultaneously, or a non-selective medium is combined with one or two selective media, or two selective media with different degree of selectivity are used.

Also, specific known **limitations in the application** of the medium are mentioned here.
For most of the media, further tests are necessary to obtain final identification of the organisms isolated.

REFERENCES
Here, the literature cited in the document is given.

PACKAGING/AVAILABILITY
Here, the available **catalog numbers**, **package sizes** and, if applicable, different formats are mentioned.

FURTHER INFORMATION
Here, further information, including the manufacturer's address (symbol for "factory"), is given. Finally, trademarks mentioned in the specific documents are given.

REFERENCES CITED IN THIS GENERAL INSTRUCTIONS FOR USE DOCUMENT

1. Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on in vitro diagnostic medical devices. Official Journal L 331 , 07.12.1998, p. 0001 - 0037
2. Directive 67/548/EEC of the European Parliament and of the Council of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances Official Journal P 196, 16.08.1967, p. 0001 – 0098.
3. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 98/391/EEC). Official Journal L 262, 17.10.2000, p. 0021-0045.
4. Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS). Standard M22. Quality assurance for commercially prepared microbiological culture media. Wayne, PA, USA. *Search for latest version at* **www.clsi.org**
5. DIN EN 12322. 1999. Culture media for microbiology – performance criteria for culture media. Beuth Verlag Berlin.
6. Council of Europe. European Pharmacopoeia. European Pharmacopoeia Secretariat. Strasbourg/France. *Search for latest version at* **www.pheur.org**
7. U.S. Pharmacopeial Convention. The U.S. Pharmacopeia /The national formulary. U.S. Pharmacopeial Convention, Inc., Rockville, Md. *Search for latest version at* **www.uspnf.com**
8. Thomson, R.B. 2007. Specimen collection, transport, and processing: bacteriology. *In:* Murray, P. R., E. J. Baron, J.H. Jorgensen, M.L. Landry, and M. A. Pfaller (ed.). Manual of clinical microbiology, 9th ed. American Society for Microbiology, ASM Press, Washington, D.C.
9. Murray, P. R., E. J. Baron, J.H. Jorgensen, M.L. Landry, and M. A. Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, ASM Press, Washington, D.C.

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