

Spring 2004

BD Catapult

Propelling applications development
in the areas of Biopharm Production,
QA/QC & Environmental Monitoring



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Automating Media Preparation and Dispensing

Bill Richman
Microbiology International, LLC

One of the most tedious and time-consuming tasks in the modern microbiology laboratory is the preparation and dispensing of culture media. Although many laboratories have modernized their testing methods and techniques, most labs are still preparing and dispensing media with stir plates, flasks, autoclaves and peristaltic pumps. This manual preparation process provides for a greater likelihood of media contamination, along with safety risks to the technicians preparing the media (transferring heavy, hot flasks from the autoclave to the bench top).

There are now reliable solutions to these process challenges in the form of high capacity automated media sterilizers and plate pourers. Microbiology International is now able to provide a total laboratory solution by supplying a full range of media sterilizers with sizes from 9 to 110 liters, along with a complete range of BD Difco™ and BBL™ quality dehydrated culture media through its distribution partnership with Becton Dickinson. The microprocessor-controlled sterilizers provide a simple and safe media preparation process.

Using Microbiology International's S8000 (an automated media preparation system), the technician simply adds



*Microbiology International's APS 300
Automated Pourer and Stacker*

dehydrated culture media and water to the sterilization vessel within the media preparation system, defines the sterilization parameters (i.e. sterilization temperature, sterilization time and pouring temperature) and presses start. The media preparation system will automatically mix, sterilize and cool the culture media. The entire process is monitored by the microprocessor and important parameters such as time, pressure and temperature are recorded using a ticket printer or computer for batch-to-batch traceability. The media preparation system also allows for the addition of thermolabile components such as blood and antibiotics.

The next step in the media preparation process is dispensing into Petri plates, tubes or bottles. Microbiology



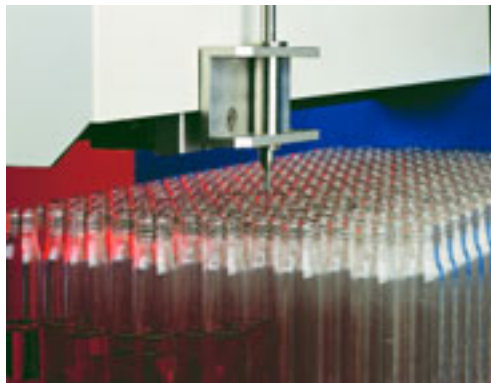
Automating Media Preparation and Dispensing

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We are pleased to announce that **BD Diagnostics**, Sparks, Maryland and **Microbiology International**, Frederick, Maryland have formed a distribution partnership to better serve the needs of the microbiology community. This partnership provides customers with a single source for BD Diagnostics products and Microbiology International's automated equipment. BD products include our premium line of dehydrated and prepared culture media, as well as other laboratory products. Microbiology International offers instruments to automate the process of preparing and dispensing microbiology culture media. We look forward to working with you as a valued supplier for all your microbiology needs.

International's APS300 plate pourer/stacker and XY500 tube/bottle dispenser provide turnkey solutions to automating the process of media dispensing. These instruments take full advantage of the latest technology in optical and torque sensors to ensure reliable "walk away" operation. The microprocessor monitors the presence or absence of a plate along with its orientation so that filling errors do not occur. With direct connection to the media sterilizer, the operator simply defines the volume to be poured, presses start and walks away. Fresh sterile media is evenly dispensed at a rate of up to 700 plates per hour. Optional automated pour plates and cooling accessories are also available for increased productivity.

For those laboratories that are diluting their samples, the gravimetric Dilumat (a sample diluter) is an ideal instrument to ensure accurate sterile dilutions. The technician simply adds a random weight of sample to be diluted, defines



Microbiology International's
XY500 Automated Robotic Dispensing System

the dilution value and presses start. The Dilumat will automatically dispense the proper volume into the sample container quickly and accurately. With direct connection to a media sterilizer, a fresh constant supply of broth is readily available for use. Laboratories that employ this automated technology enjoy significant increases in media quality and production efficiency as well as greatly reduced labor costs.

The table below provides a cost justification that illustrates how reduced labor costs can be realized using an automated media preparation system. The figures are based on a technician earning an hourly rate of \$27.00/hour. The laboratory in this illustration prepares 2.25 liters of media per day, with a total of 21 working days per month. In this example, for one liter of medium,

it takes the technician three hours to prepare, autoclave, mix and pour the plates manually. In addition, it takes one hour per day to clean glassware. The approximate cost of the \$8000 (an automated media preparation system) and the APS300 (an automated pourer/stacker system) is \$43,900.00.

These tools help improve efficiency, operator safety and media quality. With the ever-increasing demand to do more with less, these automated solutions to media preparation and dispensing are becoming a necessity for the modern successful microbiology testing laboratory.

About the Author

Bill Richman is co-founder and co-CEO of Microbiology International, LLC, a sales and distribution company founded in 1997. Mr. Richman graduated from the Philadelphia College of Pharmacy in 1989 with a B.S. in Toxicology. Mr. Richman worked for the Mobil Oil Environmental Health laboratory prior to establishing Microbiology International. Mr. Richman lives in Frederick, Maryland with his wife and three children.

Manual Media Preparation Costs		Media Preparation Costs Using APS300 and S8000	
Daily cost to prepare 2.25L and pour plates (2.25L x 3 hr. x \$27.00/hr.)	\$182.25	Daily labor cost to prepare 9L of media, start S8000, load APS300 (0.25 hr x \$27.00/hr.)	\$6.75
Daily cost of cleanup of glassware (1 hr. x \$27.00/hr.)	\$27.00	Daily cost to clean S8000/APS300 (0.25 hr x \$27.00/hr.)	\$6.75
Total daily cost of manual media prep (prep costs + cleanup costs)	\$209.25	Total daily cost of automated media prep/pouring (prep costs + cleanup costs)	\$13.50
Monthly cost to manually prepare plates (\$209.25 x 21 days)	\$4,394.25	Monthly cost to prepare plates (\$13.50 x 21 days)	\$283.50
Monthly cost savings (labor only) by using an automated media preparation system (\$4,394.25 - \$283.50 = \$4,110.75)			
Instrument payback time (labor only) (\$43,900.00 ÷ \$4,110.75 = 10.68 Months)			

Biopharm Production

Production Enhancement Through Media Optimization

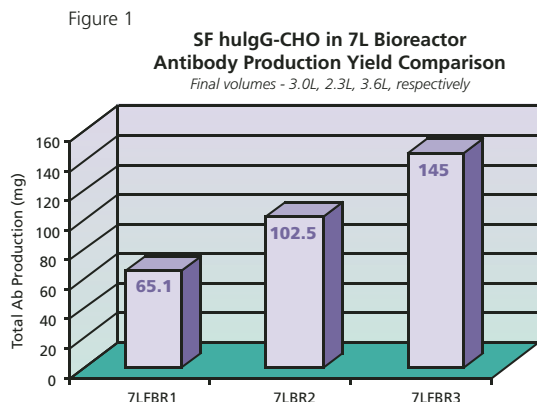
Cindy Hunt, BD Senior Scientist
AutoNutrient™ Media Design Service

Cells used in therapeutic protein production have different nutritional requirements for optimal proliferation or expression of the protein product, making it virtually impossible to develop a universal medium for all cell culture applications. Media optimization is a process that can be used to identify the ideal growth and production environment of a cell, resulting in optimal cell performance.

Complete optimization involves the balancing of each medium component, resulting in a completely defined formulation. A partial optimization uses animal-free peptones when complete optimization is not required or when media development time is limited. Both strategies provide a way to increase culture performance while decreasing or eliminating the amount of undefined or animal-derived materials used in cell culture media. Screening methods are employed to evaluate a wide range of animal-free peptones in order to select the best peptone or combination of peptones and their respective optimal concentration(s). Methods for complete or partial optimization can be automated to further accelerate development time, increase assay reproducibility and reduce laboratory costs.

Further enhancement to production can be obtained through process development. From the aspect of media development, the optimization of a feed solution for a fed-batch process will assist in increasing protein production.

Research scientists at BD Diagnostics and Laureate Pharma LP conducted studies to develop a fed-batch process for improved production of a Chinese Hamster Ovary (CHO) cell line producing



human IgG (huIgG) in BD Cell™ MAb Animal Component Free Medium.¹ The huIgG line was successfully adapted into BD Cell MAb Animal Component Free Medium without fetal bovine serum (FBS) supplementation through stepwise reduction in FBS over several serial passages. A spent media study was conducted in another set of experiments in order to develop a feed medium for the huIgG-CHO cell line in BD Cell MAb Animal Component Free Medium. Based on the results of this study, a complete chemically-defined medium feed solution with specifically fortified amino acids was selected and used for the next set of studies.

Three process development runs were performed at the 7-liter (7L) stirred tank bioreactor level to verify the feed. Further analysis showed that the glucose levels were insufficient in the feed medium. The feed medium was modified to increase glucose levels and the feed strategy was changed to allow for two feeds rather than a single feed. This fed-batch run resulted in a 40% antibody yield enhancement when compared to the original batch run (Figure 1).

A scaled-up fed-batch run was then conducted of the huIgG-CHO cell

line in BD Cell MAb Animal Component Free Medium in a 300L bio-reactor using the optimized protein-free feed medium. Figure 2 illustrates the cell densities obtained when the batch is fed at two intervals, which are indicated by black arrows, and the resulting cell densities achieved. Figure 3 compares antibody production yields in grams that are achieved when three batches of the huIgG-CHO cell line are scaled up

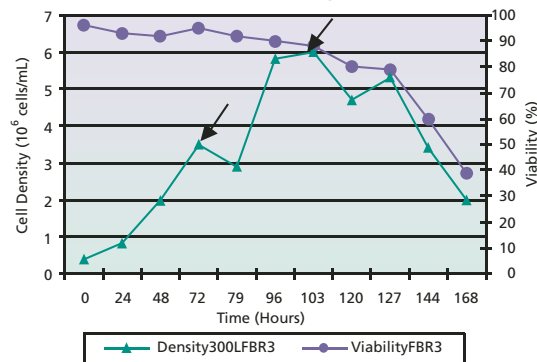
to 200L in a bioreactor: BR#1 is the BD Cell MAb Animal Component Free Medium with 2% FBS; BR#2 is the BD Cell MAb Animal Component Free Medium; and BR#3 is the fed batch of BD Cell MAb Animal Component Free Medium with optimized feed medium.

The development of the fed-batch process resulted in a 35% increase in monoclonal antibody (MAb) yield. Overall, an approximate twofold increase in peak cell density was achieved between BR#2 and Fed-BR#3.

In the next series of studies, the goal was to develop a medium that would provide superior production performance over the current growth medium.

Figure 2

SF huIgG-CHO in 300L Bioreactor
Fed Batch Run #3, 210L



Continued on page 4

Biopharm Production

Production Enhancement Through Media Optimization

Continued from page 3

During the initial medium development, sister clones labeled Clone 1 and Clone 2 were cultured in 80 chemically defined media. Cell proliferation and antibody production were evaluated. The testing was set up in 96-well microtiter plates using alamarBlue™ for cell proliferation determinations and enzyme linked immuno-sorbant assay (ELISA) for antibody production yields. From the 80-media library screen, several media formulations were selected to further evaluate at the shaker flask level. The shaker study focused on the viable cell density of each clone in a 21-day batch culture as well as production on days 14 and 21. Antibody quantification was conducted by high performance liquid chromatography (HPLC).

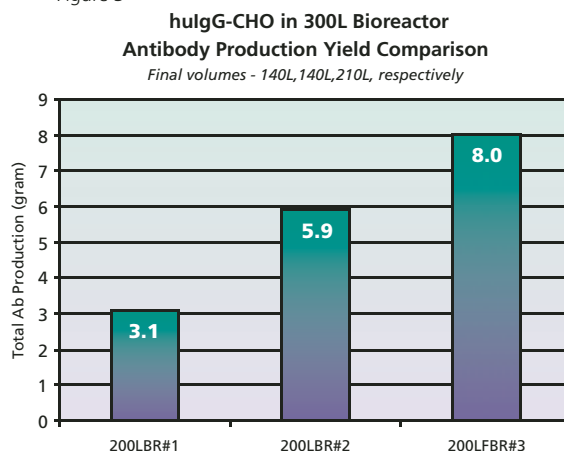
Testing identified chemically defined Medium 24 and Medium 44 as candidates to be used in the next phase of peptone studies. Further testing was conducted to determine the best peptone or blend of peptones for enhancement of protein production and viable cell density. The five animal-free peptones tested were:

- Difco™ Select Soytone
- BBL™ Phytone™ UF
- DS (Difco™ Springer) 100 Soy Peptone UF
- Difco™ TC Yeastolate UF
- Difco™ Yeast Extract UF

These peptones were titrated into Medium 24 and Medium 44 at concentrations ranging from 0–17 g/L and the performance of Clone 1 and Clone 2 was evaluated.

Proliferation readings were taken on days 0, 3, 4, 5, 6, and 7. ELISA was performed on supernatants obtained from day 7. Based on these results, a statistical design of two-peptone blends and three-peptone blends was then generated. The resulting 82 peptone blend

Figure 3



formulations were evaluated against Clone 1 and Clone 2. The results showed that Medium 24 consistently performed better than Medium 44. In addition, Clone 1 gave higher antibody yields than Clone 2 in both test and control media.

Based on these experiments, the following media were selected to test the antibody production of the specified clone at the shaker flask level:

Clone 2

- Blends 30, 39 and 79
- Medium 24 with 7 g/L TC Yeastolate UF
- Medium 24 with 1 g/L Select Soytone

Clone 1

- Blends 139, 143, 169 and 210
- Medium 24 with 1 g/L Select Soytone
- Medium 24 with 3 g/L TC Yeastolate UF
- Medium 44 with 5 g/L Yeast Extract UF

Medium 24, Medium 44 and Project 5589 CHO Medium (control) were used as control media. Terminal cultures were set up for both clones. Cell counts were performed on days 5,

10, 12, 13 and 19. Cultures were harvested on day 19. Antibody yield was determined by radial immuno-diffusion (RID) on samples obtained from days 14 and 19.

The results obtained from these experiments showed that Medium 24 with 3 g/L of TC Yeastolate UF and 3 g/L of Yeast Extract UF gave the highest antibody yields for both Clone 1 (Blend 169) and Clone 2 (Blend 39). Clone 2 achieved a twofold production increase over the corresponding control medium.

Clone 1 achieved a 26% production increase over the corresponding control medium. The selection of a new base and peptone blend resulted in an approximate two-fold increase in MAb titer at the shaker flask scale.

Media optimization provides the ideal growth and production environment for a cell. Optimization may involve complete base medium formulation development in order to eliminate undefined or animal-derived components, partnering the best base medium with the best combination of peptones and/or developing a feed medium and strategy. The approach to be taken is highly dependent on the current status of the customer cell line and the time constraints for media development.

BD scientists will work with customers from scale-up to full scale manufacturing of their tissue culture media needs. For more information regarding cell culture media optimization and the BD AutoNutrient™ Media Design Service, please fill out the request for information card or contact your local BD Sales Representative.

References

1. S. Holdread, C. Hunt, T. Oshunwusi, K. Fritchman and J. Lee. Submitted for publication.

Environmental Monitoring

HACCP and Air Sampling

(Reprinted with permission from Bioscience World Newsletter, Vol. 4, No. 1)

Daniel Y. C. Fung, Ph. D.

Professor of Food Science
Kansas State University

The Kansas State University air quality scale for food processing environment is as follows:

- Air with less than 100 colony forming units (CFU)/cubic meter is clean and acceptable
- Air with 100 to 300 CFU/cubic meter is marginal
- Air with more than 300 CFU/cubic meter is not acceptable and needs corrective action

I recommend that all food-processing plants develop air-monitoring programs to detect possible contamination sources as part of their regular Hazard Analysis Critical Control Points (HACCP) program. Weekly air monitoring for meat, dairy, juice and other food processing plants is advisable. When implementing a system for monitoring air quality, food processing plants should first establish the location of risk areas in both raw and finished product areas. Then, the plants should monitor these risk areas weekly and determine whether the risk is real. These risk areas might be near filtering systems, windows, HVAC (heating, ventilation and air conditioning) systems or doorways where cold air, heat or workers might pass. In some plants, double doors might be a remedy.

At commercial food processing plants, including meat, pasta and juice plants,



To interpret the data from the contact plates after air sampling, simply count the number of colonies from the agar plate after overnight incubation, convert the number to Most Probable Number (MPN), for example 12, then divide the number by the volume of air flow through the unit (for example 60 L) and multiply by 1000. The result is the CFUs/cubic meter.

we used the SAS™ (Surface Air System) Microbial Air Sampler and quickly pinpointed the source of contamination and suggested corrective action. Sometimes, it took only one visit to locate problem areas.

We also have extensively studied the air quality of our new meat processing plant in our teaching laboratory fourteen weeks before completion of the building and fourteen weeks after occupancy using the SAS Microbial Air Sampler. There was a high count of microbes in the air during construction as expected. The air quality in the rooms after occupancy was good but subject to changes as people and meat products moved in and out of the rooms. The SAS air sampler is a great tool to monitor influence of human beings on microbial air quality in our meat processing plant.



For information on viable air sampling options available through BD, contact your local BD representative, or mark the appropriate box on the request for information card.

Daniel Y. C. Fung, Ph.D.

As a world-leading applied microbiologist in Rapid Methods and Automation in Microbiology, Dr. Fung has published nearly 600 publications and received many awards, including the International Award from The Institute of Food Technologists. In addition to teaching, research and university services,

he has initiated and directed the internationally renowned workshop in Rapid Methods and Automation in Microbiology from 1981 to the present. For reprints on research generated from the SAS Microbial Air Sampler or information on the workshop, contact Dr. Fung at dfung@oz.oznet.ksu.edu

QA/QC

Advanced Chromogenic Media Formulations in Rapid Microbiology

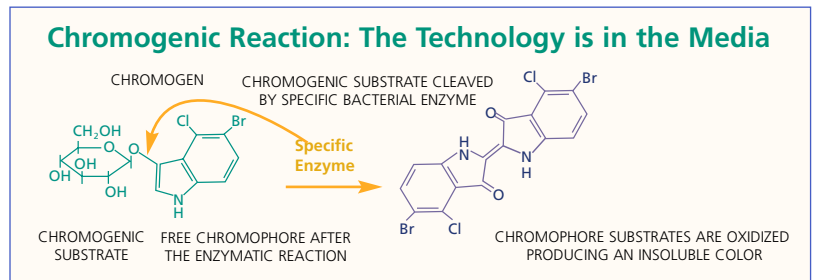
George Wilson, BS, MT(ASCP), MBA
BD Industrial Marketing Manager

Microbiology has evolved from traditional methods in the isolation, cultivation, and identification of microorganisms by conventional plated and tubed media methods to more advanced rapid methods. In the 1970s, miniaturized identification systems such as the BBL™ Minitek™ and Enterotube™ systems provided identification results in 24 to 48 hours. These systems later evolved into computerized instrument identification systems such as BD Phoenix™, providing results in 24 hours or less. In the 1980s, immunological methods were developed; i.e., BBL™ Strep Grouping and BBL™ Staphyloslide products for rapid identification of *Streptococcus* spp. and *Staphylococcus aureus*, respectively. Today, lateral flow immunoassays, polymerase chain reaction (PCR) and other advanced technologies are being developed to assist microbiologists in rapid detection of microorganisms from a variety of sources.

With all of the technological advancements made in microbiology, for a laboratory planning to incorporate a new test method, basic fundamental questions have not changed. Does the current test method meet our laboratory needs? What will the laboratory gain by changing to a new test methodology? Is the new test method faster, easier to use with improved workflow, or less expensive than the current method? Does the new test or method provide better sensitivity and specificity? Is confirmatory testing required and if so, what is the added expense?

In addition to these basic questions, what is the outcome of a delayed response or having erroneous results? What is the financial impact of high false-positive or false-negative rates? What is the added cost per test due to unnecessary sampling, testing, or the cost of recalling food products, shutting down a water distribution system or well closures? Of major importance, what are the health risks and financial implications when reporting out false-negative results whether testing food, drinking water or recreational water?

Microbiologists studying various chromogenic and fluorogenic substrates alone, or in combination, and their unique reactions through enzymatic metabolism by microorganisms has resulted in significant changes in once traditional methods. Chromogens and fluorogens are color- or fluorescence-producing compounds, respectively, that can



be used to detect specific bacterial enzymes. The substrate complex is hydrolyzed by a specific enzyme produced by the specific bacterium resulting in the formation of a colored precipitate or fluorescence when viewed under ultraviolet (UV) light.

With this knowledge, how could chromogens or fluorogens work in culture media? Can the total test time be reduced to 48 hours or less? With the specific enzyme(s) produced by the bacteria and their unique activity on certain chromogenic or fluorogenic substrates, does the medium provide presumptive or confirmatory results?

These substrates can be incorporated into primary selective media, thereby permitting the enumeration, detection and identification directly on the primary isolation plate. The interaction of the microorganism and the substrate can vary. It depends on the enzyme present in the microorganism and the structure of the chromogen or fluorogen used. This specific reaction may eliminate the need for subculture or additional biochemical tests, providing a more rapid detection and identification of the microorganism. Brenner et al.¹ demonstrated that a medium formulation can be developed to detect a single genus or species of bacteria using a chromogen through a color reaction, or a group of bacteria through the use of a fluorogen. The development of MI Agar exemplifies the use of the antimicrobial cefsulodin to allow for the selective isolation of certain microorganisms and the use of chromogenic and fluorogenic substrates to detect and differentiate *Escherichia coli* (*E. coli*) and Total Coliforms (TC) in water samples. The chromogen indoxyl- β -D-glucuronide (IBDG) detects the enzyme β -glucuronidase, resulting in a blue/indigo colored colony that is produced by 94-96% of *E. coli*. The fluorogen 4-methylumbelliferyl- β -D-galactopyranoside (MUGal) detects β -galactosidase, an enzyme produced by coliforms.

Compared to other membrane filtration methods requiring 48 to 72 hours of incubation, MI Agar, with this chromogenic-

QA/QC

Advanced Chromogenic Media Formulations in Rapid Microbiology

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fluorogenic combination, permits the simultaneous detection and enumeration of *E. coli* and TC in water by membrane filtration in 24 hours. In addition, MI Agar requires only one incubation temperature of 35°C and eliminates membrane transfers. The test results are final, requiring no additional confirmatory testing. This new formulation improves the workflow by reducing the procedural steps, labor, and other associated costs a laboratory incurs when using other membrane filtration methods.

The reported detection limit for MI Agar is one *E. coli* and/or one TC per 100 mL sample volume or dilution tested. The false-positive and false-negative rates for *E. coli* are both 4.3%. The false-positive and false-negative rates for TC are 4.6% and 8.8%, respectively. The specificity for *E. coli* is 95.7% and for TC is 93.1%. MI Agar recovered 1.8 times more TC than mEndo Agar, which demonstrated a false-positive rate of 19.1% and a false-negative rate of 15.3%.^{1,2}

MI Agar, with the low false-negative rates compared to mEndo Agar, minimizes health risk concerns by reporting



accurate, quantifiable results in ≤ 24 hours. With the low false-positive rates, this medium reduces unnecessary testing and the cost of testing in water systems, well shutdowns and treatment.

Advanced chromogenic media formulations offer microbiologists a valuable cost-effective alternative in the rapid isolation and detection of pathogenic microorganisms. BD Diagnostics currently has developed and released a wide variety of chromogenic media for the detection of pathogens from a variety of water types and food products.

For technical information on BD Chromogenic Media fill out and return the request for information card or visit our web site at www.bd.com/industrial/products/chromogenic_media.

References

- Brenner, K.P. 1993. New medium for the simultaneous detection of total coliforms and *Escherichia coli* in water. *Appl. Environ. Microbiol.* 59:3534-3554.
- U.S. Environmental Protection Agency. 2002. Method 1604: Total Coliforms and *Escherichia coli* in water by membrane filtration using a simultaneous detection technique (MI Medium). EPA-821-R-02-024. USEPA Office of Water (4303T), Washington, D.C.

BD BBL™ Chromogenic Prepared Plated Media			Cat No.	Unit	 <p>BBL™ MI Agar</p> <p>BBL™ Modified mTEC Agar</p> <p>BBL™ mEI Agar</p> <p>BBL™ CHROMagar O157</p> <p>BBL™ CHROMagar Salmonella</p> <p>BBL™ CHROMagar Staph aureus</p>
BBL™ MI Agar Plates (15 x 60 mm plate) For the simultaneous chromogenic/fluorogenic detection and enumeration of Total Coliforms and <i>E. coli</i> in water by the membrane filter technique. Conforms to USEPA Approved Method 1604.			214896 214895	Pkg. of 20 Ctn. of 100	
BBL™ Modified mTEC Agar Plates (15 x 60 mm plate) For the chromogenic detection and enumeration of <i>E. coli</i> in water by the membrane filter technique. Conforms to USEPA Approved Method 1603.			215044 215046	Pkg. of 20 Ctn. of 100	
BBL™ mEI Agar Plates (15 x 60 mm plate) For the chromogenic detection and enumeration of enterococci in water by the membrane filter technique. Conforms to USEPA Approved Method 1600.			215045 215047	Pkg. of 20 Ctn. of 100	
BBL™ CHROMagar™ O157 Plates (15 x 100 mm plate) For the selective isolation, cultivation, and chromogenic detection of <i>E. coli</i> O157:H7 in food.			214894	Pkg. of 20	
BBL™ CHROMagar™ Salmonella Plates (15 x 100 mm plate) For the selective isolation, cultivation, and chromogenic detection of <i>Salmonella</i> in food.			214893	Pkg. of 20	
BBL™ CHROMagar™ Staph aureus Plates (15 x 100 mm plate) For the selective isolation, cultivation, and chromogenic detection of <i>Staph aureus</i> in food.			214892	Pkg. of 20	
BD Difco™ Chromogenic Dehydrated Culture Media		Cat. No.	Unit	Approximate Plate Yield (15x90 mm @ 5mL)	
Difco™ MI Agar		214882 214883	100 g 500 g	555 2775	
Difco™ Modified mTEC Agar		214884 214880	100 g 500 g	438 2190	
Difco™ mEI Agar		214885 214881	100 g 500 g	275 1375	

Regulatory Corner

Detection of BSE Attributed to USDA Surveillance Program

The United States Department of Agriculture (USDA) has had an active surveillance program for the detection of Bovine Spongiform Encephalopathy (BSE) in place since May 1990.¹ The program was designed to ensure detection and swift response in the event that an introduction of BSE were to occur in the United States (US). As a result of this surveillance, the USDA made a preliminary diagnosis of BSE in a dairy cow from Washington State in December 2003.

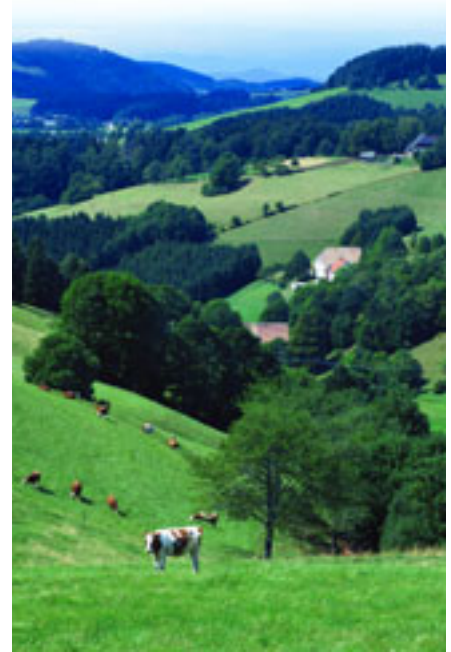
Brain samples were taken from a Holstein cow that had been slaughtered on December 9, 2003 as part of the USDA's Animal and Plant Health Inspection Service (APHIS) BSE surveillance program because the cow was nonambulatory at slaughter.² The samples were sent to the National Veterinary Services Laboratory in Ames, Iowa for histopathology and immunohistochemical testing. The presumptive diagnosis was made on December 23, 2003. The USDA, in collaboration with state and other federal animal and public health agencies, industry representatives and the Canadian Food Inspection Agency (CFIA), initiated investigations of potentially exposed cattle and regulated products. On December 24, 2003, the Food Safety and Inspection Service (FSIS) issued a Class II recall of meat from the group of 20 animals slaughtered in the same plant on the same day as the BSE-positive cow.³ Meat products manufactured from the recalled meat were distributed primarily to locations in Oregon, Washington, Idaho, California, Montana and Nevada.

An international reference laboratory in Weybridge, England confirmed the diagnosis of BSE on December 25, 2003. On December 30, 2003, Agriculture Secretary Ann Veneman

announced additional safeguards to bolster the US protection system against BSE and to further protect public health, including the immediate banning of non-ambulatory (downer) animals from the human food supply.⁴ The FSIS submitted three rules and one notice for publication in the Federal Register on Monday, January 12, 2004.⁴ The rules and notice were:

- A final rule declaring that the specified risk materials (SRM) such as the skull, brain, trigeminal ganglia, eyes, vertebral column, spinal cord and dorsal root ganglia of cattle 30 months of age or older, and the small intestine of all cattle are specified risk materials and prohibited in the food supply.
- A final rule expanding on the prohibition of central nervous system tissues in advanced meat recovery products.
- A final rule to prohibit air injection stunning.
- A notice announcing that FSIS inspectors will not mark ambulatory cattle that have been targeted for BSE surveillance testing as “inspected and passed” until negative test results are obtained.

By January 5, 2004, both the APHIS and CFIA had confirmed with a high degree of certainty by preliminary trace-back based on an ear-tag identification number that the 6½-year-old BSE-positive cow was imported into Oroville, Washington on September 4, 2001 from Canada and originated from a dairy farm in Alberta, Canada. This line of investigation indicated that the BSE-positive cow was one of 81 animals that entered the US in this shipment. The cattle from the source herd led to the epidemiological investigation of a total of 189 premises in



the US.⁵ Complete herd inventories involving the examination of the identification on more than 75,000 animals were conducted on 51 of these premises in three states—Washington, Oregon, and Idaho.

The BSE-positive cow gave birth to two live calves while in the US. The first was a yearling heifer on the same farm as the BSE-positive cow. The second, a bull calf, was in a group of calves at another location, a calf-breeding operation that also was under a state hold order. Because the bull calf could not be identified definitively, all the calves at this site were eliminated on January 6, 2004.

A total of 255 animals of interest (animals that were or could have been from that source herd in Alberta) were identified on 10 premises in Washington, Oregon, and Idaho.⁵ All 255 animals were sacrificed and BSE testing was negative on all of them. Included in the 255 animals of interest were 29 that were positively identified as being part of the group of 81 animals that entered the US, including the positive cow. The remaining 220 animals couldn't be excluded and might have been part of that 81.

Regulatory Corner

Detection of BSE Attributed to USDA Surveillance Program

Continued from page 9

In accordance with international trade agreements, the USDA notified the international animal health governing body, the Office of International Epizootic's (OIE), of the positive BSE detection. Subsequently, the OIE added the US to its list of "Countries/Territories having reported cases of BSE in imported animals only." Because the cow was not native born, and because the US continues to have strong systems in place to prevent a BSE epidemic according to an independent study by Harvard University, the USDA did not add the US to 9 CFR 94.18, where it maintains a list of countries containing or at risk of containing BSE.

When BSE was discovered in a Canadian born cow in May 2003, BD Diagnostics excluded Canadian sources of bovine materials from our products following our BD Diagnostics Animal Origin Quality Policy. With the December 2003 news of the BSE-positive cow imported into the US, we began to re-evaluate our Animal Origin Quality Policy and perform a risk assessment. Based upon this assessment, we have determined that our current animal origin policy and practices for BSE risk reduction, as well as the complement of products and services to meet the requirements of our various customer types, are both ample and sound.

However, looking to the future, for our current animal origin formulations we have determined that our policy of sourcing only from BSE-free countries becomes untenable if the US status were to change from its current designation. BD Diagnostics offers a portfolio of thousands of finished goods containing bovine origin raw materials, a large number of which include US bovine sourcing. The process of identifying, contracting and validating raw materials from new suppliers for all of our finished goods containing bovine

origin material would be both lengthy and costly. Our evaluation showed that substantially increasing the costs of most of our products in the future by switching to New Zealand and/or Australia only sources was not desirable for the majority of our diagnostic microbiology customers and for many of our other customers, as well, since it yielded only limited risk-reduction and had an insignificant impact on safety for those applications.

In order to make a continuous supply of products available to our varied customers, we are making two changes to the BD Animal Origin Quality Policy that will allow us to manage BSE risks in accordance with a given product's intended use.

- We will continue to source bovine materials only from BSE-free countries and the US, Australia and New Zealand, regardless of their designation, for our IVD (in vitro diagnostic) labeled products and for our standard FLU (for laboratory use) labeled products. We will also continue to use multi-use equipment in the manufacture of all formulations, other than those designated "Animal Free" that are produced in our DCM (dehydrated culture media) Plant's dedicated manufacturing suite and equipment.
- We will provide an elevated level of control, including use of certified herds (i.e., either certified organics or tested for BSE), in BD Diagnostics products known to be used in the manufacture of human and animal therapeutics (i.e., drugs, vaccines and/or medical devices excluding IVDs).

For our bioprocessing clients, we have created a variety of additional options for reducing BSE risks, including but not limited to:

- Animal-free formulations
- AutoNutrient™ Media Design Service
- Dedicated processing for animal-free formulations
- Select country of origin sourcing (i.e., Australia and/or New Zealand only)
- Bovine-free meat based formulations
- Certified organic bovine (i.e., never fed animal protein) formulations
- BSE tested bovine formulations
- Category IV⁶ (no detectable infectivity) only bovine formulations
- Other custom products as requested

For information on our animal-free or custom products, please send your inquiries by e-mail to BSE_Inquiry@bd.com. For more details concerning the BD Diagnostics Animal Origin Policy, please see our Animal Origin Position Statement available at www.bdregdocs.com or contact BD Technical Services at 800.638.8663, selection 2.

References

1. Bovine Spongiform Encephalopathy (BSE). 22 Feb. 2004. <<http://www.aphis.usda.gov/pa/issues/bse/bse-surveillance.html>>.
2. Bovine Spongiform Encephalopathy in a dairy cow – Washington State, 2003. 22 Feb. 2004. <<http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5253a2.htm>>.
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4. USDA Issues New Regulations to Address BSE. 23 Feb. 2004. <<http://www.fsis.usda.gov/oa/news/2004/bserregs.htm>>.
5. February 9, 2004 Technical Briefing on BSE with Dr. Ron DeHaven. 23 Feb. 2004. <<http://www.usda.gov/news/releases/2003/06/0196.htm>>.
6. EMEA/410/01 rev 1, 31 May 2001, "Note for Guidance on Minimizing the Risk of Transmitting Animal Spongiform Encephalopathy Agents Via Human and Veterinary Medicinal Products" from the Committee for Proprietary Medicinal Products (CPMP) and the Committee for Veterinary Medicinal Products (CVMP) of the European Union. Category IV – No Detectable Infectivity – includes the following: blood clot, feces, heart, kidney, mammary gland, milk, ovary, saliva, salivary gland, seminal vesicle, serum, skeletal muscle, testis, thyroid, uterus, fetal tissue, bile, gone, cartilaginous tissue, connective tissue, hair, skin and urine.

Regulatory Corner

New Media Introduced for Detection of Polluted Waters

Summertime brings with it increased use of recreational waters, which include freshwater swimming pools, whirlpools and naturally occurring fresh and marine waters.¹ A variety of diseases are associated with the use of recreational waters. Recently the United States Environmental Protection Agency (USEPA) published regulations describing methods and media for the testing of ambient water for bacterial indicators of pollution.² These methods include:

- **Method 1600: Enterococci in Water by Membrane Filtration Using membrane-Enterococcus Iron Agar (mEI).**³

The primary use of this medium is for the detection of enterococci in recreational water, fresh or marine. This test can be applied to potable, fresh, estuarine, marine, and “shellfish growing” waters. It employs a selective chromogenic medium.

The new medium was developed as a single-step membrane procedure replacing a two-step membrane transfer procedure employing two media, mE Agar and Esculin Iron Agar, to differentiate enterococci. Following filtration, the membrane containing the bacterial cells is placed on the medium and incubated for 24 hours at $41^{\circ} \pm 0.5^{\circ} \text{C}$. Observation of a blue halo around colonies is confirmatory for the presence of enterococci.

- **Method 1603: *Escherichia coli* (*E. coli*) in Water by Membrane Filtration Using Modified membrane-Thermotolerant *Escherichia coli* Agar (Modified mTEC).**⁴

The primary use of this medium is for the detection and enumeration of thermotolerant *E. coli* in recreational fresh water samples.

On the selective chromogenic medium, *E. coli* form red (magenta) colonies. The procedure employs a single membrane, replacing a two-step membrane transfer procedure. Final results are read after 24 hours and are confirmatory.

- **Method 1604: Total Coliforms and *Escherichia coli* in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium).**⁵

The primary use of this medium by certified drinking water laboratories is for the microbial analysis of potable water. Other uses include testing recreational, surface or marine water, bottled water, groundwater, well water,



treatment plant effluents, water from drinking water distribution lines, drinking water source water, and possibly foods, pharmaceuticals, clinical specimens (human and veterinary) and other environmental samples (e.g., aerosols, soil, runoff, or sludge).

On the selective chromogenic and fluorogenic medium, *E. coli* form blue or indigo colonies under normal/ambient light. Plates are then exposed to long-wave ultraviolet light (366 nm) and all fluorescent colonies counted (total coliform count).

In this single membrane technique, plates are incubated for 20-24 hours at 35°C and the results are confirmatory.

While these media were introduced after publication of the 20th edition of *Standard Methods for the Examination of Water and Wastewater*, the USEPA has stated that “the *Drinking Water Certification Manual*, EPA Method Numbers, and the *Federal Register* take precedence over *Standard Methods*” (on file, BD Diagnostics).

BD Diagnostics offers mEI Agar, Modified mTEC Agar and MI Agar as dehydrated culture media and prepared plated media. For more information, mark the appropriate box on the request for information card.

References

1. Clesceri, Greenberg and Eaton (ed.). 1998. *Standard methods for the examination of water and wastewater*, 20th ed. American Public Health Association, Washington, D.C.
2. Environmental Protection Agency. July 21, 2003. Guidelines establishing test procedures for the analysis of pollutants; analytical methods for biological pollutants in ambient water; final rule. Fed. Regist. 68 (No. 139):43272-43283.
3. USEPA. 2002. Publication EPA-821-R-02-022. USEPA Office of Water, Office of Science and Technology, Washington, D.C.
4. USEPA. 2002. Publication EPA-821-R-02-023. USEPA Office of Water, Office of Science and Technology, Washington, D.C.
5. USEPA. 2002. Publication EPA-821-R-02-024. USEPA Office of Water, Office of Science and Technology, Washington, D.C.

FYI

BBL™ Sensi-Disc™ Line Maintains Focus on Release of New Antimicrobics

The BBL™ Sensi-Disc™ line has the most complete offering of antimicrobial susceptibility test discs to meet your testing needs. BD Diagnostics works closely with pharmaceutical manufacturers during the development of drugs for treatment of human and veterinary pathogens. This close link allows BD to incorporate new antimicrobics into the BBL Sensi-Disc line soon after drug development is completed. This continuous development of discs provides you with the ability to perform antimicrobial susceptibility testing for the latest antimicrobics on the market.

Serving the needs of hospital, reference, public health and veterinary laboratories, our discs contain over 100 antimicrobics. Many antimicrobics that are



used for treating human pathogens are also used for animal pathogens. However, several compounds are unique to veterinary use only. Our offering of discs for veterinary use only includes:

- Ceftiofur
- Enrofloxacin
- Sulfadimethoxine with Ormetoprim
- Tilimicosin

Reference and public health laboratories often receive mycobacterial isolates for identification and susceptibility

testing. The BBL Sensi-Disc product line includes the following antimycobacterial drugs:

- Ethambutol
- Ethionamide
- Isoniazid
- p-Aminosalicylic Acid
- Rifampin
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All of your antimicrobial susceptibility testing needs are met with the BBL Sensi-Disc line.

For a complete listing of our products, contact your local BD Sales Representative or call Customer Service at 800.675.0908 for a copy of our Clinical and Industrial 2003-2004 Product Catalog, or visit our web site at www.bd.com/ds/. For a copy of our BBL Sensi-Disc Antimicrobial Susceptibility Test Discs Wall Chart, listing antimicrobial agents by their generic and trade names and other useful information, contact your local BD Sales Representative.

New E-Mail Addresses for Regulatory Documentation

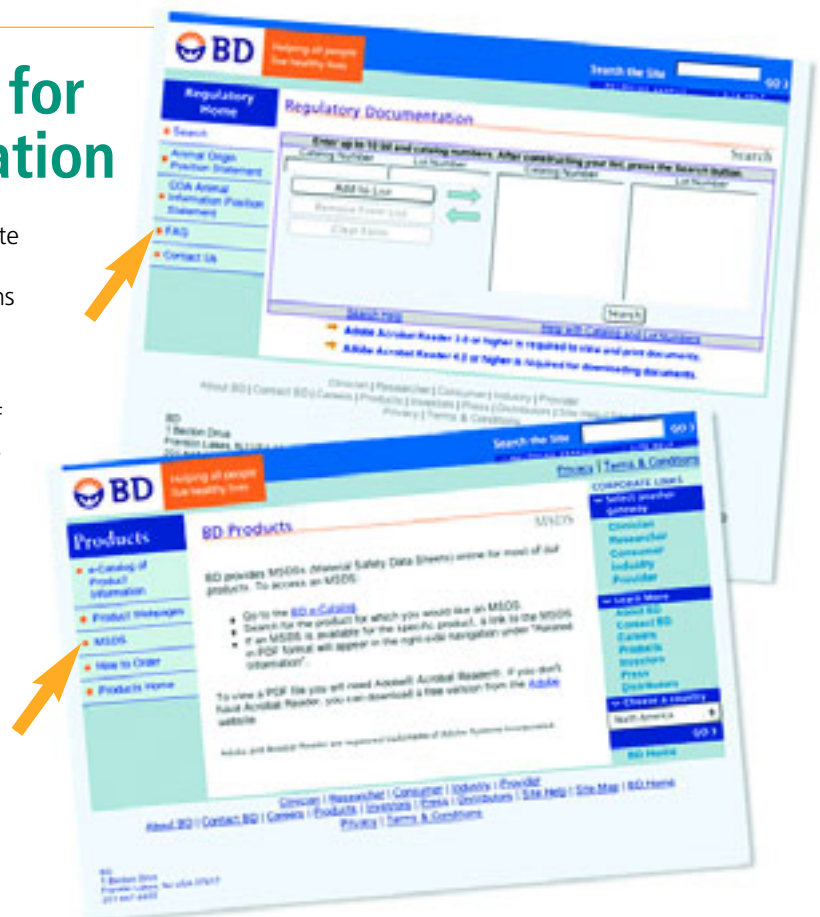
Introducing two new e-mail addresses for your Certificate of Analysis and Material Safety Data Sheet requests! With these new addresses, you now have several options to meet your documentation needs.

To request a Certificate of Analysis (CofA):

- Go to www.bdregdocs.com to access Certificates of Analysis and Certificates of Origin documents. On this site, click the link to FAQ under "Regulatory Home" on the left side of the screen for detailed instructions on how to access these documents, or
- Send your e-mail request to CofA@bd.com, or
- Contact Technical and Informatics Services at 800.638.8663, option 2, followed by option 1

To request a Material Safety Data Sheet (MSDS):

- Go to www.bd.com/products/msds.asp to access MSDS documents, or
- Send your e-mail request to MSDS@bd.com, or
- Contact Technical and Informatics Services at 800.638.8663, option 2, followed by option 1



FYI

Experience Lagniappe at ASM—BD Style

Hey Cher! This year's 104th General Meeting of the American Society for Microbiology will be held in N'Awlins, Louisiana from May 23 through 27 and BD is looking forward to giving y'all a little lagniappe¹.

We'll feature our instrumented systems including the BD ProbeTec™ ET, BD Viper™ Sample Processor, BD Phoenix™ Automated Microbiology System, BD BACTEC™ LX², BD Affirm™ VPIII and BD EpiCenter™ systems, as well as the latest rapid testing developments with the BD Directigen™ EZ RSV, BD Directigen™ EZ Flu A+B and BD Directigen™ EZ Strep A test kits. We'll highlight the new additions to our BBL™ CHROMagar™ family of culture media products and the BBL™ Sterile Pack media line. We're also offering *a little something extra* in the way of seminars—right at our booth—led by leading microbiologists on important topics.

BD wants y'all to learn what lagniappe means to us in the way we do business.

So spend some time visiting with us in Booth #745 at ASM. It won't exactly be a fais do do³, but you'll get to see product demonstrations, talk with guest speakers, and learn how we incorporate lagniappe into all our products and services.

See ya there Cher! And here's a tip: don't let nobody bet they can tell you where you got dem shoes!

¹ (lan-yap) Cajun for a little "something extra".

² For investigational use only. The performance characteristics of this product have not been established.

³ (fay-doe-doe) Cajun-speak for party.



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Contributions or suggestions on topics of interest for future editions of the newsletter are welcome. Please send comments, suggestions or articles by mail to the attention of Sharon Miller, Mail Code 632, by fax to 248.888.8382, or by e-mail to Sharon_Miller@bd.com. Send address changes and mailing list additions to the attention of Marketing Communications at Mail Code 634.

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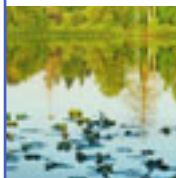
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Calendar of Events



• May 2-5, 2004

The Waterside Conference
9th Annual Meeting
Beverly Hills, CA



• May 23-27, 2004

American Society for
Microbiology (ASM)
104th General Meeting
New Orleans, LA



• June 18-25, 2004

Rapid Methods and Automation
in Microbiology XXIV
Kansas State University
Manhattan, KS



• August 8-10, 2004

IAFP (International Association
of Food Protection)
Phoenix, AZ

• October 17-20, 2004

24th Food Microbiology Symposium
University of Wisconsin
River Falls, WI