bioFood

n°3 December 2006

The international newsletter for bioMérieux agri-food partners

BIOMÉRIEUX INDUSTRY

editorial

I think you will find the third issue of bioFood, the newsletter for bioMérieux agri-food partners worldwide, particularly enriching. The focus of this edition is sample preparation. Progress has been made in many steps of the analytical process such as the specificity and sensitivity of detection, result interpretation or simply time-to-result. Not enough attention, however, has been paid to sample preparation and bioMérieux believes that more has to be done to bring a fully integrated solution to food safety professionals.

The quality of analyses, whatever the technology being used, begins with and depends upon the quality of sample preparation. In his article, Dr. Freier explains the challenge of ridding the sample of food matrix interferences – whether they be physical, chemical or biological – without removing, altering, damaging or destroying the target pathogen.

In another look at sample preparation, Ingrid Wanninger of Profos, a German biotechnology company, reveals how Profos' phage ligand technology, which uses bacteriophages (bacterial viruses) that specifically bind to a bacterial species or strain, is applied to sample preparation. With this revolutionary new technology, a specific species or strain from a mixture of bacteria in a complex food matrix can be "caught" and isolated by phage proteins, even if there are very few cells of the target present. We are so impressed by this technology that will reduce detection time, enhance performance and ensure better security for the Food Industry, that we will be integrating it into bioMérieux's Food Safety range as part of an R&D collaboration.

We hope that bioFood no. 3 achieves its goal of providing you with a forum for exploring Food Safety and Quality issues in an increasingly complex environment. Your feedback is vital. Think of what you would like to see developed in future issues and share your experience with other readers.

Alexandre Mérieux
Corporate Vice President
Industrial Microbiology

at stake

Sample Preparation and Food Matrices

AUTHOR: TIMOTHY A. FREIER, PH.D.
FUNCTION: DIRECTOR OF GLOBAL FOOD SAFETY TECHNOLOGIES
COMPANY NAME: CARGILL
COUNTRY: MINNEAPOLIS, MINNESOTA, USA

Recent technological breakthroughs in nucleic acid manipulation, protein chemistry, micro fluidics and nanotechnology have created incredible advances in food-borne pathogen detection. This article is not to address the significant advancements in detection methods. Instead, this article is about the rather mundane but incredibly important and too-often ignored operation of delivering the target organism into the technological wonder detection device. In other words, we will be putting the cart (detection) after the horse (sample preparation). Engineers can build an entire PCR system that fits on a postage stamp, but how are we going to get a single cell of *E. coli* O157:H7 from the center of a 375 g ball of ground beef into the microtubule that feeds into the "lab on a chip"?

Once the sample has arrived at the laboratory, the process of trying to deliver the target into the detection device begins. Some special challenges include thawing frozen samples without killing or growing the target, and how to aseptically open the package and retrieve the sample. The next critical step is deciding how best to get the target out of or off of the food sample or sampling device. This can include rinsing, shaking, stomaching, vortexing, masticating, sonicating, filtering, stirring, dissolving, blending, grinding, diluting, or my personal favorite, gently massaging the sample within a plastic bag.

Some day, we will hopefully have true real-time pathogen detection methods. These methods will require even more emphasis on sample preparation, since the micro-organisms will need to be extracted from the food matrix and delivered into the detection device. Currently, most bacterial food pathogen testing involves enrichment. This process allows the target bacteria to grow out of the sample and multiply to higher numbers in the enrichment broth. An aliquot of the enriched sample can then be transferred into the testing device. Eliminating this need for enrichment, while allowing real-time pathogen detection, also introduces new challenges such as distinguishing dead cells from live ones, and of course, getting the organisms out of the food and into the test device. Methods for bacterial detection will need to borrow from methods currently used for viral and protozoan detection, where enrichment is typically not an option. Many of these methods rely on centrifugation, filtration or immunomagnetic concentration. Researchers are beginning to make progress on improving these techniques through automation and miniaturization but there remains a great need for further research in this area.

Whether the sample is going into a real-time assay, or a more traditional enrichment, issues of the food matrix confounding the pathogen detection need to be considered. For example the *Salmonella* chapter in the FDA Bacteriological Analytical Manual (www.cfsan.fda.gov/~ebam/bam-toc.html) lists numerous special sample preparation procedures specific to food categories, such as eggs, nonfat dry milk, casein, soy flour, spices, and those all-important categories, frog legs and rabbit carcasses. Reasons for these food matrices to be singled out for special handling include excessive physical clumping, antimicrobial substances, pH extremes, excessive swelling or gelling when water is added and high fat content. The need for special sample prep extends to other types of pathogen testing, but these have not been as well developed as they have for *Salmonella*. No doubt, matrix interference is causing a significant, but unknown number of false positive and false negative food pathogen assays.

These food matrix interferences can be grouped into three major categories: physical, chemical and biological. Physical interference is usually caused by the food coating or encapsulating the target pathogen, inhibiting contact with the enrichment medium, thereby slowing or even prohibiting growth. Another physical interference is temperature. This is especially problematic with large sample enrichments and when there is a large sample to growth medium ratio. These conditions extend the time for the enrichment to warm to optimum growth temperature thus extending the target's lagphase. Chemical interferences suppress the growth of the target pathogen when in concentrated form, but growth may progress when the inhibitors are blocked or diluted (a common phenomenon with certain spices and plant extracts, or presence of residual sanitizers). Chemical interferences can also interact with enzymes such as

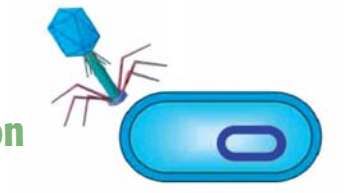
continued on page 2

continued from page 1

at stake

polymerases used in the detection technology. Biological interferences are primarily background flora competing for nutrients, or producing antimicrobial compounds such as fatty acids or bacteriocins. Biological interferences could also include organisms closely related to the target that can cross-react with the detection technology, causing false positive results. The overall challenge for sample prep is to remove these interferences, without removing, altering, injuring or killing the target pathogen.

Proper sample preparation is critical to obtaining accurate, reliable microbiological test results. Much research remains to be done to optimize this step, providing job security for future generations of microbiologists. While significant gaps exist for current cultural technologies, the need for practical, efficient and efficacious sample preparation methods will become even more crucial with the eventual advent of true real-time pathogen detection.



INTERVIEWEE: INGRID WANNINGER
TITLE: DIRECTOR OF MARKETING AND SALES
COMPANY NAME: PROFOS AG
COUNTRY: GERMANY

bioMérieux and Profos recently announced the signing of an agreement by which the two companies will jointly develop innovative solutions for food-borne pathogen testing. Under the terms of the agreement, bioMérieux will market these future solutions for the food industry and service laboratories.

bioFood: Tell me about Profos and its activities?
Ingrid Wanninger: Profos is a German biotechnology company located in Regensburg, in the centre of one of the most active regions for biotech in Germany. We are focused on the development of products for the capture and detection of bacteria and bacterial components (endotoxins) based on advanced, proprietary phage-ligand technology.

bioFood: What is the principle of your technology and know-how?
I.W.: Our technology is based on the ability of bacteriophages (bacterial viruses) to specifically bind their bacterial hosts. The know-how of Profos is to screen for bacteriophages specific to a bacteria species or strain, then isolate the respective proteins of the phages – the proteins that recognize the bacteria, and finally recombinantly produce the proteins responsible for the specificity and sensitivity of phages.
Subsequently, we plug these proteins to a separation technology (magnetic beads) in order to have a solution which is capable to purify bacteria.

bioFood: What are the advantages of this technology? How is it innovative?
I.W.: Phage proteins are very specific. They are able to isolate a specific species or strain from a mixture of bacteria, like in the situation of an enrichment culture of a complex food matrix. Phage proteins have a very high affinity as well. Even if a very low number of cells is present in an enrichment, these few bacteria can be caught by phage proteins. Compared to other purification strategies, our AMS (affinity magnetic separation) technologies will bring exceptional specificity and sensitivity features.

bioFood: Why did you chose bioMérieux to be your partner?
I.W.: bioMérieux is a world leader in Food Safety and Quality testing, with solution like VIDAS®, TEMPO®, and a complete range of Chromogenic Media. Phage ligand technology from Profos AG will provide new benefits in sample preparation, that will reduce detection time, enhance performance, and therefore ensure better security for the Food Industry.
It was a very good opportunity to bring our technology to the food labs with such an international partner.
We think that bioMérieux will be able to embed our AMS technology in their range of platforms to offer new benefits and complete workflow concepts to food microbiologists.

bioFood: Could you tell us more about your AOAC approval?
I.W.: We obtained the AOAC® approval for a *Listeria* sample prep. system test (*Listeria* Capture Kit) we launched this year. The performance of Profos *Listeria* Capture Kit method was evaluated in a comparison study using the ISO11290-1:1996 as the reference method. Results of the method comparison clearly showed that the *Listeria* Capture Kit is capable of rapid selective enrichment of *Listeria* from the selected food types after a pre-enrichment of 18 hrs, which reduces the total time for sample preparation by 75 % compared to ISO11290-1:1996. The performance of the kit was verified at an independent testing laboratory. The reduced background flora on plates of the *Listeria* Capture Kit method compared to the plates of the reference method makes the isolation and identification of *Listeria spp.* easier and more reliable.
For us, it was a proof that a Food Microbiology lab can benefit from our technology in order to increase the accuracy of their work.

bioFood: What do you think will the future of this technology be?
I.W.: First, we would like to increase the list of bacteria species, in order to cover all bacteria responsible for food poisoning. Then we will further adress clinical applications, e.g. the ability to specifically detect a clinically relevant species or a strain which will bring new value to the world of infectious disease diagnostics.

Bacteriophages - New Applications in Food Microbiology(*)

Karolina Heed, Ingrid Wanninger. Profos AG

Summary
Microbiological contamination is a serious problem facing the food industry and *Listeria monocytogenes* is one of the most hazardous microorganisms. Major food-borne pathogens have low incidence rates in food and are not equally distributed. This property of nature creates a need for sophisticated test methods that are able to find even very small amounts of bacteria in food. Profos focus is to provide innovative solutions for rapid food quality testing. The company has developed and patented a technology where special binding proteins from bacteriophages are used for a targeted capture and isolation of bacteria.

Technology
Bacteriophages are highly specific viruses that only infect bacteria. They use adhesion structures to bind to their bacterial hosts and inject their genetic material into the cytoplasm. In the lytic lifecycle (Fig 1.) the host's metabolism is taken over and the cell starts to produce new phages until the cell is lysed and the new phages are set free. The specificity of bacteriophages is defined over a line of their own proteins, which are important for the binding or lysis of the target cell. Meanwhile, a range of such proteins are available for research and production. Profos AG makes use of bacteriophage proteins for the binding of gram negative bacteria such as *Salmonella*, and *E. Coli* as well as gram positive organisms such as *Listeria* and *Clostridium*.
Lysins from bacteriophages are ideal for the specific and rapid lysis of bacteria. It is a high-affinity binding with an affinity constant in the nanomolar range and a substrate specific action. Lysins are currently available for *Listeria spp.* for all serovar types. Suitable applications are rapid testing with subsequent detection of ATP or other enzymatic activity, or antimicrobial action as an alternative for bacteria control.

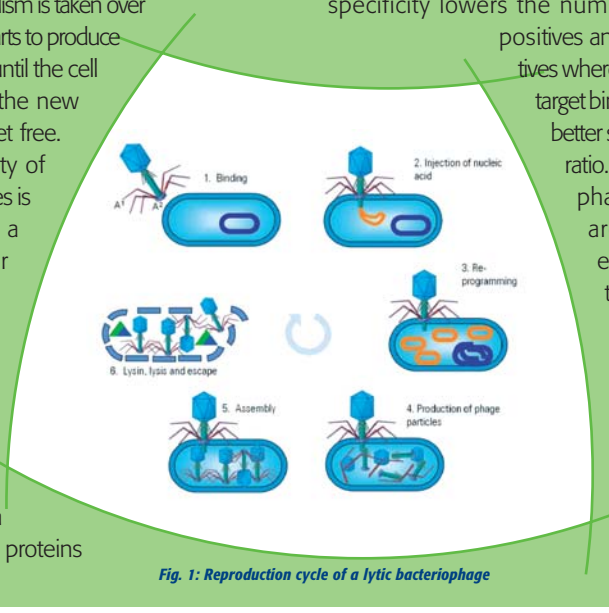


Fig. 1: Reproduction cycle of a lytic bacteriophage

Phage Advantages vs. Antibodies
Phage derived ligands offer a number of advantages over antibodies when used in microbiological test systems, such as superior specificity and superior binding. The higher specificity lowers the number of false positives and false negatives whereas the better target binding gives a better signal to noise ratio. Furthermore phage proteins are simple to engineer; they can be immobilized on any surface and can easily be coupled to other molecules (e.g. fluorescent markers). Phage proteins have been proven to provide robust performance in many different applications, even when challenged with the most demanding and complex food matrices.

Species	No. of Different Strains	No. of Positive Results
L. grayi	2	2
L. innocua	9	9
L. ivanovii	2	2
L. monocytogenes	38	38
L. seeligeri	5	5
L. welshmeri	4	4
Total	60	60

Table 1: Inclusivity Testing of all six Listeria species - all 60 isolates were detected, no false-negative results were obtained.

Products & Applications
The first results of Profos research effort are new Bacteria Capture Kits: *Listeria* Capture Kit, *Salmonella* Capture Kit and *E. coli* Capture Kit.
The Kits are based on the highly specific interaction of naturally occurring bacteriophages (short phages) with their bacterial host. Profos *Listeria* Capture Kit is a novel product for the fast and specific concentration of Listeria out of a pre enrichment broth based on magnetic bead separation with subsequent detection on chromogenic media (e.g. Fig. 2 OAA chromogenic agar for *Listeria* supplied by bioMérieux SA). The Profos *Listeria* Capture Kit method achieves higher sensitivity levels so the needed time for pre enrichment is reduced with more than 75 %
.

Profos *Listeria* Capture Kit Granted AOAC PTM Status
Profos *Listeria* Capture Kit has been validated within the AOAC Performance Tested Methods SM program (Certificate No.: 080604) and has been found to be an effective, reliable and time-saving method for testing on *Listeria* in the selected food types smoked salmon, salami and Camembert cheese. Results of the method comparison clearly showed that the *Listeria* Capture Kit is capable of rapid selective enrichment of *Listeria* from the selected food types after a pre-enrichment of 18 hrs, which reduces the total time for sample preparation with more than 75% compared to ISO11290. The reduced background flora on plates of the *Listeria* Capture Kit method compared to the plates of the reference method makes the isolation and identification of *Listeria spp.* easier and more confident. Internal and independent method comparison studies was performed: 60 different *Listeria* strains were tested with the Profos *Listeria* Capture Kit according to the kit protocol and gave positive results with all tested strains (Table 1). To evaluate the ability of Profos *Listeria* Capture Kit to differentiate *Listeria* from similar micro organisms, 42 *non-Listeria* isolates were tested (e.g. *Bacillus* and *Proteus*). None of the tested strains gave positive results with the Profos *Listeria* Capture Kit method. The statistical analysis using chi-square and Kappa Index of Agreement showed that the Profos *Listeria* Capture Kit method performed as well as the reference method ISO 11290-1:1996/AMD 1:2004 (E).

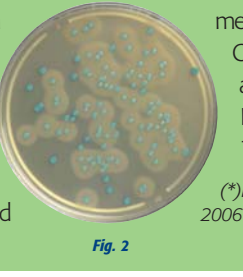


Fig. 2

(*)Poster presented at AOAC Annual Meeting 2006 - Minneapolis, USA

news

Sixth Food Industry Microbiology congress – May 16, 2006

Building on the successes of previous years, bioMérieux France's Industry Division convened over 120 participants from the food processing industry and analysis service laboratories at its Marcy l'Étoile for a day exchange with their peers..

Presentations from eight experts covered a wide variety of topics, including how to apply the new European microbiological criteria regulations, the problems posed by emerging bacterial and viral pathogens or the accreditation process for industrial laboratories.The other highlight of the day was the new TEMPO automated quality indicator testing system. Participants had the opportunity to learn from the experience of a service laboratory which has added this system to its routine tests and discuss accreditation-related issues.

The top-quality scientific presentations and the lively discussions which ensued were key factors in the event's success. Plans are already under way for next year's edition, scheduled for spring 2007.

First in the World

bioMérieux is the first manufacturer worldwide to be officially recognized as compliant with the XP CEN ISO/TS 11133 standard "Guidelines on preparation and production of culture media".

Audited in July 2006 by AFAQ/AFNOR certification, bioMérieux's Craponne plant officially received the certificate of evaluation. This standard concerns all laboratories which manufacture culture media for their own use as well as for commercialization. It enables laboratory testing results to be more reliable by guaranteeing a minimum level of culture media performances. It's the first standard for microbiology of food and animal feeding stuffs that is not only related to laboratories but also to manufacturers.

This compliance didn't only concern documentation but required scientific validation of several batches of each manufactured product. For $1^{1/2}$ years, a team of over 20 people from R&D, Quality Control and Marketing at our Craponne plant worked to modify and validate 150 control procedures, leading to a successful validation of our compliance with the standard.

For our customers, this recognition demonstrates that bioMérieux is constantly involved in standardization and takes applicable standards into account to guarantee compliance of its culture media. This guarantee means that customers will no longer need to systematically test each product lot of culture media upon reception, representing cost savings and reinforcing their confidence in the quality of our products.

The evaluation certificate officiallly given by M. Jounot (AFAQ/AFNOR) on the right, with the presence of Alexandre Mérieux and Alain Cotte-Pattat (Quality Operation Manager at bioMérieux Craponne) on the left.

products & services

bioMérieux Brings Improved Quality, Workflow and Savings with the IUL Pinch Dilutor

The Pinch Dilutor* is distributed in exclusivity by bioMérieux in some European countries.

Ready-to-use dilution broth is automatically delivered from a flexible bag into a sterile homogenizer bag containing the sample. Because samples are usually solid, semi-solid, paste, etc., gravimetric dilution has been chosen and has been incorporated into a weighing cell that is the central part of the system.

The Pinch Dilutor constantly monitors the weight in the homogenizer bag and consequently distributes the right quantity of diluent into the bag. Once the programmed dilution ratio has been reached, the system notifies the operator that the process is complete.

(*) Manufactured by the IUL Company. Consult your bioMérieux representative for availability.

expert opinion

Automatic sample preparation in ARIAS y ANGULO manufacturing plants, bongrain group.

INTERVIEWEE: BELÉN ZAPATA
TITLE: LABORATORY MANAGER
COMPANY NAME : MANTEQUERIAS ARIAS (BONGRAIN GROUP)

Introduction of the Company

ARIAS and ANGULO are companies belonging to the French multinational Bongrain Group (a family-held company, listed on the Paris stock exchange). The group is involved in the manufacture and sale of dairy products.

- 89 plants in 21 countries (Europe, Asia, South America, etc.)
- Milk, cheese, butter, creams, industrial products, etc.

ARIAS was founded in 1848 as a family enterprise dedicated to the production of "Mantequerías ARIAS" butters. In 1976, Mantequerías ARIAS was acquired by the Bongrain Group.

ANGULO GENERAL QUESERA was founded by the Angulo family in Burgos in 1949. Its primary source of business was the sale of traditional fresh cheeses from Burgos. Subsequently, it developed a broader assortment of fresh cheeses, with products like Cincho, Villalón, Requesón, fresh goat's milk cheeses, etc. Angulo is currently a company of the Bongrain Group.

Preparation of Samples in the Laboratory

The preparation of samples is the first and most important step in carrying out a proper analysis, since all the results depend on the preparation of samples.

Until not long ago, the diluent was prepared entirely by hand, from weighing the dehydrated medium to autoclaving and subsequent quality control, which involved time, labor and expense.

For the preparation of all samples analyzed, the same protocol is followed, performing counts of indicator micro-organisms, or searching for pathogens. The sample is weighed and the diluent is added. Due to the great volume of samples analyzed, time for us was a determining factor.

Experience with the PINCH DILUTOR

Following a brief presentation by our supplier, bioMérieux, we have become familiar with and tested the "Pinch" automatic diluter. The results were excellent, leading us to install three such diluters in our laboratory at Vegalencia.

Before, work was done manually. Currently, the diluent is added automatically using the Pinch Dilutor, saving handling time and eliminating dispensing errors. A sterile, commercial diluent prepared in individual 3 liter bags by bioMérieux (Fraser and BPW) is used.

Conclusions

With the prepared medium and the diluter, the laboratory has been able to eliminate medium preparation time, to reduce sample handling time and ensure that a correct analysis result is obtained.

We can almost speak of 100% advantages, since all our needs have been met, we have saved time (and therefore, expense), we have achieved greater accuracy in dilution, greater hygiene due to less handling, comfort in the workplace, less risk of contamination, a decrease in lost volume of diluent, etc.

In summary, the Pinch Dilutor equipment has provided us with a considerable improvement at one of the most critical points of the microbiological analyses. the preparation of samples, permitting us to optimize the entire process, from the use of prepared media to its automatic dispensing.

news

bioMérieux "Craponne": Europe Biggest Facility for Prepared Plate Media Production

In 2005 bioMérieux made a significant investment in its manufacturing facility for prepared culture media (PPM). The plant in Craponne, France (already the largest European plant for prepared plate media) significantly increased its yearly capacity to more than 150 millions plates. This capacity increase was achieved by installing a completely new manufacturing line operating in a cleanroom environment (ISO 5/CLASS 100 at critical process points).

The line is a unique model designed by bioMérieux's Engineering Group to meet our quality expectations. Its design benefited from bioMérieux's 40 years of experience in prepared culture media production. In just nine months it was fully installed, validated with complete IQ-OQ-PQ and operating at full speed. Among its features are optimized ergonomics and process control to reduce operator intervention, limiting the risks of errors and contamination in a critical process.

The new line and the Craponne plant were successfully audited by the AFAQ (French Agency for Quality Assurance) which renewed its ISO 9001 v2000 certification. Moreover, several of the largest global food and pharmaceutical companies and many food labs have noted the quality of the installation and the validation. Worldwide today, bioMérieux operates five state-of-the-art manufacturing sites dedicated to high quality PPM manufacturing.

news

Successful Latin American VIDAS Symposiums

Last summer, several symposiums were held in Brazil, Mexico, Argentina and Colombia. The high quality of participants and speakers made these events a great success with an average of 100 participants at each symposium, comprising representatives from the leading agri-food companies in the region, as well as Key Opinion Leaders.

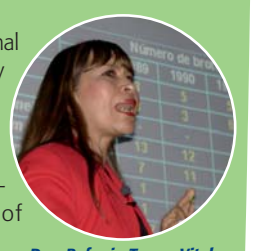
Each country designed a special program adapted to its local context. The symposiums featured key topics such as: Free Trade Agreements, sanitary issues for exporters and new microbiological solutions for food safety. For the latter, bioMérieux presented its latest innovations including VIDAS LDUO and new protocols for Easy *Salmonella*,… Renowned speakers, local sanitary authorities, former USDA staff and university professors took part in what all qualified as an interesting and lively debate.
The Latin American symposiums are part of an ongoing initiative to foster exchange between our customers and Key Opinion Leaders.

Argentinian participants in a splendid theatre

4th Food Symposium in Mexico City

The objective of the event, held last June 22nd in the Universidad Nacional Autónoma de México (UNAM), was to build a bridge between the different actors in the food chain and share the experience of local and international opinion leaders. A full auditorium of more than 160 attendees from the main companies, universities and regulatory agencies for food safety, listened to the following lectures:

- "Evaluation of a Rapid Method for Pathogen Testing in Dairy Products", by Dra. Refugio Torres Vitela – University of Guadalajara – Mexico
- "Surveillance of Animal Origin Food Safety Products by the Laboratory Network of SAGARPA" by Dra. Ofelia Flores – Mexican Ministry of Agriculture
- "Innovation in Pathogen Testing" by Fabrice Lesault - Food Safety Product Manager – bioMérieux France
- "Importance of AOAC Methods for International Food Trade: Why AOAC?" by Dr. Michael Brodsky - ex president of AOAC, IAFP & Ontario Food Protection Association – Director of Brodsky Consultants - Canada



Dra. Refugio Torres Vitela

We are glad to say that our goal was fully achieved.

VIDAS validation update

AFNOR Renewals
fully compliant with ISO 16140

VIDAS SLM single path : BIO-12/10–09/02
dual path : BIOP 12/01-04/94

VIDAS LIS : BIO 12/02-06/94

products & services

VIDAS®, the User's Point of View

With over 2500 systems installed worldwide, VIDAS is #1 for pathogen testing.

With continuous innovation, for both media and immunoassays, VIDAS brings accuracy to all laboratories. Constantly working to improve the performance, ruggedness and workflow of VIDAS solutions, bioMérieux delivers its customers the best balance between reliability and ease of use. 30 international validations confirm the excellence of this solution.
By proposing new, alternative approaches to pathogen testing, bioMérieux wants to make laboratory organization easier, taking into account the increasing demand for faster turn-around time.
Preparing the future, bioMérieux continues to innovate and enrich its VIDAS offer to meet the needs of all customers who enjoy working everyday with this leading solution.

expert opinion

INTERVIEWEE: LILIA PEÑA
TITLE: LABORATORY COORDINATOR - QUALITY ASSURANCE DEPARTMENT - MONTERREY PLANT
COMPANY NAME: QUALTIA ALIMENTOS
COUNTRY: MEXICO

Qualtia is a company which manufactures processed meat and cheese, with three facilities: Monterrey, México and Querétaro.
The company is the second most important processed meat manufacturer in Mexico.

bioFood: What are the main activities in your laboratory?
Lilia Peña: We test the environment, machines and personnel. We also check that the finished products fulfill the specifications of official and internal norms, which are more strict than the official ones. The air and water used in processes must also be verified.
Besides, we are responsible for the physico-chemical analysis of the raw materials and finished products. In the plant we have inspectors who verify the fulfillment of the process specifications.
For finished product, we test total viable count, yeast and molds, total coliforms, *Listeria monocytogenes*, *Salmonella* and *E. coli* O157.
For the environment, we test total viable count and *Listeria spp*.
Regarding the personnel, we test viable count, total & fecal coliforms. We also receive dairy samples from the Querétaro Plant and test for *Listeria monocytogenes* and *Salmonella*.

bioFood: Speaking of pathogen testing (Salmonella, Listeria, E. coli 0157:H7), what are your expectations?
L.P.: The system must be reliable, provide rapid results, easy to use by any technician and cost affordable. Before starting to work with bioMérieux, we used an automated molecular biology system (Bax) for around four years. Once we compared both methodologies, we found miniVIDAS® protocols much easier to use, with shorter time in the system, allowing us to perform several runs during the day and faster time to result with a reasonable price.
Now that we are using miniVIDAS we can tell that the sample treatment is simple, the protocol is very easy to perform and we get faster results.
The previous system (Bax) was more complicated, where you must perform a lysis step, which takes more than an hour. We also had to use very small volumes (only a few microliters) which increases the uncertainty and complicates the sample treatment. Normally an enrichment that was ready at 8 a.m. took us around 3 hours to get it ready to run in the system, and then wait for results at about $3^{1/2}$ hours. So, we were able to place only one run per day (our lab works 8 hrs/day)

bioFood: What are the benefits beyond the lab?
L.P.: The key repercussion of a faster result is for us in the production facilities. They have a high frequency of disarming and cleaning the machinery. With these faster results (24 or 48 hrs), we provide the maintenance, quality and production departments a more efficient disarming and cleaning program. Of course we will also be able to detect early any pathogen presence in our environment.

bioFood: What are your challenges for the future?
L.P.: Several months ago, the USDA approved one of our facilities for finish product export to the U.S.. This ambitious project will lead us to go even further in our quality approach.

expert opinion

INTERVIEW

NAME & TITLE: BEGOÑA MALDONADO (LAB SUPERVISOR), ADRIANA TREJO (MICROBIOLOGY TECHNICIAN), ROSA MARIA FLORES (MICROBIOLOGY TECHNICIAN) AND MIRIAM CURIEL (SALES REPRESENTATIVE).
COMPANY NAME: BUFETE QUIMICO
COUNTRY: MEXICO

bioFood: Miss Begoña, could you describe your Company?
Bufete Químico was founded 35 years ago; our company is an external testing laboratory with three divisions: Food, Environment and Toys testing.

bioFood: For the Food division, who are your main customers?
We offer analytical services to all kind of food industries - dairy, meat, fruit and vegetable producers, supermarkets - covering all types of food matrices.

bioFood: Does Bufete Quimico have a specific authorization and/or accreditation for your microbiology lab?
We have authorization from the Ministry of Health to perform Mexican Official Norms (NOM) and we also have accreditations from EMA (Mexican Institute for Accreditation) for the following Mexican Official Norms: total viable count, yeast and molds, *Listeria monocytogenes* and *Salmonella*.

bioFood: Do you offer environmental testing for the agri-food industries?
Yes we do. We can perceive a growing trend in environmental testing among our customers. Most of the time, we send qualified personnel to do the sampling, but some customers bring their samples to us.

bioFood: You have been using miniVIDAS for several months; as a laboratory supervisor, what are the advantages of using this system?
We consider the fact to have the system a great competitive advantage as a lot of customers need faster results in microbiology testing, and with miniVIDAS we can offer next day or 48 hr results. We have plans to obtain in the near future EMA accreditations for the following VIDAS protocols: *Salmonella*, *Listeria monocytogenes* and *Staph enterotoxins*.

bioFood: Adriana and Rosa, why did you choose this system?
We were searching for rapid methods, as many customers needed quicker results. First we evaluated a manual method; the problem was the amount of time for manipulation of the several steps and the fact that the technician had to spend several hours attending the procedure.
Then we evaluated miniVIDAS, and after two months of routine analysis we adopted it, mainly because the equipment does the entire job, eliminates handling errors and we don't need to monitor the time of the reaction. The reliability we have achieved on the system after several months of routine use supports our confidence in it.

bioFood: As a regular miniVIDAS user, which strengths do you see?
To be able to run different tests at the same time, time-to-result and ease of use. Since adoption, miniVIDAS is our first choice for pathogen analysis for all samples, unless the customer specifies that he needs an official norm.

bioFood: Miriam, as a Sales Representative, how does miniVIDAS impact your offer?
Several food producer customers, need faster results, so I can propose them better service than many of my competitors. This is bringing new business to us. Our plans to submit several miniVIDAS protocols for accreditation, will give even more confidence to my customers.

news

TEMPO is launched in North America!

bioMérieux introduced its latest innovative product for quality testing, TEMPO®, and hosted a number of educational events at the International Association for Food Protection (IAFP) 93rd Annual Meeting in Calgary, Alberta, Canada. bioMérieux addressed topics having an immediate impact on the food quality industry during the "Innovation@Work for Quality Indicator Testing" Symposium. Additionally, bioMérieux showcased the interactive forums for food safety and protection, as well as a roundtable discussion with industry leaders.

The Quality Indicator Testing Symposium opened with a keynote address from Bala Swaminathan, Centers for Disease Control and Prevention focusing on a 20-year journey of progress in food safety. The event was followed by a round table presentation from several of the food industry's leaders sharing their first experiences with TEMPO. Attended by companies including Nestle USA and Tyson Foods, the discussion provided firsthand reports on workflow, performance data, and laboratory implementation of the new system.

Pr. Bala Swaminthan

As part of the innovation@work initiative, bioMérieux's Innovation in Motion 93-city tour made a stop in Calgary, Canada for IAFP. The show attendees had the opportunity to experience the tour bus, which includes a presentation theater and working mobile laboratory, with demonstrations of the BacT/ALERT® 3D bacterial identification system, mini VIDAS®, VITEK® 2 Compact, culture media, and TEMPO. The bus is currently making its way across the US on an eight-month tour to provide laboratory technicians, microbiologists and physicians, as well as quality assurance and production management professionals across the country with innovative diagnostic technologies.

TEMPO official Validations

TEMPO EB
Enumeration of *Enterobacteriaceae* in 24 hours Including confirmation - AFNOR in progress

TEMPO EC
Enumeration of *Escherichia coli* in 24 hours
AFNOR/ISO 16140 TEMPO EC-BIO 12/13-02/05
AOAC-RI 080603 harmonized (1st step AOAC-OMA)

TEMPO TVC
Enumeration of aerobic mesophilic total flora in 40-48 hours
AFNOR/ISO 16140 TEMPO TVC-BIO 12/15-09/05
AOAC in progress

TEMPO TC
Enumeration of total coliforms in 24 hours
AFNOR/ISO 16140 TEMPO TC-BIO 12/17-12/05

TEMPO CC
Enumeration of coliform count in 24 hours
AOAC in progress

products & services

TEMPO® EB

TEMPO® EB, the first automated test for *Enterobacteriaceae* enumeration in food products in 24 hours including confirmation.

TEMPO EB is a new automated test associating an innovative card with a selective medium, to ensure rapid enumeration of *Enterobacteriaceae*. TEMPO EB delivers an *Enterobacteriaceae* count as early as 22 hours, confirmation included, compared to 72 hours for the reference method EN-ISO 21528-2 which requires a two-step confirmation. This easy-to-use method offers significant time savings especially when large numbers of positive samples are processed, and allows faster reporting of results.

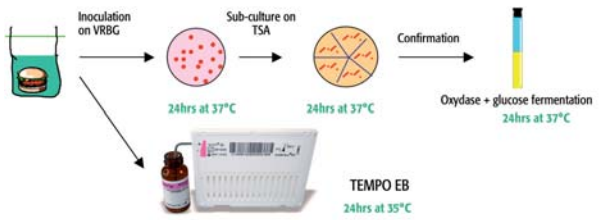
With TEMPO EB, bioMérieux reinforces the current TEMPO menu which already contains TEMPO TVC (Total Viable Count), TEMPO EC (*Escherichia coli*), TEMPO TC (Total coliforms) and TEMPO CC (coliform count), providing an enlarged menu for wider market fit. TEMPO EB is currently undergoing validation by AFNOR according to the ISO 16140 standard.

Increasing emphasis on a total quality approach in food production, HACCP plans and Risk Assessment procedures enhance the role that quality indicators such as Total Viable Count, Coliforms, *Escherichia coli* and *Enterobacteriaceae* have in monitoring the hygienic and commercial quality of food.

Comparison of the Automated TEMPO® System with Conventional Plate Counts for the Enumeration of *Enterobacteriaceae* in Food Products (*).

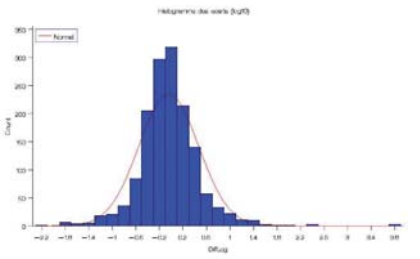
The 2073/2005 European regulation on microbiological criteria for foodstuffs highlights the importance of the Enterobacteriaceae as an indicator of food microbial quality.
The goal of this study was to evaluate the performance of TEMPO EB in comparison to the ISO 21528-2 reference method.

MATERIAL AND METHODS:



559 different samples (raw, processed and cooked) including meat (227), poultry (43), seafood (60), vegetables (85), dairy (63), bakery & confectionary (17) and miscellaneous (64) were tested on 4 different sites.
Ten grams of these naturally contaminated products were homogenized in 90 ml of peptone salt or Buffered Peptoned Water (primary 1/10 dilution) in a specific TEMPO bag with filter, and analyzed by TEMPO and standard ISO methods in parallel.

RESULTS:



Distribution histogram of differences in Log10 observed between both methods

Table2: Percentage of agreement and discrepancies rate for within and below/above range data

		Rate of Discrepancies			
		Less than – 1 Log_{10}		More than + 1 Log_{10}	
	Proportion of Agreement [3]	Proportion	95% confidence interval	Proportion	95% confidence interval
Within range data [1]	94.91 %	2.88 %	[2.09 ; 3.86]	2.21 %	[1.53 ; 3.09]
Below/above range data [2]	96.81 %	1.06 %	[0.66 ; 1.62]	2.12 %	[1.53 ; 2.86]

1 - numerical data which can be transformed in Log10 for both methods
2 - data for which at least one of the two methods gives results "less than" or "more than"
3 - difference (in absolute value) calculated between both TEMPO and reference methods which is less than or equal to 1 Log10

In terms of accuracy, enumerations obtained by TEMPO EB and the reference method ISO 21528-2 were similar. The proportion of agreement for the within range data is around 95% with a bias close to 0. The proportion of discrepancies lower than 1 Log10 or greater than 1 Log10 were both lower than 5% : respectively 2,88% and 2.21%.

CONCLUSION:

No deviation is observed between the TEMPO EB method and the ISO 21528-2 plate count method: around 95% of agreement.
The automated method produced accurate results for the enumeration of *Enterobacteriacea* in a variety of foods in 24 hours instead of 3 days for the ISO method.
The TEMPO EB method is proved easy-to-use and represents an alternative for the enumeration of *Enterobacteriaceae* in food.
It offers improved standardization as well as automated reading and recording of results, and important cost savings in terms of labor, by eliminating the need for serial dilutions and confirmation testing.

(*)Summary of the poster presented at IAFP Europe congress 2006 held in Barcelona (Spain)

expert opinion

INTERWIEE: **JEAN-YVES FRANÇOIS**
NAME & TITLE: **TECHNICAL MANAGER**
COMPANY NAME : **QUALITY PARTNER, SERVICE LABORATORY**
COUNTRY : **BELGIUM**

bioFood: Could you describe the activities of your laboratory?
Quality Partner is an independent service laboratory, specialized in food, ESB detection and cosmetic testing. It is a spin-off of the Liege University and therefore reaches an high scientific level.
Currently, 12 analysts proceeds ~75 000 food tests per year, including ~50 000 Quality Indicator tests.

bioFood: What decided you to move from the traditional method to TEMPO?
Today, we use the spiral method. However, the Belgium agency for food protection will not authorise anymore methods which are not ISO 16140 validated, *stricto senso*. It means that we will not be able to use anymore a spiral inoculation as we are proceeding now.

As we provide a high quality service, we wanted to work with a knowledgeable company in food testing. We work with VIDAS® for the detection of pathogens and therefore were very confident in the quality of bioMérieux products. So we decided to look at TEMPO which is validated according to the ISO 16140 standard.

TEMPO is first a financial choice. TEMPO will help us to increase our client portfolio without increasing our staff. Indeed, TEMPO will allow us to save significant time all along the analysis stages. So we are happy to move our routine to TEMPO

bioFood: Is TEMPO well accepted by your staff?
Yes. Our technicians are pleased with the idea of not having to count the plates anymore, as it is a work which takes lots of time and has no added value. My staff knows that TEMPO will help them to increase the number of samples without having to do additional hours.

bioFood : You recently performed an evaluation of TEMPO EB, which allows the enumeration of Enterobacteriaceae, what are your feelings on this new parameter ?
Actually, to enumerate *Enterobacteriaceae*, we follow the ISO standard 21528-2, using VRBG plates, with spiral inoculation and two steps of confirmation. TEMPO EB gives equivalent results to the ISO reference method within 22 hours, confirmation included, compared to 72 hours for the ISO 21528-2 method.

The ISO 16140 TEMPO EB validation is in progress. We will use TEMPO EB in routine as soon as the validation is completed.

news

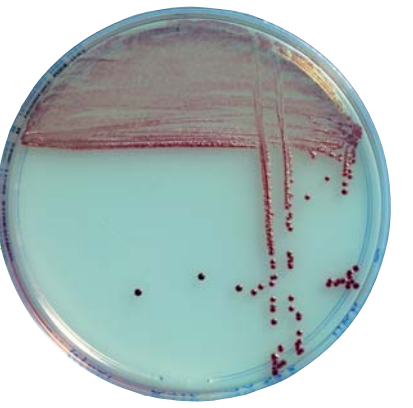
2006 bioMérieux Belgium Award for Excellence in Pathogen Research

bioMérieux Belgium was proud to present on September 14th & 15th its 9th Pathogen Research Award at the Eleventh Conference on Food Microbiology, that was held by the University of Ghent.
The 2006 edition of the award ceremony welcomed some 300 participants from Belgian authorities, reference labs, public and private food labs and auto-control labs.
A jury of experts led by Peter Van Landschoot, Sales and Marketing Manager - Industry at bioMérieux selected Mrs Van Coillie's team from CLO Melle from among 30 abstracts. .
François Donald Monroe, Food Market Manager Industry - Europe at bioMérieux, presented the award and a 1,000 € check to the team, conveying a strong message about bioMérieux's commitment to innovation in the field of food safety. For bioMérieux Belgium, it was important to support the organiser of this important congress.

science & technology

Counting campylobacter: Performance Comparison of two Selective Agars (*).

Lisa. K. Williams and Tom. J. Humphrey. University of Bristol UK



Colonies of Campylobacter jejuni

There is currently no consensus on the selective agar to use for the isolation and enumeration of Campylobacter spp. The UK favours modified charcoal cefoperazone deoxycholate agar (mCCDA) whereas in the United States, for example, Campy-cefex is widely used. Many of the Campylobacter selective agars contain similar components and the most commonly used antibiotics are polymyxin B, rifampicin and cefoperazone; these allow the growth of Campylobacters by suppressing contaminants. The agars currently available usually have a charcoal or blood base and this can make identification of campylobacters more complex. bioMerieux has developed CampyFood ID, which was launched in 2006. It is a pre-poured agar that contains a specific combination of selective antibiotics with a colour indicator, which assists in the identification of Campylobacter spp.

This study carried out by the University of Bristol, UK compared the UK reference method of mCCDA with CampyFood ID agar (CFA), for the isolation of campylobacters from naturally contaminated samples of animal, farm and food origin.

Materials and Methods

Experiments were conducted under standard laboratory conditions using the International Standard Organisation Microbiological method for the examination of food and animal feeding stuffs (ISO 10272). Naturally contaminated fresh and frozen samples collected from various locations in South West England, were examined. A total of 173 samples were tested, consisting of 25 unpasteurised milk (RM); 43 natural surface waters (NSW); 26 fresh chicken carcass rinses (CRW); 30 fresh chicken neck skins (CS); 27 fresh chicken faeces (CFS) and 22 frozen chicken carcass rinses (FCRW). Samples were prepared in either Maximum Recovery Diluent (MRD) or Buffered Peptone Water (BPW). Samples were diluted according to sample type, with one dilution for RM and NSW. In contrast, several decimal dilutions were made after rinsing fresh or frozen chicken carcass with 225 ml of BPW or MRD and CS and CFS samples were diluted as follows: add 90 ml of MRD or BPW to 10g of CS and homogenise; add 9 ml of MRD or BPW to 1 g of CFS and homogenise. BPW samples were only plated on to CFA, with MRD samples being plated onto CFA and mCCDA. Plates were incubated in a microaerobic atmosphere (5% O2 ,10% CO2, 2% H2 balanced in N2) for up to 48 h. The study was divided into two sample groups. In group 1, CFA plates were counted at both 24 and 48 h, in group 2, CFA plates were read at the later time only. In both groups mCCDA plates were read at 48 h only.
Presumptive campylobacters-positive colonies from both agars were confirmed by microscopy, biochemical tests (oxidase, catalase) and species identified using PCR and API® campy.

Comparison	Number of isolates	Median number of campylobacter colony forming units (cfu)					p value
		CFA				mCCDA	
		MRD 24 h	MRD 48 h	BPW 24 h	BPW 48 h	MRD 48 h	
CFA MRD 24 h vs. mCCDA MRD 48 h	14	2000	-	-	-	1400	0.391
CFA MRD 48 h vs. mCCDA MRD 48 h	30	-	717	-	-	575	0.394
CFA BPW 24 h vs. CFA MRD 24 h	7	5600	-	9100	-	-	0.297
CFA BPW 48 h vs. CFA MRD 48 h	14	-	1800	-	1350	-	0.761

Table 1: The number of campylobacter present in samples from each medium, analysed by incubation time and diluent used.

Comparison	Number of isolates	Median number of *campylobacter* cfu			p value
		CFA		mCCDA	
		MRD 48 h	BPW 48 h	MRD 48 h	
CFA MRD 48 h vs. mCCDA MRD 48 h	17	540	-	170	0.963
CFA BPW 48 h vs. MRD 48 h	7	31000	44500	-	0.031

Table 2: The number of campylobacter present in samples based on each medium analysed by incubation time and diluent used.

Results

Of the 134 samples tested in Group 1, 43 were positive on one or more agar, 39 on mCCDA read at 48 h, 16 on CFA read at 24 h and 33 on CFA read at 48 h. In Group 2 39 samples were tested, of these, 23 were positive on 1 or more agar, 17 on CFA read at 48 h and 22 on mCCDA read at 48 h.

Group 1

For each comparison there was no significant difference ($p > 0.05$) observed between the two media, irrespective of the diluent used. These results are summarised in Table 1.

Group 2

For each comparison, there was a significant difference ($p = 0.03$ Wilcoxon test) observed between the diluents BPW or MRD, but there was no significant difference between the CFA and mCCDA with MRD (Table 2).

Species identification

The predominant species isolated was *C. jejuni* with *C. coli* being rarely detected either by PCR or API campy.

Conclusions

For group 1, mCCDA and CFA had equivalent performance when both agars were read at 48 h. Reading CFA at 24h decreased the number of positive samples obtained. The diluent used did not affect the ability to isolate campylobacters on CFA. Analysis of enumeration and selectivity results from the two agars identified no significant difference between them, or the diluents.
For group 2, only limited sample numbers were tested, which restricted the interpretation of the results by statistical analysis. There was no difference identified between the diluents or the agars. Analysis of enumeration results identified a statistically significant difference between BPW and MRD, indicating that BPW was better at recovering campylobacters cells.

In conclusion, CFA was designed as an isolation agar for campylobacter. However, this study highlights that it is suitable for enumeration and could be used in parallel with mCCDA. The use of a suitable second selective agar is advised in the ISO 10272 protocol. The coloured colonies produced on CFA, although not campylobacter-specific assisted in the identification of these bacteria. CFA had comparable performance at 48 h with the reference method using mCCDA for the enumeration of Campylobacter spp. from naturally contaminated samples of animal, farm and food origin.

(*)This study was presented as a Poster at the IAFP (International Association for Food Protection) 2006 annual meeting.

bioMérieux applications

As the world leader in microbiological control, bioMérieux provides a unique range of microbiology quality assurance testing solutions, covering ready-to-use culture media, identification testing, quality indicators, pathogen testing and molecular biology. We are committed to helping food industry partners provide safe products with complete confidence.

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