OXOID

THE LATEST DEVELOPMENTS IN FOOD MICROBIOLOGY

setting standards update

The BAX® System Q7 from DuPont Qualicon provides rapid and reliable detection of food-borne pathogens and other organisms

• In this special Update of Setting Standards we discuss the importance of microbiology to the food industry. Dr Roy Betts of Campden and Chorleywood Food Research Association sets out the pros and cons of PCR whilst Alec Kyriakides of J Sainsbury plc gives a detailed opinion on *Salmonella* in ready-to-eat foods. • Learn how the BAX® System Q7 has helped the DVFA and BioMar in their quest for providing safer foods, and Christian Hansen to gain control of food spoilage by using the BAX® System Yeast and Mould assay.

The Changing Face of Microbiological Food Testing

At a time when food poisoning outbreaks are occurring with alarming regularity and product recalls are seriously affecting some of our best known brands, huge expectations are placed on food companies to ensure the food we eat is safe. Fortunately though, microbiological methods are also evolving.

Some of the most advanced technologies, such as the polymerase chain reaction (PCR) technology found in the DuPont Qualicon BAX® System Q7, are now accessible to routine laboratories for the detection of food-borne pathogens and other organisms, offering new levels of convenience, speed and, above all, confidence.

Evidence of control

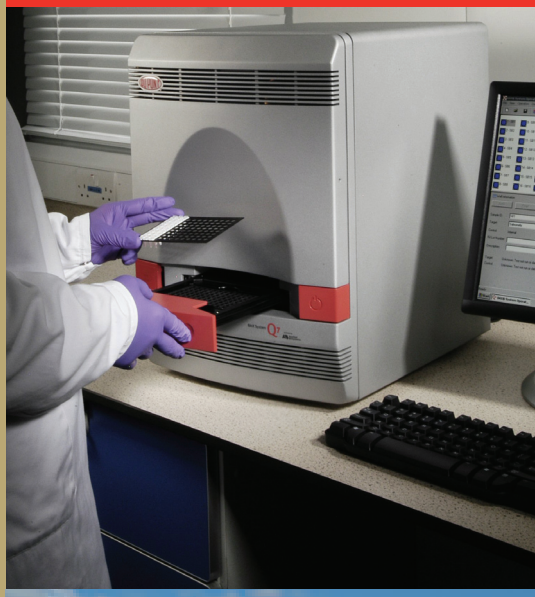
In 2006, new requirements came into force for food manufacturers and food processors in Europe, based on the microbiological criteria published in the European Commission Regulation 2073/2005. The criteria in this regulation refer to food safety and to process hygiene. They are designed to ensure that foods on the market do not present a health risk to consumers and that food safety management procedures are functioning correctly. Similarly strict criteria are enforced by other regulatory bodies around the world.

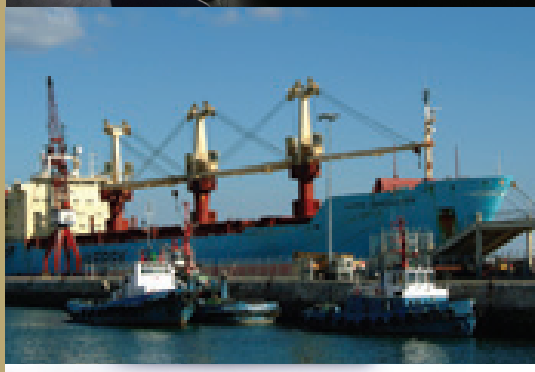
Microbiological testing of products and raw materials, combined with strict environmental monitoring programmes such as Hazard Analysis Critical Control Point (HACCP), plays an important role in providing evidence of compliance with such regulations and preventing food poisoning. Whilst this can pose a problem for smaller producers with limited facilities, there is no better way of illustrating that products are safe and processes are in control.

The longer a company has to wait for the all-clear, the longer products are on hold and productivity is suspended.

Microbiological testing is not without its challenges, particularly for companies with perishable, short shelf-life products. With some traditional testing regimes taking several days to provide an answer, there simply isn't time to wait before releasing products. In addition, these delays are unacceptable when it comes to pinpointing sources of contamination and resolving problems. The longer a company has to wait for the all-clear, the longer products are on hold and productivity is suspended.

The reference methods cited in Regulation (EC) 2073/2005 can take upwards of 3 days to perform and may require further testing to confirm the result. The regulation does, however, give scope for companies to consider alternative methods, particularly more rapid methods, providing they can be shown to give equivalent results [Regulation (EC) 2073/2005: article 5.5].





Thanks to this flexibility, and the proven performance of the BAX® System Q7, routine food microbiology laboratories can now experience the reliability, speed and convenience that PCR has to offer. The BAX® System has been validated for a wide range of food types and environmental samples.

The BAX® System Q7

It's the easy to use software and walk-away automation of the BAX® System Q7 that makes it ideal for use in a routine laboratory. Following sample enrichment and cell lysis, up to 96 samples may be loaded into the BAX® System Q7 at a time. The system then automatically performs the PCR thermocycling process, with results available in 3½ hours or less. All the necessary reagents are supplied conveniently as a small tablet, which is pre-loaded in the base of the reaction tubes. This standardises the whole process and reduces the number of pipetting steps that would otherwise be required, making the BAX® System Q7 possibly the easiest PCR method on the market.

The BAX® System Q7 offers a single testing platform for a wide range of food-borne pathogens and spoilage micro-organisms (Table 1).

Table 1: Assays available for the BAX® System Q7

ORGANISM	PCR TYPE	TABLETTED REAGENTS	TIME TO RESULT (VARIES ACCORDING TO SAMPLE TYPE AND TARGET LOAD)
Salmonella	End Point	Yes	Next day
Campylobacter	Real Time	Yes	Protocol offers same day, next day or 2 day results.
Listeria monocytogenes	End Point	Yes	2 days
Listeria genus	End Point	Yes	2 days
E. coli O157:H7	End Point	Yes	Same day
Enterobacter sakazakii	End Point	Yes	Next day
Yeast and mould	End Point	Yes	Protocol offers same day or 2 day results.
S. aureus	Real Time	Yes	Next day

To increase the flexibility of the system, some of these - *Salmonella, Listeria* genus and *L. monocytogenes* for example - can be processed together in the same run.

With same day or next day results for most pathogens, and same day to 48 hour results for *Listeria* and yeasts and moulds, the BAX® System Q7 can reduce the time it takes to confirm the presence or absence of these micro-organisms in foods by two days or more compared to conventional culture methods[1].

Furthermore, the highly accurate PCR technology, combined with an integral internal positive control, ensures that these rapid results are reliable, providing peace-of-mind and reducing the need for further testing. Such speed and confidence in results can provide enormous benefits to food companies, especially those manufacturing short shelf-life products or those that want to positively release product.

Summary of benefits:

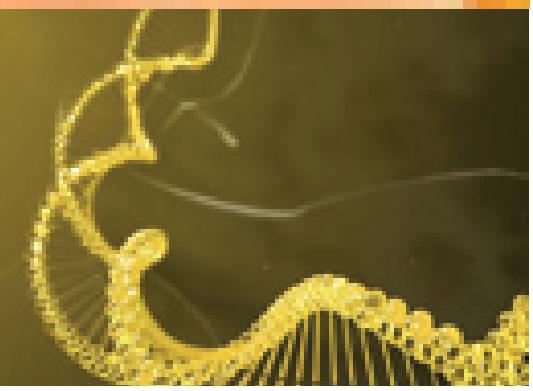
- products and raw materials can be released sooner, reducing storage costs and increasing productivity
- response times can be improved, reducing downtime and minimising the impact of contamination problems
- fewer false positives ensure unnecessary action is avoided
- companies may be able to progress to a positive release program for their short shelf-life products.

The experiences of some BAX® System customers are included in this special edition of Setting Standards. Please read on to understand what benefits the method has brought to many laboratories throughout Europe.

For more information on the BAX® System Q7, please speak to your local Oxoid representative, visit www.oxoid.com or use the reply paid card at the end of this edition.

Reference: 1. Shearer, A.E.H., Strapp, C.M. and Joerger, R.D. (2001) J Food Protection 64 (6):788-795

What is PCR?



The polymerase chain reaction (PCR) enables the amplification of target-specific DNA sequences to detectable levels. The process requires primers (short sequences of DNA that are complementary to the target DNA), DNA polymerase (an enzyme to catalyse the reaction) and free nucleotides, that are used in the extension of the amplified DNA. This process is strictly controlled by raising and lowering the temperature and is repeated 30 - 40 times to allow amplification. This process is known as thermocycling.

Traditionally, target specific DNA is extracted from target cells by heating the sample with a lysing agent. Cell walls are broken down, and the double strands of DNA are denatured (unwound and separated by heating to 90-96ºC) into single strands. After being cooled to 55ºC, primers in the reaction tube anneal to the separated target DNA. The temperature is then raised to 72ºC and DNA synthesis occurs as the polymerase extends a new sequence along the single strand of target DNA, using free nucleotides as DNA building blocks. This results in two double strands of DNA - identical copies of the original target DNA. Multiple repeats of this denaturation, annealing and extension process result in an exponential increase in the concentration of target DNA (figure 1). This results in sufficient genetic material for accurate and reliable detection in a matter of hours.

In the past, polymerase had to be added for each cycle as the high temperatures required for separating the DNA strands also destroyed the enzyme. However, the discovery of a thermostable enzyme, Taq polymerase, allowed PCR to be automated and thus used in routine applications throughout the scientific world.

Today, automated cycler-detectors have permitted the development of walk-away pathogen detection systems, such as the DuPont Qualicon BAX® System Q7. Developments in PCR detection techniques have also ensured that the amplified DNA is contained within the test unit for the duration of the process. This reduces the risk of cross contamination and eliminates the requirement for a separate work area.

There are two types of PCR detection systems currently available for routine applications in the food industry:

- Real-time PCR offers a quantifiable result, relating back to the amount of target DNA in the specimen as it was loaded into the cycler-detector. Real-time PCR detects the amplified DNA during the highly precise exponential phase of PCR using special fluorescent probes. It collects data at the end of each cycle, providing a faster method for DNA quantification. Use of different fluorescent probes also enables real-time PCR to differentiate between multiple targets.

- End-point PCR offers a qualitative result. Detection takes place when the PCR process is complete. Intercalating dyes, which fluoresce when incorporated into a double stranded section of specific DNA, are used in the detection process. A simple yes/no result is given at the end.

The BAX® System Q7 is an automated PCR system which allows both end-point and real-time assays to be run. It offers a real-time assay for the detection, differentiation and quantification of *Campylobacter* species (*C. jejuni, C. coli* and *C. lari*) in the same species sample; detection and threshold quantification of *Staphylococcus aureus* (see page 9); and clear end-point results for other important food-borne pathogens.

The BAX® System is used successfully in many different food microbiology laboratories. It has been validated by AFNOR and AOAC, and adopted by other national bodies to test a wide variety of food products (depending on the target organism), including dairy, meat, seafood, chocolate, fruit and fruit juices, vegetables, salads, and animal feeds.

Full details of validations, approvals and adoptions are available from Oxoid.

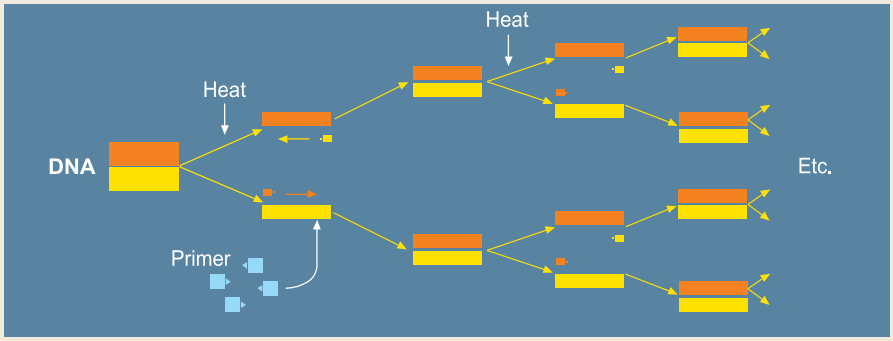


Figure 1. The Polymerase Chain Reaction (PCR).

Improved Salmonella Testing With the BAX® System

The Danish Veterinary and Food Administration (DVFA), Southern region, has recently been accredited by DANAK (according to ISO 17025:2005) to test for *Salmonella* in different foods using the BAX® System. Microbiologist in the Southern laboratory, Dr Birgitte Nauerby, explains how the BAX® System has improved the *Salmonella* testing service that they provide.

In the DVFA Southern laboratory, we analyse different kinds of food for the presence of pathogenic, as well as non-pathogenic, micro-organisms. In addition to samples from private customers, we process about a third of all samples received by the DVFA. We recently switched to the BAX® System for analysing samples for the presence of *Salmonella* - mainly in meat samples, but also in other food types. We received accreditation for this on the 12th March this year and, since then, we have used the BAX® System for most of our *Salmonella* analyses.

Faster results

The BAX® System is a big improvement on our previous method (an automated immunoassay system) as it has made it possible for us to obtain negative results 24 hours earlier. This is important for the food companies we are dealing with, as it means that negative results can be received a day earlier than before.

In addition, the BAX® System is quicker and simpler to use, reducing 'hands on' time in *Salmonella* analysis.

Reliable method

It is vital for us to have confidence in the results. Comparing the BAX® System to our previous automated immunoassay method convinced us that the BAX® System is a better method for the analysis of *Salmonella*. Since we are an official control laboratory, we simply cannot afford to get things wrong. We must isolate all *Salmonella* positives for sero-typing, so false positives can create a great deal of unnecessary work. As for missing a *Salmonella* - a false negative - well that is simply not acceptable.

Obtaining DANAK accreditation was an important and necessary step for our laboratory. For this we compared the BAX® System to our previous method. We analysed numerous samples that were both naturally contaminated and spiked, including chicken, meat and a variety of other matrices (mainly fish-feed). BAX® identified two positives which were missed by our previous method, both of which were confirmed. This indicated to us that BAX® is a more sensitive system and enabled us to incorporate it into routine use within our laboratory.

For more information on the BAX® Salmonella assay, please speak to your local Oxoid representative, visit www.oxoid.com or use the reply paid card at the end of this edition.

Left to right: Jane Hallmann, Jytte Warming, Anette Petersen, Birgitte Nauerby.

Focus on Denmark

The Danish Veterinary and Food Administration (DVFA)

The DVFA is part of the Ministry for Family and Consumer Affairs in Denmark and has three regional control authorities in the North, South and East of the country. One of the main purposes of the Administration is to ensure the safety and high quality of foods, setting national limits for the content of undesirable bacteria, such as *Salmonella* and *Campylobacter*.

DANAK

DANAK is the Danish national body for accreditation whose role is to ensure that laboratories meet the requirements of international standards.

Why use PCR?

By **Dr Roy Betts**, Head of Microbiology, Campden and Chorleywood Food Research Association

There are an increasing number of methods available to food microbiologists for the identification of food-borne pathogens, so what are the real benefits of PCR, and why should a food testing laboratory consider using this method?

Misconceptions

PCR isn't a new method. The technique has been around for many years but, being a molecular technique, it is still often considered by testing laboratories to be a research tool confined to specialist laboratories. There are often concerns that it is too complicated for the routine food testing laboratory. Happily, however, this is no longer the case, and the food testing laboratory now has a choice of well-validated PCR based methods in kit form that they can confidently use within their laboratories.

The first PCR kits were quite complicated and labour intensive, relying on gel electrophoresis as an end-point and requiring a degree of expertise from the laboratory user. However, as the technique has developed, PCR has become much easier to perform and, with the development of automation, it can offer a fast, fairly user-friendly approach to microbiological testing.

PCR kits are no longer the domain of research laboratories but are now in a format where they can be used relatively easily in routine food testing laboratories.

History is important with food testing methods. The longer a method has been used successfully, the more confidence laboratories will have in it. We can see this with both cultural and immunological methods being used today. PCR now has a history. Many PCR kits offer a proven technology, and laboratories can have more confidence in the method.

Advantages of PCR

PCR kits are available from a number of manufacturers for the most important food-borne pathogens and for certain food spoilage micro-organisms such as yeasts and moulds. The methods can be used as a screening tool or for rapid confirmation, depending on the needs of the laboratory, and can offer advantages over other methods.

Speed

PCR kits generally offer a faster test time than many other methods and are considerably faster than reference methods. Depending on the test type and the level of detection required, PCR can give a result in a matter of hours. Typically, however, PCR allows a next-day result. This can be a great advantage to food companies, especially those that operate a positive release of products. Companies don't need to hold stock for so long while they wait for clearance. PCR can save a company at least 2 days over traditional methods, allowing products to be released sooner and potentially decreasing warehousing costs.

Speed is also important in the investigation of contamination problems. Rapid results minimise factory downtime and ensure that interruptions to supply are as brief as possible.

Confidence

Often reference methods allow the growth of organisms with a similar colonial appearance to the target organism on the culture plate. This necessitates the use of further tests in order to confirm the result. With the development of more accurate tests, greater confidence in the 'presumptive result' is often being achieved. PCR kits, if well researched and validated, can offer great confidence in results. With a well designed PCR kit, you can be more confident that a negative really is a negative and a positive really is a positive, potentially reducing the need for large numbers of confirmatory tests. If results are negative, you can have peace of mind as you release raw materials and finished products. Similarly, if a result is positive, although it is still recommended to isolate and biochemically confirm organisms, you can be confident in your decision to take the necessary action.

These days, laboratories are well aware of the implications of getting an incorrect result. A false positive could initiate significant action (at great cost and inconvenience) for no reason. Even a presumptive positive result, which then turns out to be negative after further testing, can put everything on hold until the confirmation comes through. On the other hand, a false negative could potentially allow the release of a product with questionable safety. The correct result is important.

Automation

Most manufacturers of PCR kits now market them with instruments offering walk-away automation. This can be very helpful for laboratories with larger sample throughputs. Multiple tests can be loaded at the same time and left as the system automatically performs the PCR cycles. In addition, results can be displayed, stored and reported in a number of formats. Many of the systems available today are very user friendly and can be adopted with minimal training. Such automation, combined with speed, is invaluable in coping quickly with the large numbers of tests that may be required in the investigation of a contamination problem. Not only this, automation can improve accuracy by reducing the potential for operator error.

Considering cost

For some companies the cost of a test will be an important factor when deciding which method to adopt. If the cost of each test is considered in isolation, then PCR may appear more expensive than some alternatives. However, it is extremely important to perform a cost/benefit analysis for the company, considering all the associated costs before any method is disregarded purely on the grounds of test/instrument price.

Some points to consider are:

- **Warehousing**

Reference methods may take 5 or more days to confirm a result, whereas PCR takes less. This is particularly important for companies that need to perform positive release. PCR can reduce the time products need to be warehoused by several days.

- **Shelf-life**

Speedy results also have implications on the shelf life of products. If products with a short shelf-life can be released earlier, they have a longer time in which to be sold.

- **Laboratory efficiency**

Automation and faster results allow more tests to be performed in the same unit of time. This adds to the efficiency of the laboratory and frees staff to perform other important tasks.

Changes in practice

With the adoption of a new method, it may be necessary to change the way work in the laboratory is performed, and so method changes should be planned carefully. There may be different 'risk' issues which have to

be addressed. Traditional microbiological methods require aseptic technique. With PCR, preventing DNA contamination is an additional concern. PCR involves amplification of a section of DNA - an amplicon - and it is this which can cause contamination problems. It is important to consider and minimise the risk of stray amplicons getting into samples and causing cross-contamination issues. That is why many PCR kit manufacturers now use the closed cap format - a convenient and effective way of addressing this issue and much more convenient and cost effective than the previous answer - a whole new PCR suite!

It is also important to think about how the methodology will fit into the laboratory - in terms of space, staffing, maintaining quality of results, timeframes, etc. All these are essential for every new method that is adopted. Additionally, if the laboratory is accredited, consideration should be given to the inclusion of the method into the laboratory's scope and what the accreditation body may require with respect to method controls, validation, servicing and calibration.

(continued overleaf)

Why use PCR?

(continued from Page 7)

Validation

Another important consideration is the validation of the method. Good validation adds to confidence in the results and helps to comply with international regulations.

It is important to choose a method that is thoroughly validated and well regarded by international validation bodies.

Organisations undertaking testing in compliance with the recent EC Commission Regulation (2073/2005) on the microbiological criteria for foodstuffs must use a reference method cited in the regulations. There is, however, scope to adopt a rapid method so long as it has been properly validated. Any laboratory wishing to adopt a new method must consider its validation status. Validation or certification by MicroVal, AOAC or AFNOR, for example, or any such third party status, gives confidence in the method. However, no matter how well validated a kit is, laboratories should also remember that they will need to do internal validation testing in their own laboratory to prove the method works in their hands.

The importance of back up

A consideration many laboratories fail to appreciate when considering the adoption of a new method is the level of service and back up they will get from the method manufacturer. When you adopt a rapid method such as PCR, unless you're planning on running it alongside another method indefinitely, you invest everything in the new system. As there will undoubtedly be problems encountered during system commissioning and routine use, laboratories must have confidence that the method supplier will give them the help and backup they need to overcome such problems. Laboratories also need to have a strategy to follow if the new method ever fails completely (e.g. due to instrument breakdown). In such cases a very rapid service/maintenance visit or provision of a replacement instrument is invaluable. Therefore, it is important to source a product from a reputable company with a proven track record - one that you can trust - as you will need to have great confidence in the support that they can supply.

Speed, accuracy and ease of use make the BAX® System Q7 a powerful part of quality control processes in the food industry.

Points to consider:

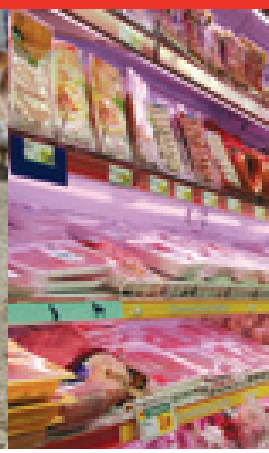
There is a wide range of new method technologies available to food microbiology laboratories. These new techniques range from new formulations of broths and agars that speed up conventional approaches, to immuno-assays and nucleic acid based tests such as PCR. These newer technologies such as PCR are now in kit-based formats that are easy to use and in many cases automated; they can offer real advantages to food testing laboratories. However, not all new methods are ideal for every laboratory situation. There is an important list of questions that any laboratory considering adopting a new test method should answer and, by answering, should gain an insight into the best type of method for their own individual circumstance:

- What are you testing for and what methods are available?
- What is your sample throughput?
- How important is a rapid result?
- How confident are you with your current method, and are you satisfied with that?
- How would automation benefit your lab?
- What is the cost of the new method (kits, instruments, annual servicing, calibration etc) and is the cost acceptable?
- Have you considered all the costs of your current test method associated with holding, testing and releasing product, and how the introduction of a rapid method may reduce these costs?
- How would the method fit into the laboratory and the working day?
- What changes would have to be made, and are these changes acceptable?
- Is the system properly validated, would it fit into an accredited laboratory?
- What support does the supplier offer?

Satisfy these and it should make the transition to a rapid method such as PCR, a very positive experience.

Fast and Accurate Campylobacter Testing

The new DuPont Qualicon BAX® System Real-Time PCR assay for *Campylobacter jejuni/coli/lari* detects, quantifies and differentiates between pathogenic *Campylobacter* species in as little as 2½ hours.



Most current screening procedures for *Campylobacter* are culture-based, taking at least three days to perform and requiring additional work to differentiate between species. The new BAX® System Real-Time PCR assay for *Campylobacter* provides a reliable answer much more quickly, allowing you to:

- Detect, differentiate and quantify the pathogenic species *C. jejuni, C. coli* and *C. lari* directly from the sample in only 2½ hours using a direct protocol.
- Detect and differentiate these pathogenic *Campylobacter* species within 48 hours from samples requiring enrichment.

The BAX® System Real-Time PCR assay for *Campylobacter jejuni/coli/lari* is the first in a series of new real-time assays for use with the BAX® System Q7. Target specific probes (linked to different fluorescent markers) are cleaved and activated during the amplification process, resulting in an increase in fluorescence. This fluorescence is measured at the end of each amplification cycle in 'real time'. Determining which fluorescent marker was detected, and when, enables the differentiation and quantification of the three pathogenic *Campylobacter* species.

Campylobacter spp. can be endemic in many poultry populations with relatively high counts of 10^4 to 10^6 per gram of carcass frequently being encountered. Couple this with an infective dose as low as 500 cells, and it is easy to understand why *Campylobacter* is the most common cause of food poisoning in many countries.

At present, there are no regulations in force on the presence of *Campylobacter* in food. None-the-less, the recognised health risks are forcing farmers, suppliers and retailers to think about *Campylobacter* and to consider ways of managing - or even eradicating - this threat.

The ability of the BAX® System to quantify and differentiate between the three pathogenic species of *Campylobacter* can help significantly in this process. Indeed, the ability to get a rapid result (in less than 2½ hours when levels are greater than 10^4 cells/g) can be very useful in assessing the effectiveness of cleaning regimes. The assay can also be useful in managing levels of contamination within poultry populations, for monitoring changes in the epidemiology of the organism and in determining the source of the contamination.

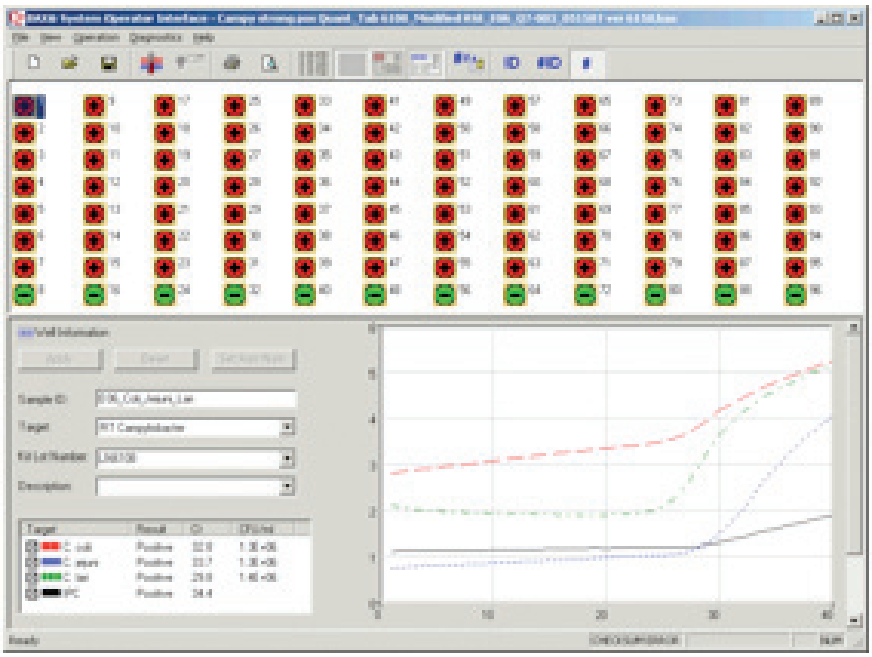


Figure 1: Results display

As with all BAX® assays, the reagents required for PCR are supplied as a single tablet inside the PCR tubes, reducing the number of pipetting steps required and simplifying the process. Results are interpreted automatically and displayed ready for reporting. This includes a positive or negative result for each of the three *Campylobacter* species, a table showing cfu/ml values for each target and a graph showing the amplification curves (figure 1). These results are easily stored, printed or retrieved as required.

The BAX® System Real-Time PCR assay for *Campylobacter jejuni/coli/lari* has been validated by the AOAC Research Institute for use on chicken carcass rinses and ready-to-eat turkey products. These studies have shown that the BAX® System assay performs as well as or better than traditional cultural methods and with a quicker time to result. Two protocols exist: highly contaminated samples can be processed directly and will give a quantified result in only 2½ hours. Samples with lower bacterial loads (e.g ready-to-eat turkey) will require enrichment, with results available in 48 hours or less.

For more information on the BAX® Campylobacter assay, please speak to your local Oxoid representative, visit www.oxoid.com or use the reply paid card at the end of this edition.

Salmonella In Ready-To-Eat Foods - a thing of the past or a re-emerging problem?

***Salmonella* species have been recognised human pathogens for well over 100 years and since the first laboratory-confirmed outbreak in the late 1880's, great strides have been made in identifying sources of the organism, routes of contamination, factors affecting growth and survival and measures to control the hazard. However, the question remains; "Are we in any better shape with regard to control of *Salmonella* in foods now than we were 100 years ago? After all, in that same time period Man has gone from developing the first internal combustion engine to landing men on the moon and contemplating a trip to Mars!"**

Reading the headlines in recent months, you would be hard pushed to think we were on top of this most publicly recognised cause of infectious foodborne disease; *Salmonella* in chocolate, salad vegetables (raw & prepared) and houmous are just a few very recent examples that demonstrate the continued challenge we all face in striving to keep the organism out of foods.

There is no doubt, however, that major strides have been made in the control of the organism, particularly in raw foods such as eggs and poultry, through significant interventions by the primary manufacturing sector. This reduction in animal carriage, reflected in reduced incidence in raw eggs and chicken, has in turn, led to a significantly reduced incidence of salmonellosis in humans since the late 1980s.

In addition, despite high profile reports of the incidence of *Salmonella* in ready-to-eat foods and occasional high profile out breaks, there is no doubt that controls applied to processed food are also at their most advanced with regard to this pathogen than they have ever been.

The adoption of food safety management control through the use of hazard analysis techniques, such as HACCP, has provided a useful framework for control of this and other hazards. However, I do have a concern about the propensity to complicate approaches to food safety management with increasingly complicated principles (and associated acronyms) being developed in an attempt to theorise simple principles. The simple fact is that control of *Salmonella*, like any other micro-organism, comes from understanding the sources of the organism and preventing these sources from contaminating the foodstuff or applying a decontamination step to the foodstuff capable of eliminating the organism.

Despite this, salmonellosis remains a significant cause of foodborne disease with nearly 15, 000 of confirmed cases in England & Wales and, if due account is taken for underreporting, an estimated disease burden of nearly 50, 000 cases per year. Of the 146 microbiological incidents managed by the UK Food Standards Agency in 2006, 43 related to contamination of food with *Salmonella,* and it remains the most common cause of identified outbreaks of infectious foodborne disease in the UK.

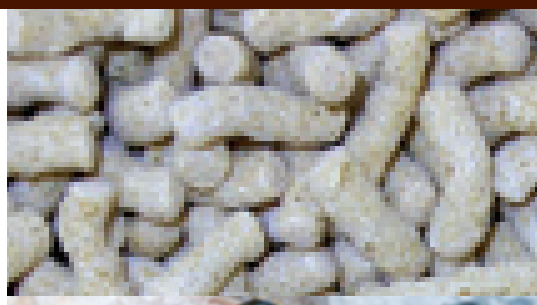
Ensuring product quality at BioMar

BioMar is an international organisation that specialises in the development and manufacture of fish feed products for farmed fish. With factories in Norway, Chile, Scotland, Denmark, Greece and France, they are one of the top three suppliers of fish feed to the aquaculture industry around the world.

We spoke to laboratory engineer, Gudrun Aksdal, who is based at the BioMar factory in Karmøy, on the south-west coast of Norway. The main function of the laboratory at Karmøy is quality control testing of the fish feed products, involving chemical, physical, biochemical and microbiological analyses. They have been using the BAX® System for about one year to test finished product and environmental samples for the presence of *Salmonella*.

"We are obliged to follow government regulations, and so we must be able to demonstrate that our products are free from *Salmonella* and other pathogens," Gudrun explains. "It is important for us to do this before products leave the factory, since they may be used very quickly. BAX® helps us to do this quickly and reliably."

Although they were very happy with their previous, rapid culture method, they have found that the BAX® System has given them a new level of sensitivity. Gudrun continues, "PCR is a very good technique with excellent sensitivity. This gives us greater confidence in the result - we know that the results are accurate, and we can release products safely in that knowledge. Also, with next day results, we are able to release products to the fish farms as soon



Alec Kyriakides

Alec Kyriakides is Head of Product Quality, Safety & Supplier Performance at J Sainsbury plc. He is a member of the UK Advisory Committee on the Microbiological Safety of Food and is co-author of books on foodborne pathogens and their control in food.

There has been much debate about the clinical significance of finding *Salmonella* in a ready-to-eat food and whether you can consider there to be an 'acceptable' level of the organism. Clearly, using modelling techniques, it is possible to mathematically predict the likelihood of suffering an infection following the consumption of *Salmonella* at different levels of exposure. However, this is where the theorist and the realist differ - in theory it would be possible to predict an incidence in food that would not cause a significant risk of foodborne disease. However, in reality, it should be apparent to any practicing microbiologist that finding *Salmonella* in a ready-to-eat food at any level of incidence is never an acceptable situation and should always be considered unacceptable and potentially hazardous. How things have changed over the years - in the 1970's, levels of *Salmonella* considered to represent a foodborne disease risk were in the hundreds of thousands, and it is only comparatively recently that very low levels have been considered to present a risk of infection, especially in foods that provide opportunity for survival of the organism while in the food and while travelling through the stomach after consumption e.g. chocolate and high fat foods.

Analytical methods are an extremely important part in the management of *Salmonella* in foods. The ability to reliably detect the organism, often at very low levels in extremely harsh food or environmental matrices and where the organism may be stressed and surrounded by high levels of competing organisms is something that calls for properly validated, robust methods. Of course, to be useful to the majority of the industry, the methods need to be simple to use and cost effective. This is why most analysts use conventional methods involving enrichment and isolation on selective agars. However, there have been significant advances in alternative techniques to both improve automation and speed of detection of *Salmonella*. While we must never rely on end product testing as a food safety control, it goes without saying that, the faster we get a result, the faster we can identify any associated implication of the presence of a contaminating organism and the more timely our risk management and communication.

The challenges facing us in the management of *Salmonella* are unlikely to become any easier in the future with significant shift in demographics towards greater consumption of food out of the home, further globalisation of the food supply chain and a dramatic increase in the proportion of elderly in the population, especially in the UK and other European countries.

We all must rise to the challenge presented by *Salmonella* and other foodborne pathogens - everyone involved in food safety management whether it is in a factory, a laboratory, in research, in government or in enforcement, has a major role to play in making sure we keep this organism under control.

Front row, left to right: Gudrun G. Aksdal, Randi-Marie H. Næsheim. Back row, from left to right: Anders Øverby, Hanne Jorun Olsen, Mette Håkonsen

as possible. This is extremely important, as we have a limited area in which to store product while waiting for a result.

When deciding which PCR system to purchase, we selected the BAX® System for several reasons: we have had very good service from Oxoid over many years and they have been very helpful in answering questions I have had; the system is extremely easy to use; and we had excellent training as the system was implemented. During our first year of running the BAX® System we have had very good support."

In addition to ensuring that finished products are *Salmonella*-free, Gudrun and her team also use the BAX® System to monitor hygiene standards within the factory, processing a wide range of environmental samples. "BAX® also helps us to ensure that our strict hygiene standards are being maintained throughout the production facilities," she adds. "If there is a potential problem, it calls for many more analyses and rapid answers. BAX® enables us to cope easily with any increases in workload and gives us the quick results that we require so that the necessary action can be taken in a timely manner."

Food safety is a high priority for the BioMar group. "The BAX® System plays an important role in ensuring the microbiological safety of our products," Gudrun concludes. "We hope to expand its use within our laboratory and are currently looking at the Yeast and Mould assay."

For more information on the BAX® Salmonella, please speak to your local Oxoid representative, visit www.oxoid.com or use the reply paid card at the end of this edition.

Yeasts and Moulds

Preventing premature food spoilage with the BAX® System

A costly business

Yeasts and moulds are common causes of food spoilage. They are abundant in the environment, and any food left exposed is susceptible to colonisation. Contamination is often not detected until the product is on the shelf or in the hands of the consumer. Clearly visible colonies or discolouration appearing on the surface of the food, unacceptable changes to the taste or smell of a food product, or bloated packaging due to the production of gas can cause consumers to lose confidence in the brand.

Premature food spoilage, i.e. while it is still within shelf-life, can be very costly to food manufacturers. It can lead to the rejection and disposal of batches of final product. It may also require investigations within the manufacturing facility and/or raw material suppliers to locate the source of contamination; factory downtime while the problem is being resolved must also be considered. If spoiled product reaches the marketplace, the implications can be enormous. Tainted aroma or flavour can have a negative effect on the brand's image and therefore future sales. It can also damage perceptions of the retail outlet where the product was purchased, which in turn can be detrimental to business partnerships.

Some food types are more prone to yeast and mould spoilage than others. Therefore, it is important that such food manufacturers take every precaution to minimise opportunities for yeast and mould contamination within their processes. This is frequently achieved by monitoring levels in raw materials and the manufacturing environment as well as end product testing. If levels start to rise above pre-defined action or alert levels, then the manufacturer can take action before any contaminated product reaches the marketplace.

Why are Yeasts and Moulds such a problem?

There are several reasons why yeasts and moulds can present a problem to food manufacturers. One of these is their ability to tolerate and grow in extreme conditions, for example:

- **Extremes of pH**
  Some yeasts and moulds are able to survive very acidic conditions, such as can be found in fruit juices and pulps.

- **Extremes of temperature**
  Yeasts are able to grow at temperatures ranging from 0 to 47ºC. The ability of some species to grow at low temperatures and low pH make them a particular problem for fermented milk products.

- **Low water activity**
  Many species are xerotolerant, allowing them to grow in environments with very low water activity, such as dried fruits, nuts, grains and spices. Other species are able to grow in environments with high osmotic pressure due to the presence of sugar (osmophilic species) or salt (halophilic species), making them a particular problem for bakery products and dried/cured meats.

- **Bactericidal treatments**
  Some species are able to withstand treatments used to minimise bacterial contamination, such as irradiation, high hydrostatic pressure or organic acid treatment. Furthermore, with the competition from normal bacterial flora reduced, yeasts and moulds are able to flourish.

Contamination may occur at any stage of the manufacturing process, and so it is important to be aware of possible routes and to minimise the risk of contamination wherever possible. Potential sources of yeast and mould contamination include:

- **Air**
  Yeasts and spores may be released into the air from soil, dust, drains, surfaces, raw materials and ventilation ducts. This last source of contamination is of particular concern to aseptic filling plants.

- **Water**
  Yeast and mould contamination may be a problem in badly maintained factory water systems.

- **Raw materials**
  Raw materials should be sourced from reliable suppliers and checked for yeast and mould contamination, especially if they are being used in the manufacture of numerous, larger batches (for example the addition of rennet during cheese manufacture, fruit pulps to yoghurts or fillings/coatings to pastries and cakes).

- **Equipment**
  Inadequate cleaning or sanitisation of processing equipment may result in contamination problems. Particular attention should be paid to parts of equipment that are difficult to clean, such as the proportioning pumps, hose connections and valves used in fruit juice plants.

- **Packaging**
  Cardboard may contain high levels of yeasts and moulds, which could cause contamination problems during packaging or storage. In addition, static (caused by the unrolling or moulding of certain packaging materials) may attract dust, which could contain yeasts and/or moulds. Decontamination of packaging is usually achieved using heat, UV irradiation, hydrogen peroxide or gamma irradiation and is an integral part of the food manufacturing process.

Enumeration of Yeasts and Moulds in Foods

Food manufacturers can obtain an indication of the levels of yeasts and moulds in their products, whether or not they are likely to cause premature spoilage, by counting the number of viable micro-organisms (or colony forming units) in samples of product. Traditionally, this has been performed using culture based methods, such as those described by the FDA Bacteriological Analytical Manual[1] (BAM method) or ISO/DIS21527 (parts 1 & 2)[2]. Plates are incubated for up to 5 days before the number of colony forming units in a sample can be counted. These counts are compared to local alert and action levels to determine if they fall within acceptable limits.

When test results for food pathogens might be available same day or next day, a 5-day wait for yeast and moulds results can be problematic for some food companies, particularly those involved in the manufacture of fresh products with short shelf-lives. Rapid methods that can give reliable results in a shorter time are therefore of great appeal to such companies.

The new BAX® System Yeast and Mould Assay offers considerable time-savings compared to current culture techniques, providing same day yeast and mould results for samples containing >500cfu/g (direct method), or 2 day results for samples with 10-500cfu/g (enriched method).

Like all BAX® System assays, the Yeast and Mould Assay is extremely easy to perform and requires little hands-on time. Homogenised sample is simply added to the disrupter tube. This is then incubated for 44 hours (enriched method), or processed immediately (direct method). DNA stabiliser is added and the tubes are agitated for 15 minutes prior to lysis and processing in the BAX® System cycler. After just 4 hours, the system displays positive (above threshold) or negative (below threshold) results. The target threshold is set according to the action levels of each individual laboratory, validated against historical plate counts for specific food products. The system detects both yeasts and moulds (including spores), ensuring accurate and reliable results. It has been tested on a variety of foods, including cheese, butter, flour, starch, syrup and nutritional supplements as well as many other products.

Internal validation studies on enriched samples have shown that the BAX® System gives comparable results to traditional culture methods[3], and the method has been submitted to the AOAC Research institute for certification.

The BAX® System is the first and only automated PCR system to offer both food safety and food quality testing on the same platform, allowing food manufacturers to be confident in both the quality and the safety of their products as they leave the factory premises.

For more information on the BAX® Y+M assay, please speak to your local Oxoid representative, visit www.oxoid.com or use the reply paid card at the end of this edition.

References: 1.Tournas, V., Stack, M.E., Mislivec, P.B. *et al.* (2001) Bacteriological Analytical Manual Online (Chapter 18). FDA Centre for Food Safety and Applied Nutrition. http://www.cfsan.fda.gov/~ebam/bam-18.html. 2. Draft ISO 21527 parts 1 & 2: Horizontal method for the enumeration of yeasts and moulds - colony count technique. 3.Data on file and available from Oxoid.

Faster results mean faster release of products for Chr. Hansen

Chr. Hansen is an international organisation providing natural ingredients, such as cultures, enzymes, colours and flavours, to the food, pharmaceutical, nutritional and agricultural industries. They are the leading manufacturer of enzymes and dairy cultures used in the production of high quality fermented milk and cheese products, supplying some of the largest dairies in the world.

As a result, quality and safety are prime considerations for Chr. Hansen, who emphasise this with a statement on their website (www.chr-hansen.com):

"Food safety is a cornerstone of Chr. Hansen. If we make a mistake, it influences the rest of the food chain, as production is based on our ingredients."

Hanne Benn Thomsen
Director Global Regulations Environment and Quality

In the laboratory at the Chr. Hansen A/S site at Graasten, Denmark, they have used the BAX® System since October 2006 to analyse rennet used in the manufacture of cheese and to analyse flavourings. Laboratory Manager, Mette Due Grohnheit, comments, "Yeasts and moulds can present a particular problem due to the nature of our products and their use as a raw material by many of our customers. With our previous, culture based method, it took 5 days to obtain results. We needed a system that would give us faster results, and so we looked at BAX®. Now we can measure yeast and mould levels in just 2 days."

Faster results have had positive knock-on effects for the business, as Mette goes on to explain, "It is very important that our customers receive products shortly after they have placed an order. BAX® has reduced our yeast and mould turnaround time from 5 days to only 2 for most of our products. This is really important because it means that we can release products for sale more quickly. It has also reduced the amount we need to hold in stock at any one time. This in turn means we can provide our customers with a better service while reducing our own storage costs.

It has taken some careful planning and modification of sample preparation by Oxoid to adopt this new method, but we are very pleased and have found BAX® to be faster and more sensitive than our previous method. In addition, we have saved time in the laboratory by reducing the need for media preparation and have also reduced the amount of waste, which is good for our environment."

Having experienced the benefits of BAX® in the area of yeast and mould testing, the laboratory has seen the potential of the system and is now expanding its use within the company. Mette concludes, "We also use the BAX® System for *Listeria* and *Salmonella* testing, and now we are working to implement the system in other areas within Chr. Hansen."

For more information on the BAX® Y+M assay, please speak to your local Oxoid representative, visit www.oxoid.com or use the reply paid card at the end of this edition.

CHR HANSEN

BAX® - meeting the demands of today... and tomorrow

In this edition of Setting Standards Update, we have explored the changing face of microbiology. Alec Kyriakides discussed how apparently little has changed over the last 100 years - the same bugs are still contaminating our food and causing us illness. Yet as he explained, things *have* progressed: manufacturers are much more aware of the dangers of food poisoning and the commercial damage they can wreak: advances have been made in the control of processes through concepts such as HACCP; hygiene has improved; sampling regimes have been refined and testing methods have now become state-of-the-art. We have seen molecular methods, such as PCR, are now becoming routine. The BAX® System Q7 is currently providing organisations, such as Biomar and the Danish Veterinary and Food Administration, with a fast, reliable and easy to use solution for food safety testing. In addition to the BAX® System assays mentioned in this issue *(Salmonella, Campylobacter*, yeasts and moulds and *S. aureus),* kits are also available for *Listeria*, *E. coli* O157:H7 and *Enterobacter sakazakii*. With the BAX® System Q7 providing the platform for quick and easy PCR, we can expect to see many other tests utilising novel methods of enrichment, real time PCR and reverse transcriptase, providing us with even more timely and accurate solutions for food safety.

Ian Sheldrake, Food and Industrial Applications Manager, Oxoid.

For more information on new BAX® System assays as they become available, please speak to your local Oxoid representative, visit www.oxoid.com or use the reply paid card at the end of this edition.

BAX® is a trademark or registered trademark of E.I. DuPont de Nemours and Company.

BAX® System assays currently available:

Salmonella

Listeria genus

Listeria monocytogenes

E. coli O157:H7

Enterobacter sakazakii

Campylobacter

Yeast and Mould

BAX® System assays soon to become available:

Listeria genus 8 hour environmental

Please Complete Our Reply Paid Card

If you would like to receive more information on the DuPont Qualicon BAX® System Q7 and/or BAX® System assays, please complete and return this reply paid card.

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BAX® System assays featured in this Setting Standards Update:
Salmonella ☐ *Campylobacter* ☐
Yeast and Mould ☐ *S. aureus* ☐

Other BAX® System assays available:
Listeria monocytogenes ☐ *Listeria* genus ☐
E.coli O157:H7 ☐ *Enterobacter sakazakii* ☐

Please keep me updated on new BAX® System assays as and when they become available ☐

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Hot News:
Staphylococcus aureus Assay Available

A new DuPont Qualicon BAX® System Real-Time PCR assay is now available for the detection of *Staphylococcus aureus* in raw minced (ground) beef and powdered infant formula.

S. aureus are common bacteria often found on human skin, in nasal passages and in the environment. Some strains of *S. aureus* produce toxins that can cause illness when ingested in contaminated food, such as cream-filled bakery products, sandwich fillings and meat and dairy products.

Traditional testing methods for *S. aureus* in food require 3-5 days (or more) for cultural growth and manual enumeration. The BAX® System *S. aureus* assay dramatically reduces testing times as it provides food companies with next-day presence/absence results and can significantly reduce costs associated with product storage, delayed raw material release and slower response times.

For more information about this assay, please speak to your local Oxoid representative, visit www.oxoid.com or use the reply paid card at the end of this edition.

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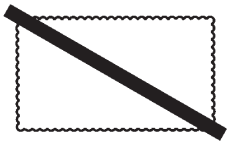

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