Microbial Challenge Testing

How challenge testing can help ensure a food's safety

BY **ANDREA TOLU**

For consumers, the concept of food's shelf life of food is quite simple: follow the storage instructions indicated on the label, and you can be reasonably sure that the product will still be good to eat at least until the expiration date. The work that goes on behind the scenes to make that happen, however, is more complicated.

There are two important measures that food manufacturers must apply to ensure microbiological safety: killing unwanted microorganisms during processing and preventing their growth during shelf life. Under the Food Safety and Modernization Act (FSMA), these measures fall into the category of process preventive controls, which are the only ones requiring written validation of their effectiveness.

How well a kill step or a food formulation will work depends on several variables. For example, the heat resistance of *Salmonella* will vary depending on the strain and the combination of macronutrients, water, salt, and pH. These intrinsic factors, together with the use of preservatives, will also have a direct effect on the growth of microorganisms during shelf life.

The interaction of these variables can make validation challenging: "Every organism has an absolute limit: it won't grow below a certain level of pH, salt content, or water activity. But when these are combined, it can be difficult to know what will happen," says Peter Wareing, a food safety consultant based in the UK. "If the pH is high enough to allow the organism to multiply, but salt content is also high and water activity is low, then those two factors might prevent the organism from growing."

Quite often, food manufacturers can use published literature to validate process preventive controls. For example, the efficacy of milk pasteurization is well established and will require no further evidence; however, for many recent products there is not sufficient data to know whether the combination of kill steps, formulation, and storage conditions will ensure shelf stability. The only way to find out is to adopt an empirical approach and conduct a microbiological challenge test.

In a challenge test, a food sample is inoculated (challenged) with a known microorganism or a surrogate, to observe how an antimicrobial treatment or the composition of the food itself will affect lethality and growth. The two main types are inactivation and growth challenge tests.

Will It Die?

In an inactivation challenge test, a food sample is first inoculated and then put through the intended kill step, such as thermal treatment or high-pressure processing (HPP), and finally analyzed to verify how many cells survived.

When this test is conducted in a lab, says Alvin Lee, PhD, associate professor of food science and nutrition at Illinois

Institute of Technology in Chicago, it's important to closely mimic the conditions the product will go through. "If you're validating the cooking process in the production of soup, for example, you'll have to apply not only the same time and temperature but also the ratios between produce and water," he adds.

Inactivation challenge tests can also be done directly at the manufacturer's plant. "On-site challenge tests allow you to use the actual processing equipment and take into account all of the unknowable variables," says Rob Limburn, group manager of industrial process microbiology at Campden BRI, a food and beverage research organization based in the UK. "In this case, we wouldn't use an actual pathogen, but a non-pathogenic surrogate with similar inactivation characteristics." Typical examples of surrogates, says Limburn, include *Enterococcus faecium* or *Pediococcus* spp for *Salmonella*, *Listeria*, or *E. coli* in dry products. In some cases, non-pathogenic strains, such as *Listeria innocua*, may be appropriate.

To make on-site test results more reliable and efficient, says Limburn, it's important to identify the points in a static system, or the path through a continuous processing line, where the product receives the least severe process: "For example, if you have a conveyor belt with several lines of products going into an oven, there's likely going to be a gradient of temperature, depending on where the fans are positioned and other factors. In that case, rather than placing more samples across the belt, the best point will be the coldest channel: If it's effective there, it will be effective anywhere else on the belt."

What Foods to Challenge

Certain types of foods are more likely to require an inactivation challenge test: "With a moist system, like sauces or ready meals, you can measure time and temperature and rely on long-established microbial inactivation kinetics," says Limburn. "But with products with low water activity, like nuts, seeds, snack bars, crisps, spices, or flour, these can be very different. *Salmonella* in particular is a lot more resistant to dry than moist heating. In those cases, microbial challenge testing would be recommended," says Limburn.

Even if a product is a good candidate for an inactivation challenge test, it doesn't necessarily need one: "Many businesses have a large portfolio of products and couldn't really do a challenge test for every single one of them. One way around that is to categorize them and conduct the challenge test on the most protective one out of a particular category. It could be the product containing a high-risk ingredient or with the lowest moisture. This way, you'll know that everything within that category would require a less severe process," says Limburn.

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Even when there is enough data available, however, performing a challenge test might still be a good idea: "No two food products are the same. When fruits are grown at different locations, the hazard or the pH could be slightly different. Even if there's an established process, we always recommend manufacturers do a challenge test, so if anything were to happen, at least they have their own data to back it up," says Dr. Lee.

A category where it wouldn't be necessary are canned and pickled products, where, says Wareing, you normally rely upon the time and temperature and the internal characteristics of the food to make sure they're safe. "If they don't survive throughout the shelf life, then it means there's something seriously wrong with the process," he adds.

Will It Grow?

In a growth challenge test, it's a finished product that gets inoculated. The goal is to find out whether the formulation provides an environment that inhibits the growth of unwanted microorganisms during shelf life. A typical target, says Wareing, are microorganisms that you already know will survive the kill step:

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"For example, if you use pasteurization to inactivate non-spore-forming pathogens like *Salmonella*, *Listeria*, and *E. coli*, you probably won't completely eliminate spore-forming *Clostridium botulinum*. Rather than applying a stronger thermal process, you might want to inoculate the food to see if the control factors, which could be a combination of pH and salt, with the addition of an antimicrobial and chilled storage, will prevent its growth over the shelf life."

Statistical Issues

Quite often, an inactivation challenge study is followed by a shelf-life test: "A typical example where we would do it is with fruit juices," says Dr. Lee. "The FDA requires that after the antimicrobial treatment there is no growth of pathogens throughout the shelf life. So, after inoculating the product and putting it through the process, we evaluate it over the shelf life, for example, 30 days, to see if anything comes back, in particular, sub-lethally injured microorganisms that may recover over time. In fact, we would prolong the period and even incorporate some abuse conditions into it, to better simulate the temperature variations the food product is likely to experience."

The reason for this additional step, says Lee, is to verify the lethality of the process without having to sample everything: "Microbiological testing can only go that far. You only need one cell for the pathogen to multiply and make food unsafe again. But that cell may not be present in the samples you analyze. With shelf-life testing, you're trying to find out whether that one cell is there or not."

The problem of the statistical validity of samples is also the main reason for doing a growth challenge study, as opposed to a shelf-life test without inoculation: "When you look at the statistical analysis of sampling, it's quite complicated," says Wareing. "If the microorganism is present only in certain parts of the batch you want to analyze, and/or at very low counts, you won't find it unless you take enough samples. By inoculating the product, you're making sure that you've got enough of it in there to show up when you take your microbiological samples after the challenge test is over."

Designing the Right Test

The reliability of a challenge test depends largely on how well it's designed. When selecting the target microorganism, a key aspect to consider is risk: "We normally use strains that are found in actual outbreaks," says Dr. Lee. "For example, we're conducting a challenge study right now that involves seafood, where we're targeting *Listeria monocytogenes* strains that were isolated in seafood products."

Once the pathogen is identified, it's always better to try and recreate the worst-case scenario: "Different strains behave in different ways," says Jeff Kornacki, PhD, president and senior technical director of Kornacki Microbiology Solutions, a firm that provides food safety and quality consulting services and is based in Madison, Wisc. "I would choose a serotype that is known to have high heat resistance for that type of food. An even better approach would be to use a cocktail of different strains, so you can cover variability of growth models."

In preparation for a challenge test, says Dr. Kornacki, there are two important checks to do to eliminate confounding factors: "One is to analyze the background microbiota in the food sample that might have the same appearance as the target microorganism on a petri dish. If *Salmonella* is supposed to form a black colony on a certain medium, and I just assume that everything that makes a black colony is *Salmonella*, the final count might be inaccurate."

Another good practice, continues Dr. Kornacki, is to consider the effect of the food sample on the target microorganism: "In an inoculated sample, the population of the microorganism might start to decline as a consequence of its interaction with the food. This, however, might create inaccurate results, because what you're measuring is the lethality of the process, not of the food itself. It's therefore best to wait until the population has stabilized before you put the inoculated sample through the process."

A useful tool for designing challenge tests is predictive microbiology, a computer-

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Logs and D-Values

The lethality of an antimicrobial process is measured in logs, which equal to a ten-fold decrease of the initial population of microorganisms; 1 log reduction means that the number of surviving cells is reduced to 10%, 2 logs to 1%, 3 logs to 0, 1% and so on. The D-value is the time required to reach 1 log reduction at a given temperature.

Typically, each food (or food category) has a target reduction established by regulations or GMPs. For example, the FDA requires 4 logs for *Salmonella* in California almonds; in ready-to-eat foods, the recommendation for *L. monocytogenes* is 5 logs, or even 6, if a higher contamination is expected.

When there's not sufficient data available on the lethality of a process, the goal of an inactivation challenge test can also be to understand the inactivation kinetics of the pathogen and define the time and temperature necessary to achieve the required reduction.

A key metric in this case is Z-value, which is the temperature required to achieve a tenfold reduction of a D-value (for example, to go from 1 log to 10 log reduction for the same hold time): "To calculate the D-value, you take different samples of an inoculated product at different times at a specific temperature," says Limburn. "When you've found it, you can repeat the test at further temperatures to obtain the Z-value. These references will be the building blocks to determine the minimal process you need to achieve a safe product."—*AT*

based system that calculates the effects of a process and food formulation, giving an indication of what microorganism might survive. "The results from a predictive model can help you narrow down the parameters for the study," says Dr. Lee. "For example, if you're trying to identify the right concentration of an antimicrobial, and the predictive modeling says that the ideal concentration is 25 ppm or above, you can limit your sampling range of the growth test to between 20 ppm and 30 ppm."

The result of a predictive microbiology test can also indicate whether it makes sense to challenge the food in the first place: "If the predictive model says that the target microorganism will grow, then there's no point in doing the study, because the result is likely to be the same," says Dr. Kornacki. "However, if the predictive model says that an organism won't grow, then you probably should do the study to make sure that it's true." ■

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Designing for Food Safety *(Continued from p. 22)*

Hygienic equipment design enhances cleanability, decreasing the risk of biological, physical, and chemical contamination. Equipment that is designed and constructed to meet hygienic principles will also be easier to maintain and will reduce the risk of physical hazards contaminating the product. Overall, the operating costs of hygienically designed equipment are usually lower than costs for equipment that has not been designed with the same level of care, and such lower running costs must be considered when comparing the investment costs of different systems.

Hygienic design principles encompass a range of different factors, such as material choice, surface finish, and construction methods, as well as the design of the equipment itself—avoiding lips, crevices, and sharp angles where cleaning chemicals or product may build up or remain after cleaning. To facilitate cleaning underneath and around equipment, it should be elevated above the floor on legs or mounted in a frame, as is the case with skid-mounted systems.

When designing equipment, different standards may be applied to food-contact and non-contact surfaces; surfaces that come into contact with product must generally be smooth, non-toxic, non-absorbent, and resistant to corrosion. For this reason, stainless steel is popular. Welding and joints are also important; continuous butt welds should be used and ground to a smooth surface, while bolts and threads used within the food contact zone must also be of a hygienic design.

It is important to maintain the movement of fluids and materials within equipment and connecting pipework, and this is equally true for products and cleaning solutions. Maintaining flow and preventing fouling is also a key priority in heat exchanger design and is one of the benefits of corrugated tube or scraped surface designs. Closed coupled connections should also be used on equipment to prevent the creation of dead spaces, and to ensure that, where necessary, equipment can be fully drained or emptied for cleaning or product changeover. Other considerations in basic equipment design and construction include avoiding the use of O-ring seals in grooves, avoiding ledges around top rims, and ensuring that shafts are sealed with double seals where necessary.

Reducing Waste while Maintaining Safety

There is a wide range of heat exchange and ancillary equipment for use in the food and beverage sectors, from basic tubular heat exchangers to fully integrated pasteurization/sterilization and aseptic filler systems, as well as a number of specialist products such as evaporators, ice crushers and melters, direct steam injection systems, air removal systems, and pumps. All of these items, and others, must be hygienically designed from the start to facilitate clean operation and prevent the types of product contamination discussed above, while meeting 3A Sanitary Standards for optimum design.

Some equipment has been specifically designed to facilitate product removal and subsequent cleaning. It has always been a challenge for food and drink businesses to implement effective and rigorous clean-in-place (CIP) regimens that meet the necessary standards in a way that minimizes the loss or degradation of saleable or useful product, but some recent designs of rotating scraped surface heat exchangers can physically remove product without the need for traditional pigging or flushing systems.

Such heat exchangers are suitable for a range of heat transfer applications, and their design enables high-viscosity products to be pumped with reduced back pressure and lower energy use. Some products use a helical spiral fitted with scrapers, which scrapes the surface of the tubes to prevent fouling in normal use, and such designs can also be run in reverse; enabling valuable product to be recovered prior to routine cleaning or product changeover. This design feature means that much of the product can be removed from the heat exchanger without the need for additional pumps or pressure systems, reducing both capital expenditure and operating expense.

Automated product recovery systems, a further development of this type of technology, combine the continual monitoring of a set parameter (Brix, pH, or viscosity) with three-way valve technology. These systems ensure that all product meeting the pre-set parameters is utilized, while only that which falls outside specification (such as product that has been diluted during CIP) is discarded. Such monitoring also helps to validate the effectiveness of CIP and ensures that, following a cleaning cycle, only product that meets the required specification is allowed to proceed.

The hygienic design and construction of food processing equipment is an essential but often overlooked aspect of controlling the safety and quality of food and drink products, playing a crucial role in preventing contamination and allowing other food safety procedures to be carried out. ■

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