An interview with Daniel Y.C. Fung, Ph.D.

Fung's Forecast on Rapid & Automated Methods: **Where Are We Now?**

As Food Microbiology Division Lecturer of the American Society of Microbiology (ASM) at the organization's national meeting in Washington, D.C. in 1995, Daniel Y.C. Fung, Ph.D. was asked to give a lecture reviewing rapid methods and automation in microbiology and provide predictions of the future of this important field in food and beverage analysis. Nearly five years later, *Food Safety* Magazine published an update of those predictions to see whether the noted food microbiologist's forecasts had achieved "real-world use" status. In that 1999 article, "Predictions of the Future of Rapid Methods in Microbiology," Dr. Fung wrote, "Predicting the future is risky business at best. Several of the predictions I made in 1995 actually became reality. Hopefully, a few of the 1999 predictions I've forecast here will become a reality in the next decade." By his next update for this publication in 2002, most of Dr. Fung's prognostications were indeed realities–before the decade even hit the midway mark.

In this 2004 *Food Safety* Magazine interview, Dr. Fung once again takes out his crystal ball (the scientific one, of course!) and analyzes his earlier predictions to see what has transpired and what has yet to transpire in the field.

Food Safety Magazine: Have rapid and automated microbiological methods expanded capabilities in the food industry?

Daniel Y.C. Fung: First, the demand for microbiological testing worldwide is increasing and encompasses many product categories, from food and beverage, to pharmaceutical and environmental, to personal care and industrial processing (see box, p. 00). In 2003, according to new market research published in Strategic Consulting Inc.'s *Industrial Microbiology Market Review, 2nd Edition,* the volume of microbiological tests was estimated to be a worldwide market of 1,136.5 billion tests conducted annually, of which 558.1 million were run in the food industry. Of the six identified industrial sectors, the food industry is the largest market for microbiological tests, representing 49% of the total volume of testing performed, followed by the pharmaceutical (206.9 million), personal care product (194.3 million), beverage (102.4 million), environmental (44.8 million) and industrial processing (30 million) sectors. An analysis of the market trend from 1993-2003 shows that the volume of microbiological testing has experienced steady growth in all of these segments and the report predicts that worldwide usage of rapid microbiological testing methods will double by 2008.

I think this market growth is an indicator that we are making progress in developing better, more usable rapid

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microbiological detection and analytical methods and technologies that can provide the speed to result that many food companies require. So, these advances really have expanded the food industry's capabilities and as I've said in the past, the reasons that food manufacturers test products are well established. These include finding out whether the raw materials coming into your processing plant are of good quality or not, and whether ingredients and raw materials meet your company's specifications for microbiological integrity and other requirements. If suppliers don't meet the specs, they will lose the business. For example, a ground beef processor supplying to fast food operation typically will have a microbial specification of 100,000 organisms and less per gram of ground beef. If you have more than 100,000 organisms per gram, the beef is not very good and will be rejected. The faster the test, the more quickly unacceptable ingredient can be prevented from entering the plant in the first place and the higher the assurance the company has in protecting itself and the health of consumers.

Of course, the next area in which the food manufacturer needs to conduct testing is in the factory itself during the processing. There are different critical points in each manufacturer's process where you want to know if you have an unusual number of organisms in the product or not, and whether the plant environment is clean and sanitary. Even with the best Hazard Analysis and Critical Control Points (HACCP) program, companies are still testing the end product before they release it, as well. Rapid and automated microbiology have really allowed food manufacturers to expand their capabilities to streamline food safety and quality assurance.

With all of these tests, the faster the better, the simpler the better. Overall, my prediction is that rapid methods will enable companies to test more and more classes of foods. We all know that the use of these methods is well established in meat, poultry, dairy and seafood but many people don't realize that a mere 10 years ago we weren't concentrating on fruits and vegetables. In the beginning, everyone thought produce was clean until we witnessed *E. coli*, *Salmonella* and other pathogen-associated foodborne illness outbreaks sourced to produce. So this is a newer area of concern and the demand for testing in this class of food will increase. Another interesting trend is the increase in consumer demand for imported ethnic foods, such as those you find in ethnic grocery stores. People expect to know exactly what is in the food they consume and these ethnic foods are not being tested regularly.

The Strategic Consulting market report I've quoted above also states that roughly 20% of all microbiology tests performed in the food industry are for pathogenic organisms such as *Salmonella* and *Escherichia coli* O157:H7, with the remaining 80% of tests falling into the routine testing category, which includes total count, coliform count and yeast and mold. I predict that in the next five years we will see the percentage of pathogen testing increase by 10%, with a corresponding 10% decrease in the percentage of routine testing as new standards, regulations and or pathogens of concern to the food industry emerge.

Similarly, I also think that the balance of where tests are done worldwide is going to shift. Currently, the total amount of tests conducted throughout the world is split at about 33% each between North America, Europe and the rest of the world. But I think that in the next 10 years, those percentages will shift to 25% North America, 25% Europe and 50% rest of the world. This is because other countries are becoming more conscientious of food safety. In China, for example, the economy is growing and with an improvement in wealth there often follows an improvement in health. Essentially, people are able to and like to eat better food. As such, many countries are looking into safer and healthier foods, so I predict that the food testing market will grow substantially in the rest of the world.

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Food Safety Magazine: Will you review your 10 predictions from 2002 and talk about whether you think these forecasts on food microbiology advances in rapid and automated methods have been realized?

Fung: It has been nearly a decade since I made the first set of predictions on advances in the development of rapid and automated microbiology methods, and again, many of these predictions have now been realized. There are exciting new developments in the field that are enabling food microbiologists to get more sensitive, more accurate and very fast results, which in turn helps food manufacturers more quickly release their products to market with a high level of assurance that the products they ship are safe and of good quality.

I think the 10 points made in my predictions are still valid but they have been refined. We have witnessed incremental advances since my 2002 update, and we are doing quite well overall in increasing speed, sensitivity and specificity of microbiological diagnostic test results.

Prediction 1. Viable Cell Counts Will Still Be Used. I would like to add to this predictive statement "and more efficiently," because there have been many developments in instrumentation that are allowing more efficient ways to obtain viable cell count. Again, the ability to tell whether cells are alive or dead in a sample is important because live cells, which can be harmful to the health of those consuming them, will grow into millions of cells in just a few hours. The viable cell count enables us to identify the spoilage potential of a food and, of course, the potential of pathogens to grow in large numbers that will make us sick. As such, viable cell count–total aerobic count, anaerobic count, differential count, and pathogenic count–is a very important parameter used to assess the safety and hygienic quality of food and beverage products. During the last few years, improvements in alternative methods, including rapid culture methods, spiral plating systems and other innovations in more efficient dilution instruments and sample preparation instruments, have made viable cell count procedures more efficient and rapid.

Some of the specific advances I've predicted in this area are closer to reality, including the improvement of vital stains using very specific dyes that differentiate living versus non-living cells in less than one hour and the development of handheld units in which a film is produced from the reaction of a sample and vital stain and automatically scanned to provide a viable cell count. A very interesting new development relates to the automation of the conventional most probable number (MPN) technique, which is difficult to run and very time-consuming. When yours truly developed a miniaturized MPN system 30 years ago, it improved on the drawbacks of the method but still had to be performed manually. Recently, the *Journal of Rapid Methods & Automation in Microbiology* published a paper detailing how my system is being automated using instruments and sophisticated statistics to increase the efficiency of the MPN method.

Something new in this area is work involving developing technology that can split a cell into many, many particles, which can be used instead of single-organism counting methods. For example, you break up one *E. coli* into several thousand pieces through sonification or some other method, and then attach to each particle some signal that can be amplified so that eventually you can detect one *E. coli* in a liquid. The cell particles react with a special compound, enabling the microbiologist to see and count all the organisms. This is actually being done: You can detect one cell. And, if you can detect one cell, imagine a scenario where you can bombard that cell and pick it up–now you've got some markers that show the cell is alive *and* markers that show the cell is pathogenic. With these multiple markers of that one cell's breakup products you can identify both the pathogenic bacteria and their numbers quickly. Thus, I would refine my original prediction to include a new prediction that the industry will see advances in rapid microbiology technology that will allow us to detect organisms and get a viable cell count at the same time. I know one company right now that has technology that can detect one *E. coli* per mL in a liquid in about 10 minutes. This is almost like science fiction, but it is being done right now.

Another of my predictions related to viable cell counts was the development of technologies for early sensing of viable colonies on agar. I stated that I believed

> *"Given the* **advances** *in microarray technology, we soon should be able to* **detect** *100 pathogens on a single microchip."*

that in the future it would be possible to use heat-sensing or colony-sensing devices to detect the development of colonies on the agar without having to "see" the colonies with one's eyes. This development would shorten the procedure to about three to four hours instead of the traditional 48 hours to count a visible colony. Well, I just saw something in Boston that really is like science fiction. A person there showed me a series of agar plates showing from one, individual cell on a plate all the way to an actual colony (which could be seen with the naked eye) by a procedure involving an antibody reaction and the amplification of specific dyes. The ability to identify one *E. coli* O157:H7 on the surface of an agar is really amazing. This type of development, which shows our ability to see cells much sooner, supports my earlier predictions.

Prediction 2. Real-Time Monitoring of Hygiene Will Be in Place. Of course, ATP bioluminescence diagnostic tests are firmly entrenched in food company hygiene monitoring and sanitation programs worldwide–and with good cause. These systems are truly rapid methods, offering food companies real-time screening results about the cleanliness of processing equipment and facilities and promoting good cleaning practices in food plants that reduce microbial contamination. As I stated in 2002, the use of catalase enzyme, surface contact plates, paddle methods, swabs, the Petrifilm contact method, impedance instrumentation and optical method automated systems all have significantly helped food manufacturers obtain very rapid indicators of hygiene.

Recently, there has been a more concerted movement to improve residue testing in terms of hygiene monitoring applications. Our ability to measure the presence of protein, fat, carbohydrate, and so on, has improved since my 2002 prediction. In particular, protein tests have moved beyond the first generation stage and today allow the user to detect protein residues in five seconds. These are instant detection tests are very simple–you take a swab, activate it, swab the target surface, and if it changes to a specified color, you know that too much protein is present, which indicates that microbes are present in unacceptable numbers.

Prediction 3. Polymerase Chain Reaction (PCR), Ribotyping, and Genetic Tests Will Become Reality in Food Laboratories. This was a bold prediction in 1995, a not-so-bold one in 1999 and a given in 2004. Today, many food companies and the federal government are using PCR to detect pathogens because the method offers the ability to detect with a high degree of specificity and rapidity several pathogens of interest. More and more food industry labs, including smaller ones, are incorporating genetic-based rapid methods and testing because the newer systems are user friendly. For example, at one laboratory I know they are using an automated instrument to perform gene sequencing to confirm *Salmonella*. The machine can do it very fast and its sophisticated automation eliminates many of the time-consuming aspects of past genetic testing protocols.

While PCR, ribotyping and other genetic-based tests have become a reality in many laboratories, their application will be even more useful if these systems can do more to determine the virulence factor. This means that the microbiologist not only can detect the organism but also can determine whether the organism possesses a pathogenic gene. This exciting advance is now being made and it will be quite useful to know that gene. We hear about the mapping of the human genome, but there also are many bacteria that have been completely genetically mapped, as well. We now have very detailed information that we can study to discover why *L. monocytogenes* or *E. coli* O157:H7 is pathogenic, which enhances our ability to develop better methods to control or eliminate that harmful pathogenicity in food. Molecular biology is here to stay and will flourish.

Prediction 4. Enzyme-Linked Immunosorbent Assay (ELISA) and Immunological Tests Will Be Completely Automated and Widely Used. ELISA is well established and highly trusted as a microbial detection technique in food testing applications. In 2002, I noted that test kit manufacturers were focusing on improving lateral flow test systems to increase their sensitivity. Since then, the systems have indeed been refined to the point that they can detect 10^5 or even 10^4 organisms and some near the 8-hour total test time mark.

Although rapid ELISA tests are indeed widely used in the food industry because of their rapidity (some provide results in 8 hours), as stated in my prediction, these immunological tests are not yet completely automated. However, automation of ELISA tests is improving for the benefit of the food industry. For example, there have been advances by some companies that offer automated ELISA systems who have begun to develop interesting new media designed for use with their systems that speed the enrichment process.

At Kansas State, we just recently completed a work studying a combination method using immunomagnetic separation and ELISA technology that can detect *E. coli* O157:H7 in 5.25 hours. In this automated system, immunomagnetic capture is combined with ELISA. Following growth of the organisms, immunomagnetic beads are placed with the antibody against the organisms and the entire 250 mL sample is circulated through the machine many times. Each time and every time it comes back to the magnetic area, organisms are captured. In approximately 30 minutes nearly all of the organisms are captured and the beads are released. The beads and bacteria go through an ELISA test. For example, a sample of ground beef is placed in the broth and incubated for 4.5 hours, circulated for 30 minutes and then undergoes the ELISA test where the reaction takes place. If you see a positive, you will confirm, and if negative, the food is safe. The speed to result of 5.25 hours is the fastest possible right now. I am trying to reduce this time even more by growing the pathogen in oxyrase and shortening the incubation to 3 hours. Perhaps we can cycle the sample faster and capture more organisms by doing this. I am absolutely sure that this type of combination technology will be able to provide results in four hours. That's what I predict and it is an exciting thought.

Prediction 5. Dipstick Technology Will Provide Rapid Answers. Many forms of dipsticks have been available for rapid screening of pathogens and today are widely used in the food industry. While they provide very fast microbial detection and screening results, I would add to this prediction that we will see advances in increasing the tool's sensitivity. By this I mean that dipstick technology will be improved in the future so that it detects not only the antigens but toxins, as well. We must realize that dipstick technology is used after preenrichment, and the methods can provide results in 10 minutes but you need to have the culture enriched to about 10^6 organisms before it will provide a sensitive reaction.

Prediction 6. Biosensors Will Be in Place in HACCP Programs. In 2002, I said that we are a few steps away from the ability to place biosensors in food processing systems for the instantaneous detection of pathogens in the food matrix. And we are still a few steps away. Again, there is no lack of sensors available, particularly if one considers monitoring of ATP, catalase, enzymes, pH, oxygen gas, hydrogen gas, other metabolic products, conductance, impedance, capacitance, microcalorimetry, and so on, as "biosensors" for microbial activities. These methods can be used successfully now, but they will not enable the user to detect specific pathogens in "real time," and thus are not fully efficient for use in the HACCP program.

The problem with biosensors is that is very difficult to capture one target pathogen in 25 grams and get it to interact with the biosensor. The biosensor itself is fantastic but if there is any interference the signal will not be accurate. We must have good separation technology in order to utilize biosensors to their fullest potential in terms of food testing.

Prediction 7. Instant Detection of Target Pathogens Will Be Possible By Computer-Generated Matrix in Response to Particular Characteristics of Pathogens. In 1995, when I first made this prediction, I didn't refer to the exact method by which this instant detection would be accomplished. Now, I know that this prediction is really referring to technological advances in microchips or microarray biochips. With these chips (one million dots on one small slide), we can study different pathogenic genes and their characteristics. We now can instantaneously flood a pure culture or preparation and very quickly get a reaction with the target organisms. Microarray technology allows you to test multiple pathogens simultaneously. Today, we can test 10 pathogens and analyze each pathogen for 10 specific genes. Given the advances in microarray technology, we soon should be able to detect 100 pathogens on one chip. With microchips that are in development right now we can detect *Salmonella*, *E. coli* and *Campylobacter* genes. The organisms will hybridize and by entering specific dyes, the organisms easily stand out.

An exciting aspect of this is that such technology is moving us in the direction where it is possible to have one test that can show us all the positives for pathogens and all the viable cell counts at once. I predict that in the future there will be microchip systems that will capture all of the important foodborne pathogens known at that point and allow for viable cell count so that the food manufacturer will be able to release product more efficiently. In many cases, product is held until all test results are in, and that means staggering the release because frequently it takes five days for yeast and mold and only two days for total count, but you cannot release the product you have the results for both. With a microchip, there is a real possibility of multiplexing, or having all pathogens and bioburden on the same platform, so that we could have a single test for coliform, total count, yeast and mold, and pathogens of interest.

Prediction 8. Effective Separation, Concentration of Target Cells Will Greatly Assist in Rapid Identification. This is the bottleneck of food microbiology right now because we do not know how to separate and concentrate target cells efficiently. We always need to enrich the organisms. Separation technology is an area of great interest for development, including immunomagnetic, filtration, centrification and electrostatic attraction, because it can save at least one day in the enrichment procedure of many pathogen detection protocols such as PCR, ELISA, gene probes and other methods. We need to identify better ways to concentrate the cell and the target organisms so that it is possible to use new technologies like microarray to analyze the sample. For example, the problem with microarray is that one meat particle will ruin the whole system. You can not have any interference particles when you analyze microarray samples. Developments that will advance separation technology is exceedingly important.

Prediction 9. A Rapid Microbiological Alert System Will Be in Food Packages. Advances are moving us closer and closer to making this prediction come true. As I stated in the updated predictions for 2002, we know that microbial cells during growth and spoilage will generate a variety of compounds that can be detected by various devices. There now are sensors in food packages that can sense CO_2, pH, ammonia, and other compounds. There is technology in which antibodies from certain pathogens are placed on the packaging film and if the pathogen is present, it reacts with this antibody and further reacts with another antibody inside a unit that automatically makes a sandwich ELISA test on the packaging film.

I also know companies that have been continuing to develop the barcode color

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change type of system that I've talked about in previous updates, in which a series of reagents is embedded in a unit within the food package and when enough of a certain gas (e.g., ammonia, hydrogen sulfide, CO_2, or pH changes) is generated, a color change will occur similar to temperature-sensitive papers to indicate a potential spoilage problem exists and at what level of spoilage the product has reached by the number of bars that have changed color. I'm convinced that this will be an excellent tool for consumers, who will be able to throw away spoiled food, as long as they are educated about the color change.

Prediction 10. Consumer Will Have Rapid Alert Kits for Pathogens at Home. It remains conceivable that rapid alert kits for food spoilage and even potential food pathogen detectors available for home use will be developed. I know of some individuals who are trying to make CO_2 detection technology into solid state technology so that people can check food packages for themselves at home. As I stated earlier, we would still need a lot of consumer education. I am also hopeful that these at-home microbiological alert systems will do more than just alert the consumer that a food is potentially dangerous. It would be very good if the kit provides information on cooking and storing foods properly so consumers can better protect themselves.

Food Safety Magazine: What are the top pathogens of concern to the food industry?

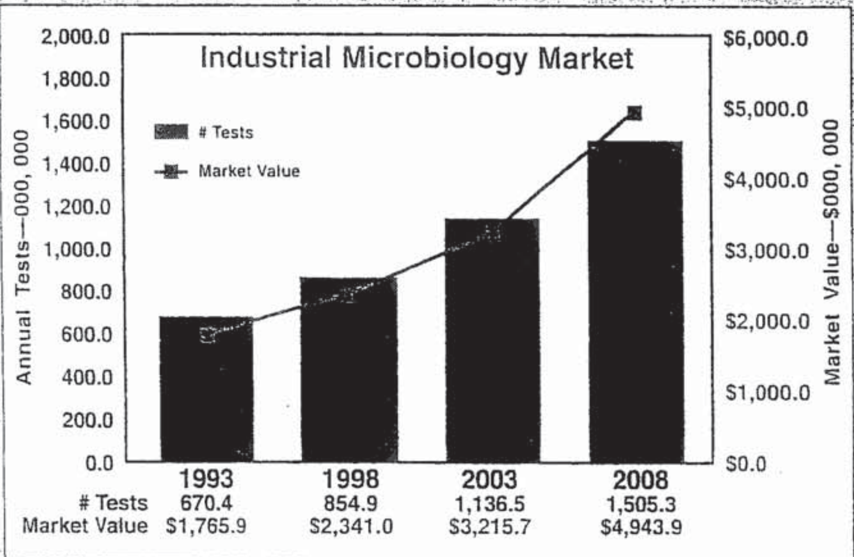
Fung: Two years ago I stated that if I had to give a top five list of microorganisms of concern to the food industry, I'd include *Salmonella, Clostridium, Staphylococcus, E. coli, Listeria monocytogenes*, and I still believe this is true. I also stated that we need to monitor the various strains of all these pathogens because these also have antibiotic-resistant and toxigenic characteristics or properties that are associated with a large number of foodborne illness outbreaks. Again, we should not jump on the bandwagon of a "top five" list; we must monitor all potentially harmful pathogens in foods.

I think that the next wave of microbes of concern will be viruses and protozoa. Viruses are very hard to detect and while we can detect them through molecular biology methods, we cannot do so with accuracy. The next major concern is how to work with viruses; in other words, how to extract them out of the food sample in order to detect them. We also are starting to worry about protozoa in water, fruits and vegetables because of irrigation methods that spread fecal material in the water and on crops. When a foodborne illness outbreak occurs, we often don't know what the source of the problem is at the outset and so we will first look for bacteria, yeast and mold. However, if the source of the outbreak is a virus or protozoa, we have no idea. So I predict that there will be more activity in food virology and food parasitology.

Because of advancements in molecular biology, these techniques are now a useful tool in the food lab and will likely help us to more easily deal with viruses. For example, PCR is useful in food virology because it can amplify DNA and the reverse-transcriptase RNA. In the same vein, we can use food molecular biology to very rapidly detect target microorganisms. At our annual International Rapid Methods Workshop here at Kansas State University, we coined another term several years ago, "Food Molecular Biology Day," dedicating one entire day of the workshop to this topic. The use of food molecular biology will definitely increase in the near future because this field encompasses the identification and detection of the genetic material of all bacteria, yeasts, molds, viruses, parasites and even higher organisms. □

Industrial Microbiology Testing to Approach $5 Billion by 2008

According to a new market report, in 2003 the worldwide industrial microbiology market generated more than 1.1 billion tests and had a market value in excess of $3.2 billion. The report includes a thorough review of the global market along with detailed examinations into its six main sectors—food processing, beverage, pharmaceutical, personal care products, environmental, and industrial process.



Industrial Microbiology Market Review, 2nd Edition: Global Review of Microbiology Testing in the Industrial Market," from Strategic Consulting Inc., projects that worldwide microbiology testing in this area will continue to grow over the next five years and will reach 1.5 billion tests in 2008 with a market value of more than $4.9 billion. *(For more information, visit www.strategic-consult.com or e-mail queries to info@straegic-consult.com).*