SPECIAL GUEST EDITOR SECTION

Predictions for Rapid Methods and Automation in Food Microbiology

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A discussion is presented on the present status of rapid methods and automation in microbiology. Predictions are also presented for development in the following areas: viable cell counts; real-time monitoring of hygiene; polymerase chain reaction, ribotyping, and genetic tests in food laboratories; automated enzyme-linked immunosorbent assay and immunotests; rapid dipstick technology; biosensors for Hazard Analysis Critical Control Point programs; instant detection of target pathogens by computer-generated matrix; effective separation and concentration for rapid identification of target cells; microbiological alert systems in food packages; and rapid alert kits for detecting pathogens at home.

Rapid methods and automation in microbiology are dynamic areas in applied microbiology. They deal with the study of improved methods for isolation, early detection, characterization, and enumeration of microorganisms and their products in clinical, food, industrial, and environmental samples. Major areas of investigation in the field include advances in sample preparation and treatments, total viable cell count methods, miniaturization and diagnostics kits, immunological testing, instrumentation and biomass measurements, genetic testing, and advances in biosensors. A recent review of this topic appears in *Encyclopedia of Food Microbiology* (1), and detailed descriptions of many rapid method tests can be found in the *Handbook for Rapid Methods and Automation in Microbiology Workshop* (2). Entis et al. (3) gives a comprehensive review of rapid methods for detection, identification, and enumeration of microorganisms. The present paper is a summary of an oral report presented in Philadelphia, PA, at the 2000 AOAC INTERNATIONAL Annual Meeting.

Present Status of Rapid Methods

By the author's conservative estimate, based on the assumption that there are 20 000 reasonably large microbiological laboratories worldwide, each performing 35 000 tests per year, about 700 million microbiological tests are conducted per year in the world. At a modest estimated cost of U.S. $2 per test, the testing market is U.S. $1.4 billion per year. Strategic Consulting, Inc. (Woodstock, VT; 4), estimated that worldwide industrial microbiological testing in 1998 amounted to 755 million tests. It estimated that 56% of the tests were for food, 30% for pharmaceuticals, 10% for beverages, and 4% for environmental water tests. Of these tests, 420 million were performed in food laboratories, with 360 million for routine tests (total viable cell, coliform, yeast and mold counts) and 60 million tests for specific pathogens (e.g., *Salmonella*, *Listeria*, *Staphylococcus aureus*, and *Escherichia coli* O157:H7). It projected that from 1998 to 2003 there will be a 25% increase in the number of tests performed, a 17% increase in price per test, and a 46% increase in total revenue of the testing market by 2003.

In its 2000 U.S. Food Industry Market study (5), it estimated that, in the United States alone, 144 million microbiological tests were performed. Processed foods constituted 36%; dairy, 32%; meat, 22%; and fruits and vegetables, 10%. The number of future tests for fruits and vegetables will certainly increase as a result of recent foodborne outbreaks related to those foods. Routine tests constituted 84%, versus 16% for pathogen tests. Of the routine tests, total viable count constituted 37% of all tests; coliform and *E. coli*, 31%; yeast and mold, 16%; and pathogens, 16%. Estimation of rapid methods versus conventional methods in these testings is difficult. However, the author estimates that about 70% of all microbiological tests use conventional methods and 30% use rapid methods. For pathogen tests, however, the ratio is about 50:50. By 2005, the ratio of conventional methods to rapid methods will also be 50:50 and to pathogen tests will be 30:70. On the international scale, about one third of the tests are performed in the United States, one third in Europe, and one third in the rest of the world. In about 10 years, because of greater awareness of the importance of microbiological testing around the world, the ratio will be one fourth in the United States, one fourth in Europe, and one half in the rest of the world.

In the past decade, the author conducted numerous rapid microbiology workshops and conferences all over the world and witnessed the momentum of the use of rapid methods in the developed, as well as the developing, countries around the world. In April 2001, the author and colleagues conducted the first rapid methods workshop and symposium in Wuhan, China. More than 200 scientists from all over China attended the meeting. Opening up a food microbiology testing market

Guest edited as a special report on "Rapid Microbiological Methods for Foods" by Eva D'Haese.
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as huge as China with 1.3 billion people will certainly increase the volume of testings.

Predictions

After working in and observing the developments of this interesting field for 30 years, the author has had the opportunity to observe the changes in this field firsthand from the very beginning. The following are 10 predictions made by the author in 1995 as the Food Microbiology Divisional Lecturer during the Annual Meeting of the American Society of Microbiology, with updated current predictions (6).

Viable Cell Counts

Viable cell counts (total aerobic, anaerobic, coliform/*E. coli*, and pathogenic) will remain an important parameter to assess the potential safety and hygiene quality of food supplies. Although current methods are cumbersome and labor-intensive, many developments have been made and will continue to be made to improve the viable cell count procedure. Special developments in early sensing of viable colonies on agar, electronic microscopic sensing of viable cells, improvements of vital stains for living cells, and more effective sensing of the most probable number (MPN) of samples will greatly improve the viable cell count procedure.

Real-Time Monitoring of Hygiene

Several exciting developments in this area, such as adenosine triphosphate (ATP) bioluminescence, catalase measurement, instant protein detection kits, and instant hygiene tests, have recently been marketed. These kits are easy to use and provide useful information in a few minutes. Catalase is a very reactive enzyme that is used to monitor the hygiene of surfaces, the microbial spoilage potential of aerobic cold-stored food, and the end-point temperature monitoring of cooked foods. These tests are simple, inexpensive, and rapid (7). Monitoring of the presence of protein, fat, and carbohydrate on food-contact surfaces also provides rapid and meaningful information on the hygiene quality of the surfaces. Recently, BioControl (Bellevue, WA) introduced a protein-testing kit called FLASH™, which detects the presence of protein on food-contact surfaces almost instantaneously. Positive surfaces change the color of the swab from yellow to green or blue in 5 s. This type of real-time monitoring system will be developed for other compounds in the future.

Polymerase Chain Reaction (PCR), Ribotyping, and Genetic Tests in Food Laboratories

Food companies, pharmaceutical companies, and related industries are now routinely using these advanced technologies to monitor the presence of normal flora, spoilage flora, and pathogens in food and other materials. These tests are becoming more and more user-friendly and convenient. For example, the first generation of the BAX™ system (Qualicon, Wilmington, DE), which used gel electrophoresis to detect PCR products, has now changed to an automatic monitoring system of the occurrence of PCR products, thus eliminating the separate operation of gel electrophoresis. The Genetrak DNA hybridization test for organisms such as *Salmonella* has changed to the detection of RNA in a microtiter format for ease of automation in RNA hybridization of target pathogens.

Automated Enzyme-Linked Immunosorbent Assay (ELISA) and Immunological Tests

After pre-enrichment of food samples (overnight or 8 h incubation), an analyst can place the sample in an automated system and monitor the presence of the target pathogen in a matter of 1–2 h. The VIDAS™ system (bioMerieux, St. Louis, MO) is a successful automated system with more than 13 000 units used worldwide. Detex (Molecular Circuitry, Inc., King of Prussia, PA) is a new system for this type of test. Automated systems will continue to be developed and used in the future.

Rapid Dipstick Technology

Many forms of dipsticks and biochips are available for screening of pathogens by lateral migration of an antigen–antibody complex. These kits can detect target organisms in about 10 min after culture enrichment. This type of technology will also continue to be developed and used in the future.

Biosensors for Hazard Analysis Critical Control Point (HACCP) Programs

A variety of biosensors are now commercially available to monitor microbes, but they are not yet suitable for use in routine testing of pathogens in the food industry. More research and development will be needed to implement this technology. The problem is not the systems themselves, but the sample preparation of target pathogens from the food matrix to be detected by the sensitive biosensors. These obstacles will eventually be overcome by diligent research work in the field. An interesting new test is the Umedik (Toronto, Canada) FAST-Q Biochip™ test kit, which uses an ingenious separation system on a biochip and an electronic instant detection system to monitor foodborne pathogens such as *E. coli* O157:H7 in as little as 6 h after sample collection. The detection time after enrichment is only about 10 min. This company is also working on a 10–15 min total viable cell count system.

Instant Detection of Target Pathogens by Computer-Generated Matrix

A much more in-depth understanding of microbial cells and the pathogenic traits of pathogens is needed before this prediction can become a reality. Completion of the mapping of the human genome and the development of proteomics (identification and quantitation of proteins and elucidation of their functions) are rapidly moving this field into practical use for food microbiology. Biochips that can detect all the major pathogens on one chip are now being intensively researched in university, industrial, and government laboratories. DNA microarrays biochips can now spot 50 000 DNA sequences on one slide to rapidly screen target particles for drug discoveries and related genomic studies.

Effective Separation and Concentration for Rapid Identification of Target Cells

A variety of approaches have been developed for immunomagnetic separation, rapid centrifugation, and concentration procedures, which will improve detection sensitivity and increase the speed of pathogen detection.

Microbiological Alert System in Food Packages

During growth and spoilage, microbial cells generate a variety of compounds that can be detected by ingenious devices such as gas and pH indicators. A series of reagents in the form of bar codes can be placed inside the packaging materials and change color as a result of temperature abuse or the development of gas (ammonia, hydrogen sulfide, hydrogen, carbon dioxides) and acid, thus indicating a potential spoilage problem. A concept involving packaging material manufactured with specific antibodies against such pathogens as *Salmonella* or other pathogens is now being tested. When the food is spoiled by *Salmonella*, the packaging material turns a specific color to warn the consumer about the potential danger.

Rapid Alert Kits for Detecting Pathogens at Home

Kits are currently available to consumers for at-home testing of urine, blood glucose, pregnancy, and even AIDS. It is possible that rapid alert kits for food spoilage and detection of food pathogens will be developed for home use. More development in this area is needed, which along with consumer education will make these kits useful.

Conclusions

Along with the prediction of rapid method tests, the following attributes are needed for an ideal automated microbiology assay system: Accuracy of sensitivity, minimal detectable limits, specificity of test system, versatility, potential applications, and comparison with reference methods; speed in obtaining results; cost of test, reagents, and labor; acceptability by the scientific community and regulatory agencies; simplicity of sample preparation, operation of test equipment as well as computer versatility; training time and qualification of operator; preparation, stability, availability, and consistency of reagents; company reputation; speed, availability, cost, and scope of technical services; and utility and space requirements.

Acknowledgments

This material is based upon work supported by the Cooperative State Research Education and Extension Service, U.S. Department of Agriculture, under Agreement No. 93-34211-8362. Contribution No. 02-117J, Kansas Agricultural Experimental Station, Manhattan, KS.

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