HISTORICAL DEVELOPMENT OF RAPID METHODS AND AUTOMATION IN MICROBIOLOGY[1]

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ABSTRACT

Rapid methods and automation in microbiology comprise a dynamic area of research and study. The field encompasses a wide variety of subjects in theoretical and applied microbiology. This article traces the major developments of this field since the late 1960s, detailed with dates and locations of major international meetings on the subject as well as listing of major books, proceedings, and articles concerning this topic. The article should be of interest to all scientists who want to know the key developments, current status, and possible future trends of the field.

HISTORICAL DEVELOPMENT OF RAPID METHODS AND AUTOMATION IN MICROBIOLOGY

Rapid methods and automation in microbiology comprise a relatively new field of study in applied microbiology. This field attempts to utilize microbiological, chemical, biochemical, biophysical, immunological, and serological methods for the study of improved methods for the isolation, early detection, characterization, and enumeration of microorganisms and their products in clinical, food, industrial, and environmental samples. The field is dynamic and has gained momentum in the past 10 years.

Medical microbiologists started to be involved in rapid methods around the early 1970s. In the 80s there was a surge of use of diagnostic test kits for microbiology

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and immunology in the medical areas. Food microbiologists are lagging about 10 years behind medical microbiologists in this regard. However, in the past 5 years, much interest has been expressed internationally by food microbiologists in adapting new and rapid methods. Surely, the field will expand considerably in the next 10 years in all areas of microbiology. With advanced technologies in computers and instrumentation, we should expect much activity in the entire field.

At the dawn of the development of bacteriology and microbiology, scientists must have slowly tried to improve efficiency in the operation of bacteriological procedures. A look at older bacteriology text books and laboratory manuals will reveal some form of improved operations for handling large numbers of culture tubes, test tubes, plates, and inoculation procedures.

Although many handbooks and manuals contained descriptions of improved methods in bacteriology and microbiology, the most important book dealing with *Miniaturization of Microbiological Methods* was written by Paul A. Hartman (1968). The cover page of the book reads "This book provides a comprehensive compilation (over 1,200 citations) of techniques for the cultivation of bacteria, fungi, protozoa, and other plants and animal cells. Emphasis is given to techniques made more rapid, convenient, or reliable than conventional laboratory methods by miniaturization." Another very useful book was compiled by V.B.D. Skerman (1969) on *Abstracts of Microbiological Methods* with almost 900 pages of valuable information on methodologies in applied microbiology. These two books are classics and should be read by all students of rapid methods and automation in microbiology.

In the early 1970's, several interesting articles appeared concerning rapid methods and automation in microbiology. Goldschmidt (1970) had a chapter entitled "Instrumentation for Microbiology: Horizons Unlimited" in the book *Rapid Diagnosis Methods in Medical Microbiology* by Graber (1970). Richardson (1972) had an article on "Automation in the Dairy Laboratory" in the *Journal of Milk and Food Technology* and Trotman (1973) wrote an article on "The Philosophy of the Application of Automatic Methods to Hospital Diagnostic Bacteriology" in *Biochemical Engineering*.

One of the earliest symposia on this topic was organized by Daniel Amsterdam in 1971 at the Annual Meeting of the American Society for Microbiology in Minneapolis, Minnesota with the title "Rapid Methods for Detection and Characterization of Microorganisms." Daniel Y.C. Fung (1971) and Millicent C. Goldschmidt (1971), the editor and associate editor of the *Journal of Rapid Methods and Automation in Microbiology*, were invited speakers at that historical symposium.

Although many smaller meetings, seminars, and symposia were held concerning rapid methods, the key identifiable start of the field was the first *International Symposium on Rapid Methods and Automation in Microbiology* held in Stockholm, Sweden, under the chairmanship of Carl-Goran Heden in 1973. There were about

500 to 600 people at that meeting. Subsequently, symposia of this series were held in Cambridge, UK (1976), Washington, DC (1981), Berlin (1984), Florence, Italy (1987), and Helsinki (1990). The seventh symposium will be held in London, UK, in 1993. The meeting has grown in size to a little more than 1,000 participants in recent years. Also the name has been changed to *International Congress on Rapid Methods and Automation in Microbiology and Immunology.* This author has had the honor to be plenary session speaker, symposium organizer, and paper presenter in all these international symposia since 1973.

The proceedings of these meetings were published by Heden and Illeni (1975a,b), Newsom (1976), Tilton (1982), Habermehl (1984), Balows *et al.* (1989), and Vaheri *et al.* (1991). Of these proceedings, the most valuable ones are the two books edited by Heden and Illeni: *Automation in Microbiology and Immunology* (1975a) and *New Approaches to the Identification of Microorganisms* (1975b). These two books, although outdated by now, contain the basic approaches and philosophies of the field of rapid methods. Subsequent volumes of the symposium series provided updated information and newer developments. All these books are valuable resources in the field.

In between these major meetings, international conferences also were held on similar topics in Kiel, Germany (1974); Ottawa, Canada (1975); Dallas, Texas (1978); Liblice Castle, Czechoslovakia (1980); Ploufragan, France (1983); Lille, France (1983); Anchorage, Alaska (1986); Taipei, Taiwan (1987); Nancy, France (1988); Singapore (1989); and other places. Some of these proceedings were published: Tolle and Heeschen (1974), Sharpe and Clark (1978), Schindler (1980), Lahellec (1983), LaClerc (1983), Kramer and Liston (1987), Hung (1987), and Galteau *et al.* (1989).

Organizations such as *American Society for Microbiology, Institute of Food Technologists, Biodeterioration Society,* etc., occasionally organized symposia on this topic through the years. The *Food Microbiology Research Conference,* organized in 1972 has had several sessions in rapid methods over the past 20 years of interactions of applied food microbiologists. Fung (1991a) compiled the "Food Microbiology Research Conference. 1972–1991. A Historical Review and Directory" to document all the topics of the 13 conferences, as well as listing names of participants of this unique conference series.

Many books and monographs on the general subject of Rapid Methods and Automation have appeared in recent years: Palmer and LeQuesne (1976), Mitruka (1976), Sharpe (1980), Eden and Eden (1984), Barry and Houghton (1986), Pierson and Stern (1986), Beerens (1987), Felix (1987), Hurst and Mortimer (1987), Erickson and Fung (1988), Houghton *et al.* (1988), Sharpe and Peterkin (1988), Adams and Hope (1989), Baltes (1989), Bills and Kung (1990), Fox *et al.* (1990), Fung and Matthews (1991), and Rossmoore (1991).

Another important development is the initiation of "hands-on" workshops concerning these rapid methods. Some of the workshops were one or two days with

lectures and limited demonstrations and "hands-on" experiences of various systems. The most comprehensive program was developed by the author in 1981. The program lasted 8 days. The first workshop had 16 participants from several countries. In 1990, the workshop celebrated its 10th anniversary with the addition of a mini-symposium. Through the years, the workshop has attracted more than 800 scientists from 30 countries and 40 states to participate in the activities. The 12th workshop will be held in July 1992 at Kansas State University, Manhattan, Kansas. A cumulative directory of all past participants of this workshop program and systems studied was issued by Fung (1991b). Similar symposia and workshops also were held on a smaller scale in Singapore; Taiwan; Malaysia; Australia; UK; Japan; River Falls, Wisconsin. Many more are planned in the coming year, such as those held in Taiwan in March and Guelph and Toronto, Canada, in May 1992.

General review articles on this subject were published by Goldschmidt and Fung (1979); Fung and Cox (1981); Cox *et al.* (1984, 1987); Fung (1980, 1984, 1989, 1991c); Fung *et al.* (1984, 1987, 1988, 1989); and Hartman *et al.* (1992). Some reviews were written in German (Fung 1975a,b), French (Fung and Lahellec 1987), Japanese (Fung 1990), modernized Chinese (Fung and Liang 1988b), and traditional Chinese (Fung and Chen 1992).

General themes of these publications on rapid methods can be grouped as follows:

Improvement in Sampling and Sample Preparations

Stomacher instrument. Emswiler *et al.* (1977); Sharpe and Jackson (1975)
Gravimeter Diluter. Manninen and Fung (1992a)
Surface sampling. Fung *et al.* (1980); Lee and Fung (1986)

Improvements of Conventional Procedures and Development of Diagnostic Kits

Miniaturized methods for:
Bacteria. Fung (1969); Fung and Hartman (1975); Fung and Miller (1970, 1972); Fung and Petrishko (1973); Lee *et al.* (1982, 1985).
Yeast and Mold. Lin and Fung (1985); Lin *et al.* (1984); Fung and Liang (1990).
Motility enrichment oxyrase system. Yu and Fung (1991a,b, 1992).
Organic dye culture media. Fung and Miller (1973); Fung and Neimaic (1977); Chein and Fung (1990); Lin and Fung (1987); Fung and Liang (1988a); Hart *et al.* (1991); Goldschmidt *et al.* (1991).
Double tube anaerobic method. Fung and Lee (1981); Ali *et al.* (1991); Ali and Fung (1991, 1992)
Diagnostic kits: API, Enterotube, R/B, Minitek, Spectrum 10, MicroID, IDS. Cox *et al.* (1977, 1984, 1985, 1987).

Modifications of Viable Cell Count Procedure

Spiral system, Isogrid, Petrifilm, Redigel. Fung *et al.* (1987); Chain and Fung (1991); Manninen *et al.* (1991); Fung and Chain (1992).

DEFT test. Pettipher (1986).

Developments of New Methods for Estimation of Microbial Populations

ATP, Electrical Impedance, Conductance, Radiometry, Microcalorimetry, Limulus amoebocyte lysate, catalase, reflectance colorimetry. Fung (1991c); Manninen and Fung (1992b).

Identification of Microbes by Sophisticated Instruments and Procedures

Vitek, Sensititre, ELISA, DNA-RNA probes, 1-2 Test, protein profiling, cell wall components, magnetic beads, Polymerase Chain Reaction, bacterial ice nucleation test, etc. Fung (1989, 1991c).

KEY ISSUES AND CONCERNS

The key issues and concerns in the development of rapid methods are summarized in the following outline.

What is "Rapid"?

A. Speed in Reaction
   Instantaneous, seconds, minutes, hours
   versus
   Hours, days, weeks
B. Numbers of Samples Per Operation
   10, 50, 96, or more
   versus
   one at a time

What is "Automation"?

A. Manual: By humans, one or more sample at a time
B. Semi-automation: By humans, with the aid of some form of instrumentation
C. Complete Automation: By robots

Major Developments

A. Diagnostic Tests for Liquid, Semi-Solid, and Solid Media
   1. Large tubes: One reaction per tube
   2. Large tubes: Multiple reactions per tube
   3. Small tubes: One reaction per tube
   4. Small tubes: Multiple reactions per tube
   5. Wells in a tray of different configurations:
      a. One type of reaction for many organisms per tray
      b. Many types of reactions for one type of organism per tray
      c. Many types of reactions for a few organisms per tray
   6. Diagnostic kits

B. Inoculations into Diagnostic Tests
   1. One inoculation per tube
   2. Multiple inoculations (manually or by instruments)
      a. liquid in a tray
      b. solid in agar
      c. agar in multiple compartments
      d. liquid dispensing to multiple wells

C. Automated Instruments for Monitoring
   1. Cell mass
   2. Cell components
   3. Cell metabolites
   4. Cell activities

D. Development of Serological and Immunological Techniques
   1. Immunoblotting
   2. Electrophoresis
   3. RIA
   4. ELISA

E. Development of Genetic Techniques
   1. DNA probes, RNA probes
   2. Polymerase Chain Reaction

Concepts Involving the Living Cell

A. Living cell versus dead cell
B. Growing cell versus nongrowing cell
C. Meaning of viable cell count
D. Correlation between total count and other parameters
E. Amplification of cells
F. Concentration of cells
G. Signal versus background
H. Sensitivity versus detection time

SUMMARY

After being involved in the field of rapid methods and automation in microbiology for 25 years, this author summarizes his ideas concerning the attributes for an ideal automated microbiology assay system as follows:

Attributes for an Idea Automated Microbiology Assay System

1. Accuracy for the intended purposes
   sensitivity; minimal detectable limits
   specificity of test system
   versatility; potential applications
   comparison to referenced methods
2. Speed in productivity
   in obtaining results
   in number of samples processed per run; per day
3. Cost
   initial, per test, reagents, others
4. Acceptability
   by scientific community
   by regulatory agencies
5. Simplicity of operation
   sample preparation
   operation of test equipment
   computer versatility
6. Training
   on site; how long
   quality of training personnel
7. Reagents
   reagent preparation-stability-availability-consistency
8. Company reputation
9. Technical service
   speed and availability
   cost and scope of technical background
10. Utility and space requirement

Although the author marvels at the great changes in rapid methods and automation in microbiology in the past 25 years, he is still of the opinion that all students of microbiology should thoroughly master the basic conventional methods first (i.e., Gram stain, aseptic techniques, pure culture isolation, test tube methods,

standard plate count method, skill in microscope utilization, etc.) before tackling all these newer concepts, instruments, and sophisticated techniques. He likes to end a discussion on rapid methods and automation in microbiology by saying "The best rapid method is still your own brain."

In conclusion, rapid methods and automation in microbiology as a field of study is relatively new. The potential is great and many exciting developments will certainly unfold in the coming years.

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