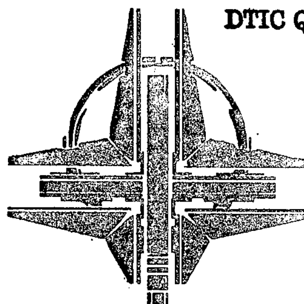
NATO ADVANCED RESEARCH WORKSHOP

RAPID METHOD FOR MONITORING THE ENVIRONMENT FOR BIOLOGICAL HAZARDS

19-22 May 1997

DTIC QUALITY INSPECTED 2



DISTRIBUTION STATEMENT A
Approved for public release;
Distribution Unlimited

19970730 087

WARSAW

NATO ADVANCED RESEARCH WORKSHOP

RAPID METHOD FOR MONITORING THE ENVIRONMENT FOR BIOLOGICAL HAZARDS

SPONSORED BY:

Scientific Affairs Division. NATO-Brussels, Belgium
Ministry of Defense, Poland
Military Institute of Hygiene and Epidemiology, Warsaw, Poland
U.S. Army Edgewood Research, Development and Engineering Center, Aberden Proving Ground, MD, USA
U.S. Army Research, Development and Standardization Group - UK, London, UK

ORGANIZATION COMMITTEE:

P.	**Stopa,**	**USA**
M.	**Bartoszcze,**	**Poland**
H.	Garrigue,	France
M.	Siebrand,	Germany
B.	Niederwohrmeier,	Germany
K.	Chomiczewski,	Poland

HOST COUNTRY ORGANIZATION COMMITTEE:

M. Kowalczyk
S. Szmigielski
E. Trafny - Secretary
B. Juńczyk
M. Pfeffer
K. Lasocki
M. Dziekiewicz
A. Patrzykąt
A. Bielawska
P. Kostrzewa
M. Maliński

---

19 -22 May 1997

WARSAW

NATO ADVANCED RESEARCH WORKSHOP

19-22 May 1997 - WARSAW

LIST OF PARTICIPATING COMPANIES

1. New Horizons Diagnostics, Inc.
   - Luminometers + Immunodiagnostics.
   Columbia, Maryland, USA
2. BIO-RAD Laboratories
   - Flow Cytometry + Confocal Microscopy.
   Hercules, California, USA
3. SCP Dynamics, Inc.
   - Air Sampling Equipment.
   Minneapolis, MN. USA
4. TSI, Inc.
   - Aerosal Generation + Measurement Equipment
   Minneapolis, MN. USA
5. DYCOR, Ltd.
   - Instrument Integration + System Engineering
   Edmonton, Alberta, CANADA
6. P.Z. HTL
   ul. Daniszewska 4, Warszawa, POLAND
7. PHU „POLEKO"
   Aparatura
   Wodzisław Śl., ul. Pszowska 900, POLAND
8. SERTOP Spółka z.o.o.
   43-100 Tychy, ul. Przemysłowa 58, POLAND
   tel: (032) 117-40-44, fax: (032) 117-48-08
9. Zakład Rolniczo Przemysłowy „FARMUTIL HS"
   64-811 Śmiłowo 68, woj. pilskie, POLAND
10. Zakłady Drobiarskie „Koziegłowy", spółka z.o.o
    62-028 Koziegłowy k. Poznania
    tel: (061) 20-57-09, fax: (061) 12-73-91
11. CONSTAR Spółka Akcyjna
    Starachowice, ul. Krańcowa 4, POLAND

PROGRAM OF NATO ADVANCED RESEARCH WORKSHOP

RAPID METHODS FOR MONITORING THE ENVIRONMENT FOR BIOLOGICAL HAZARDS.

19-22 May 1997 - WARSAW

18.5 Sunday night - Reception at the Hotel (Victoria Intercontinental)

19.5 Monday

Plenary Session

Session Chairman: Peter J. Stopa

0800-0900 Registration
0900-0915 Welcome from the Chairman - P.Stopa. Goals for the Workshop.
0915-0920 Welcome from the host country (M.Bartoszcze)
0920-0935 Polish Dignitary
0935-0945 Greetings from the US Embassy

0945-1000 **Coffee break**

1000-1015 M.Donlon, USA, ARPA
1015-1045 M.Dando, UK, „Impact On The Biological Weapons Convention (BWC) Of Sampling And Identification Techniques For Potential BW Agents".
1045-1115 J.Mierzejewski, M.Bartoszcze, Poland, MIHE - „Some Problems Concerning Biological Threats".
1115-1130 N.Schulte, NATO, Program Director, Disarmament Technologies - „Funding Opportunities within the NATO Science Program".
1130-1145 H.Egghart, USA ERO, - „Mission of the U.S. Army European Research Office"'

1200-1230 Lunch (Restaurant at the Staszic Palace)
1230-1400 Tour of the Royal Palace.

Session 1: **Classical Techniques**
Session Chairman: A.Kaprelyants, Russia, Moscow State University.

1430-1500 A.Kaprelyants - Russia, Moscow State University - „Dormancy in Non-Sporulating Bacteria: Its Significance for Environmental Monitoring"

1500-1530 V.Ivanow, Ukraine, UNU, - „Monitoring the Bacterial Neuston for Biological Hazards"

1530-1600 O.Veprintseva, Russia,- „Influence of Environmental Factors on Song Bird Songs".

1600-1630 E.Vorobyova, Russia, Soil Department of MSU, - „Subsurface Environments - Model for Detection of Relevance to Exobiology".

1630-1700 **Coffee Break**

1700-1800 Panel Discussion: TOPIC: What Do We Really Know About the Environment?

1900-2200 Welcoming Reception (Conference Center of the Ministry of Defense)

20.5 Tuesday

Session 2a: **Classical Methods of Measurements**
Session Chairman: H.Garrigue, France, CEB

0900-0930 N.Dovichi, Canada, University of Alberta, - „Ultrasensitive Protein Assay".

0930-1000 A.Kusterbeck, USA, Naval Research Laboratory, - „Environmental Applications on the NRL Flow Immunosensor".

1000-1030 V.G. DelVecchio, USA, University of Scranton, - „Development of PCR-Based Assays for the Detection and Molecular Genotyping of Microorganisms of Importance to Biological Warfare".

1030-1100 **Coffee Break**

1100-1130 M.Bartoszcze and A.Bielawska, Poland, MIHE, - „The Past, Present, and Future of Luminometric Methods in Biological Detection".

1130-1200 L.Loomis, USA, New Horizons Diagnostics, Inc., - „Rapid Methods for Monitoring Food and The Environment".

1200-1300 Lunch (Restaurant at the Staszic Palace)

1300-1500 Walking tour around the Old Town

Session 2b: **Classical Methods of Measurements - continued**

1500-1530 G. Trouiller, France, CEB, - „The ADIBio Systems: A Biological and Toxins Weapon Rapid Detection Device"

1530-1600 A. P. Snyder, USA, ERDEC, - „Biodetection by Next Generation Mass Spectrometry Techniques".

1600-1630 W. Bryden, USA, Johns Hopkins Applied Physics Laboratory, - „Tiny - TOF (Time of Flight) Mass Spectrometer for Biodetection".

1630-1700 **Coffee Break**

1700-1730 R.Goodacre, UK, University of Wales of Aberystwyth, - „Intelligent Systems for the Characterizations of Microorganisms from Hyperspectral Data".

1730-1845 Panel Discussion -.Reviev of Present Status of Technology.

2000-2300 Barbecue

21.5 Wednesday

Session 3: **Aerobiology**

Session: Chairman J.Ho, Canada, DRES

0830-0900 L.Paul, USA, TSI Inc., - „Methodology for the Generation and Measurement of Aerosols".

0900-0930 C.Petersen,USA, SCP Dynamics, Inc. - „Air Sampling Techniques".

0930-1000 Z.Krocova, Czech Republic, Medical Military Academy, - „Measurments of Biological Areosols in Areosol Chamber".

1000-1030 **Coffee Break**

1030-1100 S.Grinshpun, USA, University of Cincinnati, - „Sampling of Biological Aerosols: Physical Collection Efficiency and Microbial Survival".

1100-1130 J.Ho, Canada, DRES, - „Biological Aerosols Measurements With A Fluorescence Aerodynamic Particle Sizer: Lower Limits Of Detection".

1130-1200 B.Harper, U.S. Army Dugway Proving Ground, - „Field and Laboratory Testing at Life Sciences Division, Dugway Proving Ground".

1200-1300 Lunch (Restaurant at the Staszic Palace)

Session 4: **Emerging Technology**
Session Chairman M.Donlon, USA, ARPA

1300-1330 H.Steen, Norwegian Radium Hospital, - „Design concepts of Flow Cytometers of bacteria".
1330-1400 P.J. Stopa, USA, ERDEC, - „Field Applications of Flow Cytometry".
1400-1430 H.Davey, UK, University of Wales, Aberystwyth, - „A Portable Flow Cytometer for Detection and Identification Microorganisms".
1430-1500 H.Meier, USA, MRICD, - „The Use of Flow Cytometry to Study Sulfur Mustard's (SM) Mechanisms of Action and to Develop Diagnostic Screens and Therapeutic Treatments".

1500-1530 **Break**

1530-1600 A.Clark, USA, CBDE-Porton Down, - „Biodetection Technologies in Environmental Monitoring".
1600-1630 H.Mottl, CANADA, DYCOR, Ltd., - „A System for the Remote Control and Monitoring of Sensors and Sampling Equipment".

1700-1900 Zelazowa Wola - Chopin's Concert
1900 Departure for the Restaurant
1930-2300 Dinner

22.5 Thursday

Session 5: **DNA Probes**
Session Chairman B.Mizak, Poland, NVI.

0830-0900 J.J.Calomiris, US Air Force, - „Monitoring Waterborne Pathogens Using DNA Probes".
0900-0930 E.Henchal, USA, MRIID, - „Evaluation of 5' Nuclease Fluorogenic Polymerase Chain Reaction Assays for Identification of Biological Agents".
0930-1000 V.G. Del Vecchio, USA, University of Scranton, - „Use of Taqman, Light Cycler, and Confocal Microscopy to Detect Specific PCR Products".

1000-1030 **Coffee Break**

1030-1050 H.Meyer, Germany, FRG Laboratories, - „Specific Detection of Monkeypox Virus".

1050-1110 H.Garrigue ,France, CEB, - „Use PCR for Identification and Detection of Biological Agents".

1110-1130 D.Zegers, Netherlands,TNO, - „Single Strand Conformation Polymorphism for Detection of Pathogens".

1130-1200 Panel Discussion. Impact of DNA Probes for Environmental Analysis.

1200-1300 Lunch (Restaurant at the Staszic Palace)

Session 6: **Applications**

Session Chairman T.T. Calomiris, U.S. Air Force

1300-1330 S.G. Ignatov, Russia, - „Rapid Method for Bacterial Counting in Milk by Using Biosensor based on E.coli Cells".

1330-1400 A.P.Snyder, USA, ERDEC, - „Pyrolysis-Gas Chromatography-Ion Mobility Spectrometry Detection of Bacillus Globigii (BG) Areosol".

1400-1430 V.Seligy, Canada, - „Comparative Performance of Conventional and Advanced Molecular Methods for Environmental Exposure Mapping of Biological Aerosols: Validation Using Large-Scale Aerial Application of B.thuringiensis Kurstaki-based Commercial Biopesticides".

1430-1700 Poster Session

1700 Chairman's Wrap-Up

POSTER SESSION

1. A.V.Zakharov, Russia, Space Research Institute - „Future Missions to Mars from an Exobiology Point of View".
2. M.Smirjak and P.Filip, Slovak Republic, Csc Military Veterinary Institute - „Biological Contaminants of the Food and Health Care of the Members of the Army of the Slovak Republic in Various Conditions of Activity".
3. B.Wojton, K.Kwiatek, and H.Rozanska, Poland, National Veterinary Research Institute - :The Occurrence of Escherichia coli 0157:H7 in Food of Animal Origin in Poland".
4. J.Garcar and M.Smirjak, Slovak Republic, Military Veterinary Institute - „The control of the Incidence of the Heterogeneous Substances Residues in Bio-Areas and in the Food Chain from the Aspect of the Protection of the Members of the Army of the Slovak Republic".
5. J.Skalka and M. Smirjak, Slovak Republic, Institute of Experimental Veterinary Medicine and Military Veterinary Institute - „Toxic Plants in the Complex of the Ecosystem-Food-Human Being".
6. J.Bello and M.Smirjak, Slovak Republic, Military Veterinary Institute - „The role of the Military Veterinary Institute in the Complexity of the Protection of the Environment, Food, and Health Care of the Members of the Army of the Slovak Republic".
7. A.Lidacki, Poland, Veterinary Research Center of the Military Institute of Hygiene and Epidemiology - „Immunomagnetic Separation for the Detection of Salmonella from Food".
8. W.Palec and B.Mizak, Veterinary Research Center of the Military Institute of Hygiene and Epidemiology - „Preliminary Studies on Detection of Proteins of Clostridium botulinum by the Immuno-Blot Technique".
9. J.Bzdęga and R.Łakomy, Poland, Military Institute of Hygiene and Epidemiology (Warsaw) - „Detection of Potential Bacterial Warfare Agents by Pyrolitic Gas Chromatography".
10. Bartoszcze, H. Arciuch, J. Matras, K. Chomiczewski, A. Bielawska, and M. Cieklinska, Poland, Military Institute of Hygiene and Epidemiology - „Detection of Bacillus anthracis spores by the Luminometric Method".
11. G.Jula, Romania, Ministry of Waters, Forests, and Environmental Protection, Research and Engineering Institute for the Environment - „Some Aspect About the Biological Monitoring of a Water Supply".
12. M.Nicolae, Romania, Ministry of Waters, Forests, and Environmental Protection-Research and Engineering Institute for the Environment - „A Practical Study of the River Colentina".
13. A.P.Snyder, USA, U.S. Army Edgewood Research, Development, and Engineering Center - „Ion Mobility Spectrometry as an Immunoassay Detection Technique".

14. E.Stabnikova, Ukraine, Ukrainian University of Food Technologies - „Biodiversity of Bacillus spp. Populations Monitored by Flow Cytometry".
15. D.Paterno and P.Coon, USA, U.S. Army Edgewood Research, Development, and Engineering Center - „Luminescence Studies of Bacteria"
16. C.Boulet, G.Hung, D.Bader, P.Duck, P.Wishart and A.Lai-How, Canada, Defence Research Establishment, Suffield - „Capillary Electrophoresis Nucleic Acid Probe Identification of Biological Warfare Agent Simulants In Environmental Matrices"

M. Donlon
ARPA, USA

Abstract not available.

IMPACT ON THE BIOLOGICAL WEAPONS CONVENTION (BWC) OF SAMPLING AND IDENTIFICATION TECHNIQUES FOR POTENTIAL BW AGENTS

Malcolm Dando
University of Bradford, Dept. of Peace Studies, West Yorkshire BD7 1DP, U.K.

Following the Fourth Review Conference of the BWC in November-December 1996, work of the Ad Hoc Group charged with strengthening the convention will move to a negotiation format. The legally-binding instrument that could be agreed over the next two years seems likely to be based on mandatory declarations, on-site measures involving both non-challenge and compliance concern investigations and investigations of allegations of use.

In recent years groups such as the Federation of American Scientists, the UK Royal Society and government experts (in the VEREX series of meetings) have examined how sampling and identification techniques might be used in the process of verification of the BWC. Considerable concern has also been expressed by industry representatives over the potential loss of commercially confidential information that might be brought about by the use of such techniques.

The aim of this contribution is to assess current proposals for the legally-binding instrument, and the role that sampling and identification techniques could play, with a view to considering the potential benefits and dangers involved in the use of such techniques in strengthening the BWC.

SOME PROBLEMS CONCERNING BIOLOGICAL THREATS

J. Mierzejewski, M. Bartoszcze
The Military Institute of Hygiene and Epidemiology, 24-100 Pulawy, Lubelska 2, Poland

The article discusses problems concerning biological threats occurring naturally in the environment, as well as problems which could occur in the event of environmental contamination through man-made sources--i.e. industrial accidents, terrorist or military attacks, etc.

Additionally, emphasis will be placed on naturally-occurring diseases which although once eradicated are now once again appearing with renewed strength. Likewise, problems posed by emerging diseases will be covered in this paper. Factors which are influencing the spread of these diseases will also be presented.

One of the greatest questions which we now face is how to effectively deal with these new diseases and threats on a global scale. We will discuss the Biological Hazarads Early Warning Program proposed by the Americans (R. Zilinskas). This program would play an important role in limiting biological threats to both civilians and military personnel.

We consider the NATO Advanced Research Workshop to be one of the first steps toward a program of this type. The workshop will allow us to discuss new techniques in the field of detection and identification of different biological threats in the environment. The ideas generated from the workshop should give us valuable insight as to how to cope with both present problems as well as those which could arise in the future.

FUNDING OPPORTUNITIES WITHIN THE NATO SCIENCE PROGRAM

N. Schulte
NATO, Program Director, Disarmament Technologies

Abstract not available.

MISSION OF THE U.S. ARMY EUROPEAN RESEARCH OFFICE

H. Egghart
U.S. Army European Research Office

Abstract not available.

DORMANCY IN NON-SPORULATING BACTERIA: ITS SIGNIFICANCE FOR ENVIRONMENTAL MONITORING

[1]**Arseny S. Kaprelyants**, [1]Galina V. Mukamolova, [1]Tatyana V. Votyakova
[2]Hazel M. Davey, [2] Douglas B. Kell,
[1]Bakh Institute of Biochemistry, Russian Academy of Sciences, Leninskii Prospekt 33, 117071 Moscow, Russia.
[2]Inst. of Biological Sciences, University of Wales, Aberystwyth, Dyfed SY23 3DA, U.K.

In natural ecosystems, the total cell count obtained microscopically typically exceeds the viable count on non-selective media by orders of magnitude. The question therefore arises as to whether the "invisible", apparently nonculturable cells are dead, are killed by our isolation media, or are merely in a dormant state from which we might in principle be able to resuscitate them if only we knew how. In particular the suggested "viable-but-nonculturable" (VBNC) bacteria have been invoked to explain phenomena as divergent as the epidemiology of some infections and the persistence of genetically marked organisms in the environment (e.g. the failure to isolate Vibrio cholerae and Campylobacter jejuni from clearly implicated sources or reservoirs of infection could be accounted for on the basis of their being present in a VBNC or dormant state). Application of flow cytometry may be a useful tool to visualize bacteria without their growing and to discriminate between dead and dormant bacteria. We found that dormant Micrococcus luteus can be physically separated from dead cell by flow cytometry sorter after cell staining with rhodamine 123. Resuscitation of dormant M.luteus cells in liquid medium does not proceed in the absence of a culture supernatant from batch-grown cells. This suggests that viable cells can excrete a pheromone-like substance necessary for the resuscitation of dormant cells.

MONITORING THE BACTERIONEUSTON FOR BIOLOGICAL HAZARDS

Vladimir Ivanov
Department of Microbiology and General Immunology, Ukrainian National University,
60 Vladimirskaya Str.,Kiev 252017, Ukraine

Bacterioneuston is a microbial cenosis of the biofilm on the air-water interphase. The concentration of hydrophobic and surface-active substances and bacterial cells within the bacterioneustonic film may be some thousand times more than one in the water bulk. There are many cases when the biological hazards and water quality of natural aquatic systems is determined by the biodiversity of bacterioneuston

Biodiversity of bacterioneuston includes the distribution of bacterial specia , or genetic variants of one species, or cells within the different phases of cell cycle, or different stages of the life cycle. Specific accumulation of specia, or genetic variants, or cells of different age is determined by the hydrophobicity of their cell's surface.

The following data are some experimental examples of the mentioned above points. R- and S-genetic variants of bacteria (rough or smooth colonies) differ significantly by the metabolic activity, pathogenic properties or resistance to unfavorable environmental factors. R-variants of Bacillus licheniformis, B.thuringiensis, and Streptococcus lactis are accumulated in the bacterioneuston. Cell surface hydrophobicity of R-variants was from 3 to 5 times more than one of S-variants.

Specificities of bacterioneuston's biodiversity can be applied in monitoring, control and bioremediation of aquatic systems. Accumulation of anabiotic, resistant or pathogenic variants in bacterioneuston must be accounted in the monitoring of biohazards. This accumulation may also be used for the diminishing of risk from infections by the application into the natural aquatic system the specifically selected antibiotic-producing bacteria with high hydrophobicity of cell surface. Such biodisinfection effects were demonstrated by the application of some Bacillus spp. for the treatment of waste waters containing pathogenic bacteria.

INFLUENCE OF ENVIRONMENTAL FACTORS ON SONG BIRD SONGS

Olga Veprintseva
Veprintsev Phonotheca of Animal Voices,
Institute of Theoretical and Experimental Biophysics of RAS, 142292 Pushchino, RUSSIA

Birds are important indicators of a country's environmental health. Identification of bird species by sound is the most rapid and reliable way to survey an area or to confirm a species presence particularly where visibility is limited. "A survey utilizing sound identification can detect 97% in a forest" said Theodore Parker, a famous American ornithologist.

Library of Natural Sounds of Laboratory of Ornithology at Cornell University, US, and Veprintsev Phonotheca of Animal Voices at Institute of Theoretical and Experimental Biophysics of Russian Academy of Sciences, Russia, are among the largest wildlife sound archives in the world. Their collections of bird sound recordings are invaluable resources for rapid assessment of bird biodiversity and ecological integrity.

SUBSURFACE ENVIRONMENTS - MODEL FOR DETECTION OF RELEVANCE TO EXOBIOLOGY

Elena A. Vorobyova
Soil Biology Department, Moscow State University, Moscow, Russia

There is evidence that Mars had the early history similar to that of Earth, and the question of the origin and evolution of life on this planet follows naturally. The new methodological approach should be improved in future research aimed at biological investigations of this planet, taking into account results of the Viking missions. This approach should be based on studies of terrestrial microbial life in unfavourable conditions for revealing adaptive mechanisms inside the cells and within cell communities. Subsurface environments like deep permafrost sediments could be considered a good model for such studies. New data give strong evidence of the high quantity of microbial cells in such habitat, even under long-term (3-5 mln.yr.) cold stress.

Comparative investigations of microbial life in Arctic and Antarctic permafrost show that the salient feature of living microorganisms is their resting state that can occur as hypometabolic, or as viable but non-culturable (VBN) state. One could expect that to search the deep resting or VBN-state of microbial cells in Martian cryolythosphere could be search for biomarkers (lipids, free enzymes, pigments etc.) or micropopulative approach should be considered.

The following analysis of possible methods for life detection of relevance to exobiology is the subject of this topic.

SESSION 2A: CLASSICAL METHODS OF MEASUREMENTS

ULTRASENSITIVE PROTEIN ASSAY

N.J. Dovichi, E. Arriaga And D. Pinto, Department Of Chemistry, University Of Alberta, Edmonton, Alberta Canada And C. Boulet
Defence Research Establishment-Suffield, Alberta Canada

We report a method for the assay of proteins at concentrations lower than 10^{-10} M with as little as 200 amol of protein. High sensitivity is accomplished by derivatizing the e-amino group of the protein's lysine residues with the fluorogenic dye 5-furoylquinoline-3-carboxaldehyde (FQ) and use of a sheath-flow cuvette fluorescence detector. Most proteins have a large number of lysine residues, therefore, a large number of fluorescent molecules can be attached to each protein molecule. In general, pre-column labeling improves sensitivity but degrades resolution due to the inhomogeneity of the reaction products from multiple labeling. However, we demonstrate that, through careful manipulation of the separation and reaction conditions, high sensitivity can be obtained without excessive loss in separation efficiency. Over 190,000 theoretical plates are obtained for fluorescently labeled ovalbumin.

ENVIRONMENTAL APPLICATIONS OF THE NRL FLOW IMMUNOSENSOR

Anne W. Kusterbeck[1], Paul R. Gauger[2], Upvan Narang[3], and Paul T. Charles[1]
[1]Center for Bio/Molecular Science and Engineering, Biosensors and Biomaterials Laboratory, Code 6910, Naval Research Laboratory, 4555 Overlook Ave., S.W.
Washington, DC 20375-5348
[2]Geo-Centers, Inc., Reservoir Road, Washington, DC
[3]Georgetown University, Reservoir Road, Washington, DC

Environmental security issues continue to be of major concern for military and civilian scientists working at U.S. or foreign installations. A biosensor developed at the Naval Research Laboratory (NRL) is being tested at several U.S. Environmental Protection Agency (EPA) remediation sites to examine the utility of the technology for monitoring explosives levels in groundwater and soil. Using antibodies as the recognition element and fluourescently labelled explosive analogs as reporters of binding events, the biosensor can detect contamination at the sub-parts ber billion level in under two minutes. Unlike commercially available test kits, the system can analyze 40 or more samples with one assay cartridge, depending on concentration. A portable version of the device, known as the FAST 2000, has been engineered by Research International, Woodinville, WA, for convenient use in rapid on-site evaluation and characterization of contaminated areas. Alternatively, the device can be used to monitor treatment progress at selected intervals. Results of the field work using the NRL biosensor and other applications of the instrument will be discussed.

DEVELOPMENT OF PCR-BASED ASSAYS FOR THE DETECTION AND MOLECULAR GENOTYPING OF MICROORGANISMS OF IMPORTANCE TO BIOLOGICAL WARFARE

Vito G. DelVecchio
University of Scranton, Scranton, PA 18510

Species- or strain-specific polymerase chain reaction assays offer an extremely sensitive and accurate detection method for biological warfare agents. Several different strategies can be used for identifying and cloning unique nucleic acid sequences which can detect one type of microorganism from others found in mixed samples or the environment. Specific PCR probes can be obtained from plasmid and chromosomal libraries, ribosomal DNA genes, rep-PCR libraries, and gene sequences in DNA databases. Such probes should be conserved in the genome of the organism in question.

Conventional molecular genotyping or DNA fingerprinting is often time-consuming, laborious, and demands the full attention of a dedicated technician. Repetitive element sequence-based PCR (Rep-PCR), which amplifies regions of DNA found between repetitive elements, has been exploited as a means of genotyping. The seemingly random distribution of these repeats permit complementary oligonucleotide primers to generate fragment patterns that are specific for individual strains of a microorganism. Rep-PCR is relatively rapid, not labor-intensive, accurate, and reproducible. It can readily be adapted to any laboratory setting without great expense.

THE PAST, PRESENT AND FUTURE OF LUMINOMETRIC METHODS IN BIOLOGICAL DETECTION

M. Bartoszcze, A. Bielawska
Military Institute of Hygiene and Epidemiology, 2 Lubelska Street, 24-100 Pulawy, Poland

Bioluminescent techniques based on ATP estimates have been used for many years to regulate sanitary factors in both beer and pharmaceutical production. These techniques were limited in that procedures were time consuming and depended on factors such as the stability of reagents and lack of modern equipment. Thus bioluminescent methods were never before used on a large scale.

Now bioluminescent methods are of greater interest primarily because of increased sensitivity, speed, simplicity and portability due to miniaturization of equipment.

Bioluminescent methods now have a wide range of applications. In medicine, applications range from the ability to estimate bacterial counts in cerebrospinal fluids and urine to the determination of bacterial sensitivity to antibiotics. Additionally, they can be used to control sterilization processes in surgery.

These methods can also be used in the implementation of the HCCP system to regulate food quality and safety in production. Finally, the possibilities in research are seemingly endless. For example, they have applications in studies on microbe replication and growth which can be used together with genetic engineering methods.

Some of these new possibilities regarding improvements of bioluminescent methods, such as biosensors, will be considered in the following discussion.

RAPID METHODS FOR MONITORING FOOD AND THE ENVIRONMENT

Larry Loomis
New Horizons Diagnostics Corp., Red Branch Road, Columbia, Maryland 21045 USA

This discussion will review the development of Bioluminescence techniques with respect to the rapid detection of microbes in air, water, and food. Emphasis will be placed on sample preparation and its importance relating data reproducibility and correlation with reference methods such as aerobic plate counts. Emerging immunoassay methods utilizing bioluminescent techniques will be analyzed and compared to other assay methodologies.

Current sensitivity limits for bioluminescent detection of bacteria is @ 10^3 to 10^4 organisms. Bioluminescent immunoassay procedures can detect 10^1 to 10^2 specific organisms. A review and comparison of bioluminescent assay procedures vs other methods, i.e., ELISA, flow cytometry, and colloidal gold will be made.

Actual studies utilizing bioluminescence techniques conducted at food processing plants will be presented. The talk will conclude with a description of how bioluminescence can play an integral and practical part as a method to be used as a general screen for microbial detection and also as a method for specific analyte recognition in immunoassay protocols.

ADIBIO SYSTEM: A BIOLOGICAL AND TOXINS WEAPON RAPID DETECTION DEVICE

J. Therasse, H. Garrigue and **G. Trouiller**
Biology Department, Centre d'Etudes du Bouchet 91710, Vert le Petit - BP3 France

A biotechnology oriented program for ABO detection was conceived at Centre d'Etudes du Bouchet to provide automated and rapid identification of biological and toxinic agents under battlefield conditions.

Solid-state enzyme immunoassays based on antibody membrane immobilization are used. Specific antigen immunocomplex detection is realized by enzyme-labeled antibodies. The Enzyme Linked Tangential Immunofiltration Assay (ELIFA) has tended to increase sensitivity detection and decrease the time of ABO detection with the ADIBio system.

This device is designed to monitor the local environment by sampling ambient air continuously, by collecting and concentrating airborne particles, analyzing particles to detect biological materials, and agent identification, whatever their nature (toxins, bacteria, virus).

Present status of ADIBio, performance and limits (specificity, sensitivity) will be discussed, and the evolution and perspectives of this system will be presented. One important step actually is on trial on other agents to enhance the capabilities of ADIBio and possibility to militarize it.

BIODETECTION BY NEXT GENERATION MASS SPECTROMETRY TECHNIQUES

A. Peter Snyder
U.S. Army Edgewood Research, Development & Engineering Center,
Aberdeen Proving Ground, MD 21010-5423 USA

Biological detection necessitates more than just a simple sample introduction to the mass spectrometer (MS). Biological compounds are complex entities, and the microbiological literature documents that DNA, proteins and phospholipids/fatty acids are the only recognized biomolecules which have sufficient information for bacterial identification analyses, regardless of the analytical or microbiological technique. This presentation will emphasize two concepts. The first is the bacterial liquid processing and biomarker extraction and transfer to the MS system, and the pattern of data vs. knowledge of the identity of the ions are the second concept with respect to the MS detection. Methods which convert the neutral biomarker analytes into gas phase ions for MS analysis include electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI). Both MS techniques will be described, as well as their application to the three major bacterial biomarkers. ESI-tandem mass spectrometry also allows further information on structural identities, and phosphatidylglycerol and phosphatidyl ethanolamines will be used as examples of this procedure.

TINY-TOF MASS SPECTROMETER FOR BIODETECTION

Wayne A. Bryden, Richard C. Benson and Harvey W. Ko
The Johns Hopkins University, Applied Physics Laboratory,
Laurel, MD 20723-6099 USA

Catherine Fenselau
Department of Chemistry and Biochemistry, University of Maryland Baltimore County
Baltimore Maryland 21228 USA

Robert J. Cotter
Dept. of Pharmacology and Molecular Science, The Johns Hopkins University,
School of Medicine, Baltimore MD 21205 USA

The mass spectrometer has long been known as the most powerful analytical tool in the laboratory for analysis of a broad spectrum of chemical and biological materials. The applicability of mass spectrometers to field detection problems has been quite limited given the large size, heavy weight, and prohibitive power requirements of the instrumentation. However, this situation is rapidly changing as the need for field-portable detection systems becomes more critical. Our collaborative effort is focused on bringing small, yet powerful, time-of-flight (TOF) mass spectrometer technology to bear on the chem-bio detection problem. In order to achieve this goal, not only must small instrumentation be developed and fielded, but validated mass spectral signatures for all of the agents of interest must also be obtained. We will describe the development of a miniaturized time-of-flight mass spectrometer (Tiny-TOF) for field portable biodetection. Topics to be covered include: operating principles of the mass analyzer; miniaturization of the mass analyzer and other system components including vacuum pumps, signal acquisition boards, and power electronics; mass spectral biosignatures; signal processing paradigms; sampling and the sample interface for airborne, vapor and aqueous samples.

INTELLIGENT SYSTEMS FOR THE CHARACTERIZATION OF MICROORGANISMS FROM HYPERSPECTRAL DATA

Royston Goodacre(1*), Rebbeca Burton(1), Naheed Kaderbhai(1), Éadaoin M. Timmins(1), Paul J. Rooney(2) and Douglas B. Kell(1)

(1)Institute of Biological Sciences, University of Wales, Aberystwyth, Dyfed, SY23 3DA, U.K.
(2)Bronglais General Hospital, Aberystwyth, Dyfed, SY23 1ER, U.K.

When a pathogen is isolated in a microbiology laboratory, the time taken for subsequent culture for identification and susceptibility testing may delay the administration of the most appropriate treatment.

For routine purposes the ideal method for microbial characterization would have minimum sample preparation, would analyze samples directly (i.e. would not require reagents), would be rapid, automated and (at least relatively) inexpensive. With recent developments in analytical instrumentation, these requirements are being fulfilled by physico-chemical spectroscopic methods, often referred to as “whole-organism fingerprinting”. The most common such methods are pyrolysis mass spectrometry (PyMS), Fourier transform infrared spectroscopy (FT-IR) and UV resonance Raman spectroscopy. However, the interpretation of these multidimensional spectra has conventionally been by the application of "unsupervised" pattern recognition methods such as principal components (PCA), discriminant function (DFA) and hierarchical cluster (HCA) analyses. With "unsupervised learning" methods of this sort the relevant multivariate algorithms seek "clusters" in the data, thereby allowing the investigator to group objects together on the basis of their perceived closeness; this process is often subjective because it relies upon the interpretation of complicated scatter plots and dendrograms. More recently, various related but much more powerful methods, most often referred to within the framework of chemometrics, have been applied to the "supervised" analysis of these hyperspectral data, and arguably, the most significant of these is the application of intelligent systems based on artificial neural networks (ANNs).

A group of 60 bacteria commonly associated with urinary tract infection, or bacteriuria, were collected from the local hospital. All isolates were typed by conventional biochemical tests to belong to Escherichia coli (17), Proteus mirabilis (11), Klebsiella spp (4 K. oxytoca and 6 K. pneumoniae), Pseudomonas aeruginosa (10), and Enterococcus spp (12). All strains were grown axenically on nutrient agar and analysed by PyMS, FT-IR and dispersive Raman spectroscopies.

Direct visual analysis of these spectra was not possible, highlighting the need to use multivariate methods to reduce the dimensionality of these hyperspectral data. First the unsupervised methods of PCA, DFA and HCA were employed to cluster these organisms based on their spectral fingerprints, but none produced wholly satisfactory groupings which were characteristic for each of the five bacterial types. In contrast, for each of the spectroscopies, ANNs could be trained with representative spectra of the five bacterial groups so that isolates from clinical bacteriuria in an independent unseen test set could be correctly identified.

In conclusion, these results demonstrate that modern analytical spectroscopies, but only when combined with intelligent systems, can provide a rapid accurate microbial characterization techniques.

SESSION 3. AEROBIOLOGY

METHODOLOGY FOR THE GENERATION AND MEASUREMENT OF AEROSOLS

L. Paul
TSI, Inc., USA

Abstract not available.

AIRBORNE PARTICULATE SAMPLING AND COLLECTION: A REVIEW OF TECHNIQUES AND APPLICATIONS

Carl M. Peterson
SCP Dynamics, Inc., 7791 Elm Street, N.E., Minneapolis, MN 55432 USA

Aerosol, dust, fume, smog and bio-aerosol are but a few of the common and scientific terms synonymous to airborne particulate and which indirectly describes contaminates of the atmosphere. Regardless of what is called, the sampling and collection of particulate matter is of prime importance in ones ability to accurately define the quality of an environment and or to identify and quantify the hazardous potential of airborne contaminates. Per the subject presentation, sampler inlets and particle losses within a typical sampling train are reviewed. Collection of particulate matter by centrifugal, inertial and impaction principles are discussed.

Instrumentation which incorporates the sampling, collection, size classification and particle preservation within a single operation are presented. Instruments of this capability are of the virtual impactor or XM2 design and are currently recognized as instruments of choice by the Environmental Protection Agency and various military operations. Particulate collected or removed from the sample gas are typically presented for direct analysis to a single particle optical or UV counters or conditioned and/or preserved for subsequent analysis by wet chemical or microbiological means.

MEASUREMENT OF BIOLOGICAL AEROSOL IN AEROSOL CHAMBER

Z. Krocova, E. Henningson#, J. Krejci, M. Kroca
Institute for Immunology, Military Medical Academy, Hradec Kralove, 561 66 Techonin, Czech Rep.

#FOA Test
National Defence Research Establishment
S-901 82 Umea, Sweden

It was measured concentration of aerosol contained intracellular pathogen Francisella tularensis and distribution of particles in aerosol chamber "nose only" for mice. Concentration of culturable microbes was measured by the all-glass liquid impinger and 7-stage bioaerosol sampler with nutrient collection medium. The mass median diametr was measured by 4-stage cascade impactor according May. It was done by geometric standard deviation and counted log-normal distribution. All methods were used for performance of standard application of aerosol carried F. tularensis. This way of application of microbe will be used for immunization and immunology studies of intracellular pathogen, model F. tularensis.

SAMPLING OF BIOLOGICAL AEROSOLS: PHYSICAL COLLECTION EFFICIENCY AND MICROBIAL SURVIVAL

Sergey A. Grinshpun, Klaus Willeke, and Tiina Reponen
Aerosol Research and Exposure Assessment Laboratory, Department of Environmental Health, University of Cincinnati, P.O. Box 670056, Cincinnati, OH 45267-0056

While several dozen different techniques are currently used for the sampling of airborne microorganisms, the total and viable microbial concentrations measured by these techniques may differ significantly depending on the collection method used. Thus, there is increasing interest in understanding the physical and microbiological aspects of bioaerosol sampling and in developing reliable and accurate collection methodologies for airborne microorganisms. This paper presents the results of extensive laboratory evaluations of common bioaerosol sampling methods, such as impaction onto a solid or agar surface and impingement into a liquid. The physical collection efficiency and bioefficiency of several commercially available and newly-developed impactors and impingers have been determined with inert particles of typical bacterial range, 0.1 to 5 μm, and with several bacterial and fungal species. The effects of sampling velocity, collection medium, relative humidity and other parameters on the physical collection efficiency as well as on the microbial survival and recovery have been experimentally determined and analyzed. The findings obtained in this study contribute to the development, improvement and standardization of methods and techniques for bioaerosol sampling.

BIOLOGICAL AEROSOL MEASUREMENT WITH A FLUORESCENCE AERODYNAMIC PARTICLE SIZER: LOWER LIMITS OF DETECTION

Jim Ho
National Defence Canada, Defence Research Establishment Suffield, Box 4000, Medicine Hat, Alberta, T1A 8K6, Canada

The Fluorescent Aerodynamic Particle Sizer (FLAPS) is a unique technology able to simultaneously determine particle size and measure intrinsic fluorescence of airborne particles. It had been previously determined that the fluorescence of a single spore, excited at 340-60 nm, could be measured by flow cytometry. Based on these observations, a prototype instrument, based on an aerodynamic particle sizer, was designed to measure the fluorescence of aerosols via excitation with a CW UV laser. This first generation instrument was known as the FLAPS1. The FLAPS1 and the second generation instrument, FLAPS2, measure particle size, as well as intrinsic fluorescence characteristics of individual particles in an aerosol stream. Laser light at UV wavelength is used to preferentially excite aerosol material of a selected size range. The measured fluorescence signals represent intrinsic biological properties of the particles. By this method, it has been shown that inert particles like sand can be distinguished from biological particles like spores and vegetative bacteria. We have reported on the performance of FLAPS1 (1) used during international field trials in 1995 (2). FLAPS2 was designed and built to be more power efficient, occupy less space and be more portable. It was capable of transmitting analytical results and could be controlled via a radio modem which allowed it to function as a remote detector. This report describes some unique functional characteristics of FLAPS2 and its performance during international field trials designed to test the ability of biodetection systems under realistic field conditions. Results from these field trials suggest that this instrument is capable of practical limits of detection in the 10 Agent Containing Particles Per Liter of Air (ACPLA) region. FLAPS and FLAPS 2 have also been used to measure and characterize background aerosols which is important for the development of biodetection methods and alarm algorithms and this data will be discussed.

References:

1. P. Hairston, J. Ho, F. R. Quant. 1997 Design of an Instrument for Real-time Detection of Bioaerosols Using Simultaneous Measurement of Particle Aerodynamic Size and Intrinsic Fluorescence. J. Aerosol Sc. (In Press).

2. Boulet, C.A., J. Ho, L. Stadnyk, H.G. Thompson, M.R. Spence, G.A. Luoma, R.E. Elaine and W.E. Lee. 1996 Report on the Canadian Integrated Biological Detection System Trial Results of the Combined Joint Field Trials II, for Biological Detection. Defence Research Establishment Suffield SR 652 (Unclassified).

FIELD AND LABORATORY TESTING AT LIFE SCIENCES DIVISION, DUGWAY PROVING GROUND

Bruce G. Harper
Bio-Technology Branch, Life Sciences Division, Dugway Proving Ground, UT USA

U.S. Army Dugway Proving Ground (DPG) is located 80 miles southwest of Salt Lake City, in the state of Utah. As a vital part of the Department of Defense (DOD), Dugway is one of the major range and test facilities of the United States Army Test and Evaluation Command (TECOM), with mission responsibilities for conducting laboratory and field tests of components and/or systems used for Chemical and Biological Defense. The biological test mission is under the Life Sciences Division, and has responsibilities for Detection, Protection, Decontamination, Treaty Support, Counter-terrorism, and Contamination and Decontamination Survivability. DPG Life Sciences Division has developed two major capabilities, outdoor testing using biological simulants, and indoor testing using simulants or live agents of biological origin (ABO). Recent U. S. emphasis on developing and fielding a biological detector has led to significant increases in the number and types of laboratory and field tests. Field trials at Dugway utilize several outdoor test grids suitable for large scale test and evaluation of biological defense systems, and include the use of several different simulants (virus, bacteria, toxin), aerosol generators, aerosol collectors, and instrumentation for characterization of the aerosol clouds. Laboratory trials for testing biological collectors and detectors include both simulants and ABO materials. These tests are conducted under BL-2 and BL-3 conditions. The current facility, Baker Laboratory, will soon be replaced with a new facility, the Life Sciences Test Facility (LSTF). DPG is one of two DOD facilities with fully accredited BL-3 laboratories, which includes a large class 3 cabinet, in which detection devices may be challenged with ABO materials in an aerosol state.

This presentation will provide descriptions of both the field and laboratory biotesting at Dugway Proving Ground to give an understanding of how such capabilities are used.

SESSION 4: EMERGING TECHNOLOGY

DESIGN CONCEPTS OF FLOW CYTOMETERS FOR BACTERIA

Harald B. Steen
Institute for Cancer, The Norwegian Radium Hospital, Montebello, Oslo, N-0310 Norway

The arc lamp-based flow cytometer has some significant advantages as compared to laser-based systems, including a light source with a continuous spectrum, from which can be selected the appropriate wavelength for essentially any fluorescent dye used in bioscience, as well as optics which facilitates a small, rugged, shockproof and portable design. The main disadvantages are low excitation intensities at some wavelengths and lack of true dual focus capability. The present paper demonstrates how these shortcomings can be remedied. Excitation intensity can be enhanced by a factor of 50 by pulsing the light source so as to improve sensitivity to beyond that of most laser-based instruments. A novel optical configuration facilitates excitation in two separate foci with freely selectable wavelengths, and improves the capabilities of the light scattering measurement.

FIELD APPLICATIONS OF FLOW CYTOMETRY

Peter J. Stopa (1), Henrietta Kulaga, Patricia Anderson, and Michael Cain (2),
(1) Research and Technology Directorate, U.S. Army Edgewood Research, Development, and Engineering Center, APG, MD 21010-5423 USA
(2) Science and Technology Corporation, P.O. Box 7390, Hampton, VA 23666-0390

Flow Cytometry (FC) is a spectroscopic technique that allows one to interrogate the individual members of a sample population for information on size, shape, and specific chemical properties. FC has been the workhorse in the clinical hematology laboratory for sorting/typing blood cell populations. It has been proposed that the technique could serve as a possible detection/identification platform for biological agent detection and warning.

Preliminary results in the laboratory suggest that the technique can be very powerful. One may be able to differentiate among naturally occurring aerosols, live and dead organisms, specific chemical properties, and other properties. In addition, the instrument could serve as a possible biosensor platform for immunological and DNA probe-based detection systems.

This talk will focus on the applications of flow cytometry in the biodetection area, propose some strategies for biological detection, and present some of the laboratory and field data.

A PORTABLE FLOW CYTOMETER FOR THE DETECTION AND IDENTIFICATION OF MICROORGANISMS

Hazel M. Davey and Douglas B. Kell
Institute of Biological Sciences, University of Wales, Aberystwyth, WALES, U.K.

Flow cytometry can be used for the detection and identification of microorganisms (Davey and Kell, 1996) however traditional instrumentation is bulky and complicated to operate and align. These factors combine to make flow cytometry less than ideal for the field detection of biological agents.

Recently a new flow cytometer, the Microcyte, has been developed. The Microcyte is a battery-operated, portable instrument that is simple to use and needs no operator alignment and thus is well suited to field use. The Microcyte uses a 635 nm laser diode as the light source and we present data for a number of microorganisms stained with a variety of fluorescent stains amenable to excitation at this wavelength.

Davey H.M. and Kell D.B. (1996) Flow cytometry and cell sorting of heterogeneous microbial populations: the importance of single-cell analyses. Microbiological Reviews vol 60, pp. 641-696.

THE USE OF FLOW CYTOMETRY TO STUDY SULFUR MUSTARD'S (SM) MECHANISMS OF ACTION AND TO DEVELOP DIAGNOSTIC SCREENS AND THERAPEUTIC TREATMENTS

Henry Louis Meier
United States Army Medical Research Institute of Chemical Defense
Aberdeen Proving Ground, MD 21010-5425 USA

SM is a potent vesicating chemical warfare agent whose mechanisms of action have been studied for the last 80 years with little success until recently. Because humans react differently from animals to SM it has proved exceptionally difficult to study the biochemical mechanisms of SM-induced injury in humans. We developed a battery of in vitro assays that center around flow cytometry to study the pathology induced by SM in human tissues and to measure the efficacy of therapeutic regimens to prevent the SM-induced damage. These flow cytometry studies allowed us to investigate three aspects of SM exposure: 1. the mechanisms by which SM induces damage, 2. the efficacy of therapeutic interventions, and 3. the detection of SM exposures. This technique has enabled us to determine the time and concentration dependence of both SM-induced cell death and DNA damage using two different flow cytometric assay systems. Further, we have developed assays that have enabled us to screen >450 antivesicant compounds. This broad based screening resulted in the identification of a class of compounds that can prevent SM-induced cytotoxicity in human lymphocytes. The quantification of the assay allowed us to rank order the efficacy of the compounds and analyze structure-function relationships. At present, we are developing another flow cytometric based assay that will diagnose an individual's exposure to SM. These three flow cytometry techniques have applications to many classes of toxic compounds in the environment.

BIODETECTION TECHNOLOGIES IN ENVIRONMENTAL MONITORING

A. Clark
CBDE-Porton Down, USA

See the last page.

A SYSTEM FOR THE REMOTE CONTROL AND MONITORING OF SENSORS AND SAMPLING EQUIPMENT

H. Mottl
DYCOR, Ltd. USA

Abstract not available.

SESSION 5: DNA PROBES

EVALUATION OF 5' NUCLEASE FLUOROGENIC POLYMERASE CHAIN REACTION ASSAYS FOR IDENTIFICATION OF BIOLOGICAL AGENTS

E.A. Henchal[1], M.S. Ibrahim[1], R.S. Lofts[2], P. Jahrling1, J. Esposito[3], V.W. Weedn[4], M.A. Northrup[5], and P. Belgrader[4]

[1]U.S. Army Medical Research Institute of Infectious Diseases, [2]Armed Forces Radiobiology Research Institute, [3]Armed Forces Institute of Pathology, [4]Centers for Disease Control and Prevention, and [5]Lawrence Livermore National Laboratory USA

We evaluated 5' nuclease fluorogenic polymerase chain reaction assays with the ABI Prism 7700 (Perkin Elmer) and a microchip- based Miniature Analytical Thermal Cycler Instrument (MATCI) to identify Orthopoxvirus species and other biological agents. Both systems simultaneously detect PCR products during amplification by measuring shifts in fluorescence ratios of TaqMan probes as the hybridized probes are progressively degraded. The ABI Prism 7700 uses thermal cycler mechanics of the PE 9600 and charge- coupled device (CCD) camera fitted with optical fibers to capture and transmit fluorescence emissions from up to 96 individual reactions. Both high sample number throughput and highly precise quantitative analysis of nucleic acids are possible. The MATCI is a prototype, micro-fabricated, silicon-based thermal cycler with solid-state optical detection components and is presently configured for single sample analysis. In a blind study using DNA from four different orthopoxvirus species, both systems correctly identified one species (vaccinia virus) from the others (camelpoxvirus, cowpoxvirus and monkeypoxvirus) on the basis of a single nucleotide difference within the primer-binding sequence. The ABI Prism 7700 reached a discrimination threshold within 55 min, whereas discrimination was achieved within 30 min with the MATCI. However, the ABI Prism 7700 was at least four-fold more sensitive than the MATCI. The advantages and limitations of each system to identify biological agents in medical and environmental samples will be discussed.

USE OF TAQMAN, LIGHT CYCLER, AND CONFOCAL MICROSCOPY TO DETECT SPECIFIC PCR PRODUCTS

Vito G. DelVecchio
Institute of Molecular Biology and Medicine,University of Scranton, Scranton, PA 18510 USA

Certain amplicon-detection systems can visualize a specific nucleic acid sequence without gel analysis. The TaqMan system consists of primers for amplification of specific DNA sequences and a fluorogenic probe which contains both a reporter and quencher dye. During amplification the probe hybridizes with a target on the amplicon, and the 5' exonuclease activity of DNA polymerase hydrolyzes the probe. The separation of the reporter from the quencher results in an increase in the fluorescent emission of the reporter. This indicates the presence of the amplicon of interest. The Light Cycler™ allows real-time detection of nucleic acid amplification by measuring the increase in fluorescence due to the double-stranded DNA-specific dye SYBR® Green 1. The Light Cycler™ uses glass capillary tubes to contain 10 μl of amplification mix. This allows complete amplification and analysis in 10 - 15 minutes.

The presence of unculturable microorganisms from diverse sources can be investigated by examination of rDNA libraries. Such libraries are formed by the use of primers which will amplify rDNA genes found in all bacteria. Sequencing of each clone type permits identification or phylogenetic categorization of each organism by comparison with other rDNA sequences found in rDNA databases. Fluorophore-labeled primers specific for each rDNA (or bacterial type) can then be utilized to locate the bacteria in fixed histological sections by means of *in situ* hybridization and confocal microscopy.

SPECIFIC DETECTION OF MONKEYPOX VIRUS

H. Meyer[1], H. Neubauer[1], U. Reischl[2], C.K. Otterbein[1], J.J. Esposito[3], and E.-J. Finke[1]
[1]Institute of Microbiology, Federal Armed Forces Medical Academy, Muenchen, Germany
[2]Centers for Disease Control and Prevention, Atlanta, Georgia 30333, USA
[3]Inst. of Medical Microbiology and Hygiene, University of Regensburg, Regensburg, Germany

Smallpox was an important disease until its eradication in 1977. However, a naturally occurring relative of variola virus, monkeypox virus (MPV), is still endemic in the African rain forest. Sporadic human infections are seen clinically resembling smallpox. From 1970 to 1986, 404 cases of MPV infections (case-fatality rate 11%) have been reported by the WHO. MPV is a separate species within the genus *orthopoxvirus*. Whereas the differentiation of species has historically been achieved by biological properties the PCR technique now allows us to distinguish orthopoxviruses on the basis of sequences coding for the acidophilic-inclusion-protein (ATI) gene or the hemagglutinin gene. However, both assays use genus-specific consensus primers and therefore a differentiation relies on minor differences in size or by restriction enzyme length polymorphism of the amplicons.

In order to provide a more simple approach for a specific detection of MPV the ATI-genes of two strains (MPV reference strain Copenhagen; MPV isolate Gabun) were sequenced. In both strains ATI-genes were of identical length (2088 nucleotides) and highly conserved (homology 99.6%). A MPV-specific 8 bp deletion could be identified which allowed the selection of MPV-specific primers. Specificity of the PCR was successfully proved with DNAs from 18 MPV strains. There was no amplification with DNA of the other remaining orthopoxvirus species.

USE OF PCR FOR IDENTIFICATION AND DETECTION OF BIOLOGICAL AGENTS

H. Garrigue, G. Patra and V. Ramisse
Biology Department, Centre d'Etudes du Bouchet, 91710 Vert le Petit BP3 - France

PCR is a routine technique in many laboratories, especially in diagnostic labs for identification of viruses and bacteria. This technique can be a very useful tool for detection and identification of agents in the battlefield or in laboratories for confirmation of previous identifications. Some of the techniques which can be used are reviewed. Samples from the environment are essential to analyze, but if liquids and air do not make any problem, it is not the case of soil samples. Often inhibitors preclude amplification, even when special extractions are done. In this case, a strategy for analysis, different of simple direct or not PCR must be set. An example of a protocol used in our lab is presented. PCR can also be a tool for the control of labs, direct and associated environment. It will allow us to prevent the risk of contamination of samples to analyze. Finally, PCR could also be a tool for disarmament, especially now that more and more research is being done to distinguish strains of the same species. In the future it should be possible to differentiate strains coming from various countries and prove that a strain unusual to an area could have been introduced there intentionally.

IDENTIFICATION OF PATHOGENS USING SINGLE STRAND CONFORMATION POLYMORPHISM (SSCP) ANALYSIS

Netty D. Zegers and Myriam T.C. Offermans
TNO Prevention and Health, Division of Immunological and Infectious Diseases
P.O. Box 2215, CE Leiden

DNA-based detection methods become nowadays important tools for detection and identification of pathogens. The polymerase chain reaction (PCR) is one of them and has found widespread application. Analysis methods following PCR are multiple.

PCR and hybridization methods, however, need a huge set of pathogen-specific primers and probes for detection and identification of a large variety of microorganisms. To circumvent the need for a large number of primers or probes, a relatively simple and rapid non-denaturing nucleic acid gel electrophoresis procedure is currently adapted for this purpose. This procedure, the Single Strand Conformation Polymorphism (SSCP) technique is based upon electrophoretic separation of single-stranded DNA molecules via mobility shifts due to nucleotide sequence polymorphism within the sequence of interest. One set of a conserved primer pair can be used for PCR amplification of a DNA sequence enclosing genus-, species- or strain-specific sequences within a group of pathogens. Upon DNA denaturing of the amplicon into single strands, ssDNA is separated under native conditions. Each unique single-stranded sequence has its own conformation, resulting in a particular electrophoretic mobility, thus allowing identification.

Simultaneous identification of mixtures of amplicons is possible, being another advantage of SSCP, in contrast with sequencing. Multiplex PCR permits an increased number of pathogens identified within a single analysis. Combination of automated laser detection of fluorochrome-labeled amplicons coupled to a computer offers the possibility of generating a database of electrophoresis patterns facilitating identification.

Parameters influencing SSCP analysis will be discussed. Identification of orthopox viruses will be given as a demonstration of the SSCP technique.

RAPID METHOD FOR BACTERIAL COUNTING IN THE MILK BY USING BIOSENSOR BASED ON E.coli CELLS

Ignatov, S.G.
State Research Center for Applied Microbiology, Obolensk, Moscow Region, 142279 RUSSIA

Methods using biosensor based on E.coli cells for rapid bacterial counting in the milk have been developed. It is known that microorganisms produced lactate during their growth in the milk. Correlation between lactate concentration and bacterial enumeration in the milk was found using biosensor. To prepare lactate biosensor it is necessary to get bacterial cells with lactate activity. For induction lactate oxydase activity Escherichia coli cells were cultivated in minimal medium with lactate as C-sours. These cells were used for biochip creation. Biosensor based on such biochip was sensitive to lactate concentration in the milk. Simultaneous lactate detection by using biosensor and counting of bacterial colonies on the solid medium have been done to establish correlation between lactate concentration and bacterial enumeration in the milk. It was found that critical cell concentration (10 exp9 cells/ml of milk) determined the milk quality corresponds to 10 mM lactate.

PYROLYSIS-GAS CHROMATOGRAPHY-ION MOBILITY SPECTROMETRY DETECTION OF *BACILLUS GLOBIGII* (BG) AEROSOLS

A. Peter Snyder
U.S. Army Edgewood Research, Development and Engineering Center,
Aberdeen Proving Ground, MD 21010-5423 USA

A prototype pyrolysis-gas chromatography/ion mobility spectrometry (Py-GC/IMS) instrument was developed and field tested. The system combines a handheld GC/IMS device (Graseby/FemtoScan; model EVM II) with a platinum wire grid heater, a 1" dia. quartz fiber microfilter and a 60 liter per minute air pump. The IMS component is a modified Chemical Agent Monitor (CAM) vapor detector. The prototype Py-GC/IMS system can be operated in stand-alone mode with an air pump or in series with a particle concentrator. Fully automated collection/desorption/pyrolysis of aerosols and other particulate matter can be performed at repetition rates of up to 60 per hour by means of a special remote control and display software package (Hyphen). The prototype instrument has proved to be capable of detecting submicrogram quantities of Bacillus spores using picolinic acid and pyridine as biochemical marker compounds. Inherent background signals did not interfere with the biomarker signatures. The system also was shown to detect nerve agent simulants such as diethylmethylphosphonate (DEMP) simultaneously with that of the picolinic acid and pyridine spore biomarkers. The Py-GC/IMS system is viewed as a trigger device for biological compounds and retains the capability for chemical detection.

COMPARATIVE PERFORMANCE OF CONVENTIONAL AND ADVANCED MOLECULAR METHODS FOR ENVIRONMENTAL EXPOSURE MAPPING OF BIOLOGICAL AEROSOLS: VALIDATION USING LARGE-SCALE AERIAL APPLICATIONS OF BACILLUS THURINGIENSIS KURSTAKI-BASED COMMERCIAL BIOPESTICIDES.

V.L. Seligy[1,4], G. Douglas[1], J. Dugal[3], I. Otvos[2], G. Rousseau[3], A.G. Szabo[5], A.F. Tayabali[4] and K.van Frankenhuyzen[2]

[1]Environmental & Occupational Toxicology Division, Environmental Health Directorate, Health Canada, [2]Central (Ontario) and Pacific (British Columbia) Forestry Services, Natural Resources Canada, [3]Societe de Protection des Forets Contre des Insectes et Maladie (Quebec) and Universities of Carleton[4] and Windsor[5], Programs in Environmental Toxicology and Chemistry and Biochemistry

Biotechnology products that use natural or genetically engineered microbes as intrinsic ingredients are increasing in number, but methods and exposure/hazard databases for harmonized regulation of their use are largely under-developed. A case in point are the ´ +BT´ , products derived from fermentation of Bacillus thuringiensis subspecies which are related to, and used as models for exposure to pathogens, B.cereus and B.anthracis. Analysis of > 4000 publications reveals that ´ +BT´ , dominates the global biopesticide market, yet there is very little or no rigorous literature on product QC and methodology for quantifying occupational/bystander exposure, environmental persistence and health impact. Through federal-provincial alliances and support of the National Biotechnology Strategy (Industry Canada) we have conducted a multi-year laboratory/field assessment of several exposure/dosimetry monitoring methods directed against commercial BT product subcomponents (spores >1012/ litre product, enzymes, toxin-associated parasporal inclusion bodies and tracer dyes). This report summarizes the practical aspects (performance and cost) of conducting quantifications by conventional assays (microbe count, tracer dye detection) as well as molecular assays (quantitative bioreduction, protein and immunodetection, NA hybridization and PCR, scanning electron microscopy).

FUTURE MISSIONS TO MARS FROM AN EXOBIOLOGY POINT OF VIEW

Alexander V. Zakharov
Space Research Institute, Profsoyznaya, 84/32, Moscow 117810, Russia

One of the most perspective celestial body to search ancient or existing life is Mars. Although the Viking mission results may indicate that Mars has no life today, the possibility exists that Mars may hold the best record of the evidence that led to the origin of life. There is direct geomorphological evidence that in the past Mars had large amounts of liquid water on its surface. Atmospheric models would suggest that this early period of hydrological activity was due to the presence of a thick atmosphere and the resulting warmer temperature. From a biological perspective the existence of liquid water, by itself motivates the question of the origin of life on Mars.

Searching for ancient or existing life on Mars is one of the most important tasks for future robotics missions to this planet. The Mars-Together Program that is under the consideration now by NASA and the Russian Space Agency, implies delivery of a Russian lander with a rover on the Martian surface in 2001. The payload of the rover with a robotics arm will include some instruments what can provide data for exobiological interest.

BIOLOGICAL CONTAMINANTS OF THE FOOD AND HEALTH CARE OF THE MEMBERS OF THE ARMY OF THE SLOVAK REPUBLIC IN VARIOUS CONDITIONS OF ACTIVITY

Marian Smirjak, Peter Filip
Military Veterinary Institution, Kosice

Serious group of the natural and artificial contaminants present bacteriological means, toxins and viruses. There are references from the point of view of the importance of the ability to fight of the units, about the early and quick detection, as in areas of the permanent dislocation, also in the special areas and the operating locations of the members of the Army of the Slovak republic, about the perspective trends, related to the quantitatively rich activity even of the foreign units on the area of the Slovak Republic. A very important role from the point of view of possible biological contamination is the continual and sudden control of the civilian suppliers and producers. On the same level of importance is the protection of all of the water supplies in locations of activity of the troops from the point of view of their possible natural or artificial contamination.

THE OCCURRENCE OF ESCHERICHIA COLI O157:H7 IN FOOD OF ANIMAL ORIGIN IN POLAND

B. Wojton;, K. Kwiatek, H. Rozanska
Department of Hygiene of Food of Animal Origin, National Veterinary Research Institute, Polawy, Poland

Two hundred and ten samples of food products of animal origin were tested. The examined material included 61 samples of pork, 50 samples of beef, 25 samples of pork-beef grinded meat, 14-poultry meat, 6 samples of salami-like sausage and 54 samples of raw milk. The method used for detection and identification of pathogen was based on the multiplication of 25 g of samples of tested material in a selective enrichment broth. The obtained cultures were streaking out onto McConkey Sorbitol Agar (Oxoid). Purified, suspected colonies were biochemically tested by using Rapid 20E Test (BioMerieux) and then serologically, with E.coli 0157 Latex Test (Oxoid) and semisolid medium with H7 antiserum (Difco). The strains, which were identified on the basis of morphological, biochemical and serological features as Escherichia coli O157:H7, were isolated from 4 samples of beef (8,0%), 4 samples of grinded meat (16,0%) and 3 samples of raw milk (5,56%). This serotype was not isolated from any tested samples of pork, poultry meat and salami-like sausage. The results obtained suggest that Escherichia coli O157:H7 most frequently occur in beef and milk. The method used proved to be useful for the detection and identification this serotype E.coli in food of animal origin.

THE CONTROL OF THE INCIDENCE OF THE HETEROGENOUS SUBSTANCE RESIDUES IN BIO-AREAS AND IN THE FOOD CHAIN FROM THE ASPECT OF THE PROTECTION OF THE MEMBERS OF THE ARMY OF THE SLOVAK REPUBLIC

Jarosalv Garcar, Marian Smirjak
Military Veterinary Institution, Kosice

Military Veterinary Institution complexly carries out the long-term continual monitoring of the heterogenous substance residues in special areas under the competence of the Army of the Slovak Republic also in the food chain, from the primary production till the final food product and evaluates the dynamic records from the point of view of the quantitative activity of the individual monitored contaminants and proposes the effective measures for the normalization of the situation according to the particular legislative norms valid for this area. There are investigated new ways of the evidence and control of the admission of the new chemical contaminants to the ecosystems of the locations under the competences of the Army of the Slovak Republic and to the food chain.

TOXIC PLANTS IN THE COMPLEX OF ECOSYSTEM FOOD - HUMAN BEING

Jozef Skalka,
Institute of Experimental Veterinary Medicine, Kosice

Marian Smirjak
Military Veterinary Institute, Kosice

Poisonous plants undoubtedly belong to biological contaminants. Toxic plants may jeopardize health and life, not only of a man, but also of other populations in 3 ways:

1. Incidental presence in food: e.g. due to confusion of poisonous and non-poisonous plants or by mistake when manipulated with different plants

2. Deliberate addition to food or ingredients needed in food preparation.

3. Diversion in production ecosystems serving to food production of plant and animal origin.

Prevention seems to me the most important, first of all continuous control of flora in production areas and regions.

THE ROLE OF THE MILITARY VETERINARY INSTITUTION IN THE COMPLEXITY OF THE PROTECTION OF THE ENVIRONMENT, FOOD AND HEALTH CARE OF THE MEMBERS OF THE ARMY OF THE SLOVAK REPUBLIC

Jaroslav Bello, Marian Smirjak
Militarty Veterinary Institution, Kosice

Military Veterinary Institution as the specialized establishment of the military veterinary service of the Medical Administration of the Army of the Slovak Republic secures the protection of the ecosystems used by the army, by the preventive system of the complex continual monitoring, for the control of admission and presence of noxious agents of the bacterial, chemical and other origin, for the protection of food the chain until it reaches the consumer and aims at the search of the new, in field applicable means and methods of the detection of the biologicasl noxes and to the ways of their minimalization and/or elimination.

IMMUNOMAGNETIC SEPARATION FOR THE DETECTION OF SALMONELLA FROM FOOD

Andrzej Lidacki
Military Institute of Hygiene and Epidemiology, Pulawy, Poland

Immunomagnetic separation (IMS) has been shown to be a very functional tool for the isolation and extraction of bacteria from heterogeneous matrices. This technique employs magnetic particles coated with specific antibody to the capture of target cells.

In detection of *Salmonella*, IMS has been used as an alternative to traditional selective enrichment steps.

Fifty-one food samples were examined by Dynabeads® Anti-Salmonella (Dynal AS, Oslo) and selective enrichment. Immunoseparation gave similar numbers of true positives (60,8%) to the selective enrichment (58,9%). The overall agreement between the two methods was 90,2%.

It is possible to short about 24 hours the time of Salmonella detection by using IMS>

There is a great possibility in further development of this method. IMS linked with the new identification techniques, as well as PCR or DNA-hybridization can be very useful in the detection of pathbogenic strains of bacteria in food.

IMS is a relative inexpensive technique and does not require complicated apparatures. It is also convenient and not time-consuming.

PRELIMINARY STUDIES ON DETECTION OF PROTEINS OF CLOSTRIDIUM BOTULINUM BY IMMUNO-BLOT TECHNIQUE

Wieslawa Palec
Veterinary Research Center, Military Institute of Hygiene and Epidemiology, Pulawy, Poland

Beata Mizak, Department of Carnivora and Fur Animal, National Veterinary Research Institute, Pulawy, Poland

Bacteriological diagnosis of clostridium botulinum is difficult and time consuming. Seroneutralization test on mice is used as a diagnostic method. This test is not useful for identification of strains that have lost the ability to produce toxin, or if the amount of toxin produced is low. The application of immuno-blot technique for detection of structural proteins of Clostridium botulinum was the aim of presented studies.

The determination of structural proteins of 22 strains of Clostridium botulinum was made using 10% polyacrylamide separating gel and 4% polyacrylamide stacking gel in the discontinuous system described by Laemmli. Diluted samples were heated for 5 min at 95°C in reducing buffer (0.125 M. Tris HCl, pH 6,9; 3% SDS; 10% glycerol; 4% B-mercaptoethanol; 0,2% bromophenol blue), then run under current 50V in the stacking gel and 120V in the separating gel.

Proteins separated by SDS-Page were transferred electrophoretically (100V for 1,5 h) onto the nitrocellulose membrane (0,45 μm) by the method of Towbin et al. After transfer, gels were stained with Coomassie blue R-250 and membranes were blocked in saline buffered solution (pH 7.4) with 1% BSA (37°C for 1 h). After blocking, membranes were incubated with positive standard rabbit serum or negative rabbit serum diluted 1/50. The subsequent incubation was made with anti-rabbit IgG conjugated to alkaline phosphatase (Sigma) diluted 1/5000. The membranes were stained by the method of Harlow and Lane using bromochloroindodyl phosphate/nitro blue tetrazolium (BCIP/NBT).

All strains typed by seroneutralization test and immunodifusion test represented type B, as reference strain of Clostridium botulinum (366).

After staining the polyacrylamide gels, proteins typical for Clostridium botulinum were demonstrated. Specific antibodies reacted with proteins of molcular weight of 20 - 30 kD.

DETECTION OF POTENTIAL BACTERIAL WARFARE AGENTS BY PYROLITIC GAS CHROMATOGRAPHY

COL Jerzy Bzdega, LTC Roman Lakomy
Military Institute of Hygiene and Epidemiology, Warszawa

Pyrolysis gas chromatographic techniques have been applied to detection of potential bacterial warfare. Bacteria were investigated by gas chromatograph Chrom 5 with thermal pyrolyser. For acquisition and interpretation of microbial material gas chromatograph Chrom 5 and microcomputer IBM PC with AD/DA card online connected configuration were made. It is a basic system for acquisition and interpretation of chromatograms.

An algorithm and program called CIIRO make acquisition of chromatogram in 240 min. An algorithm and program BAZACHRO provides the following functions: recording currrent chromatogram, calculating standard chromatogram and comparing investigated chromatogram to standard chromatogram. The presented configuration and computer programs enable acquisition of analyzed chromatograms in real time.

Chromatograms of bacteria pyrolysis products were compared with standards and Pearson's correlation coefficients were calculated.

For comparing investigations *Salmonella typhimurium* was taken with plasmids Col I drd 2, Col I drd 7 and Escherichia coli with plasmids Col I drd 2, C 600 Nal^R, C 600 RP_4. In addition extra research was made from urine Escherichia coli, Streptylococcus sp, and Klebsiella sp.

DETECTION OF BACILLUS ANTHRACIS SPORES BY THE LUMINOMETRIC METHOD

M. Bartoszcze, H. Arciuch, J. Matras, K. Chomiczewski, M. Cieklinska, A. Bielawska
The Military Institute for Hygiene and Epidemiology, 24-100 Pulawy, ul. Lubelska 2, Poland

In the previous studies it was found that the luminometric method can be used for detection of Bacillus anthracis spores. An increase in the level of ATP was observed after the introduction of a combination of heat and a special medium. The main reason for this study was to examine the influence of different factors on the germination process.

The strain Sterne Bacillus anthracis was used in the study. Spores were obtained according to Titball and Manchee (1989). In order to detect bacterial ATP micro luminometer model 3550 and reagents produced by New Horizons Diagnostic Corporation (USA) were used. The results indicate the new possibilities of detecting Bacillus anthracis spores using luminometric techniques.

SOME ASPECTS ABOUT THE BIOLOGICAL MONITORING OF A WATER SUPPLY

Graziella Jula
Research & Eng. Institute for Environment, Spl. Independentei 294, sector 6, cod 77703
Bucharest 78, ROMANIA

The assurance of the quality of a water supply under the circumstances of some potential biological hazards can be performed by using the methods of biological monitoring.

These aspects are studied for a water source which is one of the most affected water supplies from our country, not only by pollution but also by hydroenergetical dams, with a high bacterial concentration, in comparison with current Romanian Standards.

A PRACTICAL STUDY OF THE RIVER COLENTINA

Mihaela Nicolae
Regia Autonoma Apele Romane (Romanian Institute of Waters), Splaiul Independentei
No. 294, sect.6, Bucharest ROMANIA

Surface waters, which represent proper environments for numerous micro-organisms different in type or number, function of physical and chemical proprieties of the water and of the geographical, climatological and biological conditions could be contaminated by air precipitation, by soil content and by residual waters.

The preserving of the quality of the surface waters (function of their destination) - in limits of some physical, chemical, biological and bacteriological parameters - represents a major concern for our society and is focused on decreasing the organized pollution sources and avoiding of pollution accidents.

This work is dedicated to the quality of water concerning hygienic and sanitary characteristics of the river Colentina, along with being arranged ten lakes used as swimming pools and for fun by the inhabitants of Bucharest. The determination of bacteriological indicators through methods of current analysis evidenced, for the period of the summer 1996, high levels of bacterians payloads.

The bacteriological results have been correlated with physical, chemical and biological indicators of the analysed water.The surveillance, from a bacteriological point of view, of the river Colentina, presents a major importance for securing the population's health, being known the fact that the ten lakes are favorite places for a large number of Bucharest and its outskirts inhabitants.

ION MOBILITY SPECTROMETRY AS AN IMMUNOASSAY DETECTION TECHNIQUE

A. Peter Snyder
U.S. Army Edgewood Research, Development and Engineering Center,
Aberdeen Proving Ground, MD 21010-5423

Conventional immunoassays employ a variety of techniques which usually have a spectrophotometric or fluorescence spectral method of detection to signal the presence of the targeted antigen in a suspect sample. As the concentration of the antigen is changed, a sigmoid-shape curve results from the response of the assay. A hand-held ion mobility spectrometer, which detects analyte ions at atmospheric pressure, was used to probe the ortho-nitrophenol (ONP) product vapor of an immunoassay reaction. Bacillus cereus organisms were employed in an enzyme-linked immunosorbent assay (ELISA) with the beta-galactosidase: ortho-nitrophenyl-beta-D-galactoside (ONPG) enzyme-substrate biochemical couple. ONP has an intense yellow color, and the ion mobility vapor responses were compared to that of the conventional spectrophotometric assay. Both detection techniques produced a sigmoid-shape curve characteristic of immunoassay experiments, and the bacterial detection limit with the ion mobility spectrometry technique was estimated at below 1000 cells for an 8 minute assay time. Thus, a commercial, hand-held chemical vapor detector is shown to have the potential of detecting the presence of microorganisms using an immunoassay format.

BIODIVERSITY OF BACILLUS SPP. POPULATIONS MONITORED BY FLOW CYTOMETRY

Elena Stabnikova
Department of Microbiology and Biotechnology, Ukrainian University of Food Technologies,
68 Vladimirskaya Str., Kiev 252017, Ukraine

Biodiversity of aquatic populations of Bacillus thuringiensis, B.licheniformis, and B.megaterium was studied by flow cytofluorometry. The distributions in size, DNA content, RNA and protein contents of the cells were measured by FACStarPlus flow cytofluotometer (Becton Dickinson). Such subpopulations of bacilli as exotrophic, endotrophic, anabiotic cells and cells within C,B, and D- periods of cell cycle were examined by flow cytofluorometry. These subpolulations greatly differ by survivability, toxigenicity, and pathogenicity. Therefore their distribution in the natural ecosystems may be applied for the monitoring of biological hazards.

The following data are some selected examples. Cells in bacterioneuston and in the bulk of water were in the same phase of DNA replication cycle. Cells in bacterioneuston were on the beginning stage of cell division cycle. They have small size and low content of protein. Anabiotic and endotrophic subpopulations are accumulated in the water-air interface. The theoretical model of cell cycle of bacilli and experimental data obtained from synchronous and asynchronous cultures show that periods C and I may be stochastically disposed within the division cycle of the individual cells, but a sum of C- and I-periods is equal to the generation time. All possible distributions on DNA content in the cells of bacilli were shown by the theoretical calculations and by experimental flow cytofluorometry analysis.

LUMINESCENCE STUDIES OF BACTERIA

D. Paterno and P. Coon
U.S. Army Edgewood Research, Development & Engineering Center
Aberdeen Proving Ground, MD 21010-5423 USA

Abstract not available.

CAPILLARY ELECTROPHORESIS / NUCLEIC ACID PROBE IDENTIFICATION OF BIOLOGICAL WARFARE AGENT SIMULANTS IN ENVIRONMENTAL MATRICES

Boulet, Camille A., Hung, Geoffrey , Bader, Douglas E. (1); Duck, Peter, Wishart, Paul, Lai-How, Angela (2).

(1) Defence Research Establishment Suffield, PO Box 4000, Medicine Hat, Alberta CANADA T1A 8K6

(2) (2) ID Biomedical Corporation, 8855 Northbrook Court, Burnaby, British Columbia CANADA V5J 5J1

The Canadian Forces require rapid, sensitive systems for identification of bacterial and viral biological warfare (BW) agents in environmental matrices such as air and water. The system must detect BW agents at extremely low concentrations with no false alarms and operate under battlefield conditions. Capillary zone electrophoresis with laser induced fluorescent detection (CE-LIF) is an attractive technology because of its ultra-low detection limits and relatively simple instrument design. Gene probes can be used to target signature sequences of BW agents for identification; Cycling Probe Technology (CPT) is a gene probe identification technique based on the use of target nucleic acid as a catalyst for the conversion of chimeric probe molecules to detectable products. In this work, a chimeric 5' $(DNA)_8(RNA)_4(DNA)_{16}$ 3' probe for *Bacillus globigii,* an anthrax simulant, was used. In a typical experiment , the 5' fluoresceinated DRES2A probe (10 fmoles/ μL) and synthetic target DNA (10^{-4}-10^{-7} pmoles/μL) were incubated at 65°C for 30 min in the presence of RNase H. A 1:10 dilution of CPT reaction mixture was then analyzed by CE-LIF. The intact probe and cleaved 5' fluoresceinated-DNA product were separated and detected using a Beckman P/ACE 5010 CE-LIF (ssDNA gel column, 7 cm L_d) in under 5 minutes. As low as 1attomole of target DNA in the CPT probe assay could be detected. This work demonstrates that a CE-LIF CPT assay can be combined to fulfil the objectives of an efficient, rapid and specific system for the identification of BW agents.

BIODETECTION TECHNOLOGIES IN ENVIRONMENTAL MONITORING

Adrian J. Clark
Detection Department Chemical and Biological Defence Porton Down, Salisbury, Wiltshire.

Chemical and Biological Defence Porton Down undertakes scientific research to ensure that the Armed forces of the United Kingdom are able to survive a chemical or biological attack, and continue to operate effectively afterwards. It provides broad band protective measures against the whole chemical and biological warfare spectrum, from classic chemical weapons through toxic industrial chemicals to biological agents. Detection forms an important activity and is concerned with the development of effective and reliable identification, monitoring and warning devices. These have been developed into robust, portable devices for use by non-specialists. Biological detection activities encompass aerosol science, particle monitoring, fluid dynamic modeling, biochemistry, microbiology, gene probe techniques and biosensor technologies. Facilities are also available for undertaking field trials and the safe handling of extremely hazardous materials. The military requirements are to develop compact, automated, on-line detectors which operate in real time. While these present some difficult challenges in biological detection, they are leading to some exciting new developments, with important applications in healthcare, environmental monitoring, detector calibration services, regulating drinking water quality and food hygiene.