NORTH ATLANTIC TREATY ORGANIZATION

SCIENCE AND TECHNOLOGY ORGANIZATION

AC/323(HFM-177)TP/552

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**STO TECHNICAL REPORT** **TR-HFM-177**

# Deployable Laboratory Applications of Nano- and Bio-Technology

(Applications de nanotechnologie et biotechnologie destinées à un laboratoire déployable)

Findings of Task Group HFM-177.

Published October 2014

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# The NATO Science and Technology Organization

Science & Technology (S&T) in the NATO context is defined as the selective and rigorous generation and application of state-of-the-art, validated knowledge for defence and security purposes. S&T activities embrace scientific research, technology development, transition, application and field-testing, experimentation and a range of related scientific activities that include systems engineering, operational research and analysis, synthesis, integration and validation of knowledge derived through the scientific method.

In NATO, S&T is addressed using different business models, namely a collaborative business model where NATO provides a forum where NATO Nations and partner Nations elect to use their national resources to define, conduct and promote cooperative research and information exchange, and secondly an in-house delivery business model where S&T activities are conducted in a NATO dedicated executive body, having its own personnel, capabilities and infrastructure.

The mission of the NATO Science & Technology Organization (STO) is to help position the Nations' and NATO's S&T investments as a strategic enabler of the knowledge and technology advantage for the defence and security posture of NATO Nations and partner Nations, by conducting and promoting S&T activities that augment and leverage the capabilities and programmes of the Alliance, of the NATO Nations and the partner Nations, in support of NATO's objectives, and contributing to NATO's ability to enable and influence security and defence related capability development and threat mitigation in NATO Nations and partner Nations, in accordance with NATO policies.

The total spectrum of this collaborative effort is addressed by six Technical Panels who manage a wide range of scientific research activities, a Group specialising in modelling and simulation, plus a Committee dedicated to supporting the information management needs of the organization.

- AVT Applied Vehicle Technology Panel
- HFM Human Factors and Medicine Panel
- IST Information Systems Technology Panel
- NMSG NATO Modelling and Simulation Group
- SAS System Analysis and Studies Panel
- SCI Systems Concepts and Integration Panel
- SET Sensors and Electronics Technology Panel

These Panels and Group are the power-house of the collaborative model and are made up of national representatives as well as recognised world-class scientists, engineers and information specialists. In addition to providing critical technical oversight, they also provide a communication link to military users and other NATO bodies.

The scientific and technological work is carried out by Technical Teams, created under one or more of these eight bodies, for specific research activities which have a defined duration. These research activities can take a variety of forms, including Task Groups, Workshops, Symposia, Specialists' Meetings, Lecture Series and Technical Courses.

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Published October 2014



ISBN 978-92-837-0208-5

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# Deployable Laboratory Applications of Nano- and Bio-Technology

## (STO-TR-HFM-177)

# Executive Summary

The expeditionary nature of the North Atlantic Treaty Organization (NATO) Response Force requires deployable laboratory capabilities leveraging advances in nano/bio-technology. As such, the NATO Science Technical Organization Panel on Human Factors and Medicine chartered the research technical group (HFM-177 RTG) to study the "Deployable Laboratory Applications of Nano- and Bio-Technology" with a focus on deployable NATO CBRN laboratory advanced technologies. The goals were:

1) To survey deployable laboratory designs, construction and materials;
2) Analyze existing instrument technology and procedures;
3) Analyze emerging nano/bio-technology for instrument acquisition; and
4) Integrate existing and emerging technologies into a deployable laboratory design.

Over 20 representatives from eight countries participated from the Republic of Georgia, Turkey, Israel, the Czech Republic, United Kingdom, United States, France and Germany. Each country discussed their CBRN deployable laboratory capabilities and challenges. The HFM-177 RTG noted the rapidly changing evolution of analytical technologies, the different roles and mission types each country has for its specific laboratory assets, and the varying levels of funds each country allots to maintain/improve laboratory operations. When collected together, these variances provide a diverse set of applications and technologies to address multiple levels of mission requirements, if agreements are placed and leveraged by NATO forces. Consequently, the group decided that a point-in-time survey of the NATO laboratories' capabilities, that would be used to narrow to a best standard set of instrument technologies would not be the appropriate approach. Instead, the RTG recognized the different approaches each country took to develop their deployable laboratory provided greater options for NATO to customize the level of response required. The team decided not to focus on a single NATO laboratory, but instead focus on providing knowledge to HFM on each country's asset strength. The survey found state-of-the-art technical advances employed in current laboratories that allow NATO to best respond with a customized response team based on the threat scenario.

This RTG consisted of highly motivated, exceptionally collaborative, and extremely knowledgeable country representatives that determine great advantages in discussing each other's capabilities and future directions. Topic discussions allowed for a much broader equipment and methodologies evaluation that would have been difficult for a single country to assess in breadth. Many countries' lessons learned were directly applicable to most others deployment laboratory activities. Each country's representatives agreed to continue to have frequent, open communications to address their ever-changing organization's field laboratory needs and emerging equipment usage to facilitate NATO needs.

The HFM-177 RTG effort was a great success. We recommend these results be forwarded to the NATO Army Armaments Group, Joint Capability Group on CBRN Defence, and the Sampling and Identification of Biological, Chemical and Radiological Agents sub-group for further consideration and development.

# Applications de nanotechnologie et biotechnologie destinées à un laboratoire déployable

## (STO-TR-HFM-177)

# Synthèse

La nature expéditionnaire de la force réaction rapide de l'Organisation du Traité de l'Atlantique Nord (OTAN) nécessite des capacités de laboratoire déployable exploitant les progrès des nanotechnologies et biotechnologies. A cet effet, la Commission sur les facteurs humains et la médecine de l'Organisation pour la science et la technologie de l'OTAN a mandaté le groupe de recherche et de technologie (RTG HFM-177) pour étudier les « Applications de nanotechnologie et biotechnologie destinées à un laboratoire déployable » en se concentrant sur les technologies perfectionnées pour un laboratoire NRBC déployable de l'OTAN. Les objectifs étaient les suivants :

1) Etudier les modèles, la construction et les matériaux de laboratoire déployable ;
2) Analyser les procédures et la technologie des instruments existants ;
3) Analyser les nanotechnologies et biotechnologies émergentes pour l'acquisition par les instruments ; et
4) Intégrer les technologies existantes et émergentes dans un modèle de laboratoire déployable.

Plus de vingt représentants de huit pays – République de Géorgie, Turquie, Israël, République tchèque, Royaume-Uni, Etats-Unis, France et Allemagne – ont participé au groupe de recherche. Chaque pays a présenté ses capacités de laboratoire NRBC déployable et les problèmes rencontrés. Le RTG HFM-177 a remarqué l'évolution rapide des technologies d'analyse, les différents types de rôles et de mission que chaque pays attribue à ses ressources spécifiques de laboratoire et les niveaux variables de financement alloué pour maintenir ou améliorer l'exploitation des laboratoires. Ces cas constituent un ensemble varié d'applications et de technologies pouvant répondre à de multiples niveaux d'exigence en mission, si des accords sont passés et exploités par les forces de l'OTAN. Par conséquent, le groupe a décidé qu'il n'était pas approprié de mener une étude ponctuelle des capacités de laboratoire OTAN pour déterminer le meilleur jeu standard de technologies instrumentales. A contrario, le RTG a reconnu que les différentes approches adoptées par chaque pays pour développer son laboratoire déployable offraient une plus large palette de possibilités à l'OTAN pour personnaliser le niveau de réponse requis. L'équipe a décidé de ne pas se concentrer sur un seul laboratoire OTAN, mais de s'efforcer d'informer le HFM sur les avantages comparés des solutions adoptées dans chaque pays. L'étude a découvert des avancées techniques, employées dans les laboratoires actuels, qui permettent à l'OTAN de réagir au mieux avec une équipe personnalisée d'intervention à partir d'un scénario de menace déterminé.

Le présent RTG se composait de représentants nationaux fortement motivés, travaillant main dans la main et extrêmement bien renseignés, qui ont décidé qu'il y avait de grands avantages à discuter des capacités et des futures orientations des uns et des autres. Les discussions thématiques ont permis une évaluation bien plus large de l'équipement et des méthodologies que ce qu'un seul pays aurait pu réaliser. Les enseignements de nombreux pays étaient directement applicables à la plupart des activités de déploiement de laboratoire des autres. Chaque représentant national a accepté que des communications fréquentes et ouvertes aient lieu pour répondre à l'évolution permanente des besoins de son organisation en matière de laboratoire sur le terrain et faire part de l'utilisation de l'équipement émergent afin de satisfaire aux besoins de l'OTAN.

Les efforts du RTG HFM-177 ont été couronnés de succès. Nous recommandons que ces résultats soient transmis au groupe armements armée de terre de l'OTAN, pour le groupe traitant de la capacité interarmées sur la défense NRBC ainsi qu'au sous-groupe d'échantillonnage et identification des agents biologiques, chimiques et radiologiques, afin qu'ils soient approfondis et développés.

# Chapter 1 – FRAMEWORK AND ACCOMPLISHMENTS

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## 1.1 BACKGROUND

### 1.1.1 NATO Needs and Committee Charter

The NATO Response Force provides a high-tech, flexible, rapidly deployable, interoperable and sustainable force, including land, sea, and air elements, capable of carrying out the full range of Alliance missions. The development of this high-readiness force serves as a catalyst for promoting capabilities improvements and ensuring continued transformation to meet evolving security challenges with greater interoperability for the Alliance military. The NATO Response Force requires a Deployable Laboratory to serve as another key element to create full readiness and ensure mission success of NATO operations and Defence Against Terrorism (DAT).

Consequently the NATO Army Armaments Group (NAAG), Joint Capability Group on CBRN Defence (JCGCBRN), Sub-group on Sampling and Identification of Biological, Chemical, and Radiological Agents (SIBCRA SG) identified the need for a Research Technology Group (RTG) as follows. "This RTG group will define the elements of a field-forward NBC laboratory and its full capabilities to aid in the analysis of samples received by JCGCBRN. The STANAG 4632 establishes the standards of proficiency for NBC deployable Analytical Laboratories (NBC-AL). This NBC-AL will be able to operate across the full spectrum of military land, air, and maritime operations. These operations may range from local security tasks in a relatively benign area to completely cross the operational spectrum for full collective defence. Crisis Response Operations may range from Peace Support Operations to Alliance Combat Operations. The NBC-AL will be capable of deploying as a whole, or in components, with missions tailored to the assessed threat and will be on 5 days' Notice-To-Move (NTM) according to NRF Readiness States."

Further, the Human Factors and Medicine Panel determined that the expeditionary nature of the NATO response force required a deployable laboratory that utilized advanced biotechnology. In 2007, Exploratory Team-066 staffed by responding country experts from the Czech Republic, Spain, Netherlands and the United States meeting in Paris tackled the task of defining specific research and technology areas necessary to develop a deployable laboratory capable of conducting theatre-level, health threat surveillance. The exploratory team identified four topics to address:

1) Deployable laboratory design, construction and materials;
2) Analysis of existing instrument technologies and procedures;
3) Analysis of emerging nano-/bio-technology for instrument acquisition; and
4) Integration of existing and emerging technologies into a deployable laboratory product.

The NATO Army Armaments Group, Joint Capability Group on CBRN Defence, and the Sampling and Identification of Biological, Chemical and Radiological Agents sub-group were all suggested recipients of this research and technology effort.

To that end, the NATO Research Technical Organization Panel on Human Factors and Medicine at the spring meeting 2008 chartered the Research Technology Group-177 (HFM-177 RTG) to study the "Deployable Laboratory Applications of Nano- and Bio-technology" with a focus on deployable NATO CBRN laboratory advanced technologies. At that time, the Czech Republic, Georgia, Germany, Hungary, Netherlands, Spain, Sweden, Turkey and the United States were Nations that were invited and/or identified as willing to participate.

### 1.1.2 Benefits to the Military

The North Atlantic Treaty Organization mission requires that forces be able to execute missions in Chemical, Biological, Radiological or Nuclear (CBRN) warfare environments. Operational success in such inhospitable conditions requires the earliest knowledge of the threat, region of use, active deployment, and if personnel were involved, the timely administration of preventative and curative medical responses in order to maintain the human force and provide enhanced protection of personnel. It is certain that these operational requirements both strategic and tactical combined with the effective use of life-saving measures are best applied using a deployed, dependable environmental surveillance asset with quick, reliable pre-clinical screening. Only this level of asset would provide the agility for the earliest, potentially individually-titrated administration of medical support.

The military force is currently transforming into a more responsive, deployable, agile, versatile, lethal, survivable and sustainable force (Future Force). Medical forces will have to be efficient, effective and capable of supporting the full spectrum of military operations. There exists a significant increase in novel emerging nano-/bio-technology science created coupled to advance technology development that when aligned and captured will create new and revolutionary medical systems. For the medical technology community to exploit these advances in science and technology and achieve significant field-forward gains, the technical and science research communities are required to be engaged in a coordinated effort to apply existing advances for combat support effectiveness en route to the Future Force Warrior. Specifically, prospective NATO medical applications of present nano-/bio-technology include advanced health and fitness monitoring, high-resolution imaging, new environmental sensor platforms, chemical/biological sensors, sensor networks, battle and human-centric data fusion and storage, and soldier therapeutics. Nevertheless, there are others areas where nano-/bio-technology development is needed:

- **Sensors:** Diagnostic and detection kits (gene-chips, protein-chips, lab-on-chips, etc.);
- **Electronics and Computing:** Bio-molecular hybrid devices for detection (arrays, biochips), bio-computing (biological models, bio-data treatment);
- **Materials:** Accurate monitoring device (biomarkers), self-decontaminating surfaces, selection of environmental ruggedized/resistant construction materials;
- **Logistic:** Miniaturization of biological devices and systems for lowest footprint (micro-electrical mechanical-based systems, nano-technologies); and
- **Diagnostics:** Novel discovery and use of multi-analyte technologies such as genomics, metabonomics, proteomics-based analysis and specific derived sub-platforms of marker monitors.

## 1.2 OBJECTIVE

The Exploratory Team-066 establish that the RTG should consider the following menu of topics regarding the detailed design and capabilities of a deployable laboratory, and application of equipment as guidelines for review of emerging nano-/bio-technologies and application to analytical and diagnostic procedures:

a) Determine the structural features/characteristics of the deployable laboratory capability, define:
   i) Fixed or mobile deployable unit.
   ii) Dimensional requirements:
      1) Transportable by air; and
      2) Adequate space for equipment/work.
   iii) Delivery by multiple means (air-droppable, tracked, etc.).
   iv) Minimal logistics/maintenance/supportability requirements.
   v) Versatile external power capability.
   vi) Adequate internal power requirements to support all instruments simultaneously.
   vii) Emergency power capability for escape.
   viii) Deployable to multiple environments/terrains.
   ix) Hardened/passive defence.
   x) External sensor capability for biological, chemical and radiological.
   xi) Easily decontaminated (both external and internal; rapid internal decontamination capability).
   xii) Manning/personnel requirements.
   xiii) Design for threat-based instrument modularity.
   xiv) Design for technological and tactical upgradeability (ex. Remote detection of biomarkers in soldiers).
   xv) Automate and remote capabilities (contained and segregated).
   xvi) Longevity of storage (pre-deployed) and operation in theatre.
   xvii) BSL-2/3+ Capable.
   xviii) External sample transfer portals (to containment and/or BSL-3 sections).
   xix) Secure internal and external communication capabilities (radio, SIPR, telecom).
   xx) System of internal engineering controls (NBC COLPRO protection (air filtration and air-conditioning Systems): HEPA, active carbon filters, positive/negative pressure, etc.).
   xxi) Apply applicable ergonomic design.
   xxii) Assure ability to use in a hot (radiological)/contaminated (BSL-4) zone.

b) Characterize and identify the deployable laboratory screening technologies:
   i) Equipment:
      1) Optimize existing equipment for type of sample (environmental or clinical), agent (chemical, biological, radiological), and dimensional restrictions;
      2) Recommend emerging technologies;
      3) Maximize use of ruggedized equipment technologies;
      4) Capable of air transport and insertion;
      5) Define transport limitations/problems;
      6) Maximize automated, self-testing/diagnostic, high through-put and rapid analyses;

7) Minimize sample manipulation (whole-sample analytical capability: time save, eliminates contaminated waste generation, eliminates sample loss, promotes safety);
8) Minimize use of fluid dependent equipment technologies and perishable consumables;
9) Focus on equipment with minimal waste generation or creation of integrated waste management;
10) Maximize resizing and application/selection of commercial-off-the-shelf technologies: Max use modular equipment in service;
11) Able to be decontaminated; and
12) Lowest power draw/supply requirements.

ii) Personnel:

1) Number minimum needed to man, maximum for highest alert status;
2) Specialty type;
3) Training requirements; key to assure quality of assay completion and full lab functionality in all threat and theatre environments;
4) Physical characteristics of individuals (height, weight, blended in ergonomic design); and
5) Consider completely automated operations.

iii) Procedures:

1) Recommend use of standardized procedures for sample collection, sample treatment, identification, and decontamination methods for deployable laboratory;
2) Evaluate, develop and validate novel/emerging analytical chemistry, biotechnology/ molecular biology, and nanotechnology integrated procedures;
3) Establish quality control, instrument function, result validation, and laboratory environmental control procedures (may include remote data validation);
4) Maximize use of automated procedures;
5) Alternative procedures (redundancy for procedure and sample assay assurance);
6) Define operation time and consider through-put limitations (the number of the samples per day);
7) Accepted restrictions in lab work, accepted limited sample preparation and identification capability, defined samples types to analyse (air, soil, water, liquid); and
8) Ensure procedures minimize fluid use.

iv) Data Treatment:

1) Real-time broadcast of data to reach-back expert laboratory;
2) Establish theatre “hardened” archive system;
3) Evaluate commercial-off-the-shelf clinical laboratory software (ex. Specialized Laboratory information software, LIMS);
4) Fully interface instrumentation, where possible, with laboratory information system; and
5) Establish stand-alone bioinformatics databases and procedures to regularly update database.

## 1.3 MEETINGS

### 1.3.1 Meeting at Edgewood Chemical Biological Center in April 2009

The HFM-177 RTG team held its inaugural meeting at Edgewood Chemical Biological Center (ECBC) in Edgewood Maryland (USA) in April 2009. Only delegates from Georgia, the Czech Republic and United States attended; the Germany representatives having planned on attending could not make this meeting date. Despite a low number of country representatives in attendance, the meeting was productive for review of both US and the Czech Republic's substantial science and engineering investments and provided a current review of deployable constructs for field laboratories and work environments. Georgia presented their position on biological agents and levied their strong support to work together to the produce deployment response applications and hardware. The meeting was opened by a welcome from Dr. Raymond Mastnjak, ECBC that was followed by a presentation from Dr. John Wade as RTG mentor who presented an RTO overview briefing. The remainder of the meeting was chaired by Dr. Schlager who followed Dr. Wade and provided a HFM-177 RTG positional briefing from the ET-066 data and the mission of the RTG. The delegates developed a professional rapport, toured multiple facilities within ECBC CBRN facilities and observed the design construction of mobile biological response laboratories utilized in the United States.

At this meeting, the delegates identified a major impediment to fulfilling the HFM-177 RTG mission. The main goal of HFM-177 RTG was to evaluate the rapid emergence of bio-and nano-technologies in NATO rapid response laboratories. However, it was quickly realized that there is no single, standard NATO response laboratory that could be used as framework to best evaluate insertion of novel technologies. The first priority objective for the RTG then became to identify the current capabilities of each Nation's laboratory to best suit these missions and determine knowledge and lessons learn to share among the Nations.

### 1.3.2 Meeting in Munich, Germany in October 2011

The second HFM-177 RTG meeting occurred in October 2011 and was hosted by the Chair with activities well supported by LtCol. Roman Woelfel in Munich, Germany. Seventeen representatives from seven countries met for one and a half days (October 29-30); immediately following the 2011 Medical Biodefence Conference, which had a session focused on deployable laboratory facilities and outbreak investigation teams. Dir. Schlager, the NATO HFM-177 chair, opened the RTG meeting with a review of the general concepts and rational for the RTG. The delegates from each country then gave formal presentations on:

1) Deployable laboratory design and construction;
2) Existing; or
3) Emerging technologies utilized in deployable labs; or
4) Product integration.

Raymond Mastnjak (United States, Army ERDEC) and Libor Pisa (Czech Republic, Central Military Health Institute) each delivered talks describing their country's large, transportable, self-contained deployable laboratories presently fielded by their respective organizations. These assets shared common themes, such as compartmentalized, hard-sided container or tent-based construction, as well as the ability to process and identify samples under BSL-3+ conditions. In contrast, Roman Woelfel (Germany, Bundeswehr Institute of Microbiology) and Daniel Parzy (France, Institut de Recherche Biomédicale des Armées) presented their country's rapid deployment responsive, modular, Pelican case-based mobile labs that met commercial airline luggage restrictions, allowing rapid material and human transportability to populated sites. Such lightweight laboratories allow small, highly specialized teams to rapidly deploy and provide environmental and diagnostic analyses to many important world threat areas to include even austere outbreak locations. Key to the effectiveness of this style for these two countries and the approach for all other laboratories is having very well trained technicians that can respond to the need quickly and can perform difficult biotechnical

procedures flawlessly, even in the most difficult environmental conditions. Gurer Budak (Turkey, Gazi University) presented a spectrum of advances in the field of nano-technology with an emphasis on medical applications, while Robert Marks (Israel, Ben Gurion University) presented his research on the development of a hand-held, fiber-optic nano-sensor device for detection applications. The BIOMEDAC Chair, Francois Thibault (France, Institut de Recherche Biomédicale des Armées), also presented his committee's progress. The meeting concluded with a general discussion on:

1) Whether the deployable NATO laboratory would be utilized for environmental surveillance following a biological warfare attack or for outbreak diagnosis and mitigation;
2) The training and educational requirements of response team members;
3) The amount of time necessary for the laboratory to be deployable and useful for response using military or commercial air transport;
4) The potential need for large, transportable, self-contained laboratory facilities; and
5) The importance of knowing regional capabilities and possessing clearly defined CONOPs prior to NATO asset deployment.

## 1.4 SURVEY DEVELOPMENT

Based upon detailed discussions at the second meeting in Munich, three key HFM-177 committee members, Drs. Woelfel, Thibault and Mastnjak volunteered to develop a comprehensive, laboratory survey to disseminate not only among the committee members but also to other NATO Nations that could not attend. The survey addressed the specific objectives developed in the charter and other concerns that evolved from the two meetings (see Annex B). To ensure optimal opportunity for our NATO Allies to receive and complete the survey, we sent the survey through two routes; one through the Human Factors in Medicine Panel Executive and second pathway to the committee members' personal contacts in other NATO Nations that did not attend.

The following countries completed the survey:

- The Czech Republic, Central Military Health Institute;
- Germany, Bundeswehr Institute of Microbiology;
- France, Medical Health Services;
- Turkey, Nano-medicine and Advanced Technologies Research Center; and
- Unites States of America, the 20$^{th}$ Support Command, CBRNE.

Table 1-1 lists the summarized finding from the survey. Our Panel pointed out the benefits and disadvantages of a highly mobile verses transportable, fixed-site response laboratory. Briefly, the highly mobile laboratory can deploy and function more rapidly but does not have the more definitive and diverse CBRN tests compared to those within the larger fixed labs. The Panel noted that both have distinct purposes in CBRN rapid response. Because each country has its own defined mission with allies in various world regions and government funding, the range and purposed target for lab deployment assets varies with each country's laboratory functions. Most of all functional needs are based upon the inherent country's capabilities, requirements and interests throughout the world. We believe this diversity and mission variance within alliance countries provides minimally a NATO deployed laboratory approach addressing at least two laboratory designs: one a very rapid expeditionary type lab, and the other, a harden, in-place lab design for longer, more CBRN complex deployment missions. We believe both designs with potential modifications for increased agility should be readily available for NATO leadership to best customize a response team, based on the specific needs from a CBRN threat or incident.

**Table 1-1: Laboratory Capability Surveys.**

| | Czech Republic | | Germany | France | Turkey | United States |
|---|---|---|---|---|---|---|
| | **Deployable Biological** | **Mobile Epi-Hygienic** | | | | **20th SUPCOM (CBRNE)** |
| *Permanent Field-Forward Laboratory* | Y | | Y | N* | Y | Y |
| *Mobile Medical Laboratory* | Y | Y | Y | | | |
| *Responsible for the Technical and Operational Management* | Medical Service | Medical Service | Medical Service | Medical Service | CBRN Defence | Medical Service |
| *Laboratory Platform* | Deployable 5 Standard ISO 1C Container (L: 6058 mm x W: 2438 mm x H: 2200 mm) | | Deployable: 15 packages (580 x 440 x 330 cm, 30 kg) | 0,49 m3-90 kg; 0,49 m3-65 kg; 0,35 m3-78 kg; 0,35 m3-80 kg; 0,22 m3-53 kg (this last package is the compressor cooler); 5 | Self-Mobile | Self-Mobile and Deployable: LMT V-Light Lab (LMEL) & MMTV for Heavy Lab (HMEL) |
| *Transportable by* | Truck, Airplane (civ/mil) Ship or Cargo Train | | Airplane (civ/mil) Ship | Airplane (civ/mil) Ship | Truck or Plane | Truck, Mil Plane, Sealift |
| *Set-Up Time at Site of Operation* | 72 hrs | | 2 hrs | 1.5 hrs | 20 min | LMEL 4 hrs; HMEL 36 hrs |
| *External Supply Requirements* | Water, Diesel Oil for Electric Generator Running, Diagnostic and Other Laboratory | | Car Battery (Electrical Power Conversion 12V) | Fuel for Generator | Generator and Water | Water and Fuel |
| *Operational Time Without External Resupplies* | 2 – 3 days | | 3 days | 2 – 3 weeks | 3 days | 3 days |
| *Scheme for Ensuring Permanent Diagnostic Capabilities (on call or duty team)* | Y | | Y | | Y | Y |
| Diagnostic Tests | B-Agents of CDC List, Categories A and B | | Suspected B-Agent Outbreaks | | | Suspected B-Agent Outbreaks |
| Capable of Serving Laboratory or Hospital Emergencies | Y | | N | N | N | N |

| | Czech Republic | | Germany | France | Turkey | United States |
|---|---|---|---|---|---|---|
| | **Deployable Biological** | **Mobile Epi-Hygienic** | | | | **20th SUPCOM (CBRNE)** |
| Intended to Respond to Specific Demands from a Surveillance System | N | | Y | | | Y |
| National or Regional Center that Assesses the Emergency and Need for Laboratory Response | Y | | Y | | Y | Y |
| Duty Time | On alert ≤ 5 days after incident | | 24/7 | | 24/7 | 24/7 |
| Network Duty System | NATO CBRN | | Bundeswehr Medical CBRN Task Force | | Y | Y |
| Number of People Involved | 12 | | 4 | 3 | 3 | Needs Dependent |
| Scientists | Microbiologist (MD, VMD, Doctor of Sciences | | Clinical Microbiologist/ Virologist (MD, PhD) | PhD/MD | 1 | CWA and BWA Analysis, Infectious Disease / MS, PhD, MD or DVM |
| | Epidemiologist (MD) | | Veterinarian Microbiologist (DVM) | | | |
| | Veterinary Epidemiologist (VMD) | | Molecular Biologist (PhD) | | | |
| Technologists | Electrician – Engineer | | Licensed Medical Technicians | Biological Technologist | 2 | CWA and BWA Analysis / BS, MS or PhD |
| | Power Generator Operator – Engineer | | | | | Quality Manager and Laboratory Operations Manager |
| | Machine Operator – Engineer | | | | | |
| | Laboratory Technician | | | | | |
| Biological Agent Testing | **P: PCR; S: Serological; M: Microscopy; C: Culture** | | | | | |
| Abrin | | | P,S | | | |
| Aflatoxins | | | P,S,M | | | |
| B. pseudomallei | | C | P | P | | P,C |
| Bacillus anthracis | S,M,C | P,S,M,C | P,S,M | P | C | P,S,C |
| Botulinum Toxine | S | P,S | P,S | S | | P,S |

| | Czech Republic | | Germany | France | Turkey | United States |
|---|---|---|---|---|---|---|
| | Deployable Biological | Mobile Epi-Hygienic | | | | 20th SUPCOM (CBRNE) |
| Brucella spp. | S | P,S | P,S | | | P,S,C |
| Burkholderia mallei | C | C | P | P | | P,C |
| CCHF virus | | | P,S | | | P |
| Chikungunya virus | | | P | S | | P |
| Clostridium perfringens toxin | | | | | | |
| Coxiella burnetii | S | C | P | | | P,S |
| Eastern-Equine-Encephalitis virus | | | P | | | P,S |
| Ebola virus | | | P | | | P |
| Escherichia coli | S,M,C | S,M,C | P | | C | |
| Flaviviruses | | | P,S | | | P |
| Francisella tularensis | S | P,S | P,S | P | | P,S,C |
| Hantaviruses | S | S | P,S | | | P |
| Influenza virus | S | S | P,S | | | P |
| Junin virus | | | P | | | |
| Lassa virus | | | P | | | P |
| Machupo virus | | | P | | | P |
| Marburg virus | | | P | | | P |
| Monkeypox virus | | | P,S | P | | P |
| Omsk virus | | | | | | P |
| Orientia tsutsugamushi | | | | | | |
| Palytoxin | | | | | | |
| Ricin | | P,S | P,S | S | | P,S |
| Rickettsia rickettsii | | S | P,S | | | |
| Rickettsia typhi | | S | P,S | | | |
| Rift Valley fever virus | | | | | | P |
| Salmonella spp. | S,M,C | P,S,M,C | | C | C | |
| Salmonella Typhi | S,M,C | S,M,C | | C | C | |
| Saxitoxins | | | | | | |
| Shigella dysenteriae | S,M,C | S,M,C | P | C | C | |
| Staphylococcal enterotoxins | S,M,C | S,M,C | P,S | C | | P,S |
| Tetradotoxin | | | | | | |

| | Czech Republic | | Germany | France | Turkey | United States |
|---|---|---|---|---|---|---|
| | Deployable Biological | Mobile Epi-Hygienic | | | | 20th SUPCOM (CBRNE) |
| Tick-Borne Encephalitis (TBE) virus | S | S | P,S | | | P |
| Trichothecene | | | P,S | | | |
| Variola virus | | P,S | P,S | P | | P,S |
| Venezuelan-Equine-Encephalitis virus | | | P | | | P,S |
| Vibrio cholerae | S,M,C | S,M,C | P,S | C | C | |
| Western-Equine-Encephalitis virus | | | P | | | P,S |
| Yellow fever virus | | | P | P | | |
| Yersinia pestis | | P,S,M,C | P,S,M | | | P,S,C |
| Others: Dengue fever virus | | S | P,S | | | |
| Others: Toxoplaxma gondii | | S | | | | |
| | | | | | | |
| Chemical Warfare Agent | t N | N | GA, GB, GD, VX, HD, Phosgene | N | N | GA, GB, GD, GF, VX, HD, L, RVX, HN-1, HN-2, HN-3 |
| Nuclear/Radiological Agent | N | N | SVG II Radiation Detector | N | N | Alpha, Beta and Gamma |
| | | | | | | |
| * France collaborates with Armaments Procurement Agency (DGA) for prototype. This laboratory is in the prototype stage and has been validated for parasitological, microbial and viral pathologies. This laboratory is in course of acquisition by the Armaments Procurement Agency (DGA) for the Medical Service. | | | | | | |

The Panel also discussed the best equipment to recommend for a NATO laboratory. After long discussions about the technology rapidly changing, the different roles that each country has for its specific laboratory and the varying funds allotted to each laboratory, we concluded that a point-in-time list of specific instruments to suggest a 'standardize' NATO laboratory (or -ies) would not be the best approach for this effort. Instead, providing a forum for frequent, open communications among the NATO laboratories about lessons learned would be best to facilitate a potential deployment and response. This approach allows an active scientific and technical address of key elements needed for the current threat combined with the ever-changing individual government organization needs, their available equipment and current country of each their laboratories. Consequently, the Chair requested that each laboratory briefly describe their laboratories in a chapter in this report as a starting point for review by the HFM Panel.

Also invited from the 2011 Medical Biodefense Conference and able to attend our meeting was Dr. Frederick Johnson from the NATO Army Armaments Group, Joint Capability Group on CBRN Defence, Sub-Group on Sampling and identification of Biological, Chemical and Radiological Agents (SIBCRA)

who participated as a SIBCRA representative. Dr. Johnson described the function/mission of his team so that we could best develop the knowledge generated from our HFM-177 RTG meetings and this report to transition to his group.

From the mission of SIBCRA, the mission is two-fold: "… to determine criteria that must be met in order to provide unequivocal proof of the first use of Biological, Chemical, and Radiological Agents to NATO political and military authorities and thus to support timely decisions concerning NATO response" and "to provide the operational commander with real time information that will lead to immediate decisions on protection that will save lives and prevent casualties".

The SIBCRA team has drafted a handbook that identifies the procedures necessary to provide NATO Command Authorities with the evidence needed for international prosecution and to maximise troop safety in cases of (suspect) B, C, or R agent exposure. Aspects of the forensic capability can also be of potential use to operational business, while these are not seen as primary tasks for SIBCRA. Potential use to operational business concerns:

- Non-forensic sampling and laboratory analysis;
- The positioning, operating posture, exposure management, tempo and manoeuvre ability of units throughout the operational spectrum;
- Support to medical services for providing the most appropriate health care to casualties and for determining the most appropriate protective actions for implementing force health protection; and
- Site decontamination and eventual remediation.

The Chair of the HFM-177 RTG sent a representative, Capt. Mark Lisanby (USAF) to present a general overview of the group's work and efforts, brief the team's progress and participate in SIBCRA's meeting on 22 May 2012 in Sweden. This NATO sub-group is working under the aegis of STANAG 4632 Deployable NBC Analytical Laboratory. This group was not aware of the HFM-177 RTG activity but appears to be the perfect recipient of this work to carry these efforts forward.

## 1.5 CONCLUSION

Overall, the HFM-177 collaborative effort was a great success. Over 20 representatives from eight countries participated (Czech Republic, France, Georgia, Germany, Netherlands, Turkey, United Kingdom and United States) over the term of the HFM directed work from Exploratory Team through the two activities involving the Research Technical Group. Each country discussed their laboratory's capabilities and challenges. The group clearly recognized that each deployable laboratory's personnel and equipment varies substantially, based upon the country's capabilities and mission. The group discussed varying scenarios of CBRN exposure and response and the pros and cons of different laboratory capabilities, while virtually all deep discussions remained within the Biological Warfare Agent threat area. The different approaches each country took to develop a deployable laboratory have actually provided greater options for NATO to address different mission and threat situations. The team decided not to provide a standard, forcing an organizational structure and equipment list but instead suggests that each country continue to focus on improving their laboratory mission strength. Each country agreed that they intend to follow state-of-the-art advancements in current technology that best support their laboratories and consequently allow NATO to consider the best respond with a customized team based upon the scenario.

Fruitful discussions focused on strategic, operational and tactical issues including:

1) The deployable NATO lab's mission after a BW attack – environmental surveillance or outbreak diagnosis and mitigation;

2) The training and educational requirements of response team members;
3) The deployment response time using military or commercial air transport;
4) The requirements for large, transportable, self-contained laboratory facilities;
5) The importance of knowing in country or regional capabilities; and
6) The need to clearly defined CONOPs prior to NATO asset deployment.

The team found great advantages in discussing each other's capabilities and future directions. The meeting approach allowed a much broader evaluation of equipment and methodologies that would be far too labor-intensive for any one country to tackle alone. Also many of the countries' lessons learned were directly applicable to the entire team. The team agreed to provide platforms for more frequent and open communications among the active deployment-ready laboratories to best facilitate the every-changing organization, equipment and mission of each country's laboratory, with focused discussions on lessons learned.

This group of representatives were highly motivated, exceptionally collaborative and extremely knowledgeable. We encourage NATO to support future collaborative efforts to best share advancements in technologies among the groups and international improvements in techniques, tactics and procedures. We also recommend that NATO Army Armaments Group, Joint Capability Group on CBRN Defence, and the Sampling and Identification of Biological, Chemical and Radiological Agents sub-group, working under the aegis of STANAG 4632 Deployable NBC Analytical Laboratory, carry these efforts forward. We suggest the HFM Panel consider creating a forum for periodic meetings (yearly) with these groups involving science/technology experts (medical clinician and researcher) to discuss tactical issues that can provide knowledge of the human factors and medicine for the NATO portfolio. Lastly, the areas of chemical (e.g., nerve, vesicant, nanomaterial), nuclear, or radiological (e.g., chemical emitter and its energy) warfare were not deeply reviewed by or found key to the participating country's response with regards to their lab assets. For future considerations by the HFM Panel and NATO regarding laboratory deployments, adding sensing/detection capabilities combined with BWA sensing and well trained personnel in a deployable lab asset would be critical for providing the fully functioning theatre laboratory that could respond accurately and swiftly to these types of insidious threats.

# Chapter 2 – CHARACTERISTICS OF THE CZECH REPUBLIC DEPLOYABLE BIOLOGICAL LABORATORY

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The Deployable Biological Laboratory (DBL) is intended for the rapid and unambiguous identification of biological warfare agents, such as pathogens registered on the United States Centers for Disease Control's category A and B select agent list, in particular. The facility is technically designed for a wide climatic range, so that it can be utilized anywhere in the world. However, it is dependent on external logistical support and therefore can only be deployed as a component of a larger operational whole. The aim of the laboratory, including selected technology, is to achieve the highest degree of protection of personnel and the environment when handling high-risk biological material in field conditions.

The layout of the facility is designed as a complex of four working sections that are connected in a unidirectional operational stream. In practice, this means that the first entry section is intended to receive samples and the preparation of laboratory personnel. The entry section also performs the function of a command and control unit. All terminals are located here. Data is collected and evaluated here. There is also a communication node located there. The core of the complex is a laboratory section. This is where laboratory tests and identification of biological agents are carried out. This space features laboratory equipment, camera system, a waste water sterilization unit and decontamination loop. Outputs are placed for air supply, allowing four people to work in pressurized protective suits. The laboratory section operates under negative pressure and airflow through the system, exhausts through HEPA and NBC filters. The air in the air conditioning unit circulates in a closed circuit.

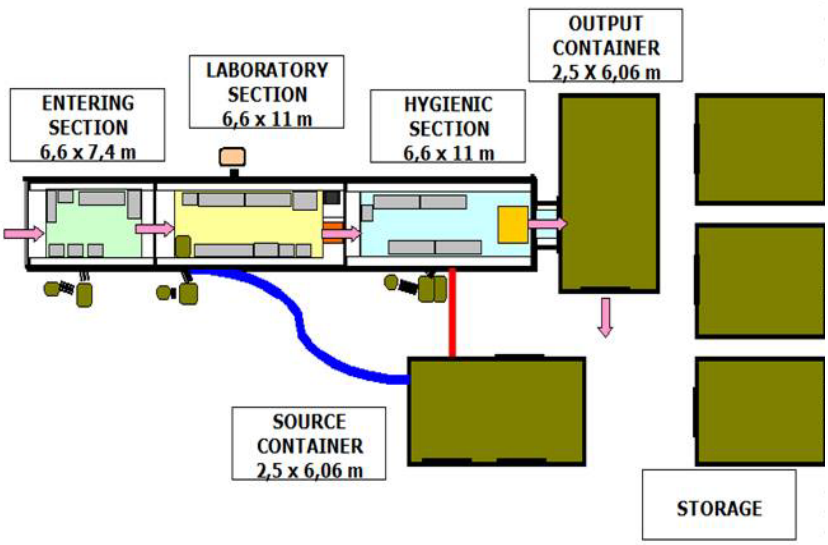
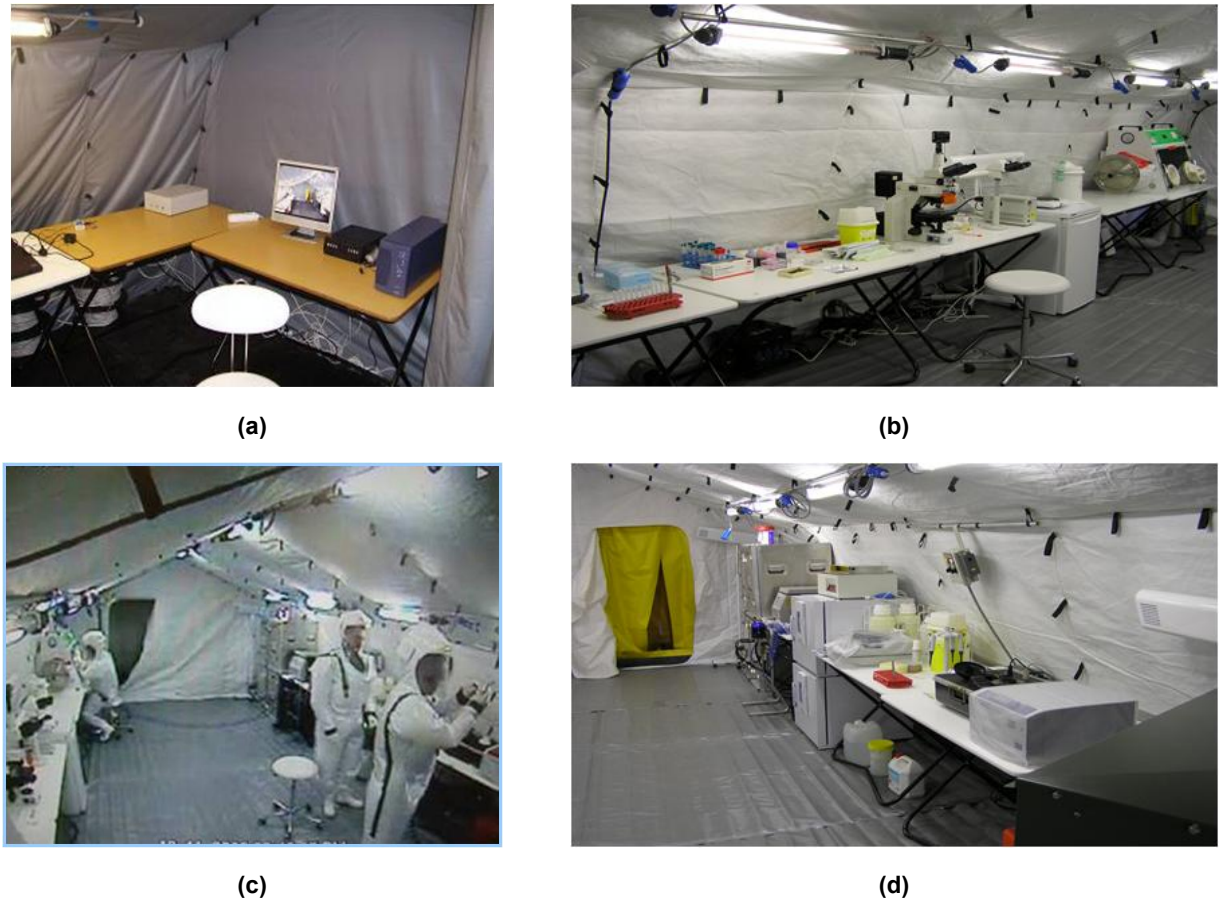



**Figure 2-1: The Deployable Biological Laboratory Complex Design Scheme.**



(a) (b)

(c) (d)

**Figure 2-2: Inside the Deployable Biological Laboratory: (a) Entry Section; (b and d) Laboratory Section Work Benches; (c) Suited Technicians Working Under Video Surveillance in the Laboratory Section.**

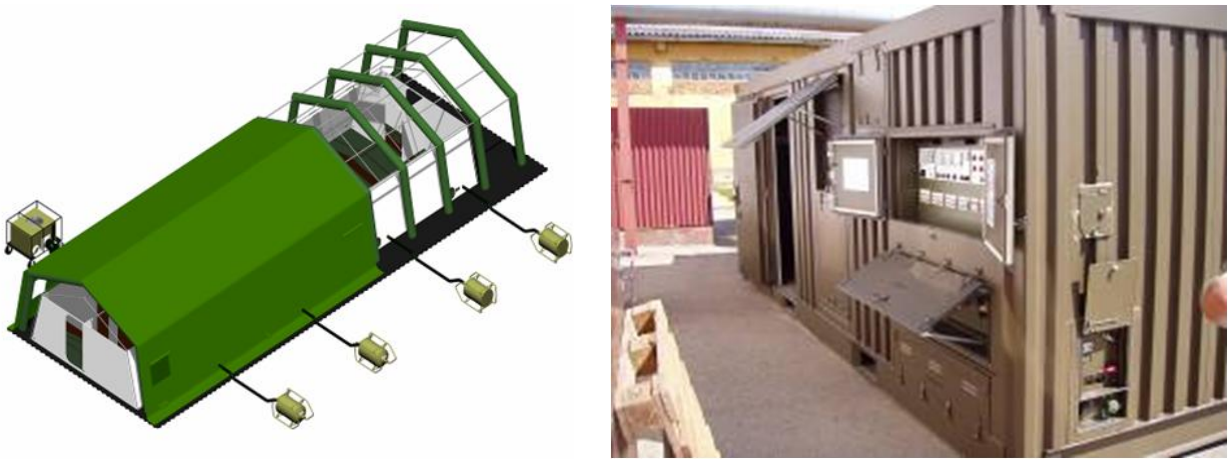
Laboratory technique allows a combination of different laboratory methods with a goal to confirm and unambiguously identify the originator. Biological agents can be identified by serological methods, by real-time PCR methods, using cultivation and subsequent microscopic and biochemical tests.

Decontamination of the laboratory section is accomplished by a combination of germicidal action of sources and application of disinfectants. The following section serves as a hygienic room where laboratory workers remove their protective suits. It is equipped with inflatable shower to perform personal hygiene.

**Figure 2-3: The Hygienic Section of the Deployable Biological Laboratory.**

The last part of the DBL is the output section. It is intended for storage of diagnostic and laboratory consumable materials. Its technical equipment allows us to store samples for examination, sub-samples for arbitrage or confirmatory and forensic tests in reference institutions for selected infectious pathogens.

The first three sections are designed as spacious tents with a special insert that forms the interior and also adjoining rooms that connect with lockable through-tunnels. The last section is placed in the container. The tents are reinforced with inflatable ribs. The insert is suspended on an aluminium frame. The floor is resistant to mechanical stress and composed of a segment system that allows for quick assembly.



**(a)** **(b)**

**Figure 2-4: Construction of the Deployable Biological Laboratory: (a) Inflatable Rib Reinforced Tents of the Entering, Laboratory and Hygienic Sections; (b) "Source" Container that Houses the Electric Generator, Fuel Tank and Compressor.**

During full operation, pressure modes are set so that the entry and hygiene sections are protected by controlled positive pressure, whereas the laboratory section is maintained under negative pressure. The heart of the facility is an energy container with a power generator unit that is equipped with a diesel motor. A compressor station provides production and storage of compressed air for the laboratory section. Although the energy unit is fully automated, it requires supervision by an engineer for each working shift. Another technician must be present to supervise the activities of air and water management. A specialist in the field of electrical devices and power distribution is essential to the operation of the DBL.

The Deployable Biological Laboratory has a 12-member staff: the Commander; 4 specialists in hygiene, microbiology, epidemiology or veterinary epidemiology; 3 laboratory technicians and 4 engineers. Designation of special laboratory equipment to field conditions requires that each staff member be completely independent and sufficiently competent in their field and at the same time be able to work as a team, especially in the construction of the complex, in full operation, in dealing with accidents or maintenance and care of some larger technological units. Construction of the complete facility can be completed within 72 hours, at which point the laboratory can be brought into a state of alert and initiate action. The deployable biological laboratory is stored in 5 standard ISO-type containers. This packaging format allows for a wide range of transportation means.

**Table 2-1: Personnel Required for Staffing the Mobile Deployable Laboratory.**

| | Post | Number |
|---|---|---|
| 1. | **Commander** | 1 |
| 2. | **Medical/biological experts (microbiologist, epidemiologist, veterinarian, doctor of natural sciences)** | 4 |
| 3. | **Laboratory technician** | 3 |
| 4. | **Engineer** | 4 |
| **Total** | | **12** |

# Chapter 3 – THE FRENCH TRANSPORTABLE MICROBIOLOGY LABORATORY

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## 3.1 CHALLENGES AND ISSUES

Our troops deployed in tropical area are exposed to major health risks likely to reduce their operational capacity. Facing the risks of natural disasters and terrorism, France has the means specific usually entrusted to civil security, but does not have all the capabilities to deploy the biological means of investigation. The Direction of the health service of the armed forces intends to strengthen its expertise on the infectious risks, in particular in the context of emerging disease, and to develop its operational research in more close proximity to the forces. This is the reason for which the military component of biological and epidemiological investigation (EMIBE) has been created. This structure should include a laboratory of microbiology developed from the experiences in field campaigns of the research teams, the concept must offer a rapid deployment regardless of the place of investigation. In the difference of laboratory shelters, it should also reduce the duration and the cost of missions. The goal is to get a quick diagnosis for a fast and appropriate response.

## 3.2 OBJECTIVES

The objective of this project was to develop a transportable, autonomous field laboratory that is quickly deployable in degraded environments. It must integrate elements for microbiological techniques, but its modular design should allow its adjustment to all situations and evolve with technological progress.

## 3.3 RESPONSES

The system is actually composed of four suitcases that are small enough and light enough to transport by car, truck, or civilian or military airplane (complies with the IATA standards). To meet the cold requirements a cooler is added to the equipment. It provides reliable service in severe-duty environments, with low maintenance.

Its design allows deployment in a short period of time (< 30 minutes) without tools. The layout of the different elements offers good ergonomics for optimal work. All the procedures and embedded software allow quality control found in the reference laboratories. Self-sufficiency in energy is provided by a generator (fuel tank capacity 4,2 L, approximately 13H00).

### 3.3.1 Constitution

- 4 composite carbon fiber cases (0,49 $m^3$-90 kg; 0,49 $m^3$-65 kg; 0,35 $m^3$-78 kg; 0,35 $m^3$-80 kg) [see Figure 3-1].

- 1 compressor cooler (- 20°C and +4°C; 0,22 $m^3$-53 kg).
- Total weight and volume: 366 kg, 1,9 $m^3$.

NB: The weight of different cases is variable depending on the supplies provided for the mission.

**Figure 3-1: The 4 Cases.**

**Figure 3-2: The Laboratory in Operation.**

### 3.3.2 Deployment

Step 1

Step 2

Step 3

Step 4

Step 5

**Figure 3-3: The Laboratory's 5 Step Assembly.**

## 3.4 MAJOR EQUIPMENT

- Laminar flux hood that ensure the protection of the biologist and samples (with a self-test security).
- Microbiology trigaz incubator with a maintenance of set-point temperature whatever the outside temperature (control of $CO_2$ and $O_2$ level by using $CO_2$ and $N_2$ compressed bottle, storage of data culture conditions).
- Micro-plaque absorbance reader and micro-plaque washer.
- Mini real-time PCR apparatus.
- Centrifuge.
- Microscope.
- Electrical board with UPS top line.
- Generator 1 kw.

The consumables and reagents are chosen according to the objectives of the mission.

### 3.4.1 Examples of Use

This laboratory has already been used in various field studies and is the subject of continuous improvement.

- Carpiagne – France

  *Climate: Mediterranean / Transport: Road / Energy: Portable Generator / Analyses: Bacteriology, Virology, Parasitology*

**Figure 3-4: Carpiagne – France.**

- N-Djamena/Abeche – Chad

  *Climate: Semi-arid / Transport: Civil and Military Airway / Energy: Portable Generator / Analyses: Bacteriology, Virology*

**Figure 3-5: Chad.**

- Bom-Bô – Vietnam / Kinshasa – DRC

  *Climate: Tropical wet / Transport: Civilian Airway and Road / Energy: Sector 110/230W-Portable Generator / Analyses: Parasitology*

**Figure 3-6: Vietnam.**

## 3.5 PERSPECTIVES

- Energy autonomy: Development of a hybrid power source integrating both a fuel cell and photovoltaic panels.
- Development of bioassays based on the luminescence technology and automated pipetted bioluminescent plate reader.
- Development of new module with integration of haematology and biochemistry devices.

# Chapter 4 – THE BUNDESWEHR RAPIDLY DEPLOYABLE BIO LAB

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Rapid and reliable identification of biological agents and other dangerous pathogens is one of the major tasks of the Department for Medical Bio-Reconnaissance and Verification at the Bundeswehr Institute of Microbiology in Munich. To fulfill this task outside of Germany a modular, rapidly deployable, microbiological laboratory was developed for field operations.

Because modern microbiological methods place high demands on the infrastructure of a lab – particularly when employed for medical or bioforensic purposes – a core capability of the rapidly deployable bio lab consists in utilizing very basic facilities for modern diagnostic investigations. By virtue of the system's modular design it is possible to bring only the equipment needed to fulfill a specific mission.

All equipment in the deployable bio lab is packed in waterproof rollable boxes. It is deployable within 72 hours in an aircraft as passenger luggage and depending on local conditions, it is operational six to twelve hours after arrival in the area of operation. The typical space requirement for the lab is approximately 20 square meters. Using different materials, several separate working areas can be created in the deployed environment. For transportation and storage of laboratory reagents and clinical samples both active and passive freezers are available. To increase safety of lab personnel, preparation of unknown biological samples can be conducted in a mobile glove box up to prevent exposure to potential pathogens.

The modular design of the deployable lab allows a mission specific tailoring of equipment and personnel to meet the needs on-site. Key features of the rapidly deployable bio lab are:

- **Modular laboratory equipment:**
  - 8 – 15 milspec boxes;
  - Weight per box: max. 31 kg;
  - Cleared as passenger baggage in commercial aircrafts; and
  - Waterproof packaging.
- **Main focus:**
  - Real-time PCR techniques; and
  - Conventional PCR as backup method.
- **In addition:**
  - (Immunofluorescence-) microscopy;
  - ELISA and immunochromatography;
  - Transport and set-up by lab personnel; and
  - Operational under resource-limited conditions.

The deployable bio lab allows the identification of bacteria, viruses, certain toxins and parasites with the aid of conventional and real-time PCR, immunological tests (ELISA and immunochromatography), as well as light and immunofluorescence microscopy. The laboratory mission, as well as all methods and documentation techniques, adhere to NATO requirements regarding rapid outbreak investigations (RDOIT) and handling and confirmed identification of biological warfare agents (SIBCRA).

At present the diagnostic spectrum of the lab covers more than twenty-eight diseases, among them anthrax, plague, tularemia, Q-fever, brucellosis, Crimean-Congo-Haemorrhagic-Fever, Ebola fever, smallpox, influenza and malaria.

The operation capacity of the lab is limited by the amount of consumables and by the number of personnel. In total approximately 50 tests can be run, with up to three different tests with 14 samples each per day. Both conventional and real-time PCR investigations can be conducted in the deployable bio lab abroad. For the confirmation of conventional PCR products DNA hybridization assays are used in the field.

By addition of modular packed supplementary equipment, it is possible to further extend both the diagnostic spectrum of the lab and also its duration of operation. This increased spectrum of application may include diagnostic methods (e.g., basic blood chemistry), additional lab equipment, and more personnel abroad to allow shift work. All necessary equipment is packed on aircraft pallets and can be deployed within a few days as air freight.

**Figure 4-1: The Deployable Bio Lab, Packed in Robust and Waterproof Transport Boxes.**

**Figure 4-2: Modern Real-Time PCR Allows Molecular Detection of Different Pathogens Within a Few Hours.**

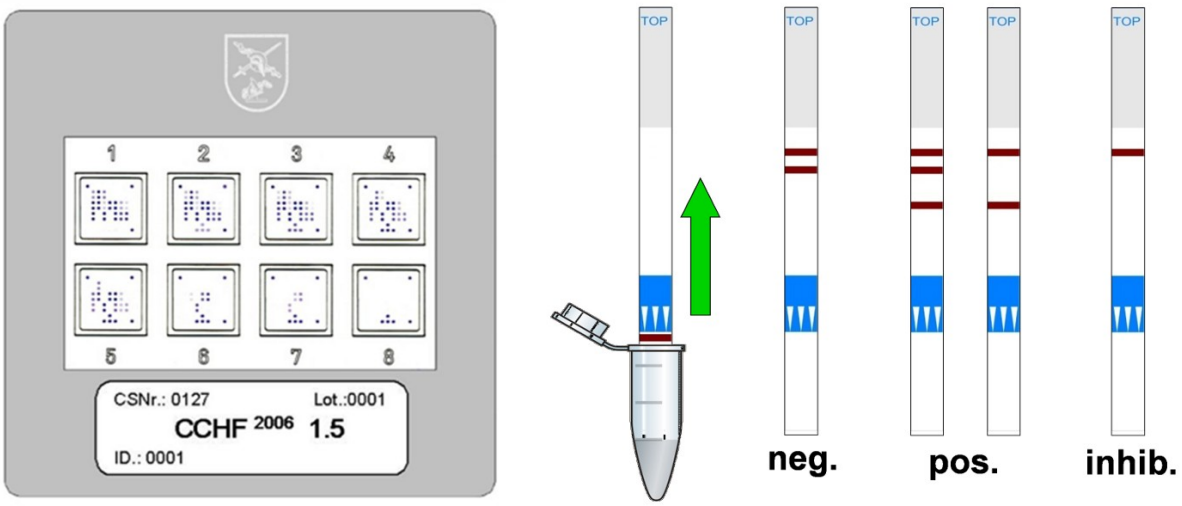


**Figure 4-3: Conventional PCR Products are Visualized and Confirmed by Either Hybridization Chip Technology (Left) or Lateral Flow Dipstick Assays (Right).**

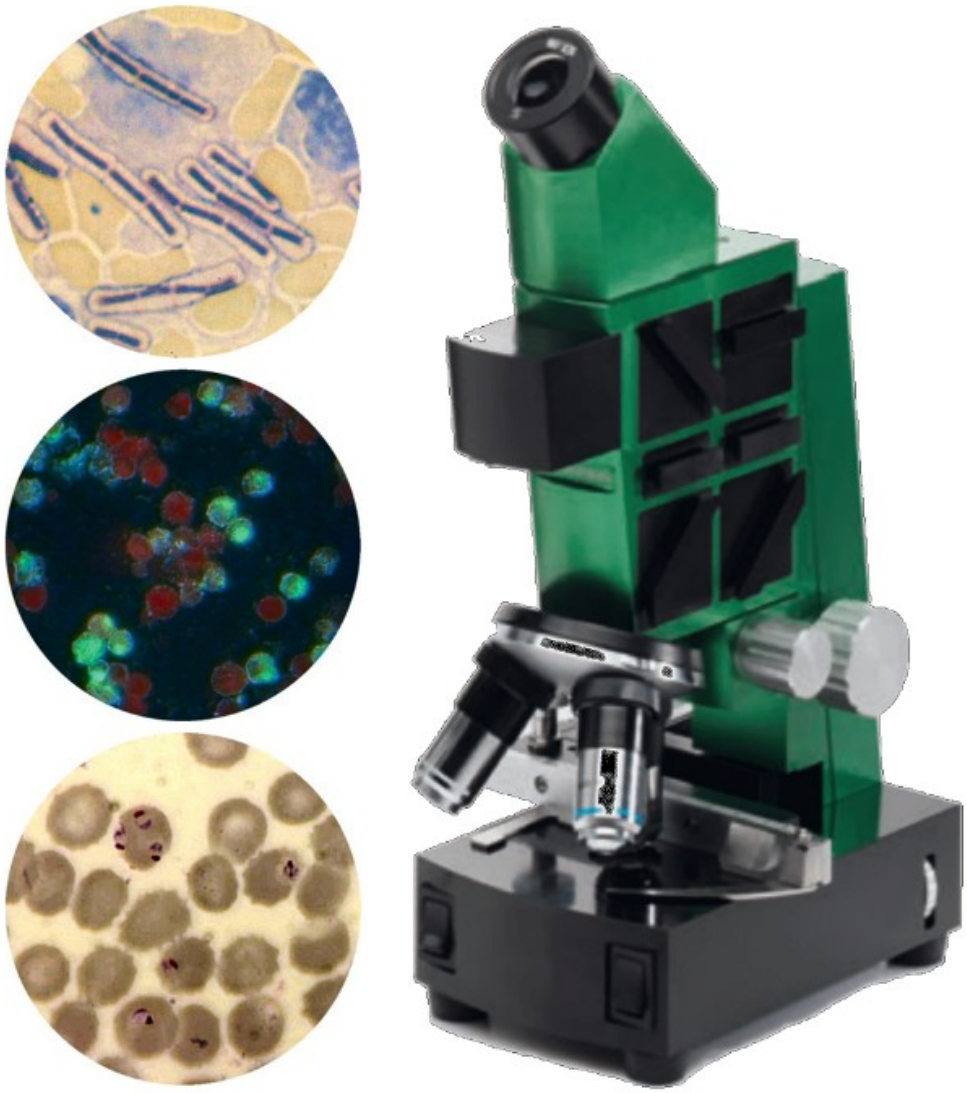

**Figure 4-4: A Mobile, Battery-Operated Microscope Allows Microscopically Investigations (e.g., Capsule Staining of *Bacillus Anthracis*, Malaria Diagnostics) as well as Serological Diagnostics by Immunofluorescence Assays.**

# Chapter 5 – NANO-MEDICINE AND NOVEL ANALYTICAL APPROACHES

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## 5.1 INTRODUCTION

Developments in nano-technology have revealed that macroscopic and nano-metric forms of organic structures possess different features in physical, chemical and biological aspects. By proving that nano-devices, which are produced at laboratory, can interact with biomolecules, both physiological processes in healthy tissues and patho-physiologic basis of diseases begin to be understood.

"Nano-medicine,'' which appeared as a new scientific interest parallel to the above-mentioned developments in nano-technology, became one of the most studied topics in the world by the reason of the fact that it leads conceptual changes in accepted and applicated medical methods up to now and presents different diagnosis-treatment alternatives.

Although nano-technology is a commonly studied field all around the world, there is still no clear consensus about what nano-scale really is. One nano-meter is calculated as one billionth (10-9). It is possible to fit 5 carbon atoms in this scale as in three dimensional forms. According to BSI (PAS 71) applications, less than 100 nm or even smaller scales are evaluated within the concept of nano-technology. While at the beginning of 2000 s, studies less than 200 nm and in smaller scale were considered as nano-medicine, today this range is accepted between 5 – 100 nm.

## 5.2 CLINICAL NANO-MEDICINE PERSPECTIVES

Currently the goal is to approach patients with diseases and diagnose and start treatment, when pathologic change is only at single-cell level. However, this is only possible by increasing the efficiency of *in-vivo* and *in-vitro* diagnosis methods. Although nano-medicine is a field presenting great opportunities in this regard, it also brings along disadvantages because it is a new, developing discipline.

In the literature, there is a wide range of research topics: everything from the discovery of new nano-biomaterials to using these materials in clinics. While searching for physical, chemical and biological applications for nano-materials it is also attempted to be understood how to use these materials on living creatures. Research want to know what adverse effects might be caused by the use of these materials specifically, effects of nano-materials on human health and environmental health. In addition, possible social and legal problems have been discussed and new ethical rules have been introduced.

Some studies are detailed, more specific and more focused on developing safer diagnostic devices. There are studies investigating different biological measuring methods with one integrated device. By using biosensors which are developed with the use of nano-electronic circuits, researchers would be able to establish micro mobile laboratories which could easily be used by patients and, if necessary, could transmit data to an external user.

Another related area of nano research studies the combination of *in vitro* monitoring techniques and *in vivo* nano-medical devices. In those studies, researchers are attempting to develop nano-structures which are able to carry specific contrast substances which will be directed from the outside. Thus, it will be possible to take detailed molecular images of target tissues.

In another study, it was researched how to combine nano-structures with pharmacological agents. Nano-structures which carry therapeutic and diagnostic agents at the same time, would be especially ground-breaking in cancer cases making it possible to administer a treatment directly on target. This cancer treatment, known as theragnostic (therapy + diagnose), is aimed to improve efficiency of cancer treatment by taking images of the target tissue at different times.

Lastly, intense studies have been conducted on the successful regeneration of diseased or injured tissues by means of nano-grafts and reproducing needed artificial organs by means of nano-scaffolds in *in vitro* conditions and then replacing diseased or injured organs with the artificial ones.

Methods which have been developed by using nanotechnology have the potential to be effective on all medical equipment. For example, developing new materials to be used in surgical implants. Nano-metric systems or minimal invasive sensors, which can be used in monitoring metabolic activities, can be considered within this regard. Nano-pumps, injectable/implantable polymer systems, liposomal drug applications and cell/gene therapy methods, can be considered for controlled drug delivery systems. Currently, half of the improvements related to new molecules all around the world are made by biotechnology companies. Therefore, over 4000 companies in the world work in an area related to drug delivery systems, tumor targeted therapy methods, or drug carrying implants.

## 5.3 INTERDISCIPLINARY FRAMEWORKS

All those efforts for understanding the development of disease at the molecular level and for treatment are very important to spread all the developments in nano-medicine to the society. Since the topic has a wide scale, different disciplines have to work together in the nano-medicine area. It can be said that for now, neither any scientific field nor areas of expertise possesses the capacity of scientific and technical infrastructure to conduct such a research by itself. To manage scientific research in such a field, it is a must to establish a well-organized ‘team’. Within such a team, conventional disciplines such as basic-clinic medical scientists, pharmacologists, physics-chemistry-electric-electronic-biomedical-computer engineers, etc., and new fields such as genome-proteome science, pharmacokinetic modeling and microscope designing, etc., should be included.

In addition to self-disciplinary nature of nano-medicine, the more the numbers of studies increase in this field the better new sub-disciplines appear. Some of these sub-disciplines are mentioned below and many studies have been conducted on each specific topic:

- Imaging: molecular, vascular, neurological, etc.;
- *In vitro* diagnosis;
- *In vivo* diagnosis and biosensors;
- Advanced biomedical materials, including “smart” and functionalized materials and surfaces;
- Regenerative medicine and tissue engineering;
- Infection control;
- Drug design and targeted drug delivery;
- Gene and cell therapy;
- Man-machine interfaces;

- Nano-toxicology;
- Nano-medicine and risk management; and
- Nano-medicine and ethics.

## 5.4 CLINICAL NANO-MEDICINE APPLICATIONS

Today, big scaled centers which conduct experimental and clinical studies are focused mainly on three fields.

### 5.4.1 Regenerative Nano-Medicine

Current 'traditional treatment' approaches seem to have limited results in many diseases or cause success of training to change from patient to patient. Both for improving the efficiency of the treatment and minimizing the side effects, methods to be used should be patient-specific characteristics.

As a result of "tissue engineering" studies, the basis of patient-specific treatments, which can be used in the regeneration and reparation of *in situ* tissues, has started.

Main implementation fields of tissue engineering, which is an interdisciplinary field, are maintaining, improving and repairing the functions of biological structures through collaboration of engineering and life sciences. By means of tissue engineering, future therapy methods will be more focused on treatment of chronic disorders by use of self-healing mechanisms of the body than targeting symptoms or reducing the development of diseases.

It is possible to evaluate regenerative nano-medicine studies into two topics as therapy and biomimesis.

### 5.4.2 Diagnosis and Imaging Methods Based on Nano-Medicine

The most important aim in the diagnosis of diseases is to diagnose disease when it is at the earliest stage, at one-cell level. To reach this aim, we must develop new *in vivo* and *in vitro* diagnosis methods based on nanotechnology. Within the scope of *in vitro* applications, studies on chemo-bio nano-sensors, ultra-sensitive biochips ("lab-on-a-chip" and "cells-on-chips" devices) have been prepared for routine medical applications.

Yet-to-be-produced nano-analyser devices will be used by patients and, at the same time, will transmit multiple types of data to clinicians. More important than that, by means of nano-biosensors, it will be possible to increase the accuracy of already used test methods. Biosensors (such as photonic crystal nano-biosensors, magneto nano-immunosensor, piezoelectric nano-sensors, resonating beam sensors, and ion-channel biosensors) harness the immensely powerful molecular recognition properties of living systems and engineer these into electronic devices to provide easy-to-use sensing devices. The most successful biosensor developed to date, is the home blood glucose sensor which is now ubiquitous world-wide. Biosensors can be used to measure disease markers, food safety, and environmental quality, to ensure safety and security.

Developments in microscopic scanning-imaging methods (quantitative-PET, MRS, d-MRI, and f-MRI), spectroscopic-spectrometric techniques (Fourier Transforms Infrared Imaging Spectroscopy – FTIR, High Performance Liquid Chromatography – HPLC-MS / HPLC-UV-Vis, and Matrix-Assisted Laser Desorption/ Ionization Mass Spectrometry-MALDI-TOF) and advanced genetic analyses (Real-Time Polymerase Chain Reaction – PCR) provide ultra-high spatial resolutions and give detailed molecular information about the complex 'functionality' of cells. Data acquired by use of quantum dots and fluorescent nanoparticles will lead developments of more innovative and stronger *in vivo* diagnosis devices. Nano-devices produced as accompanying this functional molecular imaging will be more effective and much safer.

### 5.4.3 Targeting Delivery and Releasing

Long-term aims of controlled drug delivery systems are to develop diagnosis agents with a high level of efficiency and safety, and to perform treatment, application and follow up with the same nano-system. 'Find, fight and follow', as is the concept; includes early diagnosis, treatment and monitoring of the results and also is stated as "theragnostic" (diagnosis + treatment).

Drug delivery techniques suitable for theragnostic definition are prepared in accordance with two needs. First is drugs targeting more effectively where the disease is located, with high patient tolerance and cost effectiveness, the other is to detect new methods for distribution of new types of pharmacologic agents which cannot be distributed effectively by conventional methods.

The main aim of pharmaceutical studies in this regard is to target medication to specific target tissues, at the right time, in the necessary amounts and with safe, repeatable, and controllable methods. Currently, 13% of products on the pharmaceutics market are related to controlled drug distribution systems. Nano-particle formulations are still used to increase activity without increasing surface/volume proportion. In addition, nano-particles act as drug carriers to effectively transmit therapeutics which have weak liquidity. If a therapeutic active substance is suitably encapsulated in a nano-particle, carrying this drug anywhere requested, controlled oscillation of the drug and protection from early stage activity decreases can be managed. These results will both increase the efficiency of drugs and decrease side effects dramatically. These types of nano-particle delivery systems can be used for the treatment of cancer and many other diseases.

Controlled drug delivery is based on the principle of turning pathophysiological changes, which appear basically in diseased tissue, into advantages for treatment. Because in tissues in which pathological process has already started, all physiological functionality disorders related to cell homeostasis are observed, accumulation of carriers which distribute drugs in a controlled manner will be easier. Anatomic barrier between normal and pathologic tissues and vascularisation differences will make it easier to deliver nano-carriers to diseased tissue. Thus, nano-carriers which carry therapeutic agents will reach much higher concentration in target tissue compared to doses applied with normal drug treatment.

As a result of decrease in vascular permeability and lymphatic drainage appeared especially in tissue which developed tumor and inflammatory diseases, on one hand reach of nano-structures to target tissue will be facilitated, on the other hand it will be more difficult to withdraw. By means of the opportunity created by this pathophysiological change, nano-structures can easily be accumulated in extravasations and target tissue.

By means of localization tendency of nano-carrying systems especially in RES will be considered as a huge advantage in terms of both controlled and passive distribution of drugs. This natural distribution method managed by macrophages can be used for intracellular infections of liver and spleen.

Patient-specific therapies and diagnosis have a critical role on nano-systems performing controlled distributions to reach the target. It is possible to find many nano-carrying systems having such an aim in the literature (liposomes, micellular and micro-emulsion systems, liquid crystal based formulations, nano-crystals, antibodies and conjugates, naturally occurring proteins as delivery systems, polymer conjugates and bio-conjugates, biodegradable nanoparticles/nano-capsules, virus-like particles for gene delivery, delivery of small nucleic acids or mimetics, delivery of vaccines, synthetic biomimetics, dendimers, carbon nano-tubes, etc.).

Although there have been many successful experimental studies on the topic existing today, strategies for developing new drug carrying systems aren't completely accepted yet. Efforts on this topic have been proceeding slowly because of the uncertainties about regulation and toxic side effects. It should be accepted that drug safety has to be attached as much importance as drug efficiency considering all nano-particles.

# Chapter 6 – CHARACTERISTICS OF THE UNITED STATES MILITARY DEPLOYABLE CBRNE LABORATORY

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## 6.1 INTRODUCTION

The United States Military Deployable CBRNE Laboratory interfaces with numerous units to provide Field Confirmatory and Theater Validation results. A graphic representation of the various levels of analysis is displayed below. In a field environment, rapidly deployable units are tailored to the incident and involve specialized teams with a tactical focus. These teams provide presumptive and field confirmatory results for consequence management. Mobile "Theater Validation" labs provide theater/operational level support by scientific experts to major combatant Commanders. Fixed site laboratories located in continental United States provide definitive analyses for national command authorities for strategic level decision-making. This chapter will focus on mobile laboratories providing Field Confirmatory and Theater Validation results.

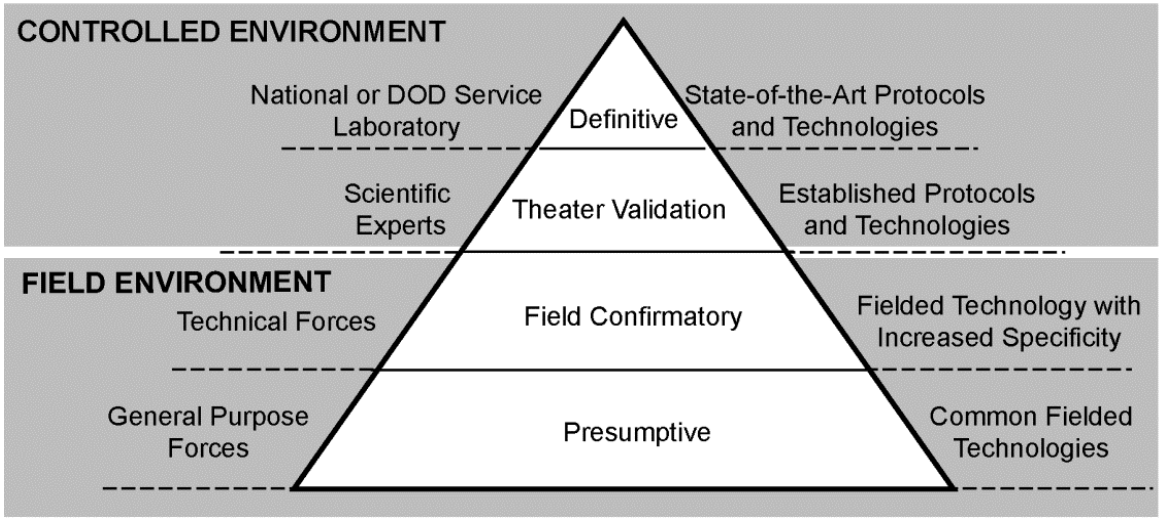


**Figure 6-1: Overview of CBRN Identification Levels.**

U.S. Army Forces Command tasks the 20th Support Command to provide specialized CBRNE solutions for DoD challenges. The CBRNE Analytical and Remediation Activity (CARA), subordinate to the 20th Support Command, deploys and conducts operations in support of regional combatant Commanders or other government agencies in order to counter CBRNE and WMD threats in support of National Combating WMD objectives.

The CARA typically deploys elements in a general support role to a theater Commander under the mission command of the JTF-HQ or mission dependent direct support allocation to designated Commanders on an

area/site specific basis. Each of these elements can also deploy separately from the JTF. In this case, these elements will most often be placed in the operational control of the supported command.

The MEL mainly operates in a permissive environment but under certain conditions can operated in uncertain environments while conducting field confirmatory identification. The MEL cannot sustain 24-hour operations unless augmented by additional technical laboratory personnel. RRTs are capable of conducting non-intrusive assessment of munitions; presumptive identification of CB materials and may collect CBRN samples if required. All of CARA deploy in mobile, modular, tailorable teams depending on the mission requirement.

## 6.2 REMEDIATION RESPONSE

CARA has four Remediation Response Teams, located at Pine Bluff Arsenal, Arkansas and Aberdeen Proving Ground, Maryland. They provide:

- Response, remediation and escort support to combatant Commanders and other Government Agencies to counter the CBRNE and WMD Threat;
- Emergency response to discoveries of suspect Recovered Chemical Warfare Materials (RCWM) in support of the Army Chemical Materials Agency's, Non-Stockpile Chemical Materiel Project;
- Remediation operations involving chemical warfare materials found at formerly used defence sites and base realignment and closure sites in support of the Army's Corps of Engineers and active military installations in support of installation Commanders;
- Stockpile and non-stockpile operations in support of the Army Chemical Materials Agency; and
- Conduct technical escorts to transport chemical and biological surety and non-surety material for various Army laboratories.

The Mobile Munitions Assessment System (MMAS) Team conducts emergency response assessments of recovered chemical warfare materiel. The MMAS serves as a command center. It has an equipment storage area and weather monitoring system. It utilizes satellite communications to transmit data back to headquarters for analysis and can remain on site for months, with a constant power supply and redundant computer systems providing added data protection.

The MMAS Operators are trained and proficient with the MMAS equipment set:

- A Portable Isotopic Neutron Spectroscopy (PINS) system: PINS accurately detects the presence of chemical elements using neutron particles to produce a unique energy spectrum given off by chemicals inside a munition;
- A Digital Radiography and Computed Tomography (DRCT): DRCT uses X-ray photography to produce high-quality images of an item's interior to show if the munition contains a liquid fill and explosive potential; and
- A Raman Spectrometer: Using a fiber optic probe and laser, the Raman identifies the contents of glass Chemical Agent Identification Sets (CAIS) bottles containing various agents and industrial chemicals once used to train Soldiers.

**Figure 6-2: Pictures of the Mobile Munitions Assessment System (from Top Left to Bottom Right): RAMANS Spectrophometer; MMAS Phase 2 System 2; Portable Isotopic Neutron Spectroscopy System; Digital Radiography / Computed Tomography.**

## 6.3 MOBILE EXPEDITIONARY LABS

The Mobile Expeditionary Laboratory is staffed with credentialed, deployable civilian scientists, both chemists, and microbiologists. It can deploy using several different packages three tactical platforms, Light Mobile Expeditionary Lab (LMEL), Heavy Mobile Expeditionary Laboratory (HMEL) and Chemical Air Monitoring Suites (CAMS). This laboratory can detect, identify, quantify and provide field confirmatory/theater validated analysis of Chemical Warfare Agents (CWA), Toxic Industrial Compounds (TIC), biological agents and select explosives; and conduct near real time air monitoring of chemical warfare agents. It supports Sensitive site exploitation, crisis and consequence management, and mitigation operations. The tactical platforms are deployable by military aircraft and ruggedized for field deployment. The modular configurations can be tailored to support needs of combatant Commanders.

### 6.3.1 Light Mobile Expeditionary Labs

**Figure 6-3: The Light Laboratory Capability.**

The light configurations are two rugged, rapidly deployable suites of modular and tailored laboratory instruments for rapid operations at the request of a ground force Commander. The platforms use a standard military Light Medium Tactical Vehicles or LMTVs and a High Mobility Multipurpose Wheeled Vehicle, better known as a HMMWV. A vestibule connects the two vehicles. The actual "lab" is inside the shelter on the back of the LMTV. The package includes one Light Medium Tactical Vehicle (LMTV) with mounted lab shelter, an environmental control unit, trailer mounted 30-kW generator, and a shelter-installed glove box; one High Mobility Multi-purpose Wheeled Vehicle (HMMWV) with tactical shelter. This package is air transportable by a C-130 or larger aircraft.

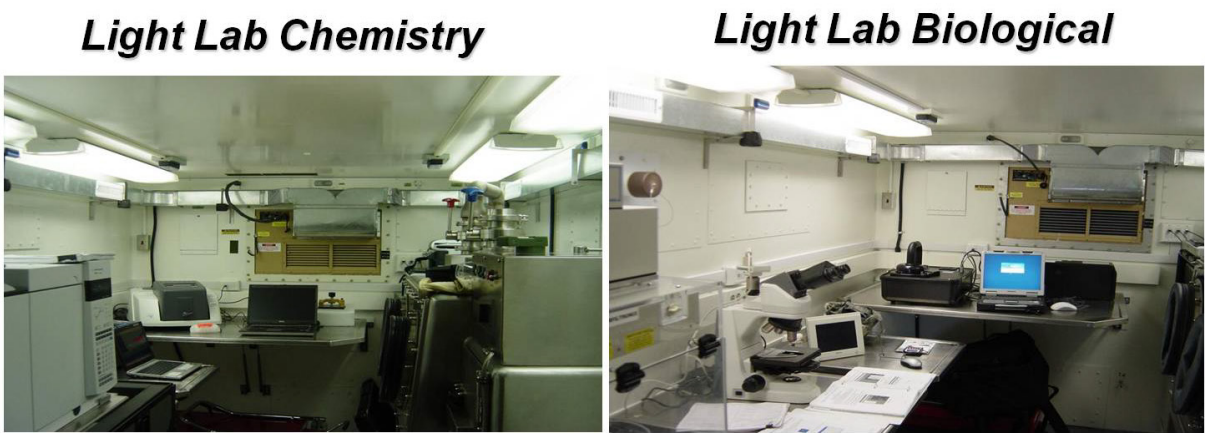


**Figure 6-4: Inside of the Flyaway Laboratory with a Chemistry and Biology Configuration.**

The inside of the light laboratory has two configurations, chemistry and biology. The analytical components include a chemical package consisting of a:

1) Gas Chromatograph Mass Spectrometer (GC/MS) to separate and identify chemical and some explosive agents in air, vegetation, solid and liquid samples;
2) Fourier-Transform Infrared (FT-IR) Spectroscopy for bulk screening of chemicals and explosives (liquid, solid, thin film);
3) Raman spectrometer for bulk screening of chemicals and explosives (liquid, solid); and
4) Radiological screening capability.

The biological package consists of a:

1) Joint Biological Agent Identification and Diagnostic System (JBAIDS) [Polymerase Chain Reaction (PCR)] for identifying specific DNA/RNA of biological weapon agents;
2) Microscope used with specific stains to determine the presence and characteristics of micro-organisms; and
3) Radiological screening capability.

The light laboratory can be configured in a combined package consisting of a GC/MS, FT-IR, PCR, and Antibody Assay, and a radiological screening capability.

### 6.3.2 Heavy Mobile Expeditionary Labs

The heavy MEL configurations are two 20-foot expandable shelter/containers that are deployed into a theater sanctuary area. The heavy MEL configuration brings a full brick and mortar lab-like facility with theatre-level confirmatory standard capabilities. A robust suite of analytical instrumentation allows for a high confidence identification of unknown hazards. The heavy MEL is deployed via C-17 and deploys with a family of medium tactical vehicles for ground transport with a 60-day supply of consumables. Each platform brings ten large containers and two refrigerators for shipment and storage.

**Figure 6-5: The Heavy Laboratory Capability.**

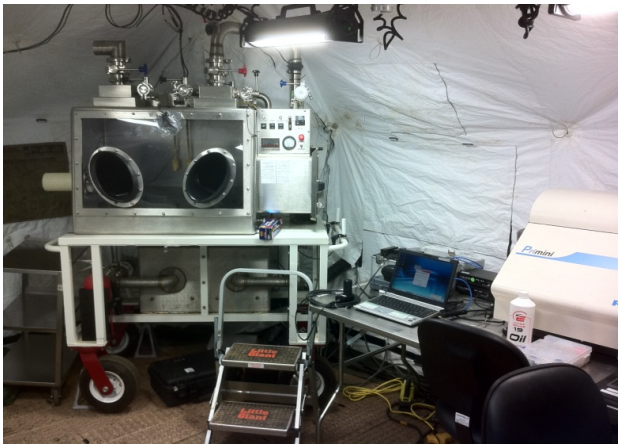

**Figure 6-6: The Heavy Laboratory Engineering Controls Include Fume Hood, Class II Biosafety Cabinet and Glovebox.**


**Figure 6-7: The Heavy Laboratory Images Inside the Sample Receipt Tent and 20' ISO Shelter.**

The HMEL deploys with a robust suite of analytical equipment to analyze CWA's, precursors, degradation products, TICs, explosives, toxins, viruses and bacteria. The chemist use the following analytical instruments:

1) Gas Chromatograph Mass Spectrometer (GC/MS) to separate and identify chemical and some explosive agents in air, vegetation, solid and liquid samples;
2) Fourier-Transform Infrared (FT-IR) spectroscopy for bulk screening of chemicals and explosives (liquid, solid, thin film);
3) Raman spectrometer for bulk screening of chemicals and explosives (liquid, solid);
4) Capillary electrophoresis to separate and detect chemical degradation products;
5) Liquid Chromatography Mass Spectrometer (LC/MS) for the detection of trace level explosive compounds and proteomics analysis;
6) X-Ray Diffraction (XRD) identifies explosive, TICs compounds with crystal structures; and
7) X-Ray Fluorescence (XRF) for post blast analysis and identification of metal alloys and elemental analysis.

The microbiologist use the same equipment listed in the LMEL but the HMEL also include limited culture capability.

## 6.4 CHEMICAL AIR MONITORING SUITES

The chemical air monitoring suites configurations are four light medium tactical vehicles with shelter and towed generator sets. The chemical air monitoring suites provide the ground force Commander with the data on occupational exposure limitations as defined by Occupational Safety and Health Administration, CDC, U.S. Army Center for Health Promotion and Preventive Medicine, and the U.S. Surgeon General during CBRN material recovery or long-term site remediation. The chemical air monitoring MEL is deployed via C-130 and carries a 90-day supply of consumables.

The CAMS equipment is transported by a 2.5-ton M1079A1 shelter-equipped Light Medium Tactical Vehicle (LMTV) towing a 30-kW trailer-mounted generator set. The term CAMS refers to the entire system, which consists of the LMTV van and on-board chemical air monitoring equipment.

The CAMS equipment consists of MINICAMS (Gas Chromatography (GC) with a Flame Photometric Detector (FPD) and GC with Halogen Specific Detector (XSD), which employs analytical and surveillance methods to continuously evaluate for the presence of low-level Chemical Warfare Agents (CWAs) in order to comply with established Short-Term Exposure Limits (STEL). Depot Air Monitoring System (DAAMS) for GC/MS monitoring of air/gas/vapor samples captured on the DAAMS tube.

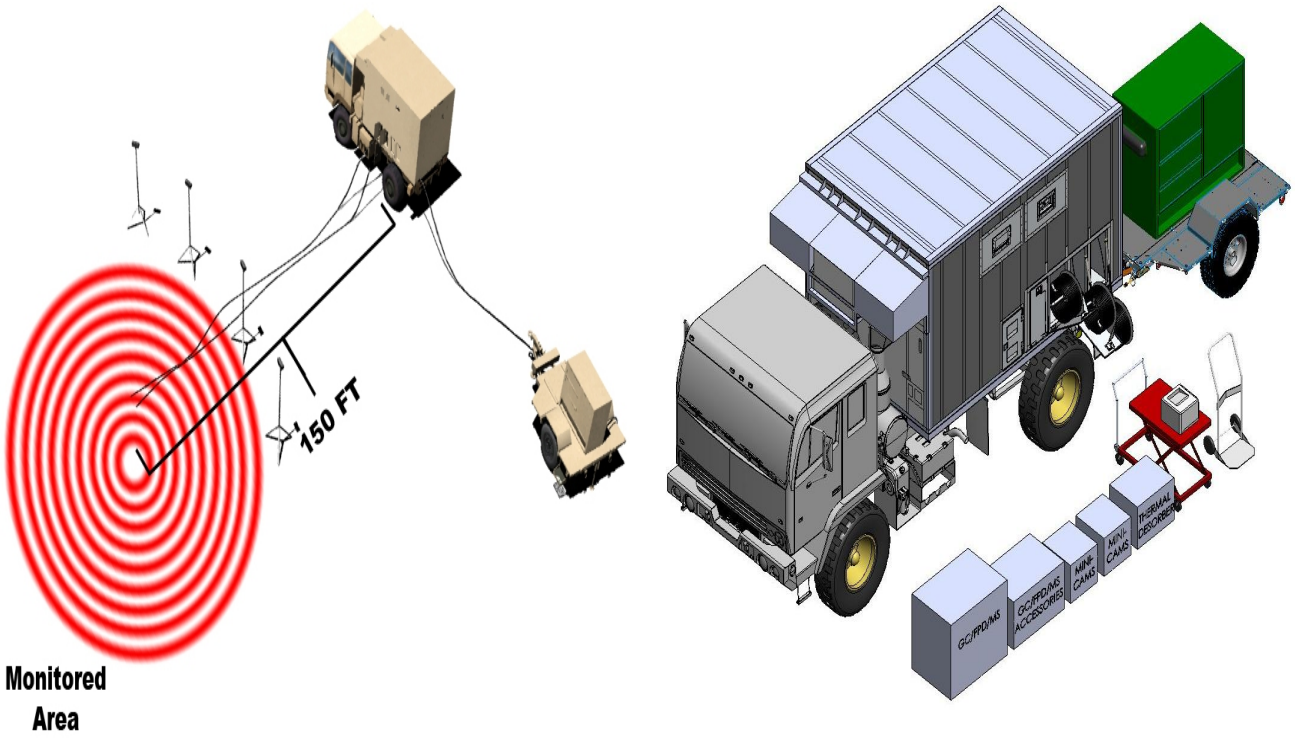


**Figure 6-8: Light Medium Tactical Vehicle with Shelter and Towed Generator. Left: Deployed air monitoring arrangement with generator; Right: Mobile configuration with stored analytical equipment removed.**

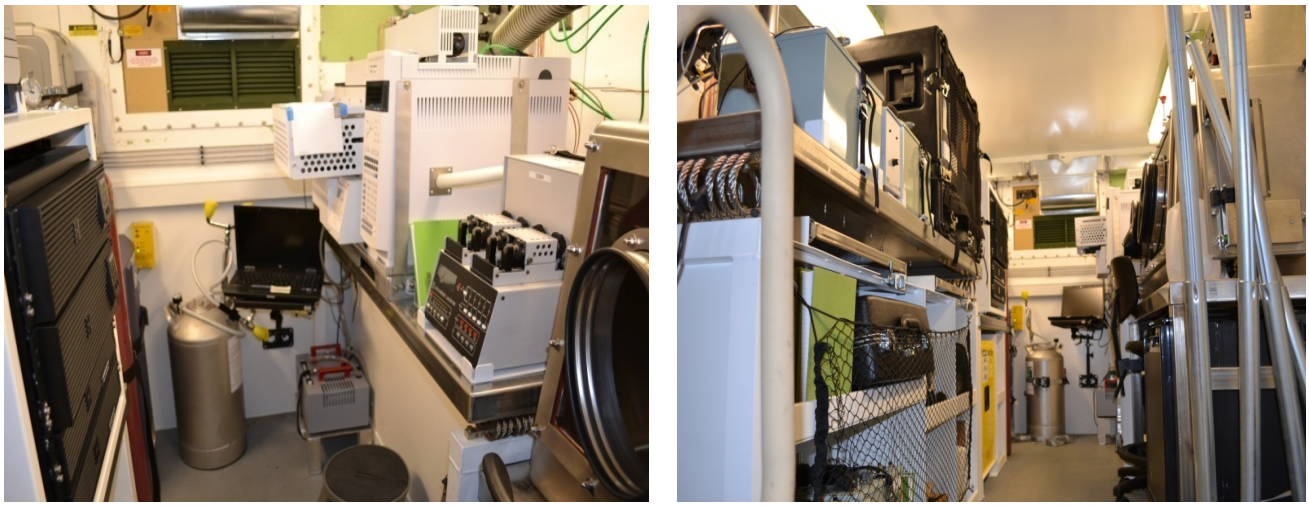

**Figure 6-9: Internal Laboratory and Storage Configuration of Light Medium Tactical Vehicle.**

The MEL brings the following capabilities to the supported Commander:

- Receipt and storage of solid, liquid, and vapor/gas samples;
- Field confirmatory or theater validation identification;
- Identification of the constituents of the solid, liquid, and vapor/gas sample;
- Characterization of the sample;
- Quantification of the sample;
- Security and positive control of samples and sample related data;
- Split samples for additional analysis as needed;
- Sensitive analytical data and results transmission; and
- Safe (and according to applicable laws, regulations, and customs) storage, transportation, and/or treatment and destruction (as needed) of any hazardous materials resulting from the laboratory operations.

# Chapter 7 – NATO JOINT CBRN DEFENCE BATTALION

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## 7.1 NATO REQUIREMENT/OBJECTIVE FOR A CBRNE DEPLOYABLE LABORATORY

The ever-growing threat of unconventional warfare requires NATO countries to be best prepared for a rapid response to a potential CBRNE event. The threat has elevated recently with the unrest in the Middle East, where terrorists and even host Nations may access CBRN stockpiles to use on their adversaries to gain the advantage. Past events that underscore the threat include the sarin attacks in the Tokyo subway, the anthrax letters in the US, and the sulfur mustard attacks on the Kurdish villages in Iraq. Most strategic analysts believe that the likelihood of such future CBRN events occurring no longer becomes a question of "if" but "when".

For CBRNE events, military Commanders need timely confirmatory analyses for crisis management to appropriately handle the immediate situation, with emphasis on appropriate decontamination, site evacuation and medical treatment for those exposed and also on avoiding exposure for anyone else. Senior political leaders require forensic confirmatory analysis for consequent management to take appropriate action on the identified perpetrator. All involved want to ensure the results are precise and accurate to make key strategic, operational, and tactical decisions in the most expeditious manner. Consequently, the need for a rapid-response CBRNE laboratory has never been more urgent.

The challenge for a NATO laboratory is complicated on several fronts. For consequence management decisions, most countries prefer obtaining advice from their own scientists/subject-matter experts. However, the skills to appropriately collect and analyze samples for a suspected CBRN incident require much training and time. As a result, forward-deployable military scientists and technicians with these required, highly-developed analytical skills are very limited in number. Maintaining a pool of military scientists for each NATO Nation with standardized, highly-technical skills would be most challenging. In addition, many countries have developed different requirements and approaches for the personnel, equipment, and missions for a deployable laboratory. The approaches are dependent upon a variety of factors to include the country's military size, political interest in the degree of retaliatory actions to be taken, and the appropriated budget to support such a laboratory. A single, standardize approach would be difficult to obtain with consensus. Consequently, the NATO deployable CBRNE laboratory has been designed to be flexible in its composition, reflecting each country's interpretation of their deployment need.

NATO's new Strategic Concept adopted at the 2010 Lisbon Summit confirmed the Alliance's commitment to further develop its capacity to defend against the threat of CBRN weapons of mass destruction and protect its populations, territory and forces. NATO developed the Combined Joint CBRN Defence Task Force (CJ-CBRND-TF) as one of NATO's key defences against CBRN events.

## 7.2 COMBINED JOINT CBRN DEFENCE TASK FORCE

The NATO CJ-CBRND-TF consists of CBRN Joint Assessment Team (JAT) and CBRN Defence Battalion. This NATO body is specifically trained and equipped to deal with CBRN events and/or attacks against NATO populations, territory, or forces.

The Battalion and the CBRN JAT, created in 2003 and declared operational the following year, is a multi-national, multi-functional team, able to deploy quickly to participate in the full spectrum of NATO operations.

The Battalion's mission is unique in that it is not only trained for armed conflicts, but is also able to be deployed to crisis situations such as natural disasters and industrial accidents, including those involving hazardous material. The CJ-CBRND-TF can also be deployed in order to support the protection of High Visibility Events (HVE) such as Olympic Games or NATO Summits.

The realization of the CJ-CBRND-TF fulfils two of the capability commitments made by Allies at the 2002 Prague Summit: a Prototype Deployable Nuclear, Biological and Chemical (NBC) Analytical Laboratory and a Prototype NBC Event Response Team. These capabilities greatly enhance the Alliance's defence against WMD.

The Battalion's mission is to provide a rapidly-deployable and credible CBRN defence capability to maintain NATO's freedom of action and operational effectiveness in a CBRN threat environment.

The CBRN Battalion may be used to provide military assistance to civil authorities when authorized by the North Atlantic Council, the Alliance's principal political decision-making body. For example, they played a key planning role during the 2004 Summer Olympics in Greece, and the 2004 Istanbul Summit, where they supported any CBRN-related contingency operations.

The CBRN Defence Battalion is capable of conducting the following tasks:

1) CBRN reconnaissance and monitoring operations;
2) Sampling and Identification of Biological, Chemical, and Radiological agents (SIBCRA);
3) Biological detection and monitoring operations;
4) Provide CBRN assessments and advice to NATO Commanders; and
5) CBRN hazard management operations, such as decontamination.

## 7.3 NATO NATIONS PARTICIPATING IN THE DEFENCE TASK FORCE

Following the agreement at the 2002 Prague Summit to enhance the Alliance's defence capabilities against weapons of mass destruction, the North Atlantic Council, in June 2003, decided to form a Multi-national CBRN Defence Battalion and JAT.

The structure of the Battalion was established at a planning conference on 17-18 September 2003. The following month, on 28 October 2003, a force generation conference was held at Supreme Headquarters Allied Command Europe (SACEUR). On 18-21 November 2003, a follow-up conference was held in the Czech Republic, the first volunteer lead country. Twelve other nations (Belgium, Canada, Hungary, Italy, Norway, Poland, Portugal, Romania, Spain, Turkey, United Kingdom and United States) have offered to provide forces for this first Multi-national CBRN Defence Battalion.

The Battalion reached its initial operational capability on 1 December 2003. Full operational capability was achieved on 28 June 2004 as declared by SACEUR at the Istanbul Summit, and responsibility was transferred into the strategic command of Allied Command Operations. From then on, the Battalion was included in the six-month rotation system of the NATO Response Force.

Some 21 NATO Nations now contribute to the Combined Joint CBRN Defence Task Force on a voluntary basis. National commitments vary depending on the rotation, but there are usually 8 – 10 Nations involved per rotation.

For the very first time a non-NATO Member Nation participated in 2010. Ukraine contributed a decontamination platoon after having accomplished a NATO evaluation and certification process very successfully.

## 7.4 OPERATIONAL PROCESS

The CBRN JAT and CBRN Battalion fall under the strategic command of the SACEUR. Operational control is delegated to a subordinate command as required.

NATO's Allied Command Transformation provides evaluation standards, supports training and determines future NBC defence requirements and develops capabilities.

The Battalion level organization is composed of personnel from a number of NATO Nations. Like the NATO Response Force, dedicated personnel are based in their countries, coming together for training and deployment.

A voluntary lead country is identified for each rotation. The lead country hosts the CBRN Joint Assessment Team and Battalion headquarters, responsible for command and control arrangements, maintaining standard operational procedures, sustaining readiness levels and for planning and conducting training. Contributing countries supply functional capabilities. This includes providing requisite troops, equipment and logistical support in accordance with mission requirements. The Defence Task Force is composed of separate but complimentary components, which can be deployed in different stages and different combinations to suit each mission.

The components are:

1) **Joint Assessment Team** – Comprised of specialists that provide CBRN-related advice and support;
2) **Headquarters Command and Control** – Tailored command and control capabilities with a robust communications package to support assigned and attached organizations;
3) **Reconnaissance** – Designed to provide route, area and point detection and identification of agents;
4) **Decontamination** – Maintains the capability to decontaminate personnel and equipment; and
5) **Deployable Analytical CBRN Laboratories**.

Designed to provide expert sampling, analysis, and scientific advice to support operational Commanders.

The Battalion has a close relationship with the NATO Response Force. While it can be deployed independently, it is consistent and complementary to the NATO Response Force. Its strength is included within the NATO Rapid Force structure, and it can deploy within 5 to 30 days.

# Annex A – HFM-177 MEETING ITINERARIES

## A.1 6-8 APRIL 2009 MEETING IN EDGEWOOD, MARYLAND, USA

**NATO RTG/HFM-177**
**Deployable Application of Biotechnology**

**6-8 April 2009**
**Conference Room 229, Building E3330**
**US Army Edgewood Chemical Biological Center**
**Edgewood, MD 21010-5424**
**United States**

*Center Host: Mr. Raymond Mastnjak, X5-2516*
*Visit POC: Ms. Marlena Long, X5-0987*

*Mil Uniform: Duty Uniform*
*Civilian: Business Attire*

**ARRIVAL DATA:**
Date: Mon 6 Apr 09
Time: 0800
Mode: POV
Loc: Bldg E3330

**DEPARTURE DATA:**
Date: Wed, 8 Apr 09
Time: 1200
Mode: POV
Loc: Bldg E3330

***ITINERARY***

| *Time* | *Event* | *Mode/Location* | *POC* |
|---|---|---|---|
| **Monday, 6 April** | | | |
| 0830 | Arrive at ECBC | Bldg E3330 / Rm 229 | Mr. Raymond Mastnjak |
| 0900 – 0930 | Introductions and Opening Comments (Mastnjak) | Bldg E3330 / Rm 229 | Mr. Raymond Mastnjak |
| 0930 – 1000 | Overview Presentation of the HFM-177 (Schlager/Wade) | Bldg E3330 / Rm 229 | Mr. Raymond Mastnjak |
| 1000 – 1030 | Overview of Czech Republic Lab Technology (Hubálek) | Bldg E3330 / Rm 229 | Mr. Raymond Mastnjak |
| 1030 – 1045 | Break and Informal Discussion | Bldg E3330 / Rm 229 | Mr. Raymond Mastnjak |
| 1045 – 1115 | Overview of the Turkey Lab Technology (Budak) | Bldg E3330 / Rm 229 | Mr. Raymond Mastnjak |
| 1115 – 1145 | General Overview of Georgia Lab Technology (Tabagari) | Bldg E3330 / Rm 229 | Mr. Raymond Mastnjak |
| 1145 – 1300 | Lunch Break | Bldg E3330 / Rm 229 | Mr. Raymond Mastnjak |
| 1300 – 1330 | Overview of US Lab Technology (Mastnjak/Schlager) | Bldg E3330 / Rm 229 | Mr. Raymond Mastnjak |
| 1330 – 1430 | Tour, STORM High Throughput Mobile Bioanalytical Lab | Bldg E3330 | Mr. Raymond Mastnjak |
| 1430 – 1445 | Break | Bldg E3330 / Rm 229 | Mr. Raymond Mastnjak |
| 1445 – 1600 | Open Discussion, Deployable Bioanalytical Laboratories | Bldg E3330 / Rm 229 | Mr. Raymond Mastnjak |
| 1700 – 1800 | Drinks / Happy Hour / Ongoing Discussion (Optional) | | Mr. Raymond Mastnjak |
| 1800 – 2000 | Dinner Outing (All) | | Mr. Raymond Mastnjak |
| **Tuesday, 7 April** | | | |
| 0900 – 0930 | Coffee and Open Discussion (All) | Bldg E3330 / Rm 229 | Mr. Raymond Mastnjak |
| 0930 – 1000 | Triage of Uncharacterized Samples (Unknowns) (Mastnjak) | Bldg E3330 / Rm 229 | Mr. Raymond Mastnjak |
| 1000 – 1030 | Field Operations in a Mobile Bioanalytical Laboratory | Bldg E3330 / Rm 229 | Dr. Carrie Poore |

| | | | |
|---|---|---|---|
| 1030 – 1045 | Break and Informal Discussion | Bldg E3330 / Rm 229 | Mr. Raymond Mastnjak |
| 1045 – 1145 | Tour, Mobile Bioanalytical Lab at Bldg E5830 (All) | Bldg E5830 | Mr. Raymond Mastnjak |
| 1145 – 1300 | Lunch | Bldg E3330 / Rm 229 | Mr. Raymond Mastnjak |
| 1300 – 1430 | Tour, US Army / FBI Sample Receipt Facility (Mastnjak) | Bldg E3330 / Rm 229 | Mr. Raymond Mastnjak |
| 1430 – 1445 | Break | | Mr. Raymond Mastnjak |
| 1445 – 1600 | Open Discussion (All) | Bldg E3330 / Rm 229 | Mr. Raymond Mastnjak |
| | * Presentation of new and existing bio- and nano-technologies | | |
| | * Discussion and consideration deployable mobility aspects, structures and technologies for lab systems | | |
| | * New concepts and consensus for mobile platforms labs | | |
| 1700 – 1800 | Drinks / Happy Hour (Optional) | | Mr. Raymond Mastnjak |
| 1800 – 2000 | Dinner Outing | | Mr. Raymond Mastnjak |

## Wednesday, 8 April (Morning only)

| | | | |
|---|---|---|---|
| 0900 – 0930 | Coffee and Open Discussion (All) | Bldg E3330 / Rm 2299 | Mr. Raymond Mastnjak |
| 0930 – 1045 | Wrap-up and discussion of preparation meeting minutes and results for NATO HFM submission (All) | Bldg E3330 / Rm 229 | |
| 1045 – 1100 | Break | Bldg E3330 / Rm 229 | Mr. Raymond Mastnjak |
| 1100 – 1130 | Concluding Comments / Open Discussion (All) | Bldg E3330 / Rm 229 | Mr. Raymond Mastnjak |

## A.2 29-30 OCTOBER 2011 MEETING IN MUNICH, GERMANY

**NATO RTG/HFM-177**
**Deployable Application of Biotechnology**

**29-30 October 2011**
**Theresia Conference Room**
**Holiday Inn Munich – Schwabing**
**Munich, Germany**

**Saturday, 29 October**

| | |
|---|---|
| 0900 – 0930 | Introductions and Overview Presentation of the HFM-177 (Schlager/Lukey)<br>– *With coffee & bagels* |
| 0930 – 1000 | Deployable Biological Laboratory of the Armed Forces of the Czech Republic (Hubálek/Pisa/Dresler) |
| 1000 – 1015 | US Army Bio-Environmental and Public Health Mobile Analytical Laboratory Capabilities (Walker) |
| 1015 – 1030 | Construction of Deployable Laboratories During Times of Reduced Funding (Mastnjak) |
| 1030 – 1045 | Break and Informal Discussion |
| 1045 – 1100 | The Role of Triage in Sample Analysis (Mastnjak) |
| 1100 – 1130 | The Bundeswehr Rapidly Deployable Bio Lab – Microbiological High-Tech Diagnostics for Outbreak Investigations Abroad (Woelfel) |
| 1130 – 1300 | Lunch Break (at the hotel) |
| 1300 – 1330 | Defence Nano-Biotechnology Applications in Turkey (Budak) |
| 1330 – 1400 | General Overview of Israel Lab Technology (Marks) |
| 1400 – 1415 | Break and Informal Discussion |
| 1415 – 1445 | General Overview of Georgia Lab Technology (Tabagari) (Optional) |
| 1445 – 1500 | Introduction to the “Biological Surveillance Collector System (BSCS)” (Howells) |
| 1500 – 1530 | NATO’s Rapidly Deployable Outbreak Investigation Team (RDOIT) – From Concept to Development, from Implementation to Deployment (Wojtyk/Thibault/Chickery) |
| 1530 – 1630 | Discussion of Presentations and Initial Ideas on NATO Lab Design Elements:<br>• Development of the deployable laboratory design, construction, and materials<br>• Analysis of existing instrument technology and procedures<br>• Analysis of emerging nano-/bio-technology for instrument acquisition<br>• Integration of existing and emerging technologies into a deployable laboratory product |
| 1830 – 1915 | Drinks / Happy Hour at the hotel (Optional) |

| | |
|---|---|
| 1930 – Ends | Dinner at "Wirtshaus zur Brezn" (All)<br>Leopoldstr.72, D-80802 München<br>http://www.zurbrezn.de/<br>+49 89 390092 |

**Sunday, 30 October**

| | |
|---|---|
| 0900 – 0930 | Coffee and Continue Open Discussion (All) |
| 0930 – 1000 | Complete Open Discussion: Development of the Deployable Laboratory Design, Construction, and Materials (All) |
| 1000 – 1030 | Complete Open Discussion: Analysis of Existing Instrument Technology and Procedures (All) |
| 1030 – 1045 | Break and Informal Discussion |
| 1045 – 1115 | Complete Open Discussion: Analysis of Emerging Nano-/Biotechnology for Instrument Acquisition (All) |
| 1115 – 1145 | Complete Open Discussion: Integration of Existing and Emerging Technologies into a Deployable Laboratory Product (All) |
| 1145 – 1300 | Lunch (at the hotel) |
| 1300 – 1430 | Discussion / Concluding Comments (All) |
| 1800 – 1900 | Drinks / Happy Hour at the hotel (Optional) |
| 1930 – Ends | Dinner at "The Big Easy"<br>Frundsbergstr. 46, D-80634 München<br>http://www.thebigeasy.de/<br>+49 89 15890253 |

# Annex B – NATO HFM-177 DEPLOYABLE LAB SURVEY

## B.1 TEMPLATE

**Country:** **Institution:**

**Point of Contact:** **Telephone:** **Email:**

| | | Yes | No | Don't Know |
|---|---|---|---|---|
| 1. | Does your Ministry Of Defence (MOD) possess permanent field-forward or mobile/deployable medical laboratory facilities for biological or chemical defence? | ☐ | ☐ | ☐ |

→ If yes, please provide descriptive information and/or documentation using an attachment. If your country has more than one deployable system, please complete this Survey for each system.

2. If your MOD does NOT possess medical biological and/or chemical defence facilities:

| | | Yes | No | Don't Know |
|---|---|---|---|---|
| | Does your MOD coordinate with any other responsible/responsive government agency (i.e., Ministry of Health) concerning the detection of infectious disease outbreaks in field containment? | ☐ | ☐ | ☐ |

→ If so, please briefly describe the arrangement:

3. Who is responsible for the technical and operational management of these field-forward or mobile/deployable medical laboratory facilities?

☐ Medical Service

☐ CBRN Defence

☐ Others?

4. The laboratory is:

☐ Self-mobile
→ Platform/type of vehicle:

☐ Deployable
→ Typical size and weight of single package:
→ Total number of packages:

☐ Others?

**Country:** **Institution:** **Point of Contact:**

5. The laboratory can be transported:

- ☐ By car
- ☐ By truck
- ☐ In a civilian airplane as passenger luggage
- ☐ In a civilian airplane as cargo only
- ☐ In a military airplane
- ☐ Others?

6. How long does it take to set up the lab after arrival at the site of operation?

7. What kind of external supplies (water, electricity, etc.) are needed to operate the laboratory?

| | Yes | No | Don't Know |
|---|---|---|---|
| 8. Is the laboratory able to work autonomously without external supplies? → If your answer is yes, how long is the laboratory operational before external supplies are needed? | ☐ | ☐ | ☐ |
| 9. Does your laboratory participate in any scheme for ensuring permanent diagnostic capacity, such as an on call or a duty team regimen? | ☐ | ☐ | ☐ |
| → If your answer is yes, is this restricted to specific diagnostic tests, or specific diseases? | ☐ | ☐ | ☐ |
| → If yes, which are they? | | | |
| 10. Is this call system also designed to serve laboratory or hospital emergencies? | ☐ | ☐ | ☐ |
| 11. Is it intended to respond to specific demands from a surveillance system? | ☐ | ☐ | ☐ |
| 12. Is there a filter, at any level (national or regional), that will receive the demand and call the system only after assessment, deciding whether or not this is needed? | ☐ | ☐ | ☐ |

**Country:** **Institution:** **Point of Contact:**

13. What is the timing of the duty?

- ☐ 24 hours a day, 7 days a week?
- ☐ Non-working hours of the day and non-working days of the week?
- ☐ Any other schedule?

| | Yes | No | Don't Know |
|---|---|---|---|
| 14. Is the duty system part of a network? | ☐ | ☐ | ☐ |

- ☐ National?
- ☐ Regional?
- ☐ International?
- ☐ Other?

| | Yes | No | Don't Know |
|---|---|---|---|
| 15. If your answer to #14 was yes, does the duty call system include a whole team? | ☐ | ☐ | ☐ |

→ How many people are involved?

→ Scientists (Experience/Degree type)?

→ Technologists (Experience/ Degree type)?

→ Others?

| | Yes | No | Don't Know |
|---|---|---|---|
| 16. Are there other relevant capabilities or features of your field-forward or mobile/ deployable medical laboratory facility which you want to mention or do you have any comments? | ☐ | ☐ | ☐ |

→ If yes, please provide this information or comments using an attachment or use the space below:

**Country:** **Institution:** **Point of Contact:**

17. Which of the following agents can be detected in the laboratory and by which technology or method?

| | Don't Know | Not Available | Molecular (PCR) Assay | Serological Assay | Microscopy (e.g. staining) | Culture | Other Methods |
|---|---|---|---|---|---|---|---|
| **Abrin** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Aflatoxins** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **B. pseudomallei** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Bacillus anthracis** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Botulinum Toxine** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Brucella spp.** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Burkholderia mallei** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **CCHF virus** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Chikungunya virus** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Clostridium perfringens toxin** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Coxiella burnetii** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Eastern-Equine-Encephalitis virus** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Ebola virus** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Escherichia coli** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Flaviviruses** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Francisella tularensis** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Hantaviruses** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Influenza virus** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Junin virus** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Lassa virus** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Machupo virus** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Marburg virus** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Monkeypox virus** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Omsk virus** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Orientia tsutsugamushi** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Palytoxin** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Ricin** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Rickettsia rickettsii** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Rickettsia typhi** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Rift Valley fever virus** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Salmonella spp.** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Salmonella Typhi** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Saxitoxins** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Shigella dysenteriae** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Staphylococcal enterotoxins** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Tetradotoxin** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Tick-borne encephalitis (TBE) virus** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Trichothecene** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Variola virus** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Venezuelan-Equine-Encephalitis virus** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Vibrio cholerae** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Western-Equine-Encephalitis virus** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Yellow fever virus** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Yersinia pestis** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| **Other (please list)** | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | |
| | | | | | | | |

**Country:** **Institution:** **Point of Contact:**

| | | Yes | No | Don't Know |
|---|---|---|---|---|
| 18. | Is the laboratory able to detect or identify Chemical Warfare Agents (CWAs)? | ☐ | ☐ | ☐ |

→ If yes, which methods or technologies are used?

→ Please identify which agents (Soman, Sarin, Tabun, VX, etc.) can be detected or identified:

| | | Yes | No | Don't Know |
|---|---|---|---|---|
| 19. | Does the laboratory have nuclear/radiological general or isotope specific survey detection systems and assays? | ☐ | ☐ | ☐ |

→ If yes, which methods or technologies are used?

→ Please identify which isotopes can be detected or identified.

# Annex C – HFM-177 MEETING PRESENTATIONS

## C.1 HFM-177 MEETING: 6-8 APRIL 2009, EDGEWOOD, MARYLAND, USA

### C.1.1 Georgia Presentation – by Sergo Tabagari


## CURRENT SITUATION

- The events that took place all over the world during last several years, have proved that the international terrorism is the biggest problem of our time.
- Bioterrorism is one of the cruelest and damaging types of terrorism.

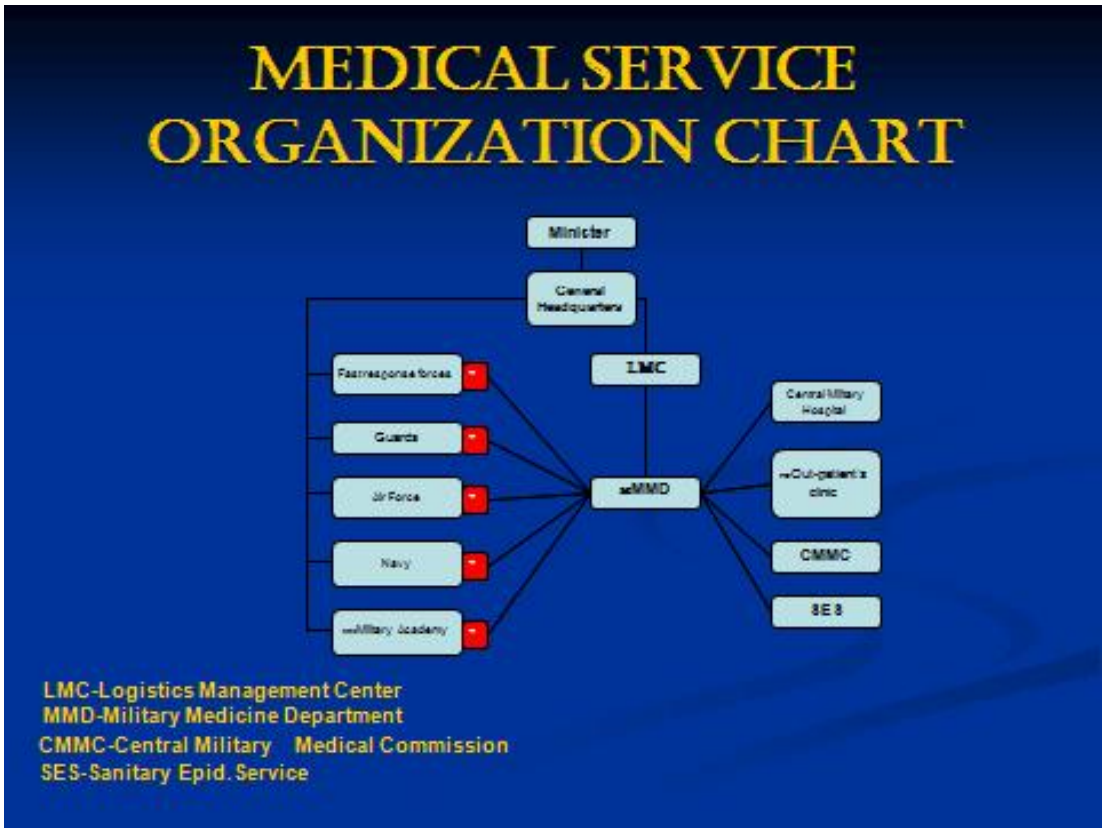
## CURRENT SITUATION

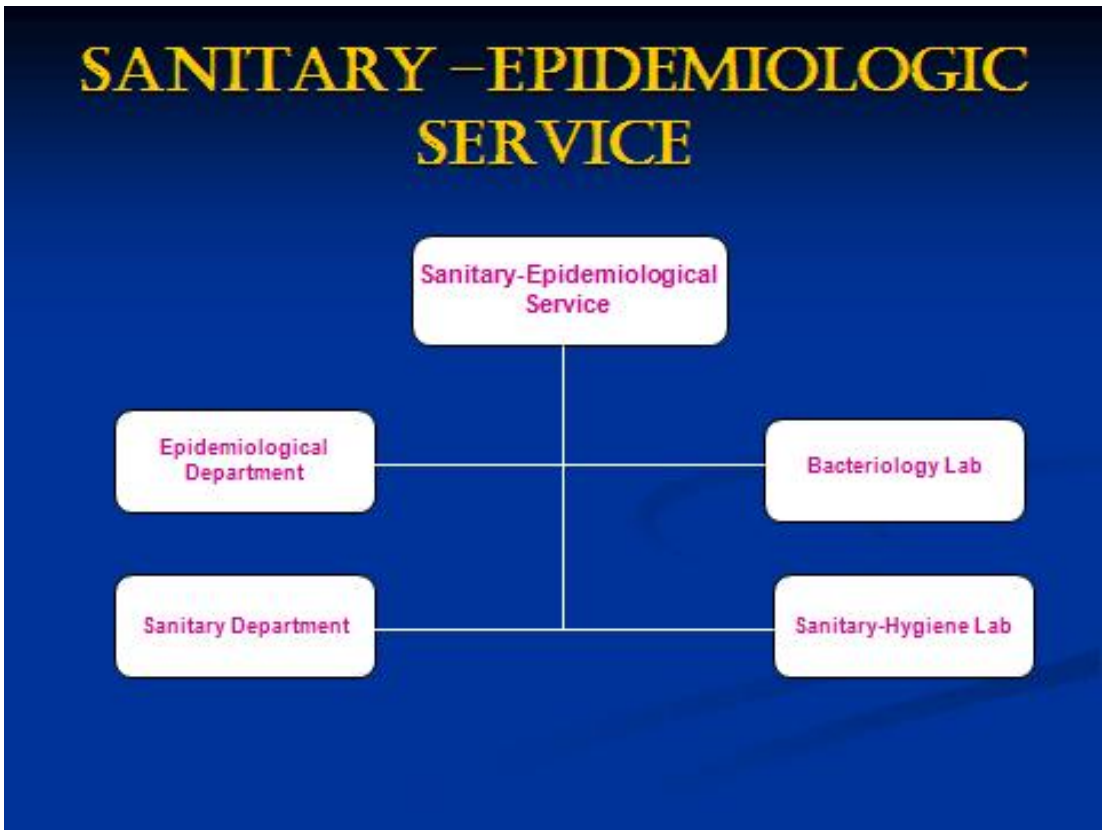
- Georgia has been in a very difficult situation since the last independence declaration more than 15 years ago.
- Georgia is located in one of the most unstable regions of the world. This causes the influx of the large numbers of refugees

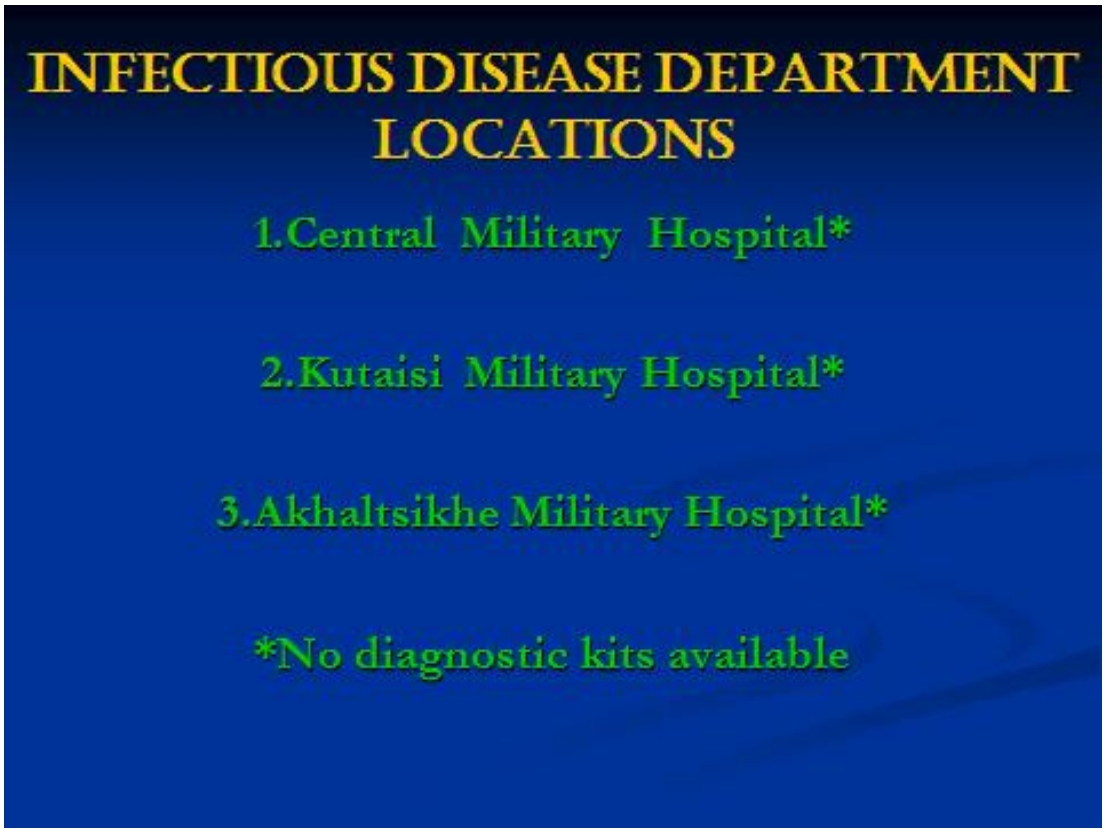
# CURRENT SITUATION

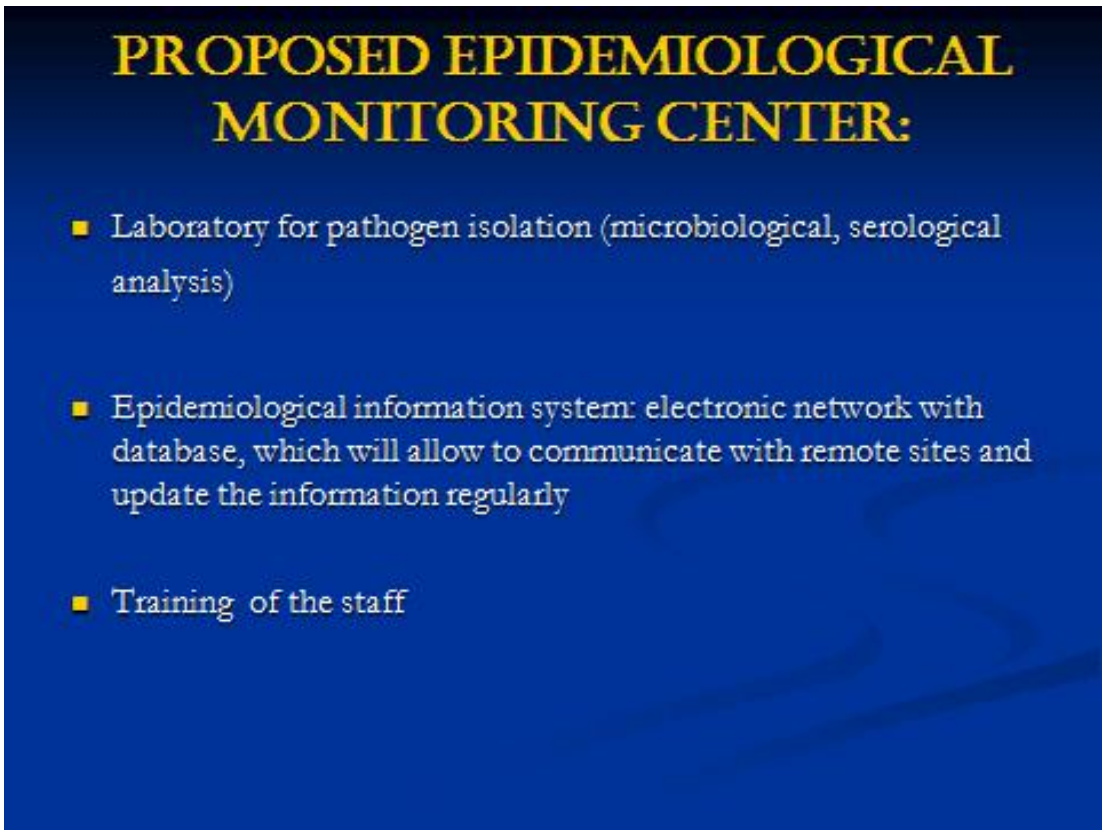
- The borders are not protected with respect to EDP detection and isolation capabilities.
- The smuggling of goods and products is widespread
- The existing water reservoirs are not protected
- There are the Natural Nidus of the most dangerous infections: plague, tularemia, anthrax etc in Georgia

# ORGANIZATIONAL CHART OF MEDICAL SERVICE OF MINISTRY OF DEFENSE


MEDICAL SERVICE ORGANIZATION CHART
Minister
General Headquarters
LMC
Guards
Air Force
Navy
MMD
Central Military Hospital
CMMC
SES
LMC-Logistics Management Center
MMD-Military Medicine Department
CMMC-Central Military Medical Commission
SES-Sanitary Epid. Service


SANITARY –EPIDEMIOLOGIC SERVICE
Sanitary-Epidemiological Service
Epidemiological Department
Bacteriology Lab
Sanitary Department
Sanitary-Hygiene Lab


INFECTIOUS DISEASE DEPARTMENT LOCATIONS
1.Central Military Hospital*
2.Kutaisi Military Hospital*
3.Akhaltsikhe Military Hospital*
*No diagnostic kits available


PROPOSED EPIDEMIOLOGICAL MONITORING CENTER:
Laboratory for pathogen isolation (microbiological, serological analysis)
Epidemiological information system: electronic network with database, which will allow to communicate with remote sites and update the information regularly
Training of the staff

## PROPOSED EPIDEMIOLOGICAL MONITORING CENTER (CONTINUED)

Staff of the Sentinel Stations:

a) Epidemiologist
b) Bacteiologist
c) 2 Paramedics
d) Disinfector
e) Veterinarians
f) Drivers

## PROPOSED EPIDEMIOLOGICAL MONITORING CENTER (CONTINUED)

- Epidemiological Monitoring Center should be located in Tbilisi

- 3 Sentinel stations :

-Eastern Georgia Sentinel station –the information from 4 garrisons will go here- Tbilisi, Gori, Telavy and Vasiani

-Western Georgia Sentinel station-the information from 3 garrisons will go here-Batumi, Poty, Kutaisi

-South Georgia Sentinel station-Akhaltsikhe garrison, location-Akhaltsikhe

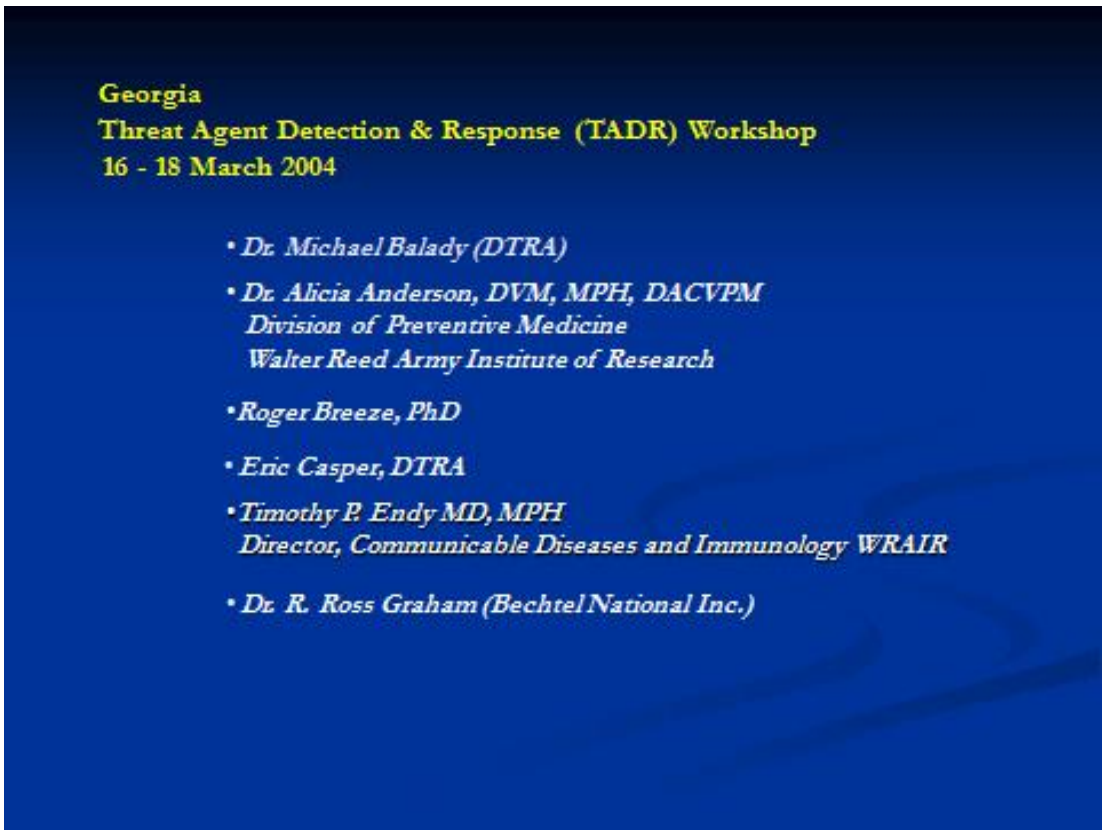
# THE PURPOSE OF SENTINEL STATIONS

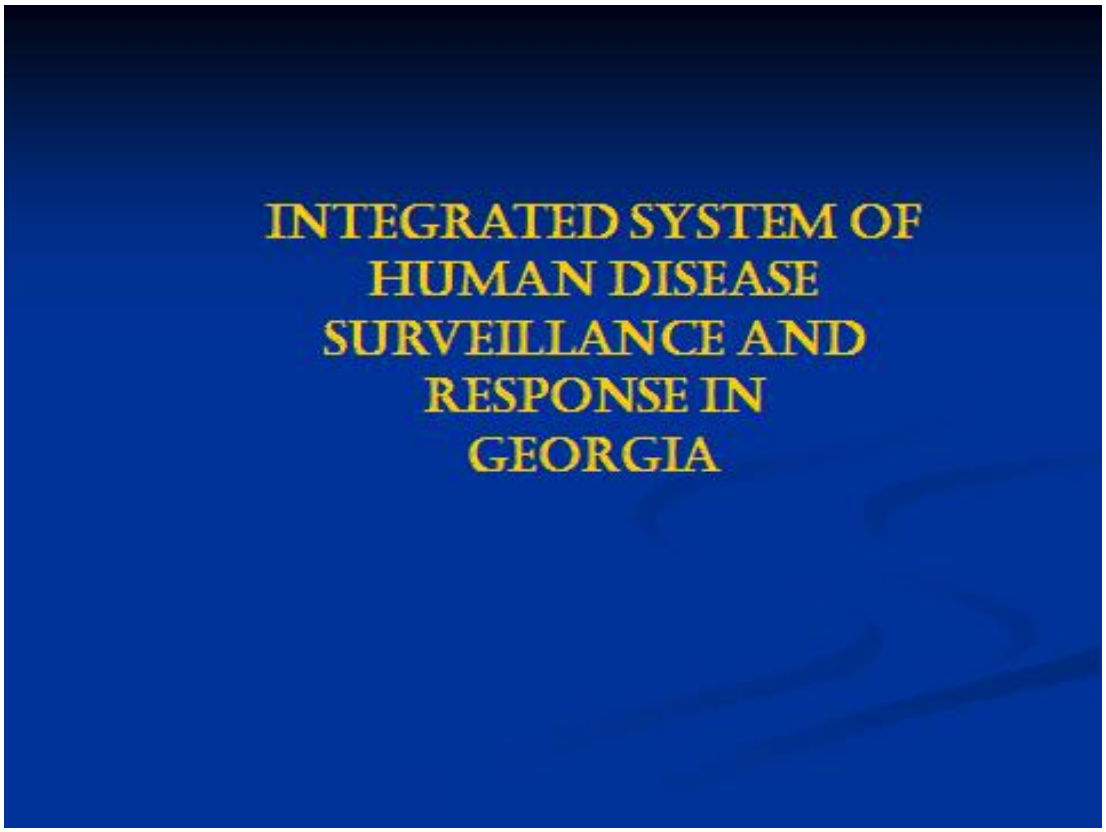
- Epidemiological surveillance of appropriate garrisons
- In cases of disease outbreak taking all necessary actions for stopping the epidemics
- Creation of the database and the analysis of received data

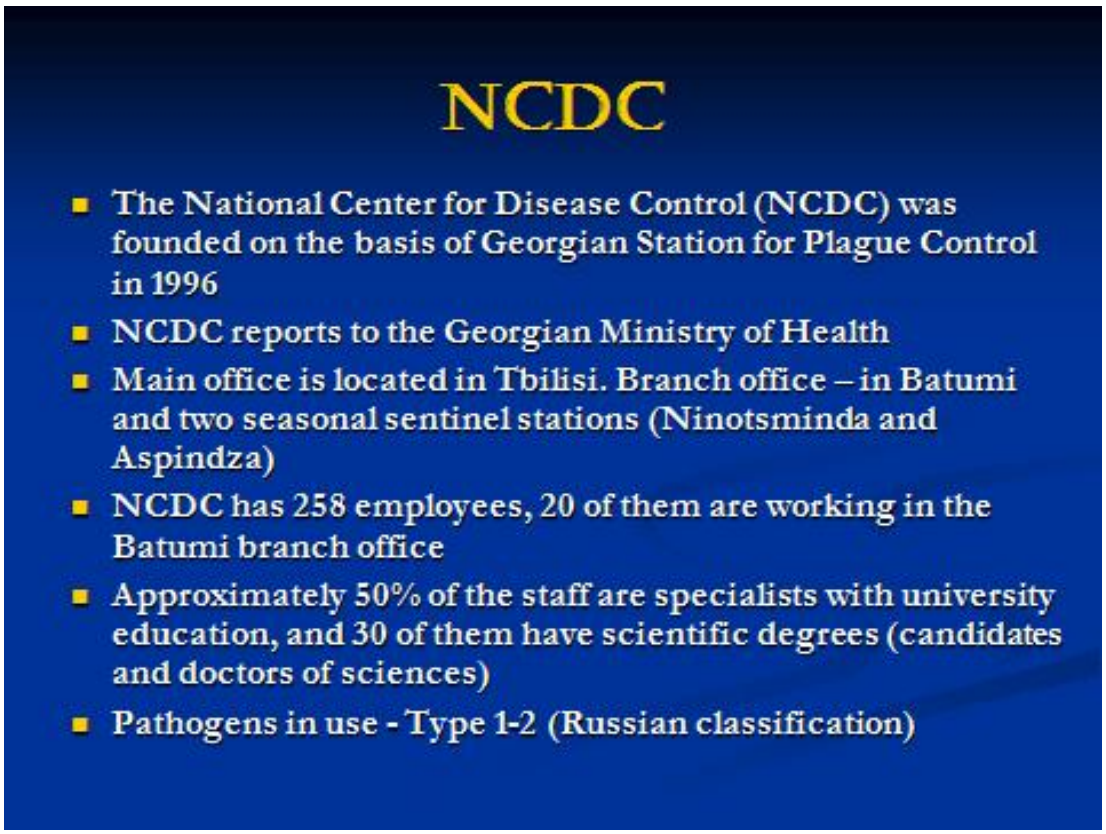
## Planning Epidemiological Monitoring System

- The 3 Epidemiological Monitoring Centers should be located in:
  - Tbilisi (Eastern Georgia)
  - Kutaisi (Western Georgia)
  - Akhaltsikhe (South Georgia)
- The 3 Mobile Groups working in nidus of Infections and on sites of bioterroristic attacks


BIOLOGICAL THREAT AGENT DETECTION & RESPONSE (TADR) IN GEORGIA
Defence Threat Reduction Agency (US)
Walter Reed Army Institute of Research (US)
Ministry of Labor Health and Social Welfare (GEO)
Ministry of Defence (GEO)
Ministry of Agriculture (GEO)


Georgia
Threat Agent Detection & Response (TADR) Workshop
16 - 18 March 2004
• Dr. Michael Balady (DTRA)
• Dr. Alicia Anderson, DVM, MPH, DACVPM
Division of Preventive Medicine
Walter Reed Army Institute of Research
• Roger Breeze, PhD
• Eric Casper, DTRA
• Timothy P. Endy MD, MPH
Director, Communicable Diseases and Immunology WRAIR
• Dr. R. Ross Graham (Bechtel National Inc.)


INTEGRATED SYSTEM OF
HUMAN DISEASE
SURVEILLANCE AND
RESPONSE IN
GEORGIA


NCDC
The National Center for Disease Control (NCDC) was founded on the basis of Georgian Station for Plague Control in 1996
NCDC reports to the Georgian Ministry of Health
Main office is located in Tbilisi. Branch office – in Batumi and two seasonal sentinel stations (Ninotsminda and Aspindza)
NCDC has 258 employees, 20 of them are working in the Batumi branch office
Approximately 50% of the staff are specialists with university education, and 30 of them have scientific degrees (candidates and doctors of sciences)
Pathogens in use - Type 1-2 (Russian classification)

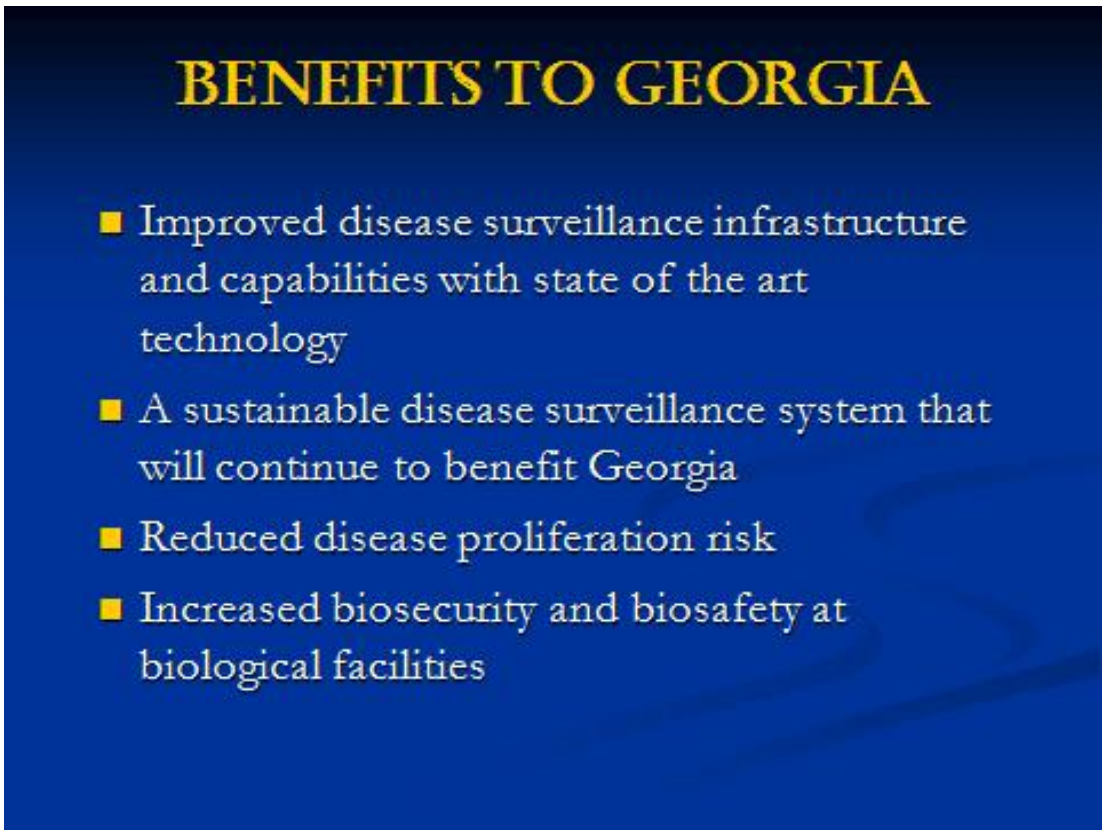
## NCDC PARTICIPATED IN THE FOLLOWING PROJECTS:

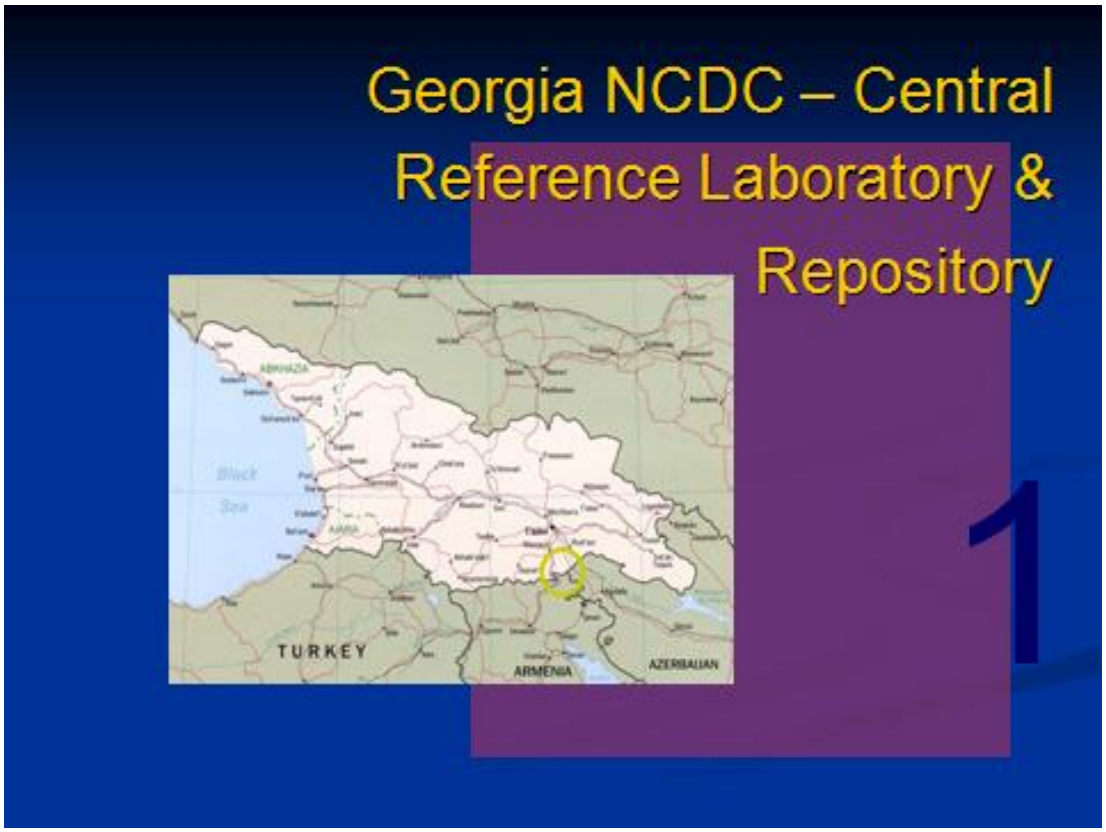
- International Training and Research in Emerging Infectious Diseases " (1997 – 2002) – Fogarthy Center, NIH
- "Establishing Epidemiological Network on the Territory of Georgia" (1997) - "Open Society Georgia" Foundation
- "Improvement of Epidemiological Network in Georgia" (1998) - "Open Society Georgia" Foundation
- Reproductive Health Survey (1999-2000) - UNFPA, UNICEF, USAID, UNHCR, AIHA , CDC
- Nutritional Status of Children Under Five Years of Age in Six Regions of Georgia (2000 – 2001) – USAID/Save the Children-US, Georgia Field Office
- Provision of Epidemiological Survey Services on Baku – Tbilisi – Ceyhan Pipeline Route – 2003, British Petroleum

## ESTABLISH AN INTEGRATED, SECURE AND SUSTAINABLE DISEASE SURVEILLANCE SYSTEM IN GEORGIA

- Support human, environmental, and veterinary disease monitoring
- Ensure close cooperation among all relevant ministries and institutes and other international organizations
- Promote potential for integration into a regional disease surveillance system
- Ensure biosecurity and biosafety of biological facilities.

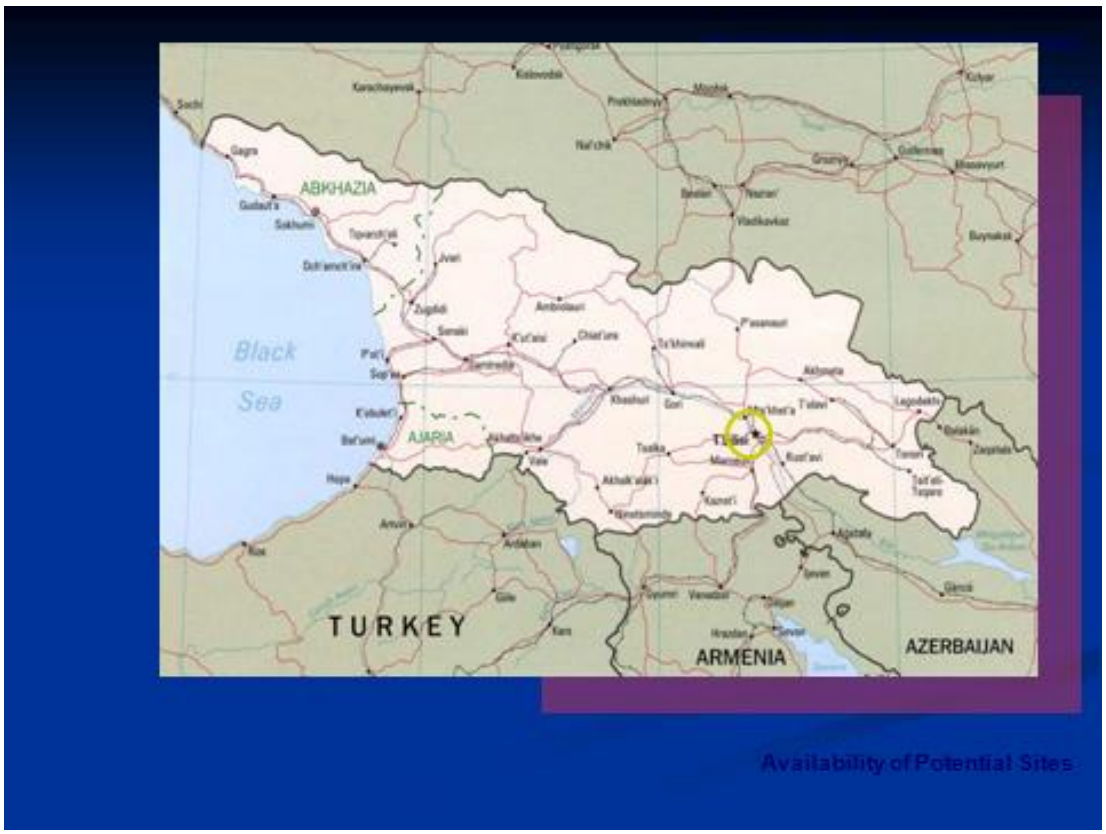

PROJECT GOALS
Laboratory
Secure and safeguarded central reference laboratories
Safe, secure, and efficient pathogen transportation systems and capabilities
Verifiable training in biosafety/biosecurity, diagnostics
Epidemiology and Surveillance
Standardized and repeatable human and animal disease monitoring systems
Mobile epidemiological response teams and secure transportation of disease elements
Verifiable epidemiology training
Communications and Information Technology
Design, develop, and deploy and sustain a robust and secure electronic communicable disease reporting system
Create and sustain secure communications and data storage systems
Rules and Regulations
Apply national regulations as they relate to BWPPP

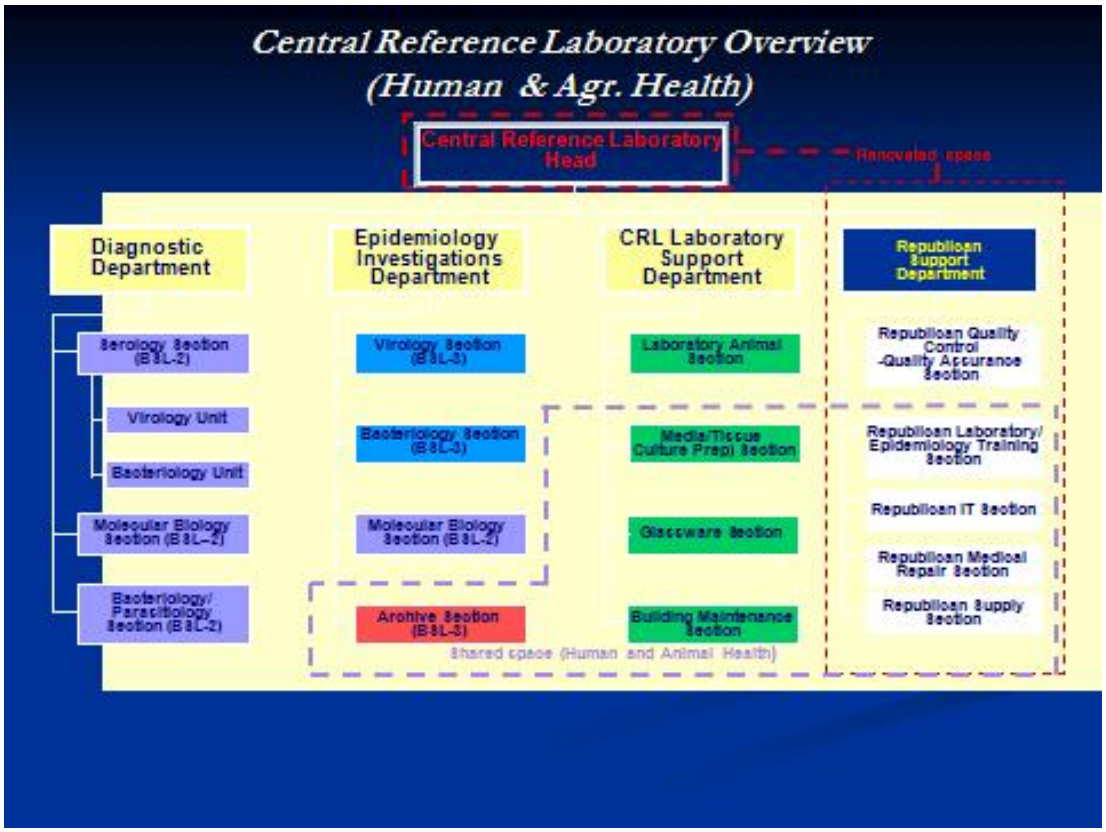

BENEFITS TO GEORGIA
Improved disease surveillance infrastructure and capabilities with state of the art technology
A sustainable disease surveillance system that will continue to benefit Georgia
Reduced disease proliferation risk
Increased biosecurity and biosafety at biological facilities

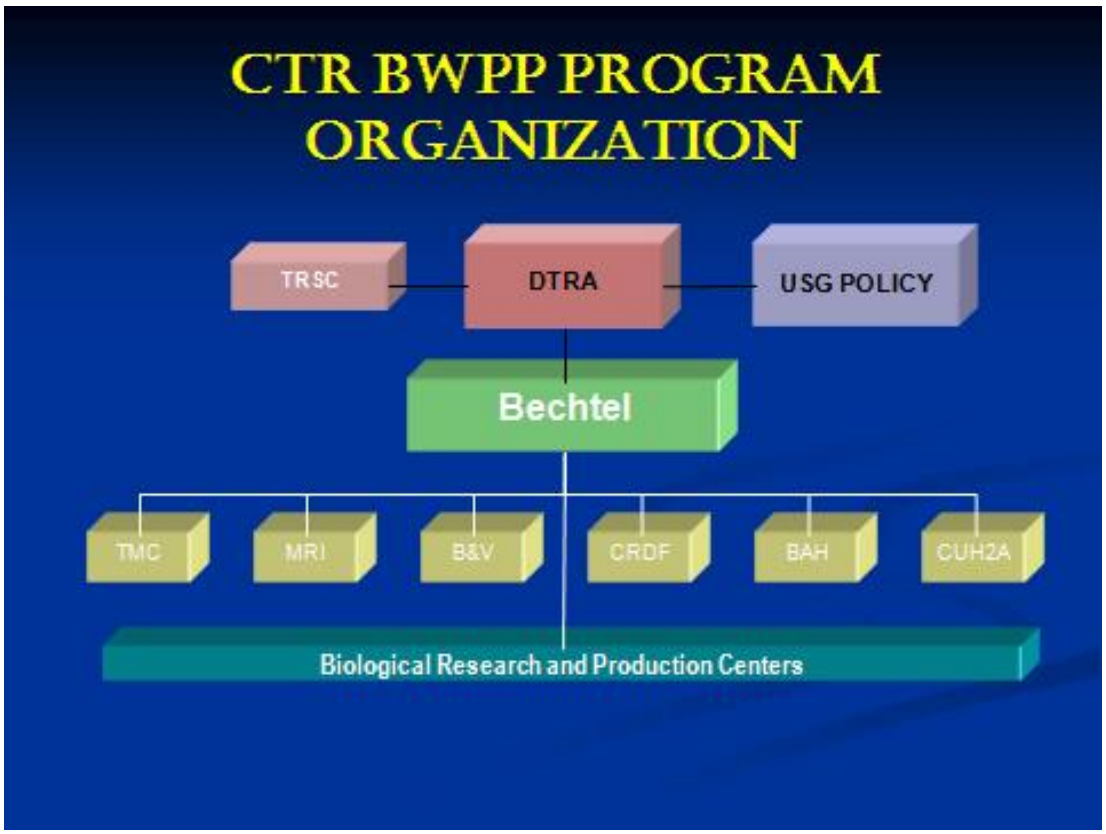

Georgia NCDC – Central
Reference Laboratory &
Repository
1
TURKEY
ARMENIA
AZERBAIJAN

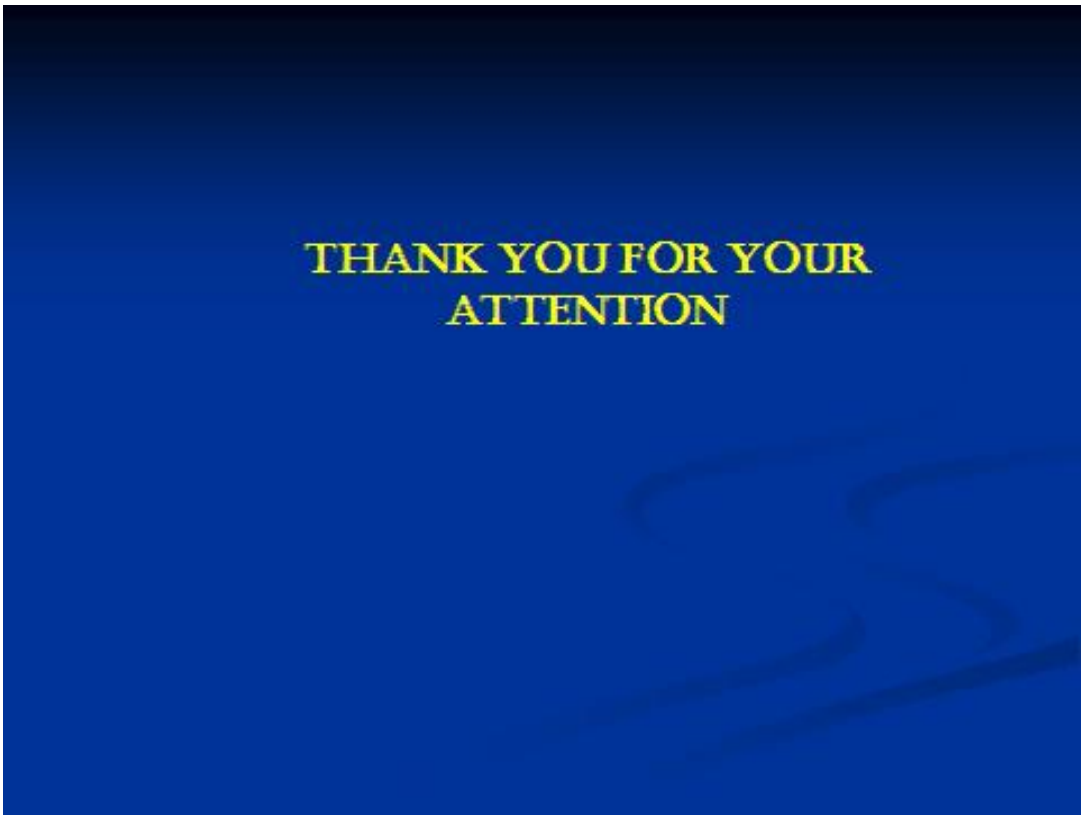

Site Opportunities & Constraints Overview


Disposition and Function of Existing Buildings on Site
Building function
1. Administration
2. Admin level 1 / lab levels 2-6
3. Lab (virus, culture production/ Polio)
4. Training / Storage
5. Vivarium
6. Warehouse shed
7. Main site electrical sub station
8. Warehouse
9. Electrical transformer
10. Water sterilization waste system
11. Vehicle garage / parking
12. Not NCDC property
13. Guard Station

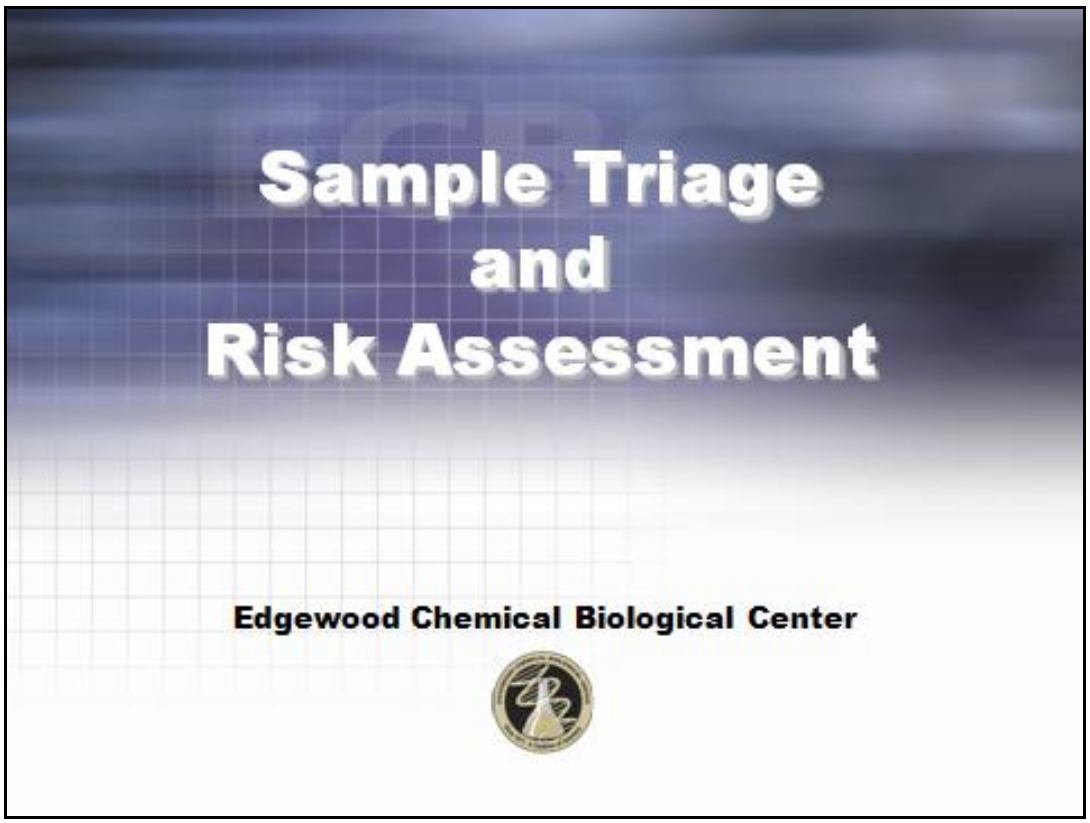

ABKHAZIA
Black Sea
AJARIA
TURKEY
ARMENIA
AZERBAIJAN
Availability of Potential Sites


Central Reference Laboratory Overview
(Human & Agr. Health)
Central Reference Laboratory Head
Renovated space
Diagnostic Department
Epidemiology Investigations Department
CRL Laboratory Support Department
Republican Support Department
Serology Section (BSL-2)
Virology Unit
Bacteriology Unit
Molecular Biology Section (BSL-2)
Bacteriology/ Parasitology Section (BSL-2)
Virology Section (BSL-3)
Bacteriology Section (BSL-3)
Molecular Biology Section (BSL-2)
Archive Section (BSL-3)
Laboratory Animal Section
Media/Tissue Culture Prep Section
Glassware Section
Building Maintenance Section
Republican Quality Control -Quality Assurance Section
Republican Laboratory/ Epidemiology Training Section
Republican IT Section
Republican Medical Repair Section
Republican Supply Section
Shared space (Human and Animal Health)


CTR BWPP PROGRAM ORGANIZATION
TRSC
DTRA
USG POLICY
Bechtel
TMC
MRI
B&V
CRDF
BAH
CUH2A
Biological Research and Production Centers




### C.1.2 USA Presentation – by Raymond Mastnjak



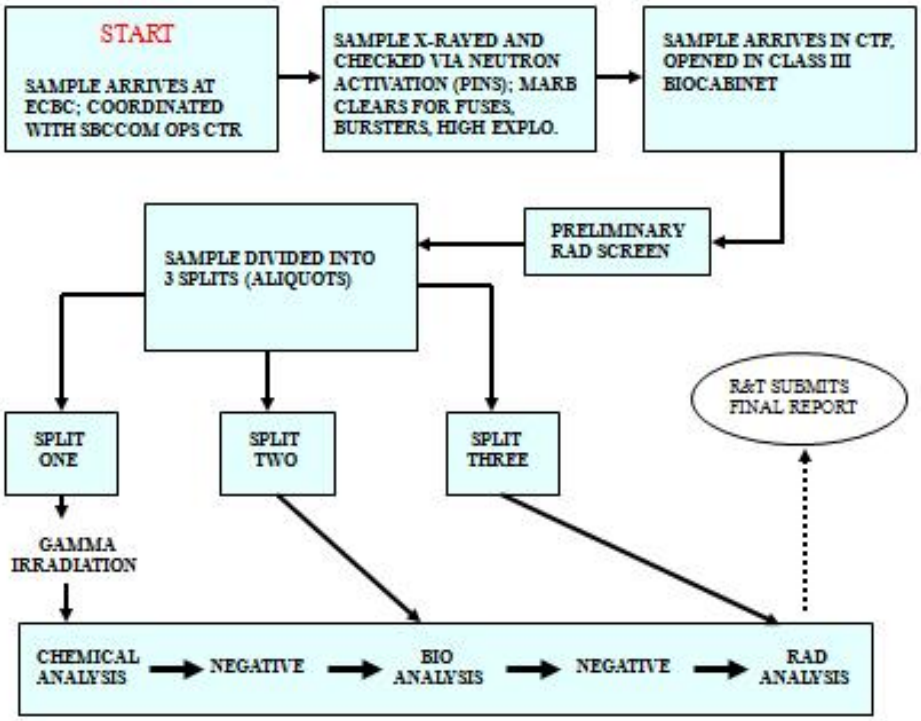
# "Unknowns" Triage Strategy

- Each Sample is Unique: Cookbook Protocols Do Not Work
- Key to Success: Desktop Risk Assessments
  - ✓ Must Include Experienced Chemists, Biologists, and Risk Management Personnel
  - ✓ Review All Available Data
- Special Concerns
  - ✓ Explosively Configured Devices / Munitions
  - ✓ Mixed Hazards (Dirty Bombs, etc)
  - ✓ Pressurized Containers
  - ✓ Protect Chemists from Biologicals, etc.



ECBC

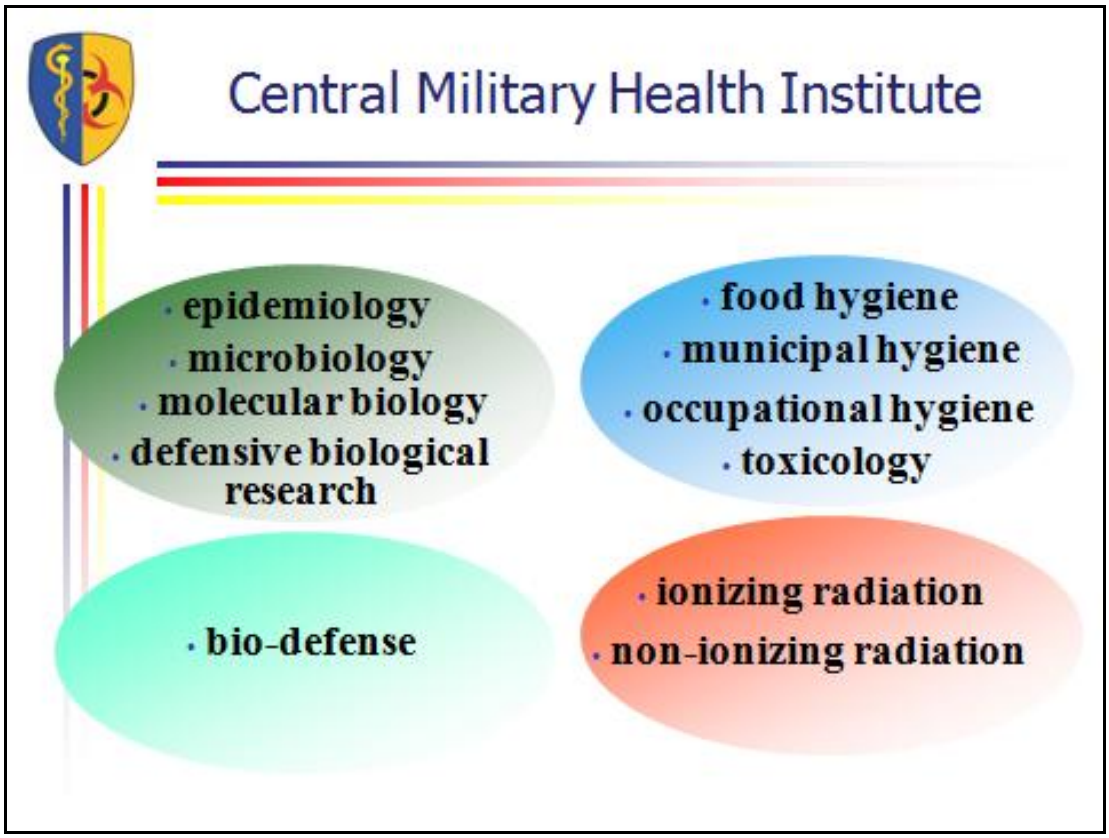
# Triage Sequence

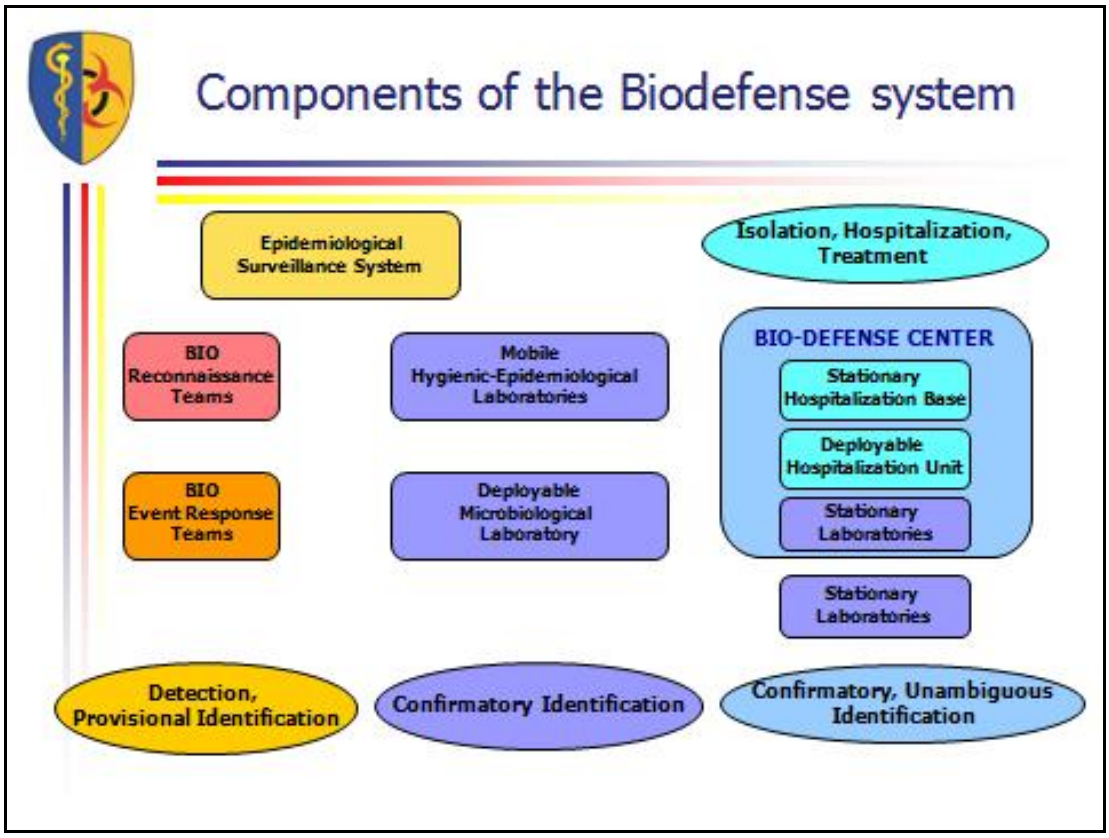



ECBC


## C.2 HFM-177 MEETING: 6-8 OCTOBER 2011, MUNICH, GERMANY

### C.2.1 The Czech Republic Mobile Lab Presentation – by Libor Pisa


Central Military Health Institute
· epidemiology
· microbiology
· molecular biology
· defensive biological research
· food hygiene
· municipal hygiene
· occupational hygiene
· toxicology
· bio-defense
· ionizing radiation
· non-ionizing radiation


Components of the Biodefense system
Epidemiological Surveillance System
Isolation, Hospitalization, Treatment
BIO Reconnaissance Teams
Mobile Hygienic-Epidemiological Laboratories
BIO-DEFENSE CENTER
Stationary Hospitalization Base
Deployable Hospitalization Unit
Stationary Laboratories
BIO Event Response Teams
Deployable Microbiological Laboratory
Stationary Laboratories
Detection, Provisional Identification
Confirmatory Identification
Confirmatory, Unambiguous Identification


Biological Event Response Team

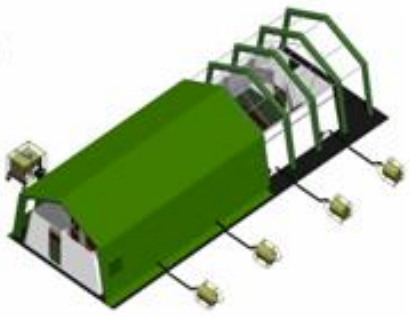

Mobile Hygienic and Epidemiological Laboratory

## Deployable Biological Laboratory

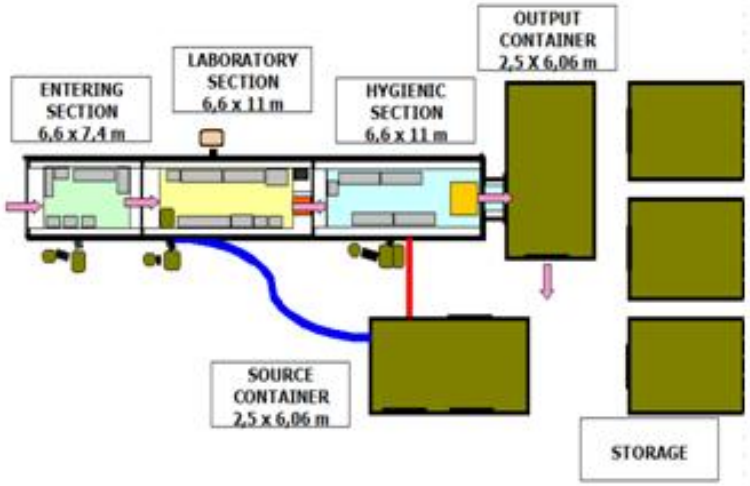
STANAG 4632
Deployable NBC Analytical Laboratory

## Deployable Biological Laboratory

- transportable by road, rail, air and ship
- required area for deployment: 50 x 25 meters
- preparatory time for examination: 72 hours
- three tents connected through tunnels
- operating without replenishment of consumables for 5 days
- own source of electricity and pressurized clean air but depends on the other logistic support
- interior is formed by three separate chambers ColPro



## Laboratory complex scheme




## Deployable Biological Laboratory

**Entering section**

- sample reception and evidence
- command and control base
- preparation of the lab personnel
- controlled pressure + 50 Pa

## Deployable Biological Laboratory

**Laboratory section**

- identification of biological agents carried out according to authorized methods and SOP
- good and safe laboratory practices
- designed for at least 2 up to 4 workers
- lab security system provides an effective protection of the staff against the infection and against escape of pathogens to the outside

## Deployable Biological Laboratory

**Laboratory section - Bio Safety Precautions**

- closed space of the lab section and transit of personnel only through chambers with air-locks
- monitoring of the lab work using camera system
- controlled under pressure -50 Pa
- filter ventilation units with NBC filter
- split air conditioning system with closed air circulation
- protective over pressurized suits with the clean air supply
- biohazard safety cabinet
- sterilizer of liquid waste (autoclave) and portable sterilizers of solid waste
- UV sterilizers of inner space

## Deployable Biological Laboratory

**Hygienic section**

- decontamination of the workers
- waste water flows to the collector and to the sterilizer in autoclave
- drying of protective suits
- controlled over pressure +50 Pa

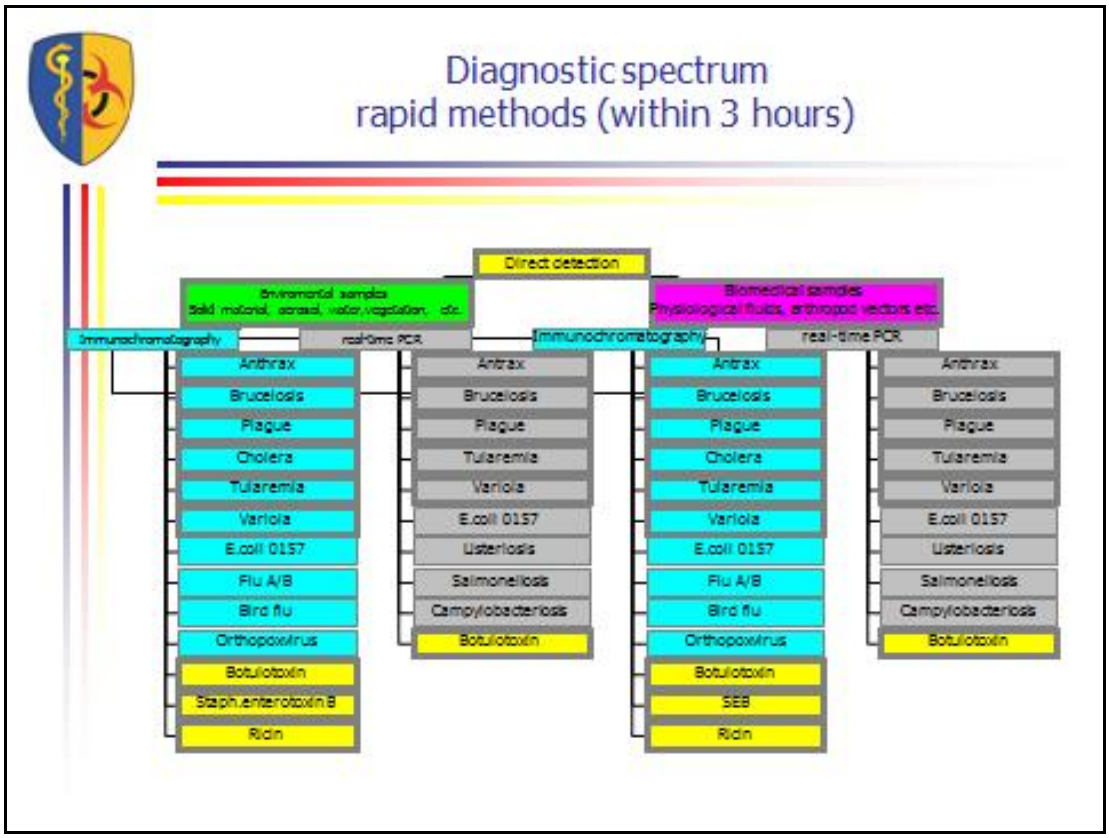
## Deployable Biological Laboratory

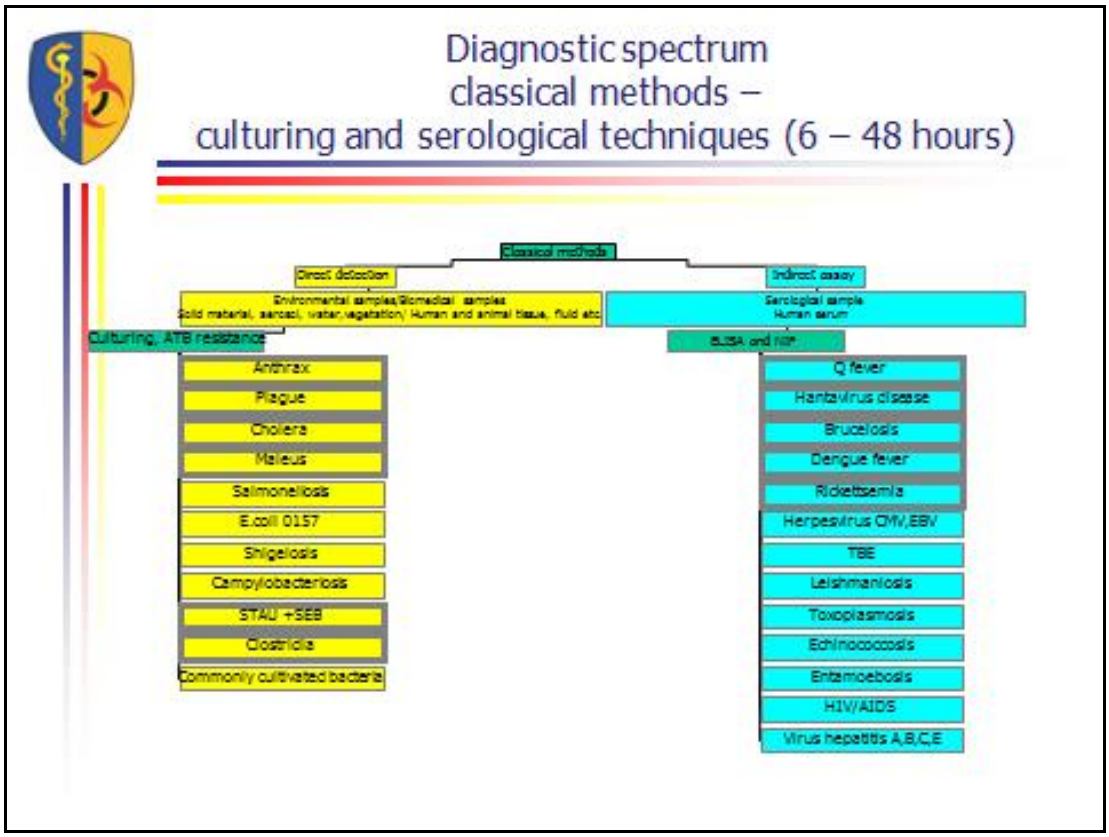
**„Source" container**

- electricity generator 60 kW
- fuel tank
- compressor

## Deployable Biological Laboratory

| | Post | Number |
|---|---|---|
| 1. | **Commander** | 1 |
| 2. | **Medical/biological experts (microbiologist, epidemiologist, veterinarian, doctor of natural sciences)** | 4 |
| 3. | **Laboratory technician** | 3 |
| 4. | **Engineer** | 4 |
| | **Total** | **12** |


Diagnostic spectrum
rapid methods (within 3 hours)
Direct detection
Environmental samples
Solid material, aerosol, water,vegetation, etc.
Biomedical samples
Physiological fluids, arthropod vectors etc.
Immunochromatography
real-time PCR
Immunochromatography
real-time PCR
Anthrax
Brucellosis
Plague
Cholera
Tularemia
Variola
E.coli 0157
Flu A/B
Bird flu
Orthopoxvirus
Botulotoxin
Staph.enterotoxin B
Ricin
Anthrax
Brucellosis
Plague
Tularemia
Variola
E.coli 0157
Listeriosis
Salmonellosis
Campylobacteriosis
Botulotoxin
Anthrax
Brucellosis
Plague
Cholera
Tularemia
Variola
E.coli 0157
Flu A/B
Bird flu
Orthopoxvirus
Botulotoxin
SEB
Ricin
Anthrax
Brucellosis
Plague
Tularemia
Variola
E.coli 0157
Listeriosis
Salmonellosis
Campylobacteriosis
Botulotoxin


Diagnostic spectrum
classical methods –
culturing and serological techniques (6 – 48 hours)
Classical methods
Direct detection
Indirect assay
Environmental samples/Biomedical samples
Solid material, aerosol, water,vegetation/ Human and animal tissue, fluid etc.
Serological sample
Human serum
Culturing, ATB resistance
ELISA and IIF
Anthrax
Plague
Cholera
Melleus
Salmonellosis
E.coli 0157
Shigelosis
Campylobacteriosis
STAU +SEB
Clostridia
Commonly cultivated bacteria
Q fever
Hantavirus disease
Brucellosis
Dengue fever
Rickettsemia
Herpesvirus CMV,EBV
TBE
Leishmaniosis
Toxoplasmosis
Echinococcosis
Entamoebosis
HIV/AIDS
Virus hepatitis A,B,C,E

## Participation of the mobile components in NRF, NATO mission and summits

**NRF and NATO missions:**

- Enduring Freedom (Kuwait, Iraq) - 2002-2003
- NRF 3 – Mn. CBRN Bn. – 2004
- NRF 8 – Mn. CBRN Bn. – 2007
- NRF 12 – Mn. CBRN Bn. – 2009
- NRF 17/18 – Mn. CBRN Bn. - 2011-2012
- ISAF (Afghanistan) – since 2009

**CBRN Defense support of summits:**

- Summit NATO in the Czech Republic – 2002
- Summit of states of Latin America, Caribbean states and European Union – Peru, 2008
- Summit of Ministers of Foreign Affairs of NATO countries – Estonia, 2010

## Thank you for your attention

## C.2.2 Germany Mobile Lab Presentation – by Roman Wölfel

# The Bundeswehr Rapidly Deployable Biolab

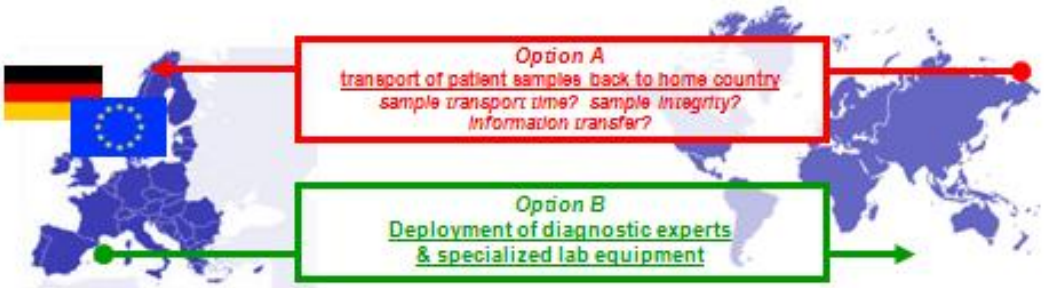
## Microbiological High-Tech Diagnostics for Outbreak Investigations Abroad

Lieutenant-Colonel MC
**Dr. med. Roman Wölfel**
MD DTMH

Bundeswehr Institute of Microbiology
Department for Medical Bio Reconnaissance & Verification
Munich - Germany

Views expressed in this presentation are those of the author and do not necessarily reflect an official position of the German Ministry of Defence

## MedBio Diagnostic Support
### Situation assessment




4 International Reference Center | 3 Regional Reference Center | 2 Stationary Field lab | 1 Health post

2

## Container-based Laboratory

3

## Modular Operations Concept

4

# Mission-tailoring

## Equipment

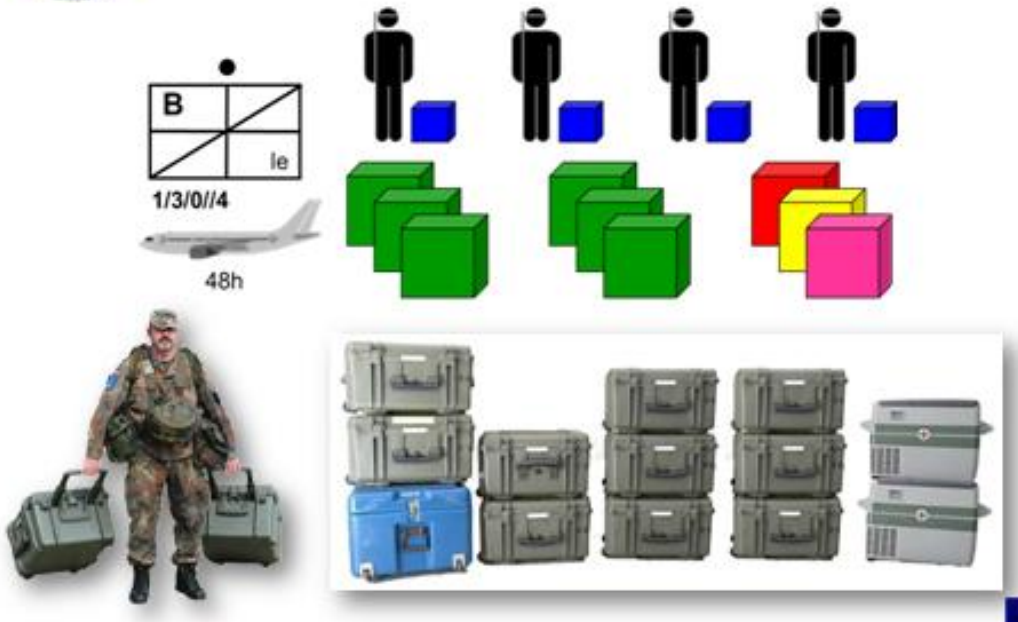
- Personal protective equipment
- MedBio sampling
- Ultra-low-temperature transport
- Rapidly deployable BioLab
- Autonomus power supply
- Inflatable Biolab environment
- Vector I (rodents)
- Vector II (ticks & insects)

## Mission requirements

pathogen known/unknown
available resources on-site
need for sustainability
transport resources
available lead-time

5

# Rapidly Deployable BioLab




6

## Rapidly Deployable BioLab

7

## Rapidly Deployable BioLab
## MECC Shelter

8

## Rapidly Deployable BioLab
## Lab Setup

9

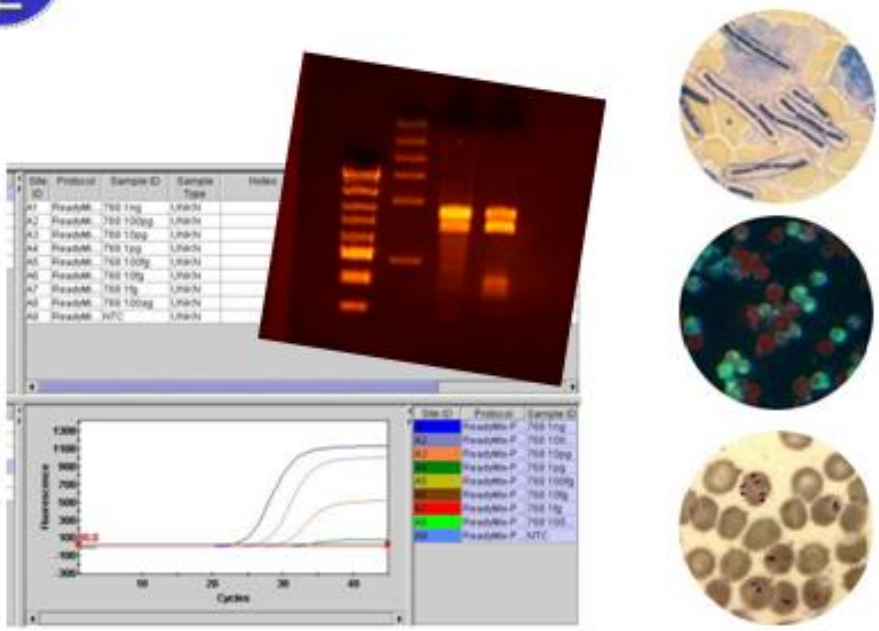
## Rapidly Deployable BioLab
## Sample Acceptance

10

## Rapidly Deployable BioLab
## Inflatable BioLab Environment

11

## Rapidly Deployable BioLab

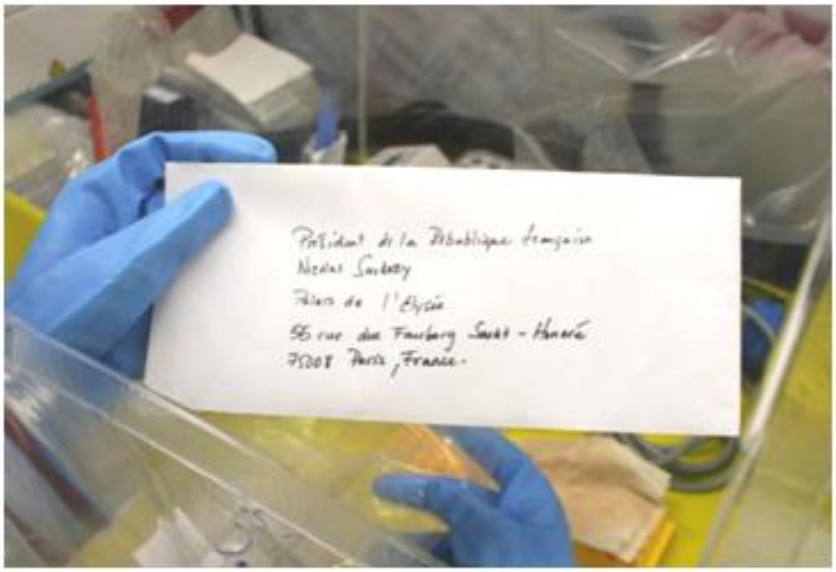


12

## Rapidly Deployable BioLab
## Sample Preparation

13

## Rapidly Deployable BioLab
## Sample Documenation

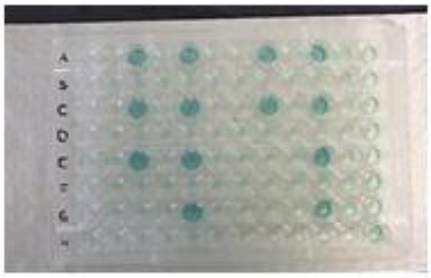



14

## Rapidly Deployable BioLab
## Sample Documentation


15

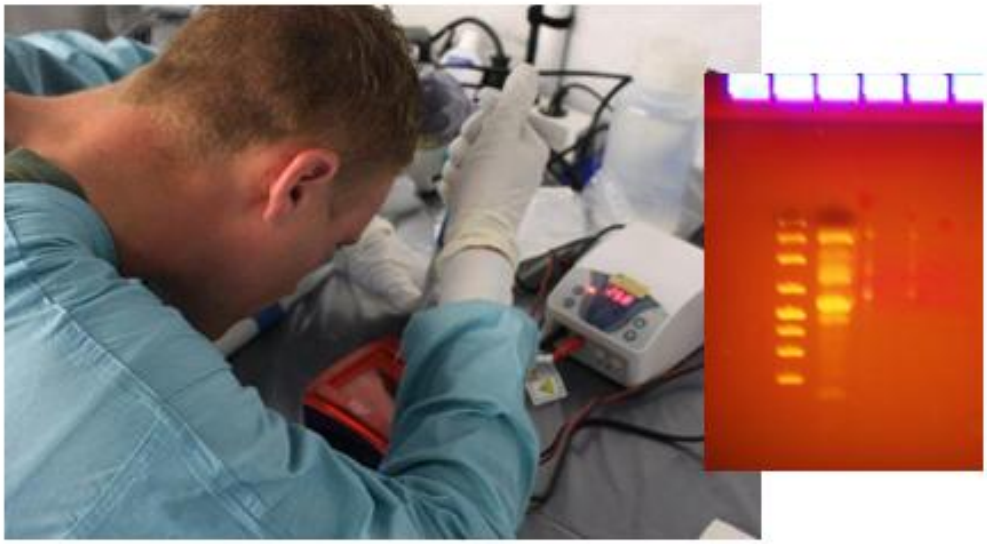
## Rapidly Deployable BioLab
## Testing Methods

16

Rapidly Deployable BioLab

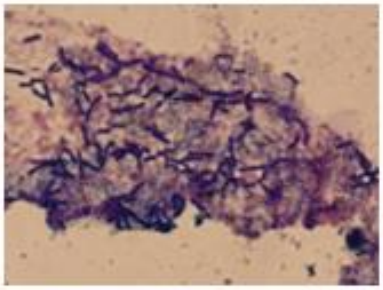
## Flash Gel Electrophoresis

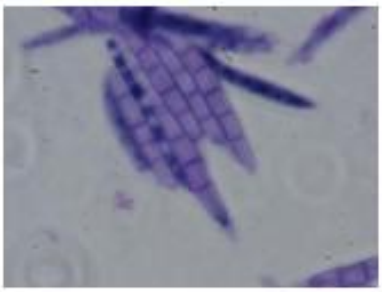


17

Rapidly Deployable BioLab

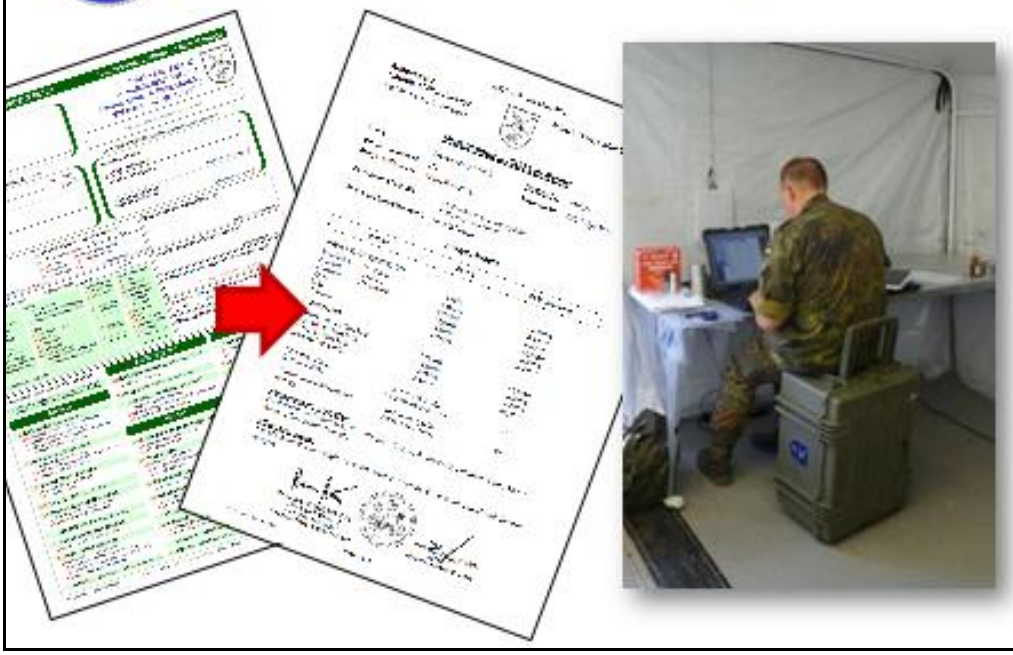
## Staining Methods





13

## Rapidly Deployable BioLab
## Individual Lab Reports



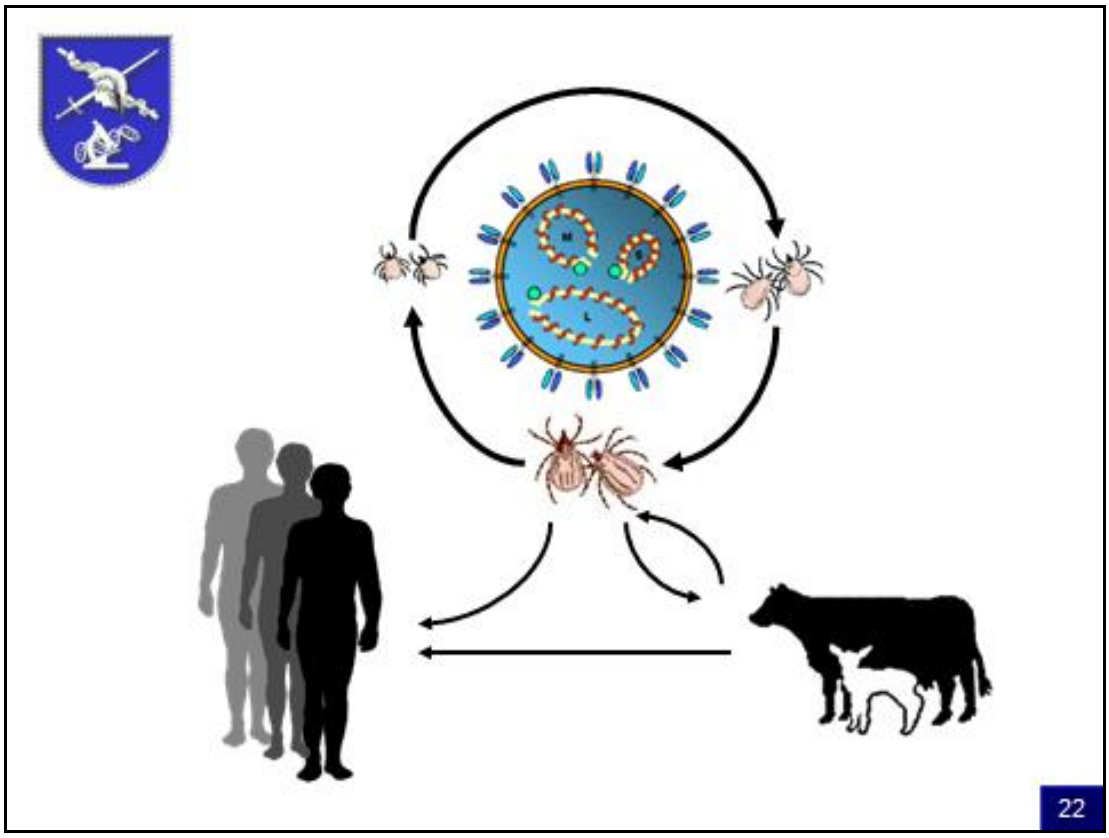
19

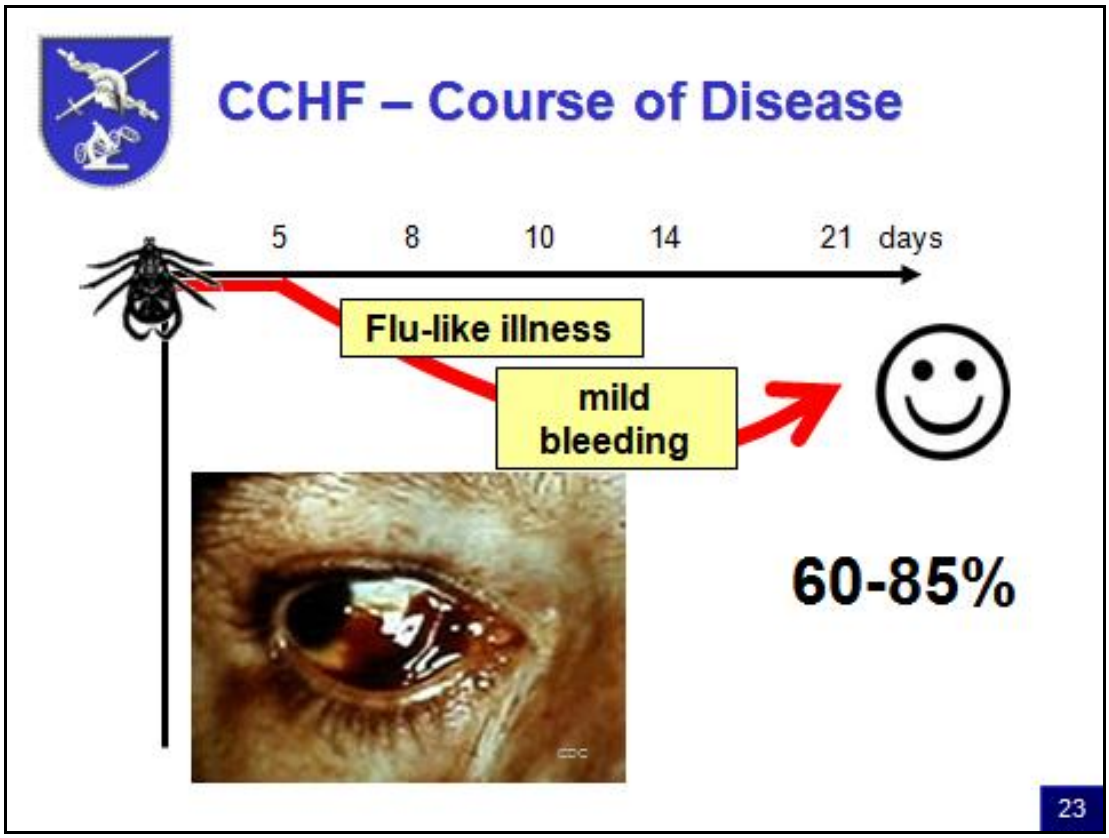
# NATO Mission Support Operations

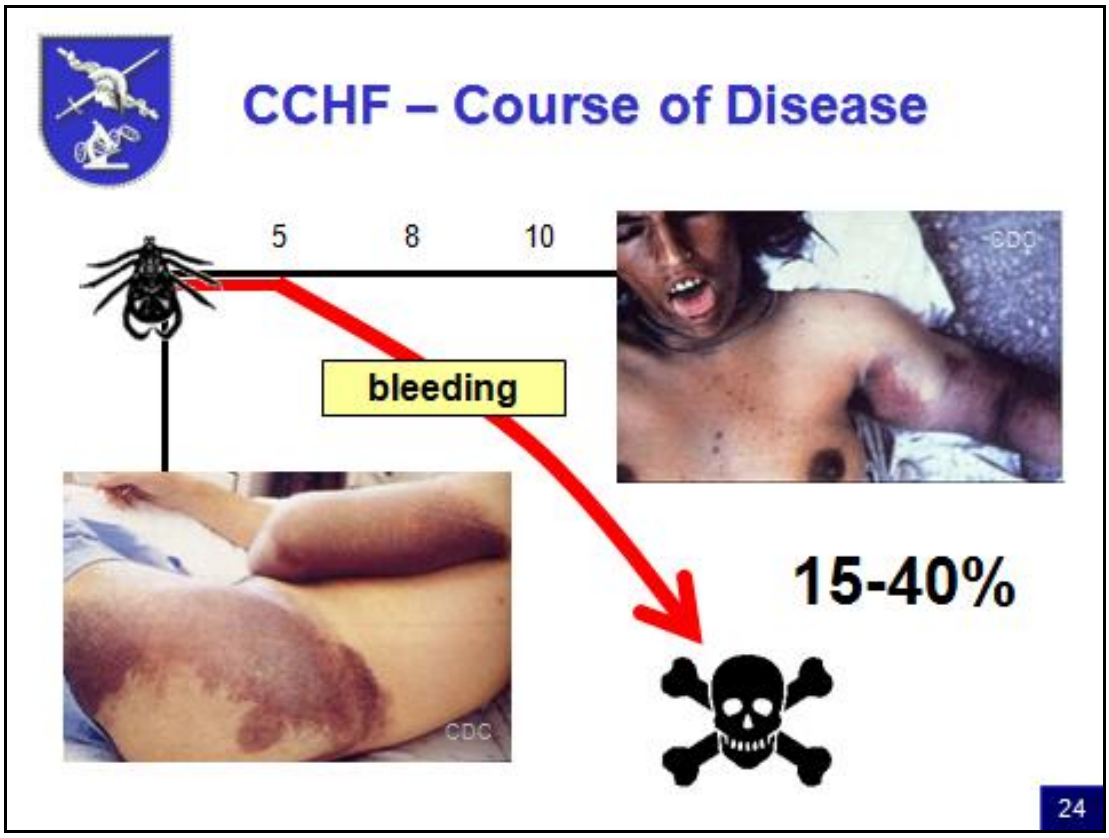
## Kosovo 2007 & 2008

20


Bundeswehr missions worldwide
Crimean-Congo Hemorrhagic Fever
21


22

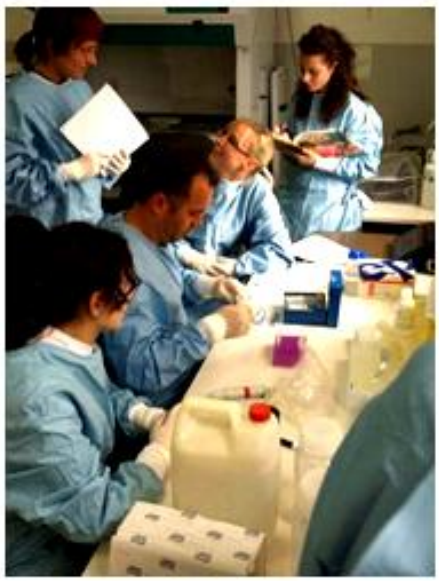

CCHF – Course of Disease
5
8
10
14
21 days
Flu-like illness
mild bleeding
60-85%
23


CCHF – Course of Disease
5
8
10
bleeding
CDC
CDC
15-40%
24

## CCHF Diagnostics in Kosovo

25

## CCHF Diagnostics in Kosovo



26

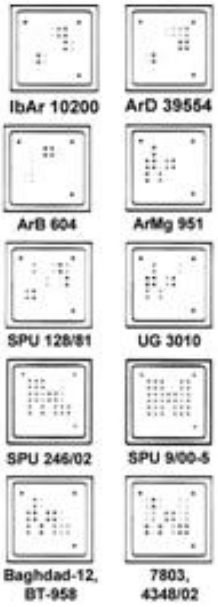
## Epizootological survey

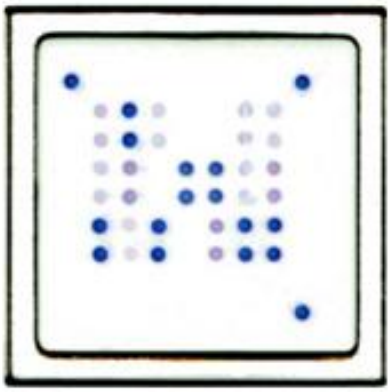
27

## CCHF Diagnostics in Kosovo

28

# CCHF Diagnostics in Kosovo

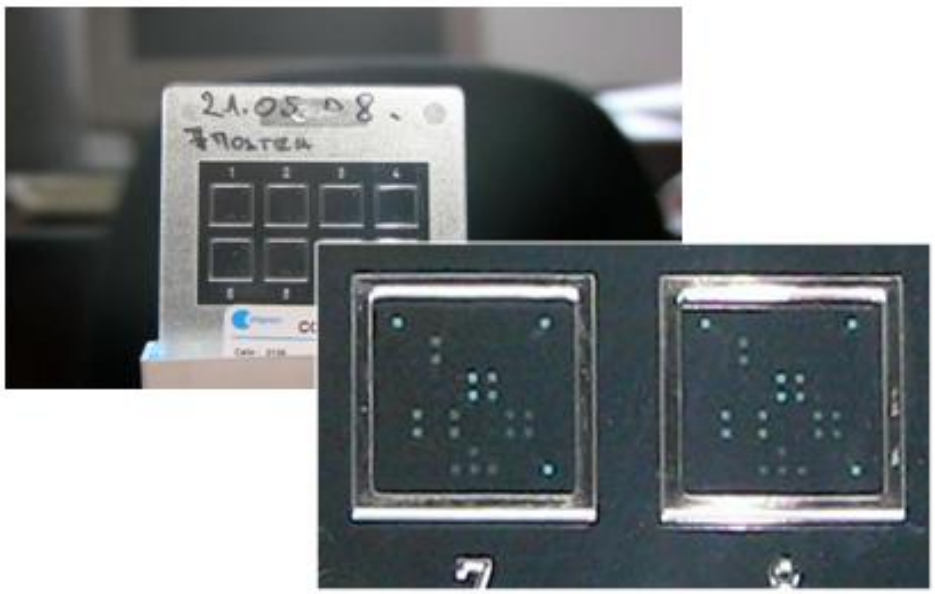


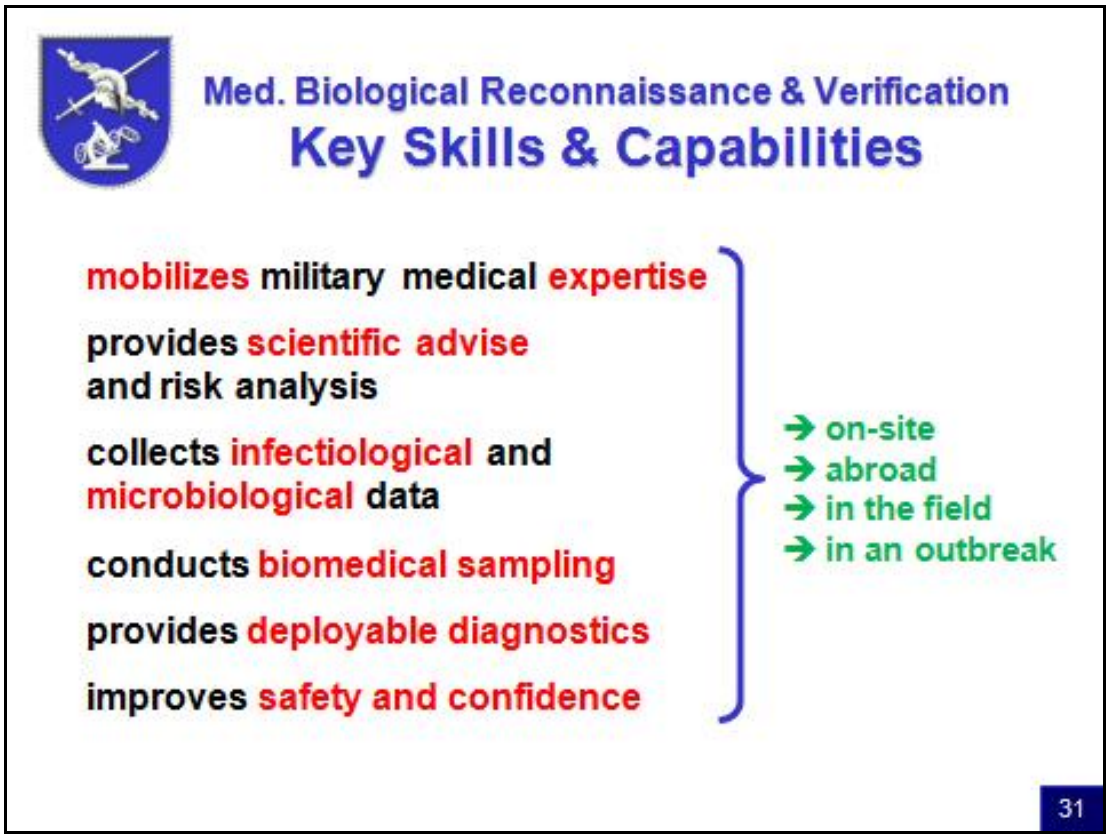
KOSOVO

29

## CCHF in Kosovo
## May 2008 & June 2009



30


Med. Biological Reconnaissance & Verification
Key Skills & Capabilities
mobilizes military medical expertise
provides scientific advise and risk analysis
collects infectiological and microbiological data
conducts biomedical sampling
provides deployable diagnostics
improves safety and confidence
→ on-site
→ abroad
→ in the field
→ in an outbreak
31


romanwoelfel@bundeswehr.org
Bundeswehr Institute of Microbiology
Department for Medical Biological
Reconnaissance & Verification
Munich – Germany

## C.2.3 United States Lab Construction Presentation – by Raymond Mastnjak

US ARMY RDECOM

Edgewood Chemical Biological Center

TECHNOLOGY DRIVEN. WARFIGHTER FOCUSED.

**HFM-177:** Construction of Deployable Laboratories During Times of Reduced Funding

29, 30 October 2011

### Construction of Deployable Laboratories During Times of Reduced Funding

- Safety/Health versus cost considerations.
- Use of CONEX type containers for mobile analytical platforms.
- Use of existing military vehicles for mobile analytical platforms .
- Dual use scenarios: Mobile laboratories as back up facilities to fixed site labs

TECHNOLOGY DRIVEN. WARFIGHTER FOCUSED.

## Safety/Health Versus Cost Considerations

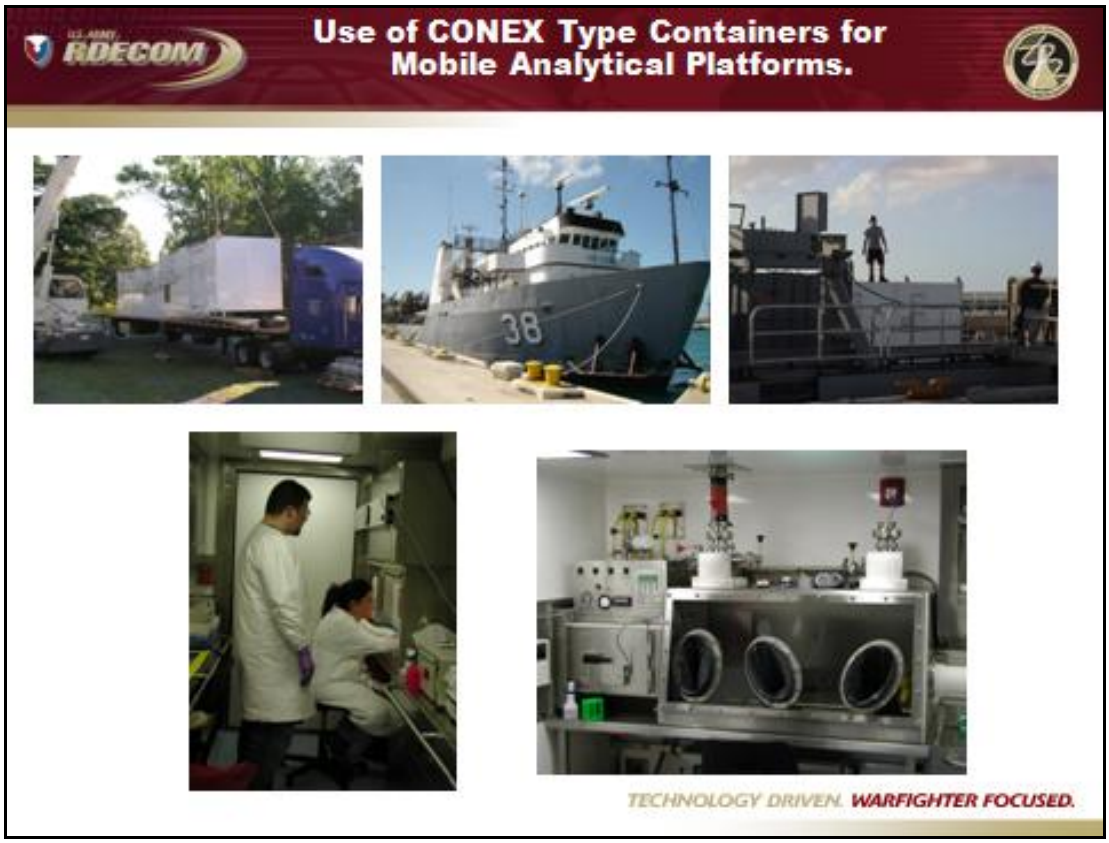
- Design should be based on actual samples anticipated, avoid over-designing.
- Biological safety level 2 (BSL-2) containment should be sufficient for most scenarios.
- Consider contributing factors such as level of screening before samples come to the deployable laboratory.
- If true unknowns are anticipated, start with a Class III biological safety cabinet or consider 100% exhaust Class II, Type B cabinet.
- Consider higher level of personal protective equipment for scenarios exceeding BSL-2.
- Consider separate portable shower out facility for scenarios exceeding BSL-2.
- Keep sample size to smallest amount needed for each assay (PCR / ECL, etc).

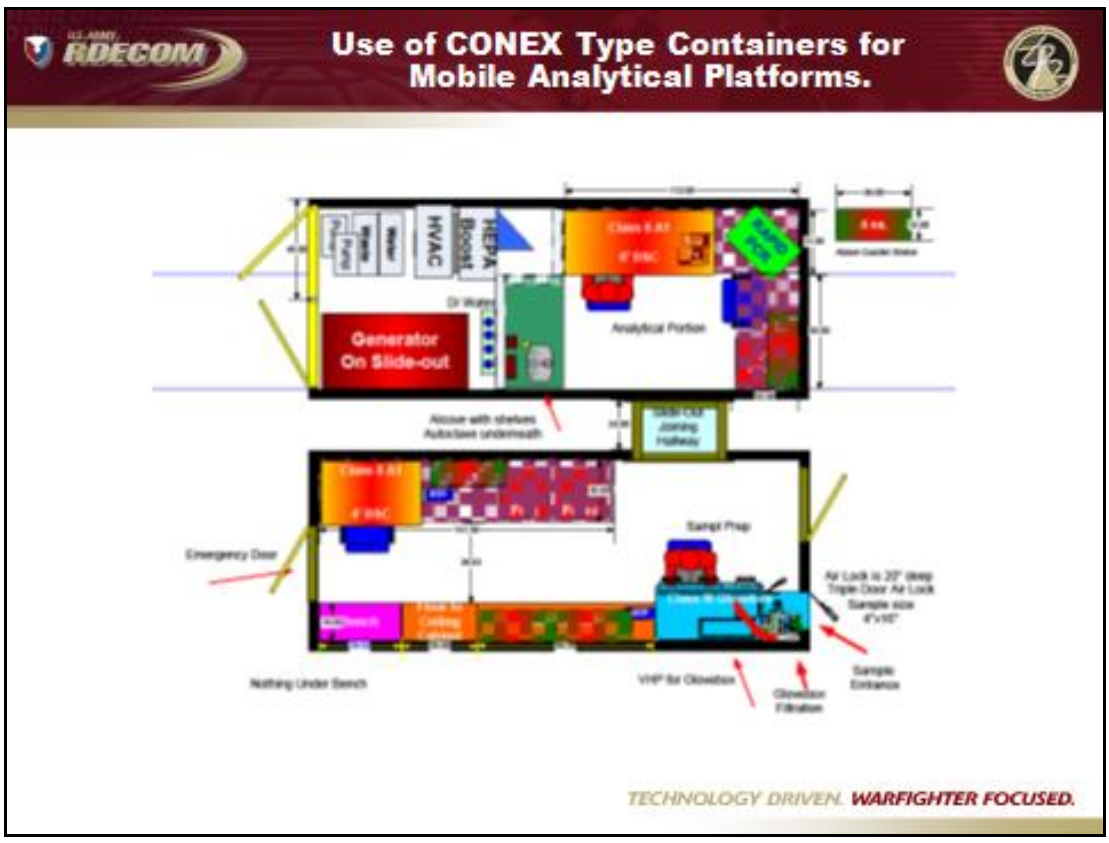
TECHNOLOGY DRIVEN. WARFIGHTER FOCUSED.

## Use of CONEX Type Containers for Mobile Analytical Platforms.

- Standard shipping containers can be used for many deployable laboratory situations.
- Containers can be handled at any marine port or airfield/airport and can easily be shipped by rail or road.
- Containers can include slide outs to accommodate larger work areas.
- Containers can be fitted with temporary wheel sets for deployment to military theatres of operation.

TECHNOLOGY DRIVEN. WARFIGHTER FOCUSED.


RDECOM
Use of CONEX Type Containers for Mobile Analytical Platforms.
38
TECHNOLOGY DRIVEN. WARFIGHTER FOCUSED.

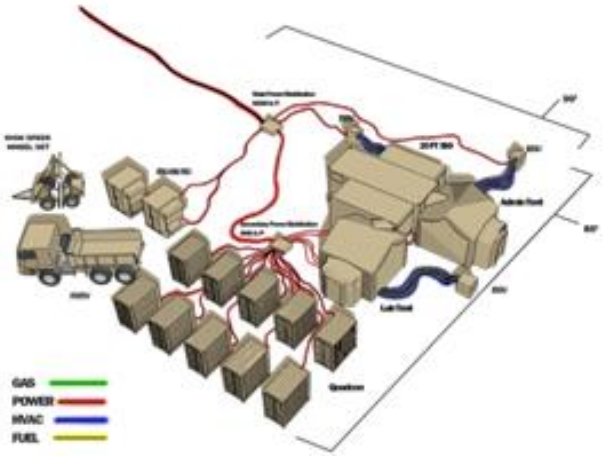

RDECOM
Use of CONEX Type Containers for Mobile Analytical Platforms.
HVAC
HEPA
Generator On Slide-out
Analytical Portion
Emergency Door
Sample Prep
Nothing Under Bench
TECHNOLOGY DRIVEN. WARFIGHTER FOCUSED.

## Use of Existing Military Vehicles for Mobile Analytical Platforms

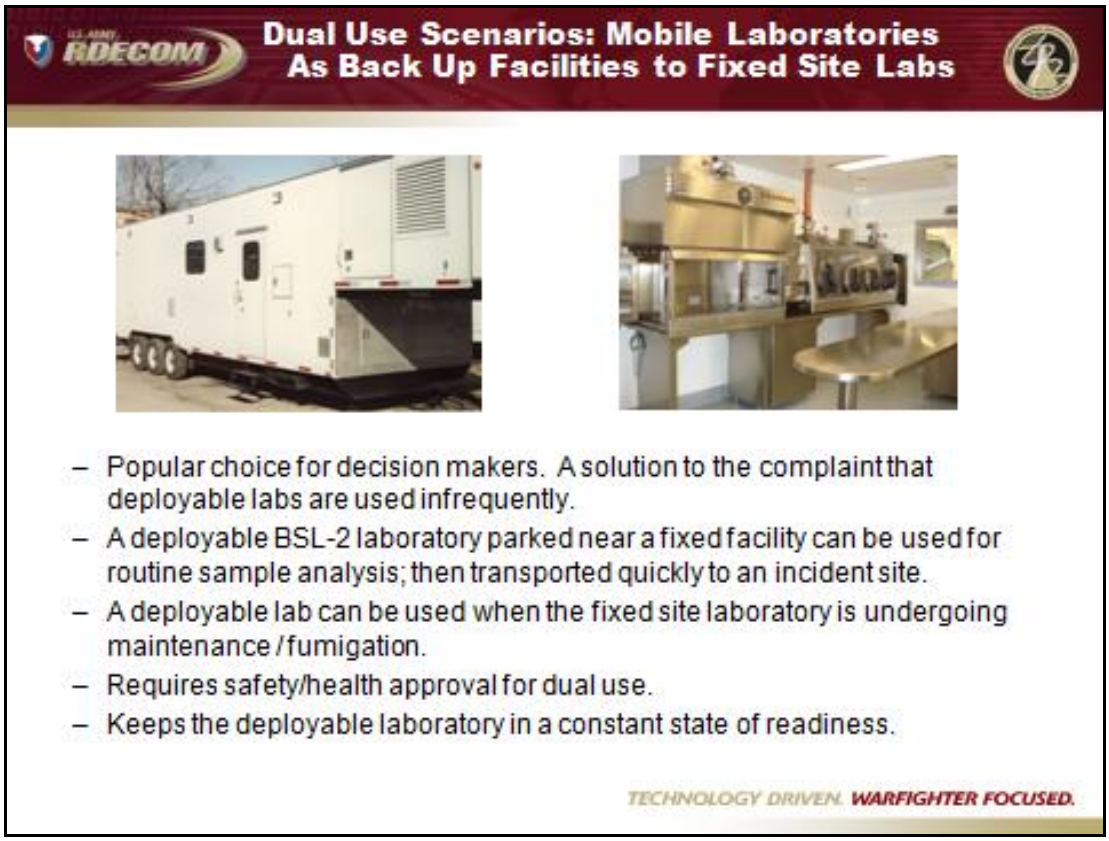
- Use of existing fleet of military trucks, containers, trailers and generators can save considerable funds.
- Design should include hardened shipping containers for analytical instrumentation.
- Integration of analytical equipment can be challenging.
- Requires ongoing training for military field technicians.
- Reachback to civilian scientists / engineers is vital.

TECHNOLOGY DRIVEN. WARFIGHTER FOCUSED.

## Use of Existing Military Vehicles for Mobile Analytical Platforms




TECHNOLOGY DRIVEN. WARFIGHTER FOCUSED.




## C.2.4 United States Sample Triage Presentation – by Raymond Mastnjak

## The Role of Triage in Sample Analysis

- Rapid risk assessment for theatre commanders / first responders.
- Sample flow in a deployable laboratory.
- Special circumstances.
- Using a triage plan to develop a deployable laboratory design.

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## Rapid Risk Assessment for Theatre Commanders / First Responders

- What is a rapid risk assessment?
- Rapid risk assessments should include experienced chemists, biologists and risk management personnel.
- The assessment should consider field screening, intelligence reports, experience of operators, engineering controls, etc.
- The assessment should sequentially notify each person in the analysis chain of actions completed and hazards.
- Special concerns include:
  - Protecting personnel from hazards they are not familiar with (such as biologists working with samples contaminated with highly toxic chemicals).
  - Explosively configured devices / samples with energetic material.
  - Mixed hazards (dirty bomb scenario).
  - Pressurized containers.
  - Regulatory requirements for field operations.



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## Sample Flow in a Deployable Laboratory

- Field screening: What is known about the sample before it comes to the deployable lab?
- Is a pass through needed? Is there a need for a sterilization station using vaporous hydrogen peroxide or 5% Sodium hypochlorite?
- Can the sample container fit the opening of the pass through or the Class III biological safety cabinet?
- Balance the safety benefits of a Class III biological safety cabinet against the difficulties of sample manipulation.
- Will a sample aliquot be transferred to a Class II biological safety cabinet?
- Movement of samples to cell culture (incubator) or to analytical equipment (PCR, ECL, sample prep robotics).
- Limitations of a deployable laboratory.

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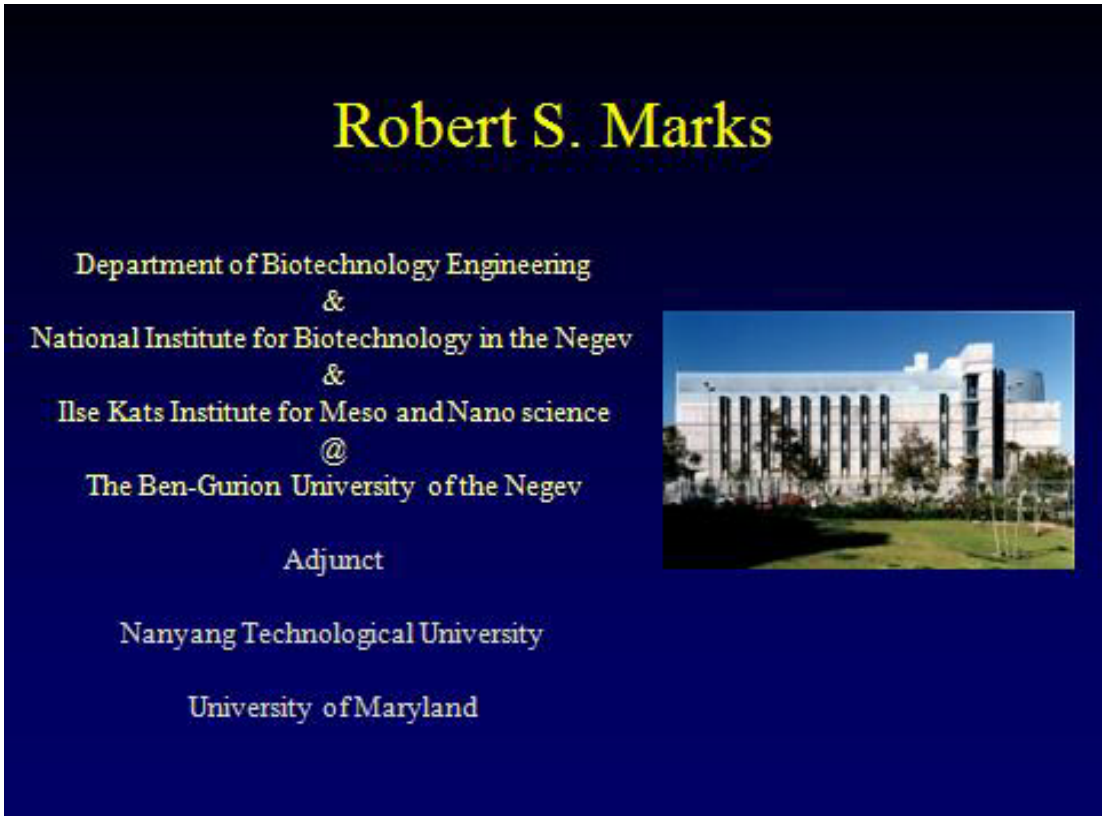
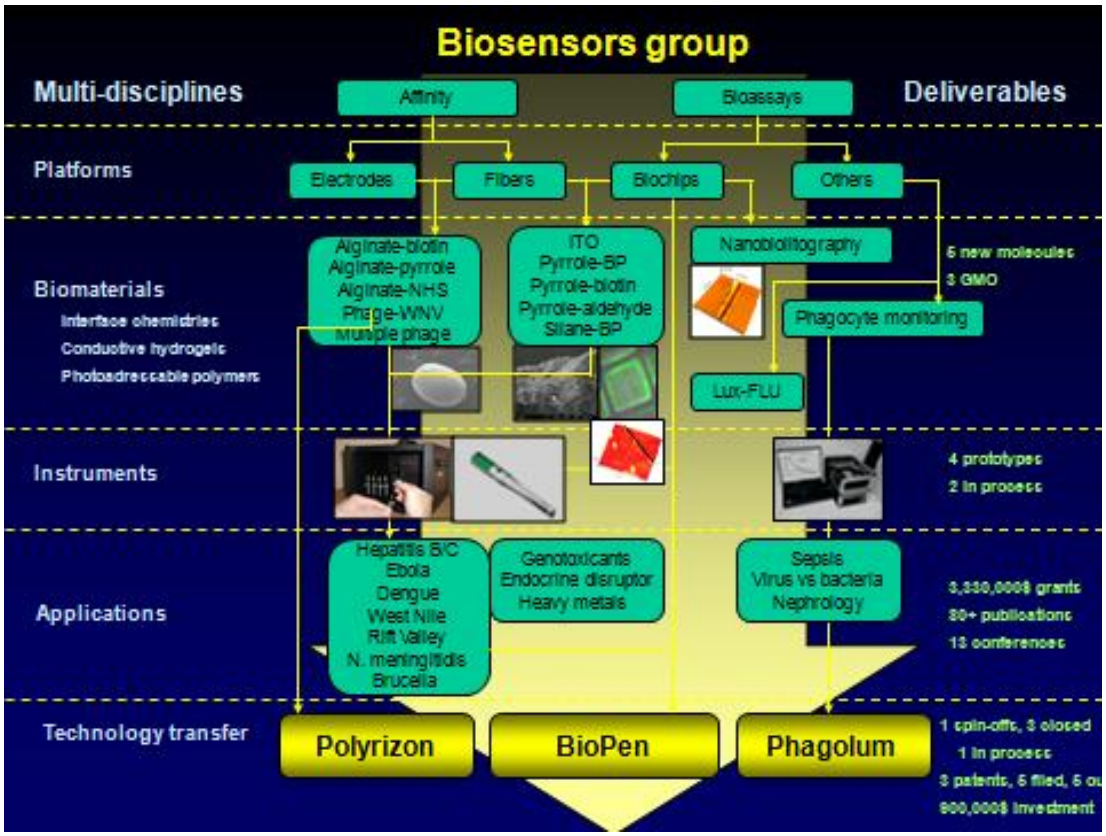
## Special Circumstances

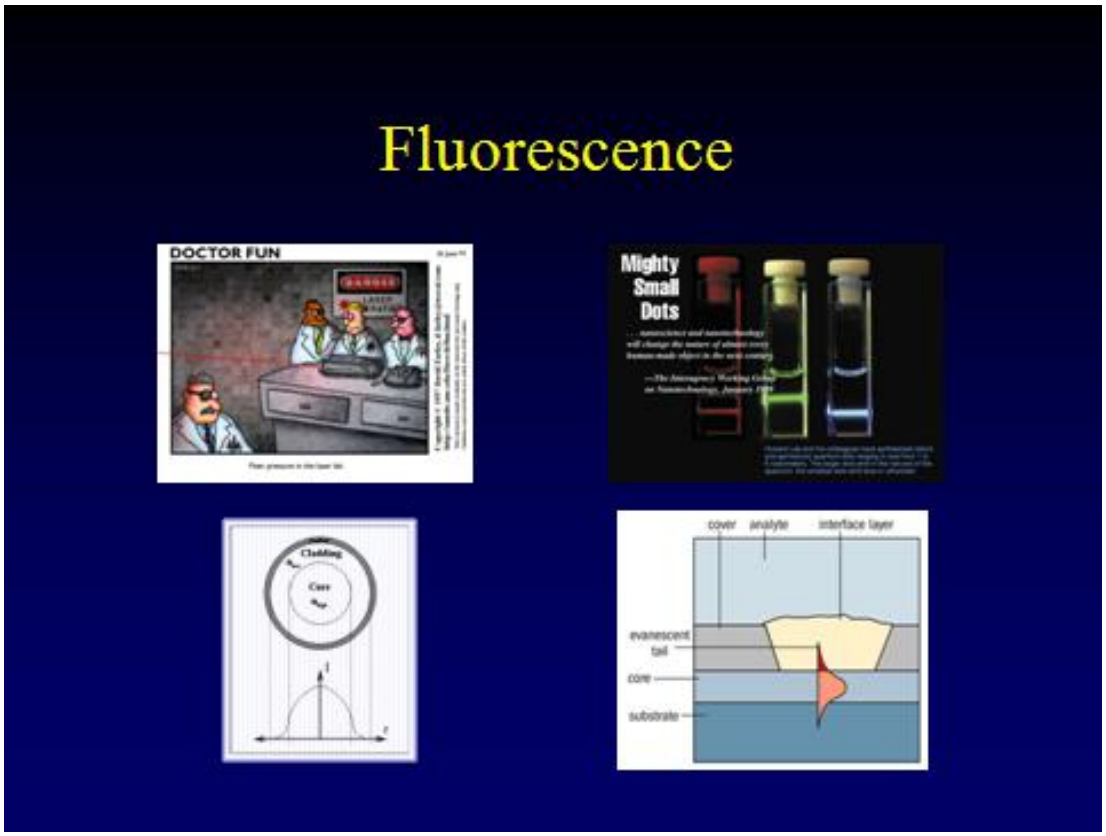
- Samples requiring invasive techniques.
- Use of portable X-ray devices, neutron activation devices and other special equipment for field screening.
- Gamma irradiation of "true" unknowns.
- Use of ancillary structures such as negative pressure tents to augment the deployable laboratory.

TECHNOLOGY DRIVEN. WARFIGHTER FOCUSED.


## C.2.5 Israel Nano-Technology – by Robert S. Marks

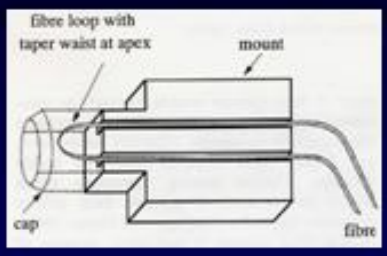


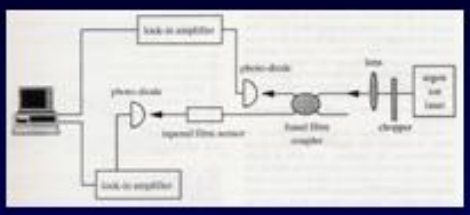

Biosensors group
Multi-disciplines
Affinity
Bioassays
Deliverables
Platforms
Electrodes
Fibers
Biochips
Others
Biomaterials
Interface chemistries
Conductive hydrogels
Photoadressable polymers
Alginate-biotin
Alginate-pyrrole
Alginate-NHS
Phage-WNV
Multiple phage
ITO
Pyrrole-BP
Pyrrole-biotin
Pyrrole-aldehyde
Silane-BP
Nanoblolitography
6 new molecules
3 GMO
Phagocyte monitoring
Lux-FLU
Instruments
4 prototypes
2 in process
Applications
Hepatitis B/C
Ebola
Dengue
West Nile
Rift Valley
N. meningitidis
Brucella
Genotoxicants
Endocrine disruptor
Heavy metals
Sepsis
Virus vs bacteria
Nephrology
3,330,000$ grants
80+ publications
13 conferences
Technology transfer
Polyrizon
BioPen
Phagolum
1 spin-offs, 3 closed
1 in process
3 patents, 5 filed, 5 ou
600,000$ investment

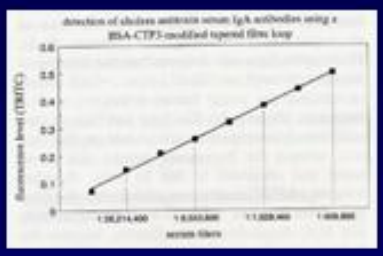

Fluorescence
DOCTOR FUN
Mighty Small Dots
cover
analyte
interface layer
evanescent tail
core
substrate

## Single-mode tapered optical fiber loop immunosensor. II. Assay of cholera antitoxin

Marks, R.S., Z.M. Hale, M.M. Levine, C.R. Lowe and F.P. Payne (1994). SPIE. 2131: 495-503

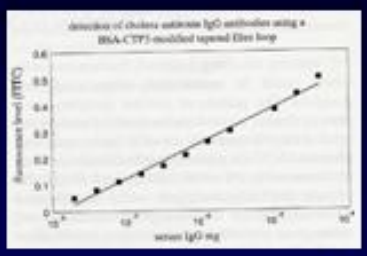


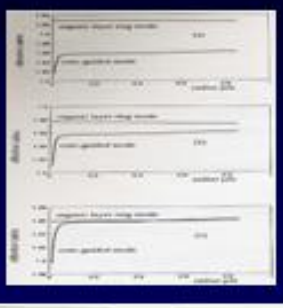



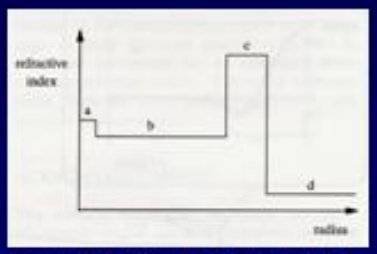


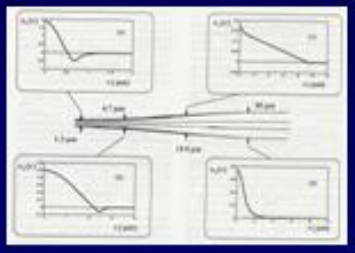
## The single mode tapered optical fibre loop immunosensor

Hale, Z.M., F.P. Payne, R.S. Marks, M.M. Levine, C.R. Lowe (1996). Biosensors and Bioelectronics. 11: 137-148





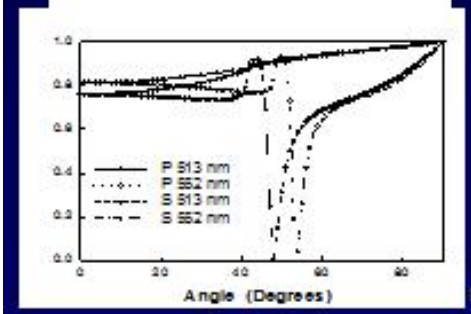


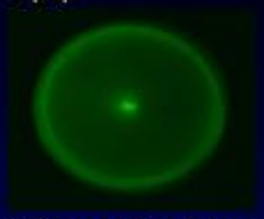



[a] core (1.469); [b] clad (1.458); [c] organic layer (1.6 est.); [d] buffer (1.32)

## Metal-enhanced fluorescence on fibers

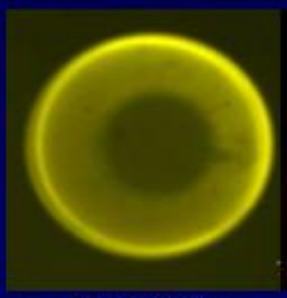
Daria Prilutsky, Evgeni Eltzov, Robert S. Marks, Leslie Lobel and Chris D. Geddes 2009 in prep



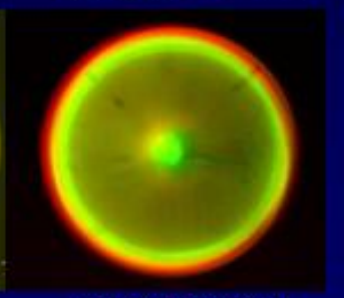



FITC non-silvered fiber, 500 nm LP filter.

Rhodamine 6G non-silvered fiber, 700 nm LP filter

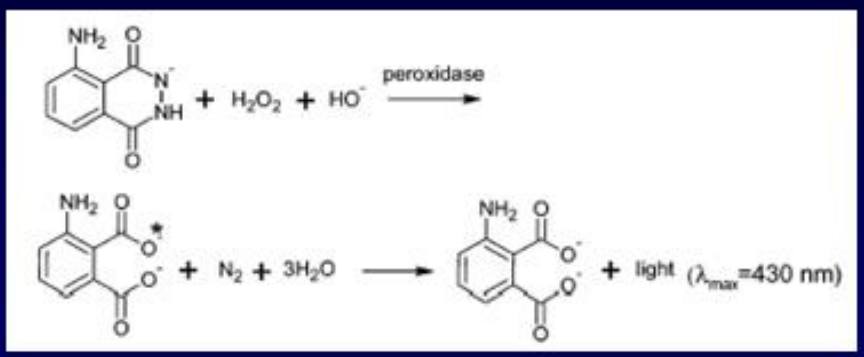


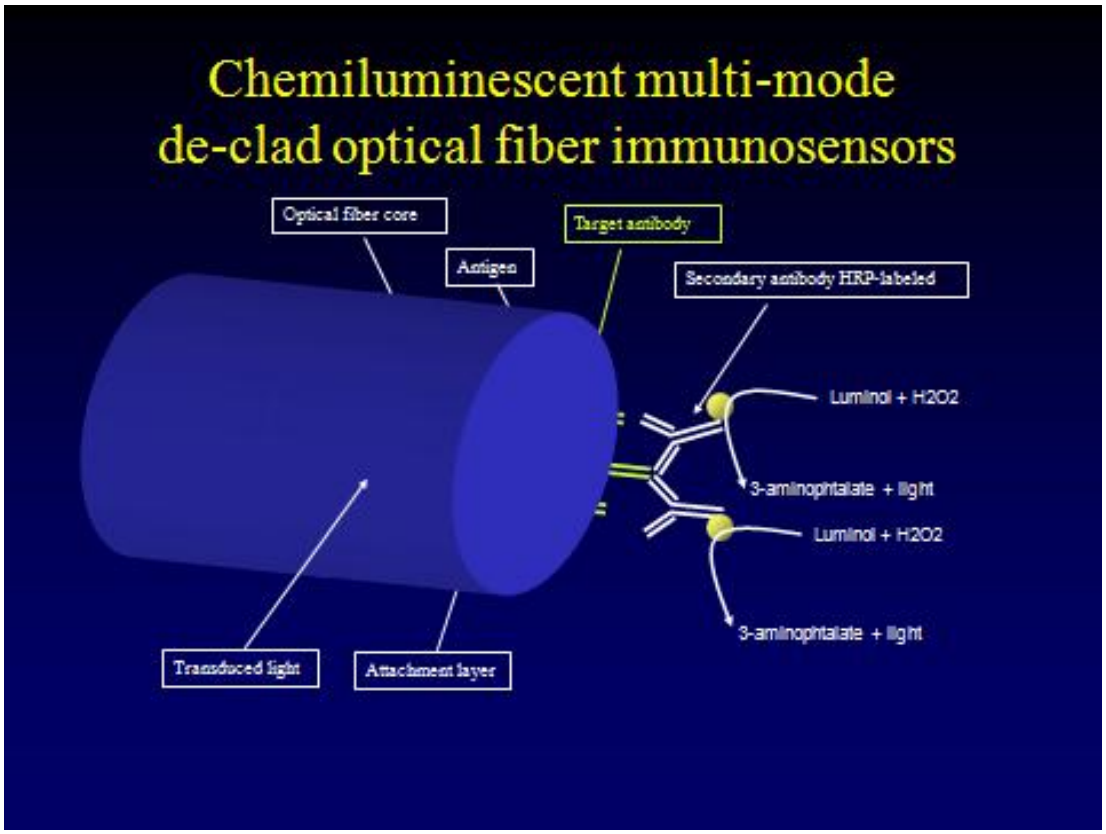
Rhodamine non-silvered fiber, 500 nm and 700 nm LP filters

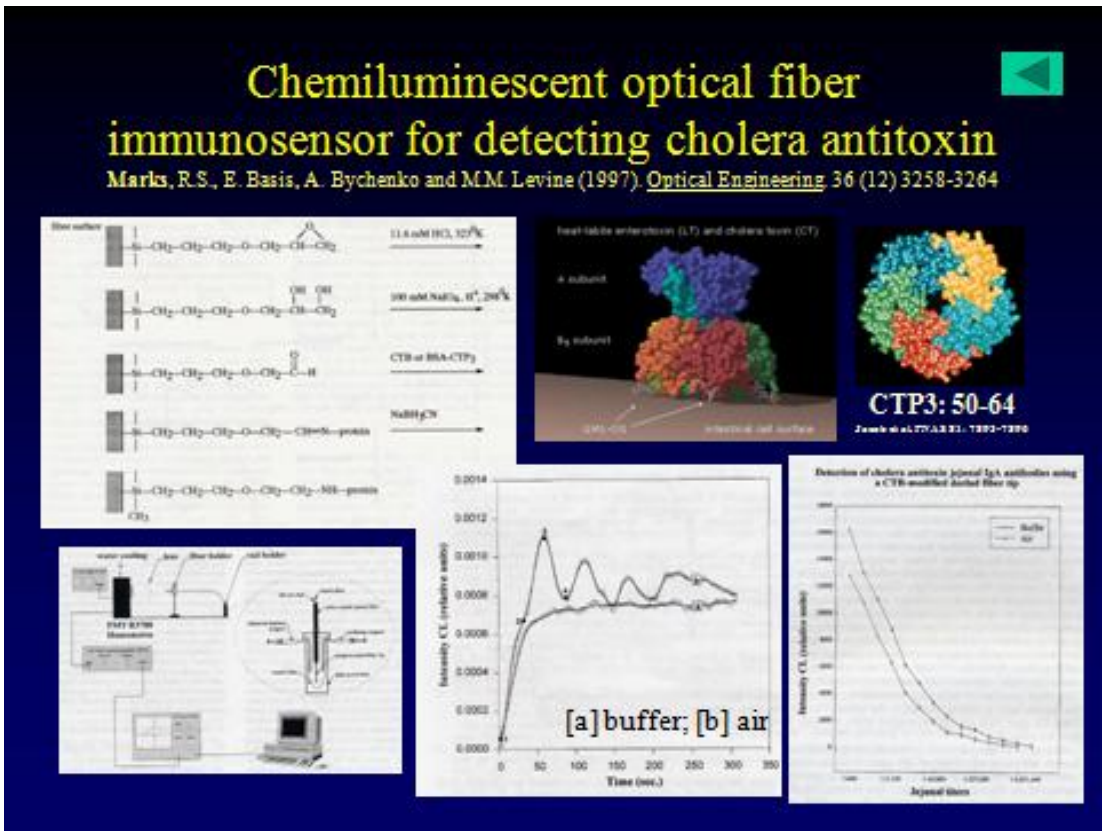


Rhodamine 6G on silvered fiber, 500 nm and 700 nm LP filters

## Chemiluminescence

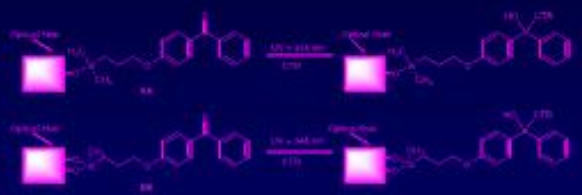


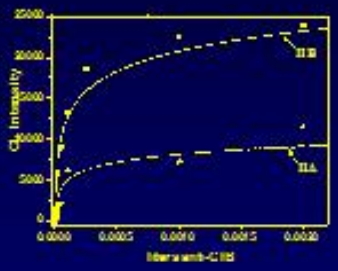

Chemiluminescent multi-mode de-clad optical fiber immunosensors
Optical fiber core
Antigen
Target antibody
Secondary antibody HRP-labeled
Luminol + H2O2
3-aminophtalate + light
Luminol + H2O2
3-aminophtalate + light
Transduced light
Attachment layer


Chemiluminescent optical fiber immunosensor for detecting cholera antitoxin
Marks, R.S., E. Basis, A. Bychenko and M.M. Levine (1997). Optical Engineering 36 (12) 3258-3264
CTP3: 50-64
[a] buffer; [b] air
Time (sec.)

## Silane-benzophenone for photochemical attachment of bio-Molecules

Leshem, B., G. Sarfati, A. Novoa, I. Breslav and R.S. **Marks** (2004) Photochemical attachment of biomolecules onto fiber-optics for construction of a chemiluminescence immunosensor Luminescence. 19: 69-77

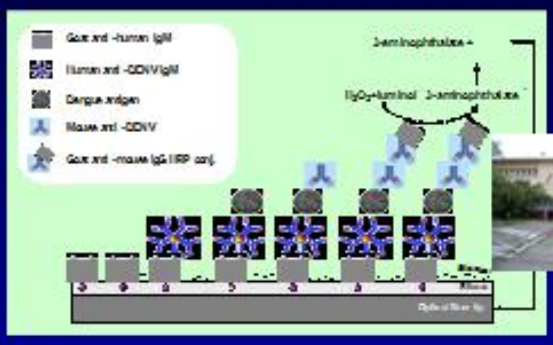


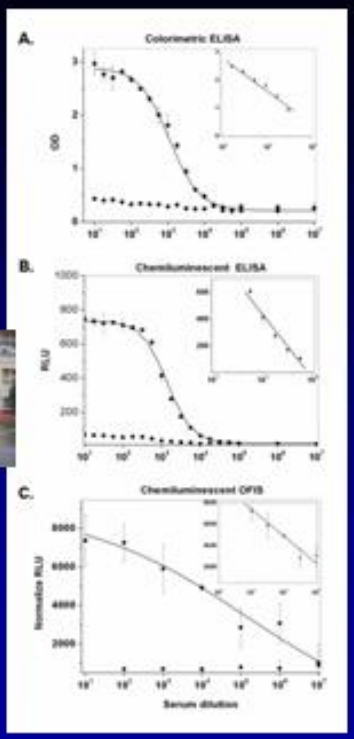


Increased bio-receptor coverage with 4-(3'-dichloromethylsilyl) propyl-oxybenzophenone

## French Guiana dengue 1, 2,3 virus immunosensor

Danit Atlas, Yael Liebes, Vered Chalifa-Caspid, Laetitia Bremand, Leslie Lobela, Robert S. Marks, Phillipe Dussart Sensors & Actuators 2005 In press




OFIS: lower detection limit: 100 x MacELISA, 10 x CL-ELISA; correlations between method's over 0.97

OFIS Specificity (87.0%) & Sensitivity (98.1%)

CL MAC ELISA Specificity (95.6%) & Sensitivity (100%)

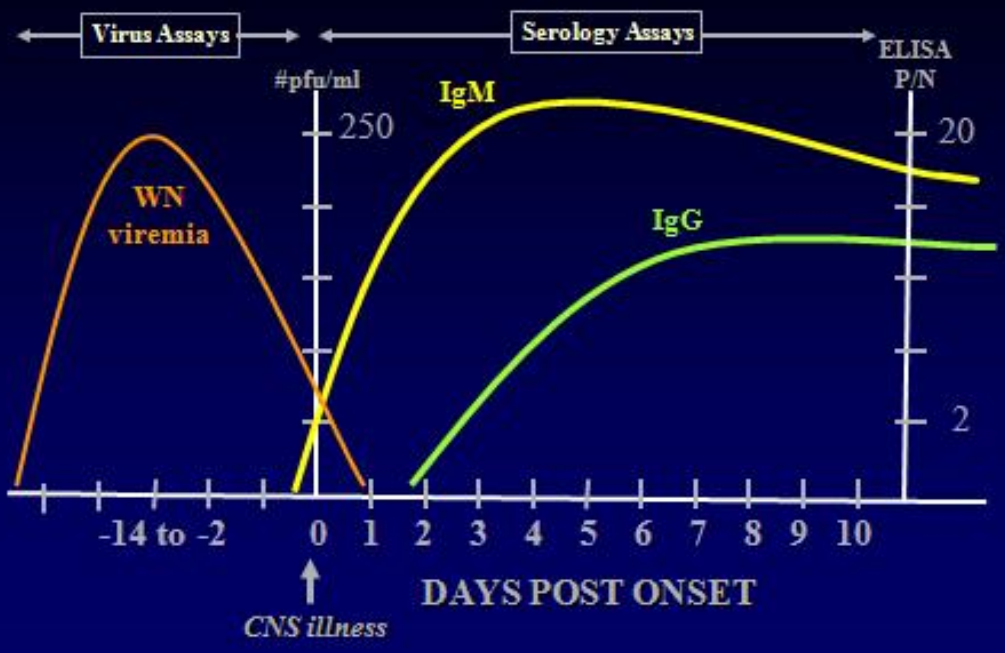
## Limits of actual diagnostic assays

| Name | ELISA | PRNT | IFA | Microsphere | NASBA | RTF-PCR | VecTest | Isolation |
|---|---|---|---|---|---|---|---|---|
| Sensitivity | + | ++ | ++ | ++ | +++ | +++ | - | +++ |
| Specificity | - | ++ | ++ | - | +++ | +++ | ++ | +++ |
| Speed | + | -- | -- | + | -- | -- | +++ | -- |
| Staffing | + | -- | --- | + | -- | -- | +++ | --- |
| Safety | ++ | -- | --- | ++ | +++ | +++ | ++ | --- |
| Portability | - | -- | -- | - | - | - | ++ | -- |
| Cost | ++ | + | + | + | + | + | ++ | + |

Serological Tests | Virological Tests

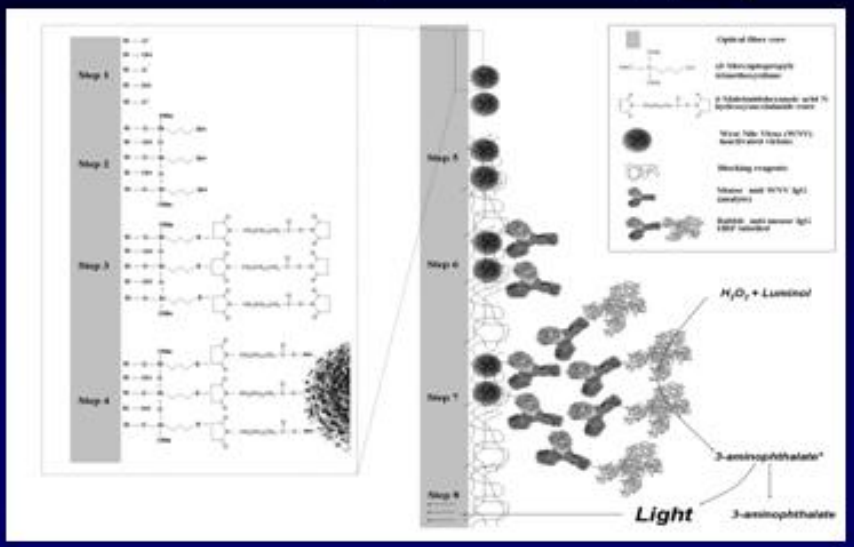

➡ Need a test enabling both high sensitivity/specificity and speed, safety and portability

## Physiological markers

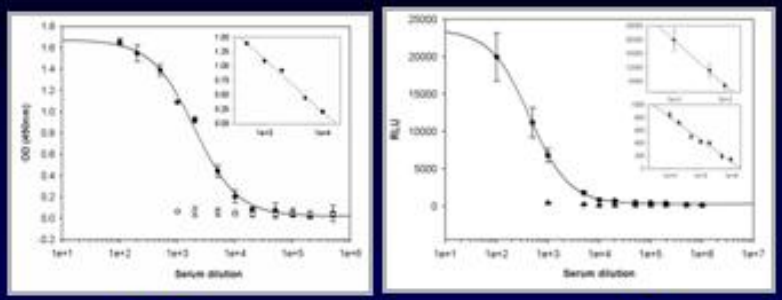


## Chemiluminescent optical fiber immunosensor for the detection of anti-West Nile virus IgG

Herrmann, S. B. Leshem, S. Landes, B. Rager-Zisman and R.S. **Marks** (2004) Talanta 66: 6-14




## The anti-WNV IgG immunosensor is more sensitive than that of ELISA

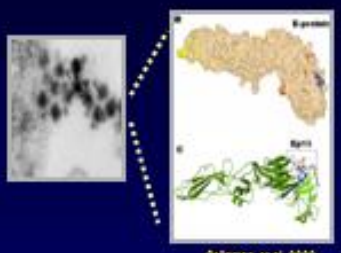



| ELISA | | OFIS |
|---|---|---|
| 3h | Speed | 1h |
| 1:10,000 | Lower detection limit | 1:1,000,000 |
| 500-10,000 | Linear range | 100-2,000<br>10,000-1,000,000 |
| 37° C | Temperature | RT |

**Chemically neutralized viruses should not enter a kit**

# T7 phage display of Ep15 peptide for the detection of WNV IgG

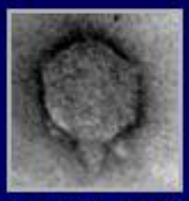
Herrmann, S., B. Leshem, L. Lobel, H. Bin, E. Mendelson, D. Ben-Nathan, P. Dussart, A. Porgador, B. Rager-Zisman, R.S. **Marks** (2007). Journal of Virological Methods. 141: 133-140



- The WNV E glycoprotein is a highly immunogenic flaviviral antigen
- Bioinformatics of conserved regions gave highly rated B-cell linear epitopes

**p10B-GGG-Ep15-GGG-Ep15**

**415 copies/phage**

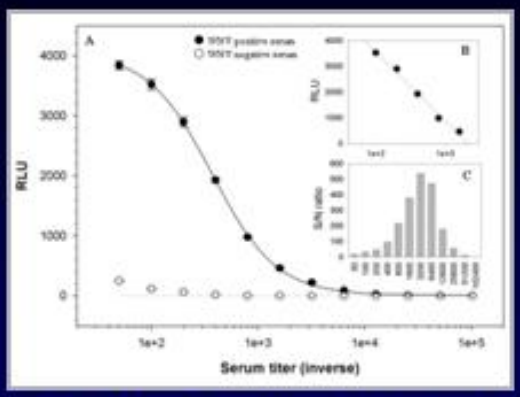


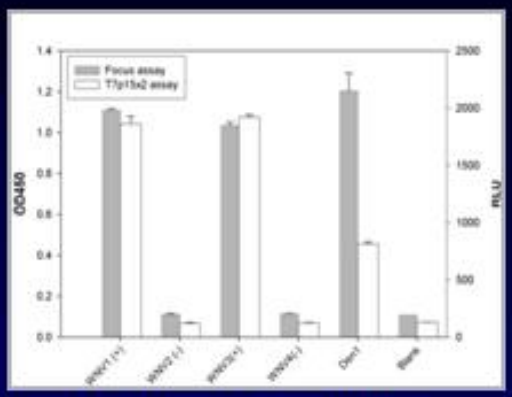
T7 phage particles displaying ~415 copies

Checking the ligation by PCR /electrophoresis

# Phage ELISA based on T7-Ep15x2

Herrmann, S., B. Leshem, L. Lobel, H. Bin, E. Mendelson, D. Ben-Nathan, P. Dussart, A. Porgador, B. Rager-Zisman, R.S. Marks (2007) T7 phage display of Ep15 peptide for the detection of WNV IgG. Journal of Virological Methods. 141: 133-140



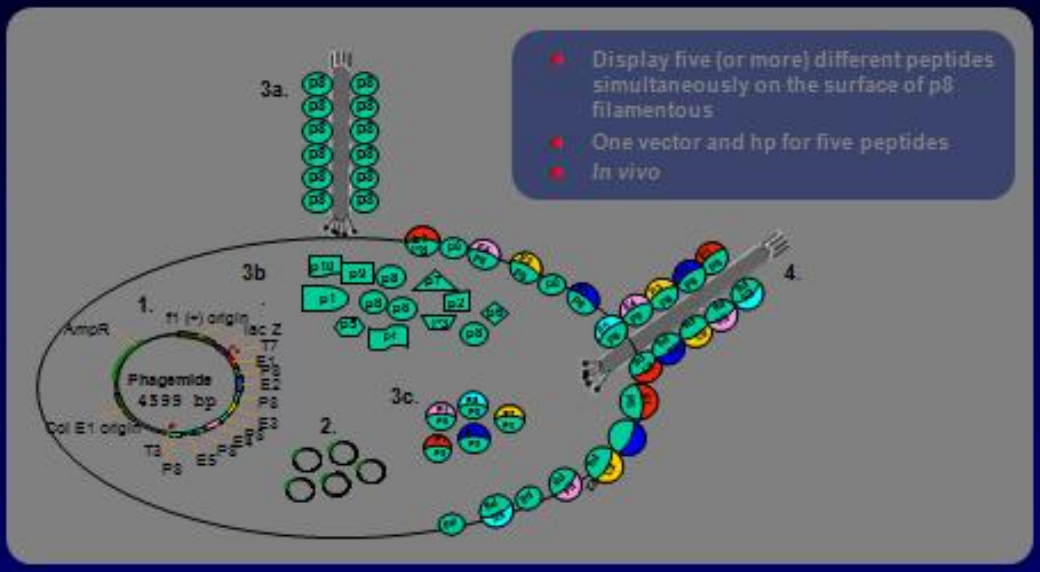




- Improved specificity as it does not cross react with Dengue virus unlike the FDA approved Focus Diagnostics kit
- **The T7-Ep15x2 ELISA** shows a **lower detection limit of 1:51,200**, a **linear range from 1:100 to 1:2,000** and the **best S/N ratio at 1:3,000**
- **Very good correlation** when testing 4 reference human sera (WNV1-4)

**Lower sensitivity**

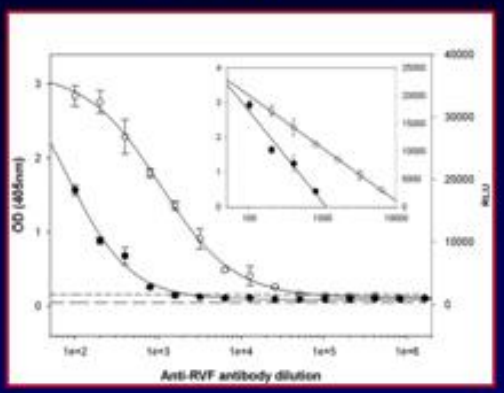
# M13 Phage displayed multiple epitopes

Atias, D., L. Lobel, M. Virta and R.S. **Marks** (2007). Marks, R.S., D. Cullen, C. Lowe, H.H. Weetall and I. Karube (eds). Handbook of Biosensors and Biochips. John Wiley & Sons Ltd Publishers



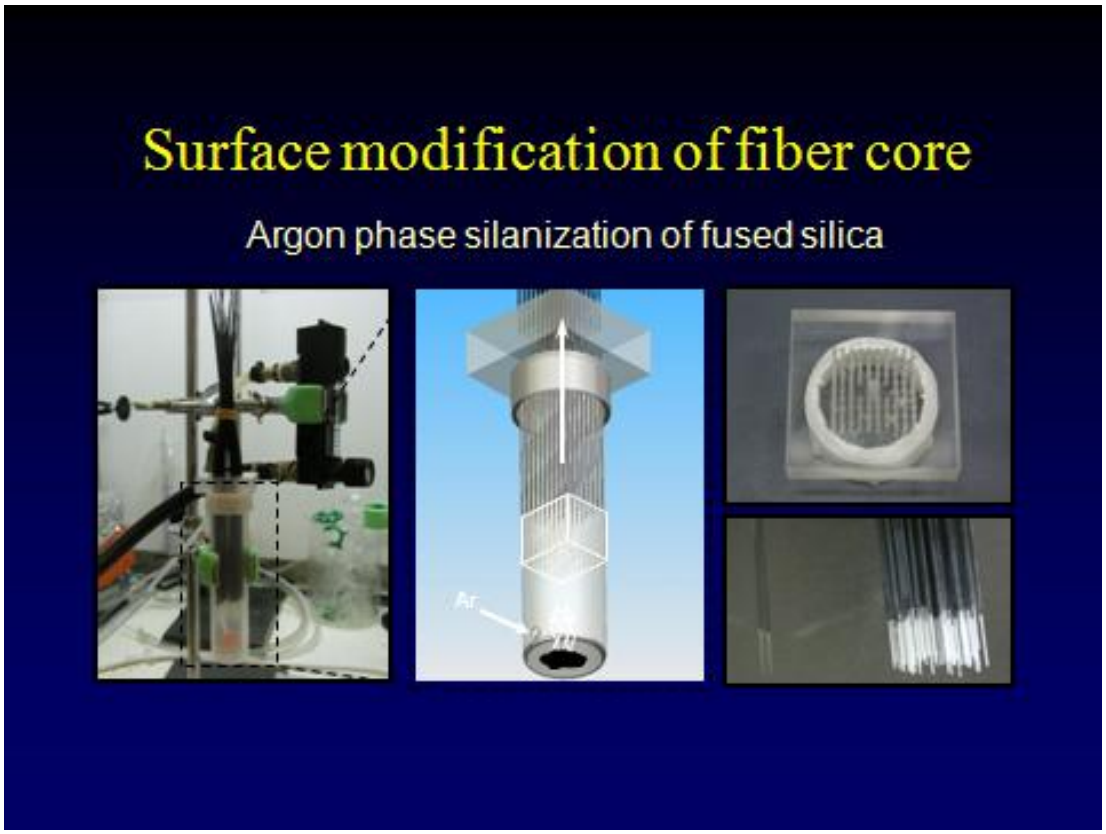

# Optical fiber immunosensor for the detection of IgG antibody to Rift Valley fever virus in humans

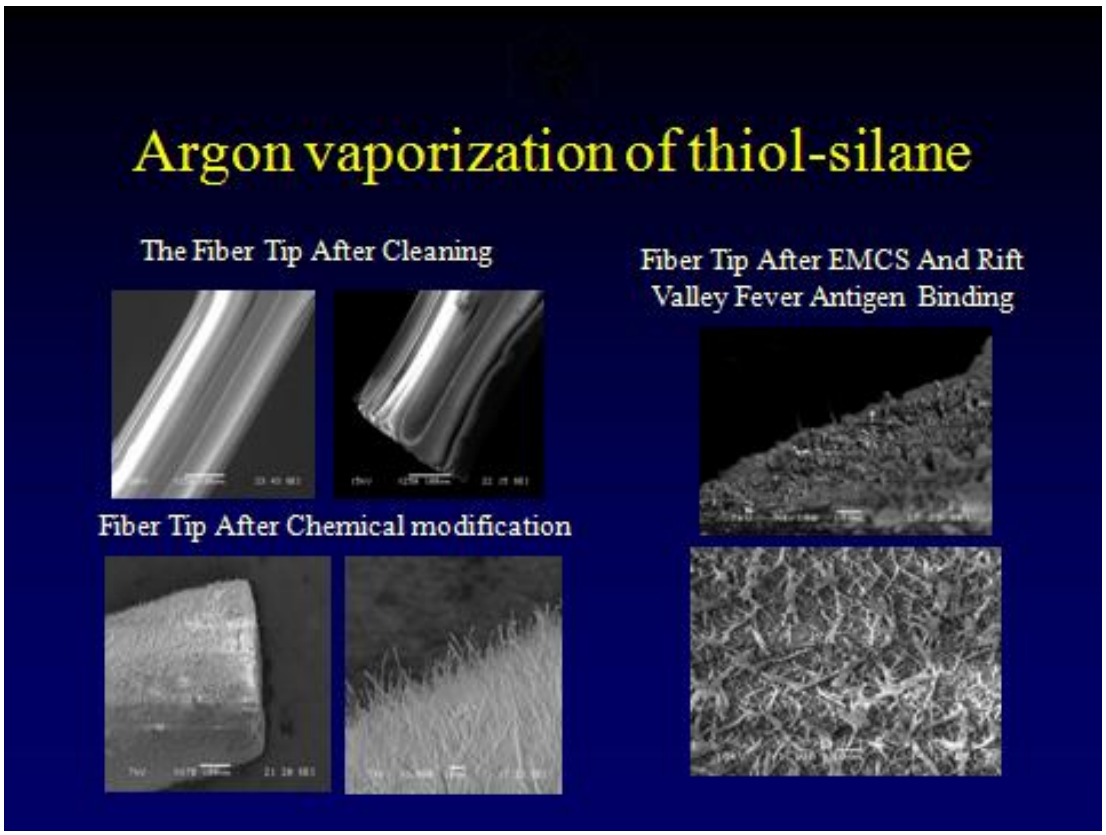
Sobarzo, A., J.T. Paweska,, S. Herrmann, T. Amir, R.S. **Marks** and L. Lobel (2007) Journal of Virological Methods 146 (1-2) 327-334

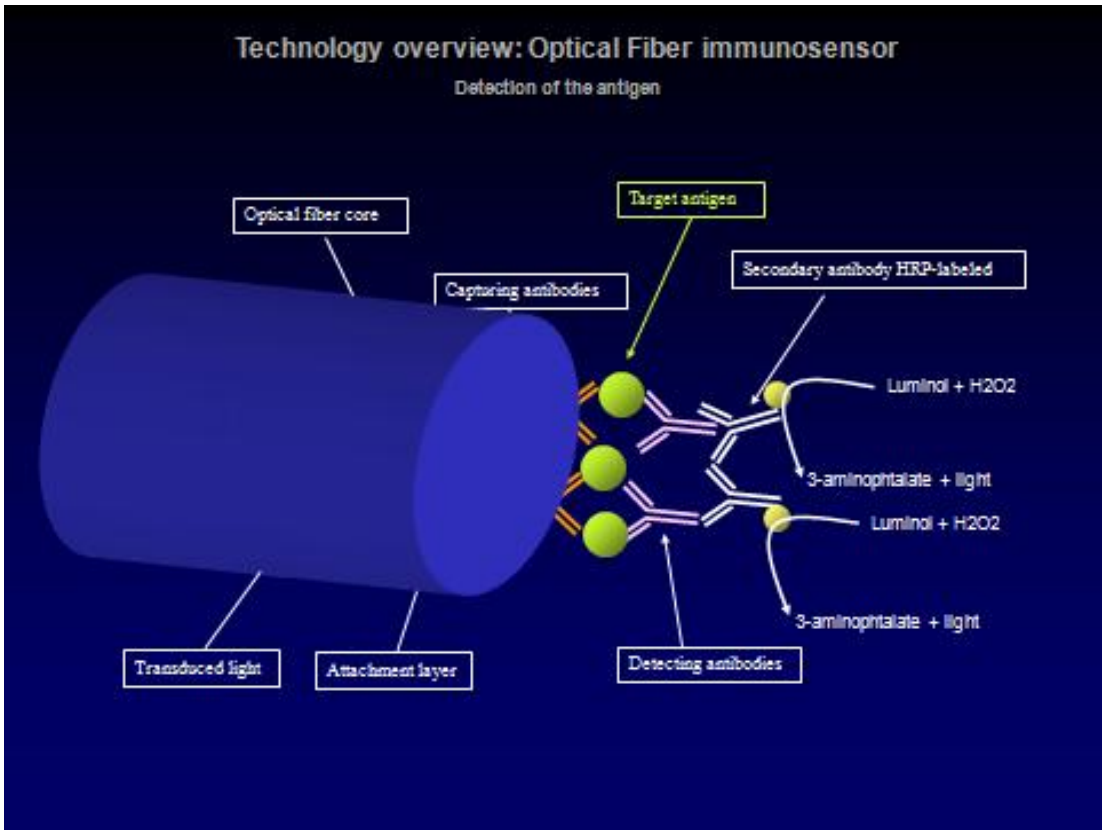


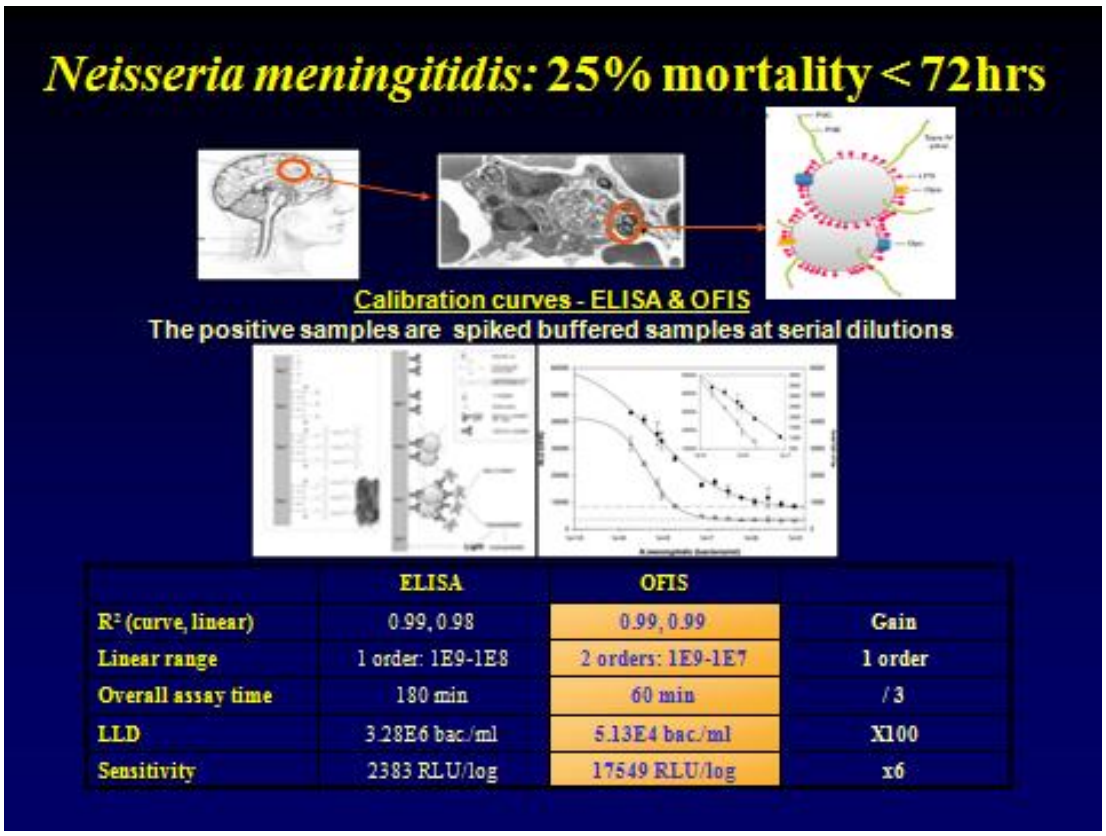

1:51,200
Vs.
1:1,638,400

On the left Titration curves for high-positive (—) and negative control serum (– –) generated by OFIS (●) and colorimetric ELISA (○). The result of the linear regression analysis of the middle part of the calibration curves for high positive control is shown upper right corner of the graph. On the right signal to noise ratio between ELISA and OFIS.

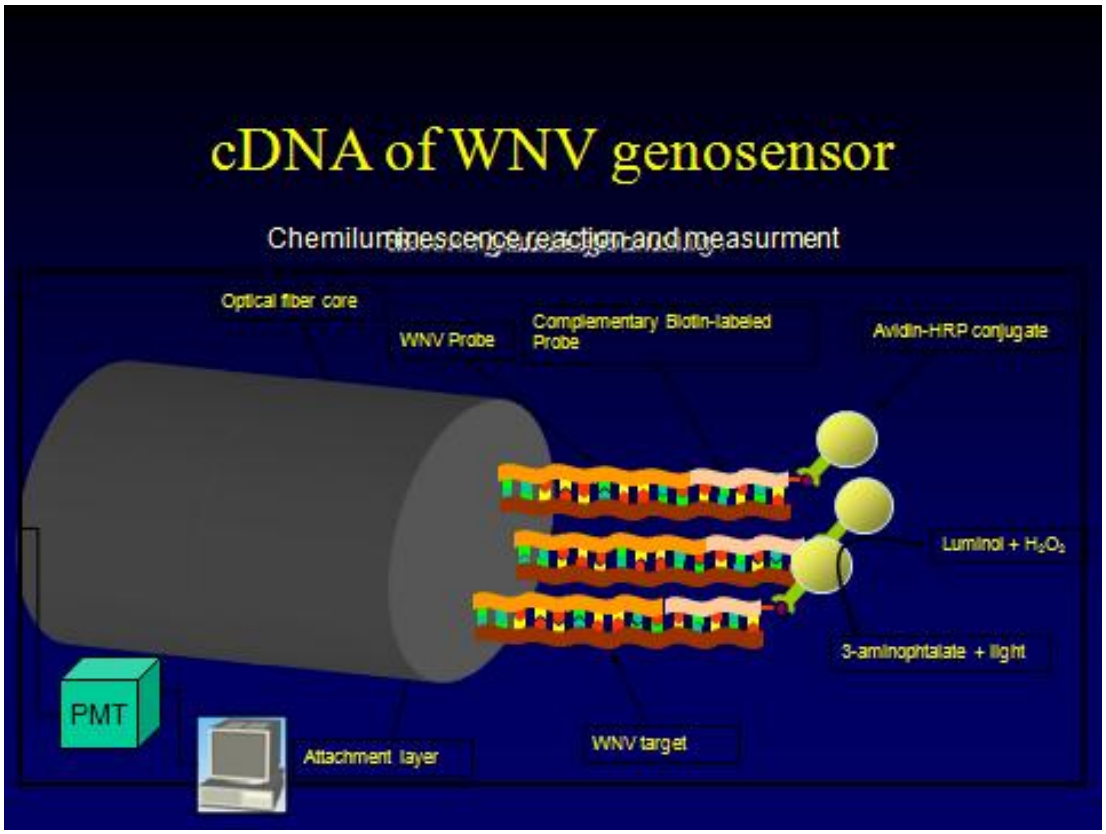

Surface modification of fiber core
Argon phase silanization of fused silica
Ar

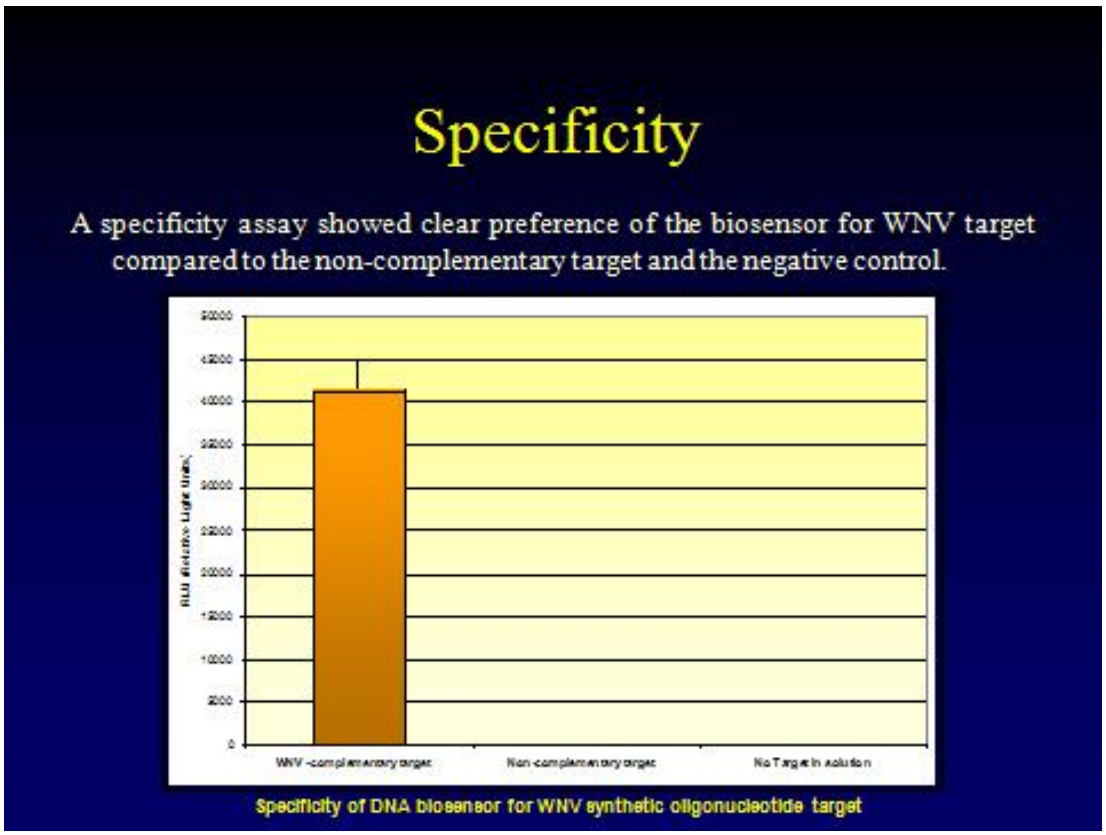

Argon vaporization of thiol-silane
The Fiber Tip After Cleaning
Fiber Tip After EMCS And Rift Valley Fever Antigen Binding
Fiber Tip After Chemical modification



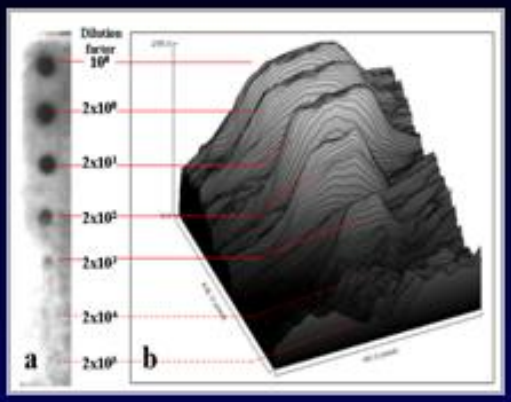



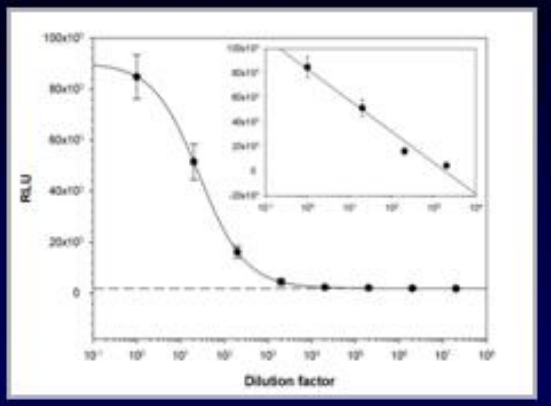

| | ELISA | OFIS | |
|---|---|---|---|
| $R^2$ (curve, linear) | 0.99, 0.98 | 0.99, 0.99 | Gain |
| Linear range | 1 order: 1E9-1E8 | 2 orders: 1E9-1E7 | 1 order |
| Overall assay time | 180 min | 60 min | / 3 |
| LLD | 3.28E6 bac./ml | 5.13E4 bac./ml | X100 |
| Sensitivity | 2383 RLU/log | 17549 RLU/log | x6 |


cDNA of WNV genosensor
Chemiluminescence reaction and measurment
Optical fiber core
WNV Probe
Complementary Biotin-labeled Probe
Avidin-HRP conjugate
Luminol + $H_2O_2$
3-aminophtalate + light
PMT
Attachment layer
WNV target


Specificity
A specificity assay showed clear preference of the biosensor for WNV target compared to the non-complementary target and the negative control.
WNV -complementary target
Non-complementary target
No Target in solution
Specificity of DNA biosensor for WNV synthetic oligonucleotide target

# Results of the sensor



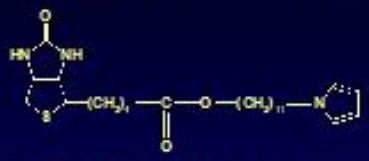




| Dot Blot | | OFDS |
|---|---|---|
| 0.5 ng | Lower detection limit | 0.5 pg |
| 0.5µg-0.5ng | Linear range | 1µg-0.5ng |

# An innovative strategy for immobilization of receptor proteins on to an optical fiber by use of poly(pyrrole-biotin)

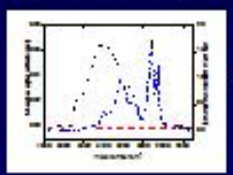
Marks, R.S., A. Novoa, D. Thomassey and S. Cosnier (2002) Analytical and Bioanalytical Chemistry. 374: 1056-1063

Pyrrole-biotin



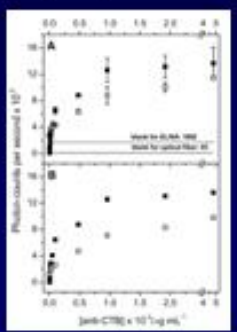
FeCl3 was selected as oxidizer since the potential of the polymerization threshold (0.67 V) is under the Fe(III)/Fe(II) reduction potential (0.77 V)

Avidin-TRICT conjugated to poly(pyrrole-biotin)



FT-IR Spectra

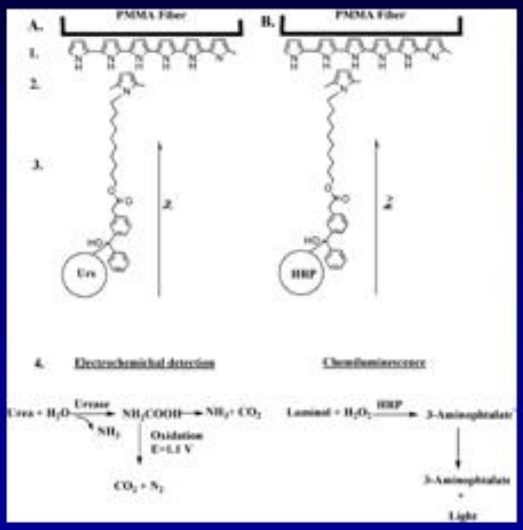
Pp-biotin) fiber optic
Pyrrole-biotin
Bare fiber-optic



Optical-fibers (■) and classical ELISA (□)

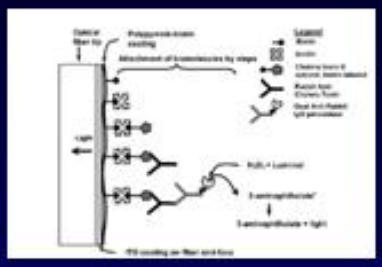
## Development of Poly (methyl metacrylate) optic-conductive fiber transducers as dual biosensor platforms

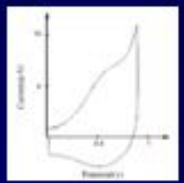
D. Atlas, K. Abu-Rabeah b, S. Herrmann, J.Frenkel, D. Tavor, S.Cosnier and R.S. Marks Biosensors & Bioelectronics (2009) 24: 3683-87

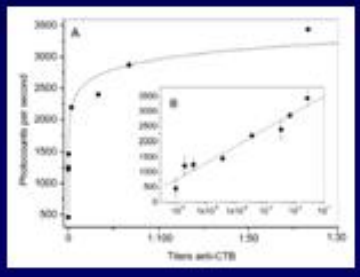



## Indium tin oxide-coated optical fiber tips for one step electro-polymerization of robust films on complex geometries

Marks, R.S., A. Novoa, T. Konry, R. Krais and S. Cosnier (2002) Materials Science and Engineering C. 21 (1-2) 189-194






Cyclic voltammogram of the electro-polymerized conducting film on the ITO surface

## Physico-Chemical studies of an ITO-coated fiber-optic biosensor

Konry, T. and R.S. **Marks** (2005). Thin Solid Films. 492: 313-321

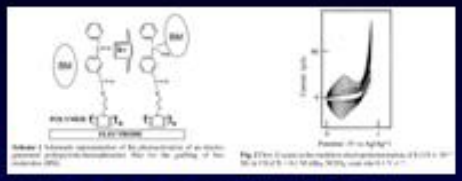
- ITO is highly transmissive
- ITO has low resistivity
- ITO is a tin oxide-doped indium oxide

| Element | Wt % |
|---|---|
| $SiO_2$ | 25.96 |
| $In_2O_3$ | 73.63 |
| $SnO_2$ | 0.41 |
| **Total** | 100.00 |

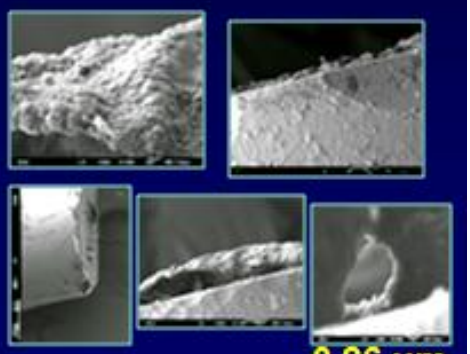
| | |
|---|---|
| **Thickness** | 260 nm |
| **Resistance** | $4.7 \times 10^{-2}$ Ω·cm |
| **Lattice constant** | 10.12 Å |
| **Average grain size <D>** | 11.5 nm |
| **Optical transmittance** | 85% |
| **Refraction index** | 2.136 |

## Optical immunosensor based on a poly(pyrrole-benzophenone) film for the detection of antibodies to viral antigen

Konry, T., A. Novoa, Y. Shemer-Avni, N. Hanuka, S. Cosnier, A. Lepellec and R.S. Marks (2005). Analytical Chemistry. 77 (6) 1771-1779

Cosnier, S.; Senillou, A. Chemical Communications 2003, 414-415.



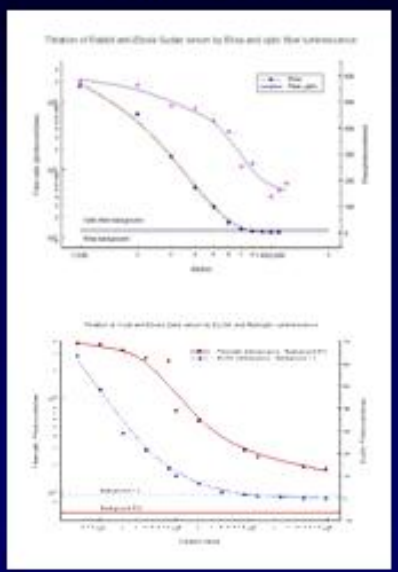
## Screening of patients with HCV infection

| Serological state of patients | Total tested | Anti-E2+ Western Blot | Anti-E2+ Immuno sensor | Western blot Detection percentage | Immun-sensor Detection percentage |
|---|---|---|---|---|---|
| Anti HCV+ RNA+ | 13 | 9 | 13 | 69 | 100 |
| Anti HCV- RNA+ | 8 | 2 | 4 | 25 | **50** |
| Anti HCV- RNA- | 7 | 0 | 0 | 0 | 0 |

*Anti HCV: Anti Core, NS5 & NS3

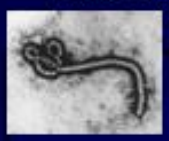
## Indium tin oxide-coated fiber optic immunosensor for the detection of antibodies to Ebola virus

Petrosova, A., T. Konry, S. Cosnier, I. Thrakt, J. Lutwama, E. Rwaguma, A. Chepurnov, E. M. Mühlberger, L. Lobel R.S. **Marks**. (2006) Sensors & Actuators. B. Chemical. B 122:578-586



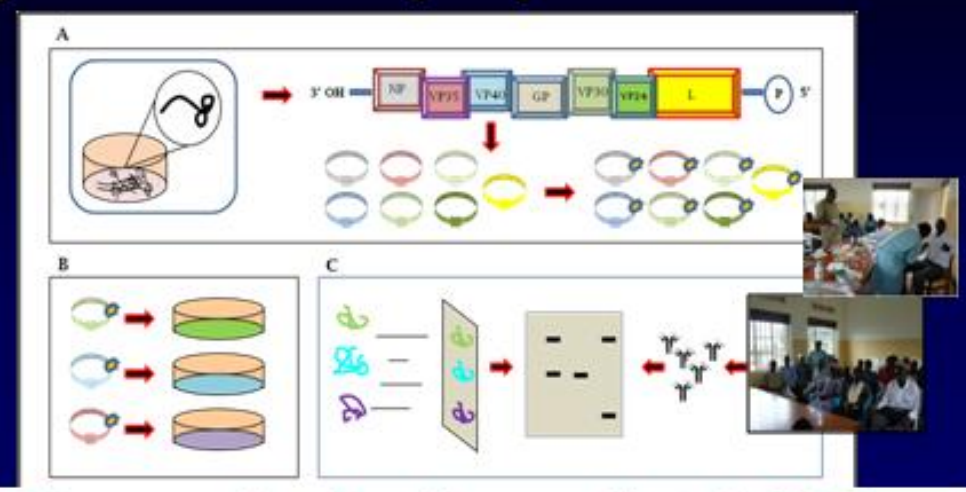
Ebola virus immunosensor vs ELISA for the detection of antibodies from Sudan and Zaire subtype strains

Calibration curve



| | Elisa | Fiber optic immunosensor |
|---|---|---|
| Linear range | 1:500 – 1:10,000 | 1:2,000 – 1:80,000 |
| Lowest detection limit | 1:20,000 | 1:960,000 |
| | | |

## Use human immune response profile to find epitope candidates




From 21 human samples with positive recognition to the NP, 20 were observed in individual survivors whereas only one in a fatal case. All .VP40 positive recognition samples were linked to survival

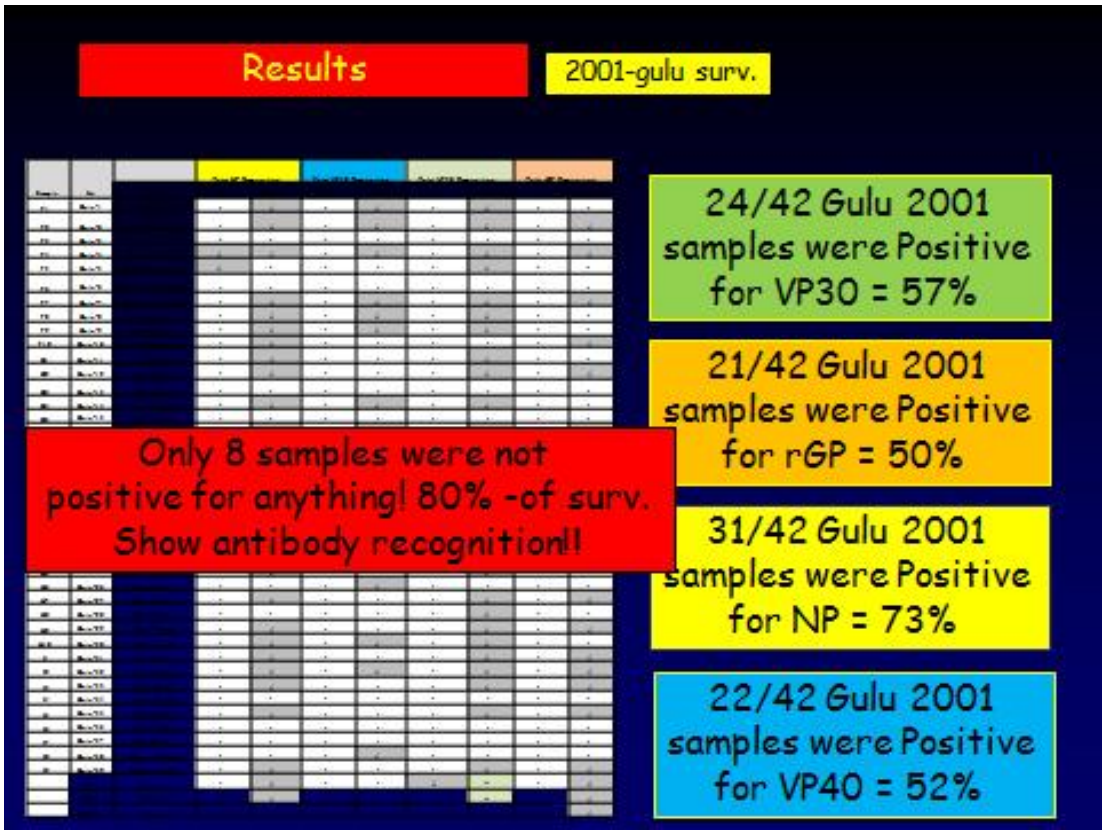
## Cloned Ebola-S proteins screening Ebola-S survivor sera

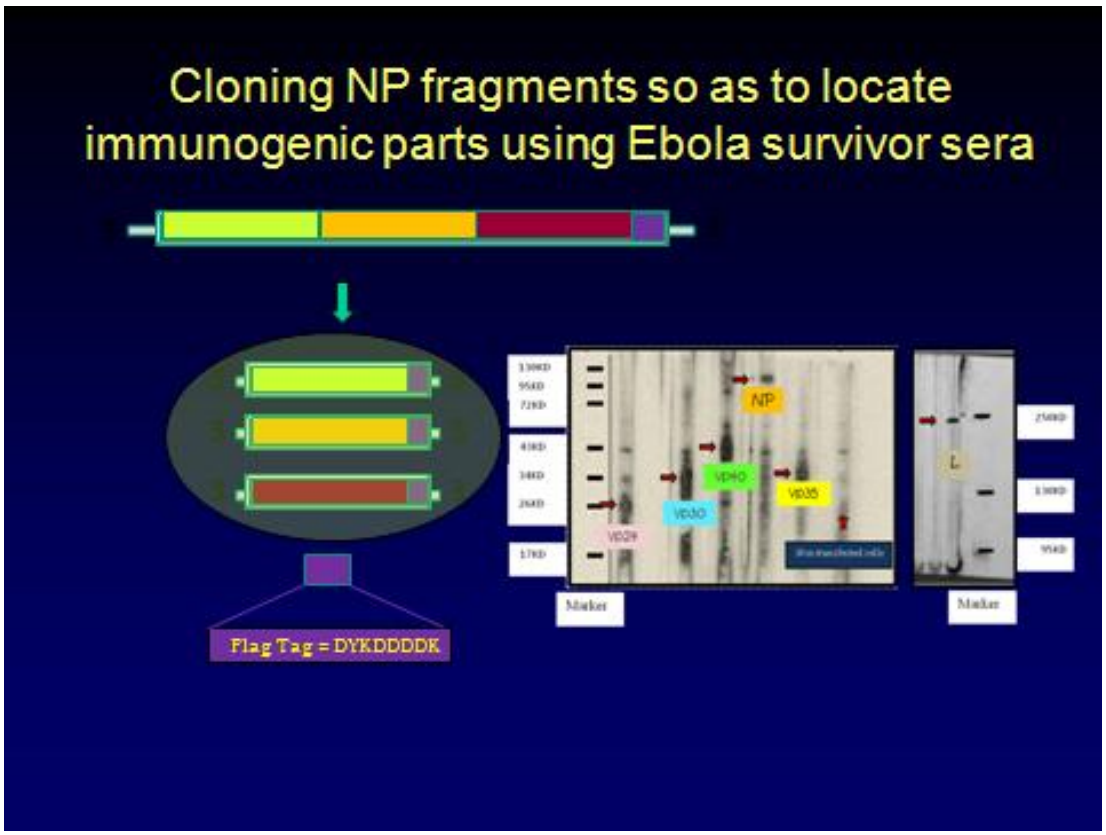
| Sample | Ebolavirus Sudan (Gulu) Recombinant | | | |
|---|---|---|---|---|
| | VP30 | VP40 | NP | rGP |
| Infected | 1/78 | 6/78 | 21/78 | 1/78 |
| Non Infected | 1/100 | 0/100 | 2/100 | 2/100 |
| Total | 2/178 | 6/178 | 23/178 | 3/178 |

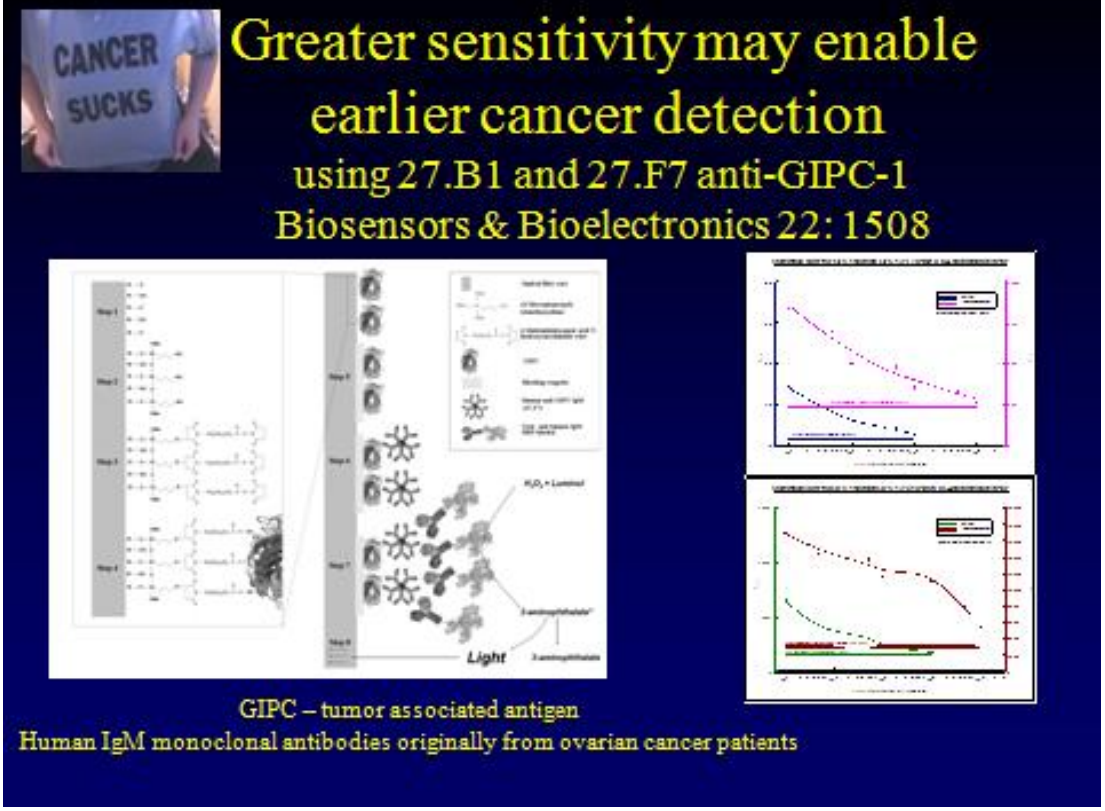
WB

ELISA

| Sample Group | Ebolavirus Sudan (Gulu) Recombinant Protein | | | | Ebolavirus Sudan (Gulu) |
|---|---|---|---|---|---|
| | VP30 | VP40 | NP | rGP | Complete antigen |
| Infected | 7/78 | 11/78 | 33/78 | 10/78 | 40/78 |
| Non infected | 1/100 | 2/100 | 5/100 | 2/100 | 2/40 |
| Total | 8/178 | 13/178 | 38/178 | 12/178 | 42/118 |


Results
2001-gulu surv.
24/42 Gulu 2001 samples were Positive for VP30 = 57%
21/42 Gulu 2001 samples were Positive for rGP = 50%
Only 8 samples were not positive for anything! 80% -of surv. Show antibody recognition!!
31/42 Gulu 2001 samples were Positive for NP = 73%
22/42 Gulu 2001 samples were Positive for VP40 = 52%


Cloning NP fragments so as to locate immunogenic parts using Ebola survivor sera
Flag Tag = DYKDDDDK
NP
VP40
VP35
VP30
Marker
Marker

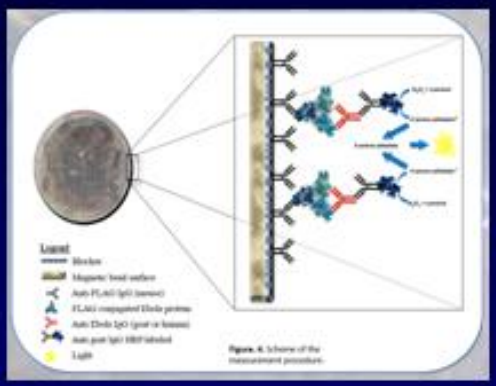



## Identification of elevated IgM anti-GIPC-1 specific auto-antibodies in ovarian and breast cancer patients

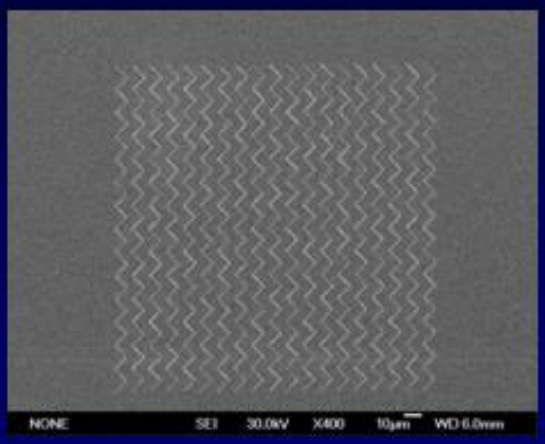
| | CL- ELISA | Immunosensor |
|---|---|---|
| Total Samples | 11 | 11 |
| - | 9 | 5 |
| + | 1 | 4 |
| ++ | 0 | 1 |
| +++ | 1 | 1 |
| Sensitivity | 18% (2/11) | 54% (6/11) |

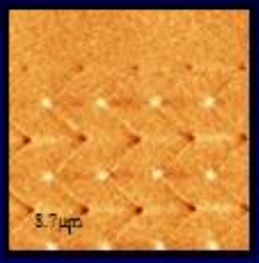
| | CL- ELISA | Immunosensor |
|---|---|---|
| Total Samples | 22 | 22 |
| - | 16 | 5 |
| + | 2 | 2 |
| ++ | 3 | 11 |
| +++ | 1 | 4 |
| Sensitivity | 27% (6/22) | 77% (17/22) |

# Liquid light guide magnetic immunosensor

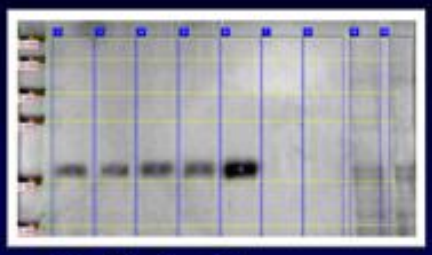


# Magnetic nanoastructures for magnetic bead capture of target immunoreagents



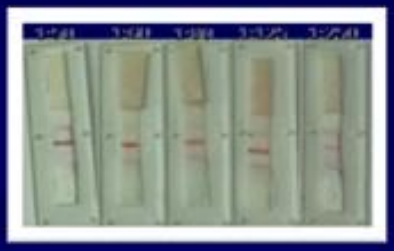
# Lateral flow immunosensor



Purify the bioreceptor

Conjugation of gold nanoparticles

Combine with transducer technology



# Viralcheck

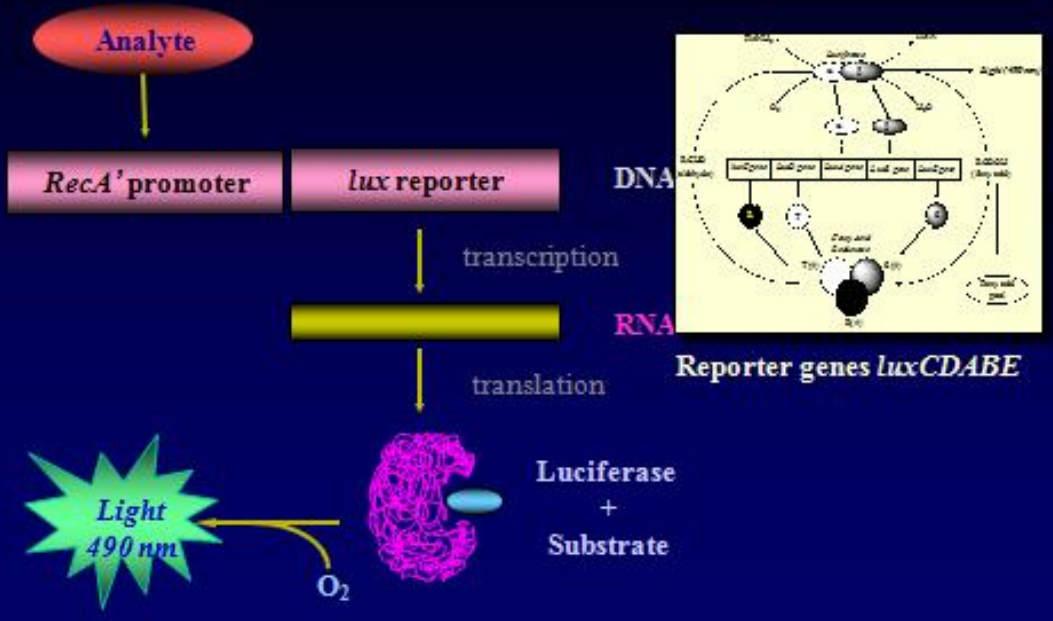
June 21-27,2008 - Dakar, Senegal

- Funding:
  - US Army Research Labs
  - US State Department
- Content
  - Tutorial
  - Hands-on workshop with blind viral samples
  - Mock outbreak
  - Symposium
- African Research Network: inauguration
- Book: Advanced diagnostics for the detection of viral pathogens

## Bioassays

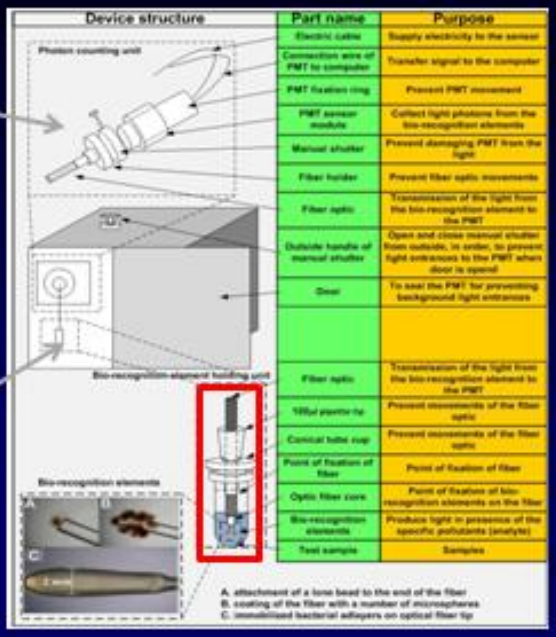
- Natural whole organisms
  - Microbial cell cultures
- Natural sub-organisms
  - Mammalian cell cultures
  - Blood sample
- Engineered organisms
  - Microbial cell cultures
  - Mammalian cell cultures

## DNA damage sensing



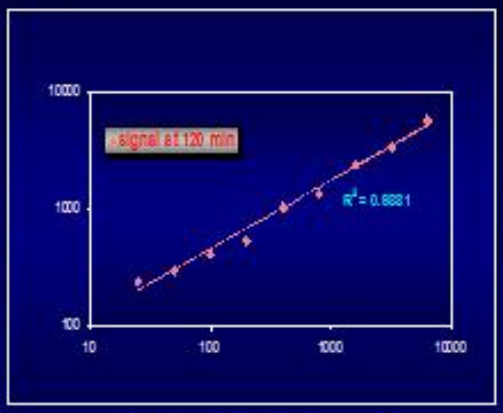
# FIBER OPTIC BIOSENSOR

Eltzov, E. et al. (2009). "Parameters to Consider in the Construction of Fiber-Optic Biosensors as Alternative Bioanalytical Tools." Ieee Instrumentation & Measurement Magazine 12(5): 10-16.



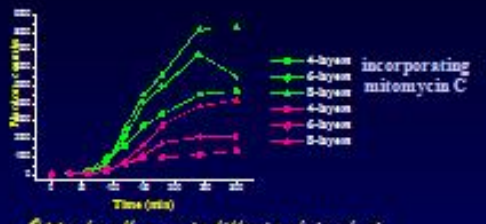

# Optical fiber bioluminescent whole-cell microbial biosensors to genotoxicants

Polyak, B., E. Bassis, A. Novodvorets, S. Belkin and R.S. **Marks** (2000). Water Science and Technology. 42 (1-2) 305-311

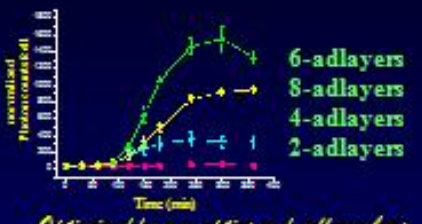


## Bioluminescent whole-cell optical fiber sensor to genotoxicants: system optimization

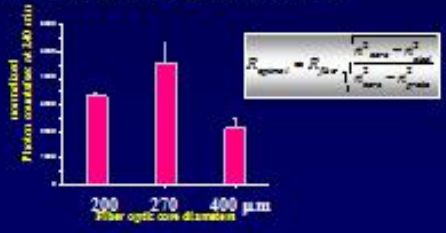
Polyak, B., E. Bassis, A. Novodvorets, S. Belkin and R.S. **Marks** (2000). Sensors and Actuators. B 3656: 1-9



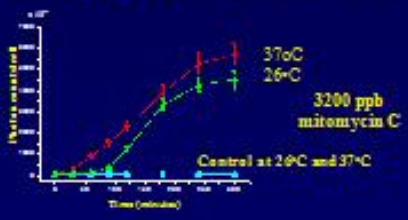

*Optrode adlayers is diffusion dependent*




*Optimized for ray optics and cell numbers*




*Data confirm formula best coupling at 270 µm*

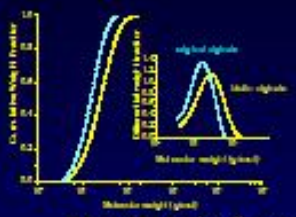



*E. coli DPD 1718 works best at 37 C*

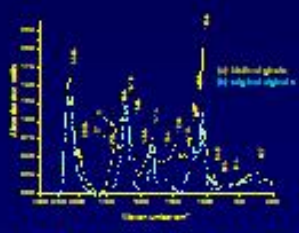
## Synthesis and characterization of a biotin-alginate conjugate and its application in a biosensor construction

Polyak, B., S. Geresh and R.S. **Marks** (2004). Biomacromolecules. 5: 389-396

Synthesis of Biotin-Alginate conjugate



MALLS-SEC (light scattering) analysis

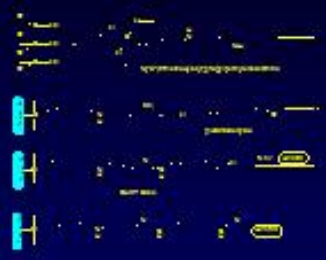


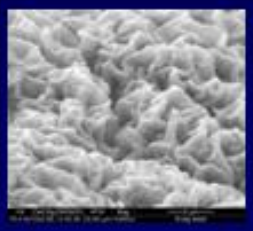
FT-IR analysis of biotin-alginate

| polymer concentration (% w/v) | biotin-alginate (13%) (cPoise) | original alginate (cPoise) |
|---|---|---|
| 1.5 | 88 | 126 |
| 2.0 | 139 | 278 |
| 2.5 | 252 | 482 |
| 3.0 | 414 | 822 |
| 3.5 | 731 | 1230 |

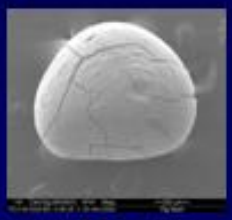
Viscosity measurements at a shear rate of 100 $s^{-1}$

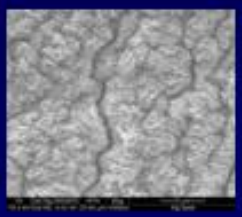
## Biotinylated microsphere attached to an avidinylated fiber-optic endface





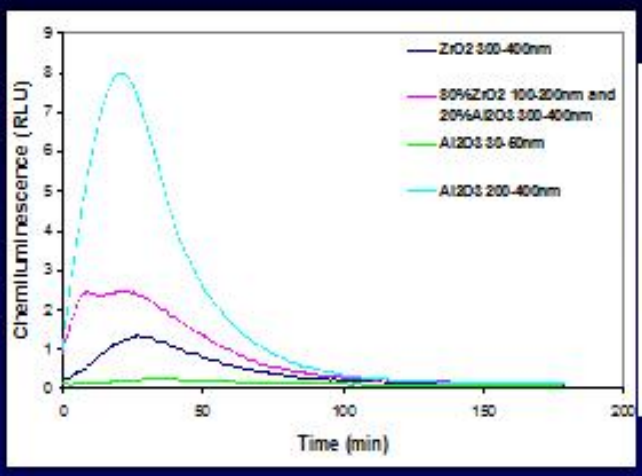
Biotin-alginate





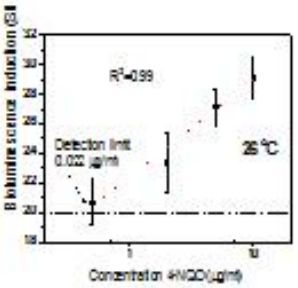
Alginate

## Phagocyte luminescence-based biotoxicity assay



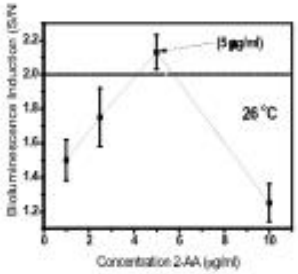

Decision tree designed with WEKA, J 48 algorithm, 60 points per graph

## Genotoxic chemicals tested at TECHNOTOX (Belgium)




Slightly genotoxic !

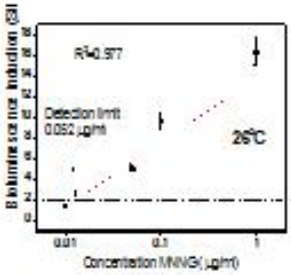
4-nitroquinoline-N-oxide (4-NQO)




2- aminoanthracene (2-AA)

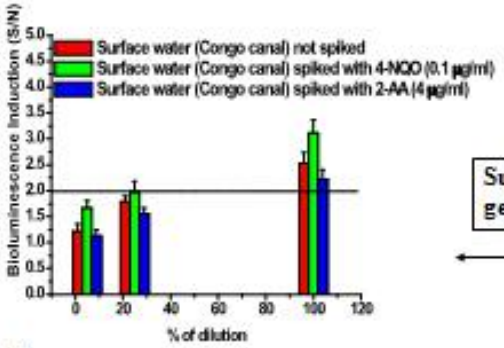
Cannot detect without metabolic activation

Highly genotoxic !

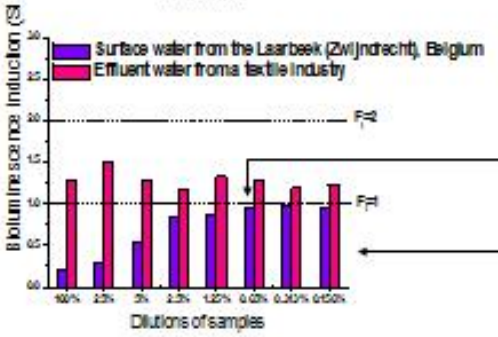



N-Methyl-N'-nitro-N-nitrosoguanidine (MNNG)

## Environmental samples tested at TECHNOTOX (Belgium)

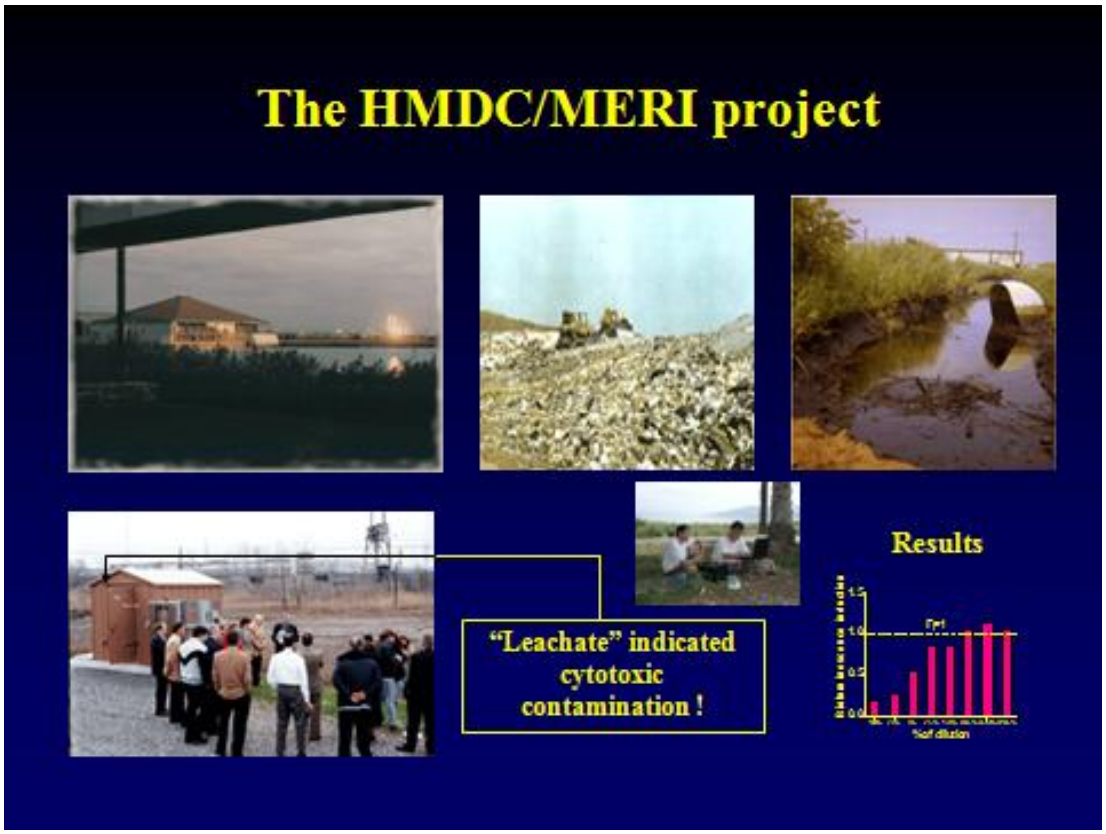


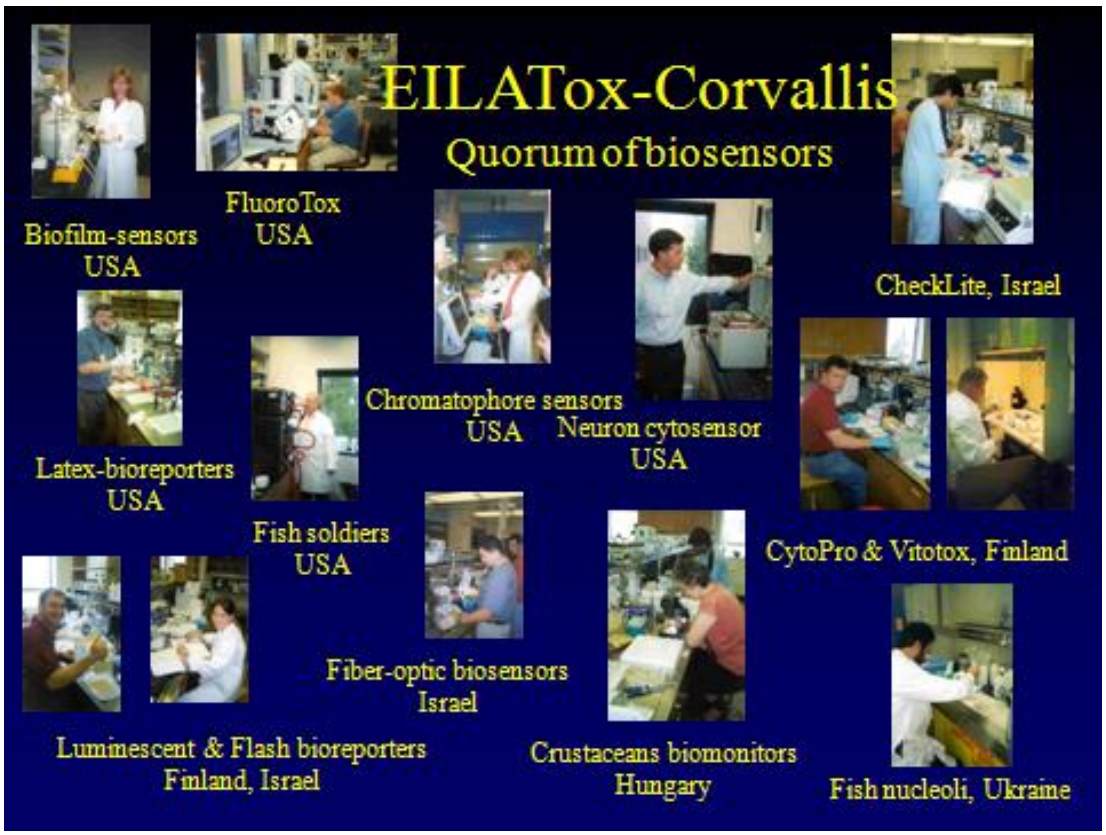

Surface water from the Congo canal showed genotoxicity in contaminated water




Effluent water from a textile industry is cytotoxic until a dilution of 1:100

Surface water from Belgium canal were neither genotoxic nor cytotoxic

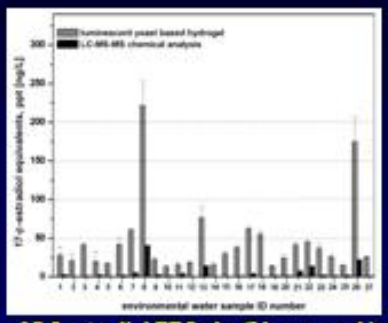

The HMDC/MERI project
"Leachate" indicated cytotoxic contamination !
Results


EILATox-Corvallis
Quorum of biosensors
Biofilm-sensors USA
FluoroTox USA
CheckLite, Israel
Chromatophore sensors USA
Neuron cytosensor USA
Latex-bioreporters USA
Fish soldiers USA
CytoPro & Vitotox, Finland
Fiber-optic biosensors Israel
Luminescent & Flash bioreporters Finland, Israel
Crustaceans biomonitors Hungary
Fish nucleoli, Ukraine

# Luminescent yeast based hydrogels for Endocrine Disrupting Compound biodetection

| Estrogenic compound | EC50 [ppb] | Relative potency ER_ | ER_ | Detection limit [ppb] (_g/L) |
|---|---|---|---|---|
| 17-_-estradiol (E2) | 0.6 | 1 | 1 | 0.05 |
| Diethylstilbestrol (DES) | 0.1 | 5.6 | 15.2 | 5.0x10$^{-3}$ |
| Ethynylestradiol (EE2) | 0.4 | 1.5 | n.a.* | 6x10$^{-3}$ |
| Estron (E1) | 2.1 | 0.2 | 0.02 | 0.05 |
| Coumesterol | 27 | 0.016 | 0.02 | 0.3 |
| Genistein | 699 | 0.0013 | 0.0035 | 18.9 |
| Bisphenol A | 632 | 0.0014 | 0.0043 | 25.6 |

*n.a=not available

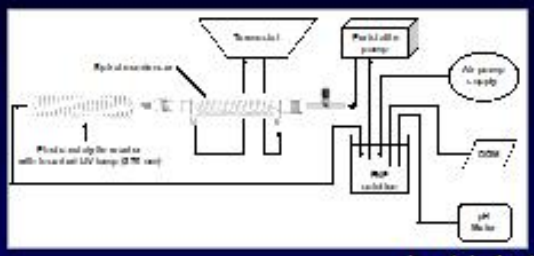


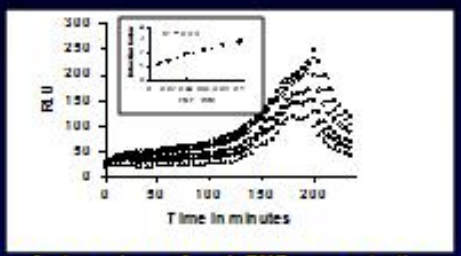

17-β-estradiol EEQs [ng/L] measured in environmental water samples from Tokyo bay

- Samples from 27 different locations in Tokyo bay metropolitan area: 8 effluents from sewage treatment plants; 19 river waters (Dr. Isao Tomokiko, National Institute for Environmental Studies, Tsukuba 305-8506, Ibaraki, Japan)
- Most estrogenic samples are similar with both measurements
- Overestimation of the luminescent hydrogels

# Monitoring genotoxicity during the photocatalytic degradation of *p*-nitrophenol

Shani Sekler, M., Y. Levi, B. Polyak, A. Novoa, PSM Dunlop, JA Byrne and RS Marks (2004). Journal of Applied Toxicology. 24: 395-400



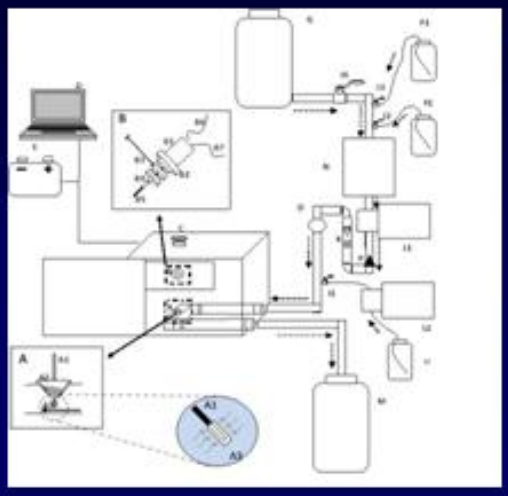


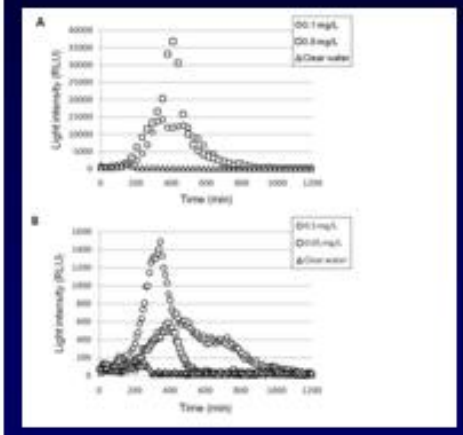

Inset: Induction factor values of each PNP concentration

- Titanium dioxide ($TiO_2$) is a semiconductor metal oxide that when irradiated with UV light radiation, it produces an electron/hole pair generating hydroxyl radicals at the surface of the particle that will oxidize pollutants.
- $TiO_2$ can be immobilized so that post treatment removal would not be necessary
- Water treatment would require monitoring its toxicity level

# Flow-through real-time bacterial biosensor for toxicants in drinking water

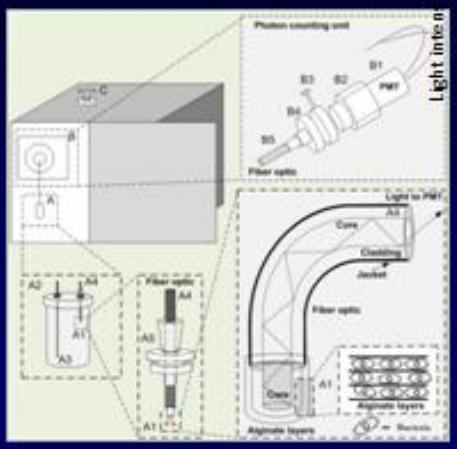
Eltzov, E. et al. (2009). "Flow-through real time bacterial biosensor for toxic compounds in water." Sensors and Actuators B-Chemical **142**(1): 11-18.

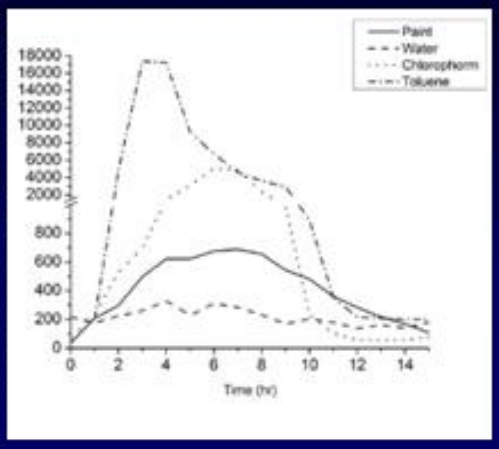




# FIBER OPTIC BIOSENSOR FOR AIR TOXICITY MONITORING

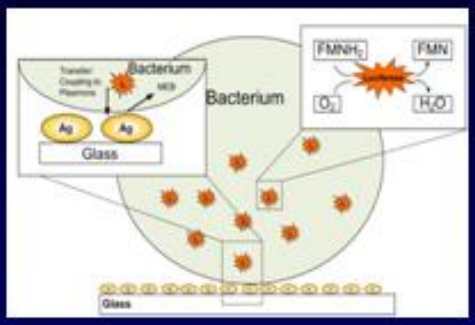
Eltzov, E. et al. (2010). "Creation fiber optic based biosensor for air toxicity monitoring." Sensors and Actuators B-Chemical, **proofs**.

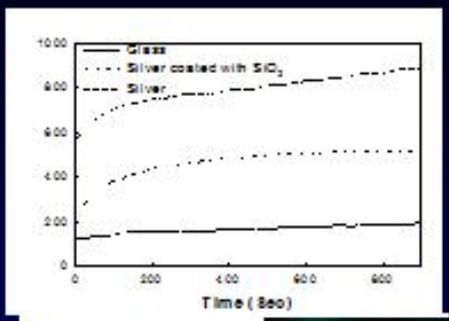


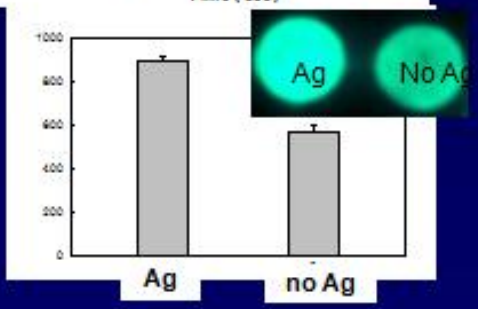


## Metal-enhanced bioluminescence

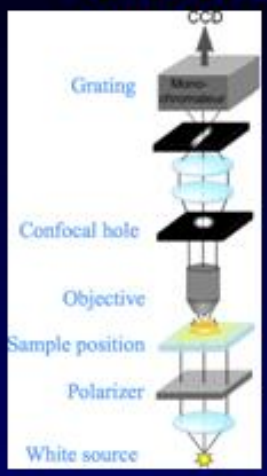
E. Ehtzov, D. Pritutsky, A. Kushmaro, R.S. Marks, C. Geddes (2009) Metal-enhanced bioluminescence: a new approach for monitoring biological luminescent process. Applied Physics Letters. 94 (5)

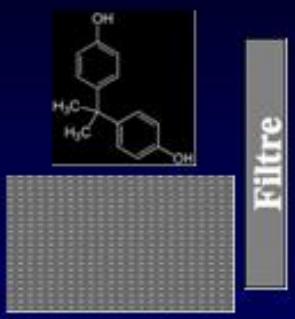


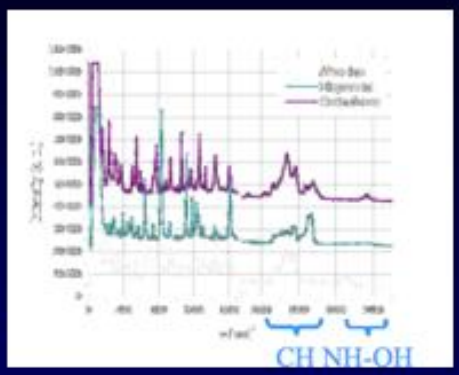




## Surface enhanced Raman scattering




Filtre




Laser illuminated molecule's bonds vibrate to scatter light at different wavelengths producing Raman spectra fingerprint of the molecule which are enhanced in the vicinity of nanoantenna (of modulable size, shape and distanc) by the oscillation of their plasmon electronic cloud

# Surface-enhanced fluorescence from metallic nano-sculptured thin films

Abdulhalim, I., A. Kanaboshvsky, C. Patzig, B. Rauschenbach, B. Fuhrmann, E. Elsov, R. Marks, J. Xu, F. Zhang, and A. Lakhtakia (2009) Surface-enhanced fluorescence from metal sculptured thin films with application to biosensing in water. Applied Physics Letters. 94 (6)

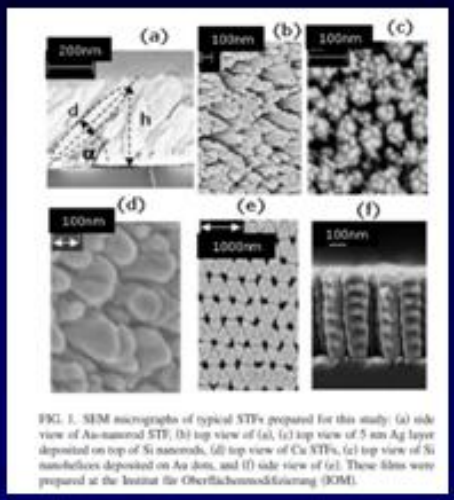
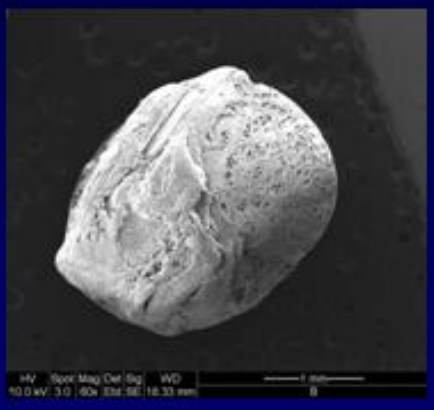


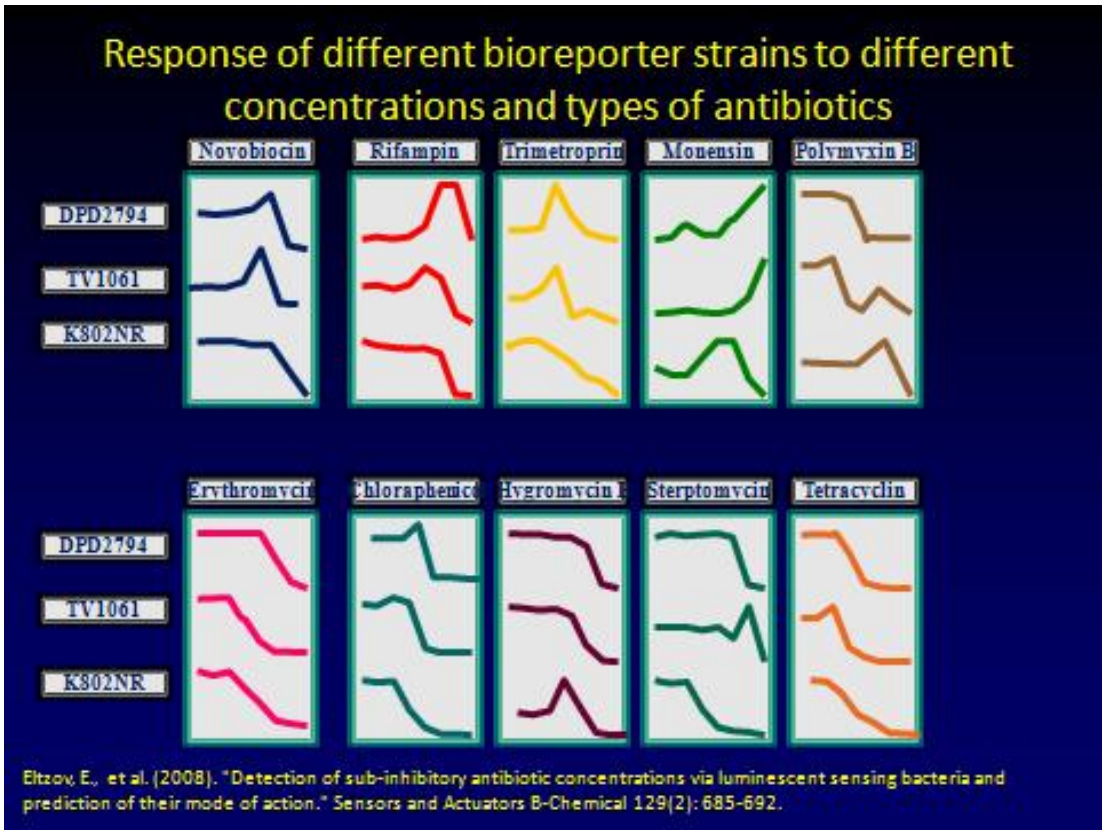

FIG. 1. SEM micrographs of typical STFs prepared for this study: (a) side view of Au-nanorod STF, (b) top view of (a), (c) top view of 5 nm Ag layer deposited on top of Si nanorods, (d) top view of Cu STFs, (e) top view of Si nanohelices deposited on Au dots, and (f) side view of (e). These films were prepared at the Institut für Oberflächenmodifizierung (IOM).

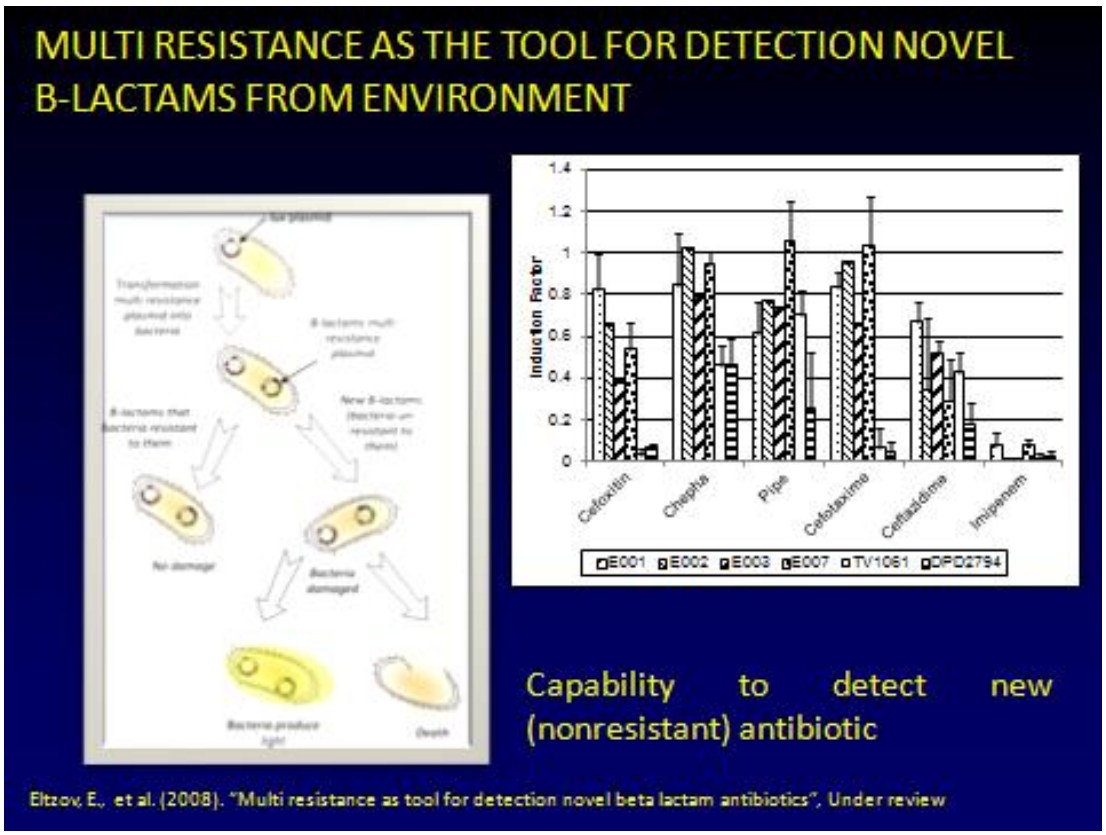
# Growing 'uncultivable' microorganisms :

## Agar Sphere Polymeric Encapsulation Technology Platform

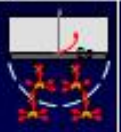
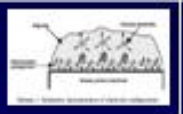
- Discovery of new antibiotics and new degraders of recalcitrant anthropogenic pollutants will lead to wealth as well as novel and economic treatment processes.




Response of different bioreporter strains to different concentrations and types of antibiotics
Novobiocin
Rifampin
Trimetroprin
Monensin
Polymyxin B
DPD2794
TV1061
K802NR
Erythromycin
Chloraphenicol
Hygromycin B
Sterptomycin
Tetracyclin
DPD2794
TV1061
K802NR
Eltzov, E., et al. (2008). "Detection of sub-inhibitory antibiotic concentrations via luminescent sensing bacteria and prediction of their mode of action." Sensors and Actuators B-Chemical 129(2): 685-692.


MULTI RESISTANCE AS THE TOOL FOR DETECTION NOVEL B-LACTAMS FROM ENVIRONMENT
Induction Factor
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Cefoxitin
Chepha
Pipe
Cefotaxime
Ceftazidime
Imipenem
E001 E002 E003 E007 TV1061 DPD2794
Capability to detect new (nonresistant) antibiotic
Eltzov, E., et al. (2008). "Multi resistance as tool for detection novel beta lactam antibiotics", Under review

## Electrochemical Biosensors Developed

| Transducer | Analyte | Receptor | Immobilization Procedure |
|---|---|---|---|
| Platinum disk electrode | Glucose *(in aqueous solution)* | Glucose oxidase | Micro-encapsulation in latex films  |
| Platinum disk electrode | Glucose *(in aqueous solution)* | Glucose oxidase biotin labeled | Biotinylated Alginate matrix  |
| Glassy carbon rotating disk electrode | Catechol *(in organic solvent)* | Polyphenol oxidase biotin labeled | Poly-pyrrol-biotin electro-polymerized film  |
| 0.9 mm HB drawing leads | Paraoxon | Molecular imprinted polymer | Imprinted template  |


- Cosnier, S.; S. Szunerits, R.S. Marks, A. Novoa, L. Puech, E. Perez, I. Rico-Lattes. *Electrochemistry Communications* 2 (2000) 851-855.
- Cosnier, S.; S. Szunerits, R.S. Marks, A. Novoa, L. Puech, E. Perez, I. Rico-Lattes. *Talanta* 55 (2001) 889-897.
- Cosnier, S., A. Novoa, C. Mousty and R.S. marks (2002) Analytica Chimica Acta 453: 71-79
- Mousty, C., A. Leppelec, S. cosnier, A. Novoa and R.S. Marks (2001) Electrochemistry Communications. 3: 727-732


## Project Biopen: a vision

Aportable device

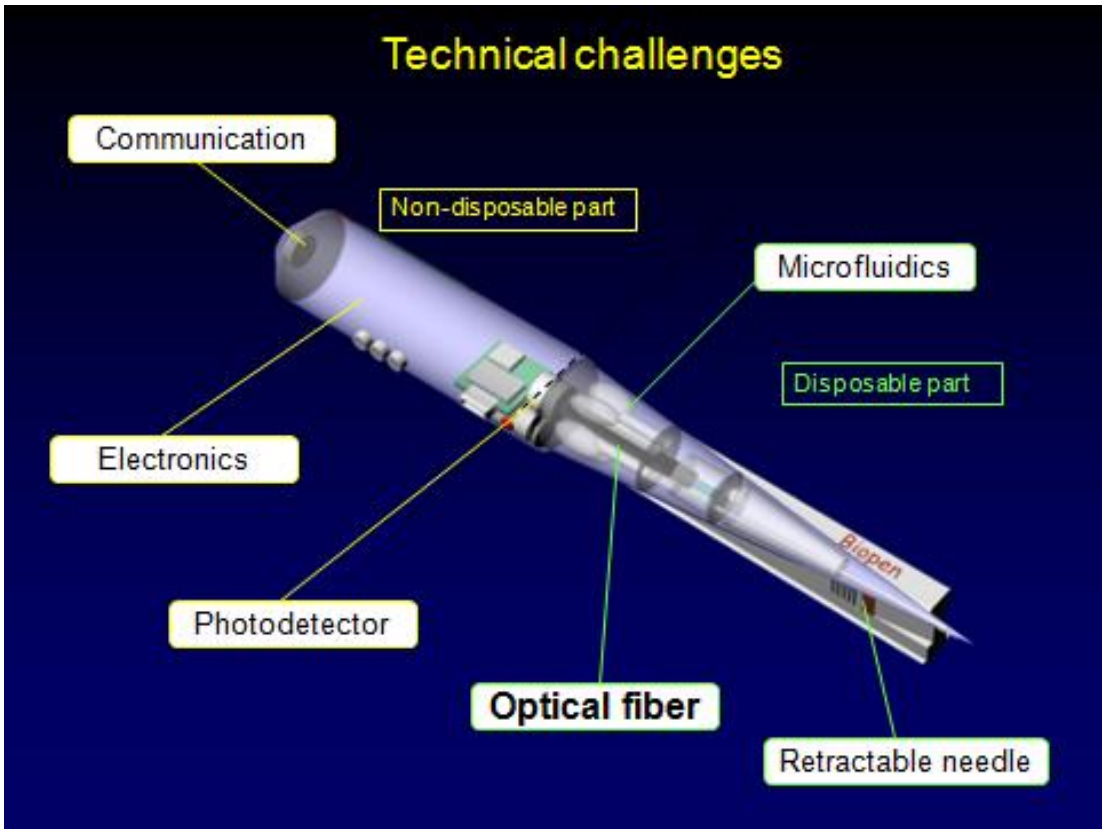
for monitoring pollutants, biotoxins and pathogens

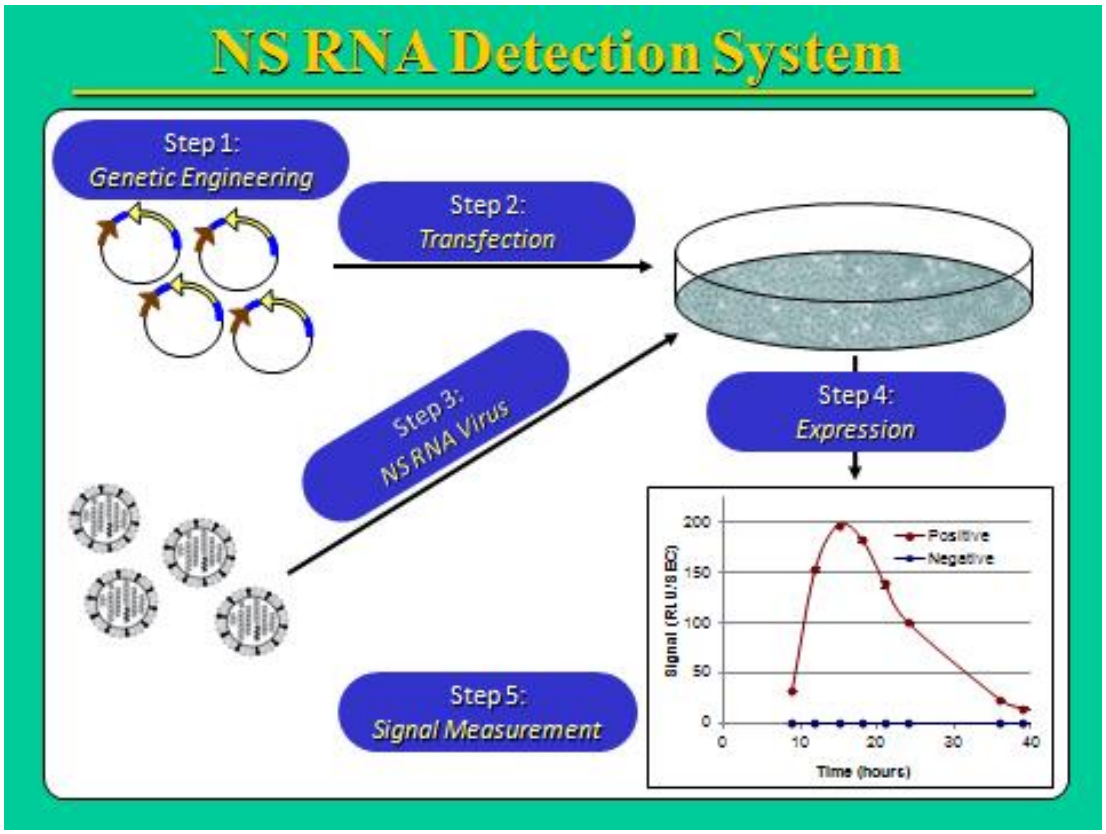
in drinking water and biological fluids

The first

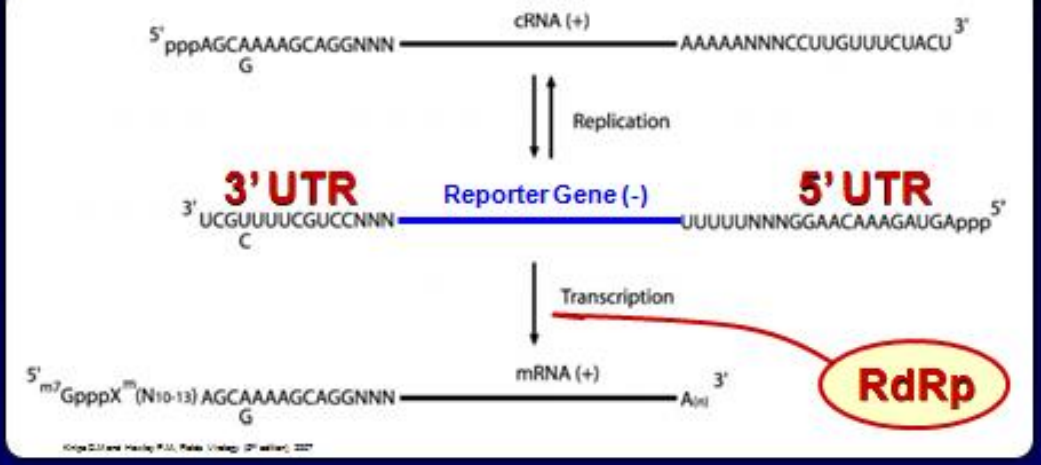
"Lab-in-a-pen"


National Institute for Biotechnology in the Negev
Ben-Gurion University of the Negev


Technical challenges
Communication
Non-disposable part
Microfluidics
Disposable part
Electronics
Photodetector
Optical fiber
Retractable needle
Biopen

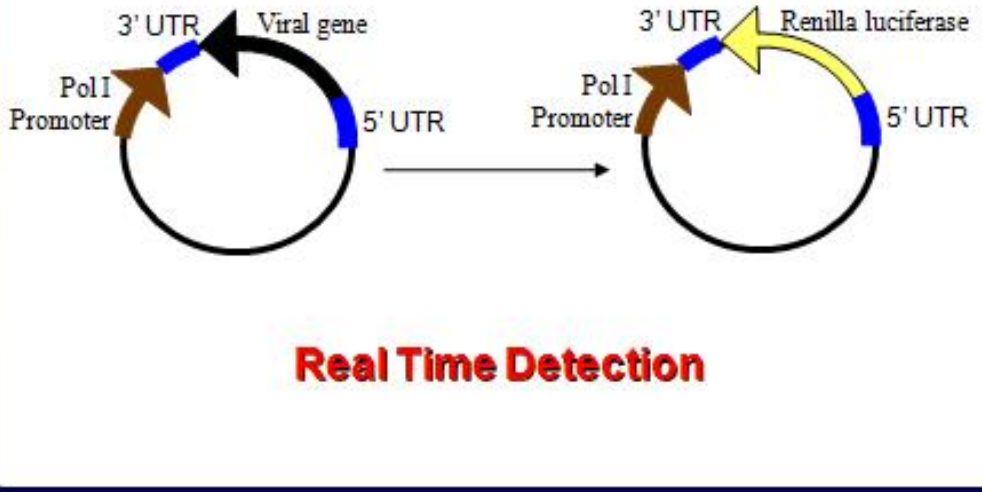

NS RNA Detection System
Step 1:
Genetic Engineering
Step 2:
Transfection
Step 3:
NS RNA Virus
Step 4:
Expression
Step 5:
Signal Measurement
Positive
Negative
Signal (RLU/9 EC)
Time (hours)
0
50
100
150
200
0
10
20
30
40

# Negative Stranded RNA Viruses



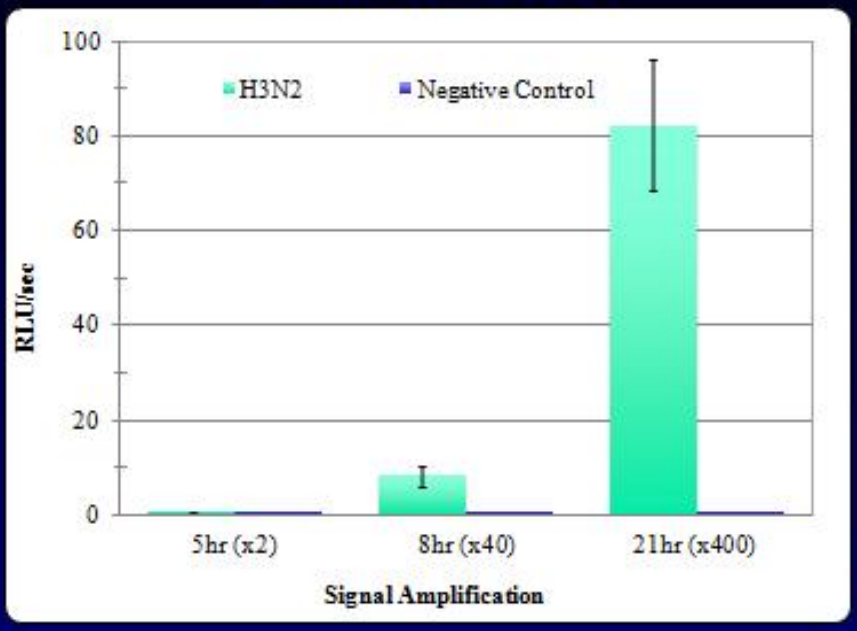

- RdRp – RNA dependent RNA polymerase
- UTRs – UnTranslated Regions

# We replace the NP viral gene with a *Luc* gene




**Real Time Detection**

## Our transfected cells report on the presence of influenza virus




## Clinical problem

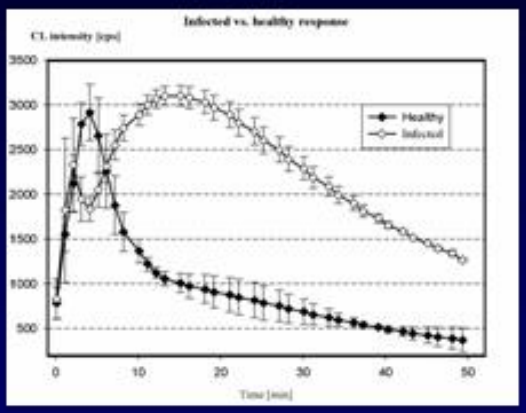
1. Clinical diagnostics are usually incomplete
2. Identification of the etiological agent ordered
3. Therapies could be directed quasi blind
4. The patient's immune reaction to the infection may be crucial to therapy

There is no diagnostic test today
providing physicians with a reflection
of the status of the insulted innate system as well as its potential course

## 'Primed' neutrophils 'mirror' the clinical status of the patient and may provide diagnostics and prognosis

- The respiratory burst is complex due to a variety of mechanisms and localizations
- Neutrophils report on the innate immune status
  - Huge numbers, so cannot miss them
  - Permanently 'broadcast' the status patient
  - First enlisted against infection
  - Neutrophil glow depends on their activation status
- The phenotypic luminescent imprints of neutrophils is modulated by:
  - the insult
  - the genetic make-up of the host
  - the environmental memory

## Fiber-optic biosensor to assess circulating phagocyte activity in whole blood by chemiluminescence




Simultaneously recorded CL responses of healthy and infected patient blood

Phagocytes circulate in pre-tuned state and can serve as a disease marker

Luminescence of phagocytes as a diagnostic tool

Patient blood samples processed

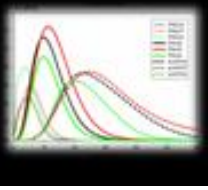
Collection of whole blood in heparinized tubes

Opsonization of zymosan (stimulant)

Preparation of the plate

Stimulation with zymosan and measurement

Analysis



# Data Analysis

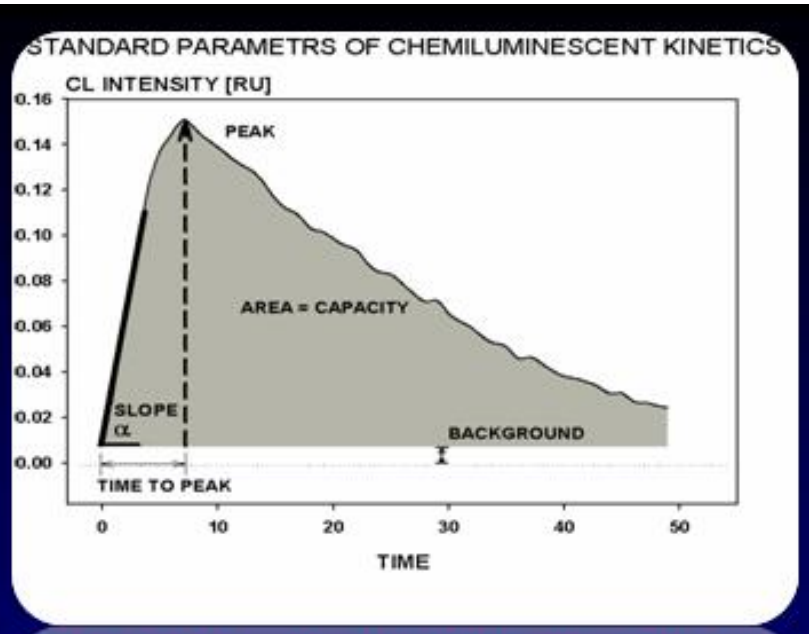
1. Measurement of total CL kinetic of circulating phagocytes

2. Decomposing the experimental curve to three components

3. Creating pool of kinetic parameters

4. Data mining and induction of decision tree classification model

5. Classification of a blind case using the induced classification model



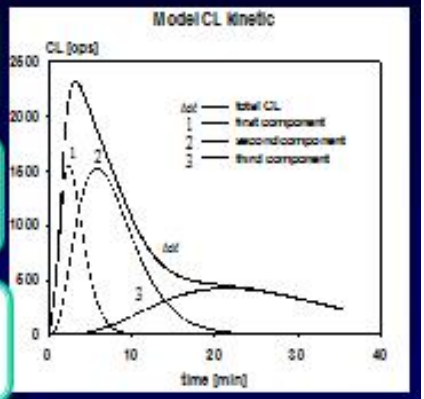

Standard parameters of LCL response used for the quantitative evaluation of PMN oxidative activity: peak intensity, time to peak, slope, area.

## Parameters driven from the CL sub-kinetics: functional states of PMNs

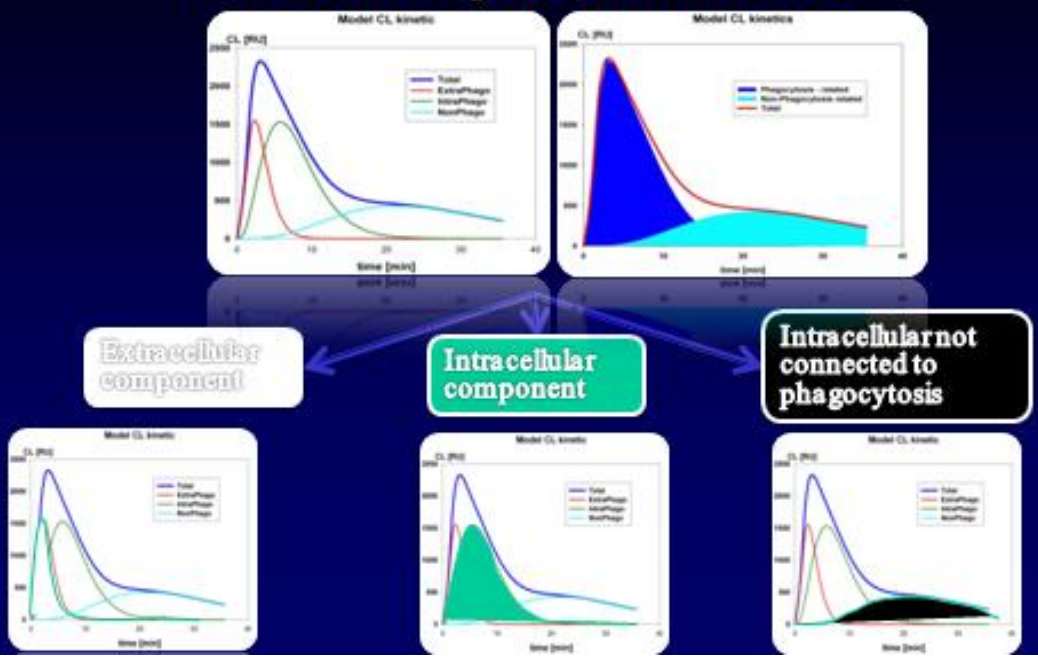
Capacity (C):
the total CL capacity of unit cells, reflecting their capability to generate ROS.

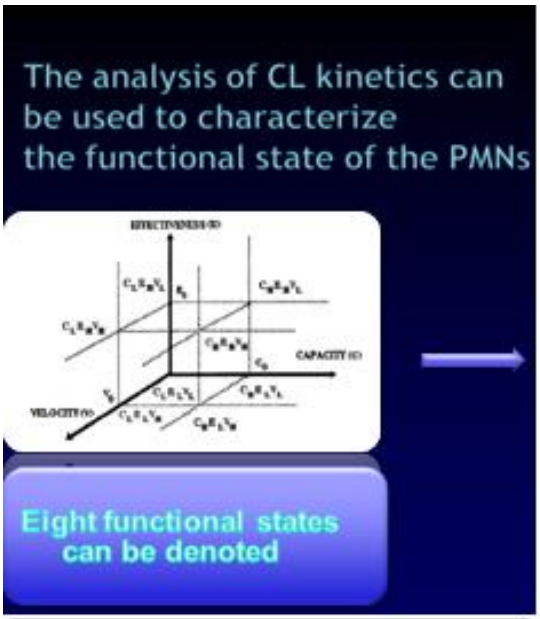
$$Effectivness = \frac{Capacity\ of\ intracellular\ component}{Capacity\ of\ extracellular\ component}$$

$$Velocity = \frac{intra + extra\ components}{third\ component}$$

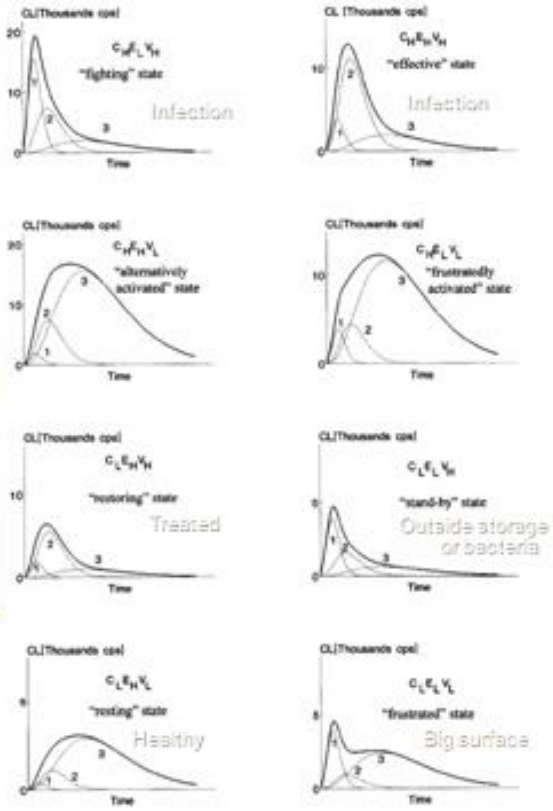



## Parallel CL imprints of intracellular & extracellular production of ROS






| Observed state | C | E | V | CEV state |
|---|---|---|---|---|
| Resting | 8.2 | ∞ | 0.1 | $C_LE_HV_L$ |
| Stand-by | 7.8 | 0.3 | 1.1 | $C_LE_LV_H$ |
| Activating (Fighting) | 67.4 | 1.0 | 1.4 | $C_HE_LV_H$ |
| Effective | 76.3 | 44 | 1.2 | $C_HE_HV_H$ |
| Restoring | 28 | 12.8 | 1.5 | $C_LE_HV_H$ |
| Frustrated | 16.6 | 1.4 | 0.8 | $C_LE_HV_L$ |

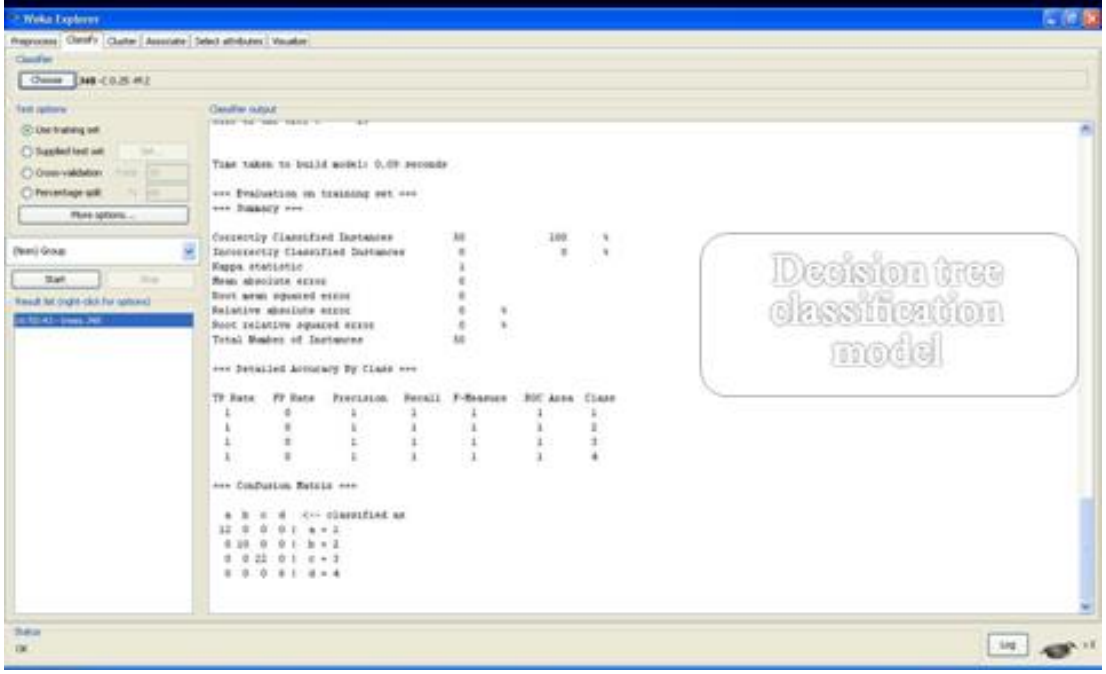



## Phagolum. 60/656,926 (2004); 20092-WO-05; 20346-WO-06

## Each patient has 82 parameters!

| Parameter | Definition |
|---|---|
| PtimeSP | Peak time of primed sample |
| Extra_SP | Extra-cellular phagocytosis-related emission of primed sample |
| CapS | Capacity of standard sample |
| RelTimeInPh_SA | Time of intracellular phagocytosis of aged sample divided by time of intracellular phagocytosis of standard sample |
| S-bkg | Background CL of standard sample |
| nonPhago_SA | Non-phago-related CL of aged sample |
| nonPhago_S | Non-phago-related CL of standard sample |
| **RelCapInPh_SP** | **Intracellular phagocytosis capacity of primed sample divided by intracellular phagocytosis capacity of standard sample** |
| **RelTimeEx_SA** | **Time of extra-cellular phagocytosis-related emission of aged sample divided by time of extra-cellular phagocytosis-related emission of standard sample** |
| RelIntranonPhago_SP | Intracellular non-phagocytosis capacity of primed sample divided by intracellular non-phagocytosis capacity of standard sample |
| RelPhago_SA | Phagocytosis capacity of aged sample divided by total capacity of aged sample |
| RelTimeInPh_SP | Time of intra-cellular phagocytosis-related emission of primed sample divided by time of intra-cellular phagocytosis-related emission of standard sample |
| CapSP | Capacity of primed sample |
| SP_vel | Velocity of primed sample |
| RelPhago_S | Phagocytosis capacity of standard sample divided by total capacity of standard sample |

## Application of J48 (C4.5) data-mining algorithm for induction of the decision tree classification model




## You can discriminate nephrology conditions using neutrophil chemiluminescence

Prilutsky, D., B. Rogachev, M. Vorobiov, M. Zlotnik, M. Last, L. Lobel, and R.S. Marks (2008) Dynamic component chemiluminescent sensor for assessing circulating morphonuclear leukocyte activity of peritoneal dialysis patients. Analytical Chemistry. 80: 5131-5138

Established classification model for diagnostics of intra-abdominal pathological processes afflicting peritoneal dialysis patients

Training set: 100% accuracy
Testing set: 84.6% accuracy

# We can differentiate between acute viral and bacterial infections

Prilutsky, D., Shneider, E., Shefer, A., Rogachev, B., Lobel, L., Last, M., Marks, R.

Algorithms used:
C4.5, Support vector Machines, Naïve Bayes

Accuracy:
Training set: 94.7%
Testing set: 88.9%

Anal. Chem., 2011, 83: 4258-65

# Feature sets and classification models for an identification of several infectious diseases were discovered

Prilutsky, D., Rogachev, B., Lobel, L., Last, M., Marks, R.


Feature selection: wrapper and filter methods

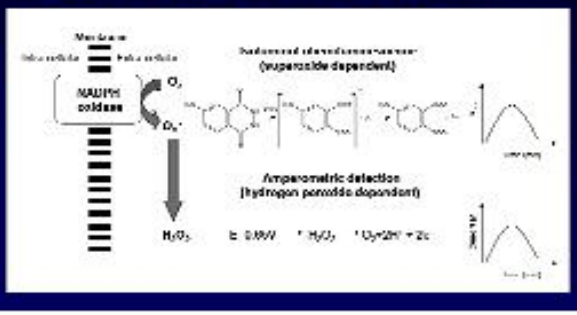
Algorithms used:
C4.5, Support vector Machines, Naïve Bayes

Artificial Intelligence in Medicine, 2011, 52: 153-63

## Reactive Oxygen Species production in neutrophil-like PLB 985 & HL 60 cells detected by electrochemistry and chemiluminescence

Ashkenazi, A. and R.S. **Marks** () Luminol dependent chemiluminescence of human phagocyte cell lines:

comparison between DMSO differentiated PLB 985 and HL60 cells. Luminescence. 24 (3) 171-7

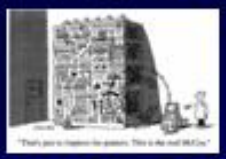



| Electrochemistry | | Isoluminol CL | | |
|---|---|---|---|---|
| DMSO induced PLB 985 | Human neutrophils | DMSO induced PLB 985 | Human neutrophils | |
| 52± 12 | 17± 6 | 32± 3 | 8± 2 | Response time (sec) |
| <500 cells | | < 500 cells | | Limit of detection |

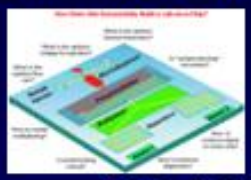
## why biochips? … go sub-micron

Diagnostic tests are mostly done in laboratories

… nanotechnology packs more!



… are usually sophisticated



… can we pack all in a biochip?

… are sometimes too complicated to run

… what we wish to replace

Small provides …

… high density of probes

… rapid reaction kinetics

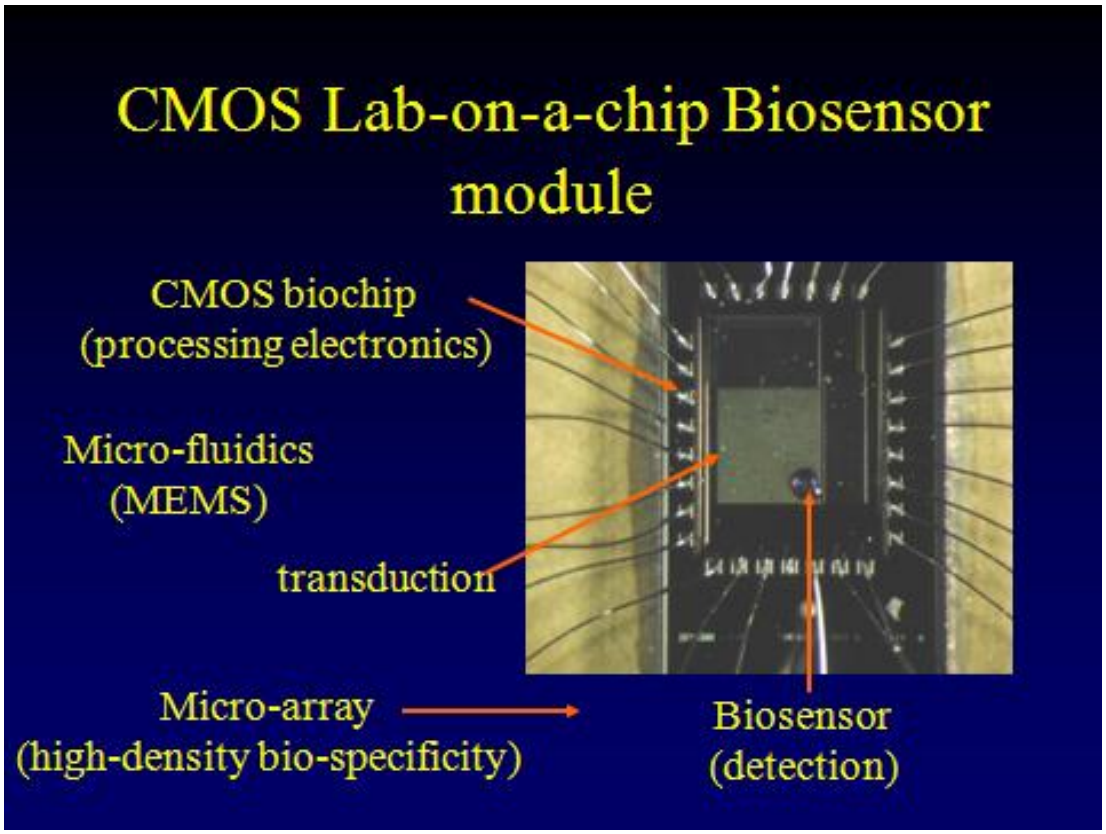
…large number of analyses on a single chip

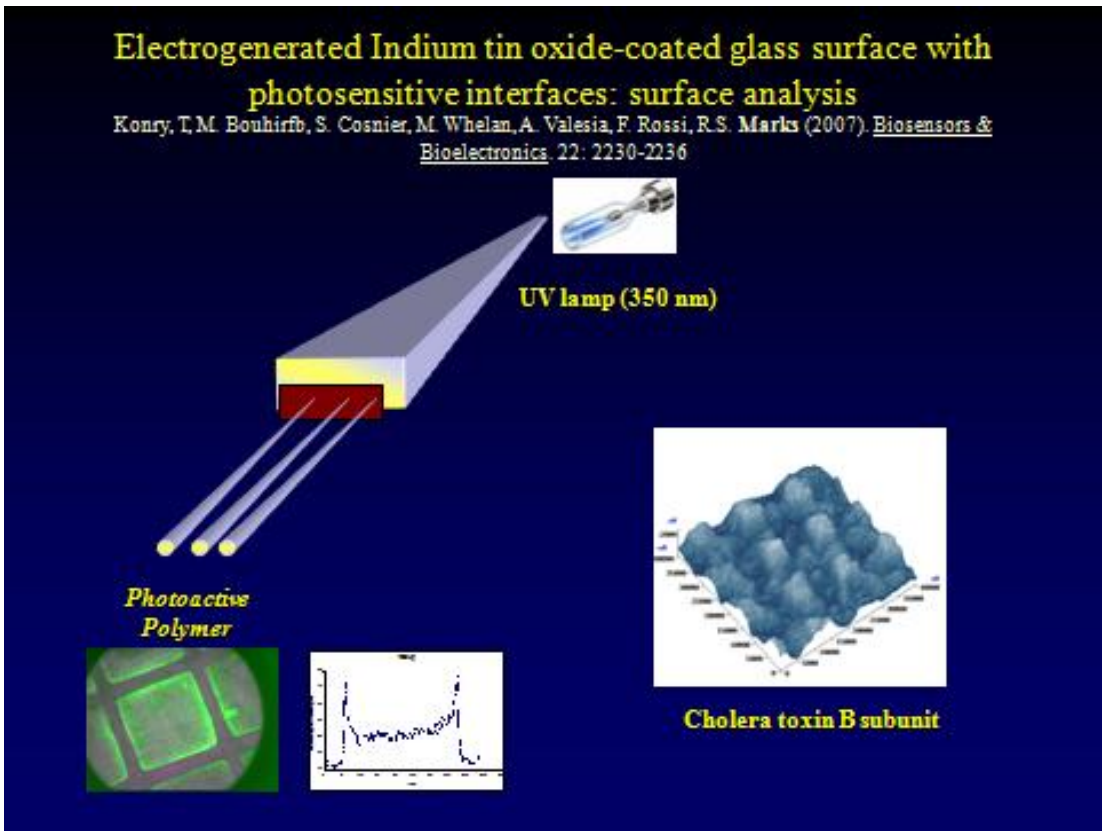
… miniaturization

… we will need sub-micron features

… but is small good?

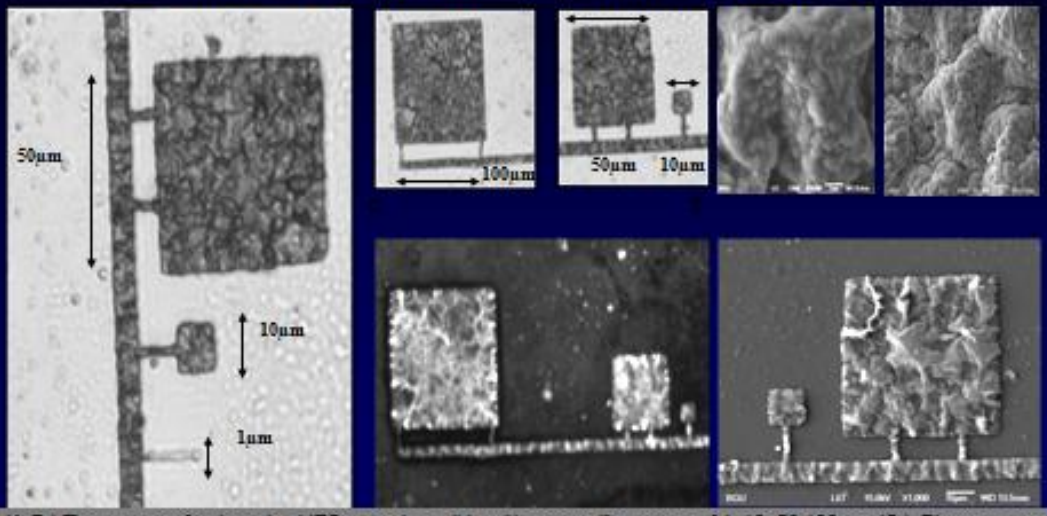
… you may miss your sample!


CMOS Lab-on-a-chip Biosensor module
CMOS biochip (processing electronics)
Micro-fluidics (MEMS)
transduction
Micro-array (high-density bio-specificity)
Biosensor (detection)


Electrogenerated Indium tin oxide-coated glass surface with photosensitive interfaces: surface analysis
Konry, T, M. Bouhirfb, S. Cosnier, M. Whelan, A. Valesia, F. Rossi, R.S. Marks (2007). Biosensors & Bioelectronics. 22: 2230-2236
UV lamp (350 nm)
Photoactive Polymer
Cholera toxin B subunit

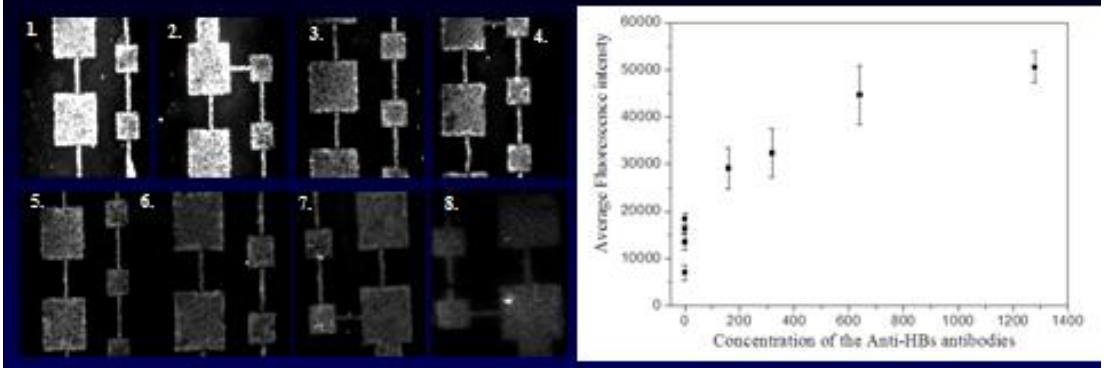
## ITO pattern fabrication of glass platforms for electropolymerization of light sensitive polymer for its conjugation bioreceptors on a micro-array

Konry, T., B. Hadad, Y. Shemer-Avni, S. Cosnier and R.S. Marks (2008) Talanta 75:564-71.




(A,B.) The pattern of polymerized ITO onto glass slide with square-like pattern of 1, 10, 50,100 µm (C.) Fluorescence images of the immuno-array designed for detection of anti-CTB antibodies (320 µg/ml). (F,D) SEM analysis of the array film.

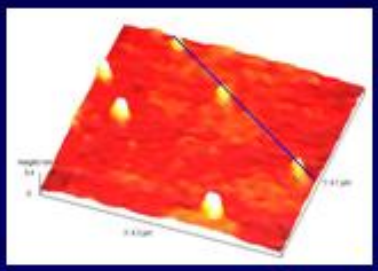
## Immuno-array behavior of the detection of anti-HBs antibodies

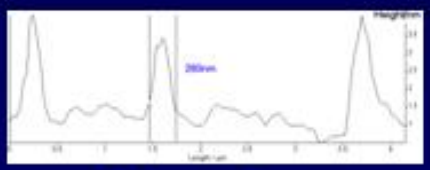



Fluorescent images of the chip surface, each panel representing a different concentration of the analyte ((1) 1,280, (2) 640, (3) 320, (4) 160, (5) 1.6, (6) 0.04, (7) 0.02, (8) 0.01 µg/ml and (9) the fluorescence image of the negative sera sample (anti-HBc -/ anti-HBs -)) on two antigen micro-array previously conjugated with antigens (HBc antigen +/ HBs antigen +).

## Protein printing with an atomic force sensing nanofountainpen

Taha, Hesham, R.S. **Marks**, L.A. Gheber, I. Rousso, J. Newman, C. Sukenik and A. Lewis (2003). Applied Physics Letters. 83 (5) 1041-1043

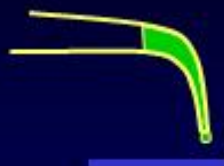


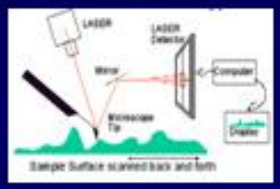


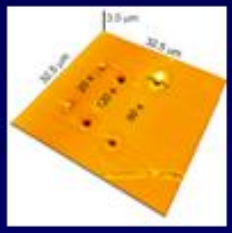
280 nm in diameter

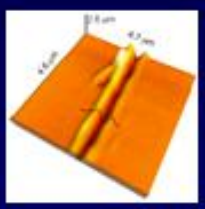
## Nanolithography Using Protease Etching of Protein Surfaces

Ionescu, R., R. S. **Marks** and L. Gheber (2003). Nano Letters. 3 (12) 1639-1642





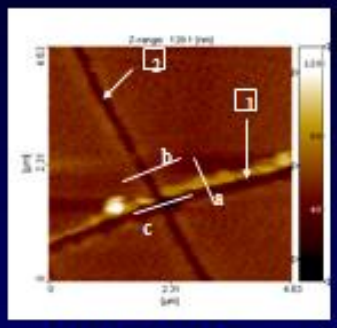





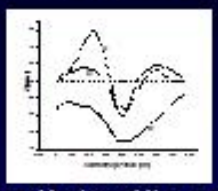
BSA surface;
200 nm pipette (wells)
50 nm pipette (groove)

## Manufacturing of nano-channels with controlled dimensions using protease nanolithography

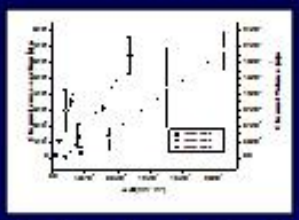
Ionescu, R.E., R.S. **Marks** and L. Gheber (2005). Nanoletters. 5 (5) 821-827




**Etching is additive**:
the depth of the depression at the intersection of 2 perpendicular channels is the sum of their depths
(same pipette; same velocity; '1' made first 60' before '2')

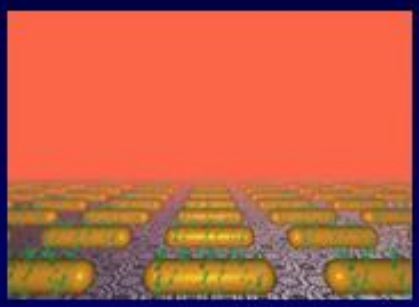


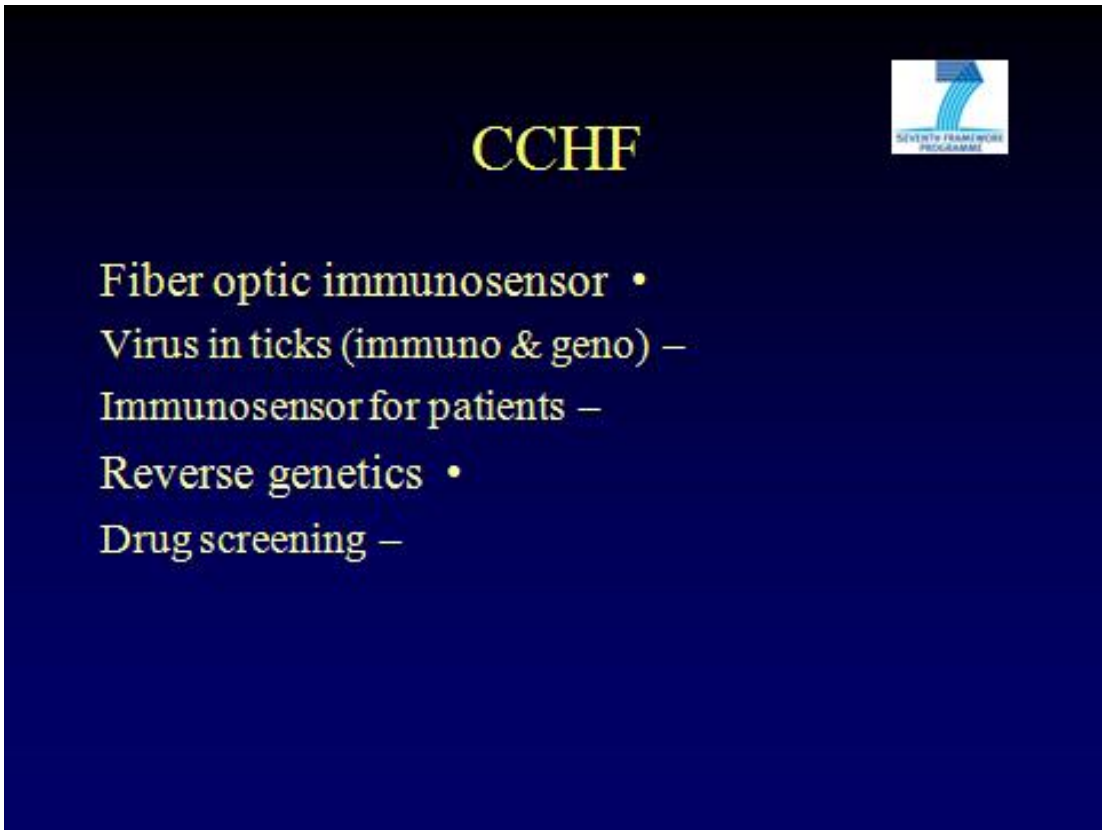
The dotted horizontal line represents the original non-swollen surface.
The channel dimensions are similar (a =17 nm; b =20 nm; c =36 nm).



The data splits into three different linear slopes for the three different pipette apertures, where the steepest one belongs to the smallest aperture (100 nm) and the shallowest one belongs to the largest aperture (300 nm).

## Nanoantenna: SEIRS & SERS




CCHF
Fiber optic immunosensor •
Virus in ticks (immuno & geno) –
Immunosensor for patients –
Reverse genetics •
Drug screening –


NRF CREATE - Singapore
Nanomaterials for Energy & Water Management
Marks, Magdassi & Ma
Ben Gurion University of the Negev
Hebrew University of Jerusalem
Nanyang Technology University

## Solid phase peptide synthesis resin as carrier and adjuvant for oral vaccination

- No cleavage from resin needed
- No purification of peptide needed
- Resin can be given as a bead and/or hydrogel formulation
- Resin belongs to GRAS

## Funding

# Buy my book!

October 2007


http//: www.wiley-vch.de/publish/en/books/specialOffer/0-470-01905-0?sID=

# Pan Stanford 'High Tech of Biotechnology'

Nanoantenna - Lamy de la Chapelle & Pucci

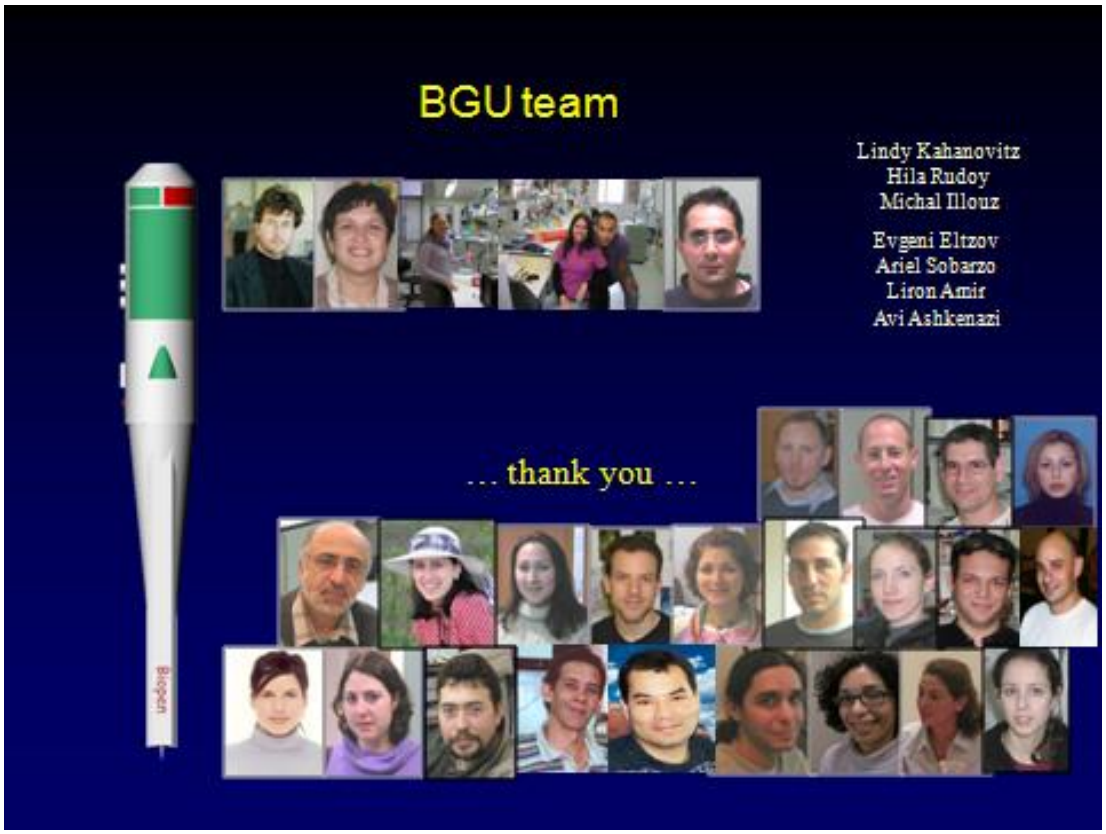
Fiber optic immunosensors - Marks

Amperometric immunosensors - Cosnier

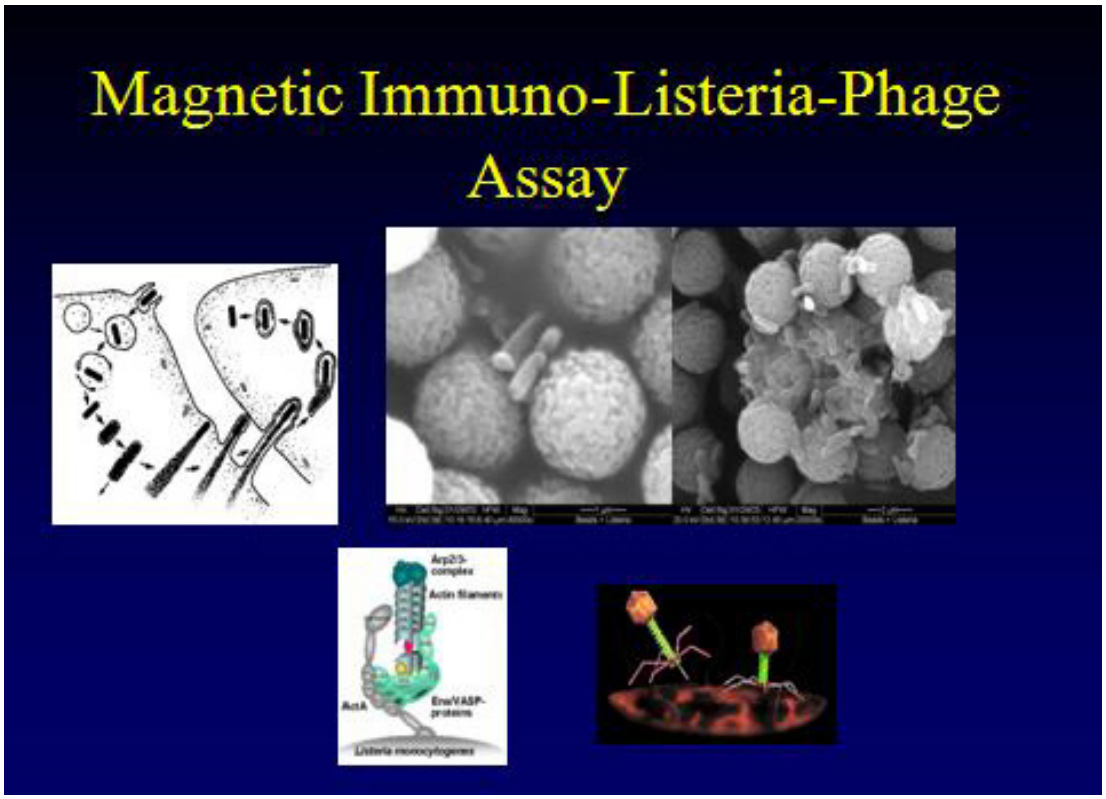
Protocols in Bioassays & Biosensors - Marks

Luminescent microbial biosensors - Thouand

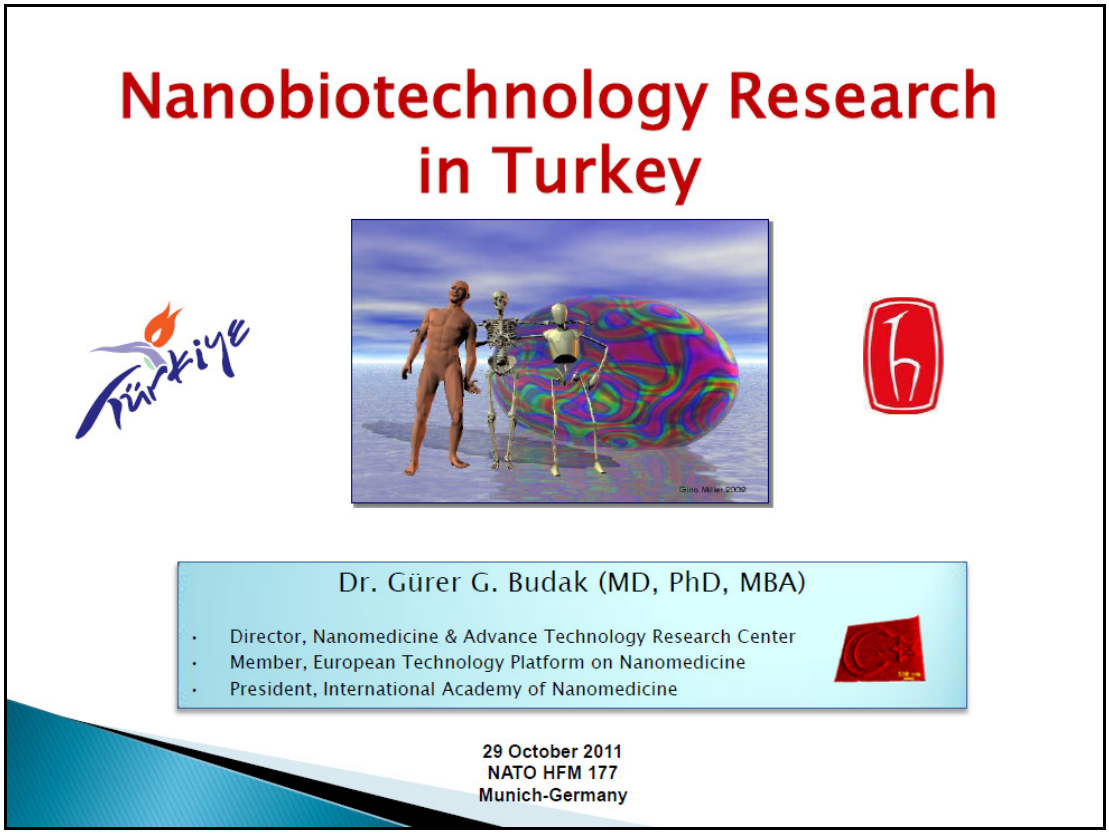
Advanced techniques in viral detection- Marks, Lobel & Sall

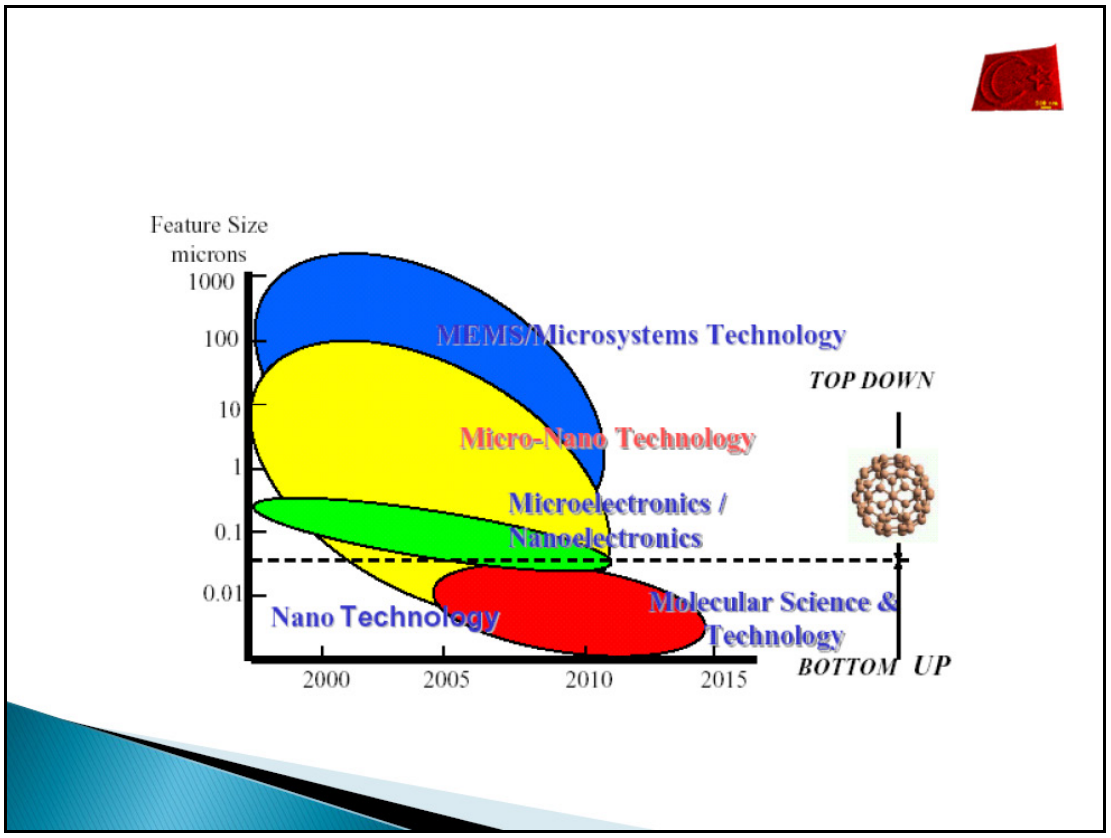

BGU team
Lindy Kahanovitz
Hila Rudoy
Michal Illouz
Evgeni Eltzov
Ariel Sobarzo
Liron Amir
Avi Ashkenazi
… thank you …

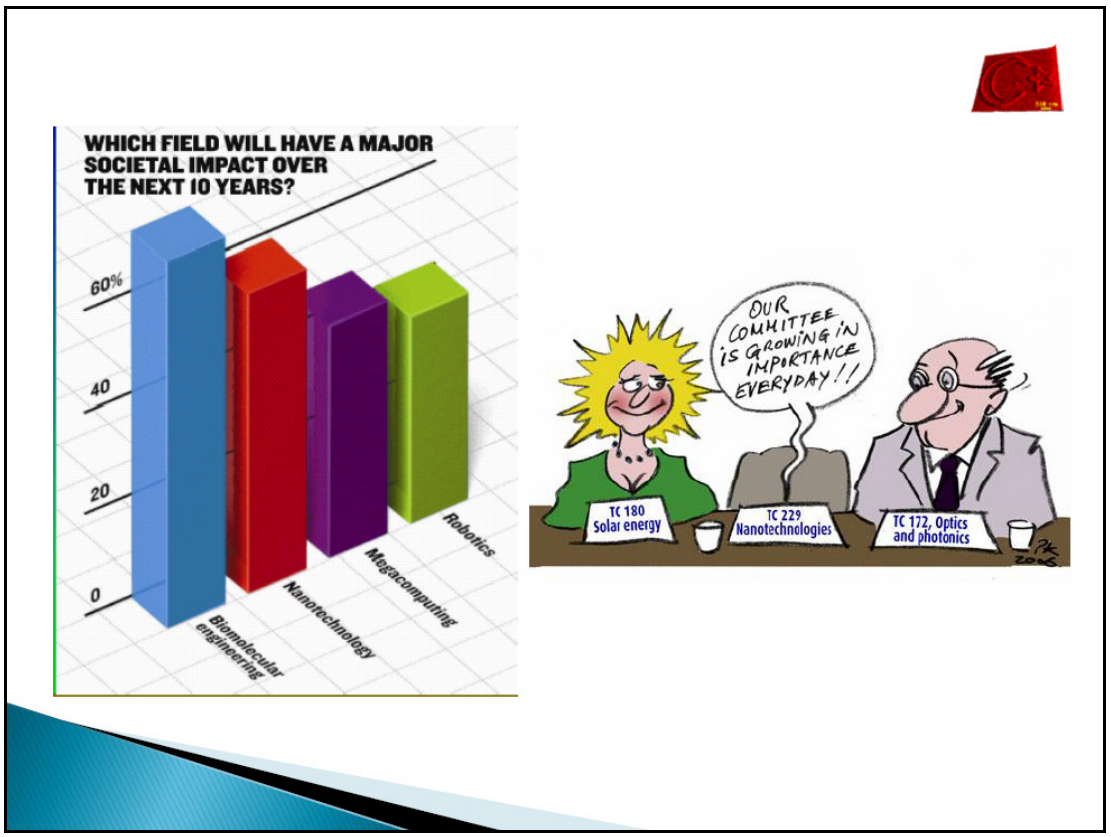

Collaborators




### C.2.6 Turkey Nano-Technology Presentation – by Gürer G. Budak




Feature Size
microns
1000
100
10
1
0.1
0.01
2000
2005
2010
2015
MEMS/Microsystems Technology
Micro-Nano Technology
Microelectronics /
Nanoelectronics
Nano Technology
Molecular Science &
Technology
TOP DOWN
BOTTOM UP


WHICH FIELD WILL HAVE A MAJOR
SOCIETAL IMPACT OVER
THE NEXT 10 YEARS?
60%
40
20
0
Biomolecular engineering
Nanotechnology
Megacomputing
Robotics
OUR COMMITTEE IS GROWING IN IMPORTANCE EVERYDAY!!
TC 180
Solar energy
TC 229
Nanotechnologies
TC 172, Optics
and photonics

-AANM

-IANM

-ETP-NM

-CLINAM

-AJNS

**DECISION OF**
**TURKISH GRAND NATIONAL ASSEMBLY**
**Decision on the approval of**
**Ninth Development Plan (2007-2013)**
**Decision No: 877 Decision Date: 28.06.2006**

16. Developing countries need to base their growth dynamics on productivity increases and on creating new comparative advantages in order to sustain and strengthen their competitiveness in the global arena. To this end, placing an emphasis on innovativeness, increasing scientific and technological capacity, improving human capital, and effective usage of information and communication technologies constitute importance. In the coming period, areas such as biotechnology and nanotechnology will come to the forefront.

482. Towards the future era; nanotechnology, biotechnology, new generation nuclear technologies and hydrogen and fuel battery technologies; research in the sectors to be given priority by the industrial policy; R&D activities that aim to transform local resources into value-added; research in the field of health to increase the quality of life, primarily vaccination and anti-serums; information and communication technologies, and defense and space technologies, will be supported as priority fields.

# Governmental Financial Support for Research

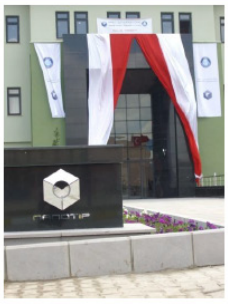
State Planning Organization of T.R.

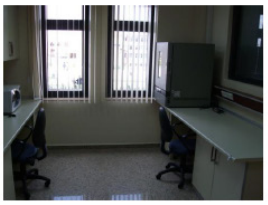
The Scientific and Technological Research Council of Turkey

# Nanobiotechnology Research in Turkey


- Nanoparticle Fabrication and Characterization Laboratory
- Histo-Pathology Laboratory
- Immunobiology Laboratory
- Molecular Biology Analysis Laboratory
- Tissue and Cell Culture Laboratory
- Biochemistry Laboratory
- Microbiology Laboratory
- Physiology Laboratory
- Experimental Animal Research Laboratory
- Computational Engineering Laboratory
- Robotics and Mechatronics Laboratory
- Simulation and Modeling Laboratory

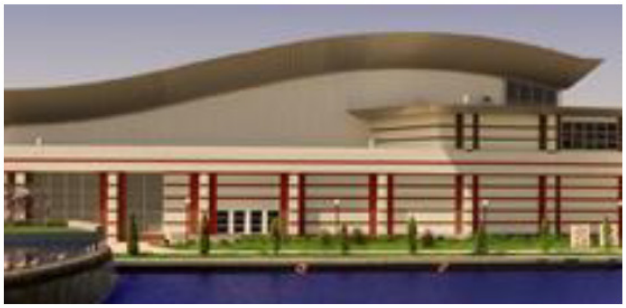
http://www.nanott.hacettepe.edu.tr/nanott.html




- Early Diagnosis and Imaging Systems
- Targeted Drug Delivery Systems
- Tissue–Material Science Engineering and Regenerative Medicine

Sabancı Üniversitesi



http//:sabanciuniv.edu.tr

BILKENT UNIVERSITY
NANOTECHNOLOGY RESEARCH CENTER

http://www.nanotr.bilkent.edu.tr/

unam

**Middle East Technical University**
**Central Laboratory**
**Molecular Biology and Biotechnology Research Center**

http://www.merkezilab.odtu.edu.tr/bio/

**ISTANBUL UNIVERSITY**
**MOLECULAR BIOLOGY-BIOTECHNOLOGY &**
**GENETICS RESEARCH CENTER (MOBGAM)**

http://www.mobgam.itu.edu.tr/

The Scientific and Technological Research Council of Turkey

TÜBİTAK MAM TÜBİTAK Marmara Research Center

INSTITUTE FOR GENETIC ENGINEERING & BIOTECHNOLOGY

TURKISH

http://www.mam.gov.tr/eng/institutes/gmbe/

KOÇ UNIVERSITY

Department of Chemical & Biological Engineering

http://www.ku.edu.tr/

Undersecretariat for Defence Industries Export Portal
International Cooperation Department

**MISSION IS:**

To meet the system requirements of Turkish Armed Forces and the government organizations those promote the national defense and security; to establish and implement the strategy and procedures for the development of defense industry

Undersecretariat for Defence Industries Export Portal
International Cooperation Department

**STRATEGIC GOAL 1: TO IMPROVE THE PROCUREMENT ACTIVITIES IN ACCORDANCE WITH THE USER REQUIREMENTS AND INDUSTRIAL GOALS**

1.1 In order to enhance procurement management capability, project management processes will be improved .
1.2 In accordance with achieving the user satisfaction, project cycle times (duration between kick-off and contract awarding) will be shortened.
1.3 Quality, test and certification activities will be improved timely and costly.

1.4 Decisions given in project management will be consistent with institutional strategies.

**STRATEGIC GOAL 2: TO RESTRUCTURE THE DEFENCE INDUSTRY TO BE ABLE TO PROVIDE UNIQUE LOCAL SOLUTIONS AND COMPETE IN THE INTERNATIONAL ARENA**

2.1 Indigenous share in expenditures for Turkish Armed Forces' defense equipment expenditures shall be enhanced.
2.2 Activities that ensure sustainability and improve efficiency in the local defense industry will be actualized.
2.3 Integration of SME's and supplier companies to defense industry shall be enhanced.
2.4 It shall be ensured that R&D Roadmap and Network of Excellences' operate effectively.

**STRATEGIC GOAL 3: TO PARTICIPATE ACTIVELY IN THE MULTINATIONAL DEFENCE AND SECURITY PROJECTS THOSE PROMOTE THE INTERNATIONAL COOPERATION**

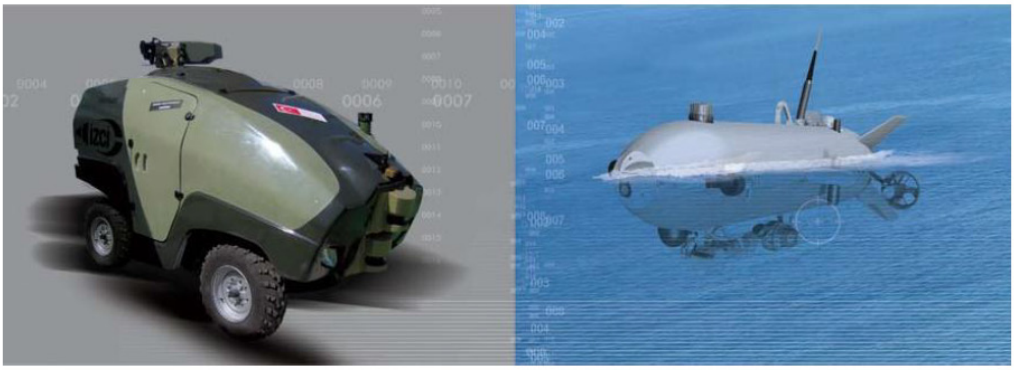
3.1 By fostering the specialization and encouraging the local industry to take place in international supply chain, strategic cooperation efforts will be promoted.
3.2 Turkish industry share in NATO defense projects shall be increased.
3.3 Export of defense and aeronautics products will be promoted and supported.

**STRATEGIC GOAL 4: TO IMPROVE THE ORGANIZATIONAL STRUCTURE**

4.1 Strategic Human Resources Program will be executed to employ highly qualified staff, to provide necessary training and basis for productive environment and to maintain organizational loyalty.
4.2 Knowledge and performance based management approach and strategic management systematic shall be institutionalized.
4.3 Governance and security of produced information and sharing of knowledge will be improved in the organization.

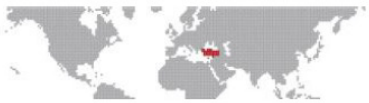
Undersecretariat for Defence Industries Export Portal
International Cooperation Department

## Center of Excellence

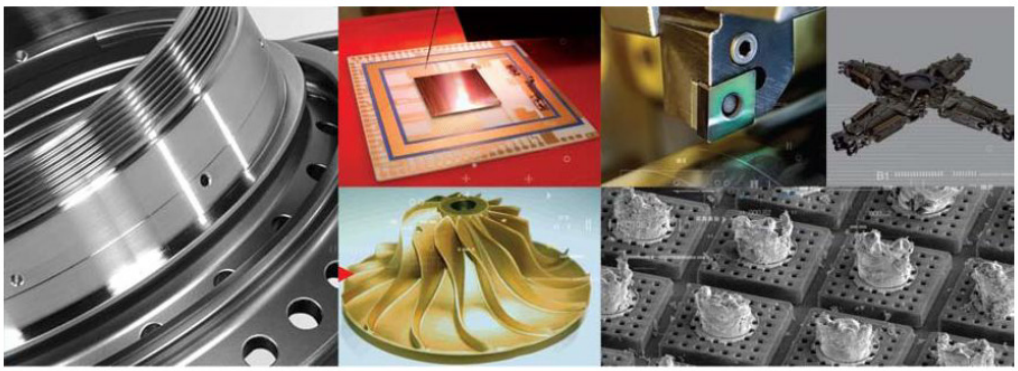


Autonomous Management

Undersecretariat for Defence Industries Export Portal
International Cooperation Department



## Center of Excellence



Advanced Materials

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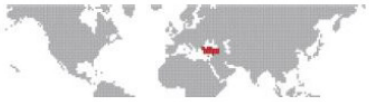

## Center of Excellence

Aerospace

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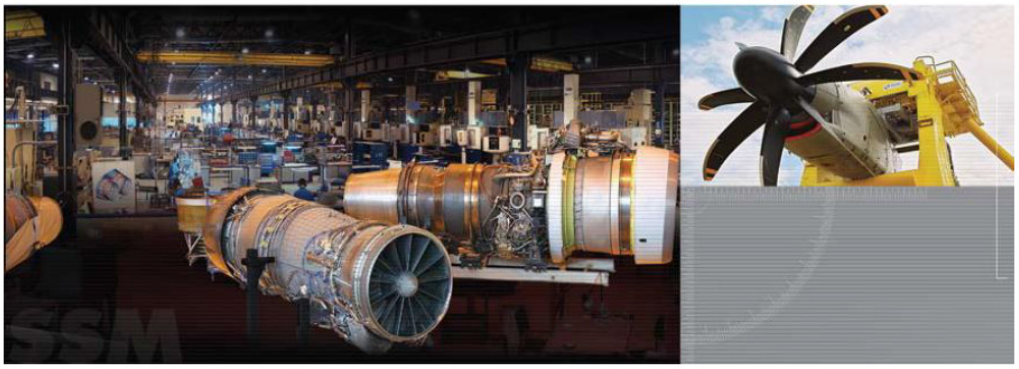
## Center of Excellence



Energy and Power Plant

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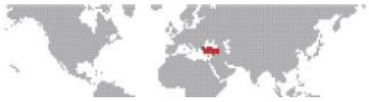

## Center of Excellence

Sensors

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## Center of Excellence

Modeling & Simulation

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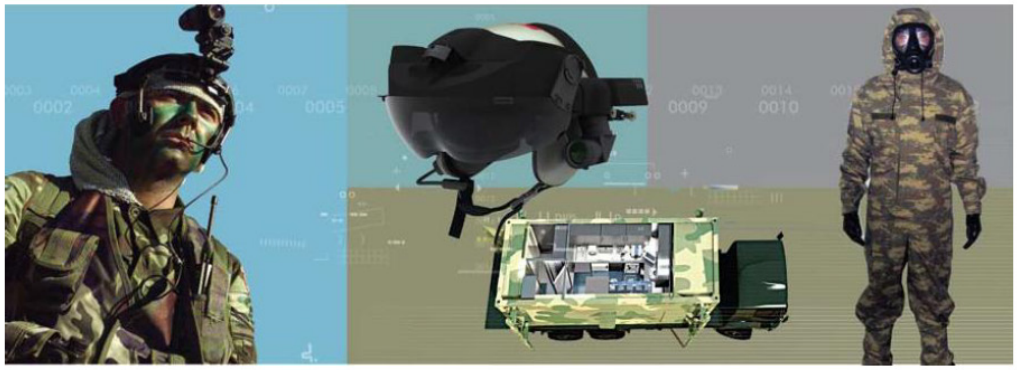
## Center of Excellence



Chemical Biological Radiological & Nuclear

Undersecretariat for Defence Industries Export Portal
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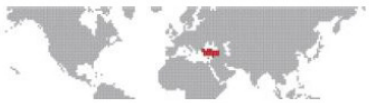
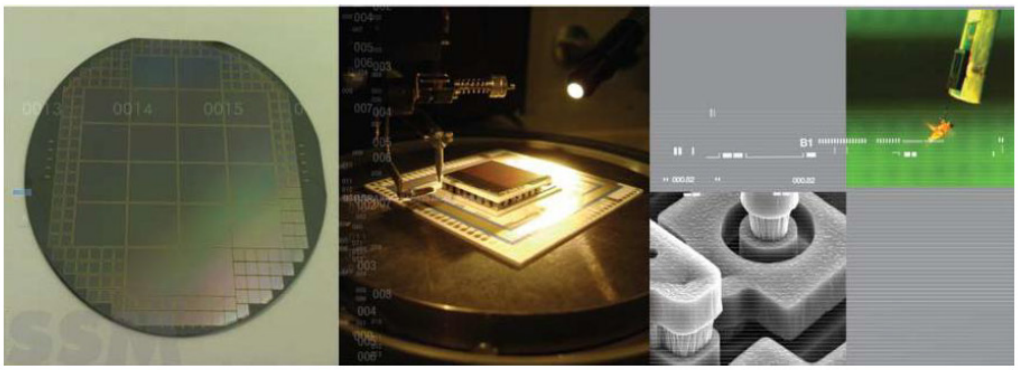
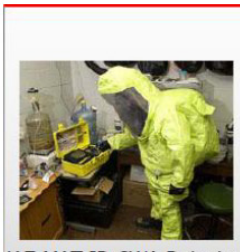


## Center of Excellence



Micro & Nanotechnologies



HAZ-MAT ID CWA Detector SensIR Technologies

Joint Biological Point Detection System (JBPDS) General Dynamic

Joint Service Lightweight Standoff Chemical Agent Detector (JSLSCAD) General Dynamic

M21 Remote Sensing CWA Detector / Alarm (RSCAAL)

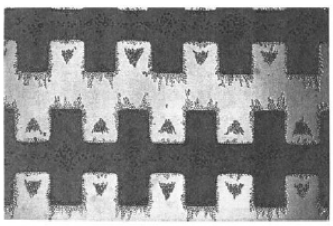
AP2C Remote Sensing CWA Detector / Alarm Proengin Inc.

UV Sentry Toxic Chemical Perimeter Monitor Ocean Optics, Inc.

# Nanobiotechnology Research in Turkey

## Applications of Biosensors

- Biosensors harness the immensely powerful molecular recognition properties of living systems and engineer these into electronic devices to provide easy-to-use sensing devices.
- Biosensors can be used more generally to measure
- Disease markers
- Food safety
- Environmental quality
- To ensure safety and security



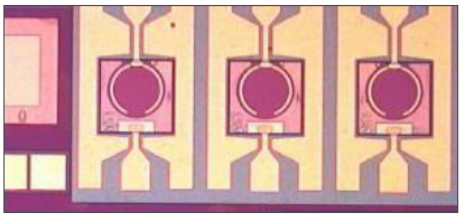
# Nanobiotechnology Research in Turkey

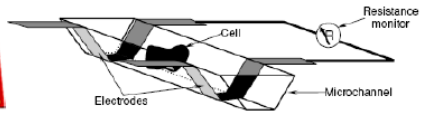
## Diagnosis and Imaging

❑ In Vitro Applications

- chemo-bio nanosensors

- ultra-sensitive biochips
  - "lab-on-a-chip" devices
  - "cells-on-chips" devices






Biomed Microdevices (2008) 10:321–328
DOI 10.1007/s10544-007-9139-2

**Zeta potential: a surface electrical characteristic to probe the interaction of nanoparticles with normal and cancer human breast epithelial cells**

Yu Zhang · Mo Yang · Nathaniel G. Portney · Daxiang Cui · Gurer Budak · Ekmel Ozbay · Mihrimah Ozkan · Cengiz S. Ozkan

Published online: 29 December 2007

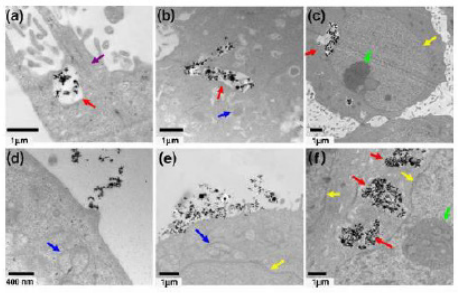




Fig. 4 Transmission Electron Micrographs of MCF10A cells that were incubated with iron oxide nanoparticles for 30 min (a), 4 h (b) and 24 h (c) at 37°C. Transmission Electron Micrographs of MCF7 cells that were incubated with iron oxide nanoparticles for 30 min (d), 4 h (e) and 24 h (f) at 37°C. All slices were treated with uranyl acetate to stain membranes and lead citrate to stain the nuclear body. *Colored arrows* represent selected cell organelles: nuclei (*yellow*), nucleolus (*green*), mitochondria (*blue*), and vesicles with iron oxide nanoparticles inside (*red*), filaments (*purple*)

ADVANCED MATERIALS

DOI: 10.1002/adma.200702863

# Synthesis and Characterization of Iron Oxide Derivatized Mutant Cowpea Mosaic Virus Hybrid Nanoparticles**

By *Alfredo A. Martinez-Morales, Nathaniel G. Portney, Yu Zhang, Giuseppe Destito, Gurer Budak, Ekmel Ozbay, Marianne Manchester, Cengiz S. Ozkan,* and Mihrimah Ozkan**

Extensively investigated and mutagenized Cow Pea Mosaic Virus (CPMV) has been demonstrated in a variety of nanoassemblies.[1–3] Iron Oxide (IO) has the potential to surpass limits of detection in bioimaging applications. Particularly $\gamma$-$Fe_2O_3$ (maghemite) is considered as one of the most desirable materials for technological and biomedical applications due to its inherent biocompatible nature.[4,5] Additionally, maghemite nanoparticles could be directed to an organ, tissue, or tumor using an external magnetic field or heated under an alternating magnetic field.[6,7] Based on the unique magnetic properties of IO nanoparticles they have been extensively used in biomedical applications, such as magnetic resonance imaging, targeting drug delivery and hyperthermia therapy detoxification and cell separation.[8–12]

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and Department of Physics
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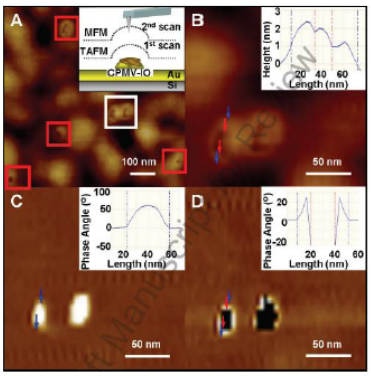
06800 Ankara (Turkey)

Combining the two systems can be devised to enhance the local magnetic field strength, by organizing monodisperse IO clusters on a CPMV-T184C mutant viral template. It is known that contrast enhancement is observed by use of superparamagnetic iron oxide nanoparticles (SPIONs) based MRI, by creating large dipolar magnetic field gradients due to their local field inhomogeneity. However, clustering a greater number of IO nanoparticles can further improve contrast beyond free particle SPIONs enhanced MRI, by creating a cumulative dipole effect.[13]

CPMV-T184C is a useful model that has a well characterized structure amenable to surface functionalization.[14] The smallest repeating structure (asymmetric unit, composed of a "small" (24kD) and "large" (42kD) subunit) displays 5 solvent exposed lysines used for IO linkage.[15] By insertion of a cysteine, at residue 184 of the small subunit, anchorage of CPMV to a self assembled monolayer (SAM) on gold substrate pathway can be employed. A previously reported SAM on Au stepwise assembly was used to integrate monodisperse CPMV-IO hybrids for characterization.[16]

It is also been shown that aggregation of Iron oxide particles can exhibit a greater magnetic dipole, and can be suited for in bio-imaging, provided certain properties are met. Harris et al.[17] demonstrated protease activated aggregation of pegylated iron oxide nanoparticles with enhanced MRI contrast to be most beneficial in improving detection limits of small tumors. Pegylation of CPMV was previously demonstrated to improve circulation times and reduce immunogenicity.[18] Also, based on enhanced permeability and retention effects (EPR),[17] the longest retention times at tumor sites for nanoparticles occurred for 60–400 nm.[19] Above 300 nm, there is vulnerability to macrophage phagocytosis,[19] and below 10 nm, nanoparticles can leave the systemic circulation via the lymph nodes.[20] Therefore, the IONs-CPMV nanoparticle hybrid system synthesized and MFM characterized in this report could be used for contrast enhanced MRI applications.

In this work the local enhancement of field strength is studied and demonstrated by magnetic force microscopy




Journal of Colloid and Interface Science 344 (2010) 528–532

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

www.elsevier.com/locate/jcis

# Size controlled synthesis of sub-100 nm monodisperse poly(methylmethacrylate) nanoparticles using surfactant-free emulsion polymerization

Sevket Tolga Camli *, Fatih Buyukserin, Oguz Balci, Gurer Guven Budak

*Nanomedicine and Advanced Technologies Research Center, Gazi University, 06830 Ankara, Turkey*

ARTICLE INFO

*Article history:*
Received 10 December 2009
Accepted 13 January 2010
Available online 18 January 2010

*Keywords:*
Poly(methylmethacrylate)
Nanoparticles
Surfactant-free emulsion polymerization
Monodisperse
Dynamic light scattering

ABSTRACT

Surfactant-free emulsion polymerization (SFEP) is a well-known technique for the production of polymeric nanoparticles that does not require post-synthetic cleaning steps. Obtaining hydrophobic particles at sub-100 nm scale, however, is quite challenging with this polymerization method. Here, we demonstrate a single step synthetic approach that yields poly(methylmethacrylate) (PMMA) nanoparticles with controlled sub-100 nm size and relatively high resultant solid content. Dynamic light scattering (DLS) was used for the particle characterization. Spherical and uniformly sized nanoparticles were confirmed by atomic force microscopy (AFM) and scanning electron microscopy (SEM). Acetone was used as a cosolvent in order to obtain monodisperse sub-100 nm diameter particles. Stable PMMA nanoparticle dispersions were obtained for all formulations where the persulfate initiator causes the negative charges on the particle surface. The effects of acetone, monomer and initiator concentration were studied to optimize average particle hydrodynamic diameter and polydispersity index of the final particles. Non-crosslinked monodisperse PMMA nanoparticles (polydispersity index less than 0.05) with diameters from 32 nm to 72 nm were synthesized by using this method.

Colloids and Surfaces A: Physicochem. Eng. Aspects 366 (2010) 141–146

Contents lists available at ScienceDirect

Colloids and Surfaces A: Physicochemical and Engineering Aspects

journal homepage: www.elsevier.com/locate/colsurfa

# Fine-tuning of functional poly(methylmethacrylate) nanoparticle size at the sub-100 nm scale using surfactant-free emulsion polymerization

Sevket Tolga Camli*, Fatih Buyukserin, Mustafa Selman Yavuz, Gürer Güven Budak

*Nanomedicine and Advanced Technologies Research Center, Gazi University, 06830 Ankara, Turkey*

ARTICLE INFO

*Article history:*
Received 1 March 2010
Received in revised form 20 May 2010
Accepted 28 May 2010
Available online 8 June 2010

*Keywords:*
Poly(methylmethacrylate)
Functional nanoparticles
Cross-linked
Surfactant-free emulsion polymerization
Monodisperse
Dynamic light scattering

ABSTRACT

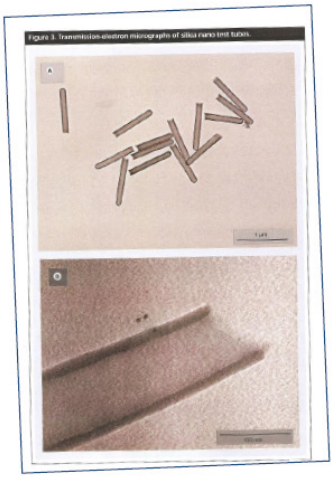
Functional poly(methylmethacrylate) (PMMA) nanoparticles are of great use in various research areas from photonic band gap materials to biomolecule delivery vehicles. Herein, we introduce a conventional surfactant-free emulsion polymerization (SFEP) method that enables the production of functional sub-100 nm PMMA nanoparticles without the need of microwave irradiation. Cross-linked PMMA latex having monodisperse size distribution can be prepared. Particle characterization studies were carried out using dynamic light scattering (DLS). Spherical and uniformly sized nanoparticles were observed by scanning electron microscopy (SEM). Stable cationic PMMA nanoparticle dispersions were obtained for all formulations where the particle charge stems from the amidine initiator. The presence of the amidine moieties was confirmed by using an isothiocyanate containing fluorophore. An important appealing feature of this method is the ability to fine-tune the resultant particle size at the sub-100 nm scale by simply varying the monomer concentration.





RESEARCH ARTICLE

## Antibody-functionalized nano test tubes target breast cancer cells



nature materials

LETTERS

PUBLISHED ONLINE: 1 NOVEMBER 2009 | DOI: 10.1038/NMAT2564

## Gold nanocages covered by smart polymers for controlled release with near-infrared light

Mustafa S. Yavuz*, Yiyun Cheng*, Jingyi Chen*, Claire M. Cobley, Qiang Zhang, Matthew Rycenga, Jingwei Xie, Chulhong Kim, Kwang H. Song, Andrea G. Schwartz, Lihong V. Wang and Younan Xia*

Photosensitive caged compounds have enhanced our ability to address the complexity of biological systems by generating effectors with remarkable spatial/temporal resolutions[1–3]. The caging effect is typically removed by photolysis with ultraviolet light to liberate the bioactive species. Although this technique has been successfully applied to many biological problems, it suffers from a number of intrinsic drawbacks. For example, it requires dedicated efforts to design and synthesize a precursor compound for each effector. The ultraviolet light may cause damage to biological samples and is suitable only for *in vitro* studies because of its quick attenuation in tissue[4]. Here we address these issues by developing a platform based on the photothermal effect of gold nanocages. Gold nanocages represent a class of nanostructures with hollow interiors and porous walls[5]. They can have strong absorption (for the photothermal effect) in the near-infrared while maintaining a compact size. When the surface of a gold nanocage is covered with a smart polymer, the pre-loaded effector can be released in a controllable fashion using a near-infrared laser. This system works well with various effectors without involving sophisticated syntheses, and is well suited for *in vivo* studies owing to the high transparency of soft tissue in the near-infrared region[6].

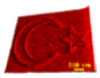
Figure 1a shows a schematic of the controlled-release system.

solution drops to 90% of the original value[8]. By incorporating acrylamide (AAm) into the polymer chain, we obtained pNIPAAm-*co*-pAAm copolymers with LCSTs being tuned to anywhere in the range of 32–50 °C (Supplementary Fig. S1 and Table S1; ref. 8). For *in vivo* applications, the LCST should be tuned to a value above the body temperature (37 °C) but below the hyperthermia temperature (42 °C). Here, we have focused on two types of polymer: pNIPAAm and a pNIPAAm-*co*-pAAm copolymer with an LCST at 32 and 39 °C, respectively. Both were prepared using atom-transfer radical polymerization[10,11].

We covalently anchored the smart polymer to the surface of Au nanocages by means of gold-thiolate linkage. One way to achieve this is to include a disulphide bond in the middle of the polymer chain by using a disulphide initiator[10] (Fig. 1b). As thiolate has a stronger binding towards the Au surface than the C=O group of poly(vinyl pyrrolidone) (PVP), we could replace the PVP on nanocages with the smart polymer. After the displacement, the absorption peak of the nanocages redshifted by ~13 nm, which could be offset during the nanocage synthesis. As shown in Fig. 1c by transmission electron microscope (TEM) imaging, the pNIPAAm-*co*-pAAm coating had a relatively uniform thickness of ~9 nm in the dry state. This result is in reasonable agreement with the value (~5 nm) estimated from the thermogravimetric analysis and gel permeation chromatography data shown in Supplementary


**EU FRAMEWORK PROGRAMME 7**
**PEOPLE SPECIFIC PROGRAMME**
**MARIE CURIE ACTIONS**
**Marie Curie International Reintegration Grants**
**FP7-PEOPLE-IRG-2009**

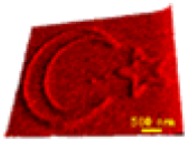
**Project Title :** MULTIFUNCTIONAL COMPOSITE SILICA NANOTUBES FOR TARGETED DELIVERY, "**MuCoSiNT**"
**Primary Investigator :** Dr. Fatih Büyükserin (PhD)
**Project Coordinator :** Prof. Dr. Gürer G. Budak (MD, PhD, MBA)
**Institution :** Nanomedicine & Advanced Technologies Research Center, Gazi University, Ankara, Turkey
**Budget :** 75.000 Euro
**Period :** 36 months (2009-2012)
**Source :** European Union

**EU FRAMEWORK PROGRAMME 7**
**PEOPLE SPECIFIC PROGRAMME**
**MARIE CURIE ACTIONS**
**Marie Curie International Reintegration Grants**
**FP7-PEOPLE-IRG-2009**

**Project Title :** SELF-ASSEMBLED THERMO-NANOPROBES ON HOLLOW GOLD NANOPARTICLES FOR THERAGNOSTIC APPLICATIONS, "**TNP-HGNs**"
**Primary Investigator :** Dr. Mustafa Selman Yavuz (PhD)
**Project Coordinator :** Prof. Dr. Gürer G. Budak (MD, PhD, MBA)
**Institution :** Nanomedicine & Advanced Technologies Research Center, Gazi University, Ankara, Turkey
**Budget :** 75.000 Euro
**Period :** 36 months (2010-2013)
**Source :** European Union

Türkiye

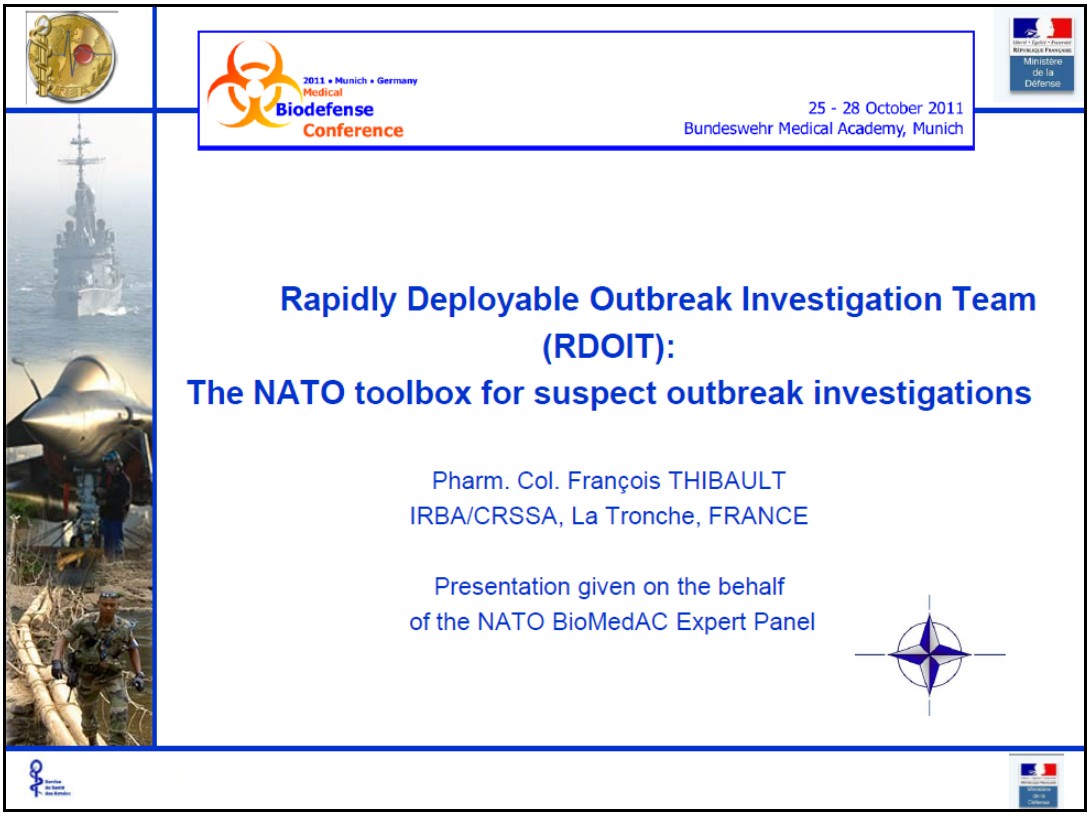


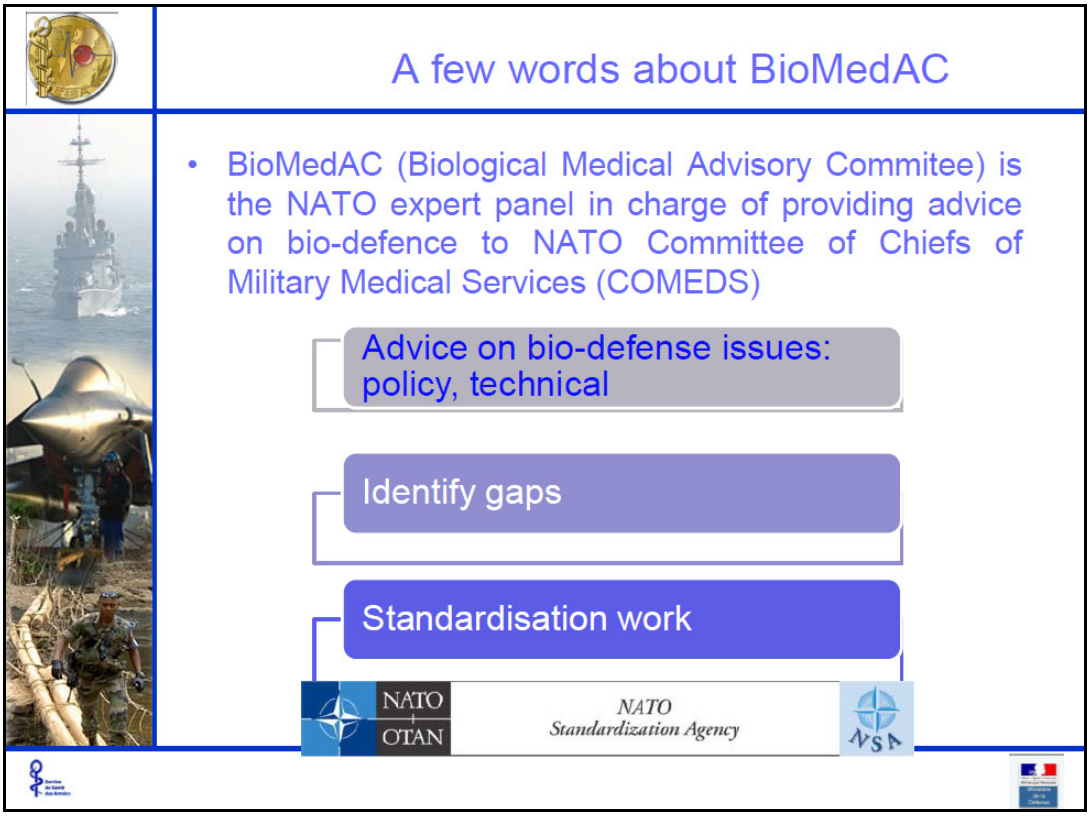
# Thank You !

Dr. Gürer G. Budak (MD, PhD, MBA)

- Director, NanoMedicine & Advance Technology Research Center
- Member, European Technology Platform on Nanomedicine
- President, International Academy of Nanomedicine

## C.2.7 BioMedAC Presentation – by François Thibault

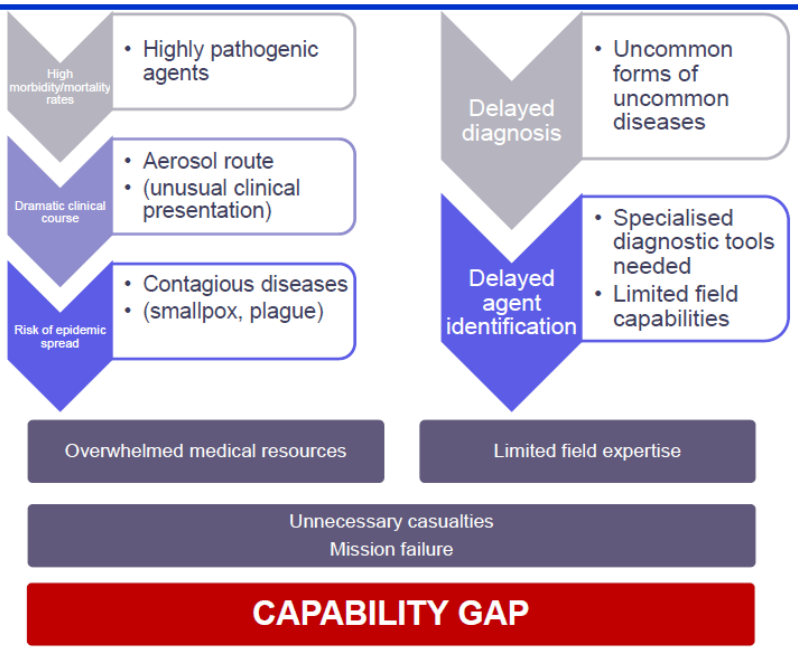


## Some BioMedAC EP deliverables

- Expert advice
  - biological threat “dirty dozen”
  - decontamination “how clean is clean”
  - smallpox vaccine, deployable laboratory,…
- Standardisation documents
  - NATO handbook on the medical aspects of NBC defensive operations (AMedP-6)
  - Concept of operations of medical support in CBRN environments (AMedP-7)
  - Planning guide for the estimation of CBRN casualties (AMedP-8)
  - Rapidly deployable outbreak investigation team (RDOIT) for suspected use of biological warfare agents (STANAG 2529)
  - …

## Impact of biological threat on NATO operations



## Rapidly deployable outbreak investigation team (RDOIT)

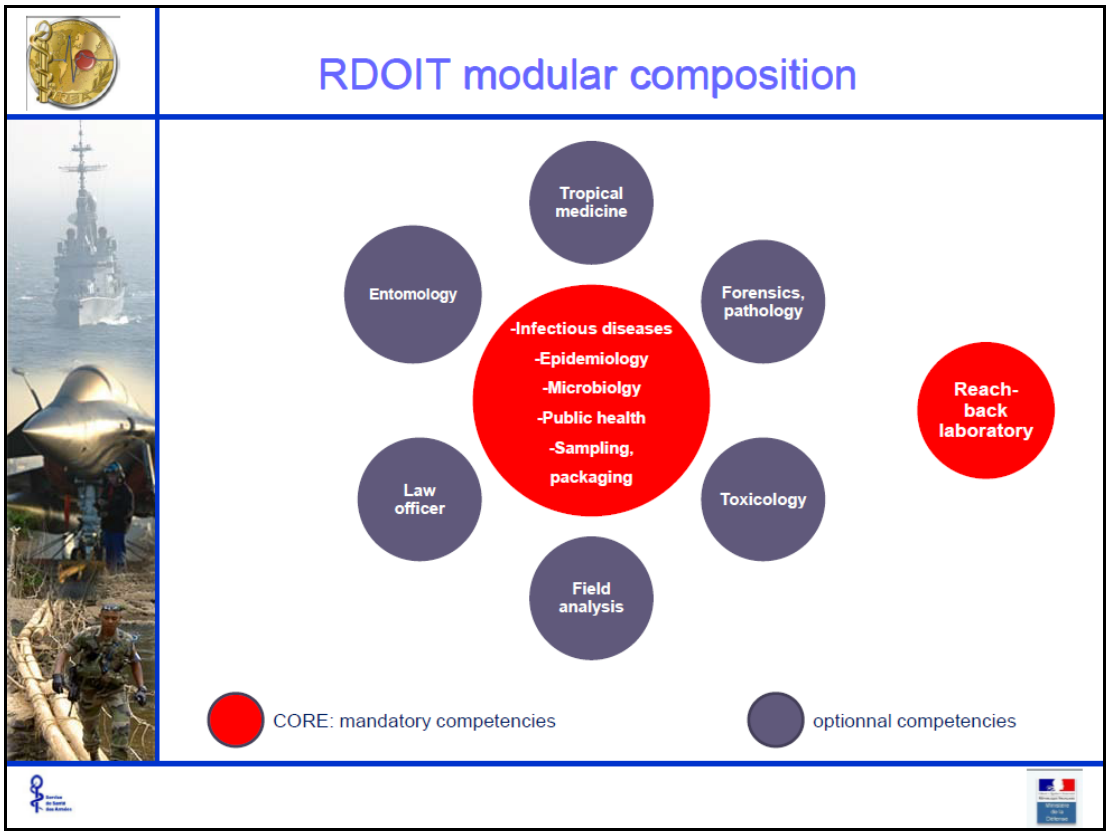
- To send highly (and adequately) specialised staff to the theater…
- …to compensate lack of expertise on the field
- …to investigate outbreak(s) or incident(s) where intentional use of biological agents (biowarfare, bioterrorism or biocrime) cannot be excluded
- …to support operational decision-making
  - advise on prevention and control measures
  - assist commander and medical authorities

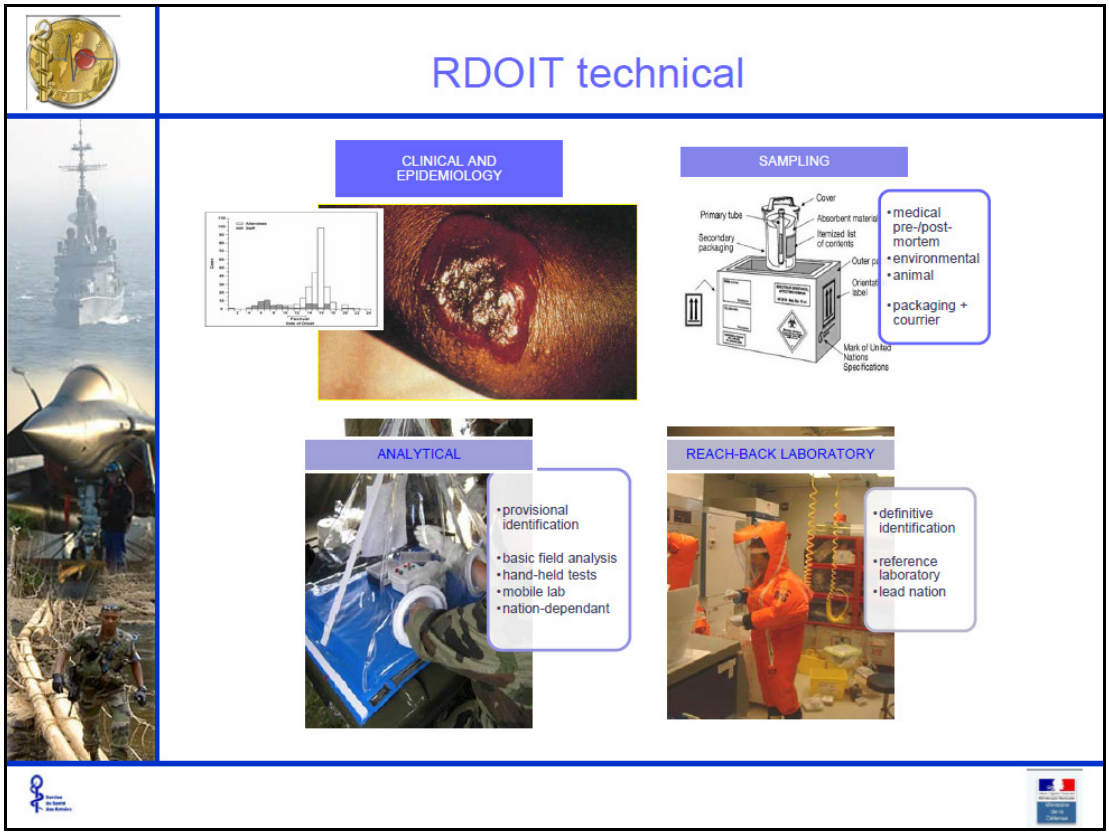
## RDOIT characteristics

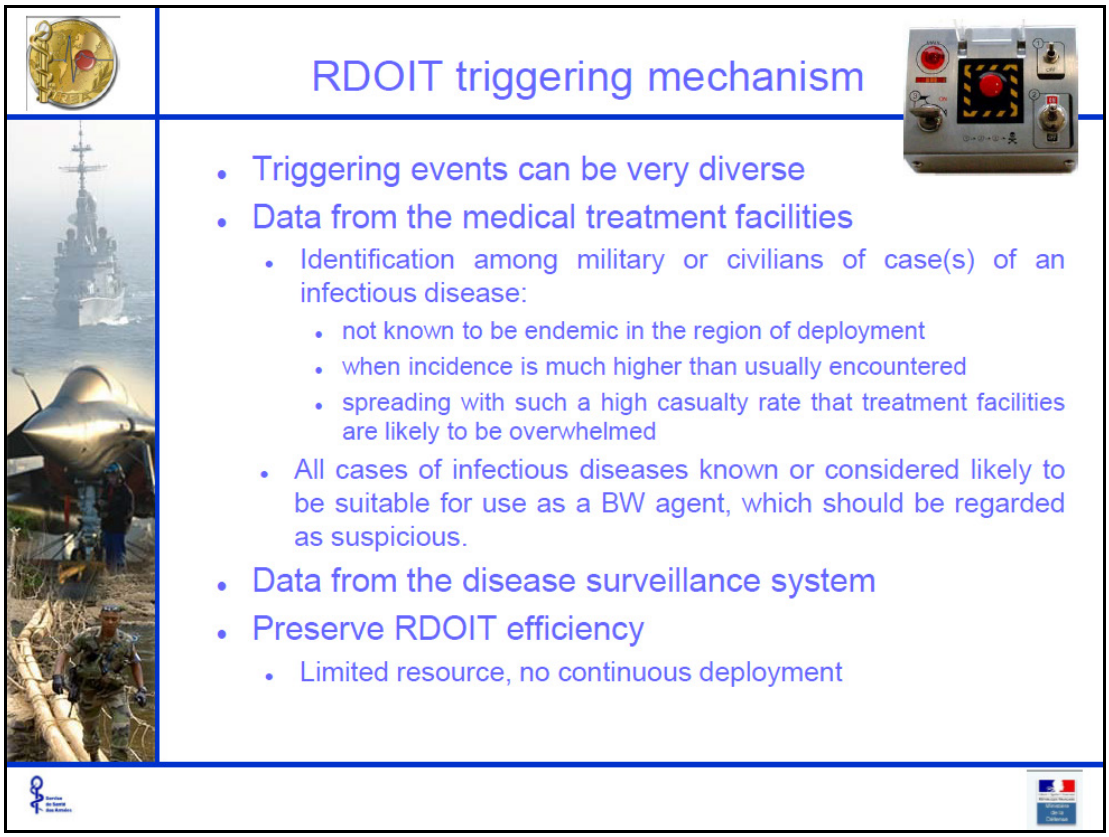
**Technical**
- Autonomous team
- Tailored to the mission
  - modularity
- Small footprint
- Mobility
- Reach-back laboratory

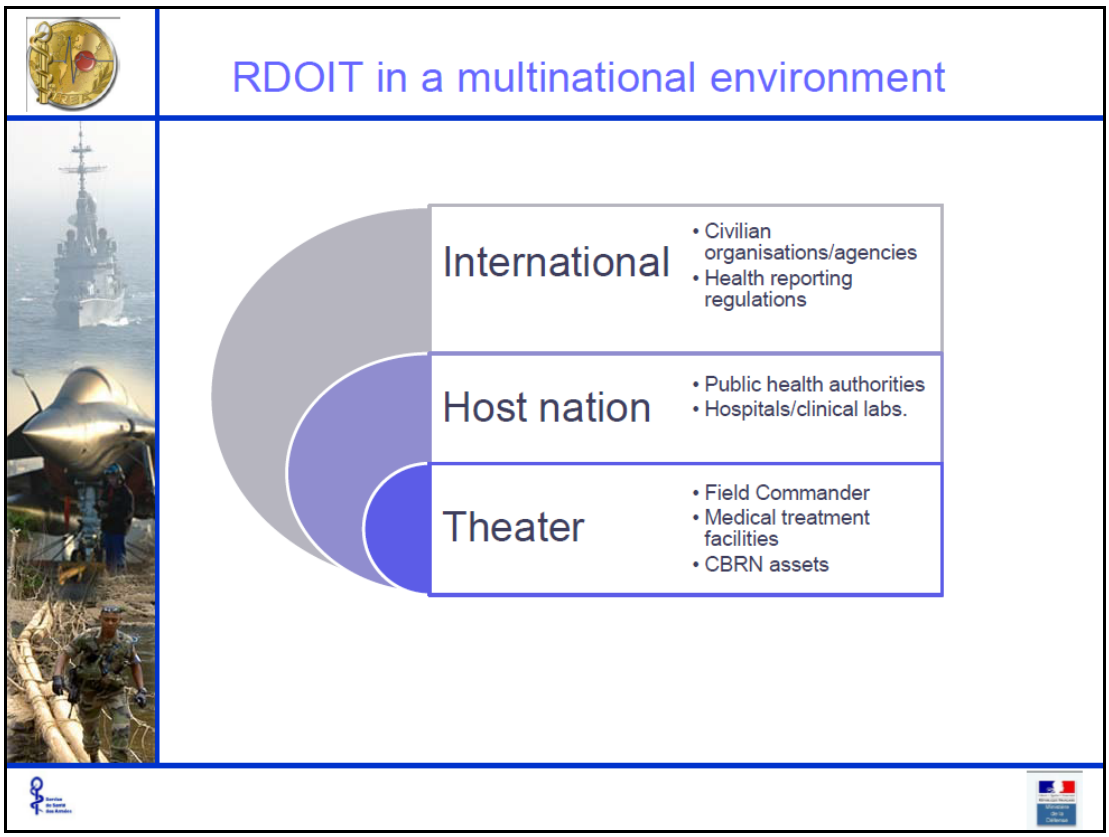
**Staff**
- High-level of expertise
- Core + optional competencies
- Operationally trained

**Operational**
- Multinational composition
  - lead nation
- 48h notice-to-move
- Op control: Theatre Commander


RDOIT modular composition
Tropical medicine
Entomology
Forensics, pathology
-Infectious diseases
-Epidemiology
-Microbiolgy
-Public health
-Sampling, packaging
Reach-back laboratory
Law officer
Toxicology
Field analysis
CORE: mandatory competencies
optionnal competencies


RDOIT technical
CLINICAL AND EPIDEMIOLOGY
SAMPLING
•medical pre-/post-mortem
•environmental
•animal
•packaging + courrier
ANALYTICAL
•provisional identification
•basic field analysis
•hand-held tests
•mobile lab
•nation-dependant
REACH-BACK LABORATORY
•definitive identification
•reference laboratory
•lead nation


RDOIT triggering mechanism
• Triggering events can be very diverse
• Data from the medical treatment facilities
• Identification among military or civilians of case(s) of an infectious disease:
• not known to be endemic in the region of deployment
• when incidence is much higher than usually encountered
• spreading with such a high casualty rate that treatment facilities are likely to be overwhelmed
• All cases of infectious diseases known or considered likely to be suitable for use as a BW agent, which should be regarded as suspicious.
• Data from the disease surveillance system
• Preserve RDOIT efficiency
• Limited resource, no continuous deployment


RDOIT in a multinational environment
International
• Civilian organisations/agencies
• Health reporting regulations
Host nation
• Public health authorities
• Hospitals/clinical labs.
Theater
• Field Commander
• Medical treatment facilities
• CBRN assets

## Where we are

- RDOIT concept has been transcribed into a Standardisation Agreement (STANAG 2529)
  - ratified by 18 nations
  - promulgated in 2009
- RDOIT in use or in development in several countries
- The way ahead:
  - planning guidance document

NATO/PfP UNCLASSIFIED

NATO STANDARDIZATION AGENCY
AGENCE OTAN DE NORMALISATION

**MILITARY COMMITTEE MEDICAL STANDARDIZATION BOARD (MCMedSB)**

29 October 2009 NSA(MED)1179(2009)CBRNMED/2529

MCMedSB

**STANAG 2529 CBRNMED (EDITION 1) – RAPIDLY DEPLOYABLE OUTBREAK INVESTIGATION TEAM (RDOIT) FOR SUSPECTED USE OF BIOLOGICAL WARFARE AGENTS**

Reference: NSA(MED)0208(2009)CBRNMED/2529 dated 18 February 2009 (Edition 1) (Ratification Draft 1)

1. The enclosed NATO Standardization Agreement, which has been ratified by nations as reflected in the NATO Standardization Document Database (NSDD), is promulgated herewith.

2. The reference listed above is to be destroyed in accordance with local document destruction procedures.

ACTION BY NATIONAL STAFFS

3. National staffs are requested to examine their ratification status of the STANAG and, if they have not already done so, advise the MCMedSB, NSA, through their national delegation as appropriate of their intention regarding its ratification and implementation.

Juan A. MORENO
Vice Admiral, ESP(N)
Director, NATO Standardization Agency

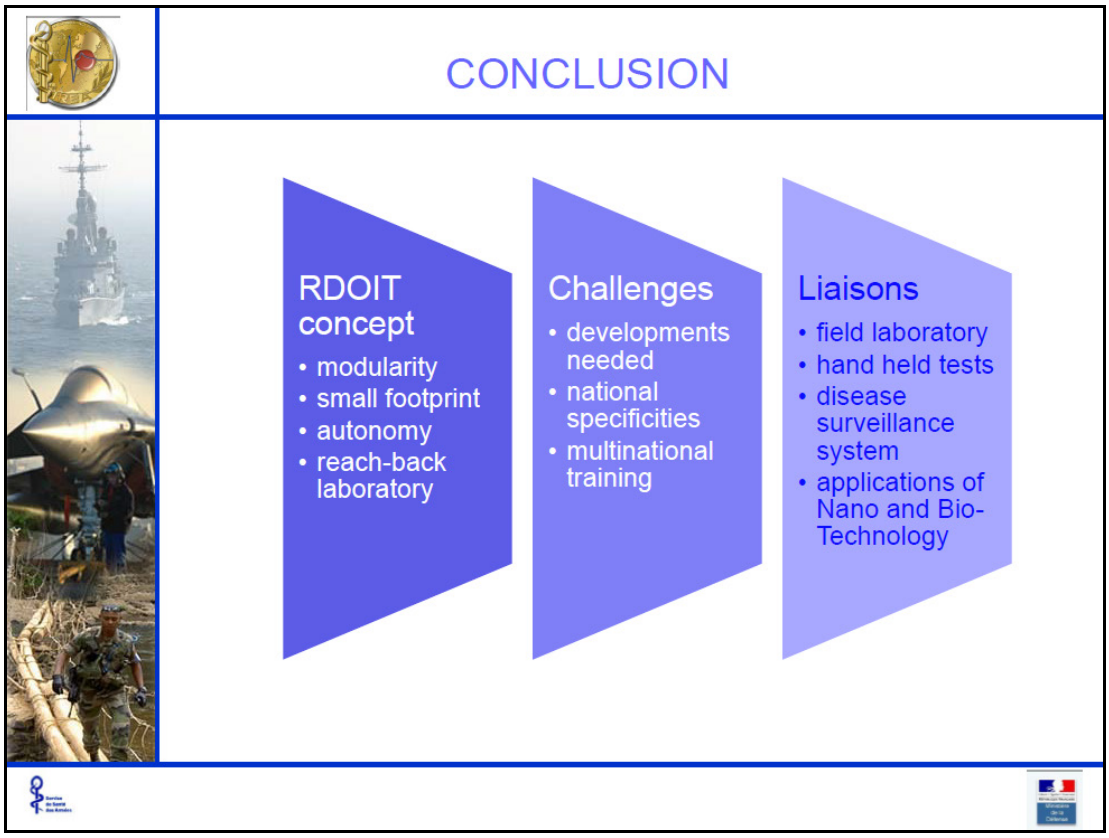
Enclosure:
STANAG 2529 (Edition 1)

NATO Standardization Agency – Agence OTAN de normalisation
B-1110 Brussels, Belgium Internet site: http://nsa.nato.int
E-mail: joint@nsa.nato.int – Tel 32.2.707.5573 – Fax 32.2.707.5718

NATO/PfP UNCLASSIFIED

## RDOIT in FRANCE : EMIBE

- "mobile element for biological and epidemiological investigation "
  - based on mobile lab for parasitology investigation
  - technical :
    - self-contained (transport boxes)
    - serology
    - real-time PCR
    - pathogen identification and culture
  - pre-development phase
    - DGA contract


CONCLUSION
RDOIT concept
• modularity
• small footprint
• autonomy
• reach-back laboratory
Challenges
• developments needed
• national specificities
• multinational training
Liaisons
• field laboratory
• hand held tests
• disease surveillance system
• applications of Nano and Bio-Technology


Thank you for your attention
Medical Biodefense Conference 2011
RDOIT

# REPORT DOCUMENTATION PAGE

| 1. Recipient's Reference | 2. Originator's References | 3. Further Reference | 4. Security Classification of Document |
|---|---|---|---|
| | STO-TR-HFM-177<br>AC/323(HFM-177)TP/552 | ISBN<br>978-92-837-0208-5 | PUBLIC RELEASE |

**5. Originator**

Science and Technology Organization
North Atlantic Treaty Organization
BP 25, F-92201 Neuilly-sur-Seine Cedex, France

**6. Title**

Deployable Laboratory Applications of Nano- and Bio-Technology

**7. Presented at/Sponsored by**

Findings of Task Group HFM-177.

| 8. Author(s)/Editor(s) | 9. Date |
|---|---|
| Multiple | October 2014 |
| **10. Author's/Editor's Address** | **11. Pages** |
| Multiple | 200 |

**12. Distribution Statement**

There are no restrictions on the distribution of this document. Information about the availability of this and other STO unclassified publications is given on the back cover.

**13. Keywords/Descriptors**

Agent
Analytic
Biological
CBRN
Chemical
Deployable laboratory
Radiological
Warfare

**14. Abstract**

The NATO STO Human Factors and Medicine panel chartered a Research Technical Group (HFM-177 RTG) to study "Deployable Laboratory Applications of Nano- and Bio-Technology", which focused on deployable NATO CBRN laboratory advance technologies. Over 20 expert representatives from the Czech Republic, France, the Republic of Georgia, Germany, Israel, Turkey, United Kingdom and United States participated. Each country discussed their CBRN deployable laboratory capabilities and challenges. The RTG recognized different approaches taken by each country in the development of their deployable laboratory for their country's mission. The review resulted in findings of potential novel options to customize the level of response required for NATO missions. A RTG sub-group developed a survey that aided documentation of state-of-the-art technical advances employed in current laboratories that may allow NATO to apply a customized response team depending upon the threat scenario. Many countries' found enhancement approaches from others' lessons-learned that were applicable to their deployment laboratory activities. The HFM-177 RTG meetings were great successes that allowed knowledge gathering resulting in a technical report addressing current capabilities and future directions. The RTG recommends that these findings be forwarded to the NATO Army Armaments Group, Joint Capability Group on CBRN Defence, and the Sampling and Identification of Biological, Chemical and Radiological Agents sub-group for their consideration of asset applications and for determining country agreement development for capability application in a NATO bio-response.

NORTH ATLANTIC TREATY ORGANIZATION

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SCIENCE AND TECHNOLOGY ORGANIZATION

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ISBN 978-92-837-0208-5