REVIEW ARTICLE

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Capabilities for identification and confirmation of bacterial biological agents

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Abstract: *Military Medical Service is able for detection, identification and confirmation of biological agents; it is part of medical protection against CBRN weapons. We are specialized capabilities for in vitro tests, under construction, the maximum containment laboratory designed for work with Risk Group Microorganisms. An efficient primary containment system must be in place, consisting of one or a combination of the following: Class III safety cabinet laboratory, passage of two doors, suit laboratory, controlled access, controlled air system. Negative pressure in the facility, supply and exhaust air must be HEPA-filtered, decontamination of effluents, sterilization of waste and materials, airlock entry ports for specimens, materials and animals must be provided etc. Complementary is an Animal facility for in vivo tests. This is suitable for work with animals that are deliberately inoculated with microorganisms in Risk Group.*

Keywords: *biological security, military capability, biological attack, biological agents, CIMIC, medical protection*

CAPABILITIES FOR IDENTIFICATION AND CONFIRMATION OF BIOLOGICAL AGENTS

Military Medical Service is abilities for the detection, identification and confirmation of biological agents. It is part of the medical protection against CBRN weapons, with mobile and stationary capabilities. MMRC participate, specifically the medical protection against biological weapons and bioterrorism with a series of microstructures: Biological mobile intervention team with mobile platform; Biological analytical laboratory CBRN defense, integrated platform for scientific and medical-military research for biological agents, with: Laboratory of Microbiology high secure, Secured animal facility laboratory for confirmation of biological agents, laboratory of molecular biology and genetics, toxicology laboratory and Annex facility. [1, 2, 3, 4]

SPECIALIZED CAPABILITIES

Stationary

The maximum containment microbiological laboratory is designed for work with Risk Group Microorganisms, for in vitro diagnostic. Operational maximum containment laboratories should be under the control of national or other appropriate health authorities. [5,

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6]

Laboratory design and facilities:

- Primary containment. An efficient primary containment system must be in place, consisting of one or a combination of the following; Class III cabinet laboratory. Passage through a minimum of two doors prior to entering the rooms containing the Class III biological safety cabinet is required; [5, 7] Suit laboratory. A protective suit laboratory with self-contained breathing apparatus differs significantly in design and facility requirements from a Biosafety Level 4 laboratory with Class III biological safety cabinets. [5]

- Controlled access. The maximum containment laboratory must be located in a separate building or in a clearly delineated zone within a secure building. Entry and exit of personnel and supplies must be through an airlock or pass-through system. [5, 8, 9, 10, 11]

- Controlled air system. Negative pressure must be maintained in the facility. Both supply and exhaust air must be HEPA-filtered. There are significant differences in the ventilating systems of the Class III cabinet laboratory and suit laboratory. [5]

- Decontamination of effluents. All effluents from the suit area, decontamination must be decontaminated before final discharge. Heat treatment is the preferred method. Effluents may also require correction to a neutral pH prior to discharge. Water from the personnel shower and toilet may be discharged directly to the sanitary sewer without treatment. [5, 8, 9, 10]

- Sterilization of waste and materials. A double-door, pass-through autoclave must be available in the laboratory area. Other methods of decontamination must be available for equipment and items that cannot withstand steam sterilization. [5, 6]

- Airlock entry ports for specimens, materials and animals must be provided. [5]

- Emergency power and dedicated power supply lines must be provided. [6]

- Containment drains must be installed. [5]

Because of the great complexity of the engineering, design and construction of Biosafety Level facilities, in cabinet or suit configuration, schematic representations of such facilities have not been included. [5] (Figure 1)

Figure 1: Microbiological laboratory for biological agents

Animal facility

This is suitable for work with animals that are deliberately inoculated with microorganisms in the Risk Group, for in vivo diagnostic. [12, 13, 14] The following safety precautions apply:

- All the requirements for animal facilities – Biosafety Level must be met. [12, 13, 14, 15]

- Biohazard warning signs (see Figure 1) should be posted on doors and other appropriate places. [12, 14]

- The facility must be designed for easy cleaning and housekeeping. [12, 14]

- Doors must open inwards and be self-closing. [12, 14]

- Heating, ventilation and lighting must be adequate. [12, 14]

- If mechanical ventilation is provided, the airflow must be inwards. Exhaust air is discharged to the outside and should not be recirculated to any part of the building. [12, 14]

- Access must be restricted to authorized persons. [12, 14]

- No animals should be admitted other than those for experimental use. [12, 14]

- There should be an arthropod and rodent control program. [12, 14]

- Windows, if present, must be secure, resistant to breakage and, if able to be opened, must be fitted with arthropod-proof screens. [12, 14]
- After use, work surfaces must be decontaminated with effective disinfectants. [12, 14]
- Biological safety cabinets (Class II) or isolator cages with dedicated air supplies and HEPA-filtered exhaust air must be provided for work that may involve the generation of aerosols [12, 13, 14, 15, 16]
- An autoclave must be available on site or in appropriate proximity to the animal facility. [12, 13, 14, 15, 16]
- Animal bedding materials must be removed in a manner that minimizes the generation of aerosols and dust. [12, 13, 14]
- All waste materials and bedding must be decontaminated before disposal. [12, 13, 14]
- Use of sharp instruments should be restricted whenever possible. Sharps should always be collected in puncture-proof/-resistant containers fitted with covers and treated as infectious. [12, 13, 14]
- Material for autoclaving or incineration must be transported safely, in closed containers. [12, 13, 14]
- Animal cages must be decontaminated after use. [12, 13, 14]
- Animal carcasses should be incinerated. [12, 13, 14]
- Protective clothing and equipment must be worn in the facility, and removed on leaving. [12, 13, 14]
- Hand-washing facilities must be provided. Staff must wash their hands before leaving the animal facility. [12, 13, 14]
- All injuries, however minor, must be treated appropriately, reported and recorded. [12, 13, 14]
- Eating, drinking, smoking and application of cosmetics must be forbidden in the facility. [12, 13, 14]
- All personnel must receive appropriate training. [12, 13, 14]

This facility is complementary to the microbiological laboratory (Figures 2, 3).

Figure 2: Animal facility for microbiological diagnosis

Figure 3: Secured animal facility laboratory

SPECIALIZED MOBILE CAPABILITIES FOR Bio-Det SYSTEM

Specialized Mobile capabilities for Bio det system:

- provides monitoring, sampling, detection and identification;
- detects long line releases and identifies up to 6000 species and sub-species (data base).
- up to 96 samples can run simultaneously, no time needed to re-deploy.
- deploy worldwide and conduct biological surveillance operations in order to provide identification of biological agent employment.
- provide information to other forces (rescue teams, medical, etc)

Bio-Det Systems

Configuration:

- Vehicle with 2 internal compartments, with power supply, specific furniture and safety cabinet class III.

For monitoring: Verotect – determines if a bio-mass is present within aerosol particles of a certain size range

For identification:

- MALDI TOF Microflex LT 20 with vehicle mount
- Water, soil and air sampling kits
- Sample preparation equipment (incubator, centrifuge, freezer, autoclave, handling tools, consumables and reagents)

Miscellaneous:

- Personal protection for the operators (suits, masks, oxygen tubes)
- Hardened laptop etc.

Mass Spectrometry

Those are complex instruments which record an ionic flow as a spectrum, in relation to both mass and the relative occurrence of the ions; Mass spectrometry is a confirmation analytical technique which allows identification of organic compounds, based on the mass/electrical charge ratio of the molecule fragments of the analyze.

The mass spectrometer consists of 4 elements: sample loading port, where a certain amount of sample is introduced at the required pressure in order to be exposed to the ion source and to be ionized at maximum efficiency. Ion source, where the neutral atoms and molecules are ionized and directed into the instrument as a monenergistic bundle.

Mass (energy) analyzer, which by electrical and/or magnetically deflections selects the ions according to the q/m values, their masses, concomitantly focusing them towards an outlet gap. Detector, comprising the system which transforms the ionic flow either into a photographic image or an electric current that can be recorded with an appropriate instrument.

MALDI TOF Microflex

Microflex LT20 is a MALDI TOF system equipped with Bioprofiler software and it is used for the identification and taxonomic appointment of the biological agents from air, water, soil and other surfaces, after a previous specific processing of the collected samples. The main element is a MALDI TOF spectrometer, with a mass domain from 2,000 to 20,000 Da.

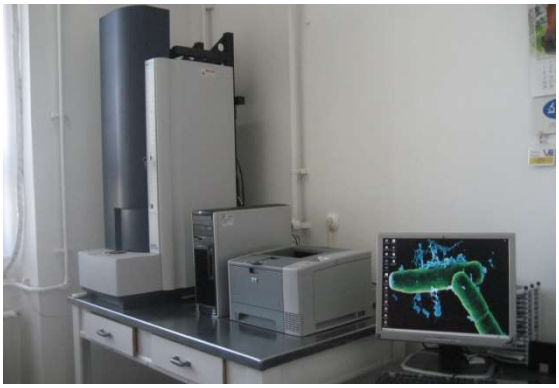
The instrument is controlled by means of a computer with Windows operating system and specialized software (Bioprofiler). The data processing system controls all acquisition, saving and storage processes of the spectral information, allowing later processing of the information or access to the data base.

Microflex LT 20 Bruker system

It has the ability to identify the biological agents from various samples (liquid, solid, gaseous). Identification is performed by comparing the obtained spectrum to the existing data base of memorized biological agents.

The software indicates the matching of the spectrum under test with spectra from the database, according to the obtained scores. Scores are generated by evaluation and comparison between spectra and are not percents. If a biological agent is not included in the data library, the agent with the closest structure is exhibited, but the score indicates that the agent under test is not included in the data base of the instrument (Figure 4).

Figure 4: Mass spectrometer for microbiological diagnosis (The Microbiology Laboratory MMRC, I. Cantacuzino)



Operation steps Microflex

Preparation of samples for MALDI TOF analysis is done by extracting 16S ribosomal proteins that can be achieved by several techniques depending on the organism analyzed. [17]

The instrument can measure mass spectra from 2,000 up to 20,000 Da, i.e. a very wide range of organic molecules originating from any kind of biological agents (bacteria, spores, viruses, fungi, yeasts, toxins). The software and its data base are capable to identify

biological agents of civil and medical-military interest.

The equipment is foreseen with a device for attachment to mobile laboratories, allowing its use in the field, either in campaigns or, in peace time, in case of biological events or crises.

The quality of the incorporated mass spectrometer as well as the structure of the soft that can be upgraded in time, strongly recommends the MALDI TOF – Bioprofiler MICROFLEX LT20 system for identifying biological agents, to be used for precise microbiologic diagnostic, species confirmation for the biological agent, medico-military research and medical defense against biological weapons or bioterrorist attacks.

Military Medical Service is able for detection, identification and confirmation of biological agents. It is part of medical protection against CBRN weapons, in civil-military cooperation (CIMIC). [17]

CONCLUSIONS

CCSMM has specific capabilities for scientific research for medical protection against CBRN WMD.

Anti-infective medical protection laboratory and Epidemiologic emergency laboratory have capability of performing detection, identification and confirmation of biological agents within the limits of available supplies.

The capacities and capabilities of health protection against biological weapons are interoperable with NATO.

Detection, identification and confirmation of the presence of bacterial biological agents are executed sequentially and modular, specialized capacities through cooperation, which must have the capability to perform these tasks.

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