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Bioterrorism and Public Health Service: Defining Management and Treatment Systems

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Abbreviations: BASIS: Detect-to-treat technology; BRICK: Bio Risk Initiative for Capacity building and Knowledge base development; BSA: Biosafety Associations; BSCL: Biosafety Cabinet; BLS- 1: Biosafety Level 1 (BSL-1); BWT: Biological and Toxin Weapons Convention; CBRN: Chemical, Biological, Radio Nuclear; CDC: Centers for Disease Control and Prevention; CWTC: Chemical Waste Treatment Centre; DSUS: Distributed Sampling Units; DNA: Deoxyribo Nucleic Acid; EBSA: European Biosafety Association; ECDC: European Centre for Disease Prevention and Control; EC: European Commission; EMEA: European Evaluation of Medicinal Products; EURONET-P4 : European Network of P4 Laboratories; EU: European Union; GMMs: Genetically Modified Micro-Organisms; GHSI: Global Health Security Initiative; HANAA: Handheld Advanced Nucleic Acid Analyzer; HSC: Health Security Committee; HSR: Health Systems Response; HEPA: High Efficiency Particulate Air filter; IFBA: International Federation of Biosafety Associations; LAI: Laboratory Associated Infections; LIDARs: Light Detection and Ranging; LIBS: Laser-Induced Breakdown Spectroscopy; NIAID: National Institute of Allergy and Infectious Disease; OSHA: Occupational Safety and Health Administration; PCR: Polymerase Chain Reaction; WHO: World Health Organization;

Introduction

Few will recall that little more than a decade ago, the possibility of biological terrorism was neither anticipated nor understood by professionals or the civilian community. The effects of a nuclear attack were documented and tangible. Chemical accidents were not uncommon, but the potential catastrophe of an epidemic following the deliberate release of a biological pathogen was difficult to comprehend. We are living in an era of uncertainty and change and the use of biological weapons is a serious problem of public health that increases the probability of "possible incidents" related and not just to bioterrorism. Some bacteria, viruses and toxin are greater problem for human health. They are employed better in agriculture, in food manufacturing and have an effect even on Environment, too. Terrorists used biological for their virulence, toxicity, transmissibility and their lethality, but what really makes those particular microorganisms used as weapons is the high pathogenicity, which can grow from a single organism or a cell. Biological agents have: the relatively low costs of production are sometimes readily available and not have significant problems regarding storage and transport. Moreover, terrorist organizations, in addition to naturally existing pathogens, may grope to use genetically modified micro-organisms (GMMs).

Experts think that toxins in the order up to a thousand, can be obtained from genetic or natural sources, but not all would be used as biological weapons; monitoring the illegal subtraction, even small quantities, it is impossible!! Pathogens are difficult to detect: they are colorless and odorless and have incubation times ranging from 48 hours for respiratory anthrax, 21 days, for Q fever. Period of incubation at the same time an advantage and a drawback; an advantage because it opens up a time-window that allows you to quarantine and treat infected individuals and vaccination of others; a drawback because often it is difficult to identify the disease.

At the initial stage many diseases present symptoms similar to flu: patients tend, thus to follow their normal rhythm of life, behavior that in case of transmissible diseases could to lead to widespread contamination. For most diseases caused by agents used in biological warfare, there are treatments and/or vaccines in order to permit the deployment of reaction mechanisms and, especially, the adoption of medical countermeasures, is essential a timely dejection of attack. Adequate background data on the natural behavior of infectious diseases facilitate recognition of an unusual event and help determine whether suspicions of a deliberate cause should be investigated. Routine surveillance systems, for epidemic-prone and emerging infectious diseases, enhance the capacity to detect and investigate deliberately caused outbreak.

Even a very small quantity of pathogen will cause disease for example the tularemia requires as few as 10 organisms to infect therefore sensors need to be sensitive for a minimal presence of pathogens. —Detect to protect" biological detection technology is currently unavailable. Available instruments are usually large, slow and expensive. The more reliable the detection instrument is the longer it takes to identify the defined threat. Thus, the main goal of biological detection is to provide sufficient warning for responders order to reduce the number of victims.

Biodefense strategies are formed by a combination of several layers of detection. A first layer is composed of standoff detectors; a second layer of protection is provided by the use of point detectors; lastly, the collection of epidemiological data can support and complement the use of biosensors. Several technologies, such as Doppler RADARs, LIDARs (Light Detection and Ranging) or LIBS (Laser-Induced Breakdown Spectroscopy), can be used for standoff detection of biological agents. They rely on radio waves or light reflectance techniques to screen clouds for airborne pathogens. However, applications for these technologies are mostly military and their efficiency is still limited.

Reality now is that biosecurity is now benefiting from the collective intense interest of political leaders or the funding commitments that followed the 2001 anthrax attacks. With the passage of time, the initial sense of urgency in efforts to shore up the nation's biosecurity

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has waned, even as it is increasingly understood that advances in the biosciences over the past decade make biological weapons ever more accessible and technically feasible, and even with evidence that terrorist groups are interested in acquiring and using them. For these reasons, organizations and laboratory directors are compelled to evaluate and ensure the:

- effectiveness of their biosafety programs;
- proficiency of their workers, as well as
- The capability of equipment, facilities, and management practices to provide containment and security of microbiological agents.

In a the strategic planning process, to protect nations by threats consequent a release, intentional or unintentional, of biological agents, is essential the support to building and growth of infrastructure of research and biodefense .The effective design and implementation of *Biosecurity's Laboratory* depends on cooperation among individuals from diverse communities, including scientists, technicians, policy makers, security engineers, and law enforcement officials.

The European Commission's Sixth Framework Programme funded the Biosafety Europe. The project (started 20006 ended on 2008) had 18 partners from 10 European countries and an overall aim of promoting European harmonization and the exchange of practices relating to biosafety and biosecurity management of biological containment facilities. Recent experience suggests we might do better in the future if we start making some achievable improvements now. Specifically, European biosurveillance systems could benefit greatly from adoption of new and better tools, greater and sustained support for existing programs, and improved integration of biosurveillance data across multiple agencies. These improvements should be prioritized given the nation's reliance on its biosurveillance systems to minimize the spread of disease, prevent unnecessary sickness and death, and reduce the economic and social harm caused by outbreaks, epidemics, pandemics, and bioterrorist attacks. In the coming years, the work of the network could offer a concrete opportunity to strengthen the global capacity of the European Community to face high-threat infections, based on reliable and validated diagnostic methods, agreed background biosafety measures and increased field investigation capability.

A potential European strategy for Biosafety or Biosecurity could be the integration of data across multiple sectors, a goal that may be more difficult to achieve but that is worth attempting. The more they can integrate healthcare and public health data with data from intelligence, law enforcement, and private sector sources, the better off we will be. Data integration could shave valuable time by weeks or even months that you can take now to identify the source of an outbreak, develop successfully a strategy of control and prevention.

In the 2005 was created the European Network of P4 Laboratories, coordinated by INMI IRCCS L. Spallanzani, (Rome) Italy. Goal of ENP4Lab is to enhance European preparedness for emerging pathogens through efforts aimed at increasing collaboration among reference P4 laboratories in different European countries. The aim was also to share such experience with other European countries, where new P4 laboratories are planned or are under construction.

The National Institute for Infectious Diseases (INMI) "Lazzaro Spallanzani with the Hospital "Luigi Sacco", in Italy, have been identified as the two poles of national for the care of any affected patients; with an investment in terms of infrastructure (hospital rooms special ambulances) for improving conditions for isolation of patients. Recently, the Italian Red Cross, in partnership with the Italian Health Ministry, showed how Italy is able to express activities and initiatives of highest excellent and great potential both nationally and internationally. The First Bio-containment Medical Transfer of Italian Red Cross may be used in every instance where they are in an emergency and management of individuals exposed of biological agents with high airborne transmissible (tuberculosis, avian influenza, SARS, meningitis, and other infectious diseases of the known international travellers) which however does not require health care or of a carriage on a stretcher, in order to minimize the possible contagion, as well to safeguarding the health of first responders that also take care transfer of exposed. Examples of use are:

- people who have shared the same restricted airspace with the index case and for this has to be made to undergo sanitary surveillance;
- who needs assistance in suitable and separated areas order to avoid further possible infection;
- Suspected / probable cases for ―safe transporting‖ at the hospital most appropriate.

Jointly at the INMI and at the Hospital L. Sacco in Italy there are:

- The Department of Infectious, Parasitic and Immune-Mediated Diseases of the National Health Service. The activity of the Department aims at protecting the human population from diseases caused by pathogenic micro-organisms, viruses and parasites, and to study the mechanisms of immune-mediated diseases. It is also responsible for the control of infectious and immunological emergencies and for the preparation of plans to respond to possible bioterrorist attacks. The Department has laboratories safety class 3, expected to work microorganisms of risk group 3 and worked for the development of diagnostic strategies for conventional and definitive identification of bacterial pathogens that could be used for the purpose bioterrorist using conventional and molecular methods.
- the Institute Zoo profilattico Puglia and Basilicata (Cerna), based in Foggia, that the Ministry of

Health has designated as a national reference Centre for anthrax. This is already was a deputy to the preparation of the two vaccines against anthrax, and Carbosap Pasteur , and prialla testing of new vaccine Sterne .The Centre has the task to test the detection anthrax spores in suspicious samples (with the exception human clinical suspicion) as part of the emergency bioterrorism, which consists in the amplification, via PCR, nucleotide sequences specific for chromosome, the lethal factor, edema factor, the antigen and the protective capsule of *Bacillus anthracis*.

Sensor Technology

Sensor technology is the most obvious example of biodetection. The fundamental challenge is that biological agents have different properties and many sensors are pathogen-specific; each test must be tailored to recognize a specific pathogen. If a biological attack were to be undertaken through the release of a biological agent into the air from a distance, advance detection would be a crucial asset to warn of the attack and allow for an organized response. Identification and clinical recognition, rely on high-quality laboratory diagnostic, tests based on validated techniques and protocols so that deliberate releases can be rapidly confirmed or excluded. Laboratory expertise and capacity must, in turn, be available to cope with high-risk agents and complex

technology and methods as well as a surge in demand in case of multiple threats or attacks.

Now they are increasing more efficient sensors which combine the collection of samples with the site PCR analysis, but for the majority of the for collection of samples device identification must be made up at least in part in the laboratory[29 30]. Standard PCR procedures take time, are expensive, heavy number of man and shall be executed by expertise; in addition, DNA-based techniques may not be used for the detection of toxins because these have not DNA. To accelerate the process, and to increase the efficiency of the detection are currently being developed technical PCR and other amplification techniques that is a smaller and versatile devices [1].

Example of the sensor, based on DNA, is the Handheld Advanced Nucleic Acid Analyzer (HANAA) biodetection system can be held in one hand and weighs less than a kilogram and was designed for emergency response groups, (s fire-fighters, police), who are often first on the bioterrorism's scene. Each handheld system can test four samples at once-either the same test on four different samples or four different tests on the same sample.

HANAA provide results in less than 30 minutes [2,3]. The commercial thermo cyclers used for standard laboratory tests are: pretty big, ranging from the size of a microwave oven to a large desk. A typical large thermo cycler takes about 3 minutes to cycle through one heating and cooling cycle, so a complete analysis requires 2 to 3 hours. In the HANAA system, the thermal cycling process occurs in tiny silicon heater chambers, micromachined by Livermore's Center for Micro technology. Each chamber has integrated heaters, cooling surfaces, and windows through which detection takes place. Because of the low thermal mass and integrated nature of the chambers, they require little power and can be heated and cooled more quickly than conventional units. The mini-chambers typically cycle from about 55˚C to 95˚C and back to 55˚C into 30 seconds [4]. Using this technique, the HANAA system could, in principle, detect as few as 10 individual bacteria in one-hundredth of a milliliter in less than 30 minutes. This system has the potential of saving many lives by saving time-anthrax, for example, is highly treatable if detected early.

Another technology is BASIS. It's designed to detect and locate an aerosol release of a biothreat organism quickly and accurately enough for an effective response. For example, the survival rate from exposure to the anthrax bacterium is high when antibiotic therapy can be administered before symptoms appear, but after symptoms manifest, the survival rate diminishes significantly [5]. BASIS is designed for indoor or outdoor use at high-profile events or around the likely terrorist targets. In 2001, the technology has been successfully tested on microbes living within a sealed chamber at Ground Dugway by the U.S. Army and was deployed for the first time during the month after the terrorist attacks of September 11. He was subsequently deployed also in Salt Lake City, for the Winter Olympics (2002), during the Olympics; BASIS has operated for 35 days: sports facilities, urban areas and transport hubs, in all, were analyzed, about 2,200 air samples.

BASIS was subsequently implemented in New York for the first anniversary of 9/11 and also the biohazard detection system of the U.S. Postal Service's Biohazard Detection System BDS, (Figure 1) combines the system of collection of samples with an analysis of the DNA by PCR [6].

BASIS includes three major components: aerosol collection hardware continually collects, time-stamps, and stores samples. A mobile field laboratory analyzes DNA from the samples and can identify and characterize a threat organism in less than half a day with a virtually zero false-alarm rate. Software designed by the BASIS team controls and integrates the operations. BASIS collects air samples at well-defined locations and at specified time intervals to help determine both the time and place of the release. Its mobile field laboratory rapidly tests samples for evidence of potentially lethal bacteria and viruses. Safeguards built into the system ensure a sample's integrity. Aerosol releases of bacteria or viruses tend to quickly become diluted as their distance from the release site increases; is designed with extremely high sensitivity for detecting the most likely threat pathogens; by identifying a pathogen within hours, allows medical response units to mobilize while law-enforcement agencies begin the search for terrorists

Figure 1: Example of BASIS in New York City seen by different side.

Air samplers, called distributed sampling units (DSUs), suction air through filters that have microscopic-size pores and collect any regional microbes onto the filters' surface. DSUs can be deployed indoors, for example, at sports arenas, airline terminals, within heating and air-conditioning systems, and outdoors at airport drop-off areas, urban commercial centers, bridges, tunnels-any area with a significant threat of bioterrorism. [DSUs are locked and password-protected to prevent unauthorized access and to guarantee the integrity of filters].

A derivative of BASIS, is now deployed in major cities nationwide under the auspices of the U.S. Department of Homeland Security. Biowatch features elements of the BASIS technology, but instead of a mobile laboratory, uses laboratories that are part of the federal Laboratory Response Network operated by the Centers for Disease Control and Prevention (CDC) [7,8].

Biological Agent

History

In the history of biological weapons are used by much of the time, chemical or nuclear weapons. Since ancient times were deliberately made to hide various objects and transmit disease agents to the enemy [9] as the use of dead bodies or animals carcasses infected to contaminate wells, cisterns and collected the water used by armies and by the people, poisons and other toxic substances found in nature or made at hoc [10,11].

In the first moments after the accident when the nature of infecting virus is unknown is important to engage in contingency planning, well experienced microbiologists (to be sent if the "field" for surveys or sampling as appropriate) that can provide more quickly and specific answers. For such events in the future, however, rescue and treatment of victims and control or containment of fire and other hazards will be greatly complicated by the fact that the site may also be contaminated with nuclear, chemical, biological or radiological substances that pose an immediate threat to the health and safety of the emergency responders. (Thousands of potentially injured and contaminated

victims may depart the scene, returning to the suburbs and satellite cities where they live, or privately seeking medical assistance) [12].

Attempts to use biological warfare agents date back to antiquity. Scythian archers infected their arrows by dipping them in decomposing bodies or in blood mixed with manure as far back as 400 BC. Persian, Greek, and Roman literature from 300 BC quotes examples of dead animals used to contaminate wells and other sources of water. In the Battle of Eurymedon in 190 BC, Hannibal won a naval victory over King Eumenes II of Pergamon by firing earthen vessels full of venomous snakes into the enemy ships. During the battle of Tortona in the 12th century AD, Barbarossa used the bodies of dead and decomposing soldiers to poison wells. During the siege of Kaffa in the 14th century AD, the attacking Tatar forces hurled plague-infected corpses into the city in an attempt to cause an epidemic within enemy forces. This was repeated in 1710, when the Russians besieging Swedish forces at Reval in Estonia catapulted bodies of people who had died from plague. The most infamous historical use of smallpox is associated with Lord Jeffery Amherst. During the French and Indian War the colonists spread smallpox to thin the native population. However, there is little evidence to support that Amherst himself actually enacted this plan. The reason most attribute spreading smallpox to the natives with him is that he mentioned the idea in a correspondence with Henry Bouquet. At the time the suggestion was made, Fort Pitt had been under siege and it was a possible strategy for relieving the siege. Unknown to Amherst and Bouquet, the men at Fort Pitt had already attempted this tactic. It is unknown how effective this was, but it has become an infamous event in North American history. Smallpox was the first agent of germ warfare [13,14].

In the twentieth century, many States developed biological weapons programs, but in recent years, these were largely dismantled or reduced through international cooperation and major investments. Over the past two decades, however, increased the threat of bioterrorism [2]. More recently, biological warfare has taken on a scientific development of modern microbiology, during the nineteenth century, has provided the opportunity to isolate and produce specific pathogens such as: *Bacillus anthracis* and *Pseudomonas mallei*. Germany developed a program of biological warfare during the First World War, infecting cattle with etiologic agents of anthrax and glanders. In the 30s all the major Europe Countries developed programs of research and defense of the bacterial spite of adherence to the Geneva Protocol (1925), which banishes (without saying anything about their production), the warfare of chemical and biological weapons [15,16].

In 1933, an aerosol of bacteria Serratia was released near a ventilation pipe of the Paris Metro, as a result of this attack a control program was developed on bacteria and viruses that that could be used in biological warfare. In the same time Britain developed its own project, focused on anthrax spores that were spread with a conventional bomb. Gruinard Island, off the coast of Scotland, was chosen as the site of the experiments and the data obtained are used by both Britain and the USA. Just after World War I people began to reflect on the danger of biological weapons and took off the diplomatic efforts to limit the proliferation and the use of weapons of mass destruction. Since the late 60's biological weapons assumed more and more marginal value; continuous research on micro-organisms, effectively, reduced to zero the "secrets" microorganisms those against that the enemy had no defense. Finally, in 1972 the international treaty signed by 160 countries and ratified by 140 countries, banned all bacteriological weapons (Biological and Toxin Weapons Convention, BTWC) [17-20].

Spite of this prohibition, in the mid-80s, the biological arms race start again. Since then the history of treaties goes hand in hand with that of experiments on biological weapons, which continue in many countries. While into the past biological weapons were designed and built especially to attack enemy armies, now the target of these weapons, released by terrorist groups, are civilians. For years, literature discusses problems of biological weapons and, more recently, of their use for terrorist purposes [6, 21]. However, despite widespread publicity of this threat, we know a few efforts to address the actual, employed by groups of terrorists, to provoke massacres among the civilian population through the use of CBRN agents [22]. An exception is the case of salmonella in the United States in 1984 and the terrorist attacks committed by Aum Shinri Kyo in Japan [23,24].

With the anthrax attacks in the U.S. in autumn 2001, and the most recent attacks on public transport networks in Madrid and London even Europe is in danger. In august 2005 the revelations that a cell of Al Qaeda was planning an attack with sarin gas against the British House of Commons, as well an incident, which occurred in May 2004 with the launch of condoms full of purple flour Prime Minister Tony Blair during question time, have demonstrated the high level of vulnerability of national parliaments and the not able to resolve this big emergency [25]. As a response to these events, on both sides of the Atlantic, were launched steps to identify appropriate methods for the detection of possible attacks with biological agents.

The U.S. has shown greater commitment with a global initiative called "Biodefense for the 21st Century", launched in April 2004 by President Bush [17]. President Obama, his senior advisors and government officials should make clear that they regard biological threats and the creation of a robust biodefense to be their top national security priorities.

During the Sixth Review Conference of the States Parties to the BTWC decided that the Seventh Review Conference is to be held in Geneva not later than 2011 and should review the operation of the BTWC, taking into account, inter alia, new scientific and technological developments relevant to the BTWC, as well as progress made by the States Parties to the BTWC in the implementation of the obligations under the BTWC and in the implementation of the decisions and recommendations agreed upon at the Sixth Review Conference [26].

What biological agents can be considered biological weapons?

Actually the Biological agents that can be used in acts of bioterrorism have already been classified according to criteria such as infectivity, virulence, persistence in the environment, the facility of manipulation and dissemination and existence of defenses to counter the spread and effects. Opinion of European Evaluation of Medicinal Products (EMEA) [27] about vaccines and health are referred to the list published by Centers for Disease Control and Prevention (CDC), US. The World Health Organization (WHO) [28] defines bioweapon: a weapon that has as its purpose to disseminate the agents which cause diseases, such as viruses, bacteria, toxins, nucleic acids infected or prion, and proposes a list of 47 agents biological that can be considered biological weapons; other lists have been proposed by the UN and NATO [29]. However, there is unanimity among the experts in finding some biological agents as biological weapons: the anthrax bacillus, the plague, the bacterium of typhoid fever, smallpox, brucellosis, *Pseudomonas pseudomallei*, and *Francisella tularensis*. WHO experts add to this catalog many other microorganisms such as *Vibrio cholera*, the *hantavirus*, (or *Korean fever virus*), the *virus of Crime* - Congo hemorrhagic fever and, Rift Valley fever, the *Russian spring – summer and summer encephalitis' virus*, the agent of dengue fever, *Japanese encephalitis virus*, *Venezuelan equine encephalitis virus* .

In the (Table 1), there are some differences among them governmental agencies about Category C agents, and a high degree of agreement for categories A and B.

Category A agents include organisms and toxins that pose the highest risk to the public and national security for the following reasons:

Category A		
	NIAID	**CDC**
Bacillus anthracis (anthrax)	Y	Y
Clostridium botulinum toxin (botulism)	Y	Y
Yersinia pestis (plague)	Y	Y
Variola major (smallpox) and other related pox viruses	Y	Y
Francisella tularensis (tularemia)	Y	Y
Viral hemorrhagic fevers	Y	Y
Arenavirus Arenaviruses LCM, Junin virus, Machupo virus, Guanarito virus Lassa Fever	Y	Y
Bunyaviruses • *Hantaviruses* • *Rift Valley Fever*	Y	Y
Filoviruses • *Ebola* • *Marburg*		
Flaviviruses • *Dengue*	Y	N

Category B		
	NIAID	
Burkholderia pseudomallei		
Coxiella burnetii (Q fever.)	Y	Y
Brucella species (brucellosis)	Y	Y
Burkholderia mallei (glanders)	Y	Y
Chlamydia psittaci (Psittacosis)	Y	Y
Ricinus communis	Y	Y
Epsilon toxin of *Clostridium perfringens*	Y	Y
Staphylococcus enterotoxin B	Y	Y
Typhus fever (*Rickettsia prowazekii*.)	Y	Y
Food- and Waterborne Pathogens	Y	Y
Additional viral encephalitide	Y	Y

Category C		
	NIAID	**CDC**
Tickborne hemorrhagic fever viruses • Crimean-Congo *Hemorrhagic fever virus*	Y	N
Tickborne encephalitis viruses	Y	N
Yellow fever	Y	N
Tuberculosis, including drug-resistant TB	Y	N
Influenza	Y	
Other Rickettsias	Y	
Rabies	Y	N
Prions	Y	N
Nipah virus	Y	N
Hhantaviruses	Y	Y

Y: yes, that is listed as a potential biological weapon; **N:** is not listed as a potential biological weapon.

Any category A, B and C are found in nature, except of *Variola major*, the causative agent of smallpox.

Table 1: Differences among them governmental agencies about Category C agents, and a high degree of agreement for categories A and B.

- can be easily spread or transmitted from person to person;
- result in high death rates and have the potential for major public health impact;
- could cause extreme concern and social disruption;
- Require special action for public health preparedness.

This category includes agents like anthrax, the agent causative agent of Black Death or plague and smallpox. Use of mail for the spread of anthrax in 2001 has detected the impact that any agent category "A" can have on the population and the impact it may have on the Health Systems Response (HSR).

CDC [30] and the National Institute of Allergy and Infectious Disease (NIAID) [31,32] categorizes bioterrorism agents according to the risk they pose to the public and depending on how easily they can be spread and the severity of illness or death they cause [33,34]. Those that pose the highest risk, because they can be easily disseminated and could result in high mortality, are classified as Category A. The CDC classifies biological agents that pose a moderate risk to the public as Category B. These agents can be spread with some ease and can cause a moderate degree of illness, but death rates due to these diseases are usually low. Category C agents include emerging pathogens that warrant monitoring because they could be manipulated and used as a weapon, are easily available, and have the potential to make a big impact.

Engineering Features of Biosafety

Biosafety containment levels

In the publication *Biosafety in Microbiological and Biomedical Laboratories (BMBL)* [35] are defined four Levels of biosafety published by the CDC and NIH. The levels, designated in ascending order by degree of protection provided to personnel, the environment, and the community, are combinations of laboratory practices, safety equipment, and laboratory facilities. Below is a summary of each biosafety level.

Biosafety Level 1 (BSL-1) is suitable for work involving well-characterized agents not known to consistently cause disease in immuno competent adult humans, and present minimal potential hazard to laboratory personnel and the environment. BSL-1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required, but may be used as determined by appropriate risk assessment. Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or a related science.

Biosafety Level 2 (BSL-2) practices, equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, and other laboratories in which work is done with the broad spectrum of indigenous moderate-risk agents that are present in the community and associated with human disease of varying severity. With good microbiological techniques, these agents can be used safely in activities conducted on the open bench, provided the potential for producing splashes or aerosols is low. *Hepatitis B virus*, *HIV*, the *salmonellae*, and *Toxoplasma spp*. are representative of microorganisms assigned to this containment level. BSL-2 is appropriate when work is done with any human-derived blood, body fluids, tissues, or primary human cell lines where the presence of an infectious agent may be unknown. (Laboratory personnel working with human-derived materials should refer to the OSHA Blood borne Pathogen Standard 2 for specific required

precautions). Primary hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures, or ingestion of infectious materials. Extreme caution should be taken with contaminated needles or sharp instruments. Even though organisms routinely manipulated at BSL-2 are not known to be transmissible by the aerosol route, procedures with aerosol or high splash potential that may increase the risk of such personnel exposure must be conducted in primary containment equipment, or in devices such as a Biosafety Cabinet (BSC) or safety centrifuge cups. Personal protective equipment should be used as appropriate, such as splash shields, face protection, gowns, and gloves. Secondary barriers such as hand washing sinks and waste decontamination facilities must be available to reduce potential environmental contamination.

Biosafety Level 3 (BSL-3) practices, safety equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents with a potential for respiratory transmission, and which may cause serious and potentially lethal infection. *Mycobacterium tuberculosis*, St. Louis encephalitis virus, and *Coxiella burnetii* are representative of the microorganisms assigned to this level. Primary hazards to personnel working with these agents relate to autoinoculation, ingestion, and exposure to infectious aerosols. At BSL-3, more emphasis is placed on primary and secondary barriers to protect personnel in contiguous areas, the community, and the environment from exposure to potentially infectious aerosols. For example, all laboratory manipulations should be performed in a BSC or other enclosed equipment, such as a gas-tight aerosol generation chamber. Secondary barriers for this level include controlled access to the laboratory and ventilation requirements that minimize the release of infectious aerosols from the laboratory.

Biosafety Level 4 (BSL-4) practices, safety equipment, and facility design and construction are applicable for work with dangerous and exotic agents that pose a high individual risk of life-threatening disease, which may be transmitted via the aerosol route and for which there is no available vaccine or therapy. Agents with a close or identical antigenic relationship to BSL-4 agents also should be handled at this level. When sufficient data are obtained, work with these agents may continue at this level or at a lower level. Viruses such as Marburg or Congo-Crimean hemorrhagic fever are manipulated at BSL- 4. The primary hazards to personnel working with BSL-4 agents are respiratory exposure to infectious aerosols, mucous membrane or broken skin exposure to infectious droplets, and autoinoculation. All manipulations of potentially infectious diagnostic materials, isolates, and naturally or experimentally infected animals, pose a high risk of exposure and infection to laboratory personnel, theCommunity and the environment. The laboratory worker's complete isolation from aerosolized infectious materials is accomplished primarily by working in a Class III BSC or in a full-body, air-supplied positive-pressure personnel suit. The BSL-4 facility itself is generally a separate building or completely isolated zone with complex, specialized ventilation requirements and waste management systems to prevent release of viable agents to the environment. The laboratory director is specifically and primarily responsible for the safe operation of the laboratory. His/her knowledge and judgment are critical in assessing risks and appropriately applying these recommendations. The recommended biosafety level represents those conditions under which the agent can ordinarily be safely handled. Special characteristics of the agents used the training and experience of personnel, procedures being conducted and the nature or function of the laboratory may further influence the director in applying these recommendations (Figure 2).

Building the suit lab

Planning a laboratory with Biosafety Level 4 (BSL-4) [36] means to apply the maximum standards out in individual segments [37-39], they are subjected to the characteristics of laboratory containment Biosafety Level 3 [40] with additions of security [41-43]. BSL-4 must be located in a separate building or in a clearly delineated zone within a secure building. BSL4 -labs have been compared to ―submarines inside bank vaults.‖ Heat, pressure, and chemical systems housed in the vault area process, or ―cook,‖ all liquid and solid wastes completely, and high-efficiency filtration with HEPA filtered breathing air. The breathing air systems must have redundant compressors, failure alarms and emergency backup [44], removes any airborne material, making all the liquid and air effluents sterile or safe before they leave the facility. Double and triple redundancies in equipment and systems help ensure that if an unexpected failure does occur, a backup is in place to maintain safety [45-48].

Entry and exit of personnel and supplies must be through an airlock or pass-through system. On entering, personnel must put on a complete change of clothing; before leaving, they should shower before putting on their street clothing [49]. The BSL-4 facility design parameters and operational procedures must be documented. The facility must be tested to verify that the design and operational parameters have been met prior to operation. Facilities must also be re-verified annually.

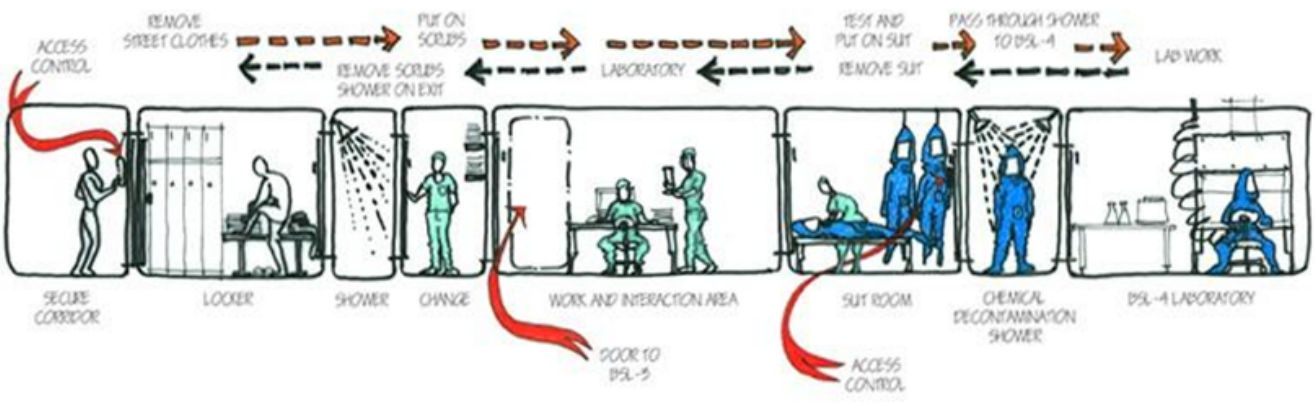

Figure 2: This illustration shows the typical process of entering and exiting a BSL-4 laboratory, though specifics might vary from facility to facility.

Verification criteria should be modified as necessary by operational experience. Appropriate communication systems must be provided between the laboratory and the outside (e.g., voice, fax, and computer). Provisions for emergency communication and emergency access or egress must be developed and implemented [50,51].

All procedures must be conducted by personnel wearing a one-piece positive pressure supplied air suit. All manipulations of infectious agents must be performed within a BSC or other primary barrier system [52,53]. Workers must wear laboratory clothing, such as scrub suits, before entering the room used for donning positive pressure suits. All laboratory clothing must be removed in the dirty side change room before entering the personal shower [54]. Consequently, laboratories having capabilities to work with biological agents, even though they do not posses select agents, are not currently subject to oversight. These laboratories also have associated biosecurity risks because of their potential as targets for terrorism or theft by either internal or external perpetrators. A laboratory outside the select agent program also represents a capability that can be paired with dangerous pathogens and skilled but ill-intentioned scientists to become a threat [54,55].

There are two models for BSL-4 laboratories:

- Cabinet Laboratory- Manipulation of agents must be performed in a Class III BSC;
- Suit Laboratory- Personnel must wear a positive pressure supplied air protective suit [56]. The BSL-4 suit laboratory consists of either a separate building or a clearly demarcated and isolated zone within a building [57,58].

Monitoring and control systems for air supply, exhaust, life support, alarms, entry and exit controls, and security systems should be on a UPS. An automatically activated emergency power source must be provided, at a minimum, for the laboratory exhaust system, life support systems, alarms, lighting, entry and exit controls, BSCs, and door gaskets [59]. A double-door autoclave, dunk tank, or fumigation chamber must be provided at the containment barrier for the passage of materials, supplies, or equipment in or out of the laboratory. Sinks inside the suit laboratory should be placed near procedure areas and be connected to the wastewater decontamination system. Walls, floors, and ceilings of the laboratory must be constructed to form a sealed internal shell to facilitate fumigation and prohibit animal and insect intrusion. The internal surfaces of this shell must be resistant to chemicals used for cleaning and decontamination of the area. Floors must be monolithic, sealed and coved. All penetrations in the internal shell of the laboratory, suit storage room and the inner change room must be sealed. Drains, if present, in the laboratory floor must be connected directly to the liquid waste decontamination system. Sewer vents must have protection against insect and animal intrusion. Services and plumbing that penetrate the laboratory walls, floors, or ceiling must be installed to ensure that no backflow from the laboratory occurs. These penetrations must be fitted with two (in series) backflow prevention devices. Consideration should be given to locating these devices outside of containment.

Atmospheric venting systems must be provided with two HEPA filters in series and be sealed up to the second filter [60].Redundant exhaust fans are required. Supply and exhaust fans must be interlocked to prevent positive pressurization of the laboratory. The ventilation system must be monitored and alarmed to indicate malfunction or deviation from design parameters. A visual monitoring device must be installed near the clean change room so proper differential pressures within the laboratory may be verified prior to entry. Supply air to the laboratory, including the decontamination shower, must pass through a HEPA filter. All exhaust air from the suit laboratory, decontamination shower and fumigation or decontamination chambers must pass through two HEPA filters, in series, before discharge to the outside. The exhaust air discharge must be located away from occupied spaces and air intakes. All HEPA filters must be located as near as practicable to the laboratory in order to minimize the length of potentially contaminated ductwork and they must be tested and certified annually. The HEPA filter housings must be designed to allow for in situ decontamination and validation of the filter prior to removal. The design of the HEPA filter housing must have gas-tight isolation dampers, decontamination ports, and ability to scan each filter assembly for leaks. Pass through dunk tanks, fumigation chambers, or equivalent decontamination methods must be provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the BSL-4 laboratory. Access to the exit side of the pass-through shall be limited to those individuals authorized to be in the BSL-4 laboratory. Liquid effluents from chemical showers, sinks, floor drains, autoclave chambers, and other sources within the laboratory must be decontaminated by a proven method, preferably heat treatment, before being discharged to the sanitary sewer. Decontamination of all liquid wastes must be documented. The decontamination process for liquid wastes must be validated physically and biologically. Biological validation must be performed annually or more often if required by institutional policy. Effluents from personal body showers and toilets may be discharged to the sanitary sewer without treatment. Gas and liquid discharge from the autoclave chamber must be decontaminated. When feasible, autoclave decontamination processes should be designed so that unfiltered air or steam exposed to infectious material cannot be released to the environment.

Biosafety and biosecurity concerns

Laboratory biosecurity is a relatively new concept that is still developing and there is currently little consensus across Europe as to what biosecurity means, even within the laboratory environment. Biosafety - Europe has used the term ―Laboratory Biosecurity‖ to describe protection against, control of, and accountability for biological material and information within laboratories, in order to prevent their loss, theft, misuse, diversion, unauthorized access or intentional unauthorized release.

EU level legislation exists that has been specifically developed to address the protection of biological agents in the laboratory from loss or willful misuse. However due to the many synergies between biosafety and biosecurity, the EU Directives developed to protect workers from exposure to GMMs address most of the issues related to laboratory biosecurity. Only a limited number of Member States have introduced special laboratory biosecurity legislation. Many facilities do implement some biosecurity controls but these are often not based on risk assessment and are often focused on physical security. Less attention is focused on information security or organizational security issues, despite the fact that internal threats from individuals with authorized access to the laboratory must be recognized. Biosafety Europe is a coordination action funded through the 6th Framework Programmed of the European Commission (EC), which aims to explore harmonization and exchange of biosafety and biosecurity practices within a pan-European network [61,62]. Effective design and implementation of Biosecurity‘s Laboratory depends on cooperation among individuals from diverse communities, including scientists, technicians, policy makers, security engineers, and law enforcement officials [63,64].

Biosafety Associations play an important role in the enhancement of biosafety and biosecurity through awareness raising, sharing of resources and the promotion of best practices. The mission of Laboratory Associated Infections (LAI) from exposure to biological agents known to cause disease is no frequent. It is critical that the microbiological and biomedical community continue its resolve to remain vigilant and not to become complacent. LAI is to be a forum for discussion and knowledge exchange in order to strengthen Biosafety in Europe by bringing together experts in the fields of biological safety, biosecurity, biotechnology, transport and associated activities [65-67].

European Network of P4 Laboratories (EURONET-P4)

In Europe, there are seven Biosafety-Level-4 (BSL - 4) able to process and confirm the presence in the samples, and specimens of high-risk agents, such: Viral hemorrhagic fevers, in five countries [68] (Figure 3).

- INMI L. Spallanzani, Rome, Italy
- Health Protection Agency, London and Porton Down, UK
- Philipps Universitat Marburg, Germany
- Bernhardt Nocht Institute of Tropical Medicine, Hamburg, Germany
- Swedish Institute for Infectious Disease Control, Solna, Sweden
- Inserm, Lab. P3/P4 Jean Mérieux, Lyon, France (as of 2007).

The European Network of P4 Laboratories (EURONET-P4) created in 2005 [69] by INMI IRCCS L. Spallanzani. Purpose of the current Euro Net P4 Biosafety and Biosecurity work package has taken into account current international and various national guidelines relating to biosafety, biosecurity and training requirements associated with the operation and management of Biosafety level 4 laboratories. This activity forms the basis of establishing a framework checklist 'that underpins a workable and agreed measurable audit and guidance system. This _checklist' system aims to provide the confidence that current and planned BSL-4 facilities comply with defined specific performance indicators and standards of essential systems that can be considered through the implementation of a robust European Audit system [70]. It also promotes harmonisation and standardisation of biosafety practices and diagnostic procedures and offers assistance to countries where new BSL-4 laboratories are being conceived, planned, constructed or comm. issioned; (to provide diagnostic services high quality ensured in all Member States, identify *Hemorrhagic fever viruses and smallpox*, establish services available 24 hours to 24 hours seven days week days, to communicate quickly with national authorities and the Commission, to develop a framework for sending / receive and treat the samples and to organize training courses o develop expertise). Nevertheless the laboratory capacity is always and still no sufficient in many Member States, so it is therefore necessary that the states with improved infrastructure should pool making them its resources to benefit Member States do not have [71].

To identify L3 laboratories click on the yellow dots, for the L4 laboratories on the red ones. (Or on the name of the cities below the picture) orange dots indicate places where L4 laboratories are planned .

L4 Laboratories: Hamburg HPA London Lyon Marburg Rome (L. Spallanzani) HPA Porton Down Stockholm

L3 Laboratories: Berlin Helsinki Munich Paris Rotterdam Thessaloniki Madrid Marseille Rome (ISS) Orense Ljubljana

Figure 3: L3/L4 Laboratories in Europe.

2011 will mark the first —Year of Biosafety in Europe‖. The European Centre for Disease Prevention and Control ECDC) together with the European Biosafety Association (EBSA) [72], have planned a special session at this year's annual EBSA conference to bring together laboratory and biosafety experts to discuss the development of effective pan-European biosafety network; Objective of the session is to bring together ECDC, EBSA, and representatives of successful national associations and delegates from countries less well networked regionally and internationally to discuss how to strengthen biosafety in the EU/EEA Member States as well as the development of a wider European Biosafety Community. Funding from ECDC, with additional support from EBSA, has been made available to MS (through designated National Microbiology Focal Points) to send a delegate to attend the conference. Facilitation of the discussions and output recommendations are under the scientific leadership of Allan Bennett (Health Protection

Agency, UK), as part of his coordination of the ECDC funded project — Bio risk Initiative for Capacity building and Knowledge base development (BRICK) [73].

European Union and Health Safety

Health Security is an increasingly important issue inside European policies both security and health. To develop European policies on Health Security has been established in 2001 for the Health Security Committee (HSC).Committee has representatives from all EU countries, its operates in 3 core areas: generic preparedness, influenza, and chemical, biological and radio-nuclear (CBRN) threats and it has a multi-year work program, closely linked to the authorities of Member States so as to improve the ability to develop concrete actions to sensitive about security health care [74]. The Commission shall be a liaison between the Global Health Security Initiative (GHSI) [75] and the Health Security Committee to ensure that coherence of the work done by these institutions.

The health aspects of bioterrorism against the EU shall be grouped in the Commission communication of 2 June 2003 [76-78], the Council and the European Parliament on cooperation in the European Union related to preparedness and response to biological and chemical terrorist attacks (health Security), which refers to problems and

challenges regarding the preparedness and response, which is in front of the health sector, on which rests the burden is to quickly detect biological and chemical agents is to identify at an early stage and treat individuals exposed to these agents. In its conclusions of 22 February 2007 on the Health Security Committee 7 the Council extended the HSC' planning in addition to its competence in the field of CBRN, and that in its conclusions of 16 December 2008 on health security 8 the Council emphasized the necessity to improve and strengthen the coordination of responses to CBRN threats; on 24 June 2009 the Commission adopted its communication on strengthening Chemical, Biological, Radiological and Nuclear security in the European Union - an EU CBRN Action Plan [79].

Standoff detection is a measure crucial to triggering the alarms been attack and to adopt measures to reaction, in the case of a biological attack with the release of a biological agent from one source rather remotely. Need to set up a mechanism for information exchange, consultation and co-ordination for the handling of health –related issues related to attacks:

- create an EU-wide capability for the timely detection and identification of biological and chemical agents that might be used in attacks and for the rapid and reliable determination and diagnosis of relevant cases;
- create a medicines stock and health services database and a stand-by facility for making medicines and health care specialists available in cases of suspected or unfolding attacks;
- Draw-up rules and disseminate guidance on facing-up to attacks from the health point of view and co-ordinating the EU response and links with third countries and international organizations.

The importance of joint action in the EU to complement national measures led to the establishment on 26 October 2001 of a Health Security Committee, comprised of high-level representatives of the Health Ministers, to serve as the main instrument for cooperation in countering deliberate releases of biological and chemical agents to cause harm and the setting up in 2002 of a Task Force of national experts and Commission officials to implement an action programme to enhance health security. To give effect to the request of the Health Ministers of 15 November 2001 the Committee agreed on 17 December 2001 a programme of cooperation on preparedness and response to biological and chemical agent attacks (health security), code-named BICHAT, comprising 25 actions grouped under four objectives including to create an EU-wide capability for the timely detection and identification of biological and chemical agents that might be used in attacks and for the rapid and reliable determination and diagnosis of relevant cases [80].

Italian Overview

Italy was one of the states that have joined:

- Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction (Biological and Toxin Weapons Convention, BTWC).
- Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on their Destruction (Chemical Weapons Convention).
- Australia Group [81,82].

In October 2001, the Italian Minister of Health was presented National Emergency Plan against the Chemical, Biological, Nuclear terrorism. Regard biological terrorism, the National Plan provides two centers: Hospital "L. Spallanzani" in Rome and the Hospital "L. Sacco" in Milan.

The emergency of bioterrorism has also mobilized the attention of researchers of Italian National Institute of Health (Istituto Superiorei di Sanità - ISS) to the improvement of diagnostic systems, for the preparation of microbiological procedures for the definition of the levels of safety in the laboratory in relation to the risk group membership of pathogens, to the training of staff working in the National Health Service. The activity of the Department of Infectious, Parasitic and Immune-Mediated Diseases aims at protecting the human population from diseases caused by pathogenic micro-organisms, viruses and parasites, and to study the mechanisms of immune-mediated diseases. The Department carries out research and provides advice and services in the field of epidemic infections caused by viruses, bacteria, fungi and parasites, and immune-mediated diseases, with special emphasis on poverty-related diseases (AIDS, tuberculosis, malaria). It is also responsible for the control of infectious and immunological emergencies and for the preparation of plans to respond to possible bioterrorist attacks. Among the many projects currently in progress are the generation, application and control of new cellular and molecular tools for the prevention, diagnosis and treatment of infectious, parasitic and immune-mediated diseases, with a special focus on vaccines and vaccination. The Department is also responsible for monitoring the efficacy and quality of existing and new vaccines and immunotherapeutic tools. Some of the current studies aim to improve the treatment of infections through the development of new antibiotics, the rational use of available chemotherapeutic drugs and measures to fight resistance to antibiotics.

The Department has laboratories safety class 3, expected to work microorganisms of risk group 3 and worked for the development of diagnostic strategies for conventional and definitive identification of bacterial pathogens that could be used for the purpose bioterrorist using conventional and molecular methods. For the *B. anthracis* were also prepared genotyping protocols useful for the identification of the source of release. (The methods used for the diagnosis and the final confirmation of *B. anthracis* were validated at the international level. It 'an ongoing research project on anthrax and other diseases by bacterial pathogens of class A). The tools for a successful attribution include genetically based-assays to determine the exact strain of isolate, aiming the individualization of the source of the pathogen used in a biological weapon. Following the 2001 anthrax attacks, genotyping of B. anthracis was done on 8 variable number tandem repeats loci (VNTR polymorphisms), with multilocus variable number tandem repeats (MLVA) method. In recent years some research groups have increased the VNTR markers number to 25 loci, while other groups have identified single nucleotide repeat (SNR) polymorphisms, which display very high mutation rates. SNR marker system allows the distinguishing of isolates with extremely low levels of genetic diversity within the same MLVA genotype [83].

The Italian Ministry of Health has been designated as national reference center for anthrax, the Institute Zooprofilattico Puglia and Basilicata (Cerna), based in Foggia. It already was a deputy to the preparation of the two vaccines against anthrax, and Carbosap Pasteur

[84], and prialla testing of new vaccine Sterne [85]. The center has the task to test the detection anthrax spores in suspicious samples (with the exception human clinical suspicion) as part of the emergency bioterrorism, which consists in the amplification nucleotide sequences specific for chromosome, the lethal factor, edema factor, the antigen and the protective capsule of *Bacillus anthracis* [86].

With reference to the operating modes, however, the Ministry of Health has ordered that the suspect samples (envelopes, letters or other material containing powders), identified at local level, are taken from the body Fire Brigade, transported to the hospital nearest equipped with an autoclave and sterilized immediately at 121°C for 45 minutes, before being sent at the center. This measure minimizes the possibility of dissemination of the pathogen in the environment and ensures the safety of the operators at all stages, from transportation to processing. At the center, in addition, has been also entrusted with the task to constantly update the map of Italian genotypes of *B. anthracis* [87,88].

Italian Red Cross Vs Biological Agent

National Institute for Infectious Diseases (INMI) and the Hospital "Sacco" during the first SARS's emergency, was identified as the two poles of national for the care of any affected patients, with an investment in terms of infrastructure (hospital rooms special ambulances) for improving conditions for isolation of patients.

Now the Italian Red Cross has adopted the first and only Italian vehicle for management of potentially exposed to highly contagious biological agents, that not requiring carriage on a stretcher, and their transfer in biosecurity [89].

This kind of technology comes from Israel and it is already in use by Italian Armed Forces and other Italian's Institutions. It allows the realization of filter pressurized chambers where the possibility of isolation is technically limited. It guarantees safety for the operator and the community. The prototype is a Fiat Ducato Combi Flex Floor 2.3 to 9 people, arranged with insulation module BETH-RL and with a unit-filter pressurized at high efficiency HEPA (High Efficiency Pa reticular Air) (Figure 4) which ensures the control of the flow of air with variable and setting according the number of people transported (maximum of negative internal pressure equivalent to 80/10/50 Pascal) and with filtering the air coming out (Figure 5). Inside the car is also present metallic structure, with functions of support and anchorage for the room in PVC, high resistance connected to the floor through suitable corridors of aluminum (Figure 6). The frame can be easily disassembled and reassembled by health care teams and can be used with different cars.

Figure 4: Fiat Ducato Combi Flex Floor 2.3 to 9 people arranged with insulation module BETH-RL and with a unit-filter pressurized at high efficiency HEPA (High Efficiency Pa reticular Air).

Figure 5: A filter unit external pressurization HEPA ensures the control of the air flow, variable and adjustable according to the number persons transported and the negative internal pressure (Pascal 80/10/50).

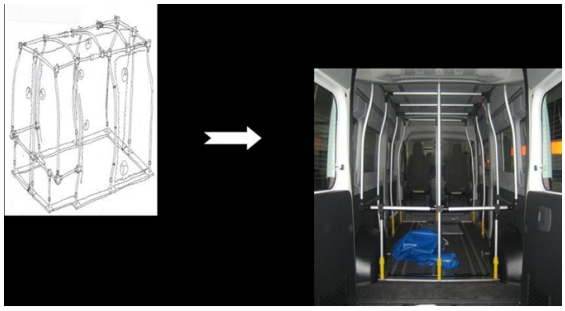

Figure 6: The metal structure arm serves as a support and anchoring room in high resistance PVC, sealed to the floor by means of special corridors aluminum.

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