



Biosafety Risk Assessment of the Severe Acute Respiratory Syndrome (SARS) Coronavirus and Containment Measures for the Diagnostic and Research Laboratories

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Abstract

At the end of 2002, an outbreak of a new viral respiratory illness, called SARS (Severe Acute Respiratory Syndrome virus), occurred in China. The disease spread over Asia, North America, Europe, and Africa. In response to the SARS outbreak, the World Health Organization (WHO) coordinated an international collaboration that included clinical, epidemiologic, and laboratory investigations, and initiated efforts to control the spread of SARS. As in other countries, Belgium has been decided to establish biosafety guidelines and recommendations with particular emphasis on handling clinical specimens associated with SARS for research, production, and clinical laboratories. Taking into account that there is so far no SARS case reported in Belgium as well as in other countries in the world, and based on a scientific risk assessment related to the contained use of biological agents, the SARS-CoV was

classified as a Risk Group 3 agent. In relation to the reported biosafety assessment, the SARS-CoV should be handled in appropriate biosafety containment levels to avoid laboratory-acquired infections and spread of the disease in the human population and the environment. Therefore, diagnostic activities with inactivated clinical specimens associated with SARS cases and with specimens originating in countries where SARS is documented but not associated with SARS cases should be performed under Biosafety Level 2 (BSL-2) conditions. Diagnostic activities involving non inactivated clinical specimens associated with SARS should be carried out under BSL-2 containment with BSL-3 safety equipment and work practices. Culture of SARS-CoV and all research activities involving SARS-CoV require a BSL-3 containment.

Introduction

Severe Acute Respiratory Syndrome (SARS) is a respiratory illness which originated in the Guangdong Province in China. The earliest known cases were identified in mid-November 2002. The syndrome spread to Asia, North America, Europe, and Africa, with 8,422 suspected and probable cases of

SARS, including 916 deaths, reported to the World Health Organization (WHO) by August 7, 2003 (WHO, Summary Table, 2003). The rate of mild or asymptomatic SARS infections is still not known.

This outbreak raised biosafety concerns and, specifically, the question of risk assessment regarding the contained use of SARS-CoV for laboratory work. This paper focuses on this risk assessment but first gives a short description of the SARS outbreak and a listing of existing guidelines related to the handling of SARS-CoV in clinical, research, or production laboratories. As a result of the risk assessment that has been performed, biosafety recommendations are set out and should be applied.

The mechanisms of SARS transmission remain unclear. However, on the basis of the reported exposures for the majority of cases (i.e., household contacts and health care workers), the primary ways that SARS appears to spread is by close person-to-person contact involving exposure to infectious droplets of respiratory secretions from an infected person, by the faecal-oral route, and likely by mechanical transmission (WHO, Severe Acute Respiratory Syndrome, 2003). Indirect transmission by aerosolised material or animal vectors such as roof rats is not excluded as suggested by the peculiar outbreak in the Amoy Gardens in Hong Kong (Ng, 2003).

In general, SARS begins with fever above 38.0°C. Other symptoms may include headache, cough or breathing difficulty, an overall feeling of discomfort, and body aches. At least 69% of SARS-infected persons have respiratory symptoms (Booth et al., 2003). In about 10%-20% of cases, patients require mechanical ventilation (WHO, Severe Acute Respiratory Syndrome, 2003). At this moment no other treatment beyond intensive and supportive care can be offered. Empiric therapy should include coverage for organisms associated with any community-acquired pneumonia of unclear aetiology.

According to the latest findings, the etiologic agent responsible for the syndrome is a previously unrecognised virus belonging to the family of coronaviruses, currently called SARS coronavirus, or SARS-CoV (Drosten et al., 2003; Ksiazek et al., 2003; Peiris et al., 2003). Although uncertainties remain regarding the exact source of the virus, palm civets (*Paguma larvata*) and possibly other small mam-

mals such as badgers (*Melogale moschata*) and raccoon dogs (*Nyctereutes procyonoides*) sold in the animal markets of Southern China have been found to be infected with SARS-CoV indicating interspecies transmission, although the natural reservoir of the virus is unknown (Guan et al., 2003).

In response to the SARS outbreak, WHO coordinated an international collaboration that included clinical, epidemiological, and laboratory investigations, and initiated efforts to control the spread of SARS. In this respect, several organisations and countries developed biosafety guidelines and recommendations, with particular emphasis on handling clinical specimens associated with SARS (WHO, Biosafety Guidelines, 2003; CDC, 2003; Health Canada, 2003; Health & Safety Executive, 2003; Ministère de la Santé, 2003; Landelijke Coördinatiestructuur Infectieziektebestrijding, 2003).

According to these guidelines, routine diagnostic tests involving samples from potential SARS cases should be performed in BSL-2 facilities, using additional operational practices aiming primarily at protecting laboratory workers from potential droplets and aerosols (manipulations to be performed in a biological safety cabinet, use of sealed centrifuge rotors or sample cups, and use of personal protective equipment). Activities involving culturing the etiologic agent should be performed in BSL-3 facilities with corresponding operational practices. Manipulations involving the inoculation of animals for potential recovery or for characterisation of the etiologic agent should be performed in BSL-3 animal facilities. Moreover, the UK Advisory Committee on Dangerous Pathogens considered that the SARS-CoV should be classified as a Risk Group 3 virus.

As of 2004, no SARS case has been reported in Belgium (Ministry of Public Health, 2003). However, to conduct SARS-CoV research work in Belgian laboratories, a risk group and the biosafety levels for SARS-CoV have to be determined, based on a thorough risk assessment.

At the European Union level, contained uses of genetically modified microorganisms, pathogenic or not, are subjected to the provisions of Directive 90/219/EEC as amended by Directive 98/81/EC (Official Journal of the EC, 1990). Activities involving human pathogens and recombinant relatives en-

ter the scope of Directive 2000/54/EC repealing Directive 90/679/EEC and regulating the protection of workers exposed to biological agents at work (Official Journal of the EC, 2000). In Belgium, Directive 90/219/EEC has been implemented in the general frame of biosafety regulation as part of the Regional Environmental Laws for classified installations; this has also been extended to the contained use of any pathogenic organism for humans, animals, and plants. These regulations describe which risk assessment procedure to perform. When performing the case-by-case assessment of an activity with a given pathogen, it is important to first take into account the classification of this agent into one of the four risk groups.

However, in order to assess proper practices and containment measures, additional factors should also be considered. These include absence of the virus in the human population, pathogenicity and infectious dose of the agent, natural route of infection as well as other routes of infection resulting from laboratory manipulations (parenteral, airborne, ingestion), the stability of the agent in the environment, concentration of the agent and volume of concentrated material to be manipulated, and presence of a susceptible host (human or animal). Additional important information includes previous cases of laboratory-acquired infection, availability of effective prophylaxis or therapeutic interventions, and planned laboratory activities (concentration, sonication, aerosolization, centrifugation, etc.).

Biosafety Risk Assessment of Laboratory Activities Using SARS Virus

An undefined coronavirus detected in patients with SARS is agreed to be the causal vector of the disease and the epidemic. The coronaviruses belong to a genus of viruses with an outer envelope bearing distinctive spikes that have a halo or crown-like appearance under the electron microscope. Presently 15 species are identified in this genus, most notably common cold viruses in humans. Coronaviruses are nonsegmented, single-stranded (+) sense RNA viruses, with a 27-32 kb-long genome. They can function as mRNAs, and the purified genomic RNA is

infectious (Lai & Holmes, 2001). The RNA genome and the viral nucleocapsid phosphoprotein form a helical nucleocapsid. The SARS-CoV belongs to a virus family characterized by a high frequency of point mutations, large deletions or insertions of foreign RNA (Holmes, 2003).

Recent studies indicate that SARS-CoV is neither a mutant of any known coronavirus nor a recombinant of known coronaviruses. It is a previously unknown coronavirus probably coming from an animal and able to infect humans (Guan et al., 2003). Today the whole nucleotide sequence of the SARS-CoV is available in the scientific community (Marra et al., 2003).

A recent report during the WHO Global Conference on SARS in Kuala Lumpur, Malaysia, identifies some small mammal pets from China (palm civet, badgers, and raccoon dogs) as a potential reservoir for the SARS-CoV. It is reasonably thought that SARS-CoV could have jumped from these animals to humans (WHO, Global Conference on SARS, 2003).

The pathogenicity of SARS-CoV is associated with a disease of the lower respiratory tract and death from progressive respiratory failure. The case fatality rate essentially depends on age and is estimated to be less than 1% in persons aged 24 years or younger, 6% in persons aged 25 to 44, 15% in persons aged 45 to 64, and greater than 50% in persons over the age of 65 (WHO, 2003). The rate of asymptomatic SARS infections is unknown but likely to be low.

The route of transmission is mainly through close person-to-person contact, exposure to droplets of respiratory secretions, the faecal-oral route, and mechanical transmission. However, some additional transmission routes remain undefined. The likelihood of SARS-CoV transmission through indirect transmission remains to be determined. SARS-CoV does not seem to be transmitted by aerosols (WHO, Consensus Document, 2003).

Coronaviruses are generally thermolabile. The length of time that the virus survives likely depends on a number of factors including the type of material or body fluid containing the virus and various environmental conditions such as temperature or humidity. Its survival under natural environmental conditions is still unknown and is presently being

researched at the CDC in Atlanta and other institutions. In animals the virus can be detected for several days to weeks, and it may survive in the environment for several days. According to WHO's reports (WHO, Laboratory Network, 2003), the SARS-CoV remains infectious 1 to 2 days in faeces and urine at room temperature. On an open plastic surface the virus survives 48 hours. The virus is more stable (up to 4 days) in stool from diarrhoea patients. The virus loses infectivity after exposure to different commonly used disinfectants and fixatives. SARS-CoV acetone fixation on glass slides for immunofluorescence assays at room temperature does not eliminate the virus efficiently unless the acetone is cooled down to -20°C (WHO, Laboratory Network, 2003).

The infectious dose of the agent is currently unknown (WHO, Severe Acute Respiratory Syndrome, 2003). The highest titre of infectious SARS-CoV is measured in a patient's sputum. Lower titres are found in blood during the acute phase of the illness. Excretions (10^6 virus particles/gram) through defecation can last 6 weeks from the onset of the disease (Landelijke Coördinatiestructuur Infectieziektebestrijding, 2003). No prophylactic or therapeutic treatment is available and no specific treatment recommendations can be made at this time. To date, no other treatment other than good intensive and supportive care can be offered.

Approximately 30% of SARS cases were reported to occur in health care workers in direct contact with patients, but until recently no SARS-CoV laboratory-acquired infections (LAI) were demonstrated (WHO, Severe Acute Respiratory Syndrome, 2003). In 2003 a doctoral student was infected by the virus in Singapore. This student worked in a virology laboratory not involved in SARS diagnosis, but he unwittingly manipulated SARS-CoV (Report of the Review Panel on New SARS Case and Biosafety, 2003). A second case of SARS LAI was reported in Taiwan (WHO, SARS Case in Laboratory Worker in Taiwan, 2003). More recently in China a Chinese laboratory-centered outbreak was reported (WHO, 2004). These cases are confirmed laboratory accidents and raise the question of biosafety for laboratories involved in SARS-CoV manipulation.

Several factors may influence the biosafety containment for laboratory manipulations of SARS.

The following factors should be taken into account in the risk assessment procedure: the laboratory activities that are planned with SARS-CoV, the length of time of exposure to the infectious agent, the concentration of the virus during exposure, the method and route of exposure, the nature of the manipulations to be performed (formation of aerosols, use of needles, lancets or sharp instruments), and also the possible changes of virulence in culture and in animals and the possible shedding of live virus by inoculated animals.

Risk Groups

Directive 2000/54/EC classifies human coronaviruses in Risk Group 2 (on a 1 to 4 scale). These coronaviruses cause up to 30% of common colds, and they rarely cause lower respiratory tract disease. In contrast, some animal coronaviruses causing devastating epizootics of respiratory or enteric disease in livestock and poultry are classified as pathogens of Risk Group 3.

With the emergence of SARS-CoV within the coronavirus family, all human coronaviruses should no longer be considered as Risk Group 2 biological agents. An interim class of biological risk for SARS-CoV should be defined and a risk assessment specifically dedicated to SARS-CoV should be conducted in response to the recent epidemic and the risk of exposure for laboratory workers.

Risk groups (also called classes of biological risk in other European regulations) are defined as follows in the Directive 2000/54/EC:

- Risk Group 2: *biological agent that can cause human disease and might be a hazard for directly exposed persons; it is unlikely to spread to the community; but there is usually effective prophylaxis or treatment available.*
- Risk Group 3: *biological agent that can cause severe human disease and present a serious hazard for directly exposed persons; it may present a risk of spreading to the community; but there is usually effective prophylaxis or treatment available.*
- Risk Group 4: *biological agent that causes severe human disease and is a serious hazard for directly exposed persons; it may present a high risk of spreading to the community; there is usually no effective prophylaxis or treatment available.*

To determine how to classify SARS-CoV into a risk group, Table 1 illustrates how Human influenza, Hantavirus and Ebola Virus, as examples, are classified. These infectious agents are compared with SARS-CoV's main known properties.

Considering the most recent available data and the whole biosafety risk assessment about the infectious agent causing atypical pneumonia, this virus should be classified as an agent of Risk Group 3. The key elements to classify the SARS-CoV in Risk

Table 1

Influenza, Hantavirus, and Ebola Virus Classification into Risk Groups Compared to SARS-CoV

Factors	Influenza (Health Canada, 2003)	Hantavirus (Health Canada, 2003)	Ebola Virus (Health Canada, 2003)	SARS-CoV
Characteristics	Orthomyxovirus enveloped (-) ssRNA	Hantavirus [§] enveloped (-) ssRNA	Filovirus enveloped (-) ssRNA	Coronavirus enveloped (+) ssRNA
Host range	Influenza A: humans, swine, horses, avian Influenza B: humans only	Humans, field rodents (mice and rats)	Humans, monkeys, chimpanzees, guinea pigs	Humans
Pathogenicity	Variable, fatality generally low	Variable, fatality generally low	50%-90% case fatality rate	14%-15 % case fatality rate
Infectious dose	Influenza A: 2 - 790 p.f.u.	Unknown	Unknown*	Unknown
Mode of transmission	By direct contact through infectious droplets, aerosols	Aerosol transmission, ingestion, contact of infectious materials via mucous membrane, bites	Intimate contact, contaminated syringes and needles, droplets and aerosols	Direct contact, droplets. Faecal oral route possible.
Reservoir	Humans and animals (swine)	Field rodents	Unknown**	Unknown
Vectors	None	None	Unknown	Unknown
Survival outside the host	Several hours in dried mucus	Sensitive to drying, several hours at 37°C in neutral solution	Several weeks at RT in blood, several weeks in corpses, does not survive after drying	Weeks in animals, 2 days on surfaces, in faeces and urine
Treatment	Neuraminidase inhibitors	Ribavirin (in early phase)	Not available	Not available
Immunization	Available for serotypes A and B	None available	None available	None available
LAI	Not documented	Yes	Yes	Yes***
Geographical localization	No	No	Yes	No
Risk Group	2	3	4	3

RT: room temperature

LAI: Laboratory-acquired infection

[§]25 antigenically distinguishable viral species

*Less than 10 units by aerosol for non-human primates

**Antibodies found in domestic guinea pigs but no evidence of transmission to humans.

***Four confirmed cases have been reported.

Group 3 and not Risk Group 4 are the moderate fatality rate and the fact that SARS-CoV seems not to be transmitted by aerosols (WHO, Consensus document, 2003). The key elements to classify the SARS-CoV in Risk Group 3 and not Risk Group 2 are the medium fatality rate and the unknowns, such as infectious dose, natural reservoir(s), and vector(s).

Biosafety Containment Levels

Primary laboratory activities are diagnosis, research, and production (manufacturing of diagnostic tests). To define the containment levels in which these laboratory activities must be performed, the biological risk associated with SARS-CoV handling is reviewed, taking into account the characteristics of these activities.

Diagnostic activities involve specimens from countries where SARS is documented and samples from suspect, probable, or confirmed SARS cases. The type of samples can be categorized as a function of the viral load. Samples with low viral load are serum, plasma, and blood. Samples with high viral load are respiratory samples containing respiratory cells and secretions (nasopharyngeal aspirates, nasal washes, combined nose and throat swabs, bronchoalveolar lavages, and sputa), urine, and stool. Other samples with variable or unknown viral loads are cerebrospinal fluid, pleural fluid, pericardial fluid, tissue biopsies, or any other specimens taken as part of the clinical diagnostic workup.

Some laboratory activities increase the risk of exposure to SARS-CoV. These include any analysis of a cell culture, where, although not intended to grow SARS-CoV, viral replication is possible and the formation of aerosols (pipetting, homogenization, centrifugation) during sample handling and other manipulations of SARS specimens such as inoculation of bacterial or fungal culture media could occur. However, other activities reduce the risk by inactivation of the SARS-CoV. These include formalin-fixation or any other inactivation of tissues for examination, manipulation of extracted nucleic acid preparations for PCR, preparation of glutaraldehyde-fixed grids for electron microscopic studies, and fixation of smears for routine staining and microscopic analysis.

Currently, diagnostic tests are available to iden-

tify SARS. These include an ELISA antibody tests to detect IgM/IgA in the serum of SARS patients, immunofluorescence assay to detect a patient's IgM antibodies in SARS-CoV-infected VERO cells, electron microscopy of biopsies, and biochips (indirect immunofluorescence test for the detection of IgM/IgG based on a paired slides test with one of the slides coated with SARS-CoV-infected cells and the other coated with noninfected cells and used as negative control. Viral cell culture also constitutes an important step for *in vitro* diagnosis. The risk of exposure when performing all the cell culture steps (cell culture initiation, pipetting, cell washing, trypsinization, centrifugation, and culture refeeding) must always be considered.

Molecular tests (RT-PCR) involve the manipulation of extracted nucleic acids from various samples (sputum, nasopharyngeal aspirate, stool, etc.). Research and development activities, "regular scale" or large scale, involve deliberate propagation of the SARS-CoV generally in much larger quantities than in diagnostic activities and need additional protection for laboratory workers and the environment.

For manipulations with infected animals the main risks include bites, scratches, exposure to body fluids, and droplets coming out of the respiratory tract or any other excretion route. It should be noted that the SARS-CoV is able to survive several days to weeks in animals. Animal models are used in the following contexts: inoculation of animals for potential recovery of the SARS-CoV from suspect or probable samples cases and all other studies where animal injections are planned with a known or putative SARS agent in research settings.

Taking into account the type of manipulation performed and the biological risk assessment presented, SARS-CoV should be handled according to appropriate containment measures in order to avoid laboratory-acquired infection and spread of disease to close contacts and to the environment.

Biosafety Recommendations

Diagnostic Activities

Diagnostic activities include all diagnostic tests mentioned above. Clinical specimens associated with SARS that have been inactivated by appropriate

and validated methods, as well as clinical specimens originating from countries where SARS is documented but not associated with suspect, probable, or confirmed SARS cases should be performed using standard BSL-2 precautions, equipment, and work practices. It should be noted that the above-mentioned inactivated clinical specimens might contain SARS-CoV infectious RNA. In any case, standard precautions should be taken to avoid accidental injection. The use of needles and other sharp instruments should be avoided when manipulating inactivated clinical specimens, or, if this is not possible, the instruments should be adequately managed to prevent or reduce the risk of percutaneous injuries. Diagnostic activities involving handling and processing of non inactivated clinical specimens associated with suspect or probable cases of SARS should be performed in BSL-2 facilities with BSL-3 safety equipment and work practices, as long as no viral cell culture or manipulation of cultured SARS-CoV is performed.

Attention should be focused on the following biosafety measures:

- Appropriate and suitable personal protective equipment should be worn when manipulating clinical specimens known to transmit SARS-CoV. Laboratory workers must wear gloves, solid-front or wrap-around gowns with cuffed sleeves, and respiratory and face mucosal protection (e.g., respirators, face shields). Appropriate systems of respiratory protection with HEPA filtration (N/R/P/95/99/100 or FFP2, 3 filter level) should be worn. For example,

particle-filtering halfmask FFP3 is recommended (CEN EN 149 norm, 2001).

- Sera should be inactivated by exposure at 58°C for 30 minutes before analysing.
- Careful attention should be given to hand hygiene after removal of gloves. The BSL-2 area must have a hands-free or automatically operated sink for handwashing and decontamination.
- All manipulations of specimens should be carried out in a certified class II biological safety cabinet. Sealed centrifuge rotors or safety cups should be used for centrifugation. These rotors or cups should be loaded and unloaded in a biological class II safety cabinet.
- Work surfaces and equipment should be decontaminated after specimens have been processed. Standard decontamination agents that are effective against lipid-enveloped viruses are sufficient.

A description of required facilities, equipment, and work practices may be found respectively in Tables 2, 4, and 5. Based on the risk assessment, all containment measures should be adequate for work with viral agents with emphasis on potential spread by droplets and/or contaminated surfaces and objects.

This specific topic gave the opportunity to highlight that Good Microbiological Practices apply to all types of handled clinical samples. Local biosafety officers must conduct appropriate personnel training and insist about the following points: good house-keeping and good personal hygiene; the consistent use of Standard Operating Procedures and guide-

Table 2

Biosafety Level-2—Laboratory Facilities: Design Features and Technical Characteristics

<ul style="list-style-type: none">• The laboratory is physically separated from other activity areas in the same building.• The access doors into the laboratory are lockable when the corridor or the area does not have restricted access.• The access doors into the laboratory are provided with an automatic closing system when they directly give access to a public area.• Furniture is designed to facilitate room cleaning as well as a control program for rodents and insects.• The laboratory has a sink for handwashing and decontamination.• The personnel have access to a change room for protective clothing. Protective and street clothing cannot be in contact.• Bench tops are easy to clean, impervious to water, and resistant to acids, alkalis, organic solvents, and those disinfectants and chemicals normally used in the laboratory for decontamination.
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Table 3

Biosafety Level-3—Laboratory Facilities: Design Features and Technical Characteristics

- The laboratory is physically separated from other activity areas in the same building or is located in a separate building.
- The entry into the laboratory occurs through an airlock.
- The first door of the airlock is lockable and controlled through an electronic system or equivalent.
- The doors of the airlock are provided with an automatic closing system. A mechanism is installed which prevents the inner and outer doors of the airlock to be opened at the same time (e.g., by an interlocking system). This mechanism does not interfere with assistance in case of emergency.
- Windows are hermetically sealed.
- Rooms are sealable to allow decontamination with a gaseous substance.
- Furniture is designed to facilitate room cleaning and decontamination as well as a control program for rodents and insects.
- The laboratory contains an observation window or an equivalent system so that occupants can be seen from outside.
- The contained area has a hands-free or automatically operated sink for handwashing and decontamination. The sink is located in the airlock or near the room exit door.
- An emergency shower may be located either in the laboratory or in the airlock.
- The personnel have access to a change room for protective clothing. Protective and street clothing cannot be in contact.
- The supply fluid conducts are provided with anti-reflux devices.
- Floor and bench tops are easy to clean, impervious to water, and resistant to acids, alkalis, organic solvents, and those disinfectants and chemicals used for decontamination.
- Back-up power is provided in case of power failure.
- The laboratory has an automatic system for fire detection and alarm.
- The contained area has an interphone, a phone, or any other system to communicate with the outside.
- The work area is permanently held at a negative air pressure relative to the pressure of the adjacent areas to avoid any transfer of contamination from inside to outside.
- A manometer controls the relative negative air pressure. It is recommended that an alarm system turns on in case of failure of the ventilation system.
- When the supply air is not provided by a system independent of adjacent areas (a dedicated system is recommended), airtight back draft dampers or a HEPA filter are installed. When the exhaust air is not discharged by a system independent of adjacent areas (a dedicated system is recommended), a second HEPA filter is installed in the exhaust system.
- Supply and exhaust air systems are interlocked to avoid a positive pressurization in case of accidental failure of the exhaust system.
- The supply and exhaust air systems can be sealed with dampers.
- Air is exhausted from the laboratory after filtration through a HEPA filter. This air cannot be recirculated within the building or within adjacent buildings or discharged near air intakes or near rooms with outside communication, unless a second HEPA filter is installed in the exhaust system.
- HEPA filters are changed after previous decontamination or in conditions that avoid any contamination, and in accordance with the constructor instructions.
- Air conducts are designed to allow room decontamination with a gaseous substance.
- Ventilation system has an emergency power supply in case of general power failure.
- An appropriate renewal air rate allows ventilation of the controlled area in order to reduce most air contamination.

Table 4

Biosafety Level-3—Laboratory Facilities: Safety Equipment

- The laboratory has at least one biological safety cabinet if open manipulations are performed. It is installed to avoid disturbing airflow equilibrium inside the work area. It is located away from doors, windows, room supply and exhaust air louvers, and heavily travelled laboratory areas. It is controlled and certified when placed, after each moving, and at least once a year.
- An autoclave, preferably a double-door one, is located in contained area.
- Biological material is centrifuged in centrifuges located in the contained area. It is placed in leak-proof tubes in rotors or cups with a hermetic closing system (“safety cups”) to contain aerosols in case of breaking or cracks in the tubes.
- Vacuum lines used for work are provided with HEPA filters.

lines; the consistent use and maintenance of personal protective equipment; and the handling of all human fluids, other materials, and cell cultures as if they were potentially infectious. Moreover, in case of initial spreading of SARS among the Belgian population, the number of laboratories performing the diagnosis of SARS should be limited.

Research and Production Activities

Viral cell culture and manipulations of cultured SARS-CoV for diagnostic, research, or production (e.g., preparation of diagnostic tests for SARS) purposes require BSL-3 facilities, equipment, and work practices. These are described in Tables 3, 4, and 5.

All activities involving inoculated animals require BSL-3 animal facilities, equipment, and work practices (Tables 6, 7, and 8). When possible, a class II biological safety cabinet must be used (e.g., inoculations of small animals).

These recommended biosafety measures for the manipulation of SARS-CoV positive clinical samples or the virus itself are comparable to those previously cited (WHO, CDC, Canada, etc.). Nevertheless, these recommendations take into account local provisions.

Storage and Transport

Before handling or transporting samples, they should be stored frozen in locked freezers with restricted entrance, clearly labelled, and packaged separately from other samples.

All biological samples (clinical specimen, cell culture) must be packed in a double packing during

transport within the facility. Primary leak-proof receptacle must be packed in secondary packaging in such a way that, under normal conditions of transport, it must be unbreakable. For transportation outside the facility, SARS samples should be packed in triple packing according to the current requirements of the International Air Transport Association (IATA) Dangerous Goods Regulations (IATA, 2003) and the recent WHO post-outbreak biosafety guidelines for handling of SARS-CoV specimens and cultures (WHO, 2003).

Conclusion

After evaluating the most recent data on the infectious agent responsible for SARS, the performed biological risk assessment, and the fact that no case of SARS has been reported in Belgium and in other countries, the SARS-CoV should be classified as a Risk Group 3 biological agent.

Samples associated with SARS cases inactivated by appropriate and validated methods, as well as clinical specimens not associated with SARS cases but originating from countries where SARS is suspected or confirmed, should be manipulated in BSL-2 facilities using standard precautions, equipment, and work practices. Diagnostic activities, involving the handling and processing of non inactivated clinical specimens considered suspect or a probable case of SARS, should be performed in biosafety containment level BSL-2 facilities, with BSL-3 safety equipment and work practices.

To avoid inadvertent dissemination of the virus

Table 5

Biosafety Level-3—Laboratory Facilities: Work Practice and Waste Disposal Management

- Access to the laboratory is restricted to persons authorized by the responsible person and advised of the potential risks. An access control system is put in place.
- The room access door is labelled with the following information:
 - ◇ Biohazard symbol and Containment level
 - ◇ Coordinates (name and phone number) of the responsible person for the area
 - ◇ Nature of the biological risk
 - ◇ The list of persons authorized to enter the area
 - ◇ Requirements for entering the contained area
- Dedicated equipment is assigned to the laboratory.
- Protective laboratory clothing is worn. Protective clothing is dedicated to the contained area and is not worn outside. It is decontaminated preferably in the contained area prior to laundering or elimination.
- Gloves are available for personnel and worn when necessary.
- Outside manipulations, viable pathogens, and/or genetically modified (micro)-organisms are contained within closed systems (tubes, flasks, etc.).
- The creation of splashes and the formation of aerosols are minimized. Their spreading is controlled by the use of appropriate equipment and practices.
- All manipulations likely to produce infectious aerosols or involving potential risks are conducted within a biological safety cabinet.
- Use of a horizontal laminar flow cabinet is prohibited for the manipulation of pathogens and/or genetically modified (micro)-organisms.
- Mechanical pipetting devices are used. Mouth pipetting is prohibited.
- Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption are not permitted in the laboratory.
- All the manipulated and stored pathogens and/or genetically modified (micro)-organisms are recorded in a register.
- Control measures and control equipment as well as protective equipment are adequately and regularly tested.
- Workers wash their hands when they leave the contained area, before beginning another activity, and each time it is proved necessary.
- Work surfaces are decontaminated with an appropriate disinfectant after work is finished and after any spill of biological material.
- Directions for use of disinfectants are available for the personnel. Depending on the purpose, instructions prescribe the kind of disinfectant to use, its concentration, and contact time.
- Instruction of personnel on biosafety aspects is conducted as well as a follow up and regular updates. The personnel are specifically trained to work in an area with containment level 3.
- A biosafety manual is prepared and adopted. Personnel are advised of special risks they are exposed to and are required to read instructions on work practice. Behaviour in case of an accident is clearly posted in the laboratory.
- The biosafety symbol is posted on incubators, refrigerators, freezers, and liquid nitrogen cryopreservators containing biological material with a BSL-2 or higher.
- An efficient control program for rodents and insects is in effect.
- Animals are not allowed in the laboratory except as part of experiments.
- Management of wastes and/or residual biological material satisfies the following conditions:
 - ◇ Contaminated wastes and/or residual biological material and contaminated disposal are inactivated by an appropriate and validated method before disposal (e.g., by autoclaving or incineration). Incineration is performed in an accredited installation. Bags and containers used for infectious waste collection are resistant, sealable, labelled with the biosafety symbol, and closed before leaving the contained area.
 - ◇ Before washing, reuse, and/or destruction, contaminated material (glassware, slides, etc.) is inactivated by appropriate and validated means.
- Effluents from sinks and showers are preferably inactivated by appropriate and validated means before final disposal.

Table 6

Biosafety Level-3—Animal Facilities: Design Features and Technical Characteristics

- The animal facility is physically separated from other activity areas in the same building or is located in a separate building.
- Entry into the animal facility occurs through an airlock.
- The first door of the airlock is lockable and controlled through an electronic system or equivalent.
- The doors of the airlock are provided with an automatic closing system. A mechanism is installed which prevents both the inner and outer doors of the airlock to be opened at the same time (e.g., by an interlocking system). This mechanism does not interfere with assistance in case of emergency.
- Windows are hermetically sealed.
- Rooms are sealable to allow decontamination with a gaseous substance.
- Building is designed to avoid accidental escape of animals.
- The room contains an observation window or an equivalent system so that occupants can be seen from the outside.
- The contained area has a hands-free or automatically operated sink for handwashing and decontamination. The sink is located in the airlock or near the exit of the contained area.
- A shower is located either in the contained area or in the airlock.
- Personnel have access to a change room for protective clothing. Protective and street clothing cannot be in contact.
- The supply fluid conducts are provided with anti-reflux devices.
- A separate room is used to store clean cages, feed, and bedding.
- Cages, work surfaces, floor, walls, and ceiling are easy to clean, impervious to water, and resistant to those disinfectants and chemicals used for decontamination.
- An installation to clean cages is available.
- Back-up power is provided in case of power failure.
- The room has an automatic system for fire detection and alarm.
- The contained area has an interphone, a phone, or any other system to communicate with the outside.
- The work area is permanently held at a negative air pressure relative to the pressure of the adjacent areas to avoid any transfer of contamination from inside to outside.
- A manometer controls the relative negative air pressure. It is recommended that an alarm system turns on in case of failure of the ventilation system.
- When the supply air is not provided by a system independent of adjacent areas (a dedicated system is recommended), airtight back draft dampers or a HEPA filter is installed. When the exhaust air is not discharged by a system independent of adjacent areas (a dedicated system is recommended), a second HEPA filter is installed in the exhaust system.
- Supply and exhaust air systems are interlocked to avoid a positive pressurization in case of accidental failure of the exhaust system.
- The supply and exhaust air systems can be sealed with dampers.
- Air is exhausted from the contained area after filtration through a HEPA filter. This air cannot be recirculated within the building or within adjacent buildings or discharged near air intakes or near rooms with outside communication, unless a second HEPA filter is installed in the exhaust system.
- HEPA filters are changed after previous decontamination or in conditions that allow avoiding any contamination, and in accordance with the constructor instructions.
- Air ducts are designed to allow room decontamination with a gaseous substance.
- The ventilation system has an emergency power supply in case of general power failure.
- An appropriate renewal air rate allows ventilation of the controlled area in order to reduce air contamination.

Table 7

Biosafety Level-3—Animal Facilities: Safety Equipment

- When a biological safety cabinet is available, it is installed to avoid disturbing airflow equilibrium inside the work area. It is located away from doors, windows, room supply and exhaust air louvers, and heavily travelled room areas. It is controlled and certified when placed, after each moving, and at least once a year.
- Animals are kept in cages or in other equivalent appropriate containment installations.
- Animals are kept in isolators with HEPA-filtered exhaust air.
- An autoclave, preferably a double-door one, is located in contained area.
- A fumigation device or a disinfectant bath is available to decontaminate the outgoing equipment.

to susceptible animal or human hosts, particular care should be taken in research and production activities. Viral cell culture and manipulations of cultured SARS-CoV for diagnostic as well as for research or production purposes (e.g., preparation of diagnostic tests for SARS) require BSL-3 facilities, equipment, and work practices (Figure 1).

Suspecting that this disease could re-emerge during the northern winter flu season, these recommendations should be updated as disease knowledge increases or changes.

Authors' Note

The authors consider that the proposed containment measures are theoretically sufficient to prevent risks to human health and the environment. However, such assessment is only indicative and without prejudice of the final provisions that could be established by competent authorities, regarding the realistic applicability and predictability of the proposed containment measures and the required laboratory practices.

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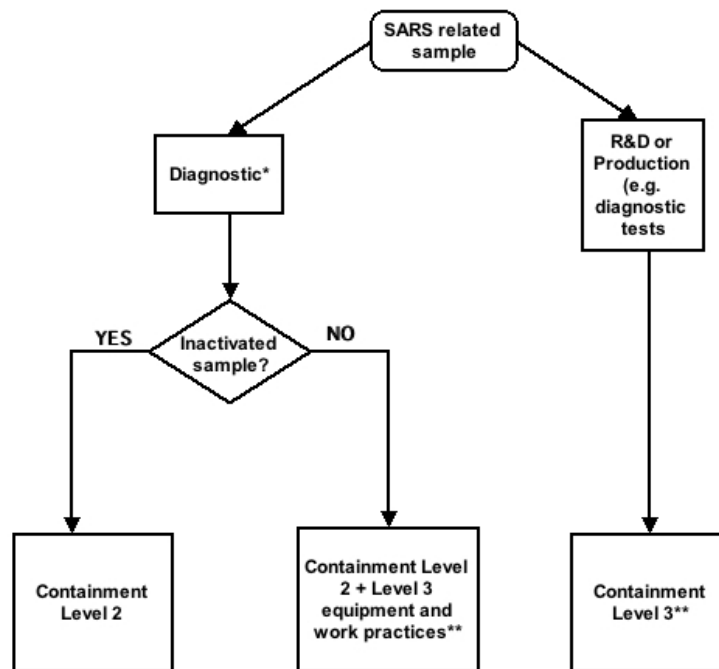
Table 8

Biosafety Level-3—Animal Facilities: Work Practice and Waste Disposal Management

<ul style="list-style-type: none"> • Access to the animal facility is restricted to persons authorized by the responsible person and advised of the potential risks. An access control system is put in place.
<ul style="list-style-type: none"> • The room access door to the animal facility is labelled with the following information: <ul style="list-style-type: none"> ◇ Biohazard symbol ◇ Containment level ◇ Coordinates (name and phone number) of the responsible person for the area ◇ Nature of the biological risk ◇ The list of persons authorized to enter the area ◇ Requirements for entering the contained area
<ul style="list-style-type: none"> • Dedicated equipment is assigned to the animal facility.
<ul style="list-style-type: none"> • Protective clothing is worn. Protective clothing is dedicated to the contained area and is not worn outside. It is decontaminated in the contained area prior to laundering or elimination.
<ul style="list-style-type: none"> • Gloves are available for personnel and worn when necessary.
<ul style="list-style-type: none"> • The creation of splashes and the formation of aerosols are minimized. Their spreading is controlled by the use of appropriate equipment and practices.
<ul style="list-style-type: none"> • Use of a horizontal laminar flow cabinet is prohibited for the manipulation of pathogens and/or genetically modified (micro)-organisms and contaminated animals.
<ul style="list-style-type: none"> • Mechanical pipetting devices are used. Mouth pipetting is prohibited.
<ul style="list-style-type: none"> • Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption are not permitted in the room.
<ul style="list-style-type: none"> • Registers record all the operations that have been performed (animal entries and exits, inoculations to animals of pathogens and/or genetically modified (micro)-organisms, etc.).
<ul style="list-style-type: none"> • Control measures and control equipment as well as protective equipment are adequately and regularly tested.
<ul style="list-style-type: none"> • Workers wash their hands when they leave the contained area, before beginning another activity, and each time it is proved necessary.
<ul style="list-style-type: none"> • Work surfaces are decontaminated with an appropriate disinfectant after work is finished and after any accidental spill of biological material.
<ul style="list-style-type: none"> • Directions for use of disinfectants are available for personnel. Depending on the purpose, instructions prescribe the kind of disinfectant to use, its concentration, and contact time.
<ul style="list-style-type: none"> • Siphons are protected with disinfectant.
<ul style="list-style-type: none"> • Instruction of personnel on biosafety aspects is conducted as well as a follow up and regular updates. The personnel are specifically trained to work in an area with containment level 3.
<ul style="list-style-type: none"> • A biosafety manual is prepared and adopted. Personnel are advised of special risks they are exposed to and are required to read instructions on work practice. Behaviour in case of an accident is clearly posted in the room.
<ul style="list-style-type: none"> • The biosafety symbol is posted on incubators, refrigerators, freezers, and liquid nitrogen cryopreservators containing biological material with a BSL-2 or higher.
<ul style="list-style-type: none"> • An efficient control program of potential vectors such as rodents and insects is in effect.
<ul style="list-style-type: none"> • Animals that are part of the experiment are isolated in a separate room.
<ul style="list-style-type: none"> • Management of wastes and/or residual biological material satisfies the following conditions: <ul style="list-style-type: none"> ◇ Contaminated wastes and/or residual biological material (carcasses, excrements, contaminated bedding, etc.) and contaminated disposal are inactivated by an appropriate and validated method before disposal (e.g., by autoclaving or incineration). Incineration is performed in an accredited installation. Bags and containers used for infectious waste collect are resistant, sealable, labelled with the biosafety symbol, and closed before leaving contained area. ◇ Before washing, reuse, and/or destruction, contaminated material (glassware, cages, etc.) is inactivated by appropriate and validated means.
<ul style="list-style-type: none"> • Effluents from sinks and showers are preferably inactivated by appropriate and validated means before final disposal.

Figure 1

Flow chart describing the required facilities for manipulating SARS related sample



* Without culture, amplification of SARS-CoV or use of cultured, amplified SARS-CoV

** HEPA-filtered mask and restricted entrance

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