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Review Article

Experience Report on Different PPE Variants used in a BSL-3 Facility for Autopsies and COVID-19-Related Research

Martina Loibner, Julia Rieger, Martin Zacharias, Kurt Zatloukal*

Diagnostic and Research Institute of Pathology, Medical University Graz, Graz, Austria

ABSTRACT

The severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) pandemic has highlighted the dependency of healthcare systems and research organizations on manufacturers and suppliers of personnel protective equipment (PPE). The need for rapid capacity building in health care and research institutions generated an unprecedented demand for PPE. The delivery bottlenecks of PPE components also required flexibility and forward-looking stocking of PPE. Well trained staff who can deal with different variations and combinations of PPE components always providing the best possible personal protection is essential. We report on advantages and disadvantages of different PPE components used in a BSL-3 laboratory for a broad spectrum of different work with SARS-CoV-2 including performing autopsies, establishing virus cultures from patients, developing and validating diagnostics, as well as virus inactivation assays to test antiviral agents and decontamination technologies. Depending on the requirements of the practical work four different types of PPE were used by laboratory workers, molecular scientists, pathologists and autopsy assistants. Both autopsies and lab work increased substantially during the pandemic and thus led to very demanding working conditions with working shifts of more than 8 hours which showed the limits for certain PPE variants but also demonstrated the benefit of using PPE with powered air-purifying respirators (PAPR).

Keywords: BSL-3 facility; Autopsies; Virus cultures; PPE variants; Powered Air-Purifying Respirator (PAPR); Chemical shower; Working shifts

INTRODUCTION

The COVID-19 pandemic resulted in a massive and rapid increase in work load for Health Care Workers (HCW) as well as in research laboratories, which led to major challenges in capacity building with regards to personnel trained to work with highly pathogenic agents and the availability of appropriate Personal Protective Equipment (PPE). New challenges were on the one hand performing work with SARS-CoV-2 with, at the beginning of the pandemic, not clearly defined risk which requires upgraded biosafety measures, and on the other hand increased workload under time pressure due to the high number of patients to be treated and the countless inquiries from organizations requiring research work performed with SARS-CoV-2. The WHO and Centers for Disease Control and Prevention (CDC) classified SARS-CoV-2 as a Risk Group 3 (RG-3) biological agent because of the high infectivity, the observed case fatality rate, and the fact that no effective treatment was available [1,2]. However, several groups in the Employment and Social Affairs Committee of the European Parliament agreed to classify SARS-CoV-2 as a RG-3 biological agent only after adding more safeguards to protect all workers [3]. This discussion mirrored the fact that mandatory requirements for biosafety level 3 (BSL-3) do not further specify PPE requirements [4].h. Although SARS-CoV-2 has been classified as a RG-3 agent, handling of patient samples for diagnostics can be performed under BSL-2 conditions.

However, working with increased virus concentrations involving propagation of the virus definitely requires a BSL-3 environment as described in the "CDC Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019" [5]. BSL-3 requirements are defined at different levels, such as guidelines published by the WHO Guideline "Infection prevention and control of epidemic and pandemic-prone acute respiratory infections in health care", and the Laboratory Biosafety Manual Fourth Edition published on 21.12.2020 [7] or the "Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work" with its consolidated version of 24.06.2020 [8] which has to be implemented in national legislation. Furthermore national legislation (e.g., in Austria) does not further specify the PPE components to be used in a BSL-3 environment, stating only that PPE has to be "adequate" or "appropriate", which is very vague and leaves many issues open.

Correspondence to: Dr. Kurt Zatloukal, Department of Diagnostic and Research Institute of Pathology, Medical University, Neue Stiftingtalstraße, 68010 Graz, Graz, Austria, Email:Kurt.zatloukal@medunigraz.at

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MATERIALS AND METHODS

PPE selection criteria

When selecting appropriate PPE it is important to consider that the best possible protection for employees must not undermine practicality and ease of use, otherwise this could lead on the one hand to low acceptance and, on the other hand, could even generate new risk factors because of improper use or impaired working conditions. Within the approved biosafety level of the facility or laboratory containment the selection of the appropriate PPE is based on the risk group and stability of the pathogen, the method of transmission and the concrete work performed that defines either the necessary level of respiratory protection, the protection of eyes, or the enhanced protection from injuries due to cutting with sharp tools and needle pricks. Furthermore, the possible exposure to the pathogen determines the selection of PPE components e.g., whether a biosafety cabinet (BSC, class II) is the main workplace or whether the work with infectious agents has to be carried out outside of a BSC e.g., when performing autopsies. In case of autopsies the PPE typically consists of multiple layers with an outer liquid-tight layer, cut protection gloves, respiratory masks and additional face shields that protect from infectious splashes of body fluids [9] and aerosols generated by using oscillating saws. The multi-layer PPE allows disposing and inactivation of the contaminated outer PPE layer within the BSL-3 laboratory. Other factors that influence the selection of PPE components are the duration of working hours which is closely related to ergonomics, limitations of vision and hearing, fine motor skills and requirements for dexterity [10,11], as well as individual requirements and needs of the working people. Thus, PPE components must be as safe as necessary, but also as comfortable to wear as possible.

Role of working conditions, human factors and skills

Within the necessary protection level, all workers should have the opportunity to test the PPE under the expected working conditions in a normal, non-infectious environment before working with pathogens in a BSL-3 laboratory. This is important because wearing PPE can cause significant physical and psychological stress such as sweating and heat stress because of tight overalls, restricted vision and freedom of movement, and the general feeling of constriction. Furthermore, working in PPE goes hand in hand with a total restriction on eating, drinking or using the toilet for several hours.

In general, four working hours are considered as being well tolerated under ambient temperatures around 20°C and reported to be strictly controlled in some institutions e.g. as stated by Lin, et al. [12]. Longer working shifts may bear the risk of medical issues such as circulatory problems [11,13] or of human failures. However, a report from the UK described a survey among 224 HCWs wearing PPE that revealed that 27% worked for 0-4 hours, 34% for 4-8 hours, 33% for 8-11 hours, and 6% worked more than 12 hours, which by far exceeds any recommendation [11].

HCWs who work in shifts for 10-12 hours in COVID-19 hospital wards have to change their PPE several times in order to prevent viral transmission from infected patients. During these changes human needs may be met only at a minimum due to time pressure in overcrowded wards. Also in biomedical research longer working periods were required because of the massive work load.

PPE training and raising awareness of the appropriate use of PPE, especially the donning process, is of highest priority because this bears the risk for contamination and infection [10,12-19]. Using checklists and regular training for donning and doffing processes should be mandatory in order to reduce risks and to avoid errors [10,15,17].

Laboratory staffs who decide to work in BSL-3 laboratories while wearing PPE should be aware of all constrictions and their physical constitution must be appropriate. They should be stress resistant, not claustrophobic and should be able to work in a team. Only after PPE training has been performed (including donning, performing simulated lab work for several hours and doffing) it can be decided whether a person is able to work in BSL-3 containment.

Furthermore, first aid and emergency training for all team members is highly recommended for several reasons. In case of emergency e.g., medical reasons, such as injuries and cardiovascular problems or contaminations with infectious material, quick and efficient help is essential. Therefore, emergency training including first aid, emergency decontamination, and recovery of persons from the laboratory has to be performed regularly. It is important that every team member is trained to help the team partner because the time period needed for an additional first aid trained person to get dressed with PPE and into the laboratory might take too long. Furthermore training includes managing the rescue chain outside the laboratory, e.g. notification and instruction of the ambulance or the fire brigade, if required.

In addition to regular training, the review und update of procedures is essential. For this it is important to implement the detailed documentation of all incidents and near misses including corrective and prevention measures. This not only leads to raising awareness and improved procedures but also increases trust within the team.

Experience obtained in establishing and operating the BSL-3 laboratory at the Medical University Graz

The BSL-3 laboratory was built to carry out autopsies, frozen section diagnostics and sample preparation for molecular pathogen diagnostics as well as for sample collection for biobanking, isolation and propagation of pathogens, and to perform research projects. The design was based on experience obtained by participating in the planning of the European Research Infrastructure on Highly Pathogenic Agents which includes most European BSL-4 laboratories and their associated BSL-3 laboratories. As a consequence, we have implemented in our laboratory several biosafety features of BSL-4 laboratories (e.g., chemical shower), which are not mandatory for BLS-3 laboratories in order to be prepared to handle pathogens of emerging pandemics and new pathogens with unknown risk.

In Austria, the national legislation for worker protection contains detailed structural specifications for BSL-3 facilities but only stipulates “adequate PPE”. Therefore we followed the more detailed WHO recommendations [20], standards and norms of PPE components, and the relevant scientific literature for PPE selection.

Before our BSL-3 laboratory became operational in 2019 limiting factors for wearing PPE in a health care environment were evaluated in a randomised study (registered at ClinicalTrials. gov, NCT03004690). Nineteen study volunteers tested randomly allocated head or full body-ventilated PPE suits equipped with Powered-Air-Purifying-Respirators (PAPR) by performing four different tasks during 6 working hours at 22°C on one day and 4 working hours at 28°C on another day. Error rates and physical parameters were determined and ergonomically related parameters were assessed. Depending on the PPE system the most restrictive factors were reduced dexterity due to multiple glove layers, impaired visibility due to flexible face shields and back pain related to the respirator of the fully ventilated suit. However, those factors had no negative impact on the working performance (speed and error rate). Heat stress and liquid loss were reported as restrictive at a working temperature of 28°C but not at 22°C [21]. The respective PPE components were then stockpiled and used for training and process validation. This preparatory work enabled a good response to the rapid increase in work load due to the SARS-CoV-2 pandemic which also led to sudden high consumption of PPE and also showed the limitations of certain PPE variants. The global shortage of PPE and delivery bottlenecks led to a critical situation but due to the earlier stockpiling of different PPE components laboratory operations could be maintained. Furthermore, the chemical shower enabled the reuse of several PPE components which allowed us to stay operational even when there was a severe shortage in the supply of PPE components.

PPE basics and interchangeable components

A typical set of PPE components used consists of coverall, respiratory protection, shoe covers and gloves with different protection categories for different applications and working processes.Private clothing is taken off and surgical scrub, consisting of shirt, pants and socks is put on. When we selected the composition of PPE components we distinguished between laboratory work performed in a class II BSC and autopsies, also considering the pathogenic agent, the amount and concentration of virus that is handled, and the decontamination and changing out processes. Propagative work on viruses with highly concentrated virus cultures (e.g. for virus neutralization assays) should be performed in a BSL-3 facility as stated by CDC and WHO [1,6,22,23]. Working with high titer virus is considered to be of increased risk in case of spills or other incidents that may cause contaminations of persons or the working area. Performing an autopsy generates other risks of direct contact with infectious organs, body liquids at organ inspection, tissue sampling, aerosol generation when an oscillating bone saw is used, and the use of sharps.

In principle, in the EU CE-certified category III coveralls i.e. chemical protection suits are used which are divided into six groups: type 1 gastight (EN 943-1:2002), type 2 not gastight (EN 943-1:2002), type 3 liquid-tight (EN14605:2005), type 4 spray-tight (EN14605:2005), type 5 particle-tight (EN14605:2005), and type 6 protection against liquid splashes (EN 13982-1.2004). Protection against infectious agents is additionally indicated by "B", e.g. type 3-B, comprising several test methods of the protective performance of the material as specified in EN 14126:2003. In the EU the standard for clothing is EN 14126, and for surgical gowns EN 13795. In the US the ANSI/AAMI PB70 2012 standard classifies surgical and isolation gowns and NFPA 1999 covers different protective clothing for emergency medical responders. However, test methods and performance requirements cannot be compared directly which makes it difficult to assign equivalency [19] (Table 1 and Figure 1).

Preferred PPE multi-layer variants for autopsies

Autopsies of cases with confirmed or suspected COVID-19 or other BSL-3 agents like Creutzfeld-Jakob disease (transmissible spongiform encephalopathy, TSE) are performed with a so-called multi-layer PPE (Figures 1a and 1b). These PPE variants are for single use and consist of multiple barrier layers that require a well specified process for donning, doffing and locking out, and all components of the outer two layers are disposed for inactivation in an autoclave with special programs for certain pathogenic agents such as prions. The components are a single-use coverall (Cat. III, type 4-B/5-B/ 6-B), a PE (polyethylene) apron with long sleeves, a double layer of PP (Poly Propylene) shoe covers worn over crocs, a double layer of differently colored gloves, e.g. green nitrile gloves as the inner layer and white gloves as the outer layer. Different colors have the advantage that a defect in the outer glove is easily visible. Autopsy assistants and pathologists who are using a bone saw or other sharps wear cut protection gloves (e.g., Kevlar) between the inner and outer gloves. For respiratory protection we have two options that depend on the pathogenic agent. When an autopsy for suspected TSE is performed, PPE 1 with filtering-face-piece mask FFP3, goggles and an additional face shield is used (Figure 1a). The European FFP3 filter standard requires removal of 99% of particles (≥ 0.3 µm). For comparison FFP2 removes 94%, the US filter standards N95 95%, and N100 99%,97%, respectively [13,24]. After completing the autopsy of a TSE case, lab spaces are cleaned with 1M NaOH with an incubation time of at least one hour because alcohol or chlorine-based and other oxidative disinfectants are not effective against prions [25]. Dried up NaOH residues are removed with water on the next day. The waste is put into the pass-through autoclave. All instruments for reuse are cleaned and immersed in 1M NaOH for at least one hour and then autoclaved on a prion program at 134°C.

The locking out of persons wearing PPE 1 from the laboratory has to follow the prescribed doffing procedure. The outer layer, outer gloves, apron, outer shoe cover, and face shield are doffed at a defined area within the BSL-3 laboratory following an exact order and with slow movements, carefully curling from the inside out and without touching the outside. All pieces are put into the bag for the autoclave. After these PPE components are removed, the laboratory can be exited from the second negative pressure stage to the first negative pressure stage of the airlock. There, the remaining parts of the PPE, coverall, inner shoe covers, goggles, FFP3 mask and inner gloves are put into a waste bag that will be closed and taken into the laboratory for autoclaving at the next entry.

Table 1: List of PPE components used for different PPE variants 1 -4. The combination of different PPE components lead to the four different PPE variants that are used for different purposes in the BSL-3 laboratory.Delivery problems of single components can be compensated and laboratory operations maintained.

PPE components	PPE 1 multi-layer	PPE 2 multi-layer with PAPR	PPE 3 liquid-tight	PPE 4 liquid-tight one-piece
Surgical scrub	✓	✓	✓	✓
Single-use coverall, Cat. III, type 4-B/5-B/ 6-B	✓	✓	-	-
Coverall Cat. III, Type 3/4/5/6	-	-	✓	✓
Apron with long sleeves	✓	✓	-	-
Inner layer of latex or nitrile gloves	✓	✓	✓	✓
Outer layer of nitrile gloves, chemically resistant	✓	✓	-	Yes, integrated with suit
Cut protection gloves	Yes, when using sharps	Yes, when using sharps	Yes, when using sharps	Yes, when using sharps
FFP3 or N100 mask	✓	-	-	-
PAPR with A2BEKP filters	-	✓	✓	✓
Crocs	✓	✓	-	✓
Rubber boots	-	-	✓	-
Double layer of PP shoe covers	✓	✓	-	-

Figure 1: Multilayer PPF (A), Multilayer PPE with PAPR (B), liquid-splash tight PPE (C) and (D)

For autopsies with SARS-CoV-2 we use the PPE 1 or the PPE 2 variant with a Powered Air-Purifying Respirator (PAPR, e.g. Jupiter™ Air Filter Unit with two A2BEKP filters and 8 hours rechargeable batteries) connected to a hood assembly providing head, face and shoulder coverage (Figure 1b). The hood and PAPR can be decontaminated by spraying disinfectant all over the surface, tube and the waist belt that contains the blower, filters and batteries, and then reused again. This is also the first step for the locking out process for this PPE variant, the next steps are the same as described for the multi-layer PPE 1 variant.

At the beginning of the COVID-19 pandemic the PPE 1 variant (FFP3 masks, goggles and face shields) were also used for the SARS-CoV-2 autopsies. However, the comprehensive tissue sampling performed for a broad range of morphological and molecular analyses resulted in long working periods. The autopsy team reported several limiting factors of using FFP3 masks and goggles for longer periods. First, the FFP3 masks have to be worn tightly fitting with a rubber seal on the skin which is appropriate for short term working periods, but after one hour becomes increasingly uncomfortable, causing pressure and heat on the face, painful pressure behind the ears, and headache. Second, the multi-layer of PPE causes sweating which might loosen the tight

fit on the face which could in turn make the goggles slip, both of which may lead to intentional touching of the face to readjust the mask and goggles, which is a possible source of infection [19,26]. Third, goggles fog up easily, which in one case led to injury of a team member by working with sharps. Moreover, FFP3 masks and goggles do not provide optimal protection since parts of the face are unprotected whereas the rest of the body is totally protected with two layers. For the above described reasons we replaced the FFP3 masks by a PAPR system in the PPE 2 variant which protects the whole head and prevents unintentional touching of the face. Another advantage reported by our autopsy team is that breathing is easier because of the lack of resistance from a mask. Lab workers also reported that the PAPR ventilating the hood is more comfortable to wear and reduces heat stress. Furthermore, the PAPR system avoids fogging of eye glasses and male workers do not need to shave as would be necessary for wearing FFP3 masks. Another advantage of PPE 2 is that some national legislation require a break after three hours of wearing FFP3 masks, which cannot always be met for some working procedures. There is no time limit by law for wearing PPE with PAPR.

PPE variants suitable for the chemical shower, PPE 3 and 4

For any research work carried out with high titres of viruses, isolation from human specimen, cultivation for stock production, and development and performance of various assays we use the liquid-splash tight PPE variants 3 or 4 (Figures 1c and 1d).

PPE 3 (Figure 1c) consists of a Tychem C2 protective suit (Cat. III, Type 3/4/5/6) which protects against dangerous biological substances, and is impermeable to liquid and particles. Outer layers of gloves and chemical protective boots are sealed with adhesive tape. The hood is ventilated by the same PAPR used in the multi-layer PPE 2 variant in Figure 1b.

PPE 4 (Figure 1d) consists of a one-piece TychemProChem protective suit (CPM F1 H L2, Cat. III, Type 3/5) with integrated boot socks and gloves, resistant against biological hazards, particles and liquids. This variant surpasses PPE 3 because it enables additional protection due to positive pressure generated by the PAPR system in the entire suit. From a practical point of view donning PPE 4 is more demanding. Crocs are worn in the boot socks. The waist belt with the powered ventilator and the battery is fixed to the suit by the externally mounted filters. This assembly makes it difficult to pull the whole suit up over the back and slip on the sleeves. For this suit the welded on gloves can be ordered in the appropriate sizes which is an advantage but this makes stockpiling more costly and difficult. The sleeves and the gloves must be fixed to the forearms; otherwise the gloves will slip off the fingers which adversely affect fine motor skills.

Both PPE 3 and 4 liquid-splash tight variants are used for a decontamination process in the chemical shower which is operated with 2% per acetic acid and takes 15 minutes. The showering process consists of fogging-in the per acetic acid, a defined incubation time, water flush and an air exchange time. This procedure ensures the complete decontamination of the outside of the suit and therefore doffing is with less effort and risk as with the multi- layer PPE 1 and 2 variants.

Table 2: Overview on usage, safety and personal perception of the four PPE variants described. The four different PPE variants offer different stages of safety, usability, and practicability within their use in a BSL-3 laboratory. Each of them requires dedicated handling, has advantages and disadvantages, and leads to different perceptions among the wearers.

Overview on usage, safety and personal perception	PPE 1 multi-layer	PPE 2 multi-layer with PAPR	PPE 3 liquid-tight	PPE 4 liquid-tight one-piece
Autopsies with suspected TSE	✓✓✓	No	No	No
Autopsies with all other BSL-3-agents	✓	✓✓	✓✓✓	✓✓✓
Lab work with propagative BSL-3 agents	✓	✓✓	✓✓✓	✓✓✓
Strictly dedicated doffing process	✓✓✓	✓✓✓	No	No
Use of chemical shower	No	No	✓✓✓	✓✓✓
Heat stress	✓✓✓	✓✓	✓	No
Working hours	✓	✓✓	✓✓✓	✓✓
Slipping	✓✓	✓✓	No	No
Fine motor skills	✓✓✓	✓✓✓	✓✓✓	✓
View	✓	✓✓✓	✓✓✓	✓✓✓
Foggy goggles	✓✓✓	No	No	No
Use of adhesive tape to seal gloves, boots or shoe covers	✓✓✓	✓✓✓	✓✓✓	No
PAPR battery exchange	Not used here	✓✓✓	✓✓✓	No
Reuse of components	None	None, except hood+PAPR	✓✓✓	✓✓✓

✓✓✓: Completely applies; ✓✓: Applies; ✓: Just about applies

Additionally, wearing solid rubber boots with PPE 3 and crocs within the one-piece suit with PPE 4 reduces the risk of slipping as compared to the double PP shoe covers worn over crocs as with PPE 1 and 2.

Although PPE 3 has the disadvantage of the separate hood and the necessity of sealing gloves and boots with adhesive tape, a major advantage is that the waist belt containing the batteries is outside the suit. This allows a battery change in case of low battery, which may become necessary during long working shifts. In general, lab workers prefer PPE variant 3 as it offers sufficient respiratory protection but donning is less complicated and there is the option to change the battery during work. Although working shifts should not take longer than four hours, the massive work load in the COVID-19 pandemic required sometimes working shifts of more than eight hours, which is possible by using PPE 3.

In general, no matter which PPE variant is used, mutual help with donning and doffing by following the respective protocols and checklists and mutual control of the equipment for completeness and intactness is necessary to mitigate risks and errors. Mirrors in the first dressing room and the airlock room where the multi-layer PPE and the chemically resistant Tychem suits and PAPR are donned also facilitate the correct donning of all components, as recommended by Ruskin, et al. [10], (Table 2).

DISCUSSION AND CONCLUSION

The experiences of the last year have shown that both preparedness and flexibility regarding PPE components and motivated people are the greatest assets to overcoming unexpected challenges. The major health organizations WHO and CDC had to update their recommendations in an urgent procedure to the currently available knowledge about SARS-CoV-2. Legislation in the EU and other nations on biosafety requirements had to be reconsidered or even newly created. Available PPE components were exploited in the best possible way and strategies for reasonable and safe reuse due to the worldwide shortage were developed. Di Marzo reported that 12% of HCWs were infected at the beginning of the pandemic [27]. This underlines the importance of proper protection of HCW and other personnel working with infectious material. Staff shortages due to illness and quarantine further exacerbated the situation. Wang, et al. analyzed twenty studies on medical staff infections and reported using BSL-3 adequate PPE equipment did not get infected and strict protection measures are essential [28]. The well-known phrase for the training philosophy "train not until you get it right but until you never get it wrong" is essential but difficult to implement due to lack of time in health care and research institutions.

The PPE 1 and 2 multi-layer variants described here correspond to the PPE used in other health care units, where wearing three layers of PPE is also reported [16]. Studies with 230 participants in the UK on adverse reactions of HCW treating COVID-19 report that 72.3% felt hot, 89.7% uncomfortable or very uncomfortable and 98.7% had increased sweating [11]. In China 94% of 129 participants felt discomfort, from those 108 nurses 74.1% indicated facial skin indentations, 59.3% respiratory difficulties, 70.4% heat stress and 52.8% dizziness [29]. Especially for the health care system the development of smart fabrics that effectively block virions and enable better heat and moisture transfer would increase comfort, convenience and acceptance, making long working shifts less physically demanding [18].

The pandemic also showed that supply chains for PPE were not ensured for months. One of our lessons learned is that personnel that is well trained with different PPE variants that were stockpiled is the best way to maintain a BSL-3 facility operative for various kinds work and requests that occur. In this context also the availability of the chemical shower played a very important role because, during the shortage of single use coveralls, types 4/5/6 and FFP3 masks, we switched to using the more expensive liquid-tight types 3/4/5/6 and the PAPR that are suitable for the chemical shower. At this time the reuse of the liquid-splash tight variants 3 and 4 with the PAPR brought an unambiguous advantage, increased the personnel protection at the same time and allowed extended working periods compared to the multi-layer variants.

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