Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Commentary

Management of bacteriological specimens of patients suffering from coronavirus disease 2019 (COVID-19)

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Introduction

Since 31st December 2019, and as of 29th September 2020, 5 011 669 cases of coronavirus disease 2019 (COVID-19) have been reported in Europe (worldwide 33 423 469, including 1 002 678 deaths). In Germany, 287 421 cases and 9471 deaths have been recorded [1]. COVID-19 presents as a mild to severe disease, and the outcome is potentially fatal [2,3]. The course of infection might be complicated by nosocomial infections (e.g. ventilator-associated pneumonia, sepsis, etc.), eventually caused by multidrug-resistant organisms (MDRO). Timely and coordinated microbiological diagnostics are crucial to ensure the best medical treatment for patients suffering from COVID-19.

In contrast to PCR-based virus diagnostics, where samples containing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are routinely inactivated (e.g. by heat or detergents), microbiology samples remain untreated to allow subsequent bacterial culture. Therefore, microbiology staff may have a relevant risk of exposure. Specific hands-on protocols are currently not available, as only general biosafety recommendations for the laboratory handling of SARS-CoV-2-positive samples exist [4–7].

Here, we report on the set-up of a diagnostic approach to provide microbiological algorithms for patients with suspected or confirmed COVID-19. Our experiences are based on 100 patients presenting between 26th March and 4th May 2020. We established a bacteriological workflow that ensures both high throughput and high-quality microbiological diagnostics for COVID-19 patients with maximum staff safety.

Risk assessment and general safety aspects of microbiological diagnostics regarding the handling of SARS-CoV-2-positive samples

Microbiology laboratories process a variety of human specimens—such as blood, serum, stool, urine, respiratory secretions, and tissue biopsies—that potentially contain a broad spectrum of infective biological agents. Laboratories should therefore follow standard practices (e.g. decontamination of work surfaces, hand hygiene etc.) and (national) guidelines for laboratory biosafety. Recently, the World Health Organization (WHO), Centers for Disease Control (CDC), and the European Centre for Disease Control (ECDC) released interim recommendations for the laboratory handling of SARS-CoV-2-positive samples [4–7]. In Germany, the Technical Rules for Biological Agents (TRBA) and the German Quality Standards for the Microbiological Diagnosis of Infectious Diseases (MiQ) reflect the state of technology and occupational hygiene regarding the handling of biological agents [8–10]. Further, the German Committee on Biological Agents (Ausschuss für biologische Arbeitsstoffe, ABAS) establishes several safety rules and adapts them to the current state of development (https://www.

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baua.de/EN/Home/Home_node.html). The German Biological Agents Ordinance (Biostoffverordnung) governs the classification of biological agents into risk groups 1–4 (in accordance with the EU-wide classification due to directive 2000/54/EC) and the corresponding protection levels 1–4 [11,12]. TRBA 100 specifies protective measures for activities involving biological agents in laboratories by differentiating specific and unspecific activities. Examples for specific activities are the handling of a biological agent that is known by species (e.g. propagation of a bacterial culture or a virus by cell culture). The processing of a primary patient specimen in the microbiological laboratory typically falls into the unspecific activity category. TRBA 462 applies to the classification of viruses. The protection levels typically meet the risk group of the biological agent. The Middle East respiratory syndrome coronavirus (MERS-CoV), SARS-CoV-1 and SARS-CoV-2 are labelled as biosafety level 3 (BSL-3) agents. They cause disease which may be fatal, are easily transmitted by an airborne route, and consequently require protection level 3 standards. On 27th March 2020 the German ABAS clarified that the unspecific handling of respiratory samples for the detection of SARS-CoV-2 is possible under BSL-2 standards, requiring a BSL-2 cabinet, a laboratory coat and, potentially, gloves, while FFP2 mask and safety glasses are only recommended [13]. Of note, this resolution by ABAS does not address the spectrum of microbiology laboratories that handle hundreds of specimens of various origins per day, with the majority of samples being processed by culture-based techniques that by nature do not allow sample inactivation. Thus, microbiologists are obliged to independently perform a site-specific (what infrastructure is available?) and activity-specific (what kind of specimen is being handled and which tests are being applied?) risk assessment and to define a detailed workflow applicable 7 days/week for the processing of SARS-CoV-2-positive samples.

In this context we reviewed the potential infection risk (reflected by the viral load) by type of specimen according to current knowledge (Table 1). Furthermore, we evaluated the spectrum of microbiological samples of the first 100 patients hospitalized at the University hospital Frankfurt due to confirmed or highly suspected COVID 19 (Fig. 1). MDRO screening samples accounted for 35% of all specimens. The majority of the clinical microbiology samples ($n = 337$; 27%) were blood cultures with a negligible laboratory risk of SARS-CoV-2 laboratory infection. Frequently, respiratory samples were submitted to the laboratory ($n = 205$; 17%).

Of note, the available reports on SARS-CoV-2 positivity and the viral load for different specimens are heterogeneous and highly dependent on disease stage. To allow a more precise assessment of specimen infectivity, studies on viral shedding need to be improved [14]. Most data rely on the detection of viral RNA. Studies determining the level of live virus are very rare, and cover only a very low number of patients, e.g. from throat swabs ($n = 2$ [15]) or stool ($n = 4$ [16]). However, among available studies there is a clear trend that respiratory secretions contain the highest viral load, while that of serum or plasma [17] is very low or even undetectable (Table 1). Respiratory secretions of patients with severe disease are proven to contain significantly higher viral loads than those of patients with mild disease. Viral RNA may also be detected in stool samples (for even longer than in respiratory samples) but typically not from urine [18]. Thus, respiratory samples from patients with COVID-19 in particular are assessed as potentially infective.

Microbiological procedures for COVID-19 patient samples

Microbiological routine laboratories handle human specimens in a 'containment level 2 laboratory' with standard protection measurements. This includes the use of personal protective equipment (PPE) such as laboratory coats, gloves and protective eyewear (both risk-adapted) and safety cabinets for activities with aerosol formation (such as quantitative plating of respiratory secretions). Routine laboratory procedures such as hand disinfection, decontamination of work surfaces and management of laboratory waste represent additional mandatory laboratory safety aspects. In contrast, containment level 3 represents an increased safety level (e.g. limited access, airlock, air filtration, autoclave on site, negative pressure area, safety cabinets) [11].

In the case of SARS-CoV-2, reports on viral kinetics and the positivity rate and viral load of clinical specimens are highly heterogeneous (Table 1). The processing of respiratory secretions (with high numbers of infective SARS-CoV-2 virus particles) poses the highest risk to microbiological staff and needs to be specifically addressed in terms of laboratory safety. Due to the risk of aerosol formation, respiratory secretions are generally handled in safety cabinets in microbiology laboratories, ensuring a high level of protection for staff. However, non-respiratory specimens such as blood/serum or stool cannot simply be categorized as 'generally non-infectious' due to the fact that small amounts of virus/RNA may be present (Table 1). The relevance of viral shedding with non-respiratory samples for person-to-person transmission of SARS-CoV-2 is still not known. Examples of routine microbiology

Table 1
Source-specific risk assessment of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infectivity of microbiological samples from patients suffering from coronavirus disease 2019 (COVID-19)

Clinical specimen	RT-PCR[a] % (n); reference	Virus load (copies/mL)
Bronchoalveolar lavage	93% (14), [16]	$<2.6 \times 10^4$ [b]
Sputum	72% (104), [16]	$<2.6 \times 10^4$
	100% (18), [19]	2.36×10^2
Nasal swab/ nasopharyngeal swab	100% (1), [20]	CT values of 18–20
	63% (8, nasal), [16]	1.4×10^6 [c]
Oropharyngeal swab	100% (1), [20]	CT values of 23–24
	32% (398, pharyngeal), [16]	$<2.6 \times 10^4$
	78% (9), [21]	4.56×10^2 to 6.77×10^4
	53.3% (15, oral), [22]	NA
Urine	0% (72), [16]	NA
	0% (18), [23]	NA
	11% (9), [21]	3.22×10^2
Stool	100% (1), [20]	CT values of 36–38
	29% (153), [16]	$<2.6 \times 10^4$
	53% (17), [24]	550 to 1.21×10^5
	53.42% (73), [25]	NA
Anal/rectal swab	22% (9), [21]	4.47×10^2 to 5.42×10^4
	38% (8, severe disease), [23]	NA
	14% (7, mild disease), [23]	NA
	26.7% (15), [22]	NA
Serum	20% (15), [22]	NA
	17% (6), [26]	NA
Plasma	15% (41, plasma), [27]	NA
	5.6% (18), [19]	1.79×10^2
Blood	1% (307), [16]	$<2.6 \times 10^4$
	22% (9), [21]	9.11×10^{-1} to 8.04×10^0
	30% (10, severe disease), [23]	NA
	15% (13, mild disease), [23]	NA
	40% (15), [22]	NA
Saliva (oropharyngeal)	50% (8, severe disease), [23]	5×10^2 (median)
	23% (13, mild disease), [23]	
Conjunctival swab	1.5 % (67), [28]	NA

NA, not available.
[a] Percentage of positive samples (total number of tested samples), reference.
[b] Mean cycle value threshold value of 30 ($<2.6 \times 10^4$ copies/mL) [16].
[c] Mean cycle value threshold value of 24.3 (1.4×10^6 copies/mL) [16].

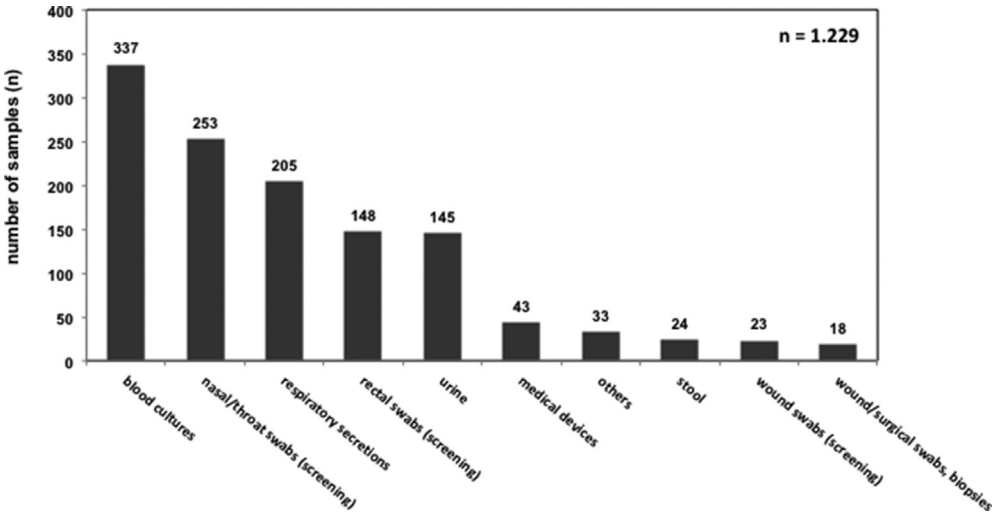

Fig. 1. Microbiological specimen distribution submitted from 100 patients with coronavirus disease 2019 (COVID-19).

Table 2
Microbiology laboratory coronavirus disease 2019 (COVID-19) specimens and corresponding procedures (as applied at Frankfurt University Hospital). Sample can be routinely processed under BSL-2 conditions. A site-specific risk analysis should be performed in accordance with available laboratory facilities

Specimen	Target test/technique	Risk assessment [a]	Measurements[b]
Respiratory secretions - bronchoalveolar lavage (BAL) - bronchial secretion - tracheal secretion - sputum	Microscopy/Gram stain (remark: dispensable procedure) (Quantitative) microbiology culture - inoculation of media - plating of serial dilutions ELISA/immunochromatographic assay target: galactomannan antigen	Low (slide preparation) (none after heat or ethanol fixation) High (high viral load/risk of aerosol formation)	BSL-3 containment [c] plus - BSL-2 cabinet - FFP-2 mask, gloves - protective glasses
Throat/nasal swabs	Microbiology culture (MDRO screening) Inoculation of media	Low (very low risk of aerosol formation when using agar-based swabs) Medium (low risk of aerosol formation when using liquid-based swabs)	BSL-2 containment plus - BSL-2 cabinet - FFP-2 mask, gloves - protective glasses (optional)
Biopsies Tissue aspirates Punctures	Microbiology culture - Specimen homogenization - Inoculation of media	High (if specimen originates from the respiratory tract) Low (if specimen does not originate from the respiratory tract)	BSL-2 containment plus - BSL-2 cabinet - FFP-2 mask, gloves - protective glasses (optional)
Blood cultures	Microbiology culture - Subculture from blood-culture bottles	None/minimal (no SARS-CoV-2 content)	BSL-2 containment plus - BSL-2 cabinet - gloves (standard precautions independent of SARS-CoV-2)
Urine	(Quantitative) microbiology culture - inoculation/plating of media Immunochromatographic assays target: *Legionella* antigen Pneumococcal antigen	Minimal (low risk of aerosol formation/no or only very low SARS-CoV-2 content)	BSL-2 containment plus - BSL-2 cabinet - FFP-2 mask, gloves - protective glasses (optional)
Stool	Microbiology culture - Inoculation of media Immunochromatographic assays target: *Clostridioides difficile* GDH *C. difficile* toxin	Low (low risk of aerosol formation/no or only low SARS-CoV-2 content)	BSL-2 containment plus - BSL-2 cabinet - FFP-2 mask, gloves - protective glasses (optional)
Rectal swab	Microbiology culture (MDRO screening) - Inoculation of media	Low (very low risk of aerosol formation when using agar-based swabs) (low risk of aerosol formation when using liquid-based swabs/no or only low SARS-CoV-2 content)	BSL-2 containment plus - BSL-2 cabinet - FFP-2 mask, gloves - protective glasses (optional)
Serum	Specific antibody detection ELISA target: *Legionella* spp. *Mycoplasma* spp. *Chlamydia* spp.	None/minimal (no or very low SARS-CoV-2 content)	BSL-2 containment plus - gloves

MDRO, multidrug-resistant organism.
[a] Regarding SARS-CoV-2 exposure.
[b] Safety level can be adjusted to standard safety precautions if robust data on the source specific risk is available (see Table 1).
[c] If available on site/ensuring room separation of high-risk sample processing and the reduction in laboratory staff to a minimum (laboratory coat, hand disinfection, decontamination of work surfaces included).

laboratory specimens and corresponding procedures are listed in Table 2.

While the primary processing of specimens from COVID-19 patients warrants special attention with regard to safety precautions, the processing of bacterial and/or fungal cultures, inactivated specimens, or the handling of extracted DNA requires standard safety precautions. Generally, inoculated agar plates can be processed under routine laboratory conditions (BSL-2).

The correct labelling of specimens from COVID-19 patients is of particular importance in order to guide these specimens in the appropriate workflow. Our experience is that adherence of clinical staff in labelling SARS-CoV-2-confirmed samples (e.g. 'COV+') in a pandemic situation is limited. Therefore, definite target sample identification in a routine laboratory setting is not practicable. Thus, assessing all samples from wards treating COVID-19 positive patients as 'COV+' is the easiest way to identify SARS-CoV-2-positive samples, although there might not be 100% coverage. Given this workflow (Fig. 2), we set up the following measures.

First, in order to separate the processing of respiratory COV + secretions with the highest risk of aerosol formation from other specimens we decided to process these samples in our BSL-3 laboratory by defined staff members ('COV + team') in accordance with recommendations of the ABAS (room separation of sample procession/reduction of laboratory staff to a minimum; Fig. 3).

Second, the use of gloves, a daily-replaced individual coat, and daily-replaced individual FFP-2 mask and eye protection supplemented the processing of all respiratory samples even from COV− wards (performed under a biosafety cabinet in our BSL-2 laboratory). Furthermore, only a defined number of well-trained staff carried out sample work-up.

Third, for additional or follow-up diagnostics, SARS-CoV-2-positive respiratory samples were separately stored for 72 h in a sealed safety box in the BSL-3 laboratory to prevent accidental staff exposure.

Fourth, the laboratory test repertoire was replaced by easy-to-process (even under a BSL-2 cabinet) point-of-care test (POCT)

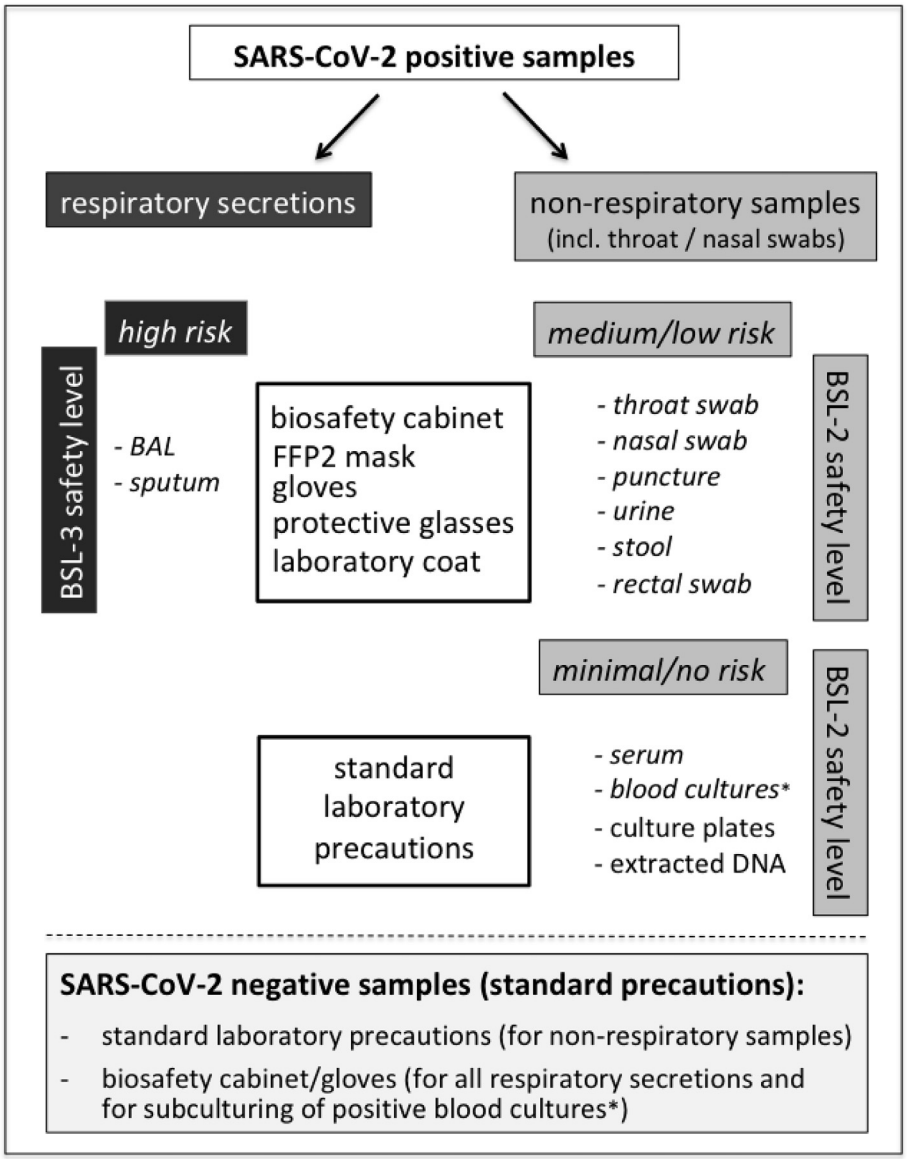

Fig. 2. University hospital Frankfurt-site specific and activity-specific workflow in the processing of severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) confirmed and/or highly suspected cases including follow-up samples of patients with proven coronavirus disease 2019 (COVID-19). Samples can be processed under biosafety level 2 (BSL-2) conditions. A laboratory site-specific risk analysis should be performed in accordance with available laboratory facilities. BAL, bronchoalveolar lavage.

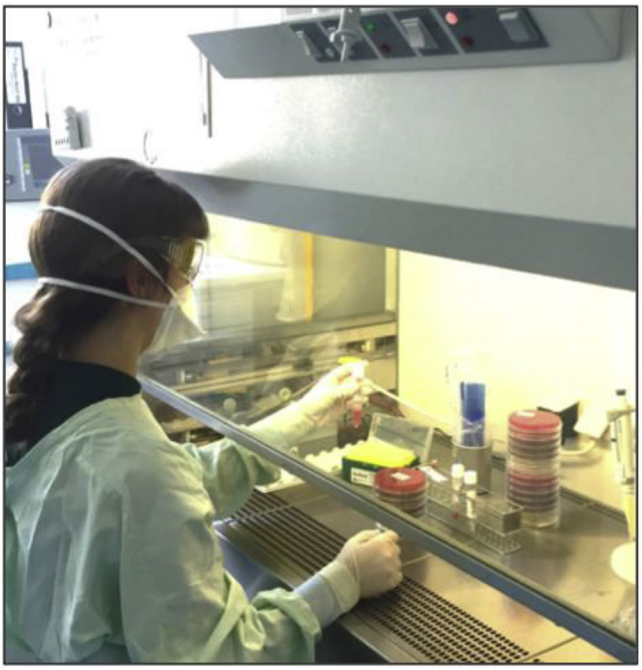

Fig. 3. Technician processing respiratory samples positive for severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) under a biosafety cabinet in the biosafety level 3 (BSL-3) laboratory. PPE: FFP2 mask, gloves, protective glasses, laboratory coat.

devices whenever possible. This includes immunochromatographic tests instead of enzyme-linked immunosorbent assays—e.g. for the detection of glutamate dehydrogenase (GDH) antigen and A/B toxins of *Clostridioides difficile* from faecal specimens or the detection of *Legionella pneumophila* and *Streptococcus pneumoniae* antigens from urine samples—to avoid aerosol formation (Table 2). This was established in the light of the high SARS-CoV-2 viral loads in respiratory samples of COVID-19 patients (see above), our function as a regional centre treating a high number of patients, and the imminent risk of a 'routine-related' reduction in attentiveness. No procedure resulted in a significantly increased workload.

In conclusion, due to the non-inactivated nature of clinical specimens, the emergence of SARS-CoV-2 results in a number of laboratory safety challenges to a clinical microbiology laboratory. Risk assessment of the laboratory work and implementation of appropriate risk control measures are important to guarantee the safety of the laboratory staff as well as the optimal processing of diagnostic specimens. Although we decided to process samples of COVID-19 patients under BSL-3 conditions, this is not a general recommendation from us. In our setting, this algorithm proved to be practicable and increased the staff's attention. A dedicated room for processing respiratory samples under BSL-2 conditions might also improve staff safety. BSL-3 safety standards are only recommended for specific handling procedures such as viral cultures. Moreover, we are not aware of any reports on laboratory infections or contaminations with SARS-CoV-2 due to the handling of positive samples under BSL-2 conditions.

Laboratory hygiene plan to avoid interpersonal SARS-CoV-2 transmission in laboratory staff

It has to be realized that an outbreak scenario in a diagnostic laboratory leads to severe constraints in a hospital's diagnostic capacity, as described recently [29], even if the primary source of the infection (staff, patient sample) might not be identified. Therefore, and in addition to the laboratory safety procedures given above, several other measures should be implemented to avoid interpersonal laboratory-associated infections. We decided that all staff must (a) wear surgical masks all through the day, and (b) indicate illness or contact with known COVID-19 patients resulting in quarantine (under the responsibility of the occupational health physicians). Moreover, the space between the laboratory working stations was increased to >1.5 meters, and finally (and perhaps most importantly) social behaviour (breakfast break, lunch break, coffee break etc.) was restructured to minimize interpersonal contact (e.g. non-overlapping breaks, etc.).

Concluding remarks

The COVID-19 pandemic is a global threat and poses a particular challenge to diagnostic laboratories. During treatment of hospitalized COVID-19 patients, clinical microbiology laboratories are faced with the processing of diverse specimens confirmed or at least suspected to contain SARS-CoV-2. Based on the high infectivity of SARS-CoV-2, its interim classification as a biosafety level 3 pathogen, and the fact that inactivation procedures are not feasible in microbiology, precise algorithms for the performance of microbiological diagnostics need to be set up. As SARS-CoV-2 specimens can be handled in BSL-2 facilities, laboratories may individually adapt their workflow. Our approach may represent a blueprint for clinical microbiology diagnostic laboratories to examine their workflow in the presence of emerging highly virulent pathogens.

Ethics approval

No ethics approval is needed for this description of a laboratory workflow.

Author contributions

Conceptualization: MH and VAJK. Data analysis: SB, LV, SG, TW, DV, DH, JS, VI. Writing original draft: MH, SB, TW, VK. Writing, review and editing: MH, VK.

Transparency declaration

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