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Methods and Compliance Adopted in Controlling Infectivity of COVID-19/SARS-CoV-2 and facilitating Sample/Specimen Handling under BSL-2 Containment for Research and Process Development Activities

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Abstract

The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has presented the healthcare and scientific research community with an uphill task in testing, controlling, and finding the prevention or cure for the pandemic. A significant amount of challenge related to testing and research activities arise as SARS-CoV-2 is a risk group 3 (RG3) agent and requires for all activities that use live virus for diagnostics, screening, surveillance, process development and scientific research to be handled in the Biosafety Level-3 (BSL-3) biocontainment facility environment. Low availability of the BSL-3 laboratories presents a daunting challenge for health care and research personnel, globally. Therefore, specific sample manipulation followed by risk assessment approach will allow the work to be carried in a Biosafety Level-2 (BSL-2) laboratory with enhanced practices. In the present scenario, this approach will maximize the testing and research activities along with minimizing the risk of infection for personnel involved in this process. The regulatory agencies have laid down interim guidelines for carrying out the work in BSL-2 laboratories but there has been a lot of confusion amongst the research community in adopting those guidelines in their basic science research laboratory set-up. This is due to the interim guidelines being mainly formulated around the way the work should be carried in medical laboratories and not necessarily in the basic science research laboratory environment. The article presents various scientific approaches that can be adopted while planning the work related to SARS-CoV-2 in a BSL-2 basic science research lab environment. This approach will allow simultaneously the health care and research personnel to develop a testing or research benchmark protocol in such a way that majority of the work can be accomplished under the BSL-2 laboratory conditions without having a need of a BSL-3 biocontainment facility environment.

Keywords: SARS-CoV-2/COVID-19 • Biosafety • Virus inactivation • Biocontainment facility • Pathogenic-microflora • Risk groups

Introduction

The SARS-CoV-2 pandemic has intrigued and challenged the scientific/medical community towards undertaking research that would identify molecular mechanisms that could prevent and treat COVID-19 infections. COVID-19 scientific interest groups have been formed to promote collaboration and exchange of the scientific information while working with clinical and environmental samples from known or strongly suspected COVID-19 positive subjects or resources [1]. Therefore, the risk of infection to the scientists, research and medical personnel is high, thus requiring the work to be conducted under Biosafety Level-3 (BSL-3) facilities, especially when virus is being isolated or propagated. However, once inactivated these samples and specimens can be safely handled at a lower biosafety level (BSL-2 with enhanced practices). To address the exposure risk associated with these materials when used in laboratory for diagnostic, screening, bench research or process development analyses and biocontainment, certain guidelines have been laid down by various regulatory agencies (CDC, NIH, OSHA, VA Office of Research and Development [ORD] and VA Office of Research Oversight [ORO]) [2-5]. According to these guidelines, we provide here a working standard operating procedure to be used at the institutional level for the researchers willing to work with biomaterials, specimens, or samples that are SARS-CoV-2 positive or suspected to be positive. The overall goal of these recommended approaches is to ensure safety of research personnel working in the research laboratory and prevent accidental release of the virus into the environment. The current approaches can also be implemented successfully for the non-research projects (surveillance, quality improvement, etc.) where the hazard level is not greater than the already approved conditions. The current article will also be very useful for various institutional research safety committees for reference purposes and to be included in their respective SOPs.

The procedures that involve or have the potential to result in isolation or propagation of COVID-19/SARS-CoV-2 virus that includes handling of clinical samples/specimens or intentional infection of animal models containing live strains of SARS-CoV-2 can only be conducted in a BSL-3 or ABSL-3 laboratory. Additionally, the NIH Office of Science Policy has posted FAQs for Interim Laboratory Biosafety Guidance for Research with SARS-CoV-2 that state: "At the present time, SARS-CoV-2 best meets the definition of a RG3 agent and Institutional Safety Committee should consider the agent to be RG3 as a starting point in their risk assessments when reviewing research subject to the NIH Guidelines" [3].

Literature Review

Manipulation of biomaterial, samples or specimens positive or tentatively positive for SARS-CoV-2 strains can be conducted in BSL-2 with enhanced practices; however, if samples are manipulated to create aerosolization of the virus or if the live virus is concentrated, the work must be conducted in a BSL-3

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laboratory [2-5]. The CDC and WHO have generated detailed guidelines for clinical diagnostic laboratories but this guidance is not always directly applicable to the research laboratories especially when the procedure is difficult to be performed under the confinement of the primary containment device (e.g. flow cytometry, microscopy studies, and few QPCR methods). The SARS-CoV-2 positive inactivated samples, fixed samples or isolated nucleic acid (RNA) can be used under BSL-2 with enhanced practices (under biosafety cabinet Type A1 or A2) for further downstream biological assays [2-5]. As SARS-CoV-2 is a positive-sense single-stranded RNA virus, it is critical that research personnel should clearly understand that the viral genome mimics a cellular messenger RNA. This makes the SARS-CoV-2 genome enough to initiate viral replication, and may cause infection in case of percutaneous, mucous membrane, or other potential exposure of research personnel [6].

The CDC considers positive-strand RNA virus genomes to be immediate precursors to virus infection and, in the case of the closely related SARS-associated coronavirus (SARS-CoV), the full-length genomic RNA is regulated as a select agent [7,8]. Thus, isolated genomic RNA of SARS-CoV-2 should be considered potentially infectious, warranting elevated precautions even when nucleic acids are manipulated at elevated BSL-2, and risk mitigation strategies should include evaluation of any potentially aerosol-producing procedures or use of sharp-devices. Handling of all genomic RNA samples should be conducted under biosafety cabinet (BSC) inside the BSL-2 settings with appropriate PPE.

The main criteria of deciding whether the laboratory work can be accomplished under BSL-2 conditions mainly depend on a site-specific risk assessment and on evaluating the process of sample and specimen manipulation for inactivating the virus. If the investigators and research personnel are not using the aerosolization steps and not concentrating live virus (centrifugation, multiple pipetting steps, trituration, etc.) then the work can be conducted under BSL-2 laboratory with enhanced practices by using automated instruments and analyzers with enhanced BSL-2 biosafety precautions. Most of the QPCR work that is conducted in a research laboratory environment does include virus concentration and multiple steps that induce aerosolization processes.

A site-specific and activity-specific risk assessment can identify risks and processes to mitigate risks involved in handling SARS-CoV-2 samples and specimens. The risk assessment determines if enhanced biosafety precautions are warranted based on situational needs, such as high testing volumes, and the likelihood to generate infectious droplets and aerosols [8].

These guidelines convey that the samples and specimens can be handled or manipulated under enhanced BSL-2 precautions only if they have been biologically inactivated and a thorough risk assessment has been conducted by the project team. This article summarizes common inactivation methods that are suitable for SARS-CoV-2 samples/specimens to permit these materials to be downgraded from BSL-3 to a BSL-2 lab setting with enhanced BSL-2 precautions. The researchers could reference this article while formulating their COVID-19/SARS-CoV-2 related research or process enhancement protocols and requesting the required approvals from their respective institutional research safety committees.

The methods described below are standard methods that are used in research labs and are based on a review of available scientific literature that supports their efficacy for inactivating SARS-CoV-2 or other members of the β-coronavirus family. These methods are specifically proposed for the research laboratory activities related to manipulated or inactivated COVID-19 strains and are not meant for clinical laboratory practices. Overall, this article provides researchers a structure through which they can formulate their SARS-CoV-2 related research or process enhancement protocols and acquire all required approvals from their respective institutional research safety committees for conducting research in their currently available BSL-2 space. As this is a novel virus, the effectiveness of these inactivation methods has not been tested yet. SARS-CoV shares 80% genome identity with the new SARS-CoV-2 [9]. Therefore, it is anticipated that the methods which were used to inactivate SARS-CoV will also work in the case of SARS-CoV-2 strain but scientifically these methods need to be validated in the case of the new viral strain.

Physical and Chemical Inactivation Methods

Heat mediated inactivation (Recommended only for RNA extraction and not for immunological assays)

It has been established that most of the mesophilic microflora (pathogenic/non-pathogenic) are inactivated when heated above 56°C is for a constant period. Likewise, members of the coronavirus family of viruses can be inactivated by applying constant heat [10-15]. Several laboratories have evaluated the best temperature range and time duration for complete inactivation of SARS-CoV-2 strains in various specimens since viral aggregate protein content of the specimens can vary as a function of time while the specimen is heated. Amongst these, the most effective and with the high reproducibility rate for complete virus inactivation has been heating the sample at 75°C for 45 minutes or 65°C for 90 minutes in a heated circulating water bath pre-calibrated ahead of the start of the process of sample heat-inactivation (although the temperature duration might vary according to the volume of the sample and the size of the primary sample holding container) [10-13].

Once heat-inactivated the samples should only be handled under Class II biosafety cabinet for further manipulations. The research or process development protocols that are adopted in basic research laboratories for handling samples and specimens tentatively having SARS-CoV-2 strains should be adopted with a more conservative approach with longer exposure times/temperature to assure complete inactivation of the viral particles present in any form (viral particle load, viral aggregates or samples with high protein content) for attaining consistency in sample/specimen batch variations. Samples containing high protein content should be validated for viral inactivation as increased protein concentration affects heat diffusion. The heat inactivation method finalized should provide more coverage in inactivating any other RG3 agents which might be co-present along with SARS-CoV-2 strains in the tested samples/specimens. Finally, a QC step should be incorporated where documentation of the temperature log of the heated water bath used for the batch inactivation process is maintained and recorded every 20 minutes to ensure that the heating was constant during the whole process. However, heat inactivation is not recommended for immunological assays as it will interfere in antibody binding for clinical samples which could potentially lead to a false-negative diagnosis.

Viral concentration method (Non-aerosol generating method)

One of the key differences between the BSL-2 and BSL-3 biocontainment facility is the use of centrifugation step for concentrating the live virus for various downstream application. Even though the samples can be heat inactivated, the assurance of full inactivation of the viral load per sample can only be evaluated by conducting a plaque assay. As the viral load in each sample can be different therefore, in the absence of a plaque assay we should be cautious and should still assume that a certain level of viral load is still active and any step that promotes aerosol generation like centrifugation should be avoided. The presence of some innovative techniques like the InnovaPrep Concentrating Pipette (CP) Select (InnovaPrep, Drexel, MO, USA) and electronegative HA filtration can be used to resolve such issues of aerosol generation during isolation of the viral strains/nucleic acid for downstream applications. As precautionary measure the elution step should be carried under class II biosafety cabinet. The retentate from both these approaches can be eluted in the recommended buffers and can be stored at -80 °C freezer until total nucleic acid isolation is completed for use in real time PCR or sequencing analysis.

Chemical/lysis buffer induced inactivation (RNA extraction for QPCR)

The QPCR assays require RNA extraction from the virus. The samples for RNA extraction are stored (room temperature or lower) in specific buffers that inactivate proteins while preserving the RNA. Detergents disrupt the lipid coat of enveloped viruses and typically do not disrupt the proteins so they can be

used in downstream procedures preserving the native structure of proteins. Likewise, in many of the workflows, the virus is also transferred in VTM (viral transfer media) which helps to keep the virus viable. The sample storage buffers used might inactivate the enveloped virus, but there is little data on the effect of storage buffers on activity of coronavirus and there are no reported studies that have investigated the exact role of storage buffers' ingredients on COVID-19 inactivation. Therefore, SARS-CoV-2 samples in storage buffers should always be considered viable. The samples transferred in VTM must be lysed before they can be brought into the BSL-2 setting. Also, the viability quality check (plaque assay) must be performed in the BSL-3 laboratory that is sending the samples and the viability check report must be submitted to the institutional research safety committees for review and final approval.

The lysis buffer commonly used by various laboratories that has been evaluated for inactivating MERS-CoV are the TRIzol and AVL buffer (Qiagen kits) [14,15]. Recently, the studies on SARS-CoV-2 showed that several lysis buffers from extraction kits like ATL (1% to 10% SDS) and VXL (30% to 50% guanidine hydrochloride and 1%-10% Triton X-100) inactivate the virus. We believe the heat-mediated inactivation method followed by the addition of the lysis buffer in the sample provides a more conservative approach in being assured that the virus will completely be non-viable and replication-deficient. This approach is vital in research laboratories where the viability check cannot be performed using the plaque assay. It has been reported that once inactivated, centrifugal columns remove cytotoxic compounds and raise the threshold of detection by approximately 10-fold [16,17].

Chemical fixation induced inactivation of COVID-19/SARS-CoV-2 (cells or tissues)

These methods preserve cell morphology and simultaneously render samples/specimens or biomaterials to be noninfectious. Majority of the studies have utilized chemical fixed monolayer of coronavirus transfected (MOI 0.001) cells (tissue culture) for studying the viral particle. The tissues that will be preserved for imaging can be stored immersed in 10% formalin or phosphate-buffered saline containing 4% paraformaldehyde (PFA) for at least 24 hours. The 24-hour fixation time is enough for tissue samples as large as 2 cm^3, although the permeability rates vary according to the tissue sample type and therefore must be validated. Reagents like TRI-zol used for RNA isolation, samples should be immersed in 3 times the reagent volume. The common chemical fixatives that have been used for histopathology and microscopy are as follows: 4% paraformaldehyde, 10% neutral buffered formalin and 1:1 methanol/acetone [18]. A 30-minute incubation in formalin or paraformaldehyde at room temperature was enough to inactivate MERS-CoV; a 60-minute incubation was required for methanol/acetone. Fixation using 100% methanol or 4% PFA is also used for downstream immunohistochemical techniques or methods that utilize to stabilize cellular viral structure. Currently, there are no peer-reviewed data on inactivation of coronavirus in tissue samples. The CDC Interim Guidance on Collection and Submission of Postmortem Specimens from Deceased Persons with Known or Suspected COVID-19 instructs that a tissue of 4-5 mm in thickness should be placed in at least 10 times the volume of the sample of 10% formalin and incubated for 72 hours for optimal fixation [18].

Paraffin embedded samples should be fully heat-inactivated because the paraffin infiltration/perfusion step requires sample to be kept at a temperature close to 65°C for around 2 hours [19]. Formaldehyde has been used for quite some time in inactivating the virus particles especially in vaccine production, such as the polio Salk vaccine [20]. An earlier study showed that the formalin and glutaraldehyde inactivation of SARS-CoV by incubating virus samples with formalin or glutaraldehyde at two different dilutions (1:1000 and 1:4000; in PBS) with incubation temperatures of 4, 25 or 37°C [21]. Both aldehydes had no effect on inactivation at 4°C but at 25°C and 37°C formalin inactivated most of the virus, close to the limit of detection of the assay, after 1 day, with some virus remaining infectious on day 3. However, glutaraldehyde completely inactivated the virus by day 2 at 25°C and by 1 at 37°C. This suggests that both formalin and glutaraldehyde can be used as efficient methods of SARS inactivation.

Effect of pH change

It has been reported earlier that a pH of 8.0 induces a conformational change in the spike protein of the coronavirus, which enables fusion of the virion with the host cell [22]. Specifically, the spike protein of SARS-CoV has been reported to form a fusion with the host cell at neutral pH. This clearly informs us that the pH change can affect the infectivity of coronaviruses. The study investigated the effects of extreme change in pH with the degree of infectivity of SARS-CoV. SARS-CoV was exposed to extreme alkaline pH conditions of pH 12 and 14 for 1 h with an outcome of complete virus inactivation. The pH change between 5 to 9 had little effect on viral titer. However, acidic pH of 1 and 3 completely inactivated the virus at 25°C and 37°C. This clearly depicts that SARS-CoV infectivity is significantly sensitive to extreme pH environments. Interestingly, this also explains that why the SARS-CoV could not survive in the human stomach (gastric secretions pH range 1.0 to 3.5) but can survive in the large intestine (pH range 7.5 to 8.0) and can get transmitted through the stool of the infected patient as it can replicate in the slightly alkaline environment of the intestines.

Quality control to assess the effectiveness of the inactivation procedure used

Before moving the SARS-CoV-2 live virus or COVID-19 clinical samples to BSL-2 laboratory with enhanced practices, care must be taken to verify that the selected inactivation method has successfully inactivated the virus making it 100% incapable of replication. For envelope viruses (like SARS-CoV-2) the plaque assay is considered the gold standard for verifying that an inactivation protocol has been fully successful or not. This is a specialized assay and requires propagation of SARS-CoV-2 as a positive control therefore could only be performed in a BSL-3 setting. Any method of inactivation is acceptable if it has been validated using the plaque assay. Tissue culture infectious dose 50 (TCID50) assay is another method commonly used and less subject to variations linked to operator counting plaques and other fluctuations associated with plaque assay controls. As TCID50 assays are performed with multiple microcultures, they allow statistical analysis and provide more robust results than plaque assays.

We all are aware that most of the research institutes in the country do not have BSL-3 laboratories or expertise to assess the success of the inactivation procedure. Therefore, under such circumstances, methods should be developed where a hybrid approach of inactivating the virus could be adopted. A process using heat inactivation along with chemical lysis is strongly recommended while isolating the RNA for QPCR or whole genome sequencing (WGS) applications. This approach will provide higher assurance that the virus particle has been fully inactivated and are safe to be used for downstream biological application under BSL-2 laboratory with enhanced practices.

Recommended additional requirements and compliance for the use of inactivation methods approved by the Institutional Research Safety Committees

The inactivation methods suggested or approved by the research committees must be followed by the investigators who are working with potential COVID-19/SARS-CoV-2 biomaterials and samples or specimens. The investigators can suggest newer methods of inactivation, but those must be approved by their institutional research safety committees. The investigators are also required to do the following:

1. All procedures including sample collection, handling, processing and storage should be mentioned in the lab standard operating procedures (SOPs). A copy of the SOP must be submitted to the research safety committee for approval.
2. If possible, all activities should be confined to one laboratory, where no other research project or process development activities are occurring.
3. Appropriate EPA (Environmental Protection Agency) approved disinfectants for coronavirus must be used to decontaminate outer areas of the containment boxes/containers, work surfaces and solid and liquid waste. Double gloves must be used when handling biomaterials, samples, or specimens. The outer glove should be discarded appropriately if contamination is suspected.

4. The biosafety cabinets used for BSL-2 settings must have been recently inspected for proper functioning along with the UV lamp tested for exact amount of UV energy delivered (>0.04 J/cm^2). The energy evaluation is primary to the time duration of UV exposure. The validation process is ascertained to ensure that no infectious virus is viable after UV inactivation. The safest approach is to replace the UV lamp with a new one at the start of the project as the energy emitted by the UV lamp deteriorates with time (especially when validation of the energy emitted by an older UV lamp cannot be evaluated in the laboratory). In recent years, the UV lamp-based method for sterilizing the class II biosafety cabinet has been a bigger issue due to the actual effective UV intensity being varying at times. As a safeguard measure the labs should also be conducting extensive chemical cleaning procedures of these biological cabinets and not just relying on UV lamps.

5. Personnel must demonstrate proficiency in carrying out the procedures successfully and, as mentioned in the lab SOP; this proficiency must be documented by the designated oversight laboratory personnel (mainly the PI or the lab manager). The enhanced BSL-2 precautions followed by the personnel must be documented and included in the lab SOP.

6. A lab COVID-19/SARS-CoV-2 Specimen Intake Form must be generated and included in the above SOP. The form must have the information of the person receiving, processing, data acquisition, and sample storage with recorded time and dates.

a) Sample acceptance and rejection criteria

b) Steps to be followed if there is sample container leak

c) Steps to be followed if the sample requires shipment outside the specific research building.

7. Any inactivation procedure that requires opening the sample container or has the potential for aerosol creation must take place inside a biosafety cabinet.

8. An amendment to the inactivation method should be updated in the SOP and the paperwork must be submitted to the institutional research committee.

9. Institutional research safety committee approval for transfer of inactivated materials from a lab should be limited to the materials and labs detailed in the lab's COVID-19/SARS-CoV-2 Specimen Intake Form and SOPs. Any changes to the materials being transferred of the recipient labs must be communicated to the research safety committees or ad hoc work group for review and approval.

10. For each batch of inactivated samples, a log must be maintained that details:

    a) Type of sample/specimen (whole blood, serum, environmental, etc.)

    b) Date of inactivation

    c) Person inactivating the sample

    d) Activation method used (specific SOP followed must be documented)

    e) The inactivation samples got transferred further to which person

11. Each batch of sample inactivation and materials transferred to the next person in the research laboratory should be documented in writing which includes a detailed method used for the sample inactivation process. By dating and timing the transfer, both the giving and receiving persons will also wet sign the transfer thereby creating a chain of custody. Whenever one person transfers a sample another, the data and time of transferred must be countersigned by both individuals and the giving individual specifies the action(s) that they performed on the sample. Such action must be directly specified as described in the research protocol.

12. If the person in-charge of the sample inactivation process suspects that a batch might have failed the inactivation process for any possible reasons then he/she should contact the PI, Research Safety Officer, and Institutional Research Safety Committee Chair immediately. The sample should immediately be placed in a BSL-2 hood and isolated pending a full review. This event should be reported as an adverse event by the PI to the research committee clarifying the remedial action plan taken to fix the current and the future issues of similar nature.

Oversight and compliance monitoring

Every 3 months the PI and the research team should be required to submit a report of the samples handled and the data generated (including protocol SOP deviations). The research safety committee should review the report and provide necessary feed-back. The research safety officer and the research safety committee members should be performing random audits of all research logs, training certifications, safety drills and security to monitor safety compliance as per the approved project SOP.

Discussion

The global presence of BSL-3 containment facilities is minimum while the burden of testing and conducting research on SARS-CoV-2 is growing exponentially. There is a dire need to develop procedures where the RG-3 agent could be handled under BSL-2 containment facilities which are more commonly present worldwide. Developing protocols that inactivate the virus and at the same time maintains the integrity of the sample for testing and research is one of the practical choices to be followed. This approach not only speeds up the testing process but also supports the much-needed research required to fight this pandemic. The inactivation of coronavirus can be achieved through several techniques, but the most important aspect of the inactivation techniques is not only to make the microorganism non-infectious but at the same time also maintain the integrity of the sample for down-stream applications (molecular, immunological, etc.) for detection and for research activities. The choice of inactivation technique should not compromise the quality of biological material (RNA, protein, etc.) extracted from the sample for further applications.

The isolated protein and RNA samples extracted from the viral particles has been the major source of testing and laboratory research. Therefore, methods that are being developed for inactivation of the viral particles should not immensely affect the quality of the isolated proteins and the RNA sample. The whole process can be divided into two steps a) Validation of the robustness of the inactivation process b) determining the quality of the biological material isolated from the inactivated samples. Given the burden of testing and conducting research on SARS-CoV-2 globally, the greatest advantage in using the heat inactivated method is that it is applicable in situations where sophisticated instrumentation is not available (remote areas) and just a timer and heated water can be used to inactivate the COVID-19 positive samples without compromising the integrity of the sample. Now, a special attention must be given for the kind of assay oneself will be conducting for achieving the study outcome. If the end goal is to perform immunological based assays/research, then the heat inactivation method should be avoided as it will inactivate viral proteins necessary for the success of the assay and will provide us with higher false negative results.

To preserve the proteins in the sample that are to be used for immunological assays, detergents and UV can be used to inactivate viruses [22]. Similarly, if the integrity of the viral genetic material is affected by the inactivation method adopted, then the chances of false negative results will increase significantly. In majority of the testing and research activities related to SAR-CoV-2, real time RT-PCR is being used as a gold standard for detection. The rate of false negative results will increase if the quality of RNA and the RNA copy number gets affected during the sample inactivation process. The reduction in RNA copy number will reduce the amount of detectable viral RNA (low sensitivity) in an assay like real time RT-PCR, increasing the limit of detection (LoD) along with expected increase in false-negative results. In a pandemic situation, we

do not want this to happen. These conditions promote false negative results, failing the whole purpose of the molecular based detection or identification of the viral strain. It was reported in a recent study that whenever possible, the inactivation step should utilize the use of TRizol reagent than heat inactivation, as the TRizol reagent had the least effect on the RNA copy number among the tested samples [23]. In the past, formaldehyde has been shown to inactivate both SARS and MERS [24] and thus is a strong candidate for disinfection and inactivation of SARS-CoV-2 [24,25]. The specificity and sensitivity of virus testing is not affected by formaldehyde inactivation in case of immunological based testing, but the molecular based virus testing is absolutely hampered whenever this reagent has been used for inactivation. Formaldehyde results in RNA degradation and modification which allows an increase in false negative cases of molecular detection [26]. Overall, in case of SARS-CoV-2, this reagent should be the choice of disinfection, but sample inactivation should only be conducted if the sample is not being used for molecular based detection.

The primary step in molecular detection or identification of any virus is the lysis step where the nucleic acids are exposed and extracted following the lysis and are used for the downstream application. Similarly, the molecular detection of SARS-CoV-2 requires the use of some of these lysis buffers for molecular based detection. Earlier the use of these lysis buffers has yielded varied results but interestingly the recent use of the AVL lysis buffer in the SARS-CoV-2 studies has provided consistent results in virus inactivation [27].

The decision-making process in picking up the method of sample inactivation process totally depends on the kind of assay that is used. A heat inactivation will work perfectly in case of molecular biology-based assays but will not be a good choice if immunological based detection assays are being planned with the sample. Sometimes it becomes impossible to maintain a balance between achieving maximum sample inactivation and to maintain sample integrity at the same time therefore, secondary inactivation methods could be adopted to resolve the issue. The heat inactivation method can be combined with the chemical inactivation method to gain control [28-30]. Another approach of inactivating samples and at the same time maintaining the quality of the RNA and the copy number is the use of the virus transport medium (inactivation medium). Life science product making companies like Clinisciences (https://www.clinisciences.com/en/buy/cat-sars-cov-2-products-for-diagnosis-5098.html) are producing SARS-CoV-2 transport medium which can be used to transport clinical samples in an inactivated condition, minimizing the threat of infection significantly. Moreover, at the same time this method protects and stabilizes the virus nucleic acids that are used for molecular downstream applications like PCR and gene sequencing for diagnosis, screening, or epidemiological data collection.

As the pandemic is not going away, the scientific community must quickly develop strategies to combat SARS-CoV-2 with the tools and environment that are currently available. The effort in developing methods to manipulate an RG3 agent in such a way that it can be handled under BSL-2 conditions is a challenge but with a scientific approach it can be achieved successfully. The availability of BSL-3 containment facilities is limited globally and the dire need to conduct SARS-CoV-2 research is increasing immensely. The current article facilitates the researchers to carve out their SARS-CoV-2 related research protocols in such a way that they would be able to carry out their research in their currently available BSL-2 containment facilities which are far more in number globally.

Conclusion

These sample inactivation approaches allows research and testing labs to collect smaller or larger volume of samples with significant lower chances of exposure during sample transportation, handling, and processing thus, allowing the labs with BSL-2 or lower capabilities to handle the growing demand of research and testing for SARS-CoV-2 worldwide. It is our understanding that the availability of good scientific tools is already there but generating space for handling RG3 agent is a challenge. The current article has been an effort towards developing a strategy in handling this challenge. This article will help the researchers to carve out their assay specific protocols that can be used to carry the much-required SARS-CoV-2 research in their available BSL-2 laboratory space. Taken together, we hope that the methods reported here will serve to expedite the SARS-CoV-2 related research activities in the largely available BSL-2 laboratories worldwide.

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Conflicts of Interest

The author declares no conflict of interest.

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