Journal of Clinical & Experimental Pathology

Saheb, J Clin Exp Pathol 2023, 13:1

Commentary Open Access

Pathology and Preclinical Coronavirus Research in a High-Containment Laboratory

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Received: 05-Jan-2023, Manuscript No. JCEP-23-94891; **Editor assigned:** 09-Jan-2023, PreQC No. JCEP-23-94891 (PQ); **Reviewed:** 30-Jan-2023, QC No. JCEP-23-94891; **Revised:** 07-Feb-2023, Manuscript No. JCEP-23-94891 (R); **Published:** 15-Feb-2023, DOI:10.4172/2161-0681.23.13.431

Citation: Saheb H (2023) Pathology and Preclinical Coronavirus Research in a High-Containment Laboratory. J Clin Exp Pathol. 13:431.

Description

Pathologic assessment of future models, as novel infections emerge a new animal models needing high-containment facilities that are established. In addition to the scientific community's ability to quickly change course in reaction to the COVID-19 pandemic, this was a watershed moment for animal models as they emerged as a crucial tool for the quick assessment of therapies, from remdesivir to monoclonal antibody therapy. Pathology data sets derived from animal models are by definition translational research and essential indicators for assessing preclinical models.

The pathology community has worked to develop technical guidelines that support best practises to support pathologic end points and to advance rigour and reproducibility in recent years, but these very procedures present difficulties when used in a high-bio containment laboratory, which is typically where coronavirus studies are carried out.

The purpose of this paper is to discuss obstacles to performing preclinical coronavirus investigations and possible solutions to include pathogenic end points. As with any studies, early preparation and coordination between researchers and pathologists, followed by openness in disclosing findings, will advance the best science and animal usage.

Laboratories with strict security

Following an evaluation of the biological safety risk, certain bio-hazardous agents are given a Bio Safety Level (BSL) at which work with these agents is permitted. There are four BSLs in the US, numbered 1-4, and as the BSL rises, so do the requirements for biosafety facilities, safety gear, operating procedures, and personal Protective Equipment (PPE). *Bacillus subtilis*, *Saccharomyces cerevisiae*, and laboratory strains of *Escherichia coli* are examples of bio-hazardous agents that are designated for work at BSL 1, which do not normally cause disease in immuno-competent people. In laboratories lacking specific primary or secondary barriers outside of a door, a hand-washing sink, and easily cleanable work surfaces, work with these agents may be undertaken utilizing fundamental safety precautions and accepted microbiological practices.

Pathogens such seasonal influenza A viruses, *Staphylococcus aureus*, and *Toxoplasma gondii* are examples of BSL2-designated agents that have the ability to inflict disease on immuno-competent people with varied degrees of severity largely through percutaneous, mucosal, or oral routes of exposure. Sharps handling requires additional safety measures, in keeping with the risks involved in working with BSL2 agents, and procedures with a high risk of splashing or aerosolization are typically carried out using Biosafety Cabinets (BSCs) or other physical containment equipment. Practices and facilities that fall under BSL3 and BSL4 are referred to as "high containment." Mycobacterium TB, SARS-CoV-2, and yellow fever virus are a few examples of agents with a BSL3 designation that have the potential to cause serious or fatal illness after aerosol transmission.

At BSL3, primary and secondary barriers are given more attention than at BSL1 and BSL2, in order to safeguard the staff, the local community, and the environment. Workers must use Powered Air-Purifying Respirators (PAPRs) or N95 masks as respiratory protection to reduce the hazards of aerosol transmission. Moreover, all work must be done in a BSC or with a primary containment device, such as a centrifuge with rotor gasket and sealable cups.

Advanced ventilation systems with negative directed airflow and access control measures are also required for BSL3 facilities. In addition to anatomic pathology, one of the challenges in acquiring and analyzing tissues from BSL3 animals is clinical pathology. A Complete Blood Count (CBC) utilizing a hematology analyzer could initially seem straightforward given the machine's tiny footprint, low cost, and regular optimization for small sample volumes obtained from rodents. Fluid samples frequently need to be evaluated shortly after collection and cannot be kept without affecting subsequent studies, necessitating the assays to be performed at the BSL3 facility.

Aerosols are produced by the analyzer as it collects blood samples, necessitating their housing in a BSC while in use. The waste produced by the instrument also needs to be decontaminated without impairing its capacity to function technically in the future. If specific housing in the BSC is not available, analyzers are compact but weigh about 12 kg, making them difficult for a user wearing PPE to maneuver within and outside of the BSC. Also, the calibration of the equipment requires expensive and time-consuming quality control reagents. The number of samples that may be tested in a day is limited by the time it takes to conduct a CBC on one sample, which is typically not financially practical. CBCs can be run on one sample at a time but can take 2 to 20 minutes. Samples should be properly stratified to minimize batch effects. An alternative is a semi-automated or automated blood film analyzer in the BSC, which represents an added expense and an extra instrument within the facility. Serum chemistry analysis is slightly more amenable to high-containment laboratory studies, as many analyses are stable for prolonged periods by frozen storage, allowing analysis to be conducted at a time convenient for staff. However, the concern for generating aerosols and limited BSC space remains, and some analyses, particularly enzymes, exhibit reduced activity with freeze–thaw cycles. Point-of-care serum chemistry analyzers are available, but cost and rodent serum volumes can be prohibitive.