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A Standardized Protocol for the Safe Retrieval of Infectious Postmortem Human Brain for Studying Whole-Brain Pathology

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Abstract: We describe a safe and standardized perfusion protocol for studying brain pathology in high-risk autopsies using a custom-designed low-cost infection containment chamber and high-resolution histology. The output quality was studied using the histological data from the whole cerebellum and brain stem processed using a high-resolution cryohistology pipeline at 0.5 μm per pixel, in-plane resolution with serial sections at 20-μm thickness. To understand the pathophysiology of highly infectious diseases, it is necessary to have a safe and cost-effective method of performing high-risk autopsies and a standardized perfusion protocol for preparing high-quality tissues. Using the low-cost infection containment chamber, we detail the cranial autopsy protocol and ex situ perfusion-fixation of 4 highly infectious adult human brains. The digitized high-resolution histology images of the Nissl-stained series reveal that most of the sections were free of processing artifacts, such as fixation damage, freezing artifacts, and osmotic shock, at the macrocellular and microcellular level. The quality of our protocol was also tested with the highly sensitive immunohistochemistry staining for specific protein markers. Our protocol provides a safe and effective method in high-risk autopsies that allows for the evaluation of pathogen-host interaction, the underlying pathophysiology, and the extent of the infection across the whole brain at microscopic resolutions.

Key Words: biohazard containment, brain fixation, brain infections, cranial autopsy, high-resolution histology, infectious disease transmission
(*Am J Forensic Med Pathol* 2023;00: 00–00)

Well-preserved postmortem preparations of the whole brain are vital for studying the pathophysiology of infectious diseases of the brain. Autopsies and subsequent extractions of the brain and other tissues help us understand the pathophysiology of brain infections with manifestations varying according to the causative pathogens and their incubation periods. However, autopsies in infectious cases come with significant occupational risks including the risk of infection.[1] Before performing high-risk autopsies and whole-brain histology studies, 2 concerns need to be addressed. First, the minimization of the risk of the spread of pathogens during the autopsy and the development of a standardized protocol for the preservation, extraction, and high-quality histology of the brain.

In the 1990s, when unusual opportunistic infections and malignancies were reported among homosexual men, autopsy studies were key in identifying the causal pathogen, human immunodeficiency virus (HIV).[2] More recently, autopsy studies have helped us study thrombotic events and other pathology among patients infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). During the 2003 anthrax bioterrorism attack, US autopsy studies provided information that was important in the management of other individuals with infection.[3]

There is significant evidence that transmission of pathogens can occur during autopsy as summarized in Table 1 (adapted from the study by Gill[4]). This underlines the need for safety protocols and facilities where high-risk autopsies can be conducted. Several methods have been proposed for handling high-risk cases including the use of personal protection equipment (PPE), high ventilation, and virtual autopsies.[5] However, there is still a lack of standardization in the methods used for isolating the body, particularly in cases of infectious diseases. Furthermore, if the medical information at the time of autopsy is incomplete or if an accurate antemortem diagnosis cannot be made, the autopsy surgeon and the team may inadvertently be exposed to pathogens and can experience profound consequences from contracting infectious diseases such as *Mycobacterium tuberculosis*, hepatitis B virus, hepatitis C virus, HIV, variant Creutzfeldt-Jakob disease, and spongiform encephalopathies.[6–8] Many of the pathogens

Manuscript received February 18, 2023; accepted June 13, 2023.
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The authors report no conflict of interest.
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This work was supported by funding from Office of Principal Scientific Adviser, Government of India and Pratiksha Trust. P.P.M. is supported by the H N Mahabala Chair Professorship of the Indian Institute of Technology Madras.
The study was conducted according to the guidelines approved by the review board and ethics committee of Christian Medical College Vellore (IRB no. 13262). All tissue processing was performed based on a protocol approved by the IITM Institutional Human Ethics Committee (EC/2021-01/MS/06) guidelines.
All autopsies were performed after due consent was obtained from the next of kin of the deceased involved in the study in accordance with the Declaration of Helsinki.
The data that support the findings of this study are available from the corresponding author upon reasonable request.
R.I.J., R.V., P.P.M., M.Si., and G.M.V. did the conceptualization. R.I.J., R.V., J. Ja., J.Js., and R.K. did the methodology. R.I.J., R.V., and J.Ja. did the formal analysis. RIJ, R.V., J.Ja., J.Js., and R.K. did the investigation. All authors did the resources. RIJ and RV. did the writing of the original draft. All authors did the review and editing. R.I.J., R.V., and J.Ja. did the visualization. G.M. V., M.Si., P.P.M., and R.V. did the supervision. G.M.V. and M.Si. did the project administration. M.Si., G.M.V., J.Js., and P.P.M. did the funding acquisition.
R.I.J. and R.V. shared first authorship.

ISSN: 0195-7910/23/0000–0000
DOI: 10.1097/PAF.0000000000000871

TABLE 1. Infectious and Noninfectious Conditions That Can Be Transmitted During Autopsies (Adapted From the Study by Gill[4])

Viruses	Human immunodeficiency virus, hepatitis C virus, hepatitis B virus, rabies, Hanta virus, West Nile encephalitis virus, lymphocytic choriomeningitis virus, human T-cell lymphotropic virus type I, Ebola virus, Lassa virus, and Yellow fever virus
Bacteria	*Mycobacterium tuberculosis*, *Yersinia pestis*, typhoid fever, cat scratch disease, anthrax, brucellosis, meningococcal infections, and melioidosis.
Fungi	Blastomycosis and coccidioidomycosis
Prions	Creutzfeldt-Jakob disease

responsible for these diseases have been observed to retain their infectivity even after the death of the patient.[9–13] SARS-CoV-2 has now been categorized under hazard group 3—along with other infectious agents such as HIV, hepatitis B virus, and hepatitis C virus—as it can cause severe disease, present serious hazards to healthcare workers, and spread to the community. Efficiently using PPE and taking adequate precautions can mitigate these associated risks.[14] Isolation in a workable chamber could provide an efficient solution in cases of virulent infection, such as Rabies, Ebola, Nipah, and SARS-CoV-2 infections.[15–17] Furthermore, in developing countries, mortuaries may not yet be equipped to safely handle such high-risk autopsies, and funding to upgrade them may not be available. Hence, a low-cost, effective, and safe strategy to handle high-risk autopsies is needed for studying highly infectious diseases. The low-cost infection containment chamber (LCICC) designed by Johnson et al[18] in 2021 was modified and used in this study for the extraction of whole postmortem brains for further evaluation. The outcomes of this effective and safe procedure have been evaluated using high-resolution histology.

In addition to the LCICC, there is also a need for developing a postmortem perfusion protocol to preserve whole brain, which is widely used in animal models. A standardized postmortem perfusion protocol to study infections of the human adult brain has not yet been fully developed because of the lack of trained personnel, specialized autopsy facilities, and control of postmortem intervals (PMIs) as well as because of the risks associated with handling infectious tissues.[19–21] In addition, histological processing of large volume whole brains in a high-throughput manner including human brains is also yet to be standardized. The tissue processing pipelines have been standardized for basic research for smaller animal brains, such as mouse[22] and the primate, marmoset[23] but not for human brains. Because of whole-brain ultrastructural studies are crucial for studying and quantifying a variety of infectious agents, there is a need for a standardized protocol to be developed. A perfusion protocol of the tissue along with as short a PMI as possible are arguably the best method for achieving high-quality tissue as it relies on the role of circulatory system rather than on diffusion.[24–26] Perfusion protocols are widely used in animal preparations where PMI can be controlled. As the control of the PMI is challenging in humans, immersion fixation is more commonly used for preserving large whole postmortem brains, particularly for brain banking. However, immersion fixation requires the tissue to remain in the fixative for a long period (4–8 weeks) making it less preferable to perfusion fixation.[21] Furthermore, perfusion fixation allows the washout solution and fixative to penetrate deeper regions of the brain. This ensures that tissues are free of blood artifacts and clots while also preserving the cellular morphology as close to the time of death. The choice of fixation technique

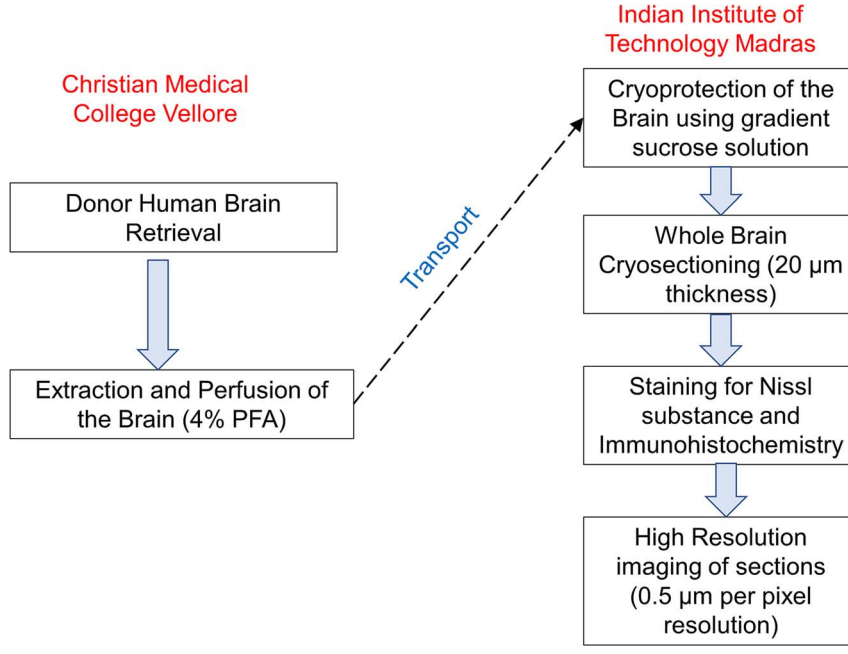

FIGURE 1. The workflow of the high-risk autopsy protocol for the extraction, fixation, and subsequent histology and imaging of whole human brains. The high-risk autopsy and brain extraction were performed at Christian Medical College Vellore and the subsequent histology and imaging of whole brains were through the histological pipeline at the Indian Institute of Technology Madras.

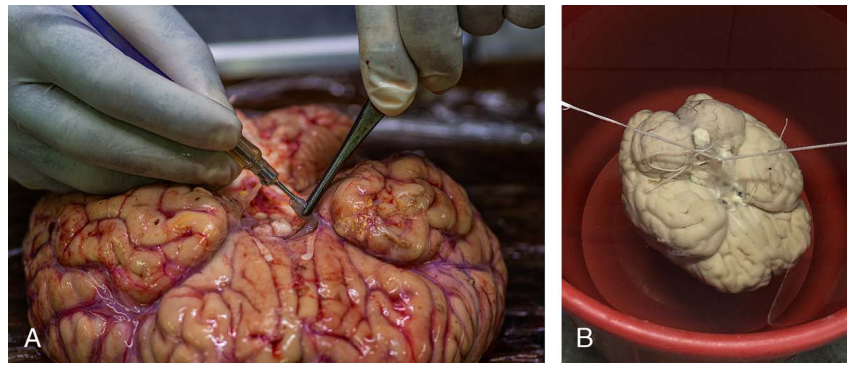

FIGURE 2. The perfusion and immersion steps after the extraction of postmortem human brains in high-risk autopsies. The left panel (A) shows the ex situ perfusion step where the major arteries are cannulated with 16–18G needles to facilitate the perfusion of the washout solution and the fixative. The right panel (B) shows the final immersion procedure where the brain is suspended in fixative with twine tied around the basilar artery perfusion fixation was done with 20% NBF after the use of washout solution (heparinized phosphate buffer solution). After perfusion, the brain was immersed in 10% NBF.

and the PMI is critical in preventing a reduction in tissue quality because of factors such as the breakdown of the cellular membrane, microbial degradation, and autolysis. The final quality outcome of histological processing of large whole human brains is dependent on high-quality sample retrieval and the standardization of the whole histology platform capable of processing large tissue samples in a high throughput in an undistorted manner that allows studying and quantifying of whole brains at cellular level. The histological data presented in this study are a part of whole human brain histology pipeline that has been developed at Sudha Gopalakrishnan Brain Centre, Indian Institute of Technology Madras, to process whole human brains. This article describes the key components of the experimental protocol for the extraction and perfusion fixation of brains in high-risk autopsies and the corroboration of outcomes in the form of cellular-level histology to demonstrate the consistent presence of high-quality tissue over large regions of the brain.

RESULTS

The first step of the processing pipeline is the autopsy and the extraction of the brain. Figure 1 shows the brain retrieval and processing pipeline where 4 autopsies were performed for standardization of the perfusion and extraction of the whole human brain at Christian Medical College (CMC) Vellore. The 4 autopsies were performed in the LCICC, on cases diagnosed with rabies (n = 1), SARS-CoV-2 and rhino-orbitocerebral mucormycosis (n = 2), and with pyrexia of unknown origin (n = 1). They were all male patients aged 25, 54, 57, and 69 years, respectively. After the extraction, the brains were perfused and immersed in formalin fixative for a minimum of 4 weeks, after which they underwent histological processing, using the Tape Transfer cryosectioing at the Sudha Gopalakrishnan Brain Centre, Indian Institute of Technology Madras (Fig. 1).

Postmortem Extraction of Whole Human Brain Using the LCICC and Standardization of Perfusion Protocol

The postmortem autopsies were carried out in the LCICC (described later). Figure 2 shows the ex situ perfusion fixation of one extracted brain (2A) followed by immersion fixation (2B). The PMI before the perfusion fixations ranged from 6 to 45 hours. The qualitative results presented here is from one of the samples. For the ex situ perfusion, the basilar and bilateral internal carotid arteries were cannulated using a 16/18-gauge needle and were perfused with heparinized phosphate buffer saline (0.01 PBS), which acted as a washout solution flushing out any blood clots that may be present in the vasculature. After the washout, approximately 750 mL of 20% neutral buffered formalin (NBF) was perfused over 15 to 20 minutes at room temperature. The end point of perfusion was determined when the blood from the vasculature was replaced with clear fluid and the outflow from the contralateral side was clear. After the perfusion, the brains were immersed in 25 L of 10% NBF and suspended for 3 to 4 weeks by twine tied around the basilar artery as shows in Figure 2B. The pH of the washout solution and fixatives used in the fixation process was maintained between 7.2 and 7.4 to avoid any tissue artifacts due to changes in osmotic concentration. Given the highly infectious nature of the diseases, such as rabies and SARS-CoV-2, retrieval of the brain was performed within the LCICC while following the recommendations given by the Centers for Disease Control and Prevention.[27] The skull was exposed by making a bimastoidal incision over the scalp and opened using an electric oscillating saw. After craniotomy and durotomy, the brain was retrieved carefully by making incisions to release the brain from its attachments (Fig. 2).

Figure 3 shows the custom-designed autopsy and containment chamber that was developed by the Department of Forensic Medicine at CMC Vellore to minimize exposure to pathogens. As

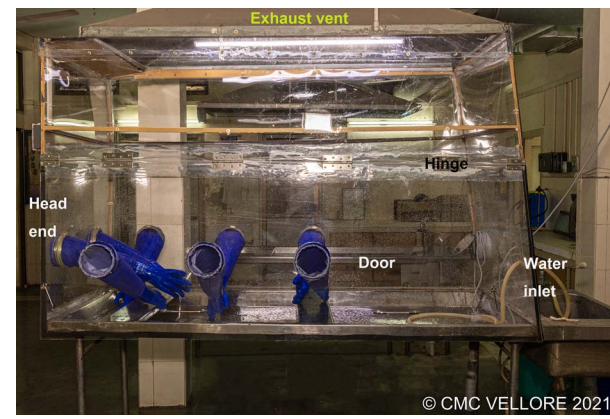

FIGURE 3. The custom-designed prototype for LCICC for high-risk autopsies. The figure shows the various components of the LCICC including the placement of the doors, inlets, and outlets.

described in Johnson et al,[18] the steps to create the LCICC are as follows:

A standard straight mortuary table measuring 2682.2 mm (length) by 914.4 mm (width) was modified using locally available materials. The mortuary table had a suction exhaust vent 1280.2 mm above its surface, which extended across the length of the table and allowed air to be released outside the mortuary.

Transparent acrylic sheets of 5-mm thickness were installed on the 4 sides of the mortuary table extending from the table to the vent above to create an enclosed space and were held in place with screws fixed to the metal plates.

1. Metal plates were screwed on to the ends of the table extending up to the exhaust vent, which act as the support for the acrylic sheets, which make the walls of the chamber.
2. The gaps between the acrylic sheets, table, metal plates, and suction vent were sealed using silicone sealant and rubber gaskets.
3. The acrylic sheet that formed one of the long walls was cut lengthwise in half, and both halves were joined with hinges to create a door, which could be lifted up to open and close the chamber.
4. A 2-mm-thick transparent plastic sheet was then glued behind the junction of the two halves to seal the hinge joint. Four-inch-wide openings were cut into the walls of the chamber, one at the leg end, two at the head end, and three on either side of the chamber.
5. Elbow-length, thick rubber gloves were fixed to these openings such that when the door was closed, procedures could be performed on the body by passing the hands through the gloves.

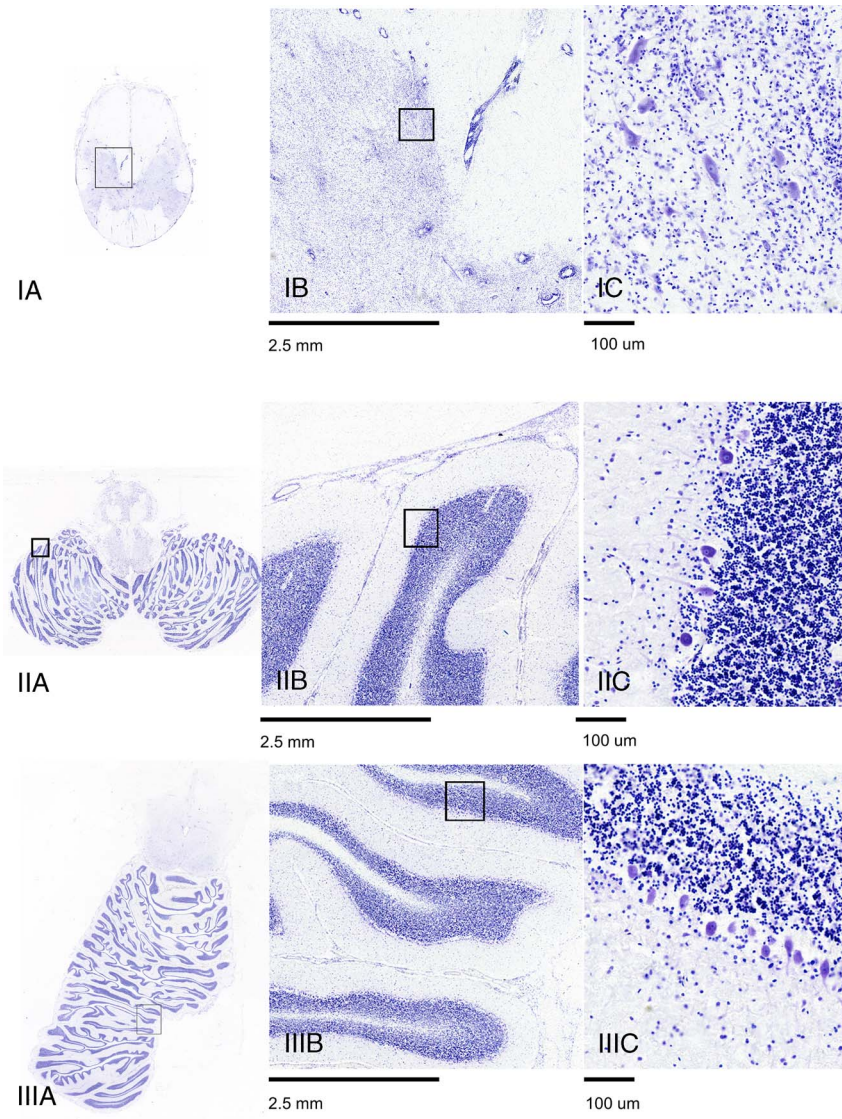

FIGURE 4. Histology of the brain demonstrating the outcome of procedure that starts with the extraction and perfusion protocol. The figure shows the transverse section of the brainstem cerebellum block (inferior-superior) at 3 different levels, which were stained for Nissl substance with thionine. I A–C show a section at the level of the medulla. B and C are high magnification images of the marked inset in (A). II A–C show a section at the level of pons of the anterior lobe of the cerebellum with the insets showing Purkinje cells at different resolutions (B, C). III A–C show a section from the superior cerebellum at the level of the cerebral peduncle with the marked inset in high resolution under high magnification showing different cells, including Purkinje cells.

6. At the leg end of the chamber, a one-way inlet valve was used to allow air to enter the chamber. Thus, when the suction was on, the air from the chamber was evacuated outside the room via the exhaust vent and room air entered through the one-way valve to balance the pressure within the chamber.
7. The autopsy surgeon could stand outside the chamber and perform the retrieval of the brain from the cadaver in the closed chamber, thus reducing the risk of exposure to aerosols.
8. The total cost of making the chamber was $410

Our LCICC is built around a standard autopsy table, which not only prevents the spread of harmful pathogens but also provides a substantial reduction in costs needed to convert the whole autopsy suite into a negative pressure room. The LCICC also minimizes contact with infected bodies, fluids, and tissues while allowing for decontamination of the body. The water inlets in the chamber allowed disinfection of the cadaver after the autopsy was completed and samples were collected before it was placed in a body bag.

Using elbow length thick rubber gloves in the LCIC results in some limitations of movements particularly for using complex instruments such as the oscillating electric saw and less flexibility compared with the sterile surgical gloves regularly used in autopsy. In addition, it also takes a longer time compared with normal brain extraction. All of the above limitations can be overcome with enough experience using the LCICC (Fig. 3).

High-Resolution Histological Data From Cerebellum Brain Stem Block

The evaluation of the effectiveness of the perfusion procedure was performed using the high-resolution digital histology of the extracted brains for Nissl (Fig. 4), hematoxylin and eosin (H&E) (Fig. 5), and immunohistochemistry (IHC) (Fig. 6). Figures 4 and 5 show inferior-superior transverse sections of the cerebellum that were stained for Nissl substance using 0.02% thionine and H&E, respectively. The middle and right panels show high-resolution images of laminar organization and individual neurons. The whole slab was approximately 530 mL in volume: dorsal-ventral, 6 cm; lateral-medial,

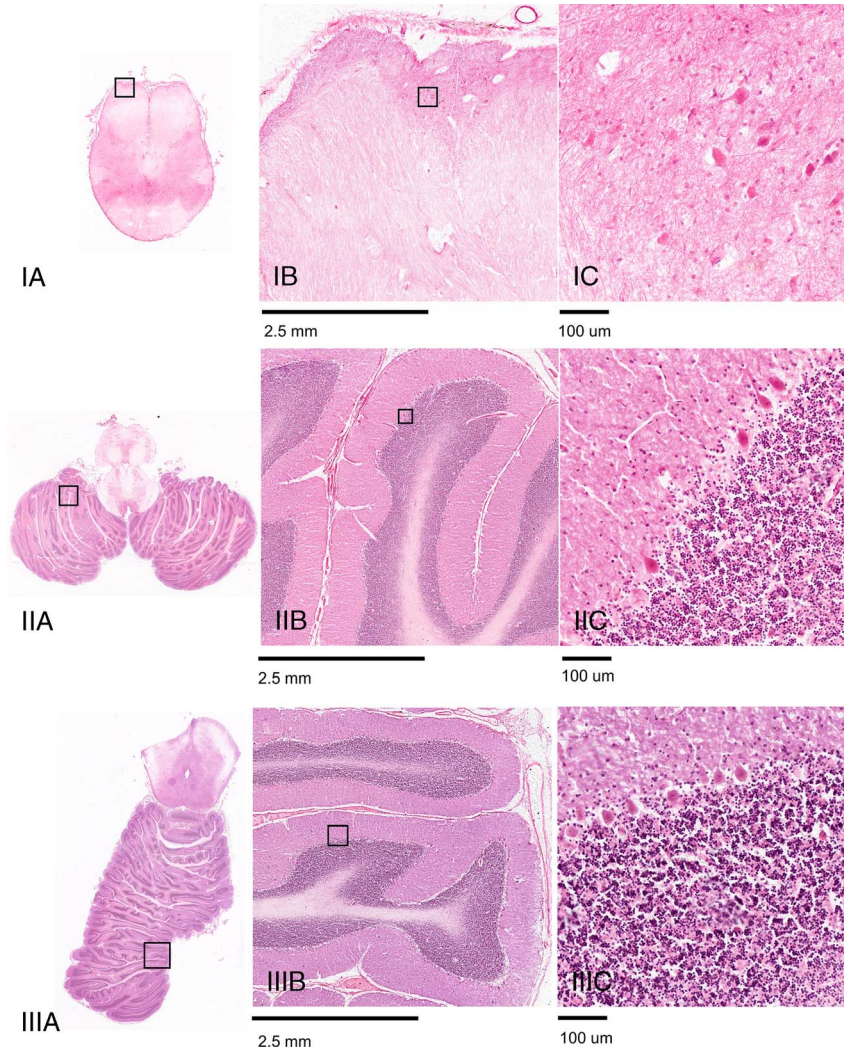

FIGURE 5. Hematoxylin and eosin–stained transverse sections of the brainstem cerebellum block (inferior–superior) at 3 different levels, similar to Figure 4. I A–C show a section at the level of the medulla, at the edge of arcute nucleus. B and C are high magnification images of the marked inset in (A). II A–C show a section at the level of pons of the anterior lobe of the cerebellum with the insets showing Purkinje cells at different resolutions (B, C). III A–C show a section from the superior cerebellum at the level of the cerebral peduncle with the marked inset in high-resolution under high magnification showing different cells, including Purkinje cells.

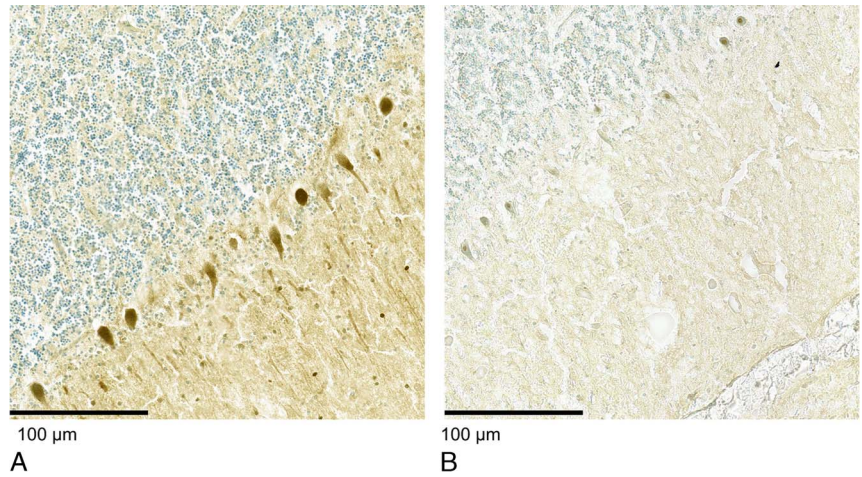

FIGURE 6. Immunohistochemistry labelling for GAD-65 and parvalbumin in the cerebellum. Panel (A) demonstrates the labelling of the Purkinje cells and their fibers for GAD-65 and panel (B) shows the IHC labelling of Parvalbumin-positive cells in the cerebellum.

11 cm; and anterior-posterior, 8 cm. The first row shows the inferior-most transverse section from the brainstem and medulla. High-resolution images of neurons in the cuneate nucleus (Nissl) and arcuate nucleus (H&E) at the level of the medulla, the anterior lobe of the cerebellum at the level of the pons, and the superior cerebellum at the level of the cerebral peduncles are seen in Figures 4 and 5:I, 4 and 5:II, and 4 and 5:III, respectively. The second row (Figs. 4, 5:II A–C) shows a high-resolution image of a section from the middle of the block at the level of the pons showing neurons in the molecular layer, Purkinje cell layer, and cellular layer of the left cerebellum. The third row (Figs. 4, 5:III A–C) shows the superior section from the block at the level of cerebral peduncles with neurons from the left cerebellum. We qualitatively assessed the protocol used based on the presence of fixation damage, osmotic shocks, freezing artifacts, mechanical damage during cryosectioning (blade and tape peeling artifacts), and staining artifacts. Across the block, including sections from 3 different levels, there was no observable evidence of perineural retraction spaces for cells or dark neurons, which are typically indicative of tissue damage during fixation.[28] In addition, the apical dendrites did not show any tortuosity, which is often present when fixation is inadequate or when the PMIs are delayed.[29] There was uniform fixation across the superficial and deeper regions of the large tissue, and Nissl staining demonstrated that there was no evidence of cellular osmotic shock (neurons appearing ruptured or bloated) or freezing artifacts (Swiss-cheese pattern), indicating that preservation was good, and histology was of high quality.[30,31] The cerebellum and brain stem slabs presented here were dissected separately by making surgical cuts at the level of the cerebral peduncles; due to large volume, the whole brain was divided into five slabs with minimal tissue damage after fixation (Figs. 4, 5).

Before cryosectioning, the samples were cryoprotected in graded sucrose (10%–30% sucrose). The solutions were changed once the tissue block sank completely. Throughout the fixation and cryoprotection process and until the block was frozen, pH measurements were performed regularly to ensure that the pH was maintained between 7.2 and 7.4. After cryoprotection, the slabs were embedded in an Optimum Cutting Temperature compound using the rapid freezing technique (−80°C) with isopentane in dry ice baths. To facilitate the rapid freezing of the large brain samples, the freezing methodology was developed and optimized on whole goat brains. This development is a necessary step as rapid freezing techniques have been implemented only for smaller animal brains.[30] The temperature of the freezing medium was maintained at −80°C to −78°C until the blocks were completely frozen. The frozen slabs were cryosectioned into 20-µm-thick sections and 3 alternating sets were stained for Nissl, immunohistochemistry, and myelin staining, using the modified tape transfer technique[22,23] on a large-format cryomacrotome (Leica CM3600, Leica Biosystems). The tape transfer technique provides an unprecedented advantage over the commonly used "brush" technique by producing geometrically undistorted and undamaged serial sections from the large tissue block.

Immunohistochemistry is a commonly used technique with a reputation of easily being disrupted if the tissue quality is less than adequate. Figure 5 shows the IHC of the cerebellum and brainstem block. The left panel, Figure 5A, shows Purkinje cells and their processes that have been labeled glutamic acid decarboxylase 65 (GAD-65). The right panel, Figure 5B, shows parvalbumin labelling that is specific to the nuclei of the Purkinje cells. Immunohistochemistry demonstrated that the techniques used for extraction, fixation, and histology were optimal to ensure the long-term viability of cells (Fig. 6).

DISCUSSION

We have developed a low-cost infection containment chamber and standardized a safe protocol for whole-brain perfusion and extraction that can be used in high-risk autopsies. The protocol has been qualitatively assessed using serial sections (approximately 3000) from a single cerebellum–brainstem block of 530-mL volume, which was then digitized at a high resolution (0.5 µm per pixel, in-plane). Studies have proven that taking safety precautions such as using the LCICC greatly limits and contain the aerosols generated during this stage of the procedure. Hasmi et al[19] devised a craniotomy box for this purpose. After the autopsy, they swabbed the surfaces of the craniotomy box and performed SARS-CoV-2 reverse transcription-polymerase chain reaction tests on them. Viral genetic material was only present within the craniotomy box proving that bone dust and aerosols generated while opening the skull carried the virus but was completely confined within the craniotomy box.

Our methodology can be adapted for in situ perfusion techniques. However, similar to previous studies,[25,26,32] the ex situ method for perfusing the brain was chosen over in situ methods as the body had to be handed over to the family within a few hours

of the autopsy. With the in situ technique, complete perfusion would take much longer as it would require careful dissection, identification, and clamping of all the vessels in the neck. Furthermore, with in situ techniques, there is a risk of suboptimal perfusion from unidentified vessel anomalies that result in high leakages. Ex situ perfusion allows accurate identification and prompt management of these challenges.

The volume and duration of perfusion with 20% NBF were similar to those in previous studies using the ex situ approach. When using the perfusion protocol, the use of a heparinized buffer solution followed by 20% NBF did not cause edema or osmotic shock at the microcellular level. Previous studies have used osmotic diuretics, such as 20% mannitol or 0.9% sodium chloride, as washout solutions to minimize edema and changes in volume.[26,33] Using a heparinized buffer solution with a pH of 7.4 eliminated clots, maintained the pH osmolarity, and reduced tissue damage at macro and micro levels. There were no signs of tissue edema as a result of perfusion fixation, or any other chemical processing used for histology in our study. Previous studies, which compared perfusion versus immersion fixation protocols, highlight the association between a short PMI and the quality of brain tissue. Hence, for this study, the PMI was kept less than 15 hours except for case 4 where it was 45 hours. Given the sensitivity of human postmortem samples, the PMI is often challenging to control, especially in infectious autopsies. Sharma et al[25] used the rapid fixation protocol of 20% NBF perfused through the lateral ventricles, followed by immersion fixation in 20% NBF for 3 to 4 days instead of the usual 4 weeks or more. In our study, the goal was to demonstrate subcellular whole-brain histology across large blocks. Hence, samples were left for immersion fixation for a longer duration until the brain was ready for processing. For whole-brain cryosectioning, we did not use a cryoprotectant during perfusion.[34] Rather, we chose to cryoprotect (graded sucrose) after immersion in paraformaldehyde to ensure good fixation before cryoprotection. After processing, the histological quality was assessed using the serial sections by comparing different regions of the brain tissue from the whole cerebellar and brain stem block. Histology demonstrated that the sections were free of fixation damage (neurons were free of perivascular space), neuronal shrinkage, osmotic shock (chromatin material in neurons were intact and did not appear ruptured), and freezing artifacts (no Swiss-cheese pattern was seen). In addition, process-sensitive methods such as IHC for GAD-65 and parvalbumin demonstrated that antigens were viable and tissue quality was good at the macrocellular and microcellular levels. Prolonged fixation with formaldehyde is known to alter tissues by causing changes in covalent bonds and antigen expression.[24] The long-term viability of tissues with our protocol was demonstrated by IHC labelling performed approximately 12 months after fixation and extraction. Nissl and IHC staining demonstrated that tissue quality was maintained with the long duration of fixation as demonstrated by other studies that have previously used these perfusion protocols.[24,25] The perfusion protocol using the partial ex situ approach along with cryoprotection and rapid freezing with tape transfer cryosectioning resulted in high-quality whole-brain histology.

CONCLUSIONS

Studying brain pathology in cases of high-risk infectious diseases requires standardized and safe retrieval of the whole brain with no risk of spread of infection and uniform good quality tissue preservation. Our methodology highlights the use of a LCICC and a perfusion protocol to not only limit the exposure to potential pathogens to the autopsy team but also ensure valuable high-quality fixation for studying the pathophysiology in the postmortem whole human brain. Combined with the high-resolution histology platform, this will be a valuable resource to investigate pathophysiology in detail, for large volume of brain samples, which are currently limited.

ACKNOWLEDGMENTS

The authors thank Dr Daniel Manoj, the technicians, and support staff from both CMC Vellore and IIT Madras for their assistance during autopsy and processing of samples.

REFERENCES

1. Claydon SM. The high risk autopsy. recognition and protection. *Am J Forensic Med Pathol*. 1993;14:253–256. doi:10.1097/00000433-199309000-00016.
2. Masliah E, DeTeresa RM, Mallory ME, et al. Changes in pathological findings at autopsy in AIDS cases for the last 15 years. *AIDS*. 2000;14:69–74. doi:10.1097/00002030-200001070-00008.
3. Centers for Disease Control and Prevention (CDC). Update: investigation of anthrax associated with intentional exposure and interim public health guidelines, October 2001. *MMWR Morb Mortal Wkly Rep*. 2001;50:889–893.
4. Gill JR. Autopsy: infectious and serious communicable diseases. *Encyclopedia Forensic Legal Med*. 2016;279–284. doi:10.1016/B978-0-12-800034-2.00039-2.
5. Liu Q, Wang RS, Qu GQ, et al. Gross examination report of a COVID-19 death autopsy. *Fa Yi Xue Za Zhi*. 2020;36:21–23.
6. Healing TD, Hoffman PN, Young SE. The infection hazards of human cadavers. *Commun Dis Rep CDR Rev.* 1995;5:R61–R68.
7. Aoun H. When a house officer gets AIDS. *N Engl J Med*. 1989;321:693–696.
8. Aoun H. From the eye of the storm, with the eyes of a physician. *Ann Intern Med*. 1991;116:335–338.
9. Henry K, Dexter D, Sannerud K, et al. Recovery of HIV at autopsy. *N Engl J Med*. 1989;321:1833–1834.
10. Ball J, Desselberger U, Whitwell H. Long-lasting viability of HIV after patient's death. *Lancet*. 1991;338:63.
11. Douceron H, Deforges L, Gherardi R, et al. Long-lasting postmortem viability of human immunodeficiency virus: a potential risk in forensic medicine practice. *Forensic Sci Int*. 1993;60:61–66.
12. de Craemer D. Postmortem viability of human immunodeficiency virus—implications for the teaching of anatomy. *N Engl J Med*. 1994;331:1315.
13. Hanley B, Lucas SB, Youd E, et al. Autopsy in suspected COVID-19 cases. *J Clin Pathol*. 2020;73:239–242. doi:10.1136/jclinpath-2020-206522.
14. Fineschi V, Aprile A, Aquila I, et al. Management of the corpse with suspect, probable or confirmed COVID-19 respiratory infection—Italian interim recommendations for personnel potentially exposed to material from corpses, including body fluids, in morgue structures and during autopsy practice. *Pathologica*. 2020;112:64–77.
15. Kantor HS, Poblete R, Pusateri SL. Nosocomial transmission of tuberculosis from unsuspected disease. *Am J Med*. 1988;84:833–838.
16. Posthaus H, Bodmer T, Alves L, et al. Accidental infection of veterinary personnel with mycobacterium tuberculosis at necropsy: a case study. *Vet Microbiol*. 2011;149:374–380.
17. Templeton GL, Illing LA, Young L, et al. The risk for transmission of mycobacterium tuberculosis at the bedside and during autopsy. *Ann Intern Med*. 1995;122:922–925.
18. Johnson LR, James RI, Rajapandian , et al. Novel, safe, indigenous cost-effective infection containment chamber for high-risk autopsies: the need of the hour. *J Forensic Med Toxicol*. 2021;38:102–106.
19. Hasmi AH, Khoo LS, Koo ZP, et al. The craniotomy box: an innovative method of containing hazardous aerosols generated during skull saw use in autopsy on a COVID-19 body. *Forensic Sci Med Pathol*. 2020;16:477–480.

20. Loibner M, Langner C, Regitnig P, et al. Biosafety requirements for autopsies of patients with COVID-19: example of a BSL-3 autopsy facility designed for highly pathogenic agents. *Pathobiology*. 2021;88:37–45.
21. McFadden WC, Walsh H, Richter F, et al. Perfusion fixation in brain banking: a systematic review. *Acta Neuropathol Commun*. 2019;7:146.
22. Pinskiy V, Jones J, Tolpygo AS, Franciotti N, Weber K, Mitra PP. High-throughput method of whole-brain sectioning, using the tape-transfer technique. PLoS One 2015, 10, e0102363, doi:10.1371/journal.pone.0102363.
23. Lin MK, Takahashi YS, Huo B-X, et al. A high-throughput neurohistological pipeline for brain-wide mesoscale connectivity mapping of the common marmoset. *Elife*. 2019;8:e40042.
24. Lyck L, Dalmau I, Chemnitz J, et al. Immunohistochemical markers for quantitative studies of neurons and glia in human neocortex. *J Histochem Cytochem*. 2008;56:201–221.
25. Sharma M, Grieve JHK. Rapid fixation of brains: a viable alternative? *J Clin Pathol*. 2006;59:393–395.
26. Grinberg LT, Amaro E Jr., Teipel S, et al, Brazilian Aging Brain Study Group. Assessment of factors that confound MRI and neuropathological correlation of human postmortem brain tissue. *Cell Tissue Bank*. 2008;9:195–203.
27. Human Rabies—Kentucky/Indiana, 2009 Available at: https://www.cdc.gov/mmwr/preview/mmwrhtml/mm5913a3.htm. Accessed October 17, 2022.
28. Sele M, Wernitznig S, Lipovšek S, et al. Optimization of ultrastructural preservation of human brain for transmission electron microscopy after long post-mortem intervals. *Acta Neuropathol Commun*. 2019;7:144.
29. Garman RH. Artifacts in routinely immersion fixed nervous tissue. *Toxicol Pathol*. 1990;18(1 Pt 2):149–153. doi:10.1177/019262339001800120.
30. Pinskiy V, Tolpygo AS, Jones J, et al. A low-cost technique to cryo-protect and freeze rodent brains, precisely aligned to stereotaxic coordinates for whole-brain cryosectioning. *J Neurosci Methods*. 2013;218:206–213.
31. Taqi SA, Sami SA, Sami LB, et al. A review of artifacts in histopathology. *J Oral Maxillofac Pathol*. 2018;22(2):279.
32. Adickes ED, Folkerth RD, Sims KL. Use of perfusion fixation for improved neuropathologic examination. *Arch Pathol Lab Med*. 1997;121:1199–1206.
33. de Oliveira KC, Grinberg LT, Hoexter MQ, et al. Layer-specific reduced neuronal density in the orbitofrontal cortex of older adults with obsessive–compulsive disorder. *Brain Struct Funct*. 2019;224:191–203.
34. McIntyre RL, Fahy GM. Aldehyde-stabilized cryopreservation. *Cryobiology*. 2015;71:448–458.