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Research Article

In-house Assembled Protective Devices in Laboratory Safety in Clinical Biochemistry Laboratory of a COVID-19 Dedicated Hospital

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Abstract

Staff of diagnostic laboratory handling COVID positive samples is at risk and need to take recommended protective measures. Many protective materials were not available in diagnostic laboratories in India when the pandemic reached the country before it was prepared adequately, forcing laboratory managers to take innovative measures to protect the laboratory personnel. In this study, we have evaluated the effect of standard operating procedure (SOP) using innovative protective devices such as face shield made from OHP sheets and cardboard boxes fitted with hypochlorite spraying device as alternative to biosafety cabinet on contamination of laboratory surfaces and rate of infection among laboratory staff with virus causing COVID-19. These were used for 14 days along with other routine safety measures like use of gloves, surgical masks, OT gowns etc. before PPE and biosafety cabinet were made available in Clinical biochemistry lab of a dedicated COVID hospital in the national capital region of Delhi, India. Laboratory technicians, residents and nursing orderlies posted in clinical biochemistry laboratory were checked regularly for signs and symptoms of COVID 19 during these 14 days and tested for virus causing COVID 19 from their nasal and throat swab by RT-PCR on 14th day. The laboratory surface, by taking swab from multiple sites, was checked on 14th day for above-mentioned virus by RT-PCR. The COVID test report was negative for the staff members (n = 10) and no surface contamination was detected. Innovative and cost effective protective devices can be built in-house with locally available resources and are effective in preventing the spread of COVID 19 among the staff working in clinical biochemistry laboratory. Laboratory managers in resource scarce areas need to be innovative to face such sudden challenges like COVID-19 pandemic.

Keywords: COVID-19; SARS-CoV 2; Biosafety; Laboratory Safety; Clinical Biochemistry Laboratory; Developing Country

The highlights of the manuscript are

- Strengthening the Basics Approaches to protect the lab personnel in dedicated COVID hospital of Low-Resource Settings.
- Designed and developed in-house standard operating procedure (SOP) to fill the gap and evaluate the effect in dedicated COVID-19 hospitals.
- Innovative protective devices made from OHP sheets and cardboard boxes fitted with hypochlorite spraying devices as alternatives to biosafety cabinets on contamination of laboratory surfaces.
- Performances of the devices were clinically validated and it can be used as alternative in low resources settings.

Introduction

Last day of the year of 2019 (December 31st 2019) was the day when the first Corona virus case was identified and came into light officially in China [1]. Even as on date there is a lot of fear and anxiety about this pandemic with drastic developments in the worldwide healthcare scenario [2]. In a low resource settings nation like India; a dense population country with limited resources, existing healthcare infrastructure and delivery system have already been facing challenges of accessibility and affordability. Unfortunately, due to COVID-19 pandemic further increased the already over burdened Health Care Workers (HCWs). The doctors, nurses, paramedics, and other hospital staff are bearing the onslaught of this infection directly. HCWs that are at the fore front of managing the disease are at grave risk of contracting the disease as it is highly contagious in nature. Besides the treating physicians and the intensivists who are coming in direct contact of the patients, laboratory personnel working in clinical biochemistry laboratories are involved in carrying out the various diagnostic tests required for management these COVID-19 patients [3]. Many such patients need to be tested for routine parameters like plasma glucose, liver function tests, kidney function tests, etc. The critical patients often need specialized tests like C Reactive protein (CRPC), Ferritin, Procalcitonin, D-Dimer etc. [4-7]. The risk of exposure of laboratory personnel to virus is substantial in view of presence of viremia although it is lesser than that of treating clinicians and interventionists. The risk of exposure of laboratory personnel to virus is substantial in view of presence of viral load although it is lesser than that of treating clinicians and interventionists [8,9].

The virus also spreads through contaminated fomites and surfaces which necessitate sanitizing all surfaces and fomites like centrifuge machine, bench-tops, switches, elevator buttons, door handles etc. [10,11]. However, major route of transmission of COVID-19 is through respiratory route and that mostly occurs through droplets produced during coughing and sneezing [12]. Many of laboratory procedures like centrifugation of samples, de-capping of sample tubes, pipetting etc. also generate aerosols that are potential sources of infection [13,14]. Various measures are recommended by CDC and WHO for preventing such spread of COVID-19 among laboratory personnel and Community [15]. Use of gloves, face masks, face shield, PPE, biosafety cabinet for aerosol generating procedure, decontamination of surfaces by hypochlorite or other solutions, proper disposal of biomedical wastes generated in diagnostic laboratories are a few of them [16]. There is sudden huge demand and an immense resource crunch on the already shoestring healthcare budget of developing countries leading to shortage of personal protective equipment (PPE), face shields, masks, caps, sanitizers etc. due to the surge in the number of COVID-19 cases. The laboratory from where we are sharing the experience did not get PPEs, face shields and biosafety cabinet on initial days when samples from COVID 19 patients started coming. It took some times to arranging this equipment. Disposable face shields were made in-house from OHP sheets. Cardboard boxes fitted with saran wrap on its upper surface, a device to spray hypochlorite and two holes to introduce gloved hands inside, those get closed on taking out the hand was made in-house and was used as substitute of biosafety cabinet for de-capping of sample tubes and pipetting of samples. Strict use of Operation Theater (OT) gowns by laboratory staffs when on duty and their daily cleaning with soapy water was ensured besides other standard precautions like use of gloves, surgical masks, regular surface decontamination of working bench and lab instruments and biomedical waste disposal. Keeping the safety of laboratory personnel paramount and taking cognizance of the limited resources available, we devised these low cost alternatives to ensure safety while handling samples in our lab. The present study presents the effectiveness of SOP using these innovative and cost effective protective devices built in-house with limited resources in preventing contamination of laboratory working surfaces and equipments and in preventing the spread of COVID-19 among the staff working in clinical biochemistry laboratory.

Materials and Methods

The experience is shared from the clinical Biochemistry laboratory of LN Hospital, New Delhi, India that has been ear-marked as an exclusive facility catering only to COVID-19 patients. The SOP developed was a part of the managerial and operational effort when lab personnel suddenly reported of arrival of blood samples in the lab for investigations from patients suffering from COVID-19 and the results presented in this article are byproduct of the activities done during this process on subsequent day still the lab could ensure standard safety measures recommended for such labs.

A three member team was formed immediately to make a SOP for Biochemistry lab processing COVID samples based on WHO/ CDC guideline, to find out the gaps/deficits in lab for implementing those standard biosafety recommendations and to recommend the best possible measures to tackle the gaps [17]. The following steps were taken:

- High risk lab tasks requiring centrifugation, de-capping and pipetting that generate aerosols were identified. Two pregnant women, one woman with history of cardiac valve replacement and one person having age more than 60 years were identified as high risk subjects who were decided not to be involved in high risk job. Hydroxy chloroquine prophylaxis was provided to all the staff members.
- In view of reduced sample load, inconvenience of commuting due to lock down and in an effort to reduce the exposure duration, only two technicians, one resident and one nursing orderly were posted in each shift and shift duration was 12hours.
- Gloves and three ply surgical masks were available. Hazmat suit was not available for lab staff. As an alternative, OT gown was obtained from surgical store and shoe covers and head cover/caps were obtained from cell culture lab. Face shield for aerosol generating procedure was essential but was not available. The face shield was prepared in-house from transparent /OHP sheets. Four holes were punched using a punch machine and a mask was attached by passing the threads of the mask through these threads. The technicians wear this shield over another mask. The protective gear for technicians were constituted with a disposable surgical gown, double gloves, shoe covers, head cover and face mask before processing the samples.
- The samples were received in ziplock pouch inside a thermcol box in the sample collection room located away from the technician and doctor resting rooms. The requisition slip and the pouch were kept on disposable plastic sheet spread on the working bench and sprayed with freshly prepared 1% hypochlorite. Photograph of requisition slip was taken and subsequently zip lock pouches and requisition slips were discarded as fomite in yellow bag.
- In view of decreased sample load, only one centrifuge and one clinical chemistry analyser, one immunoassay analyser and one coagulation analyser were used. Rest all of the equipment were shut down and covered in plastic wraps to avoid any damage due to frequent sanitization procedures.
- Only one technical staff in each shift was designated for sample processing to reduce unnecessary exposure.
- The samples are centrifuged in the dedicated instrument installed in the COVID processing room. The samples are left inside the centrifuge for 15 minutes post centrifugation to let the aerosol generated inside the vacutainer to settle.
- Indigenous biosafety box with inbuilt hypochlorite spraying device was made using readily available cardboard box. These empty cartons of supplied store items were used to prepare the box. Two sides were kept open while the other four sides were closed. Cling film or transparent plastic was used to cover one open side so as to make visualization easy. The other side was kept over the surface covered with disposable plastic sheet which contains the pipettes, samples cups (if sample volume is inadequate), tube holder made from thermocol for keeping the vacutainers upright. A plastic tube with holes was inserted inside the box connected to a motor. This helped in initiating hypochlorite spray inside the box once sample de-capping was completed. Two holes were made on one surface for inserting the gloved hands inside for processing. These were covered from outside by adjustable plastic. The vacutainers were kept in the thermocol stand and the de-capping was done inside the box. After de-capping the samples were kept inside the box for 15-20 minutes to let the aerosols settle down before the tubes were taken out. After this the hypochlorite spray was done for 5 minutes inside the box with freshly prepared hypochlorite stored in a reservoir tank. The tubings as well as the boxes were changed every 24-48 hours.
- Subsequent to sample processing in dry chemistry system (VITROS 5600), all the used microslides, pippette tubes and cups were disposed off in double yellow bags designated as 'COVID 19 WASTE'.
- All the disposable gowns, masks, head covers, shoe covers, face shields etc were disposed off in yellow bags. The processed recapped vacutainers, original requisition slips,

dry waste from VITROS 5600 (tips, cups, micro slides etc) were also disposed off in yellow bag. The bag was tied and sprayed with 1% hypochlorite solution. This was put inside another yellow bag and tied. A Sticker mentioning 'COVID 19 WASTE' was stuck outside the bag and kept in the waste room. The room was sprayed with 1% hypochlorite solution and locked. The designated officer was informed regarding the waste for its timely disposal

- The instruments were disinfected with 70% alcohol. Thrice weekly sanitization of the entire lab was done by the hospital authorities as well.
- The reports were uploaded immediately in the whatsapp groups for each specialty. Later a designated staff from the COVID wards collected all hard copy of reports.

Detection of virus causing COVID-19 by RT-PCR

Total RNA was isolated from fresh Throat and Nasal swab (TS/NS-200 ul/ Specimens) collected from laboratory staff and followed by MagGenome Xpress RNA Isolation Kit as per instruction manuals [18]. The surface swabs were also processed in a similar way. The quality and quantity of RNA were estimated by using NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and extracted RNA was stored at -20°C for further use. Quality of isolated total RNA from all samples was checked by RNaseP gene amplification which was used as internal control. Combined reverse transcription of viral RNA and PCR amplification using real-time reverse transcriptase PCR (RT-PCR) methods was done as per instruction manuals [19-22].

RT-PCR amplification was carried out in 25 µl reaction mix containing 12.5 µl of 2X Reaction mix, 1.5 µl of target specific primers and Probe (Egene, RNasep gene, RDRP gene and ORF Gene), and 5µl of total RNA isolated from clinical sample, 1.0 µl AgPath One-Step RT-PCR enzymes (Applied Biosystem). Each set of RT-PCR assays included a negative control (nucleases free water instead of RNA sample) and a positive control of COVID-19. Amplification of COVID-19 was performed in the thermal cycler (Applied Biosystems QuantStudio 5 Real-Time PCR System) using the following conditions; Reverse Transcriptions 55°C for 30 min, Taq inhibitor inactivation 95°C for 3 min, PCR Amplifications; 45 cycles of 95°C for 15s, 58°C for 30 s Data Collections) as per published protocol. In this one step RT PCR assays, set of primer and probe used are shown in table 1.

S. N.	Assay/ Use	Oligonucleotide ID	Sequence (5'–3')
	E gene	E_Sarbeco_F1	5' ACAGGTACGTTAATAGTTAATAGCGT3'
		E_Sarbeco_R2	5' ATATTGCAGCAGTACGCACACA3'
		E_Sarbeco_P1	FAM: 5'ACACTAGCCATCCTTACTGCGCTTCG3' -BHQ
2.	RNaseP gene (Internal Control)	RNaseP Forward	5'AGATTTGGACCTGCGAGCG3'
		RNaseP Reverse	5' GAGCGGCTGTCTCCACAAGT3'
		RNaseP Probe	FAM: 5'TTCTGACCTGAAGGCTCTGCGCG3' BHQ
3.	RdRp	RdRP_SARSr-F2	5' GTGARATGGTCATGTGTGGCGG3'
		RdRP_SARSr-R1	5' CARATGTTAAASACACTATTAGCATA3'
		RdRP_SARSr-P2 Specific for Wuhan-CoV	FAM-5' CAGGTGGAACCTCATCAGGAGATGC3' QSY
4.	HKU ORF gene	HKU-ORF1b-nsp14F	5' TGGGGYTTTACRGGTAACCT'3'
		HKU-ORF1b-nsp14 R	5' AACRCGCTTAACAAAGCACTC3'
		HKU-ORF1b-nsp14 P	FAM-5'TAGTTGTGATGCWATCATGACTAG3' QSY

Table 1: List of Primers and Probes Sequences used in the diagnosis of COVID-19.

Results

The residents (n = 5) and lab technicians (n = 10) did 4 to 5 days lab duty of 12 hour duration during this 14 days period. Each nursing orderlies did duty for 8 to 10 days in shifts during this period. None of the staff members reported sick during this period. The nasal and throat swab test for virus causing COVID-19 of all technical staff (n = 10), residents (n = 5) and nursing orderlies (n = 3) also came out to be negative. No surface contamination with the virus of the work area and instruments was detected.

Figure1: Spraying of hypochlorite on the samples and the requisition slips.

Figure 2: A staff member wearing the face shield.

Figure 3: A technician all geared up for sample processing.

Figure 4: De-capper with sanitization. Demonstration of the steps.

Figure 5: A segregated waste room with COVID-19 waste in yellow bags.

Discussion

This article presents the data that evaluated the effectiveness of a SOP that used a face shield made in-house from OHP sheets and innovative device made from card board fitted with hypochlorite spraying device as alternative to biosafety cabinet along with other standard biosafety measures in preventing spread of COVID-19 virus among laboratory personnel working in biochemistry laboratory in a dedicated COVID hospital.

The data shows that at the end of 14 days of following the SOP, none of the lab personnel including those who did high risk jobs that produce aerosols developed signs and symptoms of COVID infection and RT-PCR based COVID-19 testing was negative for all lab staff. This indicates that these low cost, innovative devices built in-house were effective in preventing COVID-19 spread through aerosol among lab staffs. We attribute this prevention to use of these innovative devices along with other protective gears like face mask, gloves etc. However, we do not claim that it is solely due to our innovative devices but is contributed by these devices also. However, we could not keep a control arm in our study to prove our claim. Another limitation is that most of the COVID-19 cases are asymptomatic and hence, clinical monitoring by self reporting of symptoms is bound to miss the diagnosis in most of the cases. Even diagnosis by RT-PCR is only 70-80% sensitive.

Even the lab surfaces and equipments did not show the presence of the virus by RT-PCR testing of the swab taken from the working surfaces and equipments. So it proves that transmission through surface contamination did not occur. Substitute of bio safety cabinet with innovative cardboard box might have acted as barrier for spread of aerosol although it was not having a negative pressure. However, regular decontamination of the working surfaces with hypochlorite and instruments with 70% ethanol might be major contributor of prevention of surface contamination. Chance of survival of virus inside the cardboard box was prevented by spray of hypochlorite solution after each session of de-capping and pipetting. This spraying device also helped us using the device for 1-2 days.

We conclude that in resource scarce health set up where standard CDC or WHO recommended biosafety measures cannot be totally followed, such simple, low cost and innovative devices made in-house or locally from locally available materials can be effectively used in such sudden outbreak of contagious viral infections. With understanding of basic principle of barrier method, innovations are possible and are key to success in such critical period. In addition of the continuation of the study, we expanded our knowledge and expertise about the COVID-19 pandemic and share few important finding to the scientific community [23-27].

Competing Interest Statement

The authors have declared no competing interest.

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Author Declarations

I confirm all relevant ethical guidelines have been followed, and any necessary IRB and/or ethics committee approvals were not taken because at time of studied, most of the offices were closed and/or have been obtained. The study was carried out as per the institutional ethical guidelines and approval from; Maulana Azad Medical College and Associated Hospitals, New Delhi, India. The results of the study did not influence the treatment. Grant of Ethics Clearance Certificate No. F-1/IEC/MAMC/(77)/05/2020/164, Dated :05/08/2020.

Preprint Servers

The current publication titled “In-house assembled protective devices in laboratory safety against SARS-nCoV-2 in clinical

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