COVID-19 (nCorona) Virus Outbreak Control and Prevention State Cell

Health & Family Welfare Department

Government of Kerala

COVID-19 Sample Management inside a Virus Diagnostic Laboratory
No.31/F2/2020/H&FWD dated 16th August 2020

GENERAL:

The most essential component of successfully running a covid-19 sample testing laboratory is the large number of trained manpower- trained in multidisciplinary aspects of a virus diagnostic laboratory who are prepared for shunting between the levels of screening, assay and reporting procedures.

There should be an overall lab-supervisor, who will man all the shifts judiciously, for trouble shooting, emergency procedures, and who will completely control of the entire work flow. Each shift should have a senior thoroughly trained personnel, such as a Research Officer who will rearrange duties internally for different sections as and when required, coordinate each shift and enable easy migration of shift changes and monitor on-time progress of testing in all the sections.

There are four major components in the sample testing section, and each section of these should have at least four members each (total 16) in a given shift. This must be supplemented by a manpower training program, where graduates/postgraduates in microbiology/ biochemistry/biotechnology could be invited to join (one-two trainees in each section at a given time). In addition, there should be atleast 6-8 data entry operators working on a two shift basis. Here too, 2-3 trainees from relevant disciplines could be invited to join for limited period of time. The number of data entry operators, and technical staff can be increased depending on the volume of samples handled in a lab.

The integrity of the Sample Referral Form (SRF) requires special mention. It is imperative that the forms are complete in all respects and care must be taken to avoid errors of any kind. The hospitals collecting and sending the samples to the labs should be instructed by the District Medical Officer (Health) in this respect as any error in the SRF can lead to wrong/delayed declaration of results. The District Medical Officer shall liaise with the labs for identification of

common errors in SRF and training shall be given to periphery hospitals collecting samples to avoid such errors.

Step 1. RECEIPT OF SAMPLES

Requirement: There must a specified area within or immediately outside the Virus Diagnostic Laboratory (VDL), where personnel transporting samples to the lab from outside could enter to deliver the samples. It must be on the same floor and very near to the sample processing area of the VDL. Personnel should be available to receive the samples and issue signed official receipts.

Process

- Transport boxes with samples are collected in the "sample receipt room''.
- These sample containing boxes are identified, details are entered into 'Sample Receipt Register' kept at the VDL and signed official receipts bearing the VDL Registry number, name of the person who brought and received the sample, number of boxes received, date and time of receipt, and the name of the hospital that issued the boxes to the carrier.
- If there are any Emergency sample, detailed information is to be noted down including the name and telephone number of the responsible doctor, and these are to be immediately conveyed to the Research Officer/Technician in Charge of the Aliquoting processes for speedy completion of testing of these samples.
- All samples must arrive cold. Examine it for required low temperature, and those arriving warm may be marked and the source hospital informed immediately. *Such samples may be rejected.*
- Samples are removed from the boxes and kept in the refrigerators *in the aliquoting room* till processing.

Step 2. HANDLING SAMPLES FOR ALIQUOTING

2a. Unique Laboratory ID Numbering of the samples

- The staff handling the samples should wear adequate personal protective equipment (PPE).
- When a large number of samples are needed to be processed, more personnel have to work together to speed up the process. In other words, the number of samples received would determine the number of personnel engaged at this level and at the later levels.
- The samples are taken into the biosafety cabinet and examined carefully to ensure that there are no leaks.
- Sample packings are opened one by one and labels on each one are matched with the name and number in the sample referral form (SRF).

Any discrepancy should be informed to the hospital of origin of the samples, and if these are not resolved, samples may be kept on hold in the cold chain till the issues are resolved.

- Processing of the samples will be in the following priority:
  - Emergency samples are grouped for immediate processing.
  - Pooled sentinel surveillance samples and pooled routine samples received, district-wise.
  - Routine samples that are not to be pooled.
- These are numbered with Unique Lab ID. This for easy tracking of the samples within a specific lab as these numbers are oriented, continuous but progressive, and trackable at a glance at the number. Use the SRF ID can be too cumbersome with several digits, and sections. If SRF ID is used, the numbers would be v disoriented, discontinuous but, progressive. It will take more time to examine if the lab has received a sample or not, or on what date, or track the sample in stoc, etc., at a later date.
- Distinct prefix initials will be used for pooled samples and for routine samples.
- After assigning such unique laboratory ID to each sample, one person will start labelling each of the cryovials with the Unique Lab IDs, and keep those arranged on a numbered stand, serially.
- Each sample form (SRF) must be marked with a corresponding Unique Lab ID.
- Samples that are rejected should be mentioned in the SRF citing the reason for rejection (leakage, insufficient volume, mismatch between sample label & SRF, break in cold chain, etc.)

2b. Aliquoting the samples

- Before opening the sample tube, vortex the tube.
- The swabs are carefully squeezed (using a disposable sterilized tweezer) and both are disposed of.
- Use a sterilized disposable plastic Pasteur pipette to transfer the liquid samples to the cryovials already numbered with Unique Lab ID. Using a one mL pipette may cause contamination when handling the samples, because the pipette-tips are disposable, **but the same pipetting device is reused.**
- For easy follow-up, Lab ID numbered vials may be serially kept in **batches of 12.** This is advised since the number seems to be ideal for easy and error free handling by personnel in further steps for RNA extraction.

Step 3. RNA EXTRACTION

- Depending on the machine used and the wells available for sample run, 96 or 36 or 72 (*depending on the PCR machine to be used*) cell format templates could be prepared and kept ready for each set of assays.
- Depending on the RNA extraction kit to be used, appropriate amount of sample can be used for the manual RNA extraction / automated RNA extraction from the aliquoted samples in the cryovials, taking care to keep track of the sample numbers.
- For automated RNA extraction system, a 96-cell template sheet should be used to mark the sample position. This will be used in setting the PCR run also.
- Positive controls and negative controls are to be included in the setup, whenever manageable and should be run in every test.

Step 4. MASTER-MIX PREPARATION

- The master-mix is prepared in a DNA free area for PCR (depending on the RT PCR kit) and dispensed into a 96 well plate / strip, depending on the number of samples.
- This is a very critical step which has to be done in a completely RNA/DNA free laminar flow hood. This room should not have had any exposure of biological material that could contaminate the hood or devices used.
- Master-Mix is prepared exactly as per the recommendation provided along with the kit to be used.
- As per the template that will be used in the PCR, pipetting of each well will receive the recommended volume of the Master mix.
- The plates/tubes are taken out of the Master-mix room and transported to another room where RNA is carefully added with respect to the marked templates in a Biosafety hood/Laminar flow.

Step 5. RNA ADDITION

- RNA addition is done in a separate room inside a Biosafety cabinet. Extracted RNA and the positive control is added to each of the wells exactly as per the template. **Personnel should always work as pairs at this stage to prevent any errors.**
  Caution: Keep in mind that the same template is programmed into the real time PCR machine and therefore, any mistake in the addition of a sample RNA will cause error the test results. In case there is an error, the template positions could be corrected for any error.
- The 96 well plate is sealed with an adhesive film and if tubes are used, these are closed, centrifuged in a plate rotor or a microfuge and proceeded for the PCR run.

Step 5. PCR RUN

- The PCR machine is programmed as per the PCR Kit requirements.
- The same template that is used for the RNA addition is labelled into the real time PCR machine and set the program
- Carefully recheck all the templates and identify the sample positions in the PCR before starting the run.

Step 6. PCR RESULTS

- At the end of the run, the data are analyzed based on the genes that are identified, C_T values, and control positive and negatives after setting the baseline threshold in each run.
- The values of C_T are compared with the guidance provided in the respective kits used for the genes assayed.
- The C_T values and the slope of the curve are judged for deciding, if the sample is positive, negative or out of a definite decision (indeterminate/inconclusive).

6a. Test Reports

- The results are entered on to a prepared chart useful for entering all the details such as genes and C_T values, decision on positivity, negative nature or indeterminate/inconclusive for the routine samples.
- Pooled samples are entered on a chart template for pooled samples, and the positive pools and their C_T values, and independent re-run values.
- Final lists of positive, negative and indeterminate/inconclusive samples are given to Date Entry Operators (DEOs) for accurate entry into the Government portals.
- Reporting is done as per the directions from Department of Health & Family Welfare, Govt of Kerala/ICMR, New Delhi.

WORK SHIFT ARRANGMENT FOR A LABORATORY WITH 2 SHIFTS

Lab-in-charge/Nodal Officer

Shift 1 9:00 AM- 3:00 PM
1 Research Officer
5 Lab Technicians
1Junior Lab Assistant
1Data Entry Operator
1 Cleaning Staff

Shift 2
3:00 PM- 9:00 PM
1 Research Officer
5 Lab Technicians
1Junior Lab Assistant
1Data Entry Operator
1 Cleaning Staff
Depending on the sample load, the number of shifts shall be increased with the appointment of additional staff.

Co-ordination of Duty Shifts and Sample Management.

Samples usually come to the laboratory at fixed timings from collection centres. Say a particular lab receives one lot of samples by evening and another lot by midnight (L2)

The morning shift (Day 1)

· Aliquoting samples received on Day 0 midnight, ieL2

· RNA isolation and PCR analysis of samples received on the day before(Day0 evening) ie L1 of Day0

· Entry of patient details of samples received the day before and samples aliquoted in the morning.

· Data analysis of results

· Entry of all negative data into the portal.

· Repeat test of individual samples from positive pools

· Reconfirmation of positive samples and data entry in the portal.

Afternoon Shift (Day 1)

. RNA isolation and PCR of L2 samples received in the previous midnight

. Repeat test of individual samples from positive pools

. Reconfirmation of positive samples

. Data analysis of results

. Entry of all negative data into the portal

. Aliquoting of samples received by evening(of which RNA isolation and PCR analysis will be done next morning)

. Data entry of all confirmed positive samples in the portal

Work flow shall be structured in such a way that all samples will be processed and the reports are sent within 24 hours of receival of samples in the lab.

SOP For Workflow And Sample Management Inside The Laboratory

1. Transport containers in which the Samples are brought in are sprayed with isopropanol or Bleach for decontamination, in the sample receipt room
2. Brought into the lab through the Passbox (The one-way passage of samples)
3. Ensured that the samples have maintained the cold chain
4. Containers are opened and the samples are removed and kept in the refrigerator till opened for processing.
5. The samples are handled by technicians/staff wearing adequate protective gear (PPE, googles, head cover, face shield and double gloves etc)
6. The triple layer packed samples are taken into the Biosafety Cabinet and checked to ensure that there are no leaks or breakages
7. All samples with leaks or breaks are discarded immediately and disinfected. The corresponding patient data sheet is marked to show the same.
8. Sample packings are opened one by one and each one is matched with the patient data sheet to ensure that there is no mix up.
9. The samples are given an appropriate lab ID number denoting the sample details and processing details for eg : PS for pooled samples, IC for regular samples etc.
10. Each sample is thoroughly vortexed and kept in the appropriate stand
11. The sample tube is opened and the swab is removed with the forceps
12. The sample is aliquoted into cryo tubes (Number depends on the volume of the sample)
13. An appropriate amount ranging from 150 to 200 ul (depending on the RNA isolation kit) is added to a microcentrifuge tube that is labelled with the sample ID
14. The samples are arranged in an appropriate rack
15. The viral lysing solution and the internal control (if necessary) is added to the sample and kept for an incubation, generally 10 minutes at room temperature or 70 C depending on the kit

16. The original sample tube, swabs are all put into disinfectant solution for discarding

17. After incubation the whole covered rack is taken for RNA isolation through a passbox.

18. RNA isolation is generally done in batches of 12

19. RNA isolation is done very carefully wearing gloves, masks etc as RNA is very fragile.

20. The procedure followed depends on the kit

21. For a column-based kit, each column is clearly marked with the sample ID

22. The sample with viral lysing solution and an appropriate amount of pure ethanol is added to the column

23. The column is washed with the wash solutions provided (which are reconstituted with pure ethanol) and centrifuged after each wash

24. The nucleic acids are eluted from the column using 50 ul of pure RNAse/DNAse free water

25. In a separate master-mix room (where no DNA or RNA is taken in), in a laminar flow hood, the COVID detection RT PCR kit is opened

26. Depending on the number of samples, the master-mix is prepared and carefully dispensed in a 20 ul volume (depending on the kit) into the wells of a 96 well plate.

27. One of the wells is earmarked positive control and another as negative control

28. Pure water is added to the negative control.

29. The plate is taken out of the master-mix room and the positive control provided in the kit is added in a separate area/hood

30. For each run a specific template for the PCR is set up designating which sample goes into which well.

31. The prepared plate is taken to the RNA addition room where in a biosafety cabinet the RNA is added to each of the 94 wells exactly as per the template under the watchful eyes of a senior/colleague.

32. The same template is programmed into the real time PCR machine and the program is set.

33. The 96 well plate is sealed with the optical adhesive film, centrifuged in a plate rotor and put in to the PCR machine and the run is started.

34. At the end of the run, the data are analyzed based on the instructions in the PCR assay kit used.

35. Threshold and baseline settings are done as per the machine.

36. Checked to ensure that Positive and negative controls have worked

37. The Ct value and the shape of the curve is assessed for data analysis.

(These guidelines are prepared by IUCBR & SSH Kottayam under the guidance of Director and District Collector Kottayam.)

Prl Secretary H&FW