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Brief report

Quality control of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) polymerase chain reaction (PCR) test for coronavirus disease 2019 (COVID-19) through our cross-contamination experience

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Our pathological genetic laboratory has been established in 1999, and performed coronavirus PCR testing from April, 2020. The genetic laboratory (Biosafety level 2) is isolated room of air volume 7.5×5.5m×3m. We used chemical reagents: SARS-CoV-2 Direct Detection RT-qPCR Kit® (Takara, RC300A), Positive Control RNA (US N1/N2, Takara, RC351A). Realtime PCR was done with the PCR device AriaMx® (Agilent). Coronavirus PCR test is performed on the basis of our standard operating procedure (ISO15189: 2012, SOP. bacteria and others-401, 3rd ed, 2020/12/07).

We recognized the PCR contamination in August, 2021, and our accredited laboratory was required to establish a corrective action on the basis of ISO15189; 2012.

The causes of our contamination were analyzed based on a guidance of PCR procedure and a pursuit procedure of causes presented by the reagent-manufacturing institute (Table 1). The pollution seemed to occur mainly by the incomplete isolation between a chemical reagent adjustment area and a positive control/the specimen mixture area (non-conformity: 5.2.6 facility maintenance and environmental conditions in ISO15189;2012), delicate procedure before and after opening of tube and 8-well PCR tube (5.3.1.3 equipment instructions for use), contact pollution of tube cap (5.3.1.3), incomplete 3S procedure of appliance and pollution site. 3 months were required to suppress the contamination (5.2.6). The cooperation of Takara Institute, Agilent, and AS ONE, was a great help to remove the pollution.

We can establish our prophylactic check list against PCR contamination as follows: creation of a check list (Table 2) and review of the SOP (standard of procedure) in manual, complete separation of the working areas: a chemical reagent adjustment area and a positive control/the sample mixture area, no share appliances between working areas, each working place was locked with new safety cabinet, centrifuge the tube before open and spin the intratubular droplets down, smear test of instrument/the pollution site with the distilled water, use the micropipette tip with a filter, wipe with hypochlorous acid, ultraviolet irradiation, and an education of PCR procedures. This education included the proper handling the micropipette not to contact any intra-cabinet equipment, no instant opening of any cap to reduce splashing pollution, spin tube and 8-well PCR tube down before open their caps, keep out the inner cap surface from pollution by no direct touch and placing on a torn aluminum-sheet rest for temporary installation, avoid hanging the injection tip filled with positive control or sample over or around 8-well PCR tubes, avoid opening the 8-well PCR tubes after PCR not to pollute by PCR products, and push into a plastic bag and take out from genetic laboratory. This check list will satisfy the requirements of training (5. 1. 5 in IS015189) and facility maintenance and environmental conditions (5. 2. 6), and help the revision of Standard Operating Procedures (SOP) of SARS-CoV-2 PCR test.

Key words : SARS-CoV-2, COVID-19, PCR, contamination in laboratory, ISO15189; 2012, corrective action

和　文　抄　録

短報

長岡中央病院病理部遺伝子検査室における新型コロナウイルス（SARS-CoV-2）PCR検査のコンタミネーションの経験

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2021年8月に遺伝子検査室における新型コロナウイルス（SARS-CoV-2）PCR検査のコンタミネーションを経験した。試薬調整作業台と陽性対照・検体混入作業台の区分が不完全でクロスコンタミネーションを来したこと、手技の不手際、施設・装置・備品の3S不良等が原因と考えられた。ISO15189; 2012是正措置要求

に基づく対処により、３ヶ月後に現状に復帰できた。この是正措置作業を通して、日常業務に有効なチェックリストを作成した。関連施設の指導に感謝し、品質マネジメントシステムに基づく対処の有効性が確認された。

キーワード：SARS-CoV-2、COVID-19、PCR、検査室内でのコンタミネーション、ISO15189; 2012、是正措置

Table 1. Measures for the safe inspection to remove contaminating threats

1. confirmation of the risk by the check list
2. detection of the pollution part by the smear test, negative control reacts with an unopened kit (more than N＝8), consideration of contamination in procedure step (sampling, pretreatment, reagent adjustment, addition of positive control or sample, PCR)
3. isolation of each working space
4. decontamination of the laboratory by wipe with sodium hypochlorite and ultraviolet irradiation before and after inspection
5. lay aluminum foil on working area to assume it a clean site
6. changing expendable supplies to a new article

Table 2. Check list for contamination measures

1. in general
1-1. divide working areas separately into a pretreatment area of the specimen, a reagent adjustment area, and a positive control or specimen addition area
1-2. do the operation in a safety cabinet
1-3. prepare exclusive equipment such as the centrifuge, microtube, micropipette, and hydrophobic filter-tip, and do not share it in other area
1-4. use a hydrophobic filter-tip tip
1-5. wipe every equipment and tool with a sodium hypochlorite solution before and after inspection
1-6. lay aluminum foil on working area to assume it a clean site
1-7 clean a laboratory once a week
1-8. irradiate ultraviolet if necessary

2. at pretreatment
2-1. wear the white robe, preventing the contiguity pollution by the sleeve, gloves, cap, and mask for exclusive use around this area,
2-2. wipe every equipment and tool with a sodium hypochlorite solution before and after inspection
2-3. lay aluminum foil on working area to assume it a clean site
2-4. do not touch the backside of the cover at opening a tube and 8-well PCR (polymerase chain reaction) tubes
2-5. cover tube immediately to decrease volatilization
2-6. seal up the waste in bag immediately and discard

3. at chemical reagent adjustment area, mix master fluid and dispensation
3-1. wear the white robe, preventing the contiguity pollution by the sleeve, gloves, cap, and mask for exclusive use around this area
3-2. do not bring samples and the positive control into the adjustment area to prevent contamination
3-3. wipe every equipment and tool with a sodium hypochlorite solution before and after inspection
3-4. lay aluminum foil on working area to assume it a clean site
3-5. warn a micropipette tip touching at a safety cabinet to avoid any pollution
3-6. do not touch the backside of the cover at opening a tube and 8-well PCR tubes
3-7. seal up the waste in bag immediately and discard

4. at sample or positive-control addition area, and PCR area
4-1. wear the white robe, preventing the contiguity pollution by the sleeve, gloves, cap, and mask for exclusive use around this area
4-2. do not bring samples and the positive control into the adjustment area to prevent contamination
4-3. wipe every equipment and tool with a sodium hypochlorite solution before and after inspection
4-4. lay aluminum foil on working area to assume it a clean site
4-5. warn a micropipette tip touching at a safety cabinet to avoid any pollution
4-6. spin-down with centrifuge before opening a tube and 8-well PCR tubes
4-7. do not touch the backside of the cover at opening a tube and 8-well PCR tubes
4-8. avoid adding sample over other sample tube to avoid any pollution
4-9. do not open 8-well PCR tubes after PCR
4-10. seal up the waste and PCR products in bag immediately and discard