

Recommendations for reducing nucleic acid contamination in Columbia University research laboratories

Contamination of laboratory personnel with non-infectious nucleic acid molecules encoding SARS-CoV-2 sequences that were generated in a laboratory by amplification of plasmids or by polymerase chain reaction (PCR) has led to **positive results in PCR-based SARS-CoV-2 tests**. These findings have not corresponded with evidence of actual infection. Thus, the positive SARS-CoV-2 test results are believed to be attributable to nucleic acid contamination of personnel getting tested.

Environmental nucleic acid contamination can be persistent and challenging to decontaminate. However, the following provisions may reduce the likelihood of researchers becoming contaminated with nucleic acids present in the research laboratory.

Segregate areas

- Use separate rooms or bench areas for pre-amplification set-up and post-amplification analysis/processing.
- Maintain dedicated equipment and personal protective equipment (PPE) that is used and remains located in each space. This includes laboratory notebooks, pipettors, reagents, etc.
- Consider implementing a workflow whereby individuals who have entered the post-amplification space do not return to other areas of the lab.
- Regularly communicate with all laboratory members specifically where and when SARS-CoV-2 fragments are being used and stored.

Use equipment to contain contamination

- Containment devices such as biosafety cabinets are strongly encouraged for handling post amplification PCR products. Biosafety cabinets will prevent laboratory contamination as well as sample contamination. The biosafety cabinet will contain any aerosols. Moreover, people working in a cabinet automatically have better microbiological technique; they're unlikely to put a phone or notebook next to their work and more likely to wipe down the work surface when they have finished. Only work that cannot be done in such a cabinet, e.g. running gels, may be done outside biosafety cabinets.

- Fume hoods will protect the laboratory and researcher from contamination but not prevent the contamination of the samples being opened within the hood
- Beware of “PCR hoods” or “PCR prep stations”. These often function like clean benches that provide a clean environment for your samples but actively blow any contamination directly out of the workspace and toward the researcher/into the laboratory.
- Air-cooled and refrigerated centrifuges vent air from inside the centrifuge into the laboratory environment while running, which can spread aerosolized products. Use aerosol-tight caps or lids when running centrifuges and decontaminate after each use.
- Use screw-cap tubes in place of flip-cap tubes to prevent accidental aerosolization of material on caps.
- Use positive-displacement pipettes and aerosol-resistant filtered pipette tips.

Use good microbiological technique

- Only open post-PCR samples if absolutely necessary.
- If tubes must be opened, open them carefully to avoid splashing or spraying contents or transmitting to gloves.
- Take care to avoid touching the underside of caps. Caps should never be placed face down on benches or other work surfaces.
- Keep samples capped/covered as much as possible.
- Maintain control samples at a useful concentration range for serving as controls (i.e., minimize handling of highly concentrated stocks).
- Perform quick “spin-downs” on tubes every time before opening.
- Utilize pipetting techniques that reduce splashing or spraying to reduce aerosolization.
- Change aerosol barrier pipette tips between each manual liquid transfer.
- If bench pads are used, they should be changed frequently.
- Dispose of unneeded samples in a designated regulated medical waste container.
- Decontaminate the exterior of tubes and pipettors with a product effective against nucleic acids (e.g., DNAZap™).
- Change gloves between work processes to prevent contamination. Never reuse gloves and dispose of gloves after one use. Always remove and discard gloves in the post-amplification area before leaving.
- Wash hands immediately after removing PPE and before doing desk work and/or handling frequently

Avoid trafficking nucleic acid on personal items

- It is possible for contamination to be transmitted on laboratory and personal items. Avoid bringing lab notebooks and personal items (jewelry, watches, cell phones and writing implements) into areas where amplified nucleic acid is handled.
- Gloves must never be used to touch personal items.
- Facial tissues must be stored with other personal items and outside of the laboratory.
- Store clean and dirty PPE separately and away from personal belongings.
- Avoid touching your face or mask. Follow proper doffing practices for removal of face masks and store/dispose of correctly:
 - To doff: With washed hands, grasp earloops or ties and remove without touching the front of the mask. Follow the same procedure to adjust the mask.
 - If the mask must be temporarily stored, such as while in breakrooms to eat or drink, place the mask inside a paper bag. Do not place directly on any laboratory or common surface.
 - Always wash hands before and after donning a mask or removing a mask.

Routinely decontaminate areas and equipment where contamination is likely

- Environmental nucleic acid contamination can be persistent and reducing existing contamination to undetectable levels can rarely be accomplished. Nonetheless, it is advisable to frequently decontaminate areas such as post-PCR analysis/processing spaces. Also laboratory door handles and fridge/freezer handles where post-PCR samples may be stored. Note that use of 70% ethanol will not destroy nucleic acids; a 10% solution of household bleach is recommended for nucleic acid destruction. Consider using a product specifically designed to destroy nucleic acids (e.g. DNAZap™).

Questions

Please contact biosafety@columbia.edu

Acknowledgement

Some content kindly provided by Harvard University EH&S