Detection of Environmental Nucleic Acid Contamination in a Molecular Laboratory

By Amelia Heather Garner, Microbiology, Northern Care Alliance

Introduction

The identification of respiratory and enteric pathogens' nucleic acid is achieved through multiplex tandem real time PCR. The PCR technique has a high sensitivity, 80 to 100% (AusDiagnostics 2016/2017). Therefore, a small amount of positive nucleic acid present in the molecular laboratory environment has the potential to cause a false positive result. Norovirus can be detected in the environment for 21 to 28 days when in a dried state, at room temperature (Weber et al., 2010). Whereas, Influenza A only survived for one to two hours on plastic banknotes, without a protective mucus, (Thomas et al., 2018). A study by Banasik et al., (2016) found false positive results still occurred in controls after all good laboratory practices had been applied. To be assured no contamination has occurred, Public Health England, (2018) have recommended routine environmental swabbing to be carried out, in areas where PCR is performed regularly. The current literature supported the best implement for the recovery of viruses from non porous fomites to be a polyester tipped swab (Julian et al., 2011). There was no current literature on the use of newly available cellulose sponges for the recovery of nucleic acid from fomites. The aim of the project was to verify the use of these two implements, in the detection of contamination, that could cause a false positive result. The best implement would then be used to investigate the possible presence of environmental contamination, within the laboratory.

Method-Verification of Implement

- The control area was a bench top surface, separate to the molecular laboratory.
- The control area was cleaned with methods known to remove nucleic acid before the study began.
- RNase free water was used as a negative control.
- Previous positive samples that had been archived and stored at -20°C were used as positive controls.
- Samples that were positive for Rotavirus, Influenza A and Adenovirus were selected.
- Each positive sample was diluted in a ten-fold series dilution with RNase free water.
- 0.5 ml of each control was pipetted into a 10x10cm area and then spread with a sterile spreader
- The area was left until visibly dry.
- 0.5ml of each control was saved to compare the concentration the recovery method detected.
- Blue hygine sponges in easy open stomacher pouch dosed with 0.9% saline was the first implement used.(Figure 1).
- Sterile polypropylene swabs (Figure 2) were pre-wetted in 0.85% saline and then used on separate areas to the previous implement type.
- The eluent for each implement was tested using the multiplex tandem real time PCR and the concentration was recorded.

Results- Verification of Implement

- A concentration of zero was obtained for both the expected values and the implements.

Discussion- Verification of Implement

- The literature gap was reduced as, the results showed cellulose sponges could be used to detect viral nucleic acid on fomites.
- The negative control of DNase free water obtained all negative results, this showed no contamination occurred in the study. (Figure 3 and 4).
- A limitation of the study was none of positive controls were analysed after storage at -20°C. Negative values could have been obtained for Adenovirus as it was determined if it was still present in the sample before dilution.
- Thomas et al., (2018) showed Influenza A does not survive on fomites for a long time, this could be why no concentrations were obtained for every dilution factor. (Figure 3)
- The result for Influenza A dilution factor 10^2 with a swab implement could be a anomaly. (Figure 3).There is no result for dilution factor 100. A sampling error could have occurred. To remove uncertainty the study should have been repeated.
- Sample collection values were obtained for every dilution factor of Norovirus (Figure 4). This could be due to Norovirus being detected in the environment for a long time (Weber et al., 2010)

Method-Contamination in the laboratory

- The results for the best implement to be swabs. Overall swabs obtained concentration values closer to the expected values. The results were easier to use as they are routinely used in laboratory work. This would allow training in use of the implement to be more efficient. The study findings agreed with the current literature, (Julian et al., 2011).

Results- Contamination in the laboratory

- Areas in the Molecular Laboratory were selected based on if patient samples came onto contact with the area directly or indirectly.
- The areas were then sampled for environmental contamination with the best implement, using the same method used in the verification study.

Discussion- Contamination in the laboratory

- The results of contamination within the molecular laboratory supported Banasik et al., (2016) study findings.
- It was expected more enteric virus contamination would be found as the implement yielded a greater range of concentration values in the validation study. (Figure 6).
- More enteric virus contamination could have been detected, as Norovirus is detectable for longer than Influenza A (Weber et al., 2010; Thomas et al., 2018).
- Two areas were positive for respiratory viruses (Figure 5), this was significant as the areas were pre-amplification areas. If contamination occurred the nucleic acid would be amplified and could be reported as a positive result.
- Enteric virus contamination was detected in both pre and post amplification areas. Contamination is not significant in a post amplification area as it doesn’t have the potential to cause a positive result.
- The contamination is believed to have come from different sources, as it was found in varying concentrations. If the contamination came from one sample, a small range of concentrations would be expected.
- The findings of the study have led to changes in the cleaning protocols. Areas previously not cleaned are.

Conclusion

The aim of the study was achieved. A validated method for the detection of nucleic acid was developed and put into practice. The method has proved useful in finding sources of contamination. The molecular laboratory can now be assured a positive result is due to a viral infection and not environmental contamination.

Acknowledgements

A special thank you to all the staff at the Royal Oldham Microbiology Department. Sponges provided freely by Technical Services Limited.

References


Keywords

PCR
Environmental Contamination
Swab
Sponges

Swab or Sponge?

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