



Practice Points

Mpox virus DNA contamination can still be detected by qPCR analysis after autoclaving

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Environmental sampling played an important role in evaluating levels of environmental contamination present in hospitals and outpatient settings during the mpox 2022 outbreak. This allowed validation of infection prevention and control (IPC) measures and identification of potential routes of transmission when caring for infected patients. Investigations typically focused on sampling in high-risk settings, using quantitative polymerase chain reaction (qPCR) to identify the presence of mpox virus (MPXV) DNA [1–4]. On occasion, MPXV DNA contamination was detected outside controlled areas such as corridors outside of isolation rooms. However, these occurrences usually identified extremely low levels of DNA [4]. Whereas such findings may reflect ineffective IPC measures, other explanations for detection of low levels of DNA in ‘clean’ areas include qPCR false-positivity and DNA deposition from autoclaved, reusable personal

protective equipment (PPE). Though most PPE is typically single-use, items such as autoclavable rubber clogs can be reused if suitably sterilized, thereby offering robust foot protection in addition to other benefits such as a reduction of waste and pollution, predictable availability, and economic viability [5,6].

After detection of low levels of MPXV immediately outside of a patient isolation room in the UK, we investigated whether MPXV DNA can be detected on styrene–ethylene–butylene–styrene autoclavable thermoplastic rubber clogs (Reposa, Empoli, Italy), used in this facility as part of the PPE required to treat confirmed mpox cases, after multiple cycles of autoclaving. For this study, two identical hospital clogs (clog A and clog B) were surface disinfected with sodium hypochlorite (10,000 ppm for a contact time of 10 min) and RNase AWAY (Sigma–Aldrich, St Louis, MO, USA) and inoculated with 10 µL of MPXV DNA previously extracted from an inactivated MPXV isolate from the 2022 mpox outbreak. This MPXV DNA inoculum was diluted to produce pre-autoclave swab sample with a crossing threshold (C_T) value of 25, similar to those observed on contaminated PPE in hospital settings. This inoculum was used to artificially contaminate quadrants on two clogs (Figure 1A). Two sampling approaches were utilized: the first sampled a different quadrant sequentially prior to autoclaving and after each of the three autoclave cycles (clog A; four samples in total); the other involved repeat sampling of all four quadrants before autoclaving and then after each autoclave cycle (clog B; 16 samples in total). A total of three autoclave cycles was performed with a hold time of 15 min at 2.4 bar pressure at 121 °C. Samples were taken using FLOQ Swabs (Copan, Murrieta, CA, USA) containing 2 mL viral transport media. Samples were inactivated, DNA extracted

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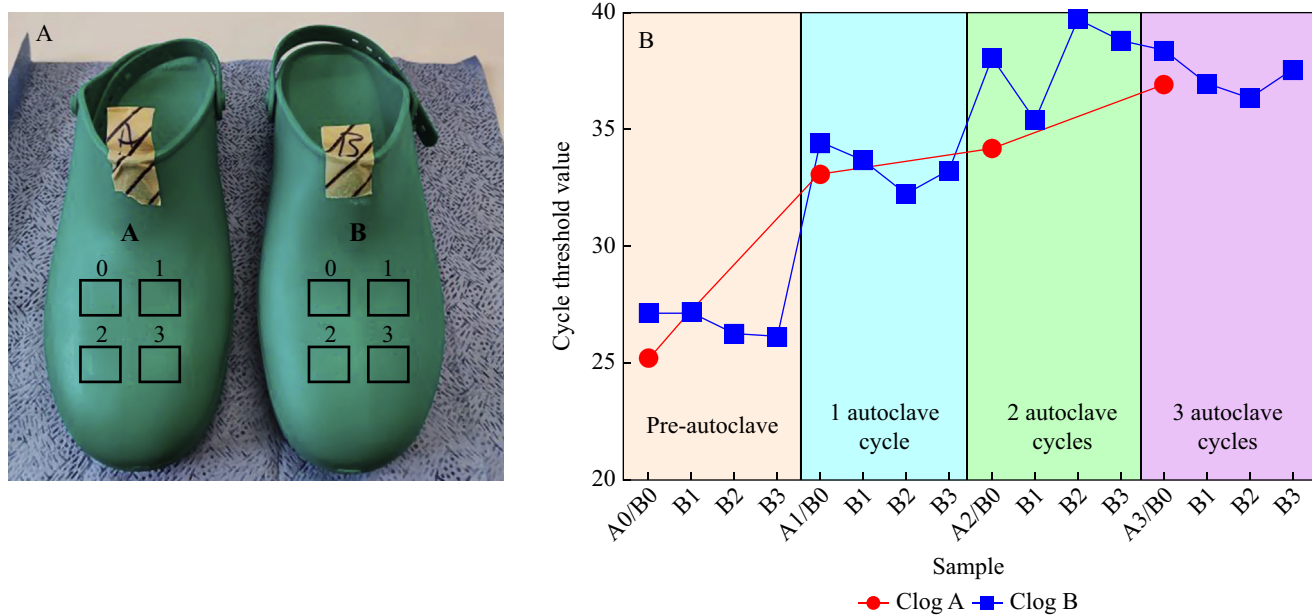


Figure 1. (A) Clog set-up. Squares on clog A were sampled once; 0, pre-autoclave; 1, post 1st autoclave; 2, post 2nd autoclave; 3, post 3rd autoclave. Each square of clog B was sampled prior to autoclaving and then after each autoclave cycle. (B) Cycle threshold (C_T) values produced by qPCR of mpox DNA recovered from hospital clog surfaces using environmental swabs before and after three autoclave cycles, e.g. B1 (B, clog; 1, quadrant on clog).

and analysed by qPCR following a previously described method used for genuine environmental samples [4]. All samples taken from clog A and clog B had detectable amounts of viral DNA before and after each autoclave cycle. However, there was a large decrease between samples taken pre-autoclave and after the first autoclave cycle on both clogs. The decrease in DNA detected was consistent in samples taken pre-autoclave and after the first and second autoclave cycles. After the second autoclave cycle, C_T values plateaued on both clogs (Figure 1B).

qPCR was used in this study to analyse the samples due to the sensitivity it provides for detecting MPXV DNA when sampling areas potentially contaminated with widespread genetic material. These data confirm that MPXV DNA can still be detected post-autoclaving, albeit with a reduction of approximately 1000-fold (3 log₁₀). The inoculum used in this study provided a C_T similar to the C_T observed on PPE worn in mpox inpatient settings; therefore the C_T of samples taken after the first autoclave are indicative of levels on sterilized PPE reused in hospitals.

There are several important implications for the data obtained from this study. First, while autoclaving is effective at sterilizing materials, it does not completely eradicate DNA and subsequent detection by qPCR is possible, even after multiple autoclave cycles [7]. In addition, it is feasible that autoclaved MPXV DNA from reusable PPE may contaminate clean areas as a result from shedding or dislodgment. Finally, as identifying DNA does not necessarily equate to either presence of infectious virus or evidence of direct contamination, careful interpretation of environmental sampling data is required to interpret results and inform IPC measures. These results highlight the importance of thorough investigation of environmental sampling results and confirm that the sterilization provided by autoclaving does not result in the complete destruction of nucleic acid.

Ethical approval

The investigations performed were a component of the urgent public health investigation performed as part of UKHSA's public health incident response to cases of a high-consequence infectious disease in the UK. UKHSA is the national health security agency for England and an executive agency of the UK Government's Department of Health and Social Care. The study protocol was subject to internal review by the Research Ethics and Governance Group, which is the UKHSA Research Ethics Committee, and was granted full approval.

Author contributions

Conceptualization and methodology: A.S., J.D., T.P., S.G., T.F., A.M.B., B.A. Investigation: A.S., I.N., J.F., J.G., J.D., S.G., B.A. Formal analysis: A.S., O.O., I.N., T.P., B.A. Writing – original draft: A.S., B.A. Writing – review and editing: all authors.

Disclosure statement

The contents of this paper, including any opinions and/or conclusions expressed, are those of the authors alone and do not necessarily reflect UK Health Security Agency policy.

Conflict of interest statement

None declared.

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References

- [1] Marimuthu K, Wong JCC, Lim PL, Octavia S, Huan X, Ng YK, et al. Viable mpox virus in the environment of a patient room. *Int J Infect Dis* 2023;131:40–5.
- [2] Atkinson B, Spencer A, Onianwa O, Furneaux J, Grieves J, Nicholls I, et al. Longitudinal mpox virus surface sampling in an outpatient setting. *J Hosp Infect* 2023;135:196–8.
- [3] Nörz D, Pfefferle S, Brehm TT, Franke G, Grewe I, Knobling B, et al. Evidence of surface contamination in hospital rooms occupied by patients infected with monkeypox, Germany, June 2022. *Eurosurveillance* 2022;27:2200477.
- [4] Gould S, Atkinson B, Onianwa O, Spencer A, Furneaux J, Grieves J, et al. Air and surface sampling for monkeypox virus in a UK hospital: an observational study. *Lancet Microbe* 2022;3:e904–11.
- [5] Mantelakis A, Spiers HVM, Lee CW, Chambers A, Joshi A. Availability of personal protective equipment in NHS hospitals during COVID-19: a national survey. *Ann Work Expo Health* 2021;65:136–40.
- [6] Khan MT, Shah IA, Hossain MF, Akther N, Zhou Y, Khan MS, et al. Personal protective equipment (PPE) disposal during COVID-19: an emerging source of microplastic and microfiber pollution in the environment. *Sci Total Environ* 2023;860:160322.
- [7] Block SS. Disinfection, sterilization, and preservation. Philadelphia: Lippincott Williams & Wilkins; 2001.