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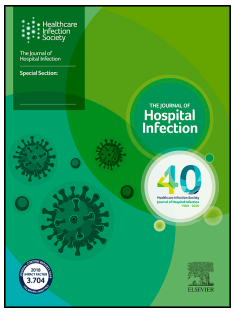

Mpox virus DNA contamination can still be detected by qPCR analysis after autoclaving

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Article type: Practice Points

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

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Running title: Investigating mpox virus DNA found outside hospital isolation rooms

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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 focussed 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). While 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). While most PPE is typically single-use, items such as autoclavable rubber clogs can be reused if suitably sterilised, 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, 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 (10,000ppm for a contact time of 10 minutes) and RNase AWAY (Sigma-Aldrich) and inoculated with 10µL of MPXV DNA previously extracted from an inactivated MPXV isolate from the 2022 mpox outbreak. This MXPV DNA inoculum was diluted to produce pre-autoclave swab sample with a Ct 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; panel A). Two sampling approaches were utilised: 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 were

performed with a hold time of 15 mins at 2.4 bar pressure at 121°C. Samples were taken using FLOQ Swabs (Copan, USA) containing 2mL viral transport media. Samples were inactivated, DNA extracted and analysed by qPCR following a previously described method used for genuine environmental samples (4). All samples taken from Clog A and 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, Ct values plateaued on both clogs (Figure; panel B).

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 $\log_{10}$). The inoculum used in this study provided a Ct similar to the Ct observed on PPE worn in mpox inpatient settings, therefore the Ct of samples taken after the first autoclave are indicative of levels on sterilised PPE reused in hospitals.

There are several important implications for the data obtained from this study. Firstly, while autoclaving is effective at sterilising materials (7), it does not completely eradicate DNA and subsequent detection by qPCR is possible, even after multiple autoclave cycles. 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 sterilisation provided by autoclaving does not result in the complete destruction of nucleic acid.

Authors' contributions*:*

Conceptualisation and methodology: AS, JD, TP, SG, TF, AMB, BA.

Investigation: AS, IN, JF, JG, JD, SG, BA.

Formal analysis: AS, OO, IN, TP, BA.

Writing – original draft: AS, BA.

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.

Statements

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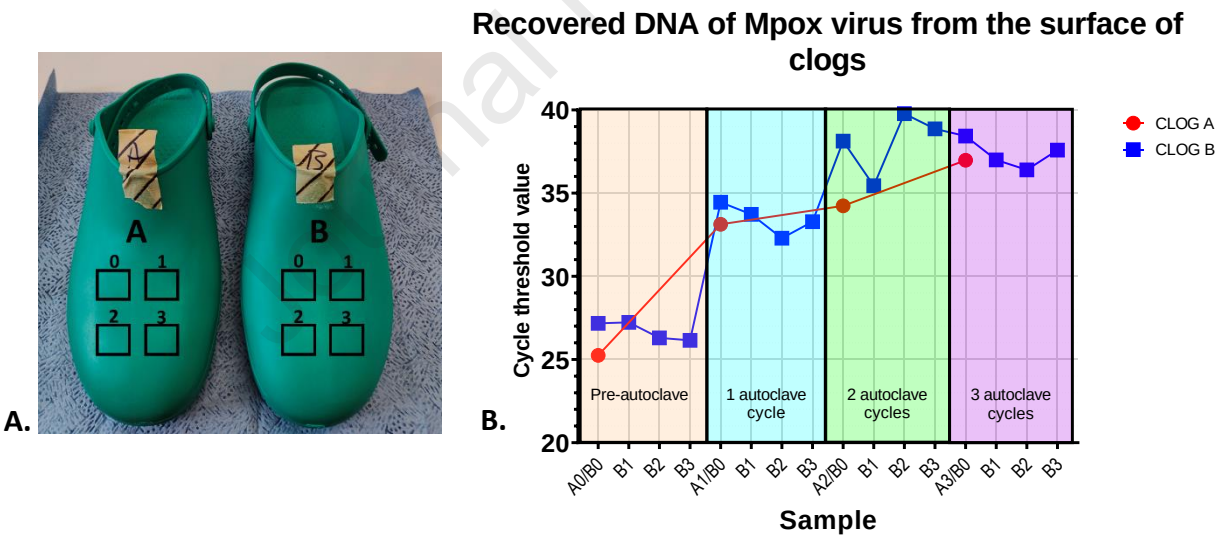
Competing interests: None declared.

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.

Figure legend

Figure: Panel A. Photo of 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. **Panel B.** Graph showing crossing threshold (Ct) 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).



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