Efficacy of MVM inactivation utilizing HTST pasteurization and suitability assessment of HTST-treated glucose feeds in CHO cell expression systems

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Supplementary Information

This document outlines additional supporting information to the viral clearance studies undertaken as part of the assessment of MVM inactivation using HTST pasteurization contained within the American Institute of Chemical Engineering's Journal of Biotechnology Progress.

This supporting information should be viewed alongside the submitted paper and provides additional detail of MVM detection in the samples post-HTST treatment.

Determination of Virus Log Reduction in 40% (w/v) glucose following HTST treatment

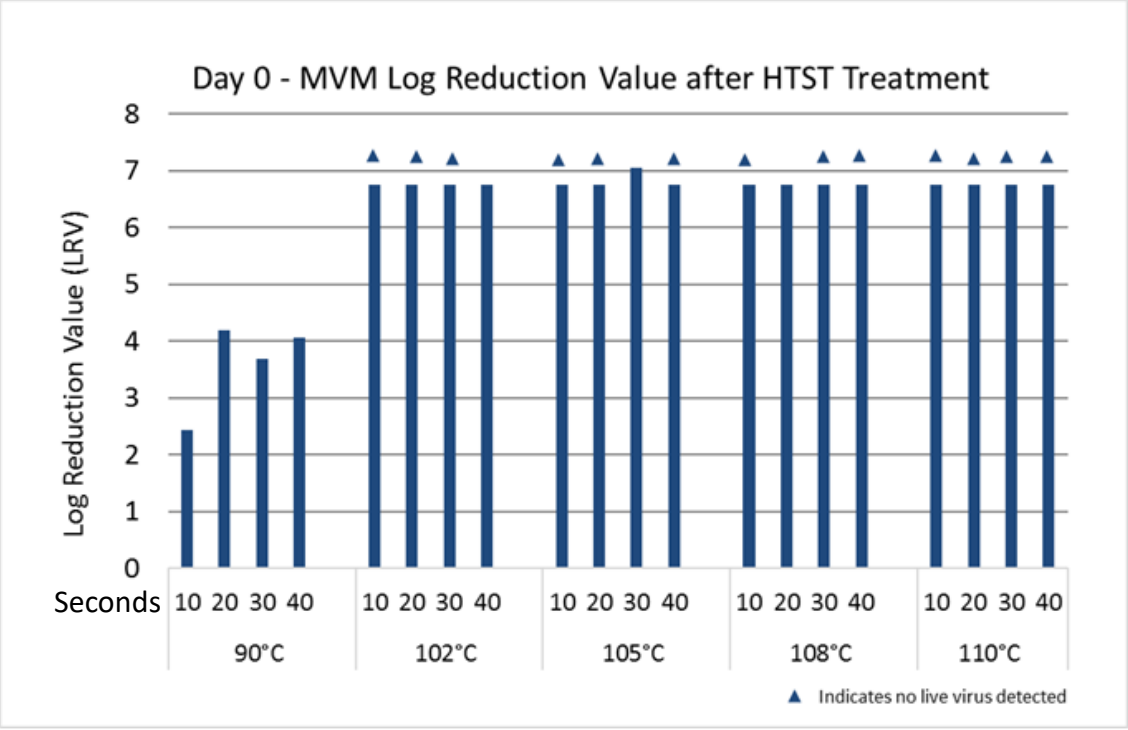


Figure S1. MVM Log reduction values for each HTST condition, Day 0

The viral clearance assay results from the Day 0 HTST treatment run of 40% (w/v) glucose data is shown in Figure S1. At 90ºC, partial inactivation of MVM was achieved with each exposure time with LRVs ranging from 2.4 to 4.2. At all higher temperatures, greater than 6 logs of MVM reduction was achieved within the 10 sec hold condition. For three samples, a low level of residual infectivity was detected (102ºC/40s, 105ºC/30 s, 108ºC/20s). However, for each of these an LRV ≥6.7 was achieved.

The triangle indicates that no live virus was detected in the lowest dilution of the sample, and the minimum LRV is calculated from the limit of detection of the assay.

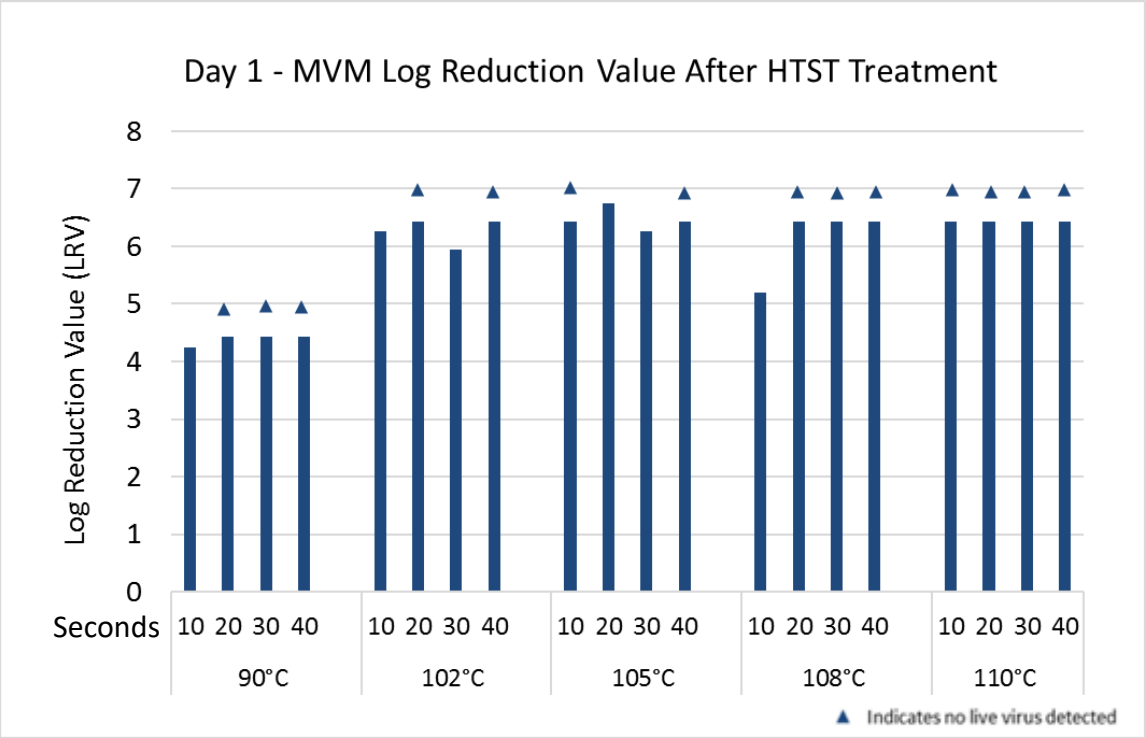


Figure S2. MVM Log reduction value for each HTST condition, Day 1

On Day 1 at 90ºC, there was inactivation of MVM (LRV ≥ 4.3) at each hold time (Figure S2). Detectable virus was only observed at the 10 sec hold condition. The maximum measurable LRV at the 90°C was limited compared to the other temperatures since this condition was assayed based on an estimate of incomplete virus inactivation (estimated LRV < 4.3). However, virus inactivation at 90°C was higher than expected. On Day 1 there was greater than 6 logs of MVM reduction at exposure temperatures between 102ºC to 1 10ºC, except at two conditions (102ºC/30s, 108ºC/10s). These conditions had lower LRVs (LRV=5.9 and 5.2 respectively), inconsistent with data generated on additional test days.

A possible explanation is that a small volume of the test solution had splashed onto the underside of the silicon cap. This drop of solution would not have been effectively exposed to the same temperature treatment as the bulk. The final temperature experienced by this detached volume would have been delayed until the headspace reached equilibrium with the bulk. Reintroduction of any detached volume into the bulk solution could result from subsequent vial handling.

Additionally, there was a very low level of residual infectivity of virus at three conditions, although LRVs were all greater than 6.3 logs (102ºC/10s, 105ºC/20s, 105ºC/30s).

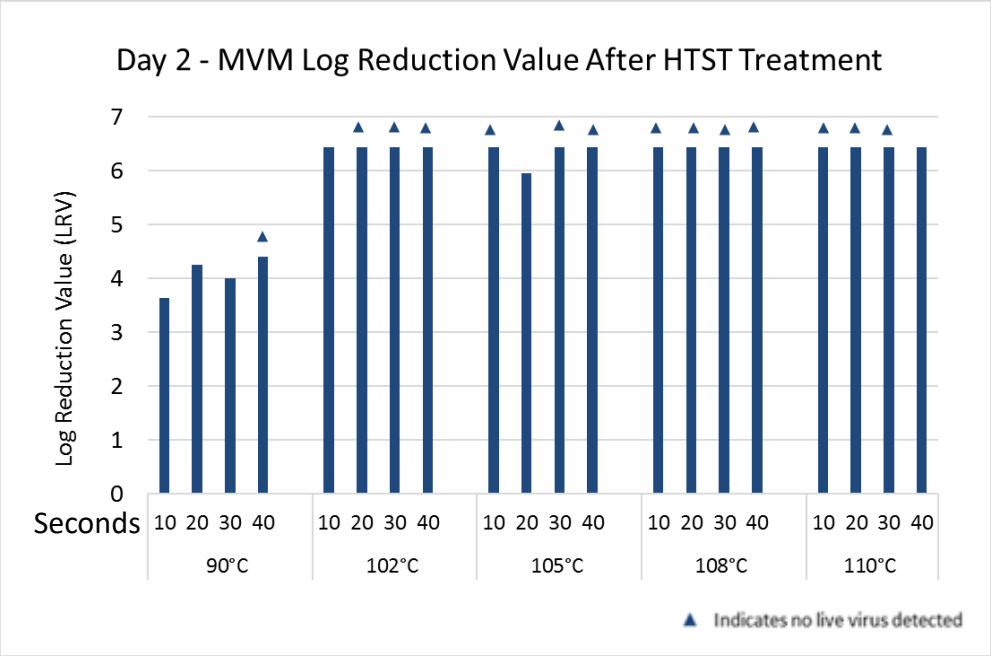
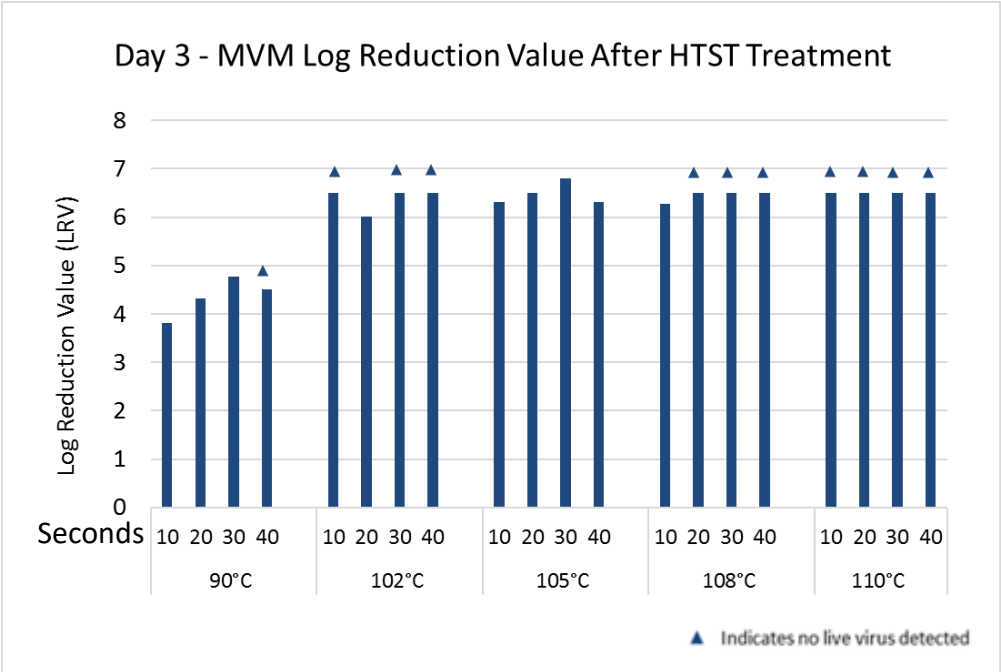


Figure S3. MVM Log reduction values for each HTST condition, Day 2

On Day 2, there was residual activity of MVM observed at each exposure time (Figure S3). LRVs ranging from 3.6 to 4.4 logs were achieved at the 90ºC exposure temperature. There was greater than 6 logs of MVM reduction at all exposure temperatures from 102ºC to 110ºC, except for one sample where 5.9 LRV was achieved (105ºC/20s). There was residual infectivity at two other conditions with LRVs of 6.4 logs (102ºC/10s, 110ºC/40s). As proposed earlier, the residual infectivity observed could possibly be due to material splashing up onto the vial cap during incubation, preventing complete exposure to the target incubation temperature.

Figure S4. MVM Log reduction values for each HTST condition, Day 3



On Day 3, there was partial inactivation of MVM at each hold time at 90ºC, with LRVs ranging from 3.8 to 4.8 logs (Figure S4). There was > 6 logs of MVM reduction from 102ºC to 110ºC within the 10s hold condition. There was a low level of residual infectivity observed at six conditions (102ºC/20s, 105ºC/10s, 105ºC/20s, 105ºC/30s, 105ºC/40s, 108ºC/10s) (Figure S4).

This study was performed to assess virus inactivation following high temperature, short time (HTST) treatment of a 40% (w/v) glucose solution. Target heating conditions were comparable to the SAFC industrial process.

Determination of Virus Log Reduction in 50% (w/v) glucose following HTST treatment

The LRV data shown in Figure S5 reflects the MVM log reduction values (LRV) for each run.

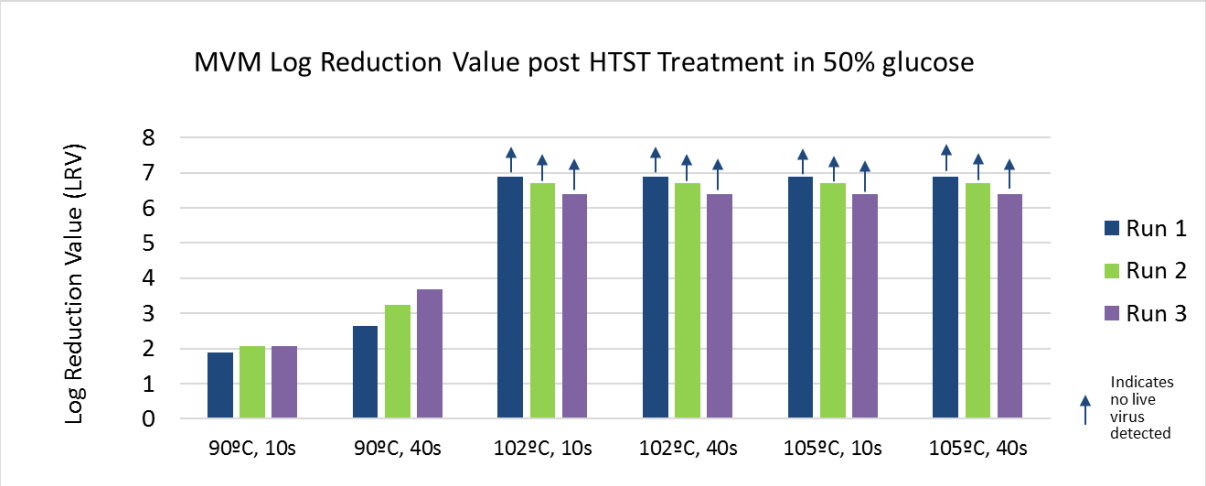


Figure S5. MVM Log reduction values for each HTST condition

There was partial inactivation of MVM at each hold time at 90ºC, with LRVs ranging from 1.9 to 3.7 logs (Figure S5). There was greater than 6.4 logs of MVM reduction from 102ºC and 105ºC within the 10 sec hold condition. All samples at 102ºC and 105ºC had no detectable virus (i.e. below assay limit of detection).