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Life Sciences

Viral Inactivation Study of SARS-CoV-2 in Lysis Buffer (LBF)

Methods

Lysis Buffer (LBF) and a PBS control buffer were incubated with SARS-COV-2 virus (150 µL Lysis Buffer (LBF) or PBS and 200 µL SARS-COV-2 sample as the RNAdvance Viral XP Instructions for Use indicate. The conditions tested were:

- 20 min Room Temperature
- 60 min Room Temperature
- 20 min heat at 60˚C

To reduce Lysis Buffer (LBF) cytotoxic effects, 0.1 mL of each sample was diluted into 0.9 mL PBS. 0.5 mL of each diluted sample was added to a 100 kDa molecular weight (MW) cutoff filter (Amicon) and centrifuged at 10,000 rpm for 5 minutes. Flowthrough was discarded and the remaining sample added and centrifuged. 0.5 ml PBS was added to the sample for buffer exchange, which was followed by another centrifugation. After the 2nd centrifugation, 0.2 mL PBS was added to resuspend the sample and 0.1 mL of the sample was used for $TCID_{50}$ assay.

In addition to $TCID_{50}$ assay, 0.1 mL of the virus/buffer mixture was transferred into a single well of a 6-well plate for each condition. Due to cytotoxicity observed, an additional $TCID_{50}$ assay was performed with the culture media in order to determine whether infectious virus could be detected in these wells.

Results

Controls

The $TCID_{50}$ for PBS control group is 7.94 E4 after Amicon filtration. This is lower than expected and there was likely some sample loss on the Amicon filters.

Heating (in PBS) at 60˚C led to undetectable levels of virus.

Lysis Buffer (LBF)

$TCID_{50}$ assay

For the LBF buffer, there was toxicity at the 10^{-1} dilution. There was no evidence of virus induced CPE at 10^{-2} dilutions and beyond. This equates to 3.16x 10^{2} $TCID_{50}$/mL. The heat condition showed the same result.

Buffer	PBS	PBS	PBS	LBF	LBF	LBF
Temperature	RT	RT	60˚C	RT	RT	60˚C
Time (min)	20	60	20	20	60	20
$TCID_{50}$/mL	7.94E4	6.31E4	3.70E1	3.16E2*	3.16E2*	3.16E2*

*maximum titer

$TCID_{50}$ (culture media)

No virus-induced CPE or cytotoxicity was observed in this assay. This equates to < 1 infectious unit present in the initial 0.1 mL inoculums of LBF-SARS-CoV-2 mixtures (10 $TCID_{50}$/mL).

Conclusions

The conclusion of this assay indicates a ≧ 7,940 reduction in viral activity, or ≧ 99.987% effective viral inactivation. These results were consistent at both room temperature and 60˚C for 20 min in Lysis buffer (LBF). The Initial $TCID_{50}$ assay indicated viral inactivation could be confirmed at ≧ 99.602% (1-(316/7.94x10^4)) and the concluding culture medial $TCID_{50}$ assay further improved the sensitivity to ≧ 99.987% (1-(10/7.94x10^4)). Confirmation of 100% inactivation is limited by the assay sensitivity due to the cytotoxic properties of Lysis Buffer (LBF).

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