

Evaluation of Viral Elimination from HIMACS by Bleach or Alcohol – Human Immunodeficiency Virus Type 1 (HIV-1)

Test Article	Active Ingredient(s)	Contact Time	Neutralizer
Diluted Bleach	0.5% sodium hypochlorite	30 seconds per submersion	RPMI 1640 + 10% Fetal Bovine Serum + 0.5% Na ₂ S ₂ O ₃
70% Alcohol	70% Isopropanol	30 seconds per submersion	RPMI 1640 + 10% Fetal Bovine Serum

Dilution medium:

RPMI 1640 + 2% Fetal Bovine Serum (FBS)

Carrier preparation, inoculation, and dry time:

The test surfaces were steam sterilized for 15 minutes at 121°C, cooled and stored at room temperature prior to testing. Surfaces were UV irradiated for ≥15 minutes per side then were inoculated with 0.4 mL of virus and dried for 40 minutes at 20°C.

Contact temperature:

Ambient room temperature 20±2°C (Actual: 20°C)

Organic load:

Virus contained 5% serum

Incubation temperature:

36±2°C in 5±3% CO₂

Media and reagents:

RPMI 1640 + 2% FBS
 RPMI 1640 + 10% FBS
 RPMI 1640 + 10% FBS + 0.5% Na₂S₂O₃ Sterile Deionized Water
 pH paper
 RPMI 1640 + 5% FBS

RESULTS

Data are presented in Tables 1 – 3.

Table 1
Titer results

Surface	Treatment	Replicate	Titer \pm 95% CL (Log ₁₀ TCID ₅₀ /mL)	Volume (mL) ^A	Viral Load (Log ₁₀ TCID ₅₀)
HI-MACS®	Diluted Bleach (30 second submersion) + Rinse (30 second submersion)	Rep 1	≤ 0.83 *	4.0	≤ 1.43
		Rep 2	≤ 0.83 *		≤ 1.43
		Rep 3	≤ 0.83 *		≤ 1.43
	70% IPA (30 second submersion) + Rinse (30 second submersion)	Rep 1	≤ 0.83 *	4.0	≤ 1.43
		Rep 2	≤ 0.83 *		≤ 1.43
		Rep 3	≤ 0.83 *		≤ 1.43
	Rinse only (30 second submersion)	Rep 1	4.93 \pm 0.12	4.0	5.53 \pm 0.12
		Rep 2	4.93 \pm 0.12		5.53 \pm 0.12
		Rep 3	4.93 \pm 0.24		5.53 \pm 0.24
	Untreated	Rep 1	5.30 \pm 0.19	4.0	5.90 \pm 0.19
		Rep 2	5.55 \pm 0.25		6.15 \pm 0.25
		Rep 3	5.55 \pm 0.16		6.15 \pm 0.16
Average Viral Load			6.08 \pm 0.20		

^A Volume refers to the volume of the virus recovery solution.

* No virus was detected; the theoretical titer was determined based on the Poisson distribution.

Table 2
Controls

Sample	Results
Neutralization/Viral Interference – Diluted Bleach	Virus detected in all wells
Cytotoxicity Control – Diluted Bleach	no cytotoxicity observed
Neutralization/Viral Interference – 70% IPA	Virus detected in all wells
Cytotoxicity Control – 70% IPA	no cytotoxicity observed
Cell Viability Control	no virus detected, cells were viable; media was sterile
Virus Stock Titer Control	6.55 \pm 0.16 Log ₁₀ Titer (TCID ₅₀ /mL)

RESULTS (continued)

Table 3
Reduction factors

Surface	Treatment	Input Viral Load (Log ₁₀ TCID ₅₀) ^B	Replicate	Output Viral Load (Log ₁₀ TCID ₅₀)	Reduction (Log ₁₀ TCID ₅₀)
HI-MACS®	Diluted Bleach (30 second submersion) + Rinse (30 second submersion)	6.08 ± 0.20	Rep 1	≤ 1.43	≥ 4.65 ± 0.20
			Rep 2	≤ 1.43	≥ 4.65 ± 0.20
			Rep 3	≤ 1.43	≥ 4.65 ± 0.20
	70% IPA (30 second submersion) + Rinse (30 second submersion)	6.08 ± 0.20	Rep 1	≤ 1.43	≥ 4.65 ± 0.20
			Rep 2	≤ 1.43	≥ 4.65 ± 0.20
			Rep 3	≤ 1.43	≥ 4.65 ± 0.20
	Rinse only (30 second submersion)	6.08 ± 0.20	Rep 1	5.53 ± 0.12	0.55 ± 0.23
			Rep 2	5.53 ± 0.12	0.55 ± 0.23
			Rep 3	5.53 ± 0.24	0.55 ± 0.31

^B Input Viral Load is the average Viral Load of the untreated samples.

Conclusion:	I A treatment procedure of a 30 second submersion in diluted bleach (5,000 ppm sodium hypochlorite) followed by a 30 second submersion in Sterile Deionized Water was able to completely inactivate HIV-1 (≥4.65 Log ₁₀ reduction) from the HIMACS material.	II A treatment procedure of a 30 second submersion in 70% Isopropanol followed by a 30 second submersion in Sterile Deionized Water was able to completely inactivate HIV-1 (≥4.65 Log ₁₀ reduction) from the HIMACS material.	III A treatment procedure of a 30 second submersion in Sterile Deionized Water alone reduced the viral load of HIV-1 from the HIMACS material by 0.55 Log ₁₀ .
<p>All controls met the criteria for a valid test. These conclusions are based on observed data.</p>			