

A new non-guanidine, non-hazardous SARS-CoV-2 inactivation medium for safe respiratory swab sample collection and transport

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Introduction

Specimens containing pathogenic respiratory viruses pose a risk of infection during handling, transportation and testing. The highly infectious nature and sheer volume of samples collected during the COVID-19 pandemic highlights a need for swab sample transport devices that inactivate viruses¹, making samples safe to handle outside of high containment facilities. Existing swab transport devices use hazardous formulations to inactivate virus, typically containing guanidine isothiocyanate, which is caustic and releases cyanide gas if not handled correctly².

Thermo Scientific™ InhibiSURE™ Viral Inactivation Medium (Figure 1) is a novel, non-hazardous formula that rapidly inactivates SARS-CoV-2 in nasal, nasopharyngeal and oropharyngeal swabs. The non-hazardous formulation, enables safe collection, transportation and processing of samples. The need for further inactivation steps at the laboratory is removed, allowing faster and more efficient specimen processing. Inactivation occurs within 30 minutes at room temperature (10-30°C) and no further processing of the sample is required ahead of RNA extraction and PCR. Viral RNA is stabilized for 10 days at ambient temperature. The medium was tested by Thermo Fisher Scientific, Basingstoke, UK and The Francis Crick Institute, London, to measure SARS-CoV-2 inactivation, RNA stability and limit of detection.



Figure 1. InhibiSURE Viral Inactivation Medium, with nylon-tipped swab

Materials and Methods

Inactivation: SARS-CoV-2 at 2.5×10^7 PFU/mL was spiked into InhibiSURE medium. After 30 minutes at room temperature, samples were purified using Amicon™ 10KDa ultracentrifugation filters (Merck) before serial \log_{10} dilution and inoculation into Vero E6 plaque assay. Triple passaging studies were also performed to confirm absence of low virus numbers.

Limit of Detection: Limit of detection (LoD) was assessed using the Thermo Scientific™ MagMAX™ MVP II kit (product code A48383) on a Thermo Scientific™ KingFisher™ Flex Purification System and TaqPath™ COVID-19 CE-IVD RT-PCR Kit (product code A48067) on an Applied Biosystems™ 7500 Fast Real-Time PCR system. Non-viable SARS-CoV-2 (SeraCare Accuplex™ SARS-CoV-2 Reference Material Kit, LGC Clinical Diagnostics, Inc.) was spiked into InhibiSURE medium and PrimeStore™ MTM medium (Longhorn Vaccines and Diagnostics LLC) at concentrations from 500 to 50 copies/mL. After 30 minutes at room temperature, PCR was conducted. LoD was confirmed by testing 25 replicates to ensure $\geq 95\%$ achieved positive determination.

RNA stability: SARS-CoV-2 was spiked into InhibiSURE Viral Inactivation Medium, Thermo Scientific™ MicroTest™ M4RT™ medium and PrimeStore MTM medium. Samples were stored at recommended temperatures and tested at 0, 3, 6 and 10 days by PCR. A cycle threshold (C_T) difference of <3 compared to day 0 was deemed to indicate RNA stability.

Results

Inactivation: No virus was detected in plaque assay or triple passage after exposure to InhibiSURE Viral Inactivation Medium, indicating complete inactivation of a high titre (7.2×10^6 PFU/mL) of SARS-CoV-2 in swab samples (Table 1).

Table 1. Plaque / passaging results showing complete inactivation of SARS-CoV-2 in InhibiSURE™ Viral Inactivation Medium

Medium	Virus Titre Detected PFU/mL	Log ₁₀ Reduction	Passage 1	Passage 2	Passage 3	Virus Detected Y/N	Observations / Notes
InhibiSURE medium with virus	0	$>7.2 \times 10^6$	No CPE	No CPE	No CPE	No	Virus not detected
InhibiSURE medium without virus	0	n/a	n/a	n/a	n/a	No	No cytotoxicity
PBS Control with virus	7.2×10^6	n/a	n/a	n/a	n/a	Yes	Virus detected

Limit of Detection: The lowest dilution at which all three samples of non-viable SARS-CoV-2 was detected was determined to be 150 copies/mL in both InhibiSURE medium and PrimeStore MTM (table 2).

Table 2. Sample determinations for non-viable SARS-CoV-2 in InhibiSURE and PrimeStore MTM media at 500 to 50 copies/mL, indicating initial LoD of 150 copies/mL.

Formulation	Concentration (copies/mL)	Positive Gene Target Calls (3 replicates)			Sample Call (3 replicates)
		N Gene	ORF1ab	S Gene	
InhibiSURE Viral Inactivation Medium	500	3/3	3/3	3/3	3/3 POSITIVE
	250	3/3	3/3	0/3	3/3 POSITIVE
	150	3/3	3/3	0/3	3/3 POSITIVE
	50	3/3	2/3	0/3	2/3 POSITIVE
PrimeStore MTM Medium	500	3/3	3/3	1/3	3/3 POSITIVE
	250	3/3	3/3	0/3	3/3 POSITIVE
	150	3/3	3/3	0/3	3/3 POSITIVE
	50	3/3	2/3	0/3	2/3 POSITIVE

25 replicates at a concentration of 150 copies/mL were tested in InhibiSURE and PrimeStore MTM media with 24/25 replicates (96%) determined as positive for SARS-CoV-2 in both media (Table 3).

Table 3. C_T values and sample determinations for SARS-CoV-2 in InhibiSURE medium at 150 copies/mL.

Replicate	C_T Values for Gene Targets			Gene Target Call			Sample Call
	N	ORF1ab	S	N	ORF1ab	S	
1	31.35	33.20	-	POSITIVE	POSITIVE	NEGATIVE	POSITIVE
2	31.37	32.56	-	POSITIVE	POSITIVE	NEGATIVE	POSITIVE
3	32.33	35.83	-	POSITIVE	POSITIVE	NEGATIVE	POSITIVE
4	32.38	32.78	-	POSITIVE	POSITIVE	NEGATIVE	POSITIVE
5	32.46	38.44	-	POSITIVE	NEGATIVE	NEGATIVE	INCONCLUSIVE
6	32.63	32.83	-	POSITIVE	POSITIVE	NEGATIVE	POSITIVE
7	32.31	32.96	-	POSITIVE	POSITIVE	NEGATIVE	POSITIVE
8	31.64	33.59	-	POSITIVE	POSITIVE	NEGATIVE	POSITIVE
9	32.77	33.52	-	POSITIVE	POSITIVE	NEGATIVE	POSITIVE
10	32.29	33.21	-	POSITIVE	POSITIVE	NEGATIVE	POSITIVE
11	32.76	32.10	-	POSITIVE	POSITIVE	NEGATIVE	POSITIVE
12	33.47	34.73	-	POSITIVE	POSITIVE	NEGATIVE	POSITIVE
13	32.68	33.55	-	POSITIVE	POSITIVE	NEGATIVE	POSITIVE
14	32.17	34.06	-	POSITIVE	POSITIVE	NEGATIVE	POSITIVE
15	32.20	32.99	-	POSITIVE	POSITIVE	NEGATIVE	POSITIVE
16	32.76	34.15	-	POSITIVE	POSITIVE	NEGATIVE	POSITIVE
17	32.18	32.24	-	POSITIVE	POSITIVE	NEGATIVE	POSITIVE
18	32.21	33.48	-	POSITIVE	POSITIVE	NEGATIVE	POSITIVE
19	31.06	33.41	-	POSITIVE	POSITIVE	NEGATIVE	POSITIVE
20	32.26	32.87	-	POSITIVE	POSITIVE	NEGATIVE	POSITIVE
21	32.17	35.54	-	POSITIVE	POSITIVE	NEGATIVE	POSITIVE
22	31.73	32.87	-	POSITIVE	POSITIVE	NEGATIVE	POSITIVE
23	30.92	32.12	36.53	POSITIVE	POSITIVE	POSITIVE	POSITIVE
24	32.12	32.72	-	POSITIVE	POSITIVE	NEGATIVE	POSITIVE
25	31.36	32.88	-	POSITIVE	POSITIVE	NEGATIVE	POSITIVE

RNA Stability: Maximum C_T value changes for SARS-CoV-2 RNA over 10 days in InhibiSURE Viral Inactivation Medium were less than 3.0 compared to day 0 and were essentially equivalent to PrimeStore MTM and MicroTest M4RT media. Data for 2°C and 30°C are shown in figures 2 and 3, respectively. SARS-CoV-2 RNA is stable in InhibiSURE™ Viral Inactivation Medium for 10 days at 2°C and 30°C.

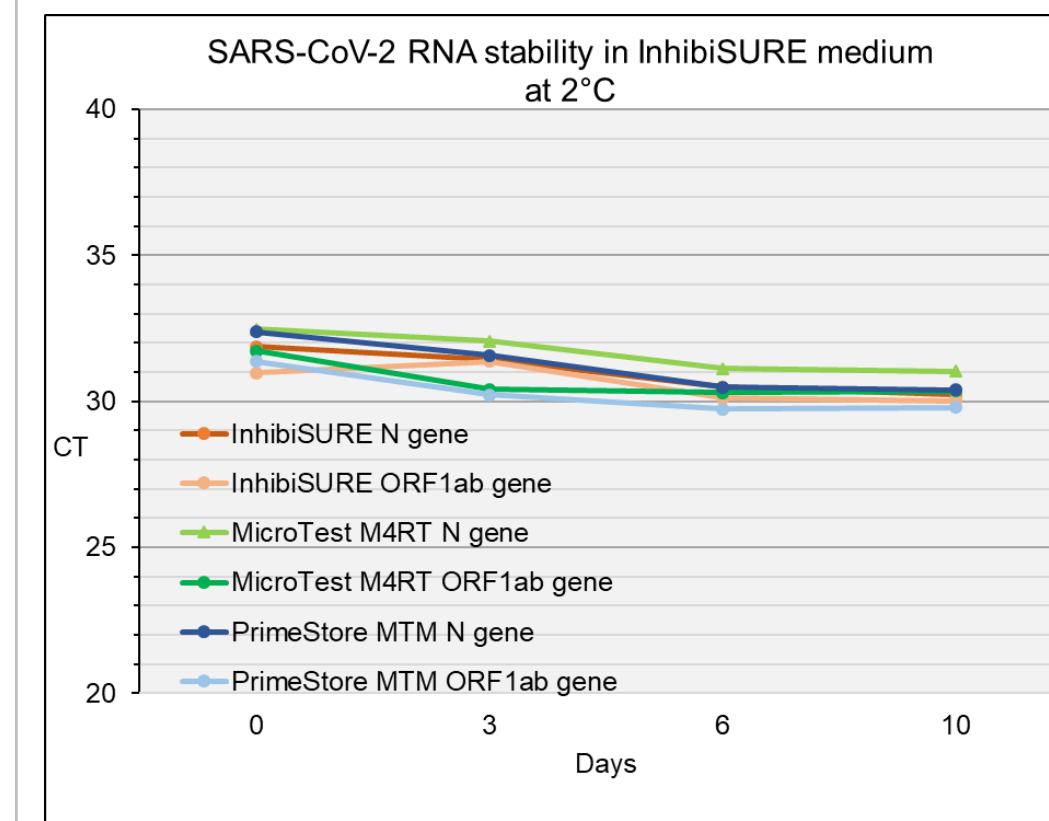


Figure 2. SARS-CoV-2 RNA C_T values in InhibiSURE, MicroTest M4RT and PrimeStore MTM media over 10 days at 2°C.

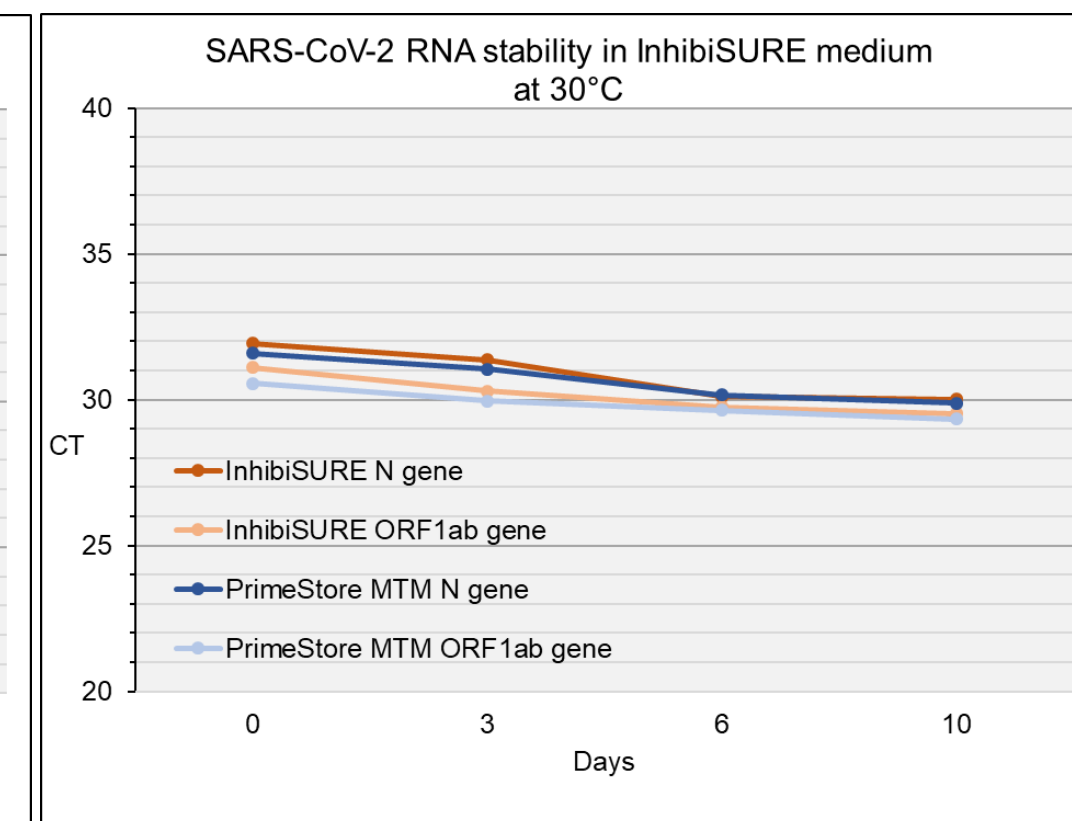


Figure 3. SARS-CoV-2 RNA C_T values in InhibiSURE and PrimeStore MTM media over 10 days at 30°C.

Conclusions

InhibiSURE Viral Inactivation Medium inactivates SARS-CoV-2 virus completely from a high titre ($>7.2 \times 10^6$ PFU/mL).

The limit of detection of SARS-CoV-2 in InhibiSURE Viral Inactivation Medium is equivalent to that of a guanidine-based inactivating transport medium, giving laboratories confidence that InhibiSURE medium does not interfere with detection of SARS-CoV-2 in the nucleic acid detection workflow.

SARS-CoV-2 RNA is stable in InhibiSURE Viral Inactivation Medium for up to 10 days at refrigerator and room temperatures. Laboratories have flexibility with sample storage and sufficient time for repeat testing if required.

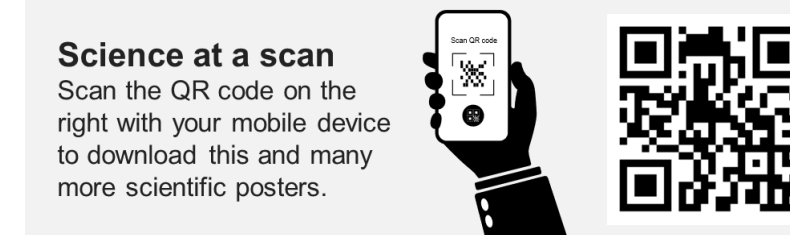
Thermo Scientific™ InhibiSURE™ Viral Inactivation Medium not only inactivates SARS-CoV-2, it also has a non-hazardous formulation, supporting the safe collection, transportation and processing of samples. As there is no need for further inactivation steps, microbiology laboratories can also expect faster throughput of specimens.

References

- Stephen R. Welch et al. Analysis of Inactivation of SARS-CoV-2 by Specimen Transport Media, Nucleic Acid Extraction Reagents, Detergents, and Fixatives. Journal of Clinical Microbiology, Vol. 58, No. 11, 2020. <https://doi.org/10.1128/JCM.01713-20>
- US Center for Disease Control and Prevention, Lab Alert: Important Update about Molecular Transport Media (MTM) and Cyanide Gas. February 2020. www.cdc.gov.

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