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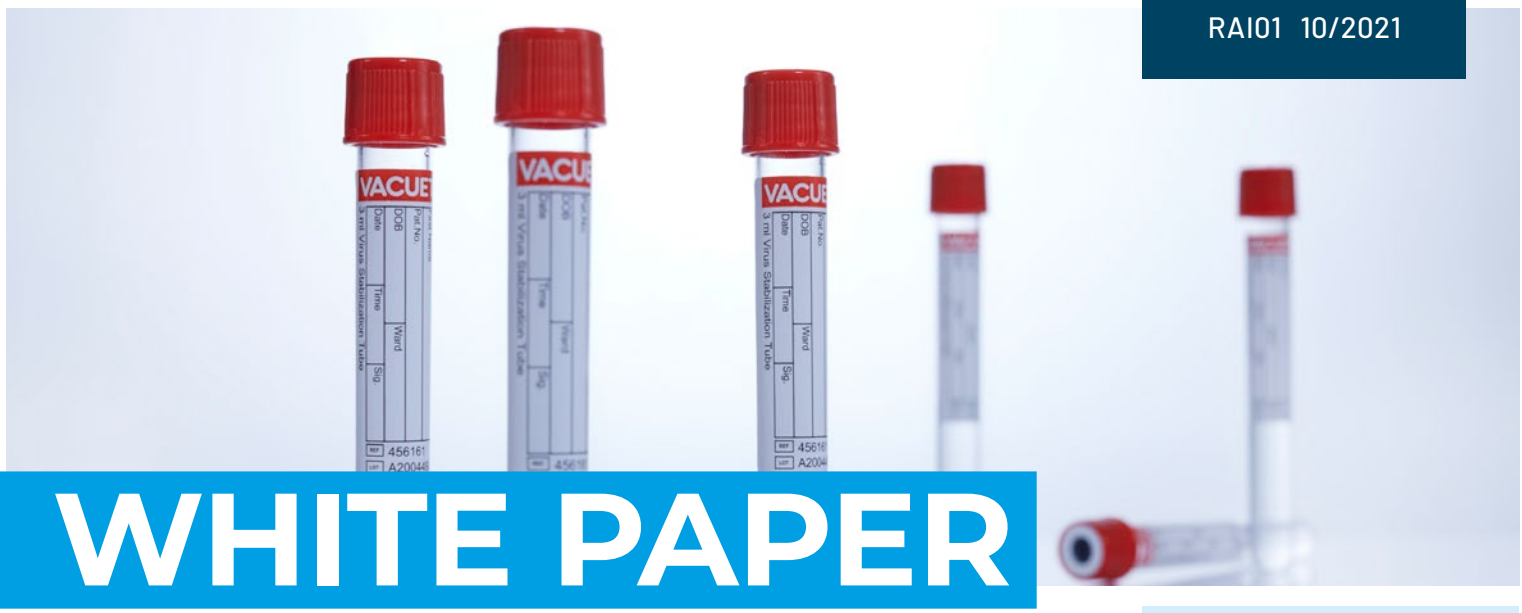

WHITE PAPER

TECHNICAL NOTES & APPLICATIONS FOR LABORATORY WORK

STABILITY TESTING OF SARS-COV-2, INFLUENZA A AND B IN VACUETTE® VIRUS STABILIZATION TUBES

CONTENT

1/ STUDY ABSTRACT

The VACUETTE® Virus Stabilization Tube (VST) was developed in response to the global healthcare system emergency caused by the COVID-19 pandemic with the intent of providing an efficient medium for transport and storage of SARS-CoV-2 as well as Influenza A and B specimens. The VST contains a predefined volume of a phosphate buffered saline solution (PBS) at a pH of 7.4, which allows the storage of nasopharyngeal and oropharyngeal specimens for up to 72h at 4 °C.

The purpose of this study was to demonstrate the stability of SARS-CoV-2 , Influenza A and Influenza B in the presence of a clinical matrix (sputum) when stored in a VACUETTE® Virus Stabilization Tube for 72 hours at 4°C (2 to 8°C). We tested the stability using freshly produced tubes (non-aged) as well as tubes at the end of their claimed shelf-life of 9 months (aged tubes).

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Our study confirmed that SARS-CoV-2, Influenza A and Influenza B are stable in the presence of a clinical matrix in VACUETTE® Virus Stabilization Tubes for 72 hours when stored at 4°C (2 to 8°C). The stability was confirmed in freshly produced (non-aged) tubes as well as tubes at the end of their claimed shelf-life (aged tubes).

2/ BACKGROUND

The COVID-19 pandemic poses a challenge to the world, especially to physicians and medical professionals, but also to manufacturers like Greiner Bio-One, who supply hospitals and entire healthcare systems with essential medical products for coping with the crisis. Greiner Bio-One provides the VACUTTE® Virus Stabilization Tube to support the vast number of tests needed to prevent uncontrolled spread of SARS-CoV-2 and the resulting disease.

However, since symptoms caused by SARS-CoV-2 resemble the symptoms of infection with Influenza viruses [1], it is of great importance to enable distinction of those two genera of viruses in order to provide the appropriate diagnosis and subsequent treatment.

3/ STUDY OBJECTIVE

To enable diagnostic laboratories to test for both SARS-CoV-2 and Influenza A and B viruses, it is necessary to store and transport samples in collection devices that guarantee stabilization of viral material from sample collection to analysis to prevent false negative results. Therefore, the VACUETTE® Virus Stabilization Tubes was developed for the purpose of stabilizing nasopharyngeal and oropharyngeal samples collected for detection of SARS-CoV-2, Influenza A and Influenza B.

4/ STUDY DESIGN AND PROCEDURE

4.1/ SAMPLE PREPARATION

The stability of SARS-CoV-2, Influenza A and Influenza B viruses was tested in freshly produced tubes as well as aged tubes (accelerated aging was performed by storing tubes at 50°C for 20 days – simulating tubes at the end of their claimed shelf life).

For the clinical background matrix, samples were obtained from a sputum supernatant spiked with ATCC reference material (SARS-COV-2 VR-1986HK Lot. 70037676, Influenza A VR-897 Lot. 58810588, Influenza B VR-103 Lot. 64295716).

150µl of the sample, consisting of the prepared matrix and virus, was pipetted on to a nasopharyngeal swab and subsequently washed in a 2 ml VACUETTE® Virus Stabilization Tube.

The Limit of Detection (LoD) for each viral target was evaluated for use in our test system before starting stability testing. Each viral target was then tested individually at concentrations of 10x LoD, 2x LoD, 0.1x LoD and matrix only samples (negative control), with 10, 20, 20 and 10 replicates respectively analyzed for each fresh and aged tube.

4.2/ TESTING FOR STABILITY – RNA DETECTION

The testing for viral pathogens requires RNA isolation, which was performed using QIAamp® Viral RNA Mini Kit (Qiagen, REF 52904). For the detection of viral RNA, VIASURE SARS-CoV-2, Flu & RSV Kit (Certest, REF VS-CFR112L or VS-CRF113L) was used in combination with CFX96 thermal cycler and CFX manager software 3.1.

The stability was evaluated immediately after dissolving the sample in a 2 ml VACUETTE® Virus Stabilization Tube (testing point 0) and following storage for 72 hours (73 hours less one hour safety margin) at 4°C (2 to 8°C).

4.3/ ACCEPTANCE CRITERIA AND STATISTICAL ANALYSIS

The results obtained with the RNA VIASURE SARS-CoV-2, Flu & RSV Kit are given in Ct values. SARS-CoV-2 is detected in the FAM channel whereas Influenza A and B are detected in the ROX channel. For each channel, cut-off Ct values were defined beforehand for the distinction of positive and negative samples. The cut-off Ct value was defined as 36.5 for the FAM channel and 37.5 for ROX. The acceptance criteria were met if the viral target of interest was detected at a Ct value at or below the defined cut-off Ct value at both testing points of 0 and 72 hours in samples of 10x and 2x LoD. For samples containing viral concentrations of 0.1x LoD or matrix only samples, Ct values had to be higher than the cut-off Ct value.

The results are shown as mean Ct value of all replicates and include standard deviation and coefficient of variation in percent [standard deviation/mean Ct value*100].

5/ RESULTS

Testing was performed for each pathogen and concentration at 0 hours (directly after spiking) and at 73 hours following storage at 4°C (2 to 8°C). The testing point at 73 hours includes a safety margin of one hour. Therefore, if acceptance criteria are fulfilled at 73 hours, stability can be confirmed for 72 hours.

5.1/ RESULTS FOR SARS-COV-2

10 of 10 and 20 of 20 analyses of spiked samples at viral concentrations of 10x LoD and 2x LoD, respectively, yielded a positive result at both time points of 0 and 73 hours when tested in aged as well as in non-aged tubes. Obtained Ct values for these two time points demonstrate stability of SARS-CoV-2 virus in both fresh VACUETTE® Virus Stabilization Tubes and tubes at the end of their claimed shelf-life (aged tubes) with maximum Ct values differences of +/- 0.26. Standard deviation as well as coefficient of variation values were low at 0.49 and 1.54% respectively.

All samples expected to be negative, meaning samples spiked with viral concentration of 0.1x LoD and matrix only samples, yielded a negative result with either Ct values > 36.5 or no amplification (n.def.) detected. This applied for both testing conditions – aged and non-aged tubes – as well as both time points – 0 and after storage for 73 hours at 4°C (2 to 8°C) (Table 1).

Target	Samples - aged tubes	Results at testing point 0				Results after storage for 73h			
		Ct mean	SD	CV [%]	Results	Ct mean	SD	CV [%]	Results
SARS CoV-2	**Sample #1 – #10** **10xLoD** SARS CoV-2 in presence of matrix in VACUETTE® Virus Stabilization Tubes	31.55	0.12	0.37	10/10 positive	31.74	0.32	1.00	10/10 positive
	Sample #11 – #30 **2xLoD** SARS CoV-2 in presence of matrix in VACUETTE® Virus Stabilization Tubes	34.27	0.43	1.25	20/20 positive	34.01	0.36	1.05	20/20 positive
	Sample #31 – #50 **0.1xLoD** SARS CoV-2 in presence of matrix in VACUETTE® Virus Stabilization Tubes	39.80	1.60	4.03	20/20 negative	40.97	1.10	2.69	20/20 negative
	Sample #51 – #60 negative samples (matrix only) in VACUETTE® Virus Stabilization Tubes	/	/	/	10/10 negative	/	/	/	10/10 negative

Target	Samples - non-aged tubes	Results at testing point 0				Results after storage for 73h			
		Ct mean	SD	CV [%]	Results	Ct mean	SD	CV [%]	Results
SARS CoV-2	**Sample #61 – #70** **10xLoD** SARS CoV-2 in presence of matrix in VACUETTE® Virus Stabilization Tubes	31.61	0.33	1.04	10/10 positive	31.87	0.49	1.54	10/10 positive
	Sample #71 – #90 **2xLoD** SARS CoV-2 in presence of matrix in VACUETTE® Virus Stabilization Tubes	33.87	0.33	0.97	20/20 positive	34.13	0.27	0.78	20/20 positive
	Sample #91 – #110 **0.1xLoD** SARS CoV-2 in presence of matrix in VACUETTE® Virus Stabilization Tubes	37.77	0.53	1.41	20/20 negative	39.26	1.52	3.87	20/20 negative
	Sample #111 – #120 negative samples (matrix only) in VACUETTE® Virus Stabilization Tubes	/	/	/	10/10 negative	/	/	/	10/10 negative

Table 1: Results for SARS-CoV-2 stability testing in aged tubes at 3 different concentrations and two different time points as well as in non-aged tubes.

5.2/ RESULTS FOR INFLUENZA A

The data in Table 2 demonstrates the stability of Influenza A virus in VACUETTE® Virus Stabilization Tubes if spiked in presence of a clinical sputum matrix at viral concentrations of 10x and 2x LoD, since Ct values of all samples allow positive interpretation of all analyses at both testing conditions (aged and non-aged tubes) and both time points (0 hours and after storage for 73 hours at 4°C (2 to 8°C)). Influenza A is considered stable since obtained Ct values differ by +/- 0.51 at most, between testing at 0 and 73 hours.

All samples containing a viral concentration of 0.1x LoD or matrix only were interpreted as negative in all testing circumstances (Table 2).

Target	Samples - aged tubes	Results at testing point 0				Results after storage for 73h			
		Ct mean	SD	CV [%]	Results	Ct mean	SD	CV [%]	Results
Influenza A	**Sample #1 – #10** **10xLoD** Influenza A in presence of matrix in VACUETTE® Virus Stabilization Tubes	32.35	0.24	0.73	10/10 positive	32.29	0.57	1.77	10/10 positive
	Sample #11 – #30 **2xLoD** Influenza A in presence of matrix in VACUETTE® Virus Stabilization Tubes	34.53	0.32	0.93	20/20 positive	35.04	0.40	1.13	20/20 positive
	Sample #31 – #50 **0.1xLoD** Influenza A in presence of matrix in VACUETTE® Virus Stabilization Tubes	39.60	1.40	3.54	20/20 negative	39.86	1.07	2.67	20/20 negative
	Sample #51 – #60 negative samples (matrix only) in VACUETTE® Virus Stabilization Tubes	/	/	/	10/10 negative	/	/	/	9/9 negative*

Target	Samples - non-aged tubes	Results at testing point 0				Results after storage for 73h			
		Ct mean	SD	CV [%]	Results	Ct mean	SD	CV [%]	Results
Influenza A	**Sample #61 – #70** **10xLoD** Influenza A in presence of matrix in VACUETTE® Virus Stabilization Tubes	33.00	0.24	0.72	10/10 positive	33.19	0.11	0.34	10/10 positive
	Sample #71 – #90 **2xLoD** Influenza A in presence of matrix in VACUETTE® Virus Stabilization Tubes	35.15	0.34	0.95	20/20 positive	35.64	0.68	1.90	20/20 positive
	Sample #91 – #110 **0.1xLoD** Influenza A in presence of matrix in VACUETTE® Virus Stabilization Tubes	39.41	1.52	3.85	20/20 negative	38.96	0.69	1.78	20/20 negative
	Sample #111 – #120 negative samples (matrix only) in VACUETTE® Virus Stabilization Tubes	/	/	/	10/10 negative	/	/	/	10/10 negative

Table 2: Results for Influenza A stability testing in aged tubes at 3 different concentrations and two different time points as well as in non-aged tubes.

* Invalid result of one of ten analyses – sample was excluded from evaluation

5.3/ RESULTS FOR INFLUENZA B

All samples spiked with Influenza B virus at concentrations of 10x and 2x LoD in the presence of a clinical sputum matrix were positive with Ct values observed at 0 and 73 hours differing by +/-0.86 at most. The standard deviation and coefficient of variation are low, indicating a good repeatability. 20 of 20 and 10 of 10 samples spiked with a viral concentration of 0.1x LoD or matrix only samples, respectively, were negative since the Ct value was 37.5 or higher (cut-off for ROX channel = 37.5), showing that our test system was specific for Influenza A and B preventing false positive results (Table 3).

Target	Samples - aged tubes	Results at testing point 0				Results after storage for 73h			
		Ct mean	SD	CV [%]	Results	Ct mean	SD	CV [%]	Results
Influenza B	**Sample #1 – #10** **10xLoD** Influenza B in presence of matrix in VACUETTE® Virus Stabilization Tubes	32.54	0.18	0.54	10/10 positive	32.69	0.24	0.73	10/10 positive
	Sample #11 – #30 **2xLoD** Influenza B in presence of matrix in VACUETTE® Virus Stabilization Tubes	34.32	0.27	0.78	20/20 positive	34.53	0.35	1.00	20/20 positive
	Sample #31 – #50 **0.1xLoD** Influenza B in presence of matrix in VACUETTE® Virus Stabilization Tubes	39.94	1.67	4.17	20/20 negative	39.34	1.63	4.13	20/20 negative
	Sample #51 – #60 negative samples (matrix only) in VACUETTE® Virus Stabilization Tubes	/	/	/	10/10 negative	/	/	/	10/10 negative

Target	Samples - non-aged tubes	Results at testing point 0				Results after storage for 73h			
		Ct mean	SD	CV [%]	Results	Ct mean	SD	CV [%]	Results
Influenza B	**Sample #61 – #70** **10xLoD** Influenza B in presence of matrix in VACUETTE® Virus Stabilization Tubes	32.50	0.24	0.73	10/10 positive	32.89	0.20	0.60	10/10 positive
	Sample #71 – #90 **2xLoD** Influenza B in presence of matrix in VACUETTE® Virus Stabilization Tubes	34.54	0.34	0.99	20/20 positive	33.68	0.97	2.87	20/20 positive
	Sample #91 – #110 **0.1xLoD** Influenza B in presence of matrix in VACUETTE® Virus Stabilization Tubes	38.90	0.97	2.50	20/20 negative	39.07	1.74	4.46	20/20 negative
	Sample #111 – #120 negative samples (matrix only) in VACUETTE® Virus Stabilization Tubes	/	/	/	10/10 negative	/	/	/	10/10 negative

Table 3: Results for Influenza B stability testing in aged tubes at 3 different concentrations and two different time points as well as in non-aged tubes.

6/ CONCLUSION

During our stability study, we confirmed that specimens containing SARS-CoV-2 and Influenza A & B remain stable in the presence of a clinical matrix (sputum) in VACUETTE® Virus Stabilization Tubes when stored at 4°C (2 to 8°C) for 72 hours. Furthermore, we showed stability in both freshly prepared tubes and tubes at the end of their stated shelf life of 9 months.

7/ REFERENCES

[1] Daum L, Worthy S, Yim K, et al. A clinical specimen collection and transport medium for molecular diagnostic and genomic applications. Epidemiol. Infect. 2011; 139: 1764-1763.

[2] Flerlage T, Boyd DF, Meliopoulos V, et al. Influenza virus and SARS-CoV-2: pathogenesis and host responses in the respiratory tract. Nat Rev Microbiol. 2021; 19: 425–441.

OUR STUDY CONFIRMED THAT SARS-COV-2, INFLUENZA A AND INFLUENZA B ARE STABLE IN THE PRESENCE OF A CLINICAL MATRIX IN VACUETTE® VIRUS STABILIZATION TUBES FOR 72 HOURS WHEN STORED AT 4°C (2 TO 8°C).

The stability was confirmed in freshly produced (non-aged) tubes as well as tubes at the end of their claimed shelf-life (aged tubes).

PRODUCT & ORDERING INFORMATION

- Primary container for the safe transport and storage of swab specimens
- Manufactured under Greiner Bio-One hygienic standard requirements
- Sterile non-evacuated PET tube with a phosphate buffered saline solution
- CE-marked product

Ever since the alarming global Covid-19 pandemic and the far-reaching effects, the focus for health-care has increasingly shifted towards detection of viruses. Greiner Bio-One can make a significant contribution to virus detection with the VACUETTE® Virus Stabilization Tube. This product supports the safe transport and storage of specimens.

For additional safety measures, the transport of this product in combination with the VACUETTE® Transport Line ensures further security in the delivery of nasopharyngeal and oropharyngeal swabs to the laboratory for SARS-CoV-2, influenza A and influenza B testing only.

VACUETTE® Virus Stabilization Tube

Item No.	Volume	Cap colour	Ring Colour	Thread type	Tube size	Label type	Inner / Outer [Qty.]
456162	2ml	red	white	PREMIUM	13 x 100	paper	50 / 1,200
456161	3ml	red	white	PREMIUM	13 x 100	paper	50 / 1,200

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