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Evaluation of Saline, Phosphate-Buffered Saline, and Minimum Essential Medium as Potential Alternatives to Viral Transport Media for SARS-CoV-2 Testing

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus disease 2019 (COVID-19), has caused a global pandemic since being discovered in late 2019. In response, clinical microbiology and public health laboratories have worked to develop, validate, and implement molecular assays to detect SARS-CoV-2 from respiratory samples. The preferred and most commonly collected specimen is a nasopharyngeal (NP) swab placed in viral transport media (VTM). As testing demand has increased, specimen collection and transportation supplies, including VTM, are decreasing nationwide. Due to these shortages of collection supplies and transport media, we assessed the feasibility of placing NP swabs in sterile 0.9% saline (Baxter, Deerfield, IL), sterile phosphate-buffered saline without calcium and magnesium (PBS), or minimum essential medium (MEM) (Corning, Corning, NY) prior to testing for SARS-CoV-2 by a commercially available (emergency use authorized [EUA]) FDA platform (cobas SARS-CoV-2; Roche Diagnostics, Indianapolis, IN) and a SARS-CoV-2 laboratory-developed test (LDT) that has been validated and submitted to the Food and Drug Administration for EUA approval. The Roche cobas SARS-CoV-2 test is performed on the cobas 6800 platform (Roche) per the manufacturer's protocol. The SARS-CoV-2 LDT is performed as described in the supplemental material, targeting the nucleocapsid (NUC) and open reading frame (ORF) regions of the virus.

For this study, samples were prepared by placing analyte-negative NP swabs (patient swabs previously tested by the LDT) into twelve 15-ml conical tubes (Corning) containing 3 ml of either M4-RT VTM (Remel Inc., San Diego, CA), MEM, saline, or PBS for a total of 48 samples. Subsequently, each sample was spiked with SARS-CoV-2-positive patient material at a concentration of 2,500 copies/ml. Two 15-ml conical tubes containing 3 ml of each medium (i.e., 8 total samples) functioned as negative controls. On day 0 (i.e., the day the samples were prepared), six contrived samples in each of the four types of media listed above (i.e., 24 samples), as well as negative controls, were tested by the Roche cobas and LDT SARS-CoV-2 methods (Table 1). Following initial testing, half of the contrived samples were stored refrigerated (2° to 8°C), while the remaining aliquots were stored frozen (−15° to −25°C). The aliquots were pulled from storage on days 1, 3, and 7 and tested by both methods. Equivalence (i.e., qualitative results as well as ±2 cycle threshold [C_T] values) and stability (±2 C_T values over 7 days) of the alternative transport media were compared to those of VTM.

The SARS-CoV-2 results of both assays showed equivalence (i.e., 100% qualitative agreement and C_T variation of < 2 cycles) when swabs were stored in MEM, PBS, saline, and VTM over 7 days under both refrigerated and frozen storage conditions (Table 1). No evidence of loss in sensitivity or stability (>2 C_T value increase) was observed for any of the transport media. One sample stored in PBS at 2°C to 8°C and tested by the LDT

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TABLE 1 Cycle threshold values for LDT and cobas SARS-CoV-2 assays for nasopharyngeal samples stored in four media[a]

Medium	Storage temp (°C)	LDT C_T value								cobas SARS-CoV-2 C_T value							
		Day 0		Day 1		Day 3		Day 7		Day 0		Day 1		Day 3		Day 7	
		NUC	*ORF*	*NUC*	*ORF*	*NUC*	*ORF*	*NUC*	*ORF*	*ORF1a*	*E*	*ORF1a*	*E*	*ORF1a*	*E*	*ORF1a*	*E*
M4	2–8	29.3	28.3	28.8	28.1	28.6	28.0	28.8	28.1	26.24	27.00	26.43	27.20	26.65	27.29	26.85	27.50
		29.6	28.6	29.0	28.2	28.9	28.2	29.6	28.7	25.92	26.84	26.37	27.31	26.66	27.47	27.01	27.80
		29.3	28.3	28.6	27.8	28.2	27.2	29.0	28.2	25.99	26.81	26.23	26.9	26.54	27.16	26.83	27.47
	−20	29.2	28.2	29.0	28.1	28.9	28.2	29.0	28.4	26.92	27.62	26.65	27.37	26.79	27.43	26.99	27.93
		28.8	27.5	29.2	28.4	27.7	27.7	29.2	28.3	26.16	27.05	26.58	27.24	26.87	27.45	26.78	27.44
		30.2	28.8	30.7	29.0	29.2	29.2	29.8	28.9	25.72	26.58	26.70	27.43	26.71	27.19	26.96	27.65
MEM	2–8	29.3	28.5	29.0	28.5	28.8	28.4	29.3	28.9	26.55	27.50	26.81	27.73	26.92	27.78	26.92	28.01
		28.9	28.1	28.6	28.2	28.4	28.1	28.6	27.9	26.00	26.83	26.89	27.76	27.22	28.02	27.27	28.04
		28.8	28.2	28.6	28.4	28.4	28.2	28.4	27.9	26.44	27.33	26.93	27.88	26.70	27.58	27.25	28.13
	−20	30.1	28.7	29.8	28.6	29.6	28.6	29.7	28.7	26.49	27.33	26.70	27.76	27.29	28.15	27.53	28.31
		29.7	28.0	29.2	28.4	27.8	26.5	29.4	28.6	26.54	27.59	26.99	27.99	27.35	28.21	27.58	28.50
		28.6	27.7	28.5	28.2	28.6	28.6	29.6	29.0	26.79	27.63	27.09	27.99	27.34	28.10	27.53	28.47
PBS	2–8	30.1	28.7	29.0	27.8	29.7	28.7	29.7	28.7	26.77	27.28	26.73	27.62	26.88	27.95	27.17	28.14
		28.0	26.9	26.2	25.5	26.8	26.0	26.8	25.9	26.42	27.24	26.79	27.53	26.80	27.57	27.22	27.99
		29.1	28.0	28.6	28.1	28.8	28.0	29.4	28.6	26.28	27.01	26.83	27.70	26.84	27.56	26.88	27.68
	−20	29.7	28.7	28.7	28.0	29.1	28.2	30.1	29.3	26.15	26.88	26.65	27.49	26.95	27.73	26.86	27.93
		29.6	28.2	29.6	28.6	29.5	28.3	29.6	28.7	26.41	27.36	26.34	27.26	26.33	27.37	26.75	27.69
		29.8	28.5	29.0	28.0	29.3	28.8	29.8	29.1	26.52	27.40	26.85	27.80	26.60	27.46	27.06	27.99
Saline	2–8	29.8	28.9	29.3	28.6	29.0	28.2	29.1	28.3	26.77	27.65	26.98	27.91	26.92	27.80	27.31	28.30
		30.0	28.9	29.5	28.7	28.6	27.5	29.8	29.2	26.48	27.47	27.06	28.00	27.14	28.10	27.41	28.41
		29.7	28.7	29.3	28.9	29.7	28.9	29.9	29.1	25.99	27.07	27.06	28.03	27.28	28.30	27.42	28.47
	−20	30.2	28.9	29.8	28.8	29.7	28.7	29.6	28.9	26.64	27.62	27.02	27.93	27.21	28.10	27.29	28.17
		29.0	27.9	29.2	28.6	30.1	29.1	29.5	28.8	26.26	27.21	26.88	27.86	27.28	28.29	27.14	28.07
		30.1	28.9	29.6	28.7	30.8	29.1	29.8	29.0	26.33	27.33	26.76	27.88	26.80	27.92	27.23	28.21

[a]The nasopharyngeal samples were stored in M4-RT VTM, MEM, PBS, or saline. Twelve samples were created for each medium, allowing for testing of 3 unique samples per assay at both storage conditions. Abbreviations: M4, M4-RT VTM; LDT, laboratory-developed test; NUC, nucleocapsid target; ORF, open reading frame target; E, envelope target; C_T, cycle threshold; MEM, minimum essential medium; PBS, phosphate-buffered saline without calcium and magnesium.

showed lower (i.e., more sensitive) C_T values on days 1, 3, and 7. This may indicate slight variation in preparing the contrived samples. Internal control results for all samples were within established quality control (QC) ranges and showed no evidence of loss in sensitivity or stability (data not shown). Negative controls were tested on day 0 and produced expected results, demonstrating that the media were free of SARS-CoV-2 contamination (data not shown). Positive and negative extraction/amplification controls run with each plate produced expected results (data not shown). These data support the use of MEM, PBS, or 0.9% saline as alternatives to VTM for SARS-CoV-2 testing.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.4 MB.