

1 Evaluation of saline, phosphate buffered saline and minimum essential medium as potential
2 alternatives to viral transport media for SARS-CoV-2 testing
3 Kyle G. Rodino, Mark J. Espy, Seanne P. Buckwalter, Robert C. Walchak, Jeffery J. Germer,
4 Emily Fernholz, Aimee Boerger, Audrey N. Schuetz, Joseph D. Yao and Matthew J. Binnicker
5 Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology,
6 Mayo Clinic, Rochester, MN 55905
7
8 Corresponding Author: Matthew J. Binnicker, Binnicker.matthew@mayo.edu
9 Phone: (507) 538-1640
10 Address: Mayo Clinic, 200 First Street SW, Hilton 4-54, Rochester, MN 55905
11

12 Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the causative agent of
13 coronavirus disease-19 (COVID-19), has caused a global pandemic since being discovered in
14 late 2019. In response, clinical microbiology and public health laboratories have worked to
15 develop, validate, and implement molecular assays to detect SARS-CoV-2 from respiratory
16 samples. The preferred and most commonly collected specimen is a nasopharyngeal (NP) swab
17 placed in viral transport media (VTM). As testing demand has increased, specimen collection
18 and transportation supplies, including VTM, are decreasing nationwide. Due to these shortages
19 of collection supplies and transport media, we assessed the feasibility of placing NP swabs in
20 sterile 0.9% saline (Baxter; Deerfield, IL), sterile phosphate buffered saline (PBS), or minimum
21 essential media (MEM) (Corning; Corning, NY) prior to testing for SARS-CoV-2 by a
22 commercially-available (emergency use authorized [EUA]) FDA platform (cobas® SARS-CoV-
23 2; Roche Diagnostics, Indianapolis, IN) and a SARS-CoV-2 laboratory-developed test (LDT)
24 that has been validated and submitted to the Food and Drug Administration for EUA approval.
25 The Roche cobas SARS-CoV-2 test is performed on the cobas® 6800 platform (Roche) per the
26 manufacturer's protocol. The SARS-CoV-2 LDT is performed as described in the supplemental
27 materials, targeting the nucleocapsid (NUC) and open reading frame (ORF) genes of the virus.

28 For this study, samples were prepared by placing analyte-negative NP swabs into twelve 15-mL
29 conical tubes (Corning) containing 3-mL of either M4-RT VTM (Remel Inc; San Diego, CA),
30 MEM, saline, or PBS for a total of 48 samples. Subsequently, each sample was spiked with
31 SARS-CoV-2 positive patient material at a concentration of 10,000 copies/mL, which is 2-fold
32 higher than the defined limit of detection of the LDT. Two 15-mL conical tubes containing 3-mL
33 of each media (i.e., 8 total samples) functioned as negative controls. On day 0 (i.e., the day the
34 samples were prepared), 6 contrived samples in each of the four media types listed above (i.e., 24

35 samples), as well as negative controls, were tested by the Roche cobas and LDT SARS-CoV-2
36 methods (Table 1). Following initial testing, half of the contrived samples were stored
37 refrigerated (2-8°C), while the remaining aliquots were stored frozen (-15 to -25°C). The aliquots
38 were pulled from storage on days 1, 3, and 7 and tested by both methods. Equivalence (i.e.,
39 qualitative results as well as +/- 2 cycle threshold [C_t] values) and stability (+/- 2 C_t values over 7
40 days) of the alternative transport media were compared to VTM.

41 The SARS-CoV-2 results of both assays showed equivalence (i.e., 100% qualitative agreement
42 and C_t variation < 2 cycles) when swabs were stored in MEM, PBS, saline and VTM over 7 days
43 at both refrigerated and frozen storage conditions (Table 1). No evidence of loss in sensitivity or
44 stability (>2 C_t value increase) was observed for any of the transport media. One sample (PBS-2)
45 showed lower (i.e., more sensitive) C_t values on days 1, 3, and 7. This may indicate slight
46 variation in preparing the contrived samples. Internal control results for all samples were within
47 established QC ranges (data not shown). Negative controls were tested on Day 0 and produced
48 expected results, demonstrating that the media were free of SARS-CoV-2 contamination.
49 Positive and negative extraction/amplification controls run with each plate produced expected
50 results (data not shown). These data support the use of MEM, PBS, or 0.9% saline as alternatives
51 to VTM for SARS-CoV-2 testing.

52

Table 1. SARS-CoV-2 results for nasopharyngeal samples stored in M4-RT, MEM, PBS or saline

Storage Temp (°C)	Media (N)	LDT C _t values								Cobas SARS-CoV-2 C _t values							
		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
2-8	M4 (3)	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	27.24	21.01	27.80
		29.3	28.3	28.6	27.8	28.2	27.2	29.0	28.2	25.99	26.81	26.23	26.90	26.54	27.16	26.83	27.47
-20	M4 (3)	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	28.6	28.6	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	30.0	30.0	29.8	28.9	25.72	26.58	26.70	27.43	26.71	27.19	26.96	27.65
2-8	MEM (3)	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	MEM (3)	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
2-8	PBS (3)	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	PBS (3)	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
2-8	Saline (3)	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	Saline (3)	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

1 N, number of samples tested; LDT, laboratory-developed test; NUC, nucleocapsid target; ORF, open reading frame target; E, envelope

2 target; Ct, cycle threshold; MEM, minimum essential medium; PBS, phosphate buffered saline