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1 # Assessment of Specimen Pooling to Conserve SARS CoV-2 Testing Resources

2 Baha Abdalhamid MD, PhD[a,b,#], Christopher R. Bilder PhD[c], Emily L. McCutchen MS[a,b],
3 Steven H. Hinrichs MD[a], Scott A. Koepsell MD[a], Peter C. Iwen PhD[a,b]
4
5 [a] Department of Pathology and Microbiology, University of Nebraska Medical Center,
6 Omaha, NE
7 [b] Nebraska Public Health Laboratory, University of Nebraska Medical Center, Omaha, NE
8 [c] Department of Statistics, University of Nebraska-Lincoln, Lincoln, NE

9 Running Head: Application of Specimen Pooling to Expand SARS CoV-2 Testing
10 Key Points: SARS CoV-2, COVID-19, Group testing

11 **[#]Corresponding Author**
12 Baha Abdalhamid, MD, PhD, D (ABMM)
13 DRC II 7035
14 Department of Pathology and Microbiology
15 University of Nebraska Medical Center
16 985900 Nebraska Medical Center
17 Omaha, NE 68198-5900
18 Phone: 402 552 9699
19 Fax: 402 559 5900
20 Email: babdalhamid@unmc.edu
21

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22 **Abstract**

23 **Objectives**

24 To establish the optimal parameters for group testing of pooled specimens for the detection of
25 SARS-CoV-2.

26 **Methods**

27 The most efficient pool size was determined to be 5 specimens using a web-based application.
28 From this analysis, 25 experimental pools were created using 50 microliter from one SARS-CoV-
29 2 positive nasopharyngeal specimen mixed with 4 negative patient specimens (50 microliter each)
30 for a total volume of 250 microliter l. Viral RNA was subsequently extracted from each pool and
31 tested using the CDC SARS-CoV-2 RT-PCR assay. Positive pools were consequently split into
32 individual specimens and tested by extraction and PCR. This method was also tested on an
33 unselected group of 60 nasopharyngeal specimens grouped into 12-pools.

34 **Results**

35 All 25 pools were positive with Cycle threshold (Ct) values within 0 and 5.03 Ct of the original
36 individual specimens. The analysis of 60 specimens determined that two pools were positive
37 followed by identification of two individual specimens among the 60 tested. This testing was
38 accomplished while using 22 extractions/PCR tests, a savings of 38 reactions.

39 **Conclusions**

40 When the incidence rate of SARS-CoV-2 infection is 10% or less, group testing will result in the
41 saving of reagents and personnel time with an overall increase in testing capability of at least 69%.

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43 Since the first detection in Wuhan, China in December 2019, severe acute respiratory
44 syndrome coronavirus 2 (SARS-CoV-2), the pathogen of coronavirus diseases 2019 (COVID-19),
45 has spread worldwide to now be considered a pandemic [1,2]. The United States (US) is experiencing
46 an acute shortage of certain reagents important for performance of assays for the detection of
47 SARS-CoV-2. Some areas of the US have stopped testing due to lack of test supplies. The ability
48 to rapidly diagnosis COVID-19 is important for evaluating the spread of disease and for tracing
49 the contacts of infected individuals.

50 The assay developed by the Centers for Disease Control and Prevention (CDC) for
51 detection of SARS-CoV-2 and approved for use under emergency use authorization (EUA) by the
52 FDA has been widely employed by public health laboratories throughout the US[3]. This assay
53 employs an extraction procedure of viral RNA from specimens collected by nasopharyngeal (NP)
54 swabs. The second step in the assay employs reverse transcription and amplification using a real
55 time PCR instrumentation. The assay therefore requires two kits, one for extraction and another
56 for amplification of the target and detection. We investigated whether a strategy used in the testing
57 of blood prior to transfusion could have application for conservation of scarce reagents for the
58 SARS CoV-2 assay [4,5]. The process of group testing that employs sample pooling is used for
59 detection of the human immunodeficiency virus, and hepatitis B and C viruses[4] in blood products.
60 Key principles for successful application of group testing involve knowledge of the limit-of-
61 detection, sensitivity and specificity of the assay, and the prevalence of disease in the population.
62 The goal of the process is to determine a pool size that provides the greatest conservation of
63 resources while maintaining the reliable performance of testing. This report describes a proof-of-
64 concept for group testing of pooled specimens for the diagnosis of COVID-19.

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65 To assess the group testing strategy, the first step was to calculate the most efficient pool
66 size using a web-based application for pooling as described at https://www.chrisbilder.com/shiny.
67 Although the prevalence of COVID-19 in Nebraska has not been specifically defined by
68 comprehensive epidemiology studies, the observed specimen positive rate within the tested
69 community has been around 5% for the past two weeks. The following parameters and
70 assumptions used in this calculation included an experimental prevalence rate of 5%, an assay
71 lower limit-of-detection of 1 to 3 RNA copies/µl, an assay sensitivity of 95% or 100%, an assay
72 specificity of 100%, a two-stage pooling algorithm, and a range of pool sizes of 3 to 10 samples[6].
73 These calculations predicted a pool size of 5 samples would provide the largest reduction in the
74 expected number of tests of 57% when compared to testing specimen separately (Figure 1).

75 The CDC RT-PCR assay was used in this study to detect SARS-CoV-2 in nasopharyngeal
76 specimens. With this assay, a positive COVID-19 result is determined when both nucleocapsid
77 targets (N1 andN2) reach a defined cycle threshold (Ct) of <40. For 158 confirmed positive
78 specimens that have been seen in the public health laboratory to date, the Ct values for N1 have
79 averaged 26.06 with a SD of 5.5 (range 15.75 to 37.96) and Ct values for N2 have averaged 26.48
80 with a SD of 5.8 (range 15.75 to 38.65). Twenty-five pools of five specimens with each containing
81 one positive patient were group tested for this study. Of these, the COVID-19 positive specimens
82 were within a range of Ct values from 18.23 to 36.74 for N1 and from 17.33 to 37.43 for N2.
83 Included in this evaluation 14 specimens were selected with low RNA concentration (Ct > 30)
84 (Table 1). Note that a low Ct values indicated the presence of higher amounts of viral RNA and
85 high Ct values indicated lower amounts.

86 Pools were created using 50 µl from a confirmed NP positive patient specimen added to 50
87 µl from each of 4 negative NP patient samples for a final volume of 250 µl. Nucleic acid (NA)

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88 extraction was performed on each pool using either the QIAGEN EZ1 Virus Mini Kit v2.0
89 (QIAGEN, Germantown, MD) or the QIAGEN manual extraction kit according to manufacturer's
90 instructions. Real-time RT-PCR was performed on the extracted NA using the CDC Diagnostic
91 Panel following the manufacturer's instructions. The results showed that all 25-pooled specimens
92 were positive within a range of 0 Ct to 5.03 Ct difference from the original samples (Table 1). To
93 examine this approach in a clinical situation, 60 specimens from individuals at risk for COVID-19
94 as determined by the public health department were separated randomly into 12 pools, which were
95 processed as described. Two of the pools were characterized as "2019-nCoV detected" by the
96 assay. All individual specimens within each of the two identified pools were re-tested with two
97 positive samples identified for an overall positive rate of 3.3%. The total reactions used were 22
98 for an overall conservation of 38 extraction kits and 38 amplification reagents.

99 Group testing of pooled samples has been successfully employed by the blood procurement
100 and infectious disease testing for many years[5]. The strategy became effective due to the
101 development of highly sensitive molecular based assays and several studies reported on statistical
102 measures to determine appropriate parameters for use[6]. This study examined whether pooling was
103 feasible using an EUA SARS CoV-2 assay in a public health setting where the desire to test large
104 numbers of individuals has been impacted by the scarcity of key resources. The predictive
105 algorithm indicated a pooling ratio of 1 to 5 was expected to retain accuracy of the test and result
106 in greater efficiency of test resources. Results of this study indicated that all positive samples by
107 the non-pooled method were detected in pools with four other negative samples.

108 The practical application of this process was confirmed with 60 samples from the
109 community resulting in the saving of reagents and personnel time that could expand testing to an
110 additional 38 samples. Assuming a consistent positivity rate, this strategy would expand testing

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111 by 133%. Table 2 summarizes the impact of different positive test rates on the overall efficiency
112 of test resources.

113 During a rapidly changing epidemic, testing strategies will need to adapt to potential
114 increases in the positive test rate. Group testing of pooled specimens also requires the use of highly
115 sensitive assays to avoid missing low positive samples. Therefore, strategies must be employed to
116 closely monitor the use of pooling as the positive rate of test specimens increases in an outbreak
117 of disease. Additionally, the impact of different extraction methods on the recovery of RNA and
118 overall test sensitivity need to be evaluated. Therefore, laboratories must perform their own
119 validation pool studies for kits used for each RNA extraction and amplification based on the
120 prevalence rate of COVID-19 in their own region. Finally, this study showed that pooling is an
121 effective approach to expand the impact of limited test resources and reagents during specific
122 stages of an infectious disease outbreak.

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128 University of Nebraska Medical Center Institutional Review Board, this study was deemed exempt
129 because it was conducted as a part of a diagnostic testing study.
130 The FDA reviewed the procedure as part of a EUA approved diagnostic procedure. All authors
131 declare no conflict of interest.

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157 **Figure 1.** Optimal sample pool size

158 Graphical comparison of initial pool size compared to expected number of tests per
159 individual using the Shiny application for pooled testing available at
160 https://www.chrisbilder.com/shiny. The optimal sample pool size was determined based
161 on the least number of tests and the following parameters: prevalence rate (5%), a lower
162 limit of detection of 1 to 3 RNA copies/µl, an assay sensitivity of either 95% or 100%, and
163 an assay specificity of 100%.

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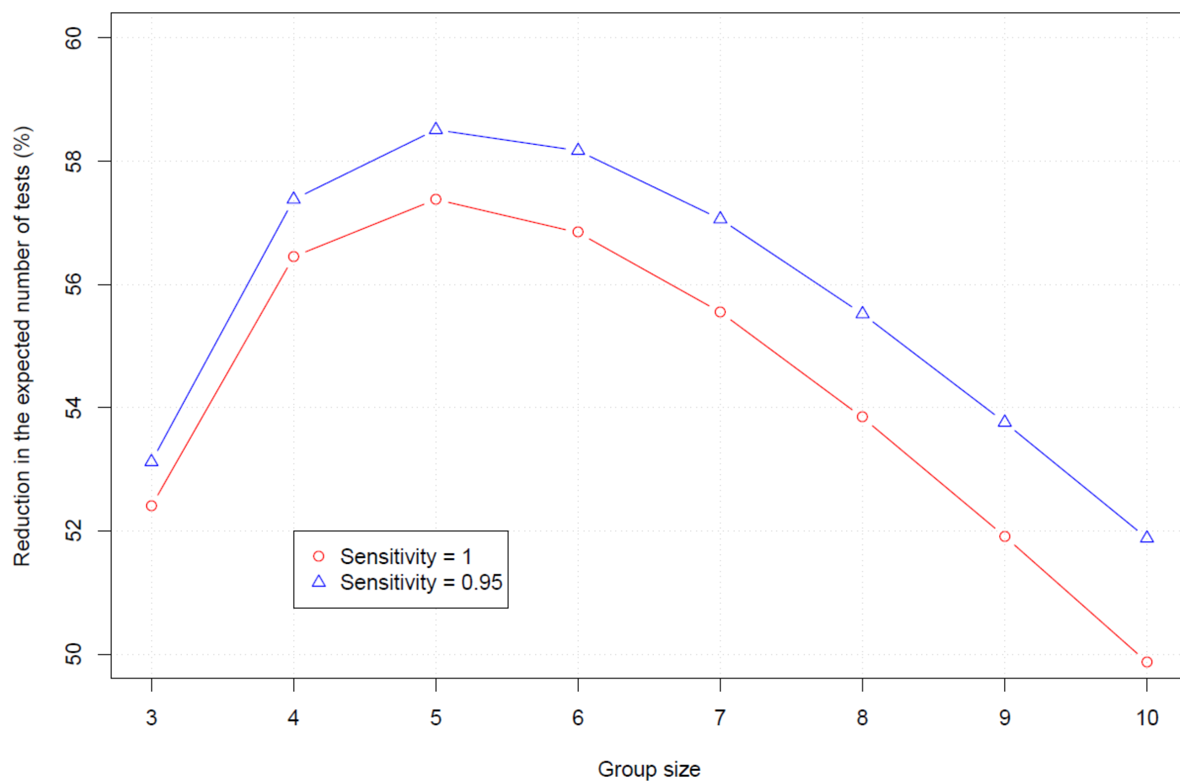

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167 **Table 1.** Comparison of threshold cycles between the original and pooled COVID-19 positive samples [a].

Specimen #	Specimen Code	N1[b](Ct)			N2[b](Ct)		
		Pooled	Original	Ct Difference	Pooled	Original	Ct Difference
1	NE-254	35.49	32.18	3.31	35.13	33.22	1.91
2	NE-284	35.27	33.33	1.94	36.5	34.23	2.27
3	NE-287	33.9	30.25	3.65	33.92	31.69	2.23
4	NE-327	33.24	30.44	2.8	32.56	30.52	2.04
5	NE-379	29.23	24.2	5.03	28.52	24.08	4.44
6	NE-393	35.5	34	1.5	36.72	35.33	1.39
7	NE-479	20.57	18.23	2.34	19.18	17.33	1.85
8	NE-464	23.93	21.2	2.73	23.07	20.95	2.12
9	NE-616	33.8	31.17	2.63	33.61	31.27	2.34
10	NE-784	33.84	32.79	1.05	34.17	32.34	1.83
11	NE-796	24.63	23.07	1.56	25.04	23.62	1.42
12	NE-822	31.57	29.71	1.86	33.29	30.06	3.23
13	NE-863	33.4	29.69	3.71	32.1	30.64	1.46
14	NE-875	23.12	21.08	2.04	23.79	21.32	2.47
15	NE-886	22.65	19.34	3.31	22.01	20.33	1.68
16	NE-892	24.65	21.4	3.25	24.68	22.83	1.85
17	NE-901	32.48	30.19	2.29	32.92	32.92	0
18	NE-907	27.7	25.01	2.69	27.91	26.34	1.57
19	NE-912	27.91	24.55	3.36	28.9	25.06	3.84
20	NE-914	33.71	30.66	3.05	33.72	31.66	2.06
21	NE-1319	36.13	32.31	3.82	36.81	33.4	3.41
22	NE-1437	36.04	34.72	1.32	37.57	33.12	4.45
23	NE-1421	37.97	35.46	2.51	39.10	36.20	2.9
24	NE-1631	39.86	36.74	3.12	39.97	37.09	2.88
25	NE-1683	35.52	33.63	1.89	37.78	37.43	0.35

168 Abbreviations: Ct, cycle threshold

169
170 [a]: The extraction platforms included both automated and manual procedures.
171
172 [b]: The N1 and N2 targets were used to detect SARS-CoV-2 during the PCR assay.
173

174 Table 2. Comparison of optimal pool size and prevalence rates on test efficiency [a].

Prevalence rate (%)	Optimal specimen pool size	Reduction in the expected number of tests (%)	Expected increase in testing efficiency (%)
1	11	80	400
3	6	67	200
5	5	57	133
7	4	50	100
10	4	41	69
15	3	28	39

175 a. The Shiny application for pooled testing available at https://www.chrisbilder.com/shiny was used for calculations.

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