toward evaluating malaria transmission reduction strategies.

Note

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Aaron M. Samuels,[1] Nobert Awino Odero,[2] Wycliffe Odongo,[2] Kephas Otieno,[2] Vincent Were,[2] Ya Ping Shi,[1] Tony Sang,[2] John Williamson,[1] Ryan Wiegand,[1] Mary J. Hamel,[1] S. Patrick Kachur,[1] Laurence Slutsker,[1] Kim A. Lindblade,[1] Simon K. Kariuki,[2] and Meghna R. Desai[1]

[1]Malaria Branch, Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, Georgia, USA, and [2]Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya

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Correspondence: A. M. Samuels, Centers for Disease Control and Prevention, PO Box 1578, Kisumu, Kenya 40100 (amsamuels@cdc.gov).

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Pool Size Selection When Testing for Severe Acute Respiratory Syndrome Coronavirus 2

To the Editor—Pooling samples has been proposed by multiple authors as an efficient way to test for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1–4]. In particular, Yelin et al [1] showed that SARS-CoV-2 can be detected in pools with up to 32 samples and potentially in pools of 64 samples. They concluded that "this pooling method can be applied immediately in current clinical testing laboratories." However, this research [1] and similar research of others [2, 3] missed answering a very important question: How does one choose the most efficient pool size relative to SARS-CoV-2 prevalence in samples? Without answering this question, laboratories cannot fully benefit from pooling. Here, we provide the answer so that laboratories can increase their testing capacity to its fullest potential.

The efficiencies from pooling samples occur when pools test negative. In general, the probability of a negative pool (θ) is given by $\theta = (1-p)^s$ for a prevalence (p) and pool size (s) [5]. For example, the most efficient pool size is 4 samples when prevalence is 10% (calculation discussed below). This will lead to 66% of the pools testing negative on average, resulting in 3 tests saved for each negative pool. On the other hand, choosing a pool size that is too large can be very inefficient. By changing the size to 32 samples in our example, only 3% of the pools will test negative. We subsequently show that there are no benefits from using this pool size with this prevalence. Similar inefficiencies occur as well when selecting pool sizes that are too small.

Yelin et al [1] identified a range of pool sizes that appear to not compromise testing sensitivity. From this range, one needs to determine the optimal pool size to perform testing most efficiently. Statistical research has shown, in general, that this is the pool size that minimizes the average number of tests on a per capita basis (A) when testing a continuous series of samples, where A is a mathematical function of prevalence [5–7]. Separate testing of each sample corresponds to $A = 1$, and pooling is more efficient when $A < 1$. Expressions for A are available [5–7], and the optimal pool size can be approximated by the next integer larger than $1/\sqrt{p}$ [8] or found exactly [9, 10].

Table 1 provides A for prevalences between 0.001 and 0.20. For example, a prevalence of 2% results in an optimal pool size of 8 and $A = 0.27$. This corresponds to a 73% average reduction in tests from pooling. Equivalently, this can mean a 264% increase in testing capacity when compared with testing samples separately. Table 1 also includes A for the same pool sizes as investigated by Yelin et al [1]. These additional results illustrate the importance of choosing pool size relative to prevalence. For example, while SARS-CoV-2 can be detected in pools of size 32, this size is optimal only for the smallest prevalence. In fact, $A > 1$ for prevalences larger than 0.10, indicating that pooling results in more tests on average than separate testing.

Notes

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Christopher R. Bilder,[1] Peter C. Iwen,[2,3] and Baha Abdalhamid[2,3]

[1]Department of Statistics, University of Nebraska–Lincoln, Lincoln, Nebraska, USA, [2]Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, Nebraska, USA, and [3]Nebraska Public Health Laboratory, University of Nebraska Medical Center, Omaha, Nebraska, USA

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Table 1. Average Number of Tests Per Capita (A) Relative to Prevalence

	Optimal		*A* for Specified Pool Size					
Prevalence (%)	Pool Size	*A*	2	4	8	16	32	64
0.1	32	0.06	0.50	0.25	0.13	0.08	0.06	0.08
0.5	15	0.14	0.51	0.27	0.16	0.14	0.18	0.29
1	11	0.20	0.52	0.29	0.20	0.21	0.31	0.49
2	8	0.27	0.54	0.33	0.27	0.34	0.51	0.74
3	6	0.33	0.56	0.36	0.34	0.45	0.65	0.87
4	6	0.38	0.58	0.40	0.40	0.54	0.76	0.94
5	5	0.43	0.60	0.44	0.46	0.62	0.84	0.98
6	5	0.47	0.62	0.47	0.52	0.69	0.89	1.00
7	4	0.50	0.64	0.50	0.57	0.75	0.93	1.01
8	4	0.53	0.65	0.53	0.61	0.80	0.96	1.01
9	4	0.56	0.67	0.56	0.65	0.84	0.98	1.01
10	4	0.59	0.69	0.59	0.69	0.88	1.00	1.01
11	4	0.62	0.71	0.62	0.73	0.91	1.01	1.02
12	4	0.65	0.73	0.65	0.77	0.93	1.01	1.02
13	3	0.67	0.74	0.68	0.80	0.95	1.02	1.02
14	3	0.70	0.76	0.70	0.83	0.97	1.02	1.02
15	3	0.72	0.78	0.73	0.85	0.99	1.03	1.02
16	3	0.74	0.79	0.75	0.88	1.00	1.03	1.02
17	3	0.76	0.81	0.78	0.90	1.01	1.03	1.02
18	3	0.78	0.83	0.80	0.92	1.02	1.03	1.02
19	3	0.80	0.84	0.82	0.94	1.03	1.03	1.02
20	3	0.82	0.86	0.84	0.96	1.03	1.03	1.02

Calculations are performed using the binGroup2 package [10] of the R statistical software environment. Abbreviation: A, average number of tests per capita.

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Correspondence: C. R. Bilder, 340 Hardin Hall North Wing, Lincoln, NE 68583-0963 (bilder@unl.edu).

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COVID-19 and the Renin-Angiotensin-Aldosterone System

To the Editor—I further Hanff and colleagues' [1] timely call for epidemiological and clinical investigations of coronavirus disease 2019 (COVID-19), including measurements of the renin-angiotensin-aldosterone system (RAAS) components, as substudies would be insightful of this pandemic. Angiotensin-converting enzyme 2 (ACE2) participates in the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) cell entry. This infection downregulates ACE2. Drugs that block RAAS also affect ACE2 expression; it is downregulated by renin inhibition and upregulated by ACE inhibitors, angiotensin receptor blockers [1], and mineralocorticoid receptor antagonists [2]. Other likely regulatory factors are age, type 2 diabetes, and sex difference [3]. These interactions would directly affect the balance between the beneficial and deleterious angiotensins (Angs), such as Ang (1–7) and Ang (1–9) vs excess Ang II. Such perturbations would also indirectly influence other RAAS components, and the coordination between circulating and local tissue expressions, as shown in Figure 1.

ACE2 is distributed throughout the body and is abundantly expressed in the lung, small intestine, and in blood vessels of many organs including the brain, heart, kidney, and testis [4]. These organs and blood vessels are potential sites of infection. The downregulation of ACE2 would reduce the production of Ang (1–7) and Ang (1–9), and concurrently prevent the reduction of Ang II, tilting