Increasing testing throughput and case detection with a pooled-sample Bayesian approach in the context of COVID-19

Rodrigo Noriega[1]*, Matthew H. Samore[2,3].

[1]Department of Chemistry, University of Utah, Salt Lake City, UT USA.

[2]Division of Epidemiology, University of Utah, Salt Lake City, UT USA.

[3]Division of Epidemiology, Veterans Affairs Salt Lake City Health Care System, Salt Lake City, Utah

*Correspondence to: noriega@chem.utah.edu

Abstract: Rapid and widespread implementation of infectious disease surveillance is a critical component in the response to novel health threats. Molecular assays are the preferred method to detect a broad range of pathogens with high sensitivity and specificity. The implementation of molecular assay testing in a rapidly evolving public health emergency can be hindered by resource availability or technical constraints. In the context of the COVID-19 pandemic, the applicability of a pooled-sample testing protocol to screen large populations more rapidly and with limited resources is discussed. A Bayesian inference analysis in which hierarchical testing stages can have different sensitivities is implemented and benchmarked against early COVID-19 testing data. Optimal pool size and increases in throughput and case detection are calculated as a function of disease prevalence. Even for moderate losses in test sensitivity upon pooling, substantial increases in testing throughput and detection efficiency are predicted, suggesting that sample pooling is a viable avenue to circumvent current testing bottlenecks for COVID-19.

22   Emerging infectious diseases pose a global hazard to public health, as exemplified by the COVID-

23   19 pandemic. Key epidemiologic strategies for control of community spread include contact tracing,

24   case isolation, ring containment, and social distancing (*1–7*). The use of microbiological testing to

25   identify disease cases is a crucially important element of these strategies. Some countries, including

26   the US, experienced a shortage of kits needed for COVID-19 diagnosis, which resulted in the

27   imposition of restrictive criteria to manage the selection of patients for testing. Constraints in the

28   supply of kits had a particularly significant impact on testing of mildly symptomatic individuals, as

29   well as asymptomatic contacts of confirmed cases. For some facilities that have been overwhelmed

30   by demand for testing as the pandemic progressed, test throughput continues to be a limiting factor

31   (*8–10*). Strategies for screening more individuals with a reduced burden on resources are highly

32   desirable. Using a Bayesian formalism, a hierarchical testing protocol based on sample pooling is

33   discussed. Anticipated benefits include easing the demand of constrained resources and enabling

34   more efficient detection of a larger number of cases.

35   Molecular assays are the predominant testing method for viral and bacterial pathogens (*11–14*).

36   Specifically, nucleic acid detection assays typically employ real-time polymerase chain reaction

37   (RT-PCR) for DNA targets and reverse-transcription real-time PCR (rRT-PCT) for RNA targets

38   (*15, 16*). The popularity of such testing platforms is due to **1)** their high sensitivity and specificity,

39   **2)** the widespread access to sequencing and synthesis technologies for the identification of nucleic

40   acid target sequences and probes, and **3)** the development of fast, user-friendly, and cost-effective

41   equipment. While nucleic acid assays have powered a revolution in diagnostics and delivery of care

42   for individual patients, their application in large-scale infectious disease surveillance is hampered

43   partly by low throughput at a population level.

44   The information content of a diagnostic test can be evaluated with a Bayesian probability formalism

45   in the context of an individual sample or for repeated sampling from the same patient (*17–19*) by

46   taking into account the probability of detecting a positive case (assay sensitivity, or identification

47   rate P_{id}) and the probability of a positive result from healthy samples (false positive rate P_{fp}).

48   Bayesian inference requires the assessment of a "prior" probability to the presence of disease in a

49   sample, $P(D)$, which is updated to a "posterior" probability given a positive or negative test result

50   with conditional probabilities $P(D|+)$ or $P(D|-)$ (**Eq. 1.a-b**).

51    $$P(D|+) = \frac{P(D) \cdot P(+|D)}{P(+)} = \frac{P(D) \cdot P_{id}}{P(+)} \textbf{ (Eq. 1a)} \quad P(D|-) = \frac{P(D) \cdot P(-|D)}{P(-)} = \frac{P(D) \cdot (1 - P_{id})}{1 - P(+)} \textbf{ (Eq. 1b)}$$

52   where $P(+)$ and $P(-)$ are the overall probabilities of the test yielding a positive or negative result,

53   respectively. These tools can be extended to the somewhat counterintuitive situation where a

54   diagnostic test is conducted on a sample pooled from multiple individuals. The motivation for

55   sample pooling is to screen multiple patients simultaneously and reduce the burden on testing

56   facilities working with limited resources. Pooling schemes have been developed since their

57   introduction in the 1940s for syphilis testing, and have been applied to screen for and estimate

58   prevalence rates of a variety of diseases (*20-24*). Here, a simple two-step hierarchical protocol first

59   introduced by Dorfman is considered: *Samples from N_p patients are collected and randomly pooled*

60   *into groups of n individual samples each. Pooled samples are interrogated with the diagnostic test.*

61   *If pooled testing yields a negative result, no further testing is conducted. If pooled testing yields a*

62   *positive result, all patients in that pool are tested individually.*

63   While statistical approaches have been focused on characterizing the performance of various

64   pooling schemes, not all of them include non-ideal test parameters (*25*). Moreover, testing

65   characteristics at the pooled- and individual-sample levels can be different. Here, the Bayesian

66   inference approach in **Eq. 1.a-b** is modified to include differences in the assay sensitivity and

67   overall probability of a positive result in pooled vs. individual tests (**Eq. S1-2**). Sensitivity loss is

68   included as a reduction in the identification rate of pooled-sample tests by a scaling factor γ. Due to

69   the exceptional specificities of nucleic acid assays, the false positive rate is assumed to remain

70 unaffected. Importantly, the posterior probability assessed from a positive pooled test is used as a

71 prior for follow-up individual tests, which yields additional information and enhances the Bayesian

72 inference assessment of those cases (**Eq. S3**).

73 For a population of N_p patients divided into sample pools of size n, the average number of tests is

74 the number of initial pooled tests plus the expected number of follow-up tests (**Eq. S4**). Throughput

75 increase χ is expressed as the effective number of individuals screened by each diagnostic test (**Eq.**

76 **S5**). The individual- and pooled-sample test characteristics determine the pool size that optimizes

77 screening throughput as a function of average disease prevalence in the tested population.

78 The advantages of pooled-sample screening are discussed in the context of the rapidly evolving

79 COVID-19 pandemic (caused by the novel coronavirus SARS-CoV-2) (*8, 26*). A recent rRT-PCR

80 assay for COVID-19 reports an identification rate of 95% and no false positives after testing 310

81 samples including other respiratory pathogens (*27*). Given reported specificities of commercially-

82 available respiratory panel assays (>99%), an estimated 1% false positive rate was included in the

83 model results reported here. A moderate reduction in the identification rate for a pooled sample

84 ($\gamma = 0.9$) was assumed – this variable is discussed in detail below. Consistent with similar

85 implementations of Dorfman-type testing algorithms (*28*), substantial increases in testing

86 throughput are predicted for low disease prevalence rates ($P(D) \leq 8\%$, **Fig. 1**), where throughput

87 more than doubles and optimal pool sizes are $4 \leq n \leq 12$. At intermediate prevalence rates $0.1 \leq$

88 $P(D) \leq 0.2$, the increase in throughput is moderate yet substantial (>30% increase in throughput)

89 and pool sizes are small ($n = 3$). For high prevalence rates, pooling yields no improvement

90 (optimal pool size $n = 1$). Average disease prevalence can be re-assessed as information is gained

91 for the tested population to re-optimize pool size. Dynamic self-tuning is a feature of Bayesian

92 inference, a significant asset when a close feedback loop is desirable.

4

93   Besides testing throughput, it is informative to assess the increased ability to detect cases using a

94   pooled-sample vs individual-sample scheme, which can be accomplished by comparing the ratio of

95   detected to missed cases in each protocol. Importantly, resource constraints are incorporated into

96   this comparison by accounting for unscreened cases in the standard 1:1 scheme (**Eq. S6-8**). The

97   relative increase in the detection-to-miss ratio between the pooled and standard 1:1 schemes

98   exhibits even more significant gains than those observed for testing throughput (**Fig. 1**).


99
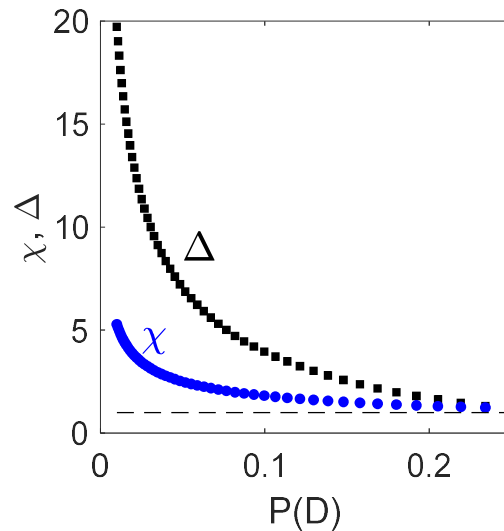
Fig. 1. Number of patients screened by each diagnostic test (χ, blue circles) and the relative increase

   in detection-to-miss ratio for a pooled scheme (Δ, black squares), as a function of average disease

prevalence. Gray line is the no-pooling scheme reference.

103   Loss in screening power associated with sample pooling is assessed by **1)** estimating the portion of

104   cases that would have been identified in an individual test but missed by pooled screening, and **2)**

105   determining the information gained for patients whose pooled screening result was negative.

106   A reduction in overall pathogen concentration due to pooling in conditions of low disease

107   prevalence can decrease the test's identification rate, although it is manageable when targeting

108   infectious diseases for which typical pathogen concentrations are non-negligible (*23*). This concern

109   is examined with a set of 186 positive rRT-PCR diagnostic test results for COVID-19 (**Fig. 2**),

110   which include nasopharyngeal swab, oropharyngeal swab, and bronchoalveolar lavage (*29*). Tests

111   have a mean cycle threshold value of $\langle C_t \rangle = 24.6$, and a positive test result is defined as a rRT-PCR

112   reaction with $C_t \leq 42$ – in agreement with early reports for nasal swab COVID-19 rRT-PCR tests

113   with $\langle C_t \rangle = 24.3$ and viral loads of 1.4×10^6 copies/mL (*30*). From these data, the portion of

114   samples that would have had a positive test result even if pooled with an entirely healthy population

115   is estimated using a pool size $n = 12$ and rRT-PCR geometric efficiencies of $\epsilon = 1.7 - 2$ per

116   cycle. A pooled screening protocol would have detected 95.7-96.8% of these cases (178-180 out of

117   186). Further support for moderate sensitivity loss can be achieved by dividing the distribution of C_t

118   values for the test-positive samples into three subpopulations. For the same pooling and rRT-PCR

119   efficiencies stated previously, >95% of the broadest, lowest-load population would be detected.

120
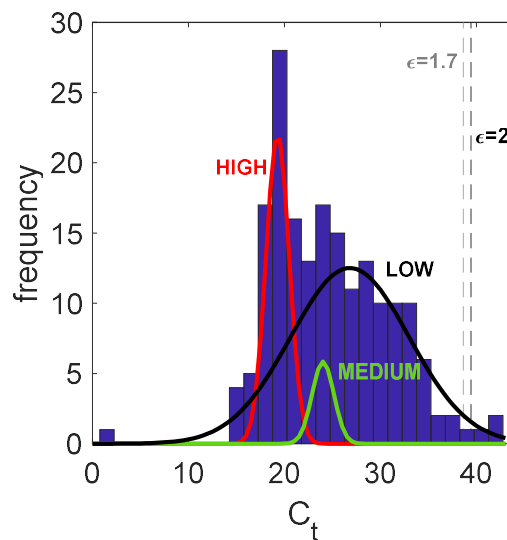
Fig. 2. Cycle threshold values for rRT-PCR reactions for confirmed COVID-19 cases (*27*),

described by subpopulations with low, medium, and high viral load. Sensitivity cutoffs shown as

vertical lines.

124   The main benefit of hierarchical pooled-sample testing protocols is the ability to screen a larger

125   portion of the population and detect more positive cases. The relative increase in case detection is

126   given by the ratio of the number of cases detected in a pooled setting and the number of cases

127   detected if the same number of tests were used on single patients – the multiplicative factor in

128   testing throughput scaled by the loss in sensitivity, $\chi \cdot \gamma$. Loss in sensitivity also leads to more

129   screened-yet-missed cases – even in the absence of pooling, diagnostic tests yield false negatives

130   and increasing those odds should not be considered lightly. In the range of disease prevalence with

131   moderate to high increase in throughput and compared to individual testing of every patient, a $\gamma =$

132   0.9 sensitivity loss leads to a relatively low increase in posterior probability of disease after a

133   negative test outcome, $P(D|-)$ (**Fig. 3**). While sensitivity losses decrease the ability to confidently

134   screen healthy individuals, the threshold for this tradeoff depends on the situation where the

135   screening protocol is deployed. Effective risk communication to screened individuals is needed to

136   prevent an outsized sense of security after a negative pooled-test result – e.g., vigilance to symptom

137   development triggers individual testing.
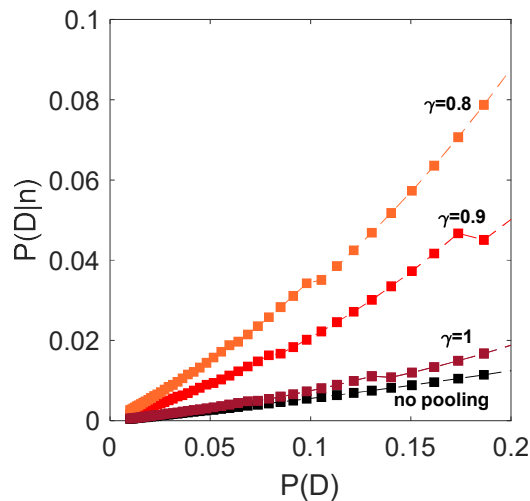
138

139   **Fig. 3.** Probability of an individual being infected even though their pooled-sample test gave a

140   negative result, as a function of background disease prevalence and for different values of

141   sensitivity loss.

142   This protocol is amenable to HIPAA regulations – in fact, pooling has been implemented by state

143   laboratories in the recent past (*31*)– and requires limited additional sample processing. Refining the

144   dependence of sensitivity loss with pool size and coupling to modeling strategies that inform

145 population sampling and prior probabilities will provide further screening improvements. From an

146 epidemiological surveillance standpoint, increased detection of positive cases in a larger portion of

147 the population denotes that a greater fraction of infectious individuals can be isolated. However,

148 pooled testing in less useful in an inpatient clinical setting where the highest sensitivity is needed to

149 minimize risk of hospital transmission from non-isolated patients. As with any change in the

150 delivery of medical care, a discussion including community stakeholders is paramount.

151 In summary, a pooled testing strategy has the potential to enhance comprehensive surveillance of

152 SARS-CoV-2 particularly when test kits are in short supply. The benefits of surveillance are

153 greatest in the early phases of community spread. Thus, improving the capacity for high-throughput

154 testing has the highest impact when prevalence is low enough that pooled sampling is most

155 beneficial. The ratio of confirmed COVID-19 cases to tests performed varies by country, but it

156 appears that aggressive testing strategies yield a low enough prevalence to benefit from pooled-

157 sample screening – e.g., South Korea's is 3% (8.3k/270k as of 3/16/2020) (*32*). While the

158 development of clinical prediction rules and non-testing screening are critical to any

159 epidemiological response, dealing with a novel disease for which data is still sparse and testing

160 capabilities are limited means that maximizing the impact of each individual test can benefit the

161 continued refinement of our strategy.

162

168   **References and Notes:**

169   1.   C. Wells, D. Yamin, M. L. Ndeffo-Mbah, N. Wenzel, S. G. Gaffney, J. P. Townsend, L. A.

170   Meyers, M. Fallah, T. G. Nyenswah, F. L. Altice, K. E. Atkins, A. P. Galvani, Harnessing

171   Case Isolation and Ring Vaccination to Control Ebola. *PLOS Neglected Tropical Diseases*. **9**,

172   e0003794 (2015).

173   2.   G. Chowell, S. Echevarría-Zuno, C. Viboud, L. Simonsen, J. Tamerius, M. A. Miller, V. H.

174   Borja-Aburto, Characterizing the Epidemiology of the 2009 Influenza A/H1N1 Pandemic in

175   Mexico. *PLoS Med*. **8** (2011), doi:10.1371/journal.pmed.1000436.

176   3.   E. P. Fenichel, C. Castillo-Chavez, M. G. Ceddia, G. Chowell, P. A. G. Parra, G. J. Hickling,

177   G. Holloway, R. Horan, B. Morin, C. Perrings, M. Springborn, L. Velazquez, C. Villalobos,

178   Adaptive human behavior in epidemiological models. *PNAS*. **108**, 6306–6311 (2011).

179   4.   R. M. May, A. R. McLean, J. Pattison, R. A. Weiss, R. M. Anderson, C. Fraser, A. C. Ghani,

180   C. A. Donnelly, S. Riley, N. M. Ferguson, G. M. Leung, T. H. Lam, A. J. Hedley,

181   Epidemiology, transmission dynamics and control of SARS: the 2002–2003 epidemic.

182   *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*. **359**,

183   1091–1105 (2004).

184   5.   D. Klinkenberg, C. Fraser, H. Heesterbeek, The Effectiveness of Contact Tracing in Emerging

185   Epidemics. *PLOS ONE*. **1**, e12 (2006).

186   6.   R. D. Smith, Responding to global infectious disease outbreaks: Lessons from SARS on the

187   role of risk perception, communication and management. *Social Science & Medicine*. **63**,

188   3113–3123 (2006).

189   7.   S. Cauchemez, N. M. Ferguson, C. Wachtel, A. Tegnell, G. Saour, B. Duncan, A. Nicoll,

190   Closure of schools during an influenza pandemic. *The Lancet Infectious Diseases*. **9**, 473–481

191   (2009).

192   8.   C. del Rio, P. N. Malani, COVID-19—New Insights on a Rapidly Changing Epidemic. *JAMA*

193   (2020), doi:10.1001/jama.2020.3072.

194   9.   M. Lin, A. Beliavsky, K. Katz, J. E. Powis, W. Ng, V. Williams, M. Science, H. Groves, M. P.

195   Muller, A. Vaisman, S. Hota, J. Johnstone, J. A. Leis, What can early Canadian experience

196   screening for COVID-19 teach us about how to prepare for a pandemic? *CMAJ* (2020),

197   doi:10.1503/cmaj.200305.

198   10. Y. Bai, L. Yao, T. Wei, F. Tian, D.-Y. Jin, L. Chen, M. Wang, Presumed Asymptomatic

199   Carrier Transmission of COVID-19. *JAMA* (2020), doi:10.1001/jama.2020.2565.

200   11. S-S. Chang, W-H. Hsieh, T-S. Kiu, S-H. Lee, C-H. Wang, H-C. Chou, Y. H. Yeo, C-P. Tseng,

201   C-C. Lee, Multiplex PCR System for Rapid Detection of Pathogens in Patients with Presumed

202   Sepsis – A Systemic Review and Meta-Analysis, PLOS ONE. **8**(5), e62323 (2013).

203   12. S. C. Kehl, S. Kumar, Utilization of Nucleic Acid Amplification Assays for the Detection of

204   Respiratory Viruses. *Clinics in Laboratory Medicine*. **29**, 661–671 (2009).

205   13. J. B. Mahony, Detection of Respiratory Viruses by Molecular Methods. *Clinical Microbiology*

206   *Reviews*. **21**, 716–747 (2008).

207   14. W. Wu, Y.-W. Tang, Emerging Molecular Assays for Detection and Characterization of

208   Respiratory Viruses. *Clinics in Laboratory Medicine*. **29**, 673–693 (2009).

15. M. J. Espy, J. R. Uhl, L. M. Sloan, S. P. Buckwalter, M. F. Jones, E. A. Vetter, J. D. C. Yao, N. L. Wengenack, J. E. Rosenblatt, F. R. Cockerill, T. F. Smith, Real-Time PCR in Clinical Microbiology: Applications for Routine Laboratory Testing. *Clinical Microbiology Reviews*. **19**, 165–256 (2006).

16. I. M. Mackay, K. E. Arden, A. Nitsche, Real-time PCR in virology. *Nucleic Acids Res*. **30**, 1292–1305 (2002).

17. H. Sox, S. Stern, D. Owens, H. L. Abrams, "The use of diagnostic tests: A probabilistic approach" in "*Assessment of Diagnostic Technology in Health Care: Rationale, Methods, Problems, and Directions*" (National Academies Press (US), 1989.

18. R. S. Ledley, L. B. Lusted, Reasoning Foundations of Medical Diagnosis: Symbolic logic, probability, and value theory aid our understanding of how physicians reason. *Science*. **130**, 9–21 (1959).

19. H. C. Sox, Diagnostic Decision: Probability Theory in the Use of Diagnostic Tests: An Introduction to Critical Study of the Literature. *Ann Intern Med*. **104**, 60 (1986).

20. R. Dorfman, The detection of defective numbers of large populations. *Annals of Mathematical Statistics* **14**, 436–440 (1943).

21. C. Pilcher, S. Fiscus, T. Nguyen, E. Foust, L. Wolf, D. Williams, R. Ashby, J. O'Dowd, J. McPherson, B. Stalzer, L. Hightow, W. Miller, J. Eron, M. Cohen, P. Leone, Detection of acute infections during HIV testing in North Carolina. New *England Journal of Medicine* **352**, 1873–1883 (2005).

22. C. Lindan, M. Mathur, S. Kumta, H. Jerajani, A. Gogate, J. Schachter, J. Moncada, Utility of pooled urine specimens for detection of Chlamydia trachomatis and Neisseria gonorrhoeae in

men attending public sexually transmitted infection clinics in Mumbai, India, by PCR. *Journal of Clinical Microbiology* **43**, 1674–1677 (2005).

23. T. Van, J. Miller, D. Warshauer, E. Reisdorf, D. Jerrigan D, Humes R, Shult P., Pooling nasopharyngeal/throat swab specimens to increase testing capacity for influenza viruses by PCR. *Journal of Clinical Microbiology* **50**, 891–896 (2012).

24. M. S. Warasi, J. M. Tebbs, C. S. McMahan,C. R. Bilder, Estimating the prevalence of multiple diseases from two-stage hierarchical pooling. *Statist. Med.* **35**, 3851– 3864 (2016).

25. H.-Y. Kim, M. G. Hudgens, J. M. Dreyfuss, D. J. Westreich, C. D. Pilcher, Comparison of Group Testing Algorithms for Case Identification in the Presence of Test Error. *Biometrics* **63**, 1152-1163 (2007).

26. S. P. Layne, J. M. Hyman, D. M. Morens, J. K. Taubenberger, New coronavirus outbreak: Framing questions for pandemic prevention. *Science Translational Medicine*. **12** (2020), doi:10.1126/scitranslmed.abb1469.

27. V. M. Corman, O. Landt, M. Kaiser, R. Molenkamp, A. Meijer, D. K. Chu, T. Bleicker, S. Brünink, J. Schneider, M. L. Schmidt, D. G. Mulders, B. L. Haagmans, B. van der Veer, S. van den Brink, L. Wijsman, G. Goderski, J.-L. Romette, J. Ellis, M. Zambon, M. Peiris, H. Goossens, C. Reusken, M. P. Koopmans, C. Drosten, Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Eurosurveillance*. **25**, p. 2000045 (2020).

28. S. M. Samuels, The exact solution to the two-stage group-testing problem. *Technometrics* **20**, 497-500 (1978).

251   29.   K. Hanson, ARUP COVID-19 rRT-PCR diagnostic testing results. Testing method is the EUA

252   approved Hologic Panther Fusion SARS-COV2 assay:

253   https://www.fda.gov/media/136156/download

254   30.   W. Wang, Y. Xu, R. Gao, R. Lu, K. Han, G. Wu, W. Tan, Detection of SARS-CoV-2 in

255   Different Types of Clinical Specimens. *JAMA* (2020), doi:10.1001/jama.2020.3786.

256   31.   J. L. Lewis, V. M. Lockary, S. Kobic, Cost Savings and Increased Efficiency Using a

257   Stratified Specimen Pooling Strategy for Chlamydia trachomatis and Neisseria gonorrhoeae.

258   *Sexually Transmitted Diseases* **39**, 46-48 (2012).

259   32.   J. Cohen, K. Kupferschmidt, Countries test tactics in 'war' against COVID-19. *Science*. **367**,

260   1287–1288 (2020).

261 **Materials and Methods**

262 <u>Bayesian inference implementation</u>

263   The process for assessing the posterior probabilities for individuals whose pooled test yielded a

264 positive or negative result (**Eq. S1.a-b**)

265 $$P(D|+) = \frac{P(D) \cdot P_{id}^{pool}}{P_{pool}(+)} \text{ (Eq. S1.a)} \qquad P(D|-) = \frac{P(D) \cdot (1 - P_{id}^{pool})}{1 - P_{pool}(+)} \text{ (Eq. S1.b)}$$

266 includes the a reduction in the identification rate of the pooled-sample diagnostic test by a factor γ

267 compared to an individual test, so that $P_{id}^{pool} = \gamma \cdot P_{id}^{ind}$. One must also consider the probability of a

268 positive result in a pooled sample. Assuming that every individual has a prior probability of being

269 infected equal to some background average $P(D)$, the probability of a positive test result in a pool

270 of size n is the probability of having a nonzero number of positive individual samples times the

271 pooled-test identification rate plus the probability of a completely-healthy sample pool yielding a

272 false positive (**Eq. S2**):

273 $$P_{pool}(+) = \left[1 - (1 - P(D))^n\right] \cdot P_{id}^{pool} + P_{fp} \cdot (1 - P(D))^n \text{ (Eq. S2)}$$

274 The posterior probability $P(D|+)$ from the pooled test with a positive outcome can be used as a

275 prior for the follow-up individual test Bayesian inference (**Eq. S3.a-b**).

276 $$P(D|++) = \frac{P(D|+) \cdot P_{id}}{P_{ind}(+)} = \frac{P(D|+) \cdot P_{id}}{P(D|+) \cdot P_{id} + (1 - P(D|+)) \cdot P_{fp}} \text{ (Eq. S3.a)}$$

277

278 $$P(D|+-) = \frac{P(D|+) \cdot (1 - P_{id})}{1 - P_{ind}(+)} \text{ (Eq. S3.b)}$$

279 where the overall probability of an individual's test being positive includes the probability of

280 correctly identifying a positive sample $P(D|+) \cdot P_{id}$ plus the probability of a negative sample

281 yielding a false positive $(1 - P(D|+)) \cdot P_{fp}$.

14

282   For a population of N_p patients divided into sample pools of size n, the average number of tests

283     $\langle N_{test} \rangle$ is given by the number of initial pooled tests plus the expected number of follow-up tests

284   (**Eq. S4**):

285
$$\langle N_{test} \rangle = \frac{N_p}{n} + n \cdot \frac{N_p}{n} \cdot P_{pool}(+) = N_p \cdot \left(\frac{1}{n} + P_{pool}(+) \right) \quad \textbf{(Eq. S4)}$$

286   The throughput increase is expressed as a multiplicative factor representing how many individuals

287   were screened by the use of each diagnostic test (**Eq. S5**).

288
$$\chi = \frac{N_p}{\langle N_{test} \rangle} = \left(\frac{1}{n} + P_{pool}(+) \right)^{-1} \quad \textbf{(Eq. S5)}$$

289

290   *Full vs. partial optimization.* In a fully optimized scheme a pooled-sample's positive result triggers

291   a cascade of smaller-sized pools, minimizing the number of tests performed. However, an

292   intermediate improvement is chosen due to possible adverse outcomes of a lengthier process with

293   several pooling and Bayesian inference steps – e.g., possible delay of necessary care, increased

294   exposure to infected individuals.

295

296   <u>Advantages in testing throughput and case detection</u>

297   We can estimate the ratio of detected-to-missed (including unscreened) cases for each protocol

298   with the following simplified analysis:

299   **In the pooled scheme**, the number of detected cases are the total number of screened individuals

300   with the disease $\langle N_{test} \rangle \chi P(D)$ multiplied by the effective detection probability $\gamma \cdot \left(P_{id}^{ind} \right)^2$, which

301   includes the sequential detection probability at the pooled stage and the follow-up individual stage.

302   The number of missed cases would thus be the difference between detected and total cases,

303     $\langle N_{test} \rangle \chi P(D) \left[1 - \gamma \cdot \left(P_{id}^{ind} \right)^2 \right]$. The number of false positives is the number of individuals in all-

304   healthy pools yielding a false positive and whose individual test is also a false positive –

305 $\langle N_{test}\rangle\chi\left(1-P(D)\right)^n P_{fp}^2$ – plus the healthy individuals pooled with a diseased sample that is

306 detected at the pooled stage and whose follow up test is a false positive, $\langle N_{test}\rangle\chi\gamma P_{id}^{ind}\big[1-$

307 $\left(1-P(D)\right)^n - P(D)\big]P_{fp}$. The detection-to-miss ratio θ_{pool} can be expressed as

308
$$\theta_{pool} = \frac{\langle N_{test}\rangle\chi P(D)\gamma\cdot\left(P_{id}^{ind}\right)^2}{\langle N_{test}\rangle\chi P(D)\left[1-\gamma\cdot\left(P_{id}^{ind}\right)^2\right]} = \frac{\gamma\cdot\left(P_{id}^{ind}\right)^2}{1-\gamma\cdot\left(P_{id}^{ind}\right)^2} \qquad \textbf{(Eq. S6)}$$

309 **In a standard 1:1 scheme**, the number of detected cases are the number of tested individuals with

310 the disease $\langle N_{test}\rangle P(D)$ times the detection efficiency of the individual test P_{id}^{ind}. The number of

311 missed cases is thus $\langle N_{test}\rangle P(D)(1-P_{id}^{ind})$. The number of false positives is $\langle N_{test}\rangle[1-$

312 $P(D)]P_{fp}$. The number of unscreened cases that carry the disease is $\langle N_{test}\rangle(\chi-1)P(D)$. The

313 detection-to-miss ratio – considering the population sampled in the pooled scheme – is given by

314
$$\theta_{ind} = \frac{\langle N_{test}\rangle P(D)P_{id}^{ind}}{\langle N_{test}\rangle P(D)\left(1-P_{id}^{ind}\right)+\langle N_{test}\rangle(\chi-1)P(D)} = \frac{P_{id}^{ind}}{\chi-P_{id}^{ind}} \qquad \textbf{(Eq. S7)}$$

315 The relative increase in the detection-to-miss ratio between the pooled and standard 1:1 schemes is

316 thus given by

317
$$\Delta = \frac{\theta_{pool}}{\theta_{ind}} = \frac{\gamma P_{id}^{ind}\left(\chi-P_{id}^{ind}\right)}{1-\gamma\cdot\left(P_{id}^{ind}\right)^2} \qquad \textbf{(Eq. S8)}$$

318 To further illustrate this, consider a disease including asymptomatic-yet-infectious individuals

319 and for which clinical predictions are at an early stage, preventing effective triage against conditions

320 presenting similar symptoms. A screening point must decide how to use limited resources (e.g.,

321 4,000 available tests) to detect the maximum number of cases in a population at 5% risk. Using the

322 test characteristics described in the main text ($P_{id}^{ind} = 0.95, P_{fp} = 0.01, \gamma = 0.9$), it is possible to

323 compare the pooled-sample screening protocol vs. standard 1:1: testing. On average, a pooled-

324 sample approach allows testing 10,000 individuals, detecting 406 cases while missing 94 and

325 yielding 18 false positives; conversely, a standard 1:1 approach would test 4,000 individuals and

16

326 detect 190 cases, miss 10, yield 38 false positives, and leave 300 positive cases untested and thus

327 undetected.

328 What if the rRT-PCR test sensitivity is much lower than expected – say $P_{id}^{ind} = 0.70$? Let us

329 also consider a much more pessimistic estimate for sensitivity loss, $\gamma = 0.8$ and no change in the

330 false positive rate ($P_{fp} = 0.01$). For the same 5% prevalence in the population, the screening point

331 with access to 4,000 tests would screen 12,550 individuals (627 of which are infected) using a

332 pooling scheme (for these new parameters, optimal pool size is $n = 7$). It would detect 246 and

333 miss 381. A 1:1 testing scheme would identify 140 cases, miss 60, and leave 427 untested. Not

334 surprisingly, the number of false negatives increases substantially in both scenarios due to the lower

335 starting point for the test's sensitivity. However, in a pooled scheme 381 infected individuals are

336 still at risk of spreading the disease in the community, while in the 1:1 scheme 487 infected

337 individuals remain at risk of further community spread. As mentioned in the main text, effective

338 risk communication is a critical component of any large-scale screening effort with imperfect tests.

339 Symptomatic individuals should be considered at increased risk even after a negative test result, and

340 other diagnostic avenues could be used (e.g., chest CT).