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Blueprint for a pop-up SARS-CoV-2 testing lab

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Although the development of vaccines and therapeutics for SARS-CoV-2 remains urgent, we must proceed with caution, using the full armory of vaccine and protein design tools at our disposal to rationally minimize the risk of ADE. ❐

Editorial note: This article has been peer reviewed.

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References

1. Taylor, A. et al. *Immunol. Rev.* **268**, 340–364 (2015).
2. Katzelnick, L. C. et al. *Science* **358**, 929–932 (2017).
3. Bardina, S. V. et al. *Science* **356**, 175–180 (2017).
4. Kuzmina, N. A. et al. *Cell Rep.* **24**, 1802–1815.e5 (2018).
5. Petersen, N. C. & Boyle, J. F. *Am. J. Vet. Res.* **41**, 868–876 (1980).
6. Vennema, H. et al. *J. Virol.* **64**, 1407–1409 (1990).
7. Yip, M. S. et al. *BMC Proc.* **5** (Suppl. 1), 80 (2011).
8. Wan, Y. et al. *J. Virol.* **94**, e02015–e02019 (2020).
9. Tetro, J. A. *Microbes Infect.* **22**, 72–73 (2020).
10. Chen, Y. C. & Wang, S. Y. *J. Virol.* **76**, 9877–9887 (2002).
11. Bosio, C. M. et al. *J. Infect. Dis.* **188**, 1630–1638 (2003).
12. Li, L. et al. *J. Clin. Virol.* **28**, 239–244 (2003).
13. Gu, J. et al. *J. Exp. Med.* **202**, 415–424 (2005).
14. Jaume, M. et al. *J. Virol.* **85**, 10582–10597 (2011).
15. Yip, M. S. et al. *Virol. J.* **11**, 82 (2014).
16. Spiekermann, G. M. et al. *J. Exp. Med.* **196**, 303–310 (2002).
17. Thul, P. J. et al. *Science* **356**, eaal3321 (2017).
18. Wang, Q. et al. *ACS Infect. Dis.* **2**, 361–376 (2016).
19. Liu, L. et al. *JCI Insight* **4**, e123158 (2019).
20. Kam, Y. W. et al. *Vaccine* **25**, 729–740 (2007).
21. Jaume, M. et al. *Hong. Kong Med. J.* **18** (Suppl. 2), 31–36 (2012).
22. Tseng, C. T. et al. *PLoS One* **7**, e35421 (2012).
23. Deming, D. et al. *PLoS Med.* **3**, e525 (2006).
24. Agrawal, A. S. et al. *Hum. Vaccin. Immunother.* **12**, 2351–2356 (2016).
25. Wang, S. F. et al. *Biochem. Biophys. Res. Commun.* **451**, 208–214 (2014).
26. Cheung, C. Y. et al. *J. Virol.* **79**, 7819–7826 (2005).
27. Yip, M. S. et al. *Hong. Kong Med. J.* **22** (Suppl. 4), 25–31 (2016).
28. Braun, J. et al. Preprint at *medRxiv* https://doi.org/10.1101/2020.04.17.20061440 (2020).
29. Rothman, A. L. *Nat. Rev. Immunol.* **11**, 532–543 (2011).
30. Larman, H. B. et al. *Nat. Biotechnol.* **29**, 535–541 (2011).
31. Price, J. V. et al. *Nat. Med.* **18**, 1434–1440 (2012).
32. Guo, N. et al. *EBioMedicine* **2**, 859–867 (2015).
33. Baerga-Ortiz, A., Hughes, C. A., Mandell, J. G. & Komives, E. A. *Protein Sci.* **11**, 1300–1308 (2002).
34. Puchades, C. et al. *Sci. Rep.* **9**, 4735 (2019).
35. Lee, J. et al. *Nat. Med.* **22**, 1456–1464 (2016).
36. Briney, B., Inderbitzin, A., Joyce, C. & Burton, D. R. *Nature* **566**, 393–397 (2019).
37. Jespersen, M. C., Peters, B., Nielsen, M. & Marcatili, P. *Nucleic Acids Res.* **45** W1 W24–W29 (2017).
38. Weitzner, B. D. et al. *Nat. Protoc.* **12**, 401–416 (2017).
39. Sahin, U. et al. *Nature* **547**, 222–226 (2017).
40. Chen, B. et al. *Nat. Biotechnol.* **37**, 1332–1343 (2019).
41. Racle, J. et al. *Nat. Biotechnol.* **37**, 1283–1286 (2019).
42. Sarkizova, S. et al. *Nat. Biotechnol.* **38**, 199–209 (2020).
43. Dash, P. et al. *Nature* **547**, 89–93 (2017).
44. Glanville, J. et al. *Nature* **547**, 94–98 (2017).
45. Zhang, S. Q. et al. *Nat. Biotechnol.* **36**, 1156–1159 (2018).
46. Kula, T. et al. *Cell* **178**, 1016–1028.e13 (2019).
47. Shaw, D. E. et al. *Proc. Intl Conf. High Performance Computing, Networking, Storage and Analysis (SC14)* 41–53 (IEEE, 2014).
48. Alford, R. F. et al. *J. Chem. Theory Comput.* **13**, 3031–3048 (2017).
49. Correia, B. E. et al. *Nature* **507**, 201–206 (2014).
50. Bhardwaj, G. et al. *Nature* **538**, 329–335 (2016).
51. Pallesen, J. et al. *Proc. Natl Acad. Sci. USA* **114**, E7348–E7357 (2017).
52. Wrapp, D. et al. *Science* **367**, 1260–1263 (2020).
53. Hu, H. et al. *Clin. Vaccin. Immunol.* **14**, 894–901 (2007).
54. Hewitt, S. L. et al. *Sci. Transl. Med.* **11**, eaat9143 (2019).
55. Hashem, A. M. et al. *J. Infect. Dis.* **220**, 1558–1567 (2019).
56. Weingartl, H. et al. *J. Virol.* **78**, 12672–12676 (2004).
57. Yang, Z. Y. et al. *Proc. Natl Acad. Sci. USA* **102**, 797–801 (2005).
58. Mestas, J. & Hughes, C. C. W. *J. Immunol.* **172**, 2731–2738 (2004).
59. Crowley, A. R. & Ackerman, M. E. *Front. Immunol.* **10**, 697 (2019).
60. Smith, P., DiLillo, D. J., Bournazos, S., Li, F. & Ravetch, J. V. *Proc. Natl Acad. Sci. USA* **109**, 6181–6186 (2012).
61. Hezareh, M., Hessell, A. J., Jensen, R. C., van de Winkel, J. G. & Parren, P. W. *J. Virol.* **75**, 12161–12168 (2001).
62. Kang, T. H. & Jung, S. T. *Exp. Mol. Med.* **51**, 1–9 (2019).
63. Tao, M. H. & Morrison, S. L. *J. Immunol.* **143**, 2595–2601 (1989).
64. Chappel, M. S. et al. *Proc. Natl Acad. Sci. USA* **88**, 9036–9040 (1991).
65. Lo, M. et al. *J. Biol. Chem.* **292**, 3900–3908 (2017).
66. Maamary, J., Wang, T. T., Tan, G. S., Palese, P. & Ravetch, J. V. *Proc. Natl Acad. Sci. USA* **114**, 10172–10177 (2017).

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Blueprint for a pop-up SARS-CoV-2 testing lab

To the Editor — On 11 March 2020, the World Health Organization declared the 2019 coronavirus disease (COVID-19) a global pandemic[1]. As of 29 May, the virus that causes the disease, SARS-CoV-2, has infected over 5,813,000 people and killed more than 360,000 worldwide (https://coronavirus.jhu.edu/map.html). The virus continues to spread around the world, and at the time of writing there are no clinically validated medical interventions to prevent or cure COVID-19. Public health measures in the United States and elsewhere focus on mitigating spread through diagnostic testing, self-isolation and shelter-in-place orders[2].

The presence of presymptomatic and mildly symptomatic individuals in the general population is a major driver in the accelerated and widespread outbreaks that have overwhelmed healthcare infrastructures worldwide, causing more deaths[2–5]. Extensive testing in countries such as Iceland, New Zealand, Germany and South Korea, among others, has proven an effective tool in controlling the spread of the disease[3–7].

At the start of our effort, on 14 March 2020, the turnaround time for testing for University of California (UC) Berkeley students through commercial labs exceeded seven days (UC Berkeley Tang Center, personal communication), and no rapid or surveillance testing was available to City of Berkeley first responders (City of Berkeley Fire Department Chief David Brannigan, personal communication) or to vulnerable populations in Berkeley, including those living in congregated settings and the unsheltered.

To address the need for expanded testing capacity, the Innovative Genomics Institute (IGI) at UC Berkeley established a clinical testing laboratory for SARS-CoV-2 in three weeks (see "Timeline of SARS-CoV-2 IGI Laboratory Establishment" on Figshare). Timely setup presented formidable challenges, including navigating state and federal regulations, supply-chain and logistic obstacles, and challenges related to serving populations beyond UC Berkeley (Table 1). To tackle these hurdles, we partnered with UC Berkeley's University Health Services (UHS) and created specialized teams to execute the technical, operations, regulatory, human resources, data management, physician interface, sample collection and sample reporting processes for the IGI laboratory (see "IGI SARS-CoV-2 Testing Organizational Chart" on Figshare).

When we began, our campus did not have a clinical testing facility that would allow our testing lab volunteers to work at the level of biosafety required by our campus for SARS-CoV-2 diagnostics, and without a medical school with an affiliated medical center, our campus had no mechanism to

Table 1 | Summary of challenges for establishment of the IGI SARS-CoV-2 testing laboratory

Category	Nature of challenge	Solution	Supporting documentation
Regulatory (CLIA)	A CLIA certificate and license for a diagnostic testing facility is required	Extend CLIA license from UC Berkeley University Health Services to the IGI testing lab	"Useful Links" on Figshare
	CLIA regulations require testing personnel to be licensed CLS	Regulations are temporarily revised, allowing non-CLS scientists to act as testing personnel with revised training requirements. IGI created an accelerated in-house CLIA training program.	"Useful Links" and "Regulatory Compliance" on Figshare
	Testing personnel must be proficiency tested	Test blinded contrived specimens provided by the American Proficiency Institute	API Proficiency Program for SARS-CoV-2
Regulatory (FDA/CLIA)	Validation study must be performed to determine LOD of LDT	Test contrived specimens with synthetic viral RNA in collection medium (semi-automated) and in clinical matrix (automated)	"Useful Links," "Limit of Detection Validation and Clinical Sample Evaluation in Semi-Automated Method" and "Limit of Detection and Clinical Sample Validation in Automated Method" on Figshare
	Clinical validation of LDT must be performed	Test panel of specimens resulted as negative and positive by a CLIA-certified laboratory with an issued EUA for a SARS-CoV-2 test. If specimens unavailable, use contrived ones in clinical matrix.	"Useful Links," "Limit of Detection Validation and Clinical Sample Evaluation in Semi-Automated Method" and "Limit of Detection and Clinical Sample Validation in Automated Method" on Figshare
	An initial batch of negative and positive specimens tested by the LDT must be tested by a third party	Submit the first 5 negative and 5 positive specimens tested under the LDT to a CLIA laboratory with an issued EUA for a SARS-CoV-2 LDT	"Useful Links" on Figshare
Regulatory (HIPAA)	Privacy and security of PHI must be consistently maintained during data handling	Develop and implement a LIMS that meets HIPAA standards for PHI privacy and security; restrict access to PHI within the LIMS to authorized personnel	"Test, LIMS and Physician Interface Development" and "Video—Semi-Automated Method LIMS Interface" on Figshare
	Testing lab and other personnel must comply with HIPAA regulations	Ensure all personnel complete an online HIPAA training class and pass the end-of-class assessment, with certificate of training placed on permanent record	The IGI team used the online HIPAA training available from Thompson Reuters
Biosafety	Specimens must be processed in a manner that complies with institutional biosafety regulations	Obtain a BUA from the university CLEB that defines how specimens are to be processed safely and what PPE the testing personnel must wear	"Regulatory Compliance" on Figshare
	Measures must be taken to reduce the possibility of SARS-CoV-2 transmission between testing team members	Require all personnel to take a self-assessment questionnaire daily before entering the building where the diagnostic lab is located	"Personnel Training and Biosafety" on Figshare
	Concerns about bringing samples with live virus on site	Develop a customized sample collection kit that uses a deactivating sample transport medium (DNA/RNA Shield)	"Sample Collection Kit—Preparation, Patient Sample, Collection and Transport" on Figshare
Healthcare partners	Challenges of serving non-campus patients	Build a physician portal into the LIMS that can be accessed by non-UC Berkeley physicians. Partner with local healthcare providers to perform swabbing of community members.	"IGI Interface with UC and Non-UC Health Partners" and "Test, LIMS and Physician Interface Development" on Figshare
	Testing kits must be made available to UHS and to third-party clinicians	Establish a kit assembly SOP. Employ a dedicated kit assembly team to generate sufficient kits on a weekly basis. Use a professional courier service to deliver the kits to the testing sites. Establish a kit use SOP and provide it to the clinicians. Establish diagnostic specimen return SOPs for every partner site.	"Sample Collection Kit—Preparation, Patient Sample, Collection and Transport" on Figshare
	The physicians requisitioning the test must receive the results in a CLIA- and HIPAA-compliant manner	As per State of California regulations, CLS report all positive results within 24 h to the requisitioning physician via a direct phone call. All test results are subsequently accessible through our CLIA- and HIPAA-compliant clinician portal.	"Test, LIMS and Physician Interface Development" on Figshare
Supply chain	A sustained supply of specimen collection components is necessary	Identify a tube and swab manufacturer with sufficient supply, and configure the LDT SOPs around it	Main text; "Test, LIMS and Physician Interface Development" on Figshare
	A sustained supply of diagnostic testing reagents and disposables is necessary	Identify a supplier (Thermo Fisher) with robust reagent production capacity; develop an LDT that uses half-reactions	

The IGI team encountered regulatory, biosafety, health partnering and supply-chain challenges over the course of our work and implemented solutions to overcome them. BUA, Biological Use Authorization; CLEB, Committee for Laboratory and Environmental Biosafety; PHI, protected health information; SOP, standard operating procedure.

provide medical services to patients from off campus. To serve populations beyond the campus, we established partnerships with community health centers and implemented an electronic portal compliant with Clinical Laboratory Improvement Amendments (CLIA) and Health Insurance Portability and Accountability Act (HIPAA) for requisitioning and providing results of tests. The portal integrates with our laboratory information management system (LIMS) (see "IGI Interface with UC and Non-UC Health Partners" on Figshare).

Three regulatory developments enabled our technical work. The first, California Governor Gavin Newsom's 4 March Executive Order N-25-20 (see "Useful Links" on Figshare), modified the requirements for clinical laboratory personnel running diagnostic tests for SARS-CoV-2 in a certified laboratory, allowing trained volunteer scientists to staff the operation (see "Personnel Training and Biosafety" on Figshare). The second, the US Food and Drug Administration (FDA) 16 March "Policy for Diagnostic Tests for Coronavirus Disease-2019 during the Public Health Emergency" (see "Useful Links" on Figshare), simplified the authorization process for a SARS-CoV-2 test, enabling quick adoption of an existing authorized test kit. The third increased the speed and flexibility of state and federal licensing procedures for clinical laboratory facilities under the CLIA program (see "Useful Links" on Figshare).

The Centers for Medicare & Medicaid Services' CLIA program regulates all US clinical laboratory testing, ensuring the accuracy and reliability of patient test results. Under the state's temporarily relaxed regulatory requirements, we obtained CLIA certification for the IGI testing lab by extension of the existing CLIA license at UC Berkeley's student health center (see "Regulatory Compliance" on Figshare). By partnering with the campus clinic, we were able to combine their medical and clinical laboratory expertise with the IGI's access to biosafety level (BSL)-2+ laboratory space, equipment, and technical expertise of IGI and UC Berkeley scientists. This combined expertise was critical for rapid SARS-CoV-2 test implementation with full regulatory oversight.

In addition to a facility license, other steps were required for regulatory compliance. First, the lab obtained a Biological Use Authorization from UC Berkeley's institutional biosafety committee (the Committee for Laboratory and Environmental Biosafety), which determined the level of biosafety and nature of personal protection equipment (PPE) necessary to receive patient samples and process them

on site. Second, while Governor Newsom's executive order allowed people other than clinical laboratory staff (CLS) to develop and staff a SARS-CoV-2 testing facility, it preserved the essential requirement of oversight by licensed CLS and documentation of proper personnel training. Partnering with UHS also allowed us to work under the guidance of their clinical laboratory director and licensed CLS, and together we developed a rigorous training program and proficiency assessment for each member of the testing team that included biosafety and assay workflow training. Testing team leads (see "IGI SARS-CoV-2 Testing Organizational Chart" on Figshare) are trained in the entire test workflow, while other team members are trained and tested specifically on the task they perform. Documentation of this training, along with proof of education in a relevant field, was sent to the California Department of Public Health to satisfy personnel requirements under the executive order. Third, in keeping with CLIA requirements for continuous proficiency assessment, our technical leads were tested in a competency assessment by processing blinded samples provided by the American Proficiency Institute. Finally, given the existence of protected health information in the testing laboratory, HIPAA compliance was observed by establishing mandatory HIPAA training for all testing personnel. In total, the complete training, testing and PPE process takes an average of ten working days (see "Personnel Training and Biosafety" on Figshare).

Additional precautionary measures were implemented to ensure the safety of our volunteer staff. Standard sample collection kits as recommended by the US Centers for Disease Control use COPAN tubes with universal transport medium (UTM), a buffer that stabilizes the virus before analysis. We developed and validated our own patient specimen collection kit, which uses a chaotropic agent (DNA/RNA Shield, Zymo) in place of UTM (Fig. 1 and "Test, LIMS and Physician Interface Development" on Figshare). This substitution not only preserves the integrity of the sample nucleic acid during transport, but also inactivates pathogens at the time of specimen collection. In this way, we minimize the potential for live virus to enter our facility. Additionally, as required by our Biological Use Authorization, our laboratory operates under BSL-2+, a higher standard of safety than the BSL-2 conditions typically employed with inactivated SARS-CoV-2. Stringent PPE requirements for all testing lab personnel include a disposable outer layer and N95 masks professionally fitted for each staff member. An added daily

self-assessment ensures that symptomatic personnel are detected as early as possible to prevent transmission within the team (see "Personnel Training and Biosafety" on Figshare).

In response to the pandemic, the FDA enacted its authority to issue Emergency Use Authorizations (EUAs), reducing the time required to devise and implement a new diagnostic test. This shift enabled companies to develop new PCR-based diagnostics for SARS-CoV-2. Rather than starting from scratch, the IGI chose to adapt the test marketed by Thermo Fisher Scientific because of its reagent availability, equipment compatibility and robust test performance in our hands. However, to increase test throughput, reduce costs, improve staff safety and make use of existing equipment, we modified Thermo Fisher's EUA workflow. Since an EUA is granted to a specific protocol implemented on defined equipment, our modifications (reduced reagent use, different sample collection kit) made our implementation a laboratory developed test (LDT) requiring a new EUA (Fig. 2). Nonetheless, our choice to adapt a test with an existing EUA allowed us to perform bridging studies, accelerating the path to our own EUA, further aided by technical support from Thermo Fisher during our effort (see "Methods" on Figshare).

To lower the cost of our test and limit reagent use in the face of supply-chain shortages, we changed the quantity of reagents used for RNA extraction and reverse transcription and quantitative PCR (RT-qPCR) after verifying that the test maintained robust performance at half the reaction volume and with targeted changes in some reagent volumes (see "Methods" and "Semi-Automated SOP" on Figshare). Additional modifications to Thermo Fisher's test included the development of a new sample collection kit with the goals of ensuring safety of our volunteer staff, as discussed above, and circumventing supply chain shortages (see "Sample Collection Kit—Preparation, Patient Sample, Collection and Transport" on Figshare) and its deployment on liquid handlers for increased testing throughput (see below).

The Thermo Fisher kit uses primer–probe pairs targeting three SARS-CoV-2 genes: *ORF1ab*, the spike protein gene (*S*) and the nucleocapsid protein gene (*N*) (Fig. 1). To return a positive result, the test must detect two of the three genes in a patient sample (see "Methods" on Figshare). Thermo Fisher's kit controls for RNA extraction and amplification by including an MS2 bacteriophage spike-in control together with the corresponding primer–probe pairs,
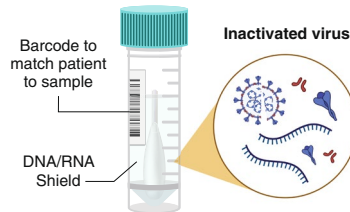
1 Oropharyngeal swab
A specimen is collected using IGI's custom kit and the provided swab. NP, OP, or NP+OP are acceptable.
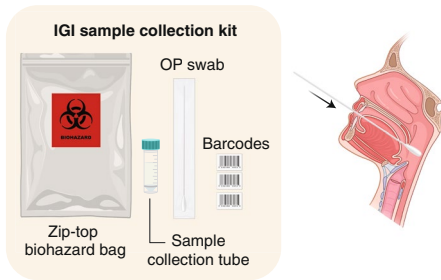
2 Collected specimen
Swab is transferred to supplied sample collection tube containing DNA/RNA Shield to inactivate the virus and stabilize the sample during transport.

3 Accessioning and arraying
Samples are placed into racks and arrayed into 96-deep-well plates by a Hamilton Microlab STARlet Liquid Handling System.
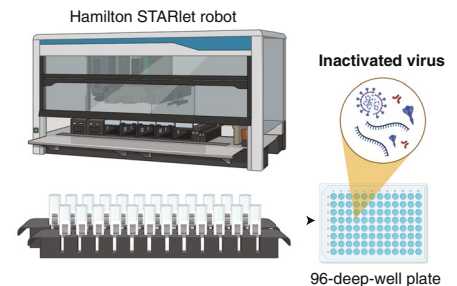
4 RNA extraction and RT-qPCR assembly
A Hamilton Vantage liquid handling system performs RNA extraction using **Thermo Fisher's MagMAX** Viral/Pathogen Nucleic Acid Isolation Kit and then assembles reactions for RT-qPCR with **Thermo Fisher's TaqPath RT-PCR COVID-19 Kit**.
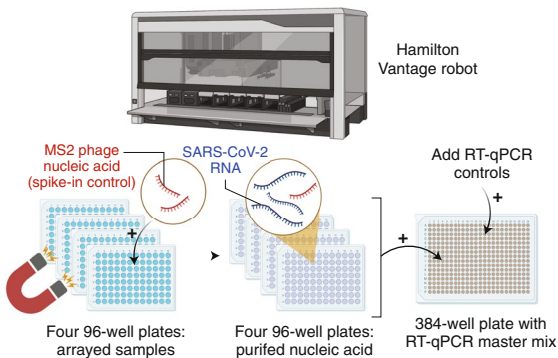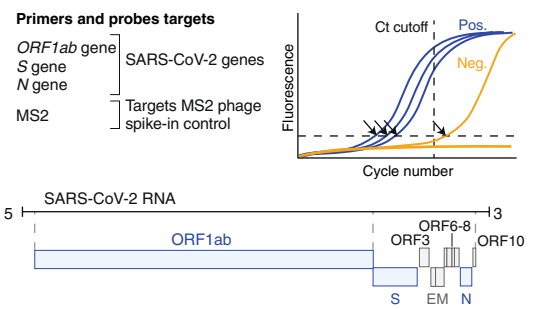
5 RT-qPCR
RT-qPCR is performed using an **ABI QuantStudio 6 Flex** Real-Time PCR System.

6 Data interpretation
Samples that cross the fluorescence threshold at a cycle number lower than the defined cutoff for two out of three SARS-CoV-2 target genes return a positive test result.

Fig. 1 | Overview of the IGI SARS-CoV-2 Testing Consortium assay. Diagram of the IGI's testing workflow and assay for SARS-CoV-2 using the automated method. Our test begins with a custom specimen collection kit for nasopharyngeal (NP) or oropharyngeal (OP) swabs (step 1). This kit uses alternative collection tubes and a chaotropic agent, DNA/RNA Shield, to inactivate the virus for transport to our facility (step 2). Barcodes are supplied to connect patient intake forms with specimens. Samples are transported to the IGI by courier where they are visually inspected for rejection criteria before being decontaminated in a biosafety cabinet and placed into trays for arraying. In step 3, a Hamilton STARlet liquid handler scans the sample barcodes, accessioning them into our LIMS, and arrays them into 96-well deep-well plates, recording the corresponding positions in the LIMS. Step 4 is conducted by a Hamilton Vantage liquid handler in our automated method and manually in our semi-automated method. The MS2 spike-in control is added and RNA extraction is performed using Thermo Fisher's MagMAX Viral/Pathogen Nucleic Acid Isolation Kit. Following extraction, the Hamilton Vantage performs RT-qPCR reaction setup using Thermo Fisher's TaqPath RT-PCR COVID-19 Kit by consolidating four 96-well plates into one 384-well plate preloaded with RT-qPCR master mix (this step performed manually in our semi-automated approach and using 96-well plates only). After the Hamilton Vantage adds positive and negative controls, the plate is moved to an ABI QuantStudio 6 Flex Real-Time PCR System for RNA detection. In our semi-automated approach, RT-qPCR is performed on an Applied Biosystems 7500 Fast Real-Time PCR system. Step 6 involves interpretation of the RT-qPCR data. Thermo Fisher's TaqPath RT-PCR COVID-19 Kit targets three SARS-CoV-2 genes shown in blue on the diagram of the SARS-CoV-2 genome (NCBI NC_045512.2). The open reading frame targeted by Thermo Fisher's kit, *ORF1ab*, encodes non-structural proteins for replication, whereas the spike (*S*) and nucleocapsid (*N*) genes encode two structural proteins. A diagram of qPCR amplification curves demonstrates the criteria by which sample data are interpreted; Ct, cycle threshold. Further criteria for data interpretation are detailed in "Methods" on Figshare, and details of the workflow are diagrammed in "Semi-Automated and Automated Workflow Equipment" on Figshare. Portions of figure created using BioRender.com.
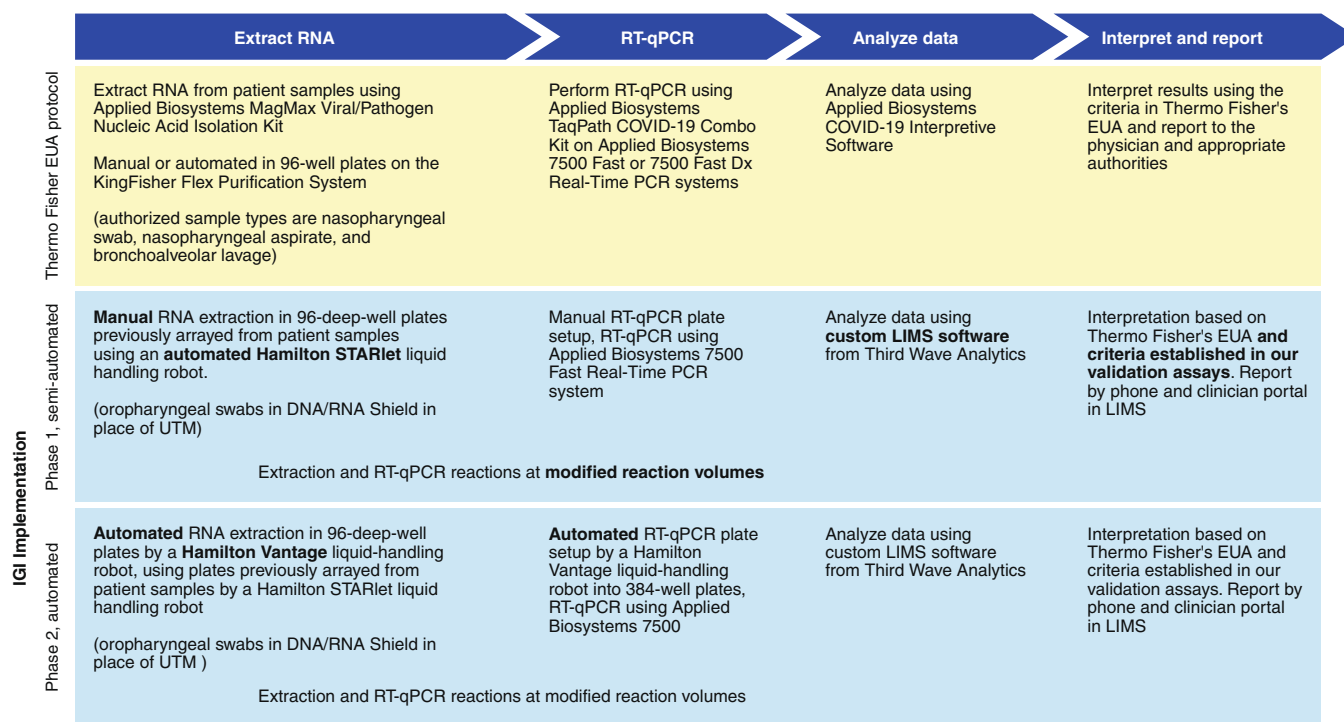
which are added before RNA extraction and RT-qPCR, respectively.

With the goal of initiating diagnostic testing at the earliest possible time, we designed our research and development workstream to begin with a semi-automated approach with a capacity of 180 tests per day that could be implemented rapidly while we

developed and validated a fully-automated assay to increase testing throughput to over 1,000 tests per day. Operating initially at reduced capacity also allowed the timely identification and resolution of process inefficiencies and technical issues, as well as the establishment of working relationships with non-university healthcare providers

to meet the needs of a larger population outside our campus.

Although no specialized equipment is required to perform an RT-qPCR assay manually, minimizing the potential for human error is essential. To this effect, we deployed a liquid handler (Hamilton STARlet) to perform patient specimen

		Extract RNA	**RT-qPCR**	**Analyze data**	**Interpret and report**
IGI Implementation	Thermo Fisher EUA protocol	Extract RNA from patient samples using Applied Biosystems MagMax Viral/Pathogen Nucleic Acid Isolation Kit Manual or automated in 96-well plates on the KingFisher Flex Purification System (authorized sample types are nasopharyngeal swab, nasopharyngeal aspirate, and bronchoalveolar lavage)	Perform RT-qPCR using Applied Biosystems TaqPath COVID-19 Combo Kit on Applied Biosystems 7500 Fast or 7500 Fast Dx Real-Time PCR systems	Analyze data using Applied Biosystems COVID-19 Interpretive Software	Interpret results using the criteria in Thermo Fisher's EUA and report to the physician and appropriate authorities
	Phase 1, semi-automated	**Manual** RNA extraction in 96-deep-well plates previously arrayed from patient samples using an **automated Hamilton STARlet** liquid handling robot. (oropharyngeal swabs in DNA/RNA Shield in place of UTM) Extraction and RT-qPCR reactions at **modified reaction volumes**	Manual RT-qPCR plate setup, RT-qPCR using Applied Biosystems 7500 Fast Real-Time PCR system	Analyze data using **custom LIMS software** from Third Wave Analytics	Interpretation based on Thermo Fisher's EUA **and criteria established in our validation assays**. Report by phone and clinician portal in LIMS
	Phase 2, automated	**Automated** RNA extraction in 96-deep-well plates by a **Hamilton Vantage** liquid-handling robot, using plates previously arrayed from patient samples by a Hamilton STARlet liquid handling robot (oropharyngeal swabs in DNA/RNA Shield in place of UTM) Extraction and RT-qPCR reactions at modified reaction volumes	**Automated** RT-qPCR plate setup by a Hamilton Vantage liquid-handling robot into 384-well plates, RT-qPCR using Applied Biosystems 7500	Analyze data using custom LIMS software from Third Wave Analytics	Interpretation based on Thermo Fisher's EUA and criteria established in our validation assays. Report by phone and clinician portal in LIMS

Fig. 2 | Workflow. Overview of Thermo Fisher's TaqPath COVID-19 Multiplex Diagnostic Solution[9,10] showing the steps as authorized in their EUA (yellow background) and IGI's implementation of this workflow (blue background). The IGI's SARS-CoV-2 testing lab was established in two phases. Phase 1 started with automated arraying of patient samples, followed by manual implementation of the Thermo Fisher kit. Phase 2 is a fully automated workflow. Bolded words indicate elements changed from the implementation above.

consolidation into 96-well deep-well plates (semi-automated workflow) and a second liquid handler (Hamilton Vantage) to perform RNA extraction and generate plates ready for RT-qPCR in the fully automated workflow (Figs. 1 and 2 and "Semi-Automated and Automated Workflow Equipment" on Figshare). Custom automation code for the Hamilton STARlet and Hamilton Vantage in the IGI setup are available on request (see "Hamilton Microlab STARlet and Hamilton Vantage Automation Process Workflow" on Figshare). Early in our process we benefited from loaned laboratory equipment (qPCR machines, liquid handlers, biological safety cabinets, and extra cold storage to enable backup in case of equipment failure, as well as our expanding testing capacity) and purchased our own as the testing continued.

With the modifications implemented to the Thermo Fisher protocol, our LDT was validated within the CLIA framework, as described below, and our EUA was submitted to the FDA as a bridging study to the original EUA awarded to Thermo Fisher. Validating an LDT requires measuring specific Centers for Medicare & Medicaid Services and FDA metrics for analytical and clinical validity and meeting or exceeding benchmarks. For our semi-automated assay,

these were (i) measuring the assay limit of detection (LOD); (ii) assessing clinical and analytical validity by running mock positive and negative samples at known concentrations, and (iii) performing our LDT on samples previously identified as positive and negative for SARS-CoV-2 provided by two local clinical diagnostic testing facilities.

To measure the LOD, the FDA recommends that "laboratories test a dilution series of three replicates per concentration, and then confirm the final concentration with 20 replicates."[8] For the purposes of an EUA, the agency defines the LOD as "the lowest concentration at which 19/20 replicates are positive." In accordance with this recommendation, we determined the LOD of our assay to be 1 genomic copy per microliter (see "Limit of Detection Validation and Clinical Sample Evaluation in Semi-Automated Method" in Figshare).

To ensure that diagnostic tests are clinically valid, the FDA recommends "that laboratories confirm performance of their assay with a series of contrived clinical specimens by testing a minimum of 30 contrived reactive specimens and 30 non-reactive specimens." We assessed the clinical validity of our LDT by creating a range of different types of contrived

SARS-CoV-2 RNA-positive and negative samples using SARS-CoV-2 positive control RNA from the TaqPath COVID-19 Control Kit, patient samples positive for SARS-CoV-2 from two local testing facilities, and human negative control RNA. Results showed 100% concordance with expected positive and negative samples (see "Limit of Detection Validation and Clinical Sample Evaluation in Semi-Automated Method" and "Clinical Sample Evaluation Assay in Semi-Automated Method (Duplicate PCR Plates)" on Figshare).

Specificity of the primer–probe pairs and the potential for cross-reactivity with other common pathogens were previously assessed by Thermo Fisher in their EUA. To further confirm specificity in our pipeline, we showed that the primer–probe sets do not cross-react with human RNA from a virus-negative cell line and that they return negative results for patient samples from alternative testing facilities that were previously identified as SARS-CoV-2 negative using orthogonal primer–probe pairs (see "Limit of Detection Validation and Clinical Sample Evaluation in Semi-Automated Method" and "Clinical Sample Evaluation Assay in Semi-Automated Method (Duplicate PCR Plates)" on Figshare).

Upon developing our automated workflow, we performed experiments to assess the LOD and clinical and analytical sensitivity (see "Limit of Detection and Clinical Sample Validation in Automated Method," "Methods" and "Automated SOP" on Figshare). Our automated method showed comparable analytical and clinical sensitivity, with an LOD at or better than that of our semi-automated method. At the time of this Correspondence, we are preparing for EUA submission to begin the switch to an automated testing platform.

To support the technical workflow of the IGI testing lab and to track patient samples through our facility, we developed a custom laboratory information management software (LIMS) system with Third Wave Analytics. Our LIMS was designed in two phases to accommodate both our semi-automated and automated approaches in a cost-effective and HIPAA-compliant manner (see "Semi-Automated and Automated Sample Workflow" and "Video—Semi-Automated Method LIMS Interface" on Figshare). In phase 1 of the LIMS build, de-identified patient barcodes are used for sample tracking. The LIMS returns de-identified barcoded results, which are sent to the UHS for integration into their electronic health system. There, the results are connected to a specific patient's file via standard unique identifier barcode matching. A template of our LIMS architecture, as customized for our semi-automated setup, is available for the cost of licensing alone, as described on the IGI website.

In phase 2 of the LIMS development, corresponding to our automated approach, we built a HIPAA-compliant clinician access portal through which tests are requisitioned and reported, supporting non-UC Berkeley patient samples (see "Test, LIMS and Physician Interface Development" and "IGI Interface with UC and Non-UCHealth Partners" on Figshare). To further enable non-UC Berkeley partnerships, we incorporated a payment interface within the portal. Partnering with non-UC Berkeley organizations enabled us to bringing testing to underserved, high-risk and other priority populations, such as the unsheltered, first responders and essential infrastructure workers. To meet these needs in our community, our partners include LifeLong Medical, the City of Berkeley, Roots Community Clinic and the State of California publicly owned utilities.

All testing results are reported to the requisitioning physician and the California Reportable Disease Information Exchange (CalREDIE) of the State of California Department of Public Health in .csv format.

Data are transferred using UC Berkeley's Google e-mail service plus Virtru encryption.

In summary, we describe here a process for creating a CLIA-certified clinical testing laboratory at a non-medical institution. Extending the license from an existing CLIA-certified facility, using online HIPAA training and adapting a commercially available FDA-authorized test saved substantial time and resources. Supply-chain bottlenecks were managed by sourcing alternative collection tubes and swabs from a provider with adequate stocks, using donated equipment, validating half-volume reactions in our assay, adopting in-house sample barcoding, and adapting materials (for example, sampling tubes) to work with available equipment. Finally, PPE including masks, gloves, and gowns was obtained by donation to our facility.

The IGI testing laboratory is currently supported primarily by philanthropy, which has enabled our rapid deployment and access to populations that would not otherwise be able to obtain testing. While the CARES Act mandates insurance coverage for SARS-CoV-2 testing, including providing coverage for the uninsured, universities without an affiliated hospital lack the insurance claims department necessary to access this financial support. We encourage government entities to consider grants to universities with testing facilities to enable these critical services to continue uninhibited and to enable expansion of the range of institutions capable of responding to this crisis.

Some observations based on initial test results are pertinent here. First, the range of viral titer in patient specimens can vary by six orders of magnitude, and we consistently detect positive specimens with a viral load that approaches our LOD. This range will affect the test sensitivity for pooled samples, raising concerns about the utility of such surveillance testing if widely implemented. Second, we observe positive specimens obtained by oral, nasal and mixed oral–nasal methods, but our data do not address the comparative sensitivity of these methods. Finally, we have detected a ~3.5% positivity rate for our total population tested, which is enriched for symptomatic or potentially exposed UC Berkeley-affiliated patients and low socioeconomic status or vulnerable community members (see "Resulting Outputs to Date" on Figshare). Although in aggregate this positive test result percentage agrees with that of other testing facilities in the San Francisco Bay Area, these positive samples are not evenly distributed among the populations we are serving. This observation, while not part of a controlled study, nonetheless underscores findings elsewhere that this

disease is disproportionately affecting communities experiencing existing health disparities[11–14]. Our observations emphasize the need for expanding access to testing and follow-up care for these communities.

To address continued large-scale surveillance needs, the IGI facility is developing saliva-based testing and eventually aims to implement serologic testing to enable better monitoring of population transmission and seroconversion rates. In keeping with our mission as a research institute, our facility also enables research on asymptomatic transmission and analysis of virus sequence evolution and provides benchmarking for new diagnostic technologies.

Although the challenges we faced were formidable, our experience and that of others demonstrates that they can be overcome. We encourage other institutions with a molecular biology department and health clinic with CLS staff to replicate or further amplify our approach and together create an invaluable resource for controlling this pandemic. ❑

Editorial note: This article has been peer reviewed.

IGI Testing Consortium*

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References

1. Ghebreyesus, T. A. WHO director-general's opening remarks at the media briefing on COVID-19, https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020 (11 March 2020).
2. Centers for Disease Control and Prevention. Implementation of mitigation strategies for communities with local COVID-19 transmission, https://www.cdc.gov/coronavirus/2019-ncov/downloads/community-mitigation-strategy.pdf (2020).
3. Li, R. et al. Science **368**, 489–493 (2020).
4. Guan, W.-J. et al. N. Engl. J. Med. **382**, 1708–1720 (2020).
5. Anonymous. An antibody test for the novel coronavirus will soon be available. The Economist https://www.economist.com/science-and-technology/2020/04/02/an-antibody-test-for-the-novel-coronavirus-will-soon-be-available (2 April 2020).
6. Bennhold, K. A German exception? Why the country's coronavirus death rate is low. The New York Times https://www.nytimes.com/2020/04/04/world/europe/germany-coronavirus-death-rate.html (4 April 2020).
7. Şimşek, A. COVID-19: Germany deaths hit 815, infection rateslows. Anadolu Agency https://www.aa.com.tr/en/europe/covid-19-germany-deaths-hit-815-infection-rate-slows/1788307 (1 April 2020).
8. US Food and Drug Administration. Guidance Document. Policy for Diagnostic Tests for CoronavirusDisease-2019 during the Public Health Emergency. Docket number FDA-2020-D-0987 (16 March 2020).
9. Applied Biosystems. TaqPath COVID-19 Combo Kit Instructions for Use. Publication Number MAN0019181, Revision B.0 (2020).
10. Thermo Fisher Scientific. TaqPath COVID-19 Multiplex Diagnostic Solution. https://www.thermofisher.com/us/en/home/clinical/clinical-genomics/pathogen-detection-solutions/coronavirus-2019-ncov/genetic-analysis/taqpath-rt-pcr-covid-19-kit.html (2020).

11. van Dorn, A., Cooney, R. E. & Sabin, M. L. *Lancet* **395**, 1243–1244 (2020).
12. Kim, E. J., Marrast, L. & Conigliaro, J. *J. Gen. Intern. Med.* https://doi.org/10.1007/s11606-020-05881-4 (2020).
13. Abrams, E. M. & Szefler, S. J. *Lancet Respir. Med.* https://doi.org/10.1016/S2213-2600(20)30234-4 (2020).
14. Godoy, M. & Wood, D. What do coronavirus racial disparities look like state by state? *NPR Shots* https://www.npr.org/sections/health-shots/2020/05/30/865413079/what-do-coronavirus-racial-disparities-look-like-state-by-state (30 May 2020).

Competing interests

The Regents of the University of California have patents issued and pending for CRISPR technologies on which J.A.D. is an inventor. J.A.D. is a cofounder of Caribou Biosciences, Editas Medicine, Scribe Therapeutics and Mammoth Biosciences. J.A.D. is a scientific advisory board member of Caribou Biosciences, Intellia Therapeutics, eFFECTOR Therapeutics, Scribe Therapeutics, Mammoth Biosciences, Synthego, Algen Biotechnologies, Felix Biosciences and Inari. J.A.D. is a director at Johnson & Johnson and has research projects sponsored by Biogen, Pfizer, AppleTree Partners and Roche.

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