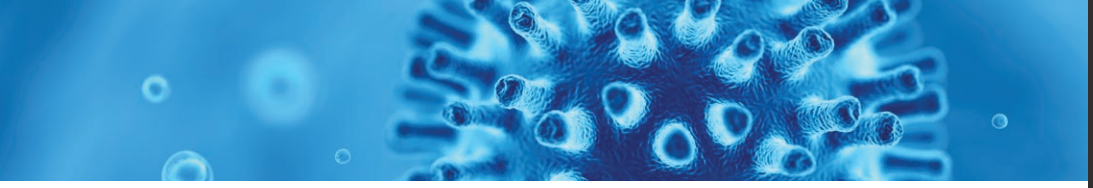

Northeastern University
College of Science

Rapid, Ultrasensitive Proximity Ligation Assay (PLA) for Potent SARS-CoV-2

Rapid, accurate, ultrasensitive, and cost-effective testing for infectious SARS-CoV-2 particles will play a critical role in controlling the COVID-19 pandemic. In particular, reliable diagnostic and detection tools that can be modified for both self-testing, and for high-throughput, population-based testing, will enable effective isolation practices to take place at the level of both the individual and the community.

BACKGROUND: Currently, the two main approaches for large-scale SARS-CoV-2 detection are: (i) Serological assays, which detect antibodies generated by infected persons by means of a blood draw or pin-prick; and (ii) Polymerase Chain Reaction (PCR) assays, which detect SARS-CoV-2 specific RNA sequences that are present in nasal swabs or blood samples.

While these approaches are useful, they have significant limitations as tools for effectively managing active COVID-19 infections. Serological tests are useful for identifying persons who have already been infected and developed immunity against COVID-19. PCR based detection of viral RNA is only 50–70 percent accurate; moreover, in many types of viral infections, viral RNA can still be detected in the body many weeks after clearance of the infectious virus.

INNOVATION: **Meni Wanunu** at Northeastern University has designed a diagnostic and detection method, *Rapid, Ultrasensitive Proximity Ligation Assay (PLA)*, which can detect the presence of intact SARS-CoV-2 virus capsids–the best possible proxy for **active, infectious virus.** This methodology is ultrasensitive (with the potential to detect even one virus per sample), draws from standard molecular biology tools and reagents, and can detect the virus from persons or surfaces. It can be formulated into detection kits that support rapid high-throughput testing, or rapid self-administered tests.

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The key innovation of the COVID-19 PLA test exploits the spiky protein structures on the virus' outer shell, and their propensity to bind to human proteins known as ACE2 proteins. The PLA approach provides a testing reagent comprised of synthetic ACE2 proteins, each attached to a single strand of DNA (an "oligo"). The single DNA strands are designed to connect to each other (to "ligate") only when they are close together. When two synthetic ACE2 proteins bind to neighboring viral proteins, their attached DNA strands will ligate together. The detection of a DNA sequence encompassing both strands of DNA indicates the presence of an intact, infectious virus **(figure 1)**.

IMPACT: Critically, this technology enables the detection of **intact virus particles** in a highly accurate manner. Test results will therefore support much better decision-making in terms of when to practice isolation and when to seek medical attention. Moreover, the simplicity of the basic testing approach allows it to be modified to a number of distinct, highly practical use cases. For example, the test could be self-administered via saliva swab, and visually detected within about 30 minutes by exposing a fluorescently activated strip to a cellphone light or sunlight. For facilities equipped with PCR expertise, potentially an airport, for example, prospective passengers could be evaluated for active virus within about an hour.

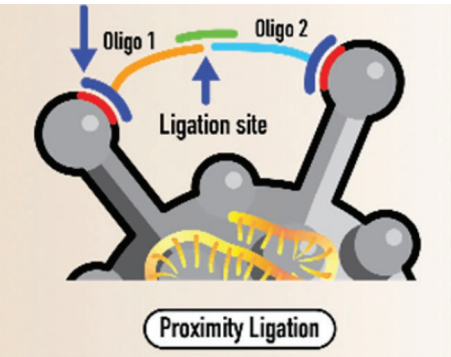

FIGURE 1

This technology could alternately be deployed on a population-scale, by collecting saliva samples across a community of potentially exposed individuals in vials that are pre-labeled with a unique DNA barcode. Samples would then be pooled at a test site and sequenced via a single high-throughput analysis, generating test results for up to millions of patients within about two days.

NEXT STEPS: With design steps completed, pilot data generation and optimization steps can be completed within six months. Testing with live virus in a BSL-3 lab is projected to require another three months. Subsequent reagent kit production and regulatory submission will require approximately another three months, for a total project timeline of one year.

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