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LLNL-TR-822650

# Final Report: Reveal-CoV Diaagnostic Platform

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May 17, 2021

## Disclaimer



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Lawrence Livermore National Laboratory

# Final Report: Reveal-CoV Diagnostic Platform

Lawrence Dugan

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Lawrence Livermore National Laboratory is operated by Lawrence Livermore National Security, LLC, for the U.S. Department of Energy, National Nuclear Security Administration under Contract DE-AC52-07NA27344.

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## Summary of work performed

LLNL designed and built a rapid, RT-LAMP-based molecular diagnostics platform as a potential tool to quickly diagnose COVID-19 in under one hour. This point-of-care testing approach involves an initial high temperature swab sample inactivation step followed by amplification of viral RNA using up to 5 control and pathogen-specific assays. Results are determined based on a discreet reaction color change from red to yellow but can also be determined using fluorescence detection. Testing of this prototype platform was conducted with synthetic viral RNA and dried, stabilized reagents. Buffer systems, swab selection, and assay stabilization formulations were evaluated for performance. Limits of detection were determined using RNA; however, testing was not performed with viable SARS-CoV-2 virus or clinical samples.

**Figure 1** shows the prototype instrument (left) developed to inactivate a sample in the Stage 1 heater and test for viral RNA with a single, pathogen-specific assay in Stage 2. The Stage 1 heating cycle required ~12 min to heat from RT to 95℃, maintain 95℃ for the required 5 min and then cool to a safe handling temperature below 45℃. Stage 2 heating is isothermal at 65℃ for 30 min and requires ~40 min from heat up to cool down to safe handling temperature.


**Figure 1. Reveal-CoV instrument development.** Left: First unit build of prototype single assay instrument consisting of two single-tube heaters for sample inactivatino (Stage 1) and detection assay amplification (Stage 2). Right: CAD model of 5 assay instrument concept consisting of a sample inactivation heater (Stage 1) and a 5-tube detection assay heater (Stage 2).

A redesigned instrument concept model is shown in Figure 1 on the right. The redesign incorporates a 5-tube heating block in Stage 2. This design change was pursued to allow us to incorporate additional assays into testing for improved specificity and potentially reduced limits of detection. Additionally, this redesign could allow testing against other pathogens, the use of independent control reactions and potentially incorporation of fluorescence detection.

Several key test parameters were studied to demonstrate improved test sensistivity and performance. Heat lysis/viral inactivation of samples was incorporated into the testing scheme based on testing performed on a surrogate human coronavirus, NL63. A 5 min hold at 95°C improved colorimetric detection ~10-fold as shown in **Figure 2** left. Selection of sampling swabs is critical such that the swab material does not interfere with the initial or final reaction color (Figure 2 center). Finally, the use of positive and negative controls along with three virus-specific assays provides added confidence that the testing performed as expected (Figure 2 right).

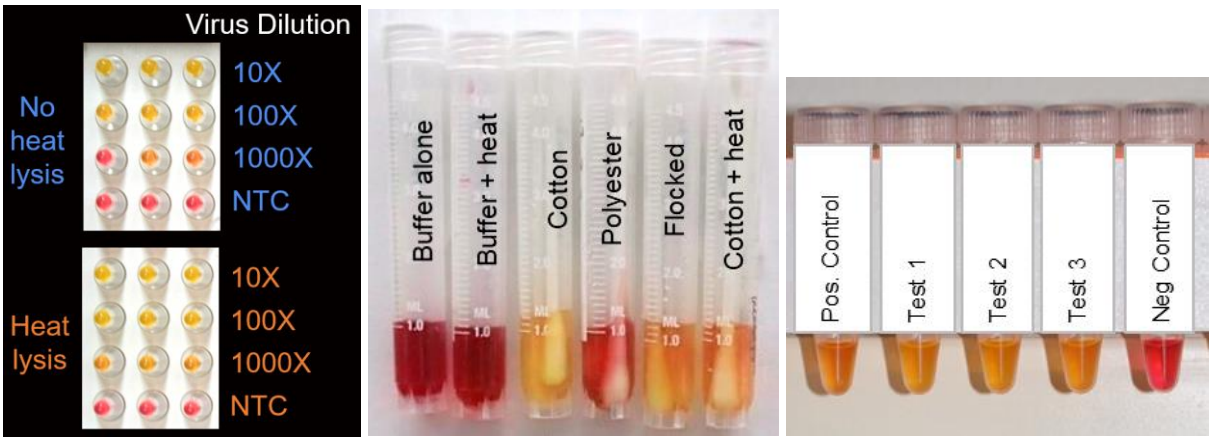


**Figure 2. Reveal-CoV test parpameter development.** Left: A pre-amplification heat lysis/sample inactivation step improves colorimetric endpoint detection ~10-fold Center: Commercial swabs were evaluated to identify products with minimal effect on reaction color. Right: A panel of 5 assays, including positive and negative controls and 3 virus-specific assays, were selected for optimal test performance.