

Electronic Supporting Information

System Description. The MAAI utilizes Autonomous Pathogen Detection System (APDS) technology, which was developed by Lawrence Livermore National Laboratory (LLNL) for the U.S. Department of Energy and the U.S. Department of Homeland Security.¹⁻⁵ The APDS platform, designed for airborne environmental bio-threat surveillance, was modified for this study to create an instrument suited for a clinical diagnostic laboratory that is capable of on-demand sample processing. The MAAI was designed to offer push-button operation, have the ability to simultaneously detect both DNA and RNA pathogens, and be self-contained with a small footprint (14" x 22"). Instrument hardware and software, the multiplex assay, and instrument operation are described below.

Hardware and Components. The MAAI fluidic platform is based on the FloPro 4P module by Global FIA, Inc. (Fox Island, WA) and consists of an X-Calibur pump (Cavro, Inc., Sunnyvale, CA) connected to three 14-port multi-position valves (VICI, Houston, TX) by tubing (perfluoroalkoxy (PFA) and Teflon® PFA HP tubing of 1/16" O.D. and 0.01", 0.02", and 0.045" I.D. - Cole-Parmer, Vernon Hills, IL and Upchurch Scientific, Oak Harbor, WA). The fluidic manifold also includes a flow-through thermal-cycler and a sequestering cell. The flow-through thermal-cycler is used to amplify the multiplexed RT-PCR reactions and to hybridize the products of these reactions to conjugated microspheres. The flow-through thermal-cycler is constructed with a heat-responsive 30 mm copper sleeve, which is similar to a previously described flow-through heater.⁶ The sequestering cell (Global FIA, Inc., Fox Island, WA) is a flow-through porous membrane that is able to retain microspheres during reporter incubation and subsequent washes.⁴

The fluidic manifold is connected to a custom engineered LX50 Luminex® analyzer that has all the capabilities of its larger LX100 predecessor (Luminex Corp., Austin, TX), but occupies roughly ½ the footprint (12.3" x 16.0"). The LX50 is equipped with a 635 nm laser for microsphere classification and a 532 nm laser to detect the presence of reporter molecules (*e.g.*, phycoerythrin and Cy3) associated with the microspheres. The Luminex® analyzer is able to accurately distinguish 100 different

classes of microspheres, each of which may be coupled to a probe that is specific to a particular pathogen.

The MAAI uses distilled water (Teknova, Inc., Hollister, CA) to move reagents through the fluidic manifold and sheath fluid (Luminex Corp., Austin TX) to prime the Luminex® analyzer and carry microspheres for analysis. These carrier fluids are held in 0.5 L and 1.5 L reservoirs, respectively. The multiplexed RT-PCR reagents, including: primer mix, enzyme/buffer mix, and streptavidin r-phycoerythrin conjugate (SA-PE) (Caltag Laboratories, Burlingame, CA), are held in amber 1.5 mL screw vials. Reagents used to clean and condition the system include: 100 mM Tris pH 8.0, 200 mM NaCl, 0.05% Triton-X 100 (TNT) buffer (Teknova, Inc., Hollister, CA), 70% isopropyl alcohol, and 1.3% sodium hypochlorite, which are held in 60 mL containers. Lastly, the microspheres, suspended by the rotational movement (400 rpm) of a stainless steel, paddled shaft driven by a Minimotor (Faulhaber, Switzerland), are held in a custom-made, light-protected 250 µL poly (methyl methacrylate) (PMMA) reservoir.

A Generation 4 Databrick computer controls the operation of the instrument and is connected to an ēlo touch-screen monitor (ēlo Touchsystems, Menlo Park, CA) via a standard serial cable. The Databrick has two serial COM ports, one for communication with the onboard LX50 analyzer and the other for communication with the X-Calibur syringe pump and multi-position valves via an RS232 - RS485 converter. The Databrick communicates with the flow-through thermal-cycler via a data acquisition (DAQ) card (National Instruments, Austin, TX). The DAQ card provides analog control for heating the resistors and reads back temperature from the resistance temperature device (RTD) within the heater.

Software and System Control. LLNL's in-house Instrument Scripting and Data Acquisition Terminal (ISDAT) software (Version 2.0.3 beta) was created using LabVIEW Version 7.1 (National Instruments, Austin, TX) and controls the system's components through developer-defined routines composed of configurable step-types and in-line commands. The ISDAT software is also used to configure assay and instrument parameters, including: thermal-cycler heating profiles, median fluorescence intensity (MFI) thresholds, selection of pathogens to be screened and their associated microsphere number, and the graphical user interface (GUI) touch-screen buttons. System scripts can

be added or modified as necessary to facilitate end-user specialized operations. The ISDAT software is used to send commands to and retrieve results from the LX50 analyzer.

References

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