**Supplementary Methods and Results**

**Optimization of nucleic acid extraction procedures**

Briefly, 400 µL whole blood or cerebrospinal fluid is inactivated with 400 µL Bacterial Lysis Buffer (BLB) (Roche Diagnostics, Indianapolis, IN, USA) and the total volume is placed in pre-filled 2mL tubes containing approximately 800 mg 0.5mm glass beads or VWR tough microorganism lysing mix that includes glass beads (VWR, Radnor, PA, USA). Next, 40 µL Proteinase K (25 mg/mL, Thermo Fisher Scientific, Waltham, MA, USA) and 400 µL Tris-EDTA (TE) buffer is added, the specimen is vortexed and incubated at room temperature for 15 minutes. The specimen is placed in the Precellys 24 Homogenizer (Bertin Corp., Rockville, MD, USA) for mechanical disruption at 5000 rpm for 60 seconds twice with a 5 second hold in-between. The specimen is centrifuged at 10,000×g for 1 min, and 700 µL of supernatant is placed in a new 2 mL tube. Rectal swab specimens are re-suspended in 1 mL of phosphate-buffered saline (PBS) and vortexed briefly. A portion of the specimen solution (400 µL) is subjected to the lysis procedure described above for blood and CSF specimens. Respiratory swab specimens (combined nasopharyngeal (NP) and oropharyngeal (OP) swabs) are inactivated by addition of BLB, but no additional lysis procedures are performed. Briefly, 350 µL transport media from combined NP/OP swabs is inactivated by addition of 350 µL BLB, 350 µL 25 mg/mL Proteinase K is added, the specimen is vortexed and incubated at room temperature for 15 minutes. Tissue specimens are immediately placed in a 2mL vial with 1 mL BLB and one 7 mm stainless steel bead; 40 µL Proteinase K (25 mg/mL) is added and specimen is vortexed and incubated at room temperature for 15 minutes. Next, the specimen is subjected to bead-beating at 4000 rpm for 90 seconds, centrifuged at 10,000×g for 1 min, and 700 µL of the supernatant is removed. Total volume of 700 µL of each specimen type is extracted for total nucleic acid (TNA) using the MagNA Pure Compact instrument (Roche Diagnostics, Indianapolis, IN, USA) with Nucleic Acid Isolation Kit I and Total NA Plasma External Lysis protocol with elution in 100 µL.

**Site-Specific Extraction Evaluation and Modifications**

Extraction protocols for use at each site were developed and optimized using BLB and the Roche MagNA Pure Compact instrument. However, lysis buffers from other manufacturers, including MagMax (Thermo Fisher Scientific) and Qiagen (Beverly, MA, USA), and other extraction platforms were substituted based on availability at each site. Extraction instruments used at CHAMPS site laboratories include NucliSENS easyMAG (bioMerieux, Inc., Durham, NC, USA) with NucliSENS lysis buffer (bioMerieux, Inc., Durham, NC, USA), KingFisher mL Purification System (Thermo Fisher Scientific) with MagMAX Viral RNA Isolation Kit (Thermo Fisher Scientific), and QIAamp DNA Mini Kit or QIAamp Fast DNA Stool Mini Kit with Buffer AL (Qiagen). Aside from the lysis buffer used, there were no deviations from the inactivation and off-board lysing protocols between sites. Following manufacturing instructions or established laboratory protocols, when necessary, additional buffer, carrier RNA, etc. was added following supernatant retrieval.

For the NucliSENS easyMAG instrument using NucliSENS lysis buffer a longer centrifugation step was incorporated following mechanical distribution due to the viscosity of the NucliSENS lysis buffer and the formation of bubbles. Carrier RNA was also added to the samples prior to extraction. For the MagMAX Viral RNA Isolation Kit there were modifications made to the manufacturing instructions for preparing the lysis/binding solution. When included in the lysis/binding solution during mechanical disruption, the isopropanol was precipitating nucleic acids and TNA was subsequently left behind in the bead-beating tube after the supernatant was removed; this was evident by a delayed TAC detection of all organisms spiked in the proficiency testing panels by 3 to 12 cycles. The solution was to leave the isopropanol out of the lysis/binding solution preparation and to add it to the supernatant in the cartridge of the KingFisher mL Purification System. Carrier RNA was also added to the supernatant with the isopropanol prior to extraction. For the Qiagen manual extraction kits, the manufacturer protocol was modified to accommodate a larger specimen input volume; due to a larger specimen volume, binding and washing steps were performed twice.

To evaluate different extraction platforms proficiency testing panels consisting of universal transport media (UTM) to simulate respiratory swab specimens, whole blood, or stool were prepared by spiking in quantified culture stocks of one or any combination of the following organisms: *Salmonella* spp. (non Typhi/Paratyphi), *Aeromonas* spp., *Escherichia coli* (non shiga toxin/vero toxin producing), *Campylobacter* spp., *Enterococcus* spp., Adenovirus, *Streptococcus* *pneumoniae*, *Streptococcus agalactiae*, *Streptococcus pyogenes*, *Haemophilus influenzae*, *Staphylococcus aureus*, *Cryptococcus* spp., Influenza A virus, *Chlamydia pneumoniae*, Parainfluenza virus 2, *Bordetella pertussis* and *Corynebacterium diphtheria*e. Specimens were extracted and tested at each site using the appropriate syndromic TAC. Results were analyzed at the site by trained laboratory staff and sent to the Respiratory Diseases Branch (RDB) at CDC for evaluation. Results were used to inform modifications to extraction procedures specific to each extraction platform and to ensure competency of laboratory staff at each site.