BioRobot EZ1 Workstation Compares Well with Manual Spin Kits for Extraction of Viral RNA from Sera and Saves Substantial Staff Time

The EZ1 BioRobot workstation is a desktop instrument for the automated extraction of nucleic acids from one to six samples. We assessed its performance, using the EZ1 Virus card, against a manual method by performing reverse transcription-PCR (RT-PCR) (RealArt Dengue LC RT-PCR kit; Artus, Germany) on a LightCycler (Roche Diagnostics, Germany) on RNA extracts that were extracted from 140 μ l (eluate, 60 μ l) and 100 μ l (eluate, 75 μ l) of sera with the Manual QIAamp Viral RNA Mini kit and the automated EZ1 Virus minikit, respectively (both from QIAGEN, Hilden, Germany). The Artus internal control (IC) was added to the master mix. RNA from the two methods was processed on the same PCR run. Two staff members tested 95 consecutive sera submitted to the diagnostic laboratory in 14 runs over a 3-week period. Interrun variation within each method was measured by testing 10 replicates; limited volumes dictated that different samples had to be used for the two methods.

Sixty-two samples were concordant and Dengue PCR positive, and 29 were concordant and Dengue PCR negative. Three discordant samples were positive with the EZ1 extract only (cycle thresholds [C_T], 31, 31, and 38), and one was positive only with the QIAamp extract (C_T , 36). Contamination was not specifically tested but seems unlikely for these discordant samples, as each one happened to be run directly after “low positive” samples that were PCR positive with a C_T of >30 . All four discordant extracts were from patients that were immunoglobulin M positive for anti-dengue antibodies (Dengue Duo IgM and IgG Rapid Strip Test; PanBio, Australia). One was from a patient strongly PCR positive on an earlier sample (C_T , 16).

The replicate standard deviations (SD) (and range) for the QIAamp Dengue PCR and IC C_T s were 0.56 (19.78 to 21.76) and 0.43 (25.61 to 26.74), respectively, and for the EZ1 were 0.36 (23.73 to 25.08) and 0.16 (27.54 to 28.05), respectively. The average of the differences between the Dengue PCR C_T values for the two methods was 0.74 cycle (SD, 1.48; range, -5.04 to 5.09); for the IC it was 0.45 cycle (SD, 0.67; range, -3.1 to 2.4).

The IC was negative in 8 QIAamp and 13 EZ1 extracts from 14 samples, although the Dengue PCR was positive for 12 of these by both methods. The remaining two sera were both PCR negative with the QIAamp, but one was positive with the EZ1. The average C_T s for QIAamp- and EZ1 Dengue PCR-positive extracts with a negative IC were quite low, 18 and 19.9, respectively, compared with the extracts with a positive IC, 26.5 and 27, respectively. The IC was negative in only 1 of the 29 concordant PCR-negative samples. The loss of IC in samples with higher viral loads suggests it is due to competition with the Dengue PCR, although the larger number of IC failures with EZ1 extracts may reflect a marginally greater propensity for inhibition. The “hands-on” time for technical staff for a run of six samples on the EZ1 was 10 min, compared to 1 h for the QIAamp kit. The runs lasted 45 min. The EZ1 did not fit inside our BioSafety cabinets. This is a drawback, as sample tubes are uncapped before being placed into the EZ1; preliminary neutralization steps are necessary if specific samples, such as sputa, constitute an appreciable biohazard.

As measured by RT-PCR results, the EZ1 delivered RNA extracts of quality similar to that of the QIAamp system, despite the smaller input and larger eluate. It is suited to a low-volume operation, but its flexibility makes it attractive for higher volume laboratories for “stat” work, as the ability to run single samples allows a quick response to priority specimens. It saves substantial staff time and reduces total test turnaround time, and its automation promises greater reproducibility.

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