



ThermoMixer-Aided Endpoint Quaking-Induced Conversion (EP-QuIC) Permits Faster Sporadic Creutzfeldt-Jakob Disease (sCJD) Identification than Real-Time Quaking-Induced Conversion (RT-QuIC)

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We recently reported a method called endpoint quaking-induced conversion (EP-QuIC) (1), which is modified from real-time quaking-induced conversion (RT-QuIC) (2–4), to aid in antemortem diagnosis of sporadic Creutzfeldt-Jakob disease (sCJD). In both QuIC reactions, the presence of minute amounts of misfolded prion protein induces the conversion of exogenous recombinant prion protein (rPrP) during intermittent shaking at 42°C (900 rpm for 90 s and rest for 30 s for each round), which is monitored fluorometrically (1–4). RT-QuIC uses a plate reader for the shaking step and needs approximately 90 h of incubation. However, shaking performed at high speed for extended periods of time increases the wear on the hardware, resulting in the need for repeated servicing of the system (1). On the other hand, EP-QuIC utilizes a benchtop shaking incubator (a ThermoMixer), and relative fluorescent units (RFU) are quantified at the beginning (RFU_{initial}; time zero) and end (RFU_{final}; time 90 h) of the reaction. While our report demonstrated that the diagnostic sensitivities and specificities of the two methods were in almost perfect agreement, it was observed that EP-QuIC reactions elicited higher RFU values at the 90-h reaction time and that the thresholds determined for the two versions of the test were distinctly different (1). This prompted us to investigate the dynamics of these two QuIC platforms.

Control material and diagnostically defined human cerebrospinal fluid (CSF) samples were tested. The negative control was artificial CSF (Harvard Apparatus), and the positive control was a 10⁻² dilution of product as seed (PAS), made from the positive QuIC reaction products from an autopsy-confirmed sCJD patient (5). Tests performed on these control samples were confirmed to be as highly reproducible as earlier tests (1). Table S1 in the supplemental material and graphs in Fig. 1 show results of testing randomly selected representative clinical samples; 6 autopsy-confirmed sCJD CSF samples and 7 diagnostically confirmed non-CJD (nCJD) CSF samples were analyzed by EP-QuIC and RT-QuIC as previously described (1), with additional manual reads of EP-QuIC plates performed at 16-, 40-, 47-, 64-, 72-, and 90-h intervals. The EP-QuIC reactions were found to take place faster, with all sCJD samples exhibiting a saturated signal at 47 h, while RT-QuIC signals from some sCJD samples were still increasing at 90 h. Single-well autoaggregation was seen with one negative control after 64 h and with two nCJD samples after 72 h on EP-QuIC and with one nCJD sample at 90 h on RT-QuIC. When the cutoff threshold at 47 h was set at a value of 2 (RFU_{final}/RFU_{initial}) for EP-QuIC, instead of the value

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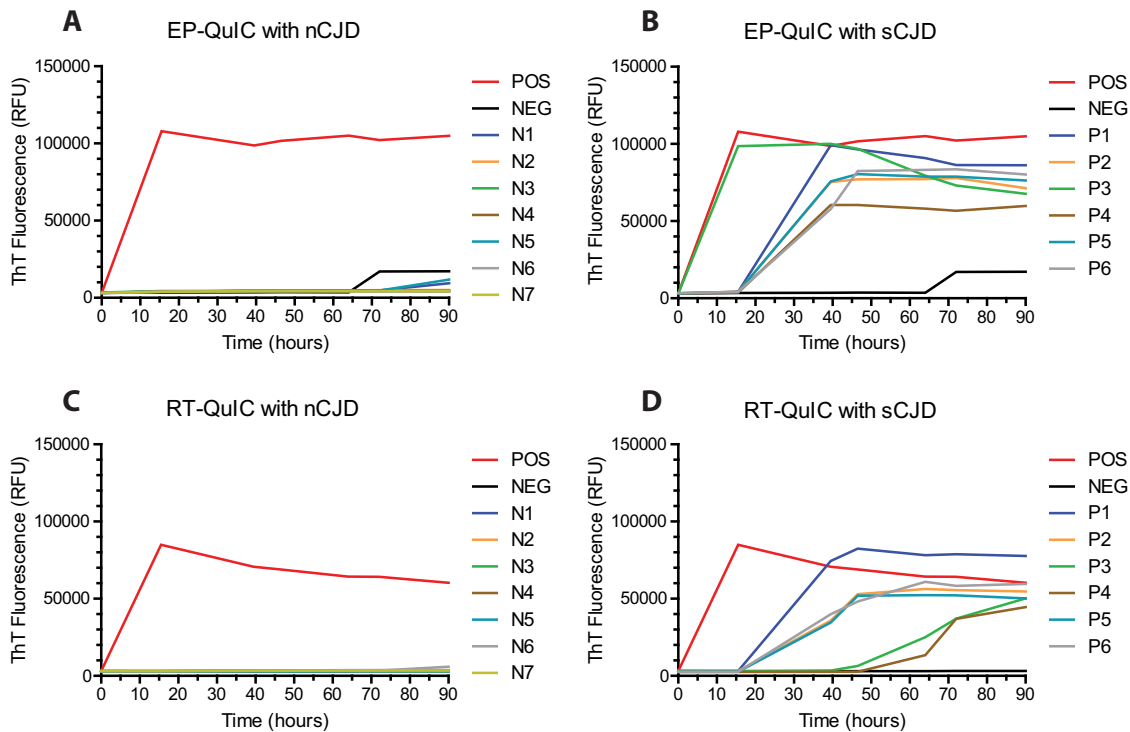


FIG 1 EP-QuIC and RT-QuIC dynamics of well-confirmed sCJD-negative (N) samples ($n = 7$) and sCJD-positive (P) samples ($n = 6$). The negative control (NEG) was artificial CSF, and the positive control (POS) was a 10^{-2} dilution of product as seed (PAS). Both EP-QuIC and RT-QuIC were performed on the basis of a method described in reference 1 (please see the main text for further details).

of 4 required for a 90-h reaction time (1), its sensitivity and specificity were same as those seen with RT-QuIC at 90 h when the same threshold was used (both were 100% sensitive and specific) (see Table S2A). Validation tests performed on 51 CSF samples (27 sCJD, 24 nCJD) confirmed this observation (see Table S2B).

Many factors affect QuIC reactions (6), and there has been no international standard determined for this test. The International Conference on Harmonisation (ICH) protocol for analytical method validation recommended by Health Canada and the U.S. FDA accepts a signal twice the level of noise (background) for qualitative analyses (7, 8). We highly recommend using EP-QuIC with daily reading for less than 48 h; if an average reading is twice the background level, a positive reaction should be called.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/JCM.00423-18>.

SUPPLEMENTAL FILE 1, PDF file, 0.3 MB.

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