Evaluation of the Altona RealStar® Parvovirus B19 PCR Kit 1.2: Comparison with the Roche LightCycler® Parvovirus B19 Quantification Kit

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Infections with human parvovirus B19 (B19) are common, and may cause severe disease in the context of immune suppression. Serology is used as a diagnostic tool and for guiding clinical care. Discontinuation of the Roche LightCycler® Parvovirus B19 Quantification Kit (Roche, Mississauga, ON) prompted a search for a suitable replacement.

Archived B19 DNA Stability: Original Roche B19 vs. Study Roche B19 Assay Qualitative Results

B19 DNA (+) and B19 DNA (-) status based on historical Roche LightCycler® Parvovirus B19 assay results.

Archived B19 DNA Stability: Original Roche B19 vs. Study Roche B19 Assay Quantitative Results

B19 DNA (+) and B19 DNA (-) status based on historical Roche LightCycler® Parvovirus B19 assay results.

Objective

Compare the Altona RealStar® Parvovirus B19 PCR kit 1.2 to the Roche LightCycler® Parvovirus B19 Quantification kit for the detection and quantitation of parvovirus B19 DNA.

Methods

Clinical specimens: Serum/plasma (n=32) and whole blood (n=5). B19 DNA (+) and B19 DNA (-) status based on historical Roche LightCycler® Parvovirus B19 assay results.

Nucleic acid extraction: B19 DNA isolated as DIC from date of extraction was used in this study. DNA was extracted using the Qiagen QIAamp DNA Mini Kit (Qagen Inc, Mississauga, ON). Amplification and detection were performed using the Roche LightCycler® 2.0 platform.

Parvovirus B19 DNA detection and quantitation: The Roche LightCycler® Parvovirus B19 quantitation kit (1) and Altona RealStar® Parvovirus B19 PCR kit 1.2 (Altona Diagnostics, Hamburg, Germany) were used as per the respective manufacturer’s instructions. Amplification and detection were performed using the Roche LightCycler® 2.0 platform.

Stability of archived B19 DNA target: Archived DNA (n=32) was re-tested using the Roche B19 assay: contemporary vs. original testing results (qualitative (+) and (-) values) were compared.

Altona RealStar® Parvovirus B19 PCR kit 1.2 performance: Source material: serial 10-fold dilutions of DNA from a high-titre clinical specimen.

Results

Altona B19 vs. Roche B19 Assay Qualitative Result Comparison

Altona CT vs. Roche CT

Altona B19 vs. Roche B19 Assay Quantitative Result Comparison

Altona B19 PCR B19 DNA (-) Assay

Intra- and inter-assay %CVs of the Altona assay were 1.3 and 0.8, respectively.

The Altona assay detected B19 in one specimen (CT 22, 1.22 X 10⁶ IU/mL) that was repeatedly inhibited (negative Roche LightCycler® Parvovirus B19 assay). This suggests the presence of inhibitor(s) selectively active against Roche kit reaction constituents.

When used as qualitative assays, results obtained by Altona and Roche were strongly correlated (R²=0.96). Overall, the Altona assay produced slightly lower CT values, generating a positive result on average 1.3 cycles earlier than the Roche assay.

Quantitation of 37 specimens showed results generated by both Altona and Roche assays were strongly correlated (P<0.01). On average, Altona viral load results were 10⁻² times (17.6-fold) higher than those obtained by the Roche assay.

The Altona assay exhibited an 8-log linear range (10⁻⁸ to 10²) with a limit of detection of 110 IU/mL.

Based on the limited evaluation of precision performed here (2 runs in triplicate of a mid-value viral load specimen), inter- and intra-assay %CVs of the Altona assay were 1.3 and 0.8, respectively.

Summary / Conclusion

Archived B19 DNA was stable upon storage at -20°C, validating the use of this material for the evaluation of the Altona RealStar® Parvovirus B19 PCR kit 1.2.

The Altona B19 assay detected B19 in one specimen (CT=22, 1.22 X 10⁶ IU/mL) that was repeatedly inhibited (negative IC, negative B19 DNA) by the Roche LightCycler® Parvovirus B19 assay. This suggests the presence of inhibitors selectively active against Roche kit reaction constituents.

When used as qualitative assays, results obtained by Altona and Roche were strongly correlated (R²=0.96). Overall, the Altona assay produced slightly lower CT values, generating a positive result on average 1.3 cycles earlier than the Roche assay.

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References

1. Roche LightCycler® Parvovirus B19 Quantification Kit User-specific conventions: 1.0±0.2 copies (95% CI 2.0±0.44). 2. Altona RealStar® Parvovirus B19 Quantification Kit REF 0323460101 product insert.