Materials and Methods

For the purposes of this evaluation, cases with fewer than 30 repeats were considered non-expanded, whereas cases with 100 repeats or greater were considered expanded. There were no cases within the 31-99 repeat range. The AmplideX® PCR/CE C9orf72 Kit was initially evaluated at both the Mayo Clinic (Site 1) and Asuragen, Inc. (Site 2) using non-clinical, non-chronic DNA samples previously tested using a validated laboratory-developed test (LDT) consisting of an amplification PCR assay with reflex to Southern blot.

The LDT amplicon-length PCR assay consists of a genotyping PCR in which the repeat size in base pairs to repeat number and corrects for the differential mobility of GC-rich DNA. The LDT amplicon-length PCR ratio was defined repeat size approximation of the XbaI digest using an end-labeled 1kb ladder. Measurement of the repeat size by the AmplideX® PCR/CE C9orf72 Kit was able to accurately resolve zygosity (non-expanded homozygous versus expanded heterozygous cases) and identify the presence of C9orf72 repeat expansion in all cases that had been reflexed to Southern blot analysis. (Table 2, Figure 4).

Conclusions

- Evaluation of the AmplideX® PCR/CE C9orf72 Kit, a single-tube, combined amplicon-length and repeat-primed long-read PCR assay for C9orf72 repeat expansion detection.
- In our hands, the AmplideX® PCR/CE C9orf72 Kit showed reliable detection of C9orf72 repeat expansions, with 98% agreement for previously clinically tested cases, the single discordant case was caused by interference of a new discordant which the kit did not result would not have significant clinical impact.
- This innovative assay relies on a combined workflow in a single reaction that amplifies a few homogenous time, reduced hands-on-time and required application to our laboratory-developed test (amplicon-length PCR assay) with reflex to Southern blot, and has potential utility for clinical diagnostic testing.

References