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Comparison and Validation of a dsDNA biomimetic Quality Control Reference for NGS based BRCA CNV analysis versus MLPA

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Abstract : Background: There remains a lack of International Standard Control Reference materials for Next Generation Sequencing-based approaches or device calibration. We have designed and validated dsDNA biomimetic reference materials for targeted such approaches incorporating proprietary motifs (patent pending) for device/test calibration. They enable internal single-sample calibration, alleviating sample comparisons to pooled historical population-based data assembly or statistical modelling approaches. We have validated such an approach for BRCA Copy Number Variation analytics using iQRS™-CNVSUITE versus Mixed Ligation-dependent Probe Amplification. Methods: Standard BRCA Copy Number Variation analysis was compared between mixed ligation-dependent probe amplification and next generation sequencing using a cohort of 198 breast/ovarian cancer patients. Next generation sequencing based copy number variation analysis of samples spiked with iQRS™ dsDNA biomimetics were analysed using proprietary CNVSUITE software. Mixed ligation-dependent probe amplification analyses were performed on an ABI-3130 Sequencer and analysed with Coffalyser software. Results: Concordance of BRCA – copy number variation events for mixed ligation-dependent probe amplification and CNVSUITE indicated an overall sensitivity of 99.88% and specificity of 100% for iQRS™-CNVSUITE. The negative predictive value of iQRS-CNVSUITE™ for BRCA was 100%, allowing for accurate exclusion of any event. The positive predictive value was 99.88%, with no discrepancy between mixed ligation-dependent probe amplification and iQRS™-CNVSUITE. For device calibration purposes, precision was 100%, spiking of patient DNA demonstrated linearity to 1% (±2.5%) and range from 100 copies. Traditional training was supplemented by predefining the calibrator to sample cut-off (lock-down) for amplicon gain or loss based upon a relative ratio threshold, following training of iQRS™-CNVSUITE using spiked iQRS™ calibrator and control mocks. BRCA copy number variation analysis using iQRS™-CNVSUITE™ was successfully validated and ISO15189 accredited and now enters CE-IVD performance evaluation. Conclusions: The inclusion of a reference control competitor (iQRS™ dsDNA mimetic) to next generation sequencing-based sequencing offers a more robust sample-independent approach for the assessment of copy number variation events compared to mixed ligation-dependent probe amplification. The approach simplifies data analyses, improves independent sample data analyses, and allows for direct comparison to an internal reference control for sample-specific quantification. Our iQRS™ biomimetic reference materials allow for single sample copy number variation analytics and further decentralisation of diagnostics to single patient sample assessment.

Keywords : validation, diagnostics, oncology, copy number variation, reference material, calibration
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