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South African Breast Cancer Mutation Spectrum: Pitfalls to Copy Number Variation Detection Using Internationally Designed Multiplex Ligation-Dependent Probe Amplification and Next Generation Sequencing Panels

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Abstract : The National Health Laboratory Services in Bloemfontien has been the diagnostic testing facility for 1830 patients for familial breast cancer since 1997. From the cohort, 540 were comprehensively screened using High-Resolution Melting Analysis or Next Generation Sequencing for the presence of point mutations and/or indels. Approximately 90% of these patients stil remain undiagnosed as they are BRCA1/2 negative. Multiplex ligation-dependent probe amplification was initially added to screen for copy number variation detection, but with the introduction of next generation sequencing in 2017, was substituted and is currently used as a confirmation assay. The aim was to investigate the viability of utilizing internationally designed copy number variation detection assays based on mostly European/Caucasian genomic data for use within a South African context. The multiplex ligation-dependent probe amplification technique is based on the hybridization and subsequent ligation of multiple probes to a targeted exon. The ligated probes are amplified using conventional polymerase chain reaction, followed by fragment analysis by means of capillary electrophoresis. The experimental design of the assay was performed according to the guidelines of MRC-Holland. For BRCA1 (P002-D1) and BRCA2 (P045-B3), both multiplex assays were validated, and results were confirmed using a secondary probe set for each gene. The next generation sequencing technique is based on target amplification via multiplex polymerase chain reaction, where after the amplicons are sequenced parallel on a semiconductor chip. Amplified read counts are visualized as relative copy numbers to determine the median of the absolute values of all pairwise differences. Various experimental parameters such as DNA quality, quantity, and signal intensity or read depth were verified using positive and negative patients previously tested internationally. DNA quality and quantity proved to be the critical factors during the verification of both assays. The quantity influenced the relative copy number frequency directly whereas the quality of the DNA and its salt concentration influenced denaturation consistency in both assays. Multiplex ligation-dependent probe amplification produced false positives due to ligation failure when ligation was inhibited due to a variant present within the ligation site. Next generation sequencing produced false positives due to read dropout when primer sequences did not meet optimal multiplex binding kinetics due to population variants in the primer binding site. The analytical sensitivity and specificity for the South African population have been proven. Verification resulted in repeatable reactions with regards to the detection of relative copy number differences. Both multiplex ligation-dependent probe amplification and next generation sequencing multiplex panels need to be optimized to accommodate South African polymorphisms present within the genetically diverse ethnic groups to reduce the false copy number variation positive rate and increase performance efficiency.

Keywords : familial breast cancer, multiplex ligation-dependent probe amplification, next generation sequencing, South Africa
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