Editorial

Nuance in Choosing Controls for RNA-Based Gene Fusion Test

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INTRODUCTION

Next Generation Sequencing (NGS) has become a widely adopted sequencing methodology in determining gene sequence changes, chromosomal structure arrangement, and even RNA transcripts alterations. Identifying the genetic variations in human cancers is especially important in determining the proper treatment regimen or disease prognosis. The quality control materials used for these NGS tests thus become increasingly important in implementing clinical- grade NGS testing. Traditionally, control materials were derived from cell line genomic DNA(s) for investigating single nucleotide variants and small indels. However, some complex genetic abnormality such as gene fusions resulted from chromosome translocation has very limited cell lines harboring designated fusion genes to use as controls. A recent FDA approved anti-cancer drug, larotrectinib, targeting NTRK gene fusion in advanced malignancies exemplifies such genetic testing may have direct impact on cancer patient care. NTRK gene fusions are relatively uncommon and only occur in 1% or less of all major solid tumors. It is necessary for a laboratory to choose the proper positive control samples to address test sensitivity and specificity in identifying NTRK fusions. Since Formalin-Fixed Paraffin- Embedded (FFPE) tissues are the most common forms of archiving oncological specimens and are tested for gene fusions, positive controls of FFPE format are the most appropriate materials to use. High quality nucleic acids extracted from non-FFPE cells, or synthetic spike-in oligonucleotides may be used initially to establish the assay performance characteristics, but they should not be used as control materials in routine clinical operation.

There are two commercial fusion gene controls composed of engineered cell lines with artificially created fusion genes, including NTRK fusions, and have underwent FFPE preparation. The first is Seraseq™v2 (Sera care, Milford, MA, USA) that contains 16 known fusion genes with the transcripts quantified by digital PCR. We have tested Seraseq™v2 and all fusion events were identified as the vender advertised. However, a laboratory does pay a premium for this control material. For long-term, routine usage of such control, the financial burden must be considered in today's challenging test reimbursement environment. Alternatively, Horizon Discovery (Cambridge, UK) HD796 provides a similar FFPE control with five known fusion genes. The fusion genes in HD796 are qualitatively, instead of quantitatively, assayed by the manufacture using end-point RT-PCR as a quality check. This control is less than half of

the Sera care's price, which provides the laboratory a cost-effective method to run positive controls in performing RNA based NGS fusion gene testing.

In our hands, the five fusion products of HD796 were all identified robustly in the past eight- month operation. Surprisingly, the NTRK1 fusion was not identified in a recent NGS run of newly purchased HD796, even when further testing a fresh, unopened tube of the same lot number. After some laborious troubleshooting procedures, we concluded that the missing NTRK1 fusion was neither due to testing personnel's bench technique, nor related to instrumentation and NGS analysis pipelines. When we contacted the vender for additional product information, the provided RT-PCR QC documentation showed an extremely faint NTRK1 fusion product compared to the previously working reagent lots. It became clear at this point that the fusion gene expression levels in this control may vary from lot-to-lot, which most likely occurred during complex processes of cell line engineering to combine multiple fusion transcripts.

Commercially available FFPE fusion controls provide convenience for molecular diagnostic laboratories to validate and routinely perform NGS-based test(s). From what we have learned, a quantified fusion control is a better choice (i.e. Seraseq™v2) if the laboratory could manage the associated higher cost. Alternatively, for most laboratories that operate on a restricted budget, fusion controls that are not quantified (i.e. HD796) could be an option with an added caution. That is, we highly recommend obtaining the RT-PCR QC results of the control samples from the vender before performing the test. Therefore, a laboratory would only run those control reagent lots with sufficient amount of mixed fusion gene products, and avoiding the unnecessary troubleshooting steps due to the control "failure". By doing so, a laboratory will ensure the proper test turnaround time and be compliance with the proper quality management program.

Conflict of Interest

The author declares no conflict of interest.