ApplicationForum

Evaluating EGFR Genotyping using HDx™ Reference Standards

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"People are always begging friends and colleagues for reference material - It is precious"

Dr. Marsha Speevak

Introduction

Excessive activation of EGFR has been shown to be associated with advanced stages of cancer and a poor prognosis found in several types of non-small cell lung cancer. Detection and identification of these mutations has become a critical factor in stratifying patients who will benefit from anti-EGFR targeted therapies and those who are less likely to respond.

In particular, non-specific and inefficient probe binding in mutations can lead to late crossing points creating these false-negatives as identified in the proficiency testing schemes. EGFR G719A/S and T790M are particular mutations that have been highlighted as areas of high error.

HDx™ Reference Standards from Horizon Diagnostics are provided as Research Use Only reference materials. Trillium Health Partners have adopted HDx Reference Standards to evaluate performance characteristics and have provided the data within this report. No financial transactions have taken place between parties for the provision of data within this report.

Methodology

Kit: Entrogen EGFR Kit (EGFR exons 18, 19, 20 and 21)

Sequencer: Roche LC480 Real Time PCR

About: Allele discriminating assays rely upon the ability of the probes to bind correctly and the software setting to correctly set a crossing point baseline. HDx Reference Standards were used to evaluate the assay and establish the detection limits for each mutation.

EGFR HDx Reference Standards as DNA were used at allelic frequencies of 50%, and matched Wild Type was used to dilute mutations down to a 2.5% allelic frequency.

Results

HDx Reference Standards were used for four applications: assay evaluation and determination of the limit of detection for each mutation; highlighting of problematic probe bindings; comparison of an assay's performance between mutations; and for the identification of mutations that are true positives but have low percentage tumor content.

Assay evaluation

Trillium Health Partners were able to quickly determine the limit of detection and assay specific crossing point cut-offs for each available mutation. The different allelic frequencies used in the assay evaluation for EGFR L861Q are highlighted in Figure 1.

Figure 1 – Assay evaluation specific crossing points cut-offs for EGFR L861Q

Highlighting problematic probe bindings

Mutation reactions can be prone to inefficient and non-specific probe binding, leading to late crossing points even in the absence of a mutation. This problem can be seen in the data gathered in this study, showing false-positive results for mutations G719X and T790M in the negative controls.

Figure 2 – Assay evaluation specific crossing points cut-offs for EGFR L861Q

Comparison of an assay's performance between mutations

The results showed a 95% confidence level in HDx Reference Standards (from 17 assays) with a mean Crossing Point: +/- 0.26 (L858R) to +/- 0.56 (G719X). The adoption of the 2.5% EGFR HDx Reference Standard allows Trillium Health Partners to evaluate the workflow to a higher degree of sensitivity in addition to the in-kit positive control.

Figure 3– Comparison of crossing points for EGFR mutations

Identification of mutations that are a true positive but have low percentage tumor content

When the mutation is in a low percentage of cells in the sample, the crossing point will be high and possibly below the 2.5% established cut-off. Although the samples' crossing points are later than that of the negative control, qualitative assessment must be used to evaluate each individual case. This can be seen in the G719X reaction example.

Figure 4 – A suggested true positive by qualitative assessment at 1-2%

Conclusions

- Crossing point values alone do not tell the whole story
- A high crossing point above the established cut-offs determined during assay evaluation can indicate true positives
- HDx Reference Standards provide support for assay evaluation to establish key performance characteristics
- Low allelic frequency Reference Standards supply a qualitative reference for true amplification

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