Detection of EGFR T790M resistance mutation: real-time allele-specific PCR versus Sanger sequencing

S.Y. Hor, K.S. Chan, E.X. Chen, M. Goh, L.L.E. Oon
Pathology, Singapore General Hospital, Singapore

Methods: Ninety-six FFPE samples sent to our laboratory for T790M mutation detection by cobas® EGFR Mutation test (Roche), between July 2014 and March 2016 were included in this study. Archived extracts were used for Sanger sequencing of EGFR exon 20 using an in-house developed protocol.

Results: T790M was detected in 49.5% (47/95) and 47.9% (46/96) of the samples by the cobas® assay and Sanger sequencing respectively. Taking cobas® assay as the gold standard for 95 samples with valid real-time PCR results, Sanger sequencing yielded a sensitivity of 95.7% (45/47) and specificity of 100% (48/48). Of the 3 discordant results, cobas® assay detected T790M in 2 samples (both with tumour contents <30%) not detectable by Sanger sequencing; while Sanger sequencing detected T790M in one sample which repeatedly yielded an inconclusive result for exon 20 on the cobas® assay. Overall, concordance rate between the two methods was 96.9% (93/96).

Conclusions: Sanger sequencing was generally comparable to, but slightly less sensitive than real-time PCR in detecting T790M. Sanger sequencing may be useful in the event of inconclusive results by the real-time assay. Otherwise, for post-treatment T790M mutation testing, where initial driver mutations had already been identified, Sanger sequencing, which requires samples with higher tumour content, offers little advantage over real-time PCR.

Legal entity responsible for the study: Singapore General Hospital

Funding: Singapore General Hospital

Disclosure: All authors have declared no conflicts of interest.