Oral Presentation

Impact of Quality and Quantity of DNA samples on KRAS Mutation Analysis

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Background: Many techniques based on polymerase chain reaction (PCR) have been utilized to detect KRAS mutation, a negative predictor of anti-EGFR treatment efficacy. Inaccurate mutation test can result from inefficient PCR amplification in poor quality DNA due to formalin-induced DNA degradation or the presence of PCR inhibitors.

Objective: This study aims to investigate the overall quality of DNA isolated from Thai colorectal cancer specimens and appropriate DNA amount for detection of KRAS mutation.

Methods: We extracted DNA from 159 formalin-fixed paraffin-embedded (FFPE) tissues submitted to our laboratory for KRAS mutation analysis. Genomic DNA was measured by spectrophotometer. We utilized quantitative PCR technique to assess the quality of DNA. Different amounts of DNA were added in each PCR reaction to evaluate proper DNA quantity for amplification.

Results: The quality of DNA isolated from FFPE tissues varies considerably from sample to sample. Most of extracted nucleic acid samples were poorly amplified. PCR amplification was simply enhanced by addition of DNA amount in three quarters of the specimens. The rest of the samples exhibited PCR inhibition when DNA quantities were increased. Ineffective PCR can not be predicted by spectrophotometry.

Conclusion: DNA quality assessment before KRAS mutation analysis in FFPE specimens is a critical and often overlooked step. DNA amount in the reaction could be adjusted in each case to rescue the chance of detecting mutation in DNA samples of poor quality and ensure optimized PCR amplification.