Background

Extended RAS testing in codons 12, 13, 59, 61, 117, and 146 in exons 2, 3, and 4 of the KRAS and NRAS genes is now mandatory before anti-EGFR therapy in metastatic colorectal cancer (mCRC) patients. BRAF and EGFR ectodomain mutation status have been shown to play a crucial role in predicting non-responsiveness in mCRC patients receiving anti-EGFR therapy. Current methods for testing of extended RAS, BRAF and the new EGFR ectodomain mutations are laborious and often require 5-20 working days.

Methods

The Idylla™ platform (BioCartis, Mechelen, Belgium) is a CE marked, fully automated sample-to-result, real-time PCR molecular diagnostics system. The Idylla NRAS-BRAF-EGFR S492R Mutation Assay (1) is an assay for formalin-fixed paraffin-embedded (FFPE) tissue samples from mCRC patients for the analysis of 18 NRAS (codons 12, 13, 59, 61, 117, and 146 in exons 2, 3, and 4), 5 BRAF (codon 600), and 2 EGFR (codon 492) mutations. The Idylla™ NRAS-BRAF-EGFR S492R Mutation Assay was compared with routine sequencing technologies at five centers (BioPath Innovations, Rigshospitalet Copenhagen, Hôpital Erasme, Hospital del Mar, BioCartis). In addition, LOD was established using cell lines embedded in paraffin containing defined ratios of mutants and dilutions thereof in a FFPE WT background.

(1) For research use only

Results

Positive predictive agreement was 97.2% (n=106), 100% (n=40) and 100% (n=1) and negative predictive agreement was 99.4% (n=348), 99.5% (n=412) and 100% (n=444) for NRAS, BRAF and EGFR, respectively. One BRAF V600E sample detected by Idylla™ only was confirmed with digital droplet PCR. No cross-reactivity was observed in KRAS positive samples (>80 samples) indicating the high specificity of the assay towards NRAS mutations. Fourteen mutations showed an LOD of ≤5%, 10 mutations of ≤5% and one mutation showed an LOD of 6% with 95% confidence.

Conclusion

Given its high sensitivity and specificity, the Idylla™ NRAS-BRAF-EGFR S492R Mutation Assay is ideally suited for rapid detection of NRAS, BRAF and EGFR mutations. Together with the Idylla™ KRAS Mutation Test (CE-IVD), sample-to-result extended RAS testing on 39 mutations can now be performed in only 2 hours.