Absolute Quantification of EGFR Activation and Resistance Mutations by Droplet Digital PCR in Circulating Nucleic Acids

**Objective:**
- To design a methodology for the detection of EGFR mutations in plasma samples.
- To evaluate the performance of droplet digital PCR (ddPCR) for the detection of EGFR T790M and L858R mutations.

**Methods:**
- Used droplet PCR technology to analyze plasma samples from a variety of sources (NSCLC, colorectal cancer, healthy donors).
- Employed a Bio-Rad proprietary ddPCR mix for amplification.
- Analyzed plasma samples for EGFR T790M mutations.
- Established ddPCR methodology for detection and quantification of EGFR mutations in plasma.

**Results:**
- ddPCR data was compared with qPCR data for accuracy.
- LLOD for the EGFR T790M, L858R, and Exon 19 del mutation assays.
- ddPCR data validated by qPCR, showing high sensitivity and specificity.

**Conclusion:**
- ddPCR is a powerful tool for the detection of EGFR mutations in plasma, offering high sensitivity and specificity for clinical applications.

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**Figure 1:** Figure 1: Droplet Digital PCR – Rare Mutant DNA in Plasma

Well as EGFR wild type colorectal cancer and healthy donor plasma samples were analyzed.

**Figure 2:** Figure 2: ddPCR Methodology and Sample Set as used in Figure 4.

To enable detection of mutations within the same sample.

**Figure 3:** Figure 3: The LLOD for the T790M assay was ~1.2% which is greater than the LLOD in any specimen, and all had 95% confidence interval for no template controls.

**Figure 4:** Figure 4: ddPCR data compared to those generated with the blinded NSCLC.

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