

## **Impact of reference materials for analytical performance evaluation of liquid biopsy NGS assays**

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### **Background & objectives**

Liquid biopsy (LB) enables the non-invasive analysis of genetic tumor variants from circulating free DNA (cfDNA) in plasma to support personalized treatment. The majority of cancer patients present with actionable tumor variants with variant allele frequencies (VAFs) <1%. Therefore, accurate analytical validation of LB assays at the lower end of the measurement scale is mandatory, especially regarding the In Vitro Diagnostic Regulation (IVDR). Selection of appropriate reference materials is the first and key prerequisite for this purpose.

We tested three commercially available reference materials for their suitability to be used for LB NGS assay validation. First, background noise was determined, which is critical for accurate assessment of specificity and sensitivity. Second, the similarity of the reference materials to native cfDNA was evaluated.

### **Methods**

NA24385-based wild-type (WT) cfDNA reference materials from SensID, Coriell, and SeraCare and 15 patient samples were analyzed using Duplex Sequencing-based LB NGS assay.

### **Results**

To investigate the background noise of the three different WT reference materials, which are important for the evaluation of specificity, the number of variants <0.1% VAF was determined and compared to patient samples. In both SensID and Coriell materials, <0.1 variants/kb were detected, whereas in SeraCare materials, 16.9 variants/kb were detected. For comparison, <0.1 variants/kb were detected in the 15 patient samples. Accordingly, both the SensID and Coriell materials showed very low background noise at low VAFs, which are in the same range as the signals observed in patient samples and are therefore highly suitable for specificity determination.

To test the closeness of agreement with native cfDNA, DNA profiles and library yields of reference materials were compared to patient samples. The SeraCare cfDNA reference material more closely resembled native cfDNA than SensID and Coriell materials, allowing for more accurate development of experimental protocols.

## **Conclusion**

In summary, careful consideration of commercially available reference materials is required for performance evaluation of LB NGS assays. While reference materials with well-defined variants are preferable for determining specificity, reference materials that closely resemble native cfDNA aid in the development of experimental protocols.