# Comparison of circulating tumor cell enrichment techniques considering tumor heterogeneity

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## Background & objectives

The quantification and characterization of circulating tumor cells (CTCs) in blood have shown to be of prognostic value in several solid cancer entities and can help to optimize individualized therapy. CTC enrichment methods are in general based on 1) the expression of markers typical for epithelial cells but not for blood cells or 2) based on physical properties of tumor cells that distinguish them from blood cells (e.g., size or deformability). However, due to tumor heterogeneity, each enrichment method is potentially biased for enriching certain CTC populations only while failing to enrich others. These limitations hamper CTC assays being utilized in the clinic. To evaluate different types of CTC enrichment techniques using different cancer entities reflecting tumor heterogeneity.

## Methods

Four different enrichment technologies were assessed, these were label-dependent (CellSearch System), size- and deformability based (Parsortix), size- and density-based (Slanted spiral microfluidics), and density-based (CTCeptor). In order to simulate tumor heterogeneity, blood was separately spiked with 7 different cell lines: two breast, three lung, one ovarian, and one CTC breast cancer cell line. These cell lines differed in EpCAM expression, cell size, and cell density.

## Results

On average, the CellSearch, Parsortix, and Slanted spiral recovered a similar number of cells, whereas the CTCeptor outperformed all other three methods with 17-21% ($p<0.001$). This difference could be attributed to the cancer entities. For breast cancer cell lines, the CTCeptor outperformed the CellSearch System with an average of 30% ($p<0.001$) and the Parsortix with 27% ($p<0.001$), but not the Slanted spiral microfluidics. However, the CTCeptor did outperformed the Slanted spiral microfluidics for lung cancer cell lines with 26% ($p<0.01$).

## Conclusion

Because of tumor heterogeneity there is probably no “one-size-fits-all” technology to enrich CTCs from blood of cancer patients. Nevertheless, we showed that the novel CTCeptor can reach high recovery rates, comparable to that of other system and even outperforming some depending on the cancer entity. Blood volume and throughput, however, remain an issue to be resolved, especially in early-stage cancer.