

## **Comparative study of streptavidin-coupled ferrofluids and magnetic beads for capture of circulating tumor cells in patients with disseminated prostate cancer.**

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### **Background & objectives**

Small numbers of circulating tumor cells (CTCs) is a key challenge in detection, and many efforts are being put into increasing assay sensitivity. Multiple publications have focused on the effect of using different types of magnetic beads in samples spiked with cancer cells, and small magnetic particles have been reported advantageous over larger beads in terms of recovery of cultured cells. However in our opinion, more comparative studies on clinical samples are warranted to aid researcher on their choice of magnetic beads for positive enrichment of CTCs.

Here, we compared two commercially-available streptavidin-coated magnetic particles of different size for isolation of cancer cells in spike-ins and prostate cancer (PCa) patient samples.

### **Methods**

To assess the capture efficiency of our assay using the two magnetic reagents, we spiked pre-stained cultured cancer cells into blood from healthy volunteers. Red blood cells were lysed, and the remaining cells were incubated with biotinylated rVAR2, which binds multiple surface proteins expressed on cancer cells. Subsequently, streptavidin-coated magnetic ferrofluids (R&D Systems) or beads (Cytvia) were added. Captured cells were paraformaldehyde fixed, stained with DAPI and manually analyzed after imaging by scanning fluorescent microscopy.

Blood from patients with disseminated PCa (n=21) was processed in a similar manner with the exception that the captured samples were permeabilized in saponin and stained with anti-cytokeratin (CK) and counterstained with a cocktail of anti-CD45/CD66b/CD16. CK<sup>+</sup>/DAPI<sup>+</sup>/CD45<sup>-</sup>/CD66b<sup>-</sup>/CD16<sup>-</sup> cells were considered as putative CTCs.

### **Results**

In technical experiments with cancer cells spiked into blood, ferrofluids resulted in a significantly higher or similar recovery when compared to magnetic beads.

For the patient samples, more putative CTCs (range 0-5 vs. 0-2 per 10 mL blood) were identified using the ferrofluids for capture when compared to magnetic beads. Similarly, putative CTCs were also detected in more individuals using the ferrofluids (52 % vs. 19 %).

## **Conclusion**

This study suggests, that using smaller magnetic particles might increase both positivity rates and numbers of CTCs detected in spike-ins and PCa patients, resulting in overall higher sensitivity of the CTC assay.