Results from an international inter-lab study of CTC enrichment and enumeration on the Genesis Rare Cell Isolation System.

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Background & objectives

There are multiple technologies for the capture and analysis of circulating tumour cells (CTCs) from blood. These utilise surface marker pull-down (e.g EpCAM) resulting in selection of either epithelial or mesenchymal CTCs, while alternatively, CTC selection is possible using size separation. These different approaches provide CTC enrichment for enumeration and downstream manipulation. In collaboration with five laboratories across Europe, all members of the European Liquid Biopsy Society (ELBS), we evaluated the performance of the Genesis Cell Isolation System from Bio-Rad Laboratories for the automated enrichment and enumeration of CTCs.

Methods

Blood samples from healthy donors were spiked with cells from the NSCLC cell lines H441 and H1563, representing model CTCs (mCTCs), in the range 50-100 cells per 10ml and added to Streck tubes for shipping and storage. Samples were generated at a central biobank in Luxembourg (IBBL) and 2 tubes (1 per cell line) were shipped to the 5 ELBS sites (Germany, France & Greece) and then processed individually at each site within 3 days. Four ml of each sample was processed through a Celselect enumeration slide on the Genesis platform prior to captured cells being automatically stained with DAPI and a combination of antibodies for CD45 and cytokeratin. mCTCs (CD45-, DAPI+, CK+) were enumerated per site and results collated across all 5 sites after analysis by the 4 following methods:

1. Automated primary mCTC selection via Image Viewer software, followed by manual secondary cell selection by an individual at each of the 5 sites.

2. Automated primary mCTC selection via Image Viewer software, followed by manual secondary cell selection by an individual at the Dusseldorf laboratory.
3. Automated primary mCTC selection via Image Viewer software, followed by manual secondary cell selection by an individual at Bio-Rad laboratories.
4. Automated mCTC selection by the ACCEPT software.

Results

mCTCs for both cell lines were identified and enumerated at all 5 sites across 3 rounds of samples. Automated processing on chip facilitated an easy and reproducible workflow for CTC capture and counting from whole blood, with minimal hands-on intervention. Mean mCTC count for the 4 analysis methods, across all 5 sites ranged from 54%-73% recovery, with no difference in mean count between $EpCAM^{high}$ cells and $EpCAM^{low}$ cells (53%-69% vs 55%-77%, respectively).

Conclusion

Validation of a new platform for the automated enrichment and enumeration of CTCs, utilising a size-based methodology, is important in the context of establishing CTCs into a translational and clinical setting. We show consistent results across 5 geographically separate laboratories, utilising size-based selection and subsequent immunocytochemical detection of mCTCs. These results highlight the capacity of an EpCAM independent CTC analysis in multi-site studies and will be of benefit in ongoing research into CTC enumeration.