**Table SI.** Summary of technologies for CTC enrichment.

| First author/s, year | Biological methods for CTC enrichment | | | | | |
|---|---|---|---|---|---|---|
| | **Enrichment Method** | **Selection criteria** | **Advantages** | **Disadvantages** | **Capture efficiency** | **(Ref.)** |
| Kowalik *et al*, 2017; Praharaj *et al*, 2018; Riethdorf *et al*, 2007 | CellSearch | ·EpCAM | ·FDA-approved·Highly robust and reproducible·Quantitative | ·Expensive to implement Failure to detect CTCs with low EpCAM expression·Inability to isolate viable CTCs | 20-80% | (29,119,120) |
| Talasaz *et al*, 2009 | MagSweeper system | ·HLA-A2·EpCAM | ·Cellular function can be kept intact without disrupting rare cellular gene expression·No need to pre-process blood samples | ·Failure to detect CTCs with low EpCAM expression·Apoptosis in captured cells | 59±27% | (63) |
| Li *et al*, 2024 | CellCollector | ·EpCAM | ·Can isolate rare CTCs in early cancer stages·No need of blood sampling·Detect CTCs *in vivo* | ·Cannot isolate EMT-CTCs·CTCs cannot be released from the wire | 41-70% | (121) |
| Ju *et al*, 2022; Liu *et al*, 2011 | EasySep | ·$CD45^+$ cells are removed | ·EMT-CTCs can be enriched·Prone to false positive results | ·Prone to false positive results | 73% | (107,122) |
| Seyfoori *et al*, 2023 | Bilayer magnetic microfluidic platform | ·EpCAM | ·Simple and low cost·Reduces the amount of manual sample processing that must be performed·Can rapidly stain cells·Enables on-chip and real-time monitoring of captured cells | ·Failure to detect CTCs with low EpCAM expression | 62-91% | (123) |
| Sun *et al*, 2023 | TDN-NanoGold Click Chip | ·CuAAC·SmaI | ·Enables enrichment and purification of CTCs with intact cellular morphology and preservation of molecular integrity | ·Larger sample size needed to further validate the feasibility of TDN-NanoGold click chip | 79.1-91.3% | (77) |
| Shen *et al*, 2021 | Magnetic-activated cell sorting | ·Affinity-based | without the need for antigen presentation·High specificity and efficiency·Can sort rare CTCs | ·Expensive·Magnetic particles can influence the phenotype and function of some cells. | 70-88% | (124) |
| Zhang *et al*, 2021 | Telomerase-based CTC detection | ·Telomerase reverse transcriptase·anti-CD45 antibody | ·Can effectively isolate EMT-CTCs·Not dependent on the expression of tumor cell surface | ·Needs to be validated in more independent research centers using larger sample | >77% | (125) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | markers such as EpCAM and GFAP | sizes | | |
| | Assays based on physical properties | | | | | |
| Ju *et al*, 2022; Vasantharajan *et al*, 2022; Zhang *et al*, 2023 | MetaCell | ·Size | ·Ability to enrich for CTCs including EpCAM negative ones·Ability to allow multiple downstream analyses | ·Membrane voids are easily clogged·Leukocyte contamination·Easy to miss small CTCs | 66.7% | (107,126,127) |
| Kowalik *et al*, 2017; Dianat-Moghadam *et al*, 2020; Lin *et al*, 2018; Pantel *et al*, 2019 | ISET technology | ·Size·Deformability | ·Easy and fast ·Relatively cheap·Isolation of EpCAM-negative CTCs·Isolation of intact CTCs | ·low specificity·CTCs smaller than leukocytes may be missed·Low recovery and purity | 75% | (29,38,128,129) |
| Zhang *et al*, 2023; Kulasinghe *et al*, 2016; Ye *et al*, 2024 | ScreenCell | ·Size | ·ScreenCell provides a rapid, easy, antigen-independent, cost-effective, and efficient technology to isolate and characterize CTCs·Isolated CTCs can be further analyzed cytologically and immunologically | ·Inability to capture CTCs smaller than WBCs | 89% | (127,130,131) |
| Philippron et al, 2021; Andrikou *et al*, 2023 | Parsortix system | ·Size·Deformability | ·CTCs with epithelial or mesenchymal features can be captured·Captured tumor cells can be used for cell culture·Consumable costs are relatively low·Simple method and operation | ·It is difficult to completely separate cancer cells from white blood cells based on their size | >90% | (132,133) |
| Çağlayan Arslan *et al*, 2022 | Dielectrophoresis (DEP) | ·Polarizability·Size | ·Can distinguish between cells of similar size but different morphological origin·Relatively low cost | ·Requires specific parameters such as battery type and electric field frequency | >90% | (134) |
| Sarioglu *et al*, 2015; Zhou *et al*, 2024 | Cluster-Chip | ·Strength of cell-cell junctions·Size | ·Label-free isolation·Ability to allow multiple downstream analyses | ·Shear stress is required to release most of the clusters from the micropillars | 30-40% | (135,136) |
| | Combined methods | | | | | |
| Boyer *et al*, 2020; Smit *et al*, 2024 | RosetteSep - DEPArray workflow | ·Negative enrichment·Density centrifugation·Charge affinity | ·Allows direct sorting of individual CTCs·Can enrich for EpCAM-negative CTCs | ·Can cause loss of CTCs | 43.6%-60% | (137,138) |

| | | | | | | |
|---|---|---|---|---|---|---|
| Karabacak *et al*, 2014 | CTC-iChip | ·Inertial focusing·Negative enrichment·Magneto phoresis·Size | ·Simplicity,·High recycling rate.·Can sort rare CTCs.·Has been developed into a clinical-grade platform | ·Expensive·Long set-up times | 97±2.7% | (139) |
| Sassi *et al*, 2024 | GEDI chip | ·Size·Immunoaffinity | ·Improved CTC capture efficiency and purity | ·Requires surface chemistry modifications | 62% | (140) |
| Wei *et al*, 2022 | CanPatrol™ CTC technology | ·Immunoaffinity·Size | ·Clusters of CTCs can be captured·Capability of multiple downstream analyses·Can sort rare CTCs | ·Small CTCs may be missed | 88% | (141) |

CTCs, circulating tumor cells; EMT, epithelial-mesenchymal transition; EpCAMs, epithelial cell adhesion molecules.

**Table SII.** Summary of CTC detection methods.

| First author/s, year | Detection method | Advantages | Limitations | (Ref.) |
|---|---|---|---|---|
| Lopresti *et al*, 2019; Kotsifaki *et al*, 2024 | Flow cytometry | ·Larger volumes of blood can be accommodated to increase test reliability and assay sensitivity while reducing potential variability·Fast and easy·The level of sensitive cell quantification that can be provided | ·Inability to maintain cellular activity·Blood samples need to be processed quickly after sampling | (142,143) |
| Ramirez *et al*, 2019; Koinis *et al*, 2023 | Immunofluorescence staining | ·Observation under a fluorescence microscope is convenient and allows determination of phenotype and morphology | ·Lack of stability in the expression of protein markers·Cannot distinguish between living and dead cells | (83,85) |
| Mazard *et al*, 2021; Soler *et al*, 2017 | EpiSpot | ·High specificity and high stability·Allows quantitative detection of biologically active CTCs | ·Requires cell culture and the accumulation of sufficient secreted marker proteins to form an immune plaque | (144,145) |
| Zeinali *et al*, 2022; Xu *et al*, 2022; Zhu *et al*, 2020 | Fluorescence *in situ* hybridization | ·Identification of CTCs at the gene level·High sensitivity and specificity | ·Possibility of interference by abnormal chromosomes from patient's blood cells·A chromosome probe can only detect one corresponding marker·FISH review and reporting of results is laborious and time-consuming | (54,146,147) |
| Kruse *et al*, 2020 | Reverse transcription-polymerase chain reaction | ·It is currently one of the most commonly used methods for detecting CTCs·RT-PCR identification of CTCs at the genetic level, including inactive CTCs | ·Transcript amplification is not patient-specific and treatment may affect expression levels, resulting in variable mRNA levels | (148) |
| Zavridou *et al*, 2022 | Reverse transcription-dd polymerase chain reaction | ·Provides simultaneous quantification of multiple targets in a limited number of samples·With high throughput and time saving·Highly sensitive, specific and reproducible. | ·Higher costs·Operation is relatively complex | (149) |
| De Luca *et al*, 2020 | Next Generation Sequencing | ·Genotyping of CTCs by identifying gene structure and gene expression status at the individual cell level.·Accurate data on DNA sequence and variation information can be provided.·Relatively low cost and short processing time | ·High demand for upstream separation technology.·Huge bioinformatics analysis challenges. | (150) |

CTCs, circulating tumor cells.