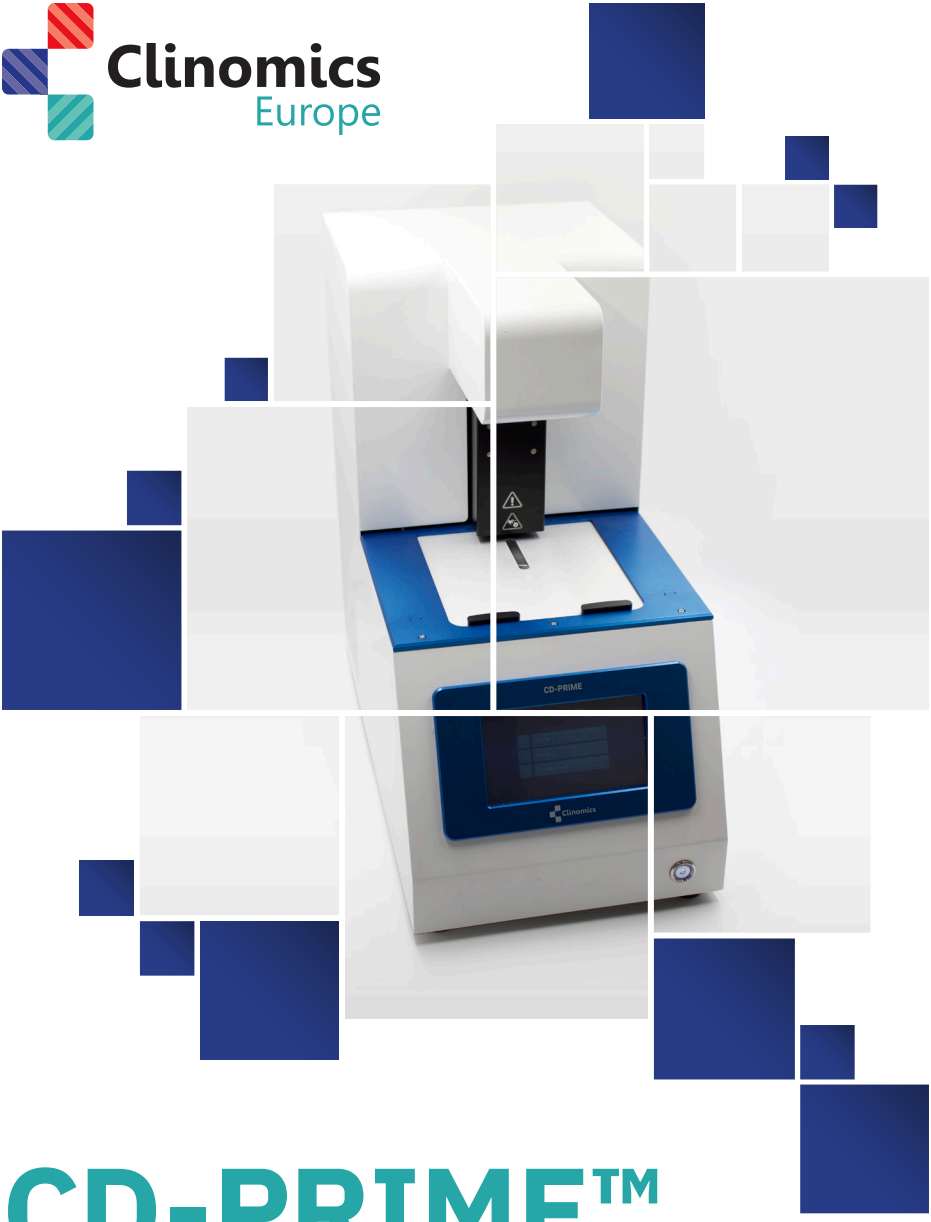
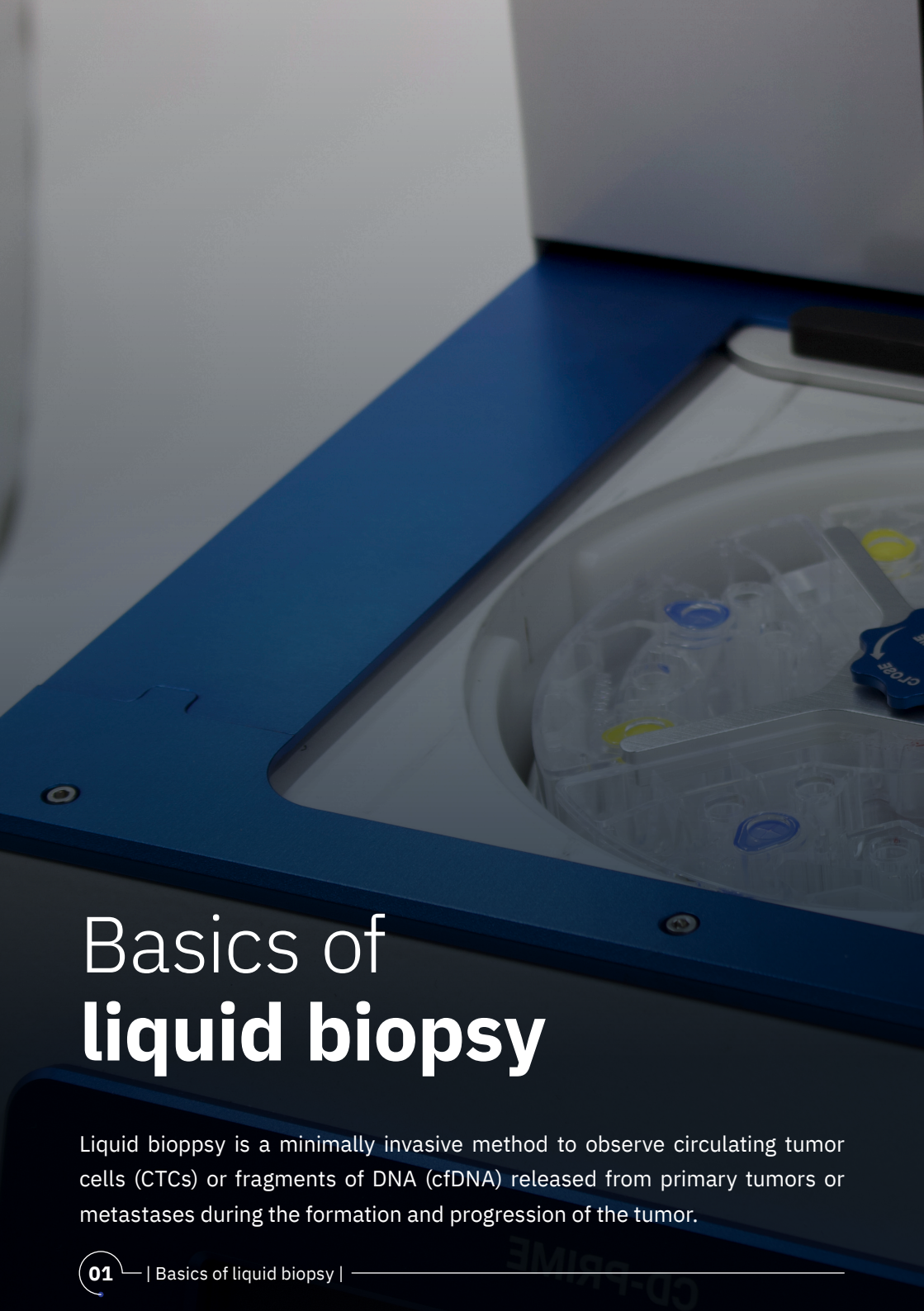
Clinomics
Europe



CD-PRIME™

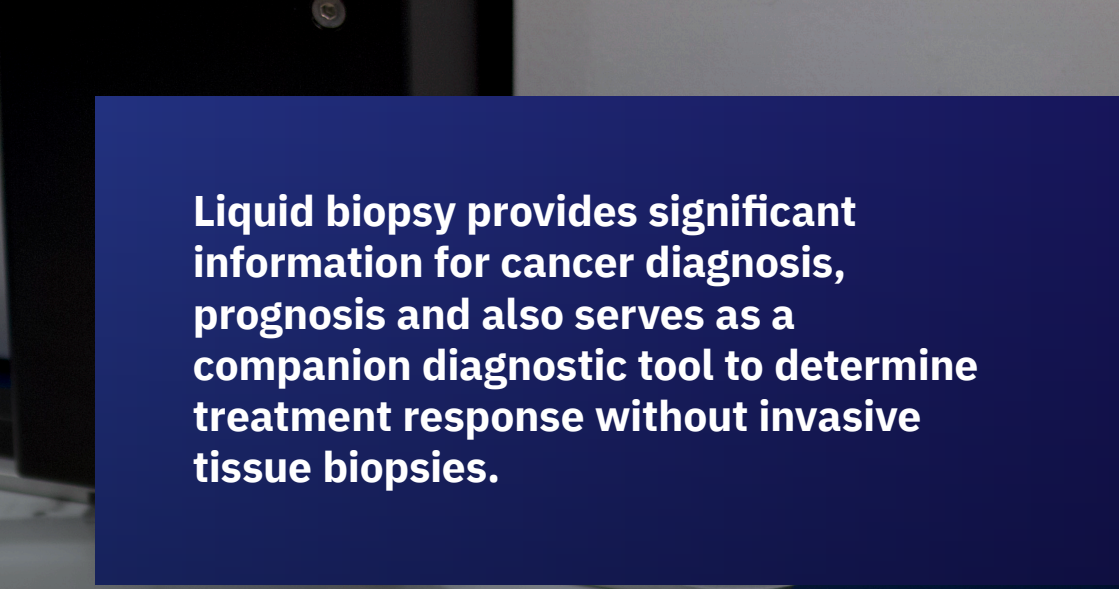
An integrated platform
for liquid biopsy



Basics of **liquid biopsy**

Liquid bioppsy is a minimally invasive method to observe circulating tumor cells (CTCs) or fragments of DNA (cfDNA) released from primary tumors or metastases during the formation and progression of the tumor.

Liquid biopsy provides significant information for cancer diagnosis, prognosis and also serves as a companion diagnostic tool to determine treatment response without invasive tissue biopsies.



Clinomics makes it possible to diagnose cancer earlier than traditional cancer diagnostic imaging modalities and more accurately than using protein tumor markers. Diagnostic delays reduces patient's chances to fight back against cancer and access timely and appropriate treatment. Our technology can be also used to get insights about patient's therapy response, assessing cancer relapse or resistance to treatment and guide oncologists in selecting the most beneficial treatment option.

CD-PRIME™ can provide much more accurate and abundant information by obtaining ctDNA (circulating tumor DNA) and CTC (circulating tumor cell) simultaneously to enhance the quality of cancer analysis.

Easy enrichment and **staining** of CTCs

Despite their key role in cancer progression CTC isolation and enumeration raise challenges because of the extreme rarity of CTCs in bloodstream in comparison with other components of blood.

CTCs are found at very low frequencies, contains about 1-40 of CTCs per ml of whole blood, but the enrichment of them can be achieved effectively with the help of advanced microfluidics technologies.

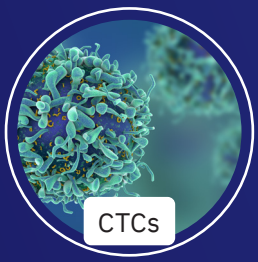
CD-PRIME™ for CTC analysis implements easy and automated protocol for CTC enrichment.

This fully automated, label-free method enriches CTCs from untreated blood samples, based on physical characteristics (cell size and elasticity), without CTC surface antigen labeling.

Whole Blood

Enrichment

CD-Prime™



CTCs

cfDNA

Downstream Analysis

Immunostaining

Downstream Analysis

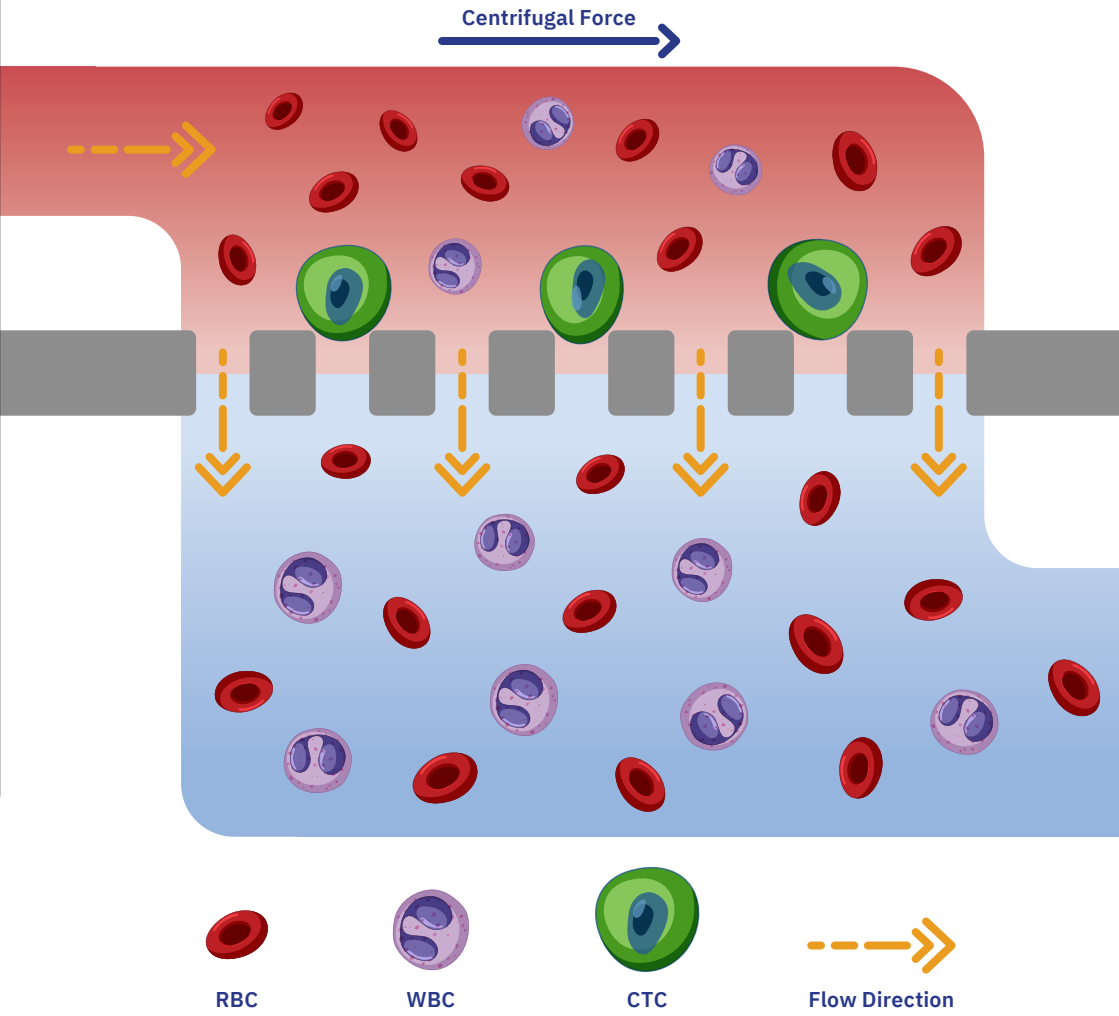
Digital PCR, NGS

High quality CTCs imaging

Core technology

CD-Prime™ uses **fluid-assisted separation technology (FAST)**. This efficient, user-friendly, and cost-effective technology enables **rapid, size-based isolation of CTCs from whole blood with high purity.**

The size-selective CTC isolation strategy is based on a polycarbonate membrane with 8 µm pores. These membrane pores are filled with steadily fixed liquid which helps in the reduction of clogging and shortening separation time. The CTC isolation process is fast, 3 mL of whole blood could be finished within 15 minutes.



Clogging is minimized by the filtration direction through the membrane, which is perpendicular to the centrifugal force, similar to the tangential-flow filtration method providing high-performance separation.

The isolation of intact tumor cells is implemented by a design which produces a uniform pressure drop across the entire membrane letting the bottom chamber to be filled with liquid.

Performance **comparison**

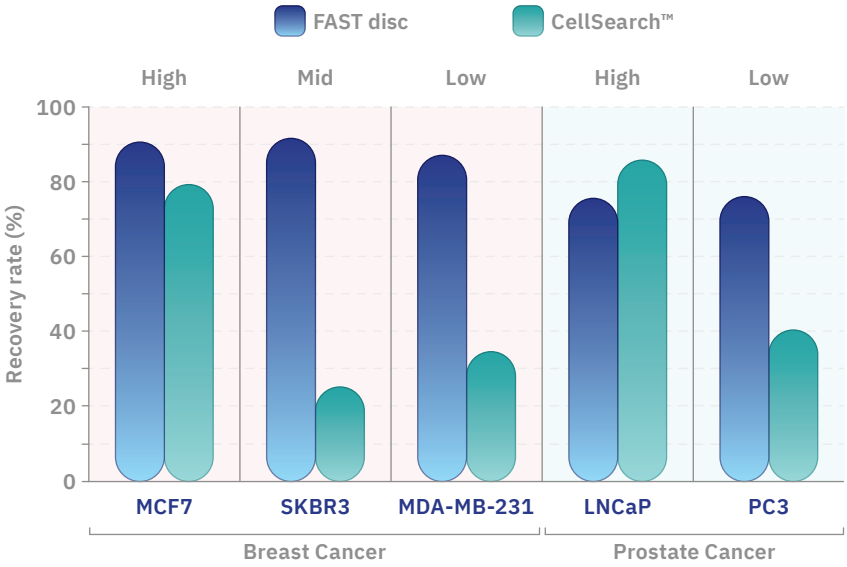
CD-Prime™ vs CellSearch™ system

Currently, only the CellSearch™ system (Menarini Silicon Biosystems) is approved by the US Food and Drug Administration (FDA) for the enumeration of CTCs in clinical use in metastatic breast, colorectal, and prostate cancer.

The CellSearch™ uses ferrofluids loaded with an epithelial cell adhesion molecule (EpCAM) antibody to capture CTCs [1, 3].

Performance comparison between **FAST disc** and **CellSearch™ system:**

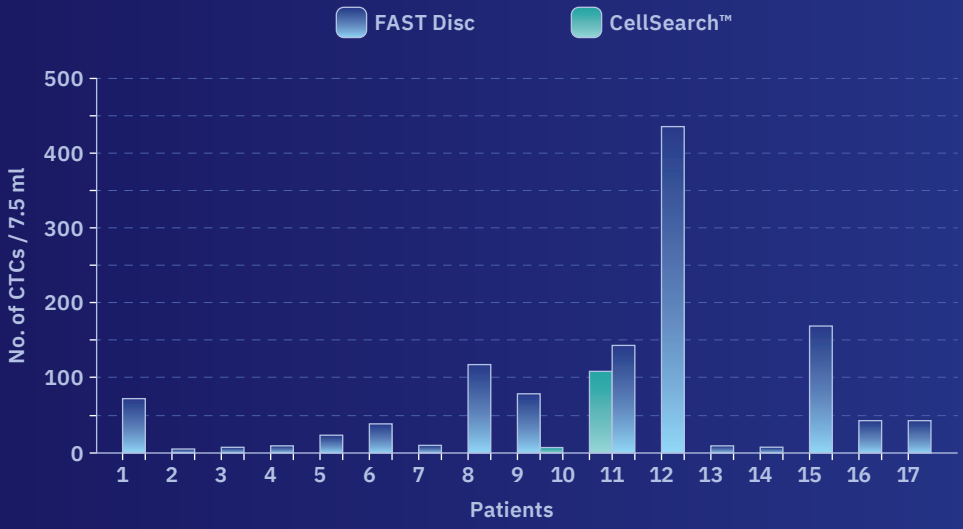
For the comparison, five cell lines with various EpCAM expression levels were used to quantify the recovery rates. The performance was confirmed using whole blood spiked with cancer cells. CellSearch™ showed a high capture efficiency only for cell lines with high EpCAM expression, but the FAST disc showed a high capture efficiency, regardless of the EpCAM expression levels in the cell lines [1].



Performance comparison of CD-Prime™ FAST disc and CellSearch™ system [1]

Comparison of **CTC enumeration by FAST disc** and **CellSearch™ system:**

In this experiment, whole blood samples were collected from 17 patients with various cancer types. The size-selective CTC isolation using the FAST disc (detection rate: 94.1%) exceeded the immunoaffinity-based enumeration by CellSearch (detection rate: 11.8%) [1].



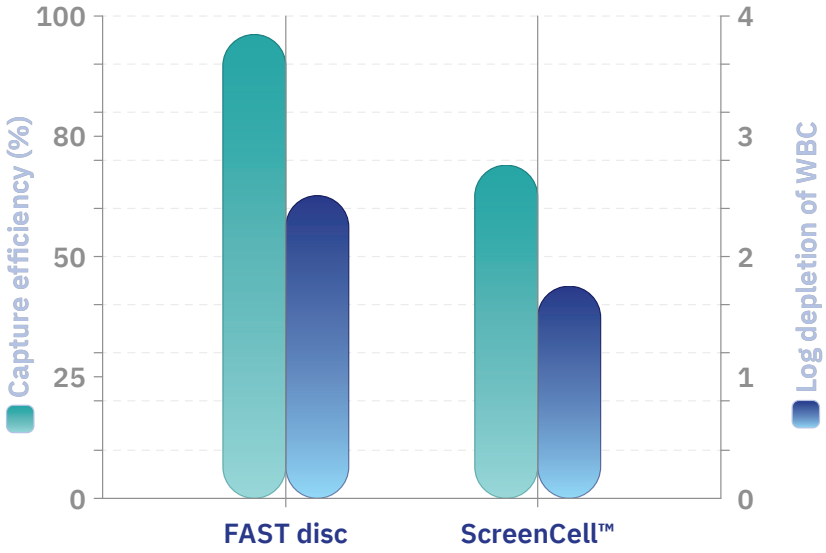
Comparison of CTC enumeration by CD-Prime™ FAST disc and CellSearch™ system [1]

Performance **comparison**

CD-Prime™ vs ScreenCell™ Cyto system

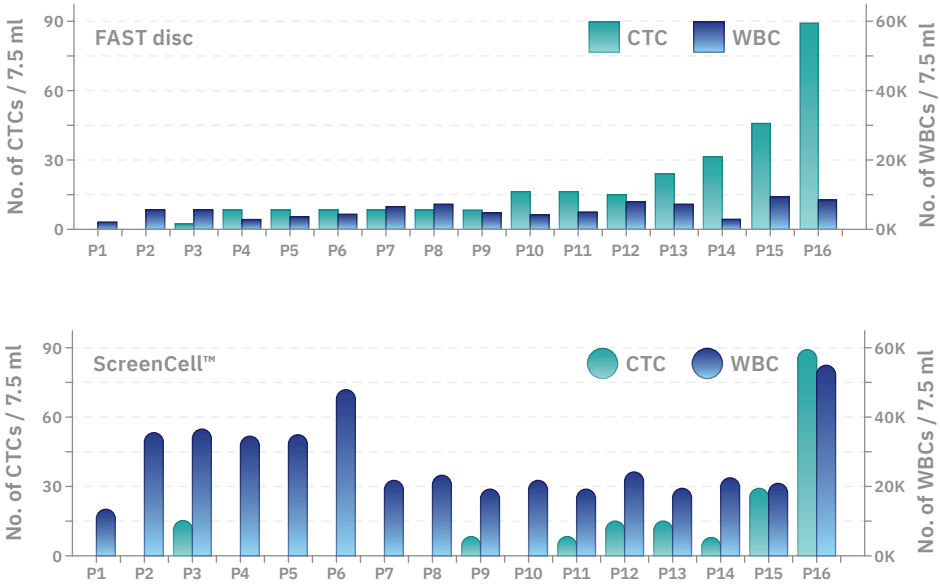
Performance comparison between **FAST disc** and **ScreenCell™ Cyto** (commercially available size-based filtration system)**:**

Capture efficiency and purity were compared, using MCF-7 cells spiked in whole blood. The capture efficiency of FAST discs was 95.9 ± 3.1% (n = 3) and 68.9 ± 10.0% (n = 3) for ScreenCell. FAST disc provides a higher purity of 2.5-log (mean: 4771 WBCs/mL, range: 4330–5483 WBCs/mL) compared to 1.7-log (mean: 32 950 WBCs/mL, range: 29 550–37 137 WBCs/mL) depletion [2].

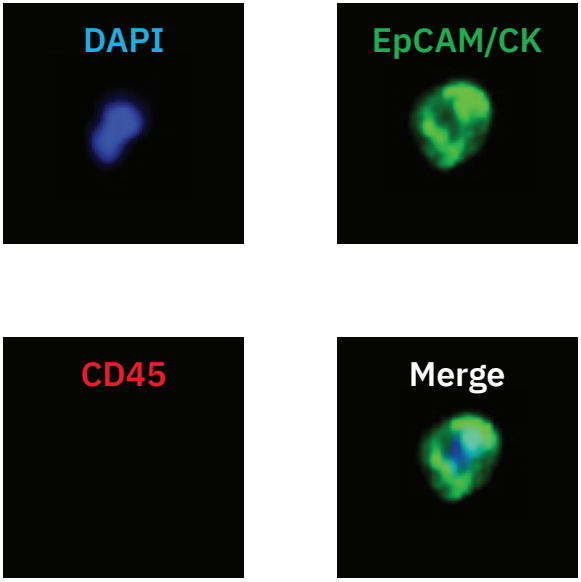


Performance comparison between CD-Prime™ FAST disc and ScreenCell™ Cyto using spiked samples [2]

Clinical samples obtained from patients with cancer of the breast (n=3), stomach (n=4), colon (n=4), bile duct (n=3), and lung (n=2) were collected. Cells that showed DAPI+, EpCAM/CK+, and CD45– were identified as CTCs, and cells with DAPI+, EpCAM/CK-, and CD45+ properties were identified as WBCs. FAST discs could detect at least one CTC from 14 out of the 16 patients (detection rate: 87.5%) while ScreenCell Cyto devices detected 8 out of the 16 samples (detection rate: 50.0%). The number of WBCs remaining on the membrane was 6.0 times higher with ScreenCell than that with the FAST disc [2].



Performance comparison between CD-Prime™ FAST disc and ScreenCell™ Cyto using clinical samples [2]



In conclusion, the experiments demonstrate that the FAST disc compared to ScreenCell could separate CTCs with a higher recovery rate and a higher level of purity [2].

References

1. Lim M, Park J, Lowe AC, Jeong HO, Lee S, Park HC, Lee K, Kim GH, Kim MH, Cho YK. A Lab-on-a-Disc platform enables serial monitoring of individual CTCs associated with tumor progression during EGFR-targeted therapy for patients with NSCLC. Theranostics. 2020 Apr 6;10(12):5181-5194. doi: 10.7150/thno.44693. PMID: 32373206; PMCID: PMC7196290.

2. Kim TH, Lim M, Park J, Oh JM, Kim H, Jeong H, Lee SJ, Park HC, Jung S, Kim BC, Lee K, Kim MH, Park DY, Kim GH, Cho YK. FAST: Size-Selective, Clog-Free Isolation of Rare Cancer Cells from Whole Blood at a Liquid-Liquid Interface. Anal Chem. 2017 Jan 17;89(2):1155-1162. doi: 10.1021/acs.analchem.6b03534. Epub 2016 Dec 13. PMID: 27958721.

3. Kim H, Lim M, Kim JY, Shin SJ, Cho YK, Cho CH. Circulating Tumor Cells Enumerated by a Centrifugal Microfluidic Device as a Predictive Marker for Monitoring Ovarian Cancer Treatment: A Pilot Study. Diagnostics (Basel). 2020 Apr 23;10(4):249. doi: 10.3390/diagnostics10040249. PMID: 32340330; PMCID: PMC7236001.

Clinical **significance**

The presence and clinical relevance of CTCs has already been shown in many types of cancer. CTCs can be directly linked to metastasis and the spread of cancer. They are responsible for >90% of all cancer-related deaths [1].

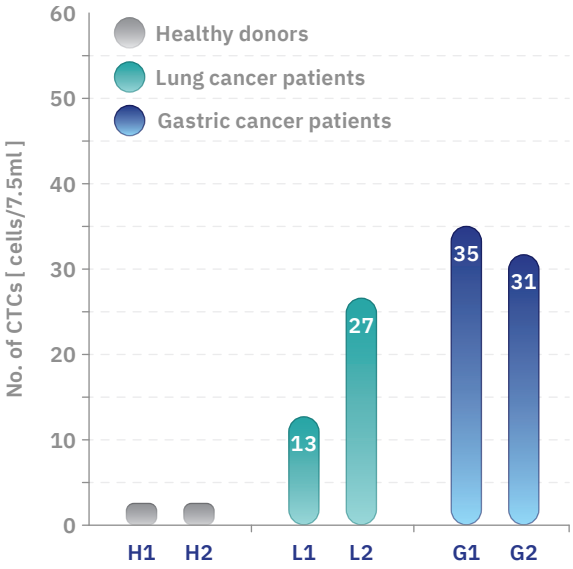
CTCs can be used in many areas of cancer diagnostics, from patient stratification to investigating new therapeutic approaches, to stopping tumor dissemination and metastasis at the very early stage [2].

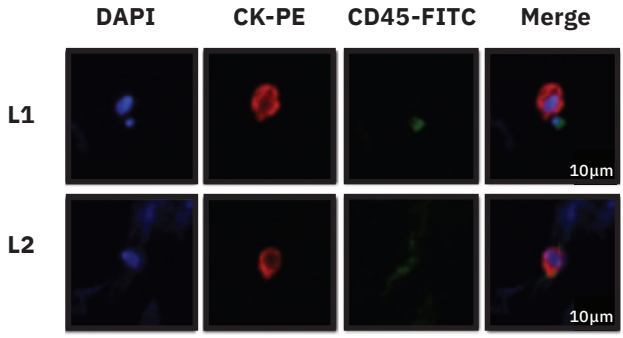
The next experiment exemplifies the usability of CTC isolation in cancer diagnostics.

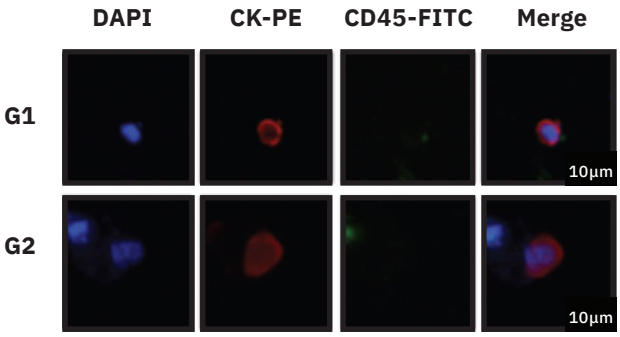
References

1. Harouaka, Ramdane A., Merisa Nisic, and Si-Yang Zheng. “Circulating tumor cell enrichment based on physical properties.” Journal of laboratory automation 18.6 (2013): 455-468.

2. Konczalla, Leonie, et al. “Clinical significance of circulating tumor cells in gastrointestinal carcinomas.” Diagnostics 10.4 (2020): 192.

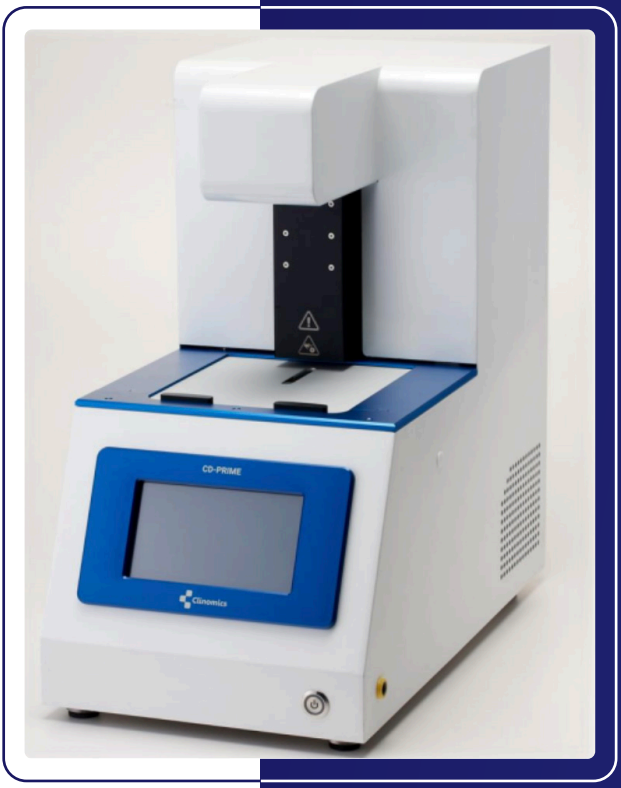

Healthy donors
Lung cancer patients
Gastric cancer patients
No. of CTCs [cells/7.5ml]
60
50
40
30
20
10
0
13
27
35
31
H1
H2
L1
L2
G1
G2


DAPI
CK-PE
CD45-FITC
Merge
L1
L2
10μm
10μm


DAPI
CK-PE
CD45-FITC
Merge
G1
G2
10μm
10μm

CD-OPR-2000™

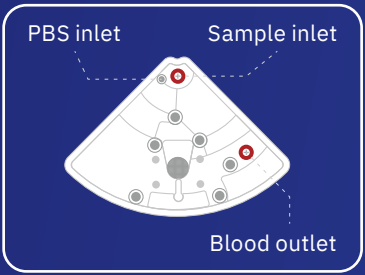
- **A fully automated rotor machine** for various optimized liquid biopsy protocols.
- **Available cartridges:**
  CD-FAST™ Auto
  CD-LBx™ 1
  CD-LBx™ 2
- Standard protocol enforced
- Practically little training is necessary



CD-FAST™ Auto

The FAST Disc is a centrifugal microfluidic device that allows label-free, clog-free, and reproducible circulating tumor cell (CTC) isolation directly from the whole blood of patients, without any pretreatment steps.

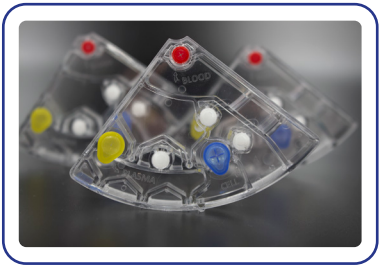
The experimental protocol of the FAST Disc is very simple: the whole blood added into the sample loading chamber is pushed into the filtration chamber where CTCs are captured by the membrane and smaller hematopoietic cells pass through the membrane into the waste chamber.

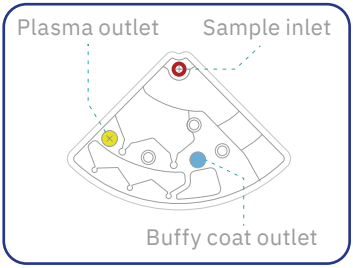


- To the maximum, four cartridges can be used as blood chamber
- Up to 3 ml whole blood can be inserted per one cartridge
- Elimination of cross-contamination (individual lid and valves block aerosol)
- Enforcement of standard protocol of fractionation
- No waste of blood sample
- Simultaneous prep of plasma (for cfDNA) and CTC
- The end-user is able to do the downstream work such as NGS and PCR analysis according to target
- Fully automatic separation by CD-OPR-2000™ device

CD-LBx™ 1

Automatically separates plasma (for cfDNA) and buffy coat (for germline DNA or enrichment CTCs) for further downstream work according to purpose.

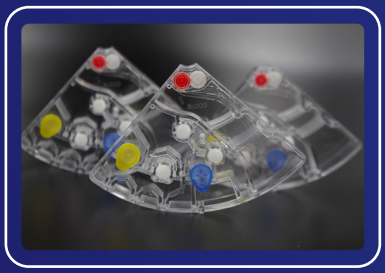


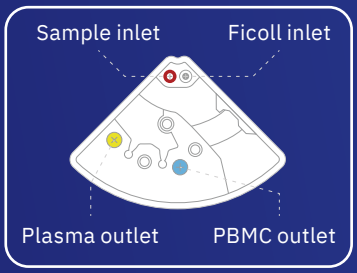


- To the maximum, four cartridges can be used as blood chamber
- Up to 9 ml whole blood can be inserted per one cartridge
- Buffy coat separated from CD-LBx™ 1 can be compatible use with CD-FAST™ Auto for CTCs enrichment
- Elimination of cross-contamination (individual lid and valves block aerosol)
- Enforcement of standard protocol of fractionation
- No waste of blood sample
- Simultaneous prep of plasma (for cfDNA) and buffy coat (for germline)
- The end-user is able to do the downstream work such as NGS and PCR analysis according to target
- Fully automatic separation by CD-OPR-2000™ device

CD-LBx™ 2

This is a plasma (for cfDNA) and PBMC (for germline DNA or enrichment live WBC) separation cartridge for further downstream work according to purpose.





- To the maximum, four cartridges can be used as blood chamber
- Up to 9 ml whole blood can be inserted per one cartridge
- Inject Ficoll-Paque and whole blood into CD-LBx™ 2 to separate PBMCs and plasma
- Elimination of cross-contamination (individual lid and valves block aerosol)
- Enforcement of standard protocol of fractionation
- No waste of blood sample
- Simultaneous prep of plasma (for cfDNA) and PBMC (for germline)
- The end-user is able to do the downstream work such as NGS and PCR analysis according to target
- Fully automatic separation by CD-OPR-2000™ device

User scenario

	CD-Lbx™ 1	CD-Lbx™ 2	CD-FAST™ Auto	CD-OPR-2000™
Product				
Required capacity	≤ 9 ml	≤ 9 ml	≤3ml or Buffy coat from CD-Lbx™ 1	
CTC			●	●
Plasma	●	●		●
Buffy coat	●			●
PBMC		●		●
Platform				

Workflow

STEP I.

Whole Blood
8-9 ml

STEP II.

Fully automated driving system

CD-FAST™ Auto

CD-LBX™ 1 : Separation of plasma & buffy coat

CD-FAST™ Auto : Separation of CTCs from buffy coat

STEP III.

CTCs
cfDNA
RT-PCR OR NGS

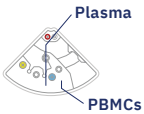
Downstream work according to purpose

STEP I.

Whole Blood
5-9 ml

STEP II.

Fully automated driving system



CD-LBX™ 2 : Separation of plasma & PBMCs

STEP III.

PBMCs
cfDNA
RT-PCR OR NGS

Downstream work according to purpose

Strengths

Full automation

Without special pretreatment of the whole blood, it is possible to enrich living CTCs.

No contamination

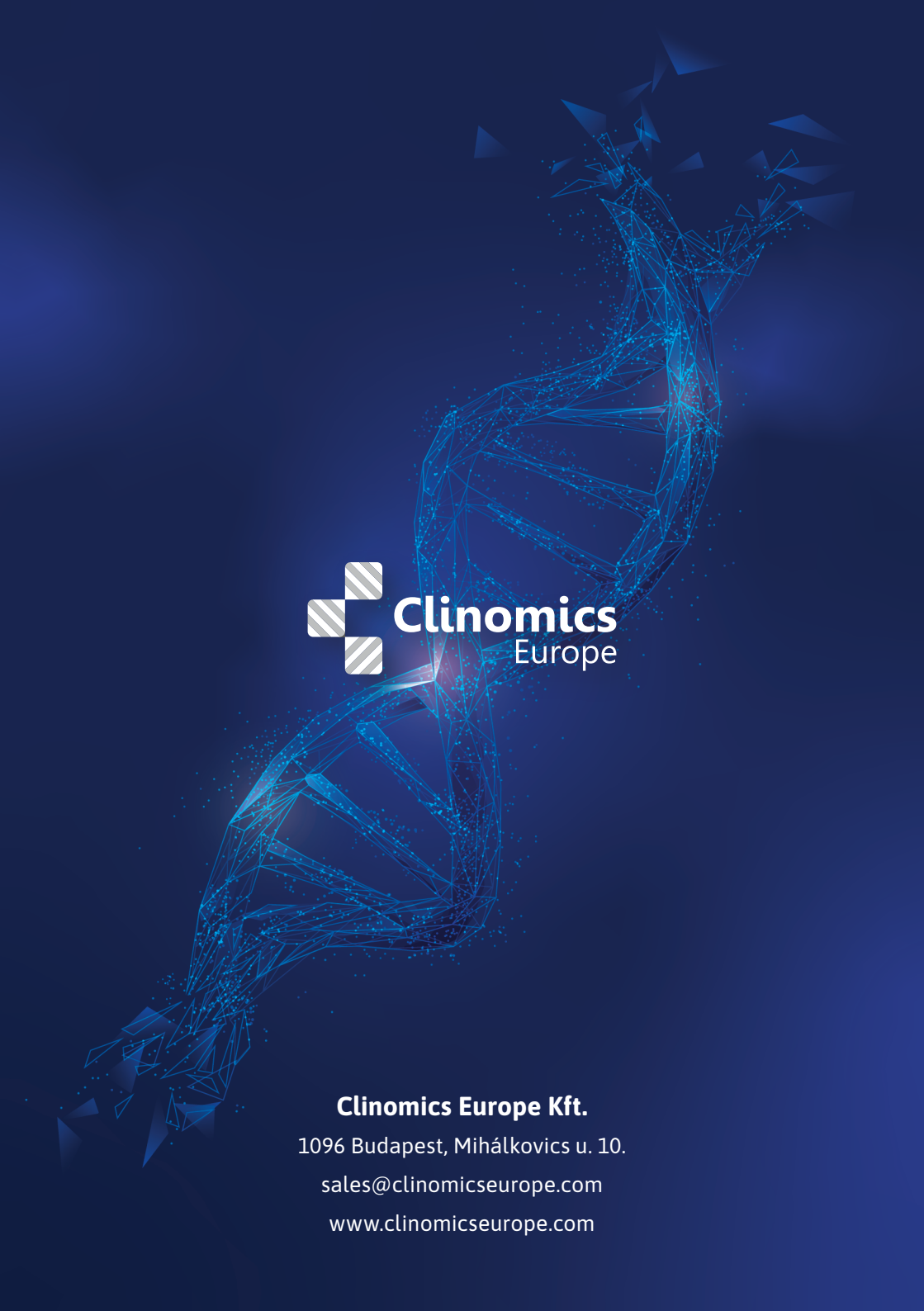
Prevents cross-contamination of samples which may occur in various clinical situations.

Easy-to-use

Full automation of blood processing procedures does not require a high-level of training, and one can obtain CTCs and plasma simultaneously for a more sensitive liquid biopsy.

Fully certified

Registered as a Class 1 medical device to the US FDA (Food and Drug Administration), CE (EU) and Hungarian National Institute of Pharmacy and Nutrition.



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