



Automated Rare Cell and Exosome Isolation Microfluidic System Accelerates the Development of Liquid Biopsy Clinical Applications

Yipeng Wang, M.D., Ph.D.
VP of Translational Medicine



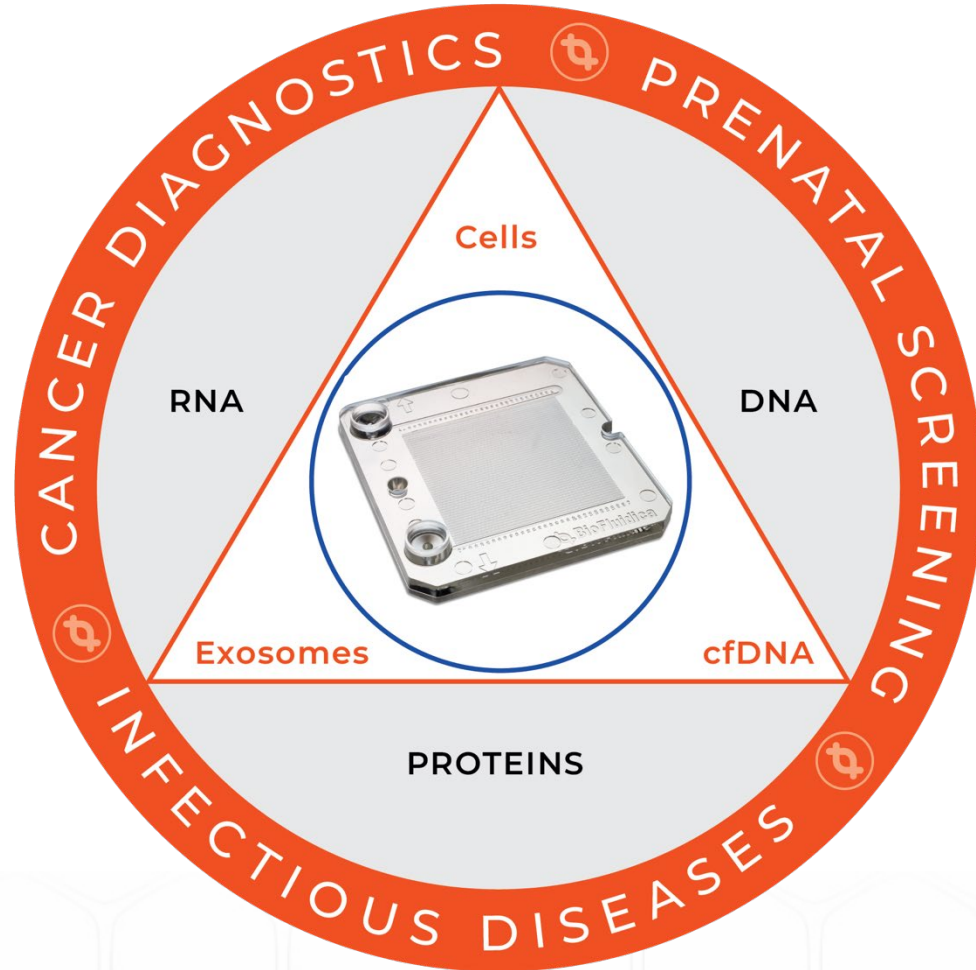
Challenges for Liquid Biopsy

- Disease cells and exosomes are rare
- cfDNA is highly fragmented and diverse among individuals
- Sample collection & transport to maintain cell viability
- Sample preparation and processing lead to biomarker loss

Biofluidica Liquid Biopsy Solution –LiquidScan™

- **Proprietary Blood Collection Tubes for room temperature overnight shipments**
 - Inhibit micro-clotting; maintain cell viability
- **No sample pre-processing**
 - LiquidScan provides affinity capture for biomarker isolation and enrichment
 - LiquidScan processes whole blood –for rare cell enrichment
 - LiquidScan processes plasma –for exosome enrichment
- **Microfluidic chip biomarker affinity catch-and-release maintains viability**
- **Completely automated**
 - Eliminate hands-on errors and manual variation
 - Precise, accurate, and reproducible
- **No tubing or sample valving used during processing**
 - Closed loop pipetting system eliminates loss during the processing of sample, capture, and enrichment of biomarkers

LiquidScan Enables Multi-Omics Analysis



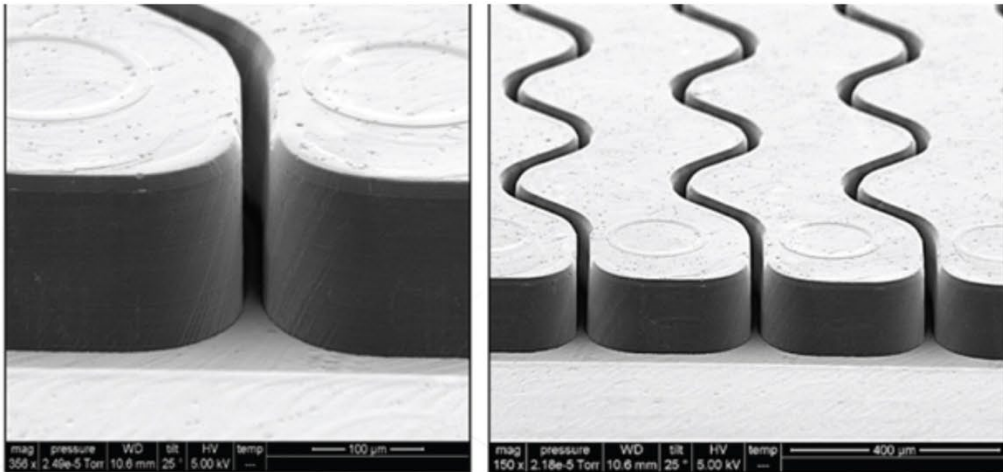
BioFluidica Proprietary Solution

LiquidScan™ Products for Collection and Processing

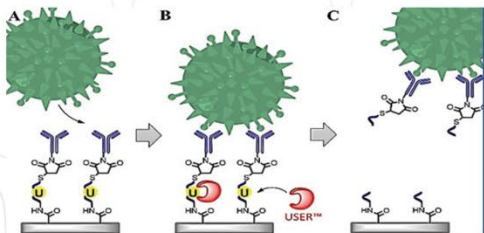
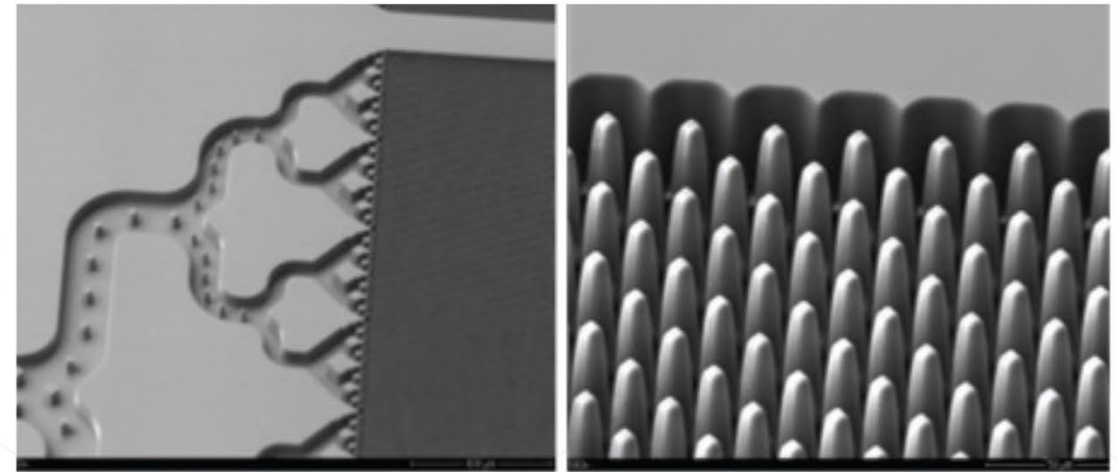
- **Blood Collection Tube** for diagnostic analysis using microfluidics
- **Consumable Kits**, including specialized **microfluidic chips**
- **Hardware & software** for patient sample processing on Hamilton Microlab STAR platforms

RARE BIOMARKER ISOLATION

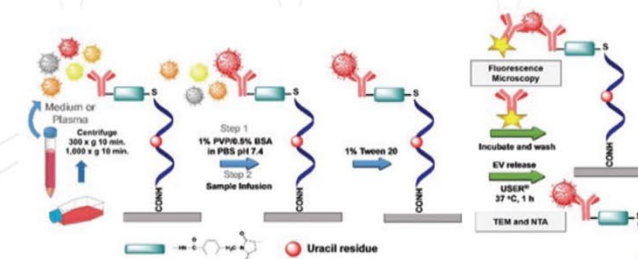
Specific Isolation of Circulating Cells



Specific Enrichment of Exosome Populations



Any cell with specific cell surface markers can be isolated using LiquidScan.



Exosome surface proteins are used to enrich sub-populations using LiquidScan.

LiquidScan Workflow

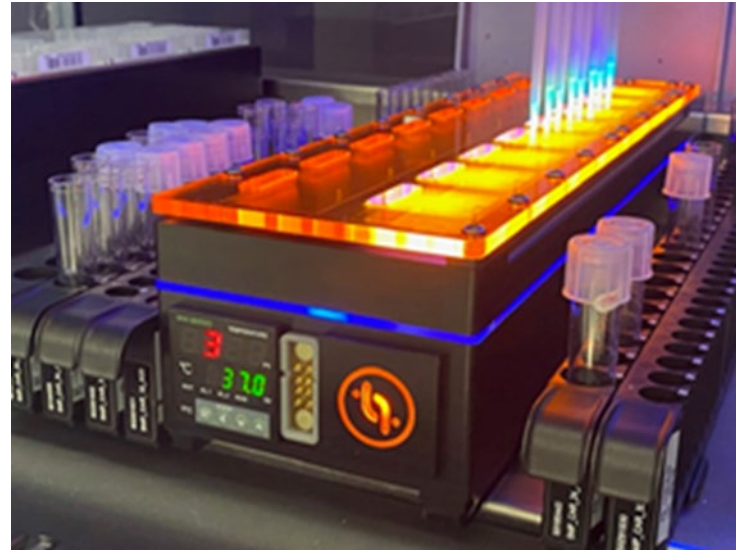


Fully automated sample processing for the isolation of rare biomarkers.

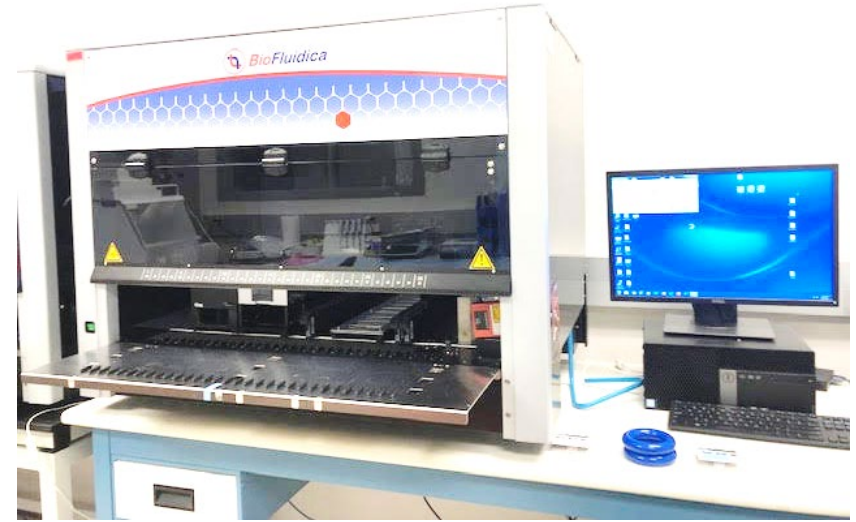
Overcoming Biomarker Losses



- ✓ Closed loop pipetting
- ✓ Synergistic activation of 2 pipettes/microfluidic chip



- ✓ Blood tube to chip without biomarker loss
- ✓ Unlimited scalability



- ✓ Custom software
- ✓ LiquidScan™ Module
- ✓ Full Automation

Transforming laboratory pipetting robots to closed loop pipetting systems with hardware & software

Full Systems Flexibility to target biomarkers

Proprietary Process for rare cells, EVs (exosomes)

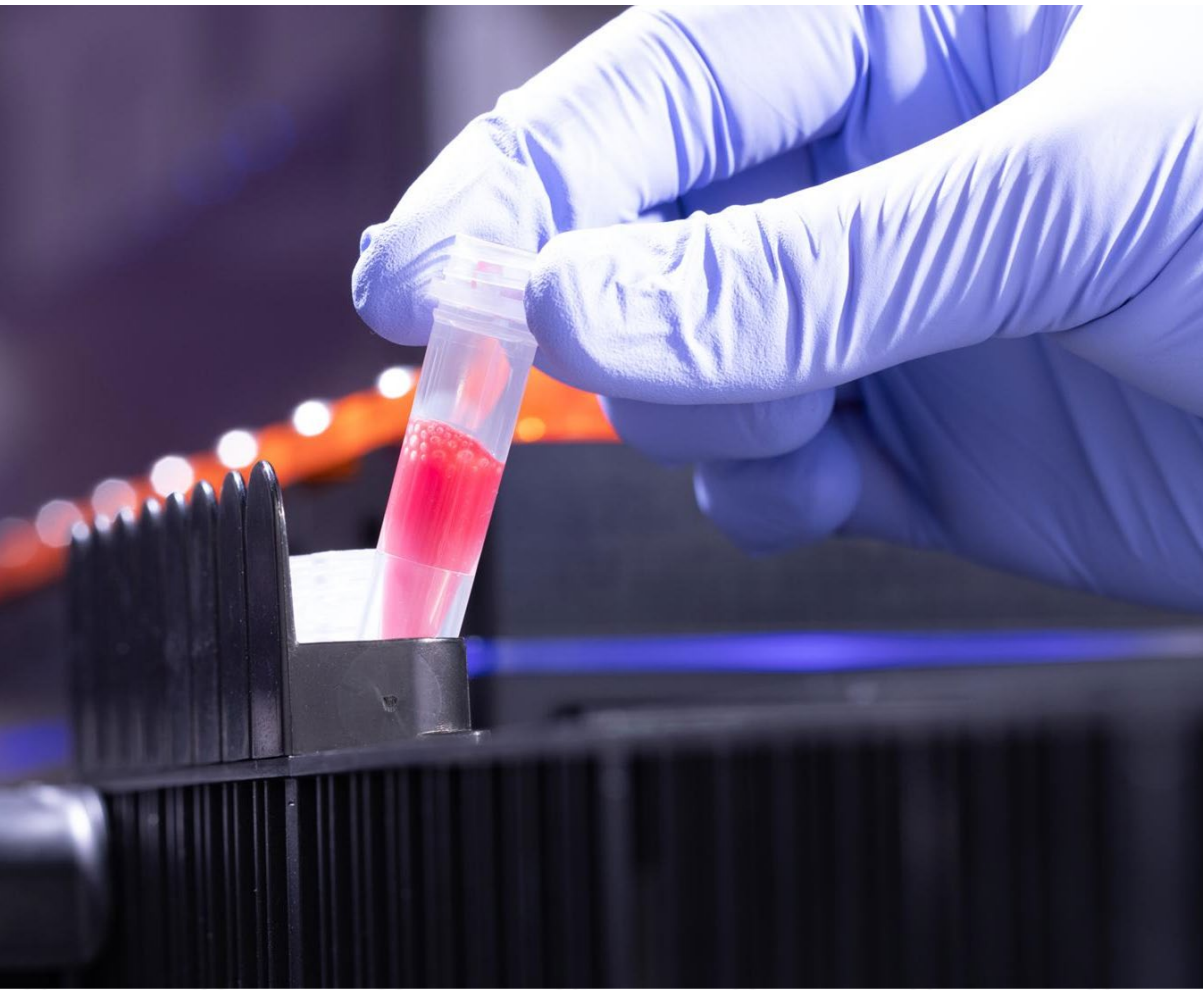


- 100ul-4ml per chip
- Speed process time: 2-3hrs dependent on loading volume
- Possible to run 8 patients per run
- From one patient possible to run multiple chips
- 3-4 runs per day per instrument

Easy and Efficient Exosome Isolation with LiquidScan

Performance Specifications	LiquidScan	Other Technologies
Purity	> 80%	10-90%
Sample Capacity	Up to 1 mL/CHIP	NA
Recovery	> 80%	5% - 80%
Sample Processing Time	2-4 hours	15 mins – 16 hours
EV Load Capacity	2.2×10^{11} Particles	NA
Tissue Specific Isolation	Yes	Yes/No

Clinical Applications



Cell-based Downstream Analysis

Cytology Analysis

- Enumeration
- ICC

Molecular Analysis

- qPCR, ddPCR
- Single Cell Sequencing: mRNA-Seq, ncRNA-Seq, Low pass WGS, Targeted Sequencing, Methylation, Gene Fusion
- FISH

Exosome Downstream Analysis

- Enumeration
- miRNA, mRNA, DNA, Protein
- Biomarker Co-localization

Indications

- Ovarian
- Colorectal
- Endometrial
- Lung
- Pancreatic
- Prostate
- Bladder
- Leiomyosarcoma
- Cholangiocarcinoma
- Multiple Myeloma
- Acute Myeloid Leukemia
- Acute Lymphoblastic Leukemia
- Prenatal
- Stroke
- SARS-CoV-2 Detection

Integrated for Clinical Research

Liquid Biopsy Core Facility

KU MEDICAL
CENTER
The University of Kansas

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ACTIVE PROJECTS
Using isolated Rare Cells
& Exosomes including:

6 solid
tumor
projects

Stroke

3
leukemia
projects

COVID-19
drug
response

Cystic
Fibrosis

Alzheimer's

Radiation
Exposure

Improved HER2+ Patient Selection with CTCs

Needle biopsy is not always feasible for late stage breast cancer patients
Liquid biopsy is an alternative or even better options for the patients

LiquidScan

Non-Invasive Isolation of Circulating Tumor Cells (CTCs)
Followed by conventional FISH diagnostics

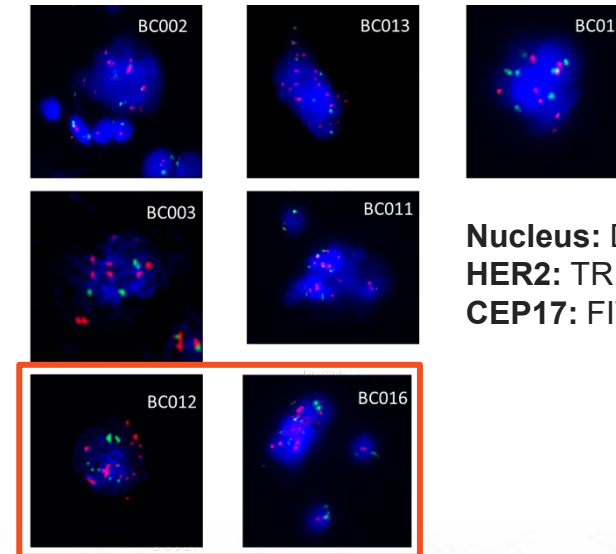
Patient ID	Her2 Status		Stage	Cellularity
	Needle Biopsy	LiquidScan		
BC002	Her2+	Her2+	Stage 4	Good
BC003	Her2+	Her2+	Stage 3	Excellent
BC011	Her2+	Her2+	Stage 3	Excellent
BC013	Her2+	Her2+	Stage 3	Excellent
BC017	Her2+	Her2+	ND	Scant
BC004	Her2-	Her2-	Stage 4	Good
BC005	Her2-	Her2-	Stage 4	Excellent
BC006	Her2-	Her2-	Stage 3 or 4	Excellent
BC007	Her2-	Her2-	Stage 3	Excellent
BC008	Her2-	Her2-	Stage 4	Excellent
BC009	Her2-	Her2-	Stage 4	Excellent
BC010	Her2-	Her2-	Stage 3	Excellent
BC014	Her2-	Her2-	ND	Excellent
BC015	Her2-	Her2-	ND	Excellent
BC012	Her2-	Her2+	Stage 3	Excellent
BC016	Her2-	Her2+	ND	Excellent

Pilot study:

100% in concordance with needle biopsy results

In addition: Identification of >25% more patients with Her2+

*Larger Study is in progress



Nucleus: DAPI (Blue)
HER2: TRITC (Red)
CEP17: FITC (Green)

Needle Biopsies are missing patients that can benefit from personalized treatments.

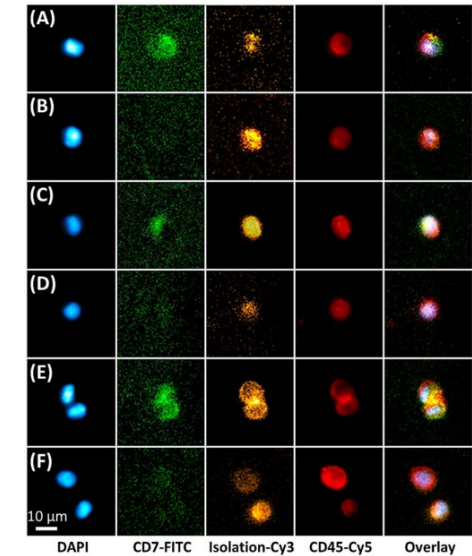
MRD Test for AML

- LiquidScan for AML CLC (Circulating Leukemia Cells) capture was developed and validated
- CLCs were detected for all AML 34 patients in low to mid range categories
- Blood samples were collected monthly for each patient
- Total success rate: 92.3% in concordance with Flow
- Detection of relapse: 83%
- Downstream NGS based targeted sequencing for AML panel is under development

Publication:

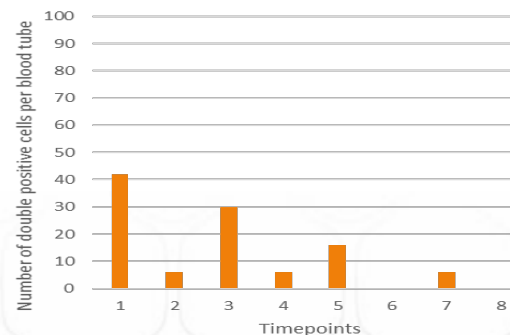
1. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4701594/>

Immunophenotyping of aberrant(+) CLCs and aberrant(-) cells isolated by targeting (A,B) CD33, (C,D) CD34, and (E,F) CD117, respectively. A



Example: Remission

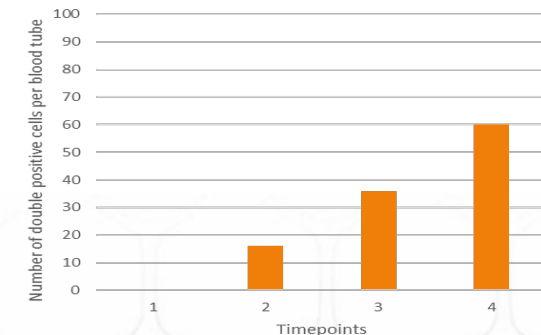
Patient 08. CD34/64. Average Double Stained Cells



Low cell count in follow up samples and downtrend

Example: Relapse

Patient 22. CD34& 13. Average Double Stained Cells



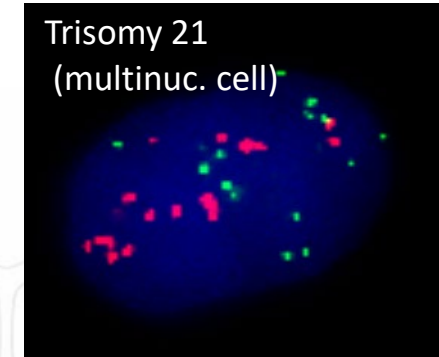
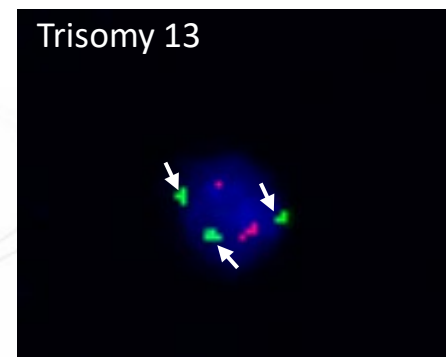
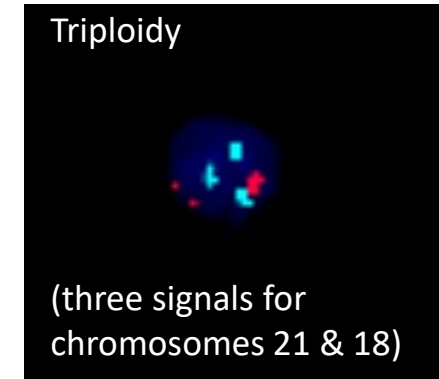
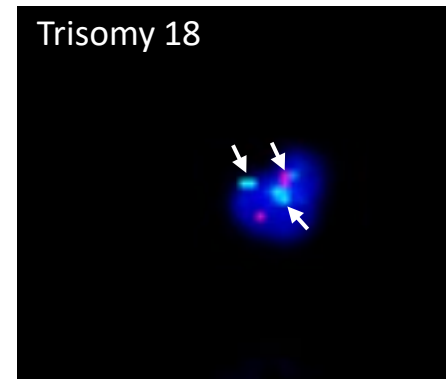
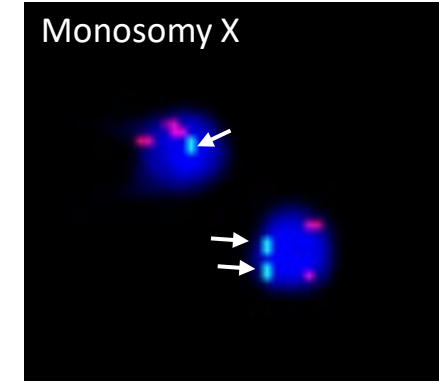
High cell count in follow up samples and uptrend

cbNIPT Clinically Feasible with LiquidScan

- Cell based NIPT (cbNIPT) has much higher sensitivity and accuracy in CNV detection than NIPT
- Major obstacle of cbNIPT development was to isolate sufficient fetal cell for analysis
- LiquidScan fetal cell CHIP was optimized with 300+ patient samples
- Average number of total fetal cells (mono. + multinucleate): 3.72/mL (74.4/20mL)
- Highest number of CTs reported previously: 0.23/mL (Ref: Panchalee et al. Prenat Diagn, 2020)

Publications:

1. <https://obgyn.onlinelibrary.wiley.com/doi/10.1002/uog.23586>
2. <https://obgyn.onlinelibrary.wiley.com/doi/10.1002/pd.6046>

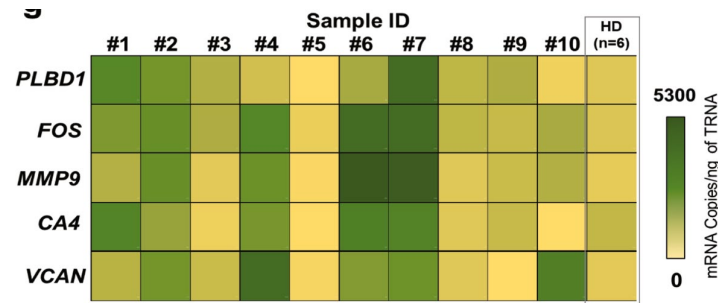


Fast Stroke Diagnosis at Point-of-Care

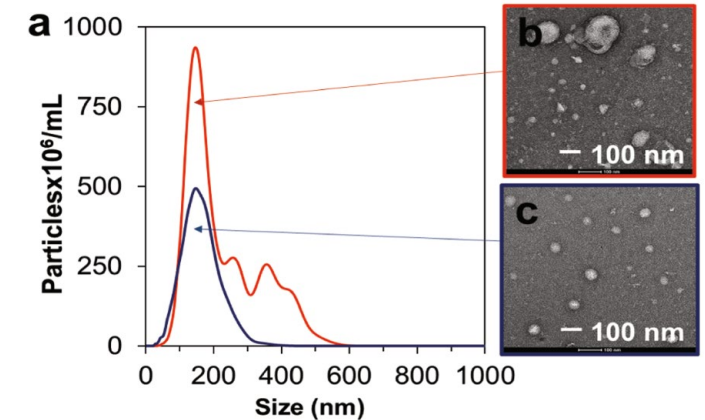
Exosome Based Diagnostics

- Acute ischemic stroke (AIS) patients
- CD8(+) T-cells mRNA biomarkers
- EVs as a source of mRNA for AIS detection
- 80% test positivity
- Samples to results in 3.7 hrs.

Publication: Wijerathne, H., *et al.* Affinity enrichment of extracellular vesicles from plasma reveals mRNA changes associated with acute ischemic stroke. *Commun Biol* **3**, 613 (2020).
<https://doi.org/10.1038/s42003-020-01336-y>

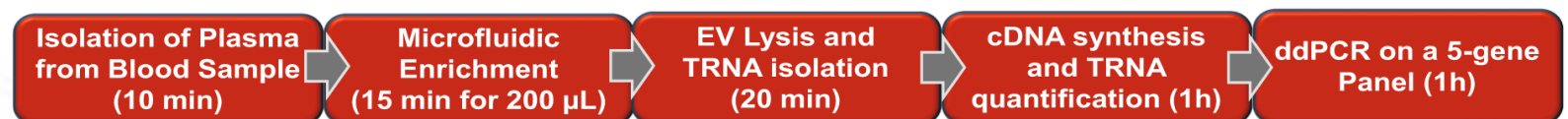


RNA expression profiling for AIS dysregulated genes in clinical samples.



Nanoparticle tracking analysis (NTA) and **b, c** TEM images of EVs isolated from clinical sample by **PEG precipitation** and **EV-MAP**

Process flow chart





Contact for more information
info@biofluidica.com

***LiquidScan™, a multi-omics Liquid Biopsy platform
for improved disease diagnostics***