

CTC technologies meeting
Sep 15th 2021

Results of the survey

Evi Lianidou

First survey CTC analysis in ELBS

CellSearch, FACS	Enumeration	28/50
Parsortix/ANGLE, ISET, Genesis/Bio-Rad, Filtration, DEPArray, VyCAP, AF4-MALS, CTCapture Chip, Magnetic bead based separation (MACS), ClearCell FX	Enrichment	35/50
RT-qPCR, BioRAD/droplet ddPCR & CFX96, QX200, QXOne Droplet Digital PCR System, WGA, SafeSEQ & OncoBEAM ctDNA mutation analysis	Molecular assays both CTCs and ctDNA	40/50

CTC analysis in ELBS

Lab#1

Enrichment /Detection technology	(Single-cell) isolation technology	DNA/RNA Amplification	DNA/RNA downstream analysis	
· 2x CellSearch (Menarini; CTC, CXC)	· CellCelector (ALS)	· Ampli1	· QIAseq Transcriptome UPX 3' Kit	Immunodetection
· Isoflux (Fluxion)	· FACS (MoFlo)	· QIAseq Transcriptome UPX 3' Kit	· DNA 2 Low Pass WGS	
· Kingfisher (Thermo)	· DEPArray (Menarini)		· DNA 2 Panel sequencing (e.g. TP53, KRAS)	
· Var2 (for capture or in CS for staining in 4 th channel])				
· EpCAM-independent antibody mix (manual capture with ferrofluid)				
· VyCAP				
· Antibody cocktail for surface proteins (Anti-CD45 [35-Z6], anti-Cytokeratin [C11/AE1/AE3], anti-EpCAM [VU1D9], anti-MUC1 [SM3])				Biophysical detection
· Parsortix (Angle)	· Nanowell (Sievewell; (Anti-CD45 [35-Z6], anti-Cytokeratin [C11/AE1/AE3], anti-EpCAM [VU1D9])			
· ClearCell (biolidics)				

Lab#2

Enrichment /Detection technology	(Single-cell) isolation technology	DNA/RNA Amplification	DNA/RNA downstream analysis	
Parsortix/four colour immunofluorescence using FITC-conjugated anti-CK (Miltenyi Biotec), Alexa Fluor 647-conjugated anti-vimentin (Abcam) () and PE-conjugated anti-CD45 (Miltenyi Biotec)	Fluidigm C1 Plate	N/A	Fluidigm multiplex RT-PCR	Biophysical detection
			Taqman RT-PCR	

CTC analysis in ELBS

Lab#3

Enrichment /Detection technology	(Single-cell) isolation technology	DNA/RNA Amplification	DNA/RNA downstream analysis	
If no defined commercial kit is used for detection, please specify Antibodies	The Genesis uses size and cell deformation to isolate CTCs from liquid biopsies. Once isolated, any collection of antibodies can be used to stain the cells in their wells for downstream imaging and analysis. We have validated staining and analysis protocols with PanCK antibodies from BioLegend (628601) and Thermo Fisher (180213), CD45 as a negative CTC marker from Bio-Rad (MCA87), and a full PDL1 staining kit from IncelIDX (T22004)	The Genesis can be used for single cell isolation and partitioning of cells for single-cell RNA isolation, but amplification of captured product occurs downstream after cell lysis on the chip.	The Genesis can be used for single cell isolation and partitioning of cells for single-cell RNA isolation, but analysis of captured product occurs downstream after cell lysis on the chip.	Immunodetection
If no defined commercial kit is used for detection, please specify Antibodies	The Genesis uses size and cell deformation to isolate CTCs from liquid biopsies.			Biophysical detection
Nucleic acid-based detection		The Genesis can stain the nucleus of captured cells and easily facilitates imaging analysis on 3 rd party microscopes		Nucleic acid-based detection

CTC analysis in ELBS

Lab#4

Enrichment /Detection technology	(Single-cell) isolation technology	DNA/RNA Amplification	DNA/RNA downstream analysis	
CellSearch	FACS	DNA: Ampli1 WGA	WES	Immunodetection
RosettSep/FACS			LowPass whole genome	
MoAB:			-Targeted PCR/NGS	
-Tumor markers:CK ('from Cellsearch), EpCAM, Hexokinase			With the cancer ampliSeq panel and one homemaid cancer panel	
-Wide panel of therapeutic targets including HER2, HER3, ALK, EGFR,gH2AX, PSMA...				
-Parsortix				Biophysical detection

Lab#5

Enrichment /Detection technology	(Single-cell) isolation technology	DNA/RNA Amplification	DNA/RNA downstream analysis	
AdnaTest Prostate cancer			RT-qPCR	Immunodetection
Immune cell depletion, then size based enrichment		Rolling Circle amplification	mRNA expression by In situ padlock probes	Biophysical detection
Cytogen Smart Biopsy				

CTC analysis in ELBS

Lab#6

Enrichment /Detection technology	(Single-cell) isolation technology	DNA/RNA Amplification	DNA/RNA downstream analysis	
CELLSEARCH Circulating Tumor Cell Kit (Menarini)	LMD (patented)	Ampli1 WGA kit (Menarini) REPLI-g WTA Single Cell Kit (Qiagen)	ddPCR (Biorad)	Immunodetection
CELLSEARCH CXC Kit (Menarini)			NGS	
CELLSEARCH Circulating Endothelial Cell Kit (Menarini)			CGH-Array	
CELLTRACKS Circulating Melanoma Cell Kit (Menarini)			Nanostring	
CELLSEARCH Epithelial Cell Profile Kit				
EpCAM- selection (ASCD-VyCap): AE1/AE3-eFluor 570, CK 7-FITC, CK 14-FITC, ALK-PE, CD45-APC, CD16-APC				
ClearCell FX1				Biophysical detection
Qiamp Circulating Nucleic Acid kit	ddPCR, One-Step RT-ddPCR, NGS			Nucleic acid-based detection

CTC analysis in ELBS

Lab#7

Enrichment /Detection technology	(Single-cell) isolation technology	DNA/RNA Amplification	DNA/RNA downstream analysis	
If no defined commercial kit is used for detection, please specify Antibodies	DEPARRAY	NGS Techonology Illumina NovaSeq	NGS	Immunodetection
			Techonology	
			Illumina	
			NovaSeq	
If no defined commercial kit is used for detection, please specify Antibodies	CellSearch Method			Biophysical detection

CTC analysis in ELBS

Lab#8

Enrichment /Detection technology	(Single-cell) isolation technology	DNA/RNA Amplification	DNA/RNA downstream analysis	
CellSearch	VyCAP	Ampli1 (Menarini)	Single CTC CNV prolifing	Immunodetection
	mAbs(clone):	In house preamp methods	Single CTC targeted seq	
	DAPI/Hoechst			
	Calcein			
	CD326(EpCAM, VD1D9)			
	CD146(MCAM, P1H12)			
	Cytokeratine(AE1/AE3)			
	Cytokeratine(C11)			
	PSMA(LNI-17)			
If no defined commercial kit is used for detection, please specify Antibodies				Biophysical detection
Negative depletion (RosetteSep)				
FACS (BD Bioscience)				
mAbs(clone): DAPI/Hoechst, Calcein, Apotracker, CD326(EpCAM, VD1D9), Cytokeratin(AE1/AE3), Cytokeratin(C11), PSMA(LNI-17), CD146(MCAM, P1H12), Melan-A(M2-7C10), Melanoma(HMB45), S100(4C4.9), Tyrosinase(T311)				

Lab#9

Enrichment /Detection technology	(Single-cell) isolation technology	DNA/RNA Amplification	DNA/RNA downstream analysis	
If no defined commercial kit is used for detection, please specify Antibodies	RosetteSep	AMPLI 1 (after DEPARRAY)	DNA	Immunodetection
	EPIDROP			
	DEPArray			
If no defined commercial kit is used for detection, please specify Antibodies				Biophysical detection

CTC analysis in ELBS

Lab#10

Enrichment /Detection technology	(Single-cell) isolation technology	DNA/RNA Amplification	DNA/RNA downstream analysis	
Magnetic beads (Dyna)	VyCAP			Immunodetection
Ficoll density gradients				
ISET				
<u>STAINING</u>				
A45 antibody/				
CK7 (thermo)				
anti-PD-L1 (Novus)				
Dapi (Invitrogen)				
Secondary antibodies (Invitrogen)				
VIM (Santa Cruz)				
CD45 (Novus)				
JUNB (Santa Cruz)				
CXCR4 (abcam)				
ISET system				Biophysical detection
VyCAP Pump				

CTC analysis in ELBS

Lab#11

Enrichment /Detection technology	(Single-cell) isolation technology	DNA/RNA Amplification	DNA/RNA downstream analysis	
Cell Search system (EpCAM-based enrichment, detection: CK8, 18 and 19 as inclusion marker and CD45 as exclusion marker)	DEPArray (Menarini Silicon Biosystems),	Ampli1 Whole Genome amplification kit (Menarini Silicon Biosystems)	Ampli1 LowPass kit (Menarini Silicon Biosystems)	Immunodetection
UT Immunomagnetic enrichment and fluorescent labelling	Puncher system (VyCAP)		Ampli1 Oncoseek panel (Menarini Silicon Biosystems)	
	Fluorescent Activated CellSorting (FACS Aria)			
Filtration, microfluidic separation				Biophysical detection
FISH, PCR, droplet PCR				Nucleic acid-based detection
FACS, Microscopy, SEM,				Other

CTC analysis in ELBS

Lab#13

Enrichment /Detection technology	(Single-cell) isolation technology	DNA/RNA Amplification	DNA/RNA downstream analysis	
CellSearch (Menarini)	DEParray (external analyses)	Ampli1 WGA	RNA: qPCR, expression arrays and Nanostring	Immunodetection
	VyCAP (external analyses)		DNA: methylation studies, ddPCR for SNV and CNV (QX200, Biorad), targeted sequencing (NextSeq, Illumina)	
	Micromanipulator			
	(internal analyses)			
Parsortix (Angle)	DEParray (external analyses)	Ampli1 WGA	DNA: ddPCR for SNV and CNV (QX200, Biorad), targeted sequencing (NextSeq, Illumina)	Biophysical detection
	VyCAP (external analyses)			
	Micromanipulator			
	(internal analyses)			
RUBYchip (Rubyanomed)			DNA: ddPCR for SNV and CNV (QX200, Biorad)	
RosetteSep™ (Stemcell)			RNA: qPCR, expression arrays and Nanostring	
Cytospin				

Lab#14

Enrichment /Detection technology	(Single-cell) isolation technology	DNA/RNA Amplification	DNA/RNA downstream analysis	
CellSearch (Menarini Silicon Biosystems, Italy)				Immunodetection
Dynabeads® Epithelial Enrich (Life Technologies, USA)				
Parsortix® (ANGLE plc, UK)				Biophysical detection
ISET (RareCells, France)				
Membrane-based affinity binding QIAGEN®, Hilden, Germany		QIAGEN®, Hilden, Germany	QIAamp Circulating Nucleic Acid Kit	Nucleic acid-based detection

CTC analysis in ELBS

Companies Technology providers

Name/Lab/COMPANY	CTC Technologies				
	Enrichment /Detection technology	(Single-cell) isolation technology	DNA/RNA Amplification	DNA/RNA downstream analysis	
nRich ^{DX} , Dr. Mayer Saidian	nRichDX will use its revolution system to capture CTCs from large volumes of biofluids up to 50mL.				Immunodetection
	EpCAM, EGFR				Biophysical detection
ALS Automated Lab Solutions GmbH, Jens Eberhardt	If no defined commercial kit is used for detection, please specify Antibodies	ALS CellCelector	NA	NA	Immunodetection
		ALS CellCelector RC			
	If no defined commercial kit is used for detection, please specify Antibodies	ALS CellCelector	NA	NA	Biophysical detection
		ALS CellCelector RC			
ANGLE S2A Laboratories, Michael O'Brien	ANGLE has an in-house set of antibodies. This will soon be available as a kit. It includes		ANGLE has an in-house technology for multiplex gene expression analysis which is able to analyse up to 100 genes at once. We have gene panels for ovarian and prostate cancer.		Immunodetection
	• CKs				
	• Mesenchymal markers				
	• Exclusion markers				
	• Nuclear stain				

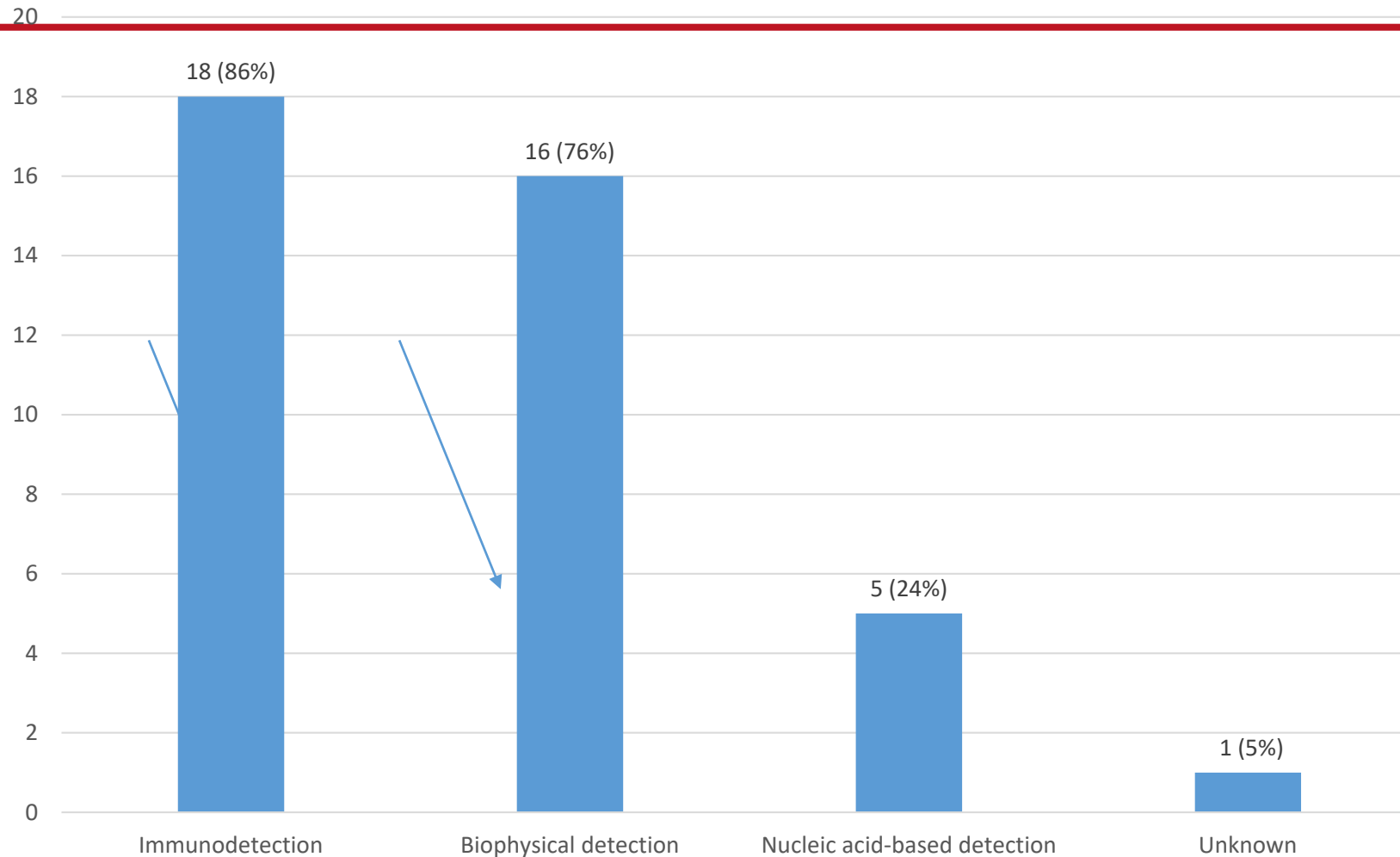
CTC analysis in ELBS

Companies/ Technology providers

Name/Lab/COMPANY	CTC Technologies				
	Enrichment /Detection technology	(Single-cell) isolation technology	DNA/RNA Amplification	DNA/RNA downstream analysis	
RareCyte, Inc., Alexandre Darmoise	AccuCyte® Sample Preparation System for simple and repeatable collection and transfer of nucleated cells from whole blood to slides with minimal loss	CytePicker® needle-based retrieval module for single-CTC isolation and downstream RNA/DNA analysis	Any choice of commercially available WGA kit, e.g. PicoPlex (Takara Bio)	Single-cell WGA, targeted sequencing, e.g. CleanPlex® OncoZoom® Cancer Hotspot Panel Kit (Paragon Genomics)	Immunodetection
	RarePlex® Staining Kits for CTC enumeration (CD45, CK/EpCAM, nucleus) and characterization (CD45, CK/EpCAM, nucleus plus any choice of 2 additional biomarkers)				
	CyteFinder® 7-channel high-speed imaging and integrated machine learning for detection and rank ordering of CTCs				

Name/Lab/COMPANY	CTC Technologies				
	Enrichment /Detection technology	(Single-cell) isolation technology	DNA/RNA Amplification	DNA/RNA downstream analysis	
QIAGEN GmbH, Siegfried Hauch / Thorsten Singer	Enrichment: AdnaTest CTC Select using immunomagnetic beads (anti EpCam, anti Her2, anti EGFR mixture)	NA	QuantiTect/QuantiFast/QuantiNova (RT-PCR) product line	AdnaTest Detect product line (RNA profiling)	Immunodetection
	AdnaTest Detect product line (RNA profiling)			AllPrep Nano CTC mRNA/gDNA purification for RT-PCR/ dPCR/qPCR and Sequencing	
	AllPrep Nano CTC mRNA/gDNA purification for RT-PCR/ dPCR/qPCR and Sequencing				Nucleic acid-based detection

CTC enrichment



8/18 CellSearch

4/16 Parsortix

CTC analysis in ELBS	Total labs: 18
Types of cancer	
breast	16 (89%)
prostate	17 (94%)
NSCLC	12 (67%)
ovarian	7 (39%)
colorectal	9 (50%)
bladder	2 (5%)
pancreatic	2 (5%)
Head and neck	5 (28%)
melanoma	7 (39%)

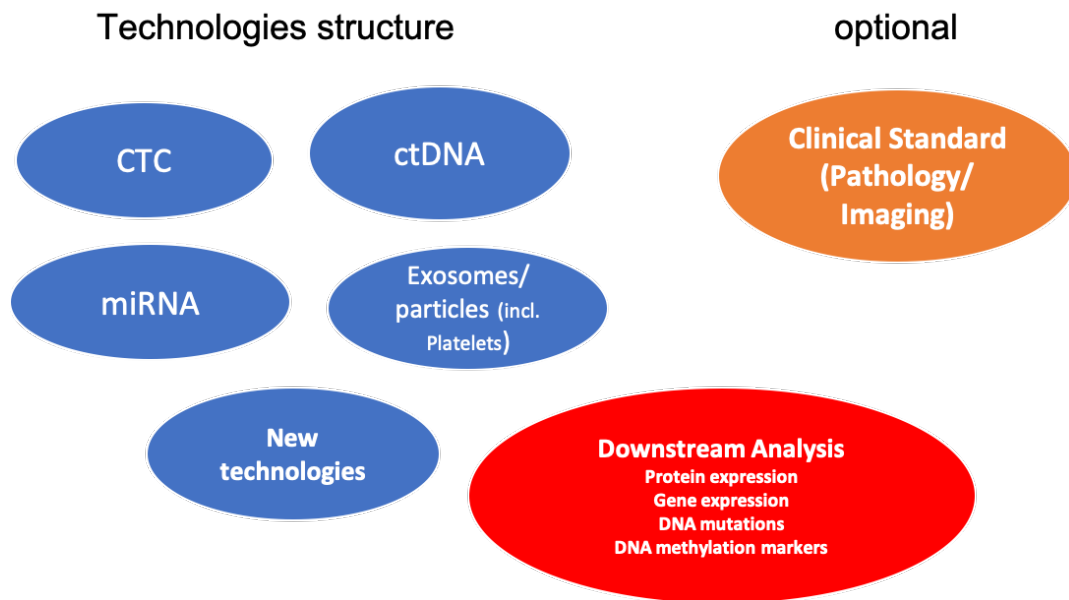
Blood volumes: 4mL- 30mL

External controls: 7/18 (cell lines)

blood collection tubes: CellSave, EDTA, Streck, PAXgene blood ccfDNA, DLA input,

Molecular assays: 5/18

Technologies



Short-term goals

- To form working groups based on the suggested structure with two (or more) responsible organizers for each working group
- To make an inventory of all technologies, analytes and biomarkers used in the labs of ELBS members (questionnaire prepared)
- **To validate novel technologies in terms of sensitivity, specificity and robustness with the platforms established within CANCER-ID and extend this beyond NSCLC**
- To establish networks with common aims to identify funding opportunities and facilitate common funding

Long-term goals

- prepare a list of validated LB technologies, based on the clinical question to be answered
- standardization of LB tests (ISO-15189) at the pre-analytical and analytical steps
- establishment of external quality control schemes in collaboration with the ELBS standardization group

CTC

Evi Lianidou

Nick Stoecklein

Working towards accreditation and reimbursement

- Markers of immediate interest for ISO15189 certification
 - Establishment of a validation pipeline
 - Distribution of protocols and support for certification
- ELBS "certified" labs

Ring trials and technology assessment

- Organization and frequency of ring trials
 - "Pre-competitive arena" for companies
 - Cross comparisons between technologies
- ELBS "approved" technologies

Networking

- "open channel" for discussion of technologies and issues
- Exchange between users and companies

High-blood-
volume analysis
(DLA)

Technology
Research

Cancer specific
technologies

CTC down-
stream
technologies

Please contribute
→ chair a task

Networking

- “open channel” for discussion of technologies and issues
- Exchange between users and companies

Plan – Online Meeting **every two months** (Wednesdays) for max 2 hrs.

Suggested topics:

- News from CTC working group members → 1-2 presentations (10-15 min) on (new) technologies by members who want to share/discuss their data
- New technologies from companies → 1-2 presentations (10-15 min) on CTC-related new developments that companies want to share with ELBS members
- Possibility to chose a certain topic (optional), e.g. pre-analytics
- Brief Journal club like presentations on new technologies from publications that could be discussed (5 min presentations)

Development of CTC reference standards

Krystyna Nahlik, PhD

Field Application Scientist, LGC Clinical Diagnostics

Summary of CANCER-ID proficiency testing



- Used 2 NSCLC cytokeratin expressing cell lines with distinct levels of EpCAM expression: NCI-H441 (EpCAM^{high}) and NCI-H1563 (EpCAM^{low}).
- Spiked 50-100 BCT fixed, stained and directly counted tumor cells into fresh healthy donor blood. Used 3 different collection tubes to conform to technology provider SOPs.
- Spiked cells shipped to labs overnight at 3 different temperature conditions.
- Tested 5 different CTC enrichment methods using size (3), EpCAM expression (1) or density (1) criteria for enrichment and CTC definition based on cytokeratin positivity (4) and /or CD45 negativity (all).
- Methods selected based on CANCER-ID membership and not use frequency
- Staff at participating sites received on-site training from technology providers

Results: Mean recovery rates ranged between 49% and 75% for NCI-H411 and 32% and 76% for NCI-H1563 and significant differences were observed between the tested methods.

Open questions



Technical

- What are the methods most commonly used by ELBS members?
- Is it possible to meet the enrichment / identification criteria for multiple methods using a single reference material?
- If not, how many different samples are required?
- Would high positive controls or challenging materials around the cut-off point be preferred?
- What should be the format of the samples and the number of cells provided?

Commercial

- How are the CTC measurements applied in academia, clinical trials, what methods are on the fore? Are there any CDx assays expected or in development?
- Number of labs likely to participate in EQAs, frequency?
- Anticipated need for reference materials outside of EQA setting

CTC reference material recommendations



ELBS perspective

- Looking for 1-2 reproducibly performing materials with broad applicability across methods and cancer types to fulfil EQA needs
- Minimum set of parameters to challenge enrichment methods, e.g. size + EpCAM expression
- Epithelial vs leukemic cells as a starting point?
- Option of selecting cells based on size criteria and transfecting / infecting with required markers or fluorescent reporters
- Advantages of CTC-derived cell lines from ELBS members? tumor genotype shown to be stable, but not phenotype

LGC SeraCare perspective

- Need to side-step shipping and specific blood tube requirements and ensure shelf life
- Looking for community feedback / collaboration in defining minimum requirements

Development challenges

Previous CTC research experience

- Accurate cell size selection is challenging and time intensive
- Fixing does not resolve all stability problems, hard fixing can affect downstream analysis – would cellular lyophilization be a viable alternative?
- Spiking very low number of cells into blood is not a reproducible process (cell loss and adherence to tubes might affect recovery)

Outstanding challenges:

- Ensuring stability of characteristics (size, epitope expression)
- Ensuring sufficient shelf life and handling ease / reproducibility
- Managing lab user training if necessary
- Cost of development and pricing of the end product

CTC project outlook



- LGC SeraCare would like to support ELBS and the CTC community
- Planning to invest into expand sourced materials and analysis capabilities
- Currently at early feasibility/investigation stage, resources to be allocated to the project in early 2022

We have previously developed a custom CTC reference material for a client, but the planned development will be completely new technology/chemistry

Reference material design process:

- Starting source material
- Genetic manipulation: transient / permanent
- In house testing method and release criteria

Clinical Lab Services

Linearity, Concordance and Custom Spike Panels

Overview

- Types of panels offered and lab application
- What is included in each panel
- How to order

Linearity

- A linearity panel establishes the range within which the assay results are linear, also known as the “reportable range”.
- It consists of multiple spike levels.
- Often used as Verification of Performance Specifications (a CLIA requirement).
- Can show precision and linearity of the assay.

Concordance

- A concordance panel establishes if an instrument is equivalent to another instrument and consists of only one spike level.
- Also referred to as an Inter-Instrument Comparison.
- Often used to compare new CellSearch system with MSB Lab in HV as part of installation.
- This panel is used by our CLIA Lab in HV to show equivalency between two AutoPreps as part of our CLIA certification.
- It can also be used to establish precision of the assay.

Custom Spike Panel

- An option for the lab to select what type of testing they would like to conduct.
- For example – To show there is no carry over from tube to tube when a large number of CTCs are present.
 - MSB sent samples (3500-4000) and no spike samples for them to process.

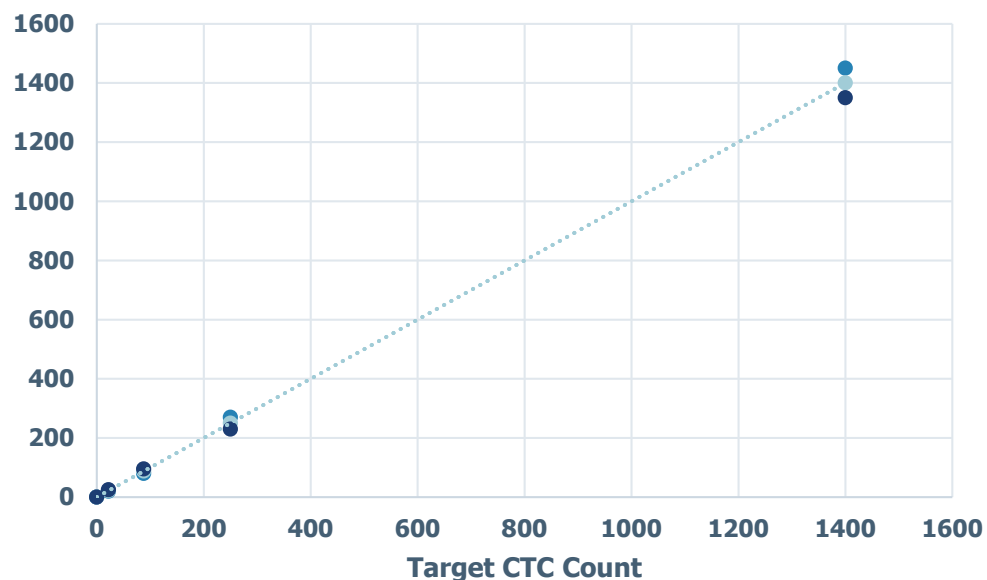
Linearity Panel Options

We offer two types of Linearity Studies.

- Panel 1 linearity - Testing in Customer Lab.
- Panel 2 linearity - Testing in Customer Lab & MSB HV Lab.
Results from MSB HV Lab sent to Customer Lab.

Linearity Panel Contents

Linearity Panel



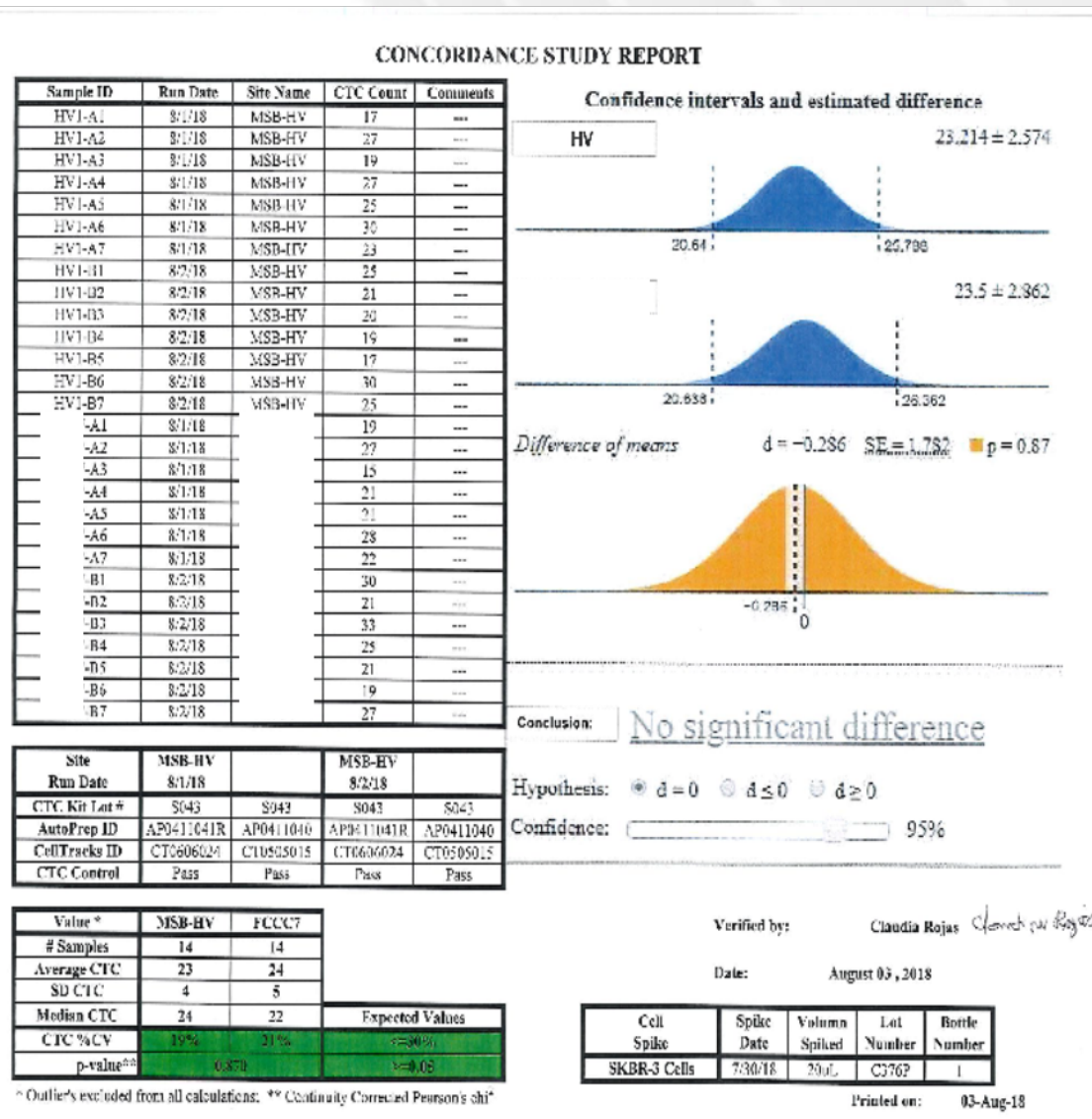
Each panel contains the following:

- 15x AP tubes containing 6.5ml of buffer spiked with fixed cells (SKBr3 for CTC testing)
- Target concentration dilutions ($\frac{1}{4}$ dilutions) (1400, 350, 88, 22, 0) 3 tubes for each dilution, shipped 2-8C
- 14x CellSave collected normal donor samples (to be pooled by site) shipped ambient temp
- Customer is responsible for supplying the reagents and agrees to perform their own data analysis and acceptance criteria

Concordance Panel Contents

- 28x AutoPrep tubes filled with 6.5 mL of buffer will be spiked with approximately 25 SKBr3 cells/tube
- The customer will receive:
 - 14 of these AutoPrep conical tubes (shipped 2-8C)
 - 14 CellSave collected normal donor blood samples (shipped ambient)
 - The lab in HV will also process 14 tubes as above.
 - It is recommended to process 7 tubes and one control on each of two days.
 - The customer is responsible for providing their reagents and will send their data to HV for analysis.
 - MSB provides one page data summary back to Customer (based on MSB established acceptance criteria)

Sample Concordance Report



* Outliers excluded from all calculations. ** Continuity Corrected Pearson's chi²

Custom Spike Panel

- Up to 18 spiked buffer tubes .
- 1 tube of CellSave collected normal donor blood for each buffer tube.
- Up to 6 different concentrations .
- Customer is responsible for data analysis and acceptance criteria.

Product Codes

Product Code	Description	Quantity / Description
85000063	Linearity Panel For Customer Only	Shipped to Customer Lab: 15x buffer samples Shipped to Customer Lab: 14x 10mL normal donor blood shipped
85000062	Linearity Panel For Customer and MSB	Shipped to Customer: 15x buffer samples Shipped to Customer: 14x 10mL normal donor blood MSB HV Lab: 15x buffer and blood samples processed
85000064	Concordance Panel For Customer and MSB	Shipped to Customer: 14x Samples shipped MSB HV Lab: 14x Samples processed
85000102	Custom panel Customer Only	Shipped to customer Lab: 18X buffer samples 18x 10mL normal donor blood shipped

How to order

- Contact Sales team for quotation. [\(info@siliconbiosystems.com\)](mailto:info@siliconbiosystems.com)
- All samples are prepared and shipped from MSB HV Lab in USA
- Once a PO is received, MSB will contact customer to schedule the study and send the customer a protocol.
- Samples will be shipped on Mondays.

www.siliconbiosystems.com

Email: info@siliconbiosystems.com