Medicines & Healthcare products Regulatory Agency

The proposed 1st WHO International standard for ctDNA diagnostics

2nd ELBS ctDNA Technology Meeting

Leandro Lo Cascio, PhD

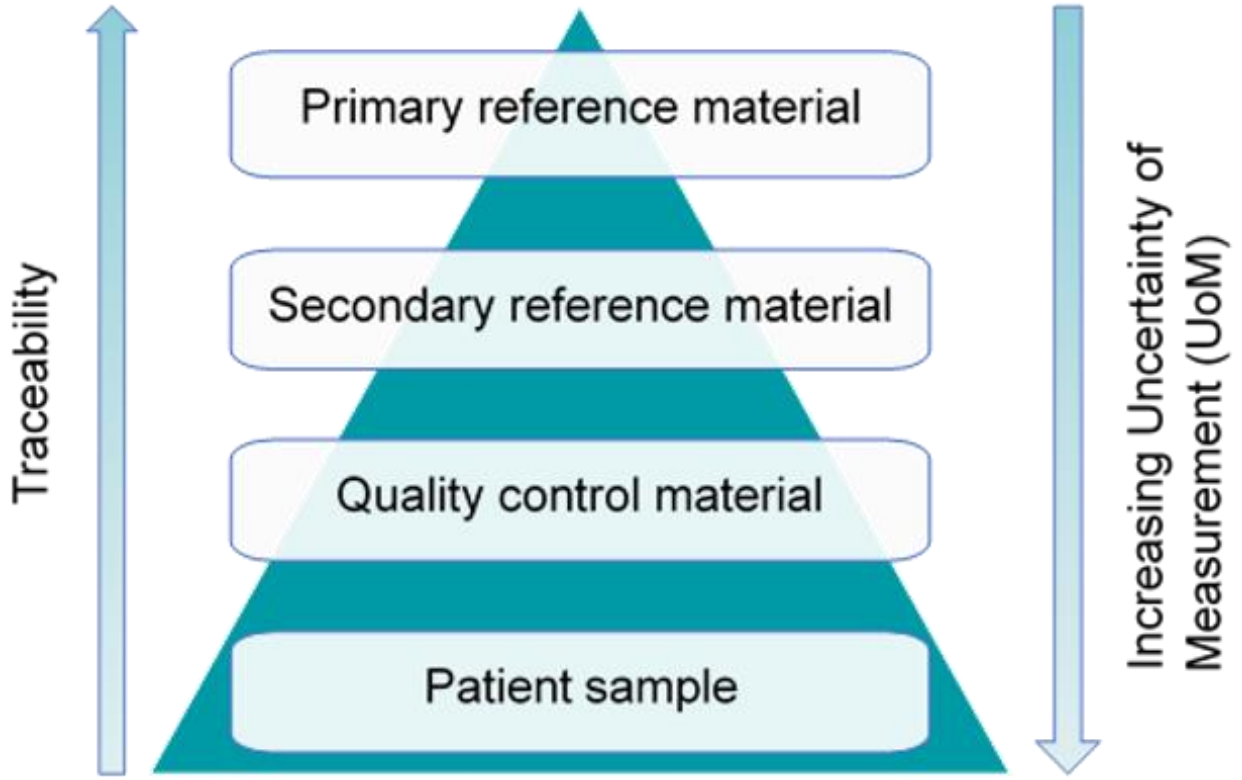
July 5th 2022

Centre of the UK **Medicines and Healthcare Products Regulatory Agency**

NIBSC
Celebrating 40 years of excellence

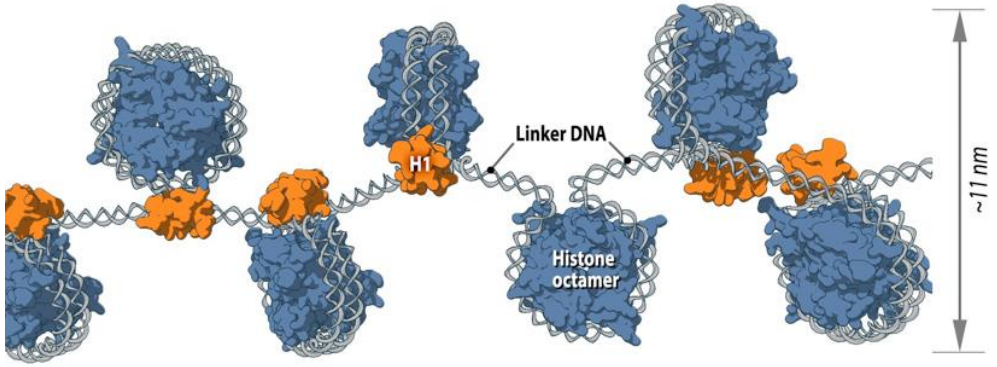
WHO ISs = Primary reference materials

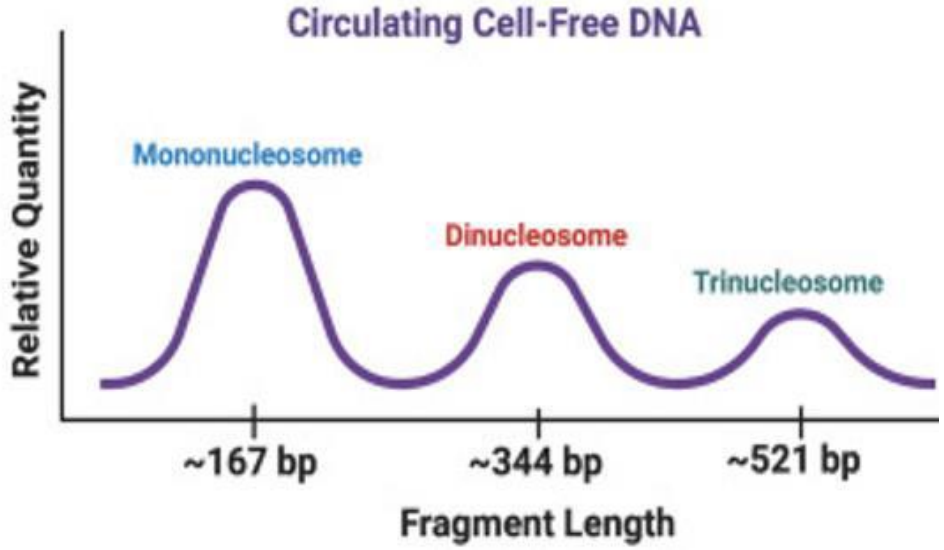
- One single batch to minimise variability
- Extensive degradation studies
- Consensus value assigned by community



Circulating cell free DNA in plasma

- Derived from apoptosis, programmed cell death
- Comprises nucleosomal DNA
- 170bp length DNA coiled around a core of histones
- Mono-nucleosomes or oligo-nucleosomes
- Longer fragments derived from necrotic cell death





Underhill: Molec Diag Therapy (2021) 25:389-408

Advantages and Technical Challenges

- Less invasive of tissue biopsies
- Can be taken regularly to monitor treatment efficacy.
- It allows the detection of multiple mutations that are linked to drug resistance and metastasis.

- ccfDNA has a short half-life
- The mean concentration in the blood stream is approximately 12ng/ml (range 9 to 19ng/ml).
- The amount of ctDNA could be as low as 1ng/ml.

Therefore it is essential to efficiently isolate and quantify ccfDNA.

Objectives

- Establish the 1st WHO international reference material for ctDNA (endorsed)
- Identify the best candidate materials
- Identify a work flow to efficiently generate a single batch
- Evaluate the long term stability of the process
- Finalise the production and QC
- Setup a collaborative study with expert of the field
- Assign a consensus value

Experimental Time Line

- Spiking-in various size double stranded oligonucleotides (Gene Blocks) into Octaplas and freeze-drying

  > synthetic DNA is very stable in freeze-dried plasma BUT does not reflect the size distribution and does not help harmonising the extraction process.

- Incubation of cell line supernatant at 37C to allow further breakdown of chromatin into mono-nucleosomes for DNA extraction

  > very low yield of DNA and very large volumes of supernatant required.

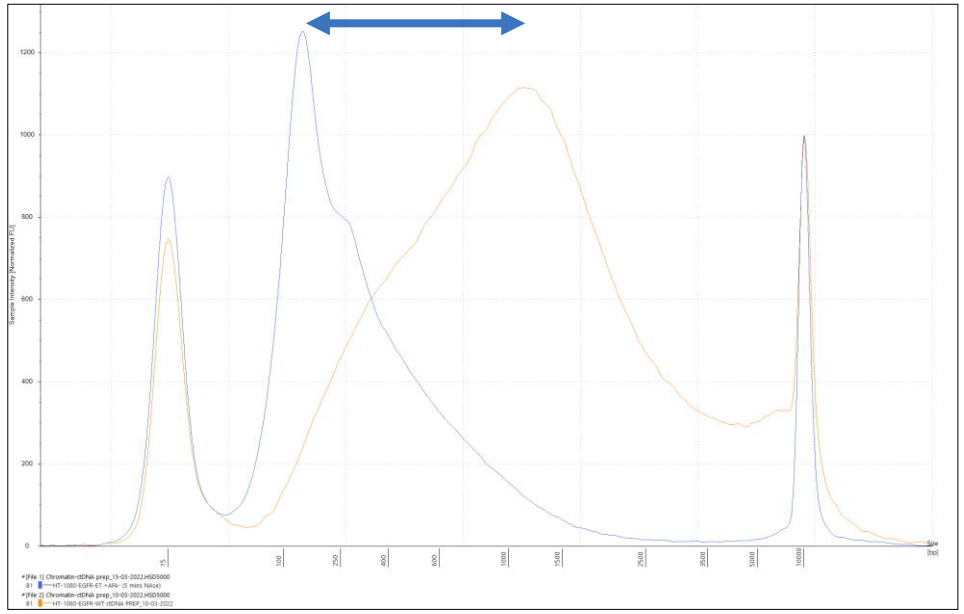
- Chromatin extraction from cell line carrying mutations of interest and Micrococcal nuclease treatment

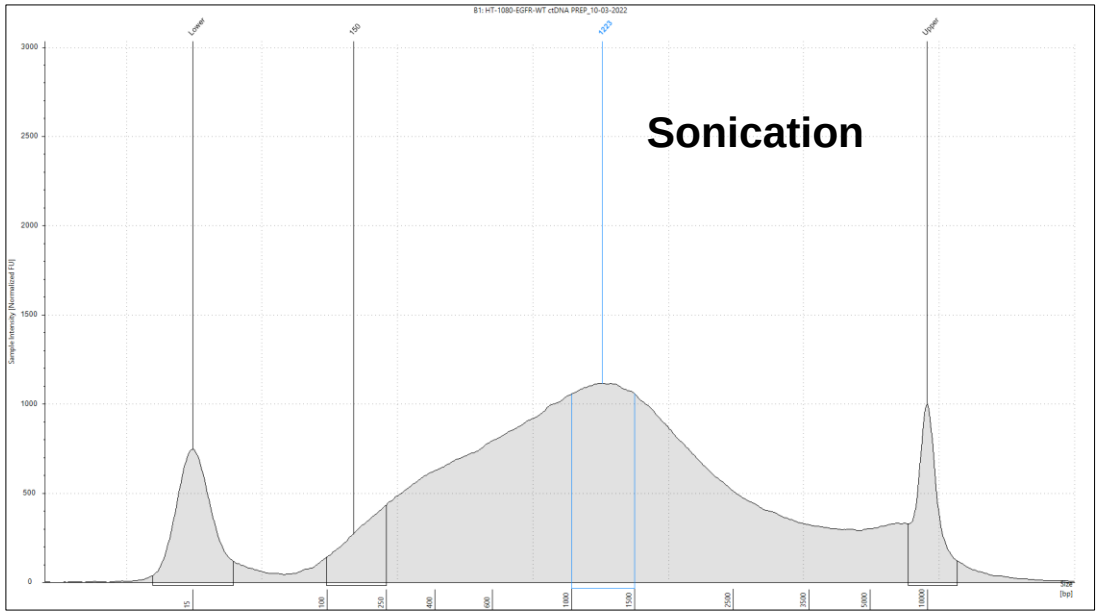
Chromatin Extraction

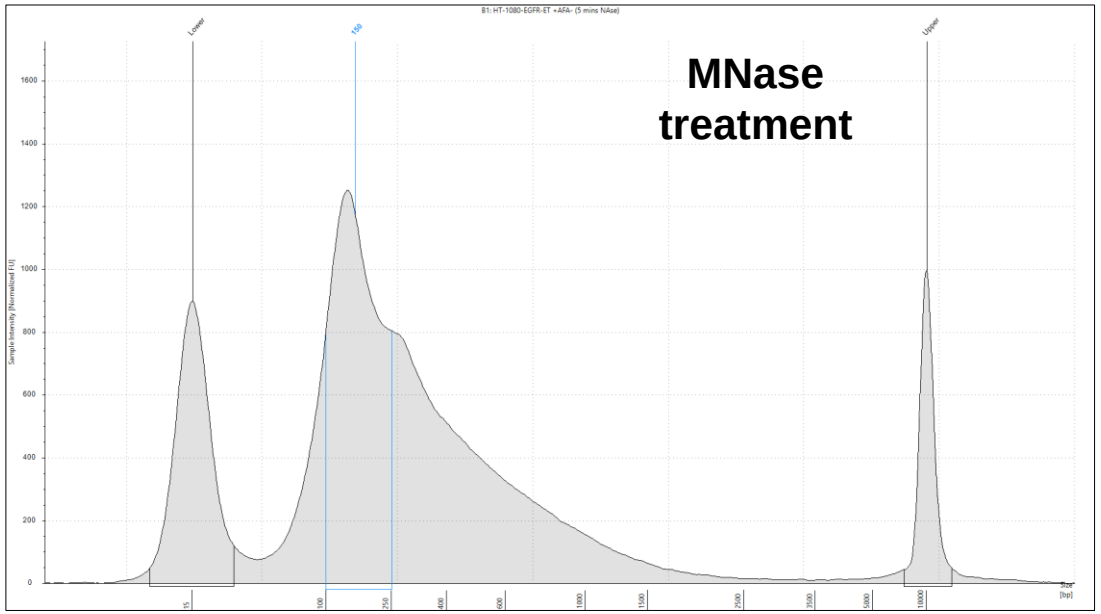
- Grow cells to the desired number
- Lyse cells to release gDNA
- Sonication to break up nucleosome links to DNA (Covaris)
- Treatment with Micrococcal nuclease
- Store at -20C

- Circulating cell free DNA extraction (Qiagen)

Chromatin Extraction







Candidate material

Developed for the EGFR variants (gDNA)

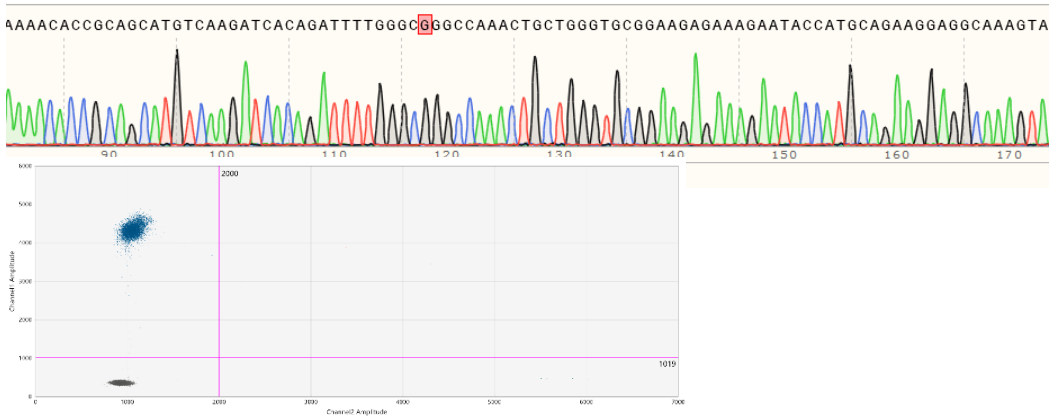
Cell line

- MSI Status: MSS
- Ploidy: 1.986
- TMB 4.7 Mut/Mb

CRISPR

- Neon Transfection System (Invitrogen)
- RNP (RiboNucleoProtein) complexes: Cas9 protein + [crRNA:trancRNA duplex]
- ssODN (single-stranded oligodeoxynucleotides)

p.L858R (C>T)



p.T790M (C>T)

Final formulation

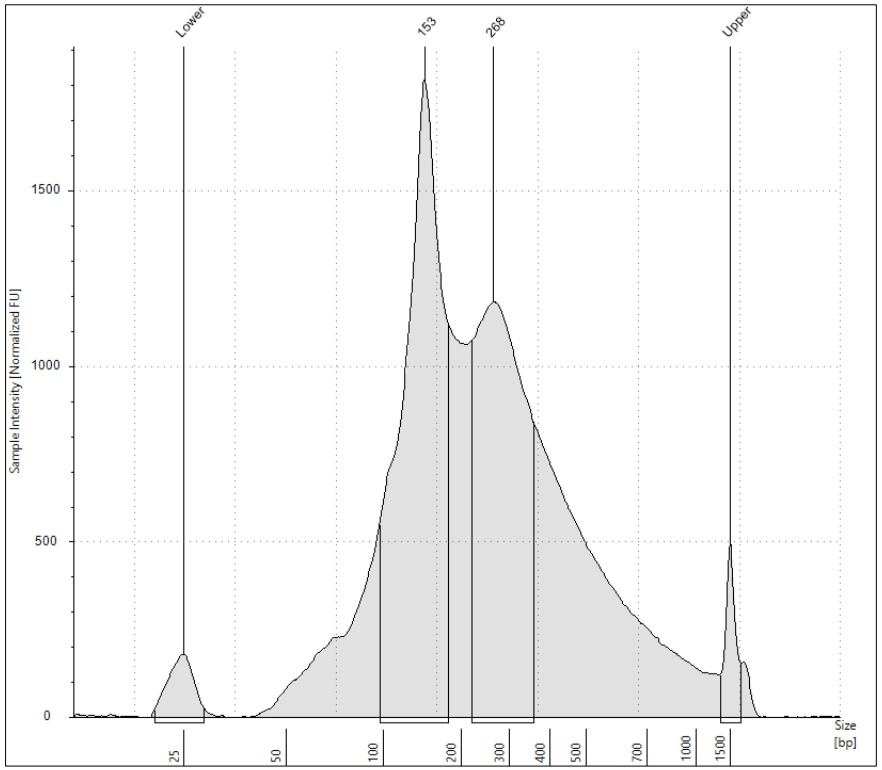
Each vial: 4ml @ 40ng/ml (160ng total) > 1500x vials

- Chromatin prep Mix
- DNA-depleted human plasma (Anchor Molecular)
- Freeze-dry cycle
- Storage -20C

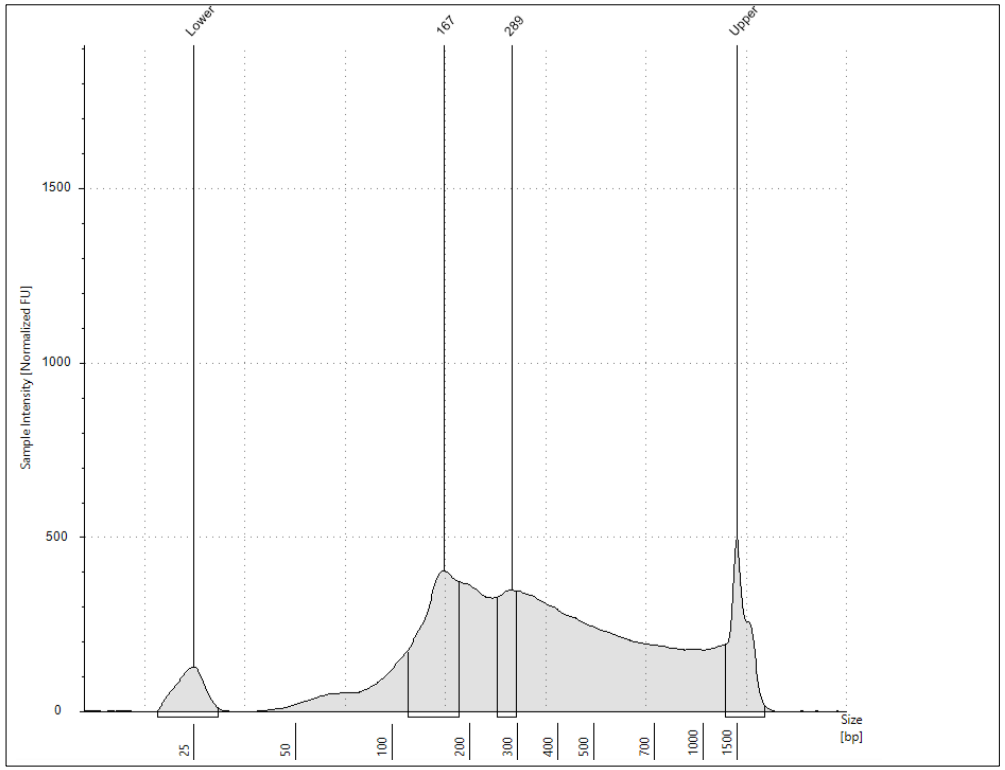
	Total (ng)	Copies DNA	%AF
L858R	8	2470	5.00
T790M	8	2470	5.00
EGFR WT	144	44460	90.00
Total	160	49400	100.00

Final formulation

EGFR WT material



EGFR mut material



WHO International standard Product life-cycle

Upcoming candidate materials

- Other EGFR variants:
  - C797S
  - Exon19del (E746_A750)

- Microsatellite instability/Tumour mutational burden (Trial filling completed)

Acknowledgments

Genomics Team
- Malcolm Hawkins (Past member)
- Angela Pia Sanzone, PhD (Past member)
- Hoda Kardooni, PhD

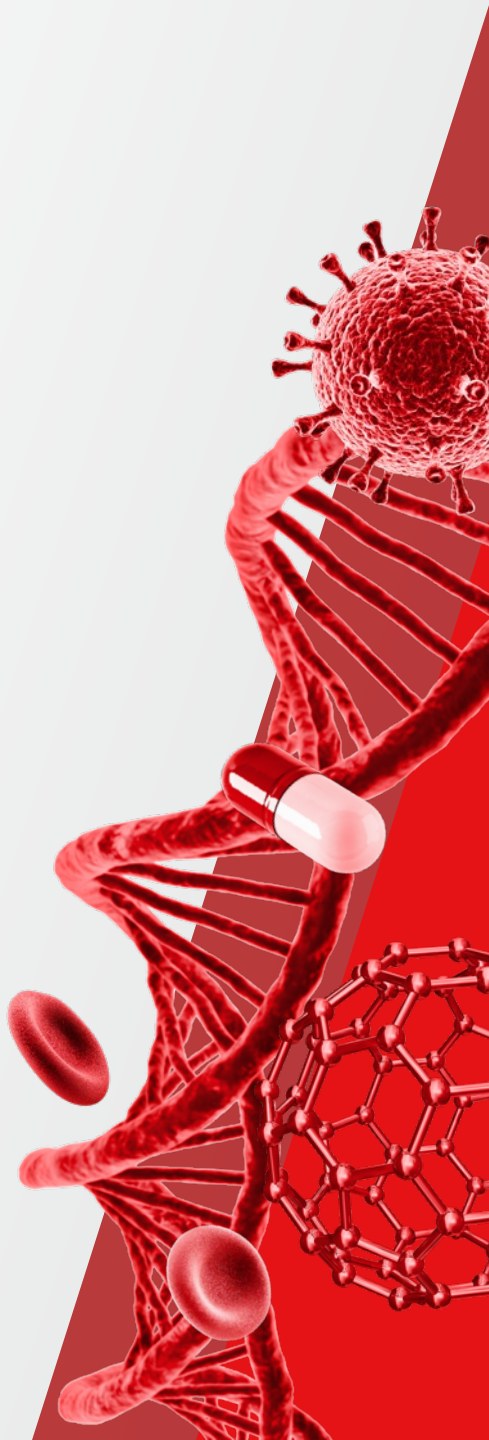
SPD Team
- Sara Jane Holmes

NIBSC TDI Team
- Paul Matejtschuk

Development of an In-Kit cfTNA Control and Other Liquid Biopsy Reference Material Capabilities

Kunal Banjara and David Chi

July 5, 2022



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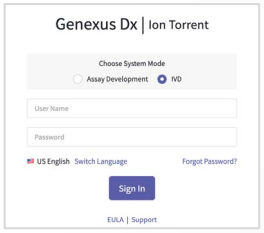
Building Blocks of a Liquid Biopsy NGS Workflow

A complete workflow that allows users to start with a sample and end with a clinically relevant report

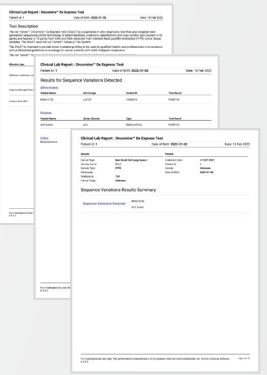
Sample

Instrument



Software

Kits



Report

Core Consumables

Panel

Control

To complete the workflow, we needed to include an **"in-kit" control** that would allow users to **monitor performance** of their liquid biopsy workflow

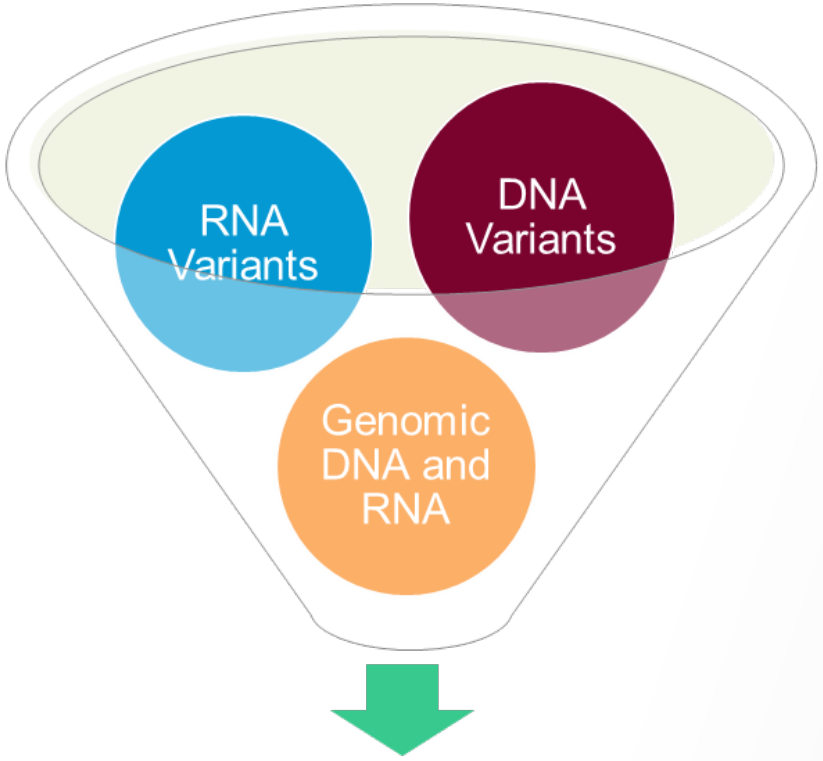
Leveraging Broader Organization to Develop a Solution

With a set of requirements; partnered with our colleagues that we knew could provide what's needed

Category	Requirement
Relevant	Design and include targets that are **common to liquid biopsy testing for clinical oncology application**
Efficient	Include BOTH DNA and RNA based targets into a single reaction; **minimizing the consumption of consumables and sequencing real-estate**
Scalable	Consistent production to utilize control run-after-run and **monitor performance based on expected vs measured variant levels** (e.g., allele frequency, copies)

Development of the “In-Kit” Control

A single control with SNV, MNV, RNA fusions, CNV, Genomic DNA and RNA



Single tube of reference material for liquid biopsy workflow performance monitoring

DNA Variants
19 SNVs, MNVs, Indels + 2 CNV

RNA Variants
7 Common to liquid biopsy RNA fusions.

Genomic DNA and RNA
GM24385 genomic DNA and RNA background well characterized by Genome in a bottle Consortium (GIAB)

AcroMetrix™ Multi-Analyte ctDNA Plasma Control A - E

SKU Part Number: 957563

- Hotspot DNA: Single to highly multiplexed variants (SNV, MNV, Indel) allows less sample input.

- gDNA: well-characterized GM24385 allows known germline mutations.

Normal Human Plasma + **ctDNA control (Buffer)** = **ctDNA control (Plasma)**

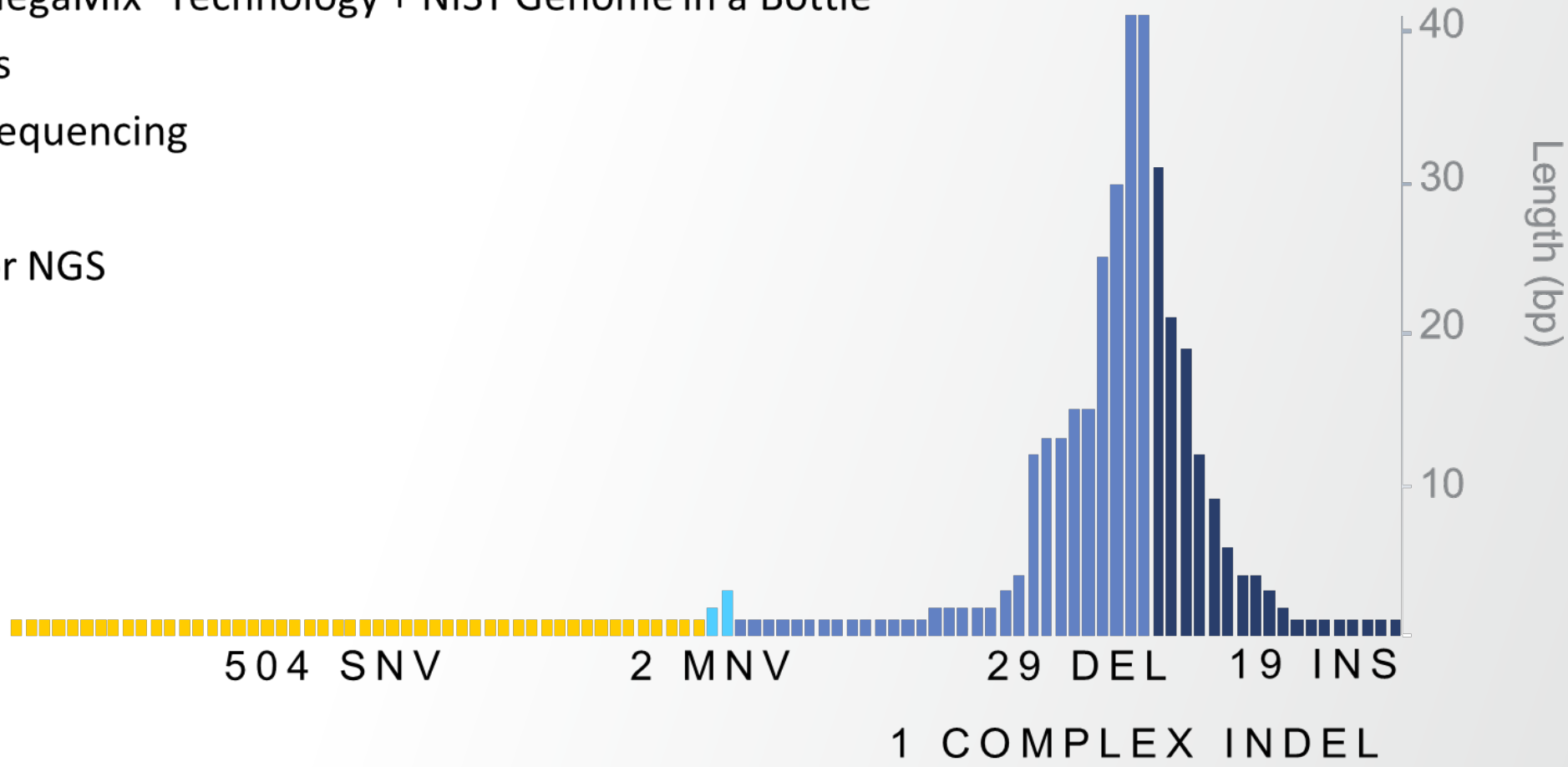
- Free of BBP
- Free of target mutations

- Frequency check
- DNA size check
- Concentration check

- Extraction yield
- Frequency check
- Full process control

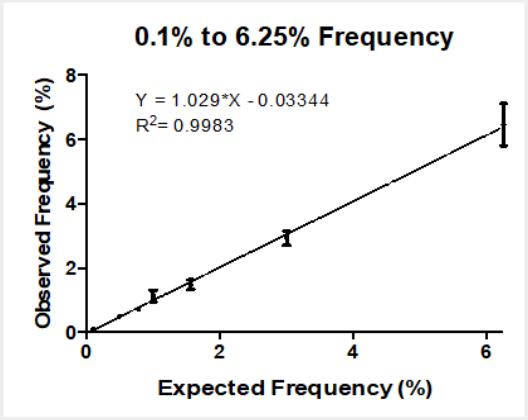
AcroMetrix™ Oncology Hotspot Control

- Built on AcroMetrix™ MegaMix™ Technology + NIST Genome in a Bottle
- 550+ Common Variants
- Confirmed by Sanger Sequencing
- Platform Agnostic
- The only IVD control for NGS



For In Vitro Diagnostic Use

Custom Solution Capabilities



Circulating Tumor DNA in Human Plasma *for new assay development*

Liu et al. **Evaluation of ctDNA extraction methods and amplifiable copy number yield using standardized human plasma-based ctDNA control materials .** ***AMP 2018.***

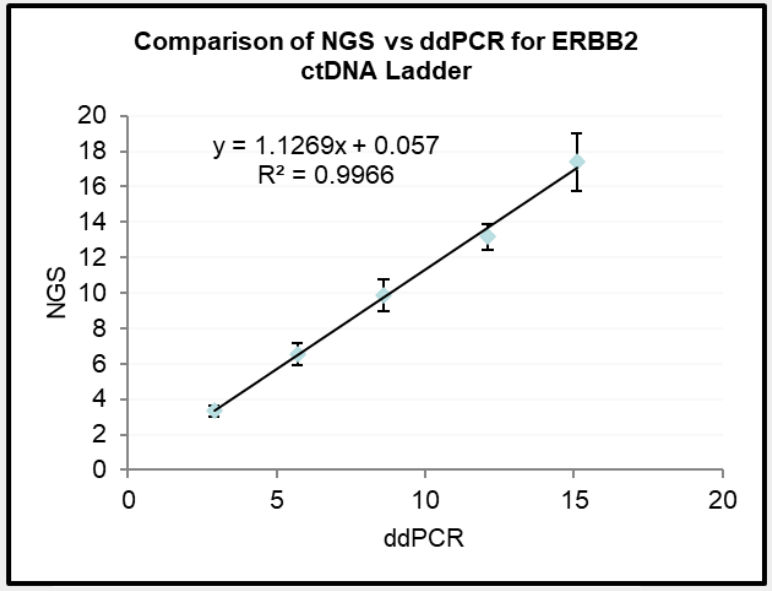
ctDNA Hotpsot Daily Run Control

Concentration: 200 ng- few ug
AF% : as low at 0.1%
Variants: AOHC or single variants
Matrix: buffer or plasma
QC: ddPCR

ctDNA Hotspot Frequency Ladder (VnV)

Concentration: 20ng/vial or can be customized according to the customer's needs
AF% : each kit contains one vial of 5%, 2.5%, 1% and 0.1%
Variants: AOHC
Matrix: buffer or plasma
QC: ddPCR

Custom Solution Capabilities



Banjara et al. **Development of 17 Novel Copy Number Variation (CNV) Reference Materials based on the Genome in a Bottle**. ***AACR 2019.***

Multiple Variant Types *for full understanding of performance*

ctDNA CNV Daily Run Control

Concentration: 200 ng- few ug
Copy Gain : as high as 15 copies
Genes: MET, ERBB2, ERBB3, PIK3CA, EGFR, BRAF, FGFR1, FGFR2, FGFR4, KIT, KRAS, MYC-N, PDGFRA, MDM2, CD274, MYC-L and MYC
Matrix: buffer or plasma
QC: ddPCR

ctDNA CNV Frequency Ladder (VnV)

Concentration: 20ng/vial or can be customized according to the customer's needs
Copy Gain : each kit contains one vial of 2, 3, 6, 9 and 12 copies
Genes: MET, ERBB2, ERBB3, PIK3CA, EGFR, BRAF, FGFR1, FGFR2, FGFR4, KIT, KRAS, MYC-N, PDGFRA, MDM2, CD274, MYC-L and MYC
Matrix: buffer or plasma
QC: ddPCR

Horizon Discover

Quality Manufacturer of Molecular Reference Standards

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PerkinElmer has a comprehensive portfolio of screening and diagnostic solutions that enable early clinical insights for a pro-active approach to healthcare.

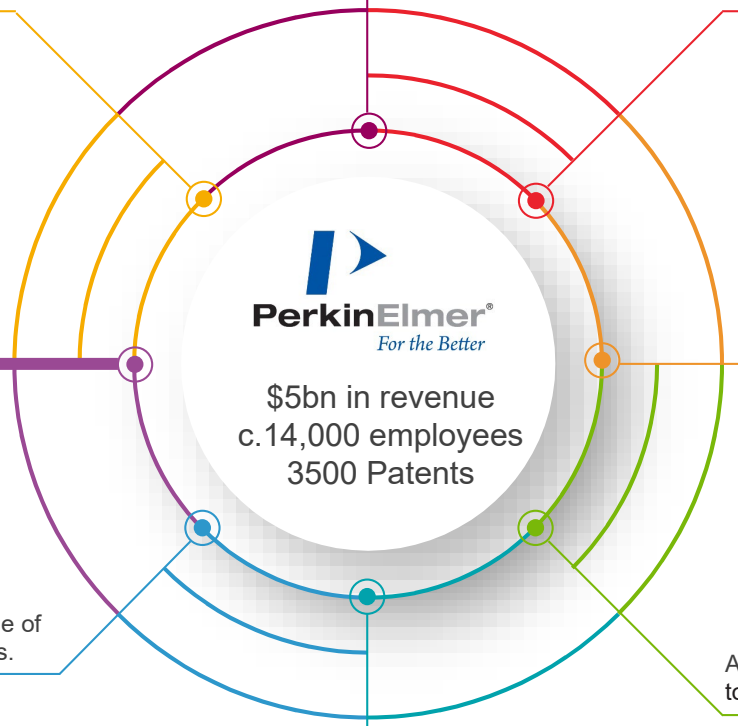
DIAGNOSTICS

Horizon Discovery, Oxford Immunotec, Bioo Scientific, Tulip Diagnostics & Euroimmune are complimentary brands within the PerkinElmer organization.

PERKINELMER BRANDS

Genomic workflow solutions that eliminate challenges associated with genomic analysis by providing complete, single source solutions from sample to solution.

APPLIED GENOMICS



PerkinElmer Genomics is a state-of-the-art biochemical and molecular genetics laboratory that provides newborn screening and genomic testing service.

PERKINELMER GENOMICS

Supporting drug development from basic research and drug discovery to the development & manufacturing of new, drugs and clinical treatments.

LIFE SCIENCE

Enterprise-wide laboratory solutions that deliver a range of innovation managed lab and adjacent support services.

ENTERPRISE

A range of solutions across industrial and environmental sectors, designed to provide the highest levels of detection, accuracy, and sensitivity.

ANALYTICAL

PerkinElmer's Informatics platforms empower customers to gain critical insights from data analytics, accelerating informed decisions by unifying data and activities across R&D, translational research and clinical trial operations.

INFORMATICS

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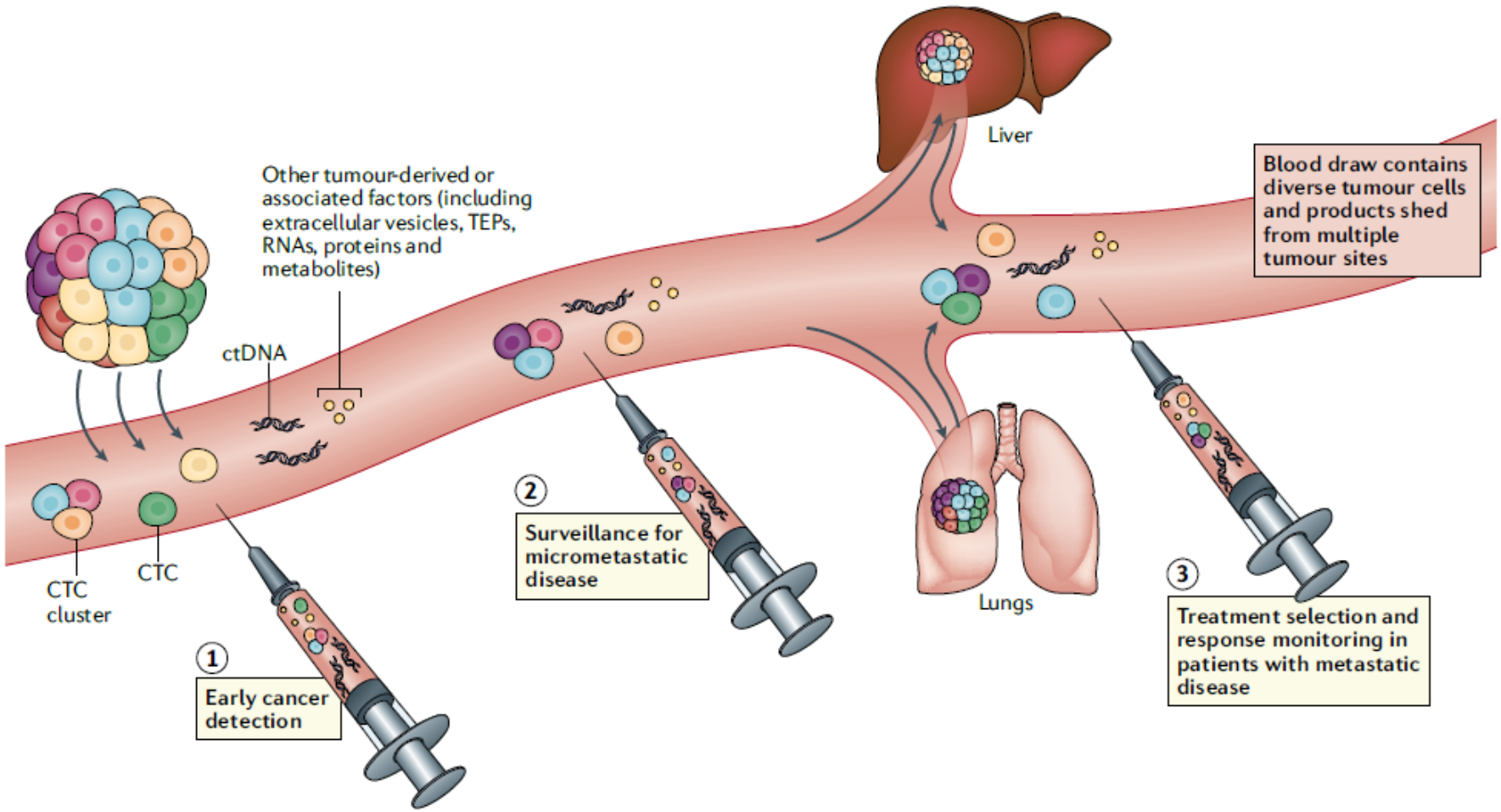
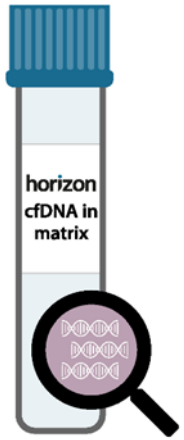
Liquid Biopsy – clinical applications





Liquid Biopsy enters the clinic- implementation issues and future challenges
Michail Ignatiadid, George W. Sledge and Stefanie S. Jeffrey
Nature Review Clinical Oncology, 18, 297-312 (2021)

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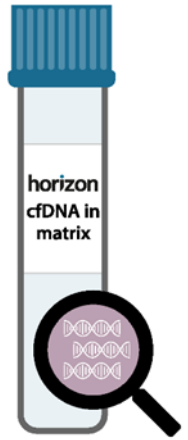
How do our customers use our standards?

- **Quality Control**
  - Assess impact of changes (e.g. new reagent, equipment move)
  - Assess user competency (lab staff training)
  - Provide evidence of clinical diagnostic quality for lab audits
  - Run controls – **ensuring accuracy with every run**
- **Assay development**
  - Calculate **accuracy**, **sensitivity** and **specificity**

  - Evaluate assay robustness
  - Limit of Detection (LOD) determination
  - Analysis Pipeline testing
- **Analysis**
  - Ensure confirmed variants are detected
  - Determine LOD
  - Provide allelic frequency confirmation
  - Assess coverage
  - Calculate False Positive & False Negative error rates
  - Orthogonal validation



Format: Fragmented DNA
For: Cell-free DNA liquid biopsy assays
- Fragmented to 160-170 bp
- Available in buffer or synthetic plasma
- *Multiplex I, Structural Multiplex, EGFR Multiplex*

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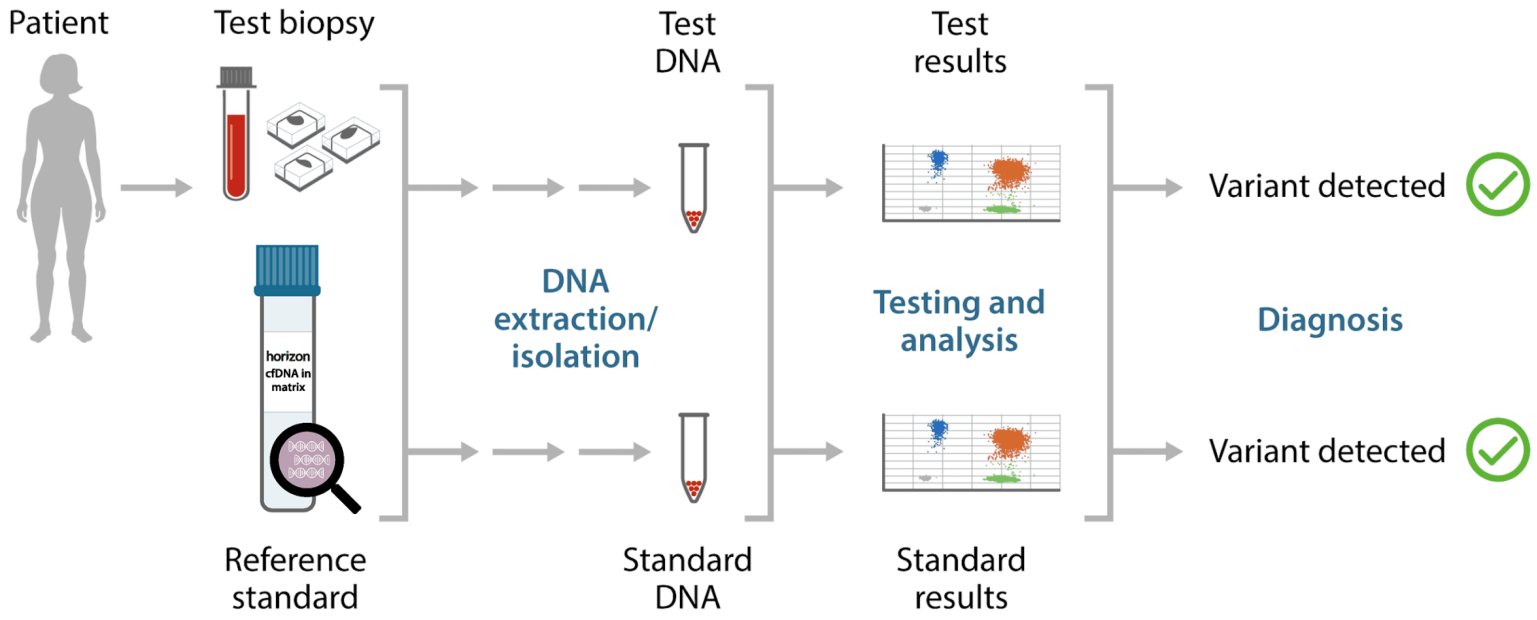
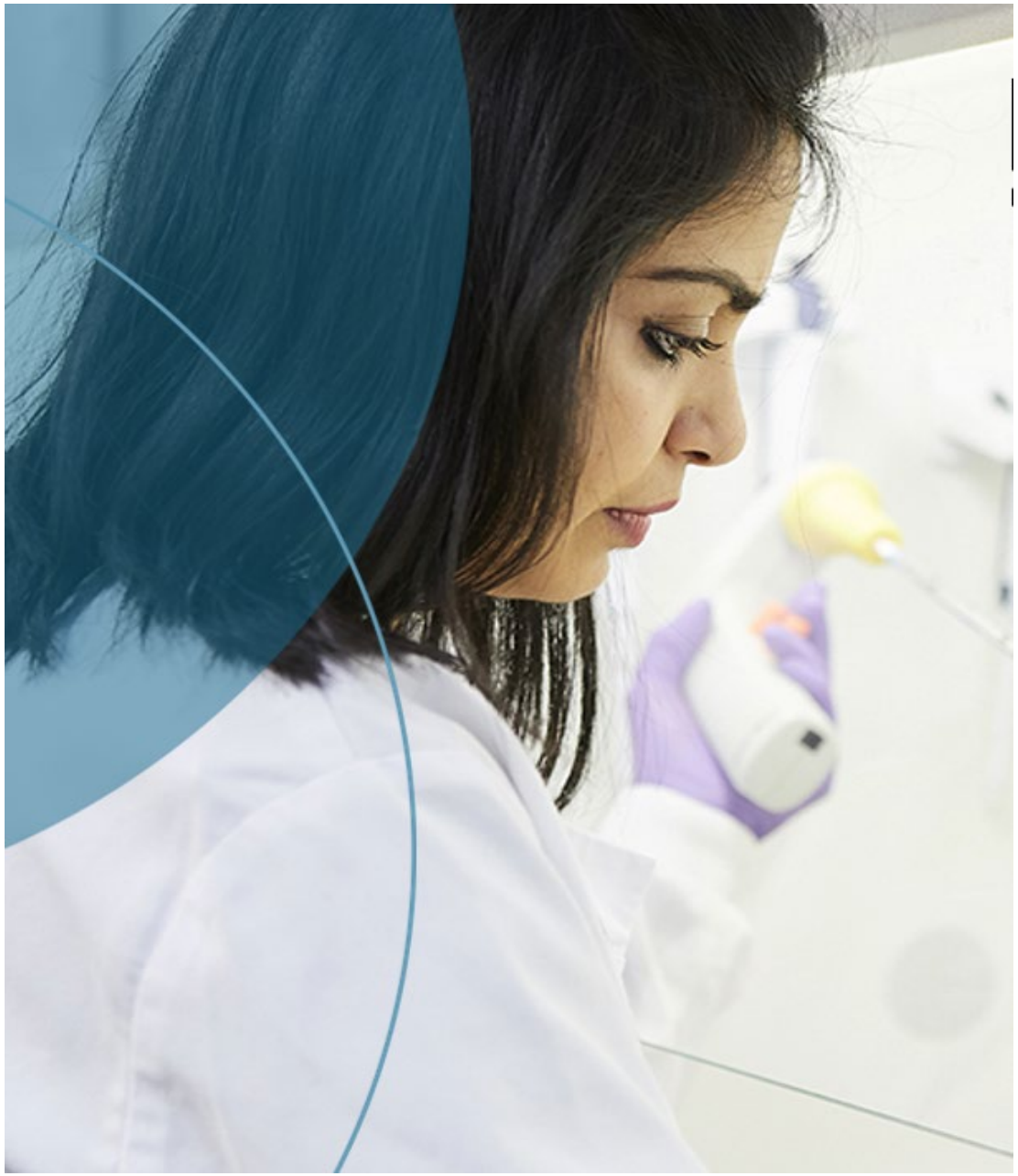
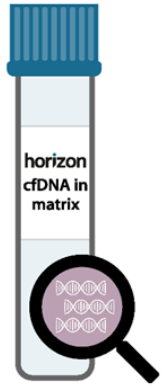
Reference standards as supporting tools for assay development and monitoring



How do we manufacture our product ?





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Liquid Biopsy reference standards

Multiplex I cfDNA HD917

Variant	Gene	ID	HGVS_PROTEIN	Allele Frequency
1	*EGFR*	COSM6240	p.T790M	0.1%-5%
2	*EGFR*	COSM6223	p.G746_A750del	0.1%-5%
3	*EGFR*	COSM6224	p.L858R	0.1%-5%
4	*EGFR*	COSM26352	p.V769-D770insASV	0.1%-5%
5	*N-RAS*	COSM221918	p.Q61K	0.1%-5%
6	*N-RAS*	COSM578	p.A59T	0.1%-5%
7	*PIK3CA*	COSM763	p.E545K	0.1%-5%
8	*K-RAS*	COSM1135366	p.G12D	0.1%-5%

EGFR Multiplex HD825

Variant	Gene	ID	HGVS_PROTEIN	Allele Frequency
1	*EGFR*	COSM26352	p.V769-D770insASV	0.1%-5%
2	*EGFR*	COSM6240	p.T790M	0.1%-5%
3	*EGFR*	COSM6224	p.L858R	0.1%-5%
4	*EGFR*	COSM6213	p.L861Q	0.1%-5%
5	*EGFR*	COSM6493937	p.C797S	0.1%-5%
6	*EGFR*	COSM6252	p.G719S	0.1%-5%
7	*EGFR*	COSM9396332	p.S464L	0.1%-5%
8	*EGFR*	COSM6223	p.G746_A750del	0.1%-5%
9	*EGFR*	COSM6110417	p.G465R	0.1%-5%
10	*EGFR*	COSM6241	p.S768I	0.1%-5%

Structural Multiplex cfDNA HD786

Variant	Gene	ID	HGVS_PROTEIN	Allele Frequency
1	*GNA11*	COSM52969	p.Q209L	5%
2	*ROS1*	n/a	translocation	5%
3	*RET*	n/a	translocation	5%
4	*MET*	n/a	amplification	4.5 copies
5	*MYC-N*	n/a	amplification	9.5 copies
6	*AKT1*	COSM33765	p.E17K	5%
7	*EGFR*	COSM6223	p.G746_A750del	5%
8	*PIK3CA*	COSM763	p.E545K	5%

Coming soon

Prostate Multiplex (15 variants including SNVs, indels. DNA repair defects, AR amplification and TP53 pathway aberrations)	cfDNA in buffer

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Diagnostics Reference Standards: Pan-Cancer *OncoSpan HD833 (cfDNA in Buffer)*

Specifications:

Format	cfDNA in Buffer
Buffer	Tris-EDTA, pH 8.0
Unit Size	350 ng
Concentration	20 ng/µl
Fragmentation Size	160bp
Allele Frequencies	1% - 92.5%

Quality Control:

Quantitation	Qubit
DNA Integrity	Tapestation
Allele Frequencies	Droplet Digital PCR™

Variant	CHR	Gene	ID	HGVS_PROTEIN	Allele Frequency
1	chr1	*NRAS*	COSM580	p.Q61K	12%
2	chr10	*RET*	COSM4418405	p.L769L	63%
3	chr12	*KRAS*	COSM1140132	p.G13D	15%
4	chr12	*KRAS*	COSM1135366	p.G12D	6%
5	chr13	*FLT3*	COSM1366185	p.P986fs*>8	10%
6	chr13	*BRCA2*	COSM6048457	p.K1691fs*15	33%
7	chr17	*TP53*	COSM250061	p.P72R	93%
8	chr2	*ALK*	rs10625834	UTR_3_PRIME	10%
9	chr3	*CTNNB1*	COSM5673	p.S33Y	32%
10	chr3	*CTNNB1*	COSM33668	p.S45del	10%
11	chr3	*PIK3CA*	COSM763	p.E545K	9%
12	chr3	*PIK3CA*	COSM775	p.H1047R	17%
13	chr4	*KIT*	COSM1314	p.D816V	10%
14	chr4	*KIT*	COSM1325	p.L862L	7%
15	chr4	*FBXW7*	COSM34018	p.S668fs*39	33%
16	chr5	*APC*	COSM3760869	p.T1493T	35%
17	chr7	*EGFR*	COSM6252	p.G719S	24%
18	chr7	*EGFR*	COSM6223	p.G746_A750del	2%
19	chr7	*EGFR*	COSM1451600	p.Q787Q	15%
20	chr7	*EGFR*	COSM6240	p.T790M	1%
21	chr7	*EGFR*	COSM6224	p.L858R	3%
22	chr7	*MET*	COSM1579080	p.L238fs*25	7%
23	chr7	*MET*	COSM150378	p.A1357A	7%
24	chr7	*BRAF*	COSM476	p.V600E	10%
25	chr9	*NOTCH1*	COSM3216060	p.P668S	30%

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1200+ customers

Trusted by manufacturing/diagnostic companies across the globe

150+ Clinically Relevant changes available

Clinically relevant variants available including CNVs, INDELs, SVs & fusions

1500+ Custom projects completed
>35K Catalog products shipped

Quality products manufactured under ISO9001:2015 & ISO13485:2016 standards

Ref Std that maintain biological complexity

Cell based materials best mimics real samples from patients

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For more information:

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Thank you!

Challenging the Limit of Detection in Liquid Biopsy with Seraseq® ctDNA Reference Materials

Krystyna Nahlik, PhD

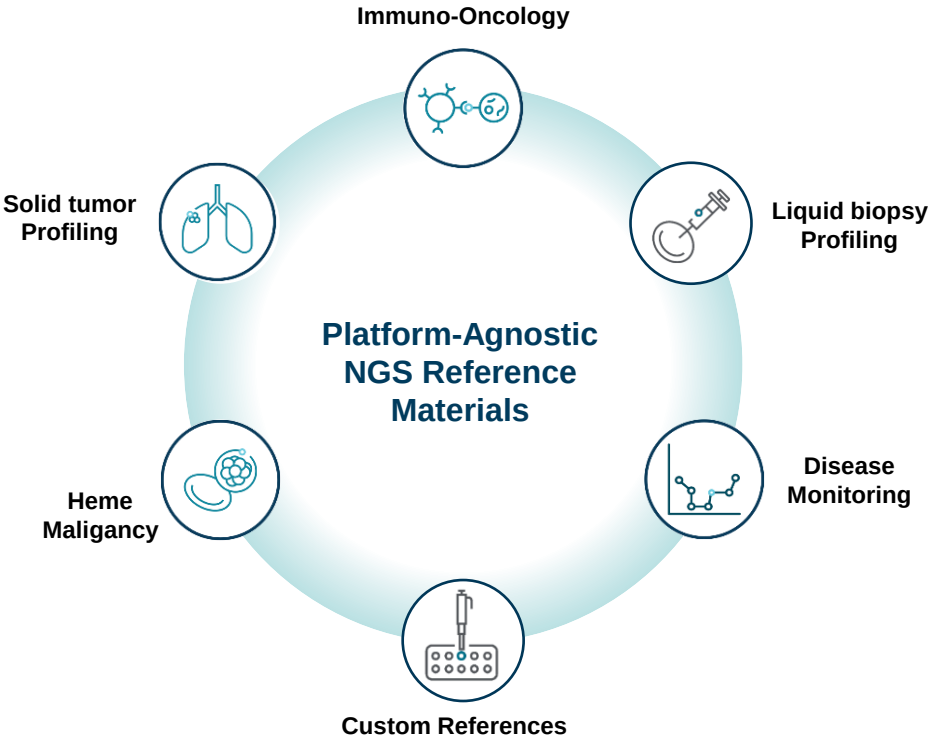
Senior Field Applications Scientist

5 July, 2022

SeraCare is now part of
LGC Clinical Diagnostics

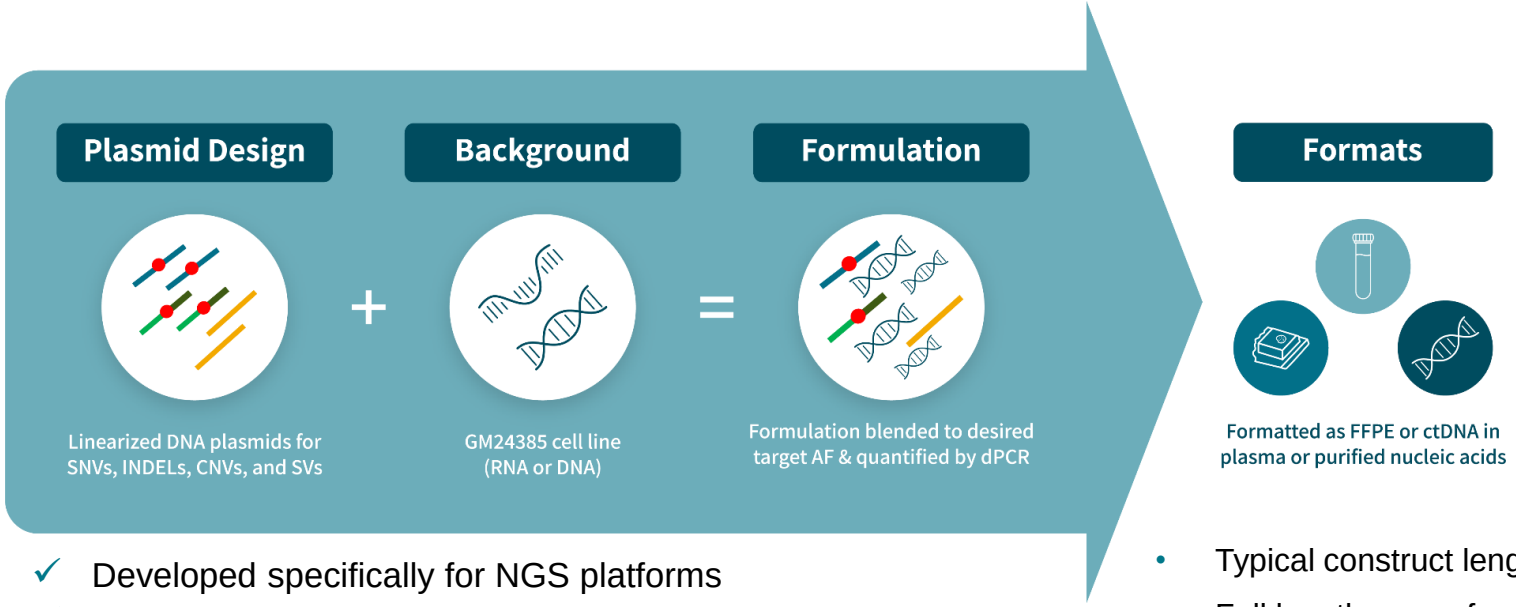
Seraseq® Products for Somatic Cancer

- **Biosynthetic spike-in technology**
  Any clinically relevant variant and variant types: SNV, INDEL, CNV, SV or RNA fusion
- **Multiplex controls**
  Provide a rich source of data in a single run at low cost
- **Patient-like material**
  Performance similar to clinical samples in multiple formats (DNA/RNA in buffer, ctDNA in buffer/plasma, FFPE), proven in a wide range of NGS and molecular assays
- **Highly customizable**
  Expedite assay development, analytical validation, QC and Proficiency testing (EQA)



Unique I-O reference portfolio supports patient stratification based on TMB and MSI

Seraseq® Reference Material Design



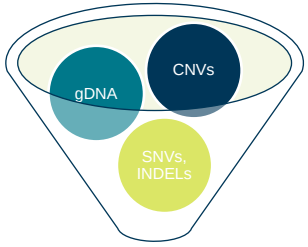
- ✓ Developed specifically for NGS platforms
- ✓ Well-characterized GIAB GM24385 genomic background
- ✓ cGMP manufactured to ISO 13485 standards
- ✓ US-FDA audited manufacturing facilities
- ✓ Global IVD product manufacturer for >30 years

- Typical construct length is 800 bp
- Full length genes for CNVs
- IVT plasmids for fusions and variant transcripts

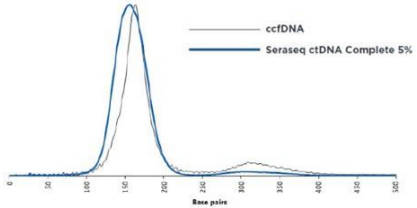
All variants quantified with ddPCR and NGS

**Seraseq® products are designated as Research Use Only.*

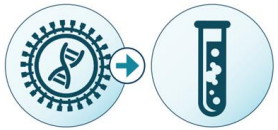
Seraseq® ctDNA Reference Materials



Sheared and size selected to ~170 bp



Encapsulated in synthetic plasma



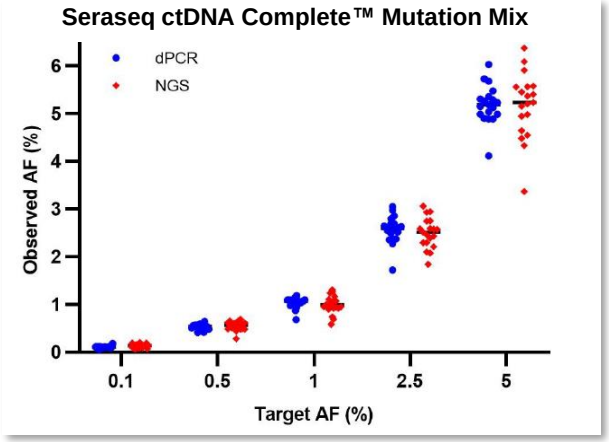
Variant AF range (SNVs/INDELs)

ctDNA v2:

WT (0%), 0.125% 0.25%, 0.5%, 1%, 2%

ctDNA Complete™:

WT (0%), 0.1%, 0.5%, 1%, 2.5%, 5%



Concordance of dPCR and NGS measurements

Mutation Mix (MM)

- ✓ Purified ctDNA in TE buffer, 25 uL at 10 ug/uL
- ✓ Suitable to use directly in library prep together with extracted patient samples
- ✓ Excellent for analytic validation and LoD studies

Reference Material (RM)

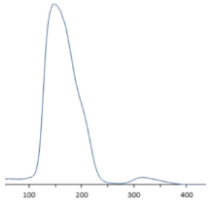
- ✓ ctDNA encapsulated in synthetic plasma, 5 mL at >25 ng/uL yield, stable at 4ºC
- ✓ Suitable as a full-process control, handle and extract like patient plasma
- ✓ Useful for validating and comparing extraction protocols

Seraseq® ctDNA Product Content

ctDNA Mix v2 / Plasma

- 28 pan-cancer genes; 40 variants
- SNVs, INDELs, & SVs
- VAFs: 0.125%, 0.25%, 0.5%, 1%, 2%, WT

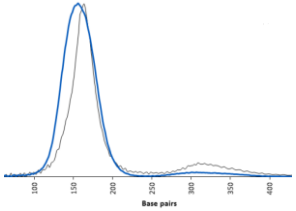
AKT1	JAK2
APC	KIT
ATM	KRAS
BRAF	MPL
CTNNB1	NCOA4-RET
EGFR	NPM1
ERBB2	NRAS
FGFR3	PDGFRA
FLT3	PIK3CA
FOXL2	PTEN
GNA11	RET
GNAQ	SMAD4
GNAS	TP53
IDH1	TPR-ALK



ctDNA Complete™ Mix / Plasma

- 16 solid tumor genes; 25 variants
- SNVs, INDELs, CNVs, & SVs
- VAFs: 0.1%, 0.5%, 1%, 2.5%, 5%, WT

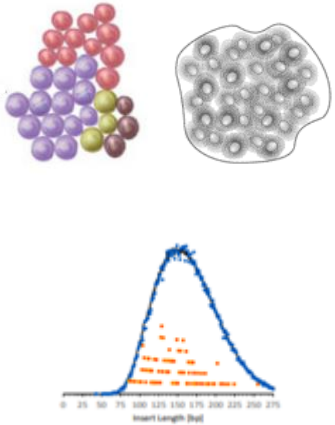
AKT1
ALK
BRAF
EGFR
KIT
KRAS
NRAS
PIK3CA
BRCA1
BRCA2
ERBB2
MET
MYC
CD74-ROS1
EML4-ALKv1
NCOA4-RET



ctDNA MRD Panel Mix

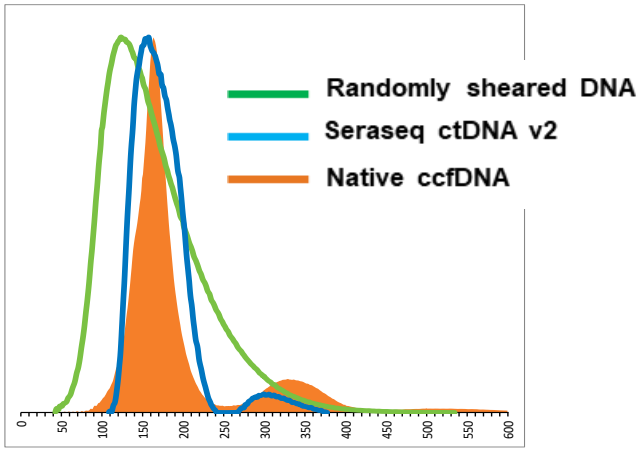
- >600 variants (solid tumor cell line + synthetic variants)
- SNVs, INDELs, CNVs
- Tumor fractions: 0%, 0.5%, 0.05% and 0.005%

★ Industry first

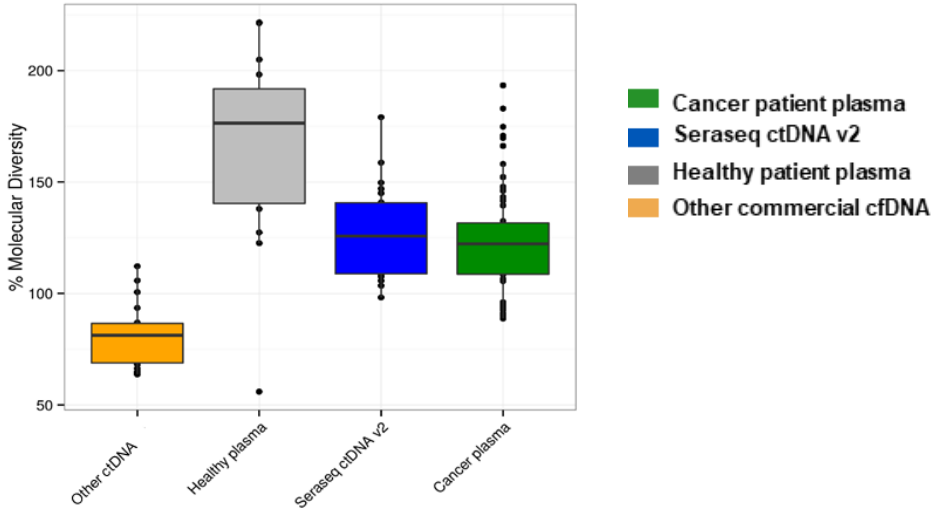


AKT1
ALK
BRAF
EGFR
KIT
KRAS
NRAS
PIK3CA
BRCA1
BRCA2
ERBB2
MET
MYC
CD74-ROS1
EML4-ALKv1
NCOA4-RET

Seraseq® ctDNA is a patient-like reference material



Molecular diversity



The Seraseq ctDNA Mutation Mix v2 reference standards and cancer patient plasma samples have comparable post-sequencing molecular diversity of barcoded molecules relative to mass input. (J Larsen, et. al., Poster#: 5574, 2018 AACR Meeting). Data courtesy of Asuragen, Inc.

Seraseq® ctDNA Product Differentiators

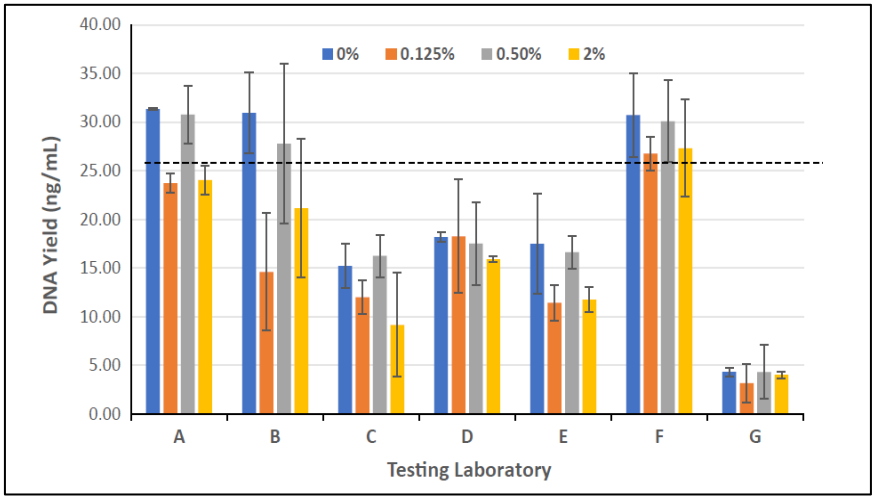
Liquid biopsy reference material features

- Expert-designed, NGS-focused, highly multiplexed reference samples for cancer profiling, monitoring, and immuno-oncology
- Most patient-like reference materials covering all genomic events: SNVs, INDELs, CNVs and Structural Variants
- Focusing on clinically-relevant and challenging variants
- All allelic frequencies validated by dPCR and NGS down to 0.1%
- Customizable to meet the needs of proficiency testing programs
- Innovative first-to-market products based on highly characterized tumor cell lines: blood TMB and ctDNA MRD

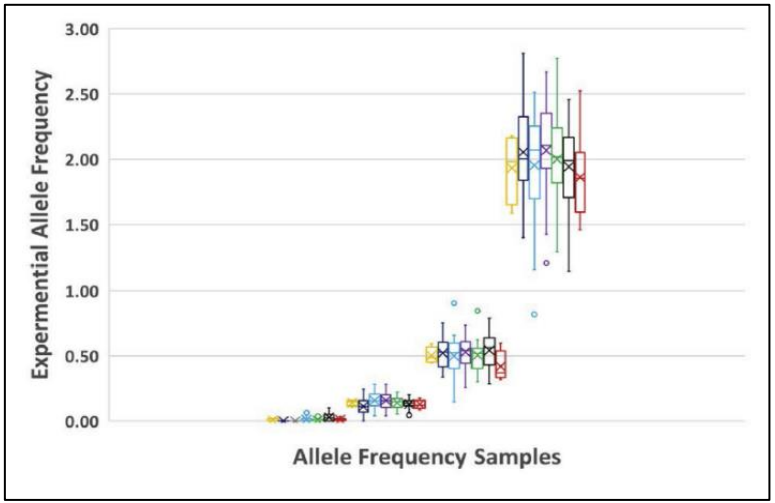
Benefits

- Expedite assay development and validation
- Monitor the performance of the assay over time
- Challenge the full analysis workflow to ensure confidence in the NGS test results
- Support global QC standards to allow for inter-lab and inter-platform comparisons

Seraseq® ctDNA v2 Multi-Lab Assessment



DNA Yields for Reference Material Samples



Comparison of NGS results of analysis of blended AF versus observed AF values

He, HJ et. al., J. Mol. Diag., 2019, Vol 21, No 4, 658-676

Multi-Lab/Platform Evaluation Study using Seraseq ctDNA Complete™

Study led by Medical University of Graz within the CANCER-ID consortium

cancers

MDPI

Article

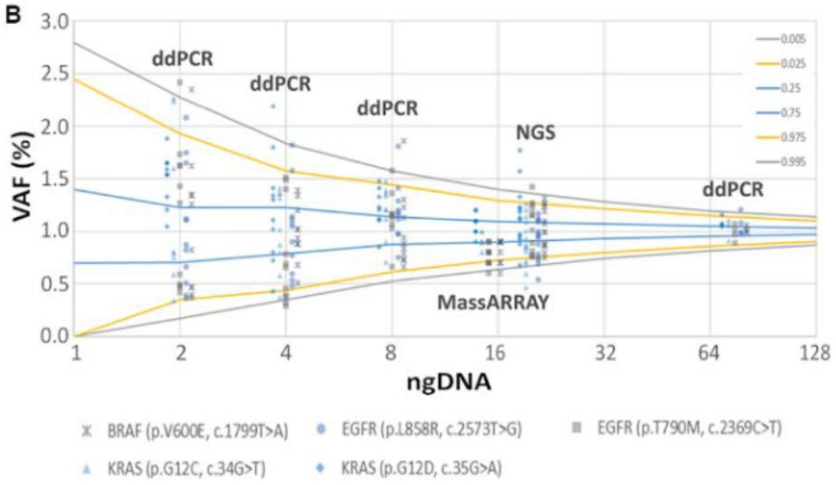
Technical Evaluation of Commercial Mutation Analysis Platforms and Reference Materials for Liquid Biopsy Profiling

Multi-lab study to investigate:

- Utility of contrived materials across clinical oncology assays, primarily solid tumor
- Compare various platform chemistries against a common reference material

Conclusions:

- Biosynthetic reference materials are useful for assessment of mutation analysis platforms
- Variability of NGS assays highlights importance of extensive validation before implementing in clinical practice



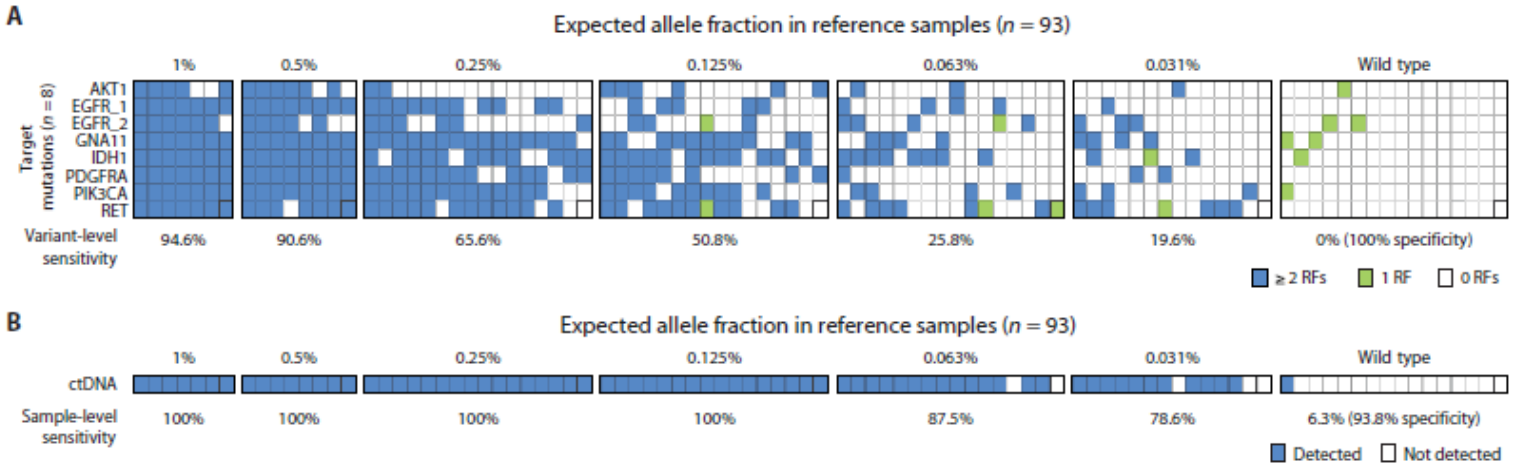
Tumor-informed MRD assay validation using Seraseq® ctDNA v2 Mutation Mix

Natera Signatera™

- Sensitivity at VAF <0.1% of cfDNA, sensitivity threshold: able to detect **at least 2 out of 16 mutations** per panel
- Analytical validation performed using Seraseq ctDNA v2 AF 0.5%, **titrated down to AF 0.005%**

TARDIS

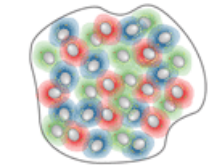
- Analytical validation at low ctDNA concentrations using a panel targeting 8 or 16 mutations in Seraseq ctDNA Mutation Mix v2 at 1, 0.5, 0.25, 0.125, **0.063, and 0.031%** AF (titrated down)



McDonald *et al.*, *Sci. Transl. Med.* **11**, eaax7392 (2019)

Seraseq® ctDNA MRD Panel Mix Development

Intended use: To support development, LoD validation and monitoring the performance of patient-informed solid tumor ctDNA MRD assays in cancer patients undergoing therapy



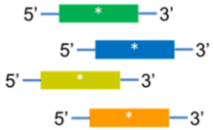
Tumor cell line (gDNA)

WES

>600 variants

Variant Calls (VCF)

0.5% tumor



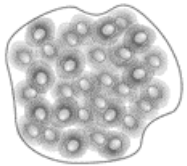
DNA constructs

Targeted panel

22 variants

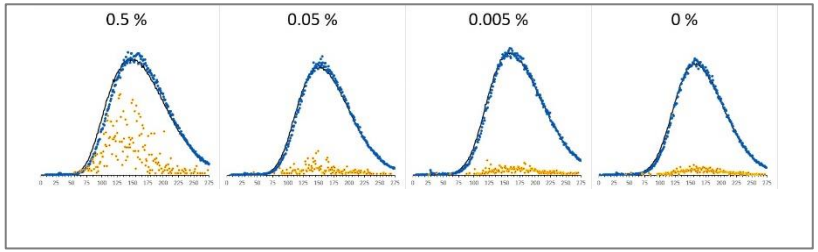
Variant Calls (VCF)

0.25% AF variants



Matched normal cell line (gDNA)

0.5% tumor blend is diluted to 0.05% and 0.005%, all TF including 0% are fragmented & sized



Blends of tumor-derived and synthetic ctDNA MRD variants

Custom targeted panel for 100 tumor variants at each TF

Variant Calls (VCF)

Design a MRD assay using a subset of tumor and synthetic variants – mix & match

Seraseq® Custom Solutions

Liquid biopsy custom options

- Variants selected from VariantFlex custom library (>400 variants across >60 genes) or synthesized to order, VAF customization
- GM24385 or tumor cell line background, tumor fraction blending
- Multiple fragmentation options: shearing / enzymatic, amplification-free
- Amount and concentration, matrix (buffer / plasma)

Partnerships and Collaborations Advancing Development and Adoption

National Cancer Institute	CRADA for biosynthetic solid tumor spike-in technology, MATCH, ctDNA
NIST	CRADA, ctDNA performance testing, dPCR vs NGS, DNA methylation
BloodPAC	ctDNA NGS assay standardization & data commons, MRD
CANCER ID	ctDNA standardization, CTC reference materials
THE EUROPEAN LIQUID BIOPSY SOCIETY	
FNIH THE biomarkers CONSORTIUM	ctDNA reference material commutability, NGS analytical validation, clinical validation of 14 biomarkers, FDA

Liquid biopsy EQA programs using Seraseq ctDNA materials

- ✓ UK NEQAS NSCLC and colorectal cancer pilot EQA schemes
- ✓ Taiwan Society of Pathology ctDNA proficiency testing
- ✓ CAP past and ongoing ctDNA proficiency programs since 2018

Thank you!

Science
for a Safer World

- **Krystyna Nahlik, PhD**
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  Krystyna.Nahlik@LGCgroup.com
- **Mark Rogers**
  BDM – EMEA
  +44 781-802-8348
  Mark.Rogers@lgcgroup.com

Quality Systems

Registrations and Certifications

ISO 13485 Certified	cGMP Compliant	EC 1069/2009 and EU 142/2011 Approval	FDA Medical Device Establishment Registration	FDA Blood Establishment Registration

bsi.

Certificate of Registration

QUALITY MANAGEMENT SYSTEM - ISO 13485:2016

This is to certify that:

LGC Clinical Diagnostics, Inc.
37 Birch Street
Milford
Massachusetts
01757
USA

Facility ID Number: F000389

Holds Certificate No: MDSAP 689354

Statement of Conformity: The company listed on this certificate has been audited to and found to conform with the following criteria: ISO 13485:2016 and Australia - Therapeutic Goods (Medical Devices) Regulations, 2002, Schedule 3 Part 1 (excluding Part 1.4) – Full Quality Assurance Procedure; Canada - Medical Devices Regulations – Part I – SOR 98/282; USA - 21 CFR 820, 21 CFR 803, 21 CFR 806, 21 CFR 807 - Supports A to D

Please see scope page.

For and on behalf of BSI:

Gary E Stack, Senior Vice President – Medical Devices

Original Registration Date: 2019-02-07 Effective Date: 2021-10-03 Expiry Date: 2024-10-02

Page: 1 of 3

MDSAP

...making excellence a habit.

- ✓ Every process is documented, and records are maintained according to these regulations
- ✓ Seraseq products are completed traceable from sourcing, through processing, to delivery – providing a high level of confidence, quality and safety

Molecular Diagnostics Reference Materials:

cfDNA Reference Materials and uses thereof

www.sens-id.com

Contact information

BusDev: Jens Beator (PhD)

jens.beator@sens-id.com

LinkedIn

Technical questions: Björn Nowack

bjoern.nowack@sens-id.com

LinkedIn

Focus on 1 Thing

Reference Materials for Molecular Diagnostics

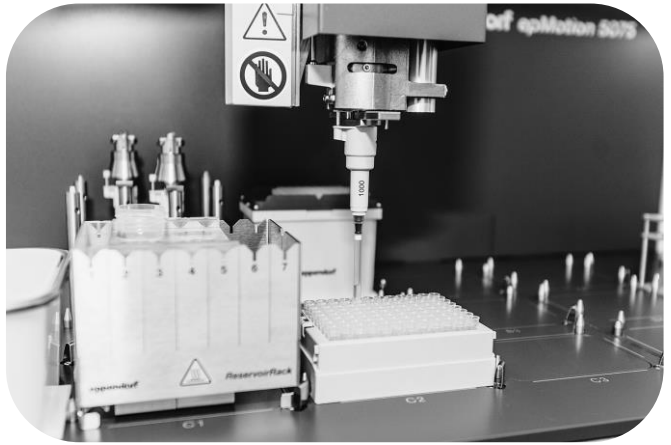
Competence from Consultation to Product supply

Consulting

Regulatory & quality requirements

Development

Development according to customer needs



Manufacturing

For assay development & diagnostic methods, OEM

A broad Range of Product Categories

DNA/RNA in Buffer

gDNA / RNA

cfDNA/ctDNA

cfDNA & Plasma

Plasma + cfDNA/ctDNA

Certified DNA free human Plasma

FFPE

Blocks, Sections, Cells, DNA/RNA in buffer

Ask for methylation reference materials!

Liquid Biopsy/gDNA range of standard products

cfDNA & Plasma

- No Template Control: Plasma 2x (human-tech) DNA free Set
- Wildtype: Ashkenazim son cfDNA in Plasma
- BRAF/KRAS/PIK3CA/AKT1/ERBB2; AF: 0%; 0.1%; 1%; 5%
- EGFR-Multiplex Set cfDNA in Plasma; AF: 0%; 0.1%; 1%; 5%

cfDNA/gDNA in Buffer

- Wildtype: Ashkenazim son cfDNA
- PIK3CA-11 mutations 12.5% AF cfDNA
- AKT1/BRAF/ERBB2/KRAS/PIK3CA; AF: 0%; 0.1%; 1%; 5%
- EGFR-Multiplex Set cfDN; AF: 0%; 0.1%; 1%; 5%

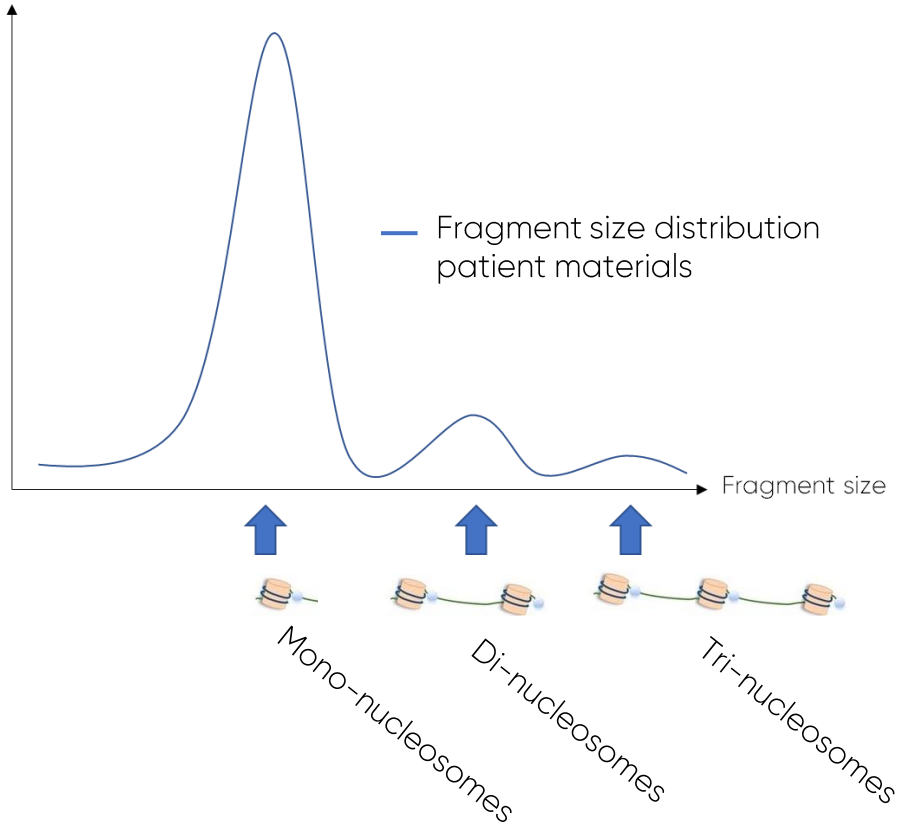
- DPYD Multiplex Set gDNA

For further products visit sens-id.com

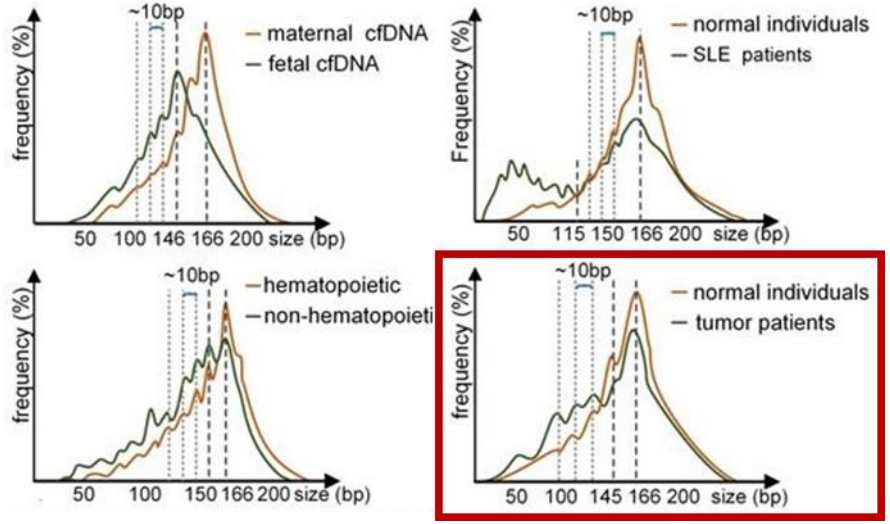
DNA from Liquid Biopsies is a complex Subject

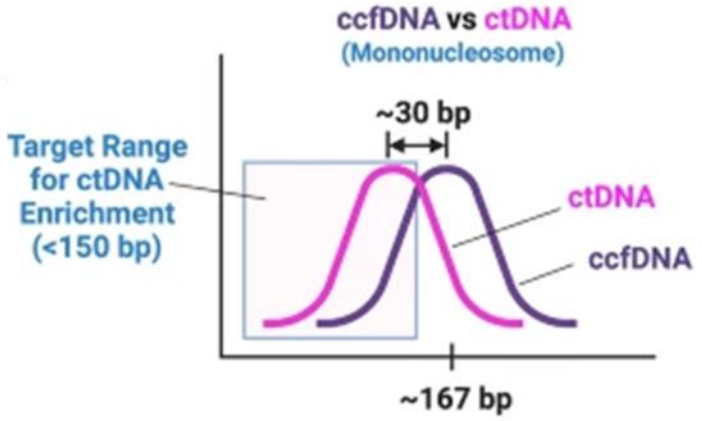
concentration

The theoretical patient model system (simplified)



Real patient samples vary with health status

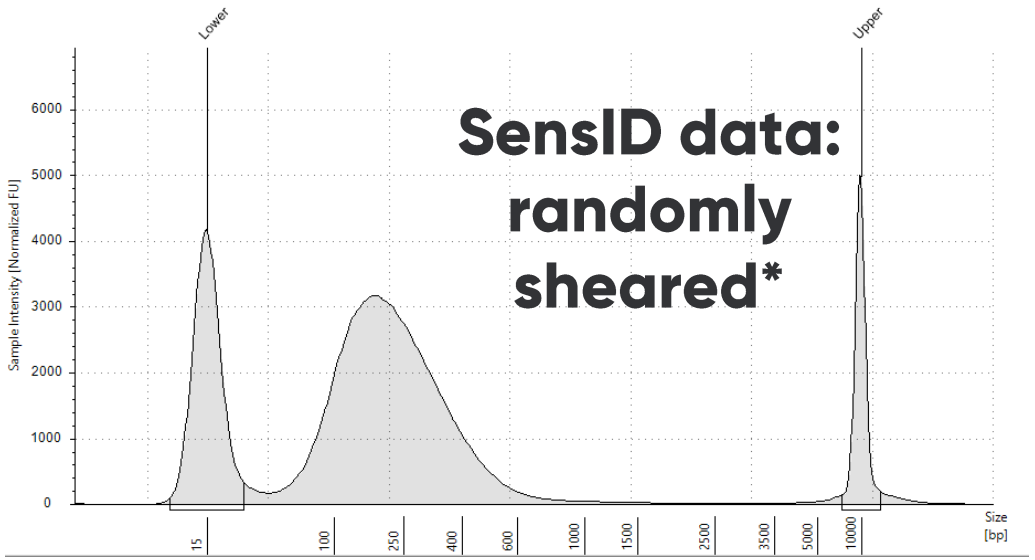


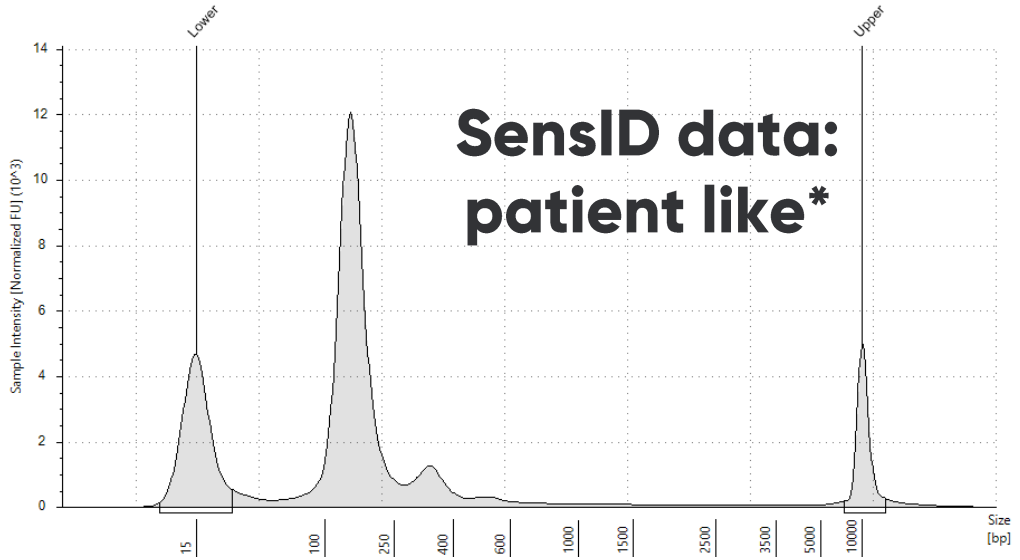


ctDNA < wt cfDNA

- ct DNA: 134–144 bp for mononucleosomes
- "embedded" in wt cfDNA background

Reference Material for Liquid Biopsies (cf/ctDNA)





As in kit control, early development

For technical validation, clinical trials

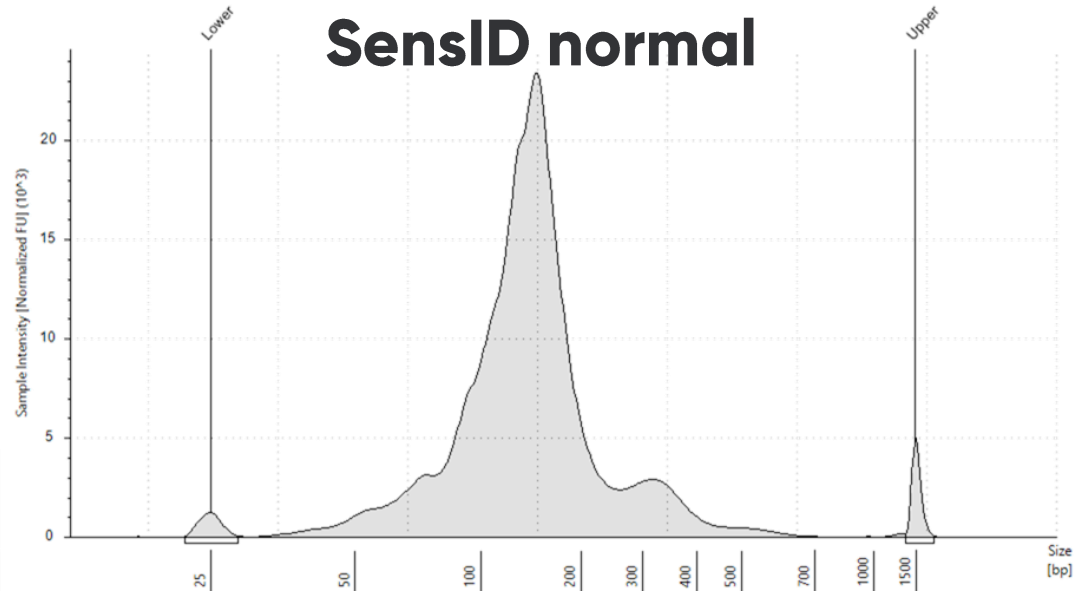
1. Metrologically traceable, tailored number of mutant copies (**No plasmids** / **Not amplified**) for IVDR / FDA compliance

2. Mutations at precise variant allele frequency 0,1 – 100% (SNV's, indels, fusions, …)

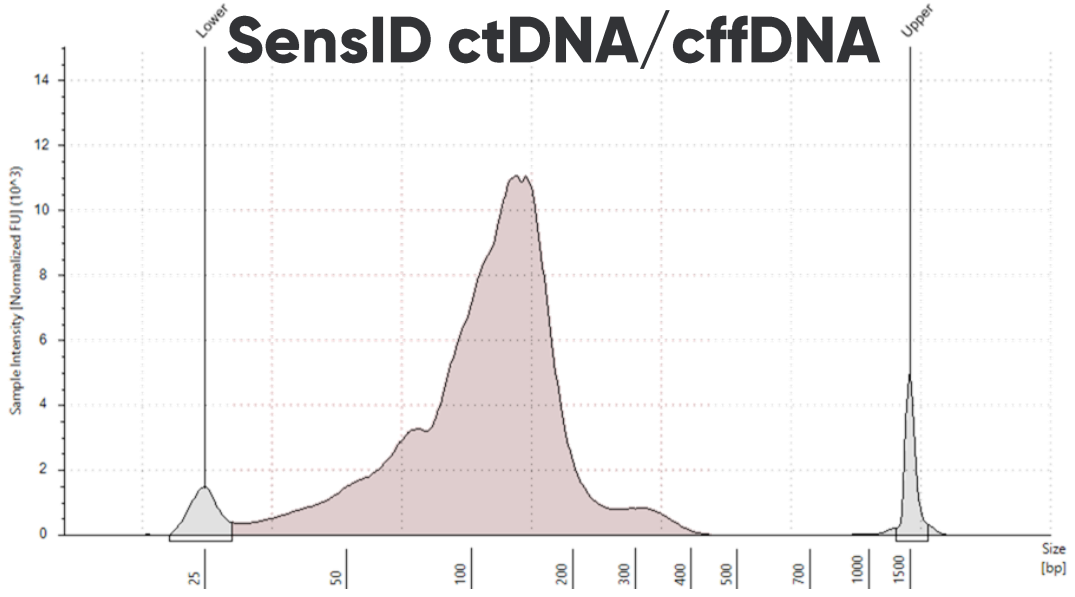
* Peak distribution adjustable

SensID can make patient-like ct/cf DNA material

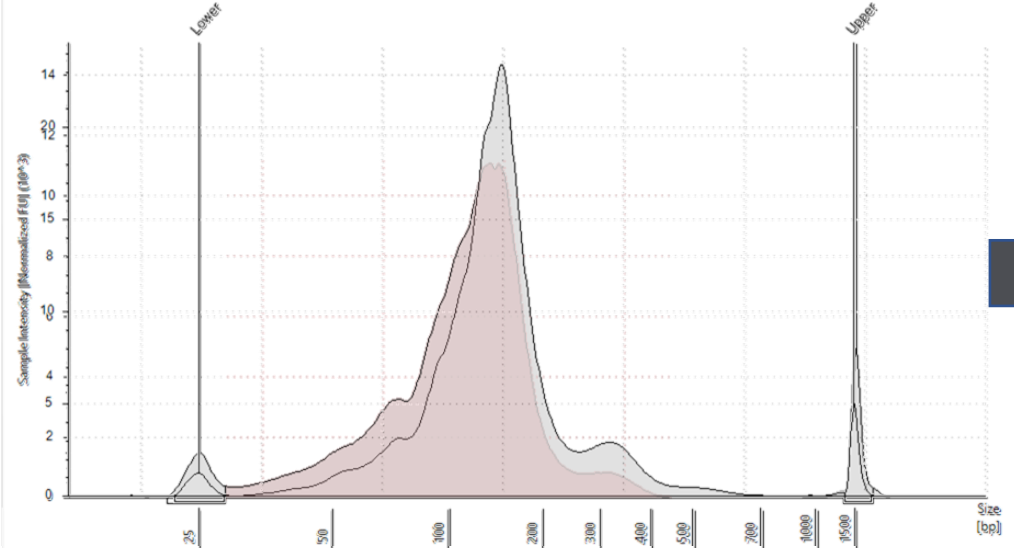
SensID normal



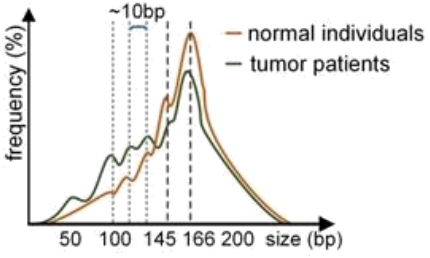
SensID ctDNA/cffDNA



Peak adaptation possible



Literature comparison



Executed & ongoing Reference Material projects

SensID materials are used in:

- **EQA schemes**
- **in-kit-controls incl. CDx**
- **Clinical trials**
- **Validation**
- **…**

Fit for your regulatory Compliance

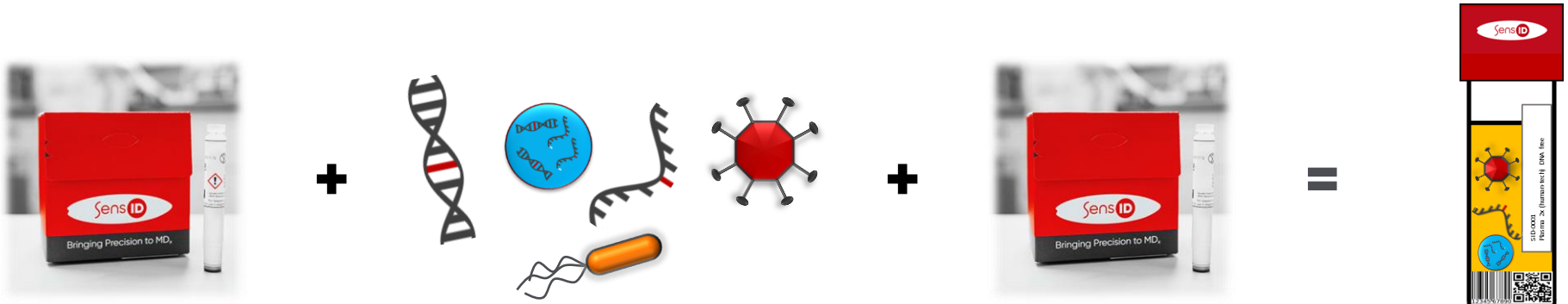
Regulatory compliance according to customer/project/market needs!

- **RUO (research use only)**
- **CE-IVD**
- **FDA compliant**

Additional information

Certified DNA free Plasma – possible use cases

How to prepare your own plasma quality control



Plasma (human) (2-fold concentrated)

Your DNA/RNA/ Exosomes/ Virus/ Bacteria / ...

Dilution buffer

Your Plasma!

Ready to use!

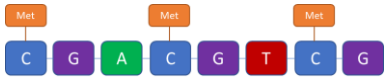
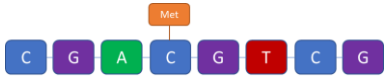
Use Dilution buffer & fill up to desired volume

For more products visit our website: www.sens-id.com/plasma-human-tech-dna-free/

Possibilities for cfDNA in buffer/plasma

product	Fragment size	Usable for technology	Recommended for
cfDNA in buffer or in plasma	Randomly sheared	Platform agnostic	In kit control when exact copies per mutation are not needed
		Platform specific	In kit control, parts of technical validation requiring metrological traceability (e.g. IVDR)
cfDNA in buffer or plasma + gDNA background		Platform agnostic	➢ technical performance evaluation (e.g. IVDR) ➢ Clinical performance evaluation (e.g. IVDR) ➢ EQA schemes ➢ validation control (e.g. IVDR) ➢ marketing purposes
		Platform specific	
cfDNA in buffer or in plasma + *optional* gDNA background	Patient like	Platform agnostic	➢ technical performance evaluation (e.g. IVDR) ➢ Clinical performance evaluation (e.g. IVDR) ➢ FDA compliance ➢ EQA schemes ➢ validation control (e.g. IVDR) ➢ marketing purposes ➢ …
		Platform specific	

Methylation specific Reference Material Options

Possible Methylation options for reference material		
• 0 – 99.9 % of all CpG motifs		Full workflow control including bisulfite conversion testing
• 0 - 99.9 % of individual CpG motifs		
• 0 - 99.9 % all CpG motifs		Sequencing/PCR only control without bisulfite conversion
• 0 - 99.9 % individual CpG motifs		

Provided as:

- gDNA
- cfDNA / ctDNA
- cfDNA/ctDNA in plasma

- Project example on specific targets:

Target sequence	Ratio methylated/ not-methylated	Methylated CpG motifs
Promotor sequence A	50/50	5
Promotor sequence A	10/90	All
Promotor sequence A Promotor sequence B	0/100 0/100	0
Promotor sequence A Promotor sequence B	50/50 75/25	5 All

Can't find what you are looking for?

Tailored, Jointly Designed Products

Licensing / OEM Options

Standardized Daily Run Controls

Contact us: info@sens-id.com

Droplet Digital PCR

Improving Nucleic Acid Quantification Across The Board

BIO-RAD

Outline

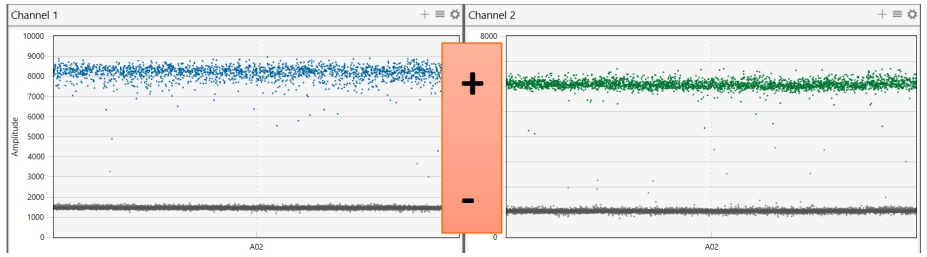
- ddPCR- how does quantification work.
- Absolute quantification by metrology institutes
- Covid and the challenges of qPCR
- Viral metrology and EQAs
- BCR-ABL and ddPCR

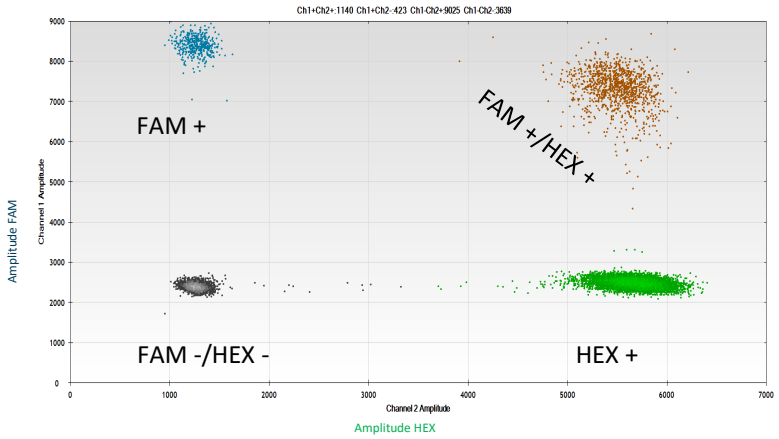
Key Benefits of Droplet Digital PCR

Easy Data Analysis: 1D or 2D

Channel 1 (FAM, EvaGreen)

Channel 2 (HEX, VIC)





ABSOLUTE QUANTIFICATION

- No standard curve required
- **Unaffected by non-optimal PCR efficiencies**

UNPARALLELED PRECISION

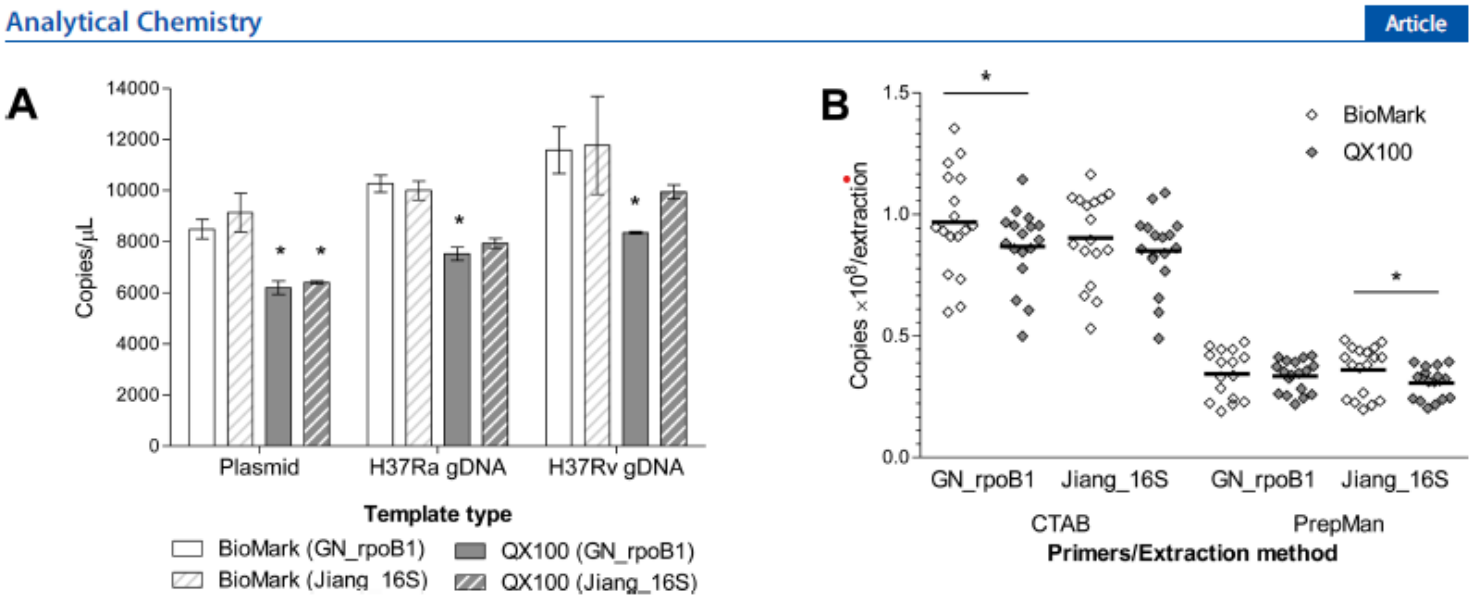
- **Precision down to ±10%**
- Very high data reproducibility

HIGHER SENSITIVITY

- **Single DNA copy resolution in high background**
- Analyze large sample volumes

Interplatform reproducibility by digital PCR- M.tuburculosis

- Comparison of chamber dPCR to droplet digital PCR
- Complex gDNA to plasmid

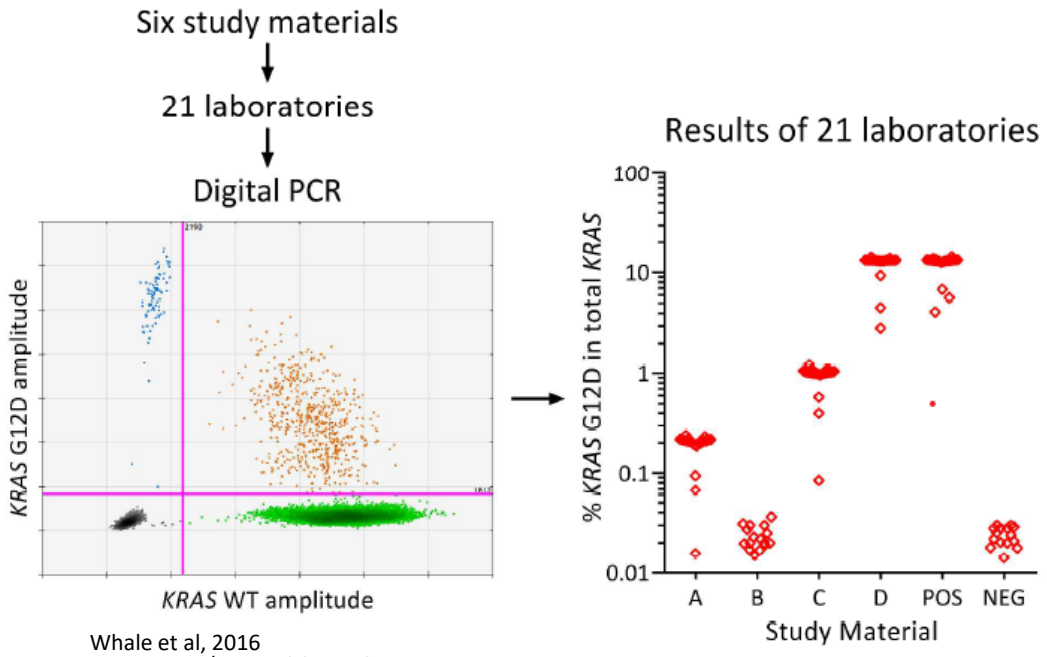


- Absolute quantification of nucleic acid
- Plasmid or complex gDNA
- 20-30% difference between:
  - Different platforms
  - Different templates
- Significant differences between extraction methods

Devonshire et al, Anal. Chem. 2015, 87, 3706−3713
DOI: 10.1021/ac5041617

Interlab reproducibility- KRAS dual probe SNV quantification

Comparison between 21 independent labs
Comparison of % VAF and Absolute Quantification



Whale et al, 2016
DOI: 0.1021/acs.analchem.6b03980

Material		Characterisation				Inter-labor
Name	Target	Nominal*	Mean*	U_{rel}	Median*	% difference$
	[WT]	2000	2400	1.0%	2267.00	6%
A	[G12D]	3	4.9	5.9%	4.74	3%
	% G12D	0.17	0.20	6.0%	0.21	5%
	[WT]	2000	2300	1.7%	2197.00	4%
B	[G12D]	0	0.58	16%	0.42	28%
	% G12D	0.00	0.025	16%	0.02	20%
	[WT]	2000	2300	1.4%	2273.78	1%
C	[G12D]	18	22	4.5%	22.67	3%
	% G12D	0.90	0.96	3.5%	1.01	6%
	[WT]	1760	2100	1.5%	1975.00	6%
D	[G12D]	240	310	1.6%	300.80	3%
	% G12D	12.00	13.00	0.92%	13.16	1%
	[WT]	2000	2300	1.7%	2264.67	2%
NEG	[G12D]	0	0.58	16%	0.49	16%
	% G12D	0.00	0.025	16%	0.02	19%
	[WT]	1760	2100	1.5%	1990.00	5%
POS	[G12D]	240	310	1.6%	294.67	5%
	% G12D	12.00	13.00	0.92%	13.10	1%

"Our findings show that dPCR has demonstrable reproducibility across a large number of laboratories without calibration. Applicable for both high (2000) and low (1-3) copy numbers and rare event proportions (0.17-12%)"

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Primary Reference Measurement Procedure-KRAS SNV

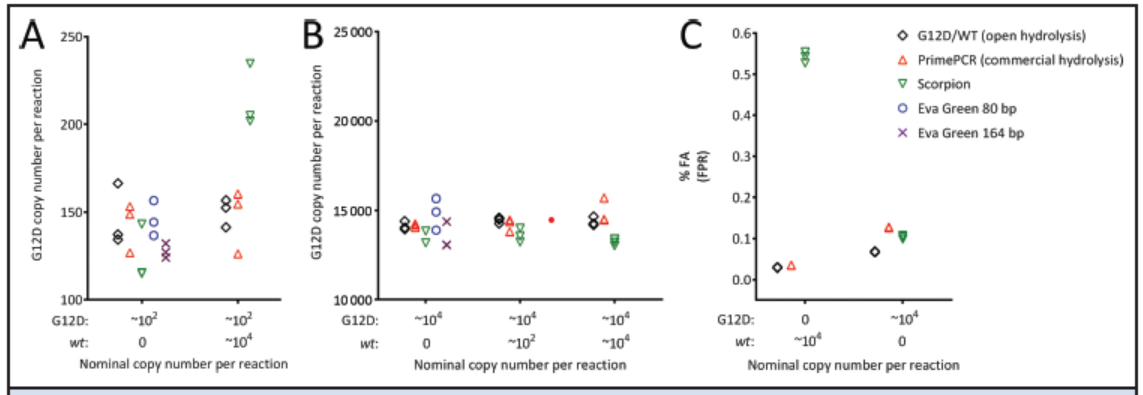

Fig. 2. dPCR assay selection and evaluation of molecular dropout and analytical specificity.

Quantification of samples containing *KRAS* G12D molecules measured at 2 nominal concentrations 2-logs apart at (A) approximately 10^2 and (B) approximately 10^4 copies per reaction using 5 dPCR assays (each shown as different symbol and color data points). *KRAS* G12D molecules were measured with and without a background of 10^4 *wt* molecules. (C), FPR for G12D and *wt* assays. Sample composition is shown on the *x* axis. Data points show the mean value of each of the 3 experiments, each of which was performed with triplicate reactions.

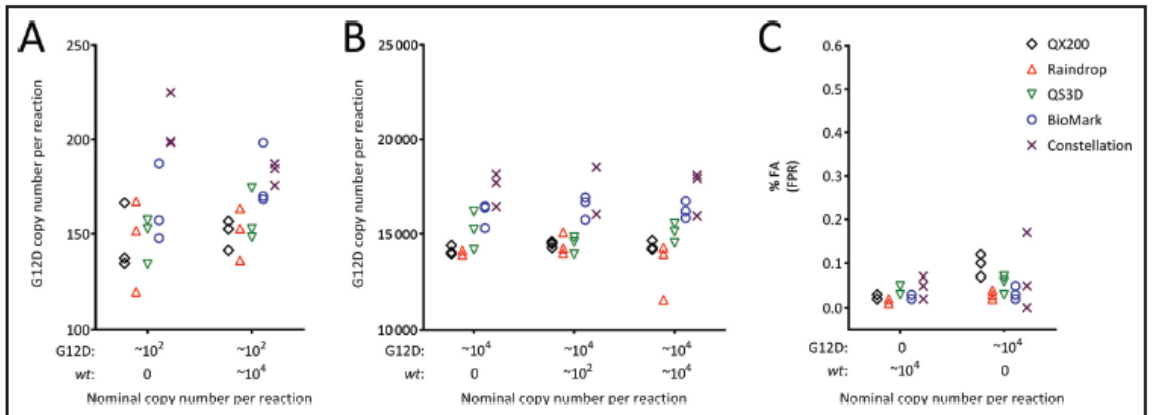

Fig. 3. Digital PCR platform comparison.

Quantification of samples containing *KRAS* G12D molecules at (A) approximately 10^2 or (B) approximately 10^4 concentration using 5 dPCR platforms. These are the same samples as those described in Fig. 2. *KRAS* G12D molecules ere measured with and without a background of *wt* approximately 10^4 molecules. FPR for G12D and *wt* assays (C). Sample composition is shown on the *x* axis. Data points show the mean value of each of the 3 experiments. Replicate reactions within an experiment were n = 3 (QX200, BioMark, and Constellation); n = 1 (QS3D and RainDrop).

- 5 chemistries
- QX200
- <1.2fold variation

Comparison between 5 assay chemistries

Comparison between 5 digital PCR platforms

- 5 platforms
- High and low concentrations
- <1.5 fold variation

Whale et al, Clinical Chemistry 64:9 1296–1307 (2018)

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Improving Nucleic Acid Quantification Across The Board

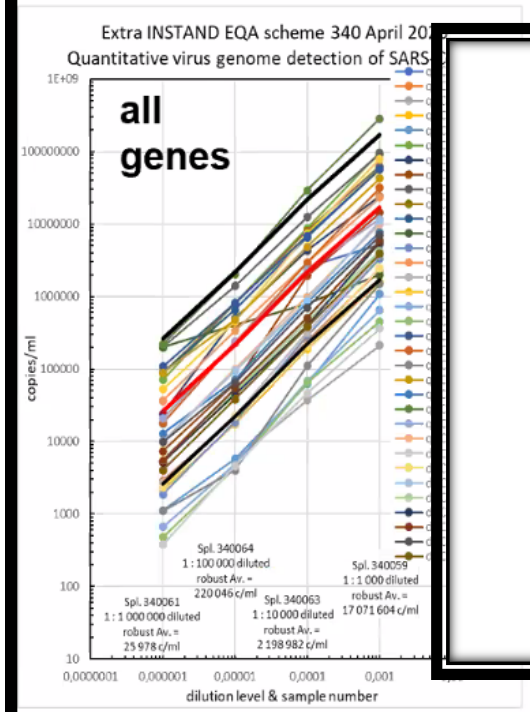
- Reproducibility within labs and between labs
- Reproducibility within platforms and between platforms
- Reproducibility within chemistries and between chemistries

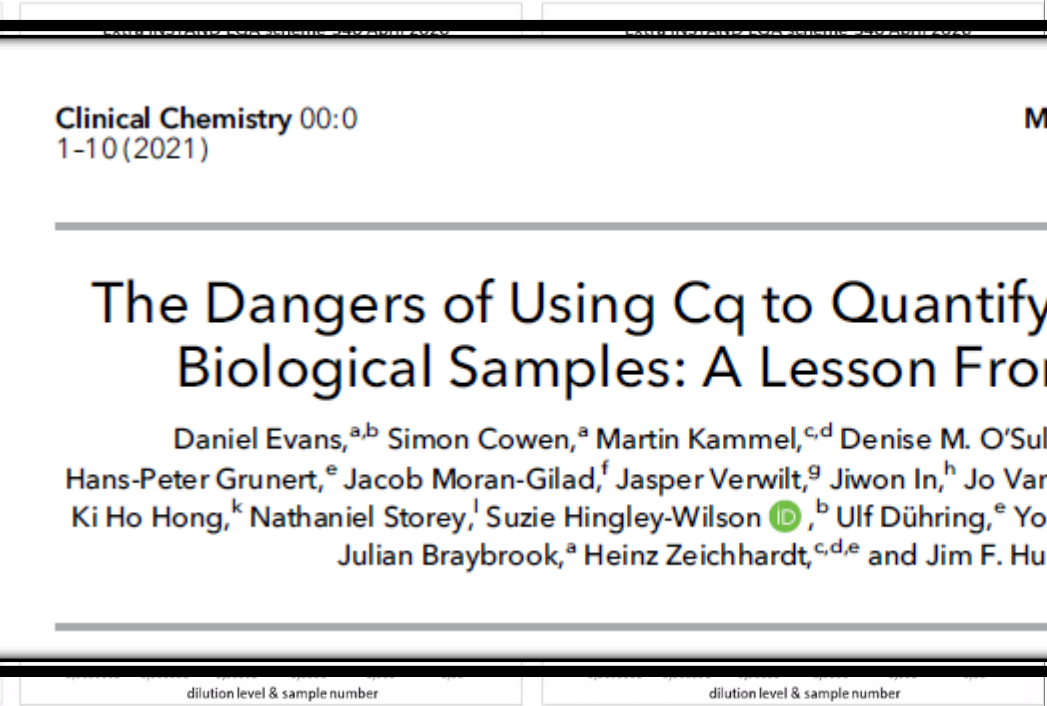
- **Without any standards or standard curves**

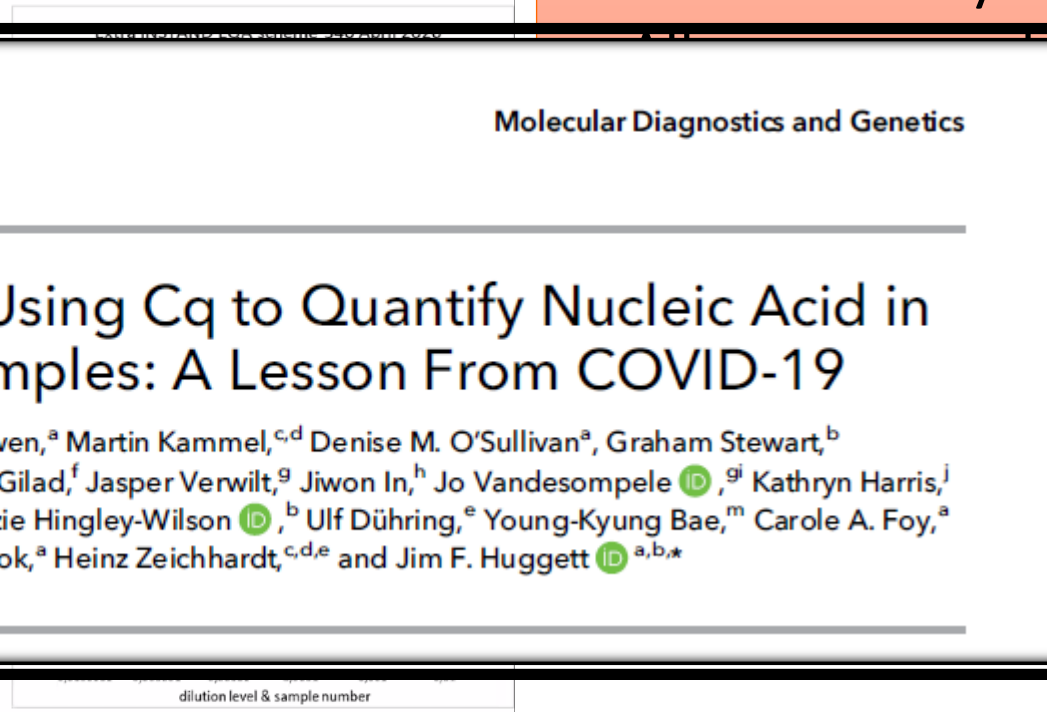
Linearity qPCR vs dPCR, gene by gene

Comparison qPCR vs dPCR

qPCR dPCR







Clinical Chemistry 00:0
1-10 (2021)

Molecular Diagnostics and Genetics

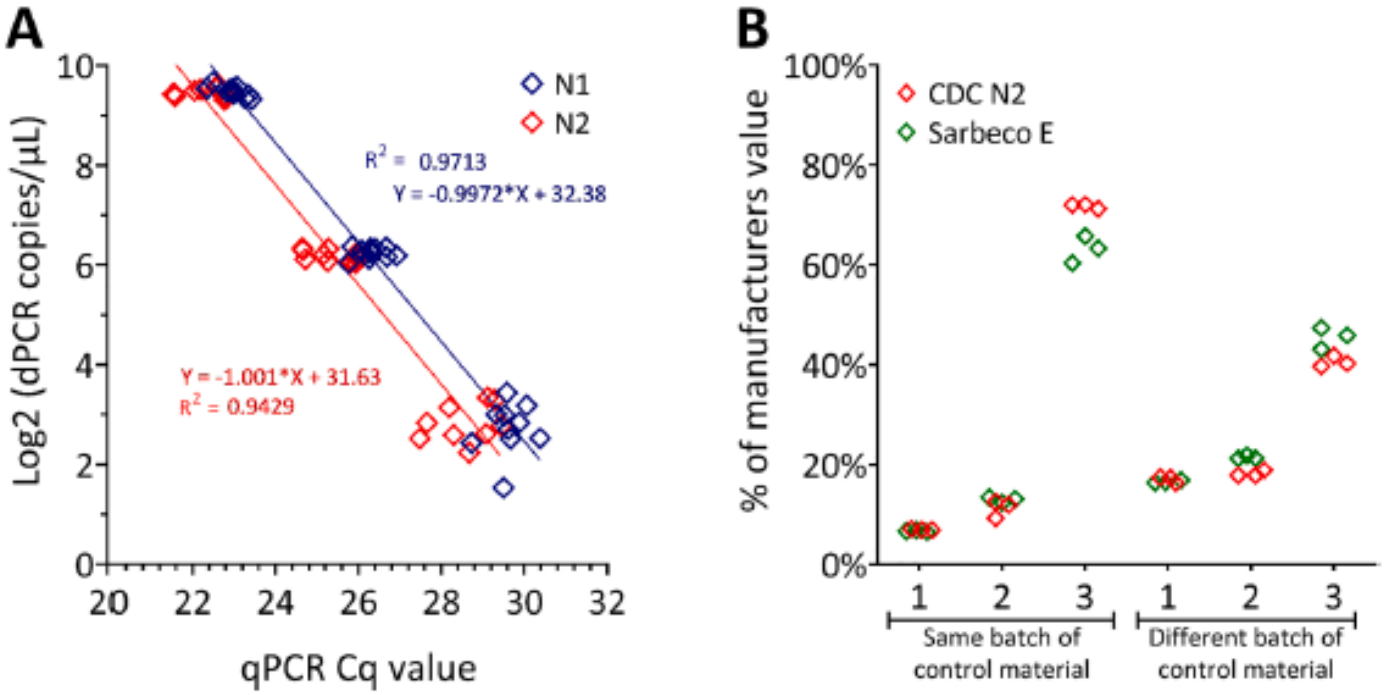
The Dangers of Using Cq to Quantify Nucleic Acid in Biological Samples: A Lesson From COVID-19

Daniel Evans,[a,b] Simon Cowen,[a] Martin Kammel,[c,d] Denise M. O'Sullivan[a], Graham Stewart,[b] Hans-Peter Grunert,[e] Jacob Moran-Gilad,[f] Jasper Verwilt,[g] Jiwon In,[h] Jo Vandesompele ,[gi] Kathryn Harris,[j] Ki Ho Hong,[k] Nathaniel Storey,[l] Suzie Hingley-Wilson ,[b] Ulf Dühring,[e] Young-Kyung Bae,[m] Carole A. Foy,[a] Julian Braybrook,[a] Heinz Zeichhardt,[c,d,e] and Jim F. Huggett [a,b,*]

- Good linearity- dPCR and qPCR
  - ad spread
  - ad spread
  - row spread

Note that the overall mean is skewed higher by the qPCR results.
The dPCR values (in green) trend below the collective mean, but with very similar quantities.

Commercial controls for Covid testing

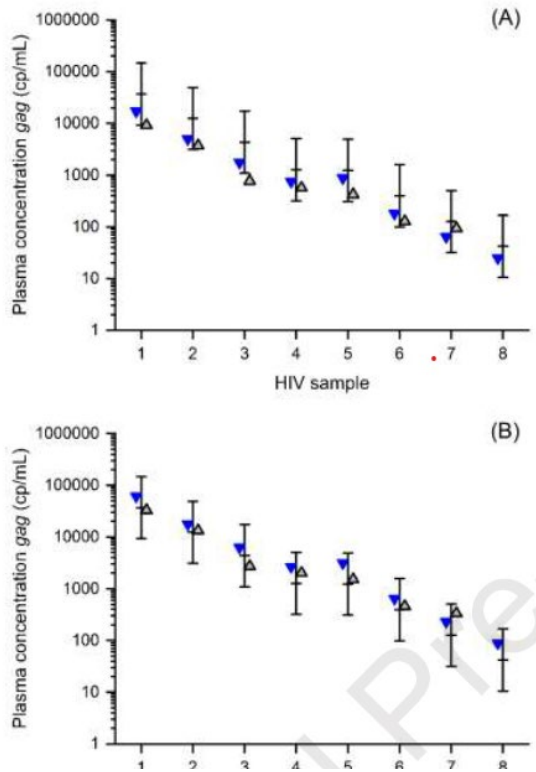


Repeatability with Commercial IVT RNA control materials

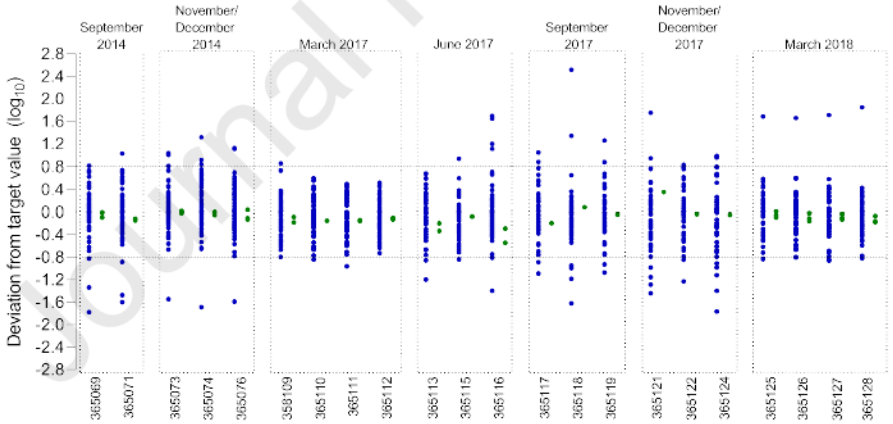
Whale et al, 2021. doi.org/10.1016/j.ymeth.2021.08.006

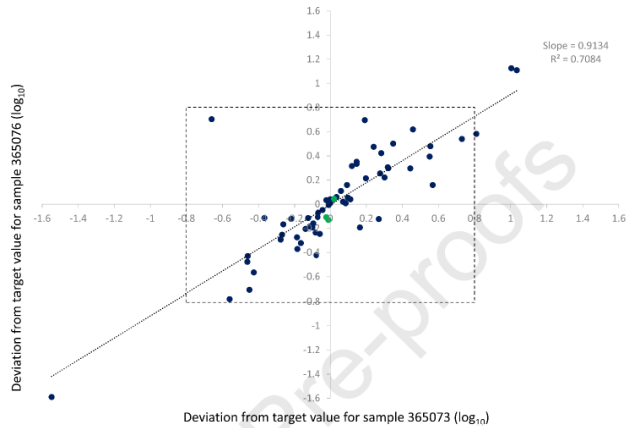
Good concordance between Cq values with RT-qPCR and cp/ul with RT-dPCR.
Poor absolute quantification of control materials from 3 separate batches of IVT RNA control material.

dPCR in virology- RMP for HIV and CMV metrology



Falak et al, 2021. doi.org/10.1016/j.ymeth.2021.03.006





Milavec et al 2021. doi.org/10.1016/j.ymeth.2021.03.016

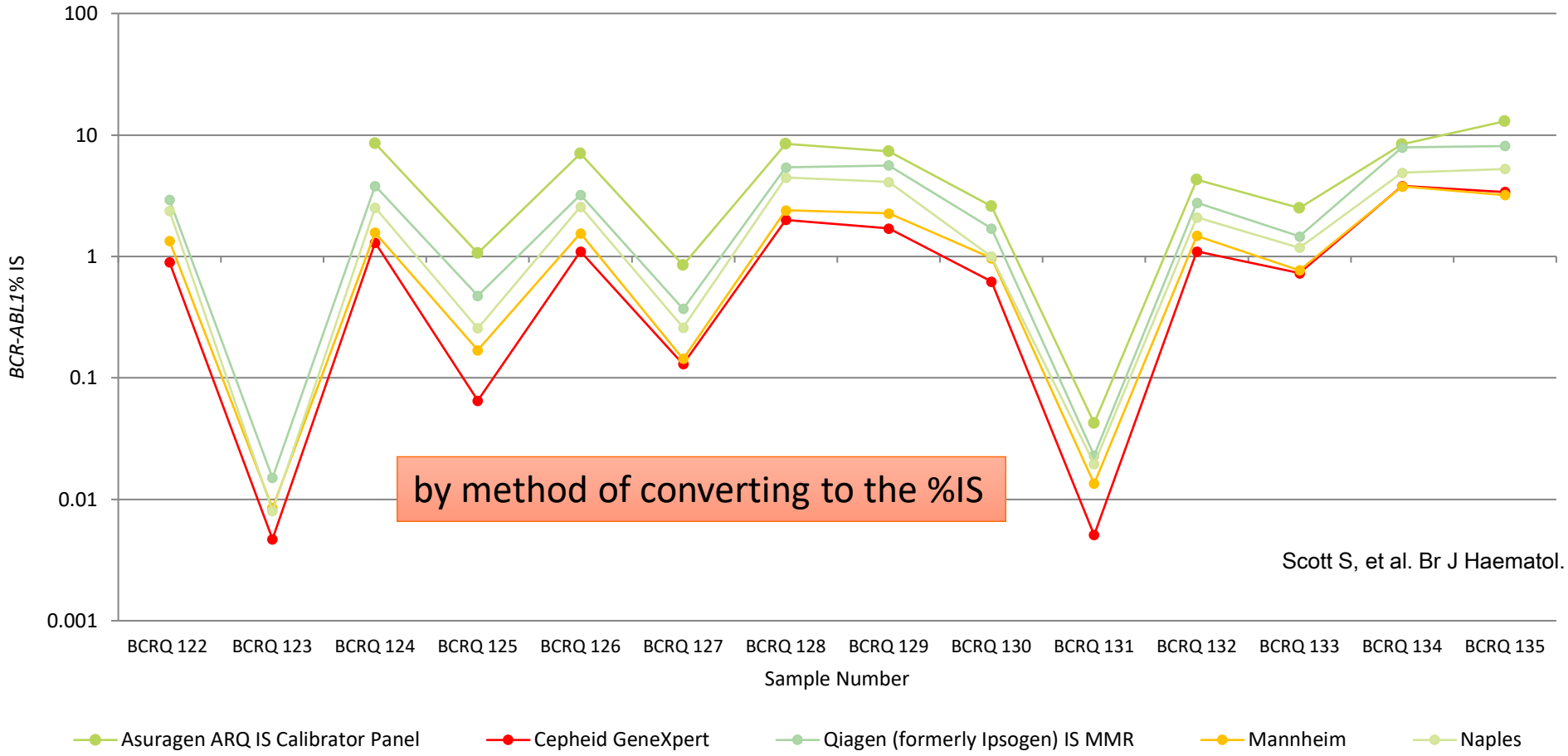
qPCR deviations over 7 years for hCMV EQA compared to dPCR

Comparison of 2 repeats analysis of the same sample: qPCR vs dPCR

Absolute quant of HIV within an international EQA before and after alignment to the WHO 4th HIV standard.

- Analysis of samples by dPCR aligns very well with qPCR mean va
- qPCR results show broad variation
- ddPCR is independent of standards

Median *BCR-ABL1* IS results submitted to UKNEQAS, >4 years



Scott S, et al. Br J Haematol. 2017 May;177(3):414-422.

This illustrates an underlying issue with qPCR for BCR-ABL monitoring:
The reliance on standards and the requirement to generate a conversion factor.

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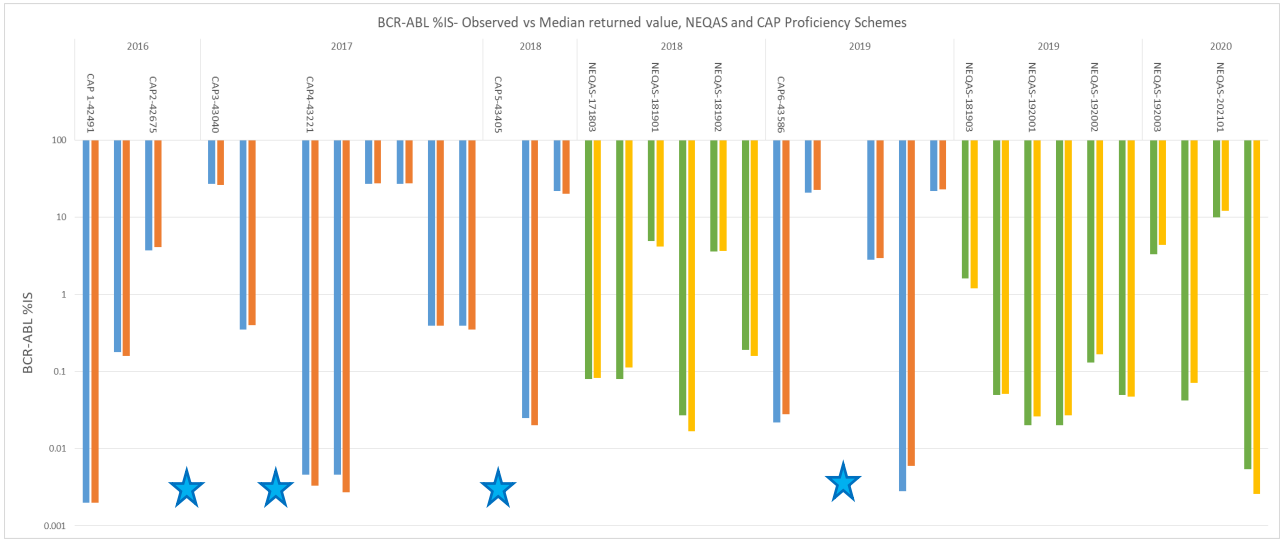
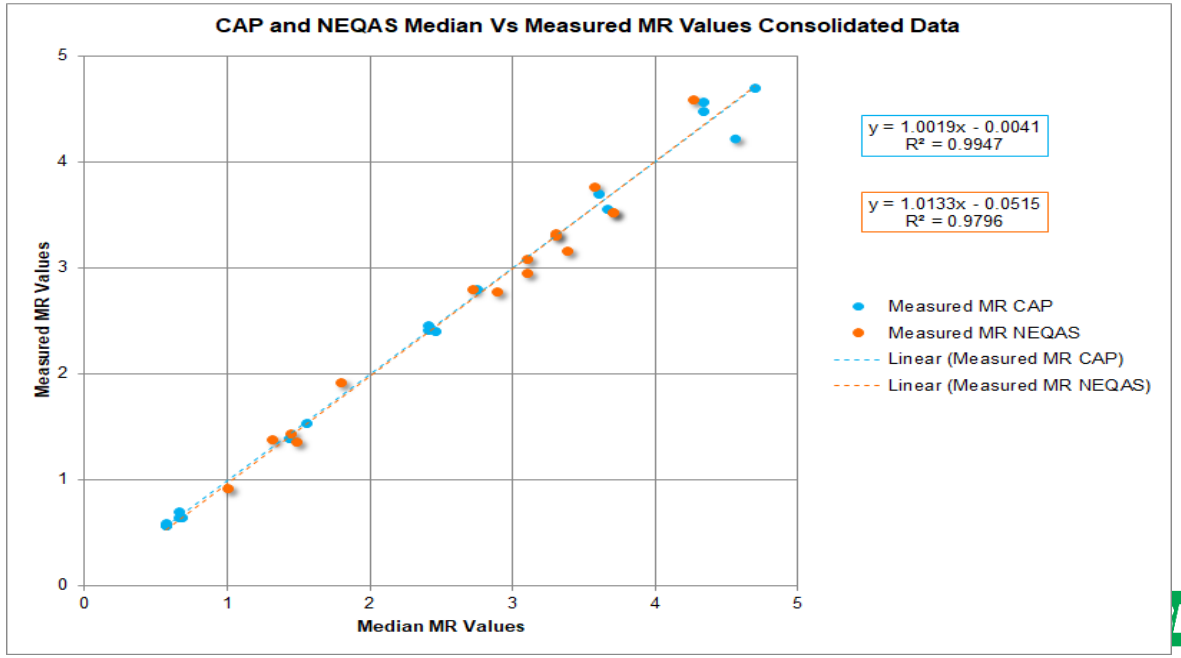
The CAP & NEQAS data



Over a 5 year period, alignment to CAP and NEQAS schemes was excellent.

Linearity of the kit capabilities was highly consistent.



The conclusions

ddPCR can provide absolute quantification and is highly reproducible across platforms, chemistries and input quantities without standards.

The SARS-CoV 2 pandemic has highlighted a need for inclusion of ddPCR into the generation of controls and standards, to reduce bias and over interpretation of results from other technologies.

ddPCR has the potential to become a reference measurement procedure.

But is user friendly and so can be implemented locally and broadly.

The take home message

Metrology labs and Standards manufacturers are using ddPCR for nucleic acid quantification.

EQA organisations and ultimately clinical labs should consider ddPCR as a foundational technology too.