UK NEQAS
International Quality Expertise

GenQA Genomics Quality Assessment
Member of UK NEQAS consortium

Assessing cfDNA somatic variant testing

Prof Sandi Deans
Consultant Clinical scientist, Director of GenQA

1st The European Liquid Biopsy Society ctDNA Technology Meeting, 23rd March 2022

IQNPath cfDNA pilot EQA Group

2016

Online survey of plasma ctDNA testing practice – 167 labs

Deans ZC, *et al.* Virchows Arch. 2017 Dec;471(6):809-813.

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- Pilot EQA delivered to 32 clinical testing labs
- 5 samples for *EGFR* testing and/or 5 samples for *KRAS*/*NRAS* testing
- Artificial samples - 3 mL of human plasma with 20 ng/mL of fragmented ctDNA
- VAFs of 1 and 5%

✓ Demonstrated feasibility of delivering a cfDNA EQA
Identified need for EQA due to high error rates (20%) in detecting low VAF clinically relevant variants
Keppens C, *et al. BMC Cancer* 2018 18;804

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2018

2019

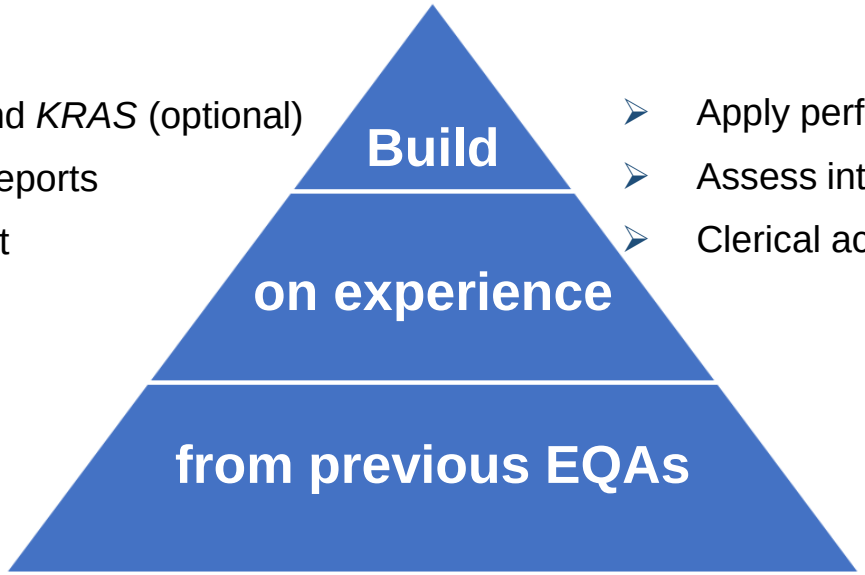
- EQA provided to 264 laboratories from 45 countries
- 5 Cases with *EGFR* exon19 dels, p.(T790M), p.(L858R) & combinations - VAFs 0.5%, 1%, 5%
- Artificial plasma samples containing 250ng of fragmented ctDNA
- Genotyping accuracy high for VAFs (94%) >1%

Results of a worldwide External Quality Assessment of cfDNA testing in Lung Cancer
Fairley JA, *et al.* (2022) Virchows Arch. Submitted

EQA for cfDNA testing for *EGFR* variants in Lung cancer EQA 2020 (n = 258)

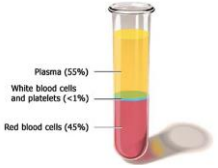
- Centralised sample sourcing and validation of EQA materials
- Include common and clinically relevant variants
- Include challenging samples with low frequency allele variants
- Submission of clinical reports and data collection form

- *EGFR* (mandatory) and *KRAS* (optional)
- Individual laboratory reports
- EQA Summary Report



- Apply performance criteria for genotyping
- Assess interpretation of the results
- Clerical accuracy of the reports

Format of EQAs



Registration

Sample sourcing & validation

EQA distribution

Results submission

Marking critiera defined

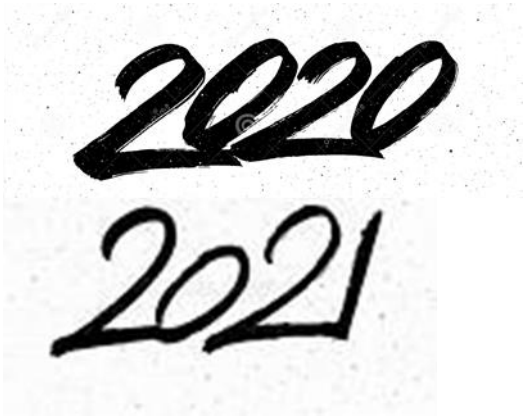
Assessment

Collation of results

Individual EQA Provider Scheme reports plus laboratory reports

Appeals process

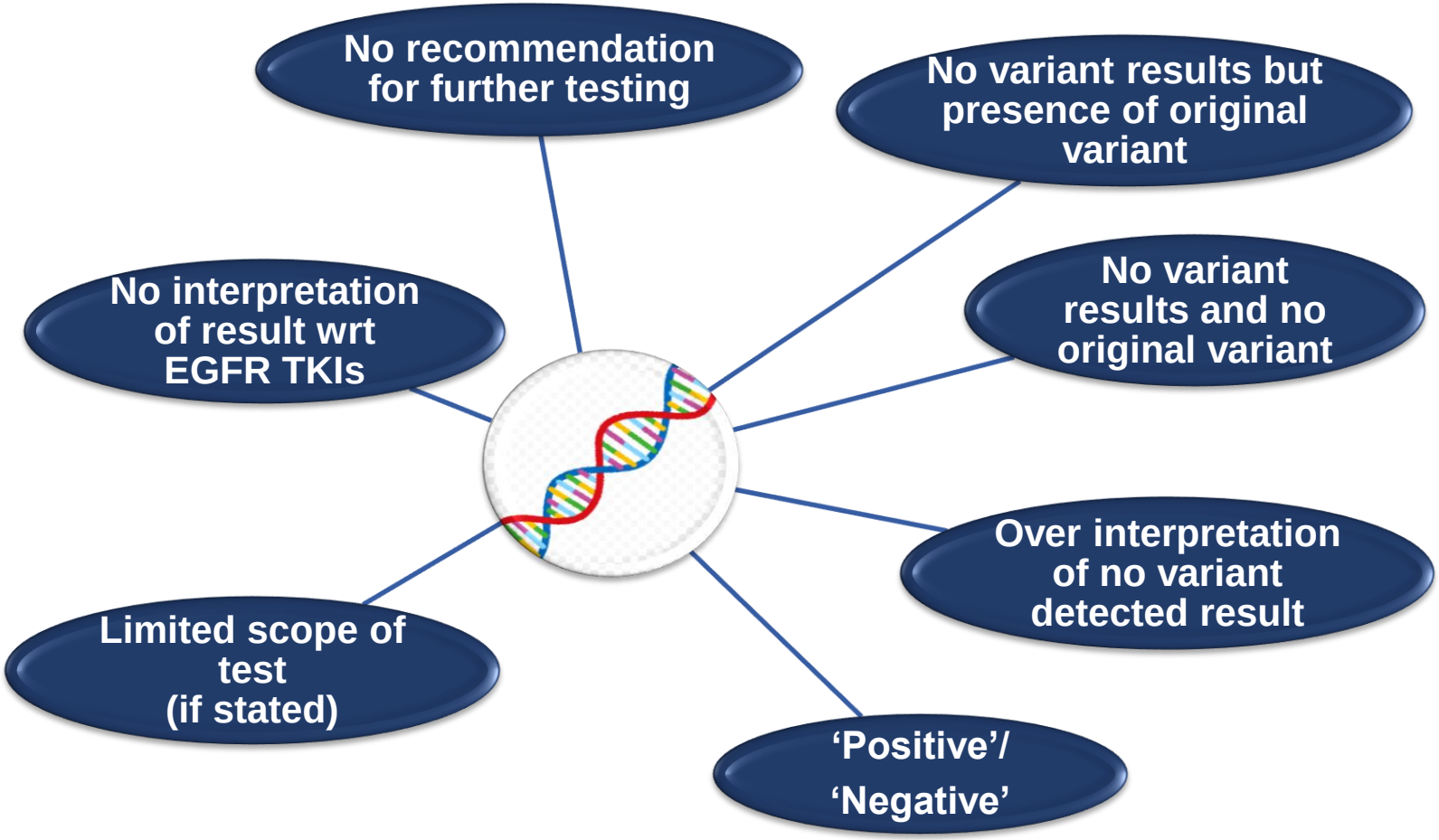
Finalise reports and apply performance critiera for genotyping



Genotyping

Case	Genotype	VAF	Critical genotyping errors	False positives	False negatives	Incorrect variant reported	Potential sample swap
1	No pathogenic variants	N/A	10 (4%)	9 (3.5%)	0	0	1 (0.4%)
2	*EGFR* c.2155G>A p.(Gly719Ser)	5%	27 (10%)	0	23 (9%)	3 (1.2%)	1 (0.4%)
3	*EGFR* c.2236_2250del p.(Glu746_Ala750del) and *EGFR* c.2369C>T p.(Thr790Met)	2% 1%	37 (14%)	0	17 (7%)	3 (1.2%)	2 (0.8%)
Note: 42% reported either one or no variants due to LOD of assays							

Interpretation issues



2021 EQA UPDATE

METHODS

>60 different ones!

173 labs using real-time PCR methods

- most common is COBAS EGFR mutation kit v2 (76)

97 labs used NGS with LOADS of different panels.

- most common being Oncomine lung cfDNA assay (14)
- all others <5 labs using them.

44 labs using ddPCR

There are still laboratories using kits not really suitable for cfDNA testing – e.g. Therascreen EGFR pyro kit, Ion Ampliseq panels, Trusight Tumour 15.

Number of errors dramatically reduced from last year.

Acknowledgements

IQNPath cfDNA pilot EQA Group

Assessor	Location	Role
Rachel BUTLER	UK	Assessor
Caroline CLARK	UK	Assessor
Rachel DODDS	UK	Assessor
Hongxiang LIU	UK	Assessor
Dietmar LOHMANN	Germany	Assessor
Manisha MAURYA	UK	Assessor
Sophie MCHAFFIE	UK	Assessor
Hood MUGHLAASI	UK	Assessor
Thierry NOUSPIKEL	Switzerland	Assessor
Cathal O'BRIEN	Ireland	Assessor
Anca ONISCU	UK	Assessor
Markus RECHSTEINER	Switzerland	Assessor
Matthew SMITH	UK	Assessor
Philippe Taniere	UK	Assessor
Harri WEBB	Australia	Assessor
Melanie CHEETHAM	UK	Scheme Organiser
Jenni FAIRLEY	UK	Scheme Organiser
Simon PATTON	UK	Scheme Organiser
Nicola WOLSTENHOLME	UK	Scheme Organiser

Jenni Fairley
GenQA

Mel Cheetham
EMQN

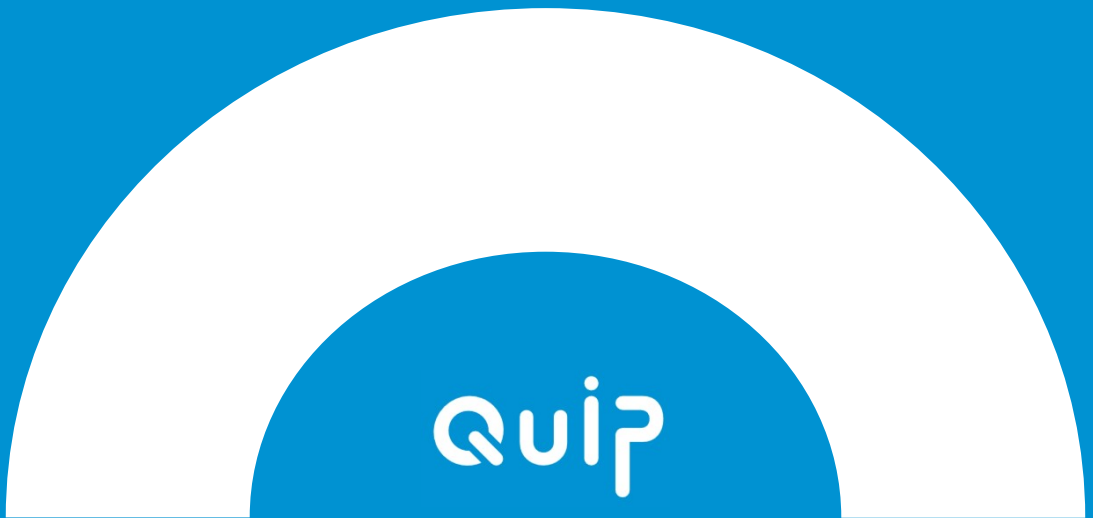
1st ELBS ctDNA Technology Meeting of 2022

23 March 2022

Agenda

- Introduction - QuIP
- QuIP's ctDNA - EQA Program
- *EGFR* Mutation Analysis Liquid Biopsy EQA 2022

QuIP – Who are we?



QuIP- Shareholders

BUNDESVERBAND DEUTSCHER PATHOLOGEN e.V.

Federal Association of German Pathologists e.V.
Bundesverband Deutscher Pathologen e.V.
(BDP)

DEUTSCHE GESELLSCHAFT FÜR PATHOLOGIE E.V.
Seit 1897 – dem Leben verpflichtet

German Society for Pathology
Deutsche Gesellschaft für Pathologie e.V.
(DGP)

QuIP - History

2009

Cooperation with Referenzinstitut für Bioanalytik* (RfB)

*Reference Institute for Bioanalytics

2008

First proficiency test (RAS) in molecular pathology (Munich), followed by numerous other molecular proficiency tests

2004

Founding of the quality assurance initiative QuIP as a subsidiary of the German Society of Pathology and the Federal Association of German Pathologists e.V.

2004

Proficiency testing of predictive factors in breast carcinoma including in situ hybridization (Hannover, Germany).

2000

Immunohistochemical (IHC) proficiency tests (PT) "2000" in the context of an IAP seminar, followed by more than 20 further IHC PT

1999

First proficiency test among university pathology institutes in Germany

2014

European Society of Pathology (ESP) and QuIP agree on mutual recognition of their certificates

2016

Founding of QuIP GmbH by BDP and DGP

2021

31 proficiency tests (42% **IHC** and 58% **molecular pathology**) were offered. International PT were introduced.

2022

ESP Foundation-QuIP EQAs will be rolled out.

QuIP's ctDNA - EQA Program

QuIP's Liquid Biopsy Program

2018	2019	2020	2021	2022
EGFR Exon 19-21	*EGFR* Exon 19-21	*EGFR* Exon 19-21 *PIK3CA* Mutation Analysis	*EGFR* Exon 20 Ins *PIK3CA* Mutation Analysis	*EGFR* Exon 19-21 *MET* Exon 14 Skipping

EGFR Mutation Analysis Liquid Biopsy EQA 2022

EGFR Mutation Analysis: Design

2018 - 2020

- 9 ml blood + fragmented cell line DNA
- Min. 1 % allele frequency (exon 19-21)
- 10 samples

+ Samples resemble real-life samples
- Stability of samples
- Limited number available
- “Production” very work-intensive
- Internal testing at the same time as open proficiency test

2022

- Samples will be produced by SensID GmbH
- Min. 1 % allele frequency (exon 19-21)
- 7 samples

+ Unlimited availability
+ Stability of samples > 1 year
+ Internal testing before open proficiency test
- Artificial samples
- 1st step of the workflow (isolation of plasma) is skipped

EGFR Mutation Analysis 2018-2022:
Case-specific questions

EGFR Liquid Biopsy 1

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
Mutationsstatus Exon 20 (T790M)										
Wildtyp	○	○	○	○	○	○	○	○	○	○
Mutiert	○	○	○	○	○	○	○	○	○	○
nicht auswertbar	○	○	○	○	○	○	○	○	○	○
Mutationsstatus Exon 19 (EGFR)										
Wildtyp	○	○	○	○	○	○	○	○	○	○
Mutiert	○	○	○	○	○	○	○	○	○	○
nicht auswertbar	○	○	○	○	○	○	○	○	○	○
Mutationsstatus Exon 21 (EGFR)										
Wildtyp	○	○	○	○	○	○	○	○	○	○
Mutiert	○	○	○	○	○	○	○	○	○	○
nicht auswertbar	○	○	○	○	○	○	○	○	○	○

Quip

Thank you for your attention.

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