ctDNA Technologies Meeting: Minutes on Discussion Points

Steering Committee: Michael Speicher, Ellen Heitzer, Ed Schuuring, and Patrizio Giacomini

1. Summary and opening of Discussion:

Ellen Heitzer:

- Which reference materials are best suited? All EQA providers use different reference materials
- High variability in cfDNA recovery and also in cfDNA quantification/concentrations
- High number of false negatives and false positives
- All EQAs presented here tested for variants with VAFs of around 1 % or higher and even there the results were partly inconclusive – in the real world we commonly see VAFs lower than that
- How is the data reported to the clinicians?

2. Discussion Point: Reasons for false negative and false positive reports

Rodrigo Toledo:

- We should open the black box of NGS analysis. Potentially the high percentage of false negatives/false positives could be partially routed in how the bioinformatics are done.

Ellen Heitzer:

- We are actually thinking of initiating a ring trial within the ELBS to address exactly this. There is a big difference in results depending in which type of analysis pipeline you are using
- Simon and Sandy – in your EQAs are the analysis pipelines reported by the participants?

Simon Patton (*Managing Director, EMQN CIC*):

- Yes, this data is collected. However, many tests and bioinformatics pipelines are verified on tissue and not cfDNA this may lead inability to detect variants in a clinical process

Ellen Heitzer:

- Do you see improvements in performance/results from labs that use a very defined end to end workflow?

Sandi Deans (*Director of GenQA, member of UK NEQAS consortium*):

- Overall yes. On the Bioinformatics end, it is great when this process is an automated “black box” for some labs but for others labs with a lot of expertise it can be limiting as to what they can do.
- Why false positives/false negatives? This could be anything from sample handling upfront to how extraction is done (e.g. use of DNA extraction kits not suitable or intended for cfDNA extraction), kits not properly applied, incorrect controls, sample swaps, data swaps. However, we do see a huge improvement following our workshops and when root cause analysis was performed.

Simon Patton (*Managing Director, EMQN CIC*):

- No matter how automated the process becomes, frequently the errors are of human cause.

Ellen Heitzer:

- When you see false positives are they generally with VAFs in low range or also high range (higher than 1,2,5 %)?

Sandi Deans (*Director of GenQA, member of UK NEQAS consortium*):

- Both. This is influenced by sample swaps in EQA samples or clinical samples, contamination, use of particular kits that have higher false positive rates than others etc.

Ellen Heitzer:

- When you think of performing EQAs with very low-level samples do you see such EQAs being possible at all due to the challenge of sampling bias?

Sandi Deans (*Director of GenQA, member of UK NEQAS consortium*):

- We do go below 0.5% and see a huge variation 1) in understanding what limit of detection is for certain labs and 2) extra layer of complexity – what do they report to the clinicians (some do not report mutations below certain VAFs) meaning the assay may have detected the variant but bioinformatics hasn't called it (or it has been called but not reported)

3. <u>Discussion Point: Reporting guidelines</u>

Ellen Heitzer:

- Do you provide any guidance on what should be contained in a report to the clinicians? I am not aware of such thing for cfDNA.

Sandi Deans (*Director of GenQA, member of UK NEQAS consortium*):

- There are guidelines out there for solid tumor reporting and European guidelines for constitutional genomic testing.
- We have put out consensus statements on liquid biopsy testing guidelines in 2016/2017 following workshop. Would it be of interest to rerun this type of workshop?

Ellen Heitzer:

- Yes, I believe we all agree on that.

Patrizio Giacomini:

- EQAs is a complicate issue and not comparable to standard EQAs for tissue testing for example
- Potentially we should look at separate modules for EAQ reports in the future: pre-analytics sample handling, wet lab, bioinformatics - instead of focussing solely on the results all together
- Need to do this for 20-50 alterations that we commonly see in clinical practice

Marc Eccleston (*Volition*):

- What impact will IVDR enforcements have on this starting May 2022?

Simon Patton (*Managing Director, EMQN CIC*):

- The implementation/enforcement has been phased.

Sandi Deans (*Director of GenQA, member of UK NEQAS consortium*):

- This is country specific: each country has its own guidelines as to when the IVDR will be implemented

Klaus Pantel:

- What I take out of the last two discussion points is that awareness of existing variables is crucial for the clinical and research community and education on this is a highly important way to improve the accuracy and quality of test results.

4. <u>Discussion Point: For the EQA providers – How can ELBS help?</u>

Niels Pallisgaard:

- I fully agree with Patrizio, we should form different small groups to work and identify what aspects of the workflows work and which do not. This is the big possibility within this network and will allow us to learn from the existing EQA schemes.
- Standardization is crucial and should not be started to late. We should collaborate and move towards the middle to walk forward, otherwise we will have a wild west
- I would suggest performing ring test to identify and provide guidelines on: how to sample/ how to purify / which nGS providers and Kits work well, etc.
- We have the chance to move fast as we have many participants and institutions.
- Lastly we should work on standardizing the reporting to clinicians

Ed Schuuring:

- There are different steps of testing and I agree that we should harmonize. The question is how to do this? We are not implementing cfDNA testing in general but have a clinical question behind it – this needs to be brought into picture of clinical implementations.

Ellen Heitzer:

- Yes, reporting depends on various scenarios. A large gene panel is different from targeted detection of single mutations, there is different levels of genomic coverage used for the testing and different reporting criteria.

Simon Patton (*Managing Director, EMQN CIC*):

- EQA is specifically designed to evaluate and compare the entire process of a laboratory test. It is not appropriate to break it up into separate little bits. However, in the context of ring trials it definitely makes sense to look at and compare specific elements of the test.
- What we are sending the laboratories is a clinical referral and we are asking them to interpret it and test that sample appropriately with whatever technology they like and report results back. What we are looking at is a report that summarizes how they have done the process and what they are saying to the clinicians and that is very different from asking a very specific question- of which technology is best to use.
- The role for this group could be to improve the quality of individual tests as a research type activity.
- Definitely a role for this group to work on guidance for labs: what is the best way to approach DNA extraction/ reporting. However, that is not what EQA does.

Patrizio Giacomini:

- Yes, EQAs give a framework for labs to self-check if they are doing it right

- Large part of the application is not clinical it is diagnostic – the main issue is whether the test that is used gives reliable results. Therefore, I would remain as general as possible

Rodrigo Toledo:

- The educational part is crucial. If there is a failing in a lab, breaking down what can go wrong can drastically improve the results. The main objective is to get better

Niels Pallisgaard:

- I agree. We should not point at one specific issue but provide guidance on which technologies work well for which type of analysis. The EQA is then the "final exam" that every step is working and you are getting the right results.

Klaus Pantel:

- ELBS could bring the efforts together
- We should take stock. Where are we now and what are the activities today, what can we say in terms of guidance today, where are the open questions.
- We also need to make people aware that when going into low VAFs (MRD field) problems intensify and false negatives will increase

Ed Schuuring:

- How about organizing a workshop: bring together users, companies
- We have enough issues and could drive this forward → would this be something ELBS could do?

Sandi Deans (*Director of GenQA, member of UK NEQAS consortium*):

- We would be fully supportive of this. Ideally face to face with a general session and breakout groups on separate elements of the pathway.

Niels Pallisgaard:

- We should also involve clinicians who will receive the results and hear what they need, so that we can later educate in what they get.

Klaus Pantel:

- BloodPAC – could be great to link this with them

Ed Schuuring:

- We will provide a small summary of what we discussed and collect ideas for the workshop. We will share the ideas with the group