2nd ctDNA Technologies Meeting: Minutes on Discussion Points

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

- 82 participants

1. Questions to speakers from the chat / Discussion:

Christa Nöhammer: Did I get it right that you use for your reference controls plasma which has not only be depleted from DNA but also from Ig etc.?

- Kunal Banjara (*Thermo Fisher Scientific*): Yes, that is correct. The plasma is screened for absence of other DNA and also antibodies.

Abhishek Sharma (*Qiagen*): For the WHO standard project, you plan to start a collaborative study in September. Is it a local site sample collection or different sites and what is the method?

- Leandro Lo Cascio (*NIBSC*): We are trying to organize a collaborative study that will cover the globe (WHO requirement). Apart from Europe, USA, China and Japan, we will include diagnostic labs that come from developing countries and more remote places. As we intend a non-profit use, we will try to reach as far as we can. All this is on volunteer basis. As far as technology, our reference materials are usually agnostic meaning they work with ddPCR, NGS approaches (targeted sequencing and exome sequencing,), etc.

Ellen Heitzer: What is the allele frequency of mutations in your samples?

- Leandro Lo Cascio (*NIBSC*): 5 % VAF

Ellen Heitzer: Are you also planning to do something on the CNA side in addition to mutations?

- Leandro Lo Cascio (*NIBSC*): We have a running project focussing on this but it is not as advanced yet.

Patrizio Giacomini: I see some conversions in the provided standards. There seem to be 2 basic type of products: purified DNA that can be spiked or plasma that is already spiked. It appears as though we have everything we need for everyday lab life. However, looking to EQA schemes, how is the feeling of the group, are we sure we can run EQA schemes without resorting to human material/clinical samples?

- Ellen Heitzer: The biggest issue I see is the amount of sample we can get from a patient. This might be sufficient for 3-5, using DLA maybe 10 patients and will therefore not be enough for large EQA schemes. In additional, the samples need to be comprehensively characterized in advance and then distributed to many labs.
- Björn Nowack (*SensID*): I agree, patient material will never be enough for many labs to participate. One could think about combining multiple samples. However, you then get massively different genomic background and polymorphisms. This is difficult for labs using NGS approaches. You could also think of amplifying the DNA material once it has been analysed but again you could introduce mutations. For 40+ labs this is currently not an option.

- Patrizio Giacomini: How about planning a mixed scheme? Sending a backbone of synthetic DNA shared among all of the EQA participants and then batch 10 labs per clinical sample (sample A/B/C/D). This might be a winning strategy.
- Björn Nowack (*SensID*): This definitely sounds like a good strategy. I wonder if it would be feasible and affordable considering all work and cost going into sample analysis and preparation.

Ellen Heitzer: Do the providers actually use artificial plasma or real human plasma?

- *SensID/LGC SeraCare/ Thermo Fisher Scientific & NIBSC* – all use normal human plasma samples from blood banks, which are subsequently modified for use.

Klaus Pantel:

- Is the plasma heat inactivated, chemically modified or simply checked for pathogens?
- Have you ever seen batch-to-batch variations?

Industry standard providers:

- This is unfortunately proprietary information. However, no significant variations are seen.

Ellen Heitzer: To David and Kunal regarding the in-kit control by *Thermo Fisher Scientific*. Is this product specific or platform agnostic? Do you use this control in every single run as a separate reaction?

- Kunal Banjara (*Thermo Fisher Scientific*): It was developed for this specific NGS platform. However, we are able to customize it for other uses and it is generally platform agnostic and could work on other library prep/NGS workflows as well.
- David Chi (*Thermo Fisher Scientific*): It is a control that is used for every single run. We mandate the use of the control and our software automatically analyses it. Certain window of results need to be detected for the control for the run to be valid. This is especially useful for customers with less experience to simplify bioinformatics pipeline.

Ellen Heitzer: I like the idea and it is surely very useful for hotspot panels. However, it might be too expensive to do in large gene panels.

2. <u>Poll on reference materials amongst ELBS members</u>

Questions:

- Are you using a ctDNA mutation detection kit in our lab?
  - Yes/No
- Do you use ctDNA mutation detection in routine diagnostics?
  - Yes/No
- Which assay do you use?
  - ddPCR/ NGS single target/ NGS panel (commercial)/ NGS panel (LDT)/ Other
- Did you assess the performance (sens/spec) of the kit?
  - Yes/No
- Did you use commercial reference materials?
  - Yes/No
- Did you use in-house generated reference materials?
  - Yes/No

Results – Ellen Heitzer:

- Most of us are performing mutation detection.
- Approx. half use mutation detection for routine diagnostics
- Most of you are doing digital PCR, but NGS-usage is coming up.
- Almost all of you assess the sensitivity and specificity of the kit, which is great. 2/3 use commercially available reference materials & about half also uses in house generated reference materials.

Ellen Heitzer: What kind of in-house reference materials do you use?

- Patrizio Giacomini: We use sonicated genomic DNA and quantified PCR products. This is a very crude type of assessment but it gives valuable first estimates.
- Niels Pallisgaard: We use two types of in-house materials. PCR fragments amplified from patient material (isolated DNA from FFPE tissue amplified using ddPCR primers) and we amplify PCR products with mutations and spike into normal DNA.
- Ariane Hallermayr: Sonicated DNA and quantified VAFs. We tend to combine commercial reference materials and in-house materials to increase repetitions.

Tu Truong: When developing reference materials out of cell lines and randomly fragmented DNA, do you experience any loss of integrity or that quantification is not consistent? I could imagine that when you detect using PCR you could face loss of primer detaching segments?

- Bernice Freeman (*Horizon*): We do see small drop due to the nature of the procedure. We use ddPCR as QC good recovery and our manufacturing pipelines are such that these are reproducible within a very narrow window.
- Björn Nowack (*SensID*): You will receive some breaking points and not have complete meteorological traceability. We can currently provide either complete meteorological traceability or precise allele frequency. It is not possible to provide both for now.

Tu Truong: Does anyone happen to have experience in comparing the presented extraction methods? As Leandro presented the chromatin extraction only from mono-nucleosomes this method might increase reproducibility of results and could be more close to actual patient ctDNA.

- Krystyna Nahlik (*LGC SeraCare*): We have tried this and it did not lead to expected results, especially in NGS analysis. Maybe the approach of combining initial fragmentation and nucleosomal digestion as presented earlier would be optimal.
- Leandro Lo Cascio (*NIBSC*): The NGS compatibility is technology dependent and in based on the size of the fragments.

Conclusion:

Ed Schuuring: I personally feel, it would be helpful for users to have an overview to identify good reference materials. We could think of developing a catalogue to inform users on how to choose the right reference material and the differences between providers.

Ellen Heitzer: This is a great idea.