Medicines & Healthcare products Regulatory Agency

# Novel Primary Reference Materials to Support Standardisation in Liquid Biopsy

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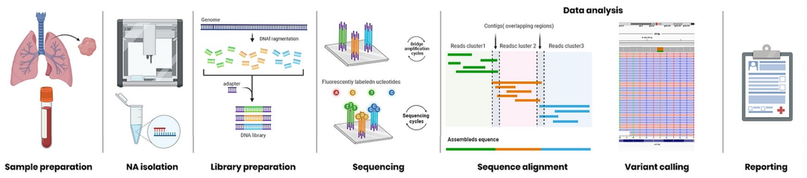
## 1. BACKGROUND

### Genomic Testing




**Needs for the harmonisation of measurement of genomic testing**[1]

- **Primary reference materials**
- **Primary reference measurement procedures**
- **Framework** for determining the measurement uncertainty in quantitative genomic data

[1]https://www.genomemet.org/

## 2. AIM

### Development of a candidate WHO reference material for *EGFR* variants in Liquid Biopsy

**Principles used by WHO for biological standardisation**[2]**:**

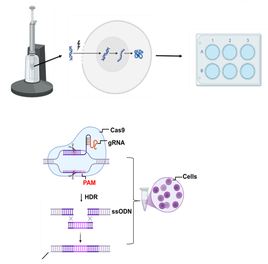
- the reference standard should be assigned a value in arbitrary rather than absolute units;
- the unit is defined by a reference standard with a physical existence;
- in the establishment of the standard, a variety of methods is usually used and that the value assigned to the standard, and therefore the definition of the unit, is not necessarily dependent on a specific method of determination;
- the behaviour of the reference standard should resemble as closely as possible the behaviour of test samples in the assay systems used to test them.

| COSMIC ID | CDS Mutation | AA Mutation |
|---|---|---|
| COSV51765161 | c.2573T>G | p.L858R |
| COSV51765492 | c.2369C>T | p.T790M |
| COSV51765119 | c.2235_2249del | Del E746_A750 |

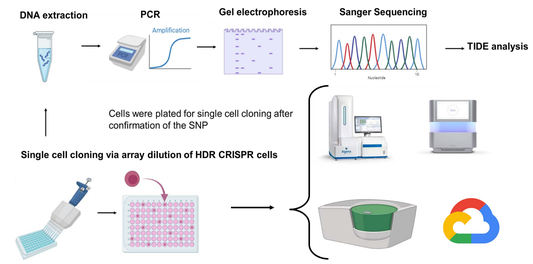
[2]Annex 2, WHO Expert Committee on Biological Standardization. Fifty-fifth report. WHO Technical Report Series, No. 932, 2006.

## 3. CANDIDATE MATERIAL

### A. Genome editing

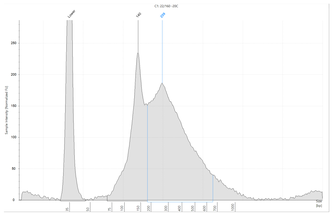



### B. Validation

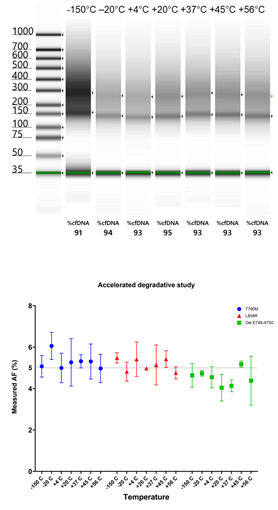



### C. Formulation and Production

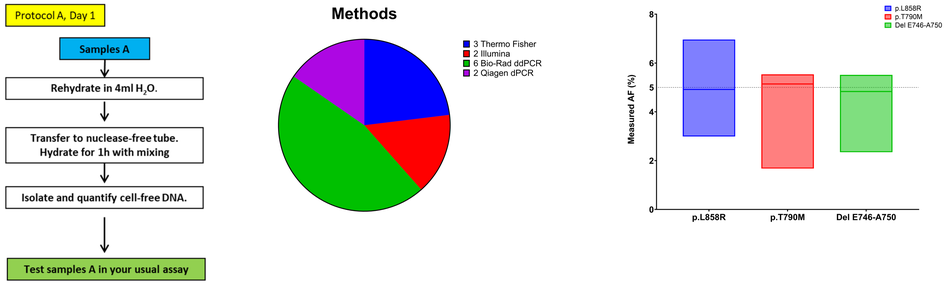
- **Formulation**
  - 160 ng total DNA
  - 4 ml total volume
  - Each variant at ~5% AF
- **Production**
  - Chromatin extraction
  - Sonication
  - MNase treatment
  - Dilution in DNA-depleted human plasma
  - Freeze-dry cycle
  - Storage -20°C



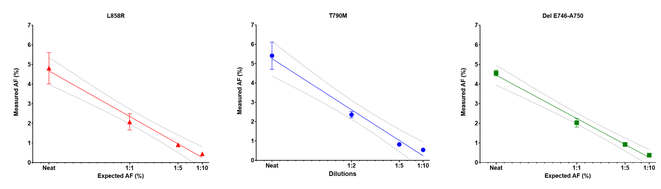
### D. Accelerated degradative study




## 4. COLLABORATIVE STUDY




## 5. DILUTION STUDY




**Collaborative study design and preliminary results**. (Left) Representative protocol of the collaborative study. Each participant received three vials of the same material to be tested according to their established pipeline. (Middle) Four different methods were included: Bio-Rad droplet digital PCR, Qiagen digital PCR, Illumina NGS and Thermo Fisher NGS. (Right) Representative median Allele frequency (AF %) values obtained for each of the *EGFR* variants. Floating boxes represent minimum and maximum values. Median values are identified by thick line.

**Dilution study results**. Representative data of serial dilutions. Candidate material was diluted at different ratio with a similar *EGFR* wild-type material. Each *EGFR* variant was assesses independently by Bio-Rad digital PCR. Four ratios were tested: Neat, 1:1. 1:5 and 1:10. Each data point represents the mean of 3 wells (Standard deviation is indicated by whiskers). Dotted lines represents calculated linear regression with CI 90%.

**Material Production**. A target cell line was modified by introduction of selected EGFR variants. Each single clone was validated orthogonally by a Sanger sequencing, NGS sequencing, digital PCR and Mass Array. The final formulation was empirically determined to contain nucleosomal DNA in human plasma matrix. Material was lyophilised, stored at –20°C and subject to periodical stability testing.

## 6. CONCLUSIONS

Here we describe the workflow for the generation of WHO Primary reference materials for harmonisation of measurement of genomic testing in oncology diagnostics. The implementation of these type of materials will expedite the development of primary reference measurement procedures as well as secondary reference materials. Ultimately, the development of a strong framework for precise and robust measurements of genomic variants, will benefit cancer patients worldwide.

## 7. ACKNOWLEDGEMENTS

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