WHITE PAPER

Why using cell line-derived reference material is critical for reliable genetic testing in oncology.

Navigating oncological tests can be challenging. The intricate nature of the tests combined with the complexity of the human genome means that technical errors can occur at any stage of the testing workflow – from sample preparation through to sequencing and analysis.

Fortunately, these technical errors can be mitigated through the utilization of high-quality reference material. Employing the best standards for your assay not only aids in calibrating your results, but also facilitates the evaluation of diagnostic performance.

With advancements in affordable technologies, there are now a multitude of reference types to choose from, ranging from patient material to cell line-derived standards and synthetic spike-ins. In this whitepaper, we spotlight cell line-derived standards and the distinct advantages these have when used for oncology assays.

What are cell line-derived reference standards?

Cell line-derived reference standards, or controls, are developed to closely mimic the genomic complexity found in patient samples. Cells containing genetic variations, such as insertions and deletions (INDELs), fusions, single nucleotide variants (SNVs), and copy number variants (CNVs) are blended to produce multiplex controls to support oncology assays.

These variants are constructed from both existing cell lines harboring the variant of interest and by engineering mutations into cell lines through genetic engineering techniques such as rAAV and CRISPR. By blending different cell lines, variants are created at specified frequencies in clinically relevant groupings. This approach supports both cancer-specific testing and pan-cancer analyses (Figure 1).

revvity

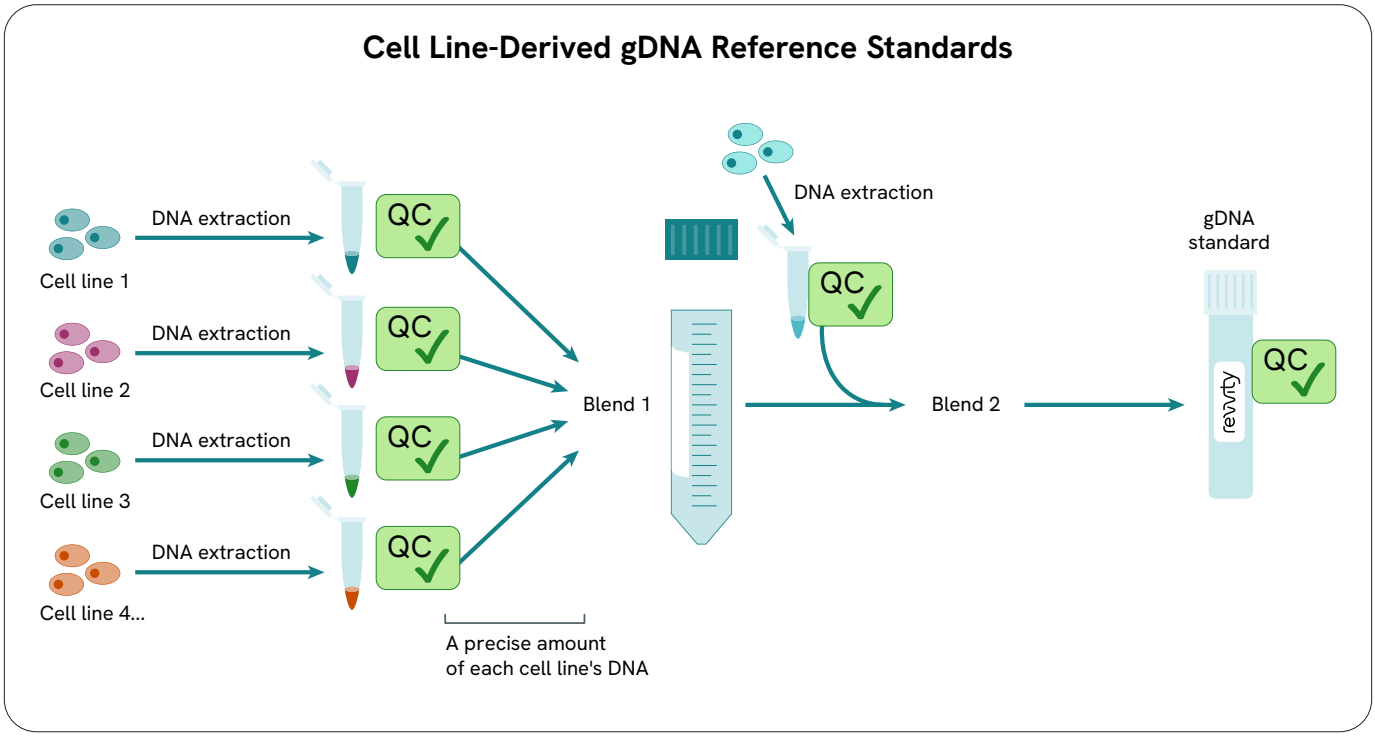

Figure 1: Characterized cell lines containing variants are blended to create standards with a few or multiple variants of known allelic frequencies. The blended cell mixture is then subjected to quality control analysis and processed to be available in a variety of formats.

How does reference material mimic the patient sample workflow?

Reference materials can be used alongside any patient sample, ensuring the accuracy and reliability of various assays (test methods). They can be used to verify results and confirm the integrity of the entire workflow – from DNA/RNA isolation through to analysis. Reference standards can also be used to troubleshoot any workflow issues, ensuring consistency and reproducibility in experimental outcomes (Figure 2).

Given the high sensitivity of techniques such as next-generation sequencing (NGS) and digital PCR for detecting low-frequency mutations in somatic tumors, it is paramount to test each step of the process to ensure result reliability. Reference standards can be used for verifying every stage of the process:

- Extraction control for gDNA/cfDNA/FFPE DNA/FFPE RNA extractions
- DNA/RNA quality controls
- Assay quality controls (NGS/qPCR/ddPCR/Sanger/Microarray)
- Analysis controls – to help filter false positives or false negatives.

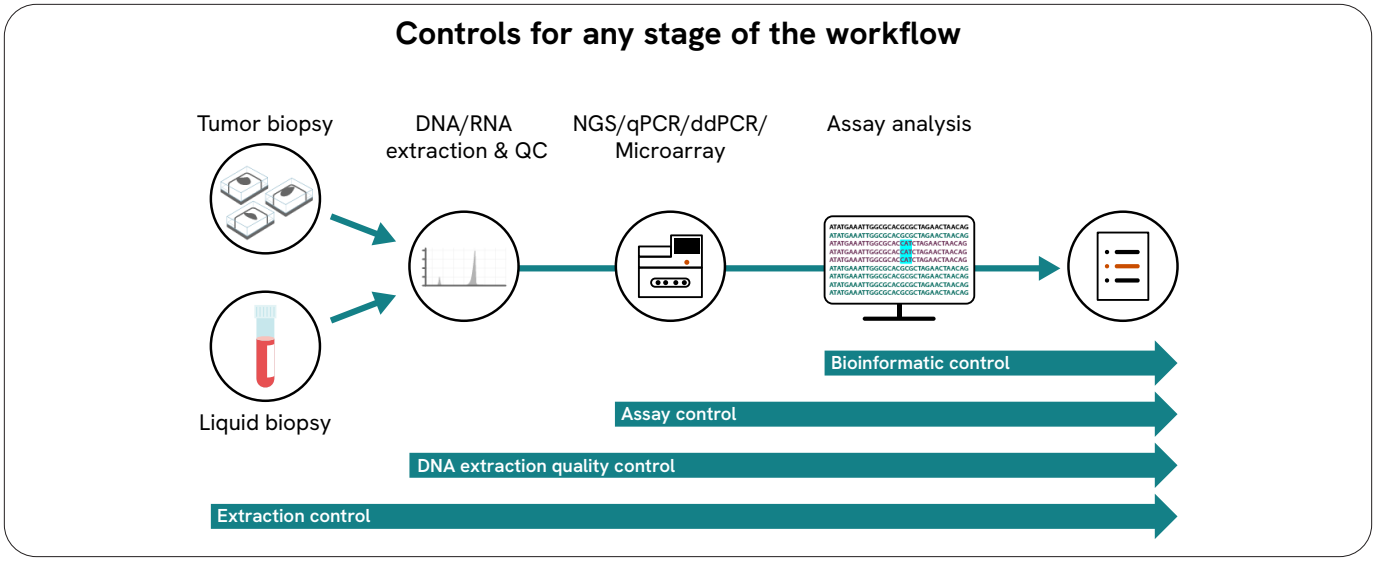

Figure 2: Different reference standard formats can act as a control for distinct stages of a workflow.

What are the advantages of cell line-derived controls for oncology assays?

1. **Representation of full genomic complexity**

   With the right approach and by leveraging cell lines, controls can be developed and manufactured with biologically relevant cancer mutations across the full genomic landscape, at the natural genetic loci with neighboring sequences.

2. **Reproducible source of material**

   Under the right conditions cell lines can be easily maintained, allowing cell line-derived reference materials to continuously be reproduced to high standards. This provides a limitless source of consistent and reliable reference material. In comparison, real FFPE patient material is often available in limited quantities, increasing the risk it will run out, and of variable quality throughout the block. Consequently, there is a possibility its genetic profile may vary between sections.

3. **Long shelf life and easy storage**

   Cell line-derived reference material can be stored for up to 44 months for gDNA @ 50ng/µl (although gDNA at 25ng/µl is only 27 months), requiring minimal maintenance compared to other standards. This longevity is a considerable advantage over real patient material, especially in the field of liquid biopsy. Real blood and extracted plasma samples from patients have limited storage time due to the degradation of the DNA by endogenous vascular enzymes. Some cell line-derived standards are available in synthetic plasma, which is stable for up to 13 months and free from contaminating analytes.

4. **Defined mutation allele frequency**

   In oncology, driver mutations can be present at varied allele frequencies (AF) depending on the percentage of patient cells containing that mutation. If the mutation is present in the germline DNA (most likely inherited from one or both parents, or arising as a novel mutation during meiosis or early zygogenesis), it will be present at either 50 or 100% AF. Alternatively, mutations generated during random mutagenic events within somatic cells at any point during the patient's lifetime will be present at a much lower AF (typically below 10% AF).

   The challenge for technology developers is to improve assay sensitivity, enabling the detection of low-frequency somatic oncogenic mutations. This capability enables the early detection and treatment of new cancers or new resistance mutations that evolve during the monitoring of an existing tumor. While the limit of detection (LOD) of current clinical NGS assays is around 1% AF, this can vary depending on the assay and the amount of data generated per sample (depth/coverage in NGS context). In fact, best clinical practice dictates that the LOD needs to be calculated using a reference standard during the introduction and validation of every new gene panel

prior to patient testing. Cell line-derived reference standards are produced to contain mutations spanning a broad range of AFs to challenge labs to correctly identify their accurate LOD.

5. **Copy number variations (CNVs)**

   In addition to mutation AF, assessing copy number is an increasingly important criterion in clinical oncology. Many cancers are distinguished by having an abnormal number of copies of key oncogenes, most likely caused by genome instability and large-scale chromosomal rearrangements in neoplasms. Technology developers are improving their capabilities in this area, with many panels now detecting CNVs in several actionable genes. To test the accuracy and guide the necessary optimization of these challenging new techniques, cell line-derived reference standards are provided with known copy number information. This enables users to verify their CNV assay in a controlled manner, a task that is not always possible with real patient material due to the frequently undetermined CNV status in real tissue samples.

6. **A range of format types to match patient samples and workflow steps**

   The reference standards used should mimic the patient sample as closely as possible and correspond to the workflow steps. Therefore, they need to be available in a range of formats. This allows technology developers and testing labs to choose the relevant format type for assay development and validations.

Table 1: Formats of cancer reference standards offered by Revvity. Standards can be used as a technical control for the assay or as a process control for a workflow (Figure 2).

Reference standard formats	
gDNA	Genomic DNA from cell lysates
ctDNA	Circulating tumor DNA in buffer or synthetic plasma
fcDNA	Formalin compromised DNA
FFPE DNA	Formalin-Fixed and Paraffin-Embedded DNA
FFPE RNA	Formalin-Fixed and Paraffin-Embedded RNA

7. **Platform agnostic**

   Reference material must be compatible with different oncological testing methodologies. Cell line-derived reference materials can be used across various genetic testing platforms such as qPCR, ddPCR, Sanger sequencing, Microarray, and NGS, contributing to advancements in precision testing.

Conclusions

The field of genetic diagnostics has witnessed remarkable growth due to its substantial contribution to cancer diagnosis and prognosis. This progress has fueled the development of more precise and sensitive diagnostic tests aimed at minimizing false positives and negatives, thereby improving patient care. These diagnostic tools play a pivotal role not only in detecting cancers but also in predicting cancer recurrence, which relies on the detection of very low concentrations of ctDNA (low-frequency mutations) in patient plasma (defined as MRD – Minimal Residual Disease).

To develop reliable tests, where precision and accuracy are paramount, the use of reliable reference materials is essential for advancing research, guiding decisions, and ultimately improving human health. Consortiums, such as the Medical Device Innovation Consortium, have recognized the need for reliable and consistent reference standards to improve the validation and regulatory review process in cancer testing.

With a wide range of off-the-shelf sample formats and cancer-specific controls available, Revvity's Mimix™ reference standards are cell line-derived, maintaining genomic complexity and faithfully mimicking patient material from sample preparation through downstream analysis. For research use only. Not for use in diagnostic procedures.

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