Non-invasive tumour diagnostics: validating reference standards

Using LGC's expertise in digital PCR (dPCR) and measurement science, LGC worked with Horizon Discovery Group to develop methods for the evaluation and application of their new Cell Free DNA (cfDNA) Reference Standards which contain cancer-related DNA mutations

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The requirement

Performing tissue biopsies to assess tumour genetics and monitor disease progression is highly invasive. For some types of cancer it is not even possible due to anatomical restrictions or the poor health of the patient. However, as tumour cells break down, they release small amounts of DNA in to the bloodstream of the patient. The ability to detect the presence of mutations in these trace amounts of DNA could enable highly specific diagnoses to be performed based only on a blood test (a so-called 'liquid biopsy') rather than an invasive biopsy. In addition, the response of the tumour to treatments could be regularly monitored.

As the quantity of mutant DNA present can be very low compared to normal DNA (0.1 % and lower) and the mutant DNA often differs by only one base compared to the normal sequence of nucleic acids, there is a need for high quality reference materials to measure the accuracy, sensitivity and specificity of cell free (cf) DNA assays for tumour detection.

Horizon Discovery, a world-leading gene editing company that designs and engineers genetically-modified cells for research and clinical applications, has produced new cfDNA Reference Standards to support the implementation and standardization of cfDNA technologies. These products contain DNA mutations that occur in lung, colon and breast cancer and the most frequently occurring mutation in malignant melanoma.

To ensure customers obtain the maximum quantitative information from assay evaluation or quality control experiments performed with these materials, the values assigned to the low concentration of mutants present must be highly accurate.

The solution

Scientists at LGC collaborated with Horizon in developing assays and data analysis parameters for low concentration measurements of cancer mutations by digital PCR. LGC performed droplet digital PCR (ddPCR) analysis using alternative assays to validate Horizon's quality control parameters and provide independent verification of the copy number quantity and frequency measurements for the first two Reference Standard products released[a].

LGC's ddPCR measurements confirmed the accuracy of the stated composition of the mutant compared to the non-mutated gene copy (wild-type sequence) including at the lowest level, where only ~10 copies of the mutant sequence were present per microlitre (0.1 % allelic frequency). Evaluation of the negative control materials, composed of 100% wild-type DNA, also demonstrated their suitability for defining the specificity and limit of detection of assays.

LGC also developed approaches for using the Horizon cfDNA Reference Standards as whole process controls to monitor the full liquid biopsy process from blood sample to diagnostic test result. The Reference Standards were mixed with blood plasma from healthy donors followed by DNA extraction and ddPCR analysis, enabling the recovery of tumour-related DNA to be evaluated.

The impact

Independent validation of these Reference Standards, as performed at LGC, adds confidence in the use of these standards for assessing the performance of high throughput platforms.

Horizon's cfDNA Reference Standard can be applied to a wide range of cfDNA NGS (Next Generation Sequencing) and qPCR (quantitative PCR) assays aimed at providing therapy guidance and monitoring by analysing the genetic makeup of the tumour from the patient's blood. These materials are now being used by Horizon Discovery customers to routinely monitor their workflows by analysing the sensitivity and specificity of their diagnostic assays, in particular to determine their limits of detection.

Since their release, these Reference Standards have been one of Horizon's best selling products. They are being used by clinical and academic labs, technology manufacturers and proficiency scheme providers from all over the world to identify pre- and post-analytical errors.

LGC's work testing the Reference Standards as whole-process controls for testing of DNA extraction efficiency contributed to Horizon launching a further cfDNA product line, the Reference Standards in Synthetic Plasma, which closely mimics real human plasma, with the advantage of greater stability and reliability.

These materials will continue to support the development of new cfDNA technologies to improve disease diagnosis and monitoring.

> *"Horizon's Reference Standards offer a source of genetically defined, quantitative, sustainable and independent third party reference material. The validation of our cf DNA standards by a high quality organisation like LGC confirms their value in supporting the critical role of assay validation and routine performance monitoring for this important new class of assays."*
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> ***Dr. Karin Schmitt,***
> ***Director Reagent Products, Horizon Discovery***

[a] *Devonshire AS et al. Independent study of Horizon Dx Cell Free DNA Reference Standards demonstrates strong concordance in the assessment of key material parameters. (2016) Horizon Discovery Technical Note.*

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