AcroMetrix™ Oncology Hotspot Control

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IVD

Read Highlighted Changes: Revised February 2024

For In Vitro Diagnostic Use

REF 969056 AcroMetrix Oncology Hotspot Control

Rx Only

Intended Use

The AcroMetrix™ Oncology Hotspot Control is intended for use with next generation sequencing (NGS) assays that are designed to identify somatic mutations in DNA from human samples. The control is intended to provide a means for assessing day-to-day test variation and may help in identifying increases in random or systematic error, such as reagent lot changes, operator-based deviations, and instrument malfunction. This product is for *in vitro* diagnostic use.

Summary and Explanation

The use of next-generation sequencing (NGS) methods in the clinical laboratory setting is increasing, affecting the need for standardization of procedures, protocols, and materials to ensure consistent testing results across laboratories, instrument and assay platforms, and laboratory operators.

Various regulatory agencies, professional societies, and public initiatives have responded to the rapid growth of NGS clinical testing by issuing guidance on aspects of quality control (QC) in clinical laboratory testing. For example, the CAP molecular pathology checklist requires that clinical labs use positive controls in each run[1]. Others, like the New York State guidelines, specify low frequency controls containing multiple variants of each kind[2] while the CDC Nex-StoCT workgroup recommends the use of materials that contain most or all variants[3].

As a highly-multiplexed, proprietary DNA quality control, the AcroMetrix Oncology Hotspot Control is designed to enable labs to control the hundreds of amplicons targeted by NGS panels. It contains over 500 mutations from the Catalogue of Somatic Mutations in Cancer (COSMIC) database, and has five variant types of varying nucleotide lengths. The 53 genes represented in the AcroMetrix Oncology Hotspot Control are: ABL1, AKT1, ALK, APC, ATM, BRAF, CDH1, CDKN2A, CSF1R, CTNNB1, EGFR, ERBB2, ERBB4, EZH2, FBXW7, FGFR1, FGFR2, FGFR3, FLT3, FOXL2, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, KRAS, MAP2K1, MET, MLH1, MPL, MSH6, NOTCH1, NPM1, NRAS, PDGFRA, PIK3CA, PTEN, PTPN11, RB1, RET, SMAD4, SMARCB1, SMO, SRC, STK11, TP53, VHL

Principles of the Procedure

The AcroMetrix Oncology Hotspot Control consists of a mixture of synthetic DNA and genomic DNA in a stabilizing buffered solution. The genomic DNA is derived from the same cell line (GM24385) that is used for the development of a NIST Genome in a Bottle reference material. The synthetic DNA, which is present at low frequencies, introduces hundreds of variants that are frequently found as somatic mutations in cancer. These 521 variants have all been confirmed by Sanger sequencing. The AcroMetrix Oncology Hotspot Control is traceable to an internal higher order standard. In addition, this higher order standard is value assigned to ensure each synthetic DNA is present at the relevant allelic frequency.

Table 1. Distribution of variant types in the AcroMetrix Oncology Hotspot Control

	Synthetic Variants		Genomic Variants
Target frequency range	5-15%	15-35%	N/A
Single Nucleotide Variants	317	155	32*
Multiple Nucleotide Variants	0	2	0
Deletions	15	14	0
Insertions	9	8	2*
Complex Variants	0	1	0

* Variants detected in the genomic DNA were confirmed using publicly available whole genome sequencing information for GM24385. Genomic variants will be updated as information becomes available from the Genome in a Bottle reference material.

Control Reagents

Catalog Number	Control Name	Quantity
969056	AcroMetrix Oncology Hotspot Control	3 x 25 µL

⚠ Precautions and Warning

WARNING: The AcroMetrix Oncology Hotspot Control contains unencapsidated DNA. Although unencapsidated DNA is not known to be dangerous, it is recommended that the product be handled as potentially biohazardous. Observe universal precautions for prevention of transmission of infectious agents when handling these materials.[4,5,6]

H412 - Harmful to aquatic life with long lasting effects.

Avoid release to the environment. Dispose of contents/container to location in accordance with local/regional/national/international regulations.

Do not pipette by mouth. Use personal protective equipment, including lab coats, gloves and safety glasses. Do not eat, drink or smoke in areas where specimens are handled.

Disinfect liquids, materials or spills with a 0.5% sodium hypochlorite solution. Dispose of all materials and liquids used in the procedure as if they contained pathogenic agents.

This product contains 0.0095% ProClin™ 950 as a preservative.

Storage Instructions

Store at -20°C or below. Do not use this product beyond the expiration date printed on the label.

To avoid contamination use standard techniques and good laboratory practices when handling the product.

Instructions for Use

Thaw the AcroMetrix Oncology Hotspot Control at room temperature, vortex gently, and pulse-spin in a centrifuge (4-6 seconds) to collect the contents at the bottom of the tube.

Introduce the control DNA as an independent sample at the library preparation step in the NGS workflow. The AcroMetrix Oncology Hotspot Control should be handled similarly to genomic DNA extracted from routine samples and run in parallel with routine samples.

The AcroMetrix Oncology Hotspot Control may be subjected to a maximum of five freeze-thaw cycles. Additionally, the product may be stored at 2-8°C for 6 days with up to four uses.

Interpretation of Results

Variant detection results of the AcroMetrix Oncology Hotspot Control may differ according to the library preparation method, sequencing method, and the bioinformatics pipeline. In order to establish a baseline performance, the user should incorporate results from multiple runs under different conditions (e.g., operator, run, day) to create an expected variant list specific to his or her laboratory. Once the range of performance is understood, the expected variant list can be used to compare each subsequent run result of the AcroMetrix Oncology Hotspot Control.

Expected Results

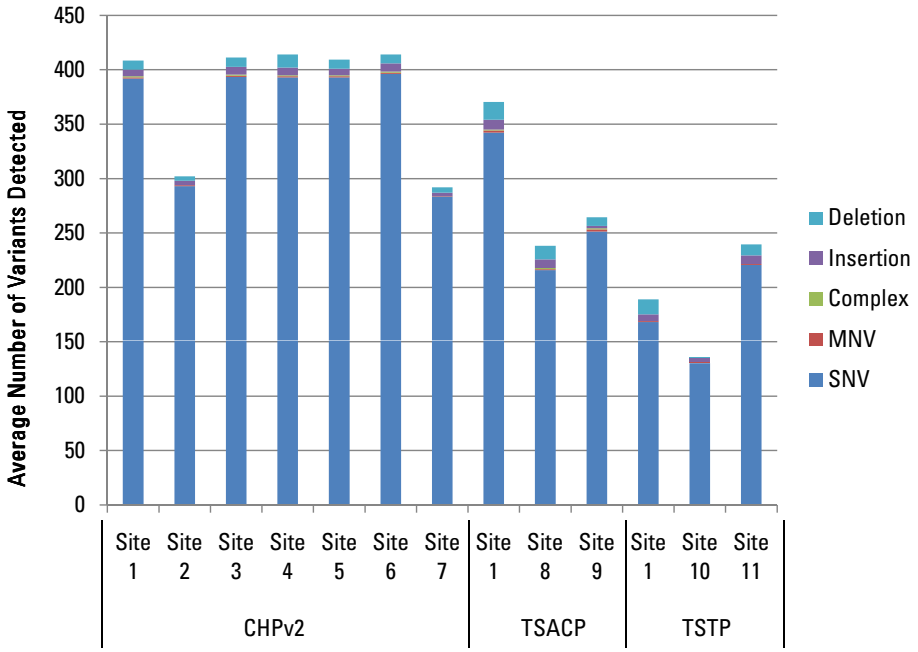
To test how many variants on the AcroMetrix Oncology Hotspot Control could be detected by NGS, three different library preparation test panels were used: the Ion AmpliSeq™ Cancer Hotspot Panel v2 (CHPv2) on the Personal Genome Machine™ (PGM™), the TruSeq™ Amplicon Cancer Panel (TSACP) on the MiSeq™, and the TruSight™ Tumor Panel (TSTP) on the MiSeq.

The following input volumes of the control were used in each library preparation test panel:

Library Preparation Test Panel	Volume of AcroMetrix Oncology Hotspot Control
Ion AmpliSeq Cancer Hotspot Panel v2	2.5 µL
TruSeq Amplicon Cancer Panel	5 µL
TruSight Tumor Panel	10 µL (into each Library Preparation)
Oncomine Comprehensive Assay v3	2.5 µL
Oncomine Comprehensive Assay Plus	2.5 µL
Oncomine Myeloid Assay	2.5 µL
Oncomine Focus Assay	2.5 µL
Oncomine Prevision Assay	2.5 µL
Oncomine Tumor Mutation Load Assay	5 µL
Oncomine Pan Cancer Panel	5 µL

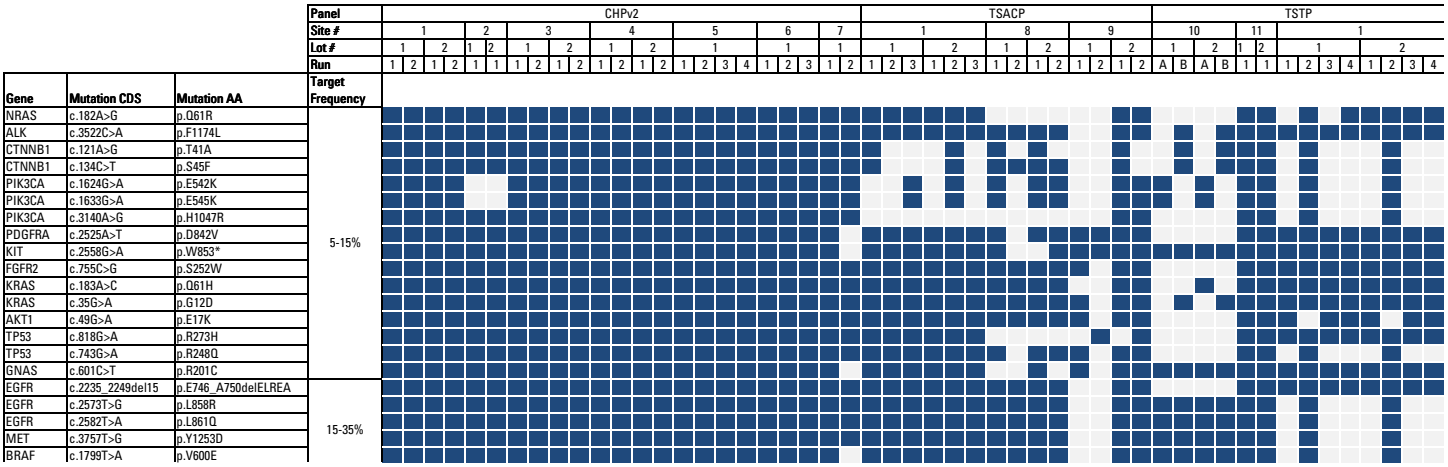
For each panel, two lots of the AcroMetrix Oncology Hotspot Control were tested in duplicate, in at least two sites. Additional sites only tested one of the lots at least twice or both lots once. Sources of variation between sites may include different instruments, operators and general workflows. Also, variation in bioinformatics pipelines may have contributed significantly to variation in performance results.

Figure 1. Performance across different sites and panels
The average number of variants of different types detected in the AcroMetrix Oncology Hotspot Control are reported by site and grouped by panel. Note: The total number of variants of each type is different for each panel.



To assess the detection of specific variants across different panels, twenty-two clinically-relevant variants that were targeted by three panels were selected.

Figure 2. Detection of 22 selected variants across panels
Analysis was conducted with data from sites that tested two lots of the control at least once or one lot at least twice. Detection is indicated in navy blue and absence indicated in light gray. Site-to-site differences are apparent, even amongst those utilizing the same library preparation method, indicating the likelihood of the bioinformatics pipeline having a strong impact on performance.

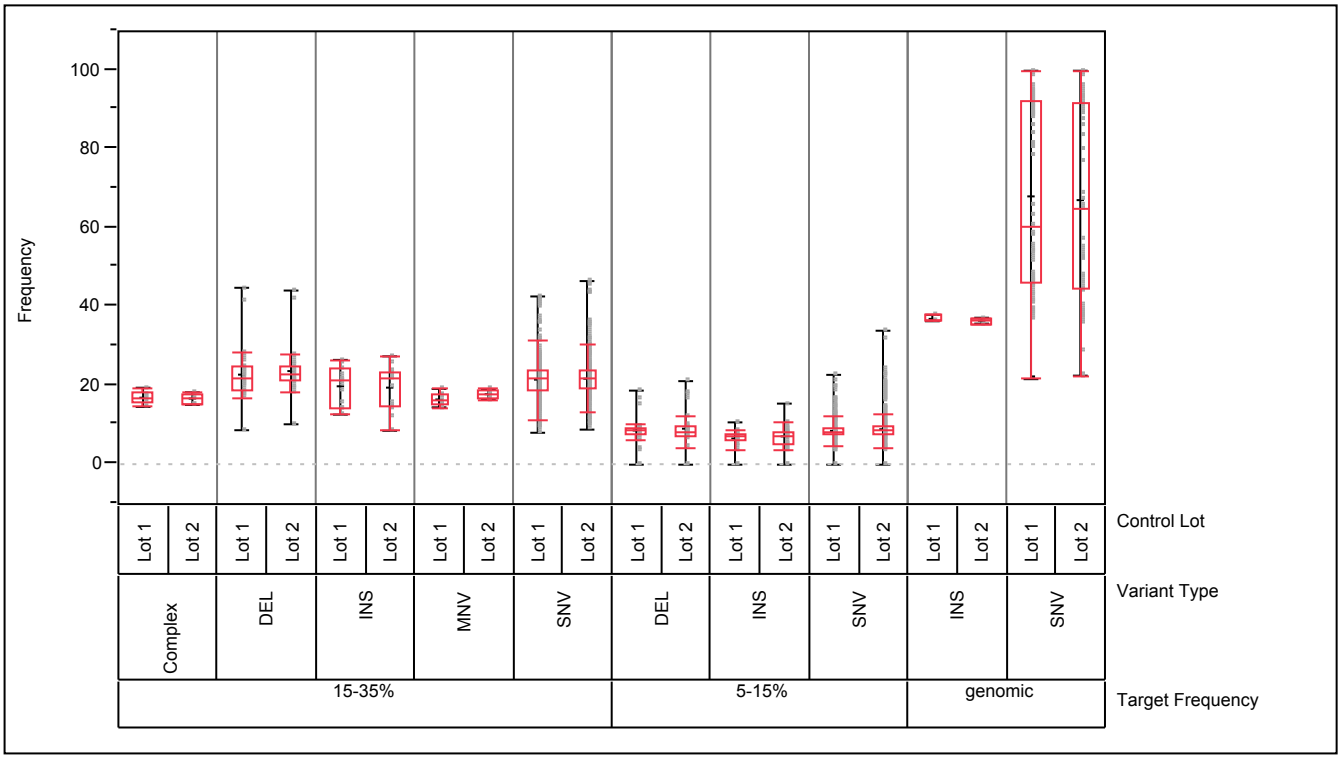


A summary of performance across methods for all variants is provided on the product page on the Thermo Scientific website: www.thermofisher.com/Acrometrix

Results provided by the Manufacturer are for informational purposes only. The end user is responsible for establishing their own performance criteria.

To assess the consistency between production lots of the AcroMetrix Oncology Hotspot Control, two lots of the control were made and tested at four sites using the Ion AmpliSeq Cancer Hotspot Panel v2. Each lot was tested 1-2 times in each site.

Figure 3. Lot-to-lot consistency
Box plots for the observed frequencies by NGS are grouped by target frequency and variant type. Similar upper quartiles, lower quartiles, and median (indicated in red) between Lot 1 and Lots 2 for each variant type demonstrate the consistency of detection between different lots of the same control.



Limitations

The AcroMetrix Oncology Hotspot Control is intended for *In vitro* diagnostic use. The AcroMetrix Oncology Hotspot Control is not intended for use as a substitute for the internal controls provided by *In vitro* diagnostic kit manufacturers.

The control is intended for use in clinical laboratories.

All quality control requirements should be performed in conformance with local, state and/or federal regulations or accreditation requirements.

References

1. College of American Pathologists (CAP) Laboratory Accreditation Checklist
2. New York State Clinical Laboratory Evaluation Program (CLEP) Laboratory Standards.
3. Gargis AS, et al. Nature Biotechnology. 30, 1033–1036 (2012).
4. Centers for Disease Control (CDC). Recommendations for prevention of HIV transmission in health care settings. MMWR 1987; 36 (supplement no. 2S).
5. Centers for Disease Control (CDC). Update: Universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus, and other bloodborne pathogens in health-care settings. MMWR 1988; 37:377-388.
6. Centers for Disease Control (CDC). Guidelines for prevention of transmission of human immunodeficiency virus and hepatitis B virus to health-care and public-safety workers. MMWR 1989; 38(S-6): 1-36.

Glossary:

http://www.thermofisher.com/symbols-glossary

Important Notice

Any serious incident that has occurred in relation to the device shall be reported to Microgenics Corporation and to the National Competent Authority in which the user and/or the patient is established unless differently instructed by such National Competent Authorities.

The summary of safety and performance is on EUDAMED and available upon request.

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For insert updates go to:
www.thermofisher.com/Acrometrix

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