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研究テーマと研究計画

QUALITY ASSURANCE OF NEXT GENERATION SEQUENCING BASED CANCER GENE PANEL TESTS THROUGH THE COMMUTABILITY OF RESULTS

はじめに **(INTRODUCTION)**

Next-generation sequencing (NGS) enables faster and more comprehensive DNA sequencing compared to traditional Sanger sequencing. This advancement has significantly expanded the clinical applications of DNA sequencing, particularly in the genotyping of conditions such as cancers, immune disorders, and hereditary diseases, thereby increasing its importance in healthcare.

Over the past six years, our research team has conducted a pilot study involving on-site evaluations as part of an external quality assessment (EQA) for ISO 15189 accreditation of laboratories performing NGS testing. While participating laboratories generally demonstrated good performance, failures were observed in both the wet-lab and dry-lab steps of NGS testing. Additionally, concerns were raised regarding the commutability of EQA samples used in the study.

The commutability of reference materials is essential for ensuring the accuracy and reliability of NGS in oncology testing. Properly evaluated commutable reference materials help standardize sequencing results across different laboratories and platforms, reducing systematic biases that could impact clinical decision-making. Key reference materials, such as Horizon’s OncoSpan and Acrometrix hotspot controls, are derived from well-characterized tumor cell lines and designed to closely mimic the genomic complexity of patient samples.

To confirm their commutability, these materials undergo rigorous validation, including sequencing across multiple NGS platforms and comparison with orthogonal methods such as digital PCR. Evaluating reference materials involves assessing their ability to produce consistent variant detection and allele frequency measurements across different measurement procedures. By integrating commutable reference materials into EQA and proficiency testing, laboratories can enhance the reproducibility of NGS oncology testing, ultimately improving confidence in cancer diagnostics and treatment decisions.

Variants are identified using various measurement technologies, including digital PCR and NGS-targeted sequencing. To further assess commutability, we compared amplicon-based sequencing and hybrid capture sequencing methods, involving commercial laboratories as EQA participants in the accreditation program under ISO 15189. This initiative aims to ensure the reliability and validity of NGS oncology tests, thereby enhancing the accurate interpretation of results and supporting more effective clinical decision-making.

１年目の研究活動実績 Methods:

Participants: Participants enrolled in this study were twelve medical laboratories that provided laboratory services based on NGS. As a platform laboratory used amplicon-based and hybrid-capture sequencing methods, respectively. Standard samples include Acrometrix Oncology Hotspot Control DNA and OncoSpan reference gDNA. AcroMetrix Oncology Hotspot Control DNA, purchased from Thermo Fisher Scientific, contains over 555 synthetic DNA variants with 48 INDELs in 53 genes from the COSMIC database. It was developed using human genomic DNA from the GM24385 cell line. OncoSpan reference gDNA, obtained from Horizon Discovery Ltd., is a blend of tumor cell lines with 386 variants and 30 INDELs across 152 cancer genes, including 249 variants with a COSMIC ID. It was developed using human genomic DNA from the NA12878 from the Genome-In-A-Bottle (GIAB) project.

The samples were transported in specific conditions: Acrometrix Oncology Hotspot Control DNA below −20°C, and OncoSpan reference gDNA between 2 and 8°C. Instructions and reporting forms, including library quality, enrichment platforms, reagents, sequence analysis software, reference data, and quality metrics, were provided with the EQA samples for participant laboratories.

Value assignment of the EQA samples: The EQA samples incorporated manufacturer-assigned reference values. The Acrometrix Oncology Hotspot Control DNA had 373 variants at 5-15% allelic frequency (AF) and 182 variants at 15-35% AF. Oncospan

reference gDNA included 1-100% AF variants, with 52 variants at ≤ 20% AF for LOD determination.

Evaluation of the performance: Laboratories were instructed to conduct NGS analysis on provided samples using their routine procedures and equipment. The reporting sheet included questions about library quality, sequencing systems, reagents, analysis software, reference data, and quality metrics. The obtained NGS results were assessed for performance using specified indicators, and feedback was given to the laboratories through onsite evaluations. Comparisons of commutability between sequencing methods were made using reference materials.

Results: The participant laboratories detected 21 to 466 variants in the genome-based Acrometrix hotspot control sample and 4 to 119 variants in the genome-based OncoSpan reference material, depending on their respective panels and sequencing systems.

The primary cause of false negative (FN) results was linked to bioinformatics processing, particularly software criteria related to low Variant Allele Frequency (VAF) and the filtering of variants in complex genomic regions. Most false negatives were attributed to low VAF thresholds, influenced by sequencing method limitations. In amplicon sequencing, a Single Nucleotide Polymorphism (SNP) within a primer site inhibited target amplification, leading to lower VAFs. Similarly, in hybrid capture sequencing, suboptimal probe design contributed to reduced VAF detection.

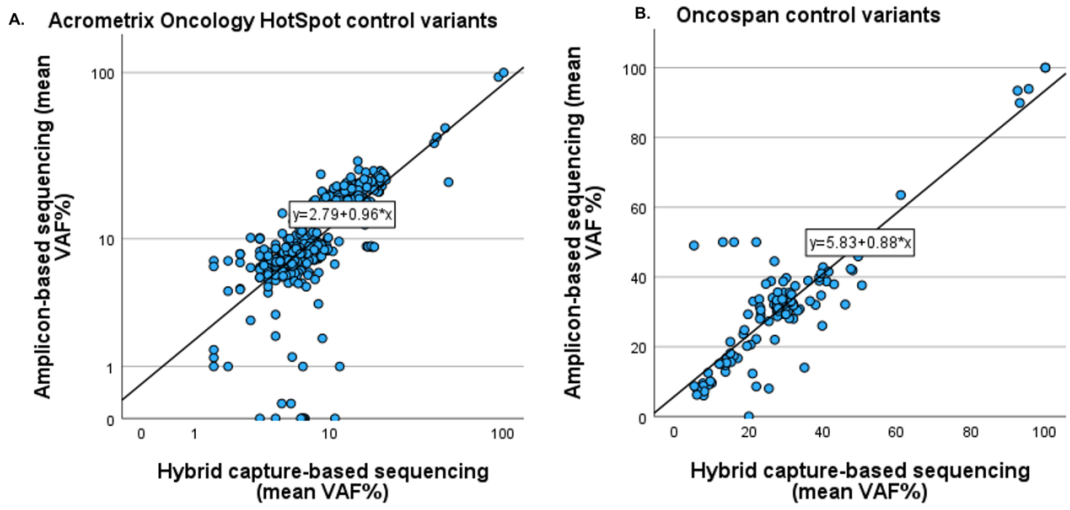
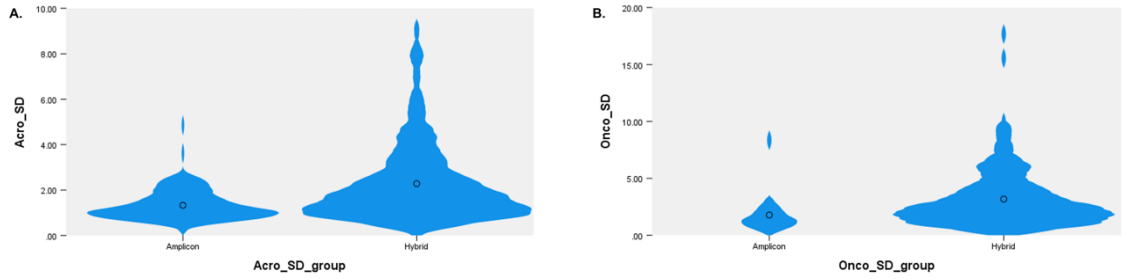


Figure 1. Correlation between amplicon-based sequencing and hybrid capture sequencing results for the Acrometrix hotspot control DNA (A) and the OncoSpan reference gDNA (B).

Additionally, some closely located variants may appear with lower VAFs depending on the stage of the wet lab process, including library preparation, sequencing, and probe or

primer design. Addressing the variability in VAF% distributions is essential for ensuring the reliability and comparability of results.

To evaluate the correlation between amplicon-based sequencing and hybrid capture sequencing, our study compared results using the Acrometrix hotspot control DNA and the OncoSpan reference gDNA. Both sample types exhibited a strong positive correlation between the two sequencing methodologies, demonstrating consistent detection trends and confirming commutability between amplicon-based and hybrid capture sequencing results.



Pigure 2. VAF distribution between amplicon-based sequencing and hybrid capture sequencing results for the Acrometrix hotspot control DNA (A) and the OncoSpan reference gDNA (B).

Furthermore, our analysis of standard deviation between the two sequencing methods revealed a higher variance in hybrid capture sequencing compared to amplicon-based sequencing across both samples ($p<0.001$). This finding highlights the need to address variability in VAF distributions to improve result reliability and comparability.

Additional Observations

- No significant differences were observed in quality metrics and general results between amplicon-based sequencing and hybrid capture sequencing across both reference samples.
- No significant differences were found between research use (RUO) and in vitro diagnostic (IVD) use in terms of quality metrics and general results.
- However, significant differences were observed in uniformity and FN rates between first accreditation and second accreditation across both reference samples.

Conclusion

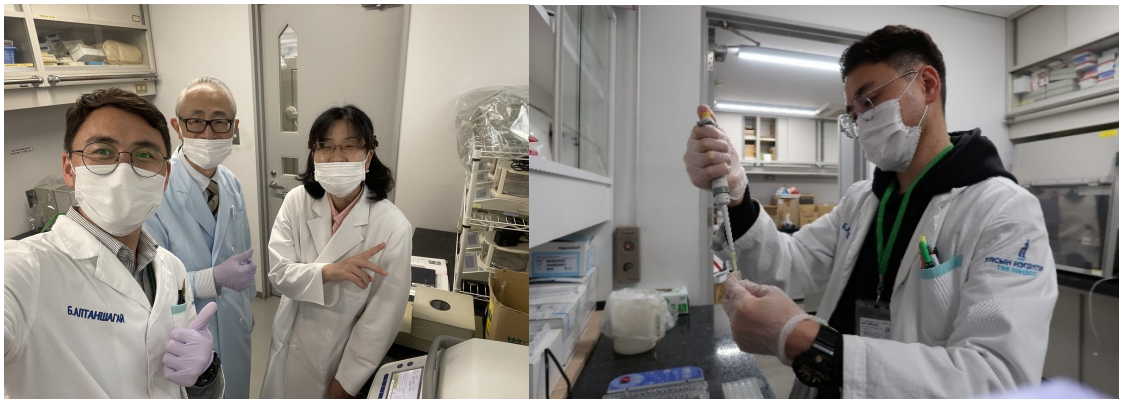
Establishing commutability is essential for ensuring the reliability and validity of NGS oncology tests, as it allows for confident interpretation of results and facilitates effective

clinical decision-making. The strong positive association between amplicon-based and hybrid capture sequencing across different sample types supports commutability, reinforcing the reliability of NGS-based cancer gene panel tests. Addressing factors contributing to variability in VAF distribution, particularly in hybrid capture sequencing, is crucial for ensuring consistent and high-quality genomic analysis in both clinical and research settings.

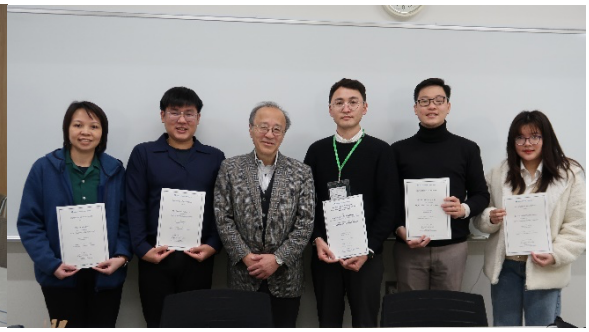
留学先での生活・交流の様子

During my last 2 years, I learned how to work in a research laboratory and basic techniques to handle cell culture, DNA, RNA, and protein samples. Also, I have learned to work on new technologies such as NGS and other emerging medical instruments. Besides, I have learned how to improve to risk management for medical laboratory

Besides I did lecturer and advisor in the ISO course on the NGS oncology testing under the Asian Clinical Trials Network for Cancers Project (ATLAS project). I presented lectures about Basic principles of risk management for NGS oncology testing and evaluation feedback report of NGS oncology testing. I attended lectures and all session of Major changes to the fourth edition for ISO 15189, session of Basic knowledge for NGS Oncology test, session for On-site evaluation of NGS Oncology test for ISO 15189 accreditation and session for self-evaluation of NGS analysis results in 2024 and 2025.



Picture 1. Working on Laboratory with professor

Picture 2. ISO International course 2024


Picture 3. ISO International course 2025

Picture 3. Participated International congress 2024 (ICBMT2024 and APBMT2024)

【謝辞】

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