Consistency of Analytical Performance Characteristics and Other Observed Trends from the Validation of Comprehensive Genomic Profiling LDTs by Diverse Laboratories

Renee L Sears, Michael J Evenson, Laura L Buttrum, Erin Buck, Angela L Williams, William T Moller, Amber Leigh, Rakesh Nagarajan, and Andrew J Bredemeyer

Velsera Boston • St. Louis • Hasselt • Belgrade • Pune

INTRODUCTION

Validation of comprehensive genomic profiling (CGP) assays on next-generation sequencing (NGS) platforms is challenging, time-consuming, and expensive. Successful on-time and on-budget validations require detailed planning and keen attention to how assay limitations and reportable range intersect with the clinical need of the laboratory's patient population. Velsera supports NGS assay validations for many laboratory customers including planning, analytical and clinical performance evaluation, and documentation services. We describe here the scope and aggregated analytical performance characteristics from CGP validations completed collaboratively by Velsera and its client laboratories.

METHODS

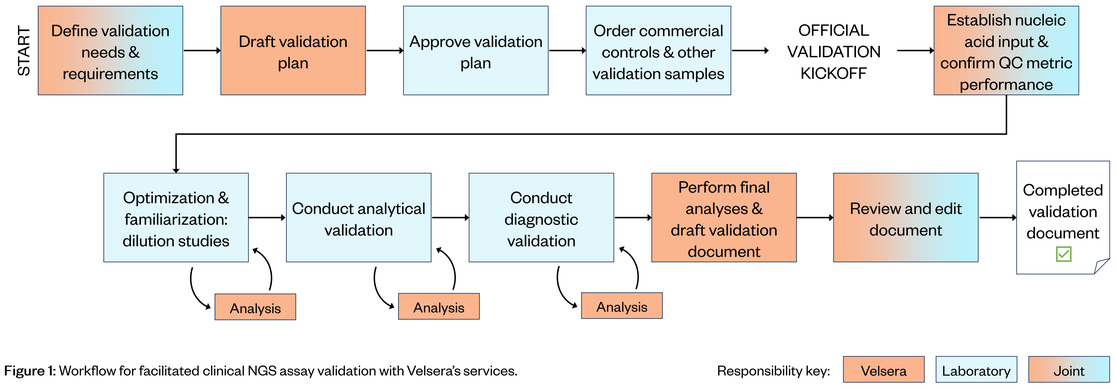


Figure 1: Workflow for facilitated clinical NGS assay validation with Velsera's services.

Solid tumor CGP validations at academic, hospital, and commercial laboratories were facilitated by Velsera's clinical assay validation service. Velsera helped create a validation plan and identify appropriate analytical and clinical samples for validation. Laboratories or their service providers performed wet lab steps including DNA and RNA library preparation and sequencing. Laboratories or Velsera performed secondary analysis, and tertiary analysis was performed on Velsera's Clinical Genomics Workspace software platform. Velsera performed comparative analyses between samples and runs, calculated performance characteristics, and wrote a detailed validation report that complies with regulatory standards. See **Figure 1**. Validation duration was calculated from the official validation kickoff following the planning phase, marked by completion of a successful initial QC run, to completion of the validation document.

RESULTS

Of 32 completed validations performed by laboratories in collaboration with Velsera since 2019, 11 were tissue-based solid tumor CGPs, representing 3 commercially available assays. On average, 207 total libraries (standard deviation, SD = 84.2) were sequenced per validation, including an average of 76 unique clinical validation samples (SD = 39.0). The average validation was completed in 248 days (SD = 145). (See **Figure 2** below.) Validation duration was calculated starting after a planning phase, at validation execution kickoff, and ending at completion of the validation document (see **Figure 1**), excluding interruptions such as reagent shortages or pandemic-related reprioritization. Validations achieved the analytical performance characteristics represented in **Table 1** below.

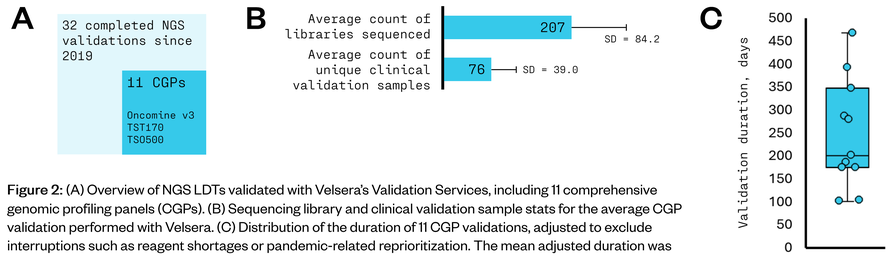


Figure 2: (A) Overview of NGS LDTs validated with Velsera's Validation Services, including 11 comprehensive genomic profiling panels (CGPs). (B) Sequencing library and clinical validation sample stats for the average CGP validation performed with Velsera. (C) Distribution of the duration of 11 CGP validations, adjusted to exclude interruptions such as reagent shortages or pandemic-related reprioritization. The mean adjusted duration was 248 days.

Table 1: Analytical performance characteristics, by variant type, of 11 comprehensive genomic profiling NGS panel LDTs validated with Velsera's clinical assay validation services. ND, not determined

			SNV		MNV/Indel		Copy Gain		Fusion/Rearrangement		Splice Variant	
Validated test ID	**Laboratory ID**	**Assay**	**Sensitivity**	**PPV**	**Sensitivity**	**PPV**	**Sensitivity**	**PPV**	**Sensitivity**	**PPV**	**Sensitivity**	**PPV**
LDT 1	Lab A	TruSight Tumor 170	98.4%	97.5%	94.2%	81.1%	91.8%	73.3%	79.2%	97.9%	79.2%	97.9%
LDT 2	Lab B	Oncomine v3	92.1%	95.6%	78.2%	86.9%	67.3%	80.0%	96.4%	100.0%	ND	ND
LDT 3	Lab C	TruSight Tumor 170	98.0%	96.6%	91.8%	99.3%	92.9%	100.0%	92.7%	96.0%	100.0%	100.0%
LDT 4	Lab D	TruSight Oncology 500	99.6%	98.8%	99.4%	97.5%	100.0%	100.0%	94.0%	95.8%	100.0%	100.0%
LDT 5	Lab E	TruSight Oncology 500	99.1%	98.9%	99.5%	98.3%	94.8%	94.2%	96.2%	98.8%	95.0%	100.0%
LDT 6	Lab F	TruSight Oncology 500	95.0%	98.8%	96.1%	99.5%	92.2%	94.0%	97.5%	100.0%	100.0%	100.0%
LDT 7	Lab G	TruSight Oncology 500	98.5%	99.1%	99.6%	95.9%	ND	ND	ND	ND	ND	ND
LDT 8	Lab H	TruSight Oncology 500	99.9%	99.5%	97.7%	95.5%	100.0%	100.0%	ND	ND	ND	ND
LDT 9	Lab I	TruSight Oncology 500	99.7%	99.6%	96.0%	98.2%	96.2%	99.6%	96.8%	98.2%	100.0%	100.0%
LDT 10	Lab J	TruSight Oncology 500	99.5%	99.9%	96.8%	99.2%	100.0%	97.5%	97.6%	100.0%	100.0%	100.0%
LDT 11	Lab K	TruSight Oncology 500	99.2%	98.9%	98.1%	95.9%	90.5%	96.2%	97.8%	100.0%	100.0%	100.0%
		Mean performance	**98.1%**	**98.5%**	**95.2%**	**95.2%**	**92.6%**	**93.5%**	**94.2%**	**98.5%**	**96.8%**	**99.7%**
		Standard deviation	*2.3%*	*1.3%*	*5.8%*	*5.6%*	*9.1%*	*8.8%*	*5.6%*	*1.6%*	*6.9%*	*0.7%*

CONCLUSIONS

Across the cohort of laboratories whose validations are reported here, validation duration was highly variable. Shorter durations were associated with labs with previous NGS experience and with higher sequencing capacity. Despite this, analytical performance was similar across validations and was robust. This is attributable to the execution of controlled secondary analysis pipelines with empirically determined lower limits of detection by variant type and consistent laboratory practices including defined minimum nucleic acid input amounts, rigorous quality assessment through all steps of the workflow, and written standard operating procedures.

Based on Velsera's experience collaborating with numerous laboratories on CGP validation, the following practices have emerged as beneficial for completing an NGS assay validation according to schedule.

1. Collect clinical validation samples well ahead of beginning your validation. Understand the clinical demand for your assay and seek the needed specimen and variant types accordingly. Unavailability of sufficient numbers of clinical samples is the most common cause for delay of validation completion in our experience.
2. Be selective with clinical validation samples, despite the challenge of collecting enough. Low quality specimens or unreliable orthogonal results can be a net detriment to your validation.
3. Dedicate effort of experienced NGS technologists, data analysts/bioinformaticians, and scientific/clinical staff to the validation.
4. Determine validation priority and timeline with the input and buy-in of lab and institutional leadership.
5. Create a validation run plan that considers staff and instrument availability. Track progress against it and make it visible to your stakeholders.
6. Check quality as you go, run-by-run, to catch issues quickly. Avoid triggering a set of parallel sequencing preps and runs that may all fall prey to the same quality issue, leading to expensive repeat runs and lost weeks.

VELSERA