Implementation of NIS4 Test for NASH in Covance Central Laboratories

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Introduction

Approximately 25-30% of the global adult population have non-alcoholic fatty liver disease (NAFLD), a chronic disease characterized by the accumulation of excess fat in the liver.[1,2] Between 11-40% of individuals with NAFLD will progress to non-alcoholic steatohepatitis (NASH), in which inflammation, hepatocyte ballooning and fibrosis markedly effect liver function.[1,2] Active development of novel therapeutic agents for NASH are mirrored by the search for novel, noninvasive tests to further enable identification of patients, including those with active NASH (NAS≥4) and fibrosis (F≥2).[3] This "Progressing NASH" patient population is at highest risk for poor clinical outcomes, and in greatest need of therapeutic intervention. As such, this population is the frequent focus of NASH clinical trial recruitment. Genfit's Non-invasive Test 4 or "NIS4" has emerged as a promising diagnostic tool for identifying patients with progressing, fibrotic NASH. NIS4 is an algorithm-based diagnostic test that incorporates the values of 4 circulating markers: miR-34a-5p, Alpha 2 Macroglobulin (A2M), Chitinase 3-like Protein 1 (CHI3L1 or YKL40), and HbA1c. This test has shown promising results in retrospective analyses of prospectively collected samples in Genfit's NASH clinical cohorts GOLDEN and RESOLVE-IT. Broader understanding of the utility of NIS4 as a tool in NASH drug development warrants further evaluation. A licensing agreement between Genfit and Covance/LabCorp, finalized in January 2019, will enable NIS4's availability to the broader clinical trial community. Full implementation of NIS4 within Covance Central Laboratory Services (Covance CLS), described here, requires correlation assessment of each component assay, as well as the test-defining algorithm.

Procedure Overview

A. Established Assays (HbA1c, A2M)

- Compare methodologies in use by Genfit, Covance CLS including instrumentation, reagent source
- Conduct correlation as needed

B. Assays in Development/Validation (CHI3L1, miR34a-5p)

- CHI3L1: compare methodologies, conduct split sample correlation study using matched batch reagents, finalize CAP/CLIA validation
- miR-34a-5p: establish correlation between instrumentation and optimized methodology/reagents, conduct split sample correlation study, finalize CAP-CLIA validation

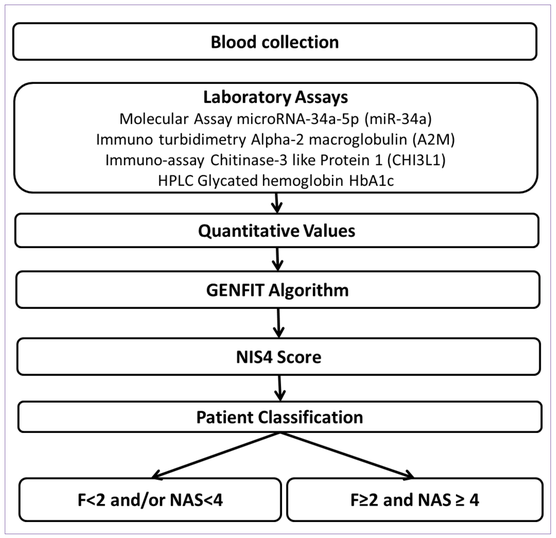

Figure 1. Overview of NIS4 testing.

Figure 2. Estimated timeline for NIS4 global implementation.

Table 2. Key Parameters of Covance CLS-Validated CHI3L1 Assay — CHI3L1

Analyte	Method	Instrument	Specimen	Intra-Assay Precision CV	Inter-Assay Precision CV	Reportable Range	Reference Ranges
CHI3L1	ELISA	Elx 800	Serum	<3.5.%	<5.1%	3125-200,000 pg/mL	< 77,200 pg/mL

CHI3L1 represents a new serum test within Covance CLS, supported by vendor-supplied commercial kits and validated to CAP/CLIA standards. Similarly to HbA1c, split sample testing is being included as part of NIS4-associated CHI3L1 assay implementation (data pending). Shown here are key Covance CLS validation-derived assay parameters including precision and reportable range.

Quantitative PCR assay for detection of miR-34a-5p levels in patients clinical samples was originally developed and optimized by Genfit on Bio-Rad CFX96 Thermal Cycler. Following assay transfer to Covance CLS, the assay will be validated to CAP/CLIA standards on QuantStudio 12K Flex Real-Time PCR System. Figure 3 and Table 3 show correlation experimental data generated by the Covance CLS-Genfit team demonstrating equivalency between these two instruments.

miR34a-5p

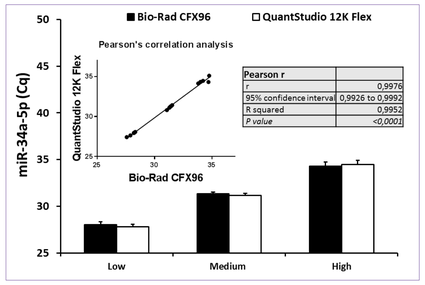

Figure 3. hsa-miR-34a-5p PCR test correlation experiment between Bio-Rad CFx96 and QuantStudio 12K. For each instrument, data are presented as mean hsa-miR-34a-5p Cq level expression +/- SD of 5 low, 5 medium and 5 high hsa-miR-34a-5p level samples measured in triplicate. All data were normalized using a pool of standard serum at a Cq cut-off of NASH diseased patients for hsa-miR-34a-5p. Correlation between Bio-Rad CFx96 and QuantStudio 12K measures was assessed using Pearson's correlation analysis.

Table 3. hsa-miR-34a-5p Inter-Measurement Variability

	has-miR-34a-5p Inter-Assay Variability (% RSD)		
	Bio-Rad CFX96	QuantStudio 12K Flex	Inter-Overall Instrument Measurements
High samples	1.071	0.923	1.077
Medium samples	0.620	0.769	0.742
Low samples	1.366	1.331	1.349
Reference sample	0.499	0.136	0.327

Data from Bio-Rad CFx96, QuantStudio 12K or both instruments. For each instrument 5 low, 5 medium and 5 high hsa-miR-34a-5p level samples were measured in triplicate each. Variability inter-assays is presented as % Relative Standard Deviation (RSD). Reference sample is a pool of standard serum at a Cq cut-off of NASH diseased patients for hsa-miR-34a-5p.

Summary

Genfit's NIS4 is a novel, multivariate index assay that is based on the measure of four individual analytes:

- miR-34a-5p (serum); alpha-2 macroglobulin (A2M) (serum); chitinase-3 like 1 protein (CHI3L1) (serum); HbA1c (whole blood)

Genfit data supports use of NIS4 as a progression and prognostic biomarker. These results support the further exploration of NIS4 in NASH clinical trial context.

- NIS4 has been validated by Genfit for the identification of NASH patients at higher risk of progression to cirrhosis (NAS≥4 and F≥2).

NIS4 is being implemented in Covance CLS to enable wide availability of the test for clinical trials.

- Implementation leverages existing, globally available A2M and HbA1c assays and requires establishment of CHI3L1 and miR-34a-5p assays.

Uptake and performance in clinical trials will help inform the downstream use of NIS4 in NASH patient clinical care.

Table 1. Summary of Existing A2M and HbA1c Assays (identical between Genfit and Covance CLS) — HbA1c, A2M

Analyte	Method	Instrument	Specimen	Certification	Intra-Assay Precision CV	Inter-Assay Precision CV	Reportable Range	Reference Ranges
HbA1c	HPLC	BioRad Variant II turbo	Whole blood EDTA	NGSP level 1	<1.9%	<3.77%	3.6 – 17.4%.	<6.5%
Alpha 2 Macroglobulin	Nephelometry	Siemens BN II	Serum	Not Applicable	<6.2%	<5.3%	20.5 - 656.0 mg/dL	110.0 – 276.0 mg/dL

HbA1c and A2M are globally implemented as CAP/CLIA-compliant assays based on In Vitro Diagnostic (IVD) approved reagents within Covance CLS. Comparisons of methods between Covance CLS and Genfit for A2M led to the realization that sample analyses for this analyte were being conducted using identical instrumentation and reagent sources for both companies.
*For HbA1c, IVD reagent supply differences between Covance CLS and Genfit did necessitate performance verification via split sample testing (data pending).

Table 4. Summary of NIS4 Assays' Matrix Volume Requirements and Known Stabilities

Analyte	Matrix	Required Volume	Standard Shipping Condition	Standard Testing TAT	Stability		
					Ambient	Refrigerated	Frozen
HbA1c	EDTA Whole Blood	1mL	Ambient	1 day	6 days*	7 days*	2 years (-70°C)*
Alpha 2 Macroglobulin	Serum	600µL	Ambient	1 day	7 days*	7 days*	3 years (-70°C)*
CHI3L1 (YKL40)	Serum	50µL	Frozen (-70°C)	30 days	7 days*	7 days*	1 year (-20°C)^
miR-34a-5p	Serum (SST)	220µL	Frozen (-70°C)	TBD	2 hrs^	2 hrs^	1 week (-20°C)^ 2 years (-70°C)^

*Established by Covance CLS during assay validation
^Established by Genfit during internal assay validation; Covance CLS validation confirmation in progress
TBD: to be determined

References

1. Z. Younassi, et al, Hepatology 2018 Sep 4. doi: 10.1002/hep.30251 [https://www.ncbi.nlm.nih.gov/pubmed/30179269]
2. https://www.niddk.nih.gov/health-information/liver-disease/nafld-nash/definition-facts
3. D. Kleiner, et al, Hepatology 2005 Jun;41(6):1313-21 [https://www.ncbi.nlm.nih.gov/pubmed/?term=Hepatology.+2005+Jun%3B41(6)%3A1313-21.]

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