Vibrant Digestive Panel I Validation Report

1 Intended Use

The Vibrant Gut Digestive panel I assay is a microarray chip-based assay designed for the measurement of Calprotectin, Pancreatic elastase-1 and Beta-glucuronidase extracted from stool samples.

2 Test Principle

The Vibrant Gut Digestive panel I assay follows the principle of competitive binding system. Competition occurs between the enzyme labelled antigen and unlabeled antigen present in stool for a limited number of antibody binding sites on the specific coated microarray chip. This is followed by stringent wash cycles to remove any non-specific binding. This is followed by detection using an enzyme based chemiluminescent substrate. The array is scanned using a Chemiluminescence imager and the raw intensity for each chip is calculated. The intensity of the raw data is inversely proportional to the concentration of the antigen in the sample since it follows the competitive binding scenario. The quantitative test results for each chip are then determined by comparison with a standard curve established using the known concentrations of calibrators.

3 Performance Characteristics

1. Performance Characteristics

The kit comprises of calibrators, positive and negative controls which are used for each assay performed. The validation studies are analyzed after each plate tested passes the acceptance criteria for controls and calibrators ranges assigned during assay design.

A. Accuracy/Trueness

Accuracy of this assay will be inferred from determining the concordance between the known quantitative results for each analyte obtained from commercial sources and the microarray chip. The test results of 120 samples with known results across the analytical measuring range are unblinded and compared to the expected results to determine the total concordance. Slope, intercept and R^2 (coefficient of correlation) are used to establish accuracy.

The results are summarized below:

Test Name	Slope	Intercept	R^2
Calprotectin	1.002	1.5138	0.9996
Pancreatic Elastase-1	1.028	0.6521	0.9978
Beta-glucuronidase	1.007	0.7826	0.9955

B. Repeatability/Reproducibility

The simple precision (repeatability) of Vibrant Gut Digestive Panel I kit was determined by running 5 samples 10 times within the same run and analyzed for each analyte tested. The complex precision (reproducibility) of Vibrant Gut Digestive Panel I kit was assayed in 2 replicates of 5 samples twice daily over 5 days and analyzed for each analyte tested.

Test Name	Sample	Simple Precision CV
Calprotectin	Sample 1	3.9%
	Sample 2	2.9%
	Sample 3	0.9%
	Sample 4	0.9%
	Sample 5	5.1%
Pancreatic Elastase-1	Sample 1	4.0%
	Sample 2	2.6%
	Sample 3	1.3%
	Sample 4	1.1%
	Sample 5	3.4%
Beta-glucuronidase	Sample 1	3.4%
	Sample 2	4.6%
	Sample 3	1.8%
	Sample 4	1.1%
	Sample 5	2.7%

Test Name	Sample	Complex Precision CV
Calprotectin	Sample 1	3.6%
	Sample 2	2.5%
	Sample 3	1.4%
	Sample 4	0.9%
	Sample 5	7.2%
Pancreatic Elastase-1	Sample 1	4.0%
	Sample 2	1.8%
	Sample 3	1.3%
	Sample 4	0.7%
	Sample 5	3.2%
Beta-glucuronidase	Sample 1	8.8%
	Sample 2	9.6%
	Sample 3	5.4%
	Sample 4	3.2%
	Sample 5	2.8%

C. Analytical Specificity / Interference substances

6 different samples were tested for cross-reactivity with endogenous and exogenous compounds including fecal fat (2.5 mg/ml), mucus (2.2 mg/ml), human blood (1:1), PMN-elastase (150 ng/ml), hemoglobin (1000 µg/g), alpha-1 anti-trypsin (1000 µg/g), and

pancreatin (800 ng/ml). No reportable change in result was observed at the concentrations tested.

D. Analytical Sensitivity / Limit of Blank / Limit of Detection / Limit of Quantitation

A blank stool matrix sample was tested in 21 replicates per run to determine the mean and standard deviation of blank to determine the limit of blank (LoB). Low concentration sample was tested in 21 replicates to determine the limit of detection (LoD). 5 samples at different levels (low, medium and high) were tested in 21 replicates per run to determine deviation of sample in comparison to each run and calculating the limit of quantitation (LoQ).

Analytical Sensitivity	LoQ (µg/g)
Calprotectin	7.43
Pancreatic Elastase-1	9.66
Beta-glucuronidase	7.85

E. Reference Range and Interpretation

Calibration - The calibrator within this test system are prepared using known standards of antigen spiked in a stool matrix and has been assigned a Correction Factor (CF) for the generation of calibrator value and spike recovery.

Reference Ranges - Reference ranges have been established using a sample cohort comprising of 192 relatively healthy stool samples. The cut-off for the healthy reference range is set between 2.5% to 97.5% percentile, and the high-risk range is set to greater than 97.5% percentile and less than 2.5% percentile as applicable. For each analyte tested, the healthy reference ranges are assigned as follows:

Result	Calprotectin (µg/g)	Pancreatic Elastase (µg/g)	Beta-glucuronidase (µg/g)
Normal	<= 50	>=200	<=2300
Moderate	50.1-119.9	100.1-199.9	N/A
High	>= 120	<=100	>=2301

F. Sample Stability

A group of 35 stool samples were tested for sample stability by running the samples which were stored at recommended temperature after shipment for up to 7 days to determine the percentage deviation from the original sample. The results are as below:

Test Name	Day 1	Day 2	Day 3	Day 5	Day 7
Calprotectin	7.8%	6.2%	4.9%	6.8%	7.2%
Pancreatic Elastase-1	3.0%	5.6%	5.5%	3.9%	5.3%
Beta-glucuronidase	7.7%	6.6%	6.8%	4.2%	9.3%

4 Conclusion

This report documents the process results and process parameters obtained during the validation of the standard operating procedure for Vibrant Gut Digestive panel I assay. All validation test results meet their required specifications set by the laboratory.