Vibrant Digestive Panel II Validation Report

1 Intended Use

The Vibrant Gut Digestive panel II assay is a LC/MS based assay designed for the measurement of bile acids and short chain fatty acids extracted from stool samples.

2 Test Principle

The Vibrant Gut Digestive panel II assay follows the principle of Liquid Chromatography - Mass Spectrometry (LC/MS) system. The first step comprises of the process of extraction of bile acids and short chain fatty acids from the stool sample. Derivatization of molecules are then performed as applicable for easier identification during mass spectrometry. This is further followed by detection and quantification of individual bile acids and short chain fatty acids using liquid chromatography, mass spectrometry and calibration using a standard curve formed by known concentrations of each molecule analyzed. The bile acid panel comprises of all major bile acids including cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), lithocholic acid (LCA) and ursodeoxycholic acid (UDCA). The short chain fatty acid panel comprises of all major short chain fatty acids including acetate, butyrate, propionate, valerate, isobutyrate, isovalerate, 2-methyl butyrate, 2,2-dimethylbutyrate, 2-ethylbutyrate, 4-methyl valerate, caproate, and pivalate.

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 using 20 stool samples spiked with varying concentrations of bile acids and short chain fatty acids. The variation of the recovered result is calculated compared to the spiked concentration.

The results are summarized below:

Test Name	Max. % Deviation
Cholic acid	8.2%
Chenodeoxycholic acid	10.5%
Deoxycholic acid	8.3%
Lithocholic acid	9%
Acetate	9.9%

Test Name	Max. % Deviation
Butyrate	10.3%
Propionate	10.5%
Valerate	9.9%
Total short chain fatty acids	10.6%

B. Repeatability/Reproducibility

The simple precision (repeatability) of Vibrant Gut Digestive Panel II kit was determined by running 3 samples 12 times within the same run and analyzed for each analyte tested. The complex precision (reproducibility) of Vibrant Gut Digestive Panel II kit was assayed in 2 replicates of 3 samples twice daily over 5 days and analyzed for each analyte tested.

Simple Precision	Sample 1 CV	Sample 2 CV	Sample 3 CV
Cholic acid	4.4%	4.2%	5.7%
Chenodeoxycholic acid	3.3%	1.6%	2.3%
Deoxycholic acid	1.8%	2.1%	2.7%
Lithocholic acid	4.9%	5.0%	8.6%
Acetate	0.7%	0.6%	0.8%
Butyrate	2.4%	1.7%	0.7%
Propionate	2.2%	1.5%	1.5%
Valerate	5.2%	2.8%	3.3%
Total short chain fatty acids	5.5%	6.4%	5.2%

Complex Precision	Sample 1 CV	Sample 2 CV	Sample 3 CV
Cholic acid	10.5%	11.2%	6.4%
Chenodeoxycholic acid	5.7%	4.9%	4.9%
Deoxycholic acid	3.1%	4.6%	2.9%
Lithocholic acid	6.7%	9.6%	5.2%
Acetate	1.1%	2.0%	2.4%
Butyrate	10.2%	10.0%	9.7%
Propionate	3.5%	3.7%	3.4%
Valerate	9.0%	9.6%	7.6%
Total short chain fatty acids	10.5%	10.6%	11.1%

C. Analytical Sensitivity / Limit of Quantitation

A set of 7 samples spiked with different concentrations of individual bile acids and short chain fatty acids were tested in 21 replicates per run to determine deviation of sample for calculating the limit of detection (LoD) and limit of quantitation (LoQ).

Analytical Sensitivity	LoQ (umol/g)
Cholic acid	10.1
Chenodeoxycholic acid	10.4
Deoxycholic acid	20.3
Lithocholic acid	60.7
Acetate	12.5

Analytical Sensitivity	LoQ (umol/g)
Butyrate	2.4
Propionate	0.1
Valerate	0.06

D. Reference Range and Interpretation

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 5% to 95% percentile, and the high-risk range is set to greater than 95% percentile and less than 5% percentile as applicable. For each analyte tested, the healthy reference ranges are assigned as follows:

Reference Range	Normal	Units
Cholic acid	<=0.36	%
Chenodeoxycholic acid	<=1.25	%
Deoxycholic acid	24.25 ~ 75.84	%
Lithocholic acid	24.16 ~ 75.75	%
Acetate	60.2 ~ 72.7	%
Butyrate	5.1 ~ 12.4	%
Propionate	15.4 ~ 30.3	%
Valerate	0.8 ~ 3.5	%
Total short chain fatty acids	45.4 ~ 210.1	umol/g

E. Sample Stability

A group of 35 stool samples were tested for sample stability by running 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
Cholic acid	7.8%	8.2%	4.9%	6.8%	7.2%
Chenodeoxycholic acid	3.0%	8.6%	5.5%	3.9%	5.3%
Deoxycholic acid	7.7%	6.6%	6.8%	4.2%	4.3%
Lithocholic acid	8.6%	2.5%	2.3%	4.2%	6.8%
Acetate	9.7%	5.9%	4.3%	2.6%	2.3%
Butyrate	7.3%	4.4%	6.1%	2.5%	5.8%
Propionate	9.3%	4.5%	2.5%	3.9%	3.0%
Valerate	8.0%	3.4%	2.8%	9.3%	3.9%
Total short chain fatty acids	6.1%	6.6%	1.3%	1.0%	6.3%

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 II assay. All validation test results meet their required specifications set by the laboratory.