

Vessel Card Reproducibility and Comparison Study Design.

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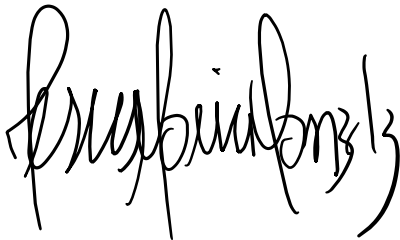
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Abstract:

The vessel testing card has been evaluated internally under several lighting conditions and with concentrations covering the range of each one of the assays. To further validate the card performance, a third-party study has been commissioned to test the card response using synthetic urine samples and to compare that response to a predicate device or gold-standard method when appropriate.

Keywords:

Vessel card 2.0, third party study, reproducibility, comparison.



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Introduction

The vessel testing card consist of 11 biomarker assays affixed to cassette designed to be exposed to urine samples. The assays are a combination of 8 colorimetric assays involving both enzymatic and chromophore reactions, and three rapid immunochromatography assays (Lateral flow). In addition, the card contains a timer reaction strip designed to measure the speed of an immune reaction and mark time accordingly.

The card performance has been previously evaluated under several lighting conditions including incandescent, fluorescent, and LED light sources and bathroom layouts representative of typical homes (1). To further validate the card performance, a reproducibility and comparison study has been commissioned with Bio IVT, a third-party entity specialized in the collection of biological samples from the public.

Bio IVT will perform the test using one phone, 2 lots of cards, 10 replicates per lot. 6 levels of urine controls (n=120). For each card five photos will be taken and analyzed for a total of 600 photos. The urine controls will be analyzed by BioIVT using a predicate device (pH, Specific Gravity) and standard orthogonal techniques (Vitamin B7, Calcium, Creatinine, and Cortisol).

Objective

1. Measure the precision of the Vessel App in a third-party environment.
2. Measure the Reproducibility of the Vessel Card/App combination.
3. Compare the results of the Vessel App/Card combination to a predicate device or accepted standard analytical technique.

Materials and Methods

- P-200 pipette
- P-1000 pipette
- Vessel cards
- 2 Clinitek Status readers.
- Siemens Multistix 10SG.
- Microwell plate reader.
- Vitamin B7 Assay kit.
- Calcium Assay kit.
- Cortisol Assay kit.
- Creatinine Assay kit.
- Urine controls levels I – VI. Solution concentrations for each of the reagents are listed in the column “ITW Accuracy Concentrations” in the [specification](#)
[Calibration values for 2.0 cards for migration to a 3.5min app.](#)
- Smartphone running the Vessel app.

Procedure

Analytical techniques

The techniques used to analyze the Vessel biomarkers are specified below:

1. Vitamin B7 – Assay kit.
2. Specific gravity – Clinitek reader/Siemens multistix 10SG.
3. pH – Clinitek reader/Siemens multistix 10SG.
4. Cortisol (LFA) – Assay kit.
5. Calcium – Assay kit.
6. Creatinine – Assay kit

Experimental procedure

1. Downloads the Vessel app using an iPhone at vesselhealth.com/go.
2. Create an account on the Vessel app.
3. Record the name and email used in the Vessel account created in step 2.
4. Practice taking photos with the app and determine five testing locations. These will be the locations where subsequent photos will be taken.
5. Remove the Vessel card from the Sachet and place the card into an empty collection cup.
6. Apply 450 μL of the LFA sample to the sample pad port in the wellness cassette.
7. Press “Start” in the app
8. Apply 12 μL of sample to each one of the colorimetric pads.
9. When the timer in the app indicates, position card in location 1 previously determined in step 4. Position the phone on top of the card until a photo is taken. Record the sample ID displayed on the screen.
10. When the photo in location 1 has been taken, place the card in location 2 previously determined in step 4.
11. Press “start” in the app and then press “skip”. Position the phone on top of the card until a photo is taken. Record the sample ID displayed on the screen.
12. Repeat step 10-11 for each one of the three remaining locations chosen.
13. Repeat steps 6 - 12 for all additional replicates/levels.
14. Analyze all the urine controls using the appropriate analytical techniques.

Results**A. pH****Background**

The pH value is the measure of acidity/alkalinity of a person’s urine; this data relates to a person’s overall health and liver function.

The pH tests are currently sourced from a commercial manufacturer as singulated pads. The pads are orange when dry and turn green/blue at low pH, and light

yellow at high pH. Control solutions are prepared in house and evaluated with a calibrated pH meter.

The spectral response for this test is shown in Figure 1.

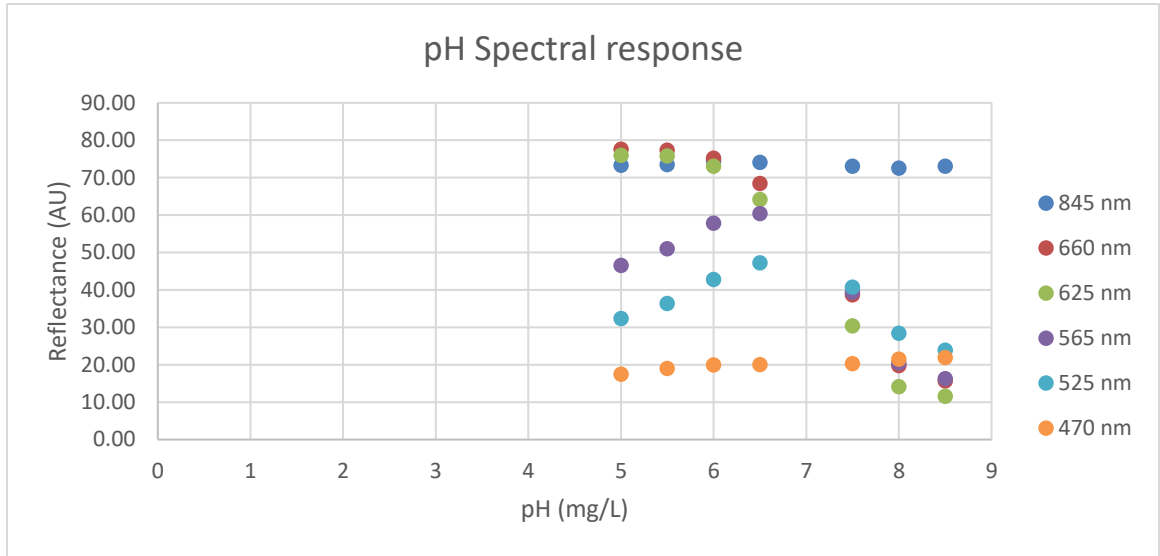


Figure 1 - pH spectral response

The pH pads exhibit a dual response, undergoing high reflectance changes 565 nm for low pH values and 660 nm and 60 seconds for the high pH values. A calibration curve for high pH values is shown in Figure 2.

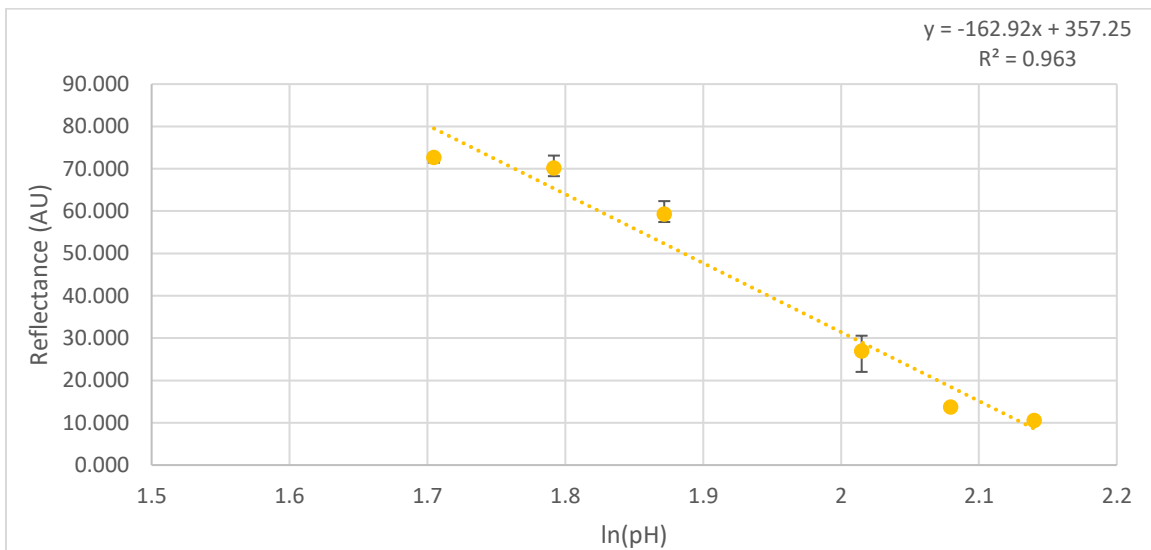


Figure 2 - pH calibration curve

Third party Vessel card performance evaluation

pH	Bucket	Vessel		Predicate	
		Exact match	Within 1	Exact match	Within 1
5	1	94%	100%	100%	100%
6	2	100%	100%	100%	100%
6.5	3	99%	100%	90%	100%
8	4	100%	100%	100%	100%

Comparison with similar products in the market

pH	Bucket	Vessel		Predicate		Acon device		Cobas device		Healthy.io	
		Exact match	Within 1	Exact match	Within 1	Exact match	Within 1	Exact match	Within 1	Exact match	Within 1
5	1	94%	100%	100%	100%	76%	91%	89%	100%	99%	100%
6	2	100%	100%	100%	100%	88%	98%	92%	100%	100%	100%
6.5	3	99%	100%	90%	100%	82%	94%	78%	100%	100%	100%
7	4					93%	98%	89%	99%	90%	100%
7.5	5					90%	98%				
8	6	100%	100%	100%	100%	100%	100%	86%	100%	100%	100%
9	7							85%	100%	100%	100%
Total		98%	100%	98%	100%	88%	97%	87%	100%	98%	100%

Remarks

The vessel and Predicate device were tested simultaneously. The data for other devices were obtained from 510k submissions.

The Vessel systems performed similarly to other commercial systems, exact matches were 94% or higher and within matches were measured at 100%

B. Specific Gravity
Background

Specific Gravity is a measurement of the density of the person's urine sample and is proportional to the amount of salt in someone's urine/diet.

The specific gravity tests are sourced from a commercial manufacturer as singulated pads. The pads are initially orange and turn green/blue when exposed to low specific gravity solutions and yellow with high specific gravity solutions.

The control solutions are prepared in house and evaluated with a calibrated clinical instrument.

The spectral response for this test is shown in Figure 3.

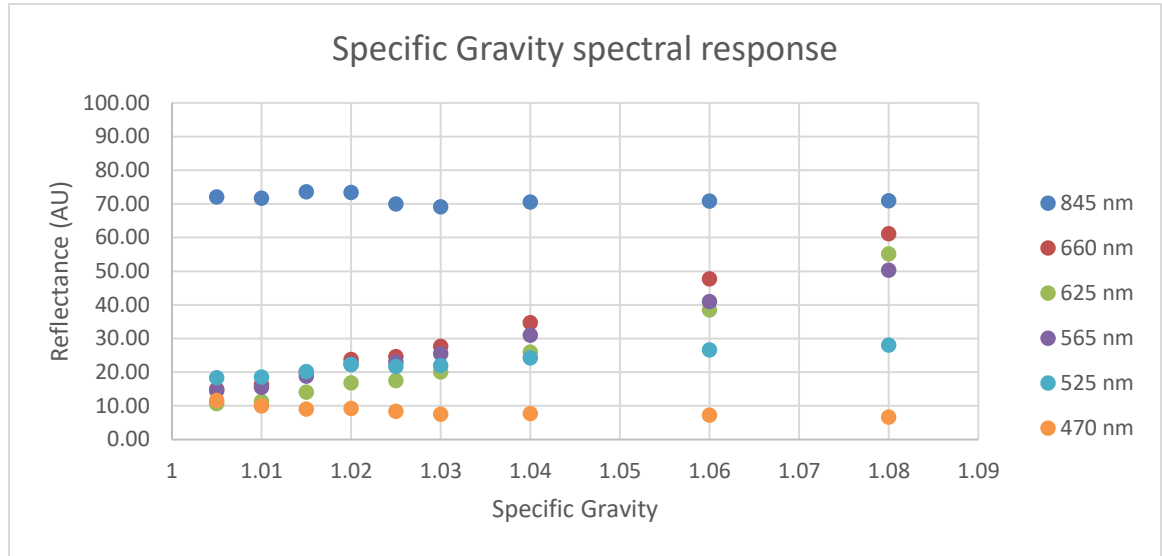


Figure 3 – Specific Gravity spectral response

A calibration curve for high Specific Gravity is shown in Figure 4.

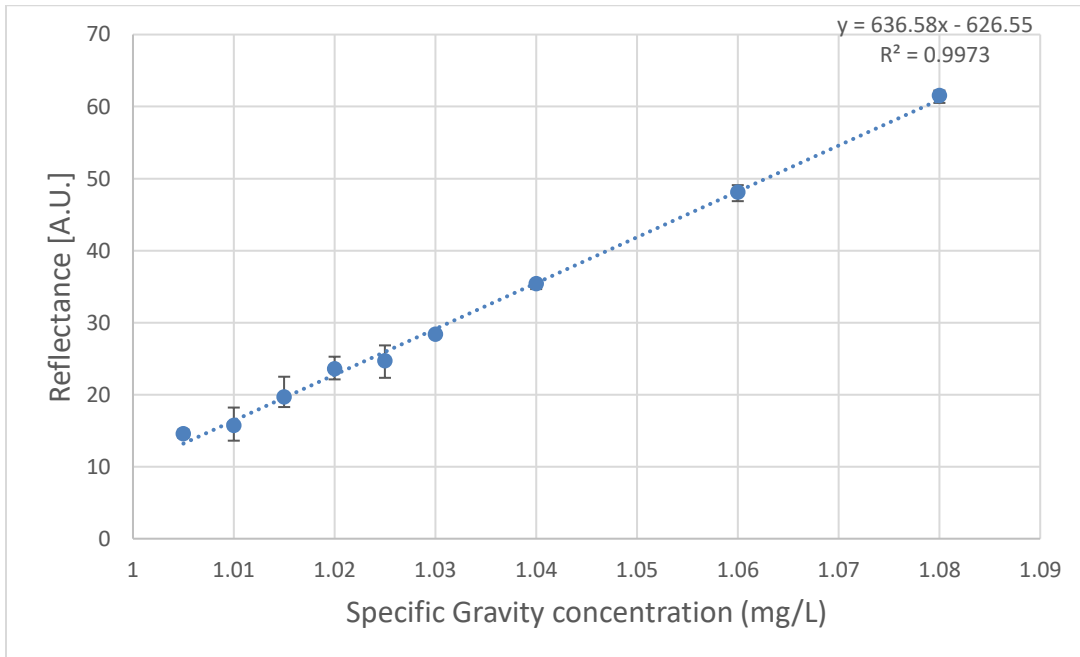


Figure 4 – Specific Gravity calibration curve

Third party Vessel card performance evaluation

SG	Bucket	Vessel		Predicate	
		Exact match	Within 1	Exact match	Within 1
1.000	1	94%	100%	100%	100%
1.005	2	60%	100%	70%	100%
1.010	3	40%	98%	100%	100%
1.015	4	84%	100%	100%	100%

Comparison with similar products in the market

SG	Bucket	Vessel		Predicate		Acon device		Cobas device		Healthy.io	
		Exact match	Within 1	Exact match	Within 1	Exact match	Within 1	Exact match	Within 1	Exact match	Within 1
1.000	1	94%	100%	100%	100%	100%	100%	69%	100%		
1.005	2	60%	100%	70%	100%	98%	100%	78%	99%	37%	91%
1.010	3	40%	98%	100%	100%	88%	98%	87%	99%	76%	97%
1.015	4	84%	100%	100%	100%	83%	97%	75%	99%	62%	100%
1.020	5					85%	95%	80%	98%	76%	100%
1.025	6					72%	94%	85%	100%	61%	100%
1.030	7					88	95	93%	100%	55%	100%
Total		70%	100%	93%	100%	92%	99%	77%	99%	58%	96%

Remarks

The vessel card performs adequately within one bucket; however, the exact match suffers and compares unfavorably with similar products for two out of the four buckets evaluated. There are several factors that could contribute to this:

- a) Spectral response that is incompatible with the Vessel app: The spectral response for specific gravity shows a wide response from 565 nm to 660 nm. This width overlaps well with typical CMOS sensors present in commonly used cell phones; therefore, the contribution to failure from this factor seems small.
- b) Unstable test formulation: Prior to implementing the specific gravity tests in the Vessel system, three lots were evaluated resulting in typical calibration

curves with low error bars and high linearity (Figure 4). The contribution of this factor to low exact match results is negligible.

- c) Quality issues associated with the material lot used in this study: This seems the most likely cause for the low exact match results observed. Better evaluation and QC procedures need to be implemented followed by reevaluation.

C. Cortisol

Background

The Cortisol test is an immunochromatographic assay that detects the presence of free cortisol in urine.

This test is manufactured in house, the final form is a strip that develops a test line that is inversely proportional to the concentration of free cortisol.

The control solutions are prepared in house from analytical standards.

A Typical dose response for cortisol is shown in Figure 5.

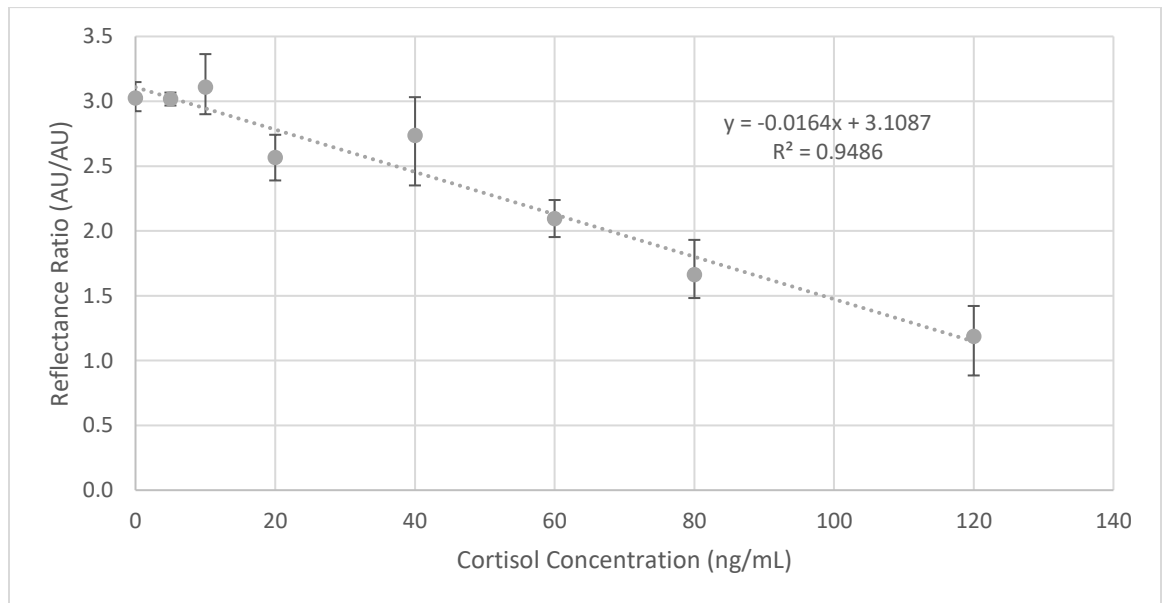


Figure 5 – Cortisol – calibration curve example

Third party Vessel card performance evaluation

Cortisol (µg/L)	Bucket	Vessel		Assay kit	
		Exact match	Within 1	Exact match	Within 1
0	1	23%	88%	100%	100%
85	2	65%	98%	35%	100%
135	3	42%	96%	0%	100%
200	4	84%	100%	100%	100%
Total		54%	96%	59%	100%

Remarks

The Vessel system is capable of distinguishing low, moderate, and high Cortisol values. Overall, the performance is similar to the one from a commercial kit system.

D. Magnesium
Background

Magnesium is a mineral present in a several of food sources and drinking water. It serves a wide variety of functions in the body. It is estimated that between 40% – 50% of Americans have suboptimal Magnesium levels.

The Magnesium pads are manufactured for Vessel and have a pale-yellow appearance that changes to magenta in the presence of aqueous magnesium. The control solutions used to evaluate the Magnesium tests are prepared in house from analytical standards.

The spectral response of the pads is shown in Figure 6.

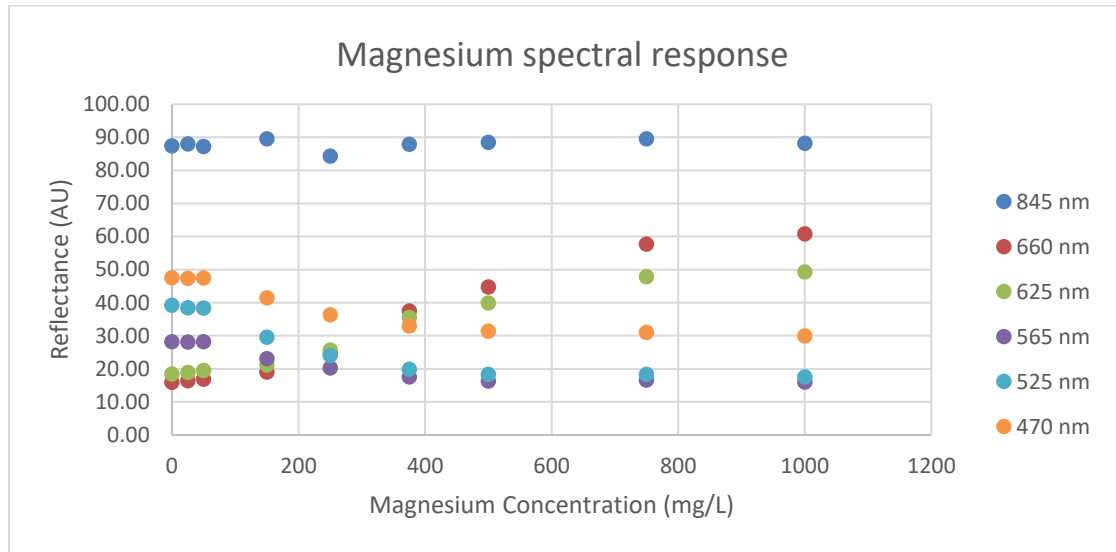


Figure 6 – Magnesium spectral response

A typical calibration curve is shown in Figure 7

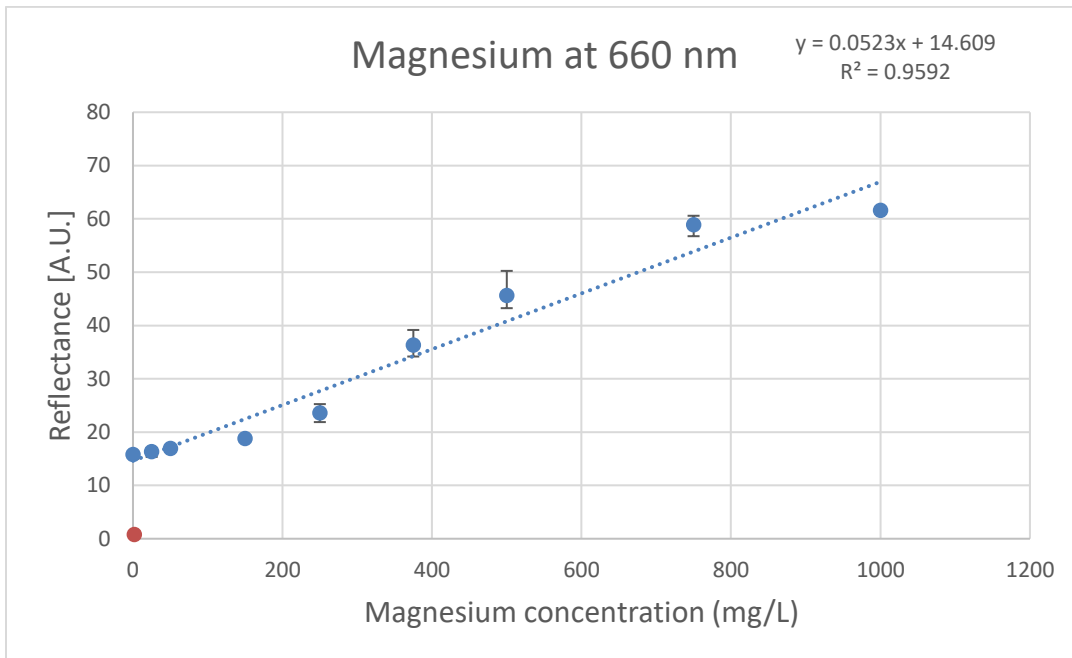


Figure 7 - Magnesium typical calibration curve

Third party Vessel card performance evaluation

Magnesium (mg/L)	Bucket	Vessel	
		Exact match	Within 1
0	1	100%	100%
50	2	82%	100%
150	3	92%	100%
250	4	90%	100%
500	5	74%	100%
Total		88%	100%

Remarks

The Vessel system shows an acceptable performance with good distinction between buckets and 100% within one bucket allocation. The evaluation of the samples with a commercial kit based on an enzymatic system was attempted but the response was found to be inadequate. Alternative orthogonal must be evaluated and deployed to compare to Vessel.

E. Ketone B
Background

The Ketone B test measures Beta-hydroxybutyrate (BHB). This is one of the three ketone bodies produced by the body as a result of ketosis.

Beta-hydroxybutyrate pads are manufactured for Vessel in singulated form. The pads turn dark purple in the presence of free BHB.

The spectral response of the pads is shown in Figure 8.

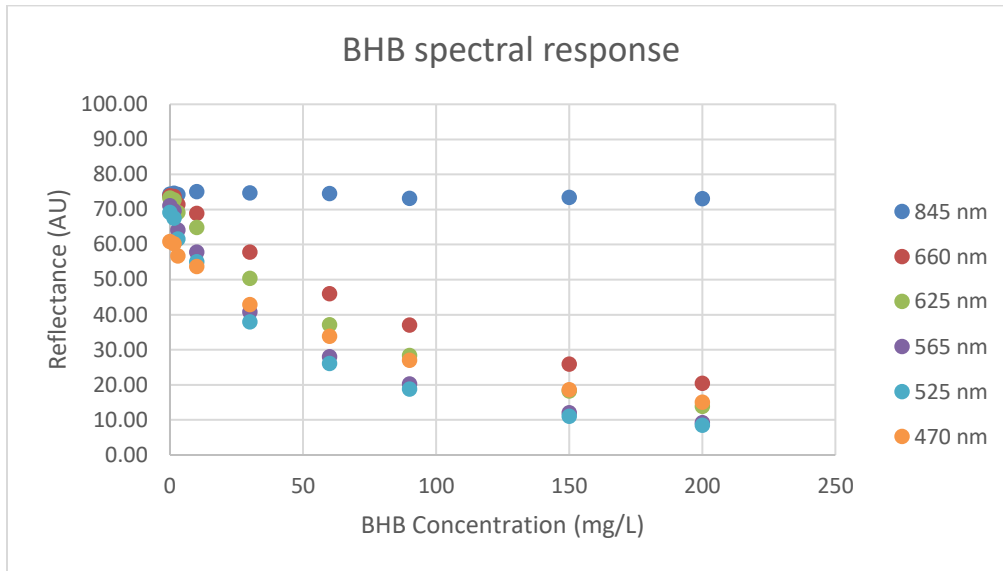


Figure 8 – BHB spectral response

A typical calibration curve is shown in Figure 9.

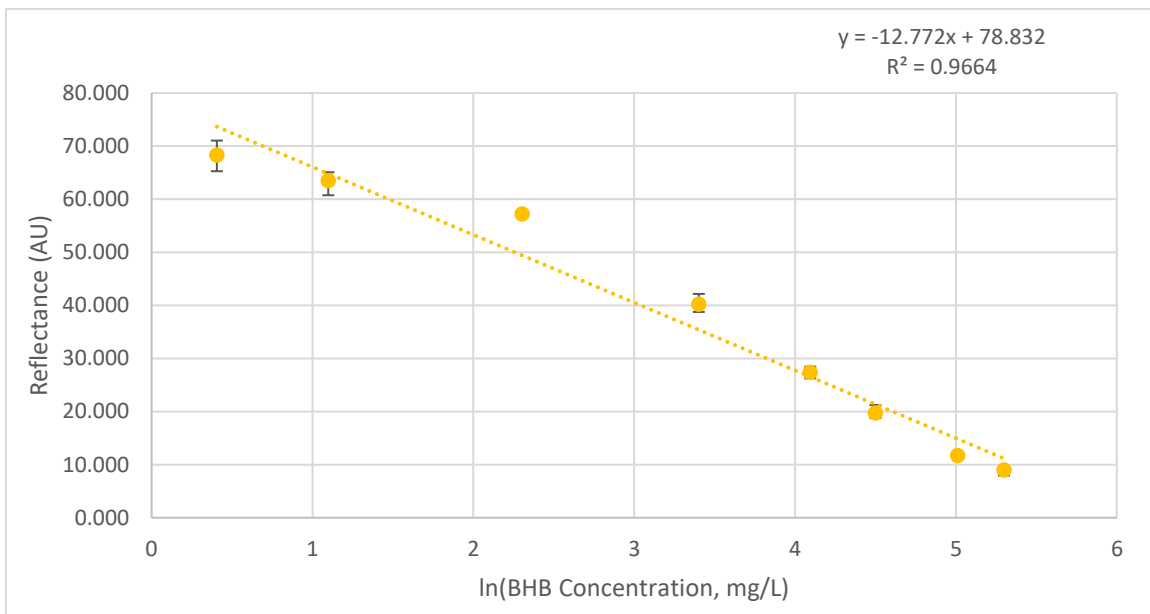


Figure 9 - BHB typical calibration curve

Third party Vessel card performance evaluation

Ketone B	Bucket	Vessel	
		Exact match	Within 1
0	1	98%	98%
35	2	100%	100%
100	3	76%	80%
180	4	51%	100%
Total		81%	95%

Remarks

The Vessel system can adequately distinguish between low, moderate and high levels of BHB. No reliable detection system is currently available in the market.

F. Vitamin C
Background

The vitamin C test detects the presence of Ascorbic Acid in urine. This nutrient is obtained from dietary sources and serves important functions due to its antioxidant properties.

The Vitamin C pads are manufactured for Vessel as singulated pads that turn bright green in the presence of aqueous ascorbic acid. The spectral response of the pads is shown in Figure 10.

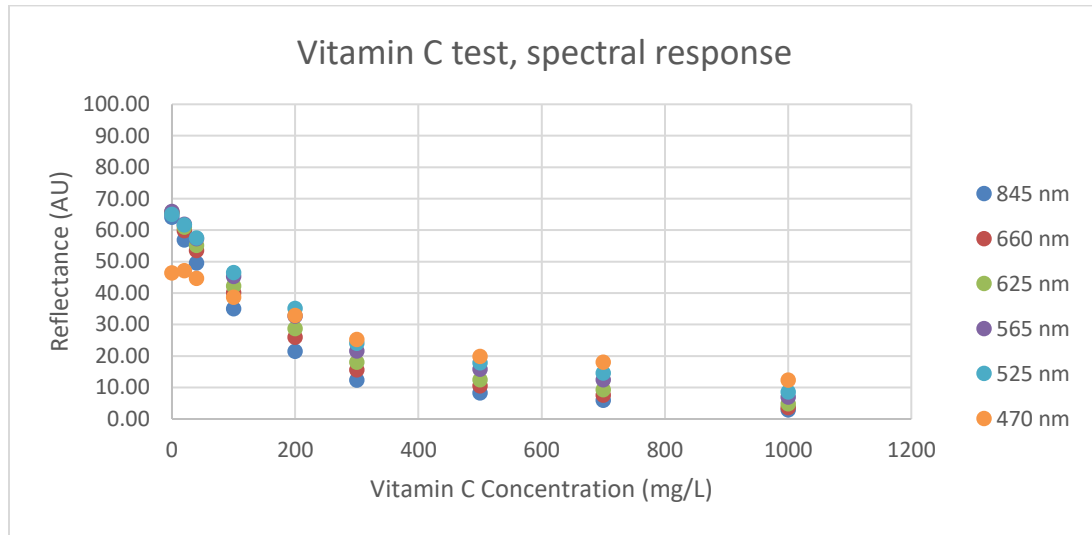


Figure 10 – Ascorbic acid spectral response

A typical calibration curve is shown in Figure 11.

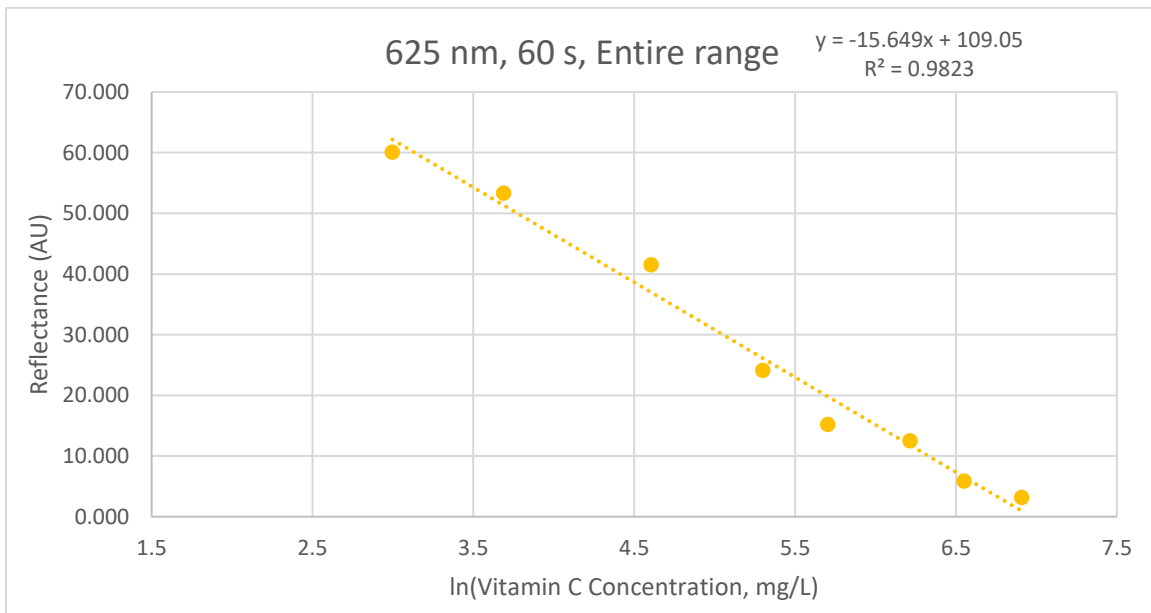


Figure 11 – Ascorbic acid test typical calibration curve

Third party Vessel card performance evaluation

Vitamin C	Bucket	Vessel	
		Exact match	Within 1
0	1	94%	100%
50	2	66%	100%
120	3	66%	98%
450	4	82%	95%
1000	5	100%	100%
Total		82%	99%

Remarks

The Vessel system can adequately distinguish ascorbic acid levels covering 5 buckets.

G. Vitamin B7
Background

The vitamin B7 test is an immunochromatographic assay that detects the presence of free Biotin in urine.

This test is manufactured in house, the final form is a strip that develops a test line that is inversely proportional to the concentration of free Biotin.

The control solutions are prepared in house from analytical standards.

A Typical dose response for vitamin B7 is shown in Figure 12.

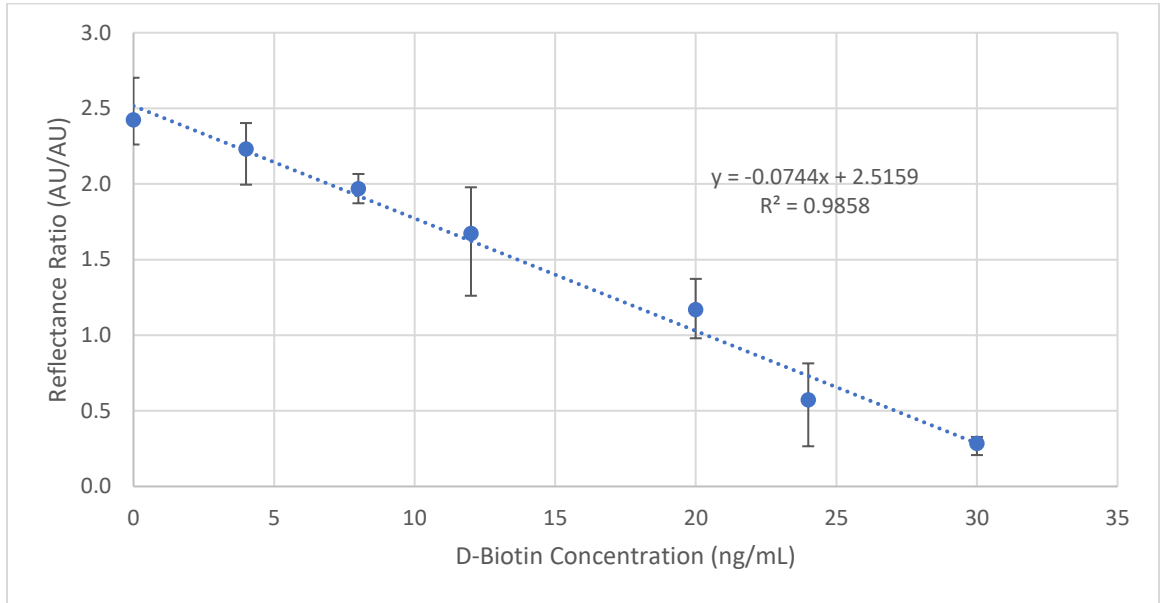


Figure 12 – Vitamin B7 – calibration curve example

Third party Vessel card performance evaluation

Vitamin B7 (µg/L)	Bucket	Vessel	
		Exact match	Within 1
0	1	41%	75%
10	2	54%	83%
30	3	54%	84%
Total		50%	81%

Remarks

The exact-match and within-one results for the vitamin B7 test are below expectations. The most likely reason for this is exposure to high/moderate humidity during manufacturing/testing. New manufacturing procedures have been implemented and instructions are provided to users to open the sachet just before use. These measures along with fine tuning of the LFA formulation will result in higher accuracy results.

H. Vitamin B9
Background

The vitamin B9 test is an immunochromatographic assay that detects the presence of free 5-Methyl tetrahydro folic acid (5-MTHFA) in urine.

This test is manufactured in house, the final form is a strip that develops a test line that is inversely proportional to the concentration of 5-MTHFA.

A Typical dose response for vitamin B9 is shown in Figure 13.

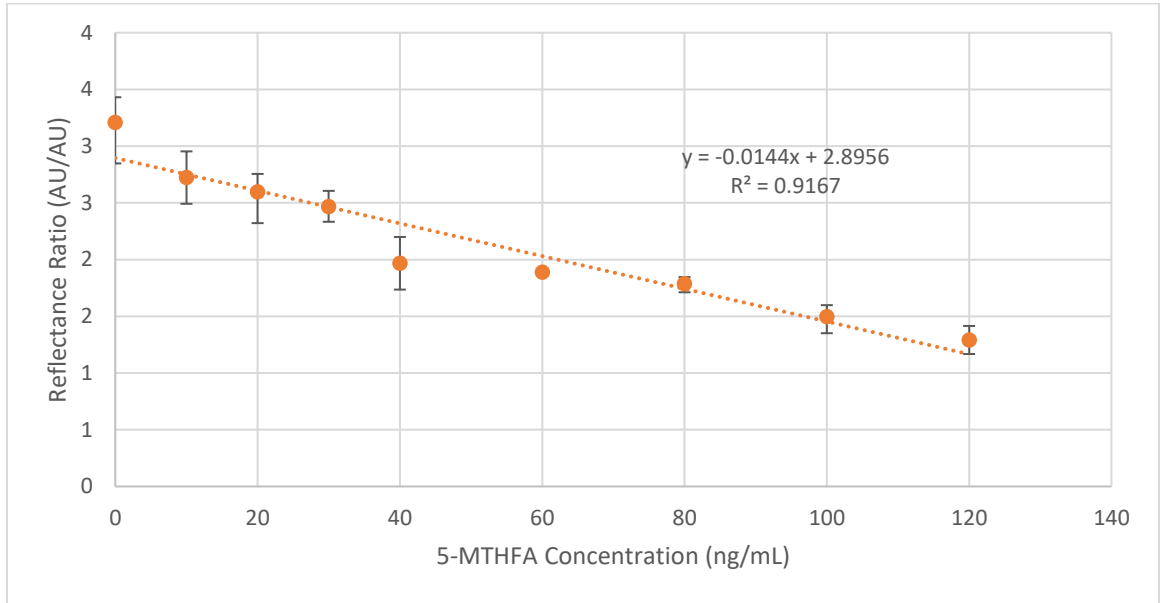


Figure 13 – Vitamin B9 – calibration curve example

Third party Vessel card performance evaluation

Vitamin B9 (µg/L)	Bucket	Vessel	
		Exact match	Within 1
0	1	61%	82%
60	2	70%	90%
135	3	11%	27%
Total		47%	66%

Remarks

The vitamin B9 test is performing below expectations, specially the last bucket. Since the last bucket is associated with faint lines, it is sensitive to fluidic and software issues. Several strategies have been proposed to correct this behavior. These strategies are currently under investigation.

Conclusions

- The Vessel system is capable of adequately detecting low moderate and high levels of Magnesium, vitamin C, Cortisol, BHB, specific gravity and pH, as shown in Table 1.

Table 1 - Overall exact match and within-one results from a third-party study for the

Overall results			
Assay	Exact match	Within one	Number of buckets
pH	98%	100%	4
Magnesium	88%	100%	5
Vitamin C	82%	99%	5
Ketone B	81%	95%	4
Specific Gravity	70%	100%	4
Cortisol	54%	96%	4
Vitamin B7	50%	81%	3
Vitamin B9	47%	66%	3

- Additional tuning of manufacturing conditions and formulation are required to improve the vitamin B7 and vitamin B9 performance.

Next Steps

- Deploy analytical techniques to evaluate the markers currently in the Vessel wellness card for which there are no commercial tests available.
- Run the cards with samples collected from live subjects and simultaneously evaluate them with orthogonal analytical techniques.

References:

1. Gonzalez, Jesus. (2020). Vessel Card Internal Repeatability study. Vessel Internal report [SC#023](#).