

RAMP[®]; High Accuracy From Immunochromatographic Assays by the Use of Internal Control Ratios. Cloney, L.P., Spiller, L.J., Fong, W.K., Harris, J.E., Harris, P.C.

RAMP[®] (Rapid Analyte Measurement Platform) is a platform technology that can be adapted to accurately quantify virtually any immunologically active substance. This patented technology incorporates an internal control that is run and measured concurrently in every assay allowing the **RAMP** System to compensate for test-to-test and Reader-to-Reader variations. **RAMP** tests have been developed for use in both clinical and environmental applications. The System consists of two components: a disposable Test Cartridge that houses an analyte-specific immunochromatographic strip, and a portable fluorescence-based Reader.

A sample is applied to the sample well of the Test Cartridge and as it migrates along the strip, fluorescent-dyed latex particles coated with antigen-specific antibodies bind to antigen if present in the sample. Antigen bound particles are immobilized at the Detection Zone by specific antibodies, and excess particles are immobilized at the Internal Control Zone by anti-immunoglobulins. The unique feature of the **RAMP** technology is that the Reader measures fluorescence emitted by the complexes bound in the Detection and Internal Control Zones and calculates a Ratio between these measurements. The **RAMP** Ratio corrects for inherent variability associated with earlier generation lateral flow assays and fluorescence-based instruments.

To demonstrate the broad application of this technology, samples were prepared containing different amounts of each of six separate analytes over their respective diagnostically relevant ranges. Replicates of each of the three cardiac markers (Troponin I, CK-MB and Myoglobin) and three biowarfare agents (*Bacillus anthracis*, Ricin and Botulinum Toxin) were tested in the **RAMP** System. The reproducibility of test results for each analyte was determined from the **RAMP** Ratio and compared to results determined from the binding of particles at the Detection Zone only.

In all of the assays the data demonstrates that the Internal Control Zone can effectively correct for assay variability due to the inherently large variations in binding at the Detection Zone. Analysis of data from replicates in each of the six analytes using the **RAMP** Ratio shows average Coefficient of Variation (CV) ranging from 3.3% to 7.8%, while the CV using only the Detection Zone ranged from 7.5% to 19.1%. The improvement in CV using the Ratio compared to the Detection Zone ranged from 1.6-fold to 5.8-fold. For example, the average CV of replicate Myoglobin samples run at 50, 100 and 200 ng/mL was improved from 19.1% when analyzed using only Detection Zone binding to 3.3% when analyzed using the **RAMP** Ratio. The data demonstrates the utility of the **RAMP** technology for rapid and accurate quantification of a broad range of analytes for both clinical and environmental applications.

INTRODUCTION

Inherent variability is associated with traditional lateral flow assays. Factors contributing to variability include environmental conditions, lot-to-lot membrane variability, Reader-to-Reader differences, and sample flow characteristics due to viscosity.

RAMP incorporates a quantitative Internal Control that is run and measured concurrently in every assay to compensate for these variations.

The **RAMP** System consists of two components: a disposable Test Cartridge that houses an analyte-specific immunochromatographic strip, and a portable fluorescence Reader.

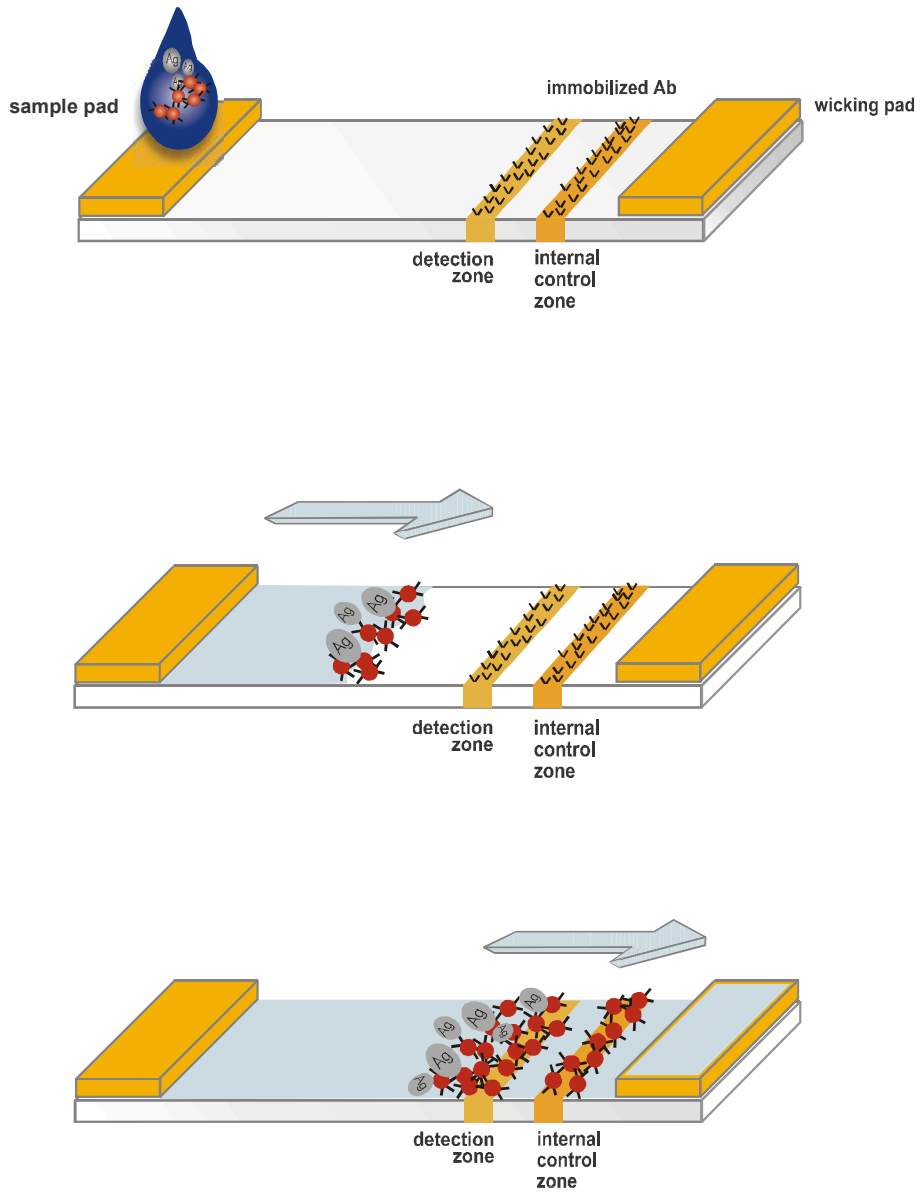
The Test Cartridge uses latex particles that are fluorescently labeled and tagged with antibodies against the analyte of interest.

Antigen bound particles are immobilized at the Detection Zone by specific antibodies and a portion of excess particles are immobilized at the Internal Control Zone.

The Reader measures fluorescence emitted by the complexes bound in the Detection and Internal Control Zones and calculates a Ratio between these measurements.

The **RAMP** Ratio corrects for inherent variability in the assay.

The RAMP Test Strip



METHODS

Serial dilutions of standards of each of six different analytes, *Bacillus anthracis* (Anthrax), Botulinum Toxin, Ricin, Myoglobin, CK-MB, and Troponin I were prepared and aliquots tested using the corresponding **RAMP** Test. Triplicates were tested for each level of Botulinum Toxin, Ricin and Anthrax and replicates of five were tested for Myoglobin, CK-MB and Troponin I at several antigen concentrations. Aliquots of 70 μ L of each sample were delivered to **RAMP** Test Cartridges and the Cartridges were inserted into the Reader. The Reader measured fluorescence (F-units) emitted by the complexes bound in the Detection and Internal Control Zones. Mean, standard deviation and %CV were calculated for each standard at the two zones. The ratio of fluorescence measured at the Detection Zone relative to that measured at the Internal Control Zone (the **RAMP** Ratio) was also determined for each sample and the %CV of the Ratios are reported.

RESULTS AND DISCUSSION

Figure 1: RAMP Test Strip Fluorescent Data

The **RAMP** Reader measures fluorescence emitted by complexes bound in the Detection and Internal Control Zones. Data from 5 replicates of 50 ng/mL standards tested in the **RAMP** Myoglobin Assay were selected to show that even extreme differences in binding at the Detection Zone can be corrected by use of the **RAMP** Internal Control Zone. Note that the replicate with higher levels of F-units at the Detection Zone also show higher levels of F-units measured at the Internal Control Zone. The **RAMP** Ratio calculated between these two zones corrects for test-to-test variations (CV=3.8%). Without the Ratio absolute fluorescence at the Detection Zone would be measured alone leading to extremely variable results (CV=35.1%).

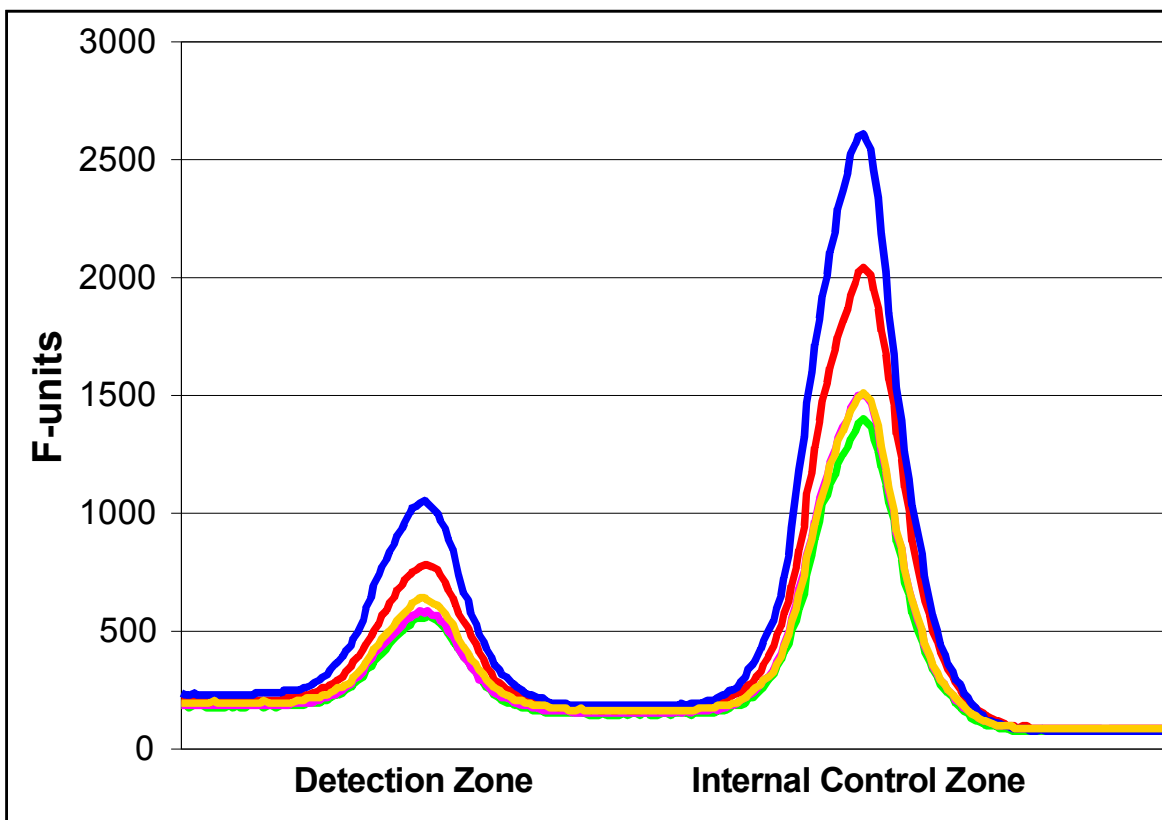


Table 1. Reproducibility of Anthrax Test Results

Anthrax (Spores /Test Sample)	Detection Zone Only (%CV)	Internal Control Zone Only (%CV)	RAMP Ratio (%CV)
586	7.9	2.7	5.2
1172	5.2	2.6	2.9
2344	5.8	6.6	1.2
4688	11.1	16.7	5.8
Average	7.5	7.2	3.8

The average %CV of Anthrax test results across a range of 586-4688 spores/test is improved from 7.5%, using Detection Zone binding only, to 3.8% when the Internal Control Zone binding is used to calculate the **RAMP** Ratio. This is a 2-fold improvement in reproducibility.

Table 2. Reproducibility of Botulinum Toxin Test Results

Bot Tox (ng/Test Sample)	Detection Zone Only (%CV)	Internal Control Zone Only (%CV)	RAMP Ratio (%CV)
3.8	6.3	5.1	1.2
7.5	10.1	10.2	2.3
15	3.1	6.7	7.3
30	14.2	19.3	4.4
Average	8.4	10.3	3.8

The average %CV of Botulinum Toxin test results across a range of 3.8-30 ng/Test is improved from 8.4%, using Detection Zone binding only, to 3.8% when the Internal Control Zone binding is used to calculate the **RAMP** Ratio. This is a 2.2 fold improvement in reproducibility.

Table 3. Reproducibility of Ricin Test Results

Ricin (ng/Test Sample)	Detection Zone Only (%CV)	Internal Control Zone Only (%CV)	RAMP Ratio (%CV)
75	6.2	5.3	5.1
150	14.6	13.2	8.5
300	13.1	4.7	7.9
Average	11.3	7.7	7.2

The average %CV of Ricin test results across a range of 75 - 300 ng/Test is improved from 11.3%, using Detection Zone binding only, to 7.2% when the Internal Control Zone binding is used to calculate the **RAMP** Ratio. This is a 1.6 fold improvement in reproducibility.

Table 4. Reproducibility of Myoglobin Test Results

Myoglobin (ng/mL)	Detection Zone Only (%CV)	Internal Control Zone Only (%CV)	RAMP Ratio (%CV)
50	35.1	32.0	3.8
100	5.7	6.6	2.7
200	16.5	12.9	3.5
Average	19.1	17.1	3.3

The average %CV of Myoglobin test results across a range of 50-200 ng/mL is improved from 19.1% using Detection Zone binding only, to 3.3% when the Internal Control Zone binding is used to calculate the **RAMP** Ratio. This is a 5.8 fold improvement in reproducibility.

Table 5. Reproducibility of CK-MB Test Results

CK-MB (ng/mL)	Detection Zone Only (%CV)	Internal Control Zone Only (%CV)	RAMP Ratio (%CV)
2.5	21.7	20.6	9.2
5	20.2	16.5	8.0
10	21.9	25.0	5.5
20	6.5	9.4	8.2
Average	17.6	17.9	7.8

The average %CV of CK-MB test results across a range of 2.5-20 ng/mL is improved from 17.6%, using Detection Zone binding only, to 7.8% when the Internal Control Zone binding is used to calculate the **RAMP** Ratio. This is a 2.3 fold improvement in reproducibility.

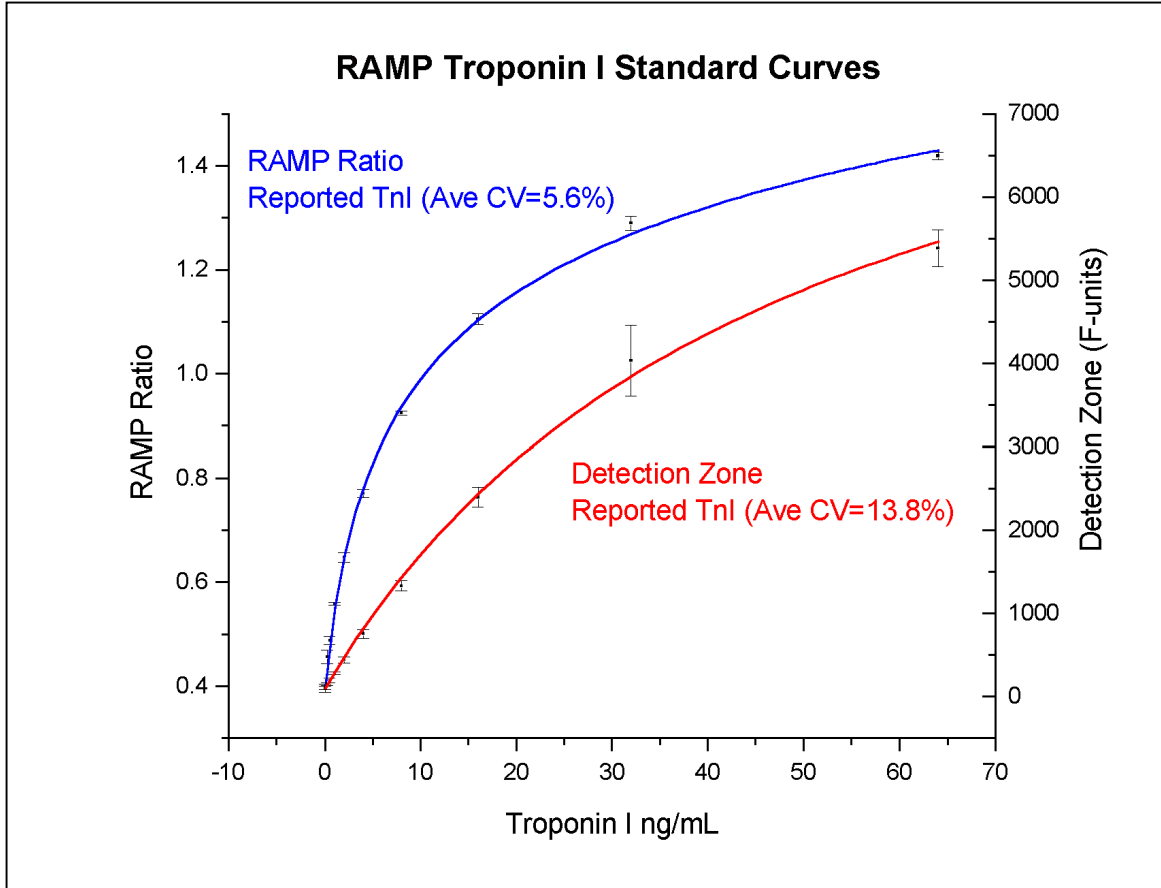
Table 6. Reproducibility of Troponin I Test Results

Troponin I (ng/mL)	Detection Zone Only (%CV)	Internal Control Zone Only (%CV)	RAMP Ratio (%CV)
0.5	14.9	11.2	4.7
1	7.0	8.1	2.8
2	9.1	5.7	4.3
4	7.2	2.8	5.3
Average	9.6	7.0	4.3

The average %CV of Troponin I test results across a range of 0.5-4 ng/mL is improved from 9.6%, using Detection Zone binding only, to 4.3% when the Internal Control Zone binding is used to calculate the **RAMP** Ratio. This is a 2.2-fold improvement.

Figure 2. Troponin I Standard Curves

RAMP Troponin I standard curves were plotted using either Detection Zone F-units or the **RAMP** Ratio over the reportable range of 0-64 ng/mL. The concentration of Troponin I was interpolated from the curves for standards (n=5) ranging from 0.5-32 ng/mL. The average %CV for reported Troponin I results was improved from 13.8%, using the Detection Zone only, to 5.6% when interpolated from the Ratio standard curve. For example, replicates of the 1 ng/mL standard were reported between 0.78 -1.04 ng/mL from the Detection Zone standard curve and between 0.98 - 1.06 ng/mL from the **RAMP** Ratio standard curve.



Troponin I ng/mL	Detection Zone Only (%CV)	RAMP Ratio (%CV)
0.5	32.4	8.3
1	11.3	3.2
2	12.3	6.5
4	8.9	6.5
8	5.7	3.7
16	6.9	4.5
32	19.2	6.3
Average	13.8	5.6

CONCLUSIONS

- Results demonstrate the **RAMP** Ratio effectively corrects variability in fluorescent particle binding at the Detection Zone by standardizing the signals against the Internal Control Zone.
- Results from 6 different analytes demonstrate that the reproducibility of **RAMP** System results is improved in all cases by use of the Internal Control Zone.
- **RAMP** demonstrates improved CVs using the **RAMP** Ratio rather than simple absolute measurements of fluorescence at the Detection Zone.
- Inherent variability associated with traditional lateral flow assays, such as environmental conditions, sample flow characteristics, membrane lot-to-lot variability and Reader-to-Reader differences are corrected using the **RAMP** Ratio method.
- **RAMP** technology can be used for rapid and reproducible quantification of a broad range of analytes for both clinical and environmental applications.