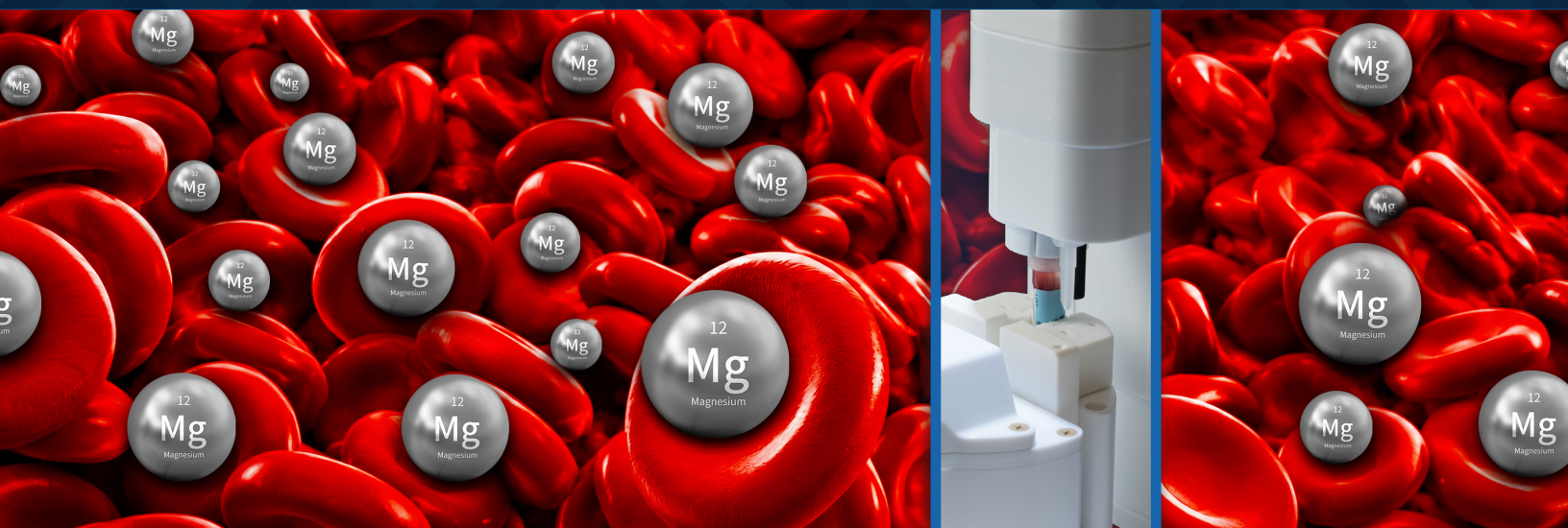




Authors: Patrick Messina, Owen Madigan, Libbie Smith

TRAXStation Clinical – Fully Automated Preparation of Red Blood Cell (RBC) Samples for the Analysis of Magnesium

Introduction

Trace element analysis plays a critical role in modern clinical diagnostics, providing essential information for evaluating nutritional status, toxic exposures, and metabolic disorders. Traditionally, the workflow for preparing and analyzing clinical samples by inductively coupled plasma mass spectrometry (ICPMS) has relied heavily on manual liquid handling steps. These steps – such as uncapping, rocking, pipetting, and dilution are time-consuming, prone to operator variability, and present ongoing risks of contamination, especially at trace and ultra-trace concentrations. As clinical demand grows, laboratories processing hundreds to thousands of patient samples per day face the mounting pressure to increase throughput while maintaining uncompromising analytical quality.

The **TRAXStation** Automated Multifunctional Liquid Handling System directly addresses these challenges by automating routine sample preparation tasks within a controlled, contamination free environment. Constructed from chemically inert materials, the platform is engineered to reliably process complex clinical matrices while minimizing external contamination and manual handling variability. Advanced capabilities including automated vial uncapping, liquid-level detection, barcode tracking, sample mixing, and precise

dilution routines ensure consistent and reproducible preparation across large sample sets.

In addition, the integrated SampleConfirm functionality verifies each aspiration event in real time, detecting incomplete or failed liquid handling steps and preventing compromised samples from advancing to analysis. For high-volume laboratories, this combination of automation and verification improves reproducibility, reduces operator workload, and provides a reliable, traceable pathway from raw sample to analysis-ready specimen.



Figure 1. TRAXStation Clinical for RBCs and clinical sample metals analysis prep.

Introduction *(Continued)*



Figure 2. TRAXStation Clinical takes up a 16 µL RBC aliquot for automated preparation to a final volume of 4000 µL.

TRAXStation Clinical automates the full preparation workflow for red blood cells (RBCs) collected and prepared in blood draw collection tubes or pour-off plastic vials. The compact

platform integrates all key steps into a single system, improving laboratory efficiency, traceability, and operator safety while ensuring high-quality, reproducible results.

TRAXStation Clinical Workflow

Barcode Reading & Sample Tracking

Integrated barcode scanning provides complete traceability and seamless connection to instruments and LIMS.

Automated Uncapping

Hands-free uncapping reduces manual handling, contamination risk, and biohazard exposure.

Sample Level Detecting

Sound waves are used to detect sample liquid levels. The probe will aspirate from just below the liquid surface.

SampleConfirm™

Sample integrity and verification function designed to confirm sample presence and total sample volume.

Mixing and Tube Preparation

Automated mixing ensures consistent sample readiness for analysis.

Precision Sampling & Dilution

High-accuracy pipetting enables aliquoting into tubes with programmable dilutions for flexible assay workflows.

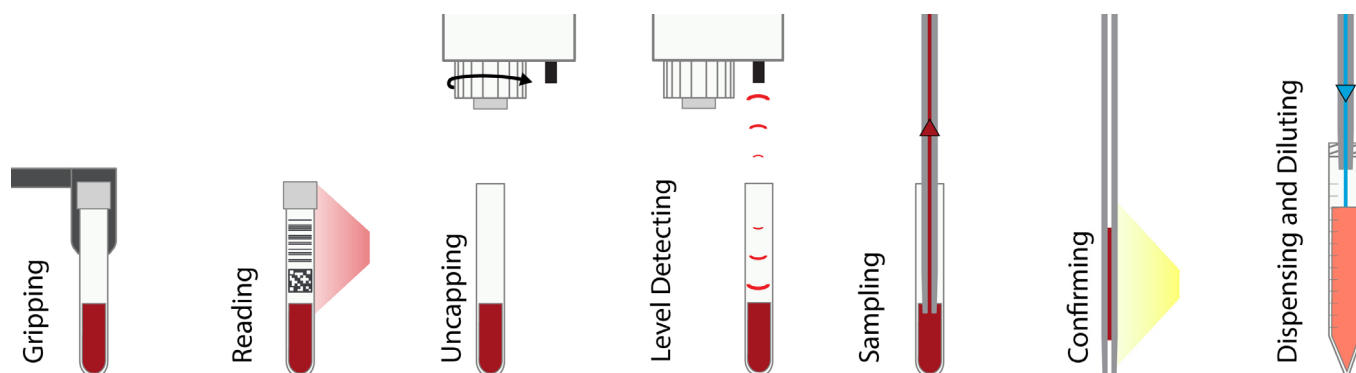


Figure 3. TRAXStation Clinical automated workflow for RBC sample preparation.

Background

Magnesium is a critical cofactor in hundreds of enzymatic reactions, playing a central role in energy metabolism, neuromuscular function, and cellular stability. While magnesium is routinely measured in serum and urine, these matrices primarily reflect extracellular and short-term status, which may not accurately represent total body magnesium stores. In contrast, red blood cell (RBC) magnesium provides a more direct assessment of intracellular magnesium, offering greater insight into long-term magnesium balance and tissue-level sufficiency. This distinction is particularly important in clinical scenarios where serum magnesium remains within normal limits despite underlying deficiency.

Serum magnesium testing is widely used due to its simplicity and rapid turnaround; however, it represents only a small fraction of total body magnesium and is tightly regulated, limiting its sensitivity to early or subclinical deficiency.

Urine magnesium, typically assessed through 24-hour collections, is valuable for evaluating renal handling and magnesium excretion, aiding in the differentiation of gastrointestinal versus renal losses. However, urinary results are influenced by recent intake and renal function, making interpretation context-dependent.

RBC magnesium analysis complements these approaches by providing a more stable and physiologically relevant indicator of intracellular status. Importantly, it may better correlate with clinical symptoms of deficiency such as fatigue, muscle cramps, neuromuscular irritability, and arrhythmias that can occur even when serum magnesium remains within the normal range. As such, RBC magnesium is increasingly recognized as a valuable tool in comprehensive magnesium assessment, particularly when integrated with serum and urine measurements to provide a more complete picture of magnesium homeostasis.

Table 1. Magnesium in red blood cell reference ranges.

Reference Range	Concentration	Interpretation
Normal Range	4.0-7.0 mg/dL*	Laboratory and methodology dependent. *Represents an average range from multiple U.S. laboratories
Deficiency	<4.0 mg/dL	Low RBC magnesium values are generally considered the most clinically relevant finding. Symptoms include cramps, tremors, fatigue, weakness, and cardiac irritability/arrhythmias, even when serum magnesium levels are normal.
Elevated	7.1-7.5 mg/dL	May be associated with supplementation.
Toxicity	>7.5 mg/dL	True intracellular magnesium toxicity is poorly characterized. Elevated serum levels have been associated with hypotension, bradycardia, respiratory depression, and cardiac arrest.

Experimental – TRAXStation Automated Sample Prep

Evaluation that the combination of **TRAXStation** Clinical preparation & ICPMS measurement produces acceptable precision (repeatability and reproducibility) and accuracy (bias vs assigned concentrations) for magnesium across the clinical range using three pooled sample levels: Low (below normal levels), Normal (clinically relevant mid-range), and High (above normal range that may represent toxicity).

Table 2. Materials and manual preparations.

Item	Description
Matrix	Packed RBCs from bovine whole blood EDTA (centrifuged, plasma removed, and pooled)
Pool Levels	Low, Normal, and High (based on reference range) prepared and homogenized in three independent blood pools
Target Conc.	Low $\approx$ 1.00 mg/dL Normal $\approx$ 5.00 mg/dL High $\approx$ 8.00 mg/dL
Aliquoting	Aliquot each pool into 25 identical tubes
Calibrators	Aqueous – 6 Levels from 0.25 mg/dL to 15.00 mg/dL
Diluent	1% Nitric Acid (v/v) 2% Methanol (v/v) 10 ppb Rhodium (Internal Standard)
Additional Materials	UPW, Calibration Standards, Blanks, and Negative

Table 3. Run structure and experimental design.

Item	Description
Samples per Run	83 (25 each level + Calibration)
Total Runs	25 (1875 data points) Multiple runs per day
Run Format	Blank, Calibrators, Negative, 25 Low, 25 Mid, 25 High (After centrifugation, packed cells were not “rocked” prior to aspiration)
Data Collection	Accuracy and precision monitored within and across runs
Data Analysis	Inter- and intra-assay performance presented as a percent CV. (See Data Analysis Criteria below)

Table 4. TRAXStation Clinical sample automation.

Item	Description
Rinse 1	0.4% TMAH, 1% EtOH, 0.05% TritonX
Rinse 2	DI Water
Diluent	(See Table 2 for prep) Connect and prime system
“Source” Samples	Load all calibrators and RBC samples to “source” racks
Destination Racks	Load all destination racks with plastic conical tubes
Dilution Factor	250x
Aspiration Volume	16 μ L sample
Diluent Volume	3984 μ L (TV = 4000 μ L)

Data Analysis Criteria

For magnesium in red blood cells (RBCs) testing, good precision and accuracy are demonstrated through rigorous statistical validation consistent with CLIA, CAP, and CLSI guidelines. Precision is evaluated by repeated analysis of RBC pools at low, normal, and high concentrations relative to reference ranges, with 25 replicates per level over 25 runs/batches and expressed as coefficient of variation (%CV). For trace metals such as magnesium, intra-assay and inter-assay %CVs should generally be $\leq 5\%$ at normal and high levels and $\leq 10\%$ at low levels. Accuracy is verified by comparison with mean recovery required to fall within $\pm 10\%$ of target values.

According to CLIA proficiency testing criteria, there is no established allowable total error (TEa) for magnesium in red blood cells (RBCs); however, serum TEa is defined by CLIA at 25%. We will place a tighter TEa of 20% for acceptability. Both short-term and long-term reproducibility must be assessed across runs, days, and operators to ensure robustness. Adherence to Good Laboratory Practice (GLP) principles and use of trace-element-free collection and processing materials are essential to minimize contamination. Together, these statistical and procedural controls ensure that magnesium RBC values are analytically valid, reproducible, and compliant with regulatory standards for clinical testing.

Instrument Prep and Parameters

All samples were prepared using **TRAXStation Clinical**, and analyzed using **FASTFluidic FilterProbe** with 4DXCi autosampler in combination with a single quad ICPMS. Magnesium was quantified at m/z 25 to minimize spectral interferences relative to the more abundant isotopes. While ^{25}Mg has lower natural

abundance than ^{24}Mg , the inherently high concentration of magnesium (ppm or mg/dL) in the sample matrix provides sufficient signal, making sensitivity limitations negligible. In fact, selecting m/z 25 improves analytical robustness by reducing interference contributions without compromising quantitation.

Table 5. Instrument information.

Parameter	Value
Sample Introduction System	FASTFluidic FilterProbe with 4DXCi Autosampler
Instrument	Single Quad ICPMS
Valve	Magnetically-coupled Inert 6-Port Valve
Probe	Carbon Fiber 0.8 mm ID Probe SC-5037-3755-100
Nebulizer	High Solids PFA Microflow Nebulizer ES-2030-79
Rinse 1	1% Nitric Acid
Carrier	1% Nitric Acid (v/v) + 2% MEOH (v/v)
Peripump Tubing	Black/Black
Method Time	19 sec sample-to-sample analysis

Table 6. Instrument parameters.

Parameter	Value
Analyte	Mg (m/z 25) First Ionization Energy: 7.64
ISTD	Rh (m/z 103) First Ionization Energy: 7.46
ICPMS Mode	KED Mode with He at 3.5 mL/min
Sweeps per Replicate	20
Replicates per Sample	3
Total Acquisition Time	3.78 sec



Figure 4. The AutoBench4 platform.

Magnesium Calibration – Analytical Measurement Range (AMR) 0.25-15.00 mg/dL

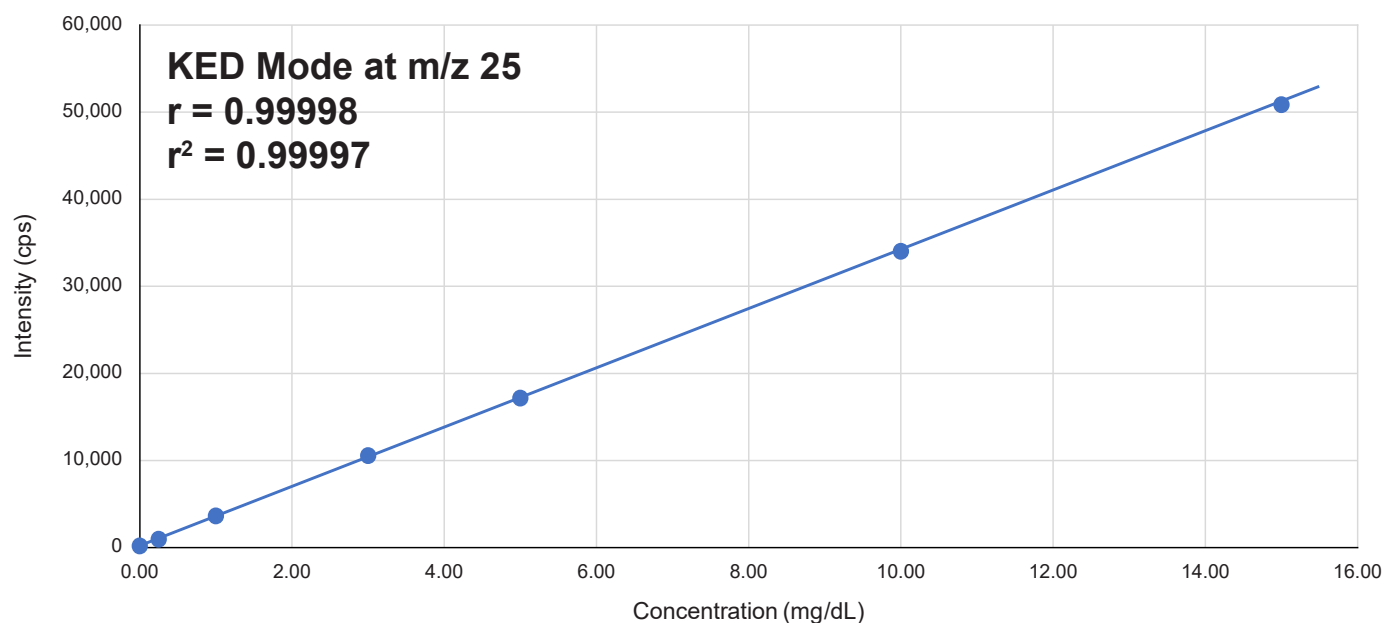


Figure 5. Calibration results demonstrated excellent precision and accuracy for magnesium when aqueous calibrators were prepared using the **TRAXStation** Clinical automated platform. The inclusion of 2% methanol in the diluent provided a consistent carbon load, improving matrix equivalency between aqueous calibrators and RBC samples while minimizing carbon-related differences in analytical response. Measured concentrations closely matched target values, with all calibration correlation coefficients of 0.999 or better, indicating excellent linearity, minimal bias, and strong agreement with expected results.



Figure 6. FASTFluidic FilterProbe ICPMS automation with 2 racks of 280 samples. The system also accommodates microwell plates.

Results Summary

Magnesium at m/z 25 in packed red blood cells was quantified using a validated ICPMS method following automated sample preparation on the **TRAXStation Clinical** platform. The automated workflow provided highly consistent 250x dilutions, minimized manual handling, and reduced contamination risk throughout the study. Despite the challenging RBC matrix, analytical performance was exceptional, with measured magnesium concentrations closely matching target values and demonstrating excellent

precision, accuracy, and reproducibility across the reportable range. The high dilution factor effectively minimized matrix effects, enabling aqueous calibrators to perform equivalently to RBC samples while maintaining robust internal standard performance and long-term analytical stability. These results demonstrate the **TRAXStation Clinical** platform's ability to deliver precise, reproducible, and contamination-controlled sample preparation that supports high-quality ICPMS analysis of complex clinical matrices.

Table 7. Magnesium in RBCs results.

	Level 1 Concentration	Level 2 Concentration	Level 3 Concentration
Average (mg/dL)	0.84	4.71	7.84
Standard Deviation (mg/dL)	0.02	0.14	0.21
Intra-Assay Precision	4.11%	3.00%	2.74%
Inter-Assay Precision	2.69%	3.66%	3.59%

The **TRAXStation Clinical 221** platform is also available. This compact version delivers the full power of automated sample preparation in a small footprint – ideal for laboratories with limited bench space. It automates uncapping, mixing, pipetting, and dilution steps to eliminate manual variability, improve reproducibility, and protect sample integrity through contamination-free, metal-inert processing. With intuitive LabSymphony software, barcode tracking, and flexible rack configurations, the **TRAXStation Clinical 221** integrates seamlessly into existing ICPMS workflows while reducing labor, consumable costs, and operator exposure. Despite its small size, it provides high-throughput performance, superior consistency, and enhanced laboratory safety – all in a compact, efficient design.

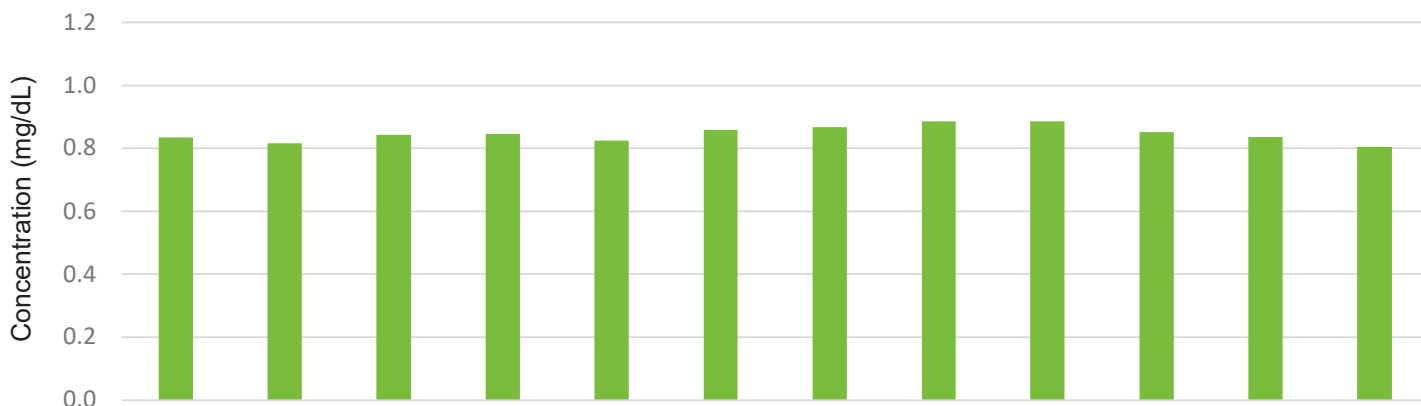


Figure 7. TRAXStation Clinical 221 for automated RBC and clinical sample prep for metals analysis.

See raw data on the following pages.

Low Level Magnesium Concentrations at 250x Dilutions (1875 data points)

(Each bar represents the average of 25 data points)



	1	2	3	4	5	6	7	8	9	10	11	12
	0.83	0.88	0.79	0.94	0.86	0.86	0.84	0.90	0.90	0.89	0.88	0.83
	0.88	0.82	0.80	0.87	0.87	0.88	0.83	0.88	0.90	0.81	0.78	0.75
	0.89	0.88	0.82	0.79	0.84	0.79	0.88	0.90	0.88	0.85	0.85	0.81
	0.83	0.87	0.85	0.80	0.84	0.85	0.85	0.94	0.92	0.88	0.87	0.82
	0.86	0.80	0.88	0.79	0.87	0.87	0.94	0.88	0.88	0.82	0.82	0.75
	0.84	0.82	0.89	0.79	0.86	0.87	0.87	0.91	0.85	0.84	0.79	0.82
	0.81	0.75	0.86	0.79	0.78	0.85	0.80	0.85	0.86	0.85	0.82	0.80
	0.84	0.78	0.88	0.83	0.81	0.90	0.85	0.89	0.91	0.81	0.82	0.81
	0.78	0.89	0.91	0.82	0.76	0.89	0.85	0.91	0.91	0.89	0.92	0.77
	0.77	0.84	0.79	0.90	0.77	0.91	0.90	0.85	0.94	0.84	0.86	0.77
	0.79	0.79	0.80	0.81	0.80	0.93	0.88	0.89	0.89	0.81	0.82	0.81
	0.80	0.84	0.81	0.92	0.85	0.85	1.00	0.90	0.86	0.90	0.89	0.85
	0.87	0.77	0.85	0.83	0.82	0.90	0.89	0.85	0.95	0.85	0.79	0.83
	0.81	0.80	0.80	0.86	0.80	0.83	0.90	0.84	0.89	0.90	0.79	0.80
	0.82	0.78	0.85	0.86	0.84	0.82	0.87	0.89	0.94	0.84	0.83	0.80
	0.84	0.77	0.90	0.82	0.83	0.81	0.85	0.86	0.85	0.89	0.84	0.80
	0.77	0.79	0.83	0.82	0.84	0.85	0.86	0.86	0.95	0.84	0.88	0.81
	0.80	0.83	0.90	0.87	0.82	0.81	0.89	0.88	0.86	0.78	0.85	0.82
	0.84	0.81	0.83	0.84	0.81	0.86	0.81	0.90	0.92	0.85	0.83	0.80
	0.86	0.88	0.88	0.81	0.82	0.82	0.82	0.86	0.96	0.88	0.81	0.85
	0.87	0.84	0.83	0.97	0.76	0.91	0.86	0.95	0.87	0.85	0.82	0.79
	0.85	0.81	0.80	0.88	0.92	0.83	0.86	0.90	0.87	0.86	0.76	0.80
	0.85	0.82	0.83	0.84	0.80	0.87	0.83	0.90	0.80	0.83	0.86	0.82
	0.92	0.77	0.86	0.86	0.84	0.89	0.89	0.86	0.81	0.89	0.84	0.79
	0.83	0.79	0.83	0.82	0.82	0.85	0.85	0.90	0.79	0.85	0.86	0.82
Ave.	0.83	0.82	0.84	0.85	0.82	0.86	0.87	0.88	0.89	0.85	0.84	0.80
SD	0.04	0.04	0.04	0.05	0.04	0.04	0.04	0.03	0.05	0.03	0.04	0.03
CV	4.46%	4.81%	4.49%	5.67%	4.46%	4.28%	4.76%	3.04%	5.18%	3.67%	4.40%	3.25%

Low Level Magnesium Concentrations at 250x Dilutions (1875 data points)

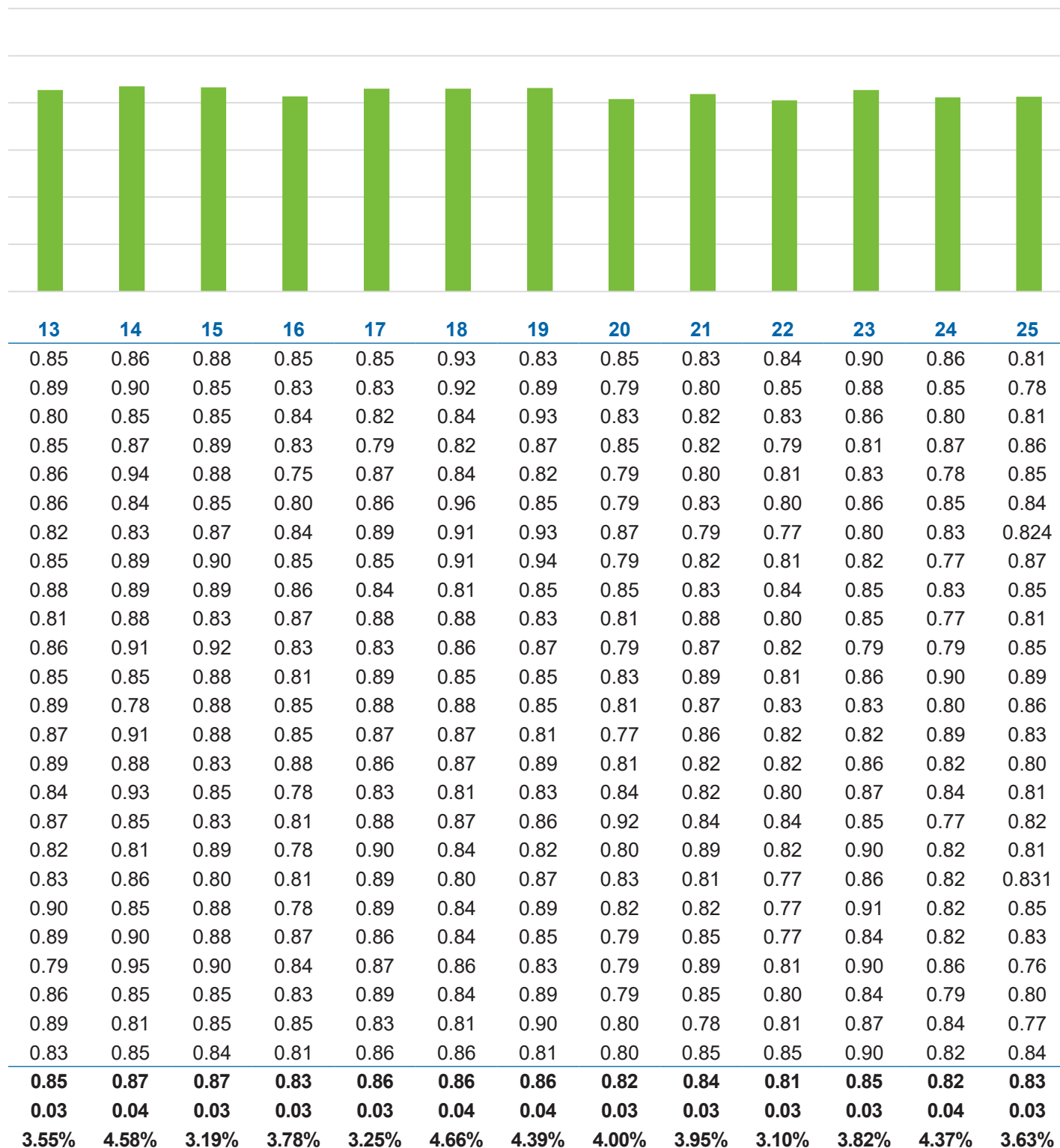
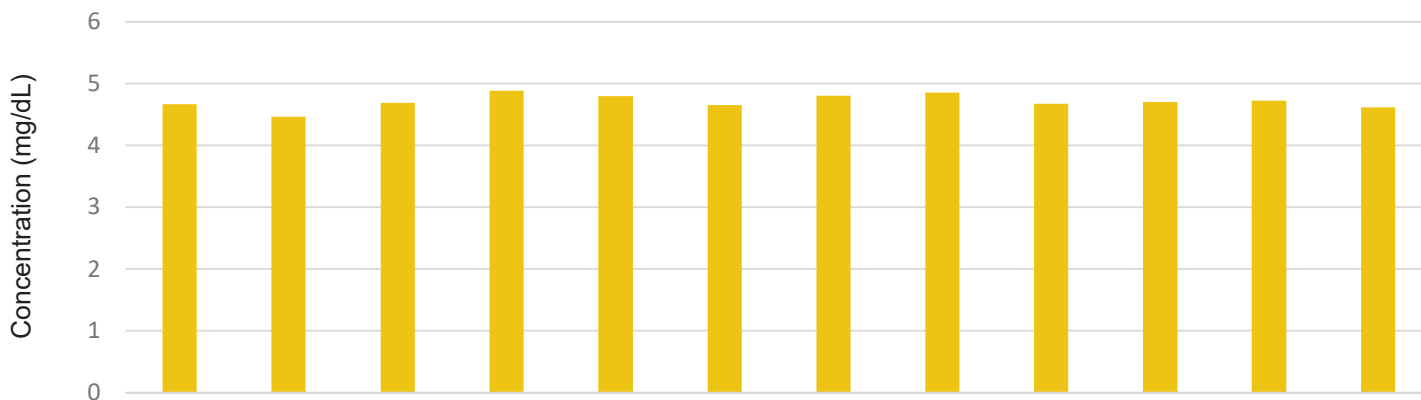


Figure 8. Low concentrations for magnesium measured by ICPMS (m/z 25). Each number 1-25 corresponds to a batch. The graph above shows the average, and the numbers below show the individual sample data from the batch.

Normal Level Magnesium Concentrations at 250x Dilutions (1875 data points)

(Each bar represents the average of 25 data points)



	1	2	3	4	5	6	7	8	9	10	11	12
	4.72	4.50	4.70	4.83	4.69	4.68	4.63	4.82	4.69	4.87	4.79	4.92
	4.60	4.47	4.73	4.90	4.73	4.69	4.87	4.80	4.60	4.86	4.98	4.67
	4.82	4.42	4.76	4.87	4.98	5.01	4.88	4.77	4.57	4.86	4.67	4.63
	4.87	4.47	4.77	4.84	4.73	4.72	5.02	5.05	4.60	4.80	4.86	4.93
	4.63	4.53	4.66	4.94	4.81	4.39	4.70	4.95	4.62	4.89	4.67	4.67
	4.90	4.45	4.81	4.79	4.73	4.97	4.89	4.91	4.59	4.76	4.61	4.69
	4.66	4.66	4.32	5.02	4.79	4.52	4.57	4.91	4.99	4.89	4.65	4.59
	4.54	4.52	4.65	4.89	4.81	4.70	4.90	4.78	4.45	4.87	4.71	4.56
	4.56	4.29	4.56	4.58	4.78	4.46	4.72	4.94	4.57	4.82	4.71	4.43
	4.54	4.68	4.24	4.75	5.08	4.52	4.45	4.83	4.59	4.82	4.79	4.35
	4.41	4.37	4.53	4.77	4.75	4.54	4.59	4.80	4.60	4.81	4.75	4.66
	4.82	4.53	5.26	4.74	4.79	4.80	4.89	4.73	4.75	4.81	4.71	4.58
	4.63	4.52	4.75	4.86	4.92	4.59	4.72	4.93	4.75	5.02	4.73	4.65
	4.68	4.37	4.81	4.75	4.72	4.46	4.89	4.76	4.67	5.00	4.88	4.75
	4.77	4.45	4.84	4.82	5.06	4.51	4.99	4.70	4.85	4.93	4.67	4.56
	4.67	4.51	4.53	5.17	4.64	4.91	4.92	4.83	4.76	4.95	4.71	4.42
	4.88	4.52	4.64	4.84	4.61	4.33	4.99	5.17	4.69	4.94	4.66	4.27
	4.65	4.32	4.52	5.06	4.79	4.88	4.83	4.63	4.70	4.72	4.76	4.58
	4.69	4.34	4.68	5.22	5.03	4.57	5.13	5.13	4.73	4.61	4.99	4.73
	4.40	4.36	4.81	4.81	4.74	4.54	4.77	4.84	4.75	4.78	4.48	4.45
	4.67	4.61	4.67	4.97	4.85	4.89	4.78	4.82	4.55	4.81	4.53	4.40
	4.70	4.31	4.58	4.76	4.50	4.60	4.76	4.76	4.69	4.98	4.88	4.65
	4.68	4.43	4.84	4.86	4.87	4.62	4.47	4.84	4.67	4.99	4.81	4.76
	4.50	4.59	4.95	5.04	4.94	4.72	5.07	4.82	4.69	4.90	4.63	4.82
	4.66	4.51	4.59	4.95	4.63	4.76	4.62	4.77	4.60	4.92	4.46	4.66
Ave.	4.67	4.47	4.69	4.88	4.80	4.65	4.80	4.85	4.67	4.70	4.72	4.61
SD	0.13	0.10	0.20	0.14	0.14	0.18	0.18	0.12	0.11	0.09	0.13	0.16
CV	2.77%	2.33%	4.18%	2.86%	2.93%	3.80%	3.69%	2.53%	2.31%	1.97%	2.76%	3.49%

Normal Level Magnesium Concentrations at 250x Dilutions (1875 data points)

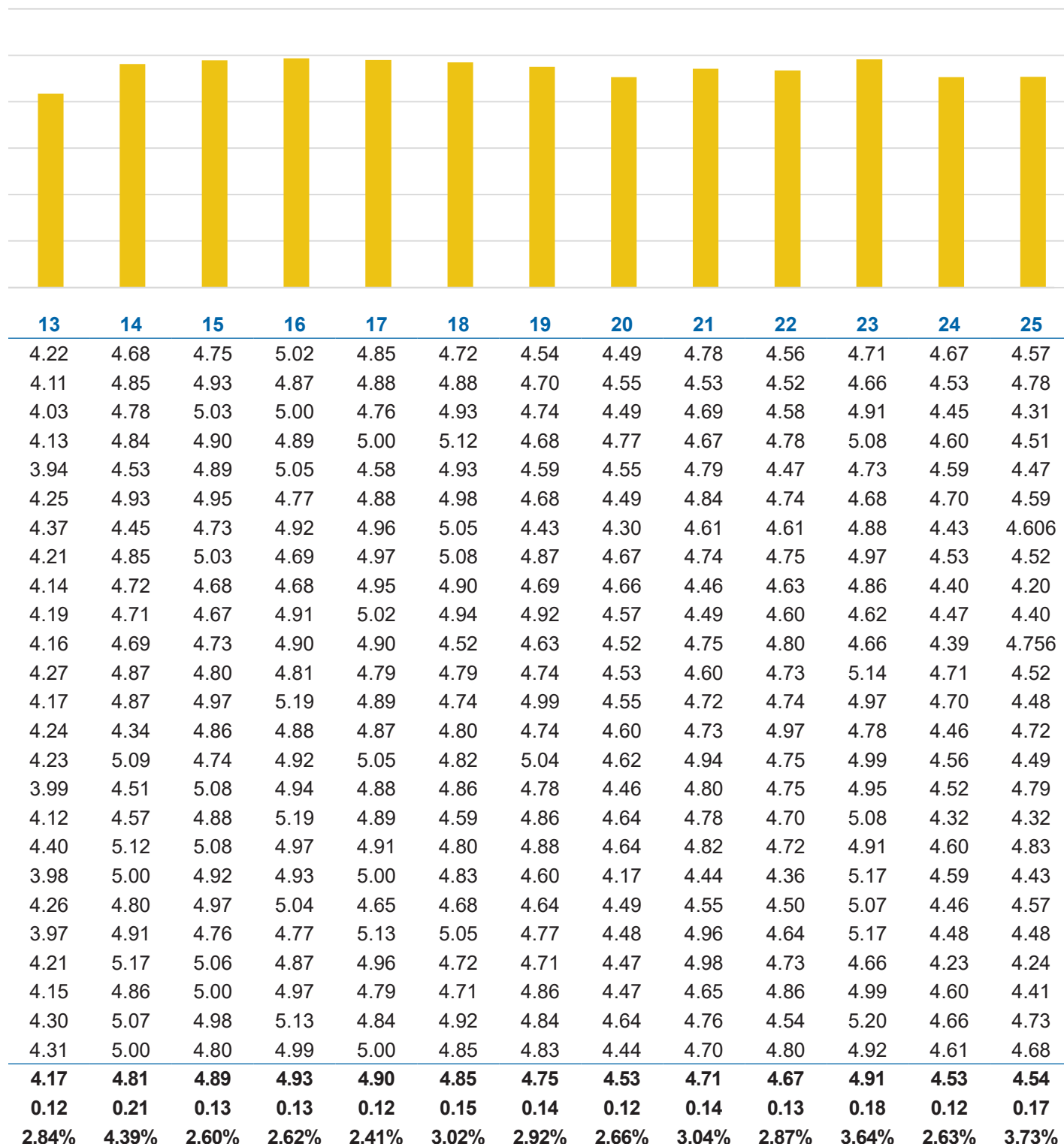
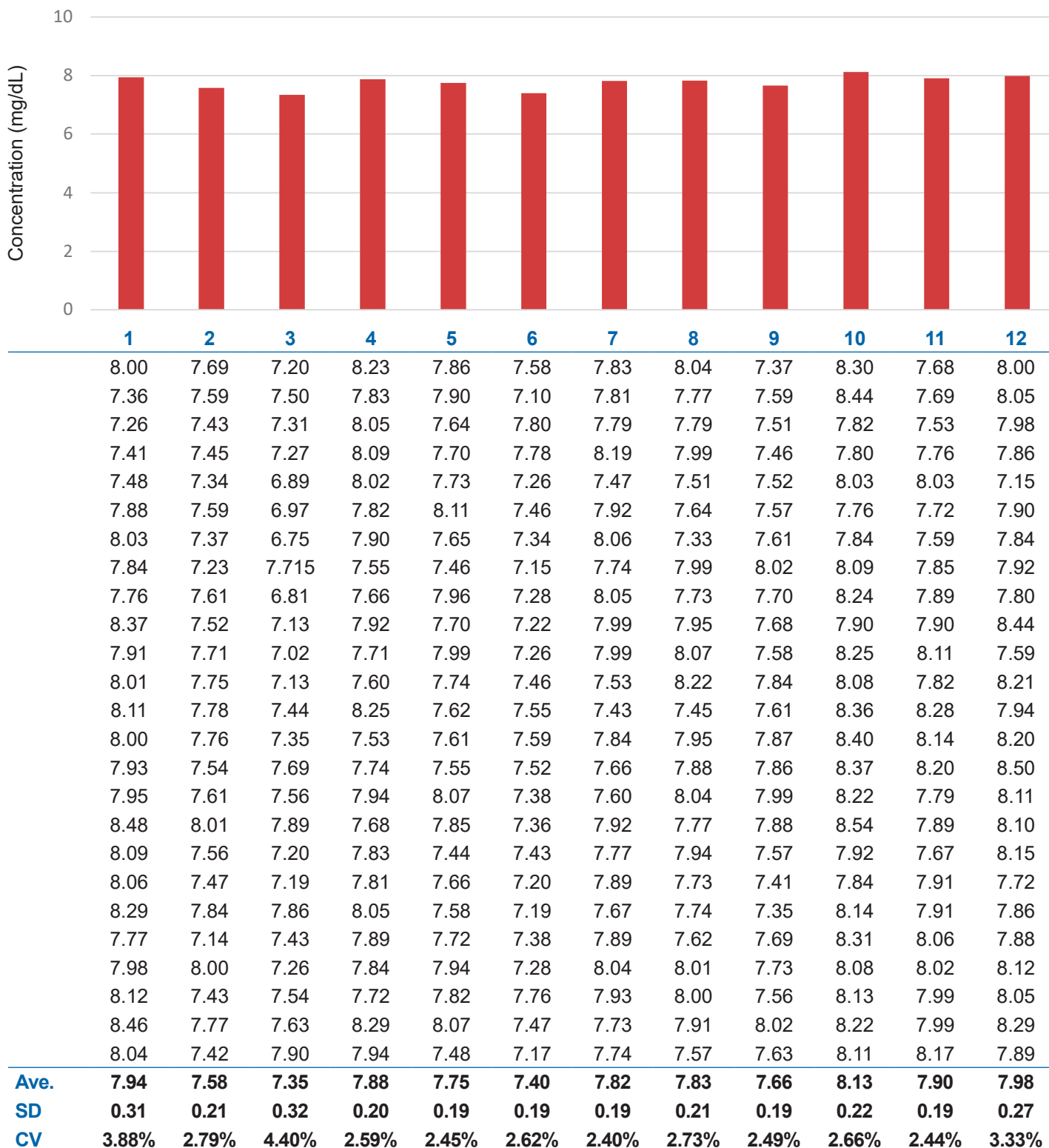


Figure 9. Normal concentrations for magnesium measured by ICPMS (m/z 25). Each number 1-25 corresponds to a batch. The graph above shows the average, and the numbers below show the individual sample data from the batch.

High Level Magnesium Concentrations at 250x Dilutions (1875 data points)

(Each bar represents the average of 25 data points)



High Level Magnesium Concentrations at 250x Dilutions (1875 data points)

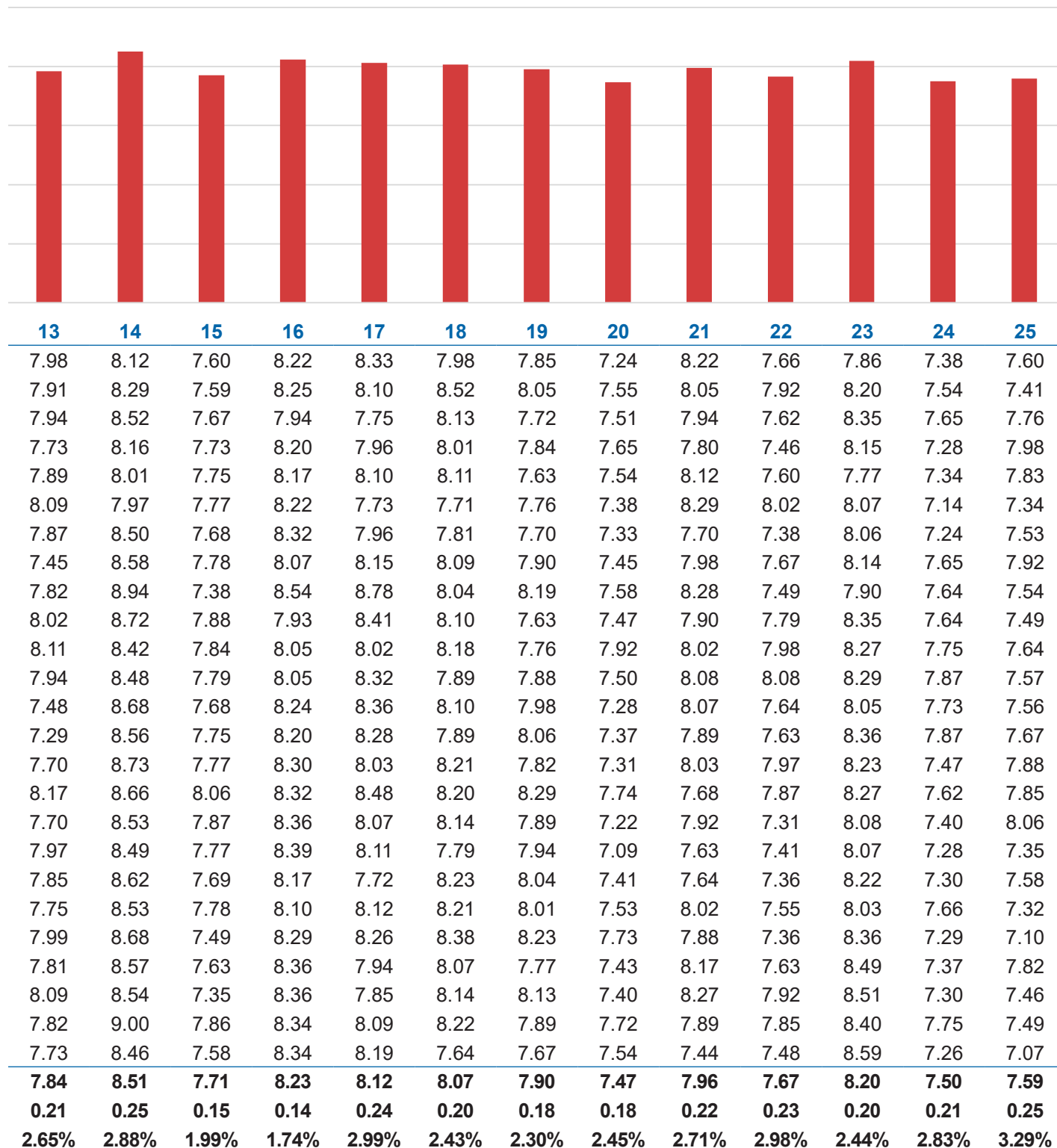


Figure 10. High concentrations for magnesium measured by ICPMS (m/z 25). Each number 1-25 corresponds to a batch. The graph above shows the average, and the numbers below show the individual sample data from the batch.

Conclusion

The **TRAXStation Clinical** Automated Multifunctional Liquid Handling System demonstrated performance that surpassed expected analytical targets for both accuracy and precision. Measured values consistently aligned with target concentrations over extended periods of time, indicating minimal systematic error and confirming high method accuracy. Replicate

analyses showed very low variability, with relative standard deviations well below acceptance criteria, reflecting exceptional precision. Collectively, the data provides strong evidence of method robustness and reliability, exceeding established quality benchmarks and reinforcing confidence in both the measurement process and resulting conclusions.

TRAXStation Clinical Features

ULPAClean 10

- ULPA filtered environment

TRAXGrip

- Transfers sample vials to **TRAXRock**, Staging Rack, and **TRAXLink**

TRAXRock

- Gently mixes whole blood, serum, or other sample types
- Configurable rocking speed and duration

Staging Rack

- Optimizes throughput by pre-staging and resting the next vial

TRAXLink

- Automatically uncaps sample vials, scans barcodes, and re-caps
- Detects liquid level
- Dynamic probe depth adjustment

Waste Management Cabinet

- Clean exchange of waste bottle – no drips
- Simplified waste line management
- Level and leak sensors

TRAXDisplay

- Easily run methods with LabSymphony software
- Library stores unlimited number of methods
- Export data as .csv or Excel file to LIMS

StationPump

- Precise sampling as low as 10 µL
- Rapid and precise dispensing

TRAXShield

- Clean sample environment
- Easily access samples from all four sides

Dual Rinse Station

- Ultra-low cross-contamination
- Low rinse consumption
- Customizable rinse solution

SampleConfirm

- Optical verification of aspirated sample volume

♦ = Feature exclusive to **TRAXStation Clinical**

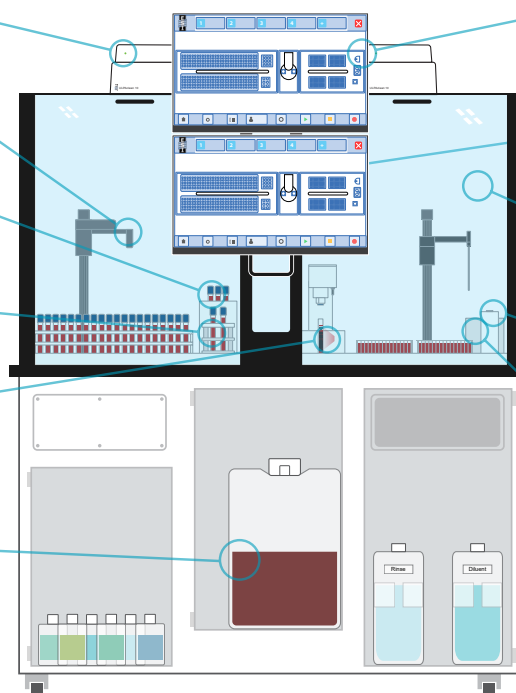


Figure 11. TRAXStation Clinical features diagram. **TRAXStation Clinical** is available in several models and configurations to match laboratory methods and throughput requirements. These include **TRAXStation 221**, **422**, and **442** models.

Notes

This image shows a full page of blank, lined paper. It features approximately 20 evenly spaced horizontal grey lines across its entire width, providing a guide for handwriting or typing. The background is a solid off-white color.



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