1.0 PURPOSE

To validate parameters for the MicroNutrient assay using LC/MS and ICP/MS.

2.0 SCOPE

This report covers performance characteristics summary of fat soluble and water soluble vitamins in serum and WBC and trace elements in serum, WBC and RBC including accuracy, simple precision, complex precision, normal range, sample stability at 4°C, Interference and reportable range using LC/MS and ICP/MS.

3.0 RESPONSIBILITIES

3.1. Clinical Laboratory Scientist (CLS): Responsible for following the procedure and reporting results to the LIS.

3.2. Laboratory Assistant: under the direct and constant supervision of the CLS, is responsible for following the procedure. The laboratory assistants may perform the assay following initial transfer of diluted patient samples to the plate.

3.3. Laboratory Director: Ensure that proper training and competency is achieved and maintained for all staff performing this procedure.

4.0 ABBREVIATIONS

4.1. DI Water – deionized water

4.2. WBC – White Blood Cells

4.3. RBC – Red Blood Cells

4.4. CLS – Clinical Lab Scientist

4.5. CLIA – Clinical Laboratory Improvement Amendments

4.6. SEa – Allowable Systematic Error

4.7. REa – Allowable Random Error

4.8. TEa – Allowable Total Error

4.9. CV – Coefficient of Variation

5.0 MATERIALS AND METHOD

5.1. Assay performance was evaluated with 1 reagents lot and 1 calibrator lot. The following elements were tested:

- Accuracy – Spike and Recovery
- Simple precision (Three human serum levels extracted in 12 replicates each and analyzed)
- Complex Precision (Three human serum samples extracted over five days, two runs per day in quadruplicates)
- Normal range

- Reportable range
- Sample stability at 4ºC
- Interference
- Limit of Quantitation

6.0 ACCEPTANCE CRITERIA

6.1. Accuracy – Spike and Recovery

% recovery from spiked sample is between 85 and 115%.

6.2. Simple precision:

CV is ≤ 20%

6.3. Complex precision:

Within Run, Between Run and Between Day CVs are ≤ 20%

6.4. Normal Range:

Apparently healthy control group samples regardless of age and gender were chosen and values within 5 and 99 percentile cut-off were documented.

6.5. Reportable Range:

CLSI protocol evaluator allowable total error < 20%.

6.5   Interference:

Variation from non-spiked sample < ± 15%, test using three concentration levels.

6.6   Sample Stability at 4ºC:

Five-days Sample Stability. Variation from day 0 < ± 15%.

7.0 RESULT SUMMARY

7.1. **Accuracy – Spike and Recovery**

Acceptance criteria: % recovery from spiked sample is between 85 and 115%.

Analytes	Theoretical value			Measured value			% recovery		
	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3
Choline (nmol/mL)	14.32	28.65	42.97	15.58	29.75	42.93	109%	104%	100%
Serine (nmol/mL)	19.03	38.06	57.09	19.73	36.63	49.87	104%	96%	87%
Cystine (nmol/mL)	3.75	7.5	11.25	3.54	6.94	9.89	94%	93%	88%
Nicotinamide (ng/mL)	40	80	120	39.31	82.23	121.37	98%	103%	101%
Asparagine (nmol/mL)	15.14	30.28	45.41	14.39	28.79	45.2	95%	95%	100%
Glutamine (nmol/mL)	273.7	547.4	821.1	288.9	570.4	829.6	106%	104%	101%
Carnitine (nmol/mL)	18.72	37.44	56.16	16.29	32.64	48.55	87%	87%	86%
Pyridoxine (ng/mL)	40	80	120	40.55	83.63	124.09	101%	105%	103%
VitC (mg/dL)	0.3	0.6	0.9	0.26	0.54	0.91	87%	90%	101%
VitB5 (mcg/L)	40	80	120	33.32	75.28	110.24	83%	94%	92%
VitB1 (nmol/L)	59.3	118.6	177.9	59.8	123.3	191.7	101%	104%	108%
Glutathione (nmol/mL)	1.7	3.4	5	1.44	3.29	5.65	85%	97%	113%
VitB2 (mcg/L)	40	80	120	37.88	80	118.11	95%	100%	98%
MMA (nmol/mL)	4.2	8.5	12.7	4.74	9.36	13.76	113%	110%	108%
Myo-Inositol (nmol/mL)	22.2	44.4	66.61	21.79	41.16	60.17	98%	93%	90%
Pyridoxal (ng/mL)	40	80	120	36.93	76.85	116.39	92%	96%	97%

Analytes	Theoretical value			Measured value			% recovery		
	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3
VitA (µg/dL)	90.0	103.3	116.6	80.0	91.7	102.5	89%	89%	88%
VitD3 (µg/mL)	0.8	1.6	2.4	0.8	1.5	2.4	95%	95%	100%
VitE (mg/L)	3.3	6.6	13.2	3.1	6.0	15.0	93%	91%	113%
VitK1 (ng/mL)	42.0	84.0	126.0	47.1	89.8	123.0	112%	107%	98%
VitK2 (ng/mL)	42.0	84.0	126.0	48.3	89.4	121.7	115%	106%	97%
CoQ10 (µg/mL)	0.8	1.6	2.4	0.7	1.6	2.3	87%	98%	96%

Analytes	Theoretical value			Measured value			% recovery		
	level 1	level 2	level 3	level 1	level 2	level 3	level 1	level 2	level 3
Ca 44 (mg/dL)	10.14	11.18	13.06	10.3	11.2	12.2	101%	100%	94%
Cu 63 (ug/mL)	2.69	3.71	5.66	2.7	3.7	4.7	100%	100%	101%
Zn 67 (ug/mL)	4.49	6.25	5.95	4.4	6.3	8.0	99%	100%	104%
Se 82 (ng/mL)	243.88	278.43	179.79	243.3	277.3	313.0	100%	100%	99%
Cr 52 (ng/mL)	113.00	171.52	0.94	116.9	171.9	230.0	103%	100%	114%
Mn 55 (ng/mL)	83.22	122.88	8.89	84.0	124.3	162.5	101%	101%	99%

	Theoretical value			Measured value			% recovery		
Analytes	**Level 1**	**Level 2**	**Level3**	**Level 1**	**Level 2**	**Level3**	**Level 1**	**Level 2**	**Level3**
Mg 24 (mg/dL)	3.19	6.20	9.42	3.13	6.39	9.42	98%	103%	100%
Fe 56 (mg/dL)	22.95	44.87	68.08	23.41	45.77	66.04	102%	102%	97%

Conclusion: Accuracy of the micro nutrient assays using LC/MS and ICP/MS yielded values within the acceptance criteria.

7.2. **Simple Precision**

Acceptance criteria: CV is ≤ 20%

Each level was ran in 10 replicates each and analyzed using LC/MS and ICP/MS. The results were analyzed using an EP Evaluator and are summarized as follows:

Instrument	Analyte	Sample	N	Mean	SD	CV	Accept
Xevo TQ-XS	✔ CoQ10	Sample 1	12 of 12	0.6970 / --	0.0334 / 0.1046	4.8% / --	_____
	✔ CoQ10	Sample 2	12 of 12	1.57 / --	0.09 / 0.24	5.5% / --	_____
	✔ CoQ10	Sample 3	12 of 12	2.28 / --	0.11 / 0.34	4.9% / --	_____
	✔ Vitamin A	Sample 1	12 of 12	79.97 / --	3.99 / 12	5.0% / --	_____
	✔ Vitamin A	Sample 2	12 of 12	91.66 / --	5.17 / 13.75	5.6% / --	_____
	✔ Vitamin A	Sample 3	12 of 12	102.48 / --	5.19 / 15.37	5.1% / --	_____
	✔ Vitamin D3	Sample 1	12 of 12	0.76 / --	0.05 / 0.11	6.8% / --	_____
	✔ Vitamin D3	Sample 2	12 of 12	1.53 / --	0.09 / 0.23	5.7% / --	_____
	✔ Vitamin D3	Sample 3	12 of 12	2.38 / --	0.15 / 0.36	6.4% / --	_____
	✔ Vitamin E	Sample 1	12 of 12	3.07 / --	0.14 / 0.46	4.7% / --	_____
	✔ Vitamin E	Sample 2	12 of 12	5.98 / --	0.38 / 0.9	6.4% / --	_____
	✔ Vitamin E	Sample 3	12 of 12	14.95 / --	0.99 / 2.24	6.6% / --	_____
	✔ Vitamin K1	Sample 1	12 of 12	47.12 / --	2.20 / 7.07	4.7% / --	_____
	✔ Vitamin K1	Sample 2	12 of 12	89.78 / --	4.92 / 13.47	5.5% / --	_____
	✔ Vitamin K1	Sample 3	12 of 12	123.03 / --	5.42 / 18.46	4.4% / --	_____
	✔ Vitamin K2	Sample 1	12 of 12	48.3520 / --	2.1597 / 7.2528	4.5% / --	_____
	✔ Vitamin K2	Sample 2	12 of 12	89.40 / --	4.24 / 13.41	4.7% / --	_____
	✔ Vitamin K2	Sample 3	12 of 12	121.73 / --	6.06 / 18.26	5.0% / --	_____

Instrument	Analyte	Sample	N	Mean	SD	CV
TQ-XS (WBA0127)	✔ Asparagine	Level 1	10 of 10	23.633 / –	4.631 / 4.727	12.1% / --
	✔ Asparagine	Level 2	10 of 10	56.000 / –	8.243 / 8.4	14.7% / --
	✔ Asparagine	Level 3	10 of 10	71.549 / –	8.899 / 10.732	12.4% / --
	✔ Carnitine	Level 1	10 of 10	17.322 / –	0.701 / 2.598	4.0% / --
	✔ Carnitine	Level 2	10 of 10	33.974 / –	1.075 / 5.096	3.2% / --
	✔ Carnitine	Level 3	10 of 10	48.932 / –	2.107 / 7.34	4.3% / --
	✔ Choline	Level 1	10 of 10	15.649 / –	0.569 / 2.347	3.6% / --
	✔ Choline	Level 2	10 of 10	30.215 / –	0.910 / 4.532	3.0% / --
	✔ Choline	Level 3	10 of 10	43.594 / –	1.753 / 6.539	4.0% / --
	✔ Glutamine	Level 1	10 of 10	387.801 / --	59.467 / 77.56	10.2% / --
	✔ Glutamine	Level 2	10 of 10	779.324 / --	102.368 / 116.899	13.1% / --
	✔ Glutamine	Level 3	10 of 10	1089.201 / --	63.765 / 163.38	5.9% / --
	✔ Glutathione	Level 1	10 of 10	3.438 / –	0.509 / 0.516	14.8% / --
	✔ Glutathione	Level 2	10 of 10	6.759 / –	0.620 / 1.014	9.2% / --
	✔ Glutathione	Level 3	10 of 10	9.515 / –	1.152 / 1.427	12.1% / --
	✔ L-Cystine	Level 1	10 of 10	2.718 / –	0.332 / 0.408	12.2% / --
	✔ L-Cystine	Level 2	10 of 10	5.915 / –	0.621 / 0.887	10.5% / --
	✔ L-Cystine	Level 3	10 of 10	9.127 / –	0.625 / 1.369	6.9% / --
	✔ L-Serine	Level 1	10 of 10	14.779 / –	1.059 / 2.217	7.2% / --
	✔ L-Serine	Level 2	10 of 10	29.935 / –	1.858 / 4.49	6.2% / --
	✔ L-Serine	Level 3	10 of 10	46.372 / –	4.249 / 6.956	9.2% / --
	✔ MMA	Level 1	10 of 10	5.91 / --	0.48 / 0.89	8.1% / --
	✔ MMA	Level 2	10 of 10	11.49 / –	0.34 / 1.72	3.0% / --
	✔ MMA	Level 3	10 of 10	16.48 / –	0.83 / 2.47	5.0% / --
	✔ Myo-Inositol	Level 1	10 of 10	23.772 / –	1.905 / 3.566	8.0% / --
	✔ Myo-Inositol	Level 2	10 of 10	48.440 / –	4.918 / 7.266	10.2% / --
	✔ Myo-Inositol	Level 3	10 of 10	76.155 / –	8.440 / 11.423	11.1% / --
	✔ Nicotinamide	Level 1	10 of 10	47.599 / –	1.967 / 7.14	4.1% / --
	✔ Nicotinamide	Level 2	10 of 10	94.580 / –	3.562 / 14.187	3.8% / --
	✔ Nicotinamide	Level 3	10 of 10	137.960 / --	5.408 / 20.694	3.9% / --
	✔ Pyridoxal	Level 1	10 of 10	49.361 / –	3.549 / 9.872	7.2% / --
	✔ Pyridoxal	Level 2	10 of 10	95.540 / –	4.015 / 19.108	4.2% / --
	✔ Pyridoxal	Level 3	10 of 10	135.612 / --	7.059 / 27.122	5.2% / --
	✔ Pyridoxine	Level 1	10 of 10	46.909 / –	1.405 / 7.036	3.0% / --
	✔ Pyridoxine	Level 2	10 of 10	92.887 / –	4.260 / 13.933	4.6% / --
	✔ Pyridoxine	Level 3	10 of 10	140.176 / --	4.333 / 21.026	3.1% / --
	✔ VitB1	Level 1	10 of 10	57.963 / –	5.917 / 8.694	10.2% / --
	✔ VitB1	Level 2	10 of 10	114.153 / --	2.109 / 17.123	1.8% / --
	✔ VitB1	Level 3	10 of 10	167.148 / --	6.533 / 25.072	3.9% / --

Instrument	Analyte	Sample	N	Mean	SD	CV
	✔ VitB2	Level 1	10 of 10	48.982 / --	2.433 / 7.347	5.0% / --
	✔ VitB2	Level 2	10 of 10	106.503 / --	5.136 / 15.975	4.8% / --
	✔ VitB2	Level 3	10 of 10	176.026 / --	8.594 / 26.404	4.9% / --
	✔ VitB5	Level 1	10 of 10	65.472 / --	6.680 / 9.821	10.2% / --
	✔ VitB5	Level 2	10 of 10	129.098 / --	10.349 / 19.365	8.0% / --
	✔ VitB5	Level 3	10 of 10	190.374 / --	15.669 / 28.556	8.2% / --
	✔ VitC	Level 1	10 of 10	0.68 / --	0.10 / 0.1	5.2% / --
	✔ VitC	Level 2	10 of 10	1.44 / --	0.17 / 0.22	11.9% / --
	✔ VitC	Level 3	10 of 10	2.07 / --	0.28 / 0.31	13.7% / --

Instrument	Analyte	Sample	N	Mean	SD	CV
NexION 2000	✔ Cr	Level 2	10 of 10	116.881 / --	6.328 / 17.532	5.4% / --
	✔ Cr	Level 3	10 of 10	171.927 / --	2.031 / 25.789	1.2% / --
	✔ Cr	Level 4	10 of 10	230.049 / --	2.807 / 34.507	1.2% / --
	✔ Cu	Level 2	10 of 10	2.697 / --	0.025 / 0.405	0.9% / --
	✔ Cu	Level 3	10 of 10	3.714 / --	0.026 / 0.557	0.7% / --
	✔ Cu	Level 4	10 of 10	4.727 / --	0.051 / 0.709	1.1% / --
	✔ Mn	Level 2	10 of 10	83.958 / --	1.338 / 12.594	1.6% / --
	✔ Mn	Level 3	10 of 10	124.328 / --	1.585 / 18.649	1.3% / --
	✔ Mn	Level 4	10 of 10	162.544 / --	1.279 / 24.382	0.8% / --
	✔ Se	Level 2	10 of 10	243.222 / --	5.970 / 36.483	2.5% / --
	✔ Se	Level 3	10 of 10	277.252 / --	6.381 / 41.588	2.3% / --
	✔ Se	Level 4	10 of 10	315.679 / --	11.385 / 47.352	3.6% / --
	✔ Zn	Level 2	10 of 10	4.426 / --	0.067 / 0.664	1.5% / --
	✔ Zn	Level 3	10 of 10	6.258 / --	0.055 / 0.939	0.9% / --
	✔ Zn	Level 4	10 of 10	8.022 / --	0.096 / 1.203	1.2% / --

Instrument	Analyte	Sample	N	Mean	SD	CV
NexION 2000	✔ Fe	Level 1	10 of 10	19.625 / --	0.839 / 2.944	4.3% / --
	✔ Fe	Level 2	10 of 10	36.995 / --	1.704 / 5.549	4.6% / --
	✔ Fe	Level 3	10 of 10	53.456 / --	1.623 / 8.018	3.0% / --
	✔ Fe	Level 4	10 of 10	68.820 / --	5.162 / 10.323	7.5% / --
	✔ Mg	Level 1	10 of 10	0.865 / --	0.116 / 0.13	13.4% / --
	✔ Mg	Level 2	10 of 10	1.631 / --	0.110 / 0.245	6.7% / --
	✔ Mg	Level 3	10 of 10	2.445 / --	0.103 / 0.367	4.2% / --
	✔ Mg	Level 4	10 of 10	3.140 / --	0.166 / 0.471	5.3% / --

Conclusion: Simple precision yielded values within the acceptance criteria.

7.3. **Complex Precision:**

Acceptance criteria: Within Run, Between Run and Between Day CVs are ≤ 20%

Each level was extracted in quadruplicates once a day for five days and analyzed using LC/MS and ICP/MS twice. The results were analyzed using an EP Evaluator and are summarized as follows:

Instrument	Analyte	Sample	Days (Tot/Excl)	No. Runs with Outliers	Within Run SD/CV	Total SD/CV
TQ-XS (WBA0127)	✔ Asparagine	Sample 1	5 / 0	None	2.28 / 15.8	2.84 / 19.7
	✔ Asparagine	Sample 2	5 / 0	None	3.40 / 11.8	5.00 / 17.4
	✔ Asparagine	Sample 3	5 / 0	None	5.75 / 12.7	6.41 / 14.2
	✔ Carnitine	Sample 1	5 / 0	None	0.77 / 4.7	1.71 / 10.5
	✔ Carnitine	Sample 2	5 / 0	None	2.13 / 6.5	3.72 / 11.4
	✔ Carnitine	Sample 3	5 / 0	None	2.47 / 5.1	6.32 / 13.0
	✔ Choline	Sample 1	5 / 0	None	0.64 / 4.1	1.15 / 7.4
	✔ Choline	Sample 2	5 / 0	None	1.26 / 4.3	2.58 / 8.7
	✔ Choline	Sample 3	5 / 0	None	1.17 / 2.7	3.29 / 7.7
	✔ Cystine	Sample 1	5 / 0	None	0.46 / 13.1	0.71 / 20.0
	✔ Cystine	Sample 2	5 / 0	None	0.57 / 8.2	1.04 / 15.0
	✔ Cystine	Sample 3	5 / 0	None	0.61 / 6.1	1.59 / 16.0
	✔ Glutamine	Sample 1	5 / 0	None	24.74 / 8.6	52.64 / 18.2
	✔ Glutamine	Sample 2	5 / 0	None	39.38 / 6.9	88.48 / 15.5
	✔ Glutamine	Sample 3	5 / 0	None	68.55 / 8.3	114.33 / 13.8
	✔ Glutathione	Sample 1	5 / 0	None	0.23 / 16.3	0.34 / 13.9
	✔ Glutathione	Sample 2	5 / 0	None	0.51 / 15.4	0.60 / 18.1
	✔ Glutathione	Sample 3	5 / 0	None	0.66 / 11.6	0.82 / 14.5
	✔ MMA	Sample 1	5 / 0	None	0.31 / 6.5	0.48 / 10.2
	✔ MMA	Sample 2	5 / 0	None	0.59 / 6.3	0.74 / 7.9
	✔ MMA	Sample 3	5 / 0	None	0.49 / 3.6	1.38 / 10.0
	✔ Myo	Sample 1	5 / 0	None	4.18 / 19.2	5.18 / 13.8
	✔ Myo	Sample 2	5 / 0	None	6.45 / 15.7	8.56 / 20.1
	✔ Myo	Sample 3	5 / 0	None	8.16 / 13.6	11.99 / 19.9
	✔ Nicotinamide	Sample 1	5 / 0	None	2.76 / 7.0	3.73 / 9.5
	✔ Nicotinamide	Sample 2	5 / 0	None	5.72 / 7.0	7.82 / 9.5
	✔ Nicotinamide	Sample 3	5 / 0	None	7.44 / 6.1	11.13 / 9.2
	✔ Pyridoxal	Sample 1	5 / 0	None	3.17 / 8.6	4.81 / 13.0
	✔ Pyridoxal	Sample 2	5 / 0	None	5.69 / 7.4	6.94 / 9.0
	✔ Pyridoxal	Sample 3	5 / 0	None	4.07 / 3.5	4.97 / 4.3
	✔ Pyridoxine	Sample 1	5 / 0	None	2.96 / 7.3	3.65 / 9.0
	✔ Pyridoxine	Sample 2	5 / 0	None	5.45 / 6.5	7.72 / 9.2
	✔ Pyridoxine	Sample 3	5 / 0	None	8.28 / 6.7	10.72 / 8.6
	✔ Serine	Sample 1	5 / 0	None	2.25 / 11.4	2.76 / 14.0
	✔ Serine	Sample 2	5 / 0	None	2.20 / 6.0	3.94 / 10.8
	✔ Serine	Sample 3	5 / 0	None	2.85 / 5.7	5.22 / 10.5
	✔ VitB1	Sample 1	5 / 0	None	4.99 / 8.3	7.96 / 13.3
	✔ VitB1	Sample 2	5 / 0	None	6.42 / 5.2	10.24 / 8.3
	✔ VitB1	Sample 3	5 / 0	None	9.72 / 5.1	13.59 / 7.1

Instrument	Analyte	Sample	Days (Tot/Excl)	No. Runs with Outliers	Within Run SD/CV	Total SD/CV
	✔ VitB2	Sample 1	5 / 0	None	2.40 / 6.3	3.26 / 8.6
	✔ VitB2	Sample 2	5 / 0	None	5.31 / 6.6	8.17 / 10.2
	✔ VitB2	Sample 3	5 / 0	None	5.27 / 4.5	13.70 / 11.6
	✔ VitB5	Sample 1	5 / 0	None	3.99 / 12.0	4.86 / 14.6
	✔ VitB5	Sample 2	5 / 0	None	10.77 / 14.3	10.84 / 14.4
	✔ VitB5	Sample 3	5 / 0	None	15.56 / 14.1	16.65 / 15.1
	✔ VitC	Sample 1	5 / 0	None	0.03 / 11.3	0.06 / 13.6
	✔ VitC	Sample 2	5 / 0	None	0.06 / 11.6	0.11 / 11.0
	✔ VitC	Sample 3	5 / 0	None	0.07 / 7.5	0.17 / 18.4

Instrument	Analyte	Sample	Days (Tot/Excl)	No. Runs with Outliers	Within Run SD/CV	Total SD/CV
TQ-XS (WBA0127)	✔ CoQ10	Level 1	5 / 0	None	0.05 / 7.2	0.07 / 10.2
	✔ CoQ10	Level 2	5 / 0	None	0.05 / 3.3	0.09 / 5.8
	✔ CoQ10	Level 3	5 / 0	None	0.08 / 3.2	0.15 / 6.2
	✔ VitA	Level 1	5 / 0	None	7.60 / 8.9	11.12 / 13.0
	✔ VitA	Level 2	5 / 0	None	6.25 / 6.6	7.68 / 8.1
	✔ VitA	Level 3	5 / 0	None	5.11 / 4.6	6.51 / 5.9
	✔ VitD3	Level 1	5 / 0	None	0.10 / 11.6	0.15 / 17.9
	✔ VitD3	Level 2	5 / 0	None	0.14 / 8.2	0.17 / 10.4
	✔ VitD3	Level 3	5 / 0	None	0.14 / 5.4	0.18 / 7.2
	✔ VitE	Level 1	5 / 0	None	0.33 / 9.4	0.55 / 15.7
	✔ VitE	Level 2	5 / 0	None	0.42 / 6.4	0.67 / 10.2
	✔ VitE	Level 3	5 / 0	None	0.74 / 5.0	0.77 / 5.2
	✔ VitK1	Level 1	5 / 0	None	4.43 / 9.0	6.24 / 12.6
	✔ VitK1	Level 2	5 / 0	None	6.01 / 6.5	7.95 / 8.6
	✔ VitK1	Level 3	5 / 0	None	4.61 / 3.5	7.17 / 5.5
	✔ VitK2	Level 1	5 / 0	None	4.55 / 8.5	5.72 / 10.7
	✔ VitK2	Level 2	5 / 0	None	5.25 / 5.4	6.64 / 6.9
	✔ VitK2	Level 3	5 / 0	None	4.83 / 3.6	7.18 / 5.3

Instrument	Analyte	Sample	Days (Tot/Excl)	No. Runs with Outliers	Within Run SD/CV	Total SD/CV
NexION 2000	✔ Chromium	Level 2	5 / 0	None	3.856 / 3.4	6.870 / 6.1
	✔ Chromium	Level 3	5 / 0	None	3.719 / 2.1	5.123 / 3.0
	✔ Chromium	Level 4	5 / 0	None	3.311 / 1.4	5.402 / 2.4
	✔ Copper	Level 2	5 / 0	None	0.026 / 1.1	0.144 / 5.9
	✔ Copper	Level 3	5 / 0	None	0.020 / 0.6	0.158 / 4.6
	✔ Copper	Level 4	5 / 0	None	0.037 / 0.9	0.233 / 5.4
	✔ Manganese	Level 2	5 / 0	None	1.755 / 2.2	3.642 / 4.6
	✔ Manganese	Level 3	5 / 0	None	1.051 / 0.9	3.874 / 3.2
	✔ Manganese	Level 4	5 / 0	None	1.469 / 0.9	5.133 / 3.3
	✔ Selenium	Level 2	5 / 0	None	6.317 / 2.5	20.194 / 8.0
	✔ Selenium	Level 3	5 / 0	None	5.008 / 1.7	19.972 / 6.9
	✔ Selenium	Level 4	5 / 0	None	4.707 / 1.5	19.043 / 6.0
	✔ Zinc	Level 2	5 / 0	None	0.080 / 1.8	0.177 / 3.9
	✔ Zinc	Level 3	5 / 0	None	0.079 / 1.2	0.212 / 3.3
	✔ Zinc	Level 4	5 / 0	None	0.083 / 1.0	0.253 / 3.1

Instrument	Analyte	Sample	Days (Tot/Excl)	No. Runs with Outliers	Within Run SD/CV	Total SD/CV
NexION 2000	✔ Fe	Level 1	3 / 0	None	0.574 / 2.7	1.615 / 7.5
	✔ Fe	Level 2	3 / 0	None	0.690 / 1.7	4.425 / 10.9
	✔ Fe	Level 3	3 / 0	None	1.155 / 2.0	5.780 / 9.9
	✔ Fe	Level 4	3 / 0	None	1.424 / 1.9	9.068 / 12.0
	✔ Mg	Level 1	3 / 0	None	0.040 / 4.2	0.167 / 17.7
	✔ Mg	Level 2	3 / 0	None	0.035 / 1.9	0.229 / 12.8
	✔ Mg	Level 3	3 / 0	None	0.058 / 2.2	0.249 / 9.5
	✔ Mg	Level 4	3 / 0	None	0.122 / 3.5	0.427 / 12.3

Conclusion: Complex precision yielded values within the acceptance criteria.

7.4. **Normal Range:**

140 apprantly healthy human samples (drawn 24-48 h before analysis) were extracted and tested. 5 and 99 percentile were used as cut-off to establish reference ranges.

Conclusion: The normal range samples tested using LC/MS and ICP/MS yielded values within the acceptance criteria.

7.5. **Reportable Range:**

Acceptance criteria: CLSI protocol evaluator allowable total error < 20%.

Three intermediate levels were prepared from these two levels Low and High as level 1 and level 5.

Level 2: 3 part Low + 1 part High
Level 3: 1 part Low + 1 part High
Level 4: 1 part Low + 3 part High

The linearity set prepared above was tested using LC/MS and ICP/MS in triplicates.

Conclusion: The linearity set results are linear and meet the acceptance criteria.

7.6 **Interference**

Acceptance criteria: Each point < ± 15 %, tested using three concentration levels prepared by spiking appropriate vitamin into human serum for bilirubin and hemoglobin.

Bilirubin - 0, 10, 20, 30 and 40 mg/dL, 5 levels added.
Hemoglobin - 0, 125, 250, 375 and 500 mg/dL, 5 levels added.

Conclusion: micro nutrient assay is not affected by bilirubin less than 40 mg/dL and by hemoglobin less than 500 mg/dL.

7.7 **Sample Stability at 4°C:**

Acceptance criteria: variation from day 0 must be < ± 15%.

Conclusion: Based on stability studies, sample stability at 4°C for up to 2 days for all analytes.

7.8 **Limit of Quantitation**

A dynamic LOQ has been set for the micro nutrient assay. A signal-to-noise (peak-to-peak) ratio of > 3 for all reported values has been set.

7.0 **CONCLUSION**

This validation study has been reviewed, and the performance of the method is considered acceptable for patient testing.