

Clinical Evaluation of Chromium, Cobalt and Titanium by ICP-MS For Metal on Metal Implants



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OVERVIEW

Purpose

- To develop and validate a rapid and accurate method for the concurrent quantitation of chromium, cobalt, and titanium in human whole blood and serum samples for the purpose monitoring metal ion levels in patients with metal prosthetic implants.

Method

- Analysis via inductively coupled plasma mass spectrometry (ICP-MS) utilizing an octopole reaction system with helium gas successfully eliminates potentially interfering species within the matrix.
- Samples are loaded onto and processed within 96 well plates providing significant efficiency gains over test-tube based techniques.
- Samples are directly analyzed from 96-well plates using a CETAC ASX-7400 autosampler coupled to an Agilent 7700 ICP-MS.

Results

- The method has been fully validated to meet the regulatory requirements of pharmaceutical bioanalytical analysis (GxP) to concurrently measure chromium, cobalt, and titanium in whole blood and serum samples.
- Normal circulating levels of chromium, cobalt and titanium in a non-exposed population are slightly below the LLOQ of this method.
- 2,000+ samples from metal on metal (MOM) implant recipients have been successfully analyzed.

INTRODUCTION

- Long term wear of a metal-on-metal hip implant may lead to metal leaching into surrounding tissue and blood. Serum and blood levels have been shown to correlate with metal ion levels in the joint synovial fluid. Thus these matrices are considered good surrogates for monitoring MOM wear and metal ion leaching.¹ Continued clinical monitoring of metal ion levels has been recommended for recipients of MOM prosthetics. However, the health implications of elevated circulating levels of chromium, cobalt, and titanium have not been elucidated.

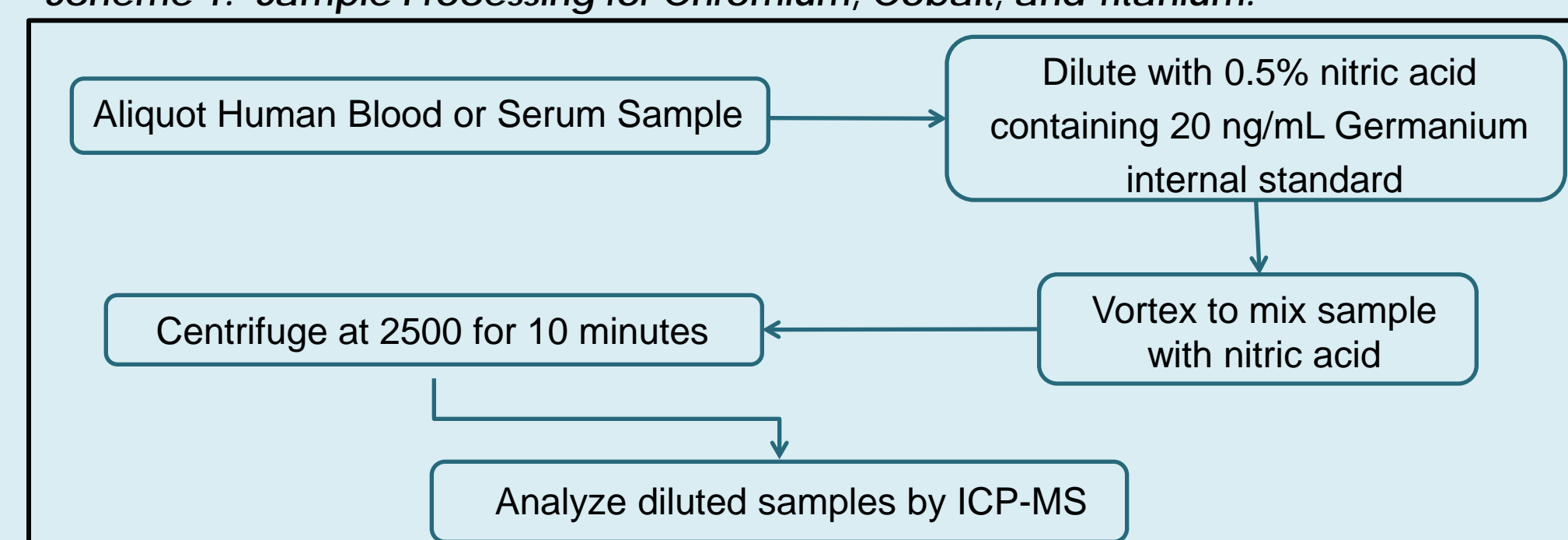
METHODOLOGY

Experimental

Calibration standards are prepared in 0.5% nitric acid spanning the range of 1.0 – 100.0 ng/mL for chromium and cobalt and 5.0 – 500.0 ng/mL for titanium. The use of a non-matrix matched calibration curve is necessary to avoid contributions from low background analyte levels seen with most matrices. Sample and standards are loaded, diluted and analyzed within a 2mL 96-well plate. Negative blood and serum is analyzed with each batch to ensure the elimination of potentially interfering species from the sample matrix. Sample preparation is illustrated in Scheme 1.

METHODOLOGY CONTINUED

Scheme 1. Sample Processing for Chromium, Cobalt, and Titanium.



Instrument Parameters

ICP-MS analysis utilized an octopole reaction system optimized to reduce polyatomic interferences and maximize analyte signal. Analysis was split between standard helium gas mode for optimal analysis of chromium and cobalt and high energy helium mode for the analysis of titanium. The assay utilized the HMI, reducing oxide formation during sample introduction. Rapid sampling of approximately 100 seconds was achieved using the integrated sample introduction system (ISIS) of the instrument. Instrument parameters are outline in Table 1.

Table 1. Instrument parameters

Assay Parameters	Chromium & Cobalt	Titanium
Points Per Mass	3	3
Readings/Replicates	1/3	1/3
Tuning File	He.u	Hhe.u
Cell Gas	Helium	Helium
Gas Flow	4.6 mL/min	10.0 mL/min
Total Acquisition Time	26.77 seconds	

Evaluation of Collection Procedures

The potential for analyte contamination from the sample collection procedure was evaluated by subjecting whole blood QC samples to various sample collection systems. Elevations in chromium and cobalt levels were then attributable to sample collection. Results are displayed below. Trace metal collection tubes and straight needles were found to be necessary to minimize contamination. However, no difference was seen between the 1st and 2nd draw. Results (shown in Table 2) are consistent with a previously published report comparing plastic cannula vs. metal needles.²

Table 2. Collection Device Evaluation Results

Contribution from Collection	Tube Type ^a				Needle Type ^b		Draw Sequence ^c	
	Trace Metal K ₂ EDTA	K ₂ EDTA	Serum Separator	Blood Clot Serum	Butterfly 23G	Straight 21G	1 st	2 nd
Chromium (ng/mL)	0.17	0.52	1.15	0.24	0.34	0.17	0.17	0.16
Cobalt (ng/mL)	0.20	0.31	0.24	0.34	0.35	0.20	0.20	0.23

^a First draw through a straight 21G needle. ^b Trace metal free K₂ EDTA tube first draw. ^c Trace metal free K₂ EDTA tube with a straight 21G needle.

RESULTS AND DISCUSSION

- The assay validation included three days of simultaneous analysis of chromium, cobalt, and titanium in each matrix. The validation included assessments of assay linearity, lower limit of quantitation (LLOQ), method detection limit, assay precision and accuracy, selectivity, and dilution. Previous validations using 15 mL Sarstedt tubes included normal range analysis, room temperature stability (≥ 7 days), refrigerated stability (≥ 12 days @ 4°C), freeze-thaw stability (at least 3 cycles), and frozen stability (≥ 19 days @ -20°C). Inter-run precision and accuracy results from the validation are presented in Table 3.

Table 3. Inter-run Precision and Accuracy Results for Chromium, Cobalt and Titanium in Whole Blood and Serum

QC Level	Chromium			Cobalt			Titanium		
	Low	Mid	High	Low	Mid	High	Low	Mid	High
Target (ng/mL)	3.00	15.00	80.00	3.00	15.00	80.00	15.00	80.00	400.00
Whole Blood									
Precision (%CV)	3.8%	3.3%	3.1%	2.8%	3.2%	2.6%	3.2%	3.2%	2.4%
Accuracy (%)	94.7%	96.1%	93.8%	95.7%	97.1%	94.1%	107.4%	105.3%	101.4%
Serum									
Precision (%CV)	5.8%	1.6%	1.6%	3.4%	2.0%	1.6%	4.1%	1.9%	2.0%
Accuracy (%)	104.0%	101.9%	101.4%	97.3%	97.1%	92.2%	106.5%	106.1%	105.1%

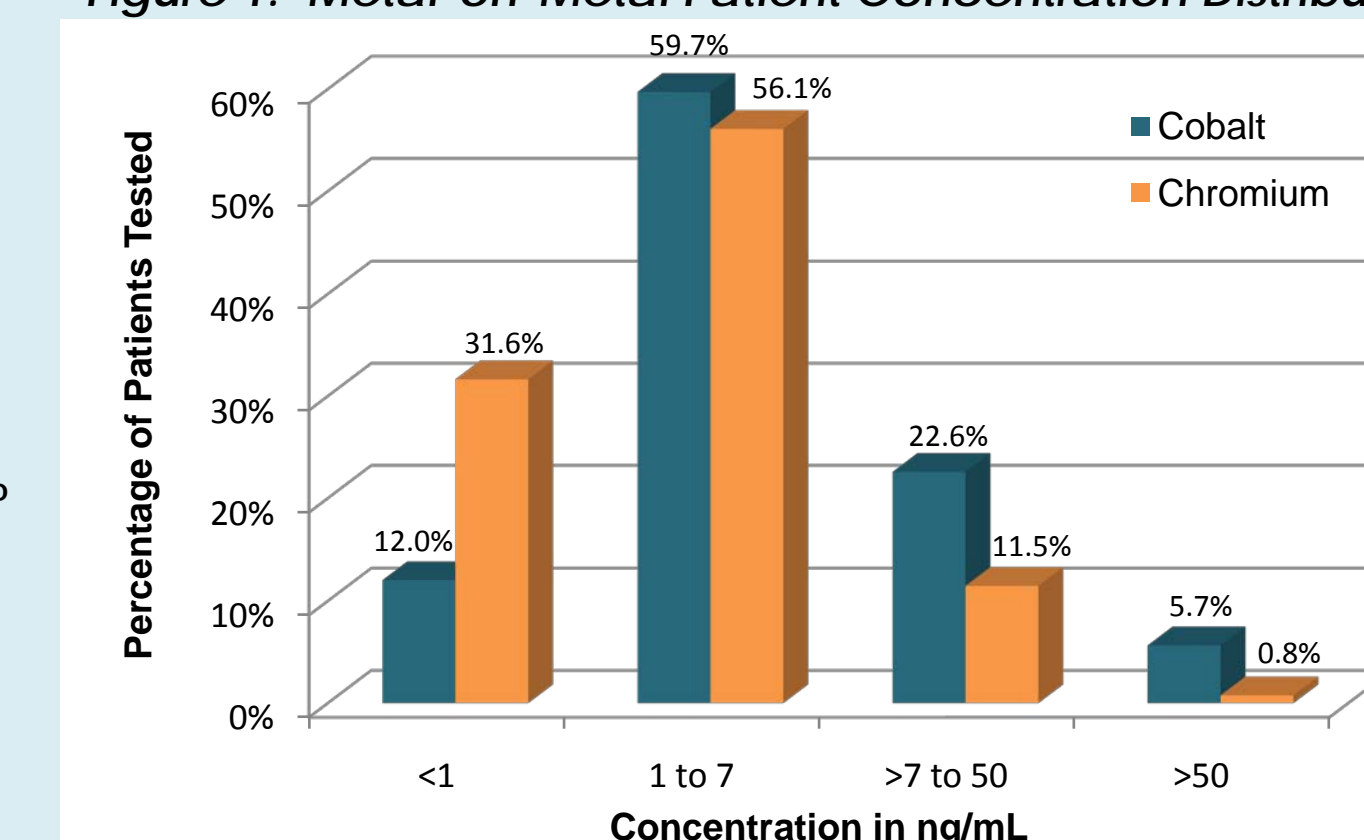
- Method detection limits(MDL) were calculated with a 98% confidence interval using 15 LLOQ replicates over three days to determine the lower limit of detection for each metal. Results are shown in Table 4.

Table 4. Statistic Results of MDL

Analyte Name	Chromium	Cobalt	Titanium
Spiked LLOQ Concentration (ng/mL)	1.00	1.00	5.00
Standard Deviation (ng/mL)	0.152	0.150	0.648
MDL (ng/mL)	0.40	0.39	1.70

- Approximately 2,000 patients with metal-on-metal recalled implants were measured for both chromium and levels in whole blood samples. Of the patients evaluated 88% had a chromium levels of less than 7 ng/mL and 72% had a cobalt levels of less than 7 ng/mL. However a few patients had levels in excess of 100 ng/mL for chromium and 500 ng/mL for cobalt. The distribution of results is shown in Figure 1.

Figure 1. Metal-on-Metal Patient Concentration Distribution



CONCLUSIONS

- Rapid simultaneous analysis of Cr, Co, and Ti is suitable for both clinical occupational monitoring of patients with metal-on-metal total hip replacement and for bioanalytical (GxP) analysis utilizing a CETAC ASX-7400 96 well plate compatible autosampler coupled to an Agilent 7700 ICP-MS employing an octopole reaction system to eliminate background interference.
- The assay has a method detection limit (MDL) for Cr of 0.40 ng/mL, Co of 0.39 ng/mL, and Ti of 1.70 ng/mL.
- Additional work is currently being performed to reduce the lower limit of the assay to 0.50 ng/mL for Cr to accurately measure normal, unexposed circulating levels.

REFERENCES

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