

## Supplementary Material

### Definition of 'SOTP'

We have previously used absolute values as a cut-off for suboptimal taper performance (SOTP): a linear wear depth of  $> 5 \mu\text{m}$  in combination with a volume loss  $> 0.5 \text{mm}^3$ .<sup>1</sup> This was not felt appropriate for this study, as the relative contribution to the total material loss (with the bearing included) was key to the analysis.

We therefore used an arbitrary value of 10% of the total material loss to define the 'SOTP' group. MoM bearings have been shown to wear at rates lower than  $1 \text{mm}^3$  per year in simulator studies. Our own analysis of explanted components has confirmed that these low wear rates are indeed possible. Assuming a 'normal' wear rate of  $1 \text{mm}^3$  per year, a 10% contribution from the taper would be approximately  $0.1 \text{mm}^3$  per year. Given the average time to revision surgery of around five years, we felt that a 10% cut-off for 'SOTP' was justified.

### ALVAL grading system

We used the binary distinction 'severe aseptic lymphocyte-dominated vasculitis-associated lesion (ALVAL)' versus 'non-severe ALVAL' for the various analyses described in the paper. This approach was chosen after consideration of two factors:

- 1) In the grading system used at our hospital, severe ALVAL is defined by, among other features, severe extensive necrosis affecting 100% of the synovial membrane. We have found a strong correlation between severe ALVAL and the severe soft-tissue destruction identified by the attending surgeon at revision. We believe the clinical significance of severe ALVAL to be unquestionable.
- 2) ALVAL is associated with delayed clearance of cobalt (Co) and chromium (Cr) from the synovial fluid. There is a pronounced change in the progression from moderate-to-severe ALVAL with respect to this effect, as shown in Figure a.

### In vitro study of the serum partitioning of different species of cobalt and chromium ions

Included below is a full breakdown of the tests carried out by our collaborator, Andrew Taylor, centring on the analysis of Co and Cr concentrations in serum, blood, and erythrocytes using inductively coupled plasma mass spectrometry (ICP-MS). This formed part of an MSc entitled 'An in vitro assessment of the leaching of metals from replacement hip-joints' completed in Faculty of Health and Medical Sciences, University of Surrey in September 2009 by Sudeep Rana.

**Summary.** The release of Cr and Co into the body from the prosthesis of a total hip arthroplasty and a hip resurfacing arthroplasty has been a concern because of the potential toxicity. The work of Walter et al<sup>2</sup> and

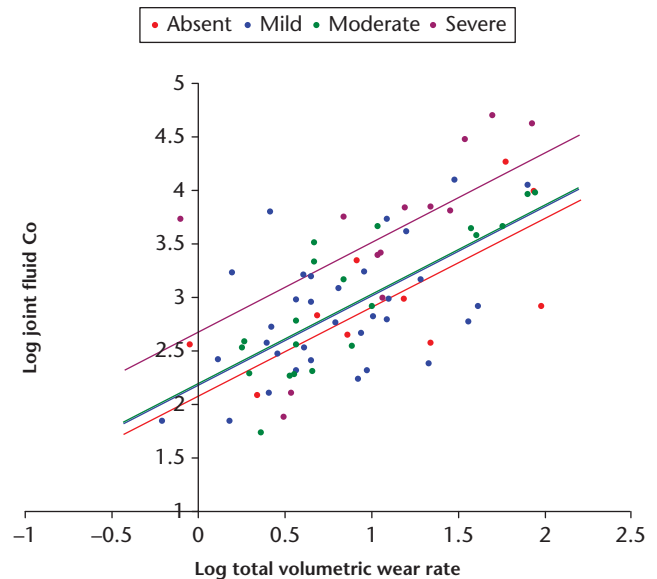


Fig. a

Best-fit regression lines for the relationship between logged values of fluid cobalt (Co) and volumetric wear, for each grade of aseptic lymphocyte-dominated vasculitis-associated lesion in a group of Articular Surface Replacement (ASR; DePuy Synthes, Raynham, Massachusetts) resurfacing patients. Reproduced from **Langton DJ, Sidaginamale RP, Joyce TJ, et al.** Aseptic lymphocyte-dominated vasculitis-associated lesions are related to changes in metal ion handling in the joint capsules of metal-on-metal hip arthroplasties. *Bone Joint Res* 2018;7:388-396.

Afolaranmi et al<sup>3</sup> suggests that it may be more useful to measure red blood cell (RBC) concentrations rather than whole blood when monitoring the levels of Cr. If so, it is important to ensure that the appropriate collection procedure is followed for both Cr and Co, and to determine if there are practical issues relevant to routine monitoring. The aim of this study was to determine the effect of anticoagulant, and the time between collection and separation, on the distribution of Cr and Co in blood fractions. Blood samples were collected in ethylenediaminetetraacetic acid (EDTA), heparin (Hep), and plain tubes. Blood samples were spiked with clinically relevant concentrations of Cr (III), Cr (VI), and Co (II) (0 ng/ml to 40 ng/ml), incubated for 45 minutes, four hours, 24 hours, and 48 hours at room temperature, and the RBCs and plasma/serum separated before the analysis of plasma/serum and RBCs by ICP-MS. Our results for the 45-minute time period before separation of the blood samples and then measurement were consistent with that of Afolaranmi et al.<sup>3</sup> There is now strong evidence that Cr, released as the hexavalent form, partitions predominantly into RBCs. Blood withdrawn into EDTA tubes was observed to have a lower partitioning of Cr (VI) concentration into RBCs compared with the Hep. Co, like Cr (III), is mostly found in the plasma fraction, indicating that Co is firmly bound to the plasma proteins and is poorly transported to the RBCs. No significant difference was found when comparing the results

**Table i.** Red blood cell (RBC) fraction and plasma fraction measurement results for cobalt (II)-spiked blood in presence of ethylenediaminetetraacetic acid (EDTA)

| Time of separation after addition of metal solution; blood collected in EDTA tubes | Concentration, ng/ml | RBC, ng/ml | Plasma, ng/ml |
|--|----------------------|------------|---------------|
| 45 mins  | 0.0                  | 0.42       | 0.47          |
|  | 2.0                  | 0.50       | 3.21          |
|  | 5.0                  | 0.50       | 8.56          |
|  | 10.0                 | 0.64       | 17.60         |
|  | 40.0                 | 1.39       | 68.70         |
| 4 hrs  | 0.0                  | 0.25       | 0.50          |
|  | 2.0                  | 0.39       | 3.57          |
|  | 5.0                  | 0.97       | 9.01          |
|  | 10.0                 | 0.47       | 17.70         |
|  | 40.0                 | 1.31       | 73.30         |
| 24 hrs   | 0.0                  | 0.25       | 0.52          |
|  | 2.0                  | 0.28       | 2.96          |
|  | 5.0                  | 0.44       | 9.16          |
|  | 10.0                 | 0.42       | 17.50         |
|  | 40.0                 | 1.17       | 65.30         |
| 48 hrs   | 0.0                  | 0.31       | 0.53          |
|  | 2.0                  | 0.56       | 5.96          |
|  | 5.0                  | 0.44       | 9.60          |
|  | 10.0                 | 0.53       | 17.80         |
|  | 40.0                 | 1.75       | 75.30         |

**Table ii.** Whole blood measurement results for cobalt (II)-spiked blood in presence of ethylenediaminetetraacetic acid (EDTA)

| Concentration, ng/ml | Measured concentration, ng/ml |
|----------------------|-------------------------------|
| 0.0                  | 0.40                          |
| 2.0                  | 2.13                          |
| 5.0                  | 5.44                          |
| 10.0                 | 9.71                          |
| 40.0                 | 38.20                         |

from the different time periods for Co (II)-, Cr (III)-, and Cr (VI)-spiked blood.

**Aims.** To determine the effect of metal species, anticoagulant, and the time between collection and separation on the distribution of Cr and Co in blood fractions.

**Materials and Methods.** Principle of measurement of Cr and Co by ICP-MS: Co and Cr in serum, plasma, and RBC fractions can be measured by ICP-MS, following dilution of the samples in 0.5% nitric acid diluent. In this method, the samples were diluted 1 in 50 with diluent and germanium (Ge) internal standards added to a final concentration of 1.5 ppb. The samples were assayed against a calibration curve matrix matched with endogenous calf serum.

Blood samples were collected in EDTA, Hep, and plain tubes. Blood samples were spiked with clinically relevant concentrations of Cr (III), Cr (VI), and Co (II) (0 ng/ml to 40 ng/ml), incubated for 45 minutes, four hours, 24 hours, and 48 hours at room temperature, and the RBCs and plasma/serum separated before analysis of plasma/serum and RBCs by ICP-MS.

**Results.** All the RBC values were corrected for haematocrit. Tables i and ii show the distribution of Co (II) into

**Table iii.** Red blood cell fraction (RBC) and plasma fraction measurement results for cobalt (II)-spiked blood in presence of heparin (Hep)

| Time of separation after addition of metal solution; blood collected in Hep tubes | Concentration, ng/ml | RBC, ng/ml | Plasma, ng/ml |
|---|----------------------|------------|---------------|
| 45 mins   | 0.0                  | 0.33       | 0.56          |
|   | 2.0                  | 0.31       | 3.44          |
|   | 5.0                  | 1.17       | 7.90          |
|   | 10.0                 | 1.19       | 17.20         |
|   | 40.0                 | 5.78       | 68.30         |
| 4 hrs   | 0.0                  | 0.36       | 0.70          |
|   | 2.0                  | 0.31       | 3.29          |
|   | 5.0                  | 1.56       | 8.81          |
|   | 10.0                 | 2.14       | 14.70         |
|   | 40.0                 | 5.97       | 68.40         |
| 24 hrs  | 0.0                  | 0.36       | 0.65          |
|   | 2.0                  | 0.25       | 2.98          |
|   | 5.0                  | 1.61       | 8.51          |
|   | 10.0                 | 2.11       | 16.30         |
|   | 40.0                 | 6.11       | 58.30         |
| 48 hrs  | 0.0                  | 0.47       | 0.65          |
|   | 2.0                  | 0.75       | 3.37          |
|   | 5.0                  | 1.72       | 8.64          |
|   | 10.0                 | 2.47       | 16.50         |
|   | 40.0                 | 7.42       | 67.10         |

**Table iv.** Whole blood measurement results for cobalt (II)-spiked blood in presence of heparin

| Concentration, ng/ml | Measured concentration, ng/ml |
|----------------------|-------------------------------|
| 0.0                  | 0.47                          |
| 2.0                  | 2.17                          |
| 5.0                  | 5.39                          |
| 10.0                 | 9.25                          |
| 40.0                 | 39.00                         |

RBCs, plasma, and whole blood in blood stabilized in EDTA. The distribution of Co (II) into RBCs and plasma, and the effect of different times of separation after the addition of metal solution, are also shown. More Co (II) concentration has partitioned into plasma than into RBCs, and there seems to be no effect on the time of separation after addition of Co (II) and the Co (II) concentration partition into plasma or RBCs.

Tables iii and iv show the distribution of Co (II) into RBCs, plasma, and whole blood in blood stabilized in Hep. The distribution of Co (II) into RBCs and plasma, and the effect of different times of separation after the addition of metal solution, are also shown. More Co (II) concentration has partitioned into plasma than into RBCs, and there seems to be no effect on the time of separation after addition of Co (II) and the Co (II) concentration partition into plasma or RBCs (Fig. b). However, a higher partitioning into RBCs was observed in Hep-stabilized blood compared with the EDTA-stabilized blood.

Table v shows the serum measurements for Co (II)-spiked blood in presence of no anticoagulant. The results suggest that minimal Co (II) concentration has partitioned into the RBCs and that most, if not all, of the Co (II) concentration has partitioned into the serum fraction.

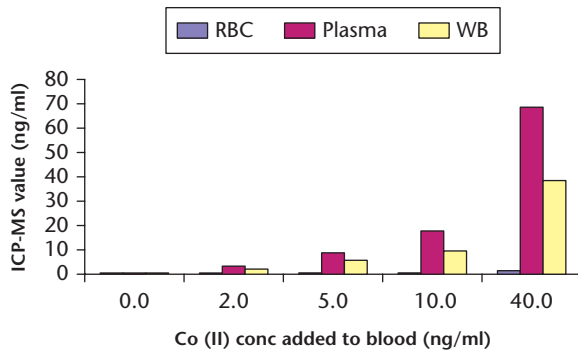


Fig. ba

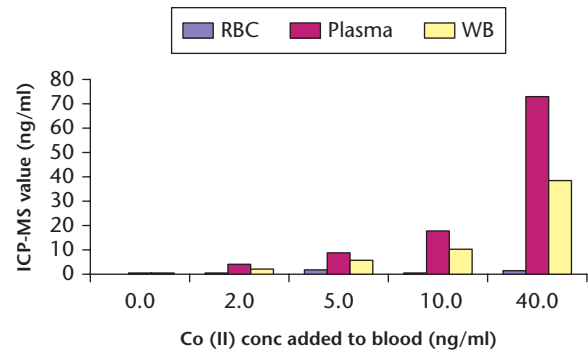


Fig. bb

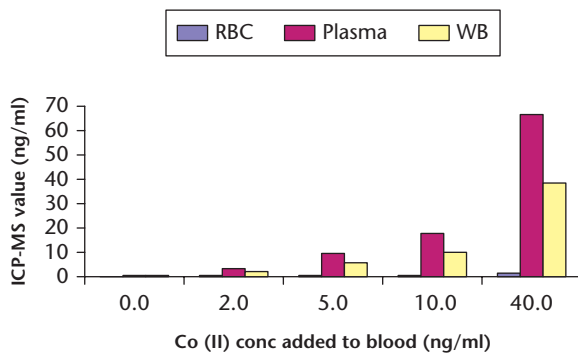


Fig. bc

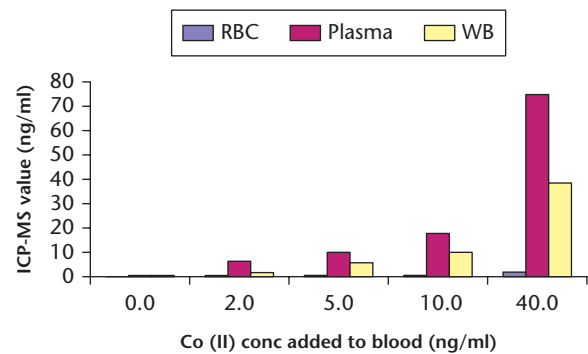


Fig. bd

Concentrations of cobalt (ng/ml) partitioned into blood fractions in the presence of ethylenediaminetetraacetic acid (EDTA) determined by inductively coupled plasma mass spectrometry at: a) 45 minutes; b) four hours; c) 24 hours; and d) 48 hours. RBC, red blood cell; WB, whole blood.

**Table v.** Serum measurement results for cobalt (II)-spiked blood in presence of no anticoagulant

| Concentration, ng/ml | Serum, ng/ml |
|----------------------|--------------|
| 0.0                  | 1.35         |
| 2.0                  | 5.74         |
| 5.0                  | 8.77         |
| 10.0                 | 13.10        |
| 40.0                 | 46.90        |

Tables vi and vii show the distribution of Cr (III) into RBCs, plasma, and whole blood in blood stabilized in EDTA. The distribution of Cr (III) into RBCs and plasma, and the effect of different times of separation after the addition of metal solution, are also shown. More Cr (III) concentration has partitioned into plasma than into RBCs, and there seems to be no effect on time of separation after addition of Co (II) and the Cr (III) concentration partition into plasma or RBCs.

Tables viii and ix show the distribution of Cr (III) into RBCs, plasma, and whole blood in blood stabilized in Hep. The distribution of Cr (III) into RBCs and plasma, and the effect of different times of separation after the addition of metal solution, are also shown (Fig. b). More Cr (III) concentration has partitioned into plasma than into RBCs, and there seems to be no effect on time of separation after addition of Cr (III) and its partition into

**Table vi.** Red blood cell fraction (RBC) and plasma fraction measurement results for chromium (III)-spiked blood in presence of ethylenediaminetetraacetic acid (EDTA)

| Time of separation after addition of metal solution; blood collected in EDTA tubes | Concentration, ng/ml | RBC, ng/ml | Plasma, ng/ml |
|--|----------------------|------------|---------------|
| 45 mins  | 0.0                  | 4.68       | 2.16          |
|  | 2.0                  | 4.73       | 5.24          |
|  | 5.0                  | 4.52       | 10.90         |
|  | 10.0                 | 4.09       | 20.60         |
|  | 40.0                 | 6.61       | 76.60         |
| 4 hrs  | 0.0                  | 4.64       | 1.57          |
|  | 2.0                  | 4.68       | 4.78          |
|  | 5.0                  | 4.50       | 9.40          |
|  | 10.0                 | 4.39       | 20.20         |
|  | 40.0                 | 6.34       | 75.10         |
| 24 hrs   | 0.0                  | 4.27       | 1.73          |
|  | 2.0                  | 4.59       | 5.12          |
|  | 5.0                  | 5.07       | 10.80         |
|  | 10.0                 | 4.48       | 19.60         |
|  | 40.0                 | 6.20       | 74.60         |
| 48 hrs   | 0.0                  | 4.25       | 1.62          |
|  | 2.0                  | 3.57       | 4.94          |
|  | 5.0                  | 4.25       | 7.12          |
|  | 10.0                 | 4.59       | 21.40         |
|  | 40.0                 | 6.70       | 76.70         |

plasma or RBCs. However, a higher partitioning into RBCs was observed in Hep-stabilized blood compared with the EDTA-stabilized blood. Figures c and d show the results for Cr (III)-spiked blood plotted on a chart.

**Table vii.** Whole blood measurement results for chromium (III)-spiked blood in presence of ethylenediaminetetraacetic acid (EDTA)

| Concentration, ng/ml | Measured concentration, ng/ml |
|----------------------|-------------------------------|
| 0.0                  | -0.06                         |
| 2.0                  | 4.43                          |
| 5.0                  | 7.44                          |
| 10.0                 | 12.60                         |
| 40.0                 | 40.80                         |

**Table viii.** Red blood cell fraction (RBC) and plasma fraction measurement results for chromium (III)-spiked blood in presence of heparin (Hep)

| Time of separation after addition of metal solution; blood collected in Hep tubes | Concentration, ng/ml | RBC, ng/ml | Plasma, ng/ml |
|---|----------------------|------------|---------------|
| 45 mins   | 0.0                  | 4.55       | 1.81          |
|   | 2.0                  | 5.14       | 5.20          |
|   | 5.0                  | 4.48       | 9.81          |
|   | 10.0                 | 9.52       | 14.90         |
|   | 40.0                 | 8.86       | 66.70         |
| 4 hrs   | 0.0                  | 4.57       | 1.41          |
|   | 2.0                  | 4.34       | 4.94          |
|   | 5.0                  | 4.50       | 10.60         |
|   | 10.0                 | 6.25       | 17.60         |
|   | 40.0                 | 9.25       | 70.40         |
| 24 hrs  | 0.0                  | 9.66       | 1.91          |
|   | 2.0                  | 4.59       | 5.30          |
|   | 5.0                  | 4.34       | 11.00         |
|   | 10.0                 | 6.25       | 18.40         |
|   | 40.0                 | 8.30       | 71.30         |
| 48 hrs  | 0.0                  | 5.70       | 1.03          |
|   | 2.0                  | 5.09       | 4.54          |
|   | 5.0                  | 4.70       | 11.00         |
|   | 10.0                 | 6.18       | 18.40         |
|   | 40.0                 | 8.16       | 71.90         |

Table x shows the serum measurements for Cr (III)-spiked blood in presence of no anticoagulant. The results suggest that minimal Cr (III) concentration has partitioned into the RBCs and that most, if not all, of the Cr (III) concentration has partitioned into the serum fraction.

Tables xi and xii show the distribution of Cr (VI) into RBCs, plasma, and whole blood in blood stabilized in EDTA. The distribution of Cr (VI) into RBCs and plasma, and the effect of different times of separation after the addition of metal solution, are also shown. More Cr (VI) concentration has partitioned into plasma than into RBCs, and there seems to be no effect on time of separation after addition of Co (II) and the Cr (III) concentration partition into plasma or RBCs. However, a higher proportion of Cr (VI) has partitioned into the RBCs compared with plasma than Co (II) and Cr (III), confirming that Cr (VI) more readily enters the RBCs than Co (II) and Cr (III).

Tables xiii and xiv shows the distribution of Cr (VI) into RBCs, plasma, and whole blood in blood stabilized in Hep. The distribution of Cr (VI) into RBCs and plasma, and the effect of different times of separation after the addition of metal solution, are also shown. More Cr (VI) concentration has partitioned into RBC fraction than into

**Table ix.** Whole blood measurement results for chromium (III)-spiked blood in presence of heparin (Hep)

| Concentration, ng/ml | Measured concentration, ng/ml |
|----------------------|-------------------------------|
| 0.0                  | 2.22                          |
| 2.0                  | 4.43                          |
| 5.0                  | 7.38                          |
| 10.0                 | 12.50                         |
| 40.0                 | 39.50                         |

plasma, and there seems to be no effect on time of separation after addition of Cr (III) and its partition into plasma or RBCs. However, a higher partitioning into RBCs was observed in Hep-stabilized blood compared with the EDTA-stabilized blood. Figures e and f show the results for Cr (VI)-spiked blood in chart form.

Table xv shows the serum measurements for Cr (VI)-spiked blood in presence of no anticoagulant. The results suggest that minimal Cr (III) concentration has partitioned into the RBCs and that most, if not all, of the Cr (III) concentration has partitioned into the serum fraction for concentration 0 ng/ml to 10 ng/ml. At 40 ng/ml, there is a much clearer suggestion of Cr (VI) concentration partitioning into the RBCs.

### Discussion

Our results for the 45-minute time period before separation of the blood samples and then measurement were consistent with those of Afolaranmi et al.<sup>3</sup> There is now strong evidence that Cr, released as the hexavalent form, partitions predominantly into RBCs. Cr (VI) readily binds to and crosses cell membranes into cells, where it is rapidly reduced to the less biologically active Cr (III).<sup>2</sup> Cr (III) does not partition as much as Cr (VI) into RBCs and is found mostly in the plasma fraction. Cr (III) is significantly bound to plasma proteins and is poorly transported across the cell membrane into the RBCs.<sup>2</sup>

In addition to Cr (VI) being predominantly partitioned into the RBC compared with the plasma fraction of blood, the extent of partitioning depends on the choice of anticoagulant into which the blood is withdrawn. Blood withdrawn into EDTA tubes was observed to have a lower partitioning of Cr (VI) concentration into RBCs compared with Hep. This difference may mean that everyone should use one type of anticoagulant to collect the blood to avoid these discrepancies if measuring plasma or RBCs fractions.

Co, like Cr (III) is mostly found in the plasma fraction, indicating that Co is firmly bound to the plasma proteins and is poorly transported to the RBCs.

In our study, we can conclude that the time of storage (45 minutes to 48 hours) has no effect on the distribution of the metal concentrations into the plasma and RBC fractions. No significant difference was found when comparing the results from the different time periods for Co (II), Cr (III), and Cr (VI).

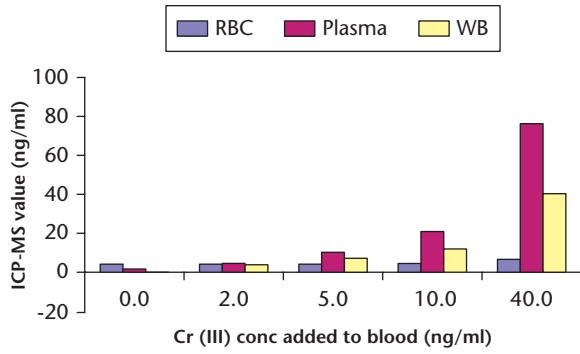


Fig. ca

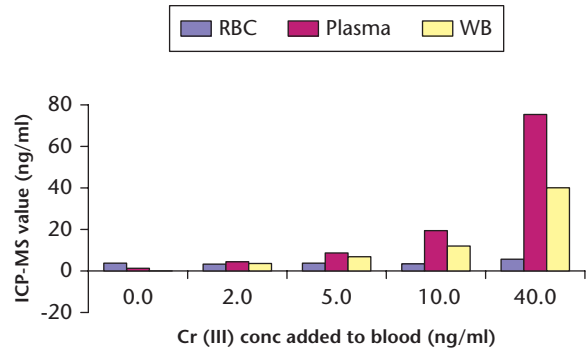


Fig. cb

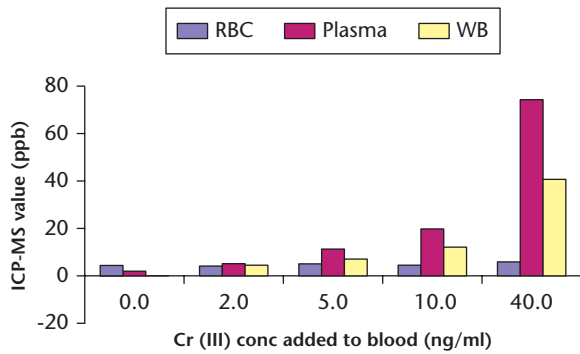


Fig. cc

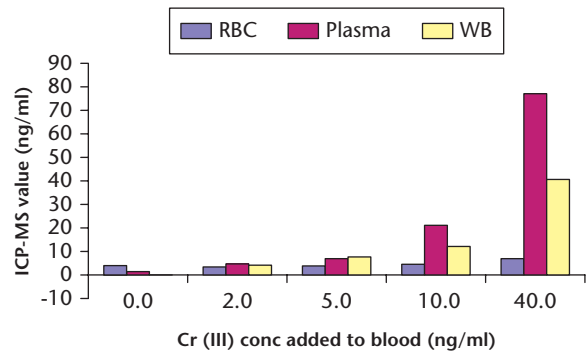


Fig. cd

Concentrations of chromium (III) (ng/ml) partitioned into blood fractions in the presence of ethylenediaminetetraacetic acid (EDTA) determined by inductively coupled plasma mass spectrometry at: a) 45 minutes; b) four hours; c) 24 hours; and d) 48 hours. RBC, red blood cell; WB, whole blood.

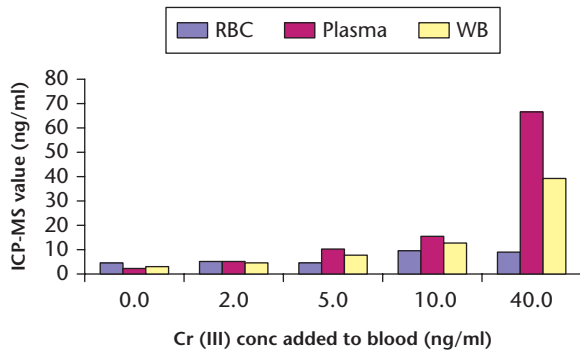


Fig. da

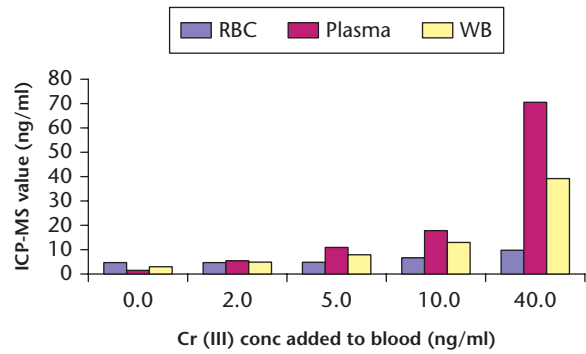


Fig. db

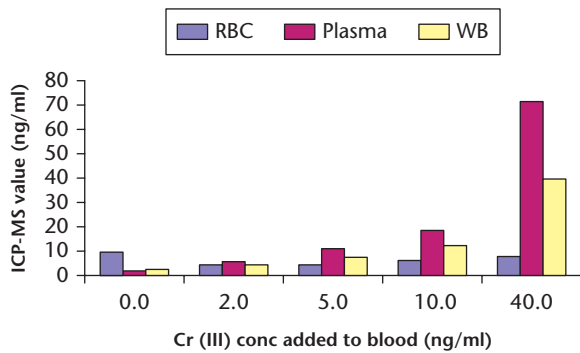


Fig. dc

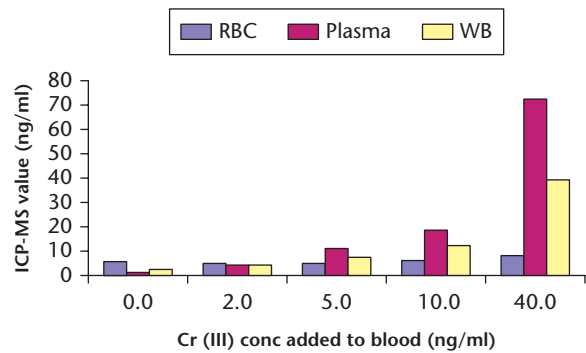


Fig. dd

Concentrations of chromium (III) (ng/ml) partitioned into blood fractions in the presence of heparin determined by inductively coupled plasma mass spectrometry at: a) 45 minutes; b) four hours; c) 24 hours; and d) 48 hours. RBC, red blood cell; WB, whole blood.

**Table x.** Serum measurement results for chromium (III)-spiked blood in presence of no anticoagulant

| Concentration, ng/ml | Serum, ng/ml |
|----------------------|--------------|
| 0.0                  | 1.66         |
| 2.0                  | 6.11         |
| 5.0                  | 10.00        |
| 10.0                 | 21.20        |
| 40.0                 | 50.40        |

**Table xi.** Red blood cell fraction (RBC) and plasma fraction measurement results for chromium (VI)-spiked blood in presence of ethylenediaminetetraacetic acid (EDTA)

| Time of separation after addition of metal solution; blood collected in EDTA tubes | Concentration, ng/ml | RBC, ng/ml | Plasma, ng/ml |
|--|----------------------|------------|---------------|
| 45 mins  | 0.0                  | 4.33       | 1.33          |
|  | 2.0                  | 5.56       | 3.06          |
|  | 5.0                  | 8.44       | 6.08          |
|  | 10.0                 | 9.62       | 10.70         |
|  | 40.0                 | 23.41      | 33.70         |
| 4 hrs  | 0.0                  | 3.97       | 1.29          |
|  | 2.0                  | 5.03       | 3.33          |
|  | 5.0                  | 6.08       | 5.71          |
|  | 10.0                 | 8.82       | 10.90         |
|  | 40.0                 | 22.36      | 38.00         |
| 24 hrs   | 0.0                  | 3.46       | 1.40          |
|  | 2.0                  | 4.85       | 3.08          |
|  | 5.0                  | 6.36       | 6.22          |
|  | 10.0                 | 8.46       | 10.80         |
|  | 40.0                 | 18.64      | 34.90         |
| 48 hrs   | 0.0                  | 4.23       | 1.30          |
|  | 2.0                  | 6.08       | 3.43          |
|  | 5.0                  | 6.08       | 6.17          |
|  | 10.0                 | 8.44       | 11.30         |
|  | 40.0                 | 28.97      | 37.50         |

**Table xii.** Whole blood measurement results for chromium (VI)-spiked blood in presence of ethylenediaminetetraacetic acid (EDTA)

| Concentration, ng/ml | Measured concentration, ng/ml |
|----------------------|-------------------------------|
| 0.0                  | 2.57                          |
| 2.0                  | 3.81                          |
| 5.0                  | 6.02                          |
| 10.0                 | 10.10                         |
| 40.0                 | 30.60                         |

The distribution of the Co concentration was not the same across the concentration range (0 ng/ml to 40 ng/ml) for the EDTA-collected blood. The ratio of RBC to plasma went down as the concentration of Co (II) added to blood sample went up. The ratio did not have any linear decline. This pattern was the same for Cr (III)- and Cr (VI)-spiked blood samples. The RBC to plasma ratios were higher for Cr (VI)- than for Cr (III)- and Co (II)-spiked blood. The RBC to

**Table xiii.** Red blood cell fraction (RBC) and plasma fraction measurement results for chromium (VI)-spiked blood in presence of heparin (Hep)

| Time of separation after addition of metal solution; blood collected in heparin tubes | Concentration, ng/ml | RBC, ng/ml | Plasma, ng/ml |
|---|----------------------|------------|---------------|
| 45 mins   | 0.0                  | 4.51       | 1.91          |
|   | 2.0                  | 7.26       | 2.25          |
|   | 5.0                  | 10.69      | 3.46          |
|   | 10.0                 | 18.38      | 5.06          |
|   | 40.0                 | 60.00      | 19.50         |
| 4 hrs   | 0.0                  | 4.46       | 1.10          |
|   | 2.0                  | 6.77       | 2.16          |
|   | 5.0                  | 13.33      | 3.63          |
|   | 10.0                 | 17.18      | 5.28          |
|   | 40.0                 | 60.26      | 18.60         |
| 24 hrs  | 0.0                  | 3.08       | 1.83          |
|   | 2.0                  | 6.05       | 3.06          |
|   | 5.0                  | 9.26       | 3.52          |
|   | 10.0                 | 15.95      | 7.07          |
|   | 40.0                 | 46.15      | 17.90         |
| 48 hrs  | 0.0                  | 3.00       | 0.71          |
|   | 2.0                  | 5.64       | 2.26          |
|   | 5.0                  | 8.90       | 4.40          |
|   | 10.0                 | 14.15      | 5.18          |
|   | 40.0                 | 48.46      | 18.20         |

**Table xiv.** Whole blood measurement results for chromium (VI)-spiked blood in presence of heparin

| Concentration, ng/ml | Measured concentration, ng/ml |
|----------------------|-------------------------------|
| 0.0                  | 2.55                          |
| 2.0                  | 3.95                          |
| 5.0                  | 5.72                          |
| 10.0                 | 9.56                          |
| 40.0                 | 32.10                         |

plasma ratios were higher for Cr (III)- than for Co (III)-spiked blood.

The distribution of the Co concentration was not the same across the concentration range (0 ng/ml to 40 ng/ml) for the Hep-collected blood. The ratio of RBC to plasma went down as the concentration of Co (II) added to the blood sample went up. The ratio did not have any linear decline. This pattern was the same for Cr (III)- and Cr (VI)-spiked blood samples. The RBC to plasma ratios were higher for Cr (VI)- than for Cr (III)- and Co (II)-spiked blood. The RBC to plasma ratios were higher for Cr (III)- than for Co (III)-spiked blood. The only significant difference between EDTA and Hep-collected blood is that of the ratio for the Cr (VI). The ratios were exceedingly higher due to the Cr (VI) concentration partitioning significantly more into the RBC fraction than the plasma fraction.

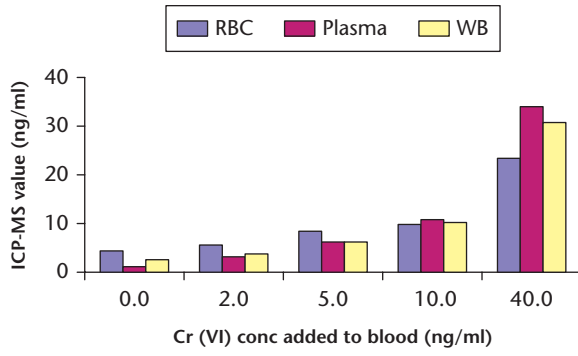


Fig. ea

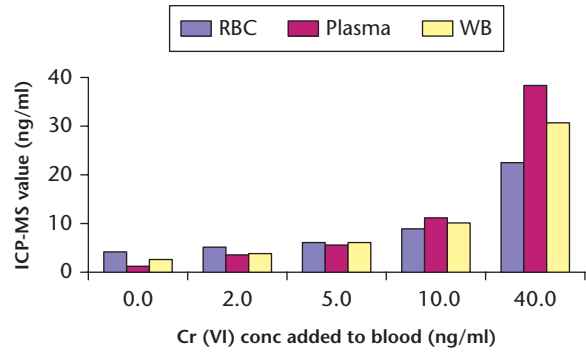


Fig. eb

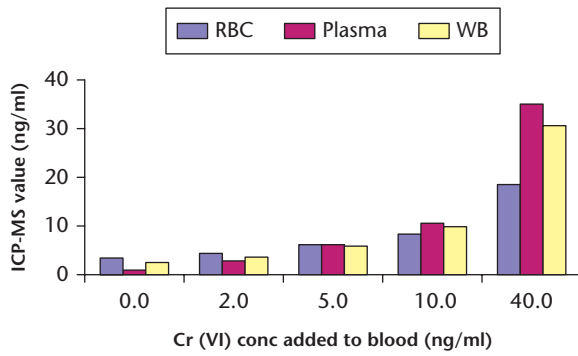


Fig. ec

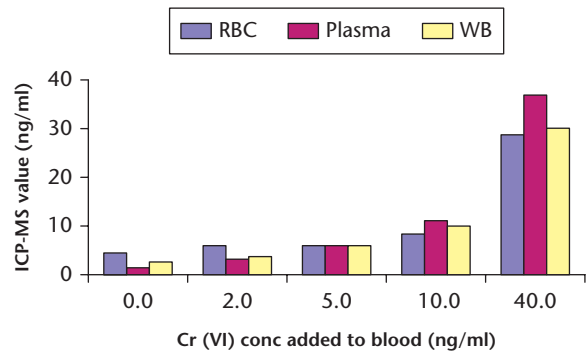


Fig. ed

Concentrations of chromium (VI) (ng/ml) partitioned into blood fractions in the presence of ethylenediaminetetraacetic acid (EDTA) determined by inductively coupled plasma mass spectrometry at: a) 45 minutes; b) four hours; c) 24 hours; and d) 48 hours. RBC, red blood cell; WB, whole blood.

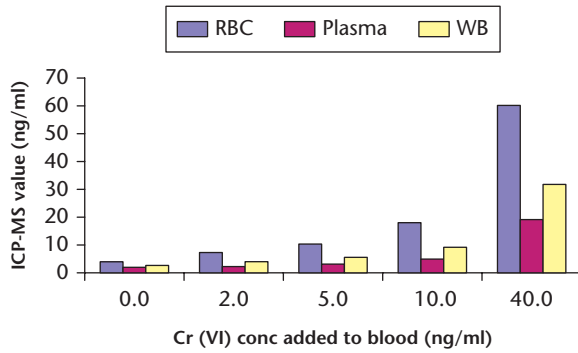


Fig. fa

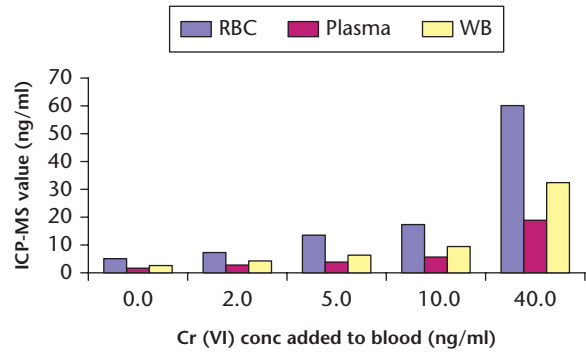


Fig. fb

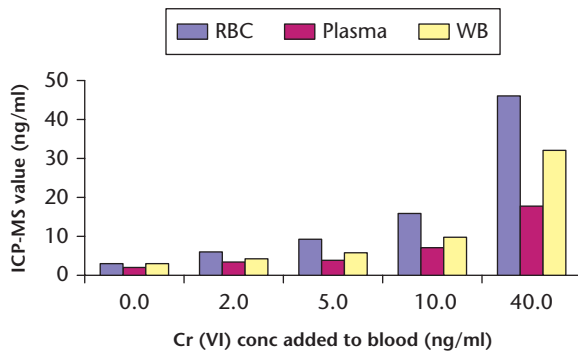


Fig. fc

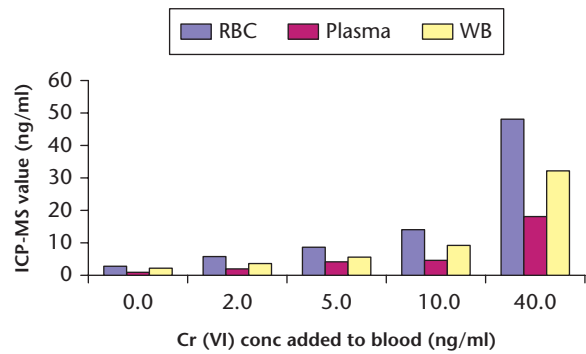


Fig. fd

Concentrations of Cr (VI) (ng/ml) partitioned into blood fractions in the presence of heparin determined by inductively coupled plasma mass spectrometry at: a) 45 minutes; b) four hours; c) 24 hours; and d) 48 hours. RBC, red blood cell; WB, whole blood.

**Table xv.** Serum measurement results for chromium (VI)-spiked blood in presence of no anticoagulant

| Concentration, ng/ml | Serum, ng/ml |
|----------------------|--------------|
| 0.0                  | 1.23         |
| 2.0                  | 3.41         |
| 5.0                  | 5.49         |
| 10.0                 | 13.00        |
| 40.0                 | 26.00        |

**Table xvi.** The red blood cell (RBC) to plasma ratios for cobalt (II)-, chromium (III)-, and chromium (VI)-spiked blood (in ethylenediaminetetraacetic acid (EDTA) and heparin (Hep)), and after 45 minutes before separation of plasma and RBC fraction

| Time of separation after addition of metal solution | Concentration, ng/ml | RBC, ppb | Plasma, ppb | RBC:plasma |
|---|----------------------|----------|-------------|------------|
| Co (II) EDTA, 45 mins                               | 0.0                  | 0.42     | 0.47        | 0.887      |
|   | 2.0                  | 0.50     | 3.21        | 0.156      |
|   | 5.0                  | 0.50     | 8.56        | 0.058      |
|   | 10.0                 | 0.64     | 17.60       | 0.036      |
|   | 40.0                 | 1.39     | 68.70       | 0.020      |
| Co (II) Hep, 45 mins                                | 0.0                  | 0.33     | 0.56        | 0.595      |
|   | 2.0                  | 0.31     | 3.44        | 0.089      |
|   | 5.0                  | 1.17     | 7.90        | 0.148      |
|   | 10.0                 | 1.19     | 17.20       | 0.069      |
|   | 40.0                 | 5.78     | 68.30       | 0.085      |
| Cr (III) EDTA, 45 mins                              | 0.0                  | 4.68     | 2.16        | 2.168      |
|   | 2.0                  | 4.73     | 5.24        | 0.902      |
|   | 5.0                  | 4.52     | 10.90       | 0.415      |
|   | 10.0                 | 4.09     | 20.60       | 0.199      |
|   | 40.0                 | 6.61     | 76.60       | 0.086      |
| Cr (III) Hep, 45 mins                               | 0.0                  | 4.55     | 1.81        | 2.51       |
|   | 2.0                  | 5.14     | 5.20        | 0.99       |
|   | 5.0                  | 4.48     | 9.81        | 0.46       |
|   | 10.0                 | 9.52     | 14.90       | 0.64       |
|   | 40.0                 | 8.86     | 66.70       | 0.13       |
| Cr (VI) EDTA, 45 mins                               | 0.0                  | 4.33     | 1.33        | 3.26       |
|   | 2.0                  | 5.56     | 3.06        | 1.82       |
|   | 5.0                  | 8.44     | 6.08        | 1.39       |
|   | 10.0                 | 9.62     | 10.70       | 0.90       |
|   | 40.0                 | 23.41    | 33.70       | 0.69       |
| Cr (VI) Hep, 45 mins                                | 0.0                  | 4.51     | 1.91        | 2.36       |
|   | 2.0                  | 7.26     | 2.25        | 3.23       |
|   | 5.0                  | 10.69    | 3.46        | 3.09       |
|   | 10.0                 | 18.38    | 5.06        | 3.63       |
|   | 40.0                 | 60.00    | 19.50       | 3.08       |

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