INTRODUCTION
Metal ion levels are used as an indirect estimate of wear and as a measure of systemic metal exposure in metal-on-metal (MM) bearing arthroplasty. Different specimens including urine, whole blood (WB), serum, plasma and erythrocyte levels have been used with various measurement techniques in different studies. In the blood, distribution of cobalt and chromium between the cells and the plasma is not uniform. The reliability of using blood fractions such as plasma and erythrocyte metal ion levels, as measures of systemic metal ion exposure, rests on whether individual variability in metal distribution in whole blood and its different fractions is within acceptable limits. Sufficient data has so far not been available to test this variability in different blood fractions. The present report is an investigation of the distribution of metal ions in concurrent specimens of WB, plasma and erythrocytes.

MATERIALS AND METHODS
In order to capture the entirely clinically relevant range, we obtained 461 concurrent specimens of whole blood, plasma and erythrocytes from a heterogeneous group of patients with large and small diameter MM hip arthroplasties after obtaining informed consent. High resolution inductively coupled plasma mass spectrometry (HRICPMS) analysis was used. 41 specimens were excluded because either cobalt or chromium was at or below the limit of detection. There were 311 male patients and 109 female patients. Of these, 276 had unilateral MM arthroplasty and 129 had bilateral and 15 were preoperative specimens. Mean age was 54 years (19-75), and mean BMI 27 (17 - 56). Agreement was assessed with scatter plots, mean differences and Bland and Altman limits of agreement.

Specimen collection was performed with a contamination-prevention protocol. Plasma and erythrocyte specimens were obtained by centrifuging whole blood at 4000 rpm for 10 minutes. The plasma layer is transferred into microtubes anduffy zone discarded. The remaining cells are mixed for ten minutes in 0.9% saline and centrifuged again for 5 minutes. The washed erythrocytes are collected in microtubes and stored frozen.

The variability between plasma, erythrocytes and whole blood levels were studied through the mean differences and scatter. Agreement was tested against whole blood levels, using the Bland limits of agreement. In order to view the relative distribution of cobalt and chromium in the plasma and erythrocytes at different levels of exposure, the cohort was subdivided into three subgroups based on their whole blood levels (< 1 μg/l, 1 to < 2, 2 to <3 and 3 or more μg/l). A p value of <0.05 was considered significant and all confidence intervals (c.i.) are quoted at the 95% level.

RESULTS
Compared to whole blood levels, the mean differences with plasma and erythrocyte concentrations were statistically significant for both cobalt (p<0.001), and chromium (p<0.0001). The scatter showed that the variability is worse with chromium compared to cobalt and that the variability of plasma chromium was worse at lower levels and that of erythrocytes was worse at higher levels. Bland analyses of whole blood and plasma showed that the limits of agreement extended from -6.2 to 75.7% for cobalt and -146 to 127% for chromium and -55% to 48.7% for cobalt and -59.8% to 56% for chromium in erythrocytes and plasma respectively.

Mean plasma and erythrocyte concentrations of cobalt and chromium were studied in the smaller cohorts as described above. With regards to the distribution of cobalt, erythrocyte levels show a slow but steadily increasing trend with increasing levels of whole blood or plasma concentration, progressively increasing from 0.3μg/l in the lowest subgroup to 2.64μg/l in the highest subgroup. Mean cobalt concentration in erythrocytes (expressed as a percentage of the sum of the mean concentrations in plasma and erythrocytes) is 28% in the lower level and stays at 29% in the subgroup with the highest level, showing only a minor variation (between 27 and 29%) throughout the range.

With regards to chromium distribution, there is no increase in the mean erythrocyte chromium with increasing chromium levels in whole blood. The mean concentration of erythrocyte chromium shows little change (0.5 to 0.65 μg/l) through the whole range. Mean chromium concentration in erythrocytes is 28% of the sum of the mean concentrations in plasma and erythrocytes in the lower range (similar to the percentage of cobalt) but progressively drops to 17%, 10% and 7% as the whole blood concentration progressively increases.

DISCUSSION
In the past, constraints in instrument sensitivity limited metal ion analysis to blood fractions such as serum or plasma. With better current instruments whole blood analysis is possible. Attempting to understand the nature of systemic exposure from MM arthroplasty; and to comprehend the in vivo kinetics of metal release, transport and clearance, from a study of one of the blood fractions has led to erroneous conclusions in the past.

The perceived wisdom with respect to chromium has been that it tends to get accumulated in the cells whilst cobalt is associated with extracellular fluid such as plasma. The mean differences in the sub-groups above, with respect to chromium highlight an important in vivo observation that has not been described before. It shows that, at higher blood levels, erythrocyte chromium does not rise with increasing concentrations in whole blood or plasma.

This is the first time this finding has been observed and suggests one of two conclusions: a) that most of the chromium produced by these bearings is of the trivalent form and being less soluble it is unable to pass into the cells. This is contrary to what has been known and described earlier that the chromium released from orthopaedic implants was mainly cell-associated and predominantly hexavalent; OR b) that there is a finite limit beyond which chromium of any valency cannot pass into the cells. Above this ceiling any increase in blood level does not lead to a proportionate increase in erythrocyte chromium.

The wide variability in the normalized scatter as well as on the Bland and Altman plots suggests that the hypotheses of agreement for both metal ions is rejected both on the basis of mean differences and the very wide limits of agreement. The finding that erythrocyte chromium does not increase linearly with an increase in either plasma or whole blood and the fact that there appears to be a cellular ceiling, beyond which metal ions remain extracellular makes erythrocyte levels particularly unsuitable as markers of systemic metal exposure.

SUMMARY AND CONCLUSION
Plasma and erythrocytes are not suitable measures of systemic metal ion exposure following hip arthroplasty with respect to both cobalt and chromium. Passage of chromium into the erythrocytes is limited by a natural ceiling, wherefore non-elevation of erythrocyte chromium cannot be taken as an indicator of reduced systemic chromium exposure.

REFERENCES
1 MacDonald SJ, Brodner W, Jacobs JJ. A consensus paper on metal ions in metal-on-metal hip arthroplasties. J Arthro 2004;19,8 Suppl:12-16