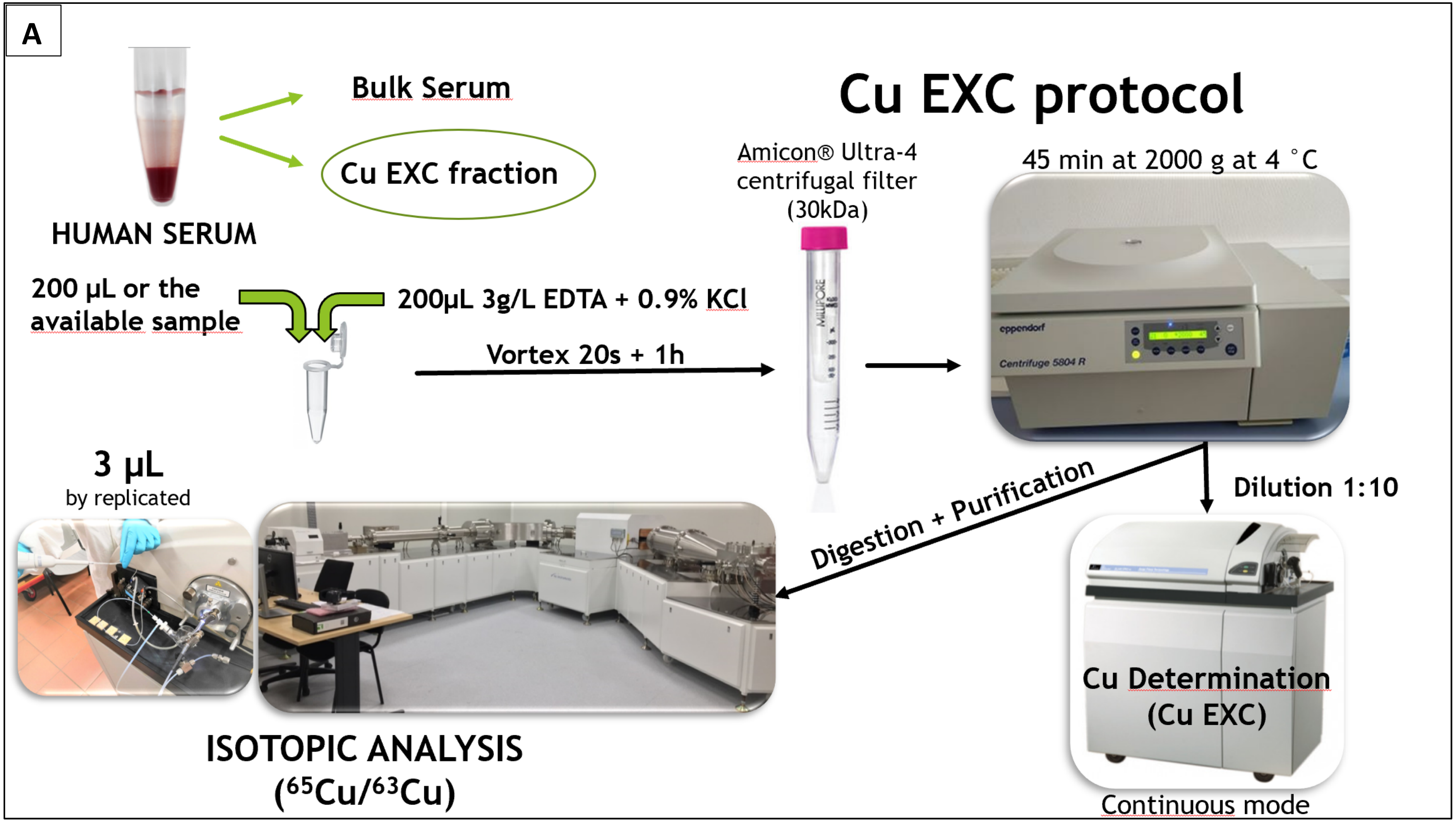
A
HUMAN SERUM
Bulk Serum
Cu EXC fraction
Cu EXC protocol
200 µL or the available sample
200µL 3g/L EDTA + 0.9% KCl
Vortex 20s + 1h
Amicon® Ultra-4 centrifugal filter (30kDa)
45 min at 2000 g at 4 °C
Dilution 1:10
Digestion + Purification
3 µL
by replicated
ISOTOPIC ANALYSIS
(65Cu/63Cu)
Cu Determination
(Cu EXC)
Continuous mode

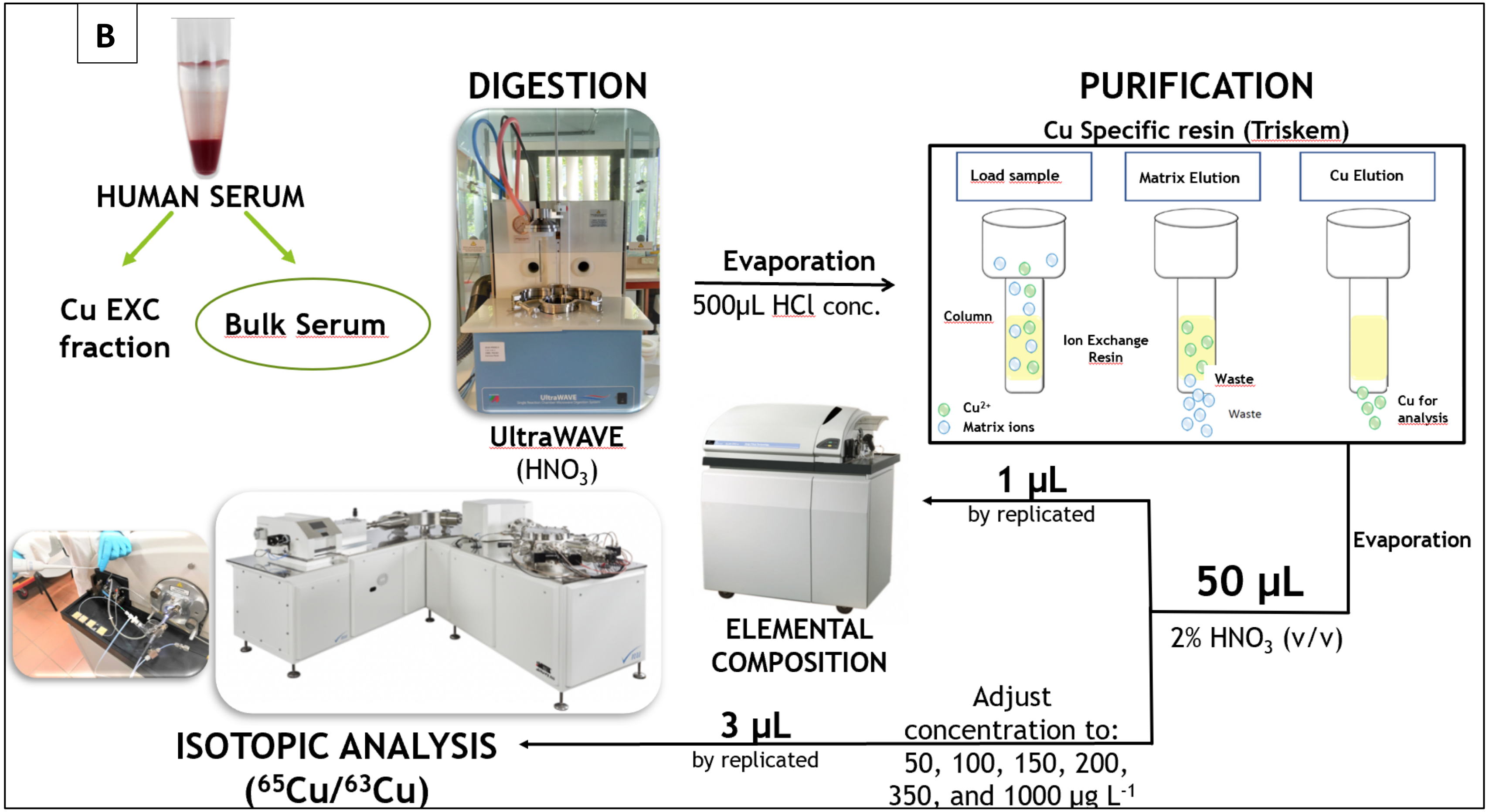


**Figure S1.** Schematics of the procedures for sample preparation for: A) Cu EXC fraction; B) Bulk fraction.

**Calculation of isotope ratios**

For mass bias correction, a combination of internal normalization according to the exponential model described by Maréchal *et al.*[41] using the $^{62}Ni/^{60}Ni$ ratio, following the equation (1S) and (2S) and the standard-sample-standard bracketing sequence with the NIST SRM 3114 were used.

$$R_{Cu} = r_{Cu} * \frac{1}{\left(\frac{65}{63}\right)^{f}} \quad (1S)$$

Where $R_{Cu}$ is the real $^{65}Cu/^{63}Cu$ isotope ratio, $r_{Cu}$ is the measured isotope ratio and f is the exponential fractionation coefficient calculated following the equation (2S)

$$f = \frac{ln\frac{r_{Ni}}{R_{Ni}}}{ln\frac{62}{60}} \quad (2S)$$

Where $R_{Ni}$ is the real of $^{62}Ni/^{60}Ni$ value and $r_{Ni}$ is the measured value (calculated by LRS).

Finally, the results were expressed as delta values (δ), *i.e.*, as a relative difference (in per mil) versus a reference (NIST SRM 3114) following equation (3S):

$$\delta^{65}Cu\ (‰) = \frac{(R_{sample} - R_{STD})}{R_{STD}} * 1000 \quad (3S)$$

where $R_{sample}$ is the $^{65}Cu/^{63}Cu$ isotope ratio determined for a given sample and $R_{STD}$ is the average $^{65}Cu/^{63}Cu$ isotope ratio determined for the standards measured before and after that particular sample.

**Table S1.** Protocol for Cu isolation with the anion exchange Cu specific resin (Triskem).

| Step | Volume (mL) | Medium |
|---|---|---|
| Resin loading | 0.5 | Cu-specific |
| Cleaning | 10 | 6 mol $L^{-1}$ HCl |
| Conditioning | 10 | 5 mmol $L^{-1}$ HCl |
| Sample load | 4 | 5 mmol $L^{-1}$ HCl |
| Matrix elution | 40 | 5 mmol $L^{-1}$ HCl |
| Cu elution | 10 | 6 mol $L^{-1}$ HCl |