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Nitrile, Latex, Neoprene and Vinyl Gloves: A Primary Source of Contamination for Trace Element and Zn Isotopic Analyses in Geological and Biological Samples

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Exogenic contamination is of primary concern for geochemical and biological clean laboratories working with sample sizes at the nanogram or even sub-picogram level. Here we determined sixty trace elements in fifteen different types of gloves from major suppliers worldwide to evaluate whether gloves could be potential sources of contamination for routine trace element and isotope measurements. We found that all gloves contain some trace elements that can be easily mobilised in significant amounts. In weak acid at room temperature, the tested gloves released up to 17 mg of Zn, more than 1 µg of Mg, Ti, Mn, Fe, Rb, Sr, Zr, Sn, Hf and Pb and between 100 and 1000 ng of Li, Sc, V, Cr, Ni, Cu, Ga, As, Se, Y, Ag, Ba, La, Ce, Nd, Tl and Th. Vinyl gloves released lower quantities of biologically and geologically important elements, with the exception of In and Sn. Isotopic analyses indicate that all gloves share roughly the same Zn isotopic composition (average $\delta^{66}\text{Zn} = +0.10 \pm 0.32\text{‰}$ (2s)). A single contact between glove and labware releases an average of ~ 6 ng of Zn, and hence can significantly shift $\delta^{66}\text{Zn}$ above the precision level when the amount of Zn determined is below 500 ng.

Keywords: gloves, contamination, zinc, isotopic determinations, trace elements.

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Trace element contents and Zn isotopic compositions are used in various research fields such as Earth and planetary sciences, biology and, more recently, medicine. The level of precision and sensitivity of such measurements has significantly increased through time mainly due to technological improvements in mass spectrometry. To date, routine analyses of Zn isotopic compositions and trace element contents reach an average precision of $\pm 0.1\text{‰}$ (2s) and $\pm 5\%$ (2s), respectively, with sample sizes as low as the nanogram to the low picogram range. To obtain accurate results, following the best analytical procedures is necessary, in particular control of exogenic contamination during sample preparation and analysis. In this context, most geochemical and biological clean laboratories carefully acid-wash all pipette tips, tubes, and other labware, and use ultra-pure water and acids that they distil themselves. While several precautions, such as measuring laboratory blanks in reagents, are taken to

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evaluate contamination levels, less attention has been given to less direct sources of surface contamination as well as their potential impact on measurement quality. Three decades ago, Hoffmann (1988) mentioned that the sources of contamination were often where one least expects to find them, for example in labware such as gloves, pipette tips, lab wipes, polyethylene bottles and spoons; an assumption that was confirmed twelve years later by Friel *et al.* (1996). Friel *et al.* (1996) found that gloves were highly enriched in several elements such as Zn, Fe and Se and recommended wearing vinyl gloves that were acid-washed before use for routine trace element work. In their study, Friel *et al.* (1996) report only approximately twenty-five trace elements in brands of latex and vinyl gloves that are mostly not used anymore in clean laboratories. The glove market has indeed significantly evolved and diversified through the past two decades. Several new types of gloves, made for example of nitrile or neoprene, are now commonly used in biological and geochemical laboratories worldwide. In addition, the scientific community has been interested in studying an increasing diversity of chemical and isotopic compositions highlighting the need to review and re-assess the risk of contamination associated with the use of recent laboratory gloves on a wide range of trace and minor elements.

In this paper, we quantitatively evaluate the risk of contamination caused by gloves on sixty trace elements commonly measured in Earth and planetary sciences, biology and medicine. We tested fifteen different types of gloves made of vinyl, neoprene, nitrile or latex from different suppliers such as Kimberley-Clark[®], Ansell[®] or Medline[®]. Since Zn is by far the most enriched trace element in gloves (Friel *et al.* 1996), we also determined their Zn isotopic compositions (henceforth referred as $\delta^{66}\text{Zn}$) to see whether glove contamination could significantly shift Zn isotopic compositions of geological and biological samples. The aims of the study are thus multiple: (1) to determine whether the gloves currently used in clean laboratories are still a major source of contamination for trace element and isotopic work; (2) which gloves offer lower levels of contamination; (3) to quantify the amount of each element that can be released from gloves into samples during preparation and analytical processes and pinpoint which elements are most susceptible to contamination; (4) to quantify the effect of glove contamination on Zn isotopic compositions of geological and biological samples.

Tested gloves and analytical methods

Tested gloves

The gloves tested in this study are among the most often used in geochemical and biological clean laboratories. These include nitrile, vinyl, neoprene and latex powder-free gloves from ten main suppliers worldwide: Kimberley Clark[®], Ansell[®], Polysem Medical[®], LCH medical[®], Microflex[®], Showa[®], Clean-Dex[®], Jet[®] and Medline[®]. Further details on the models, colours, packaging and reference numbers of the tested gloves are provided in Table 1. To avoid sample bias and allow a direct comparison between all analyses, we tested M size (7.5"–8.5") gloves only. All gloves were taken from unopened boxes to avoid external contamination. Complete duplicates were analysed for three gloves (a vinyl, a nitrile and a neoprene glove) to assess the repeatability of the results and the measurement precision.

Materials and analytical conditions

All tests were performed in a clean laboratory below laminar flow clean hoods using ultra-pure water at 18.2 MΩ cm resistivity and acids that were distilled to ensure low trace element contents.

Polypropylene tubes and pipette tips used all through the analytical procedure were not acid-washed before use to avoid any contamination by gloves worn during cleaning procedure. However, blanks were systematically run to quantify detection limits and integrate potential contamination from the material and/or the acids used to perform the experiments. Blank measurements thus eliminated the possibility that pipette tips, polypropylene tubes and reagents used in the analyses contributed to the results on for the gloves.

Experiments on gloves

We used two different techniques to test the potential contamination by gloves during analytical preparation and analyses of geological or biological samples. They are both schematically illustrated in Figure 1. The first test (Test A) consisted of soaking the gloves at room temperature for 40 hours in 20 ml of 0.4 mol l⁻¹ HNO₃ + 0.05 mol l⁻¹ HF and then analysing the content of the soaking solutions to

see which chemical elements were preferentially released in solutions. The test was done in weak acid to mimic the effects of leaching by acid fumes that may occur when handling samples and labware in the laboratory. The leaching acid also corresponds to that in which samples are commonly diluted and then introduced into an ICP-MS for trace element determination (e.g., see Chauvel *et al.* 2011). The small amount of HF ensures the stability of the solution by preventing the precipitation of high field strength elements such as Zr. The duration of the test was chosen arbitrarily and likely does not correspond to the complete leaching of the gloves. The detailed procedure consisted of putting each of the tested gloves in a 50 ml polypropylene vial being careful not to cross-contaminate the different vials in between each tested glove. We then added 20 ml of $0.4 \text{ mol l}^{-1} \text{ HNO}_3 + 0.05 \text{ mol l}^{-1} \text{ HF}$ in each vial and made sure that the whole gloves were soaked in the acid for 40 hours. The soaking solutions were pipetted out the vials and analysed on the mass spectrometer following the method described below.

The second test (Test B) aimed to evaluate how easily gloves could release trace and minor elements just through contact with disposable labware (*cf.* Figure 1). For this test, we put 3 ml of $0.4 \text{ mol l}^{-1} \text{ HNO}_3$ in a 15 ml polypropylene tube, touched the extremity of a 1 ml pipette tip with a dry glove for a second, washed the pipette tip with the solution into the tube and analysed the latter solution for trace element concentrations. In parallel, we also rubbed each glove along the interior of another 15 ml polypropylene tube for a second, added 3 ml of $0.4 \text{ mol l}^{-1} \text{ HNO}_3$, swished the acid around and then determined the trace element content of the solution. The acid used in Test B was different from that used in Test A for a technical reason. To reach higher sensitivity and improve detection limits, we measured the solutions from Test B with a different instrument (see below) that was not conditioned to analyse solutions with HF even in small amounts. Therefore, Test B was performed using $0.4 \text{ mol l}^{-1} \text{ HNO}_3$ only.

Analyses for trace element contents

Trace element mass fractions were measured in solutions from Test A using the quadrupole ICP-MS Agilent 7500 at the Laboratoire Magmas et Volcans (LMV) in Clermont-Ferrand, France. Solutions from Test B were measured using the quadrupole ICP-MS Thermo iCap-Q at the Ecole Normale

Supérieure (ENS) of Lyon, an instrument with lower detection limits. Most elements were measured in “no gas mode” except Mg, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Ge, As and Se that were determined in “He mode” using a collision cell to minimise spectral interferences caused by polyatomic ions. Mass fractions were calibrated using repeated measurements of solutions containing sixty trace elements mixed to concentrations of 0.02, 0.1, 0.5, 1, 2 and 60 ng ml⁻¹. These solutions were also used to monitor and correct the instrumental drift over one analytical sequence. Solutions from Test A had to be diluted by factors of 50 or even 500 to precisely measure Zn mass fractions and avoid detector saturation. For each test, detection limits (DL) were calculated following IUPAC guidelines for each element *i* as $DL_i = x_{bi} + k s_{bi}$ where $k = 3$ (95% confidence level), and x_{bi} and s_{bi} are, respectively, the mean and standard deviation of the number of counts measured in blanks (i.e., acids stored and handled with the same material used to test the gloves). The measurement precision of Tests A and B was estimated using complete duplicates (see online supporting information Appendices S1 and 2). Duplicated analyses are graphically represented in Appendix S3 where one can see that Test A produces repeatable results that clearly differentiate the tested gloves. The repeatability is poorer when the concentrations are low, hence closer to detection limits (*cf.* G1 and G1 Dup in Appendices S1 and 3). The repeatability of Test B is not as good as for Test A probably because the duration and surface of contact between glove and labware was variable from one tested glove to another.

Analyses for Zn isotopic compositions

Zinc isotopic compositions were measured in the soaking solutions from Test A at the Ecole Normale Supérieure (ENS) of Lyon, France following the procedure described by Maréchal *et al.* (1999). The solutions were generally not purified by ion-exchange chromatography before measurement because Zn was a hundred to a million times more concentrated than any other potential interfering element in the solutions. In a recent paper, Chen *et al.* (2016) indicate that small amounts of Ni and Ti residues could produce isobaric interferences and shift the measured $\delta^{66}\text{Zn}$ values by more than 0.07‰ if the Ni/Zn and Ti/Zn ratios were higher than 0.001 and 0.01, respectively. The Ni/Zn and Ti/Zn ratios of the soaking solutions are either close to these values or significantly lower (see Appendices S1 and 4). However, to ensure that the matrix of the solutions did not induce interferences and/or that Cu from

the gloves did not influence the mass bias correction based on Cu-doping, three vinyl gloves (plus two duplicates) with relatively high Cu/Zn, Ni/Zn and Ti/Zn ratios and one nitrile glove were processed through column chemistry. For these samples, Zn was purified on quartz columns filled with 1.8 ml of Bio-Rad AGMP-1 (100–200 mesh) anion-exchange resin by eluting 10 ml of HNO₃ 0.5 mol l⁻¹ (see Maréchal *et al.* 1999 for more details). The comparison of Zn isotopic compositions measured with and without purification by ion-exchange chromatography is discussed in the results section.

On the day of the analyses, an aliquot of each solution from Test A was evaporated to dryness and dissolved in a Cu-doped solution (Cu NIST SRM 976, National Institute of Standards and Technology, Gaithersburg, MD, USA) to reach a Zn concentration of about 300 ng ml⁻¹, which is similar within 10% to the concentration of the standard solution that was run between each sample. Zinc isotopic compositions, expressed as:

$$\delta^{66}\text{Zn}_{\text{sample}} = \left[\frac{\left(\frac{{}^{66}\text{Zn}}{{}^{64}\text{Zn}} \right)_{\text{sample}}}{\left(\frac{{}^{66}\text{Zn}}{{}^{64}\text{Zn}} \right)_{\text{standard}}} - 1 \right] * 1000 \text{ in}\text{‰} \quad (1)$$

were measured on a Nu Plasma (Nu 500) MC-ICP-MS in wet plasma conditions to avoid potential isotopic bias induced by the desolvation system on Zn isotopic ratios (Jaouen *et al.* 2013b).

Instrumental mass fractionation was corrected with an exponential law using the isotopic composition of Cu that was introduced as dopant in the analysed samples as recommended by Maréchal *et al.* (1999). $\delta^{66}\text{Zn}$ were calculated by calibrator bracketing using Zn JMC 3-0749L (also called JMC-Lyon; Johnson Matthey, Royston, UK) as reference calibrator. The latter was repeatedly measured in between each sample to correct for the instrumental drift throughout the measurement sequence.

The accuracy of Zn isotopic compositions was assessed based on the analyses of an in-house standard solution (sheep plasma; SHP) at the beginning of each measurement sequence. The average $\delta^{66}\text{Zn}$ measured for the in-house standard solution was $+0.75 \pm 0.12$ (2s, $n = 2$), which is in

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good agreement with our reference in-house value of $+0.73 \pm 0.08$ (2s, $n = 8$). Based on repeated measurements of calibrator Zn JMC 3-0749L, re-run samples and complete duplicate analyses, we estimate the precision of our measurements at $\pm 0.12\%$ (2s). The long-term precision (duration =) based on the repeated measurements of Zn JMC 3-0749L alone is better than $\pm 0.06\%$ (2s, $n = 140$).

Results

The amount of each element released by gloves in the soaking solutions of Test A is reported in Appendix S1 and shown normalised to geological and biological reference materials in Figure 2. The first important result is that all soaking solutions contain amounts of almost all elements determined that were well above detection limits. The exceptions are elements such as Ge, Te or Re (Appendix S1, Figure 2). Gloves are thus highly enriched in leachable trace elements, especially in Zn for which the amounts released in solutions are very large, between 11 μg and 17 mg (Figure 3). Large amounts ($> 1 \mu\text{g}$) were also measured for Mg, Ti, Mn, Fe, Rb, Sr, Zr, Sn, Hf and Pb. Nitrile, neoprene and latex gloves released the highest amounts of elements into solution. Latex gloves released the most rare earth elements (REE). Vinyl gloves generally released much lower amounts of trace elements, except G1 for In and Sn. Neoprene gloves released intermediate amounts of leachable elements. No systematic variation was noticed as a function of the packaging or the colour of the gloves.

Results from Test B are summarised in Appendix S2 and shown in Figure 4. The amounts of elements released by gloves when touching a pipette tip were generally higher than when touching the interior of a polypropylene tube (Figure 4). We think these features are directly related to the way the tests were performed, mostly the fact that the surface of contact between glove and pipette tip was probably larger than for the tube. In any case, results of Test B show that surface contaminants on the gloves are easily transferable from glove to labware and then to solutions by simple and quick contact. The most abundant transferable elements include Zn (up to 40 ng), Mg (up to ~ 20 ng), Fe (up to ~ 17 ng), Ba (up to ~ 4 ng) and all REE (for example, up to ~ 10 ng for Ce and 17 ng for Nd).

Amounts of Ti, Cr, Mn, Fe, Ni, Cu, Ga, Ge, As, Se, Sn, Sr and Pb > 0.1 ng were also detected for some tests. Vinyl gloves generally released lower amounts of potentially contaminant elements than all other gloves, except Sn. Note that most measurements are below detection limits (Appendix S2). This does not mean that the risk of contamination is negligible since detection limits were often > 0.1 ng or even > 1 ng for some elements and highly variable from one element to another.

The Zn isotopic compositions of the five soaking solutions analysed with and without separation by ion-exchange chromatography are compared in Appendix S4. No large difference was observed with and without purification although the two solutions with the highest Cu/Zn ratios (i.e., Cu/Zn ~ 0.002 for G8 and 0.006 for G14; cf. Appendix S4) have $\delta^{66}\text{Zn}$ values that do not totally overlap within measurement precision. Since these variations remain small and within the range of values measured for the other tested gloves, we conclude that not performing ion exchange purification of the soaking solutions makes only a very minor difference in Zn isotopic composition and its omission probably has no effect at all when Cu/Zn is < 0.002. Note that high Ti/Zn and Ni/Zn ratios (cf. G1 and G1 Dup, Appendix S4) did not bias $\delta^{66}\text{Zn}$ beyond measurement precision.

The Zn isotopic compositions of all the soaking solutions of Test A are reported in Table 2 and shown in Figure 5. For the four samples (plus two duplicates) processed through column chemistry, we reported the compositions measured after purification by ion-exchange chromatography in Table 2 (see Table caption for more details). The measured isotopic compositions span a relatively small range of values with $-0.20\text{‰} < \delta^{66}\text{Zn} < +0.34\text{‰}$. The average isotopic composition of the tested gloves yielded $\delta^{66}\text{Zn} = +0.10 \pm 0.32\text{‰}$ (2s). The most negative values were measured in nitrile and latex gloves while the most positive were measured in vinyl gloves. Within the measurement precision, there was, however, no significant difference of Zn isotopic composition between vinyl, nitrile, neoprene and latex gloves or as a function of the glove colour.

Discussion

Gloves as potential sources of contamination

Trace element contamination by gloves is of significant concern for all laboratories dealing with analyses at the nanogram to picogram levels. The potential for contamination is a function of the abundance of the element in the sample to be analysed relative to the amount of that element contributed by the glove. To take this effect into account when discussing the results, we normalised the amount of each element released by gloves into the soaking solutions (results from Test A) to the amount of that element present in 1 mg of BHVO-2 (basalt), 1 mg of CI-chondrite (meteorite), 1 g of SLRS-4 (natural river water) or 30 mg of NIST SRM 1598 (bovine serum). These amounts correspond to the approximate amounts of geological and biological test portions usually run on ICP-MS for routine trace element determinations. The normalised amounts are shown in Figure 2a–d. In Figure 3, we report the amounts of selected elements leached from the gloves in binary plots as a function of the Zn released from the same glove. The results of Test A (Figures 2 and 3) demonstrate that gloves currently used in clean laboratories are important sources of contamination for both geological and biological samples. For example, the amount of many elements leached from the gloves is comparable and often higher than the amount of those elements present in 1 mg of geological reference material BHVO-2 (basalt) (Figure 2a). In particular, the glove leaches contain up to 100,000 times more Zn; 1,000 times more Se, Ru, Ag, Cd, Sn, Ir, Au, Tl, Pb; 100 times more As, Rb, Zr, Pd, In, Sb, Te, Cs, Hf, Re, Pt, Bi, Th, U; and ten times more Li, Be, Sc, Sr, Y, REE and W than in a typically sized analytical aliquot of the geological reference material. Compared with materials with lower trace element mass fractions such as chondrite or river water (Figures 2b and 2c), enrichment factors reach higher values, for example more than 1000 for Be, Sc, Ti, V, Cr, Co, Ni, Zn, Se, Rb, Sr, Y, Zr, Cd, Sn, Cs, REE, Hf, Tl, Pb, Bi, Th and U. The situation is even worse for biological samples because their trace element contents are usually much lower than in geological samples. Figure 2d shows that the gloves can release up to 100,000 times the amounts of V, Cr, Zn and Pb present in 30 mg of biological reference material NIST SRM 1598 (i.e., bovine serum); 10,000 times the amounts of Mn, Ni, As, Cd, Cs and Tl; 1,000 times the amount of Co; 100 times the amounts of Mg, Fe, Se and

Rb; and up to ten times the amounts of Cu and Mo. Whatever the chosen reference, the results of Test A indicate that almost all elements are present at higher levels in the glove leaches than in the samples themselves, which constitutes a tremendous potential for contamination. This conclusion is supported by the results of our second test (Test B) aiming to quantify the amount of trace elements released by gloves by a single contact with either a dry pipette tip that is then used to pipette the sample or the interior of a polypropylene tube in which the sample is stored (Appendix S2 and Figure 4). This test demonstrates that high amounts (between 1 and ~ 40 ng) of Mg, Fe, Zn, Ba, La, Ce, Nd, Sm, Gd, Dy, Ho, Er, Yb and non-negligible amounts (between 0.1 and 1 ng) of Ti, Cr, Mn, Cu, Ga, Ge, As, Se, Sn, Eu, Tb, Tm, Lu and Pb can be transferred from the glove to the sample by a single contact with labware. The contamination can, however, reach much higher values if repeated contacts between labware and glove occur during sample preparation and analysis. Surprisingly, the neoprene glove G5 and the nitrile gloves G10 and G15 released comparable amounts of rare earth elements in the solutions of Test A and Test B although the two experiments were very different in design and duration. During Test A, we noted that the soaking solutions of G5 and G10 were cloudy, possibly indicating the presence of small particles in suspension. The surface of gloves G5, G10 and G15 is also rougher and more granular than the other gloves, which may be related to the easier release of particles from their surface by single dry contact. Whatever the case, Test B represents only one possible scenario of contamination in the laboratory. The behaviour of wet gloves or gloves exposed to acid fumes was not tested and could be very different. Careful handling of the samples is thus crucial to reduce the risk of contamination. Contamination of labware by gloves when acid-washing pipette tips, beakers and polypropylene tubes is, in particular, a serious concern. The operation requires several handlings with gloves that may contaminate the labware rather than clean it. We thus suggest the use of plastic tongs at every step of the acid-washing procedure to avoid direct contact between gloves and labware, and subsequent contamination of the samples.

The two tests show that all gloves contain high amounts of trace and minor elements, which is a potentially serious source of contamination for trace element and isotopic determinations. However, the amount of elements released depends on the material from which the glove is made. Except for In and Sn, nitrile, latex and neoprene gloves generally contain higher amounts of leachable elements than vinyl gloves, on average between 10 and 100 times more Mg, Ti, V, Cr, Mn, Co, Ni, Zn, Se, Rb,

Sr, Y, Zr, Nb, Rh, Cd, Cs, Ba, LREE, Hf, Pt, Tl, Pb and U (Figures 2 and 3). Similarly, results from Test B (Figure 4) show that vinyl gloves generally release less Zn and other elements (below detection limits), with the exception of Sn and Fe, by single contact with labware. The adequacy of each type of glove for handling a particular type of chemical needs to be established by rigorous testing and should not be presumed without such testing. However, the wearer needs to keep in mind that the risk of sample contamination is higher for most elements when using gloves made of nitrile, latex or neoprene to acid-wash labware and to prepare and analyse geological and biological samples.

Why are the gloves so enriched in trace elements?

The high Zn content in rubber gloves (i.e., neoprene, latex and nitrile) is very likely explained by manufacturing processes and the use of activators and accelerators such as Zn oxide and Zn organic carbamates or thiazoles (Nieuwenhuizen *et al.* 2001). These catalysts are used to “cure” the rubber (i.e., sulfur vulcanisation), an industrial process that allows the cross-linking of rubber macromolecules to confer strength, elasticity and good ageing properties to the gloves. Even if most suppliers ensure low levels of accelerators in the finished products, the certificates of analysis that we were able to consult for some of the tested gloves (nitrile and latex) indicate Zn mass fractions between 2 and 60 $\mu\text{g g}^{-1}$, which is still extremely high if the gloves are used for trace element or isotopic work. Since other trace elements are generally positively correlated with Zn (Figure 3), we suspect that they derive from the Zn catalysts used to cure the rubber. Note that the data are more scattered for Zr-Hf and rare earth elements (Figures 3d and 3g), which could be related to the type of Zn catalyst. Trace elements are probably present in different proportions in Zn oxides and Zn complexes (carbamates and thiazoles). The type of Zn catalysts added to rubber mixtures depends on industrial processes and is highly variable from one glove to another. According to the information available on the websites of some of the glove manufacturers, two nitrile gloves from the same brand can for example be made from rubber treated with Zn oxides or Zn complexes or a combination of both. Unfortunately, the exact recipe used by glove manufacturers is not available for each tested glove making it difficult to establish a direct relationship between the type of Zn catalyst used and the trace element budget of the finished product.

For vinyl gloves, the lower but still significant mass fractions of Zn cannot be accounted for the use of Zn activators and accelerators for sulfur vulcanisation since this process is used for rubber only, not for plastic such as vinyl (i.e., PVC). The presence of Ca-Zn stabilisers is however clearly mentioned in the composition of all tested vinyl gloves. Such stabilisers are used to prevent the degradation of PVC when heated to soften during the extrusion or moulding processes (Balköse *et al.* 2001, Fang *et al.* 2009). Ca-Zn stabilisers are very likely the sources of most trace and minor elements released by vinyl gloves.

For both plastic and rubber gloves, zinc and associated minor and trace elements are additives entering directly in the composition of the gloves. They are not carried in a coating or disseminated at the surface of the gloves, except possibly REEs for the neoprene glove G5 and the nitrile gloves G10 and G15. Pre-washing the gloves under water or acid-washing them before use as recommended by Friel *et al.* (1996) might slightly reduce the risk of contamination, but is probably not enough to totally rule out the risk of contamination. In our opinion, the most efficient way to minimise the contamination is to avoid as much as possible the contact between gloves and labware during washing, preparation and analyses.

Effect of glove contamination on Zn isotopic compositions

Zinc is clearly the most abundant leachable element in all types of glove, which means that glove contamination is a serious concern for the measurement of the Zn mass fraction in biological and geological samples. But, what of zinc isotopic compositions? Zinc isotopic compositions have been used both to understand Earth and planetary processes including the formation of the Solar System (Bridgestock *et al.* 2014), the origin of the Moon (Paniello *et al.* 2012), magmatic differentiation processes occurring in the bulk silicate Earth (Chen *et al.* 2013), as well as biological processes (Aucour *et al.* 2015, Cloquet *et al.* 2008, Moynier *et al.* 2009, Pichat *et al.* 2003), metabolic and pathological reactions in humans and mammals (Balter *et al.* 2010, Büchl *et al.* 2008, Moynier *et al.* 2013, Ohno *et al.* 2005, Stenberg *et al.* 2005), dietary habits (Costas-Rodríguez *et al.* 2014, Jaouen *et al.* 2013b, Van Heghe *et al.* 2012), and as markers of gender and age (Jaouen *et al.* 2013a). More recently, the use of this analytical tool is also increasing in the disease-related research field where stable isotope studies are used as new biomarkers of diverse pathologies such as breast cancer

(Larner *et al.* 2015). In these studies, the relevance of the results and the associated interpretations are based on highly precise isotopic determinations by MC-ICP-MS because the range of isotopic variation in geological samples and, in particular, in biological samples is relatively small, a few per mil at most. To date, the measurement precision on $\delta^{66}\text{Zn}$ measured by MC-ICP-MS can be lower than $\pm 0.1\%$ (2s) when performed under optimal analytical conditions (i.e., high sensitivity, stable plasma, elevated Zn mass fractions in test portions).

To quantify the impact of glove contamination on Zn isotopic compositions, we calculated the Zn isotopic composition of a binary mixture of few nanograms of Zn from gloves and the rest from geological or biological samples that have different Zn isotopic compositions (Figure 6). We re-wrote the classical mixing equation to express the Zn isotopic composition of a binary mixture as a function of the amount of Zn present in the two end-members:

$$\left(\frac{^{66}\text{Zn}}{^{64}\text{Zn}}\right)_{\text{mixture}} = \frac{m\text{Zn}_{\text{glove}}}{m\text{Zn}_{\text{glove}} + m\text{Zn}_{\text{sample}}} \left(\frac{^{66}\text{Zn}}{^{64}\text{Zn}}\right)_{\text{glove}} + \frac{m\text{Zn}_{\text{sample}}}{m\text{Zn}_{\text{glove}} + m\text{Zn}_{\text{sample}}} \left(\frac{^{66}\text{Zn}}{^{64}\text{Zn}}\right)_{\text{sample}} \quad (2)$$

where $m\text{Zn}_{\text{glove}}$ and $m\text{Zn}_{\text{sample}}$ are the amount of Zn (generally in ng) from the glove and the sample respectively. For the glove end-member, we used the average Zn isotopic composition of all gloves ($\delta^{66}\text{Zn} = +0.10 \pm 0.32\%$ (2s); Table 2) and the average amount of Zn released in Test B for both pipette tips and tubes ($= 6.0 \pm 19.3$ ng (2s); Appendix S2). Note that 6.0 ng is a minimum since the average amount of Zn released by one contact with glove was calculated taking analyses below detection limit equal to zero ng in Appendix S2. Then, we considered three different scenarios to account for different degrees of contamination: (1) glove in contact with labware once during acid-washing, sample preparation or analysis such as $m\text{Zn}_{\text{glove}} = 6.0$ ng; (2) glove in contact with labware twice such as $m\text{Zn}_{\text{glove}} = 2 \times 6.0$ ng; (3) glove in contact with labware three times such as $m\text{Zn}_{\text{glove}} = 3 \times 6.0$ ng. For each scenario, we calculated the effect of contamination on Zn isotopic compositions as a function of the amount of Zn processed through chemistry and analysed on MC-ICP-MS (i.e., 30 ng $< m\text{Zn}_{\text{sample}} < 500$ ng, cf. Figure 6).

Geological samples: In terrestrial and extra-terrestrial samples, the range of variability of Zn isotopic compositions can be quite large. Extreme $\delta^{66}\text{Zn}$ values of -7.4‰ and +6.4‰ have been reported by Moynier *et al.* (2011) and Herzog *et al.* (2009) in meteorites (EL6 chondrites) and lunar soils, respectively. Gloves have an average $\delta^{66}\text{Zn}$ of ~ 0‰. As expected, Figure 6 shows that the larger the difference is between $\delta^{66}\text{Zn}$ of glove and sample, the greater is the effect of contamination. For extreme sample compositions of -5‰ and +5‰, one contact between glove and labware during washing, preparation or analysis is enough to shift the measured isotopic compositions beyond their uncertainties (i.e., $\pm 0.1\%$, 2s) given typical amounts of Zn processed through chemistry and subsequently analysed on MC-ICP-MS (Figure 6a and h). For low amounts of Zn determined (< 50 ng), shifts in $\delta^{66}\text{Zn}$ due to contact with gloves can reach $\pm 0.5\%$ (one contact) and almost $\pm 2\%$ in the worst scenario (i.e., three contacts).

In geological materials, most $\delta^{66}\text{Zn}$ values however cluster between -1‰ and +1‰ (see for example, the values reported by Chen *et al.* (2016) for seventeen whole-rock reference materials). For these more typical Zn isotopic compositions (*cf.* Figure 6c–f), the effect of glove contamination is less important though shifts of more than $\pm 0.1\%$ can occur if less than 200 ng is processed through chemistry and three contacts between glove and labware occur during the analytical procedure. Taking into account the whole range of possible isotopic compositions for terrestrial and extra-terrestrial samples, we thus recommend that a minimum of 500 ng of Zn be processed through chemistry and determined. Extreme precaution when acid-washing labware, preparing and analysing samples is however required since any contact with glove would shift extreme Zn isotopic compositions beyond the precision level taken here at $\pm 0.1\%$. If duplicate analyses allow for a better measurement precision on $\delta^{66}\text{Zn}$ values, for example down to 0.03‰ (2s) as reported by Chen *et al.* (2016), then higher amounts of Zn should be determined to make sure the effect of glove contamination remains negligible.

Biological samples: The range of $\delta^{66}\text{Zn}$ measured in biological samples varies between -1‰ and +2‰ (e.g., Moynier *et al.* 2009, Balter *et al.* 2010, Jacouen *et al.* 2013a, b, Lerner *et al.* 2015). To our knowledge, no extreme values outside this range have been reported so far. Biological

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samples such as body fluids are often not very concentrated in Zn, for example no more than a few $\mu\text{g g}^{-1}$ in urine (Balter *et al.* 2010) and few hundred ng g^{-1} in cerebro-spinal fluids. Moreover, the amount of sample available for research purposes is restricted to extremely low quantities; hence the total amount of Zn available for isotopic determinations in a biological sample sometimes does not exceed 50 ng. Figure 6 shows that for such low amounts of Zn, one contact between glove and labware could significantly shift the measured Zn isotopic composition beyond the measurement uncertainties (i.e., $> \pm 0.1\text{‰}$, cf. Figure 6c to 6f). For instance, for a typical $\delta^{66}\text{Zn}$ of $+1\text{‰}$ (Figure 6c), one contact with a glove can bias $\delta^{66}\text{Zn}$ by $\sim 0.1\text{‰}$ and three contacts by more than 0.3‰ if the amount of Zn determined is below 50 ng. The effect of contamination becomes negligible, that is within the measurement precision of $\pm 0.1\text{‰}$, when the amount of Zn processed through chemistry and subsequently determined is higher than 300 ng. We thus recommend that, whenever possible, measurements of Zn isotopic compositions in biological samples be done on amounts > 300 ng. In the case where the measurement precision is estimated to be better than $\pm 0.1\text{‰}$, the amount of Zn determined should be increased accordingly.

Conclusions

The new generation of mass spectrometers and the curiosity of scientists to determine an increasing number of elements with high precision and sensitivity requires increasingly low procedural blanks and control on potential exogenic contamination during sample preparation and analysis. The results of this study clearly show that gloves are a primary and important source of contamination in clean laboratories for both trace element and isotope studies. Of the sixty elements determined in nitrile, latex, neoprene and vinyl gloves, very few appear to be safe from potential contamination. Soaking of gloves in weak acid ($0.4 \text{ mol l}^{-1} \text{ HNO}_3 + 0.05 \text{ mol l}^{-1} \text{ HF}$) at room temperature for several hours released significant amounts of minor and trace elements, up to 17 mg of Zn, 200 μg of Mg, and 10 to 60 μg of Ti, Fe, Sr and Zr. Compared with vinyl gloves, nitrile, latex and neoprene gloves are much more enriched in leachable elements, with the exception of In and Sn.

The tests also indicate that most elements are easily transferred from the gloves to the sample by simple contact of a pipette tip or the interior of a polypropylene tube. Acid-washing labware is in particular a potential major source of contamination since it requires several handlings with gloves.

Zinc has by far the highest risk of being contaminated by gloves. A single dry contact between labware and gloves releases an average of 6.0 ± 19.3 (2s) ng of Zn into the samples. Such contamination has variable effects on the Zn isotopic composition of the sample depending on the amount of Zn processed through chemistry and the difference between the Zn isotopic composition of the studied sample and that of the glove. All tested gloves shared roughly the same $\delta^{66}\text{Zn}$ (average $\delta^{66}\text{Zn} = +0.10 \pm 0.32\text{‰}$ (2s)). The lower the amount of Zn processed through chemistry, and the higher the difference between $\delta^{66}\text{Zn}$ of the sample and the glove, the greater is the effect of contamination. Whatever the amount of Zn determined (from 30 to 500 ng), a single contact between glove and labware is enough to bias the composition of a sample with extreme $\delta^{66}\text{Zn}$ of -5 or +5‰ beyond the measurement precision taken at $\pm 0.1\text{‰}$ here. A bias as large as $\pm 1.5\text{‰}$ can be reached for samples with extreme $\delta^{66}\text{Zn}$ of -5 or +5‰ if the amount of Zn determined is low (< 50 ng) and labware is touched repeatedly during washing, preparation or analysis. For typical $\delta^{66}\text{Zn}$ values between -1‰ and +2‰, a minimum of 300 ng of Zn should be determined to limit the potential bias due to glove contamination below 0.1‰.

We recommend the following best practices to keep glove-derived contamination in control. (1)

Recognise that gloves can be a significant source of laboratory contamination. (2) Carry out every step of washing procedures with clean tongs to minimise contact between gloves and labware (3)

Take extreme precautions when handling samples for trace element and Zn isotopic determinations with any type of glove. (4) Monitor and minimise glove contamination using Zn contents in blanks

even if Zn is not in the analysis protocol. (5) For Zn isotopic determinations, measure whole

procedural blanks routinely (i.e., in every batch of samples) and calculate the effect of contamination

on each sample using Equation (2). If the isotopic bias exceeds the measurement precision, reject the data.

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Supporting information

The following supporting information is available online:

Appendix S1. Results of Test A (gloves)

Appendix S2. Results of Test B (tube or pipette)

Appendix S3. Comparison of duplicate analyses (Test A)

Appendix S4. Comparison of zinc isotopic compositions.

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Figure captions

Figure 1. Illustration showing how Tests A and B were performed.

Figure 2. Amount of trace and minor elements released by gloves when soaked in 20 ml of 0.4 mol l⁻¹ HNO₃ + 0.05 mol l⁻¹ HF for 40 hours (results of Test A) normalised to the amount of elements present in (a) 1 mg of basalt BHVO-2, (b) 1 mg of chondrite, (c) 1 g of natural river water SLRS-4 and (d) 30 mg of bovine serum NIST SRM 1598. Enrichment factors were calculated relative to the preferred values compiled by GeoReM for reference materials BHVO-2, SLRS-4 and NIST SRM 1598.

Reference values were taken from Palme and O'Neill (2014) for CI chondrites. For panel (c) and (d), only a few elements are shown because the mass fractions of other elements in SLRS-4 and NIST SRM 1598 are not available in the literature. The grey field represents values below detection limits defined following IUPAC guidelines as the average number of counts measured in blanks plus three times the standard deviation (see section *Analyses for trace element contents* for more details). When the amount of element detected in a solution was below the detection limit (< DL in Appendix S1), it is represented as equal to the detection limit.

Figure 3. Binary plots showing the amount of chemical elements released by gloves when soaked in 20 ml of 0.4 mol l⁻¹ HNO₃ + 0.05 mol l⁻¹ HF for 40 hours (results of Test A). The grey field represents values below detection limits defined following IUPAC guidelines as the average number of counts measured in blanks plus three times the standard deviation (see section *Analyses for trace element content* for more details). When the amount of element detected in a solution was below the detection limit (< DL in Appendix S1), it is represented as equal to the detection limit: this is the case of some vinyl glove in panels (e) and (g).

Figure 4. Amount of trace and minor elements (ng) released by gloves by short contact with (a) the interior of a polypropylene tube and (b) a 1 ml pipette tip (results of Test B). The grey field represents values below detection limits defined following IUPAC guidelines as the average number of counts measured in blanks plus three times the standard deviation (see section *Analyses for trace element*

content for more details). When the amount of element detected in a solution was below the detection limit ($< DL$ in Appendix S2), it is represented as equal to the detection limit in the two panels.

Figure 5. Zinc isotopic compositions ($\delta^{66}\text{Zn}$) of the tested gloves. Error bars are fixed at $\pm 0.12\%$ based on complete duplicate analyses.

Figure 6. Effect of contamination by gloves on Zn isotopic compositions as a function on the amount of Zn processed through chemistry and determined by mass spectrometry. For this calculation, we used the average $\delta^{66}\text{Zn}$ of all tested gloves ($\delta^{66}\text{Zn} = +0.10\%$) and assumed that the amount of Zn released by gloves for one contact was equal to 6.0 ng (average amount of Zn released in Test B), 2×6.0 ng for two contacts and 3×6.0 ng for three contacts. The green field shows the isotopic composition of the sample and its measurement precision that is fixed at $\pm 0.1\%$ (2s) for this figure. More information about the calculation can be found in the main text.

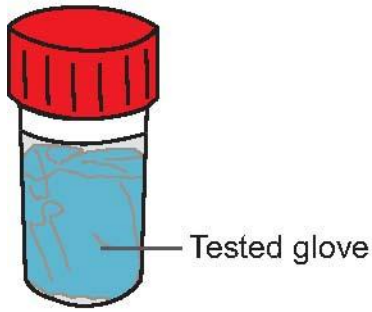
Table 1.
Description of the tested gloves

Sample Name	Type	Supplier	Model	Colour	Packaging	Reference Number
G1	Vinyl	Polysem Medical	Tactilis	Translucent	Cardboard box	GVB1200/PM
G2	Nitrile	Kimberley-Clark	KleenGuard G10	Blue	Cardboard box	57372
G3	Nitrile	Kimberley-Clark	KimTech Science Sterling Nitrile-XTRA	Grey	Cardboard box	98343
G4	Latex	Kimberley-Clark	KimTech Science Satin Plus	Pale yellow	Cardboard box	SP2330E
G5	Neoprene	Ansell	NeoTouch®	Green	Cardboard box	25-101
G6	Nitrile	Ansell	TouchNTuff®	Dark green	Cardboard box	92-605
G7	PFE Latex	Kimberley-Clark	KimTech Science	Pale yellow	Cardboard box	E330
G8	Vinyl	LCH medical	Sensinyl Free	Translucent	Cardboard box	VNX-02
G9	Nitrile	Microflex	Class 10 - Controlled environments	White	Plastic bag	CE4-313
G10	Nitrile	SHOWA	Nitrile N-DEX Ultimate	Light Blue	Plastic bag	9905PF
G11	Nitrile	Clean-Dex Best	Class 100 cleanrooms	White	Plastic bag	C9905PFM
G12	Nitrile	Kimberley-Clark	Nitrile G5 - KimTech Pure	White	Plastic bag	56865
G13	Vinyl	Jet	Non powdered natural vinyl gloves	Translucent	Cardboard box	07GV0708U
G14	Vinyl	Ansell	Synsation™ PF	Translucent	Cardboard box	4001OM
G15	Nitrile	Medline Industries Inc.	Sensicare® Ice	Dark blue	Cardboard box	486802

Table 2.			
Zinc isotopic composition ($\delta^{66}\text{Zn}$) of gloves			
Sample Name	$^{66}\text{Zn}/^{64}\text{Zn}$	2SE	$\delta^{66}\text{Zn} (\text{‰})_{\text{Zn JMC 3-0749L}}$
G1*	0.562961	1.30E-05	0.34
G1 Dup*	0.562900	1.09E-05	0.23
G2*	0.562738	1.02E-05	-0.05
G2* bis	0.562751	9.92E-06	-0.04
G3	0.562811	5.84E-06	0.17
G3 dup	0.562789	4.75E-06	0.14
G4	0.562727	8.20E-06	0.00
G5	0.562869	8.99E-06	0.19
G5 bis	0.562868	8.24E-06	0.20
G6	0.562735	1.50E-05	0.02
G7	0.562652	6.35E-06	-0.14
G8*	0.563036	4.71E-06	0.26
G8 dup*	0.562943	7.26E-06	0.07
G9	0.563050	4.42E-06	0.24
G9 bis	0.563046	4.80E-06	0.25
G10	0.563036	4.66E-06	0.18
G10 bis	0.563049	4.77E-06	0.23
G11	0.562807	4.60E-06	-0.20
G12	0.563034	5.75E-06	0.19
G12 bis	0.563039	5.03E-06	0.24
G13	0.562959	5.82E-06	0.04
G13 bis	0.562954	5.52E-06	0.03
G14*	0.562973	6.67E-06	0.12
G15	0.562895	9.60E-06	-0.13
G15 bis	0.562880	9.00E-06	-0.16
* stands for samples processed on column chemistry			
bis stands for re-run analyses			
dup stands for complete duplicate analyses			
2SE are in-run errors			

Test A

Leaching in weak acid



Test B

Dry contact with disposable labware

