Dried matrix spots and clinical elemental analysis. Current status, difficulties, and opportunities.

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Abstract

This article examines the increasing importance of dried matrix spot (DMS) specimens (such as dried blood spots, dried urine spots, etc.) in biomedical research, the challenges associated with their analysis when quantitative elemental information is aimed at, as well as the benefits deriving from the further usage of these types of samples. The article briefly reviews the historical evolution of this sampling approach in elemental clinical analysis, stressing prospective areas of applications (e.g., newborns or prosthesis control), the methodologies most recently developed to produce DMS of known volume, as well as novel strategies proposed to analyze them, often related to direct solid sampling techniques or fast lixiviation methods. Finally, the article discusses the type of information that could be obtained after isotopic analysis of DMS when targeting non-traditional stable isotopes (e.g., Cu, Fe or Zn), which can significantly help in the early diagnosis of some medical conditions (e.g. Wilson's disease).

Keywords: dried matrix spots; dried blood spots; dried urine spots; trace elemental analysis; isotopic analysis; solid sampling.

1. Introduction

In 1963, R. Guthrie and A. Susi published a seminal work describing the collection of capillary blood from the heel of newborns. Such method included the deposition of a few blood droplets on a filter paper (FP), a drying step, and, finally, the determination of phenylalanine for the screening of phenylketonuria [1].

This was actually not the first paper dealing with the microanalysis of blood collected on FP. Already in 1913, Bang described the determination of blood sugar levels by titration with a diluted iodine solution [2]. However, it can be argued that it was the work referred above by Guthrie and Susi [1] the one that laid the conceptual basis of an approach that has facilitated the diagnosis and early treatment of multiple congenital and hereditary abnormalities through newborn screening programs. Since then, analysis of the now called DBS (Dried Blood Spots) has improved or saved many lives in both developed and developing countries [3].

The number of analytes investigated in DBS samples has been steadily increasing, with applications in metabolic-endocrine diagnosis, therapeutic drug monitoring, as well as toxicological, serological and molecular biology studies [4-7]. Moreover, in recent years (see **Figure 1a**), there has been an exponential increase in the number of publications on this topic, which seemed to peak in 2014. The slow decrease in papers observed in the last couple of years does not necessarily mean that the interest in the field has decreased. Instead, it could mean that the field is reaching a state of maturity, as the pharmaceutical industry is using more and more this type of sample [8]. Also, some of the research is being transferred to the use of other biological fluids, such as urine (see **Figure**

1b), giving rise to the so-called dried urine spots (DUS), and, to a much lesser extent, to other fluids such as cerebrospinal fluid [9,10], amniotic fluid [11] or saliva [12]. Biological samples deposited onto and dried on a FP receive the generic name of DMS (Dried Matrix Spots).

The majority of this research has been focused on the monitoring of (bio) organic species *via* liquid chromatography-mass spectrometry (LC-MS). Several recent reviews covered these works [3,13-16] in detail. The current review will instead focus on the role that this field may also play in clinical elemental analysis. Obviously, some of the aspects discussed can be considered as general, regardless of the nature of the analyte, which will be noted. However, one of the key differences of clinical elemental analysis is that typically quantitative information is aimed at, as opposed to many other situations in clinical diagnosis where only qualitative or screening information is needed. This still represents an analytical challenge for DMS analysis.

2. Advantages of DMS for the analysis of biological fluids

From a purely analytical point of view, by producing DMS, liquid samples that are relatively simple to analyze after digestion or just dilution, are transformed into much more complex solid materials, which are typically not homogeneous. Therefore, from such point of view, this transformation is not so advantageous.

Moreover, deposition of other liquid materials on FP is also possible, and has been also explored in the literature [17,18], but such applications are rarely finally deployed in routine labs.

Therefore, what are the benefits of DMS to make this type of sample worthy of analytical research? There are a significant number of them, which will be discussed below.

2.1. Ease of sample collection

This advantage is particularly important in the case of blood. Development of the DBS methodology has been related to the expansion of neonatal screening programs in hospitals, because the method of collecting capillary blood on FP is very attractive for mass screening of children for the following reasons [19]: i) a minimal amount of blood is required; ii) pricking the heel (newborns) or finger (young children) with a lancet is easier and less traumatic than the conventional collection of venous blood; and iii) DBS collection can be performed by relatively untrained personnel, even by the parents or relatives at home (see **Figure 2**).

DMS are becoming also popular for this reason in a pharmacological context (e.g., experimentation with new drugs or treatments). The traditional method of collection of blood from rodents is terminal, thus requiring a new animal for each measurement. Alternatively, DBS methodology enables serial sampling from a single animal, such that pharmaceutical companies can meet the "3R's doctrine" (reduction, refinement, and replacement) as regards animal experimentation [14].

Concerning other biological fluids, use of urine or saliva permits the development of truly non-invasive methods, although the amount of sample is typically not a problem for urine. In the case of other samples, such as cerebrospinal or amniotic fluids, the advantages are not so obvious, because sampling must be carried out by specialized medical staff following the hospitalization of the patient. Still, these venues are explored because of the increasing popularity of DMS.

2.2. Ease of transportation and storage

This is another key aspect for clinical labs. Requirements for transportation and storage of biological specimens collected on FP are much less stringent than those that have to be followed for the liquid samples. For example, many urinary metabolites show little stability. Thus, urine must be frozen (at -20 °C or -80 °C) until analysis, complicating the logistics of clinical laboratories, which often have to deal with hundreds of samples on a daily basis.

In contrast, several studies indicate that the conservation of the sample dried on a FP slows down the degradation processes that analytes may undergo [20], and therefore DMS are much more stable than the original wet samples. If the analysis is to be carried out one month after collection or before, it is generally possible to preserve DMS at room temperature; for longer periods, it is recommended to keep them refrigerated under controlled humidity [5].

2.3. Improvement of welfare conditions

For the reasons discussed before, DMS methodology enables the home collection of biological fluids and their submission to the lab by ordinary mail. This aspect can certainly improve the quality of life of people who must periodically undergo tests, such as chronic patients requiring frequent controls, or those with reduced mobility or, even, bedridden, and/or those living in isolated areas.

In addition, this strategy can lead to health care and business savings, decreasing the hours of staff dedicated to the collection of the samples, as well as to administrative aspects related with the frequent attention of patients in health centers.

2.4. Increased participation in epidemiological or population-based prevention programs

Both epidemiological and population-based preventive programs make use of the measurement of biomarkers and, in such studies, the collaboration of the population is essential. This collaboration involves certain discomforts to participating subjects: sample collection can be annoying (e.g., blood collection) and it often requires a visit to a health center for collection or delivery of the sample. When the study is epidemiological, in which the participants do not see a clear benefit for their participation (in contrast to a program of prevention or of early diagnosis of a disease), these inconveniences may outweigh their collaborative spirit.

In these situations, the development of protocols for the collection of these biospecimens at home and their easy shipment should lead to greater participation [21]. As stated by McDade *et al*.: "…the burden is on the researcher to bring methods to people in the community instead of relying on select individuals willing to come to the clinic or lab" [5]. Thus, use of DMS (especially DBS and DUS) can help in these cases.

2.5. Conducting retrospective research with samples available from newborn screening programs

DBS samples are collected virtually from all newborns in many countries. Thus, this sampling strategy provides a way of preserving whole blood samples that may be used in retrospective research in clinical, epidemiological and toxicological areas, as novel techniques and methodologies are developed [22]. The creation of biobanks that can provide further information without bothering again the patient is thus another significant advantage.

Overall, to conclude this section it can be stated that the main advantages of using DMS are not analytical, quite the contrary. It is certainly easier for atomic spectrometry techniques, such as graphite furnace atomic absorption spectrometry (GF-AAS) or inductively coupled plasma mass spectrometry (ICP-MS), to deal with blood or urine samples in liquid form (diluted or digested) than with DMS. But this sample offers so many benefits for the patients and for the clinical community that is becoming widely used. It is thus the duty of the analytical community to try to meet the challenges brought by this methodology and provide analytical data as reliable as necessary.

3. Filter materials for preparing DMS

There is a variety of commercially available FPs that can be used for clinical purposes. These materials differ, among other characteristics, in weight, thickness, speed of absorption and in the presence (or absence) of some chemical compounds, added for particular applications. The key requirements of a FP to produce a DMS and achieve reproducible analytical results are that its capacity of absorption and dissemination of the sample are uniform, and that these properties are maintained between different units of both the same and different lots.

In the particular case of DBS samples, there is much more information on the performance of FPs, resulting in regulations/recommendations. The Clinical and Laboratory Standard Institute has set the requirements so that FPs can be used in the preparation of DBS specimens in neonatal screening programs. Currently, only two brands of FPs are approved as class II medical devices: Whatman 903 and Perkin Elmer 226, which are 100% made of cotton linters and contain no

additives [23]. Several studies have demonstrated that these papers are virtually identical in terms of its analytical capabilities [4].

With the level of quality accomplished, Mei *et al*. pointed out that: "The filter paper blood collection device has achieved the same level of precision and reproducibility that analytical scientists and clinicians have come to expect from standard methods of collecting blood, such as vacuum tubes and capillary pipettes. Like these devices, filter paper has associated with it some level of imprecision that can be characterized to standardize the device and to minimize the variation in measurements due to the filter paper matrix" [24].

However, despite these positive comments, the situation may still be subject to further improvements. One important aspect is that the FP material used should retain a constant amount of blood per area unit (of course, within a reasonable interval), so that for analyte quantification it may not be necessary to know the exact volume of blood that is deposited in the DBS for its formation. This is, however, not so simple to achieve, because the amount of blood (or of fluid, in general) deposited per area unit may depend to some extent on the physicochemical characteristics of every sample. For instance, it has been reported for DBS that the amount of hematocrit may have an influence on this parameter [25].

That is why this is an area of current research and there are different manufacturers investigating the use of alternative materials (frequently polymeric compounds), while also trying to simplify the procedure of sample collection.

One of such cases has given rise to the so-called volumetric absorptive microsampling (VAMS) method. In this method, in order to produce a DBS, a

puncture with a safety lancet is typically performed on the finger of the individual and a small and known volume (approx. 10.5 µL for blood) is retained on the absorptive tip of the device (see **Figure 3a**), which is made of a polymer specifically designed for this purpose [26]. Moreover, it has been shown that the collected sample volume is practically independent of the hematocrit level [26], although this value may still affect the extraction efficiency, as discussed in detail in the first review published on the use and performance of VAMS [27].

An alternative and also very recent approach is based on the so-called microfluidic-based volumetric sampling. This device incorporates several channels of a constant volume that aspirate blood (5 or 10 µL) from the finger, and further deposit it onto a clinical FP (see **Figure 3b**). Any type of FP that fits the device can be used. This approach has also been reported to enable quantitative blood analysis, regardless of the hematocrit level [28,29].

Other alternatives and modifications have been proposed in the literature, such as using precut filters to control diffusion, or adding some compounds to the filter to be used as internal standards and/or to help in retaining some species. Such cases will be commented in the next sections, focusing on approaches reported for elemental analysis only.

4. The analytical process for dried matrix spots samples

Figure 2 illustrates the typical procedure for analysis of DBS. After the sample is collected according to the recommendations [23], which can be done after puncturing the heel (newborns) or the finger, it is dried and can be sent to the laboratory for analysis.

As discussed before, the space occupied by a droplet is not always constant. This aspect is problematic for quantification unless: i) the whole DMS is completely analyzed; and ii) the volume of the droplet is known (e.g., deposited with a pipette) [30].

Very often these requisites are not fulfilled. The whole DBS is often too large for analysis and a smaller disc (diameters of 1.5, 3.2, 4.7 and 6.0 mm are standard values) is punched out of it and analyzed. Such procedure ensures obtaining sufficient sample for various measurements and also easier automation, as many of these small pieces can be pretreated (e.g., analyte extraction) simultaneously before their analysis. On the other hand, deposition with a pipette or a calibrated capillary is often considered not appropriate for home collection. In this context, the use of alternative procedures, such as those discussed in the previous section (**Figure 3**), can be beneficial.

In any case, these piece(s) of the DBS are typically submitted to an extraction procedure, and the extract is finally analyzed. When elemental information is aimed at, this is most often carried out *via* GF-AAS or ICP-MS. In the latter case, an internal standard is usually added to the extract before analysis. Examples of these procedures will be discussed in the next section.

Alternatively, the use of a solid sampling technique can make this process more straightforward, although the procedure for calibration needs to be carefully designed, and it often requires the usage of matrix-matched standards. Pros and cons of such approach will be discussed in **section 5.3.**

Finally, for biological fluids other than blood the procedure has not been yet standardized. However, a similar approach to that depicted in **Figure 2** is usually

followed, but using of a pipette for sample deposition is typical, unless the patient suffers from urine incontinency, in which case a FP can be placed inside his/her diapers. In the case of urine, using the standard 24-hour collection procedure would be preferable. Otherwise, creatinine should also be determined for normalization of the results. The particular problems arising from DUS elemental analysis will be discussed in the next section as well.

5. DMS for elemental analysis

5.1. The origin: Pb blood levels and the Delves Cup

The element most determined in DMS by a large margin is Pb. This was the first element to be investigated and still is widely monitored in DBS: of the 48 articles listed in **Table 1**, 36 are devoted to Pb, or to Pb and other elements.

There is an obvious reason for this: Pb is an important neurotoxic and its effects may be particularly grave in the case of children, who can benefit significantly from the use of DBS, as discussed before. The topic was so relevant that early in the 70s a device called the Delves Cup was commercialized, enabling fast and sensitive determination of Pb in DBS by Flame (F)-AAS. Such device used micro crucibles made from nickel foil where the samples were deposited and partially oxidized with H_2O_2. Later, they were volatilised by an air-acetylene flame into a nickel absorption tube situated in the flame [31].

The first article devoted to this topic was not dealing with DMS, but with analysis of 10 µL of blood [31]. However, direct analysis of DBS using the Delves Cup was proposed only one year later by Cernik and Sayers, with the idea of enabling easy control of workers in Pb factories [32]. These authors, nevertheless, pointed out in the conclusions the potential of this approach in paediatric practice as well.

From that moment, many other papers were published aiming at improving this methodology, for instance, including a pre-ashing step to minimize the formation of smoke [33], which could cause interferences [34].

Many of these articles discuss aspects of the methodology that can have an impact in the final results, regardless of the analytical technique chosen, such as the comparison between capillary (as obtained by finger or heel prick) and venous (as obtained by phlebotomy) blood levels. In the majority of cases, it was accepted that comparable results could be obtained for both sampling methods.

Another frequent issue is the potential contamination arising from two aspects: i) the content of Pb in the FP used; and, ii) contamination from the skin of the patient or from the atmosphere, when sampling is not performed in a clinical lab. The first case is not very relevant, in our experience, when Pb is the target, although blank FPs must always be monitored. The situation for other elements will be discussed later. For the second case, appropriate finger cleaning seems required, as well as some minimal training for proper blood deposition: the continuous application of blood is preferred over multiple discrete applications of blood drops of small volumes, which lead to non-circular geometries [35]. In any case, deposition of blood onto FPs is standardized nowadays [23].

Ever a critical aspect, the distribution of blood on the FP has been investigated in this context as well. It was already noticed in 1978 that, for a fixed sample volume, blood samples with lower haemoglobin levels spread over a larger area for a fixed volume [36]. This aspect affects the potential for achieving accurate results, at least for samples with extreme hematocrit values (most articles on DBS report/discuss on hematocrit values rather than on haemoglobin levels like ref.

[36], but both values are related) and also complicate method validation, because reconstituted reference materials also show a different consistency and tend to spread over a larger surface than real blood samples [37,38]. In any case, if the portion punched out is sufficiently large, it could be representative for Pb levels as long as the most outer circle (which is small but enriched in Pb) is not sampled [39,40].

5.2. GF-AAS and ICP-MS: responding to new elemental challenges

The popularity of F-AAS related methods was substituted by those based on GF-AAS and, later, by those deploying ICP-MS. This has led to the development of methods that are sensitive enough not only to monitor Pb, but many other elements as well.

In most cases, an extraction step is required for these techniques. It can be seen that obtaining quantitative recovery from these samples is not an easy task as the procedures proposed evolved getting more complicated and using 3 or 4 reagents [41-43]. ICP-MS adds simultaneous multi-element potential [44], but sometimes that represents further challenges to extract some analytes properly.

For instance, Hg is another clinically relevant element owing to its well-established toxicity, and up to 8 manuscripts have reported its determination in DBS. However, its accurate determination presents particular challenges, such as the typical high volatility of its compounds (even more if reduced to Hg (0)) and the tendency of some of its compounds to adhere to nonpolar surfaces (such as tubing). These factors often lead to poor recoveries as well as to memory effects [45], which require proper sample preparation and introduction strategies. For instance, Funk *et al*. [46] proposed the addition of Au in the extraction solution

to amalgamate Hg, thus enhancing recovery, as one of the improvements over the method previously proposed by Langer *et al*. [44] for multi-element analysis of DBS, method which finally did not include Hg among the elements determined due to poor reproducibility/stability. A more recent article by Nelson *et al.* [47] compares the results obtained for paired cord blood and DBS samples. This type of study is of obvious interest because the use of DBS could be ideal to establish fetal exposure to mercury, which is an important issue as even minimal amounts of Hg can have a severe effect on a still not fully developed brain and nervous system. However, the results reported show underestimated values for DBS and higher limits of detection (LOD): 0.7 μg L^{-1} for analysis of DBS *vs* 0.3 μg L^{-1} for analysis of cord blood. These results simply reflect that analysis of DBS is more challenging than traditional analysis of blood, and even more so if a difficult element needs to be determined at levels below μg L^{-1}. However, use of alternative digestion/extraction strategies or of solid sampling techniques, as will be discussed in the next section, could alleviate these issues.

It is very well known that Hg toxicity depends on the physicochemical form in which this element is present. In this regard, the first paper on Hg speciation based on analysis of DBS has been published very recently by Basu *et al.* [48]. This pioneering work uses gas chromatography coupled to cold vapour atomic fluorescence spectroscopy (GC-CVAFS) to separate Hg species and to quantify Methylmercury (MeHg) levels, after sample digestion, offering a 0.3 μg L^{-1} LOD. Still, the article states that without sample volume information it is impossible to calculate an accurate concentration. This problem has been already discussed in **section 3** and nowadays there are solutions available (see **Figure 3**), that could be tested for MeHg determination. It can also be highlighted that, for the

particular problem of Hg, CVAFS is a relatively simple but powerful technique, offering very low limits of detection and the possibility to analyze the samples directly.

In any case, the possibilities offered by ICP-MS make it feasible to investigate many other elements that have become of clinical interest, besides the traditional toxic elements, which are of obvious relevance and not only for humans, but also for other animal species. Certainly, the use of DBS is particularly interesting for small animals, as the amount of blood required is very limited. This feature has been explored to create a database collecting information on the exposure of wild birds to environmental toxicants (As, Cd, Pb, Hg, together with several organic pollutants), based on DBS analysis [49], beginning with an examination of Eurasian Griffon Vultures in Israel [50].

The importance of controlling elements related with the presence of prosthesis can be highlighted. Nowadays, large hip and knee implants are made with complex alloys also including ceramic parts, and typically containing elements such as Cr, Co, Mo, Ti or V. Monitoring these elements has become of increasing interest, not necessarily because of their toxicity, but because the appearance of an abnormally high level might indicate prostheses malfunctioning, perhaps requiring a surgical replacement. However, there is still need to develop methodologies that can reliably determine these elements (and some that are particularly relevant, such as Ti) at the low levels at which they are present in controls (below ppb level) and patients [51].

The capability of the recently introduced ICP tandem mass spectrometry (ICP-MS/MS) has demonstrated to be of particular relevance for this type of application

[52,53]. One of the main problems for the accurate determination of light (m/z below 80) elements is the occurrence of spectral overlap. ICP-MS/MS enables: i) the separation of most potential interferences in the first quadrupole; ii) a selective reaction of the ions of the target element with gases (e.g., CH_3F, NH_3 or O_2) in a cell, such that a new polyatomic species is formed; iii) the selective transmission of such polyatomic species through the second quadrupole. This constitutes the so-called mass-shift approach [54]. Thus, an element that suffers of so many potential interferences from elements that are present at high levels in biological fluids such as Ti (see **Table 2**) can be monitored and quantified practically interference-free in the form of $Ti(NH_3)_6^+$ [55].

This technique has been successfully used to determine Ti, V, Co, Sr, and Zr in blood after deposition on a VAMS device (see **section 3** for description), followed by a simple extraction step [56]. Al, Cr and Ni were monitored as well, but contamination issues affected their determination.

An alternative for still achieving sufficiently low limits of detection without using ICP-MS/MS or other more expensive ICP-MS instruments (e.g., high-resolution sector field devices) is to limit the dilution. For instance, the use of a high temperature total sample consumption system (a micronebulizer connected to a low inner volume single pass spray chamber) enhances transport efficiency to almost 100%, enabling the introduction of very small sample volumes (e.g., 2.5 µL). Thus, instead of diluting the blood deposited in the VAMS device (10.5 µL) in 1 mL to obtain enough sample for measuring at a typical aspiration rate using conventional introduction systems (0.5-1.0 mL min^{-1}) [56], with this device it is feasible to extract the target elements in only 100 µL and still be able to perform as many replicates as needed [57].

Regardless of the type of mass spectrometer or sampling introduction device used, an aspect that has to be further investigated is the contamination of the material used to produce the DMS, which probably has not been originally designed with trace and ultratrace elemental analysis in mind. As discussed before, the FP should not contain high amounts of Pb, but when other elements are being investigated, blanks may be a problem: "Adequate control of filter paper element contributions remains the primary obstacle to fully quantitative element measurement in newborn blood using NBSs (neonatal blood spots) [44]". Furthermore, blanks for different lots may vary [42]. When polymeric materials instead of FP are deployed, this problem also arises [56,57]. One solution could be the use of a precleaning step, until blanks for the target elements are sufficiently low [58,59], but always using conditions mild enough to prevent any alteration of the capacity of the materials to absorb biological fluids.

Another interesting element for DMS-based applications is iodine. Control of iodine levels are key to indicate thyroid dysfunctions. It is particularly important to control this parameter in pregnant women, to avoid that these dysfunctions can affect those children gestated in extreme conditions of iodine content.

Iodine is mostly excreted trough urine, so the iodine level in urine provides an reliable indicator of the iodine intake. In this context, after the first work of Zava *et al.* [60], in which an analytical methodology for I (and creatinine) determination in DUS was proposed, several works have used a similar procedure to investigate iodine urinary levels in pregnant women and/or children in particular areas of African countries (Burkina Faso, South Africa and Niger) [61-63], proving the suitability of using DMS for epidemiological studies, as already discussed in **section 2**.

It can be pointed out that, while the spectrophotometric method used in these articles may be fit-for-purpose, there are techniques available nowadays (e.g. ICP-MS) that can provide more selectivity and a higher detection power for iodine, when required.

5.3. Direct analysis of DMS

One alternative approach that can mitigate to some extent contamination issues, not those created by contamination of the FP but those related with sample treatment, is the use of solid sampling techniques. Besides this aspect, recovery problems can also be avoided. Finally, the results can also be obtained faster. This latter aspect, however, depends a bit on the analytical procedure used, since automated systems that enable the simultaneous lixiviation of up to 96 discs (making use of the standard 96 well plates) are available, thus also improving sample throughput.

In any case, since a DMS is a liquid that is finally transformed into a solid sample, and considering the availability of several analytical techniques capable of the direct analysis of these solid samples, it is not surprising to see that such techniques have been evaluated for DMS analysis.

In fact, as discussed before, using the Delves Cup it was possible to determine Pb in a disc punched out from a DBS, with minimal sample treatment [32]. Other techniques may, however, provide more sensitivity and selectivity nowadays. In 2007, both solid sampling (SS)-GF-AAS [40] and laser ablation (LA)-ICP-MS [37] were evaluated for Pb determination in DBS with very different results. In the case of SS-GF-AAS satisfactory quantitative results were obtained, using matrix-matched standards for calibration. These standards were prepared from Pb-free

blood samples spiked with known amounts of Pb, which were deposited onto the FP and dried, in the same way as the samples [40].

In the case of LA-ICP-MS, quantitative information could not be achieved and the differences between DBS prepared with real blood samples or reconstituted reference materials were noted [37].

The potential of SS-GF-AAS has been further enhanced during the last decade with the arrival of high-resolution continuum source graphite furnace atomic absorption spectrometry (HR CS GF-AAS) that, among other features, improves the resolution (down to approximately 1 pm) and the capabilities to detect and to correct for spectral interferences. Use of this instrumentation permitted to further improve Pb determination in DBS [64], avoiding any overlap from phosphates and enhancing the calibration range without the need for carrying out any additional measurements: signals that were outside the upper linear range could be reprocessed using side instead of central pixels, thus monitoring the wings of the atomic lines instead of the center. In such a way, it was possible to achieve a limit of detection of 1 μg L^{-1} and still determine Pb in DBS samples containing hundreds of μg L^{-1}. The method proved its robustness after analysis of more than 500 samples, and it was demonstrated that it could also be easily adapted to a screening method, able to provide a binary response in a simple way [65], as shown in **Table 3**. Discussing the statistical parameters usually calculated to evaluate the performance of a screening method is beyond the scope of the current work, although definitions are provided in the caption of **Table 3**.

HR CS GF-AAS thus offers very interesting characteristics for monoelemental analysis of DMS. In fact, HR CS GF-AAS provides some possibilities for

simultaneous multi-element analysis, although restricted to elements with atomic transitions that are sufficiently close (a few hundreds of picometers), because that is the spectral region simultaneously covered by the detector. This aspect was explored to determine Mo and Ti in DUS [51].

Focusing on DUS, one critical aspect when trying to analyze urine instead of blood is that urine spreads much more over the surface of the FP, thus leading to a more inhomogeneous distribution of the analytes (chromatographic effect) and to lower sensitivity. To prevent these issues, use of a precut FP on top of a hydrophobic surface, making it possible to deposit a larger amount of urine and letting it dry, was earlier proposed by Aramendía *et al*. [66] (see **Figure 4a**). This procedure improves the amount of urine deposited per area unit by a factor of 10, approximately. The main steps of the protocol finally used for Mo and Ti determination in DUS are shown in **Figure 4b**. A volumetric deposition (500 µL of urine) on a precut FP and the use of matrix-matched standards (prepared with real urine samples spiked with known amounts of the analytes) enabled reliable quantitative results to be obtained, with LODs (1.5 µg L^{-1} for Mo and 6.5 µg L^{-1} for Ti), possibly low enough to detect prostheses malfunctioning, but not sufficiently low to determine Ti in controls and patients with a well-performing implant [51].

To achieve more detection power, much higher multi-element potential and even isotopic information, LA-ICP-MS seems to be the technique of choice, despite the problems detected in early works [37]. These problems, however, are better understood and can be mitigated nowadays.

For instance, the work referred above by Aramendía *et al*. [66] demonstrated the potential of the technique for analysis of DUS. The usage of precut saturated DUS as the target sample is very important in this case because the inhomogeneity in the distribution of the analytes affects significantly more LA-ICP-MS, as it is a microsampling technique. Besides this point, use of a suitable internal standard (IS) is highly recommended for LA-ICP-MS. This is an important aspect and a significant difference with extraction/digestion approaches, where addition of an IS is much easier. In this work, it was shown that it is possible to deposit the IS standard solution (of Pt) on top of the dried PS-DUS. In this regard, the method proposed is thus compatible with simple DUS collection in an unsupervised context, and provided LODs of 1 μg L^{-1} or lower for 9 of the 11 elements investigated.

Blood is, however, a more complex sample than urine and thus, LA-ICP-MS analysis of DBS is *a priori* more challenging. However, the use of femtosecond LA devices operating at a high repetition rate opens some possibilities. With such LA devices, it may be feasible to ablate the blood spot completely, thus circumventing homogeneity issues and differences in spreading between real samples and reconstituted references materials, which can be appreciated even by visual inspection, as shown in **Figure 5a** [38].

Instead of the more conventional approach of using delivering high energy pulses (> 1 mJ) at low repetition rates (in the range of 1-50 Hz) and using relatively large laser spots (e.g., 80-100 μm), these devices deliver relatively low energy laser pulses (e.g., < 200 μJ) for small laser spots (10-15 μm) but at very high repetition rates (up to 100 KHz) [67]. The laser beam can be moved very fast and with high precision, such that overlapping these small spots (see **Figure 5a**) it is actually

possible to efficiently ablate a large area of any shape desired in a reasonable time (200 s for a 5 µL DBS). It can be seen in **Figures 5b** and **5c** that the distributions of Pb in the DBS of a real blood sample and of a reconstituted reference material are very different, but the integrated signals of the complete spots are comparable. This way, accurate results (and LODs below 1 µg L^{-1}) could be obtained for Cd, Co, Cu and Pb, for real blood samples as well as reference materials, relying upon the use of matrix-matched standards for calibration. In this case, since addition of an IS solution over the DBS is not straightforward (unless some dedicated approach is developed, such as the use of a piezo electric spray system [68]), the FP material was impregnated with the IS solution and dried, before the deposition of blood droplets [38].

The last paper published on this topic further demonstrates the power of LA-ICP-MS for multi-element determinations, as long as matrix-matched standards and a suitable internal standard are used [69].

A technique that has not been used in this context until very recently but provides some interesting features is X-ray fluorescence spectrometry (XRF). A novel paper evaluates the use of energy dispersive (ED)-XRF for DBS analysis [70]. While this technique is not sensitive enough for trace elemental analysis, and still requires matrix-matched solid standards for calibration, it is completely non-destructive, does offer information about the surface of the whole spot, minimizing distribution issues, and can provide complementary information for elements present at high levels that are also of clinical interest. In this regard, the importance of the determination of K can be highlighted, because it has been shown that its content can be used to correct for hematocrit variations [58,71,72]. Fast, simple and non-destructive analysis of DBS using ED-XRF can therefore

provide valuable information *per se* (e.g., helping in detect Fe deficiency anemia, the most common type of anemia), but also useful information prior to the use of other more sensitive but destructive techniques such as LA-ICP-MS (e.g., knowing the Fe level enables its signal to be used as IS; knowing the K content facilitates correcting for hematocrit variations).

Overall, despite the difficulties, it is clear that modern atomic spectrometry techniques offer enough potential to enable analysis of DMS at practically all analyte levels.

6. Obtaining further information for diagnosis: DMS for isotopic analysis

A more recent trend is the use of not only elemental, but also of isotopic information that can provide key clinical data. This trend has been related with the improvements experienced by multicollector (MC)-ICP-MS instruments, which can provide the excellent precision (down to 0.002% in the best circumstances) needed to detect the small isotopic variations expected, while offering much higher sample throughput than other traditional techniques, such as thermal ionization mass spectrometry (TIMS), and, also, the potential to produce positively charged ions from most elements in the periodic table (TIMS typically needs elements to show an ionization energy ≤7.5 eV). These aspects have opened possibilities to investigate new isotopic systems, including those of essential elements that can be relevant for biomedical applications, such as Ca, Cu, Fe or Zn [73].

It is beyond the scope of the current review to discuss this topic in detail. The readers are instead referred to a recent review by Costas-Rodriguez *et al*. [73]. It is however important, in order to understand the applications reported using

DMS, to point out that during biological processes taking place in the human body systematic isotope fractionation for the elements mentioned above can occur, and that fractionation can be measured with the methodologies available nowadays. Such fractionation may be related with some medical conditions and, thus, isotopic analysis may help in understanding better how these proceed, and even enable to diagnose them more reliably [74,75].

One paradigmatic case is Cu and Wilson´s disease (WD). WD it is a genetically determined disorder related with Cu metabolism. This clinical condition is caused by mutations in the ATP7B gene, which encodes the ATP7B protein. This protein facilitates the release of Cu ions from the liver into the bloodstream, by linking them to ceruloplasmin, but such function is impaired in WD patients. Thus, Cu accumulates in the liver tissue, among other organs, leading to grave health issues.

As a consequence of this mechanism, WD patients show Cu levels in both blood and serum that are lower than normal. Therefore, Cu determination in these fluids should permit the diagnosis of this disease. This is, however, not that simple because young infants, due to the immaturity of their liver, tend to show also Cu values that follow the same trend, even when healthy. This fact makes it difficult to diagnose this disease properly at early stages, before irreversible damage has occurred, which is unfortunate because WD is highly tractable.

In this context, the use of isotopic analysis can be helpful. Since, as discussed before, isotope fractionation typically accompanies (bio) chemical reactions, it could be expected that the protein ATP7B would interact with the two natural occurring isotopes of Cu (^{63}Cu and ^{65}Cu) with slightly different yields. This, in

practice, may be very hard to detect in the case of well-functioning proteins operating with a high efficiency, close to 100%. However, defective ATP7B proteins found in WD patients will show a much lower efficiency for interacting with Cu and incorporating it into ceruloplasmin. Therefore, isotope fractionation may become more apparent for these persons, thus enabling to differentiate WD patients from people that show low Cu blood levels for other reasons.

MC-ICP-MS analysis of serum proved that such seems to be the case [76]. The results for infants showed a Cu level lower than controls, but a similar $^{65}Cu/^{63}Cu$ value. However, patients of WD (and, to some extent, of bariatric surgery) showed not only a low Cu level in serum, but a $^{65}Cu/^{63}Cu$ value that is significantly biased low, indicating an enrichment in the lighter isotope, ^{63}Cu. Thus, there is potential to diagnose WD even in children, combining elemental and isotopic Cu determination.

The work referred above did not make use of DMS, but of digested serum samples, and obtained high precision (0.01%) after Cu isolation. However, if the goal is to be able to detect the disease in newborns, the analytical methodology should target the biological samples typically used in the screening programs for metabolic diseases already implemented throughout the world. In other words, DMS analysis would be greatly beneficial.

Such type of analysis is more challenging, particularly if direct solid sampling is aimed at, as the presence of the matrix may affect the results (e.g., $^{40}Ar^{23}Na^{+}$ overlap with $^{63}Cu^{+}$). However, some promising results have been reported. Resano *et al*. used the same fs-LA device discussed in **section 5.3.** [38] coupled to a MC-ICP-MS for Cu isotopic analysis of DUS, as a completely non-invasive sampling approach [77]. By ultrafast overlapping of small spots, this LA device

can ablate in practice any portion of the DUS, with any geometrical shape. In this work, it was decided to ablate small coronas in the rim of the filter, where Cu signal intensity was higher, because sensitivity is key to achieve sufficient precision with MC-ICP-MS using Faraday cups as detectors [77].

In the end, a method was proposed based on a) deposition of 300 µL of urine onto precut (16 mm diameter) FP; b) air drying for 4h; and, c) Cu isotopic analysis of the DUS using fs-LA-ICP-MS with the LA operating a 10,000 Hz and ablating the abovementioned coronas, the MC-ICP-MS operating in pseudo high-resolution mode to avoid spectral overlap, simultaneously admixing a Ni solution as internal standard to correct for mass bias, and finally processing the transient signals using a linear regression approach to enhance precision.

While precision values were not as good as those obtained after sample digestion and analyte isolation (0.05% RSD for urinary Cu contents of a few hundred µg L^{-1}), it was possible to appreciate that untreated WD patients show a $^{65}Cu/^{63}Cu$ biased low, while WD patients under treatment with a chelating agent show a ratio comparable to that of a control sample (very close to natural abundance). These results further prove the potential interest of the approach for diagnosis, as well as the success of the treatment, although a larger number of samples should be analyzed to confirm them [77].

Authors from the same research groups also analyzed DBS samples, in this case using the same LA device simultaneously coupled to two ICP-MS instruments: one high-resolution sector field-ICP-MS that provides excellent sensitivity and resolution for elemental analysis and one MC-ICP-MS that provides more precision for isotopic analysis [38]. Therefore, simultaneous elemental (Cd, Cu,

Co, Pb) and isotopic analysis (Cu) could be carried out, thus maximizing the amount of information that can be drawn from a single DBS specimen. The results for quantitative elemental analysis were discussed in the previous section. Regarding Cu isotopic determination, the precision of the approach was found to be limited at this point, with RSD values of approximately 0.15 %.

The last article to be published on this topic investigated the use of VAMS (see **section 3** for details) for isotopic analysis of Fe. This topic is relevant because it may provide information to achieve a better understanding of human Fe metabolism, as well for the diagnosis of Fe related disorders, such as hemochromatosis and anemia of chronic disease, among others.

After finger-prick sampling, it was feasible to extract Fe quantitatively from the VAMS into 1 mL of milli-Q water, which was followed by acid digestion and chromatographic isolation to provide the high-precision needed for monitoring Fe ratios in biomedical applications. No significant difference was observed between paired finger-prick and venous blood samples, regarding both Fe concentrations and isotope ratios [78].

While the number of groups working in this field, with access to MC-ICP-MS instrumentation and to personnel with a biomedical background and samples, is still limited, its relevance permits anticipating a growing interest and further results in upcoming years.

7. Outlook and conclusions

Despite the current importance of DMS, and of DBS in particular, as a biological specimen, this type of sample is still underutilized when aiming at obtaining

elemental information. It is, however, our view that this situation is changing, and that the arrival of new sampling devices and methodological approaches based on a constant volume make it easier to obtain reliable information on elemental contents.

In particular, the development of simple approaches that can be automated relying either on simple analyte extraction procedures or on solid sampling methodologies, are prospective areas of research. Still, the problem of contamination remains to be solved as the number of analytes to be monitored expands.

Finally, this methodology could benefit from the new type of information that can be obtained based on isotopic analysis of non-traditional stable isotopes, such as those of Cu, Fe or Zn, which can contribute to diagnose and better understand some diseases.

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Figure Captions

Figure 1. a) Publications on DBS; b) Publications on DUS. Conference papers were excluded. Source: Scopus, October 31, 2017.

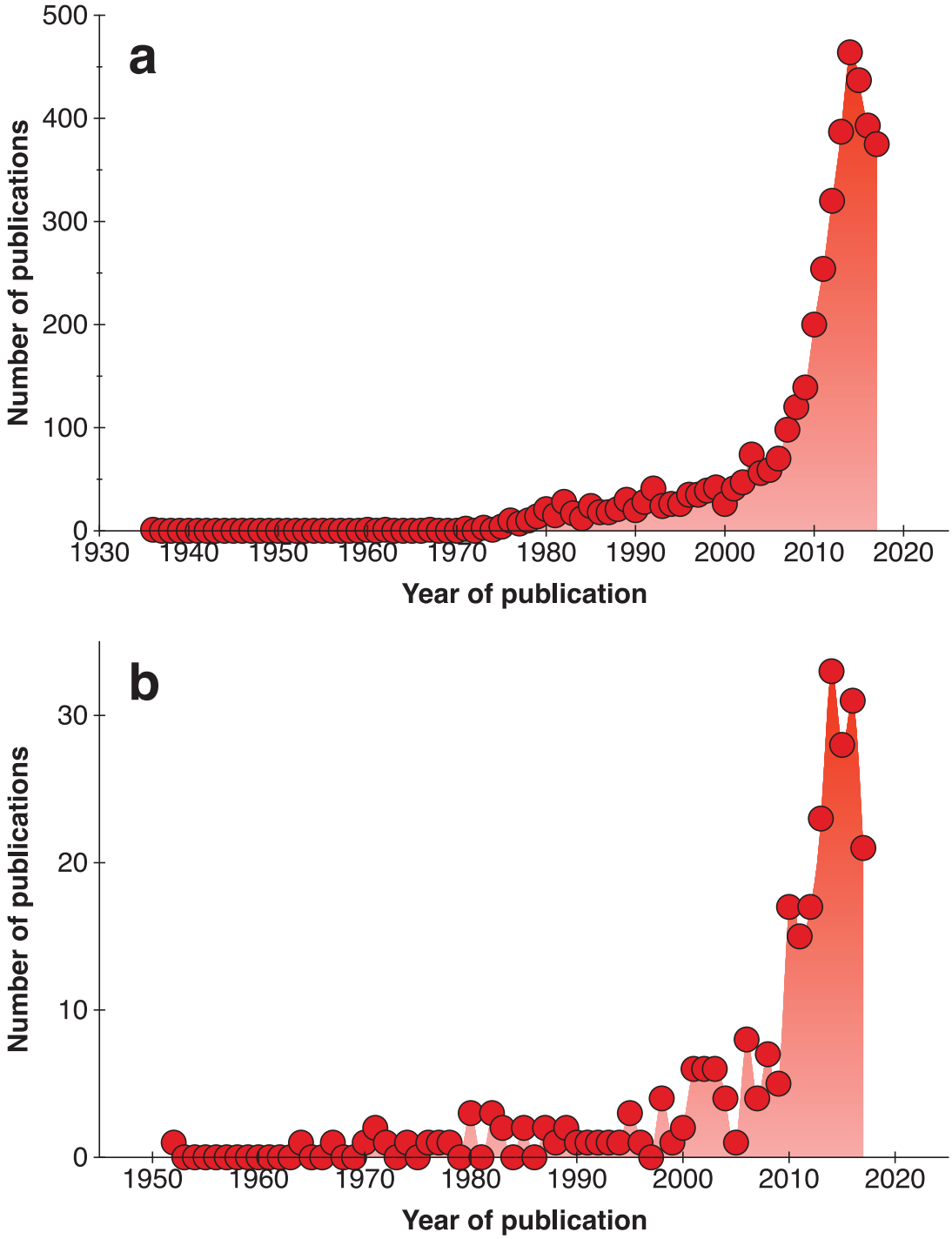
Figure 2. Biosampling using DBS. Simplified analytical procedure for DBS analysis.

Figure 3. a) Sampling blood at home using a VAMS device (left), and comparison of a VAMS device before and after sampling (right); b) Microfluidic-based device (patent WO/2013/144743 A1) for sampling blood. The sampling procedure consists of the following steps: (a) sliding the selected FP card into the cover slot; (b) filling the channels by contacting the fingertip blood drop with the inlet; (c) transferring the fixed blood volume onto the FP card by closing the cover; and (d) opening the cover and removing the FP card containing the DBS samples, which can then be processed as shown in Figure 2 (reprinted with permission from [28], copyright 2015 American Chemical Society).

Figure 4. a) Visual comparison of the sample concentration per area unit achieved when using DUS (above) and precut saturated-DUS specimens (below), respectively. DUS specimens were obtained by directly blotting the corresponding volumes of urine onto a Whatman 903 FP card with a micropipette, while precut saturated-DUS specimens were obtained by blotting 300 µL of urine onto precut FP discs (16 mm diameter), placed over a hydrophobic surface (a polycarbonate disc). Adapted with permission from [66]. Copyright 2012 American Chemical Society. b) Different steps for the direct Mo and Ti determination in DUS by HR CS GF-AAS. Adapted from ref. [51], copyright 2013, with permission from Elsevier.

Figure 5. a) Schematic representation of the ablation protocol used for complete ablation and analysis of 5 mL DBS. 5–6 mm diameter craters (depending on the DBS size) were constructed by ablating 250–285 concentric circumferences from the inside to the outside of the DBS. Approximated radial distribution of Pb in 5 mL DBS obtained from: b) a real sample; c) Seronorm level II reference sample. Each bar represents the integrated $^{208}Pb^{+}$ signal, normalized for a concentration of 1 μg L^{-1}, for the ablated corona with internal and external radius indicated in the x-axis in the figure. Adapted from ref. [38] with permission from the Royal Society of Chemistry (http://pubs.rsc.org/en/content/articlelanding/2015/ja/c4ja00313f#!divAbstract).

Figure 1


a
Number of publications
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100
200
300
400
500
1930
1940
1950
1960
1970
1980
1990
2000
2010
2020
Year of publication
b
Number of publications
0
10
20
30
1950
1960
1970
1980
1990
2000
2010
2020
Year of publication

Figure 2

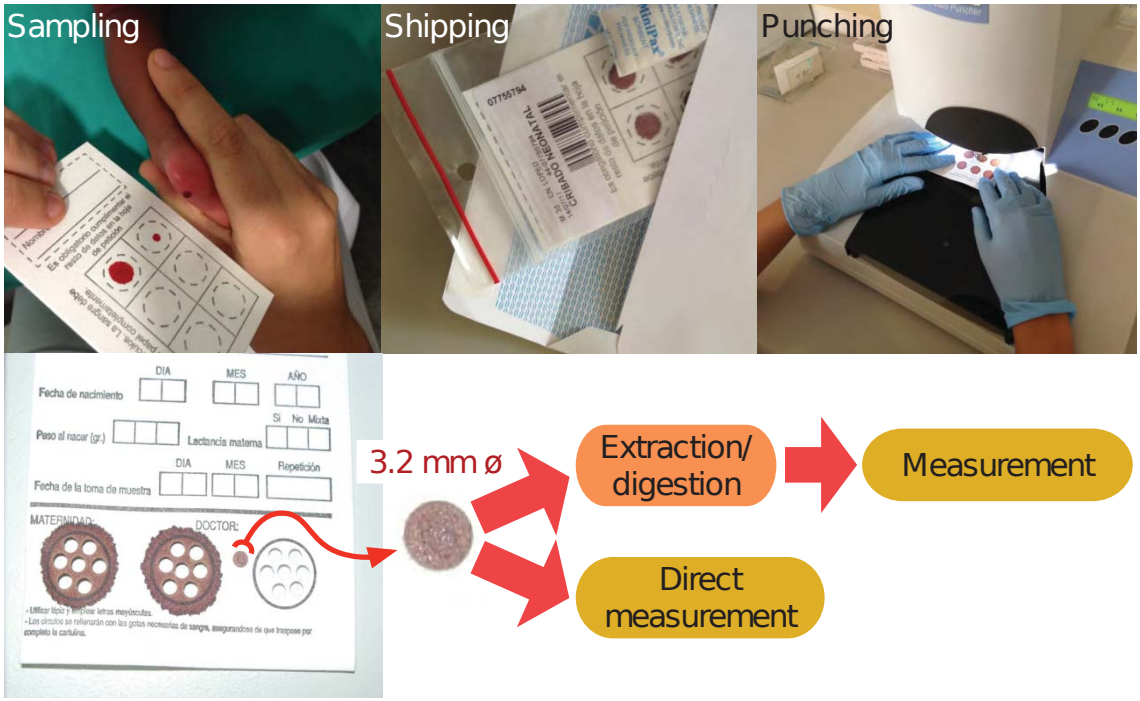
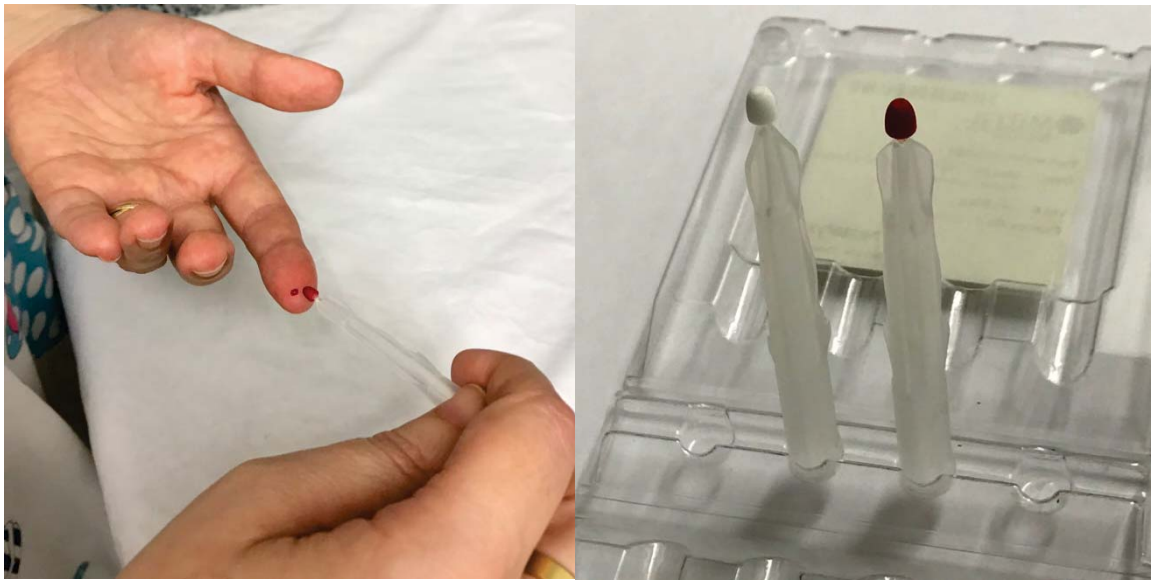

Sampling
Shipping
Punching
Fecha de nacimiento
DIA
MES
AÑO
Peso al nacar (gr.)
Lactancia materna
Si No Mixta
Fecha de la toma de muestra
DIA
MES
Repetición
MATERNIDAD:
DOCTOR:
3.2 mm ø
Extraction/ digestion
Measurement
Direct measurement

Figure 3

a)



b)

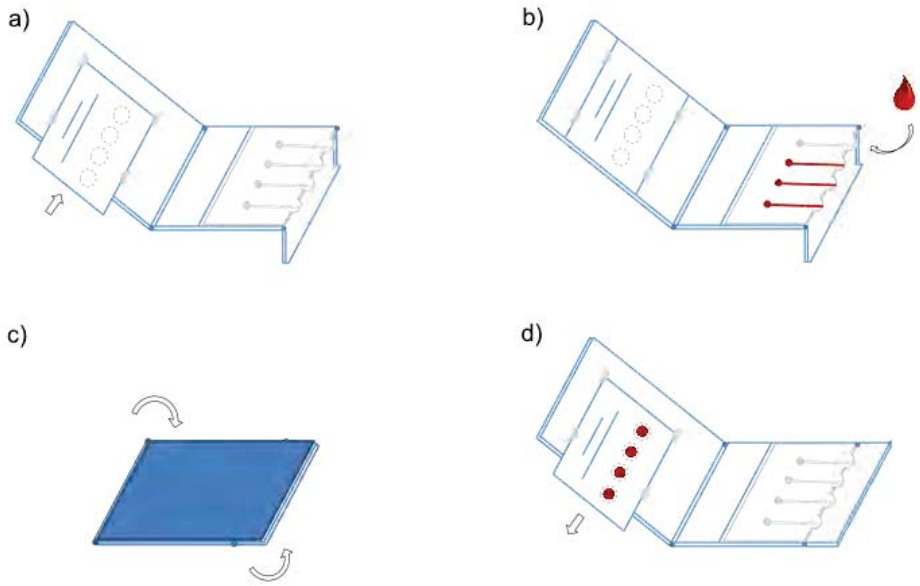


Figure 4

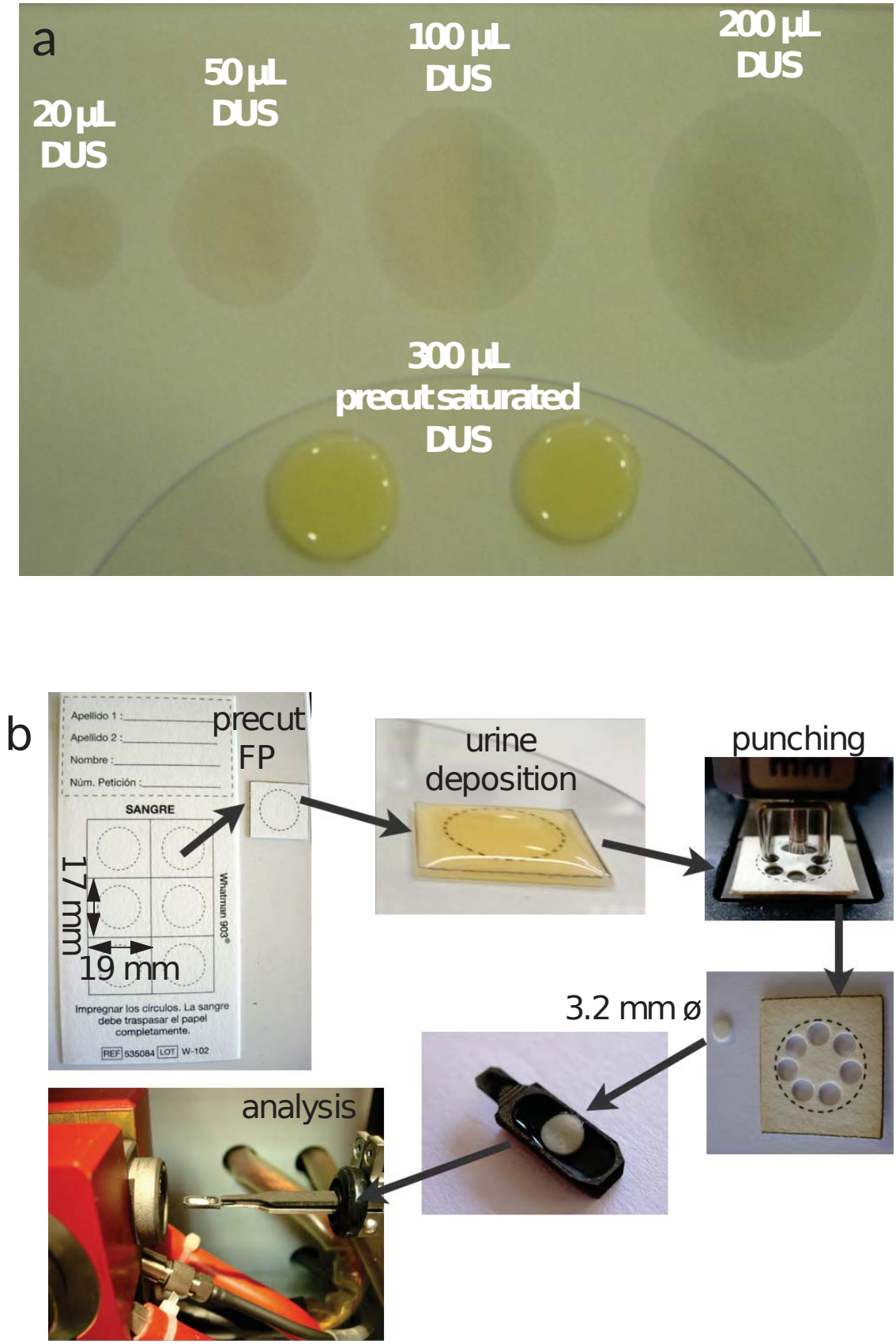

a
20 µL DUS
50 µL DUS
100 µL DUS
200 µL DUS
300 µL precut saturated DUS
b
Apellido 1 :
Apellido 2 :
Nombre :
Núm. Petición :
SANGRE
17 mm
19 mm
Whatman 903®
Impregnar los círculos. La sangre debe traspasar el papel completamente.
REF 535084 LOT W-102
precut FP
urine deposition
punching
3.2 mm ø
analysis

Figure 5

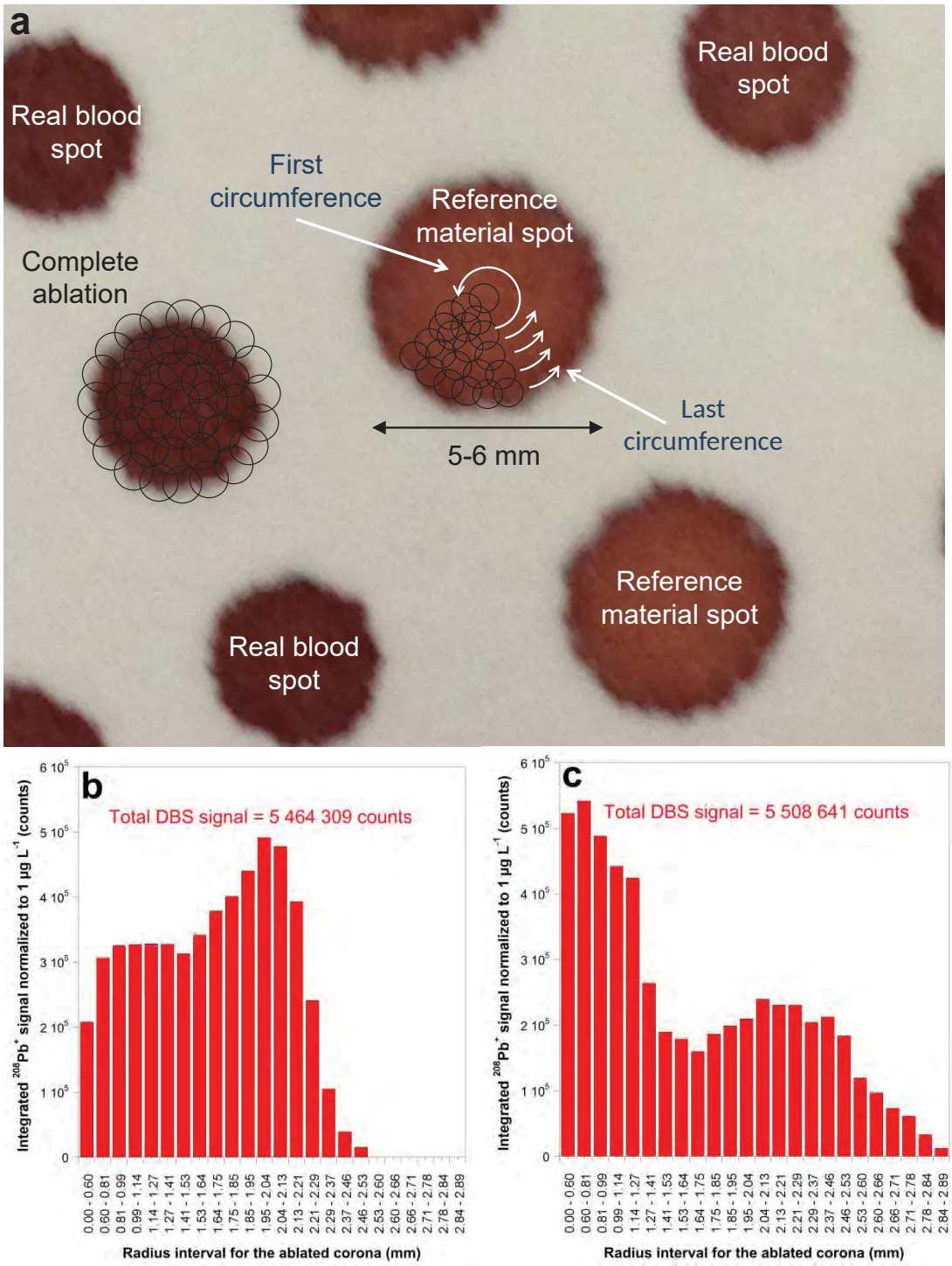

a
Real blood spot
Real blood spot
First circumference
Reference material spot
Complete ablation
Last circumference
5-6 mm
Reference material spot
Real blood spot
b
Total DBS signal = 5 464 309 counts
Integrated $^{208}Pb^{+}$ signal normalized to 1 µg L^{-1} (counts)
Radius interval for the ablated corona (mm)
c
Total DBS signal = 5 508 641 counts
Integrated $^{208}Pb^{+}$ signal normalized to 1 µg L^{-1} (counts)
Radius interval for the ablated corona (mm)

Table 1. Elemental determinations carried out in DMS samples.

Analyte	Sample	Method	Remarks	Year	Ref.
Pb	blood	F-AAS	Analysis of blood microsamples (10 µL) with the Delves Cup	1970	[31]
Pb	DBS	F-AAS	Direct analysis of DBS with the Delves Cup	1971	[32]
Pb	DBS	F-AAS	Further direct analysis of DBS with the Delves Cup. Method comparison	1973	[79]
Pb	DBS	F-AAS	Further direct analysis of DBS with the Delves Cup. Method comparison	1974	[34]
Pb	DBS	F-AAS	Delves Cup. Effect of abnormal haemoglobin values.	1978	[36]
Pb	DBS	F-AAS	Modification of the Delves method by previous calcination of the sample.	1991	[33]
Pb	DBS	GF-AAS	Extraction with $(NH_4)_2HPO_4$ and Triton X-100 solutions. Matrix-matched standards for calibration	1992	[80]
Pb	DBS	F-AAS	Direct analysis of DBS with the Delves Cup. Method comparison	1994	[81]
Pb	DBS	F-AAS	Direct analysis of DBS with the Delves Cup. Method comparison	1995	[82]
Pb	DBS	GF-AAS	Extraction with HNO_3 and Triton X-100 solutions	1995	[83]
Pb	DBS	ICP-MS	Extraction with a HNO_3 solution. Y added to the solution as IS.	1995	[84]
Pb	DBS	GF-AAS	Similar to [83]	1997	[85]
Pb	DBS	GF-AAS	Similar to [83]	1998	[86]
Pb	DBS	GF-AAS, F-AAS	Interlaboratory comparison	1999	[87]
Pb	DBS	GF-AAS	Extraction with a HNO_3 solution	2003	[88]
Pb	DBS	GF-AAS	EDTA plus $(NH_4)_2HPO_4$, HNO_3 and Triton X-100 to extract Pb	2004	[41]
Pb	DBS	ICP-MS	Influence of sampling different areas of the DBS	2007	[39]
Pb	DBS	LA-ICP-MS	Direct analysis. Feasibility study	2007	[37]
Pb	DBS	GF-AAS	Direct analysis. Calibration with matrix-matched standards	2007	[40]
Cd, Hg, Pb	DBS	ICP-MS	Extraction with HCl, 2-mercaptoethanol and L-cysteine solutions	2009	[42]
Pb	DBS	ICP-MS	Investigation of blood volumes and spot geometry	2009	[35]
28 elements	DBS	ICP-MS	Extraction with HNO_3 and Triton X-100 solutions	2011	[44]
As, Cd, Pb, Hg, Se plus organic pollutants	DBS	ICP-MS	Database for exposure to environmental toxicants *via* analysis of avian blood spots	2011	[49]

Pb	DBS	ICP-MS	Method similar to [39]. Relationship between prenatal and infant blood lead levels	2012	[89]
As, Cd, Pb, Hg, Se plus organic pollutants	DBS	ICP-MS	Examination of Eurasian griffon vultures *via* DBS	2012	[50]
Be, Bi, Cd, Co, Cu, Ni, Sb, Sn, Tl, Pb, V	DUS	LA-ICP-MS	Direct analysis. Deposition on precut-saturated DUS. Pt as IS. Calibration with matrix-matched standards	2012	[66]
As, Cd, Hg, Pb	DBS	ICP-MS	Improved procedure from [44]. Addition of Au in the extraction solution to amalgamate Hg and enhance recovery	2013	[46]
Ti and Mo	DUS	HR CS GF-AAS	Direct analysis. Urine deposition on precut-saturated DUS. Calibration with matrix-matched standards.	2013	[51]
As, Cd, Hg, Pb, Se, Tl	DBS	ICP-MS	Overnight digestion with concentrated nitric acid at 95 ºC.	2013	[90]
I	DUS	MAS	Photometric approach after Sandell–Kolthoff reaction	2013	[60]
K	DBS	Potentio-metry	Prediction of the hematocrit of DBS *via* potassium measurement	2013	[71]
As, Cd, Cu, Pb, Mo, Se, Zn	DBS	ICP-MS	Acid digestion and matrix-matched calibration with reference materials	2014	[91]
K	DBS	Potentio-metry	Correction of the hematocrit effect in DBS *via* potassium measurement	2015	[72]
As, Cd, Hg, Pb	DBS	ICP-MS	Similar procedure to [46]	2015	[59]
Pb	DBS	GF-AAS	Similar procedure to [41]	2015	[92]
Pb	DBS	HR CS GF-AAS	Direct analysis. Performance as a quantitative or as *screening* method	2015	[64]
Cd, Cu, Co, Pb	DBS	LA-ICP-MS	Direct analysis by complete ablation of 5 µL DBS. Calibration with matrix-matched standards. Pt as IS.	2015	[38]
I	DUS	MAS	Photometric approach after Sandell–Kolthoff reaction	2015	[61]
Al, Ti, V, Co, Cr, Ni, Sr, Zr	DBS	ICP-MS	Use of VAMS and extraction in water. ICP-MS/MS analysis for coping with spectral overlap.	2016	[56]
I	DUS	MAS	Photometric approach after Sandell–Kolthoff reaction	2016	[62]
Hg	DBS	ICP-MS	Underestimated values for Hg in DBS compared with cord blood. Likely due to poor extraction efficiency.	2016	[47]
11 elements	DBS	ICP-MS	Use of VAMS and extraction in water. Use of hTISIS for microsampling.	2017	[57]

15 elements	DBS	ICP-MS	Simultaneous measurement of K and Fe in DBS extracts may be used to estimate sample hematocrit.	2017	[58]
19 elements	DBS	LA-ICP-MS	Matrix-matched standards for calibration. ^{13}C and ^{32}S were used for signal intensity normalization	2017	[69]
Pb	DBS	ICP-MS	Extraction in a solution containing tetramethylammonium hydroxide, isopropyl alcohol, and ammonium pyrrolidine dithiocarbamate	2017	[43]
Cl, Fe, K, P, S	DBS	ED-XRF	Direct non-destructive analysis; matrix-matched standards for calibration	2017	[70]
I	DUS	MAS	Photometric approach after Sandell–Kolthoff reaction	2017	[63]
MeHg	DBS	GC-CV-AFS	Separation of Hg species and quantification of MeHg	2017	[48]

Table 2. Titanium isotopes with their natural isotopic abundance and the most important isobaric and polyatomic interferences (non-restrictive list). Reproduced from [55], copyright 2014, with permission from Elsevier.

Analyte	Abundance (%)	Isobaric interferences	Polyatomic Interferences
$^{46}Ti^{+}$	8.25	Ca^{+} (0.004[a])	$^{32}S^{14}N^{+}$, $^{14}N^{16}O_2^{+}$, $^{15}N_2{}^{16}O^{+}$
$^{47}Ti^{+}$	7.44	---	$^{32}S^{14}N^{1}H^{+}$, $^{30}Si^{16}O^{1}H^{+}$, $^{32}S^{15}N^{+}$, $^{33}S^{14}N^{+}$, $^{15}N^{16}O_2^{+}$, $^{14}N^{16}O_2{}^{1}H^{+}$, $^{12}C^{35}Cl^{+}$, $^{31}P^{16}O^{+}$
$^{48}Ti^{+}$	73.72	Ca^{+} (0.187[a])	$^{32}S^{16}O^{+}$, $^{34}S^{14}N^{+}$, $^{33}S^{15}N^{+}$, $^{14}N^{16}O^{18}O^{+}$, $^{14}N^{17}O_2^{+}$, $^{12}C_4^{+}$, $^{36}Ar^{12}C^{+}$
$^{49}Ti^{+}$	5.41	---	$^{32}S^{17}O^{+}$, $^{32}S^{16}O^{1}H^{+}$, $^{35}Cl^{14}N^{+}$, $^{34}S^{15}N^{+}$, $^{33}S^{16}O^{+}$, $^{14}N^{17}O_2{}^{1}H^{+}$, $^{14}N^{35}Cl^{+}$, $^{36}Ar^{13}C^{+}$, $^{36}Ar^{12}C^{1}H^{+}$, $^{12}C^{37}Cl^{+}$, $^{31}P^{18}O^{+}$, $^{31}P^{17}O^{1}H^{+}$
$^{50}Ti^{+}$	5.18	Cr^{+} (4.345[a]), V^{+} (0.25[a])	$^{32}S^{18}O^{+}$, $^{32}S^{17}O^{1}H^{+}$, $^{36}Ar^{14}N^{+}$, $^{35}Cl^{15}N^{+}$, $^{36}S^{14}N^{+}$, $^{33}S^{17}O^{+}$, $^{34}S^{16}O^{+}$, $^{35}Cl^{14}N^{1}H^{+}$, $^{34}S^{15}N^{1}H^{+}$

[a] Isotopic abundance (%) for isobaric interferences

Table 3. Diagnostic efficiency values for the screening method proposed in [64] for a critical value (threshold) of 50 μg L^{-1} Pb. Adapted from [64] with permission of Newlands Press Ltd.

	Prevalence (%)	Cut-off (μg L^{-1})	Sensitivity (%)	Specificity (%)	FNR (%)	FPR (%)	NPV (%)	PPV (%)
All samples (n = 526)	21.7	38	96.6	87.8	3.4	12.2	98.9	83.3
Newborns (n = 229)	1.3	38	100	85.8	0	14.2	100	8.6
Pregnant women (n = 185)	1.1	38	100	90.2	0	9.8	100	10.0

Prevalence: percentage of true positives in the population investigated; **Sensitivity:** % of positives correctly identified as such; **Specificity**: % of negatives correctly identified as such; **FNR**: False Negative Rate; **FPR**: False Positive Rate; **NPV**: Negative Predictive Value; **PPV**: Positive Predictive Value.

Table 4. Isotopic determinations carried out in DMS samples.

Analyte	Sample	Method	Remark	Year	Ref.
Cu	DUS	LA-MC-ICP-MS	Direct analysis. Urine deposition on precut-saturated DUS. Ni solution as IS	2013	[77]
Cu	DBS	LA coupled with both SF-ICP-MS and MC-ICP-MS	Simultaneous elemental (Cd, Co, Cu, Pb) and isotopic analysis (Cu). Ni solution as IS	2015	[38]
Fe	DBS	MC-ICP-MS	Use of VAMS and extraction in water, followed by acid digestion and Fe isolation by chromatographic separation	2017	[78]