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Sample Traceability in Toxicology

Laura Börgel Aguilera and Melissa Schulthess

Abstract

Sampling is an instrument that allows having a portion that represents a whole, and, quantitatively, it allows to measure a specific analyte or several analytes, for diagnostic, clinical, and forensic exposure or control over time, based in a pre-established and validated study plan. In clinical and forensic samples from one individual, the toxicokinetic and toxicodynamic factors should be considered in order to choose the most adequate matrix to study. In case of deceased individuals, additional matrices should be considered to the usual matrix. Sampling should be representative as for quality and quantity and should be associated to a chain of custody. Transport, storage, and analysis of samples are related to the type of matrix and the analyte to identify/measure. All samples should be traceable in any stage of the analysis and should receive an internal codification on entry. Also, the analytical method should be validated and associated to a traceable quality management system. Lastly, biosafety should consider the international recommendations for classification of mixtures and the residue management, in order to ensure the operativity of the technical working group.

Keywords: traceability, custody chain, experimental samples, clinical samples, forensic samples

1. Introduction

Sampling is an instrument that allows having a portion that represents a whole, and, quantitatively, it allows to measure a specific analyte or several analytes, for diagnostic, clinical, and forensic exposure or control over time, based in a pre-established and validated study plan.

According to the different standards, guidelines, or criteria, national and international or statistical criteria, the size of the samples will be defined. This applies specifically to studies of pharmaceutical drugs, pesticides, or productive procedures, and it also applies to clinical studies, experimental toxicology and ecotoxicology.

On the other hand, in clinical and forensic samples from one individual, toxicokinetic and toxicodynamic factors should be considered in order to choose the most adequate matrix to study.

2. Considerations

All sampling process must consider aspects related to the sampling, such as identity; name; ID or DNI, if applicable; court order; or customer's request. On the other hand, it's important to consider the type of container and specific requirements, such as moment or time of sampling. This is imperative for occupational

samples, where there is the need to assess the concentrations after a period of exposure. This also applies for the monitoring of drugs, where the time between sampling and ingestion will be important. This is relevant in cases of drugs at the workplace that may be sampled after a work accident or surprisingly as part of the company's alcohol and drug policy. The time lapse between the exposition and the sampling is also important in cases of post-environmental exposure when investigating a source of emission of a particular pollutant or part of it or in case of a drug or toxicokinetic study in which these parameters are being studied in order to achieve conclusions.

In addition to the above, it's also a necessity to specify the type of sample (nature), i.e., blood, urine, hair, gastric content, or other matrices. The latter type of sample is relevant in the forensic toxicology field, because it does not only include biological samples, but environmental, event site and clothes related to the case.

For this purpose, there must be an instrument that allows to submit the relevant information of the sampling (auditable record), with date, time, place, responsible of sampling and with its corresponding identification, circumstances or sampling objective (analyte), type of sample, quality of acceptability of this, both in quantity and added preservatives, and also rejection criteria. The person in charge of the packaging and transportation must be consigned. It's also important to register if the sample complies with the temperature conditions to proceed to quantify a certain parameter in it or, in the case of forensic samples, if the corresponding chain of custody is accompanied.

When entering the analytical area, a unique number or code of correlative sample income will be assigned. This code will be the new identity of the sample.

3. Sample selection

The choice of the sample depends on various factors to consider:

- Emergency patient in critical condition
- Monitoring of neurological, psychiatric, and chemotherapeutic treatment and dose readjustment
- Toxicokinetic and toxicodynamic factors of the agent
- Studies of environmental contaminants in work spaces and open spaces
- Quality control of productive processes of drugs, pesticides, and in general any industrial process
- Forensic studies of both the victim and the site of the event and its findings
- Laboratory equipment and validated analytical methods

There are several sources to determine quality and type of sample, where those based on validation processes stand out both in the United States and in Europe. In this context, the European Union created in 1993 the European Center for the Validation of Alternative Methods (ECVAM). This center labors it to coordinate the activities carried out in its territory and to cooperate with organizations in the United States such as the Johns Hopkins University Center for Alternatives to

Animal Testing (CAAT) (an academic institution) or the Interagency Coordination Committee for the Validation of Alternative Methods (ICCVAM). The latter is composed of representatives of the National Institutes of Health, the Environment Protection Agency (EPA), the Food and Drug Administration (FDA), and the Consumer Products Safety Commission [1].

Based on the National Institute for Safety and Hygiene and Work from Spain (INSHT) review, the following aspects should be considered for the selection of the most representative sample in the case of toxic substances [1].

3.1 Urine

The renal excretion of a toxin by urine depends on the partition coefficient of Nernst, the dissociation constant, the pH of the urine, the size and shape of the molecules, and the speed metabolic transformation in more hydrophilic metabolites and also depends on the functional capacity of the kidney.

The kinetics of the renal excretion of a toxic or its metabolite can be expressed in a curve of two, three, or four phases, depending on the distribution of the substance in the various body compartments, which present different rates of exchange with the blood.

Given the above, as an example, in the case of cocaine, it's possible to find during the first hours after single consumption only cocaine. Only after 12–24 h, its metabolite benzoylecgonine can be found, and it has a half-life in urine of approximately 72 h.

3.2 Saliva

Some drugs and metal ions can be excreted in the saliva through the mucosa of the mouth. Some examples are lead (“lead line”), mercury, arsenic, and copper, as well as bromides, iodides, ethyl alcohol, alkaloids, etc.

After its excretion through saliva, these toxins can be swallowed and reach the gastrointestinal tract, where they can be reabsorbed or eliminated in the stool.

3.3 Sweat

This applies for substances as ethyl alcohol, acetone, phenols, carbon disulfide, and chlorinated hydrocarbons.

3.4 Milk

Numerous metals, organic solvents, some organochlorine pesticides (i.e., DDT), and other persistent organic compounds (POPS) are secreted through the mammary gland in breast milk. This route is of great importance for the evaluation of risk by chronic exposure to these compounds, which can be dangerous for nursing infants.

3.5 Hair

Hair analysis can be used as an indicator of the homeostasis of some physiological substances. Exposure to some toxins, especially heavy metals and drugs of abuse, can also be assessed by this type of bioassay. In case of exposures to highly toxic concentrations of drugs, such as salicylic acids, these doses may be detected exceptionally in hair.

3.6 Other samples

The elimination of toxins from the body can be increased by using methods such as chelation tests with chelators of the type edetate monocalcium (Ca-EDTA), dimercaprol (BAL), aurotricarboxylic acid (ATA), dimercaptosuccinic acid (DMSA), or penicillamine. This method is indicated only in people under strict medical supervision. These compounds are usually administered, as a therapeutic measure, in order to remove heavy metals from the body of exposed workers. This method is also used to evaluate the total body burden and the level of a previous exposure mainly in the workplace due to chronic exposure.

The determination of toxins and metabolites present in the blood, exhaled air, urine, sweat, feces, and hair is a method increasingly used to evaluate human exposure (exposure tests) and/or the degree of intoxication. This is the reason that biological exposure limits (maximum permissible concentration (MAC) values and biological exposure indices (BEI)) have been recently established. Through these bioassays, it's possible to find the "internal exposure" of the organism, i.e., its exposure total in both the professional and general environment, and due to all entry routes. These are also referred to as "biomarkers" in "toxicology test methods."

3.7 Classic samples

In cases of patient care in emergency services, the samples of choice for toxicological studies will be blood, urine, or gastric content par excellence. One of the objectives of the care in the emergency services is to differentiate those serious processes that require immediate hospital treatment of other milder ones that can be studied or treated on an outpatient basis. The samples obtained in the emergency room are used for the diagnosis and determination of the treatment that is going to be undertaken there or to refer the patient to another service or to another institution. The decision of which analyte or analytes should be investigated depends on the physical examination and the complete clinical history, considering the patient's work history, environment, and habits. From this correlation, differential diagnoses may be proposed. All of them require confirmation or discarding, by means of specific tests. This decision is based on the toxicodynamics of the chemical substances in such a way that a series of symptoms and signs (effects) compatible with a limited number of agents can be counted. In this way, the exams must be ordered in a logical and rational manner according to the individual conditions of each patient. This achieves a reduction in the costs of medical care and increases the efficiency and effectiveness of the emergency service.

In patients who must initiate vital support measures, it is important to contemplate that the indicated therapeutic measures [2] can modify the results of the biomarkers. Therefore, it's important to proceed to the taking of samples before the beginning of these measures. As an example, if the diagnosis of carbon monoxide poisoning is being considered, the ideal is that this sample is taken before the administration of oxygen if it can be delayed for a short period. In case the treatment is initiated before sampling, due to the seriousness of the case, it should be considered that there will be interference in the results of carboxyhemoglobin. Similar situation will be in the case of the use of activated carbon in drugs with enterohepatic recirculation. In these cases, the concentrations in plasma or blood may be lower than expected by the action of activated charcoal (reduces the half-lives of drugs such as benzodiazepines, psychopharmaceuticals, oral hypoglycemic agents, and NSAID in general).

In addition, there are aspects of clinical urgency that must be pre-established in the sampling in critical patients, regarding the analyte and the characteristics that the sample collection itself must meet. These characteristics are sufficient quantity for analysis and confirmatory tests, preservative, type of syringe, venous or arterial sample, collection of urine or isolated sample, gastric content prior to the use of activated carbon and without preservatives, etc., in which the medical personnel must be acquainted.

3.8 Sampling material

It's important to have:

Order of use ^a	Type of tube/usual color ^b	Additive ^c	Mode of action	Application
1	Blood culture bottle (yellow-black striped tubes)	Broth mixture	Preserves viability of microorganisms	Microbiology—aerobes, anaerobes, fungi
2	Nonadditive tube			
3	Coagulation tube ^d (light blue top)	Sodium citrate	Forms calcium salts to remove calcium	Coagulation tests (protime and prothrombin time), requires full draw
4	Clot activator (red top)	Clot activator	Blood clots, and the serum is separated by centrifugation	Chemistry, immunology, and serology, blood bank (cross-match)
5	Serum separator tube (red-gray tiger top or gold)	None	Contains a gel at the bottom to separate blood from serum on centrifugation	Chemistry, immunology, and serology
6	Sodium heparin (dark green top)	Sodium heparin or lithium heparin	Inactivates thrombin and thromboplastin	For lithium level use sodium heparin, and for ammonia level use either
7	PST (light green top)	Lithium heparin anticoagulant and a gel separator	Anticoagulants with lithium, separates plasma with PST gel at the bottom of tube	Chemistries
8	EDTA (purple top)	EDTA	Forms calcium salts to remove calcium	Hematology, blood bank (cross-match) requires full draw
9	Blood tube (pale yellow top)	Acid citrate dextrose (ACD, ACDA, or ACDB)	Complement inactivation	HLA tissue typing, paternity testing, DNA studies
10	Oxalate/fluoride (light gray top)	Sodium fluoride and potassium oxalate	Antiglycolytic agent preserves glucose up to 5 days	Glucoses require full draw (may cause hemolysis if short draw)

ACD, acid citrate dextrose; DNA, deoxyribonucleic acid; EDTA, ethylenediaminetetraacetic acid; HLA, human leucocyte antigen; PST, plasma separating tube.
^a“1” indicates draw first, and “10” draw last (if used).
^bVerify with local laboratory in case local color codes differ.
^cGently invert tubes with additives to mix thoroughly; erroneous test results may be obtained when the blood is not thoroughly mixed with the additive.
^dIf a routine coagulation assay is the only test ordered, then a single light blue top tube may be drawn. If there is a concern about contamination by tissue fluids or thromboplastins, then a nonadditive tube can be drawn before the additive tube. The PST tube contains lithium and a gel separator; if used, draw in the order shown.

Table 1.
Recommended order of draw for plastic vacuum tubes [3].

- Blood sampling bottles/tubes of different types (anticoagulants) (**Table 1**)
- Sterile flasks with wide entry for urine collection samples and gastric content

With respect to the identification of the patient, this must be clearly stated on the label, as well as the time record, and responsible for taking the sample. This can be made by means of a simplified chain of custody (responsible for sampling, transfer, and reception in the laboratory). This is relevant in consideration of the potential legal medical aspects in cases of intoxication. It's also useful to have an additional form that consigns previous treatments to the sampling procedure and specific request of toxicological analysis.

Regarding the transport of these samples, they must be sent to the specialized laboratory with security seals that guarantee the inviolability of these and under the biosecurity norms. This means that samples must consider a primary container that corresponds to the tube or bottle, and a secondary container that corresponds to a sealed plastic bag, and polystyrene box with cooling unit.

The documentation must be complete and be presented folded outside the polystyrene box, in a sealed transparent bag, adhered to this box with tamper proof seals.

4. Laboratory

At the entrance of the samples to the toxicological laboratory, they must receive an internal coding, and caution must be maintained in the information of it. According to the type of analyte, the conditions of the sample must be verified, and the study plan established in accordance with the methodology to be used. These methodologies must be previously validated, and the procedures and instructions clearly established in the internal laboratory documents, all of them in accordance to GLP or ISO 17025. Also, it's important to have qualified and trained personnel. The results and their corresponding calculations must be registered for the review of the technical direction, approval, and issuance of the final report.

Likewise, there should be a storage system for samples and counter samples and a registry for the temperatures at which they will be maintained (5° for blood and 20° for urine and gas content). In case of other samples such as hair and nails, they do not require refrigeration [4]. These samples will be preserved for a minimum of 3 months. This is in order to support subsequent investigations of forensic type, or, in case that they are forensic samples, they must be kept, proceeding to a rigorous storage, until the specialized removal of these from the legal medical area, continuing the chain of custody initiated to the side of the patient.

The final report must contain the results, methodology used, reference values, and critical concentrations. The clinical area must be contacted, and the results communicated verbally and in writing media (email, cloud, or other previously established systems).

5. Standards for other samples

For other samples of experimental toxicological studies, the specific guidelines of the OECD should be followed according to the type of test to be developed, either studies in mammals or ecotoxicological experiments [5, 6].

For quality control samples of pharmacological or pesticide production, it's important to verify FDA, FAO, and DIN norms or the corresponding country's

standard body. The batch and date of manufacture should also be considered. In these samples, it's important to consider the number of the sample and which will be considered the control samples, that is to say, to define the universe according to these data of statistical probability. In an EPA document from 2002, there is an example of chlorpyrifos in apples (**Figure 1**) [5].

Similar situation should be considered in the sampling in the work environment, regarding the guides and sampling rules, where the sampling point, previous calibration of equipment, and analytical method to be used with the person responsible for the sampling itself should be consigned.

With regard to population sampling when possible environmental toxicology situations are investigated, a representative number of the sampling and control samples, based on epidemiological and biostatistical data, must be established (**Table 2**) [6].

For forensic samples, it must be specified if they correspond to samples from living individuals or corpses. In the case of living, blood, plasma, serum, urine, gastric content, hair, or nails will be useful (**Table 3**) [2].

For recent cadavers in which there are various organs for sampling as well as fluids, this will be available, blood, urine, and gastric content, in addition to samples of various viscera such as the brain, liver, kidney, lung, and spleen, among others, and also nails and hair and samples of bone marrow and bone [2].

Figure 1.
Inferences drawn from judgmental versus probabilistic sampling designs [5].

Sampling design/protocol	Chapter	Use
Judgmental	4	Common
Simple random	5	Common
Stratified	6	Common
Systematic and grid	7	Common
Ranked set	8	Innovative
Adaptive cluster	9	Innovative
Composite	10, 11	Common

Table 2.
Sampling designs presented in this guidance [5].

For corpses that come from exhumation or skeletons, the soft tissues may no longer be available, but there can be cadaveric fauna or the remains of dusty material inside the urn or at the site immediately to the discovery, which are also the subject of the study. In these cases, bone samples, the area of growth cartilage in children by its vascularization, and bone marrow are very important, in addition to the hair and nails [2].

5.1 Chain of custody

The chain of custody is intended to safeguard the representativeness of a sample, as mentioned in the importance of this custody from the clinical area. This also applies to any analytical process in which the traceability of the samples and their corresponding backups must be maintained. All of them are subject to quality control, such as samples in which environmental contaminants are studied, where georeferencing data with their respective matrices and characteristics of the site must be added to the chain of custody [7]. A similar situation must be considered for forensic samples, in both open and closed spaces, in which the planimetrics and photographic records of the site of the event are added.

An example of a chain of custody is the following (Figure 2).

5.2 Traceability

It extends beyond the chain of custody and is related to the quality of the study and its representativeness regarding the validity of the results. This is an extremely important point in the toxicology laboratories. This is related to the existence of more than one analytical method for confirmation and that there are dedicated laboratories focused to these topics. They consider GLP in accordance to OECD Guidelines and ISO 17025 Standards as part of their work, all of this associated with continuous improvement.

5.3 Study plan

The study plan should consider the following aspects, in addition to the aspects of the guides and standards mentioned [6] (Figure 3):

- Selection of analytical suppliers
- Certified standards and technical standards

Sample type	Preservation	Justification to conduct study	Sampling method	Packaging and storage
Blood	Use at least 1.5% sodium fluoride and potassium oxalate or EDTA	Drug analysis and/or alcoholemia in incidents with no more than 3 days (72 h) since its occurrence	7.5 mL in a 10 mL vial or two tubes with 5 mL each It's preferably that vials are no more than $\frac{3}{4}$ full In case of volatile analysis (solvent abuse or solvent inhalation), these samples must be frozen within the first hour and must be kept frozen during transit	Tubes should be kept inside sealed plastic containers and, then, put inside tamper-evident bags It's better to keep samples under refrigeration conditions, but, if not possible, samples can be frozen (only blood sample tubes that are no more than $\frac{3}{4}$ full and no more than 20 mL of urine) The samples can be kept up to 4 weeks in refrigeration, and longer times are considered for frozen samples All samples should be sent for analysis as soon as possible, considering that some analytes could be undetectable due to instability of the matrix In case of toilet tissue, it must be labeled and kept in a tamper-evident bag. It should be considered as a biological forensic sample alongside condoms/sanitary towels and not as a toxicology sample. This kind of material should be storage frozen
Urine sample	1.5% sodium fluoride	Drug analysis and/or alcohol in incidents with no more than 5 days (120 h) since its occurrence This kind of sample is also necessary in cases of suspected drug-facilitated crime in the preceding 14 days It can be complemented with hair samples, which must be submitted to the laboratory as the first analysis matrix Urine samples can be stored for future analysis, if needed, as in the case of the detection of relevant drugs in hair	It's recommended that two urine samples are taken if the incident happened in no more than 24 h In case of incidents with more than 24 h, only one sample is enough The first sample should be obtained as soon as possible after the incident, and the second sample can be taken during the next urination after the first sample. It's preferably that this second sample is taken within an hour after the first urine sample, but it can also be taken whenever it's not within this hour Ideally, 20 mL of urine must be decanted in a tube of at least 25 mL (fill up to $\frac{3}{4}$ full) Both samples can be taken prior to full medical examination Only the defendant needs witness when the sample is taken When the sample is obtained, if the individual uses toilet tissue (sometimes provided in sample taking kits), to wipe afterward, this material should also be kept as part of the evaluation	

Sample type	Preservation	Justification to conduct study	Sampling method	Packaging and storage
Hair normally: it's hair from the head, but it can be obtained from other parts of the body if needed	No specific method applies	In cases of incidents up to 6 months prior to examination, in consideration that drugs may have been eliminated through urine in times no longer than 3 weeks If in doubt of the use of this kind of sample, it's important to contact the laboratory for advice	There exist some kits which include specific instructions, or it's possible to ask for advice to the specialist in the laboratory This sample should be taken after a minimum of 4-6 weeks after the date of interest The individual should be instructed not to cut or chemically treat (dye, bleach, or perm) their hair during the intervening period In drug abuse cases, it's a wide spread of information that these interventions from the individual can tamper the results; therefore, in these cases, it's possible to take hair samples from other places of the body	Hair samples should be packaged as described in the specific hair testing kit or in sterile urine tubes The sample should be placed in a tamper-evident bag This matrix for toxicological analysis must not be frozen or refrigerated, in order to preserve it These samples must be stored in a dry environment at normal room temperature
Nail clippings	No specific method applies	Fingernail or toenail clippings can be used for drug analysis, as an alternative in cases of the absence or lack of head/body hair or as a complementary sample for head/body hair	There exist some kits which include specific instructions, or it's possible to ask for advice to the specialist in the laboratory This sample should be taken after a minimum of 4 weeks after the date of interest	This matrix should be enclosed in a suitable packaging (i.e., a specific type of packaging specified in testing kit or a folded clean paper secured with tape), and, then, this sample must be placed in a tamper-evident bag This kind of sample, for toxicology, must be stored with the same conditions as hair, namely, not frozen nor refrigerated, only dry and at room temperature

Table 3.
Toxicology [4].

- Calibration and maintenance of equipment
- Type of sampling and risk of cross contamination (Figure 4)
- Instructions and procedures (POS)
- Validation of methods and SANCO recommendations for validation dossier
- Limits of detection and limits of quantification of the method
- Repetitiveness
- Error estimation
- Interlaboratory rounds

- Biosecurity and sample management, based on the type of samples and type of tests. That is why it is very important in the management of GHS knowledge for the classification of mixtures of chemical substances in the different instructions (POPs) that are part of a study plan.

COMPANY LOGO		DG-For-007 CHAIN OF CUSTODY FORM	
1° Ed. Date: 2013.12		Edition N° 2 Date: 2016.12	Page: 1 de 1

Sample Identification	:	_____	
Internal Code	:	_____	
Project	:	_____	
Derived Sample ☐			Personalized Sample ☐
(For external samples)			(For laboratory internal samples)

FILL IN CLEAR AND LEGIBLE HANDWRITING	Responsible for packaging (Only fill for derived samples)
	Date: _____ Time: ____:____ Identity documents N°: _____ (Social Security card/Driver's license/ID card/Passport)
	Name: _____ Firm _____ Not apply for personalized samples ☐
	Responsible for Transporting: (Only fill for derived samples)
	Date: _____ Time: ____:____ Identity documents N°: _____ (Social Security card/Driver's license/ID card/Passport)
	Name: _____ Firm _____ Not apply for personalized samples ☐
	Responsible for Laboratory Reception: (Only fill for derived samples)
	Date: _____ Time: ____:____ Identity documents N°: _____ (Social Security card/Driver's license/ID card/Passport)
	Name: _____ Firm _____

Check List of Packaged Items (It is the obligation of the person responsible for the packaging to properly and correctly fill out the following data)		
	Yes	No
1 Gel pack	☐	☐
2 Green top blood tube	☐	☐
2 Gray top tube	☐	☐
1 Urine sample bottle 50 mL	☐	☐
1 Gastric contents bottle 30 mL	☐	☐
Others (Specify): _____	☐	☐
Check List of Packaging and Shipping		
	Yes	No
1. Place all samples inside the bag.	☐	☐
2. Close the container with the safety seals.	☐	☐
3. Place all annexed documents in an envelope.	☐	☐
4. Leave the envelope out of the container and be sure to include it in the shipping.	☐	☐
NOTE: Before placing the safety seals in the container, be sure to include all samples. Once sealed it can NOT be opened, since it will automatically be considered violated and invalidate the sample.		
Check List of Annexed Documents		
	Yes	No
1. Patient Data Sheet	☐	☐
2. Medical referral order (if applicable)	☐	☐
3. Chain of custody form	☐	☐
4. Others (Specify): _____	☐	☐

GENERAL DOCUMENT DOSSIER	Elaborated / Modified: Quality Coordinator	Revised / Approved: General Manager and Technical Director
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Figure 2.
Chain of custody (elaborated by L. Börgel, 2016).

Figure 3.
Strategy for securing representativity of analytical results (elaborated by authors, 2018).

Figure 4.
Life cycle of data in the EPA quality system [6].

5.4 Classification in accordance to GHS for delusions in laboratory and decision to use personal protection systems

The application of the GHS criteria and the purple book are currently standardized instruments that allow classifying the mixtures or solutions to be used in the work instructions and also allow classifying the waste, pure reagents, and certified

or technical standards. This is in order to establish their dangerousness according to the different ways of entry to the body, to adjust the danger for each type of substance or mixture, and decide the correct use of safety systems [8].

5.5 Final disposition

Waste management in the laboratory is associated with quality work. The greater the control of the procedures and the better the compliance with instructions, the lower the load of residual materials to be generated in the laboratory. These must be classified as biological waste and others as chemical waste, and, in accordance with the regulations of each country, their final disposition must be fulfilled, with the respective labeling of the United Nations [5, 9].

6. Conclusions

Therefore, according to each sampling procedure, it's important to review the recommendations, guidelines, or standards that apply, and all of them should be established in the study plan.

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Conflict of interest

Both authors declare that there are no conflicts of interest.

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