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LETTER TO THE EDITOR

The 3Rs principle applied to laboratory experiments: from waste to potential new results

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Our personal or collective efforts toward making planet Earth sustainable is something that is widely discussed and urgently needed. Based on this understanding, the policy of the 3Rs (reduce, reuse and recycle) focused on environmental ethics is gaining widespread traction worldwide. This principle of the 3Rs initially named (replacement, reduction, and refinement) was first reported by Russell & Burch (1959) for animal ethics and brought into the environmental context decades later and is currently discussed in research biological to use more efficiently of the results and samples (Dijkers 2019). The principle of the 3Rs can be interpreted in this context: creating ways to use as little as possible and reduce consumption (reduce), reusing the material or its by-products (reuse), and using the material to repeat the same analysis or use it in another (recycle).

However, do we researchers and graduate students implement this policy beyond our private lives, the laboratories, and the studies we conduct? After all, we are also responsible for generating a large amount of waste (Lopez & Badrick 2012). Can we apply the 3Rs principle not only to the consumable materials that we use and discard but also to the samples used in the tests?

In a previous article published (Leles et al. 2018), we had already alerted to the fact that methodological innovations can be useful for facilitating the optimization and reuse of rare or low-quantity samples, such as archaeological and paleontological specimens or samples from biological collections and museums. The present practices also involve several ethical issues, which have been cited for some museums and collections (Raxworthy & Smith 2021). When following the 3Rs principle, we avoid wasting samples and, importantly, preserve them for study by future generations that may apply other innovative methodologies or emerging techniques, thereby rescuing new information that cannot be collected with currently available technologies.

The following idiomatic expression is often used in archaeology: "Don't throw the baby out with the bathwater." This reflects exactly what we intend to highlight here. In Brazil, an example of preservation for future generations can be seen in Serra da Capivara National Park, a place with the highest concentration of archaeological sites in Latin America and where researcher Dr. Niede

Guidon has left a preserved part of the sites unexcavated for future generations. The same has been applied to the Collection of Paleoparasitological Specimens and Recent Animal Feces (*Coleção Paleoparasitológica e de Fezes Recentes de Animais* - CPFERA-Fiocruz), of which samples can no longer be exhausted, and aliquots of the samples processed have been preserved for future researchers.

This practice can and should also be applicable in the current context, whether for samples from collections or for patient diagnostic test samples that are low in quantity or collected on predetermined and specific dates, since it is not possible to go back in time to obtain the sample in that same context. Moreover, this practice can be applied even to epidemiological and comparative studies, such as those for determining whether an emerging infection is a reemerging one, as we have been constantly experiencing this situation with recent epidemics and pandemics.

In our study, we once again aimed to show that the reuse of samples that would otherwise be discarded is absolutely possible, since they can provide new information without affecting the reliability of the results. Such practice is in line with the principle of the 3Rs: we reduced what would have been discarded, reused the samples, and reached a new result that would not have been obtained in the previous test.

We showed that residues from DNA extraction that would be discarded can also be used in immunoenzymatic assays for the detection of parasites and not only in immunochromatographic tests, as previously demonstrated (Leles et al. 2018). We used DNA extraction residues from 11 human fecal samples positive for *Giardia duodenalis* that had been previously genotyped as genotypes A and B using the β-giardin target as well as those from an animal fecal sample positive for *Cryptosporidium* sp. that had been genotyped as *Cryptosporidium parvum* using the 18S target. These residues were tested for traces of *G. duodenalis* and *Cryptosporidium* sp., using commercial enzyme-linked immunosorbent assay (ELISA) kits (Stool Antigen Detection Microwell ELISA, IVD Research INC®, Carlsbad, CA 92010, USA). Ten of the 12 samples tested positive for the parasites in the ELISA; nine for *G. duodenalis* and one for *Cryptosporidium* sp. (CEP/UFF n.01378318.4.0000.5243 and SisGen n. AAD15E1). Therefore, this study showed that sample residues that would have been discarded in the DNA extraction process can and should be used for other laboratory tests, even in the search for another etiological agent, thereby optimizing the use of the samples and amplifying the information obtained from them.

We are clearly aware that this is a simple experiment, but going back to the basics is often necessary. The remaining residues from the DNA extraction process contain compounds of proteins that can be fully used in other diagnostic approaches that use these molecules, such as immunological assays.

Small samples are challenging, with each methodology requiring a minimum sample size; our solution involved observing the laboratory "trash" to give the samples a chance of survival. Storing post-analytical residues is a method of rescuing part of samples that would otherwise be discarded, which may be used in the future for other purposes, generating novel and relevant scientific results. Using waste from a sample that previously underwent a procedure may give rise to a false result. We propose the use of previous experimental models to validate the methodology of interest, since analyses paired with a control group (large and common samples) prevent the false result. It is essential to access the data from previous analyses and the results of these samples, which must be documented and made freely accessible to allow a comparison with the obtained data and to

minimize the chance of false results. Waste rescue and storage depends on the material analyzed and the methodology used, determined at the discretion of the researcher or custody institution. Officializing biological collections in Brazil involves teaching and research institutions, which were fostered from the implementation of the SisGen (Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado - National System for the Management of Genetic Heritage and Associated Traditional Knowledge). This is the first step in preserving this heritage for future generations, as these institutions store data obtained from the material and analyze the sample use history to discover the potential scientific benefit from the proposed research, already foreseen the return of what is not used to the collection. Additionally, unused waste can be stored or returned by the researcher. It is the role of both researchers and institutions to evaluate which samples should have waste stored. Current conservation and storage techniques stabilize residual molecules for reuse for a certain period, while other techniques are in development (Coudy et al. 2021). Furthermore, along with the physical space required to store post-analytical processing waste, some types of biological samples require the use of a freezer or ultra-freezer, an air-conditioned room, and a power generator in order to be preserved. This evidently involves high energy expenditure, which returns to the principle of the 3Rs. Brazil has played a leading role and participated in several international treaties and agendas in favor of sustainability, such as Eco 92, Rio 20, and, more recently, COP27, expressing concerns about the excessive use of non-renewable energy sources and greenhouse gas emissions, which have been significant in exacerbating climate change. Marinho (2014) states that even higher education institutions that were part of international treaties assuming a commitment to sustainability issues have not effectively put this into practice. We are aware of the difficulties in implementing alternative energy sources in institutions, which store a large portion of biological collections and biobanks; thus, this needs to be on the agenda of the supporting institutions.

However, what we aim at discussing with the readers of *Anais da Academia Brasileira de Ciências* is the value that this information can have if it reaches other researchers, including your good self who stopped to read this Letter. The idea is to lead people to rethink and perhaps change their behavior when considering sample residues, whether these are rare collection samples or modern samples of low quantity or that are difficult to obtain, among other similar situations. This practice can be promising for laboratory diagnosis and for ongoing research, in addition to contributing to the conservation and preservation of samples and, ultimately, of the environment.

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