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Diagnostic tools for the detection of animal health status in laboratory facilities

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Introduction
Molecular methodologies, such as PCR and qPCR, can represent a useful diagnostic instrument for the detection of pathogens that may threaten the health of housed animals. In accordance with 3Rs principle (1), apparent or inapparent infections must be identified to guarantee animal wellness and, also, the reliability and reproducibility of experimental data. The Federation of European Laboratory Animal Science Associations (FELASA) proposes constantly updated guidelines to be applied for health monitoring. These indications describe essential aspects such as the type of pathogen to detect, the animal or tissue to be analysed, the sampling frequency and the commonly used diagnostic techniques (2). The present study aims to implement health monitoring programs for model species such as mice and zebrafish, investigating pathogens circulating in the housing environment. The diagnosis was based on the molecular analysis of environmental samples collected from animal housing cages. This type of approach can be considered an adjunct to animal tests, which could improve, the housing conditions and, at the same time, limit the number of sentinel subjects sacrificed.

Methods
Sampling was performed at the IZSLER animal facility. Sawdust, enrichment material and faeces were collected from rodent cages. To detect zebrafish pathogens, the filtering system of the aquariums was analysed: sludge contained in the prefilter and the water cartridge filter of the recirculation system. DNA was isolated by extraction kits provided by different companies (Qiagen, Promega, Macherey-Nagel™) considered in this study. Nucleic acids were quantified by QuantiFluor™ ONE dsDNA System (Promega) or QuantiFluor™ RNA System (Promega) kits with the Quantus™ Fluorometer (Promega) instrument and finally analysed by PCR, RT-PCR, qPCR and RT-qPCR for the detection of pathogens.

Results
The amount of nucleic acids obtained with extraction kits was enough to perform investigations. The results for the tests performed on sawdust, enrichment material and faeces were negative, otherwise in the aquarium filtering system *Mycobacterium chelonae* and *Pseudocapillaria tomentosa* were weakly detected. This trend could depend on the low presence of microbial agents in the environment, their poor stability or the low efficacy of the commercial kits used. Then, appropriate sensitivity tests will have to be developed by infection of environmental matrices with known pathogens, to compare the performance of the extraction kits and to verify the presence of any PCR inhibitors.

Conclusions
This approach aims to realize screening for pathogenic microorganisms to improve control on the animal health status, and limit the number of sacrificed subjects in the experiments. Furthermore, testing environmental samples, it is possible to avoid the stress and suffering of the animals.