Research team validates new method for assessing antimicrobial efficacy of domestic cleaning products

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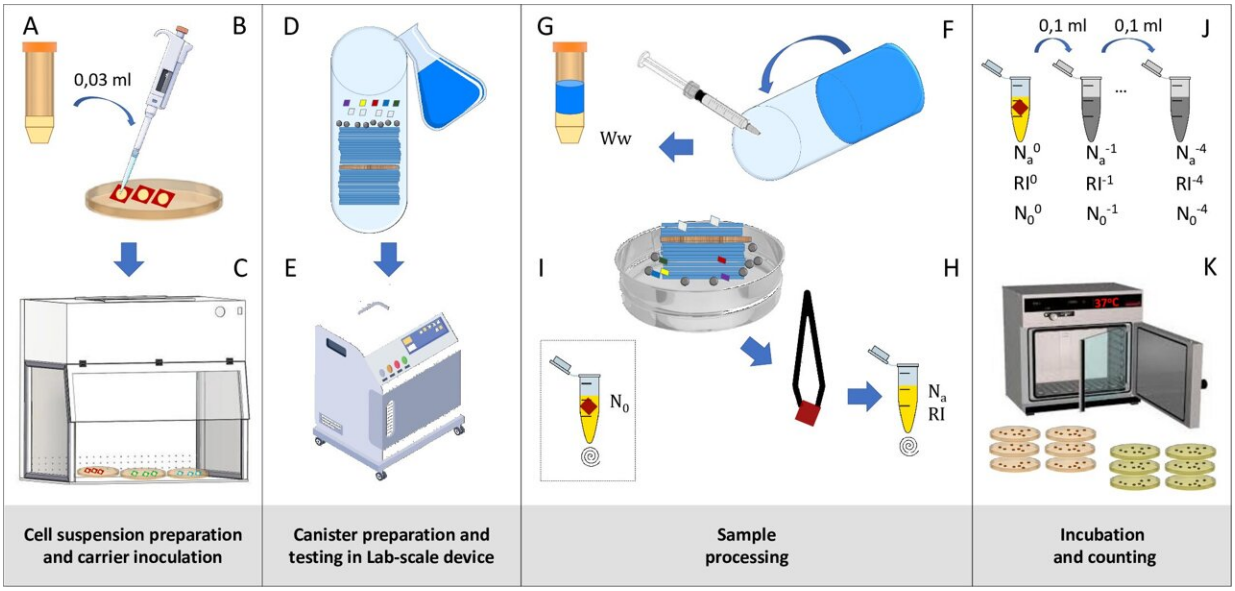

Method workflow. A: Cell suspension preparation for each strain tested. B: Carrier inoculation. C: Carrier drying process in the flow cabinet. D: Canister preparation. E: Testing time at the corresponding temperature in the lab-scale tumbling device. F: Wash water recovering and neutralizing. G: Content addressing. H: Na/RI carrier recovery and mixing in neutralizer. I: N0 mixing in neutralizer. J: 10-fold dilution preparation. K: Plate incubation and counting. Credit: Toni Monleón-Getino et al, *PLOS ONE* (2022). DOI: 10.1371/journal.pone.0269556

Environmental awareness in society is changing household laundry habits, where the use of less bleach and lower temperatures during washing machine cycles is encouraged. In this context, disinfectants added to detergents have become an essential factor to compensate for these new habits and to prevent the transmission of bacteria, fungi and viruses in the house, as well as to control the level of odor-causing microorganisms on clothes. These products must be evaluated according to standardized methods, but the current European regulations only apply to clinical settings and are restricted to the main wash cycle.

Experts from the Biost3 Research Group, led by Antoni Monleón, lecturer in the Department of Genetics, Microbiology and Statistics of the University of Barcelona (UB), have statistically validated a new method for assessing the antimicrobial efficacy of detergents and textile additives in domestic environments. The results reveal the validity of the new protocol, which has been presented to the European Committee for Standardisation (CEN) requesting it to become the European standard.

"It is very difficult to make sure that a product works and that [it] is a good disinfectant. We work with microorganisms and the results of the efficacy evaluation can be very variable depending on the method, the washing machine, the temperature and so on. That is why it is very important to publish a standardized protocol at the European level so that manufacturers of household textile disinfectants can demonstrate the efficacy of their products with a methodology that is much closer to the real situation at home," says Antoni Monleón, a member of the Research Group on Biostatistics and Bioinformatics (GRBIO) integrated in the Bioinformatics Barcelona Association (BIB).

The study, published in the journal *PLOS ONE*, has been carried out in collaboration with CEN technical committee member Michelle Cavalleri and an international consortium of industrial and testing laboratories including AC Marca, Arxada, Eurofins Biolab SRL, Henkel AG & Co

KGaA, Hohenstein Laboratories GmbH & Co. KG, Hochschule Niederrhein, FB textile-u Bekleidungs-technick. Thor Especialidades S. A. AC Marca researchers Ana Costan and Nuria Piedra have coordinated the development of the standard, the experimental phase and the data collection.

International ring trial to simulate domestic washing conditions

In response to the gap in the EU standards, the consortium organized an international ring trial to evaluate the robustness of a new method specifically designed to test the efficacy of detergents against microorganisms in a domestic environment. The seven participating laboratories were equipped with five laboratory-scale devices simulating domestic washing machines, in which seven parameters—including the removal of Escherichia coli or Staphylococcus aureus microorganisms adhering to fabrics—were evaluated at different levels of active substance and at different temperatures.

"The evaluation of disinfectant efficacy in clothing is a complex process that involves many variables and the method must allow to control aspects such as temperature, contact time, or the mechanical and chemical effect separately, so that the reproducibility is more consistent," notes Antoni Monleón.

Robust and reproducible method

This evaluation methodology not only simulates domestic laundry processes, but must be applicable as a standard procedure throughout the sector, and therefore, it must be a robust and reproducible method.

"Robustness," says Antoni Monleón, "refers to a statistical concept as a

measure of the method's ability not to be affected by small but deliberate variations in the experiment, such as using different washing machines or different temperatures.

"On the other hand, repeatability means that if you use the same method with the same procedure in another laboratory, you should get a similar result."

The task of the researchers from the Biost3 research group consisted on validating the method from a statistical point of view and verifying the robustness of the experiments carried out in the different laboratories. With this aim, they have used statistical methods to "detect highly variable values in the experiments, an analysis of strange values for when many variables are taken into account, and graphic methods that allow the joint variability of different experimental groups to be checked," says the researcher.

The results of the research have shown that the method was robust to small variations in the experiment, so the repeatability of the experiment, and of the new method, was satisfactory.

Moreover, they created a new library called Diagnobatch—a program that allows very fast calculations—in R language. The new library can be used in the industrial environment.

More realistic and cost-effective testing

The new method also reproduces domestic washing conditions more realistically compared to the current protocol, where antimicrobials are tested in industrial washing machines with markedly different characteristics.

"Furthermore," says Monleón, "previous studies indicate that a lab-scale

device is not significantly different from a domestic washing machine, but has an important advantage in terms of reproducibility and repeatability."

This new methodology also improves capacity and costs over the current protocol, in which antimicrobial efficacy must be tested separately and only one microorganism per washing machine test must be evaluated. "The new protocol allows up to 20 tests to be run simultaneously (depending on the lab-scale device) and all microorganisms are tested in the same test," says the researcher.

Pending approval by the European regulator

With these promising results, the new methodology has been sent for consultation to the European Committee for Standardisation in order to propose it as a new European standard.

"In the mid-term," concludes Monleón, "when the standard is approved by CEN, all textile disinfectants for the domestic environment to be registered will have to demonstrate their efficacy following this protocol, whose validity and robustness has been demonstrated by our team."

More information: Toni Monleón-Getino et al, International ring trial to validate a new method for testing the antimicrobial efficacy of domestic laundry products, *PLOS ONE* (2022). DOI: 10.1371/journal.pone.0269556

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https://phys.org/news/2022-07-team-validates-method-antimicrobial-efficacy.html