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Concentrating pathogenic bacteria accelerates their detection

Rapidly detecting the presence of pathogenic bacteria is essential in a number of sectors, such as the food or cosmetics industries. To guarantee the absence of these bacteria, it is necessary to block batches for 24 to 48 hours before they are put on the market, which can be a handicap. After first developing a method to count bacteria of interest, scientists in the Laboratoire de Chimie Bactérienne (CNRS/Aix-Marseille Université), the Institut de Chimie des Substances Naturelles (CNRS) and the Institut de Chimie Moléculaire et des Matériaux in Orsay (CNRS/Université Paris-Sud) are now proposing a new technique to rapidly detect and concentrate cultivable Gram-negative bacteria¹. This innovative process, which will enable the release of commercial batches within a day and be exploited by the start-up company Click4Tag, is described in *PLOS ONE* on 10 June 2015.

If pathogenic bacteria are present in a product, this is only in very small quantities. It is therefore necessary that they proliferate in order to be detected and identified. This pre-enrichment step, which forms part of microbiological control procedures, lasts between 18 and 24 hours and is a limiting factor for the release of batches of fresh, perishable products, for example. Numerous research teams have therefore been working on cutting its duration.

In this study, the scientists described an innovative method that can reduce this step to five hours by concentrating the bacteria (*E. coli*) present in a sample. To achieve this, they applied the simple principle of labeling Gram-negative bacteria that was developed as of 2012: the bacteria are fed a synthetic sugar that mimics a sugar naturally present on their surface. Cultivable bacteria assimilate the sugar, which is subsequently found on their membranes only. They have thus been "labeled". Then, using click chemistry², the scientists were able to hook magnetic beads onto the bacteria thus recognizable: using a simple magnet made it possible to concentrate the labeled bacteria.

These results show that this method can specifically detect cultivable bacteria of interest, even in the presence of dead bacteria or other organisms. Around the magnet, the scientists collected more than 90% of the bacterial targets while at the same time concentrating them more than a thousand-fold — and within a shorter period of time.

¹ The term 'culturable bacteria' refers to live bacteria that are capable of development. Their presence in a sample may be dangerous. Pathogens such as *Escherichia coli* or *Salmonella typhimurium* are Gram-negative bacteria.

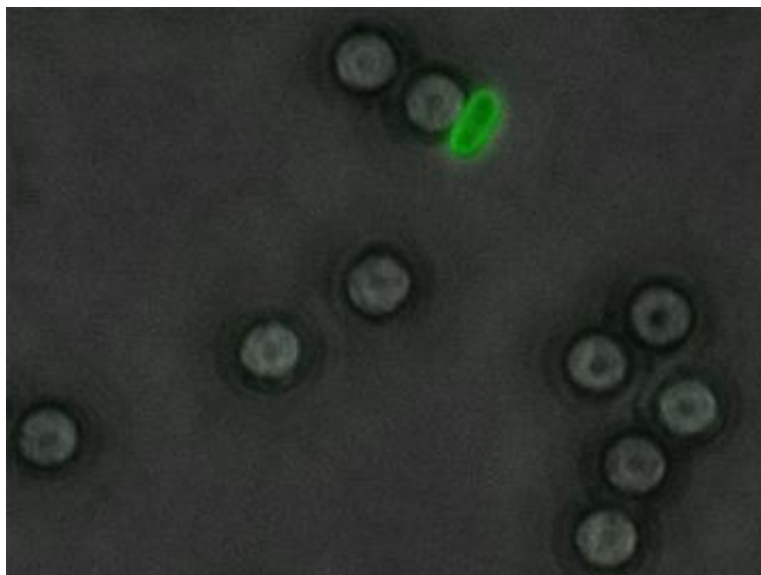
² An innovative chemical method developed in 2001. It concerns the assembly of chemical substances that enables the highly efficient combination of two molecules. These reactions are robust and biocompatible.



It is now necessary to adapt this methodology to larger sample volumes and other bacteria, with a view for the start-up company Click4Tag to optimize and market the process within the next two years. As well as providing a tool for biologists working in academic research laboratories, this technology, pending approval by the relevant authorities, could be used for microbiological quality controls, so that batches of commercial products could be released on the day of their production.

Click4Tag is the result of technology transfer exploiting the research carried out by Sam Dukan at the Institut de Microbiologie de la Méditerranée (CNRS/Aix-Marseille Université) and the Laboratoire de Chimie Bactérienne (CNRS/Aix-Marseille Université), and of Boris Vauzeilles at the Institut de Chimie des Substances Naturelles (CNRS) and the Institut de Chimie Moléculaire et des Matériaux in Orsay (CNRS/Université Paris-Sud). Sam Dukan was seconded by the CNRS and is now CEO of the start-up, while Boris Vauzeilles is scientific adviser to Click4Tag.

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Superimposition of phase contrast images (visualization of beads in grey) and fluorescent images (visualization of an *Escherichia coli* bacterium in green). The bacterium is immobilized on a bead.

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Bibliography

Rapid and specific enrichment of culturable Gram negative bacteria using non-lethal copper-free click chemistry coupled with magnetic beads separation ; Emilie Fugier, Audrey Dumont, Annie Malleron, Enora Poquet, Jordi Mas Pons, Aurélie Baron, Boris Vauzeilles, Sam Dukan ; *PLOS ONE* ; 10 June 2015 - <http://dx.plos.org/10.1371/journal.pone.0127700>

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