Microbiology

Discovery of high-order drug synergies – from impossible to dirt cheap

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This Break was edited by Max Caine, *Editor-in-chief* - TheScienceBreaker

ABSTRACT

Imagine that a super-computer integrated multitudes of biological data and predicted that a certain 10-way drug combination will have synergy. Traditional methods have been too costly and impractical to verify or refute this hypothesis. Our research unlocks the study of high-order drug synergies, measuring the synergy among any number of drugs.
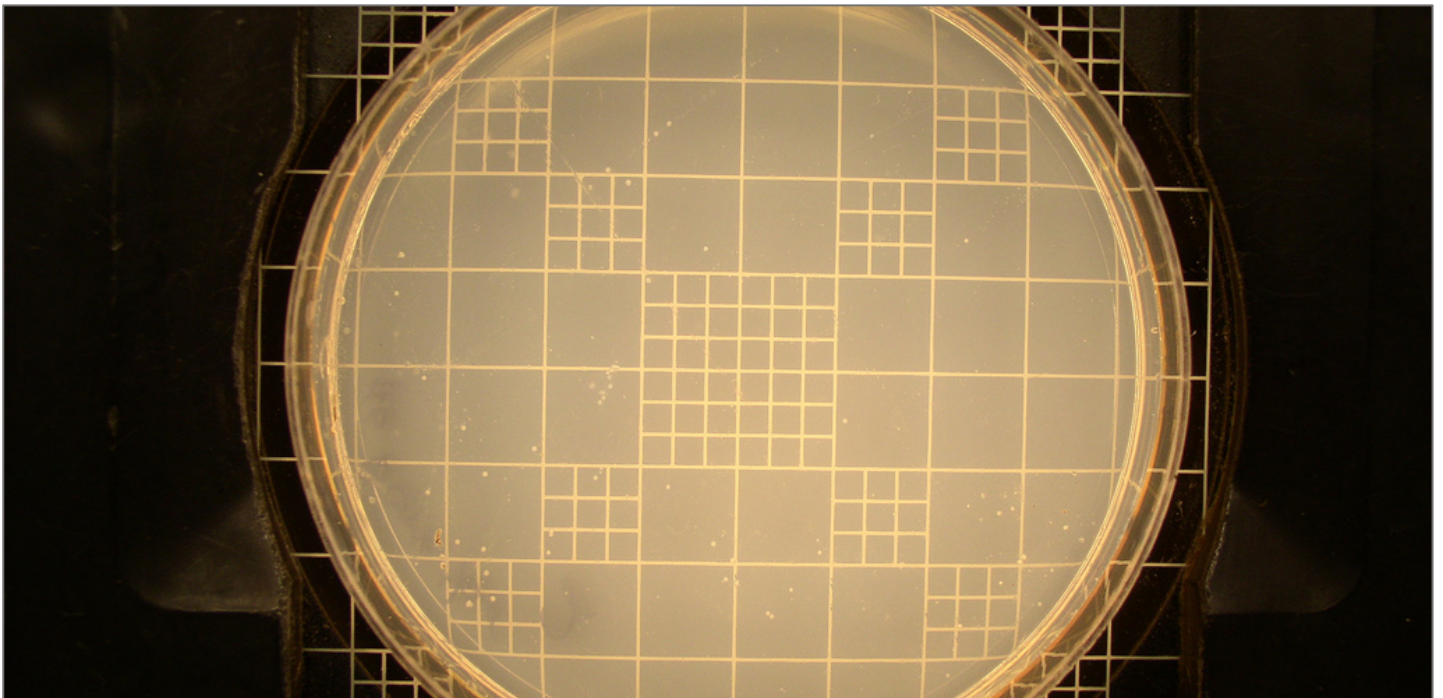
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By combining two or more drugs together (synergistic drug approach) it is possible to obtain a greater effect than with an individual drug alone. For example, the treatment of many diseases, ranging from cancer to tuberculosis to chronic diseases, depends on the use of three or more drugs together. Yet, the traditional experimental setup to discover new drug synergies does not allow to test "high-order" combinations (combinations of more than two drugs).

The traditional setup for testing of the synergy of two drug combinations has been in wide use for more than 50 years. You simply (i) prepare several concentrations of each drug and (ii) mix all concentrations of one drug with all concentrations of the second drug like a checkerboard. If, for example, you are interested in how this combination inhibits the growth of a pathogen, then you add this pathogen on all these concentration combinations and record growth. If growth is inhibited in most concentration combinations, then you would conclude that these two drugs are synergistic. If you

use only 10 concentrations per drug, this approach requires $10^2 = 100$ concentration combinations - a daunting but achievable task. However, if you are interested in the synergy among five drugs, then you would need $10^5 = 10,000$ concentration combinations, which is not feasible because of the associated cost. Consequently, there has never been a report of a synergy among five (or more) drugs in the literature. This technical limitation severely hinders the search for high-order drug synergies, which may offer health benefits such as better efficacy and decreased toxicity.

In our study, we developed a methodology to circumvent this technical limitation. With our method we have been able to collect information about the drugs combination with a simpler approach, drastically reducing the amount of experimentation required to test high-order drug synergies. Consider the following analogy for our method: Let's say you are mining gold and are trying to decide before which of the many mountains you have the most gold hidden inside. You may, of course, take each mountain, pass them through a sieve, weigh the gold and decide. But that is a lot of work! This approach is very similar to the traditional method for measuring synergy, where all possible concentration combinations are considered. Or instead, you may dig the longest straight tunnel through each of these mountains and weigh the gold you encounter in these tunnels. That would give you a good proxy for the actual gold hidden inside each mountain. This is similar to our methodology, which we dubbed the "diagonal method." Here, the researcher does not measure phenotype in all concentration combinations but instead, measures the phenotype only on the diagonal of a checkerboard. This corresponds to making an equipotent mixture of individual drugs and tracking the phenotype on increasing concentrations of this mixture.

As we have shown in our study, this approach gives a very good approximation for the synergy of high-order combinations. The increase in efficiency is striking: For a 5-way synergy test, if one uses 10 concentrations per drug, one would only need 5x10=50 conditions for individual drugs, and another 10 conditions for the 5-way combination. Therefore, only 60 conditions suffice instead of 10,000 conditions. Using this methodology, we could measure all pairwise synergies among 9 antibiotics and all 3, 4 or 5-way drug synergies among five antibiotics in *M. tuberculosis* using only a few microplates.

While our study focused on *M. tuberculosis*, the diagonal method is not specific to any organism or phenotype. Therefore, the principle behind the method is applicable to other model systems or even clinical trials. The experimental methodology is straightforward and the results are immediately interpretable by even the novice user. Indeed, in a follow-up article we presented a protocol that uses standard laboratory equipment and only three microplates to measure all pairwise and one 3-way synergy among three antibiotics (2). With this methodology, what was recently an impracticality can now be carried out with minimal cost.

References:

1. http://advances.sciencemag.org/content/3/10/e1701881

https://www.jove.com/video/57713/