

Clyde W A, Jr. Mycoplasma species identification based upon growth inhibition by specific antisera. *J. Immunology* 92:958-65, 1964.
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Advantage was taken of the fact that mycoplasma growth is inhibited in the presence of species-specific antisera to perfect a new method of speciation. Variables involved were explored and the application to unknown isolates demonstrated. [The SCF® indicates that this paper has been cited in over 610 publications. It is among the ten most-cited papers for this journal.]

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"The identification of Eaton's atypical pneumonia agents as a mycoplasma in 1962¹ led to extensive studies on the role of this organism as a cause of human respiratory disease. Cultures of patients for *Mycoplasma pneumoniae*, as the new organism was named,² yielded a variety of other mycoplasma species that had not been identified previously. Due to their small genome size (5×10^8 Daltons), mycoplasmas are much more limited in biochemical characteristics than are other conventional bacteria, and speciation consequently depends upon serological testing. The principal method that had been available was the complement fixation technique, a laborious process plagued by variable degrees of cross reaction.

"Edward and Fitzgerald in 1954³ had observed that mycoplasma growth was inhibited if homotypic antibody was incorporated into the agar medium. A modification of these studies was presented by Huijman-Evers and Ruys,⁴ who placed filter paper discs saturated with antisera on agar plates inoculated by the push-block technique. Further use of these ideas was made to simplify and better control the process, as well as to evaluate it for applicability to mycoplasma species identification.

"Antisera were prepared to eight different mycoplasma species. These organisms in broth cul-

tures were used to inoculate agar plates, and anti-serum-impregnated filter paper discs were applied. After incubation for several days to allow growth of the test organisms, zones of inhibition were observed around the homotypic, but not the heterotypic, antisera. The same mycoplasmas and antisera were studied also by the conventional complement fixation method for comparison. The growth inhibition technique proved to be much simpler and more specific as a means of speciation. However, the procedure was relatively insensitive compared to complement fixation and had no role as a serologic test.

"The growth inhibition technique was quickly adopted by other workers in the field of mycoplasma and has enjoyed extensive application subsequently. This led rapidly to the identification of a wide variety of mycoplasma species which were previously known only by strain abbreviations. A critique of the procedure together with technical variations proposed by others is the subject of a paper from the World Health Organization.⁵ A recent description of the growth inhibition technique appears in *Methods in Mycoplasmaology*.⁶

"The report in *Journal of Immunology* played a major role in establishing me in a niche in the field. Currently the number of related publications is around 80, but no others have received the same citation popularity. It is of retrospective interest that the definitive experiments on which the work was based required less than one month to accomplish.

"The description of the growth inhibition test for mycoplasma speciation formed the basis of a ten-year Career Development Award from the National Institutes of Health to evaluate the role of mycoplasmas in human diseases. In turn, this work led to editorial board appointments for the *Journal of Bacteriology*, *Infection and Immunity*, *Journal of Clinical Microbiology*, and *Pediatric Infectious Diseases*. Currently, I am chairman-elect of the International Organization for Mycoplasmaology, and will become the chief presiding officer following the forthcoming congress to be held June 24-29, 1984, in Jerusalem, Israel."

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2. Chanock R M, Dienes L, Eaton M D, Edward D G F, Freundt E A, Hayflick L, Hers J F P, Jensen K E, Liu C, Marmion B P, Morton H E, Mufson M A, Smith P F, Somerson N L & Taylor-Robinson D. *Mycoplasma pneumoniae*: proposed nomenclature for atypical pneumonia organism (Eaton agent). *Science* 140:662, 1963. (Cited 70 times.)
3. Edward D G F & Fitzgerald W A. Inhibition of growth of pleuropneumonia-like organisms by antibody. *J. Pathol. Bacteriol.* 68:23-30, 1954. (Cited 95 times since 1955.)
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6. Clyde W A, Jr. Growth inhibition tests. (Razin S & Tully J G, eds.) *Methods in mycoplasmaology*. New York: Academic Press, 1983. Vol. I, p. 405-10.