

This Week's Citation Classic

Cunningham A J & Szenberg A. Further improvements in the plaque technique for detecting single antibody-forming cells. *Immunology* 14:599-600, 1968.
[Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia]

This paper described a simple modification of earlier techniques for detecting and counting single antibody-forming cells. [The *SCI*[®] indicates that this paper has been cited over 1,215 times since 1968.]

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September 10, 1981

"Immunology in the early-1960s was moving from a preoccupation with serology to a focus on the cells responsible for immune functions. 'Cell mediated' reactions were still rather undefined, since T cells had not yet been identified as a separate group of lymphocytes, but there had been a great deal of interest for many years in the cells which produced antibody. This reached a climax with the independent development by Jerne and Nordin¹ and by Ingraham and Bussard² of simple techniques for detecting and counting single, living antibody-forming cells.

"In 1964, as a keen new graduate student, I became enchanted with the Jerne technique—areas of lysis looming up from the agar plate when complement was added, each around a single active cell which was responsible for lysis of thousands of erythrocytes. But when I tried to look at these cells more closely through a microscope, to get to know them better as it were, what disappointment! They could barely be seen through all the agar. It was obvious that the optical conditions could be improved by

making the preparations very thin, perhaps one cell thick. Cedric Minns suggested leaving out the supporting medium and just letting cells fall to the bottom of a thin chamber. I made suitable chambers by cutting microtome sections of a block of paraffin ten microns thick, with a hole through the middle, and sticking these to a microscope slide. It worked beautifully, leading to my first paper, in *Nature* in 1965.³ What excitement! (To a scientist that first paper ranks somewhere in importance between his first breath of air and his first love affair!)

"The technique was used for thesis studies quantitating cellular production of antibody in mice and sheep. Then in 1967, I went to the Walter and Eliza Hall Institute, Melbourne, for a postdoctoral year, and was enormously stimulated by many of the people there from its charismatic director, Gus Nossal, downward. They quickly persuaded Szenberg and me, working together, to make the plaque technique more robust and convenient to use. I think it was Cordon Ada who suggested the final form—two slides stuck together around double-sided sticky tape—making a simple chamber that many people have since used.

"Why is this paper cited so often? Only because it is a convenient technique for making a commonly needed measurement, and because there are a lot of immunologists. Its intellectual content is trivial, and the real technical advance was the original work—by Jerne and Ingraham. For me it was important because it led to ways of analysing the specificity of antibody released by single cells, and so to a discovery that these cells vary rapidly in phenotype.⁴ Ironically, this has proved unpopular. Perhaps there are two correlations to be derived from citation data: small, appealing advances may be highly cited, while larger, threatening leaps of the imagination are ignored."

1. Jerne N K & Nordin A A. Plaque formation in agar by single antibody-forming cells. *Science* 140:405-7, 1963.
[Citation Classic. *Current Contents/Life Sciences* 24(35): 16, 31 August 1981.]
2. Ingraham J S & Bussard A. Application of a localised haemolysin reaction for specific detection of individual antibody-forming cells. *J. Exp. Med.* 119:667-84, 1964.
3. Cunningham A J. A method of increased sensitivity for detecting single antibody-forming cells. *Nature* 207:1106-7, 1965. [The *SCI* indicates that this paper has been cited over 205 times since 1965.]
4. Evolution in microcosm: the rapid somatic diversification of lymphocytes. *Cold Spring Harbor Symp.* 41:761-70, 1977.
[The *SCI* indicates that this paper has been cited over 5 times since 1977.]