

Vesikari T & Vaheri A. Rubella: a method for rapid diagnosis of a recent infection by demonstration of the IgM antibodies. *Brit. Med. J.* 1:221-3, 1968.

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IgM antibodies characteristically appear at an early stage of immune response to viral infections, for example, rubella. These rubella-specific IgM antibodies can be "visibly" separated by sedimentation analysis, and the test can be applied for the diagnosis of recent rubella in pregnancy. [The *SC1*⁹ indicates that this paper has been cited in over 215 publications.]

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June 16, 1987

Rubella was one of the major targets of virus research in the 1960s, as the 1964 North American rubella epidemic had stimulated work both scientifically and financially. Finland became involved early, thanks to two scientists returning home to Finland from the US: Antti Vaheri from the Wistar Institute in Philadelphia and Pekka Halonen from (what is now) the Centers for Disease Control in Atlanta. Their recently acquired know-how included the growing of rubella virus to high yields¹ and the right (and tricky) conditions for the rubella hemagglutination and hemagglutination-inhibition (HI) tests.²

Thus, my first task after joining Vaheri in Helsinki as a junior worker in early 1967 was to set up a rubella HI test for diagnostic purposes. It soon became apparent that the standard serology could not solve all the diagnostic problems related to rubella in pregnancy. All too often patients posed the question, "Doctor, I had a rash two or three weeks ago, could it have been rubella?"

It had been known for a few years that IgM class antibodies are typical of early immune response to viral infections, but this information had not yet been applied to actual diagnostic virology. Rubella was a real challenge. It seemed that for studies of IgM, physical separation of IgM and IgG antibodies might be superior to other available methods. We did try other methods, however, such as treatment

of sera with 2-mercaptoethanol to destroy IgM. This created an infernal odor in the lab but did not reduce much the HI titer of a serum, as the amount of IgM antibody activity usually was only a fraction of IgG. We further attempted immunofluorescence, but we soon felt that we did not want to make decisions on abortion or no abortion with a test so much dependent on subjective interpretation.

A suitable sucrose gradient for the separation of 19S and 7S antibodies from a small serum specimen by ultracentrifugation had been worked out by J.-P. Vaerman and coauthors in 1963.³ The technique required a Spinco ultracentrifuge, an SW 39 rotor, and a gradient maker. While this equipment was readily available in our laboratory and many other virus laboratories, it was unheard of to use it for clinical diagnostics. No wonder the study was therefore a subject of debate at the department. One could perhaps compare this study to the use of the electron microscope for studies of human stools, which, while considered sacrilegious by some, a few years later led to the discovery of rotaviruses.

The sucrose gradient was usually collected in 12 fractions, just the right number to be tested for HI antibodies on a microplate. The 19S and 7S fractions were clearly separated as two peaks that could readily be seen on the microplate. It is probably this clarity, the fact that the results can be seen unequivocally by anyone, that made the sucrose gradient-HI technique such a useful (and frequently cited) method for studies of rubella IgM antibodies. The method may still be cited as a historical "test of choice" for rubella IgM.⁴ In addition, the "standard" nature of the sucrose gradient-HI method is also reflected by the fact that the test, unreferenced, may still be advocated for routine studies as a confirmatory test to complement rubella IgM RIA:s or ELISA:s.⁵

IgM antibody tests are now available for many viral infections. As rubella was perhaps the first example of useful clinical application of the IgM antibody studies, the paper has also been cited often by workers studying immunoglobulin class-specific antibody responses in other viral infections, even those seemingly remote from rubella.⁶

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