Marshall J D, Eveland W C & Smith C W. Superiority of fluorescein isothiocyanate (Riggs) for fluorescent-antibody technic with a modification of its application. *Proceedings of the Society for Experimental Biology and Medicine* **98**:898-900, 1958.

The authors compare three methods Cor preparing fluorescein conjugated globulins by using two derivatives of fluorescein amine, and recommended one for fluorescent antibody staining. [The *SCI*® indicates that this paper was cited a total of 550 times in the period 1961-1976.]

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"I find great humor in being a 'most cited author' but the real credit should go to Coons and Kaplan[1] who developed the fluorescent antibody technic and to Riggs[2] who described the use of fluorescein isothiocyanate. Our work modified the technic for the conjugation of the dye to antibody and brought-Riggs' dye to the attention of other workers.

"Perhaps you would be interested to know why and how our technic evolved. At the Armed Forces Institute of Pathology, our group was interested in the identification of microorganisms in tissues which had been processed for histological examination and were not suitable to standard microbiological processing. We were using the basic technics of Coons and Kaplan but because of potentially serious accident during the synthesis stage and the requirement for numerous high titered conjugated antisera, we were actively on the lookout for a safer, simpler, and less denaturing method.

"Coons and Kaplan published the first of a number of papers about their technic in 1950. Numerous investigators realized the value of being able to detect antigenic components at the cellular level; but the basic technic of Coons and Kaplan had three serious drawbacks: (1) the synthesis of fluorescein isocyanate required the use of phosgene gas (this alone was sufficient to deter many researchers); (2) the dye had a short shelf-life and required repeated synthesis; and (3) the method of conjugation resulted in denaturing the antibody of the finished product.

"In 1957, Riggs' masters degree thesis describing fluorescein isothiocyanate came to our attention. While the reagents used in his technic were toxic, thiophosgene was far less hazardous to handle than phosgene gas. The resultant product was quite stable, so a single lot of dye could be produced and used over several months, thereby eliminating the necessity for repeated synthesis required for fluorescein isocyanate. Earlier that year Goldman[3] had described a method for storing fluorescein isocyanate on filter paper which had the advantage of prolonging the shelf-life of the compound, alleviating one of the problems. Since fluorescein isothiocyanate was a stable dry powder, we chose to use it directly to eliminate the step of quantatively applying the dye to filter paper. Our procedure also eliminated the necessity of exposing the globulin solutions to organic solvents, reducing the possibility of denaturation of the proteins.

"We were delighted that the end result was a rather safe and simple method of preparing high titer reagents. Furthermore, our method did not compromise quality; the comparative study indicated that it had outstanding features—the fluorescein isothiocyanate was superior in stability and degree of fluorescence, and also the serum could be conjugated easily. Now our laboratory and other laboratories had a fluorescent antibody technic available which required minimum supplies, equipment, and personnel. With the increased interest, reagents soon became available commercially, which further stimulated the use of the method. We are certainly fortunate to have reported the work at a time when there was a need for the modification of this technic."

1. **Coons A H & Kaplan M H.** Localization of antigen in tissue cells. II. Improvements in a method for the detection of antigen by means of fluorescent antibody. *Journal of Experimental Medicine* **91:**1-13. 1950.
2. **Riggs J L.** Synthesis of fluorescent compounds and their use for labelling antibody. Masters Thesis, University of Kansas, 1957.
3. **Goldman M.** Staining toxoplasma gondii with fluorescein-labelled antibody. I. The reaction in smears of peritoneal exudate. *Journal of Experimental Medicine* **105:** 549-56, 1957.