Riggs J L, Seiwald R J, Burckhalter J H, Downs C M & Metcalf T G. Isothio cyanate compounds as fluorescent labeling agents for immune serum. *Amer. J. Pathol.* **34**:1081 92, 1958.

The authors describe the synthesis of two fluorescent isothiocyanate dyes, fluorescein isothiocyanate (FITC), and rhodamine B isothiocyanate (RITC), substituting thiophosgene, a less toxic substance, for highly toxic phosgene gas during the synthesis of the compounds. These two fluorescent dyes were successfully coupled with antibodies which were subsequently used in the direct and indirect fluorescent antibody procedures for staining different bacterial, rickettsial and viral antigens. [The *SCI*® indicates that this paper was cited 546 times in the period 1961-1976.]

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"It is indeed flattering to be a 'most cited author' and to have one's paper referred to as a 'Citation Classic.' The paper was the result of a portion of the work done at the University of Kansas for my master's degree in bacteriology. I had been instructed by my major professors in the Department of Bacteriology, Dr. Theodore Metcalf and Dr. Cora Downs, to attempt to synthesize some fluorescein isocyanate following Dr. Albert Coon's procedure. The synthesis was to be carried out in the Laboratory of Pharmaceutical Chemistry under the direction of Dr. Joseph Burckhalter. Dr. Robert Seiwald, a postdoctoral student in Dr. Burckhalter's laboratory at that time, and I spent many hours in the laboratory attempting to produce isocyanates of many fluorescent compounds in addition to fluorescein. It was evident, however, that the isocyanates were so reactive that they were very unstable, and many precautions had to be taken in order to couple them to solutions of proteins.

One morning Dr. Burckhalter came into the laboratory and said, 'John, why don't you try to substitute a sulfur atom for the oxygen in the isocyanate portion of the molecule? Perhaps that will stabilize the compound.' Many more hours were spent in the laboratory by Seiwald and myself attempting to synthesize the isothiocyanate by different procedures suggested by Dr. Ray Brewster, then Chairman of the Department of Chemistry at the University of Kansas. We could not at that time find a commercial source of thiophosgene, and although many commercial laboratories would custom synthesize it for us, the cost was prohibitive. We were on the verge of attempting to synthesize it ourselves when an advertisement of Rapter Laboratories of Chicago was noted which stated that they had thiophosgene available. We immediately obtained some and carried out the experiments to produce the isothio-cyanates. We were successful in producing the isothiocyanates (they were indeed more stable compounds) and, with Dr. Downs in the bacteriology laboratories, were also successful in coupling them to antibodies which were subsequently used in staining and specifically identifying different bacterial, rackettsial and viral antigens.

"Fluorescein and rhodamine isothiocyanate soon became available commercially and the fluorescent antibody procedure then became available as a tool in almost any laboratory that wished to use the technic. Quoting Dr. Coons,[1] Now it was no longer true, as Pressman had told me, that immunofluorescence was impossible because those who could do the chemistry couldn't do the histology, and *vice* versa.'

1. Coons A H. The development of immunohistochemistry. *Ann. N.Y. Acad. Sci.* 177:5-9, 1971.