How Rapid is "Rapid"? Part II. "Rapid" Simultaneous Radioimmunoassay Of Thyroxine and Thyrotropin

To the Editor:

Once again, lack of judicious editorial constraint on the part of Clinical Chemistry in allowing the inappropriate use of terms such as “more rapid,” “more efficient,” and “more prompt” to describe a new modification of pre-existing methodologies has struck a “relevancy” chord in us (1).

In this instance, the article in question describes a simultaneous radioimmunoassay of thyroxine (T₄) and thyrotropin (TSH) with ¹³¹I and ¹²⁵I as radioisotopes, respectively, for mass screening of congenital hypothyroidism in neonates (2). Given all other factors equal, we concur that simultaneous assay of both T₄ and TSH is potentially attractive, because both hormones together constitute the most efficient and reliable screen for congenital hypothyroidism. However, we strongly object to the inappropriate use of comparisons such as “rapid,” “enables more prompt therapy,” “more efficient,” “more thorough,” etc., in describing an assay which, for practical and technical reasons, is inferior to readily available existing methodologies.

Specifically, the assay described required two 24-h incubations with results available within 72 h of collection. The authors then state that “although T₄ and TSH may be assayed separately (by conventional assays) in 8 h, such a short assay is not yet available for simultaneous measurements.” The significance of this statement is a mystery to us as it must be for anyone routinely assaying T₄ and TSH.

Many accurate and reliable commercial T₄ assays are now available that can be performed in 1–2 h. Routine assay of TSH is also performed in 3.5–24 h with commercial reagents (Table 1). Therefore, we fail to see the logic of promoting an assay as permitting “more rapid identification and treatment of affected neonates than any method now in use” if it requires more time to perform than a “combined” assay does most commercial individual assays.

Table 2 shows a comparison of the published assay with an example of commercial assays for T₄ and TSH and shows that both commercial assays could be performed significantly quicker—and with comparable technical ease—than the published assay. Thus, “rapid, accurate” measurement of T₄ and TSH can already be routinely finished—using commercial reagents—within 8 h of collection vs. the 72 h stated for the simultaneous assay. In fact, one could perform tests for T₄, TSH, thyroxine binding globulin (or T₃ uptake), thyroid autoantibodies, and a thyrotropin releasing hormone stimulation test with commercial reagents in the same time that it takes to perform the new methodology.

No advantage in sample size or antibody sensitivity exists, since, by the same criterion of “practical” sensitivity expressed in the article (B/B₀ of 85%), both assays can detect between 36–40 micro-international units of thyrotropin per milliliter with a 25-µl aliquot of serum. Certainly there is no cost advantage for the new assay, since difference in technologist time to perform the assays is minimal (Table 2) and the necessary amount of all other reagents is the same, whether or not they are combined.

For practical reasons, however, the simultaneous assay is at a disadvantage. Use of ¹³¹I significantly increases the biohazard to laboratory personnel, in addition to being more inconvenient to store and quality control because of its 8-day half-life as compared with 80 days for ¹²⁵I. In addition, more frequent documentation, purchase of two different radioisotopes and increased handling precautions means considerably higher costs in material and labor to perform the simultaneous radioimmunoassay.

Thus, when serum is assayed for T₄ and TSH, the combined assay is at a significant disadvantage in comparison to the existing state of the art. Of additional consequence, however, is the difficulty in obtaining serum from newborns and the inconvenience of obtaining cord-blood specimens routinely at birth, especially for births outside of the hospital. It is for this reason that assays of filter paper spots containing absorbed whole blood from heel sticks were developed and are currently used as the sample of choice for T₄, TSH, or both, in many screening clinics.

Commercial kits for neonatal T₄ determinations using filter paper blood spots are now available from several manufacturers and can be performed in 3–24 h. TSH assays on filter paper specimens are already being performed in some screening clinics and are commercially available (4, 5).

Thus, if serum is used, well-established existing radioimmunoassay methods for T₄ and TSH offer substantial advantages of speed and economy over the published simultaneous method and with comparable accuracy and sensitivity. However, practical considerations make screening for T₄
and TSH by use of blood spotted on filter paper the method of choice (4, 6).

The advantages of the blood-spot specimens outweigh the disadvantages of a few extra days needed for patient recall and positive serum confirmation. Also, the blood-spot test for T4 is reliable and enables replacement therapy to be started before patient recall and positive diagnosis (6).

While we are not at odds with the authors for presenting a different approach to T4/TSH assay we, nevertheless, think that the editors of articles submitted to Clinical Chemistry should better understand the state of the art in radioimmunoassay so that editorial review and screening of relevant papers and their terminology can be more appropriate.

References

3. NMS Thyroxine and TSH RIA Kits, Nuclear Medical Systems, Inc., 1531 Monrovia Avenue, Newport Beach, Calif. 92663.
5. NMS Neo TSH Kit, Nuclear Medical Systems, Inc., Newport Beach, Calif. 92663.

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One of the authors of the paper in question offers the following response:

To the Editor:
Thank you for the opportunity to review the Letter of Drs. Travis and Dewhurst. Our intention was to report a method for the combined assay of T4 and thyrotropin and to suggest that it may be applicable to the screening of neonates for congenital hypothyroidism. One point possibly not adequately clarified in the text was that present screening programs measure either T4 or thyrotropin (predominantly the former). It is our opinion that all neonates should have both determinations because of the possibility of missing some infants with primary hypothyroidism in whom the T4 concentration at the time of the assay was not below the limits set to require a thyrotropin determination. Such instances do occur.

There are many modifications that can be made in terms of incubation times, volume requirements, and so forth. The reported assay has been designed so that one technician can measure T4 and thyrotropin in 100 samples by manual means during 72 h. While the commercial radioimmunoassay kits described by the correspondents require less incubation time, on a practical basis, and as reported by those directly involved in screening programs, several days elapse between completion of the T4 assay and completion of the thyrotropin assay on selected specimens. The writers do not quantitate the technician time required for two separate assays, but one suspects that it is at least as great, if not greater, than that required for the combined assay.

We agree that for mass-screening programs the assay should be performed on filter paper dried blood (and we have so adapted the assay).

We believe that the correspondents have incorrectly assessed the intent of the communication and have overstated the "art in RIA." Indiscriminate use of commercial RIA kits is fraught with potential danger, often not adequately defined.

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Antibodies to Polyamines: Some Overlooked References

To the Editor:
In a recent exchange of letters to Clinical Chemistry [23, 2171 (1977)] our research in polyanine antibodies and radioimmunoassay development was referred to by both Drs. Quash and Russell. We would appreciate the opportunity to respond, clarify and, we hope, close the matter.

First, Dr. Quash pioneered the development of polyanine immunology beginning in the mid-'60s. When we began our work in early 1973 we received not only his enthusiastic support but copies of all his published papers and all the advice we asked for concerning methods. Early on, he flew his polyanine antisera to us from the West Indies to assist in our developing polyanine radioimmunoassay. Dr. Quash is an ardent student of polyanines and a ready communicator. He could have been readily consulted by any person interested in the polyanine immunology as early as the '60s. In addition, his first and second papers referred to in his letter give enough basic information for the reader to be able to start to develop polyanine antibodies.

A generalization in Dr. Russell's letter—"so there are not, in fact, specific antibodies available for putrescine, spermidine and spermine..."—ignores our article published last May in Biochem. Biophys. Res. Commun. [75, 915-919 (1977)]. We describe how a highly specific spermidine antibody was used in comparing values generated from aliquots of normal human serum from radioimmunoassay, high-performance liquid chromatography, and gas chromatography/ mass spectrometry. The study showed the specificity, sensitivity, and accuracy of the radioimmunoassay approach.

We, like Dr. Quash, try to be good communicators. We have shared our experience in polyanine immunology with scientists at two Gordon Conferences in New England (1975, 1977), at the Pacific Northwest Polyanine Conference (Oregon 1976), and at a national Radioimmunoassay Symposium in San Francisco (1977). The high specificity of our spermine and spermidine antibodies is well known to most investigators in the field. Moreover, we have been in communication with two other laboratory groups, both of whom have excellent antispermine antibody. This suggests that the skills necessary for polyanine radioimmunoassay program development are widely held in the scientific community. Additional papers by us reporting on our high specificity antispermine and antispermidine antibodies are in preparation and in press.

We cherish the same basic scientific requirements for polyanine radioimmunoassay as Dr. Russell enumerated, namely that they should be "specific, precise, sensitive, accurate, and reproducible."

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