United States Patent [19]

Adams

[11] **3,711,247**

[45] **Jan. 16, 1973**

[54] METHOD FOR DETERMINATION OF THYRO-BINDING CAPACITY OF BLOOD PROTEINS

[75] Inventor: **Raymond J. Adams,** Anaheim, Calif.

[73] Assignee: **Curtis Nuclear Corporation,** Los Angeles, Calif.

[22] Filed: **March 2, 1970**

[21] Appl. No.: **15,342**

[52] **U.S. Cl.** .....**23/230 B,** 23/253 R, 424/1, 424/79

[51] **Int. Cl.**.....**G01n 33/16**

[58] **Field of Search**....23/230 B, 253; 250/83; 424/1, 424/79

[56] **References Cited**

UNITED STATES PATENTS

3,206,602	9/1965	Eberle	23/230 B
3,451,777	6/1969	Giulio	23/230 B

OTHER PUBLICATIONS

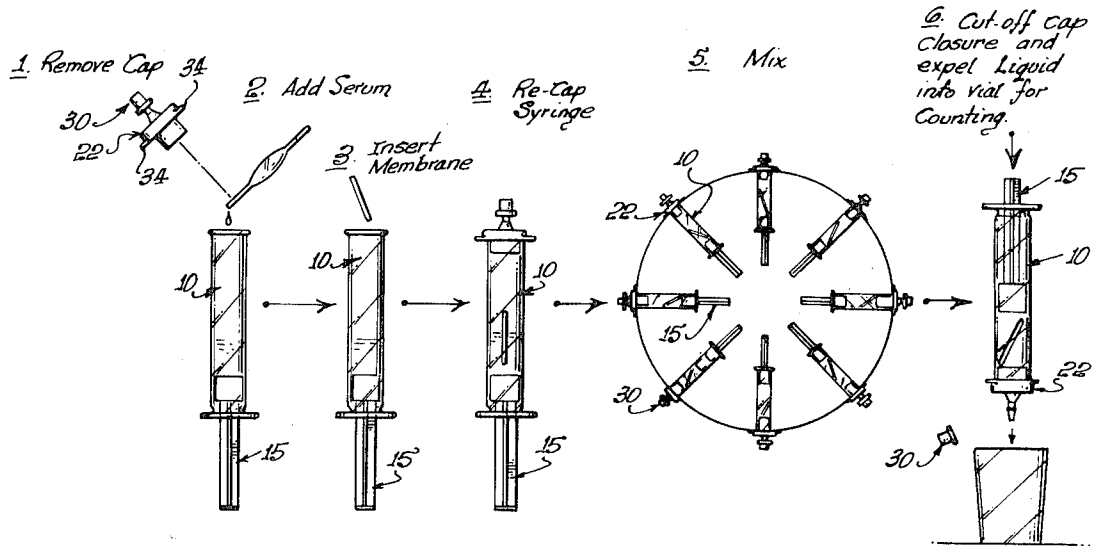
Sterling et al., "Measurement of Free Thyroxine Concentration in Human Serum," J. of Clinical Invest., No. 5, 1962, pp. 1031–1040

Primary Examiner—Joseph Scovronek
Assistant Examiner—R. E. Serwin
Attorney—Huebner and Worrel

[57] **ABSTRACT**

The ability of serum to bind additional triiodothyronine is determined by a method requiring minimal quantities of serum, of the order of 0.10 ml., and short mixing times, of the order of 40 minutes, by employment of a specially designed syringe-type reaction vial in which radioactive triiodothyronine and a buffer are prepackaged, and a resin membrane separable from the liquid reactants by syringing of the latter from the vial after mixing.

3 Claims, 3 Drawing Figures



PATENTED JAN 16 1973

3,711,247

SHEET 1 OF 2

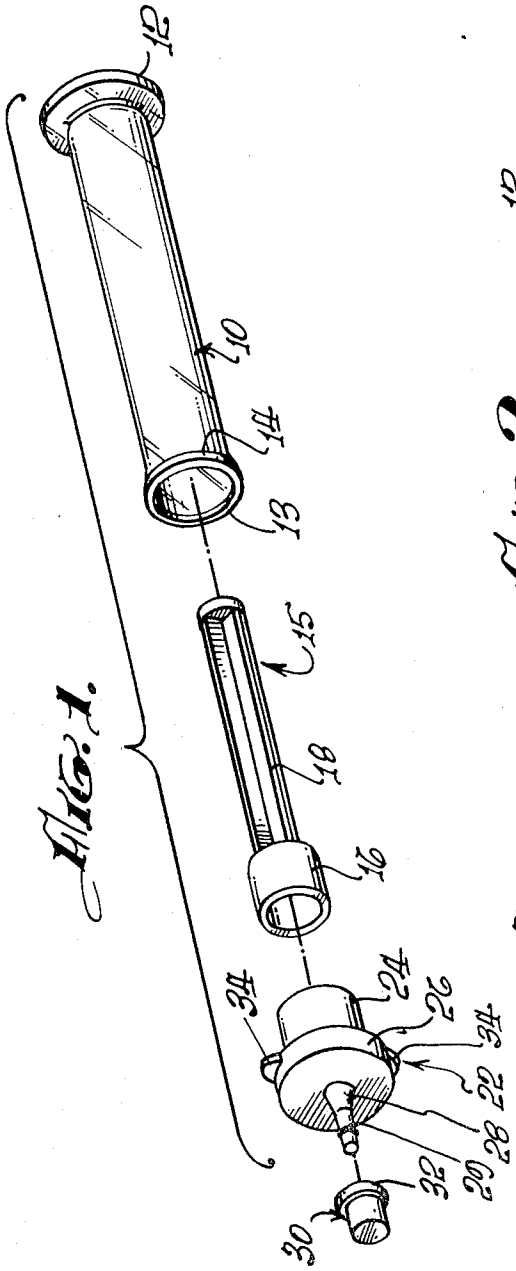


FIG. 1.

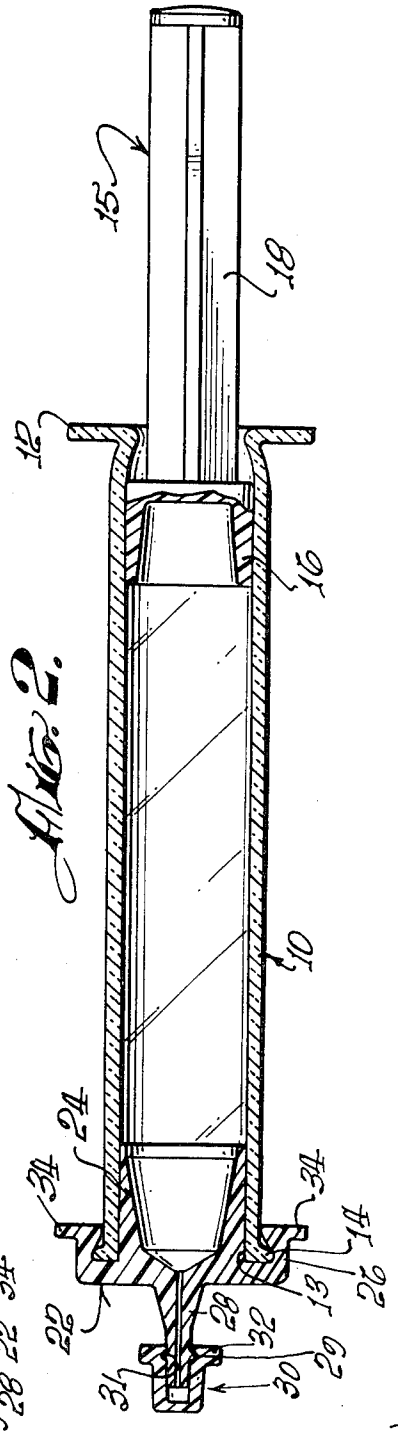


FIG. 2.

INVENTOR.
RAYMOND J. ADAMS,

By Huebner & Worrel
ATTORNEYS.

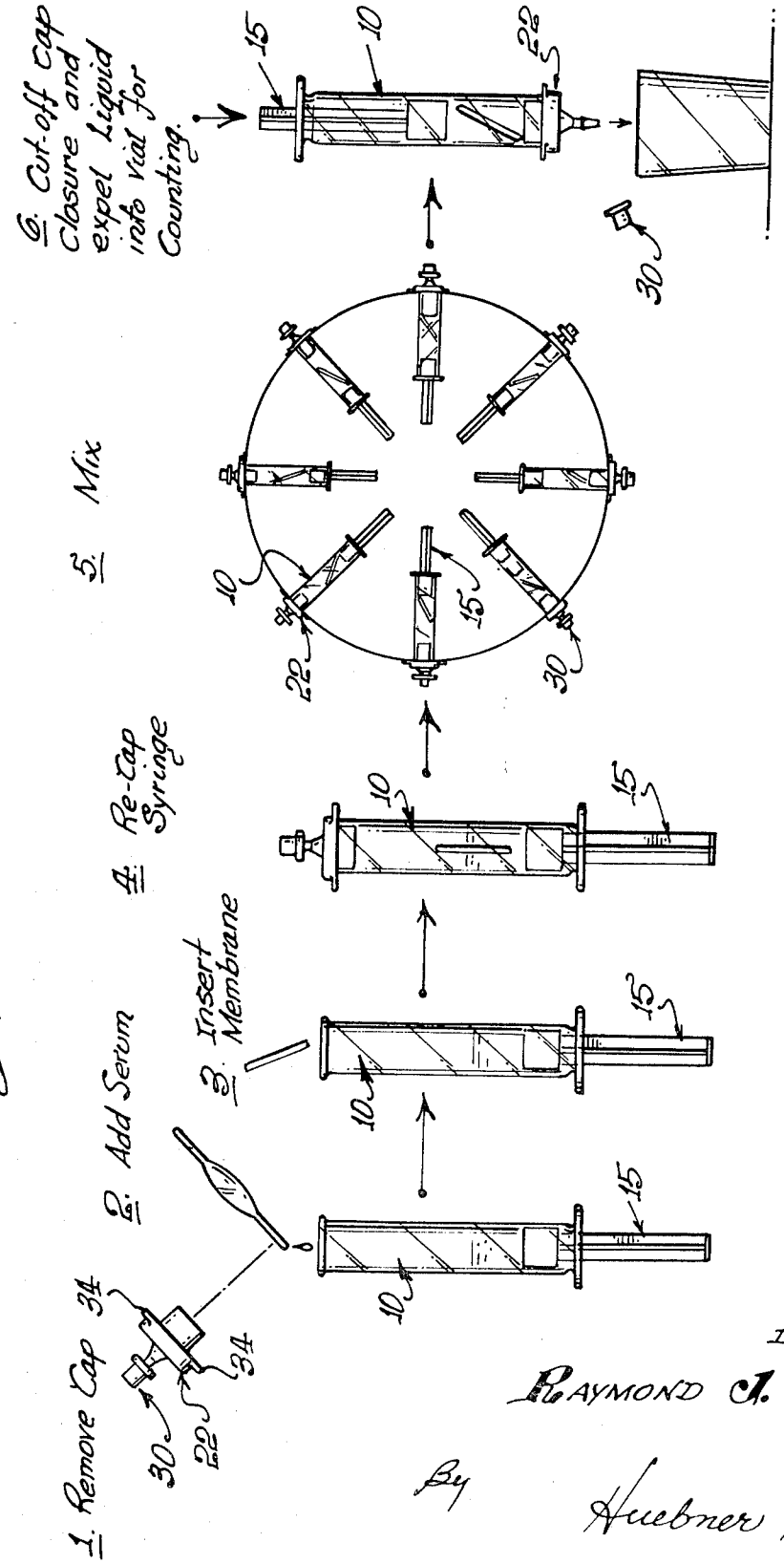


Fig. 3.

INVENTOR.
RAYMOND J. ADAMS,

By Huebner & Worrel
ATTORNEYS.

METHOD FOR DETERMINATION OF THYRO-BINDING CAPACITY OF BLOOD PROTEINS

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to an improved method and apparatus for in vitro determination of the endogenous triiodothyronine (T3) content of blood by measurement of the uptake of radioactively labeled triiodothyronine.

Assessment of the functional status of the thyroid gland by in vitro measurement of the endogenous thyroid hormones generated in the thyroid gland and circulating in the blood stream, by use of radioactive isotopes, has become a widely employed diagnostic technique in recent years.

The endogenous thyroid hormones thyroxine and triiodothyronine which circulate in the blood stream are bound to several specific sites, primarily an alpha-globulin known as thyroxine-binding globulin, or "TBG," but also including as secondary binding sites the red blood cells, or erythrocytes.

TBG and certain secondary binding sites have the same capacity to bind radiothyroxine and radio-triiodothyronine in which a radioactive isotope of iodine, such as I^{131} or I^{125}, has been substituted for the non-radioactive or stable form. Thus, by taking a blood sample in which the TBG and secondary binding sites are partially saturated with endogenous thyroid hormones, completing their saturation with synthetic radioactive hormones in the laboratory, and then measuring the amount of the radioactive hormone bound by the sample, it becomes possible to calculate the degree of hormone saturation of the original sample. This is indicative of the functional status of the thyroid gland.

2. Description of the Prior Art

A variety of in vitro procedures for measuring the uptake of radioactive hormones by blood constituents have been developed for the purpose described. Hamolsky et al. in "The Thyroid Hormone-Plasma Protein Complex in Man. II. A New in vitro Method for Study of 'Uptake' of Labelled Hormonal Components by Human Erythrocytes," J. Clin. Endrocrinol. & Metab. 17:33, 1957, described a procedure in which whole blood mixed with anti-coagulant has added to it a measure proportion of I^{131} and T3. This mixture, after being shaken, then had its radioactivity measured with a well-type scintillation counter. The plasma and erythrocytes were then separated and, after washing, the radioactivity of the erythrocytes was similarly measured or, alternatively, the radioactivity of the plasma was so measured. The ratio of the second to the first scintillation counter reading constituted the "uptake" subject to certain corrections such as for the hematocrit reading.

In order to avoid difficulties with this procedure inherent in the use of live red blood cells, ion exchange resins known to compete with the plasma for the T3 and I^{131} T3 were next used to replace the red blood cells in tests otherwise comparable to that of Hamolsky et al. described above. Scholer, in "A Simple Measure of Thyro-Binding by Plasma: A Test of Thyroid Function," J. Nuclear Medicine 3:41, 1962, and the references cited therein describe the use in this way of a labelled resin in bead form, and Mitchell et al. in

"The in vitro Resin Sponge Uptake of Triiodothyronine — I^{131} from Serum in Thyroid Disease and in Pregnancy," J. Clin. Endocrinol. & Metab., 20:11, 1960, describe the use of a resin sponge made by mixing polyurethane foam with a finely ground anion exchange resin in substantially the same way. Further refinement of the resin sponge procedure is described by McAdams et al. in "Resin Sponge Modification of the I^{131} T3 Test," J. Nuclear Medicine 5:112, 1964.

According to the preferred present practice, radioactive triiodothyronine such as I^{131} T3 or I^{125} T3 is "tagged" (bound artificially) to a quaternary amine polystyrene anion exchange resin which has been preprocessed by cleaning before tagging. Exact amounts of this resin (0.1 gram assayed radioactively) is dispensed into a reaction vial. Three milliliter tris buffer at 7.17 pH is added to the reaction vial, together with 1 ml. of the serum to be assayed. The reaction vial containing the serum, buffer and tagged resin is mixed (usually by a vial rotator) for 2 hours at approximate room temperature, and a fraction of the supernate, usually 2 ml., is removed from the reaction vial and placed into a clean vial for radioactive counting and assay.

The serum being tested is measured against a normal standard serum which has been treated and reacted in exactly the same manner as the serum being assayed. The results of this method are usually reported in terms of a "thyro binding index" which has a normal range of 0.88–1.10, but can be converted to percent resin uptake or percent red blood cell uptake and reported in these terms if desired.

SUMMARY OF THE INVENTION

The present invention has as its principal object the reduction of the required mixing time and minimization of the number of transfers of reactants in the determination of thyro-binding capacity of blood proteins.

According to the present invention, radioactive triiodothyronine in excess of the amount required to saturate the blood proteins to be assayed, and a buffer solution at the proper pH are premeasured into a specially designed syringe-type reaction vial provided with a removal tip closure and a plunger for expelling its liquid contents through that tip after removal of the closure.

To the contents of this reaction vial a very small quantity, which may be as little as 0.10 milliliter, of the serum to be assayed is added. Following this step, an anion resin in the form of a membrane of inert material impregnated with such resin is inserted into the reaction vial.

The reaction vial containing the radioactive triiodothyronine, buffer, serum and resin membrane is then mixed, usually by a conventional vial rotator, for about 40 minutes at approximately room temperature.

Following such mixing, the tip closure of the reaction vial is removed and the plunger is operated to expel the liquid contents into a separate container for counting, leaving the resin membrane in the vial. Counting and assaying then proceeds as in present practice.

The present invention thus provides a method for determination of the thyro-binding capacity requiring pipetting of only the small amount of serum to be assayed, a single transfer of reactants from one container to another, and a mixing time of only about 40 minutes

as compared with the 2-hour mixing time of the prior art.

BRIEF DESCRIPTION OF THE DRAWING

FIG. 1 is an exploded perspective view of a specially designed syringe-type reaction vial embodying the present invention;

FIG. 2 is a side view in section of the assembled reaction vial of FIG. 1; and

FIG. 3 is a flow diagram delineating successive steps of the method of the present invention.

DESCRIPTION OF THE PREFERRED EMBODIMENT

Achievement of the objects of the method of the present invention is significantly facilitated by employment of a specially designed syringe-type reaction vial, although the steps of the method may be carried out using conventional laboratory equipment.

As illustrated in FIGS. 1 and 2, the novel reaction vial of the present invention comprises a tubular glass body 10 having a flanged end 12 and provided at its opposite end 13 with a bead 14. A plunger 15 of elastic plastic material, such as polyethylene, is provided with a head 16 dimensioned for a sliding fit closely within the body 10 and a portion 18 which is preferably integral with the head and cruciform in cross-section for rigidity and which extends from the head 16 beyond the end of the tubular body 10.

The plunger 15 is assembled into the body 10 by insertion of the portion 18 first into the beaded end 13 of the body 10, the head 16 being prevented from exiting from the flanged end 12 of the body 10 by a necked-in portion 20 of the body 10.

A removable closure is provided for the beaded end 13 of the body 10 comprising a cap 22 of similar elastic plastic material formed with a thin sleeve 24 closely fitting within the body 10 and a rim 26 which may be flexed over the bead 14 and thereby retained in position on the body 10. A hollow teat 28 communicates with the interior of the sleeve 24 and is closed at its opposite end by a tip 30 of elastic material such as polyethylene. The exterior of the teat 28 is provided with an integral ring 29 and the interior of the tip 30 is provided with a complementary groove 31 so that the elastic tip 30 will be securely retained in place on the teat 28 by inter-engagement of the ring 29 and groove 31. A rim 32 on the tip 30 is provided to facilitate its removal from the teat 28 when it is desired to discharge the contents of the vial. Tabs 34 are formed integrally with the rim 26 to facilitate removal of the cap 22 from the bead 14.

According to an example of the method of the present invention, approximately 0.1 microcurie of radioactive triiodothyronine (I^{125} T3) together with 3.5 milliliters of a tris buffer at 7.0 to 7.8 pH is premeasured into a reaction vial of the kind described above, and so prepackaged are delivered to the laboratory for use within the shelf life of the radioactive isotope.

When a given sample of patient's serum is to be assayed, the cap 22 is removed from a reaction vial containing the above described mixture of radioactive triiodothyronine and buffer, as illustrated in FIG. 3 of the drawing, and approximately 0.10 milliliter of the serum to be assayed is pipetted into the vial.

Next, an anion exchange resin carried upon a mesh material to which it is sufficiently strongly attached to resist displacement during the subsequently described mixing operation is inserted into the reaction vial; the quantity of resin thus introduced being in excess of that required to bind the radioactive triiodothyronine not bound by the serum. The material manufactured by Ionac (New Jersey) and designated by it as 1 *m* 12 which is described by the manufacturer as a heterogeneous ion exchange membrane of the strongly ionized perm-selective type has been successfully used for this purpose.

Following the insertion of this membrane, the cap 22 is replaced on the reaction vial and (usually together with other identical tests vials and a control vial), the contents of the vial are mixed, usually on a vial rotator, for approximately 40 minutes at room temperature, nominally 68° F.

Following such mixing, the tip 30 of the cap 22 is removed from the teat 28, and with the vial held in the vertical position in which it is shown at 6 in FIG. 3, the liquid contents are expelled into a separate vial by operation of the plunger 15; the anion impregnated membrane being retained in the vial.

Radioactive counting of the expelled contents of the reaction vial is performed in the conventional manner, the serum being tested being measured against a normal standard serum which has been treated and reacted in exactly the same manner as the serum being assayed. The results of this method are usually reported in terms of a "thyrobinding index" which has a normal range of 0.88–1.10, but can be converted to percent resin uptake or percent red blood cell uptake and reported in these terms if desired.

While the use of triiodothyronine tagged with the I^{125} isotope of iodine has been described in the above example and is preferred because it has a shelf life of approximately 60 days, other radioactive isotopes of iodine may be used for tagging the triiodothyronine used, such as, for example, the I^{131} isotope of iodine which, however, has a shelf life of only 14 days.

Similarly, the system is not sensitive to the amount of radioactivity applied, and ranges from 2.0 to 0.07 microcuries may be employed provided that each vial utilized in the same testing cycle contains the same amount within plus or minus 1 percent.

Similarly, the volume of buffer employed may be varied within a range of from 2 to 5 milliliters provided that each vial employed in the same testing cycle contains the same amount. Buffers other than the tris buffer referred to in the above example, such as, for instance, acetate of barbitol, may be employed.

Other mesh materials and fabrics composed of paper, nylon or rayon mesh impregnated with anion exchange resins may likewise be substituted for the specific membrane described in the foregoing example. A similar membrane manufactured by Ionics (Massachusetts) under the designation 111 BZL 184 and 111 BZP 333 have been found satisfactory.

Although the invention has been herein shown and described in what is conceived to be the most practical and preferred embodiment, it is recognized that departures may be made therefrom within the scope of the invention.

What is claimed is:

1. A method of determining the degree of saturation of a serum sample by endogenous thyroid hormones which comprises equilibrating a synthetic radioactive hormone between the sample and an anion exchange resin, separating the serum sample from the resin, and then measuring the radioactive hormone uptake of the serum sample, characterized by the employment of an anion exchange resin in the form of a membrane having said resin sufficiently strongly attached thereto to resist displacement therefrom during agitation in contact with liquid.

2. An improved method in accordance with claim 1 in which the liquid reactants include 2.0 to 0.07 microcuries of radioactive triiodothyronine, 2 to 5 ml. of a tris buffer at 7.0 to 7.8 pH, and approximately 0.1 ml. of serum.

3. An improved method in accordance with claim 2 in which the mixing time is approximately 40 minutes.

* * * * *