

Technical Evaluation of the Effective Thyroxine Ratio

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Effective thyroxine ratio (ETR) test kits were produced commercially about 2 years ago by Malinkrodt/Nuclear, and they are currently used routinely in many clinical laboratories for rapid-screening in vitro thyroid-function tests. The test is not affected by changes in the concentration of thyroid-binding globulin. The test procedure consists of two identical parts, one for the standard and the other for the patient. In each part, the test is the combination of the thyroxine (T_4) competitive protein binding and "pseudo- T_4 " uptake. The effective thyroxine ratio is the ratio of the test results of the standard and the patient. In principle, no technical error will arise if identical performances for both parts are made. In practice, the results of this test, like those of others, are subject to variables such as the amount of serum and tracer used, length of incubation time, etc. Errors may arise in each step of the test. The accuracy of the test depends on the person who performs the test.

Clinical evaluation of the test has been made by several groups (1, 2, 3). In this paper, the effect of some variables is examined and the sensitivity due to the variables is assessed.

Materials and Methods

The kits were picked randomly from those purchased for routine tests. Dade Moni-Trol (American Hospital Supply Corp.) reconstituted serum and self-collected pool serum were used as normal sera. Abbott hyper- and hypothyroid sera were used as abnormal sera.

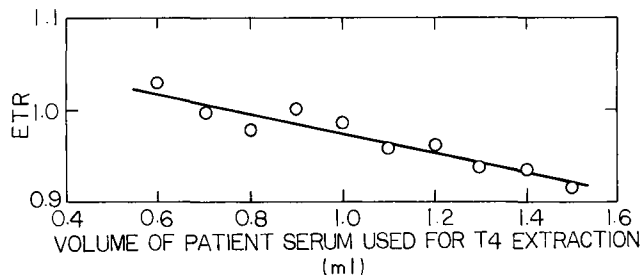
The ETR values of various samples were measured with respect to the reconstituted normal standard serum provided with the kits as functions of volume of serum used for T_4 extraction, volume of extraction alcohol supernatant, and vol-

ume of Res-O-Mat ETR solution. All the tests were performed at room temperature. Samples were counted with a Picker single-channel automatic scintillation counter.

Results and Discussion

Figure 1 shows a plot of ETR values as a function of volume of patient serum used for T_4 extraction. Its slope indicates that the ETR values decrease linearly by about 0.01 when 0.1 ml of excess serum is used; in other words, the ETR value will decrease by 0.1 if 1 ml of excess normal serum is used for extraction. If one makes an error in measuring serum volume, it does not affect the result significantly. The reason for the decrease in ETR value is that the excess serum probably causes incomplete T_4 extraction.

Figure 2 gives the relationship between the ETR value and the volume of supernatant used. The ETR value rises linearly by 0.02 in each 0.01 ml of excess supernatant used. The excess of supernatant means that much more T_4 is added to the labeled reference TBG in the first step, displacing larger amount of labeled T_4 in reference TBG. The



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FIG. 1. ETR value of reconstituted Moni-trol serum as function of serum volume used for T_4 extraction.

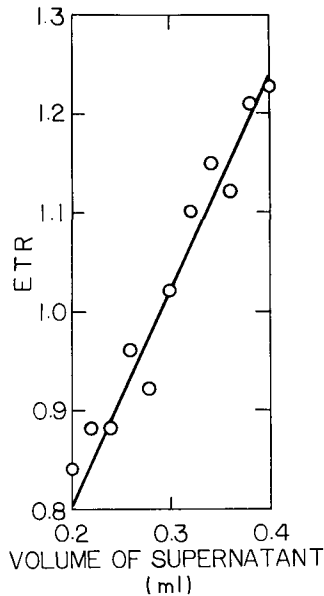


FIG. 2. ETR value of reconstituted Moni-trol serum as function of volume of ethanol supernatant.

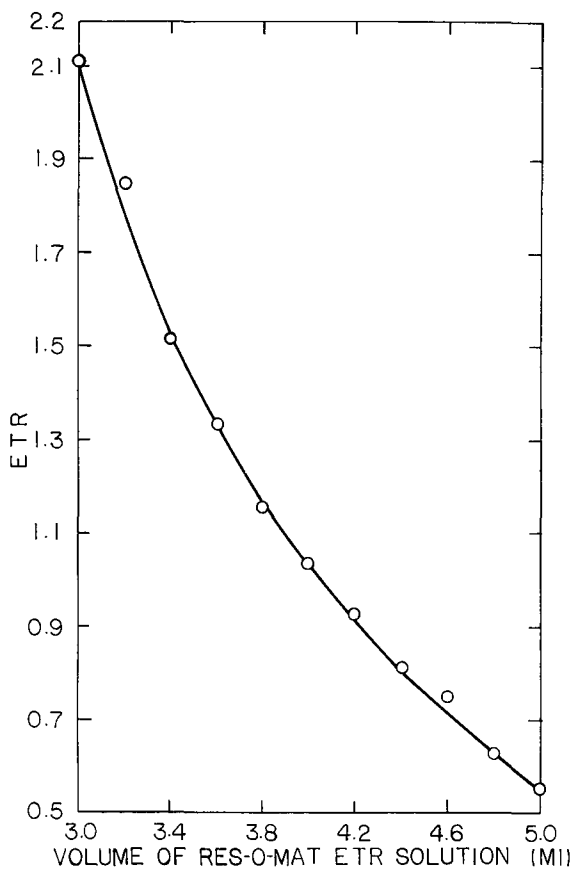


FIG. 3. ETR value of reconstituted Moni-trol serum as function of volume of Res-O-Mat ETR solution.

large amount of surplus T_4 not used in the first step was picked up by the patient TBG primarily because of the proportionality of patient T_4 and labeled T_4 . Therefore the labeled T_4 uptake by patient TBG was less and the ETR value tends to rise.

Figure 3 shows the ETR values obtained from various volumes of Res-O-Mat ETR solution labeled T_4 with TBG used for the performance of the test. The ETR value decreased exponentially as the volume of Res-O-Mat ETR solution increased. The decrease of ETR value is essentially due to the excess of labeled T_4 in the vial producing too much radioactivity. Within the range of 3.8–4.2 ml of the solution used, the decreasing rate is about 0.06 per 0.1 ml of excess labeled solution used. One commercial vial in the kit contains 4 ml Res-O-Mat solution. This variation is quite large and critical because some significant high or low counts of pretest vials were found occasionally. It is wiser to count all the pretest vials before proceeding with the test.

Figure 4 shows the change of ETR values due to different incubation times of patient samples. The solid lines show that ETR values rise linearly when the incubation time of the three samples, hyper, normal, and hypo, are over-incubated. The increment of the ETR value is worse for the hyper case. On the average, the value of ETR elevates about 0.02 for each additional incubation minute. The broken lines show that the ETR value does not change significantly when the incubation time for both standard and patient samples is the same. Apparently, the resin strip has tremendous binding sites. The longer the time it takes to incubate, the more free T_4 it picks up.

The open and closed circles are for the same sera. The only difference is that the closed circles

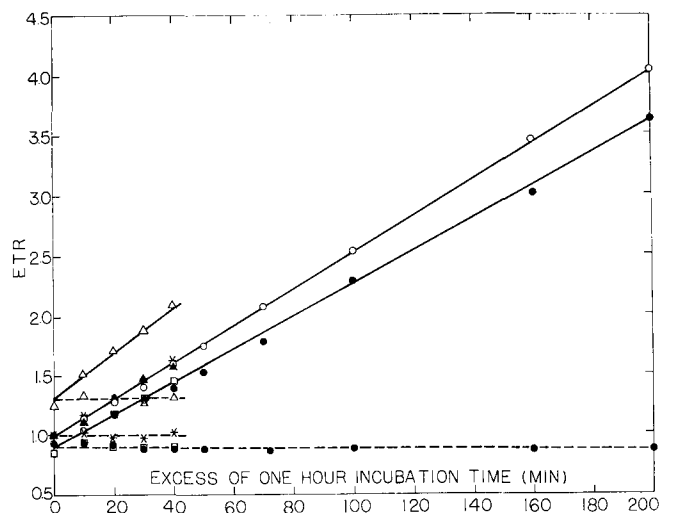


FIG. 4. ETR values of various sample sera as function of excess of incubation time. \circ represents supplied standard serum with supplied extraction alcohol and 5 λ serum aliquot, \bullet is supplied standard serum with supplied extraction alcohol and 10 λ serum aliquot, \blacktriangle is supplied standard serum with ethanol and 5 λ serum aliquot, \star is collected pool serum with ethanol and 5 λ serum aliquot, \triangle is Abbott hyperthyroid serum with ethanol and 5 λ serum aliquot, and \square is Abbott hypothyroid serum with ethanol and 5 λ serum aliquot.

represent data obtained by introducing 10 λ instead of 5 λ of patient serum in the second part of the test. The result showed hypothyroidism when it was actually euthyroidism.

The extraction alcohol provided by Mallinckrodt/Nuclear contains some percent of methanol to prevent people from drinking it. In most of the tests, pure alcohol was used for extracting T_4 ; however, identical results were obtained. Methanol is wood alcohol. It is volatile and much more toxic than ethanol if inhaled. The laboratory should be well ventilated if the extraction alcohol supplied is used.

One should be very careful in reconstituting the supplied standard dry serum. After adding 2 ml of distilled water to the dry serum, we put the vial on a rotator to rotate 12–14 rpm for at least 10 min, making sure that all small hard chunks are completely dissolved. When performing multiple test determinations, one should prepare more standard vials and place them evenly among the sample groups to reduce the differences of the incubation time.

Summary

The ETR values of various samples were measured with respect to normal standard serum as a function of volume of serum used for T_4 extraction, volume of extraction alcohol supernatant, volume of Res-O-Mat ETR solution (*T_4 ·TBG), and incubation time. The results indicated that the

ETR value decreased linearly by 0.01 per 0.1 ml of excess serum used for T_4 extraction, the ETR value elevated linearly by 0.02 per 0.01 ml of excess supernatant used, and the ETR value decreased exponentially as the volume of *T_4 ·TBG increased. Within the range of 3.8–4.2 ml of the solution used, the decreasing rate is about 0.06 per 0.1 ml of excess *T_4 ·TBG used. In some tests, ethanol replaced the supplied extraction alcohol. Identical results were obtained. The ETR value elevated linearly by about 0.015 per additional incubation minute up to 200 min. After 200 min, no further test was performed. When the incubation time for both the standard and the patient samples was the same, the variation of ETR value was negligible.

Acknowledgment

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References

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