

T₄ EXTRACTION EFFICIENCIES OF THREE ALCOHOLS

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The efficiencies of absolute alcohol, 95% ethanol, and methanol in extracting T₄ from serum have been compared. Mean values are insignificantly different but absolute alcohol is preferred because of its significantly lower standard deviation.

During a 2-year period the nuclear medicine laboratory at the University of Florida College of Medicine performed about 2,300 determinations of total serum thyroxine (T₄) by competitive protein binding (CPB) analysis using a commercial T₄ kit (1) and recently reported an analysis of the results (2). The efficiency of extraction of T₄ from serum was determined for three different types of alcohol commonly used in T₄ by CPB determinations, absolute ethanol, 95% ethanol, and methanol, according to the method outlined by the T₄ manufacturer (1).

Table 1 summarizes the results for random selections of serum samples during the 2-year period and the results recently obtained using, with each type of alcohol, the same ten serum samples whose T₄ values (uncorrected for extraction efficiency) ranged from 3.6 to 11.4 μg% with a mean of 6.1 μg%. No dependence of extraction efficiency on the T₄ level was observed. For all three alcohols the maximum and minimum extraction efficiencies were obtained

with the same two sera whose T₄ values were 5.6 and 5.3 μg%, respectively.

For statistical comparison of the results for the different alcohols, the variances, (s.d.)², were first tested in pairs for significance using the F-distribution (2). For both sets of results there is no significant difference between the variances for 95% ethanol and methanol at 0.05 probability level. For both sets of results the differences between the variances for 95% ethanol and absolute alcohol and between methanol and absolute alcohol are significant at 0.05 probability level. For both sets of results the mean T₄ extraction efficiencies for 95% ethanol and methanol were compared using the Student-distribution (t-test) and are insignificantly different at 0.05 probability level. For both sets of results the mean T₄ extraction efficiencies for 95% ethanol and absolute ethanol are also insignificantly different at 0.05 level of significance.

The first step in a T₄ by CPB assay is the denaturing of serum proteins with alcohol and extraction of T₄ from the serum sample. Most accurately T₄ extraction efficiency would be determined for each serum sample, but this is not routine practice

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TABLE 1. T₄ EXTRACTION EFFICIENCY

	Random serum samples over 2-year period			Ten serum samples		
	100% ethanol	95% ethanol	Methanol	100% ethanol	95% ethanol	Methanol
No. of determinations	42	42	22	10	10	10
Range (%)	71.3-93.4	70.9-98.6	70.6-91.5	72.0-81.6	71.3-91.8	72.6-90.9
Mean (%)	80.9	82.2	82.6	77.3	80.6	80.8
s.d. (%)	4.3	7.3	6.3	1.4	5.4	4.8

for most laboratories which either report raw T_4 values or, like our laboratory, perform T_4 extraction efficiency determinations on a small sample of sera and determine a mean T_4 extraction efficiency by which all determined T_4 values are divided, to correct for incomplete extraction of T_4 from patient sera. There are no significant differences in mean T_4 extraction efficiencies for absolute ethanol, 95% ethanol, and methanol. However, if absolute ethanol is employed, errors in determined T_4 values due to the range of T_4 extraction efficiencies will be least since in both sets of results in Table 1 the standard deviation of the absolute ethanol extraction efficiency determinations is significantly less than those of the

other two alcohols. Thus of the three alcohols studied absolute ethanol is the alcohol of choice for extraction of T_4 from serum.

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