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CONTENTS OF VOLUME II

II. STANDARDIZATION AND QUALITY CONTROL
(Session V and Session VI, Part 1)

III. APPLICATIONS

III.1. Assays for vitamins (Session VI, Part 2)

III.2. Assays for steroids and other small molecules (Session VII)

Invited review paper

III.3. Assays for thyroid-related hormones (Session VIII, Part 1)

Invited review paper

III.4. Assays for peptides (Session VIII, Part 2 and Session IX)

THE NEED FOR STANDARDIZATION OF METHODOLOGY AND COMPONENTS IN COMMERCIAL RADIOIMMUNOASSAY KITS*

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Abstract

THE NEED FOR STANDARDIZATION OF METHODOLOGY AND COMPONENTS IN COMMERCIAL RADIOIMMUNOASSAY KITS.

The problems arising from the increasing use of commercial kits in radioimmunoassay (RIA) and related fields are discussed. These problems differ according to the substance under test. The quality of individual reagents is often good, but the methodology is often not optimal and may contain short-cuts which, although commercially attractive, can lead to erroneous values and poor sensitivity and precision. Minor modifications in the methodology often lead to big improvements in sensitivity and precision. This has been demonstrated in three digoxin kits employing antibody-coated tube techniques and in four kits for thyrotropin (TSH) using different techniques. It has also been noted that with many quality-control sera imported from the USA no values are ascribed to European kits for the components listed, thus reducing these sera to the function of precision control. The study underlines the need to standardize kit components and assay methods to enable the results obtained by different laboratories with different kits to be compared.

The decentralized nature of health care in the Federal Republic of Germany (FRG) has contributed to the rapid increase in the use of commercial kits in RIA and associated techniques. The problems arising from the extended use of kits are many and they are not only concerned with methodology. The differing results obtained for certain substances with kits from different manufacturers may lead to a false diagnosis when only the absolute values are compared without reference to the given normal range. This problem arises particularly with the proteohormones.

Table I is an excerpt from the values ascribed to digoxin, TSH and insulin in different kits of commercial quality-control sera. For each component the control sera are at three concentrations with the aim of controlling low, middle and high ranges of the standard curve. For the drug digoxin, which is not normally present in serum, it can be seen from Table I that the measured values

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TABLE I. VALUES ASCRIBED TO DIFFERENT KITS FOR DIGOXIN, TSH AND INSULIN IN COMMERCIAL QUALITY-CONTROL SERA
(Taken from manufacturers' data)

Test (units)	Kit	Level 1 mean ± 2 SD	Level 2 mean ± 2 SD	Level 3 mean ± 2 SD
Digoxin (ng/ml)				
	Abbott	0.6 ± 0.16	1.7 ± 0.28	3.0 ± 0.54
	Amersham/Searle	0.7 ± 0.04	1.8 ± 0.12	3.0 ± 0.24
	Beckman	0.9 ± 0.12	1.9 ± 0.16	3.1 ± 0.38
	Burroughs Wellcome	0.8 ± 0.10	1.8 ± 0.12	3.1 ± 0.32
	Clinical Assays	0.8 ± 0.16	2.0 ± 0.24	3.0 ± 0.22
	Corning	0.8 ± 0.12	1.9 ± 0.26	2.9 ± 0.40
	Curtis	1.0 ± 0.26	2.0 ± 0.36	3.2 ± 0.28
	Kallestad ^{3}H	0.8 ± 0.24	1.8 ± 0.50	2.8 ± 0.54
	Kallestad ^{125}I	0.8 ± 0.06	1.8 ± 0.22	3.0 ± 0.24
	Micromedic	0.8 ± 0.16	1.9 ± 0.24	3.1 ± 0.48
	Dade	0.8 ± 0.08	2.0 ± 0.16	3.2 ± 0.20
	NEN ^{125}I	0.8 ± 0.18	1.8 ± 0.12	3.0 ± 0.20
	NEN ^{125}I Solid Phase	0.7 ± 0.08	1.7 ± 0.12	3.0 ± 0.30
	Radioassay Systems	0.6 ± 0.14	1.6 ± 0.20	3.0 ± 0.46
	Schwarz-Mann	0.8 ± 0.10	2.0 ± 0.28	3.4 ± 0.60
	Squibb	0.8 ± 0.20	1.6 ± 0.20	2.5 ± 0.40

TABLE I (cont.)

Test (units)	Kit	Level 1 mean ± 2 SD	Level 2 mean ± 2 SD	Level 3 mean ± 2 SD
TSH (μU/ml)				
	Abbott	5.3 ± 1.28	15.2 ± 3.10	47.3 ± 16.20
	Diagnostic Products	8.7 ± 0.76	19.6 ± 1.20	38.6 ± 2.82
	Nichols Institute	4.6 ± 0.75	15.7 ± 1.64	32.3 ± 2.90
	Radioassay Systems	4.4 ± 1.86	13.6 ± 1.62	29.9 ± 1.96
	Beckman	6.1 ± 0.72	15.5 ± 3.84	38.1 ± 13.24
Insulin (μU/ml)				
	Amersham/Searle	15 ± 3.1	39 ± 3.5	101 ± 11.1
	Corning	17 ± 4.4	38 ± 8.0	112 ± 12.3
	Curtis	6 ± 0.6	32 ± 8.0	87 ± 11.7
	Schwarz-Mann	13 ± 2.2	34 ± 4.1	105 ± 21.7
	Pharmacia	24 ± 7.5	50 ± 8.5	115 ± 27.1

TABLE II. VALUES ASCRIBED TO DIFFERENT KITS FOR T_3, CORTISOL AND T_4 IN COMMERCIAL QUALITY-CONTROL SERA
(Taken from manufacturers' data)

Test (units)	Kit	Level 1 mean ± 2 SD	Level 2 mean ± 2 SD	Level 3 mean ± 2 SD
T_3 RIA (ng/dl)				
	Beckmann	61.1 ± 11.54	163.6 ± 26.56	390.0 ± 80.46
	Abbott	107.0 ± 32.00	250.0 ± 72.00	530.0 ± 190.0
	Ames	85.4 ± 13.72	196.0 ± 21.48	428.3 ± 59.74
	Amersham/Searle	65.0 ± 8.00	170.0 ± 22.0	444.0 ± 46.00
	Corning	45.5 ± 7.84	147.6 ± 28.52	328.9 ± 60.94
	Curtis	72.4 ± 30.62	195.3 ± 31.76	384.4 ± 35.72
	Diagnostic Products	78.1 ± 8.44	165.5 ± 11.60	356.6 ± 42.16
	Maloy Laboratories	101.6 ± 10.16	245.6 ± 41.30	536.6 ± 53.40
	Nichols Institute	71.1 ± 10.58	173.8 ± 20.84	402.9 ± 34.01
	Radioassay Systems	43.9 ± 17.46	147.9 ± 20.82	349.8 ± 29.34
Cortisol (μg/dl)				
	Amersham/Searle	6.4 ± 0.60	24.3 ± 3.38	> 41
	Clinical Assays ^{3}H	4.6 ± 1.88	21.4 ± 5.16	57.8 ± 14.12
	Clinical Assays ^{125}I	5.3 ± 1.48	21.6 ± 6.96	51.7 ± 16.76
	Diagnostic Products	5.6 ± 0.62	23.4 ± 2.74	52.1 ± 4.80
	NEN	5.0 ± 0.82	21.5 ± 4.38	51.3 ± 7.32
	Micromedic Autopak	5.2 ± 0.84	18.0 ± 1.34	34.9 ± 3.22
	Micromedic	5.4 ± 1.02	20.6 ± 2.28	40.7 ± 9.04
	Radioass. Syst.	4.8 ± 1.32	17.8 ± 3.00	51.6 ± 6.89
	Schwarz-Mann	4.7 ± 1.46	17.3 ± 5.48	39.5 ± 11.00
T_4 (μg/dl)				
	Dade CPB	4.2 ± 1.08	9.3 ± 1.54	16.1 ± 1.68
	Abbott CPB	2.9 ± 1.31	10.2 ± 1.35	20.7 ± 3.70
	Abbott RIA	3.4 ± 0.60	8.3 ± 0.74	15.5 ± 2.32
	Abbott RIA-PEG	3.6 ± 0.54	8.3 ± 1.02	15.3 ± 2.32
	Ames CPB	3.6 ± 0.84	8.5 ± 1.22	16.5 ± 1.22
	Amersham/Searle CPB	4.1 ± 0.34	11.3 ± 1.04	> 19.2
	Amersham/Searle RIA	3.8 ± 0.82	9.3 ± 0.96	17.7 ± 1.56

TABLE II (cont.)

Test (units)	Kit	Level 1 mean ± 2 SD	Level 2 mean ± 2 SD	Level 3 mean ± 2 SD
	Curtis CPB	4.0 ± 1.12	8.4 ± 1.46	14.7 ± 1.48
	Curtis RIA	4.4 ± 0.64	8.7 ± 1.08	14.9 ± 2.24
	Curtis RIALIQUA	5.5 ± 0.92	10.4 ± 0.90	16.1 ± 1.38
	Oxford STAT 4	2.9 ± 0.96	5.4 ± 0.62	10.0 ± 0.84
	Oxford Column	2.2 ± 0.36	5.2 ± 0.92	9.0 ± 2.30

are relatively constant, especially at higher concentrations. Other small molecules such as T_3, T_4 and cortisol, which occur naturally in serum together with metabolites of similar structure and must first be separated from a binding protein before assay, give a different range of values, as shown in Table II. Here, the values from different kits are not so constant; in some cases the highest value is more than twice that of the lowest one for the same component in the same serum. Another point that is brought out here is that the results and precision of different kits produced by the same manufacturer for the same component may differ markedly.

Larger proteohormones show even more anomalous results, as can be seen in Table I. These anomalies are due to the assay systems used as well as to the reference preparation against which the standards are calibrated.

Many kits are delivered with methodological weaknesses which give rise to far from optimal performance. Often minor changes in the protocol give rise to major improvements in performance. Table III shows the results of such modifications in three antibody-coated tube assays for digoxin. The danger of such assays is the poor precision at low concentrations because of non-specific binding sites. Non-specific binding can be minimized by pre-incubation before labelled tracer is added. In fact, the procedure for the Clinical Assays kit includes such a pre-incubation step and good precision is achieved over the entire range of the standard curve. When this step is omitted, precision and sensitivity are poor. The Schwarz-Mann AbTRAC kit provides not only antibody-coated tubes but also lyophilised tracer already in the tubes. Here, the protocol states that first serum and then buffer should be added to each tube in turn. If, however, buffer is added to all the tubes followed by serum, then precision is achieved at lower concentrations. The Boehringer-Mannheim kit combines the tracer and buffer, thus obviating the need for pre-incubation. If, however, the tracer and buffer from the Clinical Assays kit are used with the Boehringer tubes and standards, and a pre-incubation step is included, the precision improves markedly

TABLE III. RESULTS FROM ORIGINAL AND MODIFIED METHODS FOR THREE DIGOXIN ANTIBODY-COATED TUBE KITS

Kit	Sensitivity*		50%**	Precision			CV
	$\%B_0$	ng/ml	intercept	No.	Mean	SD	
1. Clinical Assays			ng/ml				
(a)	89.8	0.24	1.09	30	1.40	0.06	4.93
				30	3.62	0.29	7.97
(b)	85.6	0.41	1.76	19	1.30	0.21	16.0
				19	3.90	0.16	4.00
2. Schwarz-Mann AbTRAC							
(a)	98.0	0.05	2.23	20	0.81	0.09	10.6
				20	3.44	0.11	3.17
(c)	95.2	0.06	1.28	22	0.78	0.06	7.27
				22	3.83	0.28	7.28
3. Boehringer-Mannheim							
(a)	99.2	0.02	2.06	20	0.84	0.23	28.5
				20	4.05	0.20	4.96
(d)	96.6	0.14	1.69	20	0.93	0.06	6.92
				20	4.24	0.17	4.15
(e)	90.4	0.40	2.64	14	0.72	0.12	16.5
				14	3.93	0.14	3.63

* Sensitivity is defined here and in Tables IV, V and VI as the value on the standard curve, 3 standard deviations from the zero standard.

** All data here and in Tables IV, V and VI were obtained from standard curves plotted using a spline function.

(a) *Assay performed according to protocol.*
(b) Without pre-incubation.
(c) Buffer added before serum.
(d) Boehringer Ab-coated tubes and standards, Clinical Assays buffer and label, with pre-incubation.
(e) As for (d) but without pre-incubation.

A further Boehringer-Mannheim digoxin kit of a different batch has been tested and has been found to give the following results under the conditions in (a). Sensitivity $\%B_0$ = 97.0 or 0.16 ng/ml, 50% intercept = 1.80 ng/ml, CV (1) n = 19, mean = 0.91 ng/ml, SD = 0.05 ng/ml, CV = 5.56%; (2) n = 14, mean = 2.89 ng/ml, SD = 0.23 ng/ml, CV = 8.03%.

TABLE IV. DIGOXIN STANDARDS FROM FIVE KITS MEASURED IN THE CLINICAL ASSAYS KIT

Standard (ng/ml)	Kits				
	Abbott	Schwarz-Mann	Squibb	Boehringer-Mannheim	Corning
0	0.05	0.08	0	0.05	0.10
0.5	0.70	0.58	0.44	0.50	0.45
1.0	1.14	1.14	0.85	1.00	0.94
1.5	–	1.60	–	–	–
2.0	2.14	2.04	1.74	2.04	–
2.5	–	–	–	–	2.35
3.0	–	2.79	2.89	3.14	–
4.0	4.80	–	–	–	–
5.0	–	5.14	4.70	5.29	5.00

at low concentrations. Omission of the pre-incubation step immediately lowers the precision. Pre-incubation has little or no effect at higher digoxin levels. This is to be expected because of the excess of unlabelled digoxin present. Pre-incubation increases the slope of the standard curve, as seen in the reduced values of the 50% intercept. The results in Table III are taken from the assay in which intra-assay precision was measured.

The reagents of the kits are often of very high quality, as shown in Table IV: the standards from five different digoxin kits were measured by the Clinical Assays kit (standard curve range 0–8 ng/ml). The question of quality is further highlighted by the results from the Boehringer kit in which the coated tubes were tested with the Clinical Assays reagents. The problems arising from proteohormone assays, which usually require a longer pre-incubation period before labelled tracer is added to increase sensitivity, concern methodology and kit components to a greater extent than in the digoxin kits. The use of standards in hormone-free serum instead of buffer for derivation of a standard curve has been described elsewhere [1, 2]. Such a curve is more realistic and gives better results, especially at lower hormone concentrations.

Four TSH kits were tested and modified to check their performance; the results are given in Tables V and VI. Table V gives the incubation details, the assay sensitivity and the 50% intercept together with the medium in which the standards were dissolved. The performance of the Kabi kit could have been improved greatly by pre-incubation before addition of the label, as shown by the sensitivity and 50% intercept values. The Schwarz-Mann kit was improved by

TABLE V. EFFECTS OF INCUBATION PROCEDURES ON SENSITIVITY AND 50% INTERCEPT IN FOUR TSH KITS

Kit	Pre-inc. (h)	Temp. (°C)	Main inc. (h)	Temp. (°C)	2nd Ab (h)	Temp. (°C)	Sensitivity (%B_0)	Sensitivity (μU/ml)	50%-intercept (μU/ml)	Kit standards
Kabi*										
(a_1)	–	–	96	4	24	4	92.9	2.89	20.9	Buffer
(b_1)	72	4	24	4	24	4	92.8	0.66	5.18	(A)**
Schwarz-Mann										
(a_1)	2	37	3	37	1.5	37	95.1	0.77	9.35	Buffer
(b_1)	18	RT	3	RT	1.0	RT	92.0	0.59	4.61	(A)
Diagnostic Products										
(a_1)	3	37	2	37	0.25	37	92.4	2.58	26.1	Protein based
(b_1)	3	37	2	RT	0.75	RT	92.8	2.85	25.9	
(b_2)	18	RT	2	RT	0.75	RT	95.9	0.96	13.7	(B)
Henning										
(a_1)	2	37	2.5	37	0.17	37	86.2	3.06	14.6	In TSH-free serum (A)
(a_2)	18	RT	2	RT	0.50	RT	92.9	0.81	6.92	

* (a_x) = Original method given with kit
(b_y) = Modified methods
** (A) = Calibrated against MRC 68/38 Standard
(B) = Calibrated against WHO 1st IRP 1974

RT = Room temperature
Pre-incubation = Incubation without labelled hormone
Main incubation = After addition of labelled hormone
2nd Ab = Incubation after addition of precipitating antiserum

TABLE VI. PERFORMANCE OF TSH KITS WITH COMMERCIAL CONTROL SERA, PATIENT SERA AND MRC 68/38 IN TSH-FREE SERUM
(Assay notation is as in Table V)

Quality control sera	Kit								
	Kabi assay		Schwarz-Mann assay		Diagnostic Products assay			Henning assay	
	a_1	b_1	a_1	b_1	a_1	b_1	b_2	a_1	a_2
RIACON 1 (7.48*)	7.48	6.27	8.39	10.27	4.59	4.46	4.79	5.72	6.51
2 (41.0)	32.0	30.1	42.4	45.3	23.5	23.6	25.7	26.6	20.5
Patient sera									
801 – 0 min TRH test	2.89	2.09	3.36	3.34	4.13	4.24	3.97	3.06	2.00
801 – 30 min	13.9	14.4	23.2	27.7	17.3	15.3	16.0	12.8	12.7
815 – 0 min TRH test	< 2.89	0.65	0.77	1.09	< 2.58	< 2.85	< 0.96	< 3.06	< 0.81
815 – 30 min	< 2.89	0.64	0.93	0.59	< 2.58	< 2.85	< 0.96	< 3.06	< 0.81
TSH Standard MRC 68/38									
0.39 μU/ml	< 2.89	0.73	1.66	1.63	3.56	3.45	2.49	< 3.06	0.84
0.78	< 2.89	1.01	1.81	2.07	4.65	3.19	3.26	< 3.06	0.98
1.56	2.83	1.65	3.11	2.94	5.81	3.89	3.76	3.52	1.74
3.12	4.57	3.05	4.17	4.94	6.99	5.70	5.19	3.66	3.04
6.25	6.91	6.43	8.85	10.1	9.05	9.19	7.97	5.44	6.08
12.5	11.72	13.3	18.2	19.2	14.5	15.5	13.2	11.0	12.6

TABLE VI (cont.)

Quality control sera	Kit								
	Kabi assay		Schwarz-Mann assay		Diagnostic Products assay			Henning assay	
	a_1	b_1	a_1	b_1	a_1	b_1	b_2	a_1	a_2
25.0	23.83	28.7	29.7	29.2	26.4	29.1	24.5	24.0	22.2
50.0	61.29	51.2	>50	>50	65.9	61.0	55.8	47.2	48.0
Normal range given by kit manufacturer mean ± 2 SD	1.5–8.0		0.4–9.2		3.1–9.6			0.5–4.0	

* Mean of 4 kits listed by manufacturer (Dr. Molter, Heidelberg, FRG).
All values in μU/ml.

overnight incubation at room termperature instead of for a shorter time at 37°C, as were the Diagnostic Products and Henning kits.

The values obtained from the patient sera, control sera and MRC Standard 68/38 dissolved in TSH-free serum differ only at lower concentrations when the sensitivity for each kit is improved. The introduction of "Quick-Tests" for TSH that require a total incubation time of only 5 or 6 hours are a commercial innovation with no clinical advantages. Although the incubation time is short and the protocol states that the results are ready the same day, the realistic assay time is somewhat different. In addition to the assay time, the patients must be attended to, the TRH tests performed and the assay tubes and kit components prepared. In addition, the tubes must be counted, the assay data analysed and the results written up. This is only possible when all the patients present themselves very early in the morning and there are enough staff on hand to perform all the required tests! With an overnight pre-incubation a hectic morning is avoided and the results are available within a few hours, with better sensitivity and precision.

The different absolute values obtained from different TSH kits often make it difficult for laboratories to compare results, and these comparisons are necessary when a patient transfers from one clinical practice to another. In such cases, false diagnoses can arise when the results are not compared with the normal range given for each method.

A final observation is that with many commercial quality-control sera imported from the United States of America, values for the components listed are ascribed to American kits but none are ascribed to European kits. This means, at best, that such sera, which take up the largest part of the market for RIA in the Federal Republic of Germany, can only be used in precision control.

The clear need for standardization of reagents and methods in kits has been stressed because of the importance of achieving inter-laboratory comparisons similar to those already achieved by the Deutsche Gesellschaft für Klinische Chemie (German Society for Clinical Chemistry) for other routine clinical chemical parameters.

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DISCUSSION

D. FULD: Don't you think that making recoveries on each sample would be better than extracting the standards, as you suggest?

W.G. WOOD: This would be better in the case of big laboratories.

D. RODBARD: I was interested in the patient sample resulting in 155 $\%B/B_0$. This increase in binding could be due to the presence of endogenous antibodies to TSH (thyrotropin). At the 1967 meeting of the American Thyroid Association and subsequently in the Journal of Clinical Endocrinology and Metabolism, M. Hays et al.[1] reported that injection of bovine TSH into human subjects could result in antibody formation with some biological neutralizing properties. Therefore, I would like to ask whether any of your subjects had previously received bovine TSH.

I would also point out that even in the case of a double-antibody radioimmunoassay, the presence of endogenous antibodies to an antigen may result in either parallel displacement and a false positive or a false negative (and elevated $\%B/B_0$), depending on the specificity of the second antiserum used (cf. PEETERS et al. Endocrinology (1977)[2]).

W.G. WOOD: This point is at present under investigation in our laboratory as well as in laboratories in Sweden and the USA. Our view is the same as yours, and until we hear anything to the contrary, we think that the "rogue factor" is either an antibody to bovine or perhaps human TSH.

As the sera were pooled from more than 100 patients according to TSH content, and not according to names, we have unfortunately no data as to whether any patient had undergone prior treatment with bovine TSH.

Your comment on the possibility of false values with the double-antibody assays in this case is very important and should be borne in mind when such cases arise. As we have not yet fully analysed the results of the TSH quality-control survey in which these two sera were assayed, we have not yet been able to assess whether the different methods or kits using double-antibody methods give different results, which would support your theory!

K. PAINTER: One can see that your results on coated-tube digoxin assays would lead you to believe that the pre-incubation step is necessary in coated-tube assays. However, you are seeing an effect, rather than a cause, of the problem. There are coated-tube digoxin assays on the market which do not behave in this way. The problem lies in the chemical structure of the iodinated digoxin derivative and in components added to the tracer buffer. The relevant studies of structure-function relationship will be published shortly. They contradict your conclusion that the reagents are good but the methodology is not.

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W.G. WOOD: I can only reiterate that our experience with coated-tube assays, not only for digoxin, but for digitoxin, T_4, cortisol and phenytoin, shows the same effect of pre-incubation on precision at low dose levels. Therefore I must stick to the results we have obtained and the logical explanation which we have derived from them. I should add that the coated tubes tested came from several different producers.