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EXTERNAL QUALITY-CONTROL SURVEYS OF PEPTIDE HORMONE RADIOIMMUNOASSAYS IN THE FEDERAL REPUBLIC OF GERMANY

*The present status**

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Abstract

EXTERNAL QUALITY-CONTROL SURVEYS OF PEPTIDE HORMONE RADIO-
IMMUNOASSAYS IN THE FEDERAL REPUBLIC OF GERMANY: THE PRESENT STATUS.

Two types of quality-control survey (QCS) of hormone assays are performed in the Federal Republic of Germany. In the one survey, the participating laboratories are requested to determine seven or eight different hormones in two lyophilized sera that are distributed several times a year. Because of the lack of reference methods for peptide hormones, the statistical evaluation of the results indicates only whether they are "correct" or subject to systematic or nonsystematic errors with respect to the findings of the other participants. In the other survey, the participating laboratories are requested to assay only one given hormone in some 20 deep-frozen sera (including standards in hormone-free sera for derivation of a standard curve) that are distributed at relatively long intervals. The statistical analysis of the data derived from these QCSs allows – together with the methodological inquiry form – detection of probable causes for discrepancies in the results.

During recent years a system has been introduced in the Federal Republic of Germany (FRG) for internal and external quality control of quantitative clinical chemical analyses. This quality control is conducted according to the guidelines of the Bundesärztekammer (Medical Association of the FRG) [1]. The guidelines are based on the Calibration Act of 1969, which requires that if the instruments used for the determination of volume are not officially calibrated, the accuracy of analytical results has to be demonstrated by means of continuous monitoring with the methods of statistical quality control.

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TABLE I. COEFFICIENTS OF VARIATION OF THE RESULTS OF THE THIRD AND FOURTH QUALITY-CONTROL SURVEY FOR HORMONE DETERMINATIONS (BONN)

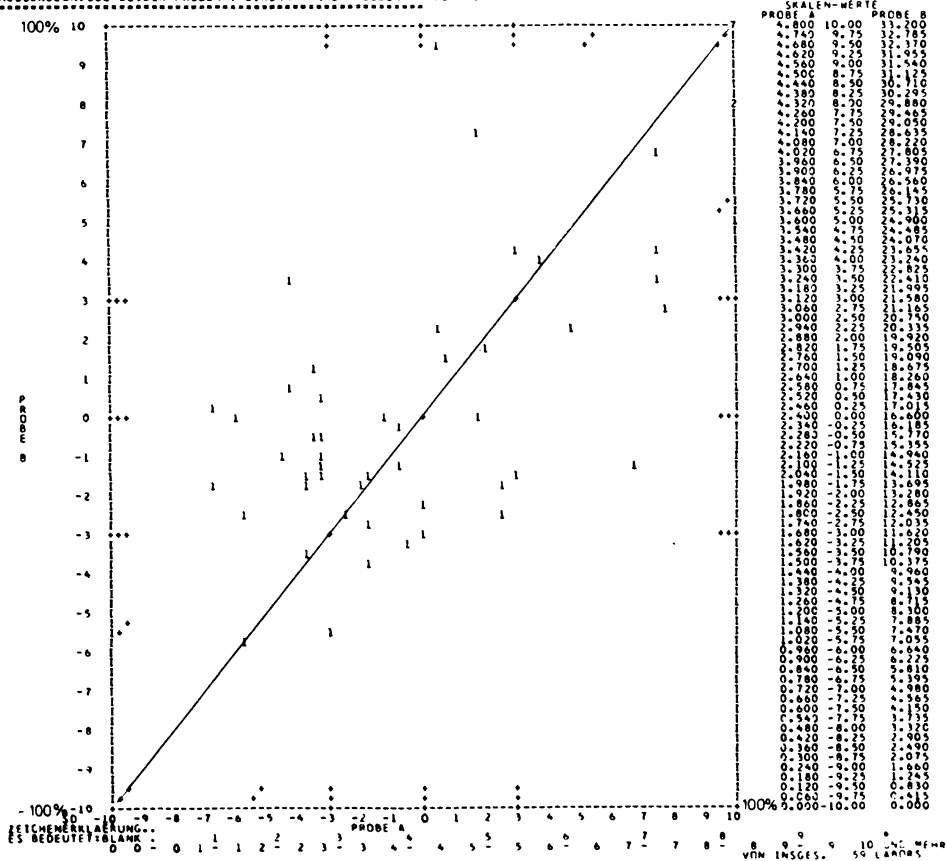
Survey	Compound	T ₃	T ₄	TSH	Prolactin	LH	FSH	hGH	Insulin
3	Number of results	66	71	50	32	44	41	29	28
	CV(%) (Sample A)	23	18	53	28	—	38	39	42
	CV(%) (Sample B)	27	18	54	31	53	33	58	51
4	Number of results	63	68	47	31	45	40	29	33
	CV(%) (Sample A)	25	24	43	42	38	34	44	49
	CV(%) (Sample B)	25	23	32	38	28	32	37	28

Results lying beyond the double value of the median were omitted.

EXTERNE QUALITAETS-KONTROLLE (DTSCH.GES.KLIN.CHEMIE I

4. HORMON-RINGVERSUCH II VOM JUNI 77

MESSERGEBNISSE BEIDER PROBEN (DIAGRAMM NACH YOUNEN) FUER TSH



PARAMETER	PROBE A MESSWERT 47	ABW. IN SD.	PROBE B MESSWERT 47	ABW. IN SD.	ANZAHL DER ERGEBN.
	2.247		16.565		MITTELMERT
	0.955		5.253		STANDARDABWEICHUNG
	42.521		31.732		VARIATIONS-KOEFF.

17 LABORS LAGEN MIT IHREN WERTEN AUSSERHALB DES BEREICHS

FIG.1. Youden plot of a QCS of TSH assays with two sera. The x-axis shows the results of sample A, the y-axis those of sample B. The expected value lies in the middle of the 45° line. Deviations along the line show systematic errors, deviations away from the line show random errors.

TABLE II. 50%, 16% AND 84% PERCENTILES (mU/litre) OF TSH DETERMINATIONS DIVIDED ACCORDING TO COMMERCIAL KITS USED BY THE PARTICIPANTS OF THE FOURTH SURVEY (BONN)

Kit (No.)	1	4	5	6	7	8	9	10	
Number of results	9	6	7	2	10	5	3	2	
SAMPLE A	50% percentile (median)	2.9	7.9	2.8	3.2	2.2	2.0	1.9	2.3
	16% percentile	1.0	1.8	1.4	—	1.4	—	—	—
	84% percentile	11.1	27.2	3.4	—	4.2	—	—	—
SAMPLE B	50% percentile (median)	16.6	27.5	22.4	13.8	16.9	12.0	13.5	13.3
	16% percentile	10.9	11.8	16.7	—	13.9	—	—	—
	84% percentile	49.6	55.8	27.2	—	29.1	—	—	—

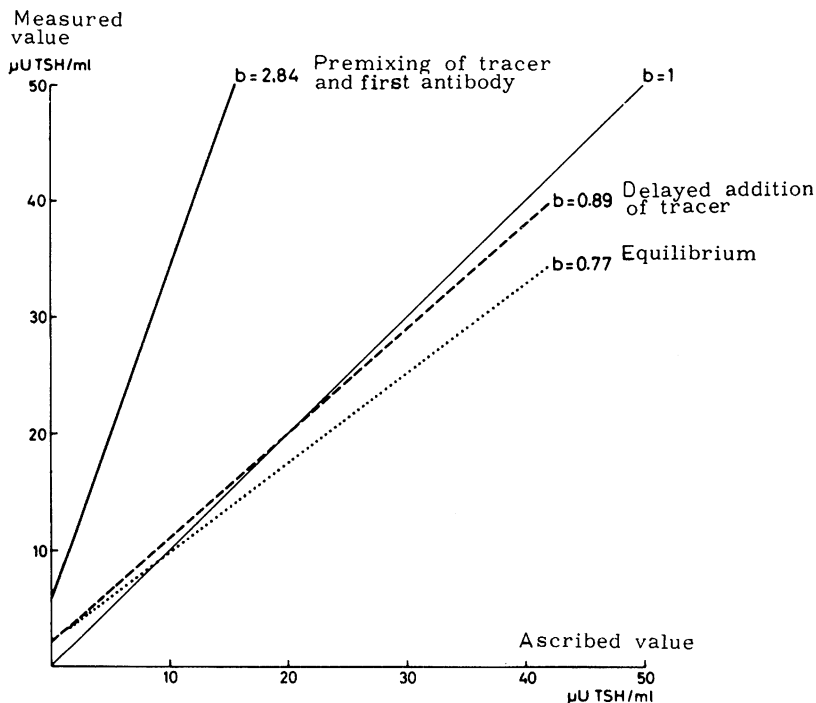


FIG. 2. QCS of TSH assays. Mean regression of all assays (separated according to incubation mode) between ascribed values and measured values in the dose range between 1.8 and 26 μU TSH/ml.

The system of external quality control for routine clinical chemical analyses is now well established [2]. In each quality-control survey (QCS) at least two specimens, differing in concentrations of the various constituents, are to be analysed by the participating laboratories. The results are evaluated on the basis of assigned values and the standard deviations, as calculated from the results of reference laboratories. A single result meets the requirements provided it lies between the limits of the assigned value plus or minus three times the interlaboratory standard deviation of the reference laboratories. The participant receives a certificate to this effect which is valid for 12 months.

In the Federal Republic of Germany there are two institutions officially authorized and acknowledged by the Bundesärztekammer that carry out external quality surveys in the field of clinical chemistry, namely the Institut für Klinische Biochemie der Universität Bonn (supported by the German Society for Clinical Chemistry) and the Institut für Standardisierung und Dokumentation, Düsseldorf.

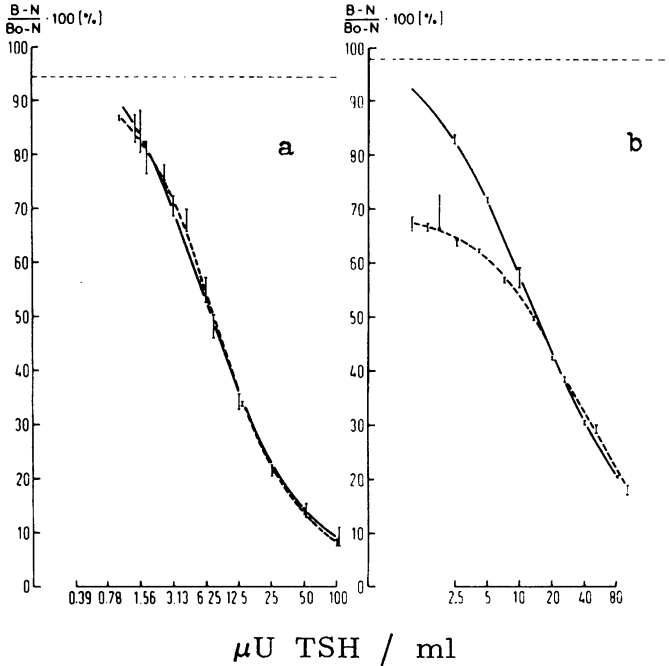


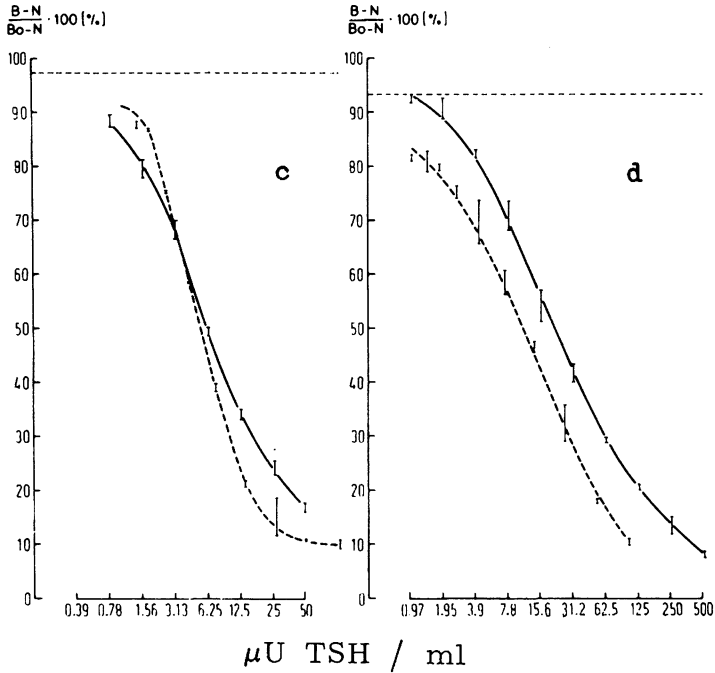
FIG. 3. Graphs from a QCS of TSH assays showing four typical relationships between the standard curves of the participating laboratories (—) and the recovery curves (---). The abscissa is logarithmic and shows the TSH concentrations ($\mu\text{U/ml}$) of the participants' standard curves.

(a) Laboratory-developed assay (non-kit) (cold preincubation, double-antibody separation). Both curves show perfect agreement.

(b) Kit assay (equilibrium, cellulose-bound second antibody separation). Good agreement only in the high dose range. High blanks.

As far as hormone assays are concerned, the legal regulations can only be partly met because of a number of technical difficulties. Thus, there are numerous techniques for the measurement of hormones in biological fluids. Although some of these methods may give satisfactory levels of precision, many of them yield unsatisfactory results, particularly with respect to accuracy and specificity.

For steroid hormone assays, however, it may be possible in the not too distant future to find a way to carry out QCSs according to the legal guidelines. The true values of the concentrations can, on the one hand, be obtained by adding defined quantities of steroids to plasma samples from which endogenous steroids have been removed; on the other hand, these low molecular hormones can be determined by a definitive method (isotope dilution-mass fragmentography). Four pilot QCSs performed on this basis by the Bonn study group have proven the practicability of this system.



(c) Kit assay (equilibrium, double antibody). Different slope of standard and recovery curve (standards in buffer instead of in hormone-free serum).

(d) Kit assay (mixing of tracer and first antibody before pipetting to save one pipetting step; second antibody separation). False high values over the whole range.

It seems to be much more difficult to create an equivalent basis for the evaluation of results of QCSs for peptide hormones. At present, no possibility exists to determine the true concentrations of peptide hormones; as long as no agreement has been reached on standardized analytical methods, values obtained by reference laboratories cannot reasonably be used for the evaluation of the results.

The efforts of the two institutions at Bonn and at Munich are directed to establish the conditions for an optimization and standardization of the determinations of peptide hormones. Up to now, the Bonn group has included six peptide hormones in their QCSs which are offered about three times a year; the form of organization of these QCSs follows the legal rules set up for clinical chemical determinations. The results of each of these surveys yield information [1] on the extent to which the analytical values of the various laboratories are comparable to each other, and [2] whether there is a relation between the

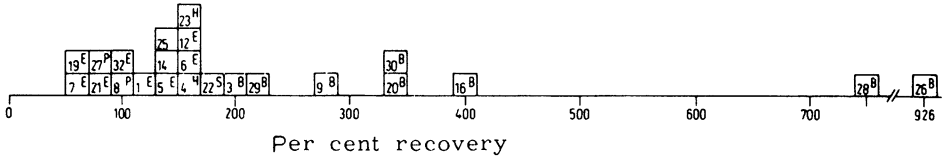


FIG. 4. QCS of TSH assays. Mean recovery of all participants in the dose range between 1.8 and 26 μ U TSH/ml. Each box contains the participant's number and a symbol indicating the method or kit used.

differing results and the reagents used. The QCSs performed by the Munich group are concerned with only one compound which is determined by the participating laboratories in a large number of samples. In this way, detailed information may be obtained about the sources of errors influencing the results.

The findings of the QCSs are demonstrated by some examples. In two surveys carried out by the Bonn group in 1977 in which more than 100 laboratories participated, the following peptide hormones were determined: TSH, prolactin, LH, FSH, hGH and insulin; in addition, tri-iodothyronine (T_3) and thyroxine (T_4) were analysed. Table I shows the interlaboratory imprecision – given as coefficients of variation – of the participants' results for each compound.

Whereas T_3 and T_4 were determined with relatively good precision, the coefficients of variation for the peptide hormones were rather high. In some cases, an improvement from the third to the fourth survey was noticed. With LH, the increase in precision was probably due to the fact that the samples of the fourth survey were supplied together with the same standard material of this hormone.

The results for each compound in each survey were analysed as a Youden plot, all pairs of results within the range of zero and the double value of the median being included. Figure 1 demonstrates this for TSH from the fourth survey. From Table II it can be speculated that the scatter of the results may depend, at least to some extent, on the origin of the kits. Laboratories that used kit No. 4 measured significantly higher values than most of the other participants. An interpretation of this phenomenon will only be possible when more information becomes available.

A second and more complex form of QCS has been carried out by the Endocrinological Study Group of the University Clinic in Munich. Here, approximately 20 serum samples are sent express in dry-ice to each participant. In these sera, a concealed standard curve in hormone-free serum, including a zero value, serves as a control to check the method and standards in use in the participants' laboratory. The remaining tubes contain interfering substances, serum from function tests, e.g. OGTT in an insulin quality control survey,

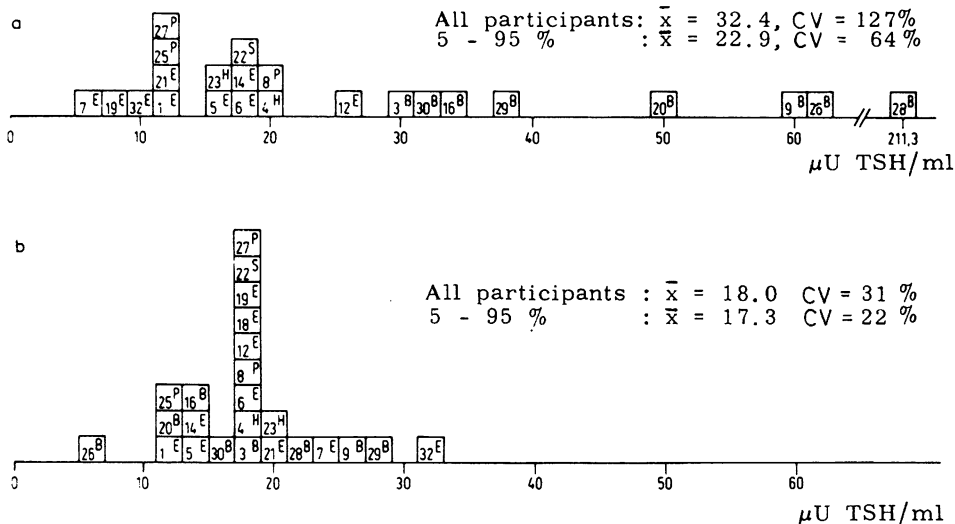


FIG. 5. QCS of TSH assays.

(a) Histogram of the results for one pooled serum (17.5 μU TSH/ml) taken from each laboratory standard curve (\bar{x} = mean value, CV = coefficient of variation).

(b) Histogram of the results for the same pooled serum taken from the recovery curves.

TRH test in TSH, an intra-assay precision control where three tubes contain the same serum (in the normal range), and sera below, within and above the expected normal range. The 20 sera are randomly numbered to keep anonymity. All sera used are human sera from volunteer blood donors. Hormone-free serum is obtained either from donors who have undergone suppression therapy (e.g. T_4 dosage to suppress TSH secretion) or from donors in whom the hormone is not present, e.g. hGH-free serum from hypophysectomised patients. All participants are asked to assay each serum at least in duplicate, and all count-rates as well as the standard curve values and test serum values obtained. A comparison of values obtained using the participants' standard curves and the hidden standard curves (recovery curves) allows a thorough evaluation of the methodology and the pin-pointing of the probable sources of error (Fig. 2). From the recovery curve, the concentrations of the participants' standard curves can be checked, and dilution errors of differences in immunoreactivity of standards detected (Fig. 3). The interfering substances show the specificity of the participants' antisera.

The results from completed QCSs of this type (three surveys for insulin, two for TSH and one each for T_3 , T_4 , hGH and cortisol) [3-5] show that it allows the causes of methodological errors to be stated with greater probability than does the aforementioned type using only two sera (Figs 4 and 5). The

results to date show that the quality of results is far less dependent on the quality of component reagents (standards, antiserum and tracer) used – whether in kits or otherwise obtained – than on the methodology, such as incubation time, temperature, extraction and separation procedures. The disadvantage of this type of QCS lies in the large number of samples sent to each laboratory and the relatively long period needed for the data-processing and feed-back of information, making it impossible to carry out frequently. A compromise might be a combination of both methods in which the control sera for the “2-sera” QCS would be determined first in a “20-sera” QCS, thus allowing a better-assigned value to be put on each sample.

REFERENCES

- [1] Dtsch. Ärztebl. **68** (1971) 2228.
- [2] RÖHLE, G., BREUER, H., OBERHOFFER, G., Dtsch. Ärztebl. **72** (1975) 883.
- [3] MARSCHNER, I., BOTTERMANN, P., ERHARDT, F., LINKE, R., LOEFFLER, G., MAIER, V., SCHWANDT, P., VOGT, W., SCRIBA, P.C., *Horm. Metab. Res.* **6** (1974) 293.
- [4] MARSCHNER, I., ERHARDT, F.W., SCRIBA, P.C., *J. Clin. Chem. Clin. Biochem.* **14** (1976) 345.
- [5] HORN, K., MARSCHNER, I., SCRIBA, P.C., *J. Clin. Chem. Clin. Biochem.* **14** (1976) 353.