RADIOIMMUNOASSAY AND RELATED PROCEDURES IN MEDICINE 1977

PROCEEDINGS OF AN INTERNATIONAL SYMPOSIUM ON RADIOIMMUNOASSAY AND RELATED PROCEDURES IN MEDICINE HELD BY THE INTERNATIONAL ATOMIC ENERGY AGENCY IN CO-OPERATION WITH THE WORLD HEALTH ORGANIZATION IN BERLIN (WEST), 31 OCTOBER – 4 NOVEMBER 1977

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INTERNATIONAL ATOMIC ENERGY AGENCY VIENNA, 1978
II. STAND\-ARDIZATION AND QUALITY CONTROL
   (Session V and Session VI, Part 1)

Round-table discussion on assay design, standardization and within-laboratory quality control, including the following papers:

Basic concepts in quality control
   \emph{R. P. Ekins}
Quality control for RIA: recommendations for a minimal program
   \emph{D. Rodbard}
Quality control and assay design
   \emph{R. P. Ekins}
The use of quality control within a laboratory
   \emph{S. L. Jeffcoate}

External quality-control surveys of peptide hormone radioimmunoassays in the Federal Republic of Germany: the present status
   \textit{(IAEA-SM-220/17) }\textit{..........................} \textbf{81}
Discussion \textit{.......................................................} \textbf{90}

Mise en place et premiers résultats d’un programme de contrôle de qualité national français en radioimmunologie \textit{(IAEA-SM-220/81) }\textit{.........} \textbf{91}
   \textit{Ch.-A. Bizollon, R. Cohen, D. Froget}
Discussion \textit{.......................................................} \textbf{102}

An elementary components of variance analysis for multi-centre quality control \textit{(IAEA-SM-220/59) }\textit{..........................} \textbf{105}
   \textit{P.J. Munson, D. Rodbard}
Discussion \textit{.......................................................} \textbf{124}

The need for standardization of methodology and components in commercial radioimmunoassay kits \textit{(IAEA-SM-220/19) }\textit{.....................} \textbf{127}
   \textit{W.G. Wood, I. Marschner, P.C. Scriba}
Discussion \textit{.......................................................} \textbf{137}

Performance of radioimmunoassays for digoxin as evaluated by a group experiment \textit{(IAEA-SM-220/7) }\textit{.....................} \textbf{141}
   \textit{A. Dwenger, R. Friedel, I. Trautschold}
Discussion \textit{.......................................................} \textbf{148}
III. APPLICATIONS

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

A novel radioassay for the determination of folate in serum and red cells and new observations on the stability of serum folate (IAEA-SM-220/32) ................................................. 171
E.P.J. Lynch, K.C. Tovey, H. Guilford
Discussion ................................................................. 176

Studies on folate binding and a radioassay for serum and whole-blood folate using goat milk as binding agent (IAEA-SM-220/45) .................. 177
R.D. Piyasena, D.A. Weerasekera, N. Hettiaratchi, T.W. Wikramanayake
Discussion ................................................................. 192

Estimation of folate binding capacity (unsaturated and total) in normal human serum and in β-thalassaemia (IAEA-SM-220/52) .................. 193
S. Moulopoulos, J. Mantzos, E. Gyftaki, M. Kesse-Elias,
V. Alevizou-Terzaki, E. Souli-Tsimili
Discussion ................................................................. 197

Assay of 25-OH vitamin D₃ (IAEA-SM-220/56) .................. 199
Ph. De Nayer, M. Thalasso, C. Beckers
Discussion ................................................................. 208

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

Invited review paper

Recent advances in steroid radioimmunoassay (IAEA-SM-220/205) ....... 213
S.L. Jeffcoate
Discussion ................................................................. 222

Radioimmunoassay of steroids in homogenates and subcellular fractions of testicular tissue (IAEA-SM-220/39) .................................. 225
S. Campo, G. Nicolau, E. Pellizari, M.A. Rivarola
Discussion ................................................................. 235
Sencillo método de dosificación de proteína transportadora de hormonas sexuales (PTHS) — sus valores en hombres, en mujeres y en el embarazo (IAEA-SM-220/100) ........................................ 237
C.A. Tafurt, R. de Estrada
Discussion ................................................................. 243

A model for evaluating steroids acting at the hypothalamus-pituitary axis using radioimmunoassay and related procedures
(IAEA-SM-220/41) .......................................................... 245
J. Spona, Ch. Bieglmayer, R. Schroeder, E. Pöckl
Discussion ................................................................. 256

Determination of estradiol, estrone and progesterone in serum and human endometrium in correlation with the content of steroid receptors and 17β-hydroxysteroid dehydrogenase activity during the menstrual cycle (IAEA-SM-220/85) ........................................ 257
M. Schmidt-Gollwitzer, J. Eiletz, J. Pachaly, K. Pollow
Discussion ................................................................. 271

Specific bile acid radioimmunoassays for separate determinations of unconjugated cholic acid, conjugated cholic acid and conjugated deoxycholic acid in serum and their clinical application
(IAEA-SM-220/4) .......................................................... 273
S. Matern, W. Gerok
Discussion ................................................................. 283

Radioimmunoassay of primary and secondary bile acids in serum with specific antisera and 125I-labelled ligands (IAEA-SM-220/87) ........ 285
O.A. Jänne, O.K. Mäentausta
Discussion ................................................................. 293

The radioimmunoassay of clomipramine (Anafranil-Geigy): a tricyclic antidepressant (IAEA-SM-220/37) ........................................ 295
G.F. Read, D. Riad-Fahmy
Discussion ................................................................. 298

The specific radioimmunoassay in pharmacokinetics: its potency, requirements and development for routine use as illustrated by an assay for Pirenzepin (IAEA-SM-220/63) ........................................ 299
G. Bozler
Discussion ................................................................. 308

The radioimmunoassay of biologically active compounds in parotid fluid and plasma (IAEA-SM-220/35) ........................................ 309
R.F. Walker, G.F. Read, D. Riad-Fahmy
Discussion ................................................................. 315
III.3. Assays for thyroid-related hormones (Session VIII, Part 1)

Invited review paper

Pathophysiological aspects of recent advances in current thyroid function testing (IAEA-SM-220/206) .................................................. 319
R.-D. Hesch
Discussion ........................................................................................................... 339

Thyroxine and thyrotrophin radioimmunoassays using dried blood samples on filter paper for screening of neonatal hypothyroidism (IAEA-SM-220/55) ................................................................. 341
C. Beckers, C. Cornette, B. François, A. Bouckaert, M. Lechat

Le dosage radioimmunologique de la thyrostimuline hypophysaire à partir d'un échantillon de sang capillaire recueilli sur papier filtre: intérêt dans le dépistage de l'hypothyroïdie néonatale (IAEA-SM-220/71) ........................................................................................................... 349
J. Ingrand, M.A. Dugue, A.M. Mamarbachi, P. Bourdoux, F. Delange
Discussion ........................................................................................................... 360

Control of treatment of differentiated thyroid carcinoma by measurement of thyroglobulin in serum (IAEA-SM-220/23) ............. 363
J. Hagemann, C. Schneider
Discussion ........................................................................................................... 368

New concepts for the assay of unbound thyroxine (FT₄) and thyroxine binding globulin (TBG) (IAEA-SM-220/92) ................................................. 369
G. Odstrchel, W. Hertl, F.B. Ward, K. Travis, R.E. Lindner, R.D. Mason
Discussion ........................................................................................................... 376

Development of a two-site radioimmunoassay for antithyroglobulin antibodies using ¹²⁵¹-thyroglobulin (IAEA-SM-220/54) ................... 379
J.P. Léonard, F. Taymans, C. Beckers
Discussion ........................................................................................................... 387

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

A radioimmunoassay of plasma corticotrophin (IAEA-SM-220/38) ........ 391
L. Hummer
Discussion ........................................................................................................... 402

Dosage radioimmunologique du fragment biologiquement actif de l'hormone parathyroïdienne humaine (IAEA-SM-220/74) .................. 405
C. Desplan, A. Jullienne, D. Raulais, P. Rivaille, J.P. Barlet, M.S. Moukhtar, G. Milhaud
Discussion ........................................................................................................... 416
Calcitonin radioimmunoassay: clinical application (IAEA-SM-220/103) .......................................................... 419
F. Raue, H. Minne, W. Streibl, R. Ziegler
Discussion ............................................................................................................................................. 426

Etude de la spécificité du dosage radioimmunologique du procollagène
de type I et de type III (IAEA-SM-220/25) .............................................................................. 427
G. Heynen, M. Broux, B. Nusgens, C.M. Lapière, J.A. Kanis,
S. Gaspar, P. Franchimont
Discussion ............................................................................................................................................. 434

Invited review paper

Tumour-associated antigens (IAEA-SM-220/207) ................................................................. 435
K.D. Bagshawe
Discussion ............................................................................................................................................. 466

A different approach to the radioimmunoassay of thyrotrophin-
releasing hormone (IAEA-SM-220/90) ..................................................................................... 469
T.J. Visser, W. Klootwijk, R. Docter, G. Hennemann
Discussion ............................................................................................................................................. 476

New immunogenic form for vasopressin: production of high-affinity
antiserum and development of an RIA for plasma arginine-vasopressin
(IAEA-SM-220/82) ............................................................................................................................ 479
G. Rougon-Rappuzi, B. Conte-Devolx, Y. Millet, M.A. Delaage
Discussion ............................................................................................................................................. 486

Radioimmunoassay of arginine-vasopressin and clinical application
(IAEA-SM-220/99) ........................................................................................................................... 489
H. Wagner, V. Maier, M. Häberle, H.E. Franz
Discussion ............................................................................................................................................. 493

Dosage radioimmunologique des enképhalines (IAEA-SM-220/67) .............. 495
P. Pradelles, C. Gros, C. Rougeot, O. Bepoldin, F. Dray,
C. Llorens-Cortes, H. Pollard, J.C. Schwartz, M.C. Fournie-Zaluski,
G. Cracel, B.P. Roques
Discussion ............................................................................................................................................. 503

Dosage radioimmunologique du facteur thymique sérique (FTS)
(IAEA-SM-220/68) ........................................................................................................................... 505
J.M. Pleau, D. Pasques, J.F. Bach, C. Gros, F. Dray
Discussion ............................................................................................................................................. 510

Chairmen of Sessions ......................................................................................................................... 511
Secretariat of the Symposium ............................................................................................................. 511
List of Participants ............................................................................................................................... 513
Author Index ......................................................................................................................................... 537
Corrigenda to Vol. I .............................................................................................................................. 541
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 RADIOIMMUNOASSAYS 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.

* Supported by the Bundesministerium für Forschung und Technologie.
TABLE I. COEFFICIENTS OF VARIATION OF THE RESULTS OF THE THIRD AND FOURTH QUALITY-CONTROL SURVEY FOR HORMONE DETERMINATIONS (BONN)

<table>
<thead>
<tr>
<th>Survey</th>
<th>Compound</th>
<th>T&lt;sub&gt;3&lt;/sub&gt;</th>
<th>T&lt;sub&gt;4&lt;/sub&gt;</th>
<th>TSH</th>
<th>Prolactin</th>
<th>LH</th>
<th>FSH</th>
<th>hGH</th>
<th>Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Number of results</td>
<td>66</td>
<td>71</td>
<td>50</td>
<td>32</td>
<td>44</td>
<td>41</td>
<td>29</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>CV(%) (Sample A)</td>
<td>23</td>
<td>18</td>
<td>53</td>
<td>28</td>
<td>–</td>
<td>38</td>
<td>39</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>CV(%) (Sample B)</td>
<td>27</td>
<td>18</td>
<td>54</td>
<td>31</td>
<td>53</td>
<td>33</td>
<td>58</td>
<td>51</td>
</tr>
<tr>
<td>4</td>
<td>Number of results</td>
<td>63</td>
<td>68</td>
<td>47</td>
<td>31</td>
<td>45</td>
<td>40</td>
<td>29</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>CV(%) (Sample A)</td>
<td>25</td>
<td>24</td>
<td>43</td>
<td>42</td>
<td>38</td>
<td>34</td>
<td>44</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>CV(%) (Sample B)</td>
<td>25</td>
<td>23</td>
<td>32</td>
<td>38</td>
<td>28</td>
<td>32</td>
<td>37</td>
<td>28</td>
</tr>
</tbody>
</table>

Results lying beyond the double value of the median were omitted.
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>
<thead>
<tr>
<th>Kit (No.)</th>
<th>1</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of results</td>
<td>9</td>
<td>6</td>
<td>7</td>
<td>2</td>
<td>10</td>
<td>5</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>50% percentile (median)</td>
<td>2.9</td>
<td>7.9</td>
<td>2.8</td>
<td>3.2</td>
<td>2.2</td>
<td>2.0</td>
<td>1.9</td>
<td>2.3</td>
</tr>
<tr>
<td>16% percentile</td>
<td>1.0</td>
<td>1.8</td>
<td>1.4</td>
<td>-</td>
<td>1.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>84% percentile</td>
<td>11.1</td>
<td>27.2</td>
<td>3.4</td>
<td>-</td>
<td>4.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SAMPLE B</td>
<td>50% percentile (median)</td>
<td>16.6</td>
<td>27.5</td>
<td>22.4</td>
<td>13.8</td>
<td>16.9</td>
<td>12.0</td>
<td>13.5</td>
</tr>
<tr>
<td>16% percentile</td>
<td>10.9</td>
<td>11.8</td>
<td>16.7</td>
<td>-</td>
<td>13.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>84% percentile</td>
<td>49.6</td>
<td>55.8</td>
<td>27.2</td>
<td>-</td>
<td>29.1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
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.
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.
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 optimalization 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...
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 (T₃) and thyroxine (T₄) were analysed. Table I shows the interlaboratory imprecision – given as coefficients of variation – of the participants’ results for each compound.

Whereas T₃ and T₄ 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,
All participants: \( \bar{x} = 32.4, CV = 127\% \)
5 - 95 % : \( \bar{x} = 22.9, CV = 64\% \)

\[ \begin{array}{cccccccccccc}
0 & 10 & 20 & 30 & 40 & 50 & 60 & 70 & 80 & 90 & 100 & 110 \\
\hline
0 & 10 & 20 & 30 & 40 & 50 & 60 & 70 & 80 & 90 & 100 & 110 \\
\end{array} \]

\( \mu U \) TSH/ml

FIG. 5. QCS of TSH assays.

(a) Histogram of the results for one pooled serum (17.5 \( \mu U \) 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