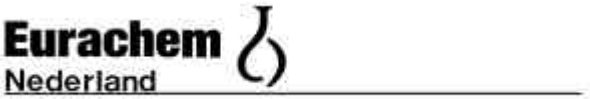
SYMPOSIUM

Use and Misuse of Reference Materials

Wednesday
22 November 2000
UFSIA-Antwerp



Reference Materials for analytical quality control: use and misuse

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Many institutions around the world are producing reference materials (RMs) and offer various kinds of certified reference materials (CRMs) ranging from technical over environmental to biological and clinical materials and their increased use has doubtless improved analytical data in important areas.

Though detailed information is provided by several CRM producers on proper use it is well known that on the one hand this information is often neglected and on the other practical application as well as evaluation and reporting of the obtained data frequently is less satisfactory (Jorhem, 1998).

The authors of this presentation have performed from January 1998 to September 2000 a detailed survey of 26 scientific journals dealing with analytical, environmental and biological studies (Stoeppler and Jenks, 2000). The initial aim of this survey was to investigate the present state of availability, use and non-use of CRMs to obtain a general overview. However, already in its earlier stages the deplorable state of reporting and misuse of RMs for many analytes and matrices was the main surprise that showed also the ignorance of journals and authors as far as correct reader information is concerned. Evaluation of the up to now gained information led us to the following conclusions:

- Despite some progress the number of certified reference materials for inorganic species and organic compounds is still not satisfactory and efforts to improve the situation should be encouraged.
- Journal editors and many authors seem unconcerned by the importance of correctly using RMs and reporting their use.
- This is underlined by the fact that only about 55% of the abstracts of the surveyed papers mention the use of RMs described in these papers. This has to be changed in the future as only abstracts of papers are easily accessible in electronic media.
- Many typical errors that can be seen in papers reporting the use of RMs are, e.g. incomplete or even wrong description of the used RM and mismatch with the material to be analyzed despite suitable RMs are available.
- Another field that must be urgently improved is the present and mainly unsatisfactory state of evaluation of the data obtained by use of CRMs in the actual literature (e.g. Jorhem et al. 2000).

In conclusion it should be strongly recommended that editors and reviewers of journals should have a closer and more critical look at the content of the submitted manuscripts dealing with the use of RMs. This is a challenge for publishers of scientific journals to provide clear recommendations how to include and at which place the description of the use of CRMs and to improve data evaluation. It this is realized it might significantly help to improve the situation.

We, the editors of a WILEY-VCH book on RMs (Stoeppler et al. 2000) further hope that our book, especially written for practitioners and thus discussing the above mentioned problems in some detail will contribute to the benefit of its readers some insight into the complex aspects of production, certification and particularly proper use of CRMs.

References:

L Jorhem (1998) Fresenius J Anal Chem 360:370-373.
PJ Jenks and M Stoeppler (2000) Fresenius J Anal Chem, submitted.
L Jorhem, J Engman and T Schröder (2000) Fresenius J Anal Chem, submitted

M Stoeppler, PJ Jenks and WR Wolf, Eds. (2000) Reference Materials for Chemical Analysis - Certification, Availability and Proper Usage. WILEY-VCH Weinheim New York, approx. 340 pages.

Reference Materials for analytical quality control: use and misuse

by

Markus Stoeppler and Peter J. Jenks

Two parts:

1) General and specific information on the RM field

2) Discussion of some results of a still ongoing literature survey on RM use in up to 26 scientific journals

- DETAILED LISTS OF ADDRESSES AND WEBSITES OF CRM PRODUCERS AND FOR THE COMAR DATABASE CAN BE FOUND IN BOTH THE ACTUAL CRM BOOKS FROM SPRINGER AND WILEY-VCH (see transparency above) and the available flyer for the WILEY-VCH book,

- The WILEY-VCH CRM book can be ordered now

CONTENTS OF THE WILEY-VCH RM BOOK

- Introduction (Historical, Theoretical basis, Technical requirements)
- From planning to production
- Certification (Certification philosophy, Methods and examples)
- Particular developments (QC/QA, Fresh materials, Micro methods, Calibrants, Radioisotopes etc.)
- RMs for "life" analysis (Microbiology, DNA, Pharmaceutical analysis)

INFORMATION ON (C)RM STATE AND PROGRESS

The Special BRM/BERM Issues (1984-2001):

- BRM-1 (1983) John Wiley Book, 1985
  (Philadelphia, USA)

The Fresenius' J Anal Chem Special Issues:

- BRM-2 (1986): Vol. 326 Number 7, 1987
  (Munich, Germany)
- BRM-3 (1988): Vol.332 Number 6, 1988
  (Bayreuth, Germany)
- BERM-4 (1990): Vol. Number 4, 1990
  (Orlando, USA)
- BERM-5 (1992): Vol. 343, Numbers 2-4, 1993
  (Aachen, Germany)
- BERM-6 (1994): Vol. 352, Numbers 1-2, 1995
  (Hawaii, USA)
- BERM-7 (1997): Vol. 360, Numbers 3-4, 1998
  (Antwerp, Belgium)
- BERM-8 (2000): to appear fall 2001
  (Bethesda, USA)

(C)RM TEXTBOOKS BASED ON SYMPOSIUM PAPERS

- REFERENCE MATERIALS for ENVIRONMENTAL ANALYSIS
  Eds. R. E. Clement, L. H. Keith and K.W.M. Siu, (CRC) Lewis Publishers, 1997 (278 p.)

  (From a symposium held at Quebec, 1993)

- THE USE OF MATRIX REFERENCE MATERIALS IN ENVIRONMENTAL ANALYTICAL PROCESSES
  Eds. A. Fajgelj and A. M. Parkany, RSC, 1999 (206 p.)

  (From a Working Party held at Berlin Spring 1999)

ACTUAL CRM BOOKS ESPECIALLY DESIGNED FOR CRM USERS

- Reference Materials in Analytical Chemistry - A Guide for Selection and Use (8 Chapters)
  Ed. A. Zschunke, Springer 2000, (238 pages)

- Reference Materials for Chemical Analysis - Certification, Availability and Proper Usage (9 Chapters)
  Eds. M. Stoeppler, W. R. Wolf and P. J. Jenks, WILEY-VCH 2000
  (324 pages with 27 figures and 45 tables)

BOTH BOOKS ARE JOINTLY DESIGNED IN ORDER TO COMPLEMENT EACH OTHER

PUBLICATIONS PROVIDING DETAILED INFORMATION HOW TO EVALUATE ANALYTICAL DATA BY USE OF CRMs

- J.K. Taylor, Handbook for SRM users, NBS Special Publication 260-100, 1985
- EURACHEM Guide, The Fitness for Purpose of Analytical Methods. LGC, 1998
- Pauwels J., How to use matrix certified reference materials. In: Fajgelj and Parkany pp 31-45, 1999
- ISO Guide 33, Uses of Certified Reference Materials, actual update, 2000
- Applications of Reference Materials in Analytical Chemistry, LGC/VAM/1999/036
- Walker R., Lumley I. Pitfalls in terminology and use of reference materials, trac 18:594-616, 1999
- LGC/VAM (C)RM Handbook, eds. R. Walker et al., anticipated to appear 2001

- General application fields (Workplace air, Clinical, Food/biological, Geology)
- Proper usage of RMs (Selection, use, abuse, Statistics)
- Availability and sources of information
- Future needs/organizational aspects

Table 1: From January 1998 to August 2000 surveyed scientific journals

Journals, full name	Abbreviations (if any)	Remarks
Accreditation and Quality Assurance	Accred Qual Assur	
Analusis	Analusis	
Analyst	Analyst	
Analytica Chimica Acta	Anal Chim Acta	
Analytical Chemistry	Anal Chem	
Analytical Communications	Anal Commun	From 99' combined with Analyst
Analytical Letters	Anal Letters	
Applied Organometallic Chemistry	Appl Organomet Chem	Only for 1998*
Applied Radiation and Isotopes	Appl Radiat Isot	
Atomic Spectroscopy	At Spectrosc	
Chemosphere	Chemosphere	
Environmental Science and Technology	Environ Sci Technol	
European Food Research and Technology (formerly Z Lebensm Unters Forsch)	European Food Res Technol	
Fresenius J Anal Chem	Fresenius J Anal Chem	
International Journal of Environmental Analytical Chemistry	Intern J Environ Anal Chem	
Journal of Analytical Atomic Spectrometry	JAAS	
Journal of AOAC International	J AOAC Int'l	
Journal of Chromatography A	J Chrom A	
Journal of Radioanalytical and Nuclear Chemistry	J Radioanal Nucl Chem	
Mikrochimica Acta (Wien)	Mikrochim Acta	
The Science of the Total Environment	Sci Total Environ	
Spectrochimica Acta B	Spectrochim Acta B	
Talanta	Talanta	
Toxicological and Environmental Chemistry	Toxicol Environ Chem	
trends in analytical chemistry	trac	
Water, Air, and Soil Pollution	Water Air Soil Poll	

Note: *Applied Radiation and Isotopes was from 1999 no more available in the visited library

Table 2: Number of papers in which CRMs are used (a) and in which their use is already mentioned in the abstract (b) i.e. a/b (with percentage of b); evaluated journals: see Table 1 for abbreviations

Journals	**1998**	**1999**	**2000 (until August)**
Accred Qual Assur	12/10 (83%)	14/11 (78.6%)	20/20 (100%)
Analusis	7/5 (71.4%)	4/2 (50%)	
Analyst	21/18 (85.7%)	24/18 (75%)	14/12 (85.7%)
Anal Chim Acta	31/20 (64.5%)	27/18 (66%)	28/16 (57%)
Anal Chem	10/6 (60%)	16/14 (87.5%)	4/4 (100%)
Anal Commun	3/1 (33%)		see under
Anal Letters	4/4 (100%)	3/2 (66%)	Analyst
Appl Organomet Chem	8/5 (62.5%)	5/1 (20%)	1/1 (100%)
Appl Radiat Isot	5/5 (100%)		2/0 (0%)
At Spectrosc	22/14 (63.6%)	16/12 (75%)	
Chemosphere	17/2 (11.8%)	25/7 (28%)	10/9 (90%)
Environ Sci Technol	9/2 (22%)	28/2 (7.2%)	12/1 (8.3%)
European Food Res	9/3 (33.5)*	1/0 (0%)	19/4 (21%)
Technol	103/88 (85.4%)	51/34 (66%)	
Fresenius J Anal Chem	4/2 (50%)	6/5 (83%)	46/33 (71.7%)
Intern J Environ Anal	73/51 (70%)	87/71 (82%)	5/2 (40%)
Chem		17/13 (76.5%)	42/32 (76.5%)
JAAS	17/6 (35.3%)	10/8 (80%)	5/4 (80%)
J AOAC Int		41/16 (39%)	10/6 (60%)
J Chrom A	5/3 (35.3%)	3/3 (100%)	66/32 (48.5%)
J Radioanal Nucl Chem	62/1 (1.6%)	86/12 (14%)	1/1 (100%)
Mikrochim Acta	24/16 (75%)	17/13 (76.5%)	53/7 (13.2%)
Sci Total Environ	20/13 (65%)	36/25 (69%)	22/13 (59%)
Spectrochim Acta B	2/2 (100%)	4/1 (25%)	18/16 (89%)
Talanta	4/3 (75%)	10/7 (70%)	6/2 (33%)
Toxicol Environ Chem	26/1 (3.8%)	19/2 (10.5%)	8/4 (50%)
trac			16/0 (0%)
Water Air Soil Poll			
Total	**498/281 (56.4%)**	**550/297 (54%)**	**408/219 (53.7%)**

Table 3: Publications in which no CRMs were used despite suitable materials were commercially available and also necessary for reliable results

Author(s)	Reference	Sample material for which no CRM was used
Soares et al.	[29]	Cr in powdered infant formulae
Niessen et al.	[30]	Total and methyl Hg in pore water
Pino et al.	[31]	PAHs in marine sediments
Antón et al.	[32]	Heavy metals in crayfish
Negoitjã and Ropota	[33]	Heavy metals in arctic soils
Oddone et al	[34]	Metals in obsidians
Ghomein et al.	[35]	Cd, Pb, Cu, Sb, Bi, Se, Zn, Mn, Ni, Co and Fe in water
Janardhana Raju et al.	[36]	Heavy metals in cigarettes
Sato et al.	[37]	Mussel watch/metals
Matek et al.	[38]	Se in various food portions (dietary daily Se intake)
Leœniewicz and ¯yrnicki	[39]	Elements in yew, pine and spruce needles
San Vicente de la Riva et al.*	[40]	Determination of trace levels of Hg in sea water
Paull et al.	[41]	Determination of Cd traces in environmental water samples

Note: * In this work not only no CRM was used, the described method is further not suitable for Hg determination in sea water because of its insufficient sensitivity for real levels

Table 4: Use of CRMs for QC in batches or within batches of samples

Author(s)	Reference	Analyte(s)/CRM(s) used
Mackey et al.	[42]	Metals/Bovine liver/Oyster tissue
Yaprak et al.	[43]	U and other elements/IAEA plant RMs
Chatterjee et al.	[44]	As/soil, Sediment and Sludge CRMs
Aksoy et al.	[45]	Metals/Peach leaves NIST SRM
Tian et al.	[46]	Lead isotope in wine, NIST isotopic SRM 981
Burger and Gochfeld	[47]	Metals in feathers of seabirds/Spiked sediments and NIST SRMs

Table 5: At least ten independent measurements of CRMs for analytical QC

Author(s)	Reference	Analyte(s)/Sample (CRM) material
Rodushkin et al.	[48]	Multi-elements/Human hair
Thakur and Deb	[49]	Cu/Alloy and rock samples
Zaichick et al	[50]	Trace elements/Animal bone
Garuti et al	[51]	Pt group and gold/Rocks, Ore tailings etc
López et al.	[52]	Al in spices and aromatic herbs/Citrus leaves

The following journals most probably belong also to the group of "worst" as they do only randomly give CRM use in the abstract of papers:

- Arch Environ Contam Toxicol
- Australian J Exp Agriculture
- Bull Environ Contam Toxicol
- Food Additives and Contaminants
- Food Chemistry
- J Agric Food Chem

Marine Poll Bulletin

The role of reference materials in analytical

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The primary purpose in performing analysis is to obtain information which can be used as a basis for making informed decisions.
It is therefore essential that the analytical data produced by laboratories is:

- fit for its intended purpose
- of the correct degree of quality for that purpose
- is capable of being benchmarked to a common reference point.

That common reference point could be, a reference method, a CRM or an SI unit.
Reference materials play a pivotal role in achieving the above objective and the presentation will explore and develop that role and will consider this in tandem with international activities (ISO REMCO) in the area of reference materials.
Areas covered will include the contribution made by reference materials to:

- method validation,
- calibration,
- quality assurance,
- comparability and traceability of data and
- metrology.

THE USE OF REFERENCE MATERIALS IN ANALYTICAL CHEMISTRY

- **Why, What, How**
- **Definitions**
- **Role of RM in analytical chemistry**
- **Brief overview of international activities and some useful references**

- **Support Government/EU Policy**
- **Protect public health**
- **Assist manufacturing industry**
- **Support world trade**
- **An aid to R & D**

- Safeguarding the quality of food and the purity of air
- developing new products and materials, such as pharmaceuticals or ceramics
- monitoring conformity assessment and product specification
- assisting a hospital physician with a medical diagnosis
- supporting the justice system in the fight against drugs and organised crime

- **providing forensic evidence for litigation including EU policies**
- **protecting the consumer against fraud and counterfeit products**
- **gathering revenue for Government (Customs and Excise)**
- **underpinning the free movement of goods within the Single Market and trade agreements with third countries such as the USA**

PRIMARY STANDARD

"Standard that is designated or widely acknowledged as having the highest metrological qualities and whose value is accepted with out reference to other standards of the same quantity, within a specified context"

SECONDARY STANDARD

"Standard whose value is assigned by comparison with a primary standard of the same quantity"

Guidelines for the In-House Production of Reference Materials, March 1997, LGC Report, UK. B. Brookman and R. Walker

Guidelines for the Preparation and Certification of Reference materials for Chemical Analysis in Occupational Health, NORDREFF, 1998, J.M. Christensen, (ISBN: 87-7904-010-1)

- **Measureand**
- **Measurement range**
- **Matrix match and potential interferences**
- **Sample size**
- **Measurement uncertainty**
- **Certification procedures**

- **for many different purposes**
- **in many different ways and**
- **in many different stages of the analytical process**

- **A certified calibrant for the calibration step**

- **A matrix reference material to control the analytical procedure**

Calibration

- primary calibrants
- calibration of equipment
- calibration of instrumentation
- calibration of transfer standards or secondary reference material
- calibration of rapid screening methods
- chemometrical calibration of instrumentation

Method development/validation/verification

- method development
- validation of test methods
- demonstration of equivalence or non equivalence of test methods
- evaluation of methods
- verify the accuracy and precision of methods
- verifying the correct use of a method

- in manufacturing industry the previous days production batch
- a reference material
- a CRM or
- an SI unit

TRACEABILITY

"Is the property of the result of a measurement or the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparison all having stated uncertainties"

- aid to establishment of an uncertainty budget
- aid to accreditation and certification
- for regulatory purposes
- for training

- **Hierarchy**
- **Calibration**
- **Promotion**
- **Accreditation**
- **Sampling**
- **Transportation and distribution of RM**

www.iso.ch

www.ilac.org

ILAC Guidelines for the Competence of Reference Material Producers, ILAC G12, 2000

ISO Guide 30: 1992, Terms and definitions used in connection with reference materials

ISO Guide 31: 1999 Contents of certificates of reference materials

ISO Guide 32: 1997 Calibration in analytical chemistry and use of certified reference materials

ISO Guide 33: 1999 Uses of certified reference materials

ISO Guide 34 Quality system guidelines for the production of RM

Analytical Production Control for the Improvement of the Quality of Reference Materials.

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For the quality assurance of analytical measurements the availability of suitable reference materials of high quality is of paramount importance. The quality of reference materials is characterised by a solid reliability of the certificate, a sufficient homogeneity and a guaranteed stability for a distinct period of time.
How can analytical production control contribute to improve the quality of reference materials? Analytical control of the materials when they arrive at the institute and during the production will reveal any contamination or losses of the element or compound in question. Additionally it shows the effect of homogenisation steps in the production like cutting, grinding and mixing on the standard deviation of repeated measurements. Particle size measurements manifest the actual size and the distribution of particles in the material.
Stability control is achieved by measurements in defined periods of time to check for the validity of the certified values. Total water analysis provides information on possible water uptake or losses in the course of time. Water activity measurements and the recording of a sorption isotherm show if the reference materials are still in the optimal conditions regarding growth of yeast, bacteria and moulds, enzyme activity, non-enzymatic browning and lipid oxidation.
Micro-analytical methods like solid sampling ZAAS and ETV-ICP-MS providing information about in-bottle and between-bottle homogeneity. From these results it is also possible to calculate the representative sample mass used for analysis and the error which will be contributed to the final result by heterogeneity of the material.

In organising a certification campaign, the selection of laboratories with proven high expertise in the field, the choice of suitable analytical methods and a statistical evaluation of the results following the guidelines are prerequisites for the achievement of a reliable certification of reference materials.

Use and misuse of (Certified) Reference Materials in trace element determination

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Certified Reference Materials (CRMs) are important tools in the Quality assurance toolkit. CRMs have many uses in the laboratory, including checks of specific results and monitoring of method performance.

The ways a CRM can be misused are maybe even more numerous. It can be misused out of ignorance as to the composition and concentration. A related misuse is bordering to a criminal offence! Hopefully it only exists in my wild imagination! There are also ways of misusing CRMs in an economical way. It must be realised that all reference materials are expensive and should thus be used with some discrimination. Statistical misuse is usually derived from the fact that there is a lot of ignorance on how to evaluate the result. This, in turn, is due to the fact that there are no useful guidelines for simple and informative evaluation of (certified) reference materials. Another troubling fact is the tremendous skill in trace element analysis demonstrated by CRM-users! How often do you see a “not so good” result in a publication?

LIVSMEDELSVERKET
NATIONAL FOOD ADMINISTRATION

Use and misuse of Certified Reference Materials

Lars Jorhem

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2000-11-30

CRM-s:

Know their weakness

Use their strength

What are the uses?
Some examples:

- Evaluation at specific times
- Monitoring analytical processes over time
- Evaluation of CRMs when used in collaborative trials
- Training of staff

More uses?

- Identification of systematic and random analytical errors
- To cross-check, ”validate”, in-house reference materials
- Calibration of instruments (?)
- Test material in Proficiency Testings (misuse??)

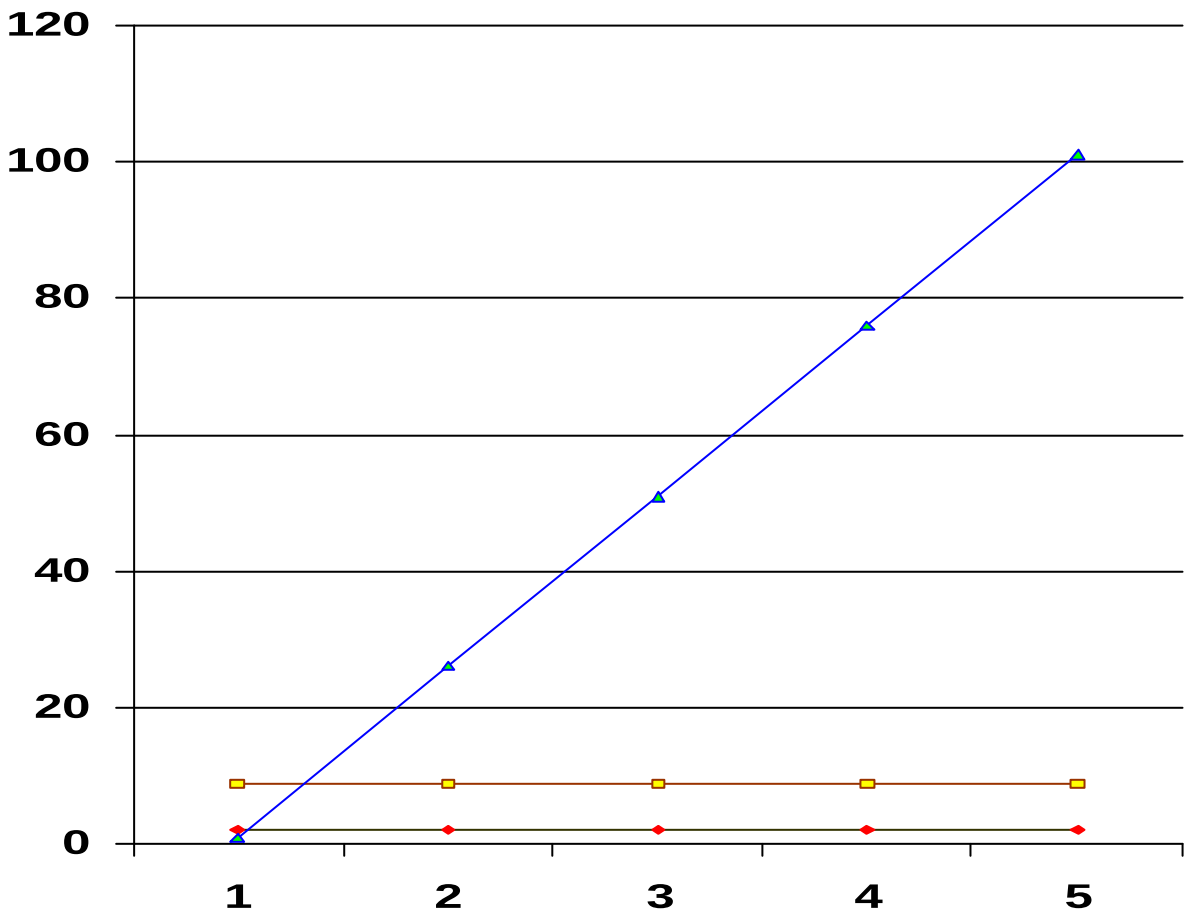
And misuses?

- Ignorant Misuse
  (incl. ”criminal” misuse)
- Economical Misuse
- Statistical Misuse

Ignorant Misuse

- ”Use of a CRM at the wrong analyte level
- Use of a CRM with the wrong matrix
- Using the wrong matrix at the wrong level

Concentration



CRM

”Criminal” Misuse

- Consciously using a CRM with the wrong analyte level and/or the wrong matrix in order to cover up incompetence

Economical Misuse

- Too frequent use
- Collaborative trials (*No evaluation procedure!*)
- (Proficiency testings) (*Maybe for certain applications*)

Statistical Misuse

- The CRM syndrome
- Recoveries
- The evaluation procedure

The CRM syndrome

- Lab's almost invariably show "good" results in reports!
  - » A randomly selected lab perform on average better than certifying expert laboratories!!
  - » Lab's get the right result even when the right result is wrong!!!

Recoveries

- Using Crm-results for recoveries may result in awkward situations:
  - » Certified: 1.00 ± 0.05 mg/kg
    Found: 0.93 mg/kg = NOT OK
    Recovery: 93% = OK
  - » Certified: 0.050 ± 0.011 mg/kg
    Found: 0.040 mg/kg = OK
    Recovery: 80% = NOT OK

The evaluation procedure

- There exists no proper evaluation procedure
- The 95% confidence interval is good for certification, but not for evaluation

The results are:

- similar to the certified-

-close to the certified-

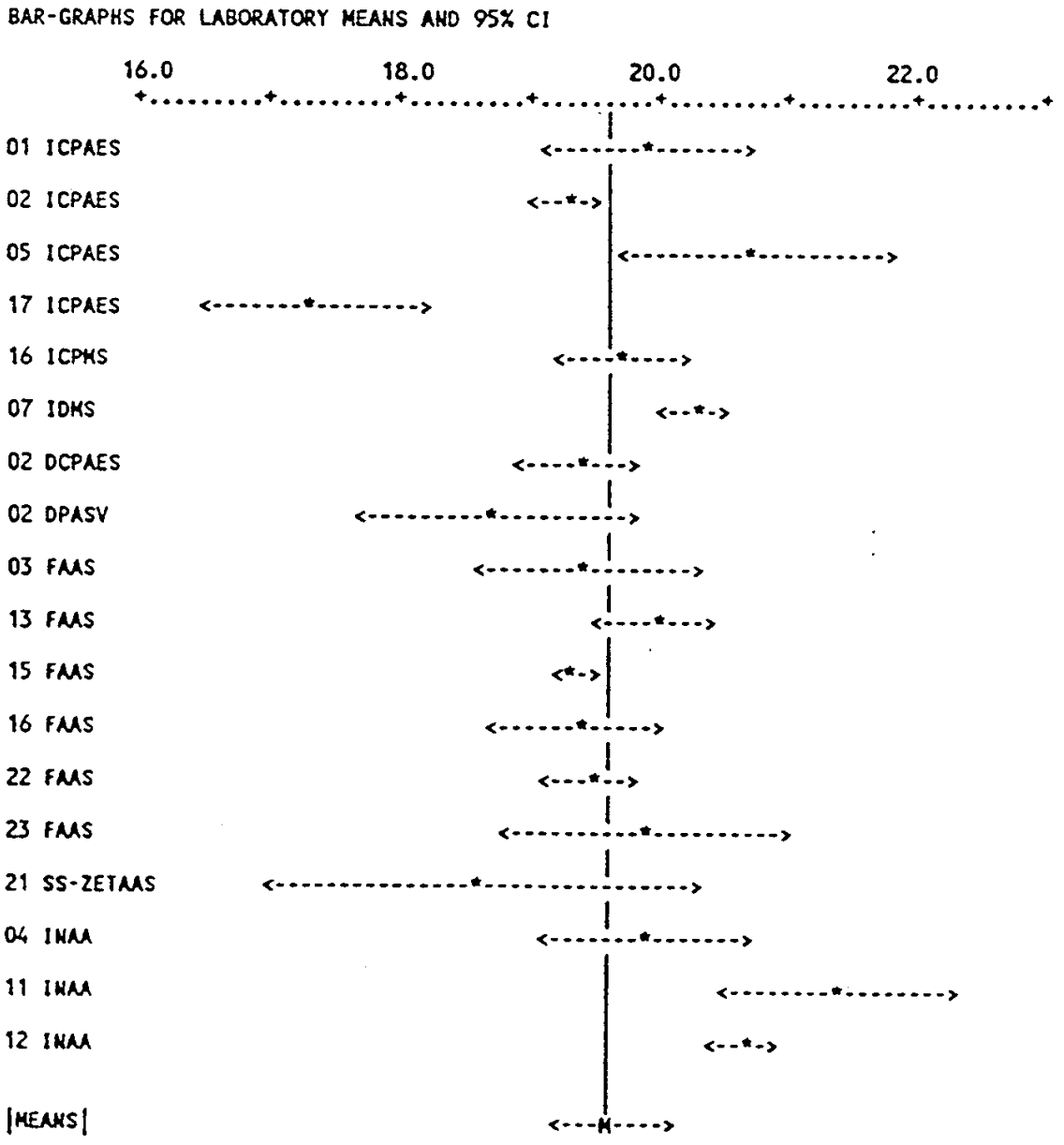
-within the certified range-

-agreed with the certified-

-verified by the certified-

-X±Y% of certified value-

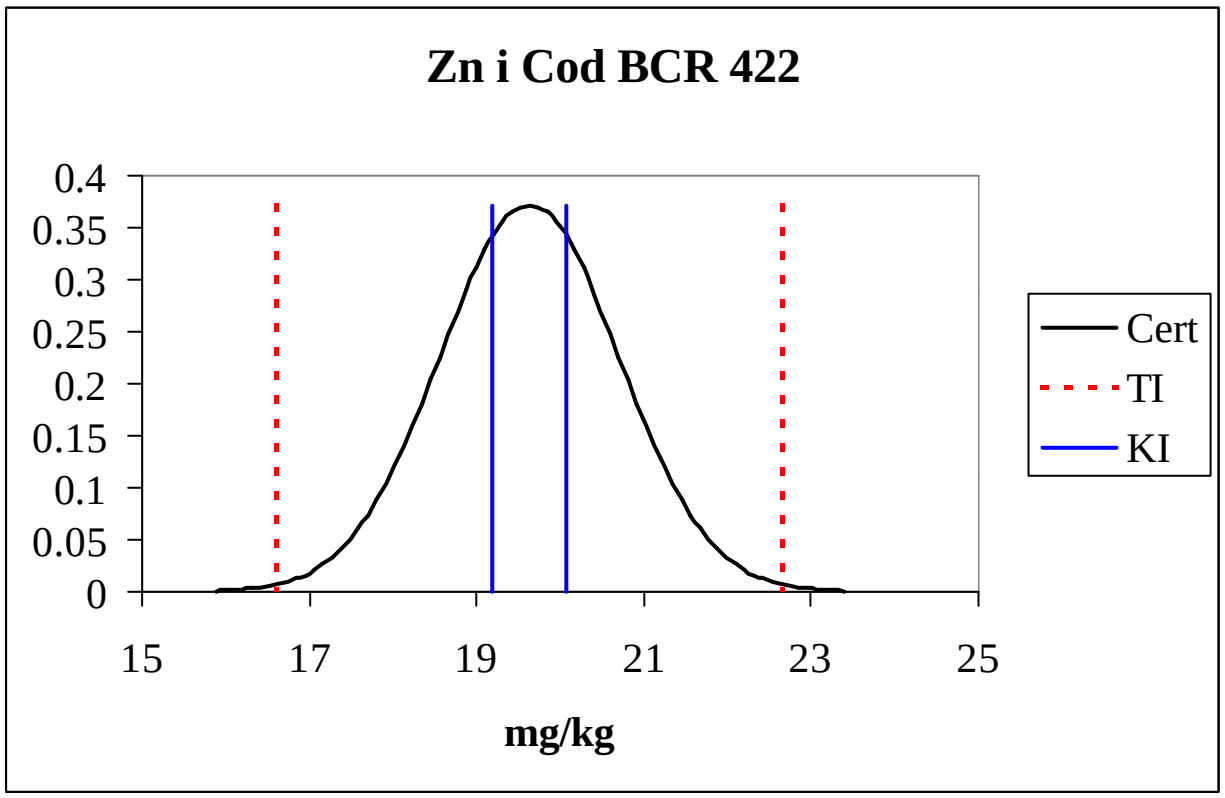
Zn in BCR CRM 422 Cod muscle



A 95/95% tolerance interval is an interval which covers 95% of individual results with a probability of 95%

TI versus CI

Based on a single result and assuming normal distribution



LIVSMEDELSVERKET
NATIONAL FOOD ADMINISTRATION

NMKL PROCEDURE No. Xx (YYYY)

Evaluation of results derived from the analysis of certified reference materials

2000-11-30

What are the needs?

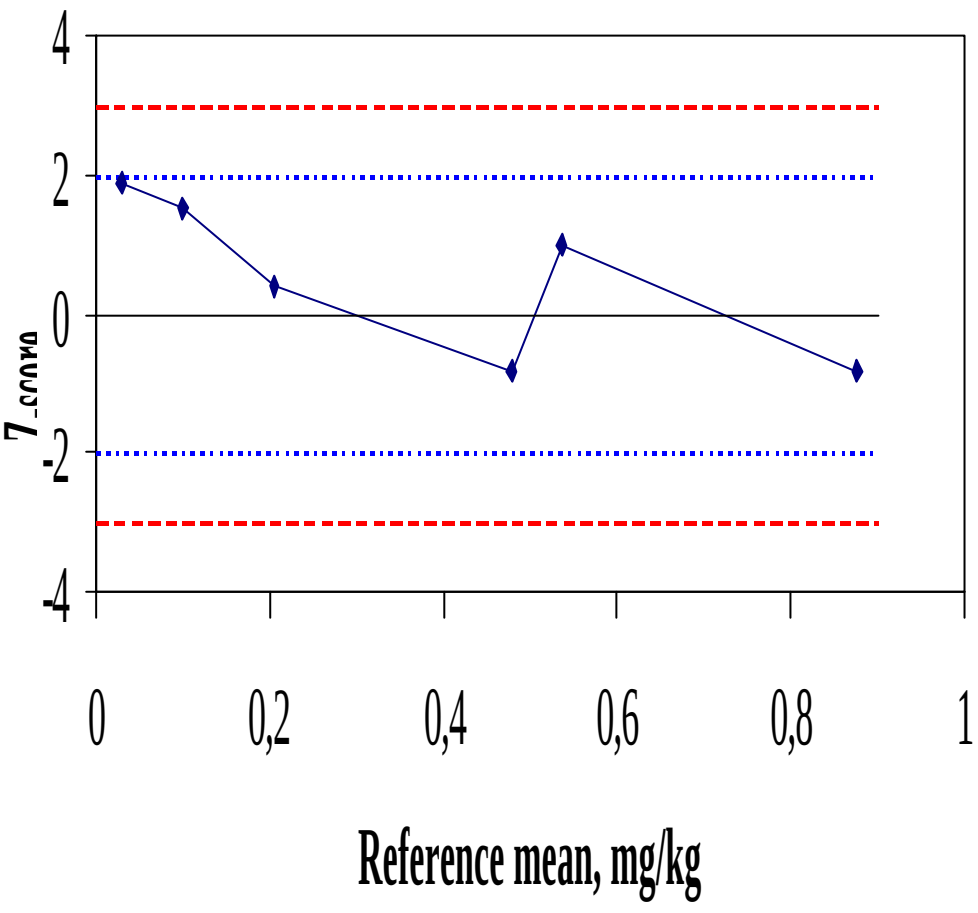
- Evaluation of single, or multiple determinations at specific times
- Identification of systematic and random analytical errors
- Monitoring analytical processes over time
- Evaluation of CRMs when used in collaborative trials

Evaluation of single, or multiple determinations at specific times

Direct/short term evaluation using Z-scores:

$$(X_{found} - X_{certified})/(SD/\sqrt{n})$$

Evaluation of Cd in six different NFA simulated diet CRMs



Wet sterilized reference materials for fish and other biological materials

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Certified reference materials (CRMs) and reference materials used in interlaboratory studies for organic contaminants such as polychlorinated biphenyls (PCBs) or organochlorine pesticides which have been made available during the last decades of the last century consisted in most case of fish oils or freeze-dried materials. Similarly, for trace metal analyses freeze-dried materials have been used most frequently. These oils and freeze-dried materials have some clear drawbacks: they cannot be used to test the extraction part of the user' s method. In addition, the oils often show unrealistically high levels of contaminants, much higher than the materials which are normally used in monitoring programmes such as lean fish, shellfish or fish liver.

During the 1990s wet sterilized reference materials were successfully used in interlaboratory studies for the marine environment (QUASIMEME programme). These fish or shellfish materials were homogenates (minces) which were sterilized and canned (for organics) or placed in glass jars (for trace metal analysis). Anti-oxydants were added prior to homogenization. Not only the homogeneity of the materials appeared to be good, also the stability of the materials was very satisfactory. This observation led to the production and certification of two candidate CRMs: PCBs in mussels and PCBs in herring. Both have been certified within the European Measurements and Testing programme (BCR).

The technique used is simple and promising. The samples can be stored at room temperature and transport is extremely simple. Other biological materials may be considered for production of CRMs in a similar way.

Wet Sterilized Reference Materials for Fish and Other Biological Matrices

Jacob de Boer

Netherlands Institute for Fisheries Research

RIVO

Packing Materials

Organics: Tins (avoiding plastic materials)

Trace Metals: Glass Jars (avoiding metals)

RIVO

Need for Reference Materials

- Use in Interlaboratory Studies
- CRMs

- Target Compounds: Organics: PCBs, PAHs, Brominated Flame Retardants, Organochlorine Pesticides

  Dioxins

  Trace Metals

RIVO

Certified Chlorobiphenyls (Mass Fractions Expressed as µg.kg^{-1}) in Fresh Mussel Tissue CRM 682

IUPAC Number	Certified Value	Uncertainty (*)
CB 28	0.30	0.07
CB 52	0.78	0.09
CB 118	2.6	0.3
CB 138	4.6	0.8
CB 149	5.7	0.6
CB 153	9.2	0.8
CB 170	0.17	0.05
CB 180	0.77	0.07

(*) half width of 95% confidence interval

RIVO

European CRMs for PCBs in biota

- Cod liver oil (349)
- Mackerel oil (350)
- Mussels (682)
- Herring
- Chub (planar CBs, planned for 2001)

Preparation

- Sampling of 2000 kg mussels Dutch Wadden Sea
- Storage and transport at 0 °C
- Heating few seconds, contractor stays in the shell
- Shaking, soft tissue loose from the shell
- Floating waterbath, removing particles
- Inspection on left visible contamination

Preparation (2)

- Mincing and milling (toothed rotary knives 3.5 mm^2)
- Addition of 0.02% BHT
- Homogenizing 10 batches of 25 kg, Stephan cutter
- Stirring of 3 batches of 75 kg
- Division over 17 trays of ca. 30 kg
- Re-homogenisation , canning, autoclaving 45 min, 122 °C

Homogeneity Study

- CBs 52, 101, 118, 153, 180
- Between-homogeneity: 20 x in 20different tins
- Within-homogeneity: 10 x in pool of 3 tins
- Determination of error in GC analysis and clean-up

Homogeneity: Results (RSD in %)

	CB 52	CB 101	CB 118	CB 153	CB 180	n
RSD GC	1.7	2.1	2.9	2.3	3.5	10
RSD Clean-up	3.3	2.9	4.1	2.5	4.3	5
RSD Within	3.5	3.7	2.4	2.2	3.6	10
RSD Between	6.7	5.3	6.0	6.0	6.8	20
RSD Inhom.	*5.7*	*3.8*	*5.5*	*5.6*	*5.8*	

$$RSD_{inhom.}^2 = RSD_{between}^2 - RSD_{within}^2$$

Moisture and Extractable Lipid

	Moisture (%) Between	Lipid (g/kg) Between	Moisture (%) Within	Lipid (%) Within
Mean	74.9	26.4	75.7	26.1
RSD	1.0	5.2	0.2	5.6
n	20	20	10	10

Stability Study, number of analyses

Temperature	T = 0	3 months	6 months	12 months	18 months	26 months
- 25 °C		1	5	5	5	5
+ 20 °C	5	1	5	5	5	5
+ 37 °C		1	5	5		5
+ 50 °C		1	5			

Stability Study

$$R_T = X_T / X_{-20\,^\circ C}$$

$$U_T = (CV^2_T + CV^2_{-20\,^\circ C})^{1/2} \times R_T$$

Certification

- 14 Expert Laboratories
- Extraction: Soxhlet, Ultra Turrax, Shaking, Ultrasonic
- Clean-up: Alumina, Silica, HPLC-PYE, Florisil, H_2SO_4
- Analysis: 12x GC/ECD, 1x GC/MS (Quad.), 1x MDGC
- Two GC columns > 50m, splitless/on-column injection

Conclusions

- Wet sterilized CRMs are a welcome addition to the available materials for interlaboratory studies and CRMs
- The properties are very close to real-world matrices
- The contaminant levels are realistic
- Spiking is possible but often not necessary
- The transportation and storage are easy

RIVO

Assuring the quality of analytical data by the use of reference materials: a case study.

The analysis of PCB's in animal fat, eggs, milk and milkproducts, food en animal feed by GC-ECD and GC-MS.

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As a result from the dioxin crisis in Belgium, a surveillance program concerning the determination of PCB's in different matrixes has been established. More than twenty laboratories were involved in these analyses. The Ministries of Public Health and Agriculture organized a proficiency study to check the quality of the analytical data. It was shown that the spread of analytical results between the laboratories was too high. Some of the analytical methods used by the laboratories were unacceptable. Therefore, both Ministries decided to develop a standardized method. The use of reference standards was mandatory. Due to this obligation, we have discovered in our laboratory a lot of hidden problems. After fixing these problems our analytical methodology has been enormously improved. Continuously improving the analytical methodology and assuring the quality of the analytical data, should be the objective of each laboratory. This can be accomplished by the use of certified reference materials in conjunction with a well developed measuring program, statistical quality control and the participation in proficiency studies.

Assuring the quality of an analytical method by using reference materials: a case study

The determination of PCB's in animal fat, eggs, milk and milk products, food products and animal feed

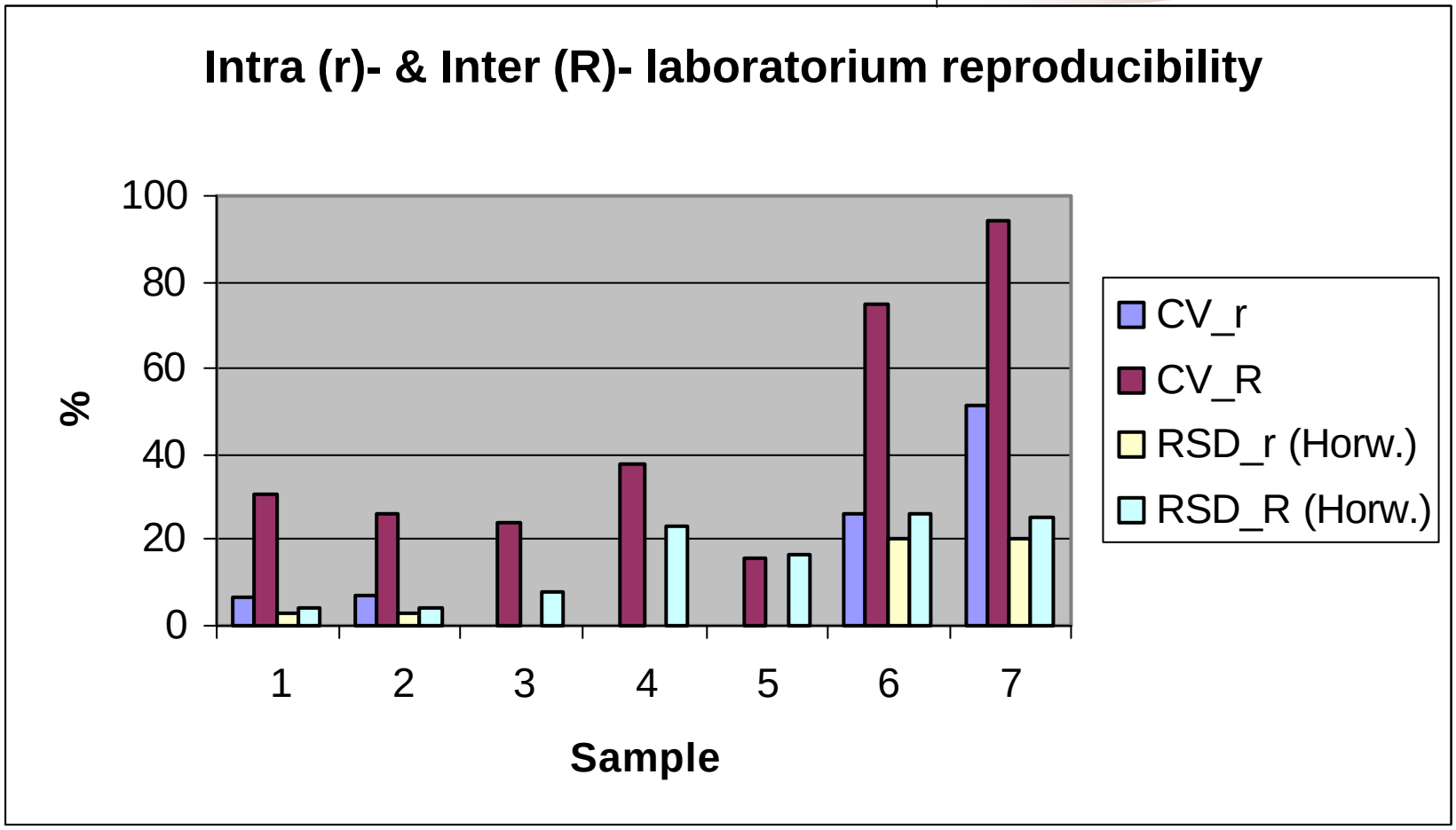
Preamble: The Dioxin Crisis in Belgium

- End of May 1999
- Discovery of Dioxin & PCB in animal feed
- Contamination of the entire food chain
- Thousands of Analysis's of food products to release the batches

Quality of the PCB determinations

- MD of June 12, 1999
  - criteria for validation
    - Recovery must be determined by using CRM's
  - criteria for quality control
    - CRM or spiked samples
- Proficiency study of July 1999

The results of the first proficiency test



The L014 standard method

- Under auspicious of BELTEST and the Ministries of Public Health and Agriculture
  - Based on the experience of the food section of the IPH
  - Based on the prEN-1528
- To be agreed for these Ministries
  - Accreditation EN 45001
  - Use of this method
  - Participation in Proficiency study
  - Other requirements
- Screening GC-ECD or GC-MS & confirmation GC-MS

The implementation of the L014 in our laboratory

- Development
- Validation
  - Accuracy
  - repeatability,
  - reproducibility,
  - Linearity
  - LOD, LOQ
- Internal Quality Control
  - Measuring Program
    - Standard solutions
    - Blanc solvent
    - Blanc procedure
    - CRM or HRM (BCR 350 & spiked samples)
    - Samples
    - Re standard
  - Blind samples
- Proficiency study

Validation of the method

- Accuracy
  - BCR 350 diluted to ± 200 µg/kg; 6x
  - Criteria: 70-130% individual congeneers, 80-110% sum of the PCB's (28,52,101,118,153,138,180)
- Repeatability
  - diluted BCR 350
  - HRM sample spiked at 50/100/200/400 ppb in fat of milk, pork, waffles, animal feed, eggs
  - Criteria: Horwitz equation (RSD_r and RSD_R)

Measuring Program

SEQUENCE

- Standard low & high
- blanc solvant
- blanc procedure
- sample spiked at LOD
- HRM

- Samples (max 10)
- Standard high

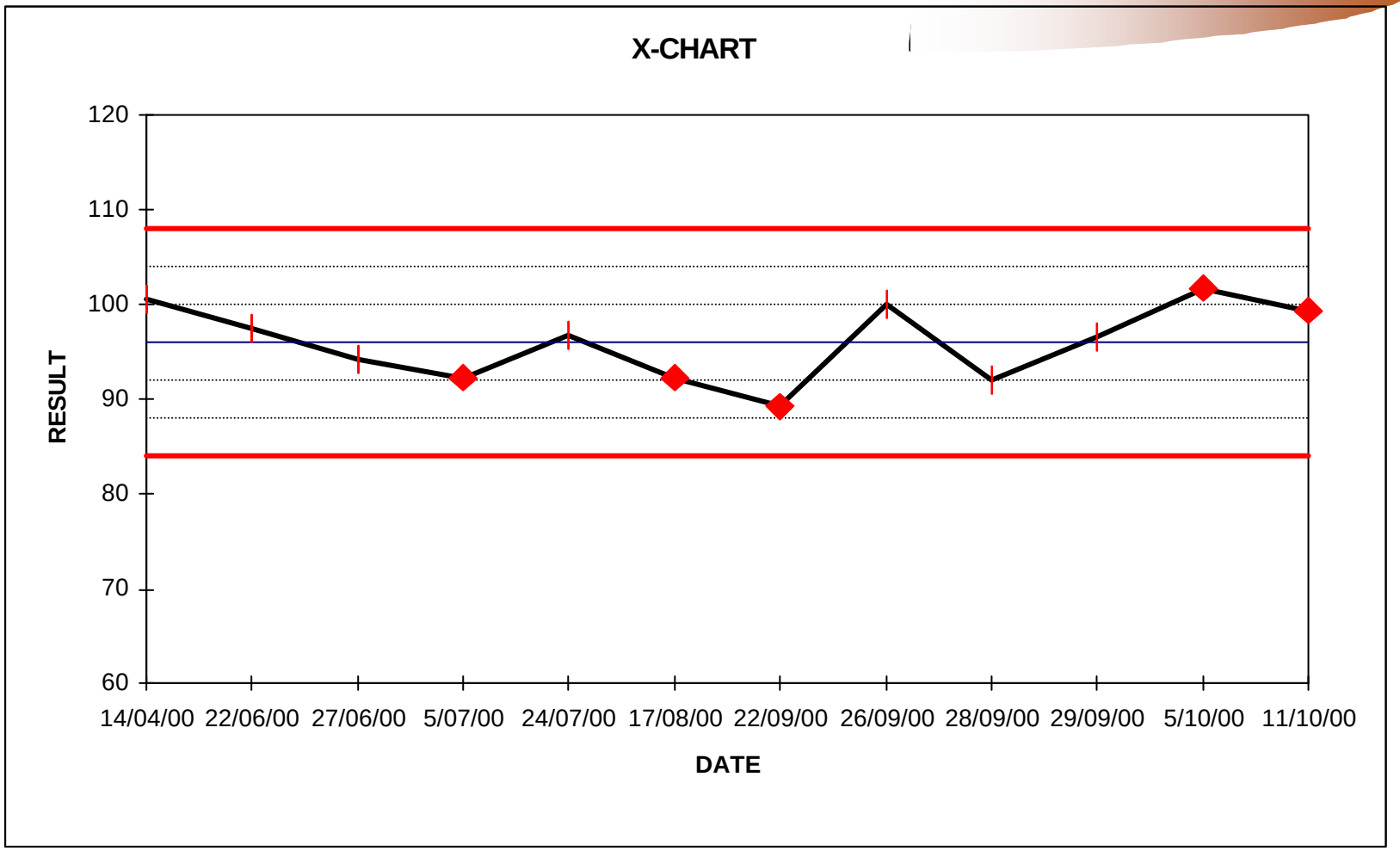
- CRITERIA
- max 10% off nominal value
- pics below LOQ

- pics > SN= 3
- Control chart
- Recov. 80-110 %, 70-130%
- RRT < 0,5%
- max 10% off
- separation PCB 28 and 31

Preparation of the HRM

- 50 g of pork fat
- Spiked stand solutions (sum = 200 ppb)
- Compared with diluted BCR 350

Control charts



The Discovery of Hidden Problems

- Non Linearity of calibration Line (GC-ECD)
  - Correlation Coefficient $R^2 > 0{,}995$
- Risk of contamination
  - Sporadic higher values for reference samples
  - Contaminated blancs
    - Due to contaminated silica
- Improving the extraction of eggs

Conclusions

- The Use of certified reference materials is absolutely necessary to evaluate the accuracy of a test method
- In house reference materials cannot replace CRM’s and vice versa
- You cannot rely on one type of IQC. Multiple approaches are necessary

Conclusions (Continuing)

- Establishing of acceptance criteria for method validation and internal quality control before fits use of the method
- Use of statistical techniques
- Try to continuously improve your methods

The development and use of microbiological reference materials

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Reference Materials (RMs) are known for quite a long time in various fields of analytical measurements but not so long for microbiological examinations. The first microbiological reference materials (RMs) were developed by the National Institute of Public Health and the Environment (RIVM). The first RM developed was a material containing *Salmonella*. Although the first investigations towards the development of this RM started in the early seventies it took until 1985 when this RM was evolved to its present form (gelatine capsules containing artificially contaminated milk powder) and was available for use by other laboratories. Later on, from 1987 onwards, more RMs were developed and evaluated on their performance with the support of the BCR/SM&T of the European Commission. This has finally lead to the development and production of 9 reference materials of which 6 were certified by BCR. These RMs are intended for use in water and food microbiology. Nowadays a few alternative producers of microbiological RMs exist although none of them produce certified RMs.

The ways in which the microbiological RMs are produced is by drying (freeze- or spray drying) or by freezing (-20 ºC or -80 ºC) the micro-organism of interest. All these processes having their advantages and disadvantages with respect to the general requirements for RMs (stability, homogeneity and representativity). The reason for the delay in the development of microbiological RMs must be found in the difficulty of producing stable and homogenous materials containing living organisms.

Microbiological examinations can be divided into two types of analyses (quantitative and qualitative examinations). Especially the qualitative examinations pose problems to the production of the reference materials, its use and the interpretation of their results. For example the number of samples that should be tested to be able to demonstrate a difference in performance of a laboratory compared to the (certified) values. Due to the lack of a "golden standard" such as an SI unit makes the values obtained for the microbiological RMs method dependent.

Currently the reference materials are mainly used in first line quality control. Some of the available RM's are an offspring from the production of materials for third line quality control (proficiency testing).

Development and use of microbiological reference materials

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Development and use of microbiological reference materials

- Introduction
- Development of Microbiological RMs (MRMs)
  - production processes
  - advantages and disadvantages of various MRMs
- Use of Microbiological (C)RMs
  - quantitative RMs and control charts
  - qualitative (C)RMs and power analysis
- Conclusions

Introduction

- RIVM first started the development of MRMs in early seventies
- Difficulties in stabilising the living organisms and homogenisation
- Stabilisation possibilities by drying or freezing
- Variation in behaviour of different m.o.
- Results are method dependant
- Financial support EU (BCR, SMT)

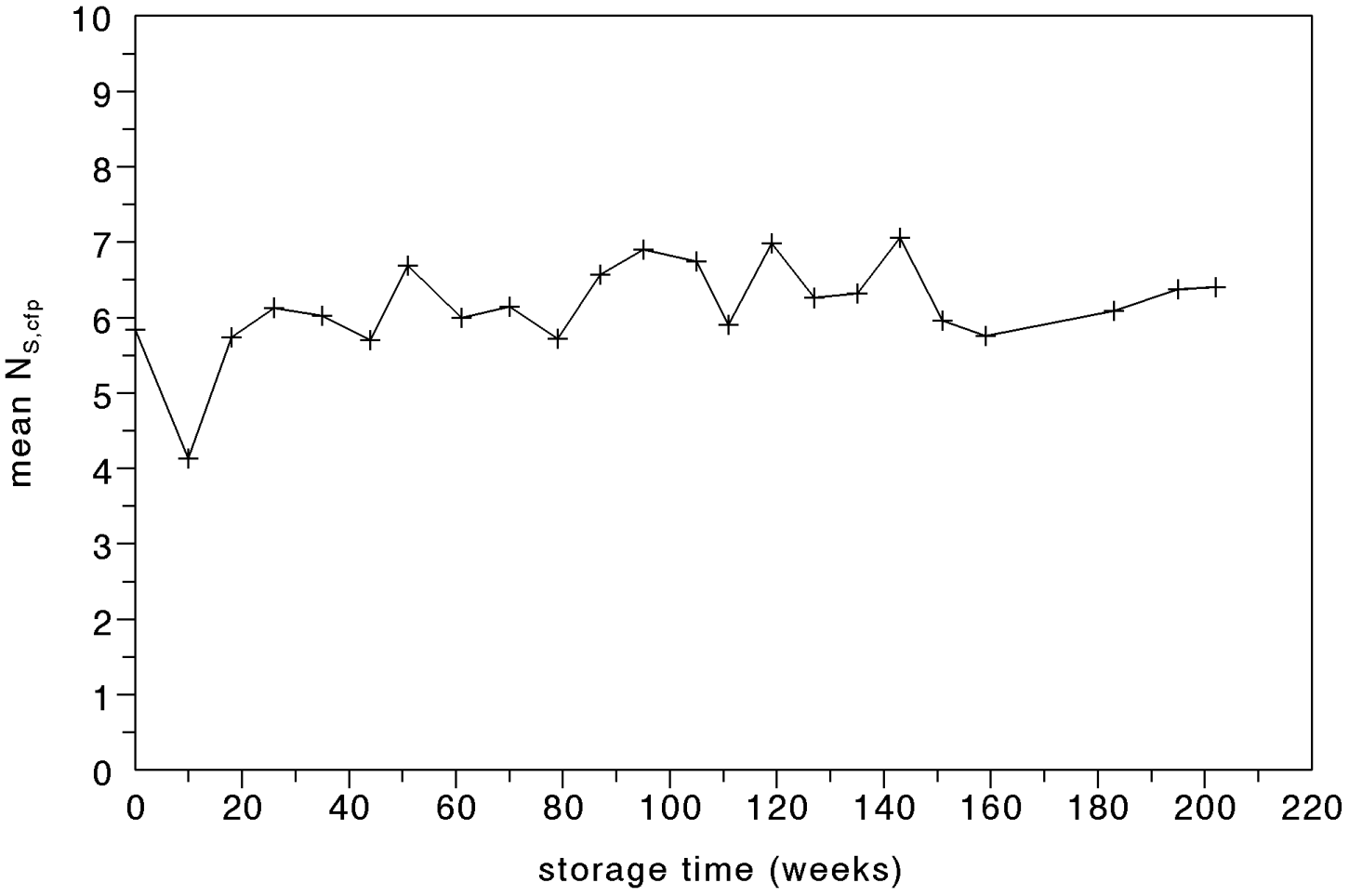
Development of MRMs

- Production process RIVM/SVM:
  - culturing and concentration of the micro-organism of interest
  - spray drying of the m.o. added to milk
  - mixing of highly contaminated milk powder with sterile milk powder
  - filling of gelatine capsules

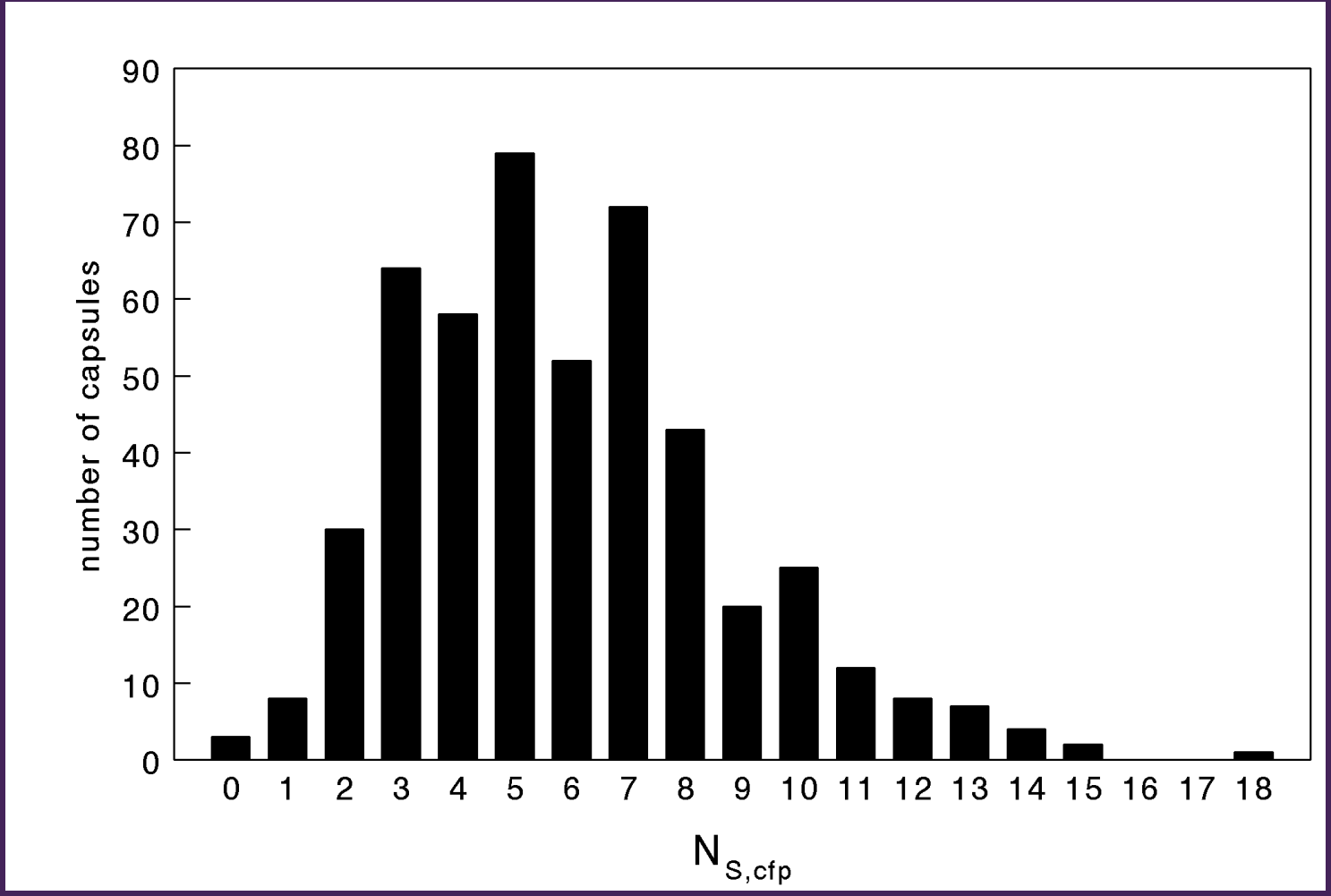
Solutions for obtaining stability and homogeneity

- Stability obtained by:
  - (spray) drying process
  - use of the highly contaminated milk powder over many years
- Homogeneity obtained by:
  - homogenise the organism in milk
  - mixing procedures for milk powder

Stability of Salmonella RM RIVM



Homogeneity Salmonella RM RIVM



Ad- and disadvantages of RMs RIVM

- Advantages:
  - stability of the materials at storage and higher temperatures
  - mixing of contaminated powder with sterile powder
  - quantitative check at low level of qualitative RMs
  - availability of CRMs

- Disadvantages:
  - limited range of materials
  - reconstitution procedure for quantitative RMs
  - price for (C)RMs

Available MRMs RIVM

- Salmonella (RM and CRM) [F]
- Bacillus cereus (RM and CRM) [F]
- Listeria monocytogenes (RM and CRM) [F]
- Clostridium perfringens (RM) [F]
- Escherichia coli (RM and CRM) [W]
- Enterococcus faecium (RM and CRM) [W]
- Enterobacter cloacae (RM and CRM) [W]
- Staphyloccus warneri (RM) [w]

F = food microbiology W = water microbiology

Other MRMs

- National Food Administration (Sweden)
- Quanti-Cult (Oxoid)
- Inspectorate for Health Protection (The Netherlands)
- Lenticules (PHLS, UK)

National Food Administration (Sweden)

- off-shoot of the production of samples for proficiency testing programme
- contains a mixture of organisms
- based on freeze drying of the samples
- limited availability, easy to use
- stable for 12 months at -20 ºC

Quanti-Cult (Oxoid)

- commercial product, expensive
- little information on level and homogeneity
- stable for 6 - 10 months stored at 5 ºC
- easy to use

Inspectorate for Health Protection (The Netherlands)

- based on freezing of the organisms at -20 ºC
- easy to use, inexpensive
- still in experimental stage
- stability varies from batch to batch

Lenticules (PHLS, UK)

- named after the shape of the materials
- off shoot from proficiency testing scheme
- based on drying of the organisms at low temperature in protective medium
- easy to use
- still in experimental phase

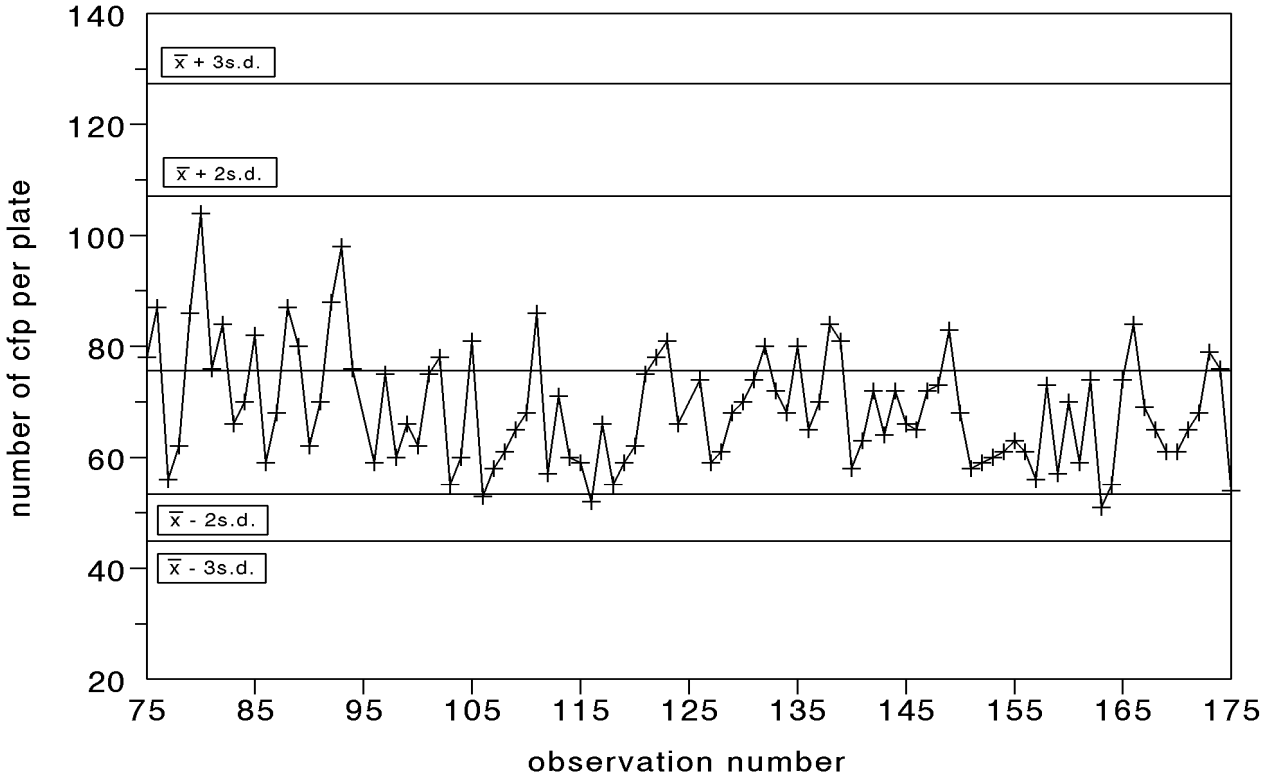
Use of MRMs

- Differentiation between qualitative and quantitative methods
- Possible uses:
  - first line quality control
  - collaborative studies
  - proficiency testing
  - method development/validation

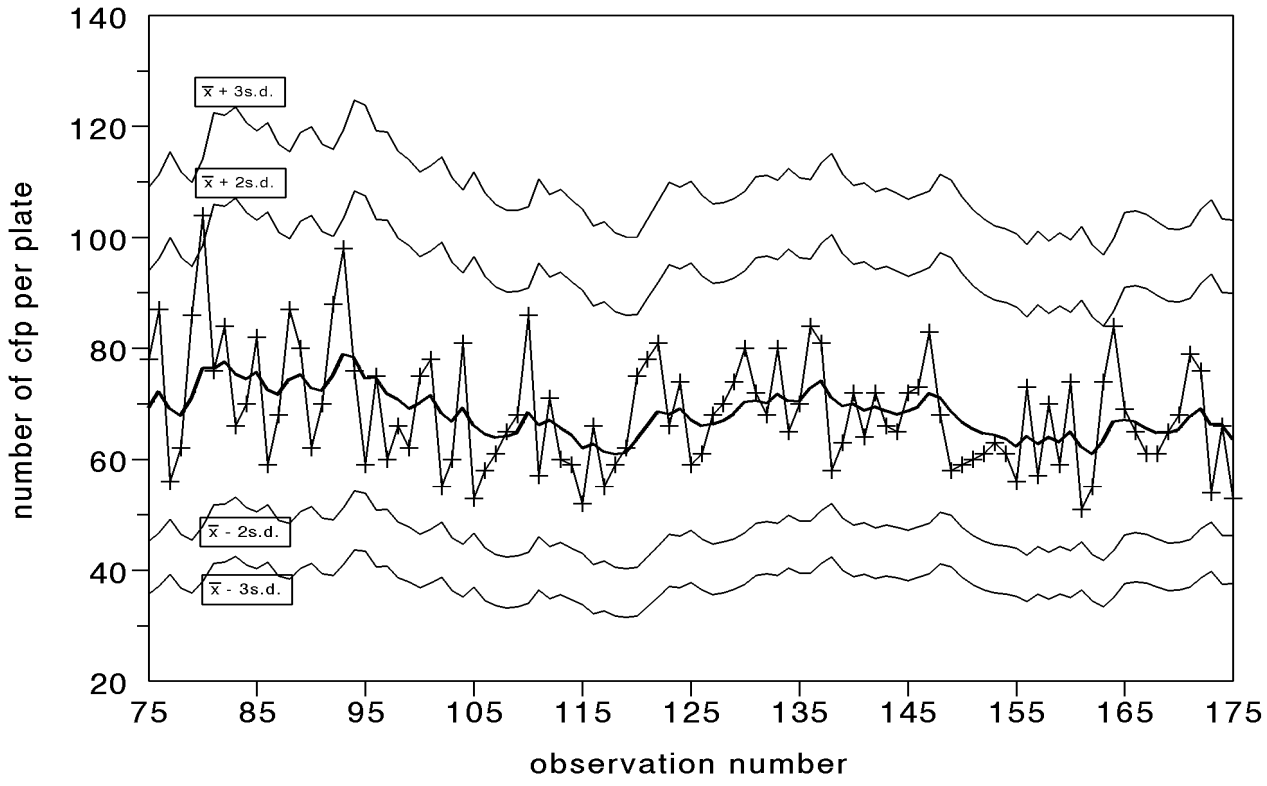
First line QC quantitative MRMs

- Use of Shewhart control charts
- Results are log-transformed before calculation of mean and s.d.
- Values for control limits recalculated back to original scale
- Use of control charts for unstable MRMs (Kalman filtering)

Shewart chart for MRM



Shewart chart using Kalman filtering for MRM



Qualitative M(C)RMs

- Contamination level of RM is compromise between:
  - requirement of method (able to detect 1 m.o. per sample)
  - fraction of negatives of the RM (ideally 0%)
- Poisson distribution:
  - mean of 1 m.o. per sample ⇒ fraction of negatives 37 %
  - fraction of negatives 0.01% ⇒ mean level is 9.2 m.o. per sample

Power analysis qual. M(C)RMs

- Power analysis defines the number of samples that should be examined in order to be able to detect a difference in the fraction of negatives observed by the lab (lab performance) and the (certified) fraction of negatives of the (C)RM.

Power analysis qual. M(C)RMs

Recommended no of samples to be examined (power ≥ 0.8)

p_{lab}	p_{neg} = 0%	p_{neg} = 1.2 % (minimum no pos.)
3 %	53	378 (370)
4 %	40	197 (192)
5 %	32	110 (107)
6 %	27	91 (88)
7 %	23	60 (58)
8 %	20	53 (51)
9 %	18	47 (45)
10 %	16	29 (28)

Power analysis qual. M(C)RMs