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6.2.4 A Nordic Reference Serum Suitable for Use as Trueness Control in the Clinical Routine Laboratory

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ABSTRACT

The described reference serum is characterized by: liquid human serum at "normal" level stored in frozen state at - 80°C; minimum damage of proteins; aseptic preparation; cryoprecipitate and excess fibrin removed; serum cleared by ultracentrifugation; pH at 7.2-7.6; available in sealed glass ampoules with inert gas (one ml serum in each); specified components among most frequently analyzed analytes; homogeneity assured and stability monitored; produced under strict rules for good manufacturing practices (GMP).

The assigned values are traceable to reference measurement procedures and reference materials of highest achievable metrological level; according to the present proposal the maximum allowable uncertainty of the assigned value is based on biological variation (shared common reference intervals); the uncertainty should ideally not exceed 1/5 of the maximum allowable bias of results obtained on patients samples (even ½ would theoretically be acceptable and, for a practical guide approximately < 1% may suffice).

The present document provides some guidance of how the reference serum could be established in practice. The document also indicates the use of the material and further extension of the concept. The present work is done as a NORDKEM project.

INTRODUCTION

This project has been initiated because the trueness of results produced in the routine laboratories nowadays seems in general to be the most important analytical problem. The aim of this project is to describe a high quality product for use as trueness control in the clinical routine laboratory, available within all Nordic countries.

The authenticity of the analytes as well as the matrix should as far as possible be identical to patient sera. The description of production and use of a high quality control material is based on: previous long term experiences with the Nordic Protein Project (1); the dry chemistry surveys organized by Labquality, Helsinki; Danish external quality assessment schemes (EQAS) using fresh frozen human sera (2) and other activities in the Nordic countries; experience of long term stability up to 10 years in Denmark.

The assigned values should be traceable to reference measurement procedures and reference materials of highest achievable metrological level. The uncertainty of the assigned values should as far as possible meet documented requirements for analytical quality.

The aim of the project is to introduce an error removal mechanism in the laboratory where relevant. The proposed material should preferably be used in a coordinated way e.g. initiated by EQAS organizers. Thus each laboratory should estimate and report their bias as it would appear after several days of measurement on the reference serum together with the internal quality control material in use.

Description of the production procedure of the reference serum

The described serum originates from blood collected from donors, knowing the purpose of the donation. Donations takes place under the auspices of a blood bank in agreement with the national donor organization. More than 400 donor portions will be used for a batch of 50 litres, intended for approximately 10 years use. Details of the procedure for collection and preparation of the serum are as follows:

- *The blood is collected* in Fenval bags (without any additions). After spontaneous coagulation (3 hours) at room temperature, the serum is isolated by centrifugation (1000 g_n for 1/4 h). After approximately 18 h of storage at 4°C the serum is centrifuged at 1000

g_n for ½ h (to remove cryoprecipitate and fibrin clots formed since the first centrifugation). The sera are pooled and stored in darkness at -80°C. Each donor portion is tested for HIV-antibodies, hepatitis C-virus antibody and hepatitis B-virus s-antigen; only pools consisting of sera with "negative" findings are used.

- The serum is *transferred in frozen state,* for further processing.

- After thawing at 4-10°C *cryoprecipitate is removed by centrifugation* (1000 g_n for ½ h). All pools are mixed carefully. Following an ultracentrifugation for 48 h at 180.000 g_n the *lipid layer is removed* by suction and the serum reconstituted to the original volume by addition of the lipid-free ultrafiltrate of a separate part of the serum pool (1). The ultracentrifugation could be carried out at Statens Seruminstitut, Copenhagen. The cleared serum pool is mixed carefully, *filled* into glass ampoules (one ml in each) under inert gas (pO_2 <1 Pa), closed by heating, and frozen immediately. The serum is stored at -80°C.

- *Requirements for the ampoules.* The ampoules must after filling and freezing not have a higher frequency of breakage than e.g. one per 1000, and they should be able to stand usual transportation on dry ice in the mail. They should not contaminate the reference serum.

- *Homogeneity test.* During the filling procedure ampoules are selected strategically for analysis; e.g. the first and last ampoules as well as ampoules at any interruption during the filling procedure; furthermore, regular sampling of ampoules during the filling process is done. Each ampoule is analyzed for e.g. Na^+, Ca(II), Fe, Zn(II), bilirubins, lactate dehydrogenase, pCO_2, pH, glucose, turbidity, and those components for which assigned values are produced. All details of the homogeneity test have to be specified.

- *Stability.* The stability of the serum is investigated during its lifetime. Critical components would be alanine aminotransferase, creatinkinase, lactate dehydrogenase, bilirubins, glucose and those for which assigned values are produced. Short time stability studies are carried out e.g. for -20°C (1 year), +4°C (1 month), +20°C (1 week). All details of the stability studying have to be specified.

- *Test for sterility is carried out.*

- The whole manufacturing procedure and testing are carried out under *strict rules for "good manufacturing pratices" (GMP).*

- All manufacturing procedures are carried out under *"aseptic"* conditions.

Requirements of the trueness of the assigned values to the reference serum

The specifications for allowable bias for assigned values of the measured quantities will be related to the goals for analytical quality necessary for laboratories that wish to share common reference intervals (3). This goal for maximum allowable bias is so specified that the use of the common reference interval (based on a large number of reference individuals) will be better than if each laboratory had estimated its own reference interval by collecting the minimum number of reference samples (n = 120) recommended by IFCC (4). This will be fulfilled when the analytical bias, B_A, in the laboratory is less than 0.25 times the biological standard deviation [$B_A \leq 0.25 \cdot (CV_{Bw}^2 + CV_{Bb}^2)^{0.5}$], where CV_{Bw} and CV_{Bb} are the biological within- and between-subject variations, respectively. Thereby, the fraction of individuals beyond each reference limit will be within the interval 1.4% to 4.4% (3).

The specifications for maximum allowable uncertainty of the assigned values must be such, that the bias of the reference serum, B_{RS}, will have no significant influence on the above mentioned concept. The goals for the assigned values of the reference serum are given as fractions of the maximum allowable bias for laboratories, B_A, and thereby, also as fractions of the biological standard deviation.

Based on this concept, the assigned values can be classified into three categories according to size of accepted bias:

Class A: $|B_{RS}| \leq 1/5 \cdot |B_A| = 0.05 \cdot (CV_{Bw}^2 + CV_{Bb}^2)^{0.5}$

Class B: $|B_{RS}| \leq 1/2 \cdot |B_A| = 0.13 \cdot (CV_{Bw}^2 + CV_{Bb}^2)^{0.5}$, but larger than for Class A

Class C: $|B_{RS}| > 1/2 \cdot |B_A|$

This means that the effect of the proposed acceptable bias for class A increases the uncertainty of the fraction of population beyond each reference limit. This fraction changes from (1.4 to 4.4% for each limit) to (1.2-4.9%) and for class B to (1.0-5.7%); details are available on request.

For quantities with assigned values according to Class A, the 90% confidence interval for the conventional true value, therefore, should be: value $\pm 1/5 \cdot |B_A|$, which has only a small influence on the concept of sharing common reference intervals.

Class B values will modify the concept to some extend, but could be counteracted by narrowing the allowable analytical bias for laboratories as compared to the reference serum.

Class C values may still be useful for laboratories, but decisions about validity must be made for each quantity.

Further classifications based on the authenticity of the serum constituents and related to traceability may improve the interpretations of the assigned values. A proposal is available on request.

HOW TO ESTABLISH THE REFERENCE SERUM

Proposed organizations who may take care of the preparation of a Nordic reference serum are EQAS organizers in the Nordic countries, National Societies for clinical chemistry, Labquality, Helsinki, Statens Seruminstitut, Copenhagen, and joint venture with certain companies.

Economical sources may be found among EQAS-organizers, clinical laboratories, manufacturers of dry chemistry analyzers and Community Bureau of Reference (BCR). For practical reasons some elements are not yet settled, e.g. proposal for ownership, budget, selection of reference laboratories, time schedule, and distributors (one distributor in each of the Nordic countries is anticipated); these matters might be better to decide upon together with the organization(s) who will take care of the preparation of a Nordic reference serum.

The present authors realize that the concepts and ideas used in this document may be difficult to *implement in practice* in all details. The organizations who will take care of the preparation of a Nordic reference serum may need to consider some compromises for economical reasons. Possible deviations from this description should, however, be done in cooperation with the authors.

Table 1. Maximum allowable analytical bias, $|B_A|$ (in percentage of the measured value) of laboratories (5), and maximum allowable bias of assigned values to reference serum, $|B_{RS}|$ (in percentage of the measured value), according to the concept of sharing common reference intervals, where the population is homogenous for the quantity.

Component in serum	$\|B_A\|$ **	$\|B_{RS}\|$ **	
		Class A	Class B
Albumin	1.1	0.2	0.6
Alkaline phosphate	6.4	1.3	3.2 *
Bilirubins	9.8	2.0 *	4.9 *
Calcium (II)	0.7	0.14 *	0.35 *
Chloride	0.5	0.1	0.25
Cholesterols ***	3.0	0.6 *	1.5 *
Cholesterols (reference interval)	4.1	0.8 *	2.1 *
Creatinine	2.8	0.6 *	1.4 *
Creatin kinase	19.8	4.0 *	9.9 *
Glucose	1.9	0.4	1.0 *
IgG	5.0	1.0	2.5 *
IgA	12.5	2.5 *	6.3 *
IgM	12.7	2.5 *	6.4 *
Iron (II+III)	8.9	1.8	4.5 *
Lactate dehydrogenase	4.1	0.8	2.1 *
Magnesium (II)	1.6	0.3	0.8 *
Phosphate	1.6	0.3	0.8 *
Potassium-ion	1.6	0.3	0.8
Sodium-ion	0.2	0.04	0.1
Thyrotropin (TSH)	8.9	1.8	4.5 *
Thyroxine (total)	4.1	0.8	2.1 *
Transferrin	2.3	0.5	1.2
Triglycerides	15.6	3.1 *	7.8 *
Urea (Carbamide)	5.3	1.1 *	2.7 *
Urate	4.0	0.8 *	2.0 *

* Probably obtainable.
** 90 % confidence interval.
*** The goal 3.0 % (6).

Planned extensions

To make the project more useful in practical work an extension is suggested: the original proposed amount of serum should be increased by 50 litres. This part of the reference serum is stored separately in e.g. gas tight plast bottles of one litre each. By addition of well characterized components, sera with several concentration levels are obtained. The additions are done one by one successively by time so experience can be gained. Organ extracts may be used. The goal is to obtain a set of modified well characterized serum batches. Serum with new modifications should be stored so all batches are available.

The described *reference sera* are intended for the control line of the quality assurance system in the laboratory. A similar procedure could be used for the production of calibration materials, and a project describing a material for supporting the *calibration line* by a set of calibration sera is anticipated.

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