NFKK Reference Serum X

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Summary
An overview of the background, manufacturing and traceability of the NFKK Reference Serum X is presented, as well as a reprint of the certified and indicative values from the official certificate. The intended use and a suggestion for the practical use are described.

Introduction
NFKK reference serum X (hereafter called X) is an unmodified fresh-frozen human serum without additives. X is a reference material and comes with a certificate [1]. X carries 14 assigned property values traceable to the highest available metrological order and 12 indicative values.

X is the result of a cooperation between NFKK, EQAnord and especially the participating laboratories in the Nordic Reference Interval Project (NORIP) [2] and in The Nordic Trueness Project (NTP)[3].

X is intended for evaluation and verification of trueness of measurement procedures, usually involving *in-vitro* diagnostic (IVD) medical devices. Primarily X is intended to work as trueness control when laboratories implement the common Nordic reference intervals. Also X could be the answer to the growing need for certified reference materials with traceable values of higher order.

X is available from each of the national External Quality Assurance (EQA) Organisers in the Nordic countries.

1 Danish Institute for External Quality Assurance for laboratories in Health Care

Links to other projects
X was first included in NORIP in 2000 as a control material, but also as the heir to CAL, the primary calibrator in the project. Later in 2002 in NTP, a Nordic expansion of the International Measurement Evaluation Programme round 17 (IMEP-17), X and CAL were measured again along with IMEP-17 Material 1 [4], a master material with assigned values of the highest available metrological order and assumed to involve none or negligible commutability problems. The measuring in parallel after common protocols enabled the transfer of values and uncertainties from the IMEP-17 Material 1 to X directly or via CAL. The transfer of the directly assigned reference measurement procedure values from CAL to X was also possible.

Background of the material
Material X is an unmodified fresh frozen human serum. The material is a pool of fresh frozen human sera isolated from donors' blood by Hjørring County Hospital in 2000. The blood was collected from healthy blood donors according to WHO recommendations. Each individual donation, as well as the final pool, was tested and found negative for anti-HIV, anti-HCV and HBsAG. The pool (~21 L) was further treated by DEKS[1] in collaboration with Statens Seruminstitut (SSI), Copenhagen, Denmark. It was left unmodified and carefully mixed and sterile-filtered (0,22 µm) before transferring 5 mL into each of 4200 polypropylene vials. The vials were closed with a Teflon-coated stopper and an outer metal seal, and stored at -80 °C [5].

The long-term stability (10 years) at -20, -80 and -150 °C is monitored by DEKS [6].

The homogeneity has been verified and found sufficient for this material [3], as well as other materials manufactured in a similar way, e.g. IMEP 17 Material 1, have also proved to be sufficiently homogenous [5;7]

No specific commutability study has been provided for X, but the material is assumed to have no or

negligible commutability problems when compared to routine results on patient sera. This assumption is supported by measurements on the similar material: IMEP 17 Material 1 [8].

X is already used in specific EQA projects: Validation of S-Creatininium results after application of a zero-point calibrator (EQUALIS 2003) and studies of the commutability of control sera, e.g. lyophilised materials (DEKS 2004).

The certificate

The certificate accompanying X is drafted according to legal necessities, demands in ISO guide 31[9], EN ISO 12286 [10], and 'the IVD directive' [11]. The certificate presents the certified and indicative values (Table I and Table II)

All NFKK board members have provided inputs to the certificate and approved it.

Traceability of the values

Certified values

The certified values and the related expanded uncertainties are traceable to SI via values assigned to IMEP-17 Material 1 by reference measurement procedures and international certified reference materials [7;12], except gamma-Glutamyltransferase which is traceable to SI via CAL[3].

Indicative values

By definition the indicative values have no uncertainty or expanded uncertainty traceable to the highest metrological order. Some of the values are traceable to reference measurement procedures performed on CAL (ALT, Bilirubin, CK, Phosphate, Protein). The only part of the uncertainty known for these values is the part relating to the transfer of values from CAL to X. The uncertainties of the reference measurement procedures are unknown, albeit expected to be smaller than the ones experienced in routine laboratories. These values are transferred as the mean of the ratio between the laboratory mean of X (meanX) and CAL (meanCAL) multiplied with the certified value of CAL (certifiedCAL) (Equation 1).

Indicative values traceable to CAL =

$$\frac{1}{N} \cdot \sum_{N=\text{laboratories}} \frac{\text{mean}_X}{\text{mean}_{CAL}} \cdot \text{certified}_{CAL}$$

(Equation 1)

For the rest of the indicative values (ALP, Amylase, AST, HDL-cholesterol, LD, Pancreatic amylase and Transferrin) the values and the indicated standard deviation of the mean are traceable to the arithmetic mean of all NORIP participants' mean measurement values using routine methodologies (Equation 2). For enzymes an IFCC 37 °C compatible methodology is used.

Indicative values traceable to NORIP participants =

$$\frac{1}{N} \cdot \sum_{N=\text{laboratories}} \text{mean}_N$$

(Equation 2)

Results

The tables are reprints from the first version of the certificate. The numbers may be subject to later changes and should in no way be used as a substitute for the values provided in the certificate.

The use of X

X is <u>not</u> a substitute for the calibrators supplied by manufactures of in-vitro medical devices. *The certified values* are intended for evaluation and verification of the performance of routine clinical measurement procedures or validation. *The indicative values* are important, because routine results can be made traceable to the common NORIP reference intervals for the Nordic countries, thus satisfying the need for traceable reference intervals.

Practical use of X

A practical suggestion to the use of X, is to:

- Measure the working calibrator (supplied by the IVD-manufacturer) 6-10 times in parallel with 6-10 measurements on X. The high number of replicates is necessary to reduce the uncertainty.
- If post calibration modifications (e.g. factors, slope and intercept) are performed locally the values should be recalculated to the original values, to ensure full traceability to the calibrator of the instrument. Calculate the arithmetic mean values. Make the following correction to the mean value of X:

$$M_X^{Corr} = M_X \cdot \frac{\text{Target}_{Calibrator}}{M_{Calibrator}}$$

where M_X and $M_{Calibrator}$ are the mean values of X and the calibrator respectively and $\text{Target}_{Calibrator}$ is the assigned value of the calibrator [13].

Table I:
Certified values for each component and their expanded uncertainties, *U* (coverage factor, *k*=2).

Component	Unit	Certified value and expanded uncertainty $U=2\cdot u_c$	
		Value	*U*
Albumin	g/L	41,5	2,7
Calcium (Ca)	mmol/L	2,325	0,008
Cholesterol	mmol/L	5,220	0,023
Creatininium	µmol/L	73,90	0,60
Iron (Fe)	µmol/L	20,00	0,56
gamma-Glutamyltransferase (GGT)	U/L	35,42	0,95
Glucose	mmol/L	4,405	0,034
Potassium (K)	mmol/L	3,732	0,022
Magnesium (Mg)	mmol/L	0,8100	0,0065
Sodium (Na)	mmol/L	140,65	0,75
Thyroxine (T4)	nmol/L	99,4	3,1
Triglyceride	mmol/L	1,287	0,038
Carbamide (Urea)	mmol/L	4,910	0,026
Urate (Uric acid)	µmol/L	309,9	5,8

Table II:
Indicative values for components in NFKK Reference Serum X, obtained in the NORIP project.

Component	Unit	Indicative value* and (standard deviation of mean of laboratory mean)# traceable to consensus mean value in NORIP [Enzymes: IFCC 37°C]	via CAL to certified reference value by DGKC 1997
Alkaline phosphatase (ALP)	U/L	72,5 (0,7)	-
Alanine transaminase (ALT)	U/L	-	24,2 (0,2)
Amylase	U/L	60,7 (1,4)	-
Aspartate transaminase (AST)	U/L	25,5 (0,2)	-
Bilirubin	µmol/L	-	8,97 (0,04)
Creatine kinase (CK)	U/L	-	133,3 (0,4)
HDL-cholesterol§	mmol/L	1,387 (0,003)	-
Lactate dehydrogenase (LD)	U/L	147,8 (3,1)	-
Pancreatic amylase	U/L	28,6 (0,4)	-
Phosphate§	mmol/L	-	1,043 (0,002)
Protein (total protein)§	g/L	-	68,7 (0,11)
Transferrin	g/L	2,709 (0,025)	-

* For values in the first column, the value is the mean of laboratory means. For values in the second column, the indicative value is the mean of factors M(X)/M(CAL) multiplied with certified reference value for CAL, where M(X) and M(CAL) is laboratory mean of X and CAL respectively.
# No expanded uncertainties can be provided for these components, however the obtained standard deviation of mean of laboratory means (SDM), from approximately 102 laboratories using routine measurement systems can be used to judge upon the reliability of the value (first column) and the reliability of the value relative to CAL (second column). For the enzymes a lower number of laboratories were involved (ALT: 86, Amylase: 24, AST: 79, CK: 81, Pancreatic amylase: 21, ALP: 23, LD: 3). For values in the second column the SDM is without the main source of uncertainty from the target value of CAL.
§ In the Nordic Trueness Project 2002, these components were re-assigned with transferred values via CAL. No significant difference between the two sets of values was observed ($p<0,05$).

- Verify that the corrected mean value of X is within acceptable limits (i.e. certified/indicative value of X ± 0.375 x total biological variation).
- If the certified values are found within stated bias goals, the NORIP reference intervals can be applied.
- If the certified value cannot be found further investigation should start; e.g. by contacting other laboratories using the same equipment to agree on a common approach or contacting the manufacturer.

If the laboratory decides to apply a factor to be able to use the NORIP reference intervals, the laboratory should be aware that it might void the legal responsibility from the IVD-manufacturer. The 'normal' or slightly low (i.e. enzymes) concentrations of the components in X could introduce difficulties for calibration of higher values, e.g. cut-off values.

References

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