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Scand J Clin Lab Invest 2004; 64: 271–284

The Nordic Reference Interval Project 2000: recommended reference intervals for 25 common biochemical properties

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Rustad P, Felding P, Franzson L, Kairisto V, Lahti A, Mårtensson A, Hyltoft Petersen P, Simonsson P, Steensland H, Uldall A. The Nordic Reference Interval Project 2000: recommended reference intervals for 25 common biochemical properties. Scand J Clin Lab Invest 2004; 64: 271–284.

Each of 102 Nordic routine clinical biochemistry laboratories collected blood samples from at least 25 healthy reference individuals evenly distributed for gender and age, and analysed 25 of the most commonly requested serum/plasma components from each reference individual. A reference material (control) consisting of a fresh frozen liquid pool of serum with values traceable to reference methods (used as the project "calibrator" for non-enzymes to correct reference values) was analysed together with other serum pool controls in the same series as the project samples. Analytical data, method data and data describing the reference individuals were submitted to a central database for evaluation and calculation of reference intervals intended for common use in the Nordic countries. In parallel to the main project, measurements of commonly requested haematology properties on EDTA samples were also carried out, mainly by laboratories in Finland and Sweden. Aliquots from reference samples were submitted to storage in a central bio-bank for future establishment of reference intervals for other properties. The 25 components were, in alphabetical order: alanine transaminase, albumin, alkaline phosphatase, amylase, amylase pancreatic, aspartate transaminase, bilirubins, calcium, carbamide, cholesterol, creatine kinase, creatininium, γ-glutamyltransferase, glucose, HDL-cholesterol, iron, iron binding capacity, lactate dehydrogenase, magnesium, phosphate, potassium, protein, sodium, triglyceride and urate.

Key words: IFCC enzyme; reference individual; reference limit; reference material; reference sample

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DOI 10.1080/00365510410006324

INTRODUCTION

Increasing national and international communication within the healthcare services has strengthened the need for harmonization of measurements and reference intervals in laboratory medicine. International organizations have established reference systems to help harmonization of analytical measurements. Results from internal and external quality assessment have shown that, despite the diversity of measurement methods in use, the quality of measurements is now generally acceptable.

Common reference intervals are important to facilitate collaboration and reduce errors caused by misunderstanding. Harmonization of analytical measurement should therefore logically be followed by harmonization of reference intervals within regions. Within the Nordic countries this has earlier been done for proteins [1]. Historically, because of method differences, but also because of population differences, the laboratories have been advised to establish their own reference intervals. To do this according to procedures recommended by the International Federation of Clinical Chemistry (IFCC) [2] is, however, a demanding task for a single laboratory. In practise, the laboratories therefore often use reference intervals from the literature or adjust old intervals when they introduce new methods in the laboratory. As many studies have shown, the result is that reference limits vary considerably from one laboratory to another.

The time was therefore more than ripe to make a joint effort to harmonize the reference intervals for the most frequently used biochemistry properties.

The Nordic Reference Interval Project (NORIP), which was intended to establish common Nordic reference intervals for 25 of the most frequently requested properties (the nomenclature of "Committee on Nomenclature, Properties and Units (IFCC&IUPAC)" is used in the text) in clinical biochemistry (Table I), was established in March 1998. The project was supported by the Scandinavian Society of Clinical Chemistry (NFKK) and organized in each participating country by project members that were elected by the respective national societies of clinical chemistry.

In addition to the biochemistry properties, Finland also sought to establish common reference intervals for commonly requested haematology properties. This project was joined by Sweden and, partly, Denmark and was included in NORIP. The conclusions of this part of NORIP are presented elsewhere [3].

The project is presented in detail on the project home site [4].

TERMINOLOGY

Naming of properties

The naming of properties is based on the NPU coding system, but in some cases abbreviations of these are used. Names used in this issue, the complete NPU names (in English) and their NPU codes are listed elsewhere [5].

Samples

To eliminate confusion between the concept "reference sample" (a sample from the reference individual) and "reference material", the term *controls* will be used for the latter.

MATERIALS AND METHODS

A detailed description of reference individuals, blood collection, treatment of samples and descriptive data from the questionnaire is given elsewhere [6]. The production and characterization of the used controls are also given elsewhere [7].

General concept

Nordic laboratories were invited to participate in NORIP according to the following terms:

- Each laboratory will collect serum-, plasma- and full blood samples from at least 25 reference individuals (e.g. healthy personnel and their healthy adult family members, see "Inclusion criteria") and freeze the samples at -80°C. The reference individuals should be evenly distributed for gender and age. Aliquots of samples should be collected for analysis at the laboratory and other aliquots for submission to a central bio-bank (7 serum aliquots, 2 plasma aliquots, 1 EDTA buffy

TABLE I. Common reference intervals for the Nordic countries suggested by NORIP project group.

Component	Unit	CAL Target value	CAL Source	NFKK Ref. Serum X Target value	Quality goal Bias	Gender	Age	NORIP Reference intervals: Calculated, Serum Low	90% CI	High	90% CI	N	Plasma (Li heparin) Low	90% CI	High	90% CI	N	Suggestions: Serum Low	High	Plasma Low	High
Albumin	g/L	40.52	NTP	41.5	2.1%	FM	18–39	36.5	36.3–36.7	47.9	47.5–48.4	1010	35.8	35.2–36.3	47.2	46.9–48.1	452	36	48		
							40–69			45.4		1248			45.4	45.1–45.9	589		45		
							≥70	34.4	33.5–34.8		45.2–45.6	450	34.5	33.8–34.9			244	34			
Bilirubin	μmol/L	8.5	DGKC	8.97	15.1%	FM	≥18	4.7	4.5–5.0	24	23.1–25.1	2738	5.1	4.7–5.4	26	24.3–28.4	887	5	25		
Calcium	mmol/L	2.266	NTP	2.325	1.4%	FM	≥18	2.17	2.17–2.18	2.51	2.50–2.52	2569	2.15	2.14–2.16	2.48	2.47–2.50	1204	2.15	2.51		
Calcium, albumin corrected[1]	mmol/L	2.282	See calcium and albumin	2.321	1.2%	FM	18–49	2.20	2.19–2.21	2.47	2.46–2.48	1385	2.17	2.16–2.18	2.46	2.45–2.49	623	2.17	2.47		
							≥50			2.53	2.52–2.54	1149			2.52	2.49–2.53	558		2.53		
Carbamide (urea)	mmol/L	4.8	NTP	4.910	7.9%	F	18–49	2.66	2.47–2.71	6.41	6.09–6.71	761	2.59	2.36–2.72	6.24	5.76–6.79	276	2.6	6.4		
							≥50	3.11	2.97–3.31	7.97	7.66–8.35	585	3.05	2.68–3.38	7.40	7.23–8.70	248	3.1	7.9		
						M	18–49	3.24	3.08–3.31	8.16	7.97–8.42	649	3.21	2.97–3.50	8.08	7.50–8.87	252	3.2	8.1		
							≥50	3.64	3.46–3.78			538	3.46	3.24–3.61	8.06	7.83–9.75	230	3.5			
Cholesterol	mmol/L	4.90	NTP	5.220	3.0%	FM	18–29	2.89	2.78–3.04	6.13	6.02–6.37	674	2.95	2.79–3.14	5.89	5.78–6.52	316	2.9	6.1		
							30–49	3.43	3.28–3.55	6.92	6.77–7.19	843	3.35	3.13–3.51	6.75	6.41–7.06	368	3.3	6.9		
							≥50	4.02	3.98–4.14	7.87	7.73–8.09	1216	3.89	3.79–4.01	7.35	7.22–7.62	618	3.9	7.8		
Creatininium[2]	μmol/L	70.6	NTP	73.90	4.7%	F	≥18	51.1	50.2–52.0	84.1	83.0–87.0	1391	50.5	47.4–52.7	87.5	84.5–90.4	647	50	90		
						M	≥18	63.6	62.8–64.3	100.0	98.7–101.8	1243	62.4	60.7–63.7	100.7	98.6–103.1	597	60	100		
Iron[3]	μmol/L	21.16	NTP	20.00	12.5%	FM	≥18	9.2	8.9–9.6	33.7	33.0–34.4	2309	9.0	8.3–9.4	33.7	32.2–35.0	1076	9	34		
Iron saturation[4]		0.311	See iron, TIBC	0.294	10.1%	F	18–49	0.11	0.08–0.12	0.50	0.48–0.58	162	0.12	–	0.61	–	56	0.10	0.50		
							≥50	0.14	0.11–0.17			133						0.15			
						M	≥18	0.16	0.14–0.17	0.57	0.53–0.61	368	0.14	–	0.59	–	80		0.57		
Glucose[5]	mmol/L	4.464	NTP	4.405	3.8%	FM	≥18	3.98	3.94–4.09	5.99	5.90–6.13	919	4.18	4.14–4.36	6.29	6.12–6.52	527	4.0	6.0	4.2	6.3
						F	≥18	3.94	3.86–4.05	5.87	5.68–5.99	482	4.13	3.97–4.18	6.12	5.91–6.30	271				
						M	≥18	4.17	4.08–4.24	6.21	5.96–6.50	436	4.47	4.34–4.55	6.54	6.19–6.99	256				
HDL-cholesterol	mmol/L	1.331	NORIP	1.387	9.0%	F	≥18	1.03	0.99–1.06	2.61	2.54–2.66	1379	1.04	0.98–1.08	2.68	2.59–2.79	644	1.0	2.7		
						M	≥18	0.83	0.79–0.86	2.13	2.05–2.16	1222	0.80	0.75–0.85	2.14	2.09–2.28	586	0.8	2.1		
Potassium[6]	mmol/L	3.74	NTP	3.732	2.3%	FM	≥18	3.61	3.60–3.63	4.64	4.61–4.66	2608	3.47	3.45–3.49	4.38	4.32–4.43	1172	3.6	4.6	3.5	4.4

TABLE I. (Continued).

Component	Unit	CAL Target value	CAL Source	NFKK Ref. Serum X Target value	Quality goal Bias	Gender	Age	NORIP Reference intervals: Calculated, Serum Low	90% CI	High	90% CI	N	Plasma (Li heparin) Low	90% CI	High	90% CI	N	Suggestions Serum Low	Serum High	Plasma Low	Plasma High
LDL-cholesterol	mmol/L	2.91	See chol., HDL-chol., trigl.	3.19	9.1%	FM	18–29	1.24	1.06–1.33	4.29	3.98–4.38	275	1.21	0.58–1.36	4.00	3.68–4.30	144	1.2	4.3		
							30–49	1.39	1.28–1.68	4.71	4.39–5.11	310	1.47	1.16–1.61	4.25	3.95–4.95	159	1.4	4.7		
							≥50	1.98	1.86–2.16	5.35	5.13–5.67	579	1.94	1.73–2.05	5.08	4.89–5.86	351	2.0	5.3		
Magnesium	mmol/L	0.797	NTP	0.810	2.6%	FM	≥18	0.71	0.70–0.71	0.94	0.93–0.95	2123	0.71	0.71–0.72	0.93	0.93–0.94	943	0.71	0.94		
Sodium	mmol/L	137.4	NTP	140.65	0.5%	FM	≥18	136.7	136.3–136.9	144.8	144.5–145.1	2642	136.7	136.4–137.1	143.6	143.4–143.9	1291	137	145		144
Phosphate	mmol/L	1.03	DGKC	1.04	5.4%	F	≥18	0.85	0.84–0.87	1.49	1.45–1.50	1365	0.76	0.72–0.78	1.41	1.37–1.45	618	0.85	1.50	0.76	1.41
						M	18–49	0.75	0.73–0.77	1.63	1.57–1.70	670	0.71	0.69–0.73	1.53	1.45–1.59	298	0.75	1.65	0.71	1.53
							≥50			1.33	1.31–1.39	558			1.23	1.16–1.31	271		1.35		1.23
Protein	g/L	67.1	DGKC	68.7	2.1%	FM	≥18	62.4	62.0–62.7	77.9	77.5–78.8	1985	64.3	63.8–64.9	79.5	79.2–80.0	877	62	78	64	79
TIBC[7]	µmol/L	68.0	NORIP (IFCC methods)	68.0	4.8%	FM	≥18	48.9	48.5–50.1	83.4	81.1–85.7	668	47.4	44.7–49.8	79.8	76.0–84.5	136	49	83	47	80
Triglyceride[8]	mmol/L	1.31	DGKC	1.287	16.4%	FM	≥18	0.47	0.44–0.48	2.60	2.35–2.86	1203	0.45	0.42–0.48	2.39	2.21–2.55	704	0.45	2.60		
Urate (uric acid)	µmol/L	290.2	NTP	309.9	7.2%	F	18–49	154	148–159	350	340–365	780	160	142–168	365	333–407	280	155	350		
							≥50			394	379–414	608			421	397–456	257		400		
						M	≥18	231	225–239	475	466–481	1232	227	213–235	482	455–502	503	230	480		
Enzymes [8]																					
Alanine transaminase	U/L	17.8	DGKC	24.2	14.1%	F	≥18	8	6.7–8.5	46	43–49	1220	7	6–8	45	37–50	482	10	45		
						M	≥18	10	8.9–10.9	68	63–74	1080	10	9–11	68	56–87	443		70		
Aspartate transaminase[9]	U/L	23.6	NORIP	25.5	7.9%	F	≥18	13	12–13	37	35–38	1128	14	13–14	36	34–38	533	15	35		
						M	≥18	14	13–15	45	43–47	1012	16	16–17	45	43–52	480		45		
Creatinekinase	U/L	118.8	DGKC	133.3	16.8%	F	≥18	33	31–35	207	180–233	1048	35	32–36	215	192–257	473	35	210		
						M	18–49	50	45–54	398	351–487	397	55	49–62	481	308–738	175	50	400		
							≥50	39	36–46	277	252–415	404	42.0	42–46	405	261–475	200	40	280		
Alkaline phosphatase[10]	U/L	64.0	NORIP	72.5	10.3%	FM	≥18	37	36–39	106	101–113	954	44.0	35–48	95	90–113	141	35	105		
γ-glutamyl-transferase	U/L	35.8	NTP	35.42	14.1%	F	18–39	10	9–11	42	34–54	283	9.0	–	42	–	113	10	45		
						F	≥40	11	10–11	77	64–81	445	9.0	3–10	77	61–92	206	10	75		
						M	18–39	12	10–13	78	56–168	244	11.0	–	117	–	104	10	80		
						M	≥40	15	14–16	114	99–134	409	13.0	10–14	109	72–127	185	15	115		

TABLE I. (Continued).

Component	Unit	CAL Target value	CAL Source	NFKK Ref. Serum X Target value	Quality goal Bias	Gender	Age	NORIP Reference intervals: Calculated: Serum Low	90% CI	High	90% CI	N	Plasma (Li heparin) Low	90% CI	High	90% CI	N	Suggestions: Serum Low	High	Plasma Low	High
Amylase	U/L	55.4	NORIP	60.7	14.7%	FM	≥18	27	25–29	118	113–124	719	24	20–29	115	99–122	311	25	120		
Amylase, pancreatic	U/L	27.0	NORIP	28.6	17.5%	FM	≥18	11	6–13	64	54–68	497	11	8–13	61	49–71	218	10	65		
Lactate dehydrogenase[11]	U/L	141	NORIP	147.8	6.3%	FM	18–69	103	90–106	204	198–210	372	–		–		0	105	205		
							≥70	114	–	255	–	87	–		–		0	115	255		

NFKK = Scandinavian Society of Clinical Chemistry; NORIP = Nordic Reference Interval Project; NTP = Nordic Trueness Project; DGKC = Deutsche Gesellschaft für Klinische Chemie; TIBC = total iron-binding capacity; IFCC = International Federation of Clinical Chemistry.

Explanations to the column labels:

"Target value": Target value for CAL is used to correct non-enzyme reference values.

"Source": NTP: Transferred value from IMEP 17 Material 1 in Nordic Trueness Project, 2002. DGKC: Reference method value from DGKC, 1997. NORIP: Median of CAL values in NORIP.

"Quality goal": The percentages are calculated for each property as 0.375 of total biological variation calculated from reference intervals in Table I as $[\ln(H)-\ln(L)]/4$ for lognormal distributions or as $0.5\cdot(H-L)/(H+L)$ for normal distributions where H (high reference limit) minus L (low reference limit) is the most narrow suggested reference interval for that property.

"LOW" and "HIGH": Low and high reference limit.

"Gender": F-female, M-male

"Calculated": The reference limits and 90% confidence intervals (90% CI) in most cases are given with one decimal more than is reasonable to use in practice.

"N": Number of reference values used to calculate reference interval.

[1]Calcium + 0.020 × (41.3 − albumin) where 41.3 g/L is the albumin median.

[2]See Table III and plot of enzymatic -, Vitros – and Jaffé methods on NORIP home site [4].

[3]Results < 6 umol/L removed.

[4]Oestrogen users and iron < 6 umol/L removed.

[5]Fasting (≥12 h).

[6]See Table II LDL-cholesterol = cholesterol − HDL-cholesterol - triglyceride/2, where triglyceride is < 4.0 mmol/L.

[7]Oestrogen users removed.

[8]Fasting (≥12 h).

[9]Results for individuals that participated in strenuous sports during the last week before sample collection are excluded.

[10]Roche Modular and Vitros for serum and only Vitros for plasma.

[11]Only Roche Modular.

coat, each 1 mL). Each reference individual should fill out a questionnaire in order to collect relevant data for compilation and evaluation of reference intervals.

- Each laboratory will receive 5 controls on dry ice: CAL, X, HIGH, LOW, P. The material CAL holds reference method target values for most properties and will be the "calibrator" of the project. Material X with transferred target values is produced for future use. Both CAL and X are unmodified fresh frozen serum pools from male blood donors. Material HIGH is a serum pool concentrated by freeze-drying and LOW is material HIGH diluted 1:2 with an aqueous solution of sodium and calcium. These two materials, HIGH and LOW, will be used to evaluate method linearity. P is a serum pool from women using oestrogen and serves as a general control with somewhat different properties than the other pools.
- Each laboratory will measure the controls (10 replicates of CAL and 3 of each of the other controls) along with the samples in at least one series. If additional series are run, 10 replicates of X will be included with the samples in each series. All laboratories will be asked to analyse the thawed samples. The laboratories are also encouraged to carry out measurements of fresh serum and plasma samples with controls in the series described above.
- Each laboratory will submit analytical data, method data and reference individual data to a central database.
- Reference intervals will be computed centrally.
- Each laboratory will contribute $600 to the project group for the whole project.
- When the project is finished, the bio- and data-bank will be administered by the NFKK.

Ethical considerations

Reference subjects participated in the project after receiving written information and giving written consent. The study was approved by ethics committees in all the Nordic countries. Owing to different rules relating to the integrity of subjects and samples, slightly different routines were used in the five countries. In Sweden, the reference individuals are allowed to retrieve samples stored in the bio-bank and also to obtain results. In the other countries, the results were made unidentifiable and not traceable to individual subjects.

INCLUSION CRITERIA FOR REFERENCE INDIVIDUALS

The reference individual should

- be subjectively healthy;
- have reached the age of 18 years;
- not be pregnant or breastfeeding;
- not have been submitted to hospital nor have been seriously ill during the past month;
- not have consumed more than 2 measures of alcohol (24 g pure alcohol) in the last 24 h;
- not have given blood as a donor in the past 5 months;
- not have taken prescribed drugs other than oral contraceptives or oestrogens during the past 2 weeks;
- not have smoked during the hour before blood sampling.

SAMPLE HANDLING

Sample collection

The reference individual should sit for at least 15 min before sample collection. The sample should be collected using the standard technique from the cubital vein with as little stasis as possible.

Preanalytical handling

Heparin- and EDTA sample tubes should be mixed by turning the sample tubes 10 times. If possible, the sample tubes should be placed in the dark in order not to influence bilirubin concentrations.

After sample collection, the primary tubes without additives should be kept at room temperature for 30 min to 1½ h before centrifugation and Li-heparin tubes should be centrifuged within 15 min, at room temperature. The samples should be centrifuged for 10 min (at a minimum of 1500 *g*).

The serum samples should be distributed to secondary sample tubes within 2 h after sample collection, and plasma within 30 min. The

samples should be placed at −80°C within 4 h after sample collection.

Analysis

Before analysis, the samples should be thawed at room temperature in the dark for one hour, and then mixed by turning the tubes 10 times. The measurements should be done within 4 h after thawing of the samples.

If fresh samples were used for analysis, the measurements should be done within 8 h after sample collection. Reference samples and controls should be run in one series, one measurement of each reference sample, and the controls according to the terms given above.

DATA

Data collected from participating laboratories

- Analytical method: method categories according to the Labquality (http://www.labquality.fi/) Clinical Chemistry Methods Guide 1999–2000: Instrument manufacturer, instrument name, method group, method name, unit. In addition: Slope and intercept ($V_s = V_i \times \text{slope} + \text{intercept}$, where V_s is submitted value and V_i is original instrument value).
- Reference individual data registered on questionnaire: identification code number (these codes are project-specific and retain the anonymity of the reference persons), age, gender, height, weight, date of first day of last menstrual period (women), ethnic origin, heredity for diabetes, number of years residing in a Nordic country, chronic disease(s), medication, strenuous exercise during last week, alcohol consumption, habitual smoking, number of hours from the last meal, date of blood sampling, total number of blood donations.
- Control analytical data: control ID, measurement date, series no. (a common number for control values and reference values measured in the same series), measurement value.
- Reference individual analytical data: project no. for the individual, measurement date, series no., material (serum or plasma), material handling (fresh or thawed), measurement value.

Database

A total of 102 laboratories participated in the project giving approximately 200 000 measurement data, 125 000 reference values (of which nearly half are on thawed serum) from 3036 reference individuals and approximately 75 000 control values.

All data are stored in a Microsoft Access relational database at Fürst Medical Laboratory, Oslo, and are administered by Pål Rustad. The database is stored on a central server and is password restricted. The content is backedupeach day on a tape and is stored in a fireproofcabinet. Back-up is available for 3 months. A copy of the database is also storedon a compact disc located in another building.

Data handling

Enzymes and non-enzymes are treated differently.

Enzymes. With respect to enzyme methodology, only results obtained by routine assay conditions at 37°C, which were compatible with and traceable to the IFCC reference methods, were included. Only complete measurement systems, i.e. reagents, calibrator and instrumentation supplied by the same manufacturer, were accepted. In cases where local adjustment of slopes and intercepts had been used, the reported values were recalculated to the original values [8].

Non-enzymes. For each series, the reference values were multiplied by the factor Target CAL/Mean CAL in that series. For series with only X (see "General concept") the factor Target X/Mean X was used.

Target values for control sera

The target values for CAL were established in three different ways depending on the property in question (see Table I):

1. Transferred value from IMEP 17, Material 1 [9] to CAL by The Nordic Trueness Project, 2002 [10].
2. Reference method values established by DGKC (Deutsche Gesellschaft für Klinische Chemie) in 1997.

3. Median of all laboratory means in NORIP (HDL-cholesterol and total iron-binding capacity (TIBC) only).

The target value for X was either established as transferred value from IMEP 17, Material 1 in The Nordic Trueness Project or as transferred value from CAL in NORIP.

Data exclusion

Data have been excluded for different reasons:

- Insufficient control data enclosed with the reported reference values.
- Same samples measured by different methods on same property.
- Material deviation: large differences between results obtained for a quantity from varying materials (fresh/thawed, serum/plasma) for the same individual—clearly deviating values were excluded.
- Exclusions of individuals (exclusion criteria): extreme values for one or more properties for one individual excluded all results for that individual:
  - Glucose ≥ 11.1 mmol/L, fasting glucose ≥ 7.0 mmol/L (fasting ≥ 12 h).
  - 5s/3s and 4s/4s rule: At least one value outside median $\pm$ 5s for one property and at least one value for a different property outside median $\pm$ 3s (5s 3s rule). The same rule has also been applied with 4s limits for both properties (s is the total biological variation based on NORIP data, logaritmic transformations).
- Method exclusions: enzyme methods not compatible with the IFCC 37°C reference method or traceability questioned. UIBC reported as TIBC method, ionized calcium reported as total calcium method, poor correlation between serum and plasma for some methods.
- Property-specific exclusions: non-fasting (triglyceride, glucose), diabetes in near family (glucose), physical activity (CK), oestrogen use (TIBC), iron values < 6 µmol/L (iron and iron saturation).
- Outliers for enzymes: results lying outside the interval mean $\pm$ 4s, where s is the standard deviation of a reference distribution, were excluded as outliers. This rule was applied to gender-specific distributions after logarithmic transformation.
- Outliers for non-enzymes: Dixon's rule as implemented in the RefVal 4.0 program [11] was used to define outliers (see "Calculation of reference intervals").

CALCULATION OF REFERENCE INTERVALS

Random number addition

If the laboratory has submitted the data with n number of decimals, then the least significant digit (LSD) $= 10^{-n}$. If the submitted reference value is R, then a random number between R $-$ LSD/2 and R $+$ LSD/2 is added. This has been done because we wanted to weight the uncertainty of rounding measurement values (e.g. when different units have been used for the same property) and to eliminate the tied (equal) value problem (when interpolating to find percentile, number of equal values are not taken into account) when calculating reference intervals.

Standard software

A simple non-parametric method has been used to calculate low and high reference limits as 2.5 and 97.5 percentiles of the distribution of reference values. Calculations have been done using the computer program Refval 4.0 [11] based on the IFCC recommendations.

Partitioning

Partitioning of distribution of reference values has been evaluated using a theory outlined by Lahti *et al.* [12] and incorporated in a special version of Refval 4.0. The criteria for no partitioning is that $> 0.9\%$ and $< 4.1\%$ of each subdistribution should be outside the 2.5- and 97.5 percentiles of the common distribution.

Gender partitioning was mostly decided by use of this program. Reasonable age limits have been estimated by "qualified guessing" prior to exposure to the partitioning program.

Clinical evaluation of reference intervals

Seven work groups consisting of clinical biochemists from different laboratories in Norway have evaluated the reference intervals for all properties based upon the information presented on the NORIP home site [4]. The results of this evaluation were presented at a one-day meeting in April 2003, in Oslo (these reports are also available on the NORIP home site). Most of the suggestions from the groups have been taken into account in the final proposal presented below.

DOCUMENTATION

Results from NORIP have been continuously updated on the NORIP home site [4]. To see specific details for each property, first select "Preliminary project data", then "Compiled data for each report", then select the specific property from the table.

RESULTS

Proposed reference intervals

See Table I.

NFKK Reference Serum X

Control X used in this project is now officially named "NFKK Reference Serum X" and is commercially available through DEKS (Danish Institute for External Quality Assurance for Laboratories in Health Care, Denmark, http://www.deks.dk/). Certified and indicative values for this serum are established either as transferred values from IMEP 17, Material 1 in the Nordic Trueness Project or as transferred values from CAL in NORIP or as median of laboratory mean in NORIP [9, 13].

Nordic Reference Interval Project Bio- and Data Bank (NOBIDA)

By establishing a bio-bank, the intention is to make the samples available for possible future projects on Nordic reference intervals for other properties than those described here. NFKK has established a group that will handle requests for data and samples from NOBIDA. The leader for the group is Pål Rustad. Guidelines for requests are published on the NORIP home site. The bio-bank, including NFKK Reference Serum X, is located at DEKS and the data are located at Fürst Medical Laboratory, Oslo.

DISCUSSION

Selection of reference individuals

Ideally, reference individuals should have been selected randomly from the population the reference intervals are intended to serve. This principle is not strictly followed in all aspects:

- Reference individuals are selected from readily available individuals in the local surroundings of the participating laboratories throughout the Nordic countries. This might be a problem, but we have no indications of possible bias or dispersion that may stem from this deviation from the ideal conditions.
- The prevalence of reference values from each country is not in concordance with the relative populations in the Nordic countries. Sweden deviates the most in this respect. As the calculations show that the differences between countries are small enough to support common reference intervals, this deviation is considered to be of minor importance.
- The age distribution of the reference individuals is not in agreement with the age distribution of the populations in the Nordic countries. This is a result of the original concept of the project to obtain samples evenly distributed in each age group.

A general theory for correction of prevalence deviations [14] and examples from NORIP for some enzymes on the relative number of reference values from each country compared to the relative country populations are presented by Lahti [15].

Analytical methods

Traceability. When NORIP started out it was not clear how the effect of the plurality of laboratories and routine measurement methods compared to the traditional use of one routine method would affect the calculated reference

intervals. In order for the laboratories and the IVD manufacturers to accept the possible deviations found for individual routine methods, it was decided that laboratory and method biases should be corrected by using control materials of the highest possible quality to be measured together with the reference samples. It was crucial to use commutable materials. It was also essential that the target values used for the project "calibrator" were traceable to the highest metrological order. Looking at the outcome, the general conclusion is that this part of the project design has been successful.

Correction. For the NORIP concept to work properly, it is crucial that the control materials are commutable. However, this demand leaves us with a project calibrator where the concentrations are low for some properties. Among the non-enzymes this could be a problem for bilirubin. For this property, however, the reference limits turned out to be nearly identical whether correction was applied or not. For the enzymes where the calibrator levels also were low, it was decided that no calibrator correction should be done, but instead strict criteria on methodology and traceability to the IFCC reference methods (37°C) was applied.

For each property and each laboratory, an evaluation on the fraction HIGH/LOW was done, following calculation of reference intervals before and after removal of laboratories with the largest deviations [16]. This did not bring about any apparent changes in the calculated reference intervals and led to the decision not to eliminate laboratories with somewhat deviating corrected control values. This is considered beneficial because the quality of the evaluation of subgroups will improve with number of reference values in each subgroup. The control P was included in the project because of the different characteristics (serum pool from females using contraceptives) than the other serum pools used as controls. Evaluation of the results on control P relative to other controls did not show any apparent differences between measurement systems.

Reference interval calculation

The Refval 4.0 software calculates reference intervals using three different methods: the simple non-parametric, the bootstrap non-parametric and the parametric methods. The parametric method uses a two-step transformation procedure to normalize a distribution. As such transformations in many cases were less successful, the project group decided that a simple non-parametric method, independent of distribution shape, should be the method of choice. Even if some researchers prefer the bootstrap method with somewhat narrower 90% confidence intervals for the reference limits, our experience is that both methods give much the same results.

During the project an improved theory on subgroup partitioning was developed [12] and used. The recommended intervals from the program have generally been adopted with few exceptions where the clinical use for partitioning was marginal.

The obvious question when making common reference intervals for several countries is whether the reference intervals are truly common [15]. This question is perhaps the most relevant for Iceland with its small and homogeneous population. Because the number of reference values is small compared with that of the other countries, the uncertainty regarding the calculated country reference intervals is greater, thus leaving this question only roughly answered. On the other hand, the influence on the overall distribution is small. Country differences are presented elsewhere [4, 5].

There are many aspects of the data that have not been investigated in detail. These include relations between reference values and information from the questionnaire, i.e. the biological, geographical and sample collection parameters of each individual. Gender, age, country differences and body mass index have been carefully investigated and to a certain extent fasting [5]. However, parameters not especially mentioned are still not systematically investigated.

Specific properties

Creatininium. As for all other non-enzymes, the reference values have been corrected to a level corresponding to the reference method value of CAL (isotope dilution GC-MS reference method) irrespective of the method used. Plots of the reference value distributions for males and females originating from the three major method groups, wet chemistry Jaffé,

wet chemistry enzymatic methods and Ortho Vitros, are shown on the NORIP home site [4]. The CAL-corrected reference intervals for the three method groups are presented in Table II. The proportional correction which is done here is not optimal for the Jaffé method because the measured value is not proportional to the true creatininium value. For the Jaffé method a non-proportional contribution is assumed because of non-creatininium chromogens. This problem may not be identified with the high and low controls because the content of non-creatininium chromogens is concentrated/diluted to the same extent as true creatininium in the production process of these controls.

It is interesting that the reference intervals originating from the three method groups are reasonably similar despite the well-known unspecificity of the Jaffé method. Therefore the project group recommends common reference intervals for creatininium (Table II) irrespective of the method used as long as the trueness of the method is according to CAL- or X level corresponding to enzyme method level.

Potassium. For potassium, NORIP, Tietz [17] and Laurell [18] all agree on separate reference intervals for serum and plasma. The plasma intervals are almost identical, but both Tietz and Laurell suggest upper limits for serum that are about 0.5 mmol/L higher than those for plasma, which is not in agreement with NORIP. As it is well known that potassium continuously leaks from cells to plasma before separation, one might suspect that Tietz and Laurell incorporate non-standard preanalytical errors in their reference interval. It might be assumed that sample treatment has been optimal in this project (see "Sample handling") compared with what is common in general practice.

Enzymes. The suggested reference intervals of the enzymes are discussed elsewhere [8]. Briefly it is concluded that: the upper reference limits for ALT, AST, CK and GT are markedly higher than those recently reported on behalf of the IFCC expert panel. Our limits for these enzymes are also somewhat higher than those currently in common use in the Scandinavian countries and in other parts of the world. Of the eight upper limits presented, the ones for CK deviate most from those commonly used, particularly that for men < 50 years of age. But several others have previously reported similarly high limits for young men.

Other properties. Descriptive data from the project and further discussion of the particular properties are presented elsewhere [5].

The haematology project is discussed elsewhere [3].

Implementation of common reference intervals

One important aspect of the project design was to engage the Nordic laboratories in the production of new reference intervals, which in turn would facilitate the implementation of the results. The Nordic societies of clinical biochemistry under the umbrella organization NFKK have taken the necessary initiatives to implement the reference intervals. By now, enzyme reference intervals have been implemented in Norway (May 2003) and in Denmark (from December 2003). Intervals for all properties will be implemented in the rest of the Nordic countries during 2004.

Harmonization of method

By using commutable controls with certified reference values, the opportunity to further

TABLE II. Creatininium reference intervals with 90% confidence limits for the three method groups.

	Female			Male		
Method	Reference interval (μmol/L)	90% confidence intervals (μmol/L)	N	Reference interval (μmol/L)	90% confidence intervals (μmol/L)	N
Enzymatic	46 – 92	41 – 50, 86 – 96	137	60 – 105	57 – 64, 101 – 109	113
Jaffé	52 – 84	51 – 53, 83 – 87	944	64 – 98	62 – 65, 96 – 100	858
Ortho	50 – 81	49 – 52, 79 – 83	298	64 – 102	63 – 66, 99 – 105	259
NORIP suggestion	50 – 90			60 – 100		

improve analytical quality by harmonization of measurement trueness when implementing the reference intervals has been the key intention of the project.

The control serum X is available through DEKS as "NFKK Reference Serum X" to facilitate the trueness validation of the local methods.

In order to implement the common reference intervals in the individual laboratories, the project group suggests that a quality goal of absolute bias relative to target values for the control X of less than 0.375 of total biological variation should be held. These quality goals are presented in Table I for each property in the column "Quality goal". This means that the laboratory ought to fulfil the criteria: $|M/T-1|<B$ where M is the measured value of X in the laboratory, T is the target value of X and B is relative bias quality goal (as suggested in Table I). As this approach only tests the method on one level, the laboratory should also ascertain that there is no concentration-independent bias relative to a reference method. This applies, for example, to creatininium. For laboratories using the Jaffé method, care should be taken to ensure agreement with the reference method over the *whole* measuring range. Concerning the Vitros method, although enzymatic in nature, this method has so far been calibrated to agree with the Jaffé method. Ortho has recently released the algorithms used for the conversions (according to Ortho, the relation between the Vitros results and their HPLC reference method is: $\text{VITROS}=0.98\times\text{HPLC}+7.96$ μmol/L.), so it should be possible to ensure traceability and compliance also for the Vitros method.

For the enzymes and some other properties with low target values for CAL and X, it might be necessary also to validate the method with respect to trueness at the levels of the clinically important reference limits.

If the criteria for the use of common reference intervals are not fulfilled, the laboratories are advised to investigate whether the reason is laboratory- or method related. If there is a method discrepancy, the IVD manufacturer should be addressed in order to collaborate on a general strategy to ensure the method complies with quality goals for implementation of the new reference intervals.

On the NORIP home site, comparisons between serum and plasma for different measurement systems are presented. These comparisons should be taken into account before reference intervals for plasma are brought into use.

ACKNOWLEDGEMENTS

The work was carried out in close cooperation with the community of clinical chemistry in the Nordic countries. The analytical and logistic work was made possible by the staffs at the participating laboratories. We thank other persons and institutions (in alphabetical order) outside the NORIP project group for invaluable contributions to the final result:

Ole Blaabjerg, Odense University Hospital, Odense, for supervision of production of the controls HIGH and LOW.

Ivan Brandslund, Vejle Hospital and Vejle Amt, for providing the project with the data registration program.

Christian Enggaard, Nalge Nunc International, for providing sample tubes free of charge.

Nils Jørgensen, Sønderborg Hospital, Sønderborg, for advice on data handling.

Minna Loikkanen, Labquality, Helsinki, for providing the project with the method database, providing us with a part of CAL and for the practical aspects concerning the project in Finland.

Gunnar Nordin, EQUALIS, Uppsala, for handling the practical aspects of the project in Sweden, also as the key person in the haematology part of the project.

Elin Olavsdottir, Landspitallin, Reykjavik, for translating the project description to English.

Inger Nørgaard, Hjørring Hospital, Hjørring, for production of the controls P, HIGH and LOW.

Kjell Torgeir Stokke, the project leader's chief at Fürst Medical Laboratory, Oslo, for allowing him to have a free hand with this project.

Petter Urdal, Ullevål University Hospital, Oslo, one of the persons to take the initiative in the project, also the organizer of the seven Norwegian groups who evaluated the NORIP results.

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Received: 15 January 2004
Accepted: 12 March 2004