NATFORLAB 2026

Reference intervals in clinical laboratory practice: from IFCC guidelines to everyday application

… why transfer is common and why local verification remains essential ...

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WHY REFERENCE INTERVALS MATTER

A laboratory result becomes useful only in context

The central 95% reference interval describes a defined reference population under specified preanalytical and analytical conditions.
It is not a diagnostic cutoff and does not replace clinical judgment.

Sources: Biochem Med 2016;26:5-16. (Ozarda Y.), Crit Rev Clin Lab Sci. 2023;60:466-482. (Doyle K, Bunch DR.)

WHY REFERENCE INTERVALS MATTER

A reference interval is not “just a range”

A number becomes clinically meaningful only after we decide what we are comparing it with.

- A transferred reference interval is a hypothesis - local verification tests whether it works
- The goal is defensible interpretation for local patients

Everyday question

“Can I safely use this reference interval for the patients population and measurement system in laboratory?”

Source: CLSI EP28-A3c (published 2010; reaffirmed as suitable without content revision in 2020).

ROUTINE LABORATORY REALITY

Why EP28-A3c remains the practical foundation

IFCC/ CLSI

EP28-A3c

Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory

Approved Guideline -Third Edition

Offers a protocol for determining reference intervals that meet the minimum requirements for reliability and usefulness.

Discusses the transfer of established reference values from one laboratory to another.

Covers the whole lifecycle:

define → establish → transfer → verify

Source: CLSI EP28-A3c (published 2010; reaffirmed as suitable without content revision in 2020).

ROUTINE LABORATORY REALITY

Most laboratories transfer an RI - then must verify it locally

Potential source	Evidence strength and transparency	Local action
Harmonized RI studies / expert group	standardized protocol broader population explicit analytical method	**VERIFY**
Peer-reviewed single - or multicenter study	defined population defined analytical system	**ASSESS + VERIFY**
Manufacturer instructions	often limited population detail may be platform- specific	**VERIFY – DO NOT ACCEPT AUTOMATICALLY**

Transfer - "where did the interval come from?" **Verify - "does it fit here?"**

Sources: Biochem Med 2016;26:5-16. (Ozarda Y), Crit Rev Clin Lab Sci. 2023;60:466-482. (Doyle K, Bunch DR), Clin Chem. 2023;69:991-1008. (Bohn MK et al.), Clin Chem. 2015;61:1012-1015. (Tate JR, Yen T, Jones GRD), Scand J Clin Lab Invest. 2021;81:264-271. (Xu P, Zhou Q, Xu J.).

DIRECT APPROACH

Direct establishment starts with selected reference individuals

1	**Define**	population / measurand / specimen/ method
2	**Recruit**	apparently healthy individuals
3	**Standardize**	collection / handling and analysis
4	**Exclude / partition**	a priori and / or a posteriori criteria
5	**Estimate**	2.5^{th} - 97.5^{th} percentiles + confidence intervals

≥ 120

per partition
for a robust nonparametric RI

Strength:
- **clean provenance**
- **controlled conditions**

Constraint:
- **recruitment**
- **time**
- **cost**
- **hard-to-access partitions**

Sources: Biochem Med 2016;26:5-16. (Ozarda Y.), Crit Rev Clin Lab Sci. 2023;60:466-482. (Doyle K, Bunch DR.), CLSI EP28-A3c (published 2010; reaffirmed as suitable without content revision in 2020).

CHANGE OF EVERYDAY PRACTICE

EP28- A3c guideline turned into an actionable laboratory process

„With as few as 20 samples from reference individuals, a laboratory can verify reasonably well the applicability of a reference interval to its own population and methodology."

„A laboratory can verify a reference interval, established by more stringent techniques elsewhere, by collecting as few as 20 samples from qualified reference individuals."

CLSI EP28-A3c sample, Section 11 context

≥ 120

per partition
for a robust nonparametric RI

Meaning ?

It is not necessary to rebuild every interval from zero. You have to show that the interval is appropriate for population and measurement procedure.

Source: CLSI EP28-A3c (published 2010; reaffirmed as suitable without content revision in 2020).

SIDE-BY-SIDE COMPARISON

Direct and indirect approaches answer the same question with different evidence

DIRECT	INDIRECT	WHAT TO CHECK
Prospective reference individuals	**Retrospective routine results**	Source population
Strong preanalytical control	**Real- world heterogeneity**	Specimen + workflow
Lower N; higher recruitment burden	**Very large N; informatics burden**	Feasibility
Transparent exclusions	**Algorithm- dependent separation**	Pathology removal
Best for de novo, well- defined studies	**Best for local, continuous RIs**	Intended use

Sources: Crit Rev Clin Lab Sci. 2023;60:466-482. (Doyle K, Bunch DR), Crit Rev Clin Lab Sci. 2023;60:427–441. (Ma S, Yu J, Qin X, Liu J.), Clin Chem. 2023;69:991-1008. (Bohn MK et al.), Clin Chem. 2023;69:945–947. (Ceriotti F, Vidali M.)

IFCC HARMONIZATION ADDS VALUE

Harmonization before local verification

The IFCC Committee on Reference Intervals and Decision Limits

C-RIDL

harmonized protocols
global studies
standardization and education
best- practice strategies

The IFCC Task Force on Global Reference Interval Database

TF-GRID

centralised global RI database
for CC/hematology
harmonized calculation tools
cross- country comparisons

Local clinical laboratory verification

confirms population fit

confirms analytical method/platform fit

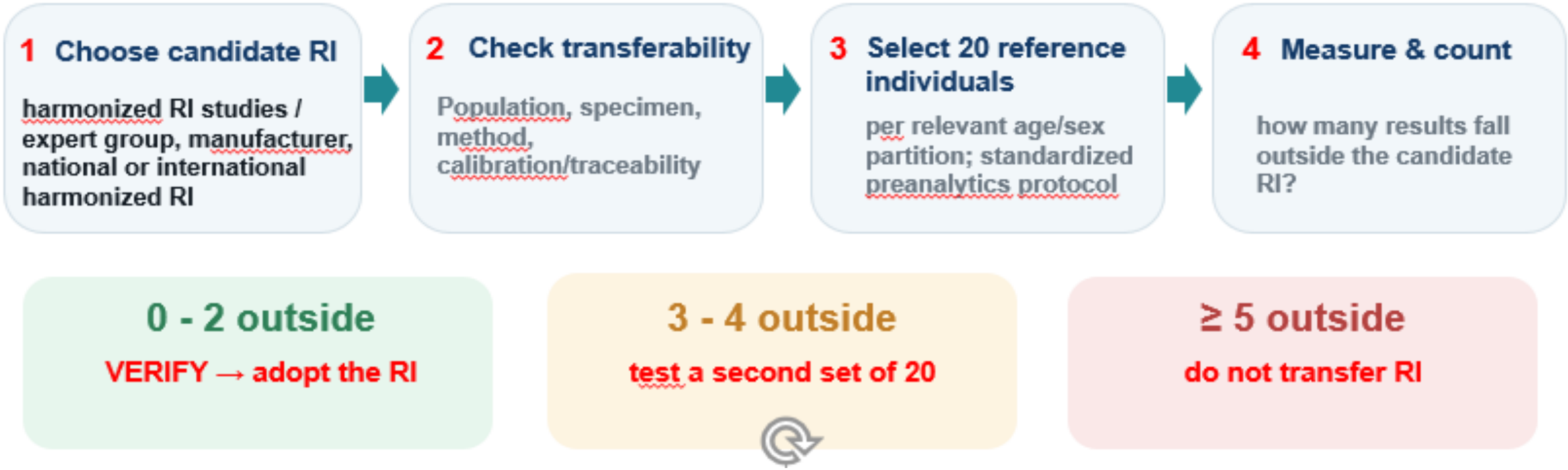
verifies before implementation in clinical practice

Verification remains esential before use in clinical practice due to population and laboratory specific conditions.

Source: https://ifcc.org/ifcc-scientific-division/sd-committees/c-ridl/ (accessed 31sth of August 2026)

A PRACTICAL EP28-A3c TRANSFER & VERIFICATION PATHWAY

Verification process flow that most laboratories can apply



If the second set also fails → investigate analytical/biological differences → choose a better source or establish an RI locally

Source: CLSI EP28-A3c (published 2010; reaffirmed as suitable without content revision in 2020)

A PRACTICAL EP28-A3c TRANSFER & VERIFICATION PATHWAY AT NATIONAL LEVEL

Croatia built a harmonized foundation and continued targeted RIs verification

1993 ● **Zagreb reference state-population**

RIs for 56 hematological and biochemical constituents of blood and serum from reference individuals

Reference intervals of 56 haematological and biochemical constituents of blood and serum for reference individuals corresponding to "reference state" - population of Zagreb, Croatia (CROSBI ID 177177)

Prilog u časopisu | izvorni znanstveni rad | međunarodna recenzija

Flegar-Meštrić, Zlata ; Tadej, Danica ; Vrhovski- Hebrang, Danijela
Reference intervals of 56 haematological and biochemical constituents of blood and serum for reference individuals corresponding to "reference state" - population of Zagreb, Croatia // Croatian medical journal, 34 (1993), 163-169

2004 2007 ● **Croatian RIs harmonization project Croatian Ris harmonization project expanded**

Croatian Chamber of Medical Biochemists (CCMB) initiative - first publications of nationally harmonized RIs for general, specialized and highly specialized biochemistry

Harmonizacija laboratorijskih nalaza u području opće medicinske biokemije (CROSBI ID 769310)

Druge vrste radova | elaborat/studija

Čvorišćec, Dubravka ; Flegar-Meštrić, Zlata ; Juretić, Dubravka
Harmonizacija laboratorijskih nalaza u području opće medicinske biokemije // Harmonizacija laboratorijskih nalaza u području opće medicinske biokemije, 2004

Harmonizacija općih pretraga iz područja opće medicinske-biokemije (CROSBI ID 44745)

Prilog u knjizi | stručni rad

Čvorišćec, Dubravka ; Flegar-Meštrić, Zlata ; Juretić, Dubravka
Harmonizacija općih pretraga iz područja opće medicinske-biokemije // Harmonizacija laboratorijskih nalaza u području opće, specijalne i visokodiferentne medicinske biokemije.
Zagreb: Medicinska naklada, 2007. str. 11-30

A PRACTICAL EP28-A3c TRANSFER & VERIFICATION PATHWAY AT NATIONAL LEVEL

Croatia built a harmonized foundation and has continued targeted verification

2000 — **Reference Center of the Ministry of Health of the Republic of Croatia for the development of reference values in the field of general medical biochemistry**

2010 2016 — **Targeted adult verification**

RIs for serum creatinine standardized IFCC AST/ALT methods with PLP

Comparative Study > Clin Chem Lab Med. 2010 Feb;48(2):231-5. doi: 10.1515/CCLM.2010.054.

Applicability of common reference intervals for serum creatinine concentrations to the Croatian population

Zlata Flegar-Mestrić [1], Sonja Perkov, Barbara Simonović, Dubravka Juretić

▸ World J Methodol. 2016 Mar 26;6(1):93–100. doi: 10.5662/wjm.v6.i1.93

Standardization in laboratory medicine: Adoption of common reference intervals to the Croatian population

Zlata Flegar-Meštrić [1], Sonja Perkov [1], Andrea Radeljak [1]

A PRACTICAL EP28-A3c TRANSFER & VERIFICATION PATHWAY AT NATIONAL LEVEL

Croatia built a harmonized foundation and has continued targeted verification

2018 — **Reference Center of the Ministry of Health of the Republic of Croatia for the development and application of biological reference intervals for medical biochemical tests**

2024 — **Harmonized neonatal RIs transfer and verification**

Transfer and verification of CALIPER RIs to the Croatian newborn population

> Biochem Med (Zagreb). 2024 Jun 15;34(2):020705. doi: 10.11613/BM.2024.020705. Epub 2024 Apr 15.

CLSI-based verification and *de novo* establishment of reference intervals for common biochemical assays in Croatian newborns

Iva Friščić [1], Sonja Perkov [1], Andrea Radeljak [1], Jasminka Stipanović-Kastelić [2], Mirjana Mariana Kardum Paro [1]

2026 — **Verification of national harmonized RIs**

*Biochem Med (Zagreb) 2026;36(2):**020706***

Verification of harmonized reference intervals in Croatia: is it time for a change?

Iva Friščić*[1], Ida Burazer[1], Andrea Radeljak[2], Sonja Perkov[1], Mirjana Mariana Kardum Paro[1]

[1]Department of Medical Biochemistry and Laboratory Medicine, Merkur University Hospital, Zagreb, Croatia
[2]Department for Clinical Laboratory Diagnostics, Children's Hospital Srebrnjak, Zagreb, Croatia

EXAMPLE 1 – FROM GUIDELINES TO EVERYDAY APPLICATION

Potential source	Evidence strength and transparency	Local action
Harmonized RI / expert group	standardized protocol broader population explicit analytical method	VERIFY
Peer-reviewed single - or multicenter study	defined population defined analytical system	ASSESS + VERIFY

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Defined population

direct *a posteriori* sampling method

inclusion criteria
Apgar index at birth 9/10 and 10/10
CRP, total bilirubin, hemoglobin and platelet count within RI

N=216

exclusion criteria
hospitalization > 7 days
maternal or fetal antibiotic therapy
serum sample hemolysis index ≥ 1

N=163

RIs transfer and verification

the first set of samples, N=20
calcium, inorganic phosphorous, glucose, urea, creatinine, total bilirubin, CRP, total protein, albumin, AST, ALT, GGT, ALP, LD

the second set of samples, N=40
sodium, chloride

***de novo* reference intervals, N=125**
potassium, magnesium, direct bilirubin

Defined analytical systems/ measurands

Automated Clinical Chemistry Analyzer (19 biochemical analytes)

Automated Hematology Analyzer (hemoglobin and platelet count)

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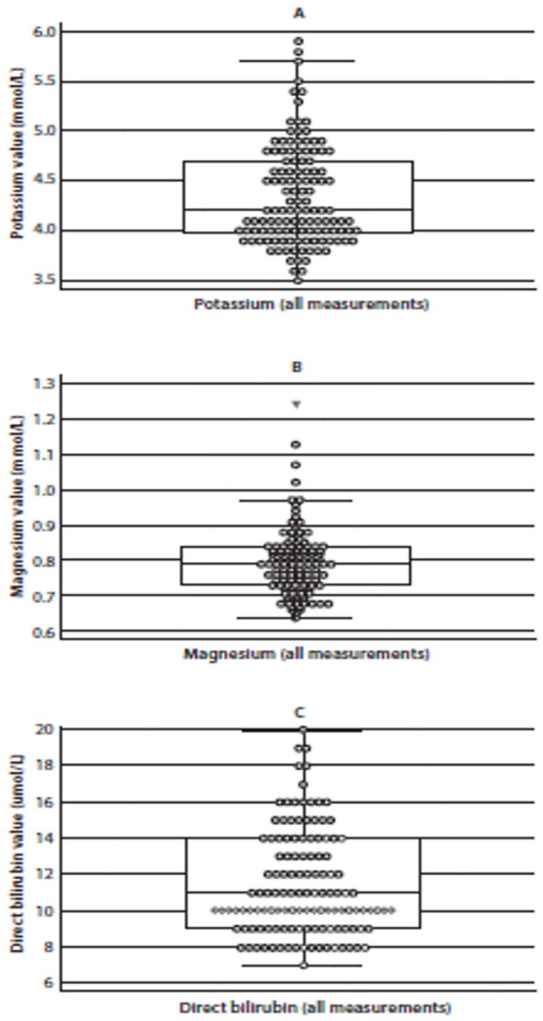

Figure 1. Box and whisker plots for potassium (A), magnesium (B) and direct bilirubin (C) before outlier exclusion. The plots show the distribution of the data. The dots outside the minimum and maximum bars are outliers and the bolded triangle in the box and whisker plot for magnesium (B) is a far-out value. There were two outliers for potassium, four for magnesium and none for direct bilirubin

Table 1. CALIPER reference interval verification results for all analytes

Analyte	Sample size	Median (IQR), first set of samples	Median (IQR), second set of samples	CALIPER reference interval	Samples within the CALIPER reference interval (first set of samples, N)	Samples within the CALIPER reference interval (second set of samples, N)
Potassium, mmol/L	40	4.1 (4.0 - 4.5)	4.1 (3.9 - 4.6)	4.2 - 6.2	13/20	12/20
Sodium, mmol/L	40	140 (139 - 142)	140 (139 - 143)	139 - 146	17/20	18/20 ✅
Chloride, mmol/L	40	107 (105 - 109)	107 (106 - 108)	104 - 109	17/20	18/20 ✅
Calcium, mmol/L	20	2.43 (2.35 - 2.51)	/	2.17 - 2.74	19/20 ✅	/
Magnesium, mmol/L	40	0.79 (0.75 - 0.83)	0.78 (0.74 - 0.83)	0.79 - 1.56	14/20	15/20
Phosphorous, mmol/L	20	2.15 (1.92 - 2.31)	/	1.76 - 3.37	19/20 ✅	/
Glucose, mmol/L	20	4.5 (4.3 - 5.0)	/	4.0 - 6.2	19/20 ✅	/
Urea, mmol/L	20	3.1 (2.6 - 3.8)	/	1.1 - 8.0	20/20 ✅	/
Creatinine, µmol/L	20	47 (39 - 61)		25 - 73	20/20 ✅	/
Total bilirubin, µmol/L	20	174 (137 - 188)	/	4 - 253	20/20 ✅	/
Direct bilirubin, µmol/L	40	11 (10 - 12)	10 (9 - 13)	3 - 8	4/20	4/20
CRP, mg/L	20	1.8 (0.9 - 3.6)	/	0.3 - 5.8	20/20 ✅	/
Total protein, g/L	20	58 (54 - 61)	/	52 - 79	20/20 ✅	/
Albumin, g/L	20	36.5 (35.4 - 38.7)	/	31 - 43	20/20 ✅	/
AST, U/L	20	47 (38 - 68)		30 - 146	20/20 ✅	
ALT, U/L	20	21 (14 - 22)	/	6 - 30	18/20 ✅	/
GGT, U/L	20	100 (76 - 132)	/	17 - 158	19/20 ✅	/
ALP, U/L	20	156 (128 - 179)	/	76 - 233	20/20 ✅	/
LD, U/L	20	544 (468 - 605)	/	256 - 1017	20/20 ✅	/

14 ✅ 2

EXAMPLE 1 – FROM GUIDELINES TO EVERYDAY APPLICATION

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TABLE 2. *De novo* reference intervals for potassium, magnesium and direct bilirubin

Analyte	Sample size (outliers excluded)	Median (IQR)	Reference interval 95th percentile	Lower limit 90% CI	Upper limit 90% CI
Potassium, mmol/L	123	4.2 (4.0 - 4.7)	3.4 - 5.2	✅ 3.3 - 3.5	5.1 - 5.3
Magnesium, mmol/L	121	0.79 (0.73 - 0.83)	0.65 - 0.92	✅ 0.63 - 0.66	0.90 - 0.94
Direct bilirubin, µmol/L	125	11 (9 - 14)	6 - 17	✅ 5 - 6	16 - 18

CI - confidence interval. IQR - interquartile range.

Transfer

"where did the interval come from?"

Verification

"does it fit here?"

EXAMPLE 2 – FROM GUIDELINES TO EVERYDAY APPLICATION

Potential source	Evidence strength and transparency	Local action
Harmonized RI / expert group	standardized protocol broader population explicit analytical method	VERIFY
Peer-reviewed single - or multicenter study	defined population defined analytical system	ASSESS + VERIFY

*Biochem Med (Zagreb) 2026;36(2):**020706***

Verification of harmonized reference intervals in Croatia: is it time for a change?

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Defined population

RIs transfer and verification

Defined analytical systems/ measurands

Population

136
apparently healthy adults
(53 M + 83 F)

a priori and *a posteriori*
exclusion criteria

100
reference persons
(40 M + 60 F)

Analytes

Hematology
23 analytes
Sysmex XN100 + iSED

Clinical chemistry
27 analytes
Beckman Coulter DxC700AU
+ Radiometer ABL90 FLEX

Coagulation
3 analytes
Sysmex CS2500

Protocol

- **standardized preanalytical conditions** (fasting time, alcohol and caffeine consumption, smoking)
- **20** reference persons per subgroup
- **CLSI EP28-A3c guidelines**

Results

- most tested reference intervals confirmed in a single sample set
- other literature sources for **total calcium** and **alkaline phosphatase** reference intervals
- need for periodical re-evaluation of reference intervals

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Table 1. Standardized questionnaire used for selection of reference individuals

Number	Question	Answer format
1	Full name	Free text
2	Age	Numeric
3	Sex	Male / Female
4	Height (cm)	Numeric
5	Weight (kg)	Numeric
6	Do you consider yourself a healthy person?	Yes / No
7	Do you suffer from any chronic diseases?	Free text
8	Have you been hospitalized in the past 3 months?	Yes (specify) / N
9	Have you had any serious illnesses in the last 3 years?	Yes (specify) / N
10	Hereditary or chronic diseases in the family	Yes (specify) / N
11	Hypertension	Yes / No
12	Regular medication, including dietary supplements	Free text
13	Physical activity	Regular (≥ 3x/week) / Oc… Never
14	When did you last exercise?	Free text
15	Smoking	Yes / No
16	Alcohol consumption	Never / Occasionally / Regularly
17	Use of addictive substances	Yes / No
18	Special diet	Free text
19	Other	Free text
20	Blood sampling in accordance with professional guidelines (filled by lab staff)	Yes / No

Table 2. Baseline characteristics of the study population used for reference interval verification

	Initial population	Final RI population	Bilirubin/liver enzymes population*	CK population†
Total	136	100	93	69
M, N (%)	53 (39)	40 (40)	39 (42)	27 (39)
F, N (%)	83 (61)	60 (60)	54 (58)	42 (61)
Age range, years (M)	26-75	26-75	26-75	29-75
Age range, years (F)	22-68	22-68	22-68	25-68

*Exclusion based on medication and dietary supplement use. †Exclusion based on physical exercise within 3 days before blood sampling. M - male. F - female. RI - reference interval. CK - creatine kinase.

EXAMPLE 2 – FROM GUIDELINES TO EVERYDAY APPLICATION

*Biochem Med (Zagreb) 2026;36(2):**020706***

Table 3. Hematology reference interval verification results

Analyte (unit)	RI partitioning	Current RI*	Median (IQR)	N	Results within the harmonized RI, N (%)
RBC ($\times 10^{12}$/L)	M	4.34-5.72	4.81(4.27-5.63)	20	20 (100)
	F	3.86-5.08	4.47 (4.24-4.72)	20	20 (100)
Hb (g/L)	M	138-175	144 (128-155)	20	20 (100)
	F	119-157	136 (125-139)	20	20 (100)
Hct (L/L)	M	0.415-0.530	0.416 (0.382-0.439)	20	20 (100)
	F	0.356-0.470	0.390 (0.370-0.406)	20	20 (100)
MCV (fL)	All	83.0-97.2	86.5 (84.9-88.7)	20	20 (100)
MCH (pg)	All	27.4 - 33.9	29.8 (29.1-30.6)	20	20 (100)
MCHC (g/L)	All	320-345	342 (338-344)	20	18 (90)
RDW (%)	All	9.0-15.0	12.7 (12.3-13.1)	20	20 (100)
Plt ($\times 10^9$/L)	All	158-424	241 (217-268)	20	20 (100)
MPV (fL)	All	6.8-10.4	9.9 (9.5-10.1)	20	19 (95)
Rtc ($\times 10^9$/L)	All	22-97	62.6 (57.2-70.7)	20	19 (95)
Rtc (Rel/10^3 RBC)	All	5.0-21.6	12.9 (11.7-14.8)	20	19 (95)
WBC ($\times 10^9$/L)	All	3.4-9.7	6.11 (5.43-7.15)	20	20 (100)
Neutrophils ($\times 10^9$/L)	All	2.06-6.49	3.34 (2.72-4.24)	20	20 (100)
Neutrophils (%)	All	44-72	54.6 (49.5-61.5)	20	20 (100)
Lymphocytes ($\times 10^9$/L)	All	1.19-3.35	2.01 (1.67-2.46)	20	20 (100)
Lymphocytes (%)	All	20-46	32.9 (26.9-38.4)	20	20 (100)
Monocytes ($\times 10^9$/L)	All	0.12-0.84	0.55 (0.42-0.60)	20	20 (100)
Monocytes (%)	All	2-12	8.3 (7.2-9.7)	20	20 (100)
Eosinophils ($\times 10^9$/L)	All	0.00-0.43	0.14 (0.09-0.24)	20	19 (95)
Eosinophils (%)	All	0-7	2.2 (1.3-4.0)	20	20 (100)
Basophils ($\times 10^9$/L)	All	0.00-0.06	0.04 (0.03-0.06)	20	20 (100)
Basophils (%)	All	0-1	0.7 (0.5-0.9)	20	20 (100)
ESR (mm/3.6 ks)	M, < 50 y	2-13	4 (3-7)	20	20 (100)
	M, ≥ 50 y	3-23	9 (6-14)	20	20 (100)
	F, < 50 y	4-24	6 (5-9)	20	20 (100)
	F, ≥ 50 y	5-28	12 (9-16)	20	20 (100)

*RIs were adopted from harmonized RIs currently in use in Croatia (8). RI - reference interval. IQR - interquartile range. M
F - female. RBC - red blood cells. Hb - hemoglobin. Hct - hematocrit. MCV - mean cell volume. MCH - mean cell haemo
MCHC - mean cell haemoglobin concentration. RDW - red cell distribution width. Plt - platelets. MPV - mean platelet volum
reticulocytes. WBC - white blood cells. ESR - erythrocyte sedimentation rate.

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Table 4. Coagulation reference interval verification results

Analyte (unit)	RI partitioning	Current RI*	Median (IQR)	N	Results within the harmonized RI, N (%)
PT (% activity)	All	≥ 0.70	1.04 (0.97 - 1.13)	20	20 (100)
APTT (s)	All	20 - 30	26 (24 - 26)	20	20 (100)
APTT (ratio)	All	0.8 - 1.2	1.0 (1.0 - 1.0)	20	20 (100)
Fbg (g/L)	All	1.8 - 3.5	2.6 (2.4 - 2.9)	20	20 (100)

*RIs were adopted from harmonized RIs currently in use in Croatia (8). RI - reference interval. IQR - interquartile range. M
female. PT - prothrombin time. APTT - activated partial thromboplastin time. Fbg - fibrinogen.

Harmonized RIs recommended by the CCMB for hematology and coagulation test were suitable for the population of apparently healthy volunteers from our clinical hospital center.

EXAMPLE 2 – FROM GUIDELINES TO EVERYDAY APPLICATION

*Biochem Med (Zagreb) 2026;36(2):**020706***

Table 5. Clinical chemistry reference interval verification results

Analyte (unit)	RI partitioning	Current RI*	Median (IQR)	N	Results within the harmonized RI, N (%)
ALP (U/L)	M	60-142	70 (60-78)§	40	15 (75)§
			68 (53-73)‖		13 (65)‖
	F; < 50 y	54-119	54 (40-67)§	40	10 (50)§
			55 (40-66)‖		11 (55)‖
	F; ≥ 50 y	64-153	77 (72-96)	20	19 (95)
GGT (U/L)	M	11-55	28 (18-34)	20	20 (100)
	F	9-35	17 (14-20)	20	20 (100)
CK (U/L)	M	< 177	109 (69-130)	20	20 (100)
	F	< 153	81 (67-113)	20	20 (100)
LD (U/L)	All	< 241	166 (156-179)	20	20 (100)
Lip (U/L)	All	13-60	19 (16-23)	20	19 (95)
K (mmol/L)	All	3.9-5.1	4.2 (4.1-4.4)	20	20 (100)
Na (mmol/L)	All	137-146	141 (140-141)	20	20 (100)
Cl (mmol/L)	All	97-108	104 (103-105)	20	20 (100)
Ca, total (mmol/L)	All	2.14-2.53	2.46 (2.38-2.53)§	40	15 (75)§
			2.47 (2.40-2.52)‖		16 (80)‖

Verification of harmonized reference intervals in Croatia: is it time for a change?

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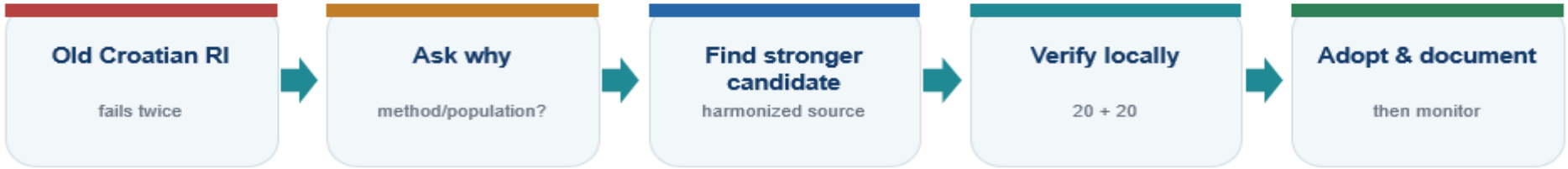
Table 6. Alternative sources reference interval verification results for total calcium and alkaline phosphatase

			International harmonized RI		Reagent manufacturer RI	
Analyte (unit)	RI partitioning	Median (IQR)	RI	Results within the RI, N (%)	RI	Results within the RI, N (%)
Ca, total (mmol/L)	All	2.46 (2.38-2.53)‡	2.20-2.60*	19 (95)	2.20-2.65	19 (95)
		2.47 (2.40-2.52)§		20 (100)		20 (100)
ALP (U/L)	M	70 (60 - 78)‡	43-115†	20 (100)	43-115	20 (100)
		68 (53 - 73)§		19 (95)		19 (95)
	F; < 50 y	54 (40 - 67)‡	33-98†	19 (95)	33-98	19 (95)
		55 (40 - 66)§		20 (100)		20 (100)
	F; ≥ 50 y	77 (72 - 96)	NA‖	NA‖	NA‖	NA‖



Verification both data sets met the predefined verification criteria for RIs from harmonized literature sources (Ca, total / UK Pathology Harmony RI and ALP / IFCC- traceable harmonized RI) as well as those provided by the reagent manufacturer.

The requirements of the EN ISO 15189 standard fit into the practice of RIs

7.3.2 VERIFICATION OF EXAMINATION PROCEDURES

Before routine use, the laboratory verifies that the examination procedure performs as intended in its own setting.

7.3.5 BIOLOGICAL REFERENCE INTERVALS AND CLINICAL DECISION LIMITS

RIs must be **periodically reviewed and reconsidered when the method or pre-analytical process changes**.

7.4.1.6 REPORTING

Reports should include RIs or decision limits when needed for interpretation, unless the laboratory documents why they are omitted.

Periodic review of RIs is needed due to changes in analytical methods and population characteristics.

It means documenting and approving whether the current RI remains fit for use.

Sources: ISO 15189:2022 overview

Verification form example 1: serum glucose in adults

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Zaštićeni broj	Oznaka dokumenta	Oznaka izdanja	List
7.3.2.7.3.5	KZMBLM-OB12.2	2	1/2

Verification of reference intervals for SOP7.1-Glucose in serum for adults (> 19 years)

Verification of reference intervals was performed according to the CLSI 28-A3 protocol by analyzing 20 samples serum of healthy volunteers. Samples with hemolysis index H < 20 were used.
Number in the table corresponds to the project number of respondents.

People aged 20 - 30 years

	Dobna skupina	Spol	Rezultat		Dobna skupina	Spol	Rezultat
3	19 – 30 godina	Ž	5,9	39	19 – 30 godina	Ž	4,8
4		Ž	4,9	45		M	5,1
5		M	5,3	48		M	4,9
6		M	5,0	54		M	4,8
14		M	4,3	56		Ž	4,9
31		Ž	5,3	58		M	4,9
33		M	4,5	60		M	4,9
34		Ž	5,0	65		Ž	5,2
35		M	5,1	70		M	5,4
38		M	4,5	72		Ž	4,7

People aged > 30 years

	Dobna skupina	Spol	Rezultat		Dobna skupina	Spol	Rezultat
2	> 30 godina	M	4,6	23	> 30 godina	M	5,3
7		M	5,7	25		Ž	4,5
8		M	5,7	28		M	4,5
10		Ž	4,8	29		Ž	5,4
11		M	5,2	30		M	6,1
12		Ž	4,7	35		M	5,5
13		M	4,9	37		Ž	4,4
15		M	4,5	40		Ž	4,4
20		Ž	4,9	46		Ž	4,4
21		M	5,1	49		Ž	4,9

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	Oznaka dokumenta	Oznaka izdanja	List
7.3.2.7.3.5	KZMBLM-OB12.2	1	2/2

The following results were obtained:

Dob	Spol	Referentni interval*	Broj uzoraka unutar kriterija	Postotak uzoraka unutar kriterija
19 – 30 godina	M, Ž	4,2 - 6,0	20	100 %
> 30 godina		4,4 - 6,4	20	100 %

*Čvorišćec D, Flegar-Meštrić Z, Juretić D. Harmonizacija laboratorijskih nalaza u području opće medicinske biokemije. Zagreb: Hrvatska komora medicinskih biokemičara. 2004.

Conclusion:

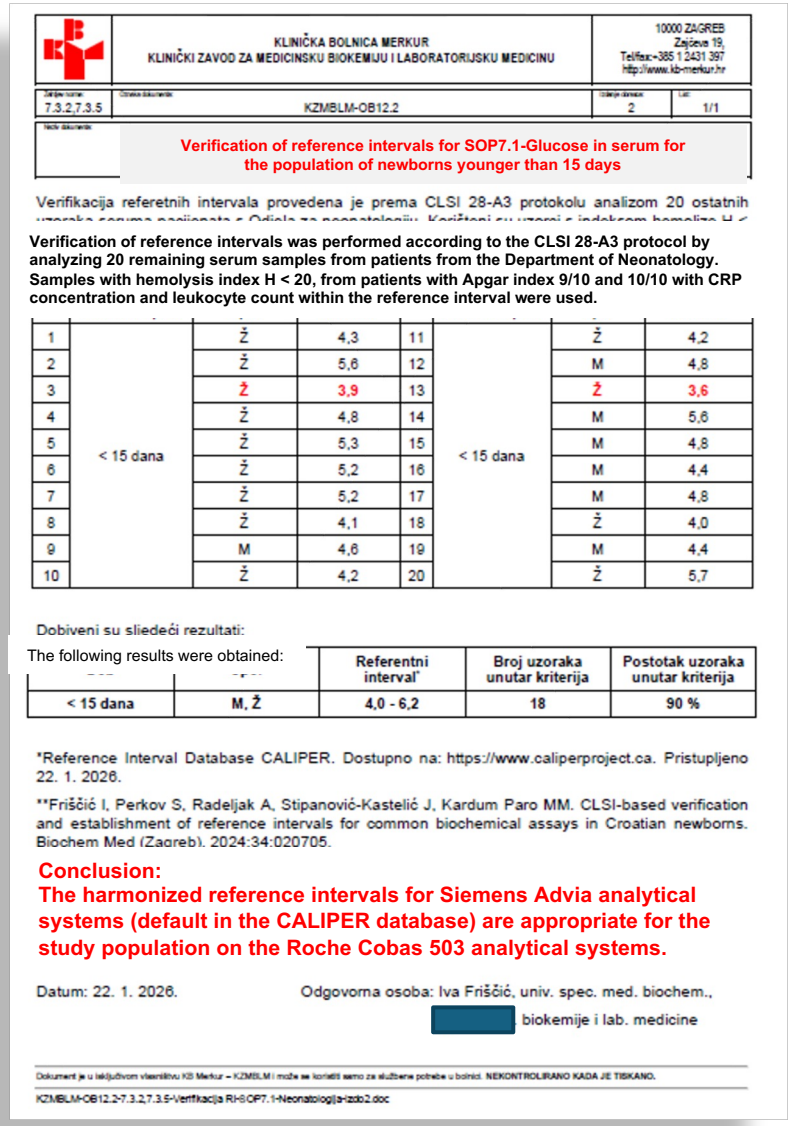
The harmonized reference intervals are appropriate for the population studied on the Roche Cobas 503 analytical systems.

Datum: 3. 3. 2026.

Odgovorna osoba: [redacted] univ. spec. med. biochem., spec. med. biokemije i lab. medicine

Dokument je u isključivom vlasništvu KB Merkur – KZMBLM i može se koristiti samo za službene potrebe u bolnici. NEKONTROLIRANO KADA JE TISKANO.
KZMBLM-OB12.2-7.3.2.7.3.5-Verifikacija RI-SOP7.1-Odrasli-Izdo2.doc

Verification form example 1: serum glucose in newborns (< 15 days)



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7.3.2,7.3.5	KZMBLM-OB12.2	2	1/1

Verification of reference intervals for SOP7.1-Glucose in serum for the population of newborns younger than 15 days

Verifikacija referetnih intervala provedena je prema CLSI 28-A3 protokolu analizom 20 ostatnih

Verification of reference intervals was performed according to the CLSI 28-A3 protocol by analyzing 20 remaining serum samples from patients from the Department of Neonatology. Samples with hemolysis index H < 20, from patients with Apgar index 9/10 and 10/10 with CRP concentration and leukocyte count within the reference interval were used.

1	< 15 dana	Ž	4,3	11	< 15 dana	Ž	4,2
2		Ž	5,6	12		M	4,8
3		Ž	3,9	13		Ž	3,6
4		Ž	4,8	14		M	5,6
5		Ž	5,3	15		M	4,8
6		Ž	5,2	16		M	4,4
7		Ž	5,2	17		M	4,8
8		Ž	4,1	18		Ž	4,0
9		M	4,6	19		M	4,4
10		Ž	4,2	20		Ž	5,7

Dobiveni su sliedeći rezultati:

The following results were obtained:

		Referentni interval*	Broj uzoraka unutar kriterija	Postotak uzoraka unutar kriterija
< 15 dana	M, Ž	4,0 - 6,2	18	90 %

*Reference Interval Database CALIPER. Dostupno na: https://www.caliperproject.ca. Pristupljeno 22. 1. 2026.

**Friščić I, Perkov S, Radeljak A, Stipanović-Kastelić J, Kardum Paro MM. CLSI-based verification and establishment of reference intervals for common biochemical assays in Croatian newborns. Biochem Med (Zagreb). 2024:34:020705.

Conclusion:
The harmonized reference intervals for Siemens Advia analytical systems (default in the CALIPER database) are appropriate for the study population on the Roche Cobas 503 analytical systems.

Datum: 22. 1. 2026.

Odgovorna osoba: Iva Friščić, univ. spec. med. biochem.,
[redacted] biokemije i lab. medicine

Dokument je u isključivom vlasništvu KB Merkur – KZMBLM i može se koristiti samo za službene potrebe u bolnici. NEKONTROLIRANO KADA JE TISKANO.

KZMBLM-OB12.2-7.3.2,7.3.5-Verifikacija RI-SOP7.1-Neonatologija-izdo2.doc

TAKE- HOME MESSAGES

1. *De novo* RIs establishment requires resources; transfer and local verification is often a realistic path for daily clinical use.
2. CLSI EP28-A3c guideline - establish when necessary, transfer when justified, verify before daily clinical practice.
3. Every RI needs a documented source, verification record and trigger for reevaluation.
4. The Croatian experience shows the point clearly: verify first, change it only when the data tell you to; a harmonized RI is useful only if it is fit for your population and measurement procedure.

Do not ask:

What RI does everyone use?

Do ask:

Does it work for our patients and our method?

Verification is the bridge to safe implementation of RIs in clinical practice.
The use of appropriate RIs provides clinicians with the proper context for interpreting laboratory test results and has a significant impact on the clinical decision-making process.

Thank you for your attention!

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