Reference Interval Harmonization in Canada

Dr. Khosrow Adeli
Dr. Christine Collier
Mary Kathryn Bohn

On Behalf of the CSCC Reference Interval Harmonization Working Group

June 5th 2022
Canadian Society for Clinical Chemists 2022 Annual Conference
Niagara Falls, ON Canada

Outline & Learning Objectives

Presentation Outline

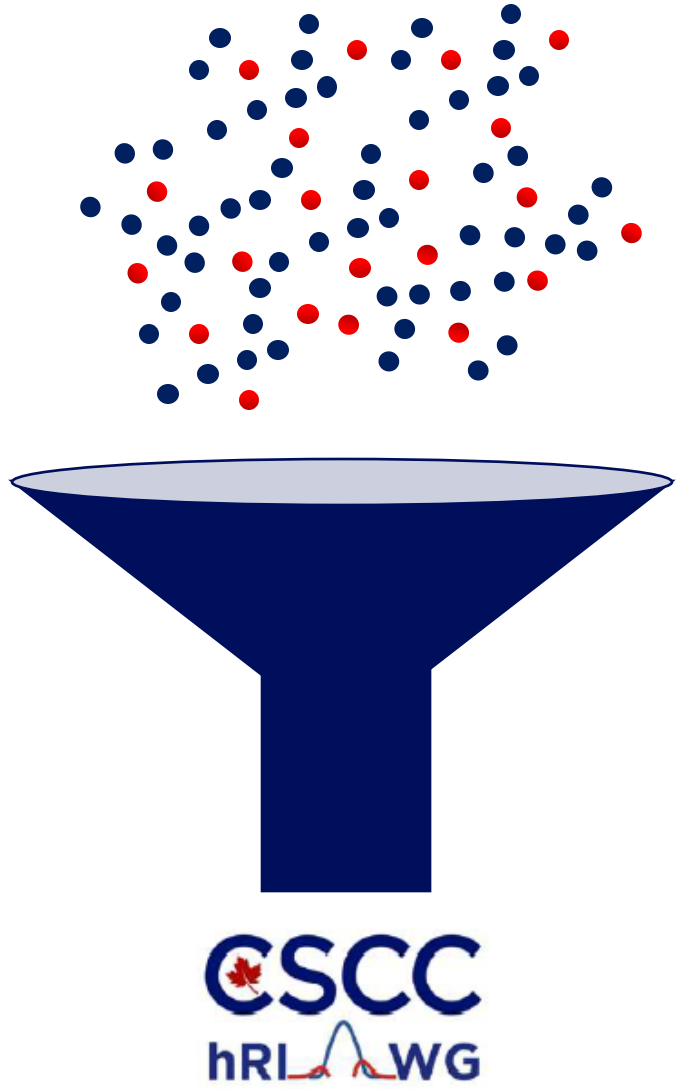
- Reference Interval Harmonization in Canada (Dr. Khosrow Adeli)
- Analyzing the Data – Approach Taken by CSCC hRI (Mary Kathryn Bohn)
- Path towards Implementation – Discussion and Input (Dr. Christine Collier)

At the end of the session, the participants will be able to:

- Outline the major gaps in reference intervals and the critical need for harmonization across clinical laboratories.
- Describe the major advances made by the CSCC Working Group on Reference Interval Harmonization.
- Discuss the development and validation of common reference intervals and their implementation across Canada.

Reference Interval Harmonization Across Canada

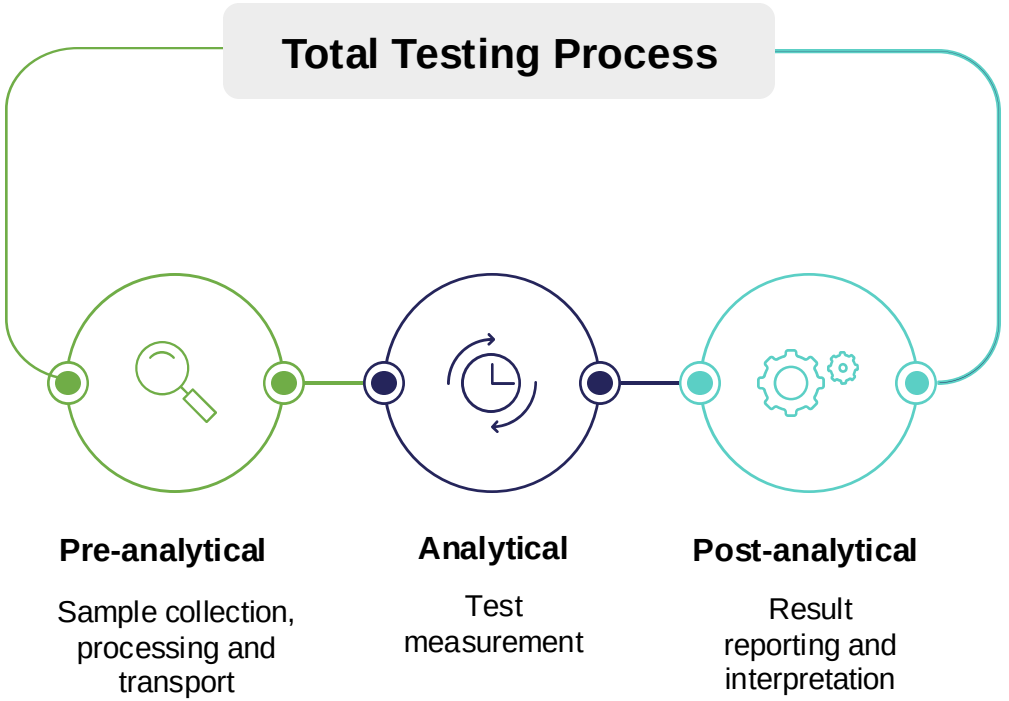
Dr. Khosrow Adeli



Harmonization in Laboratory Medicine

- Harmonization is a fundamental aspect of ensuring the analytical and clinical quality of the ***total testing process***
- Growing expectation for standardized patient care across healthcare centers
- Harmonization efforts have largely focused on the pre-analytical and analytical phase of testing, including:
    - *Standardized quality indicator goals*
    - *Increased automation*
    - *Development of commutable reference standards and improved metrological traceability*

Have similar gains been made in reference interval reporting?



Reference Interval Harmonization: Around the world

UK Pathology Harmony

Sampling: Consensus, adults
Sample Type: Serum
Statistical method: Consensus

NORIP

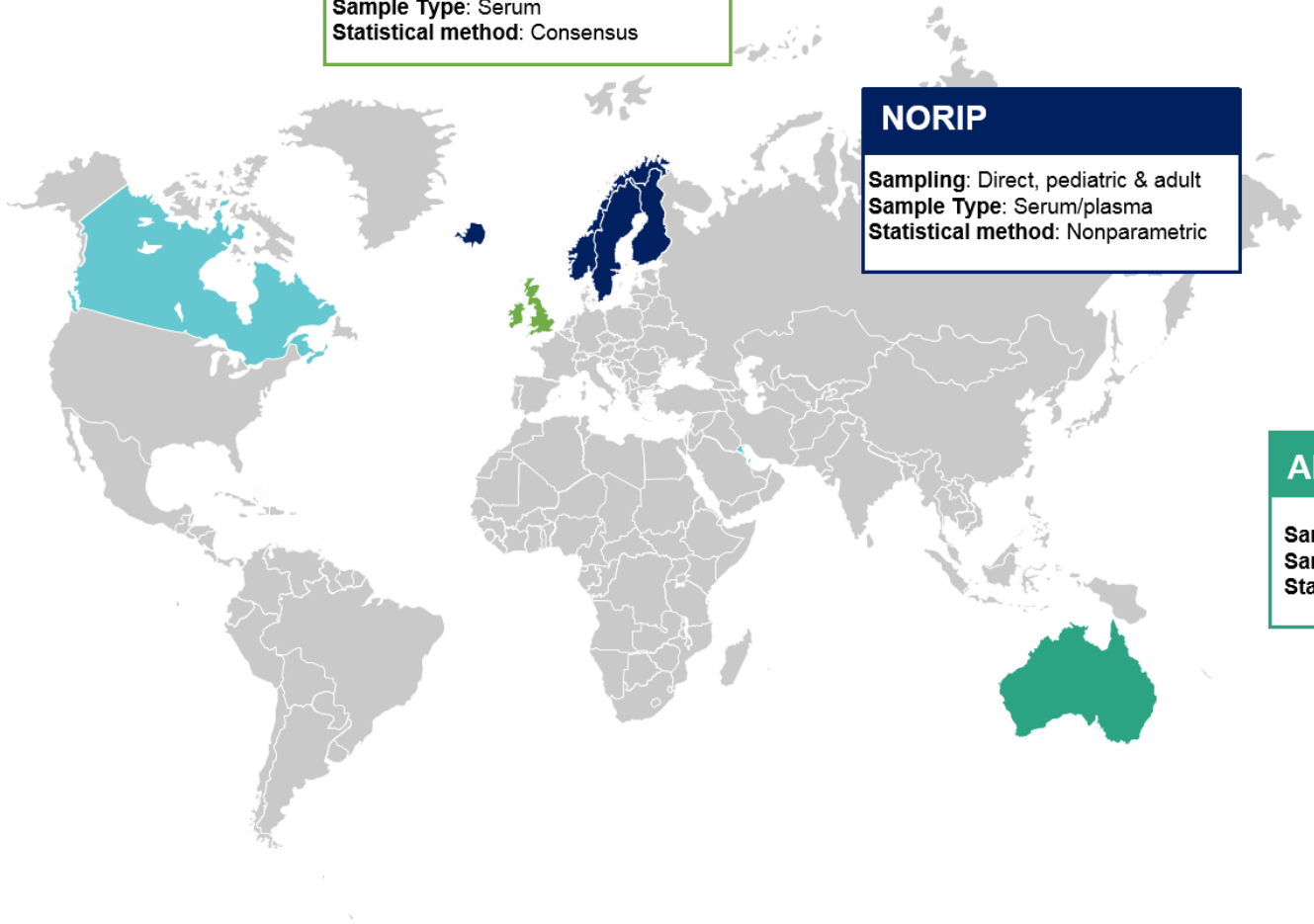
Sampling: Direct, pediatric & adult
Sample Type: Serum/plasma
Statistical method: Nonparametric

CALIPER

Sampling: Direct, pediatric
Sample Type: Serum
Statistical method: Nonparametric or robust

CSCC hRI WG

Sampling: Indirect, adult
Sample Type: Serum/plasma
Statistical method: TML method



AHRIA & AHRIP

Sampling: Combination, pediatric & adult
Sample Type: Serum/plasma
Statistical method: Combination

Harmonizing the Post-Analytical Phase: RIs & CDLs

- **Variation in reference intervals may be much greater than analytical inaccuracy of measurements**
  - i.e. The same patient result obtained by 2 laboratories using the same assay but different reference intervals, can lead to very different clinical interpretations

- **There are varying levels of quality with respect to test result interpretation:**
  1. Decision thresholds based on clinical outcomes studies (where all methods employed in the clinical setting are harmonized)

  2. Local reference interval projects aimed at harmonization
     - NORIP (Nordic Reference Interval Project), CALIPER (Canadian Laboratory Initiative for Pediatric Reference Intervals), Australia/New Zealand initiative for harmonization of reference intervals

  3. Reference intervals based on assay kit insert (lowest quality with least harmonization and little possibility of shared reference limits)

Tate JR et al, 2014. Clinica Chimica Acta 432; 4–7.

When do laboratory testing errors occur?

- **Analytic Phase**: comprise only 4-32% of all laboratory testing errors
  - Historically, more attention has been focused on this phase of testing
  - Close monitoring is used to ensure that proper testing methodologies and instrumentation are applied for every analyte
  - Stringent quality assurance and quality control procedures are employed

- **Preanalytic Phase**: Estimated to account for 32% to 75% of errors in the testing process

- **Postanalytic Phase**: Estimated to account for 9% to 55% of all errors in the testing process

***Most laboratory errors occur either before or after the actual performance of the test*

***This is most likely due to the relative disconnect between the laboratory and other parts of the health care process – lack of communication.*

Stankovic AK 2004. Clin Lab Med 24; 1023-35.

Reference intervals: Major Gaps

- Most of the available reference intervals **determined decades ago on older/less accurate laboratory instruments**/methodologies
- Most pediatric reference intervals **incomplete and out of date**
- Most available **only for Caucasian** populations
- **No data for many new and emerging disease biomarkers** of pediatric disease
- Available data from samples **collected on hospitalized adults and children**

Are Reference Intervals still a problem?

Yes → for Clinicians

- different reference intervals from different laboratories;
- confusion between **Reference Intervals** and **Decision Limits**

Yes → for Patients

- same value can be considered “normal” or “abnormal” in different laboratories.

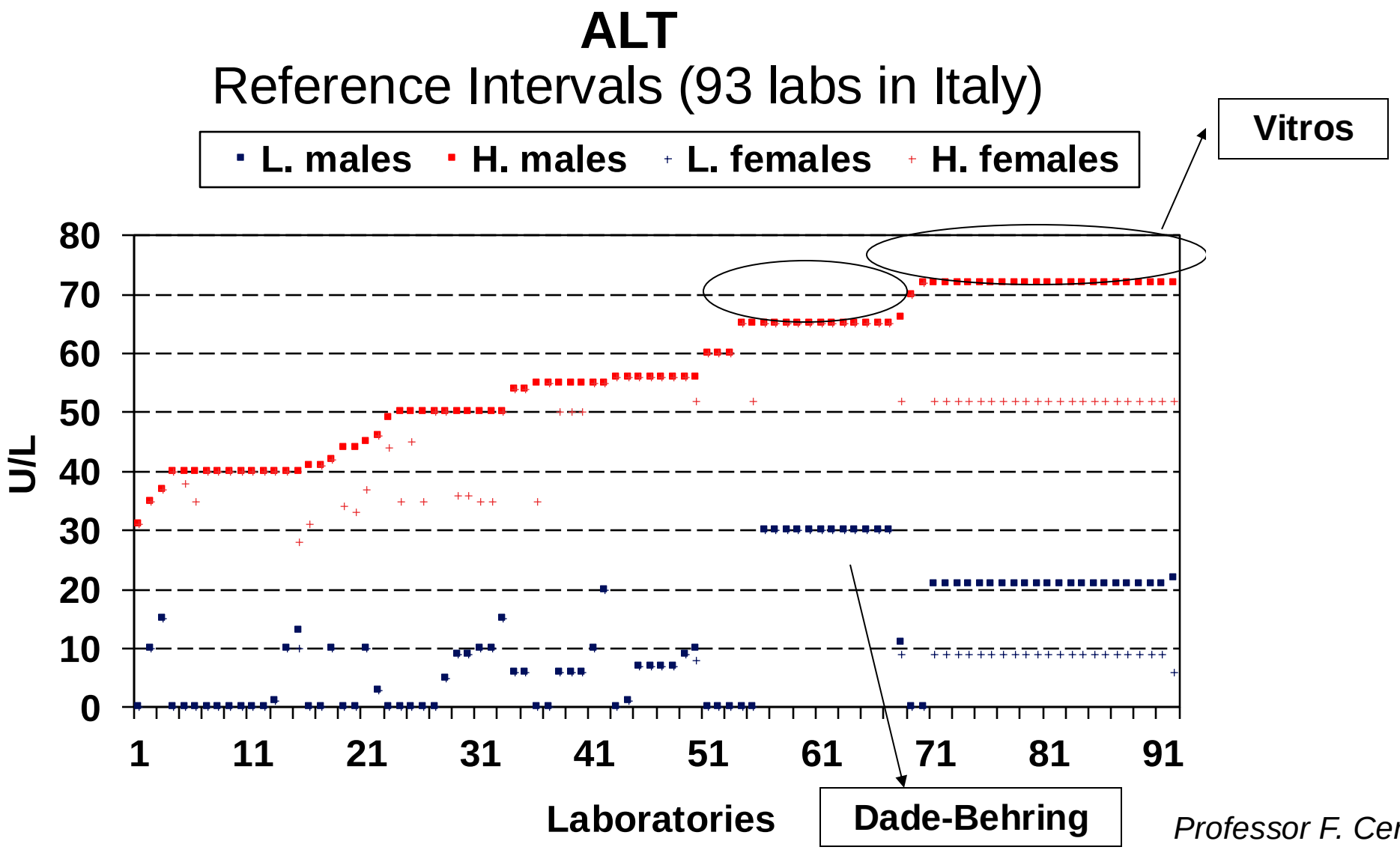
An Example: ALT Reference Intervals

From the data-base of the PROLARIT (Italy):

- 587 laboratories subdivided in 6 method groups
    1. "IFCC" optimization without P5P = 449
    2. IFCC = 47
    3. Ortho Vitros = 46
    4. Beckman = 25
    5. DGKC optimization = 11
    6. SCE optimization = 7

- 90 different Reference Intervals (R.I.) just for males
    - Most frequent RI (males) 0 – 40 U/L (150 labs)
    - 412 (70%) no lower limit
    - 255 (43%) same R.I. for males and females

An Example: ALT Reference Intervals



Professor F. Cerriotti, Italy

National Survey 2016

Reference Intervals in use across Canada: >30 Clinical Laboratories surveyed

Clinical Biochemistry (2017)

Reference Interval Harmonization in Canada: Current Gaps

- Reference interval harmonization supports consistent and standardized test result interpretation, when appropriate

- Harmonized reference intervals should only be considered when significant analytical differences are NOT observed

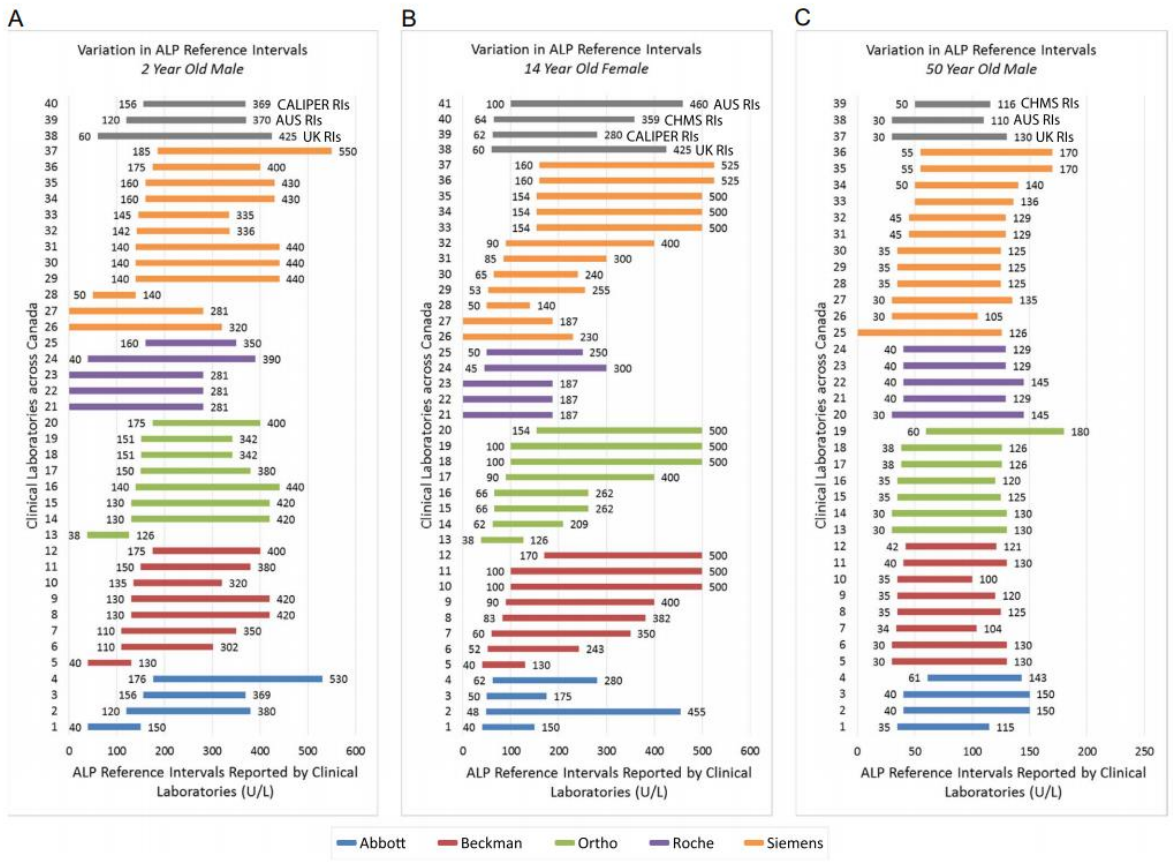
CSCC 2017 National Survey on Reference Interval Variation:

Design:

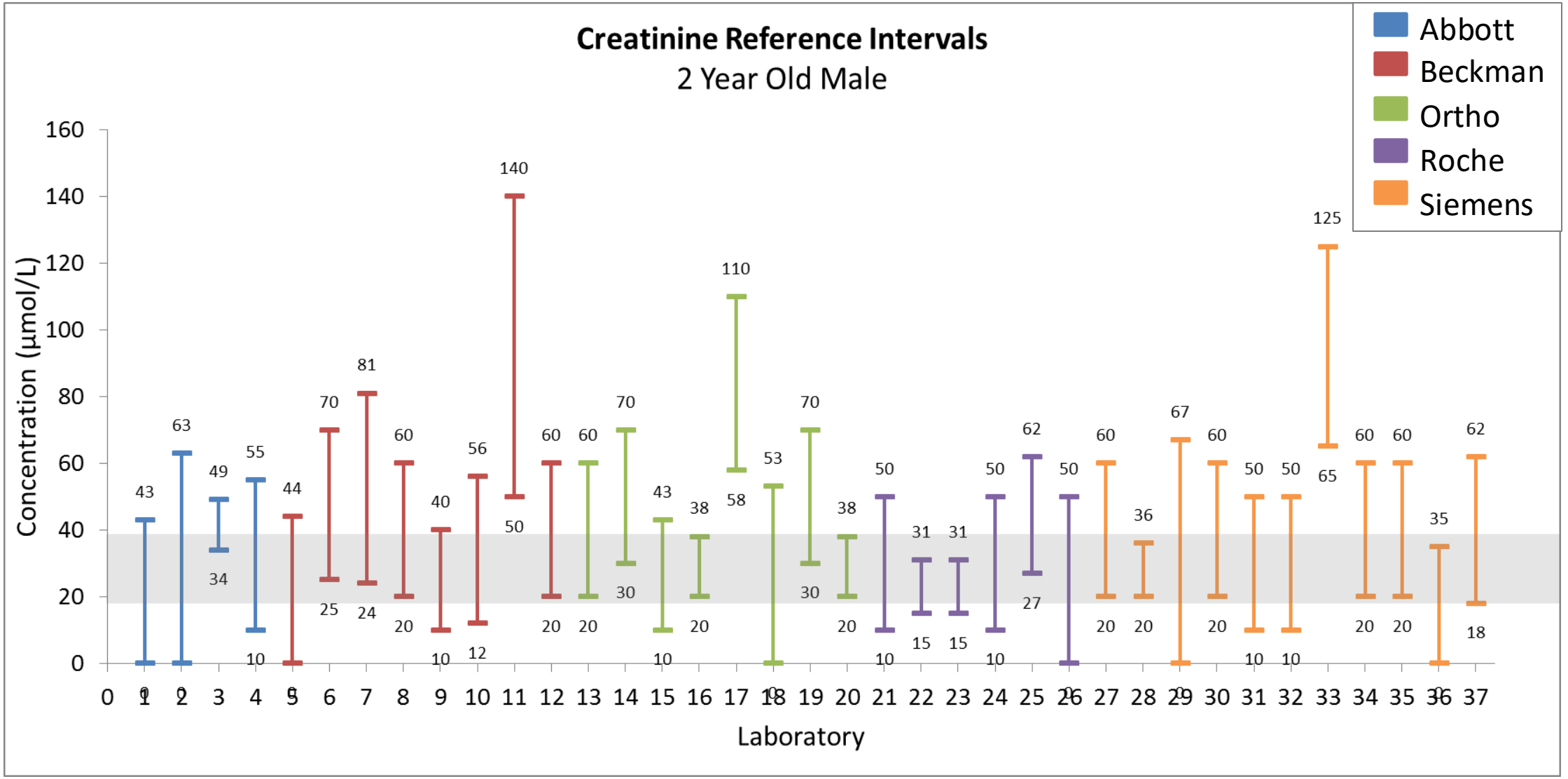
- 37 laboratories, 7 analytes: RIs for ALT, ALP, calcium, creatinine, fT4, hemoglobin, sodium
- 40 laboratories measured 6 analytes in reference samples (hemoglobin excluded)

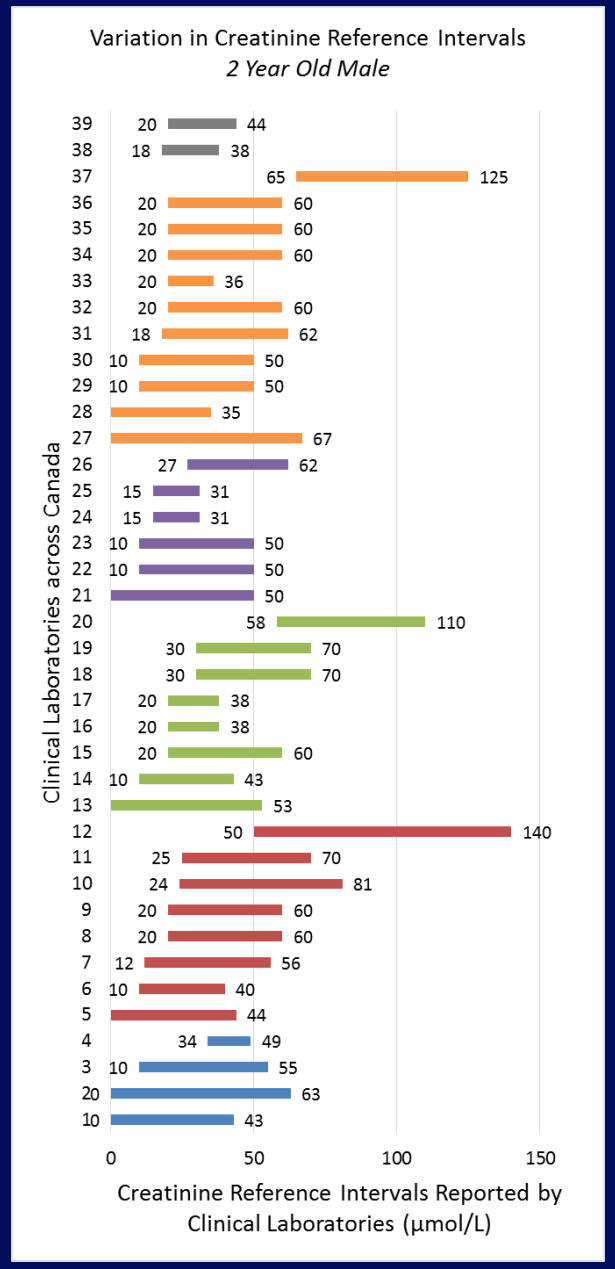
Key Findings:

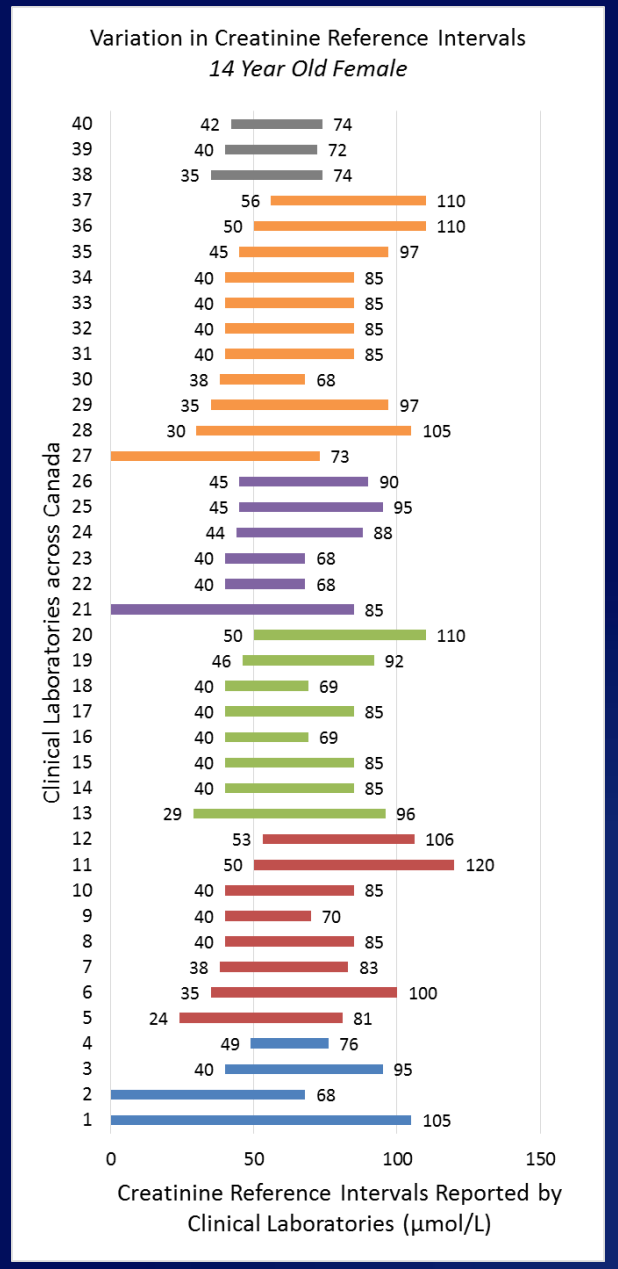
- Variability in RIs even between laboratories using the same instrumentation
- RI variability exceed test result variability

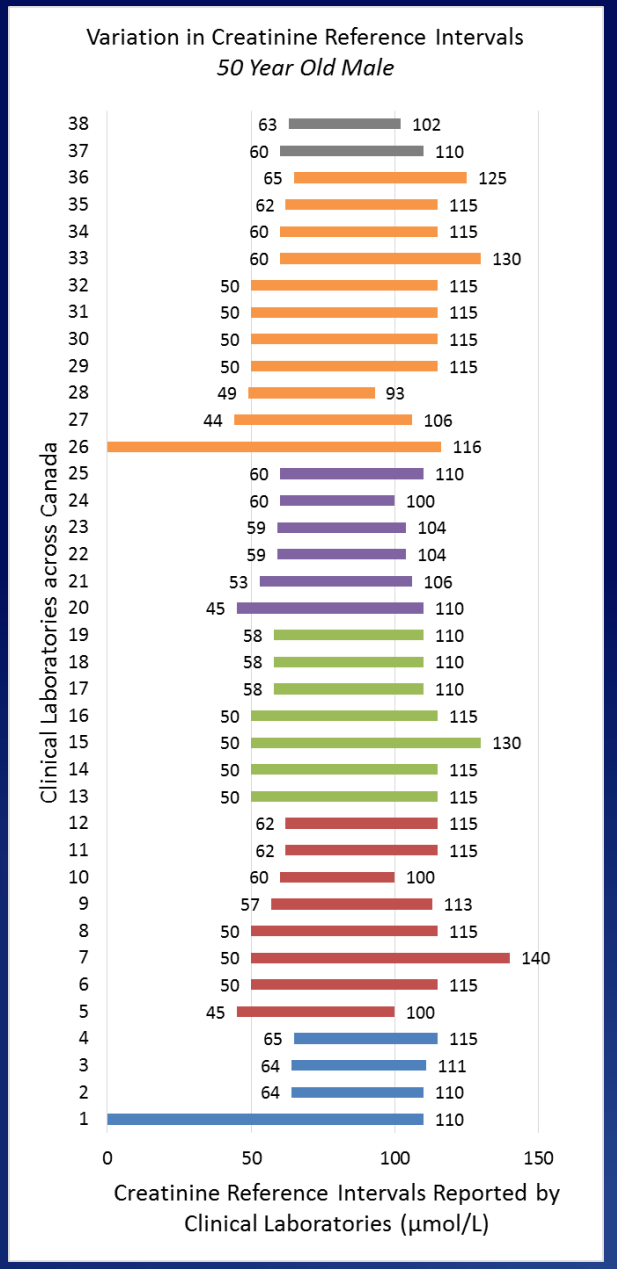


Reference Intervals in Centres across Canada: Creatinine



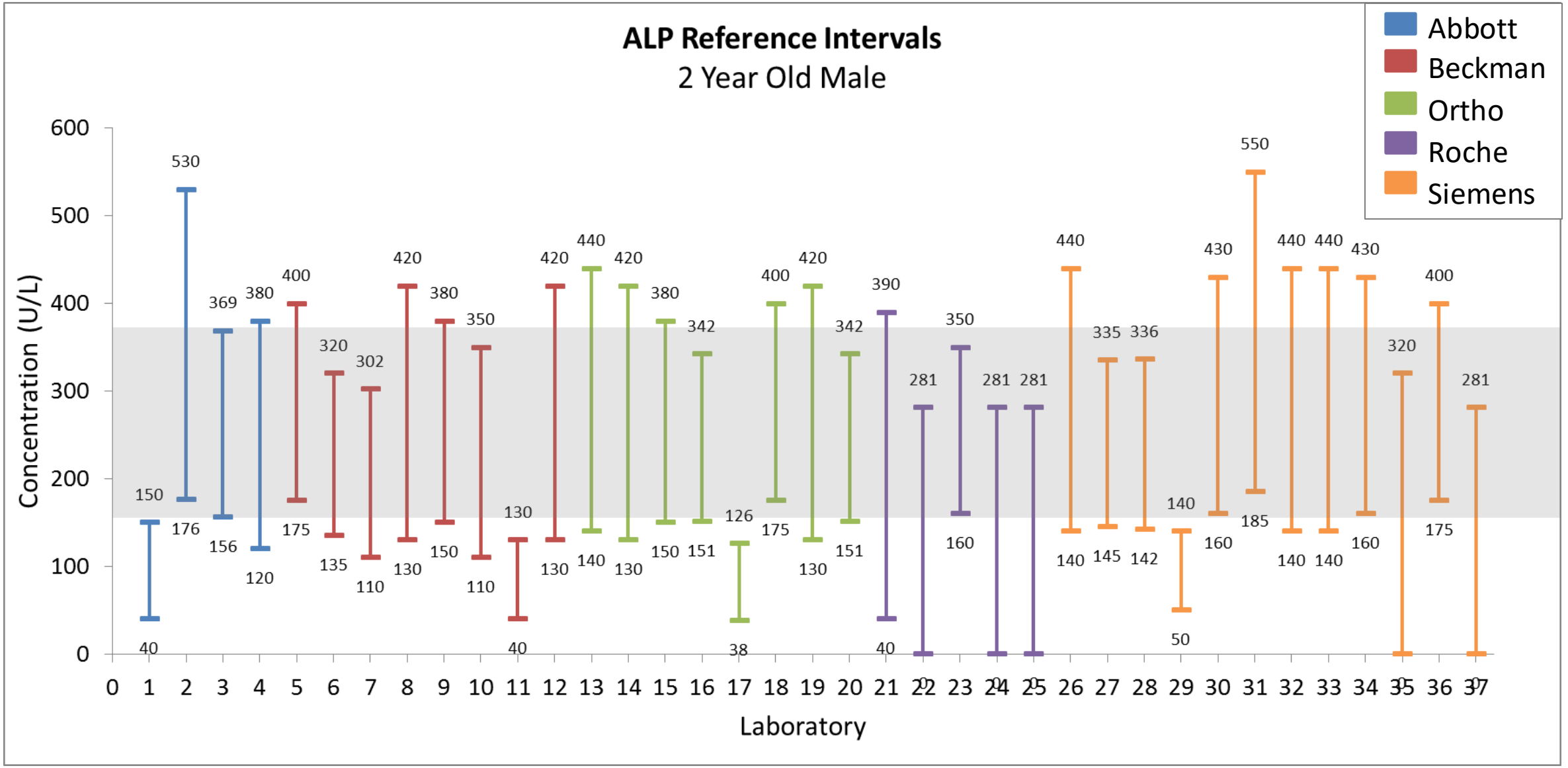

Variation in Creatinine Reference Intervals
2 Year Old Male
Clinical Laboratories across Canada
Creatinine Reference Intervals Reported by Clinical Laboratories (µmol/L)
0
50
100
150


Variation in Creatinine Reference Intervals
14 Year Old Female
Clinical Laboratories across Canada
Creatinine Reference Intervals Reported by Clinical Laboratories (µmol/L)
0
50
100
150


Variation in Creatinine Reference Intervals
50 Year Old Male
Clinical Laboratories across Canada
Creatinine Reference Intervals Reported by Clinical Laboratories (µmol/L)
0
50
100
150


Abbott
Beckman
Ortho
Roche
Siemens

Reference Intervals in Centres across Canada: ALP



Gaps and deficiencies in accurate reference intervals a serious risk to patient care and outcomes

- **Use of inappropriate reference intervals**

>>> risk of further blood collection, infection risk, pain and anxiety, lengthier stays, and unpleasant or invasive diagnostic procedures

- **Inadequate pediatric reference intervals**

>>> potentially costly and devastating, and potentially contributing to erroneous/delayed diagnosis of many diseases of childhood and adolescence

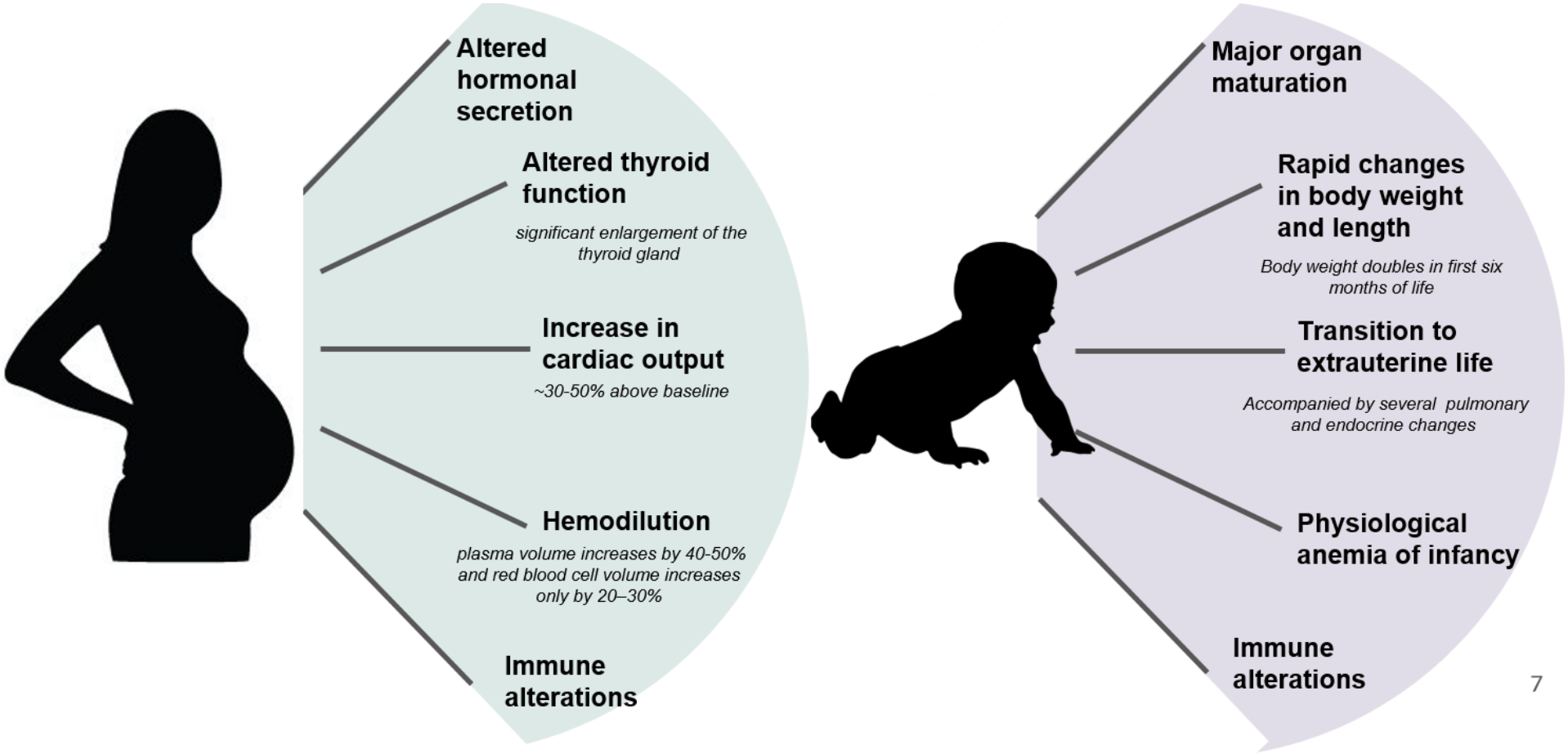
Reviewed in: *Critical Reviews in Clinical Laboratory Sciences (2017)*

Major Gaps in Available Reference Intervals:

In the face of these Gaps:

- **What is the value of a lab test result without appropriate interpretation??**
  - ➢What are the risks of using outdated, inaccurate, and inappropriate reference intervals??
  - ➢Why are most clinical laboratories ignoring the issue??
  - ➢Can we afford to ignore the issue much longer??

Major Evidence Gaps in Pediatrics and Pregnancy



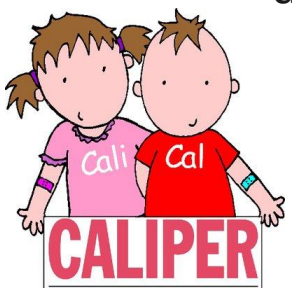
Global Reference Intervals Initiatives in Pediatrics

Study	Country	Age Range (years)	Sex	Statistical Method	Examples of Groups of Biomarkers Studied
AACB	Australia and New Zealand	All age groups	Both	Central 95%	Common blood analytes (mostly ions and enzymes)
CALIPER	Canada CALIPER 'ARM' us with the knowledge to help others	0-18	Both	Central 95%	Common biochemical markers Endocrine markers Tumor markers Vitamins Metabolic disease biomarkers Testosterone indices
CHILDx	United States	0.5-17	Both	Median, mean and central 95%	Enzymes Coagulation tests Hormones Vitamins Bone markers
COPENHAGEN	Denmark	5-20	Both	Central 95%	Common blood analytes
KiGGS	Germany KiGGS Studie zur Gesundheit von Kindern und Jugendlichen in Deutschland	0-18	Both	Median and central 90%	Nutrient deficiency markers Non-communicable diseases and lipids Immunology markers Thyroid hormones
LOOK	Australia	8, 10 and 12	Both	Median and central 95%	Cardiac Biomarker Common blood analytes

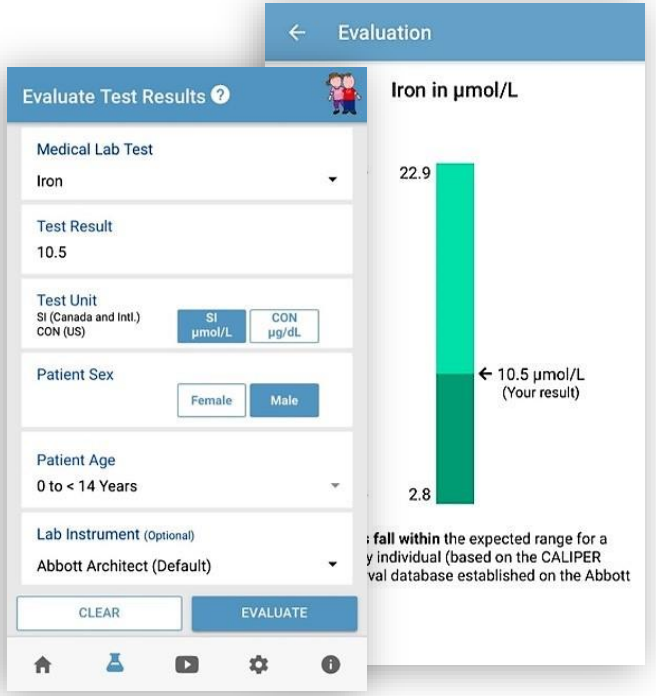
CALIPER Study of Pediatric Reference Intervals

CALIPER = Canadian Laboratory Initiative on Pediatric Reference Intervals

- **Serum Biobank:** > **12,000** samples (males/females)
- **Age Range:** Birth to 18 years
- **Health Information:** Family History, Health Status, BMI, Waist Circumference
- **CALIPER Database:** Reference standards for over 185 biomarkers from peer-reviewed publications
- **CALIPER Mobile and Web Apps** for ready access to the database of pediatric reference standards



CALIPER data for individuals aged 0 to <5 years is mostly based on outpatients from select clinics without strict exclusion/inclusion criteria



Download on the App Store

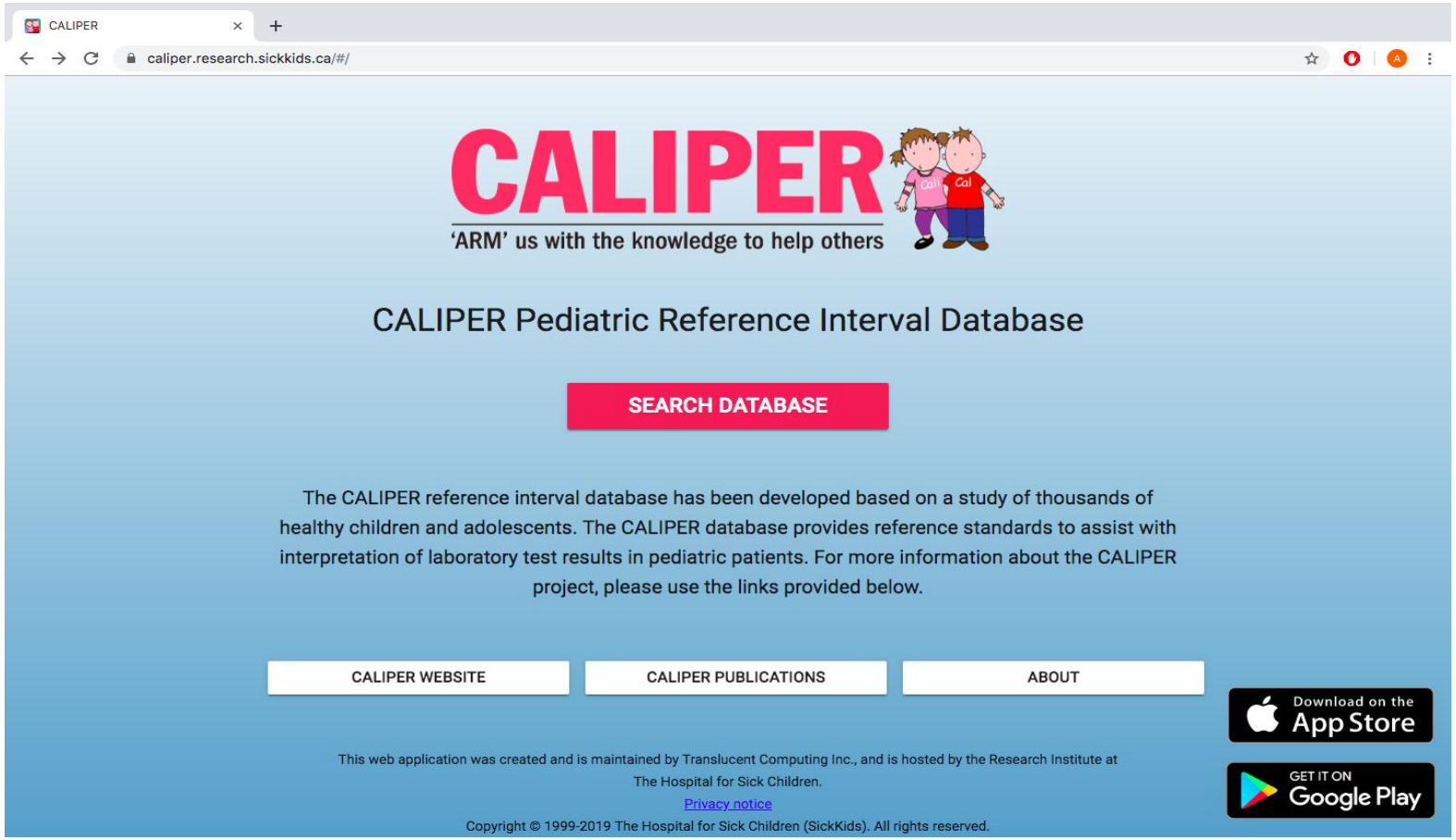
GET IT ON Google Play

visit www.caliperproject.org for more details

CALIPER Web Application

www.caliperdatabase.org

The CALIPER Web App has been developed for laboratory specialists, paediatricians, family physicians, other healthcare workers

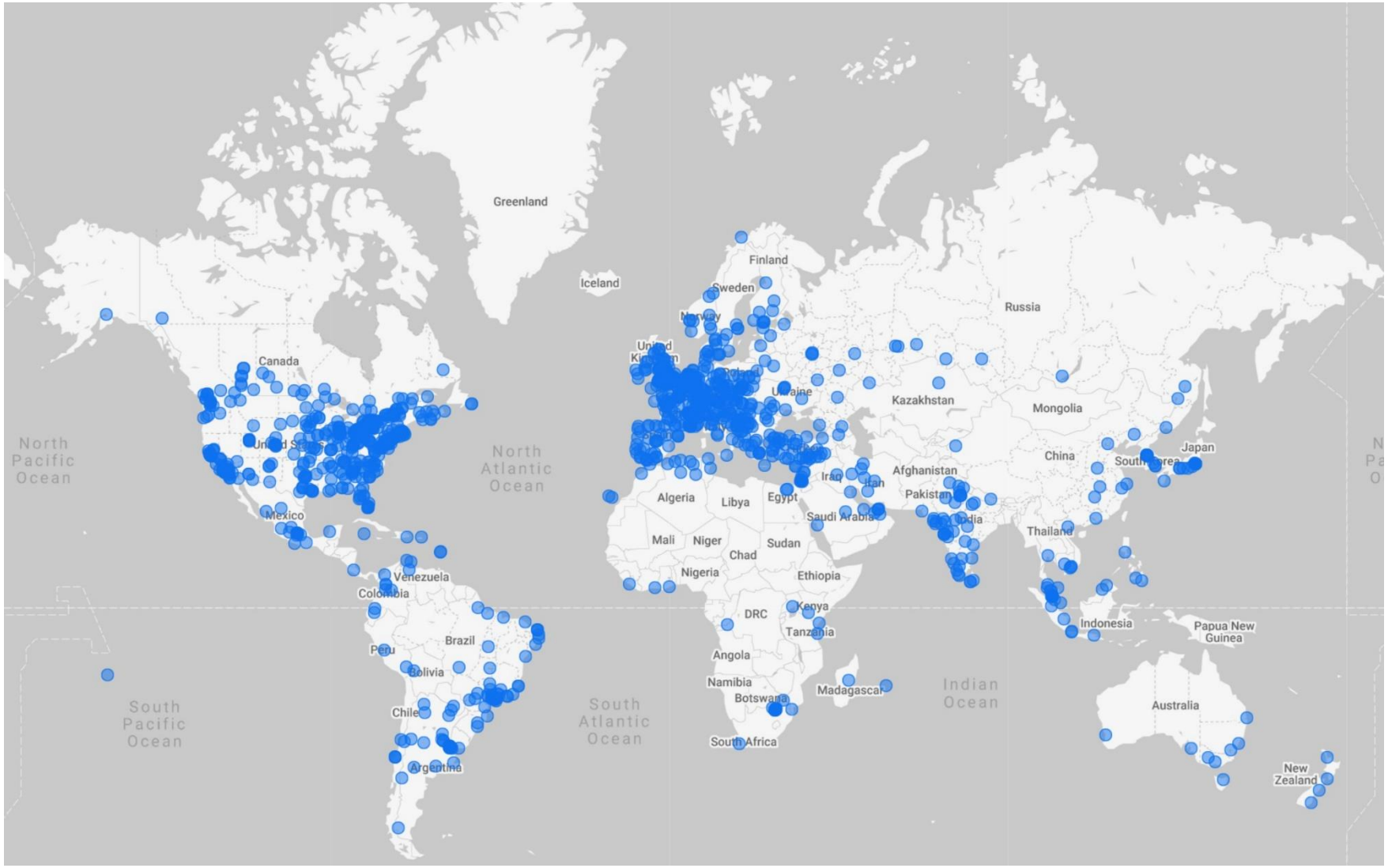


A user friendly and easy tool to view latest reference value database developed based on <u>thousands of healthy and ethnically diverse children and adolescents.</u>

Pediatric Reference Interval Harmonization

Worldwide Acceptance of the CALIPER Database

Global Access to CALIPER Database (Google Analytics 2021)



Adult Reference Interval Harmonization in Canada: The hRI Project

Harnessing the Power of Big Data Analytics

Reference Interval Harmonization in Canada: *CSCC hRI WG*

CSCC Working Group on Reference Interval Harmonization

Main Objective: Establish evidence-based harmonized/common reference intervals (hRIs) and support their implementation in laboratories across Canada.

Co-Chairs
Christine Collier
Khosrow Adeli

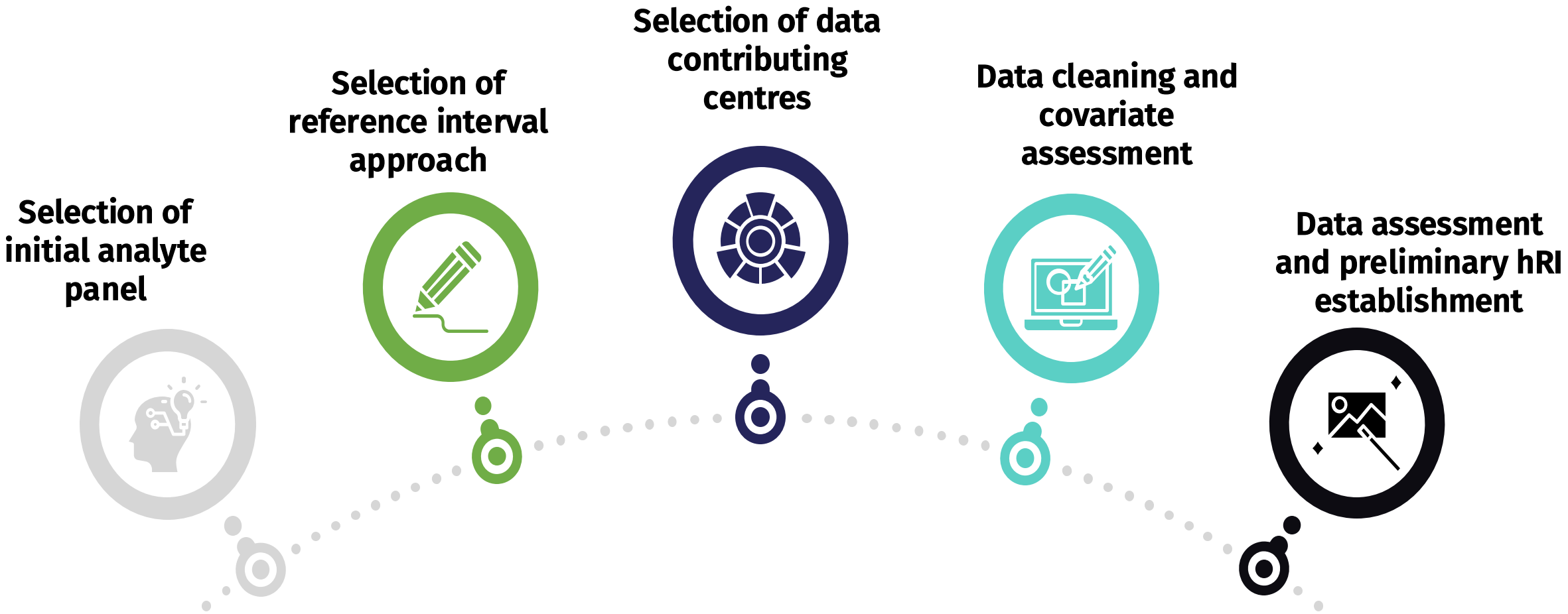
Calculations Team
Shervin Asgari
Mary Kathryn Bohn
Jake Cosme
Qing Fan
Victoria Higgins
Zahraa Mohammed-Ali
Jennifer Taher
Albert Tsui

Analysis Team
Dana Bailey
Cynthia Balion
George Cembrowski
Jim Dalton
Trefor Higgins
Benjamin Jung
Joseph Macri
David Seccombe
Julia Stemp
Alison Venner
Nicole White-Al Habeeb

Previous Members
Terence Agbor
Angela Fung
Josko Ivika
Felix Leung
Michelle Parker
Omair Sarfaraz
Julie Shaw
Janet Simons
Uvaraj Uddayasankar
Dorothy Truong

CSCC hRI WG

CSCC hRI WG: Path to Reference Interval Harmonization



CSCC hRI WG: Path to Reference Interval Harmonization

Selection of Initial Analyte Panel

- **An initial panel of 17 analytes were selected as candidates for harmonization through: literature review, manufacturer IFU review**

Electrolytes
- ✓Sodium
- ✓Potassium
- ✓Magnesium
- ✓Chloride
- ✓CO2

Hepatic
- ✓ALT
- ✓ALP
- ✓Total Protein
- ✓Total Bilirubin
- ✓Albumin
- ✓LDH

Renal
- ✓ Creatinine
- ✓ Calcium
- ✓ Phosphate

Endocrine
- ✓Free T3
- ✓Free T4
- ✓Thyroid Stimulating Hormone

CSCC hRI WG: Path to Reference Interval Harmonization

Selection of data contributing centres

- Appropriate selection of data contributing centres is essential to optimize the performance of indirect methods

Criteria for data centre contribution:

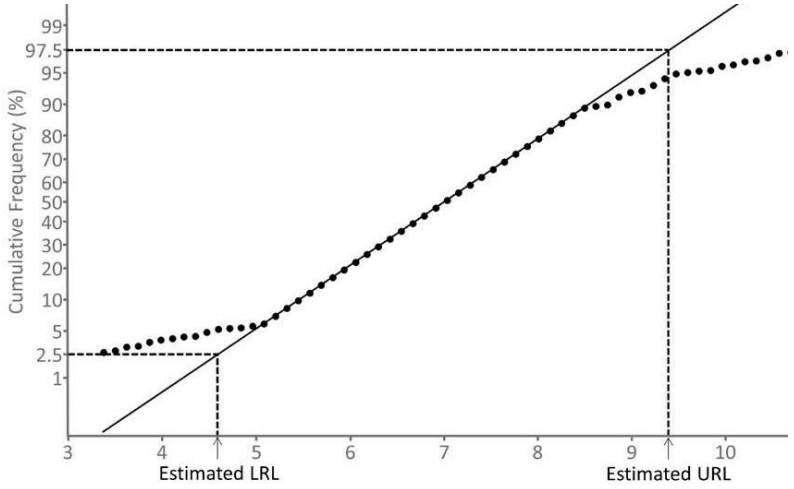
- *Large outpatient population*
- *Representative of Canadian population*
- *Representative of different analytical platforms*
- *Consistent results over time*

Formed collaborations with community laboratories to support this initiative

CSCC hRI WG: Path to Reference Interval Harmonization

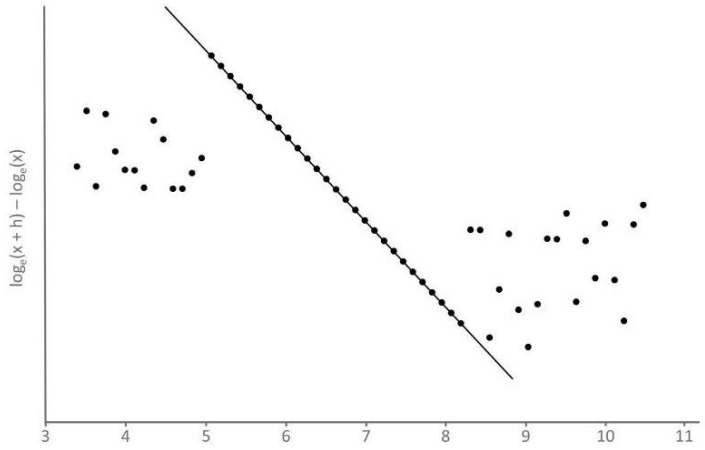
Selection of reference interval approach – available indirect approaches

Hoffman Method (1963)



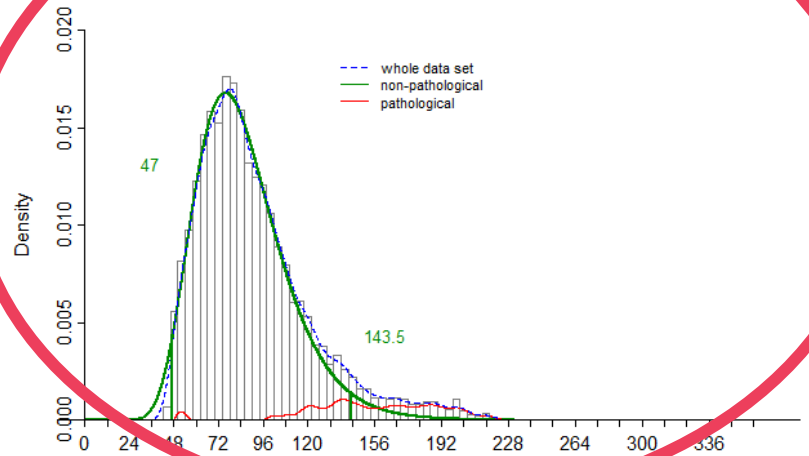
- *Plot the cumulative frequency of the distribution on a normal probability paper*
- *Reference interval extrapolated through linear regression*

Bhattacharya Method (1967)



- *Mathematical straightening of the Gaussian distribution*
- *The slope and intercept are used to determine the mean and SD, and from this, the reference interval*

TML Method (2007)



- *Modern computational power can be leveraged to derive indirect reference intervals using "maximum likelihood estimation"*

CSCC hRI WG: Path to Reference Interval Harmonization

Selection of reference interval approach – available indirect approaches

Clin Chem Lab Med 2007;45(8):1043–1057 © 2007 by Walter de Gruyter • Berlin • New York. DOI 10.1515/CCLM.2007.250

A plea for intra-laboratory reference limits. Part 2. A bimodal retrospective concept for determining reference limits from intra-laboratory databases demonstrated by catalytic activity concentrations of enzymes

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Reference limits of plasma and serum creatinine concentrations from intra-laboratory data bases of several German and Italian medical centres Comparison between direct and indirect procedures☆

Farhad Arzideh [a], Werner Wosniok [a], Rainer Haeckel [b,*]

[a] Institut für Statistik, Universität Bremen, Bremen, Germany
[b] Bremer Zentrum für Laboratoriumsmedizin, Klinikum Bremen Mitte, Bremen, Germany

Clinical Chemistry 61:7
964–973 (2015)

Pediatric Clinical Chemistry

Age- and Sex-Specific Dynamics in 22 Hematologic and Biochemical Analytes from Birth to Adolescence

Jakob Zierk,[1] Farhad Arzideh,[2] Tobias Rechenauer,[1] Rainer Haeckel,[3] Wolfgang Rascher,[1] Markus Metzler,[1] and Manfred Rauh[1*]

DGKL Deutsche Gesellschaft für Klinische Chemie und Laboratoriumsmedizin e.V.

THE DGKL | POSITIONS | ACTIVITIES | EDUCATION, TRAINING & FUR

Activities

Laboratory medicine: Confirming diagnoses and supporting treatments – quickly, effi

Home > Activities > Areas of Expertise and Divisions > Decision Limits / Reference Data

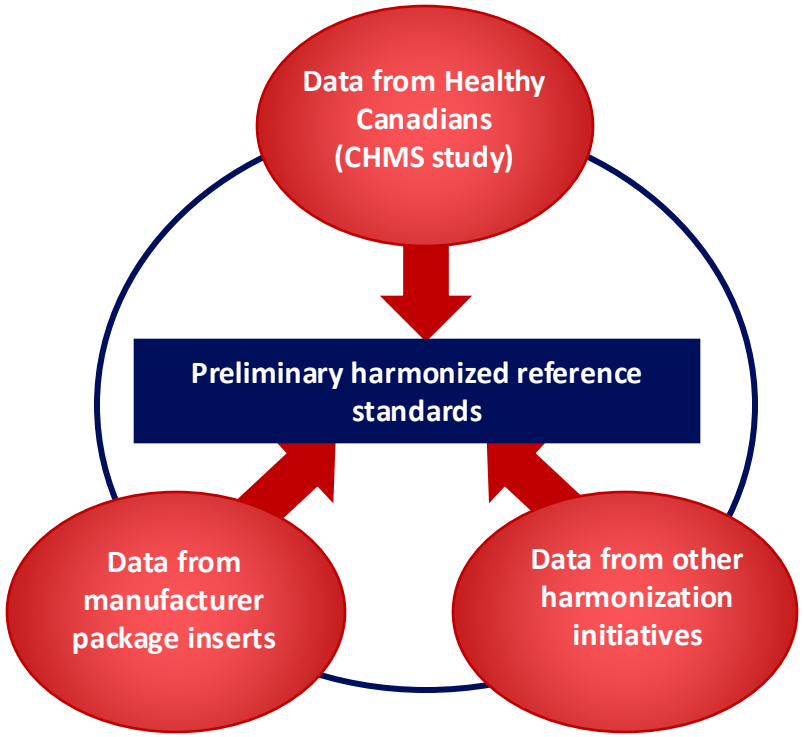
Decision Limits / Reference Data

Criteria for distinguishing between healthy and diseased

CSCC hRI WG

CSCC hRI WG: Path to Reference Interval Harmonization

Data assessment and preliminary hRI establishment



*Based on the comparison of **preliminary harmonized reference standards**, final recommendations were decided on by CSCC hRI WG members at a workshop in 2020*

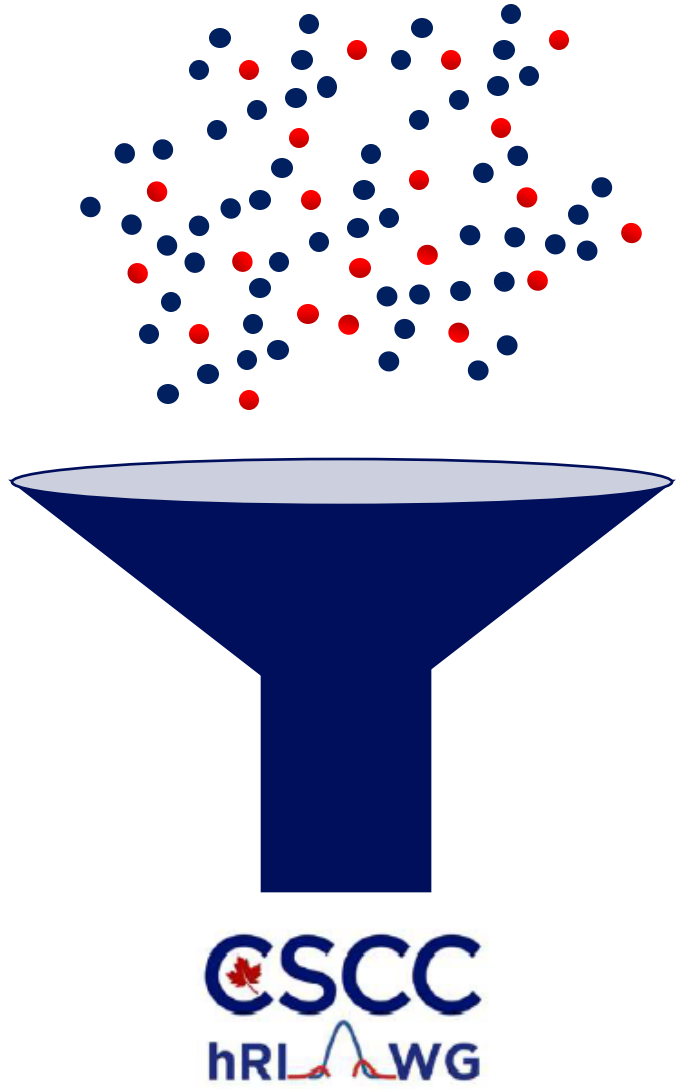
Analyzing the Data: Key Steps & Considerations

- Our team has developed novel R codes to complete the discussed analyses in combination with the RLE software released by DGKL group

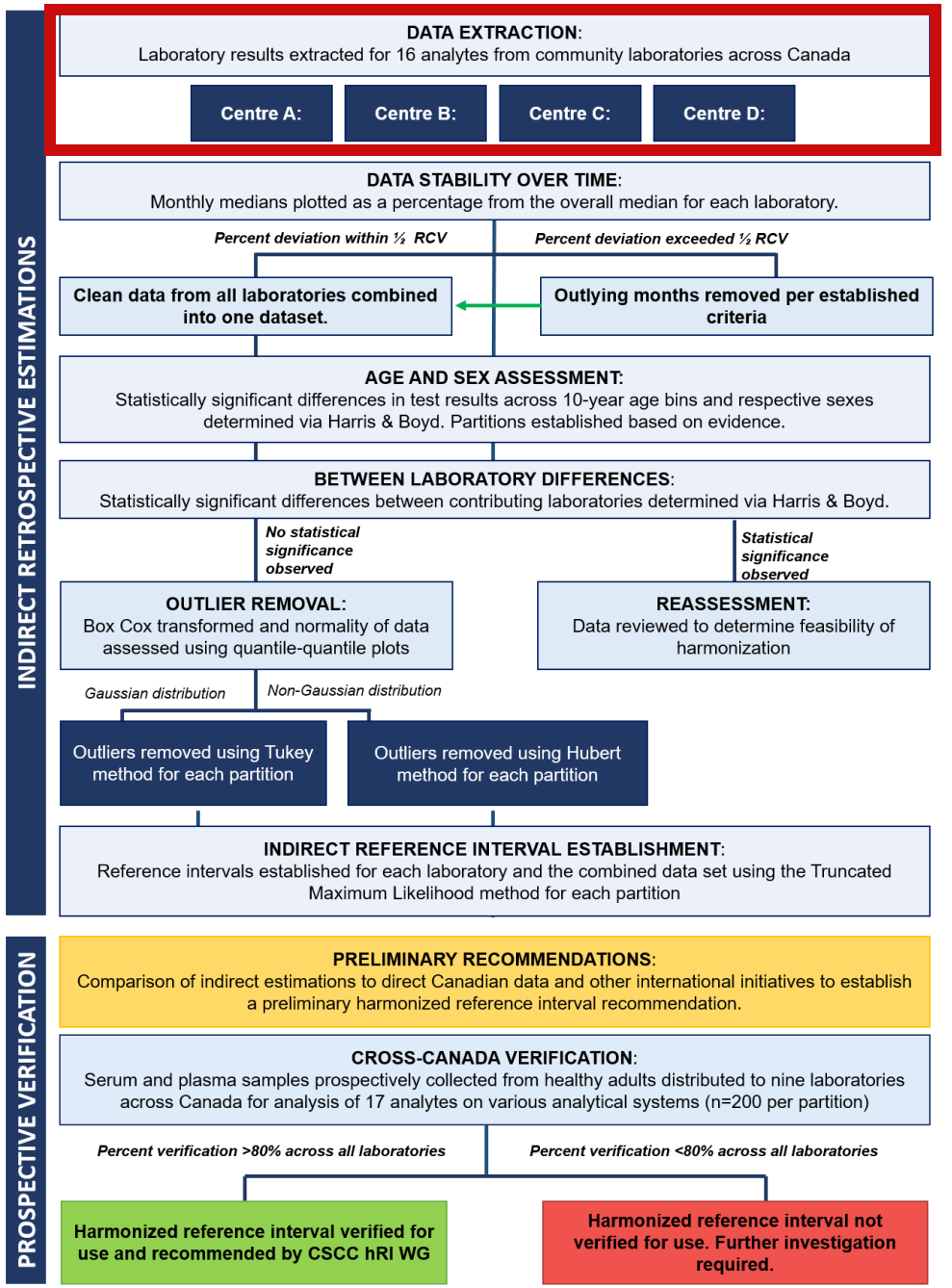
- Today, we will go through this multi-step approach for an example analyte as well as provide a preview of recommendations

Analyzing the Data

Mary Kathryn Bohn



Analyzing the Data



Analyzing the Data:
Example – Alkaline Phosphatase

1 ***Retrieve population dataset***

- ✓ **Extract data from multiple centres across two year period**
- ✓ **Remove all repeat observations**
- ✓ **Include key covariates:**
  - **Age**
  - **Sex**
  - **Date of Collection**
  - **Result**

Dynacare®

Province: Ontario
Analytical Platform: Cobas
Sample Size: 1062848

DynaLIFE MEDICAL LABS

Province: Alberta
Analytical Platform: Advia
Sample Size: 503169

LifeLabs®

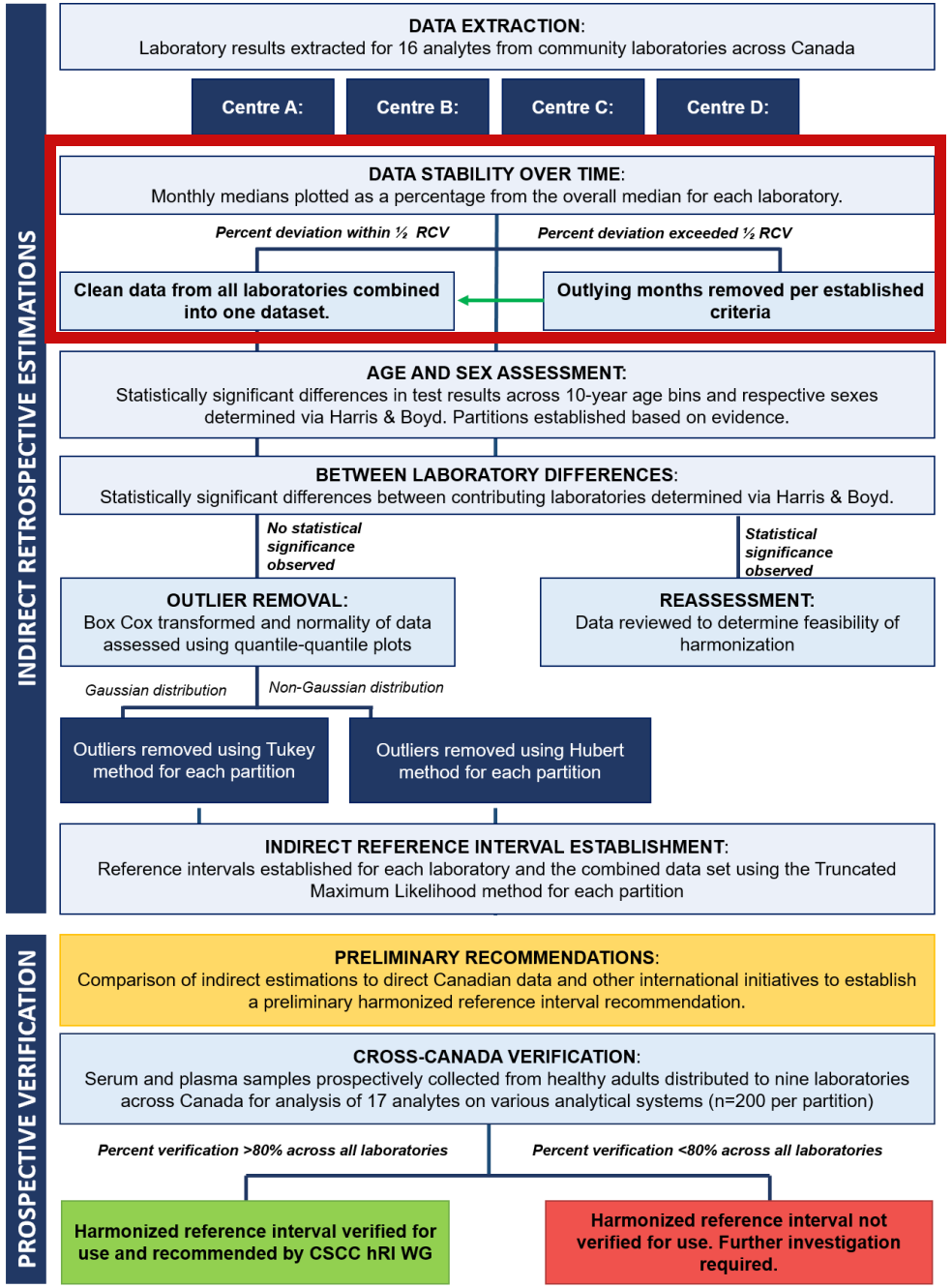
Province: Ontario
Analytical Platform: Cobas
Sample Size: 2655240

LifeLabs®

Province: BC
Analytical Platform: Cobas
Sample Size: 781171

CSCC hRI WG

Analyzing the Data



Analyzing the Data:

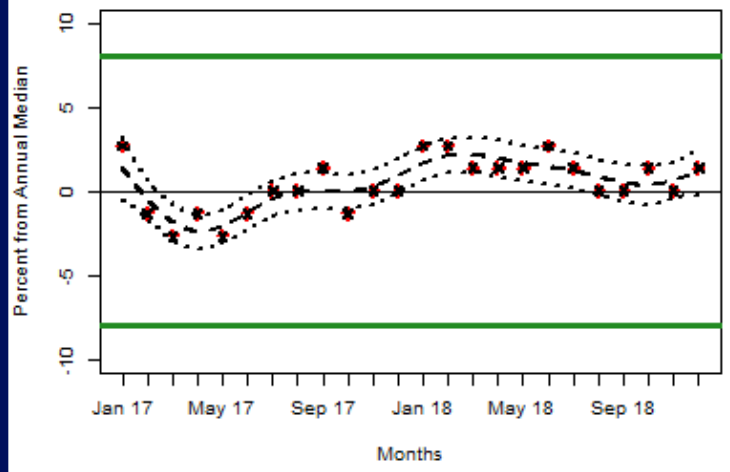
Example – Alkaline Phosphatase

2 ***Data clean up***

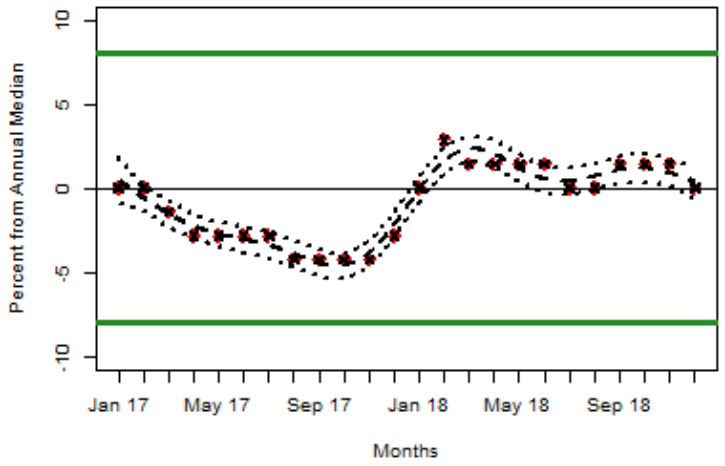
- ✓ ***Monthly stability assessed visually***
- ✓ ***Percent deviation from median compared to ½ reference change value (RCV) reported by EFLM (8%)***

Monthly Stability: Percent variation from annual median graphed for each laboratory separately and compared to ½ RCV (green line)

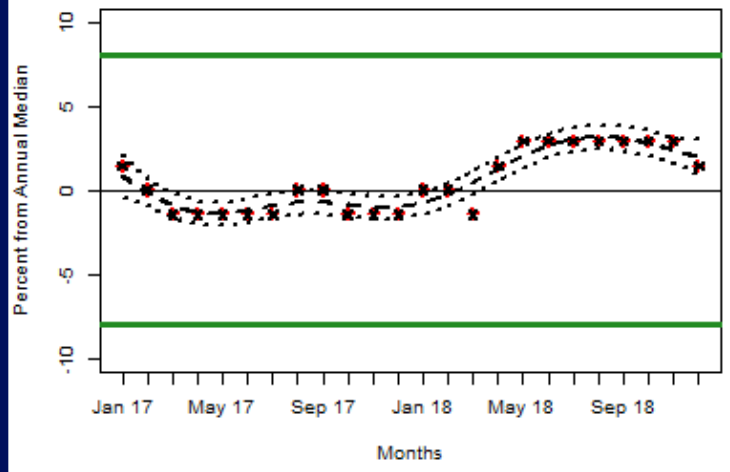
ALP (Advia – Alberta)



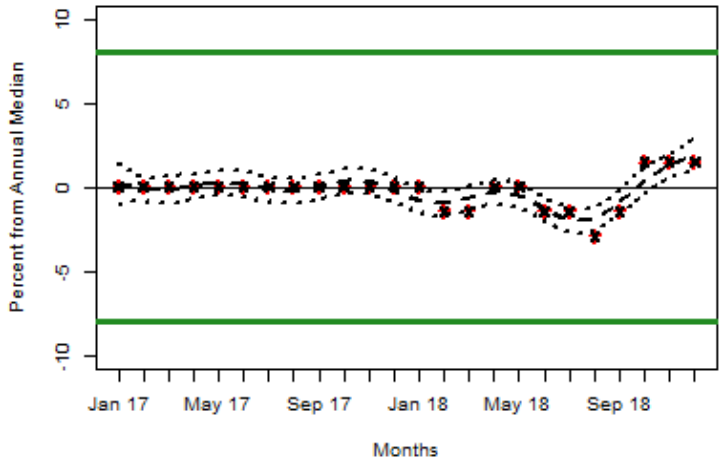
ALP (Cobas – British Columbia)



ALP (Cobas – Ontario)

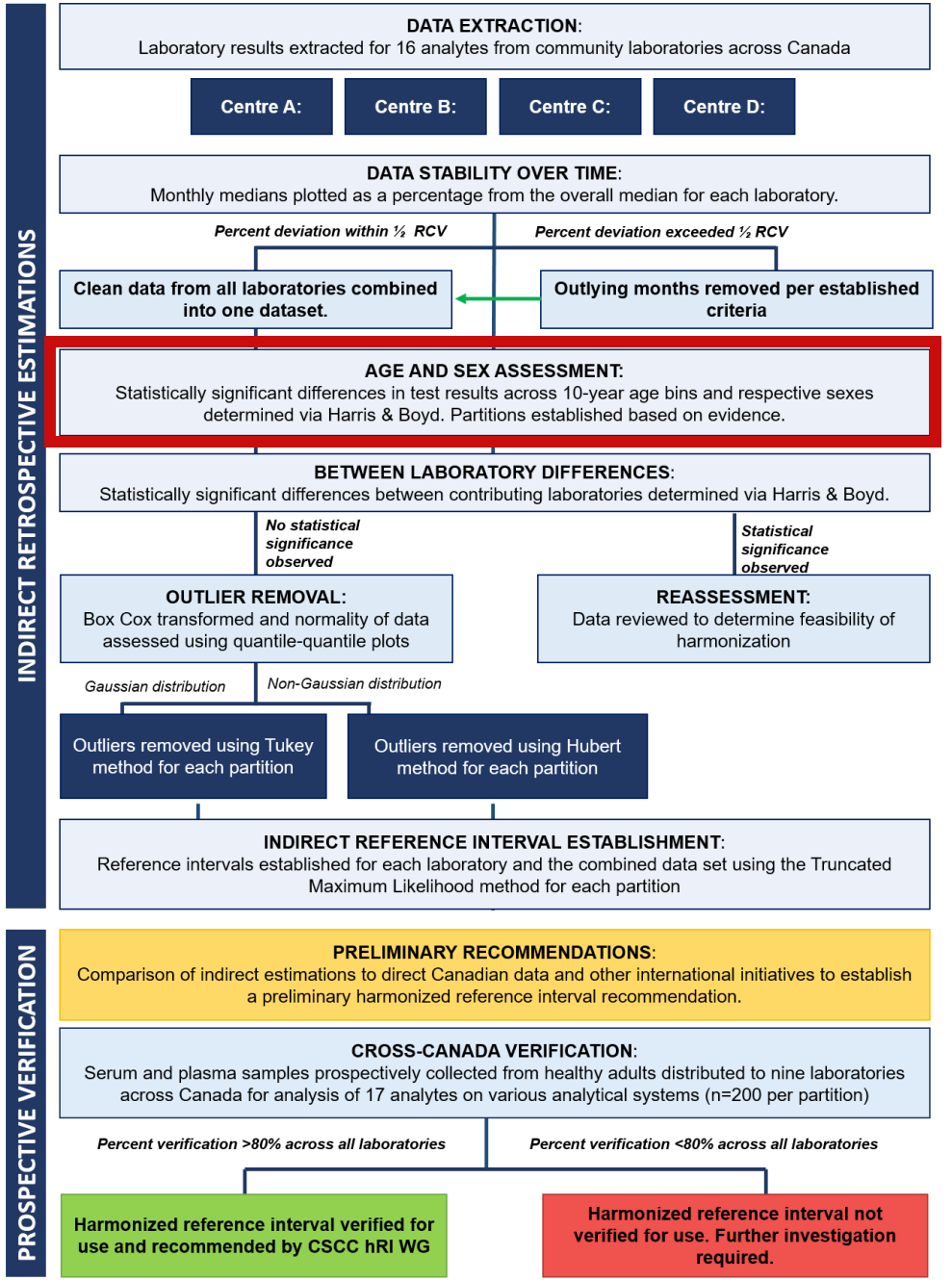


ALP (Cobas – Ontario)



No instability observed

Analyzing the Data



Analyzing the Data:

Example – Alkaline Phosphatase

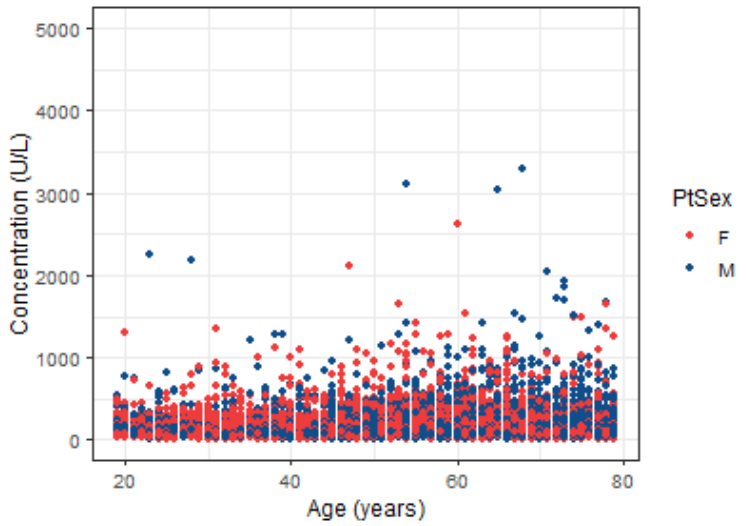
3 ***Assess age/sex differences***

- ✓ **Visually assess raw data across each centre**

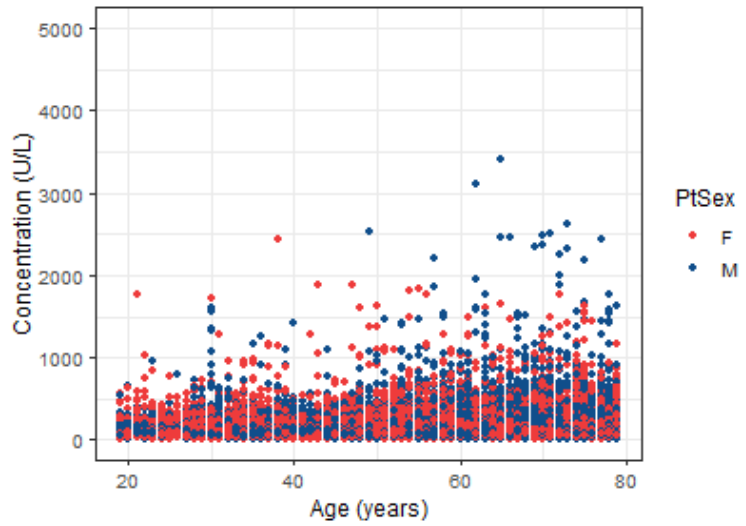
Difficult to make conclusions based on simple visualization of raw data

Scatterplot: Raw result values graphed by age and colour-coded by sex for each laboratory

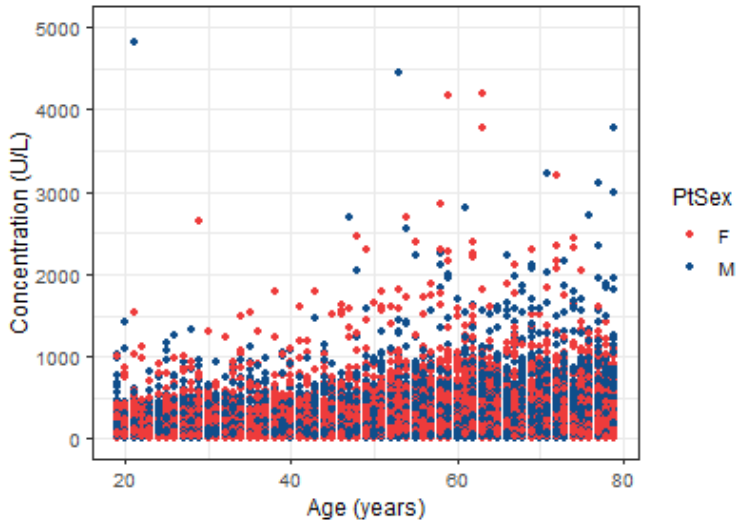
ALP (Advia – Alberta)



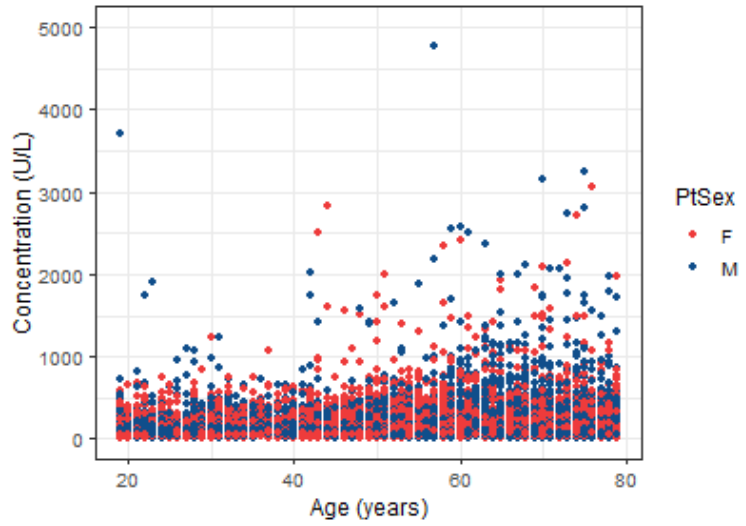
ALP (Cobas – British Columbia)



ALP (Cobas – Ontario)



ALP (Cobas – Ontario)



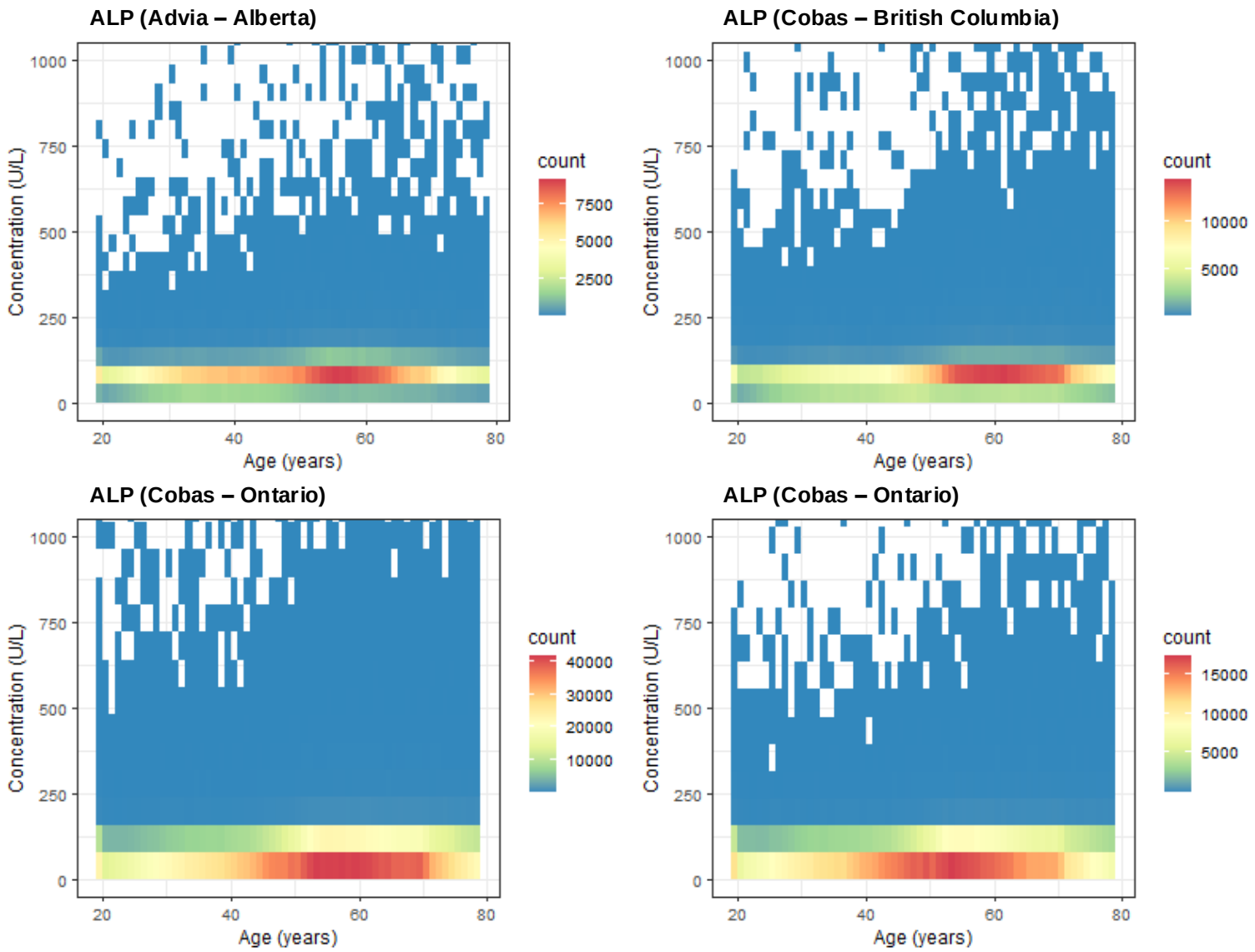
Analyzing the Data:

Example – Alkaline Phosphatase

3 ***Assess age/sex differences***

- ✓ **Visually assess raw data across each centre**
- ✓ **Compare data density across the age range for each laboratory**

Density plot: To visualize density, divides the plot area in a multitude of small fragment and represents the number of points in this fragment.



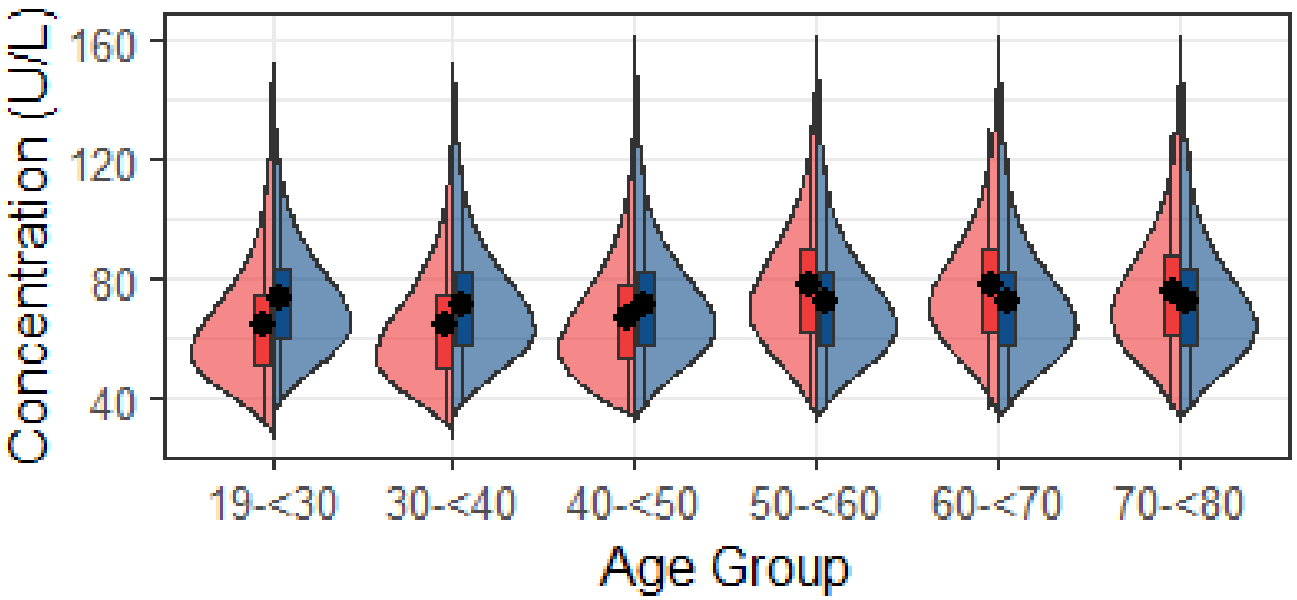
Analyzing the Data:

Example – Alkaline Phosphatase

3 *Assess age/sex differences*

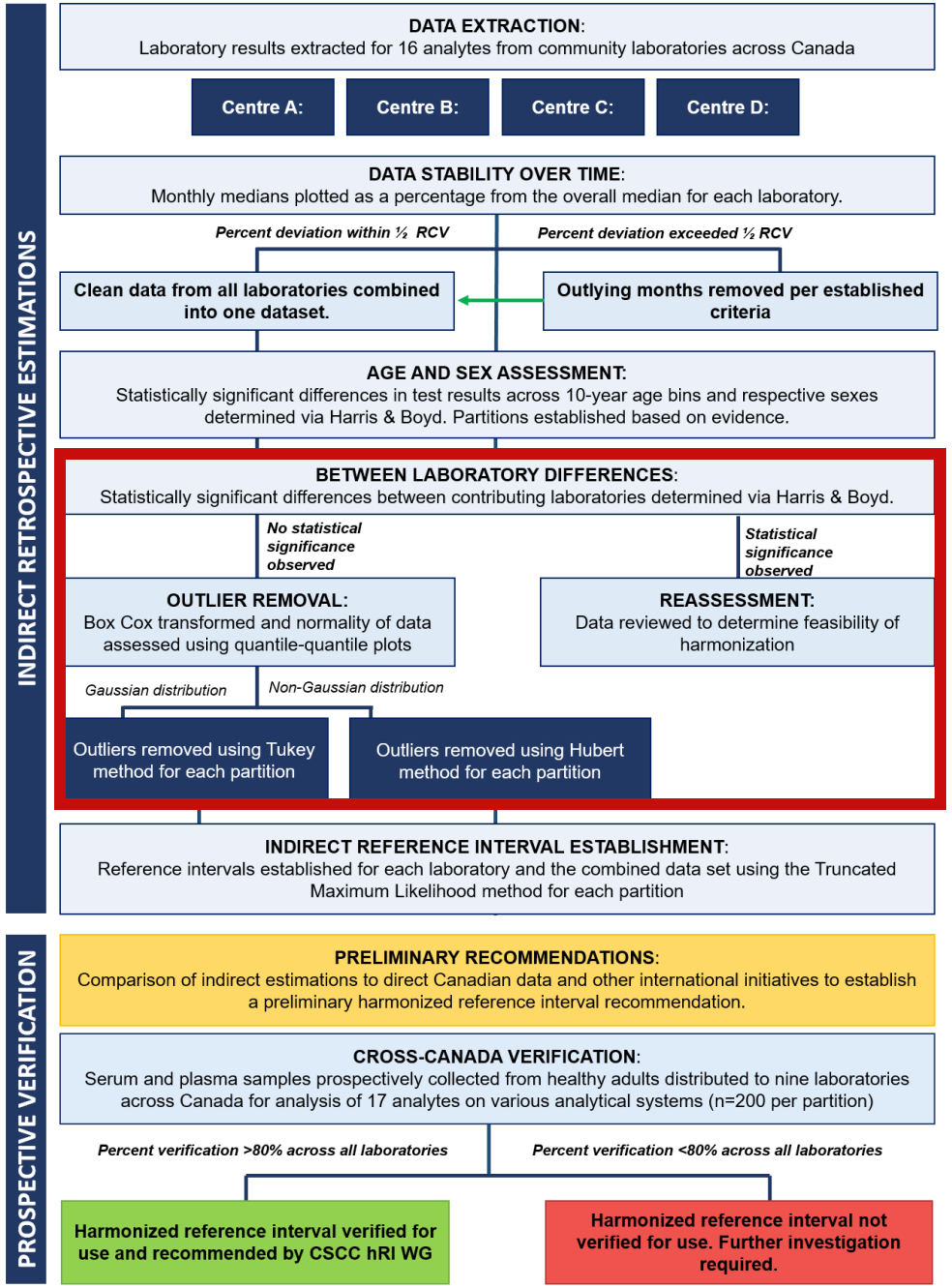
- ✓ **Visually assess raw data across each centre**
- ✓ **Compare data density across the age range for each laboratory**
- ✓ **Use specialized plots to view age- and sex-specific differences**
- ✓ **Confirm visual assessment statistically using Harris & Boyd Method**

Bean plot: Alternative to boxplot - compares the distributions of different groups by graphing a two-sided histogram (helpful to identify bimodal distributions etc.)



Established age partitions:
19-40 years M/F
40-80 years

Analyzing the Data

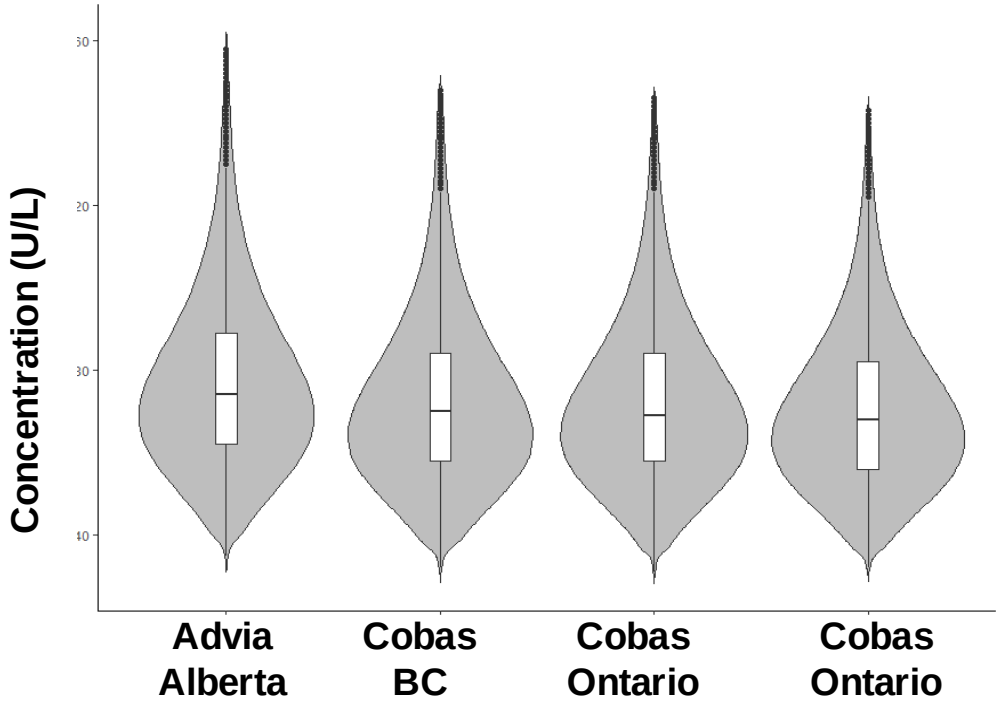


Analyzing the Data:

Example – Alkaline Phosphatase

4 *Centre-specific differences*

- ✓ ***Assess centre-specific differences using Harris & Boyd method***
- ✓ ***Combine all centres if no significant differences are observed into Canada-Wide file***



Dynacare®
Province: Ontario (Cobas)
Sample Size: 1062848

DynaLIFE MEDICAL LABS
Province: Alberta (Advia)
Sample Size: 503169

LifeLabs®
Province: Ontario (Cobas)
Sample Size: 2655240

LifeLabs®
Province: British Columbia (Cobas)
Sample Size: 781171

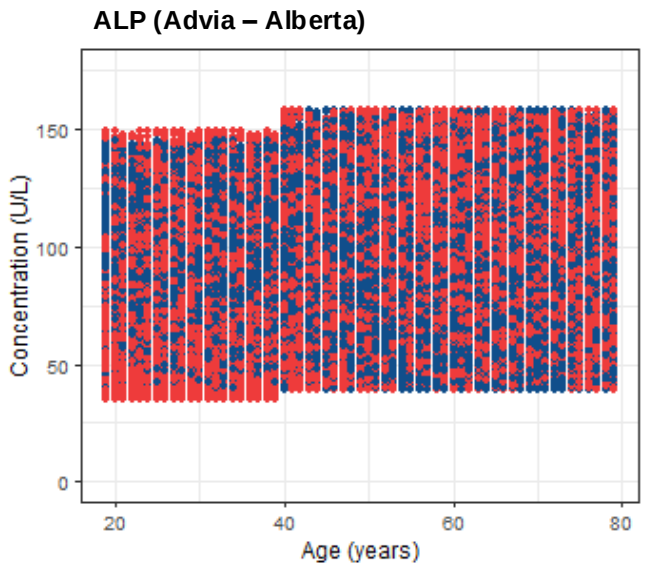
No centre-specific differences

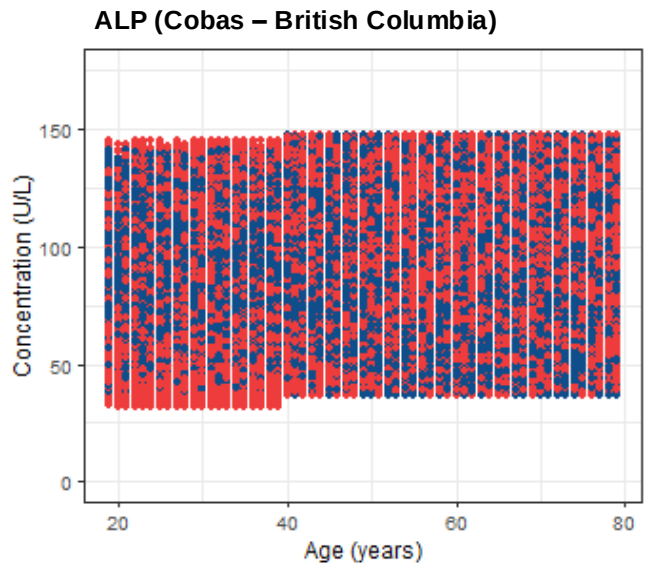
Analyzing the Data:

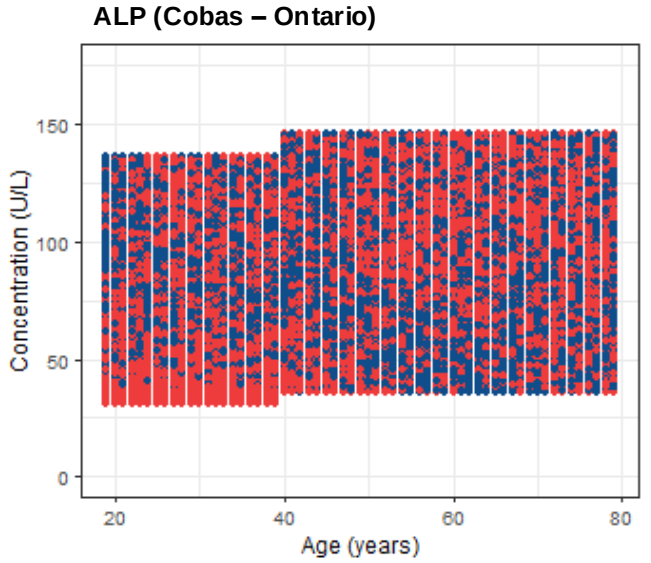
Example – Alkaline Phosphatase

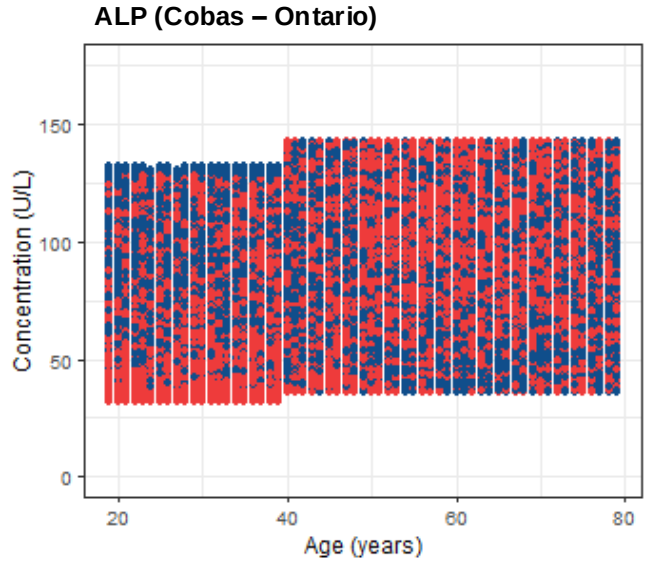
4 ***Data clean up***

- ✓ ***Remove outliers for each centre based on Tukey or Hubert method***



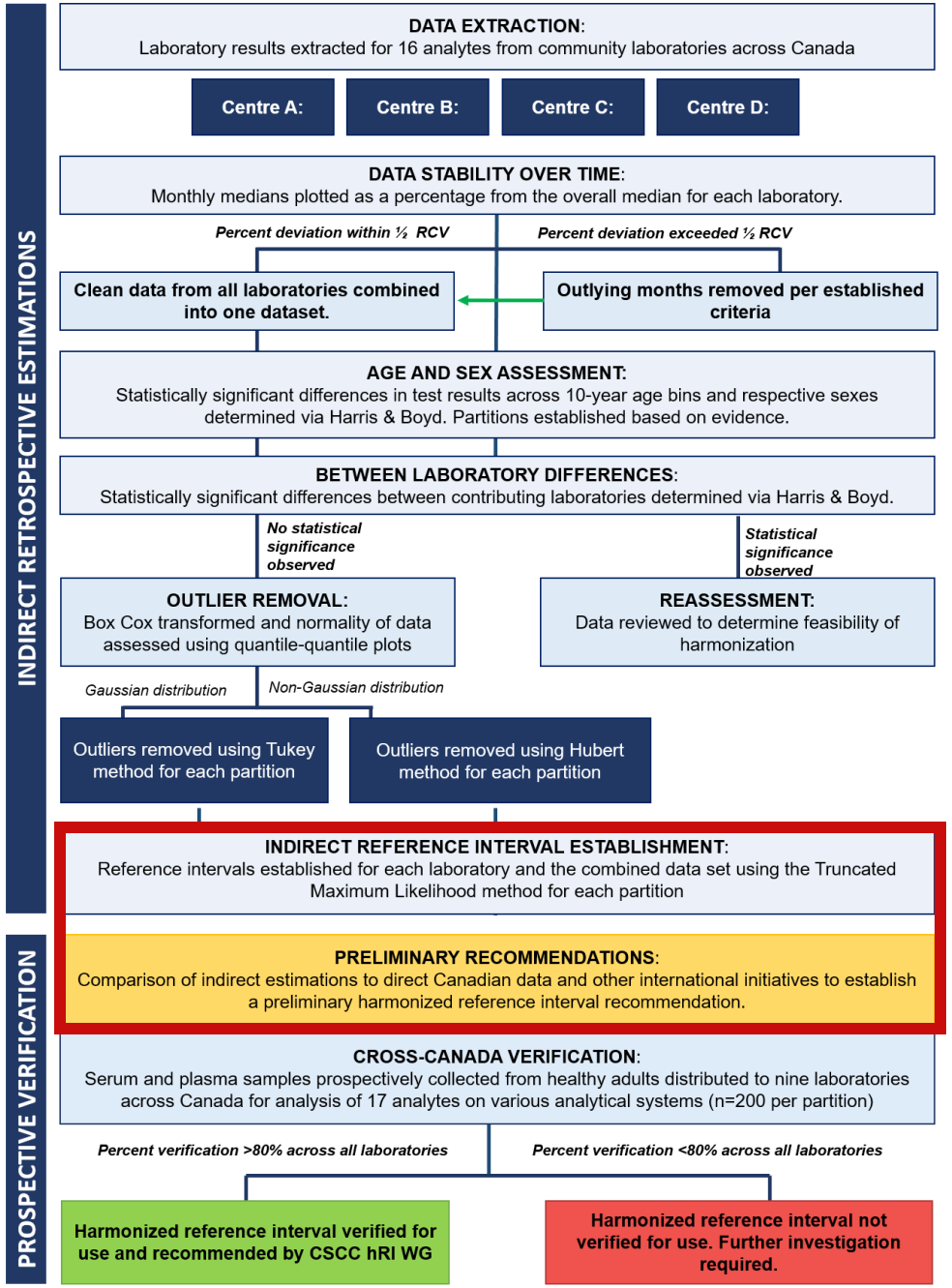






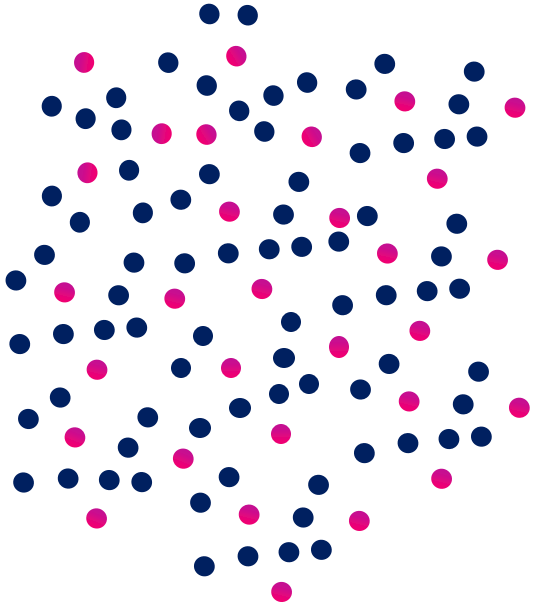
Combined sample size = 4.8 Million

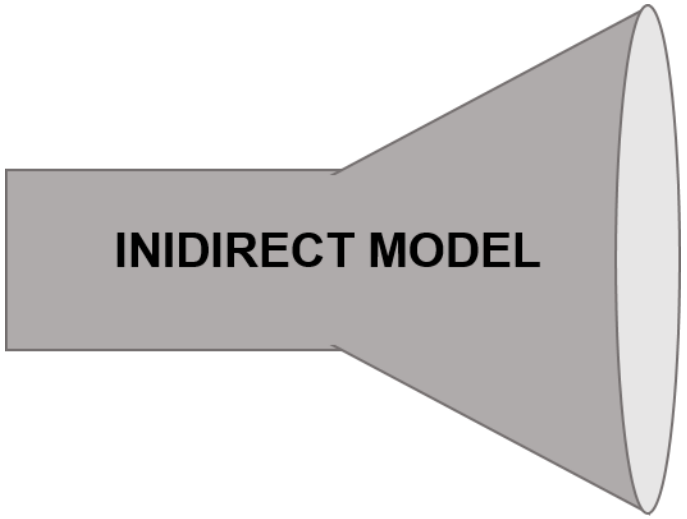
Analyzing the Data

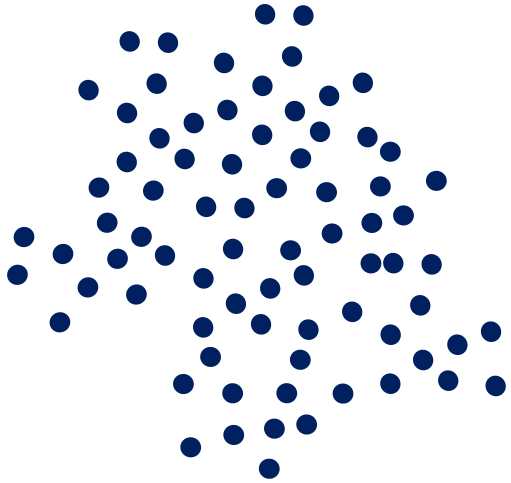


Indirect Reference Interval Estimation

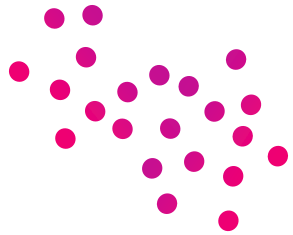
LIS







“healthy” subpopulation

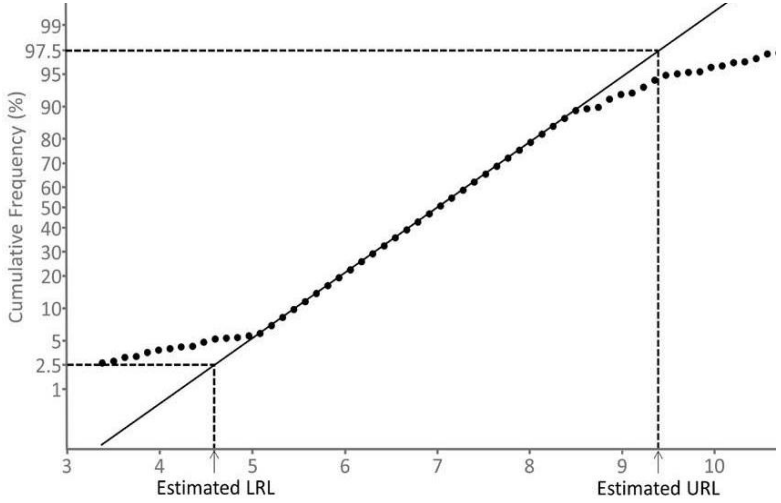


“non-healthy” subpopulation

Indirect Reference Interval Estimation

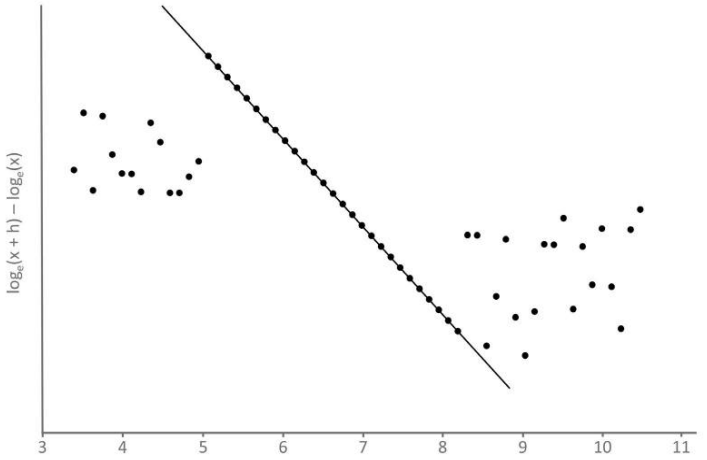
Available Indirect Methods:

Hoffman Method (1963)



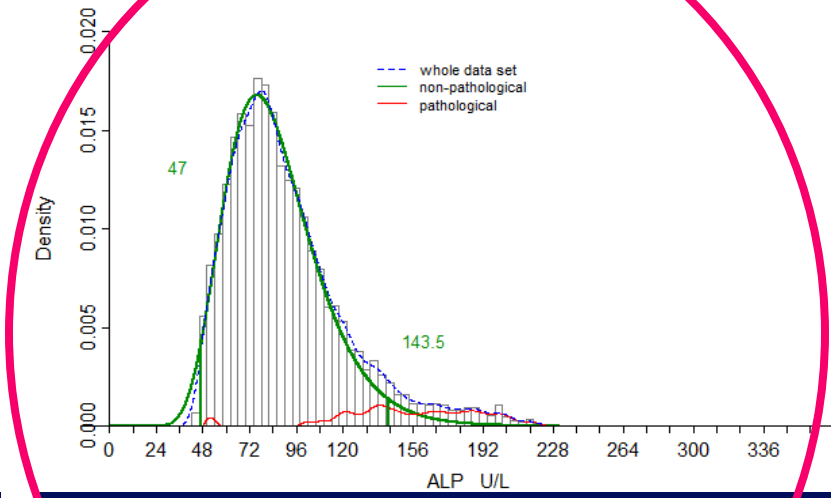
- *Plot the cumulative frequency of the distribution on a normal probability paper*
- *Reference interval extrapolated through linear regression*

Bhattacharya Method (1967)



- *Mathematical straightening of the Gaussian distribution*
- *The slope and intercept are used to determine the mean and SD, and from this, the reference interval*

TML Method (2007)



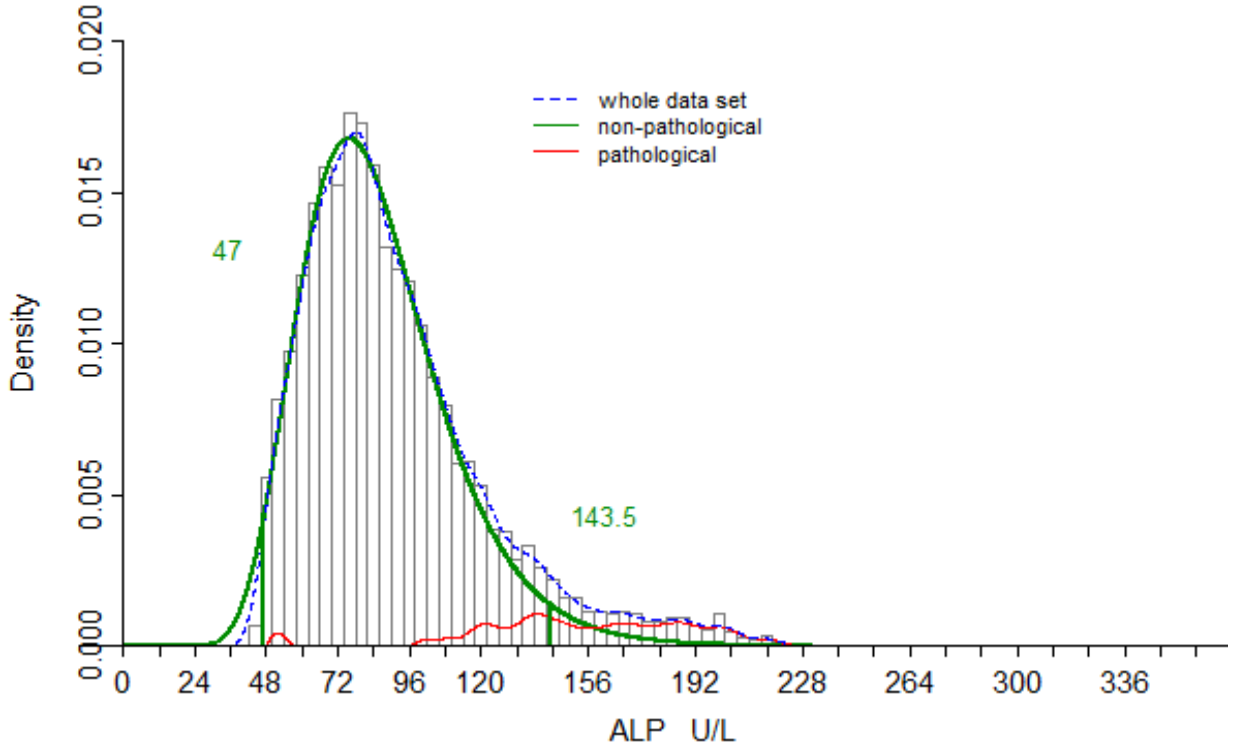
- *Modern computational power can be leveraged to derive indirect reference intervals using "maximum likelihood estimation"*

The Clinical biochemist Reviews. 2019 May;40(2):99.

Truncated Maximum Likelihood Method

TML Method:

- Developed in 2007 by Arzideh and colleagues
- Modern, non-graphical, and automatedcomputational approach
- **Key Steps:**
  - Apply a smoothed kernel density function to estimate the distribution of the entire dataset.
  - Central portion of the dataset is assumed to represent the “healthy” population and is modeled.
  - Parameters of this distribution are estimated using maximum likelihood techniques
  - 2.5th and 97.5th percentiles are then derived



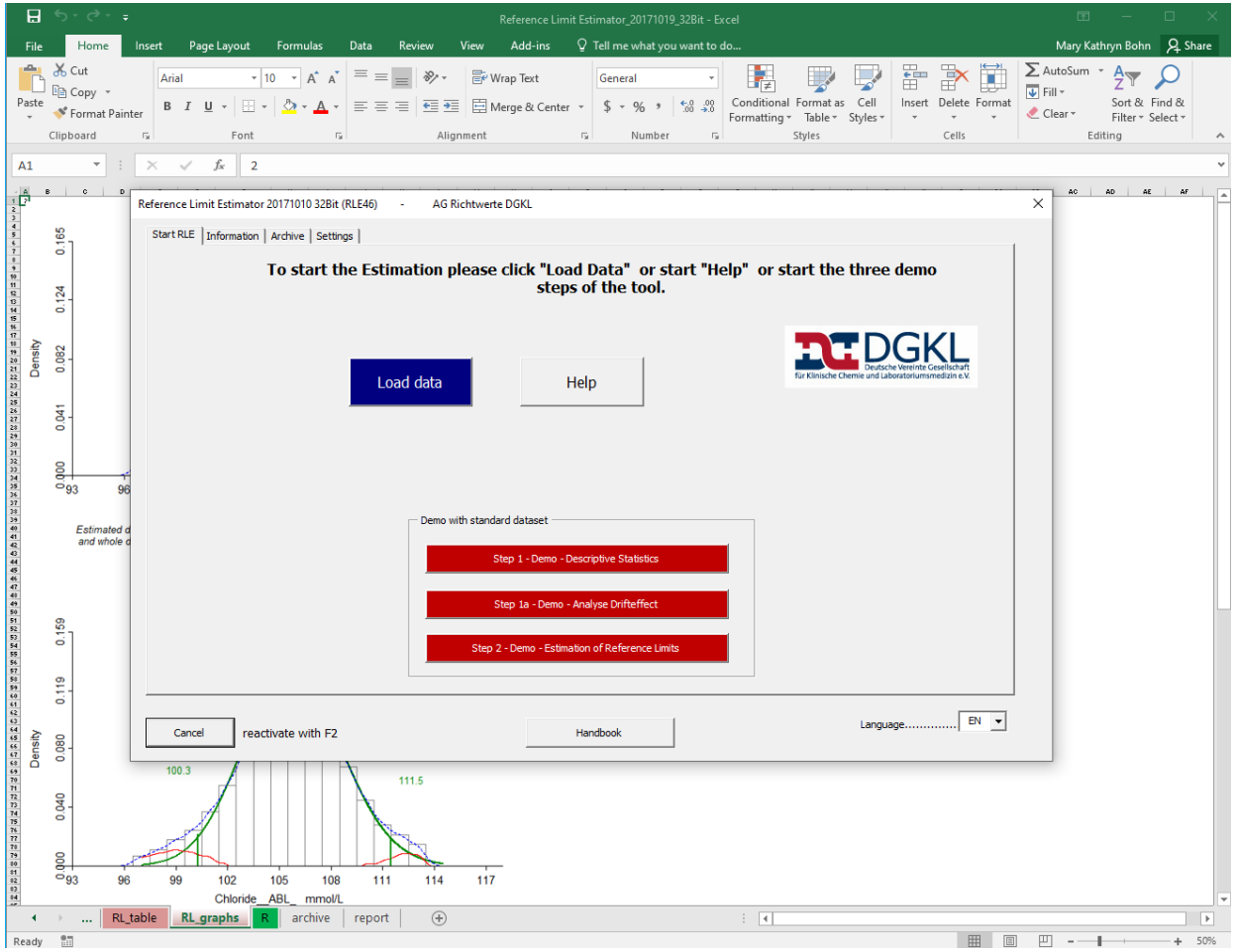
Alkaline Phosphatase Data (unpublished)

Clin Chem Lab Med 2007;45:1033–42.

Truncated Maximum Likelihood Method

TML Method:

- Computational power (millions of data points)
- Reduced subjectivity, using likelihood and fitting techniques rather than visual or manual assessment
- Makes no assumptions regarding the distribution of pathological values
- Easily executed using R or excel-based programming

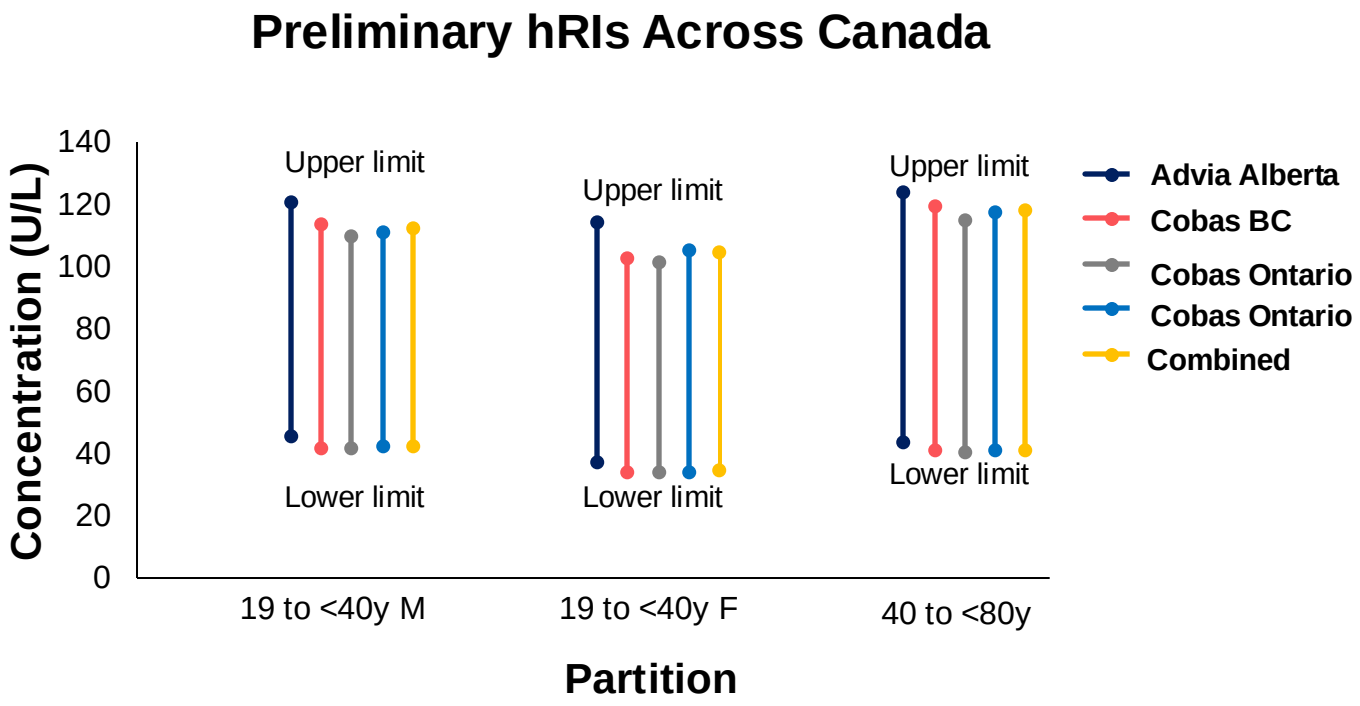


Analyzing the Data:
Example – Alkaline Phosphatase

5 ***Establish RI for each partition***

- ✓ ***Use TML method to establish reference intervals for each partition***
- ✓ ***Compare established reference intervals across provinces and reference intervals***

RI comparison plot: Lower and upper limits graphed for each dataset across partitions to identify major differences in estimations



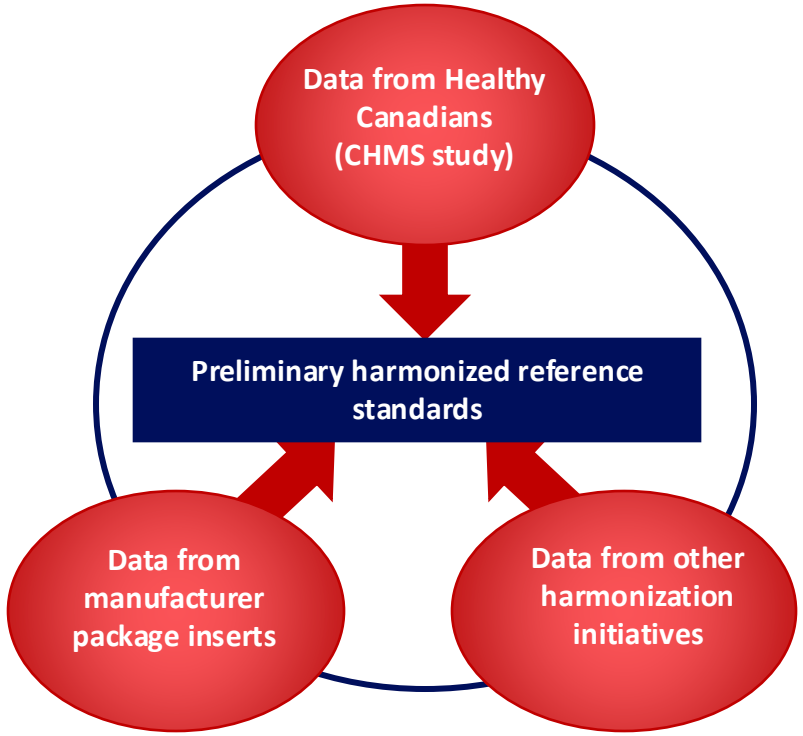
Partition	Advia AB	Cobas BC	Cobas ON	Cobas ON	Canada-Wide
19 to <40y M	46-121	42-114	42-110	42-111	42-113
19 to <40y F	37-115	34-103	34-101	34-106	35-105
40 to <80y	44-124	41-119	41-115	41-118	41-119

*displayed in U/L

Analyzing the Data:
Example – Alkaline Phosphatase

5 ***Compare and assess***

- ✓ *Compare to indirect and direct data published by international initiatives*
- ✓ *Compare to manufacturer package insert data*
- ✓ *Compare to what is currently used at each centre*
- ✓ *Internal discussion and finalization*



	Indirect Estimate	Direct International Initiatives					hRI
	All	**CHMS**	**AHRIA**	**AUSSIE**	**NORIP**	**UK**	
N	4858388						**19-39y F:** 35-105 U/L
19-39y F	35-105	**F**: 50-116	30-110	**F**: 43-111	37-106	30-130	**19-39y M:** 40-115 U/L
19-39y M	42-113						
40-79y	41-119	**M**: 46-122		**M**: 39-114			**40-79y:** 40-120 U/L

CSCC hRI WG: Preliminary Recommendations & Next Steps

Analyte	Partition	hRI
Alkaline Phosphatase (ALP)	19-39 years M	40-115 U/L
	19-39 years F	35-105 U/L
	40-79 years	40-120 U/L
Alanine Aminotransferase (ALT)	19-79 years M	<33 U/L
	19-79 years F	<25 U/L
Albumin	19-79 years	40-50 g/L
Calcium	19-79 years	2.15-2.55 mmol/L
Carbon Dioxide (total CO2)	19-79 years	22-30 mmol/L
Chloride	19-79 years	97-107 mmol/L
Creatinine	19-79 years M	65-115 umol/L
	19-79 years F	50-95 umol/L
Free Thyroxine (FT4)	19-79 years	None
Lactate Dehydrogenase (LDH)	19-79 years	120-240 U/L
Magnesium	19-79 years	0.73-1.00 mmol/L
Phosphate	19-79 years	0.80-1.45 mmol/L
Potassium	19-79 years	3.8-5.1 mmol/L
Sodium	19-79 years	135-145 mmol/L
Thyroid Stimulating Hormone (TSH)	19-79 years	0.60-4.55 mIU/L
Total Bilirubin	19-79 years M	<20 umol/L
	19-79 years F	<16 umol/L
Total Protein	19-79 years	60-80 g/L

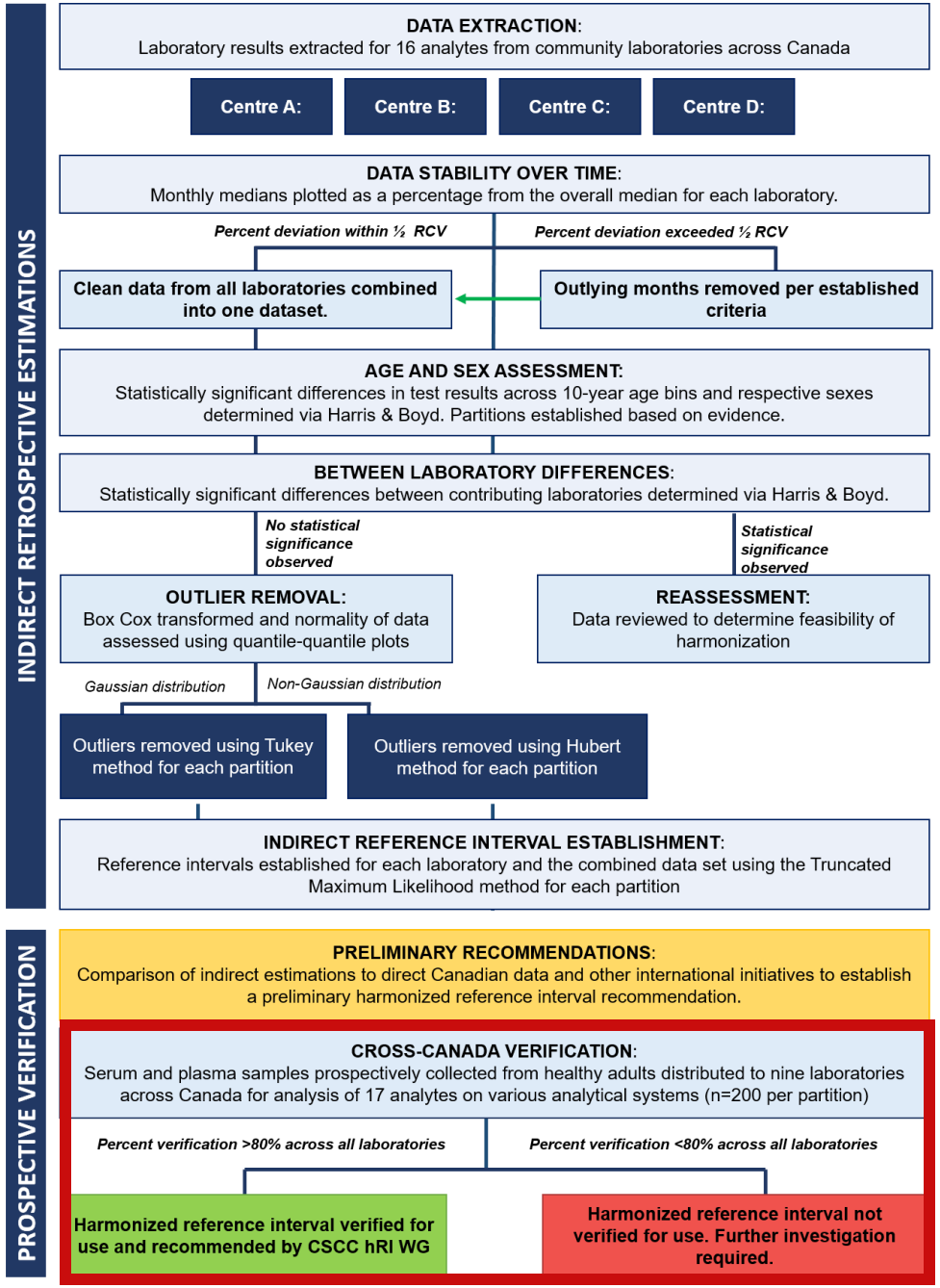
Establishment of preliminary hRIs for 16 parameters

Limitations to the current data:

- *Only three manufacturers represented*
- *Only three provinces represented*
- *All data contributing centres use serum as preferred matrices*

How can they be addressed prior to implementation?

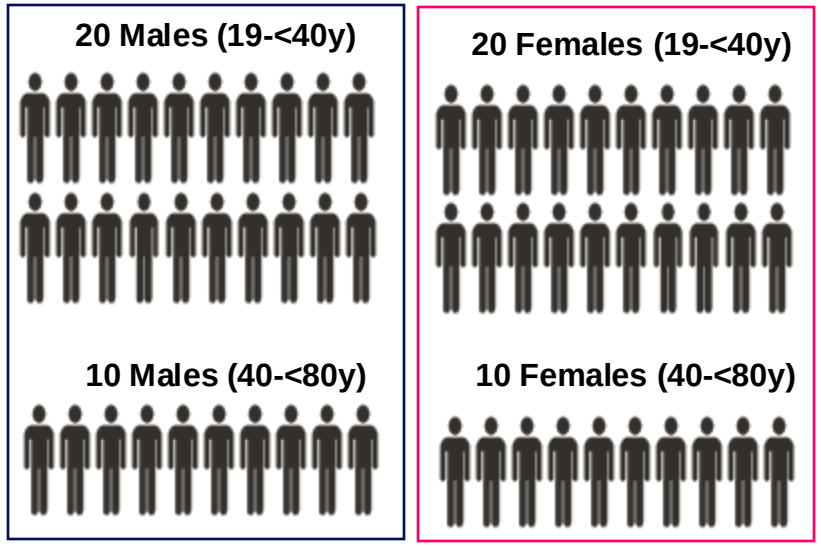
Verifying the Data



CSCC hRI WG: Cross-Canadian Verification Study

Objective: To verify proposed hRIs on major analytical platforms across Canada using serum and plasma samples prospectively collected from healthy adults.

Study Design:



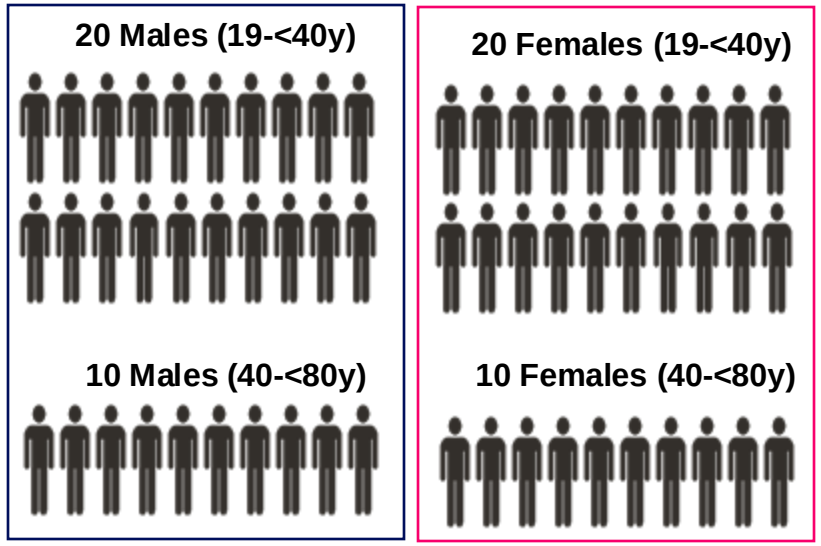
60 adult volunteers *recruited from the community with the following age/sex distribution.*

- 30 individuals were recruited from Ontario
- 30 individuals were recruited from Alberta
- Ethnic distribution is proportional to the 2016 Canadian Census
- **Exclusion criteria included**:
  - Pregnancy
  - History of chronic illness
  - History of acute illness within 7 days of collection
  - Regular use of prescribed medication

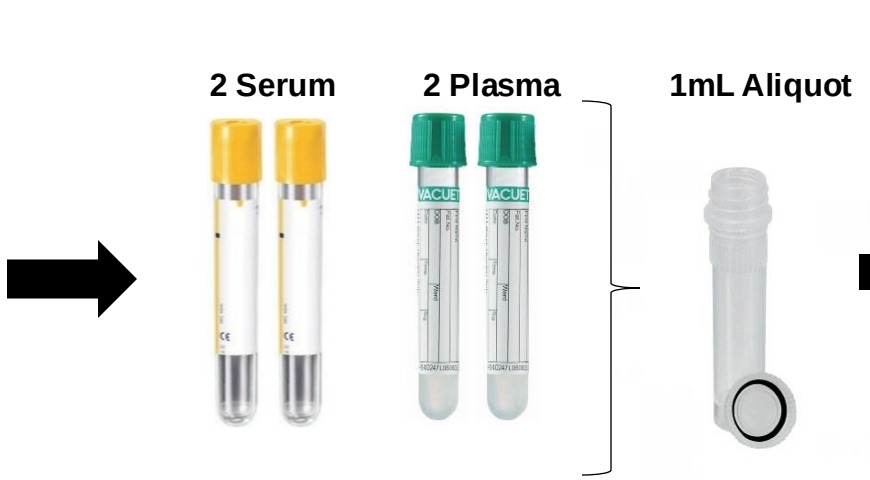
CSCC hRI WG: Cross-Canadian Verification Study

Objective: To verify proposed hRIs on major analytical platforms across Canada using serum and plasma samples prospectively collected from healthy adults.

Study Design:



60 adult volunteers *recruited from the community with the following age/sex distribution.*



2 serum and 2 plasma tubes will be collected from each participant

Distribution to 9 laboratories across Canada for analysis of all 17 analytes

CSCC hRI WG: Participating Laboratories

Province	Manufacturer
Newfoundland	Architect
Quebec	Atellica
Alberta	Cobas
Ontario	Cobas
Ontario	Cobas
Alberta	DxC
British Columbia	Integra
New Brunswick	Vitros
Ontario	Vitros

SIEMENS Roche BECKMAN COULTER Abbott Ortho Clinical Diagnostics

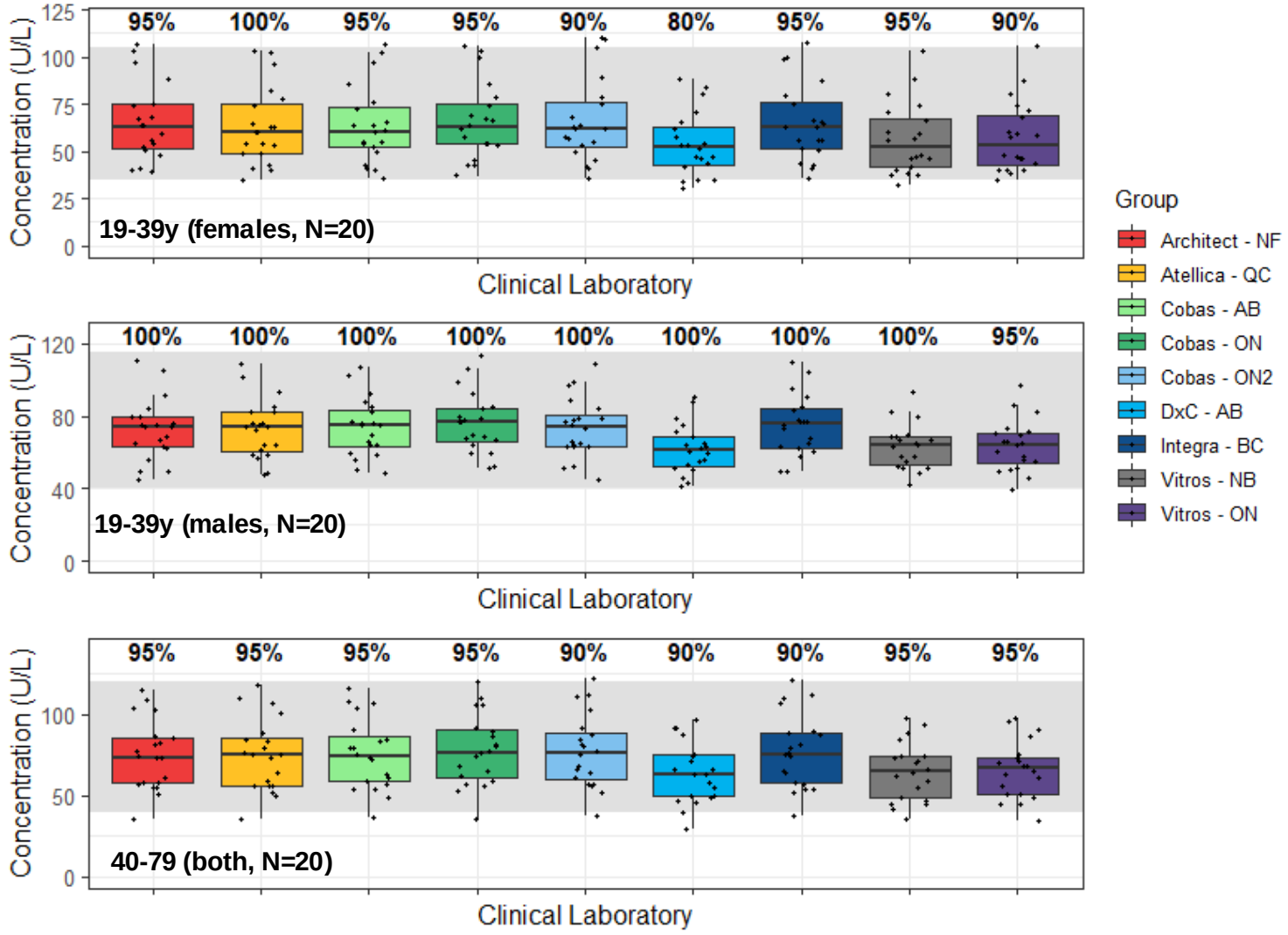
Verifying the Data:

Example – Alkaline Phosphatase

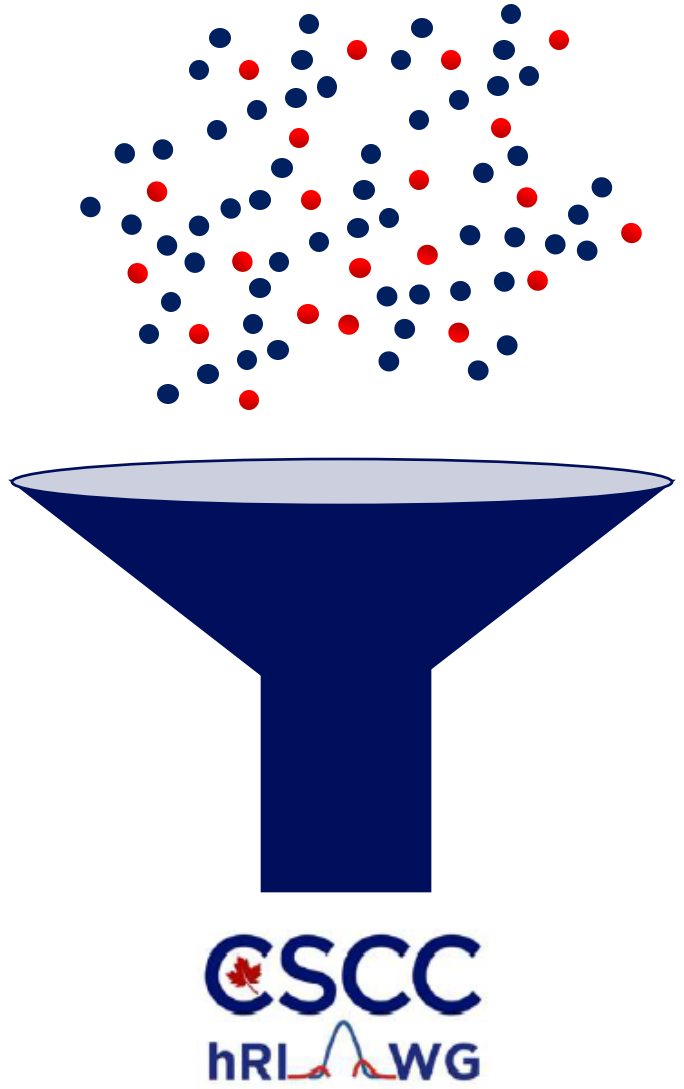
6 ***Compare and assess***

- ✓ ***Compare to indirect and direct data published by international initiatives***
- ✓ ***Compare to manufacturer package insert data***
- ✓ ***Compare to what is currently used at each centre***
- ✓ ***Internal discussion and finalization***

Verification plot: Results from samples collected for healthy Canadian adults graphed by laboratory using boxplot (median, IQR, tails: Q1/Q3+IQR). Percent verification notated for each laboratory, grey area indicates proposed hRI

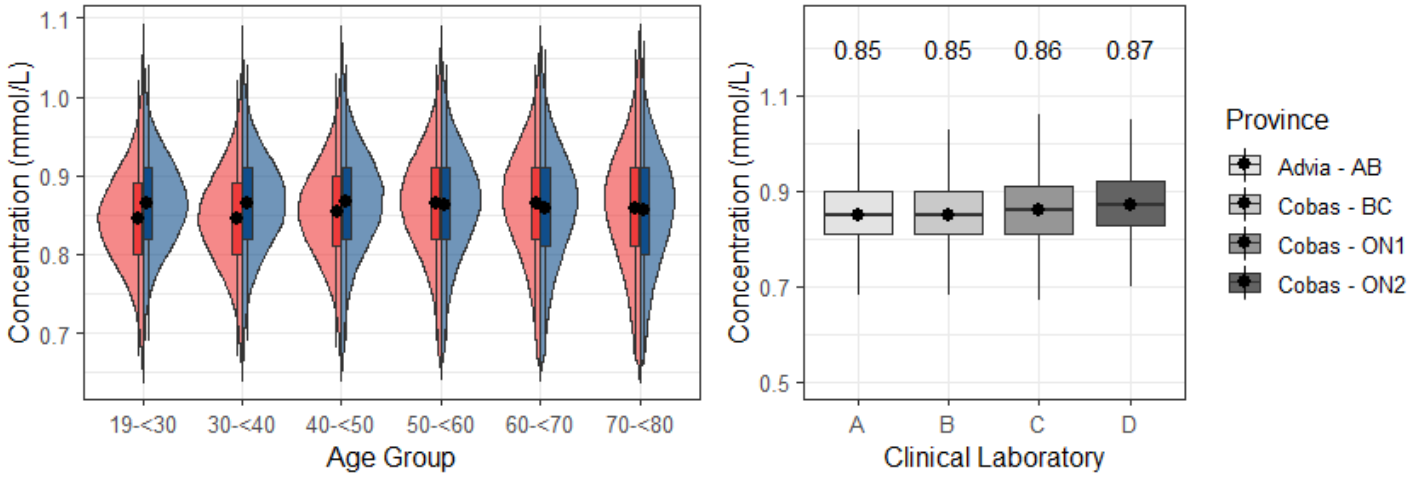


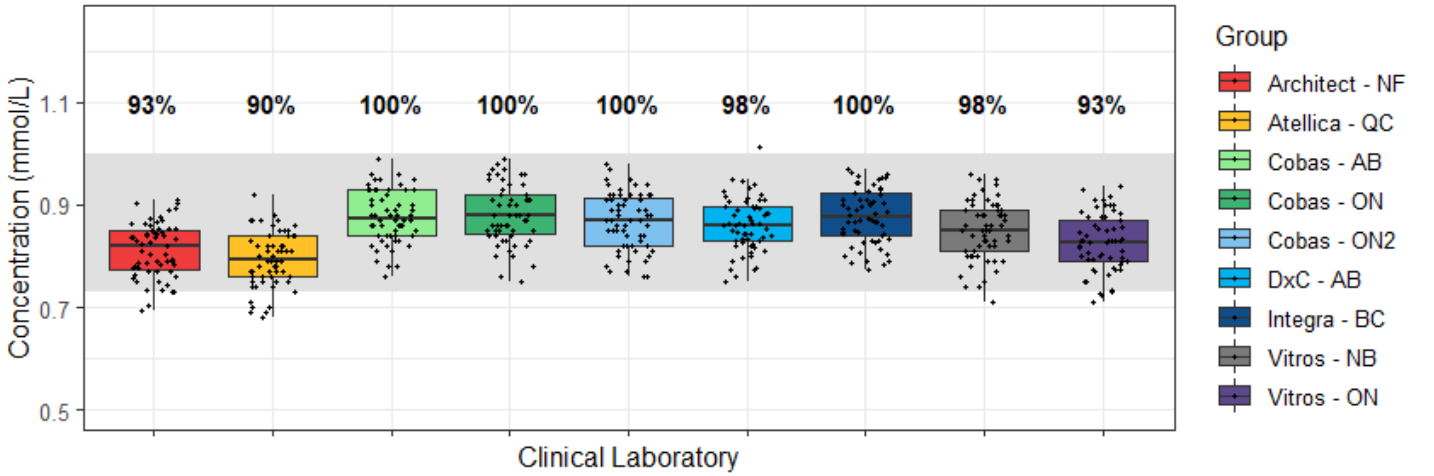
Reviewing the Data



Magnesium

Direct and Indirect Canadian Data Supports Harmonization





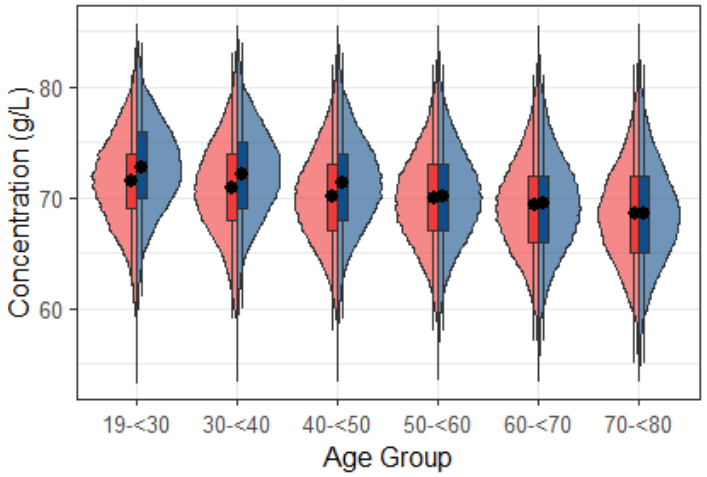
	Indirect Analysis of Provincial Data					Direct International Initiatives				hRI
	Advia – AB	Cobas – BC	Cobas – ON1	Cobas – ON2	All	AHRIA	AUSSIE	UK	NORIP	
N	201290	124417	157662	423031	906400					0.73-1.00 mmol/L
RI	0.72-0.99	0.73-0/99	0.74-1.01	0.72-1.01	0.73-1.00	0.70-1.10	0.77-1.04	0.71-0.94	0.70-1.00	

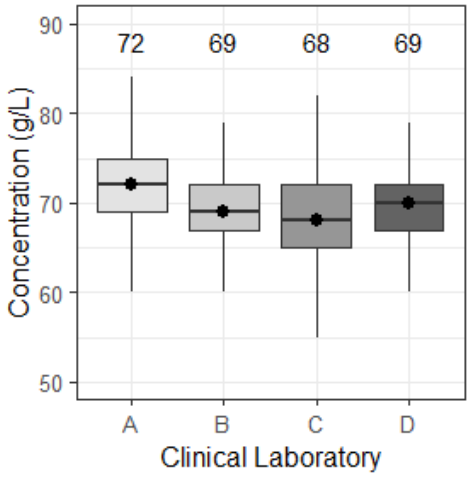
Result Summary:

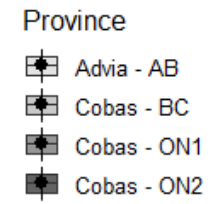
- Approximately **900,000 results** evaluated
- No age/sex-specific differences observed
- Recommend hRI verified in all nine Canadian Laboratories participating in cross-Canada verification program (*serum and plasma*)

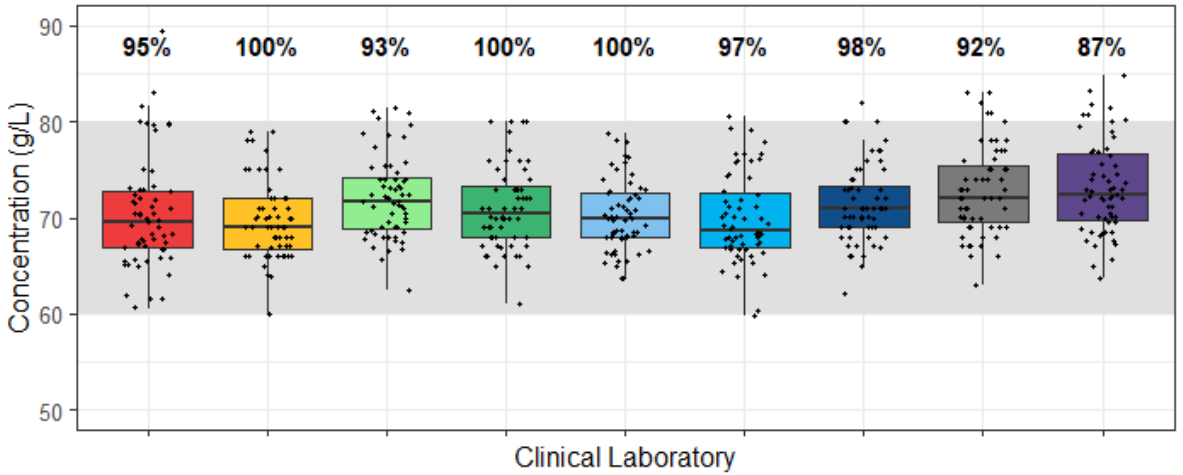
Total Protein

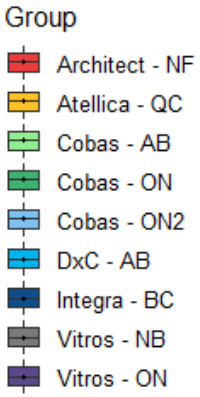
Direct and Indirect Canadian Data Supports Harmonization











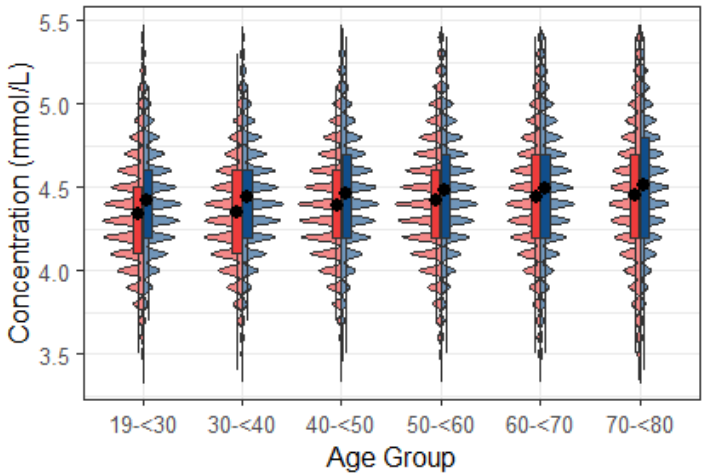
	Indirect Analysis of Provincial Data					Direct International Initiatives				hRI
	Advia – AB	Cobas – BC	Cobas – ON1	Cobas – ON2	All	CHMS	AHRIA	UK	NORIP	60-80 g/L
N	118308	160655	58144	25097	362204					
LL	64-81	61-78	60-78	61-78	61-79	65-83	60-80	60-80	63-78	

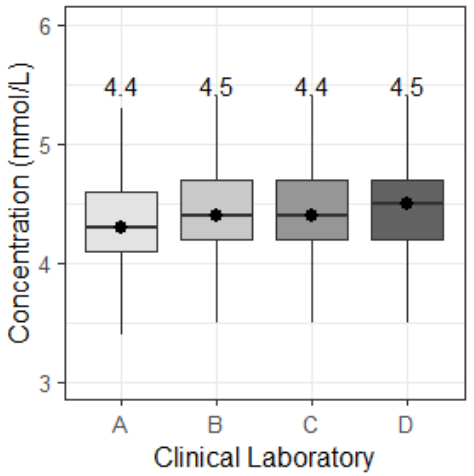
Result Summary:

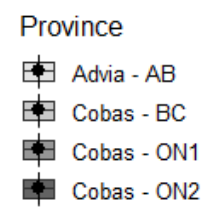
- Approximately **300,000 results** evaluated
- No age/sex-specific differences observed
- Recommend hRI verified in all nine Canadian Laboratories participating in cross-Canada verification program (*serum and plasma*)

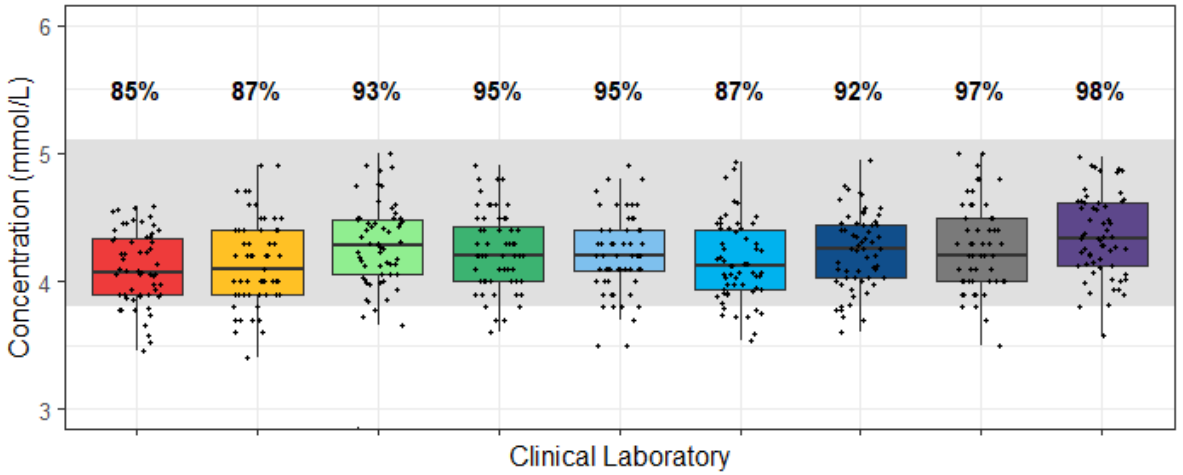
Potassium

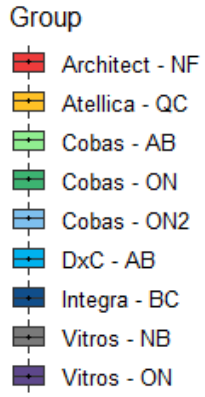
Direct and Indirect Canadian Data Supports Harmonization











	Indirect Analysis of Provincial Data					Direct International Initiatives					hRI
	Advia – AB	Cobas – BC	Cobas – ON1	Cobas – ON2	All	CHMS	AUSSIE	AHRIA	UK	NORIP	3.8-5.1 mmol/L
N	764655	1583639	3930985	1512821	7792100						
RI	3.7-5.1	3.8-5.1	3.8-5.1	3.8-5.2	3.8-5.1	3.8-4.9	3.7-4.9	3.5-5.2	3.5-5.3	3.6-4.6	

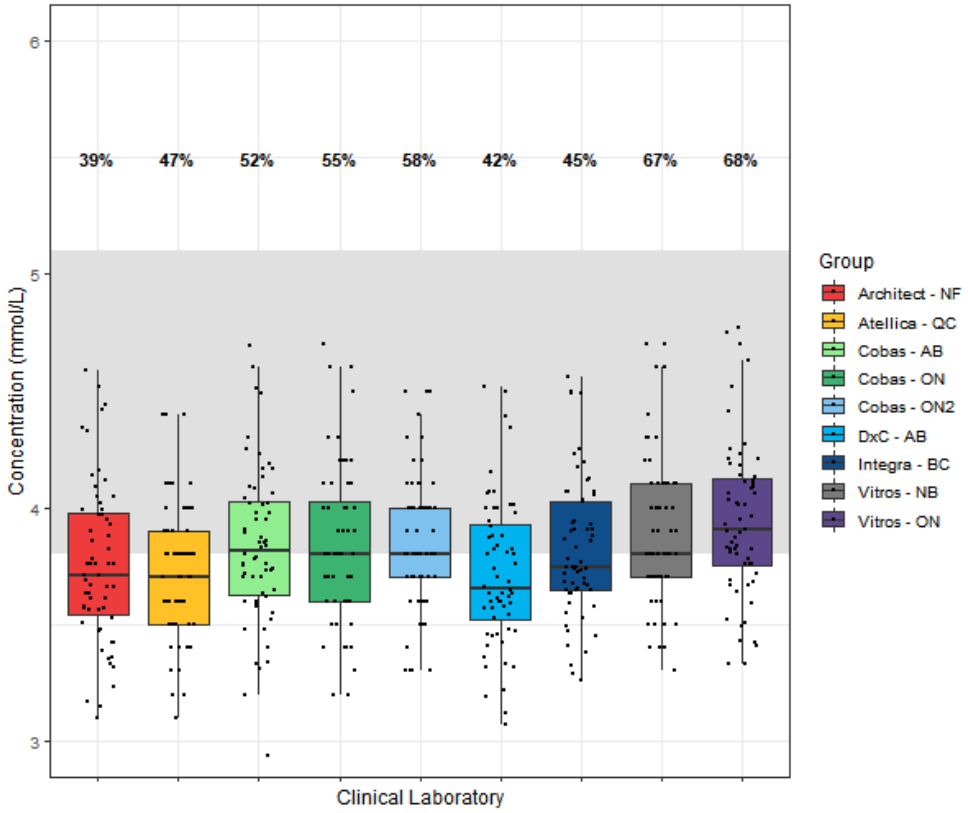
Result Summary:

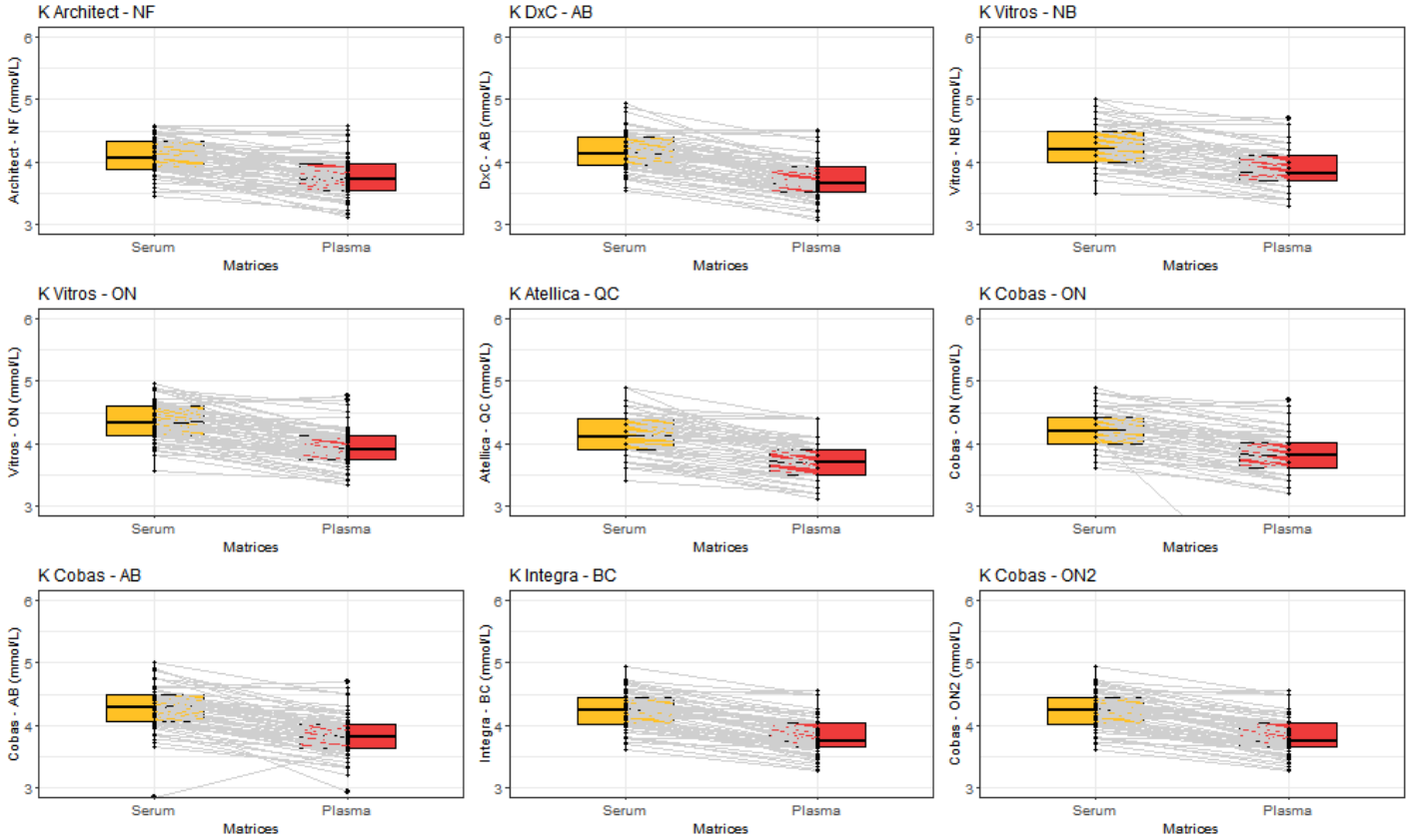
- Approximately **7.8 million results** evaluated
- No age/sex-specific differences observed
- Recommend hRI verified in all nine Canadian Laboratories participating in cross-Canada verification program in ***serum only***

Potassium

Plasma vs Serum:

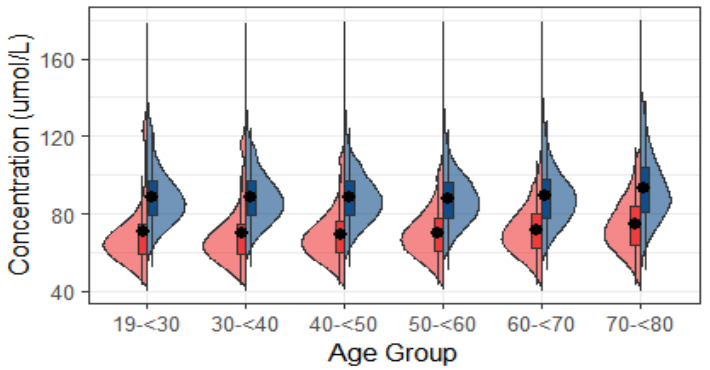
- Plasma potassium results were markedly lower as compared to paired sera
- Recommended hRI of **3.9-5.1 mmol/L** did not verify as per CSCC hRI WG criteria in plasma specimens
- A separate recommendation for plasma potassium is needed

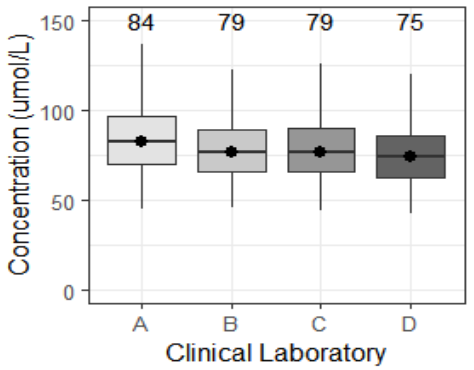


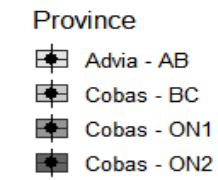


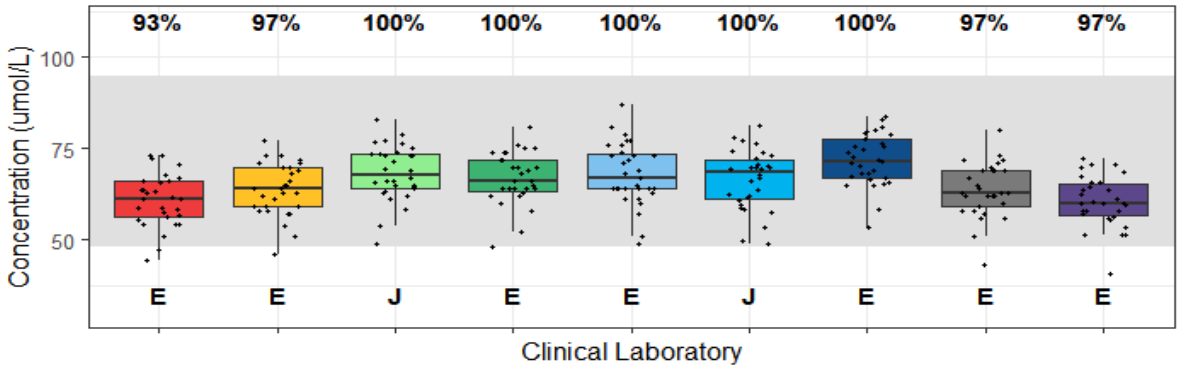
Creatinine

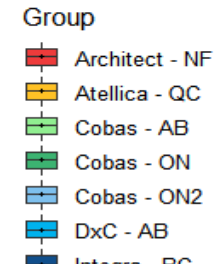
Further Data Analysis/Investigation Required

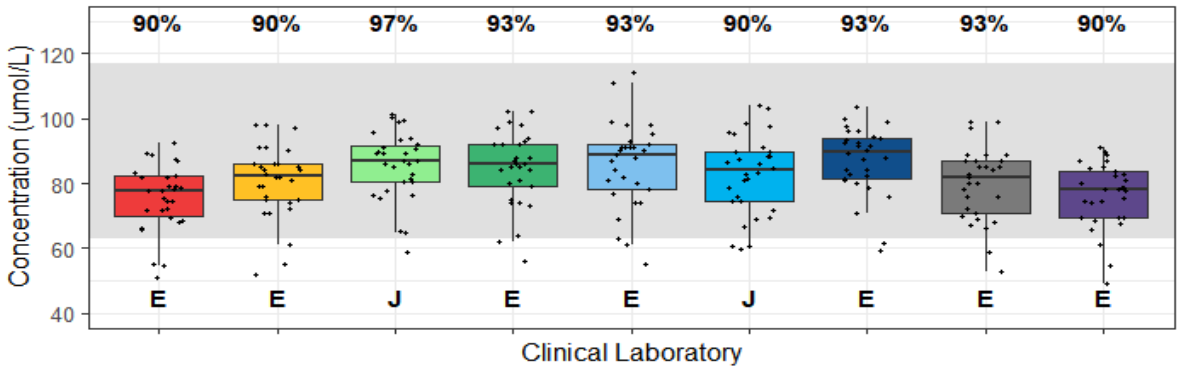


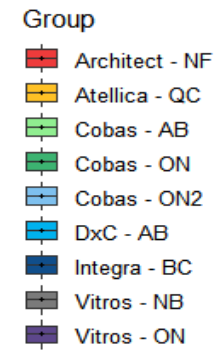












	Indirect Analysis of Provincial Data					Direct International Initiatives				hRI
	Advia – AB	Cobas – BC	Cobas – ON1	Cobas – ON2	All	CHMS	AHRIP	AUSSIE	NORIP	
N	2428982	2103632	7962147	2081579	14576340					**M:** 65-115 **F:** 50-95 **umol/L**
18-79y M	61-117	65-117	64-118	62-114	63-117	64-109	60-110	65-103	64-100	
18-79y F	47-101	49-93	49-93	46-89	48-95	56-91	45-90	54-83	51-84	

Result Summary:

- Approximately **14.7 million results** evaluated
- Statistically significant sex differences observed
  - Males higher concentrations relative to females
- **Sex-specific** recommend hRIs verified in all nine Canadian laboratories participating in cross-Canada verification program (both Jaffe and Enzymatic methods)
- Currently reviewing data to discuss validity of **upper reference limits**

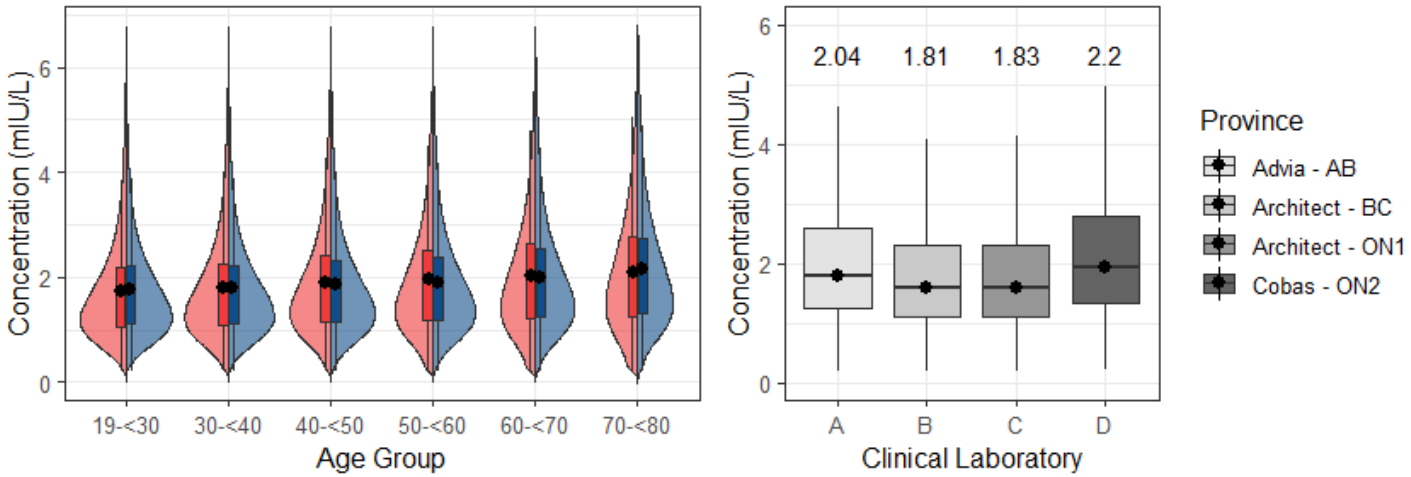
TSH

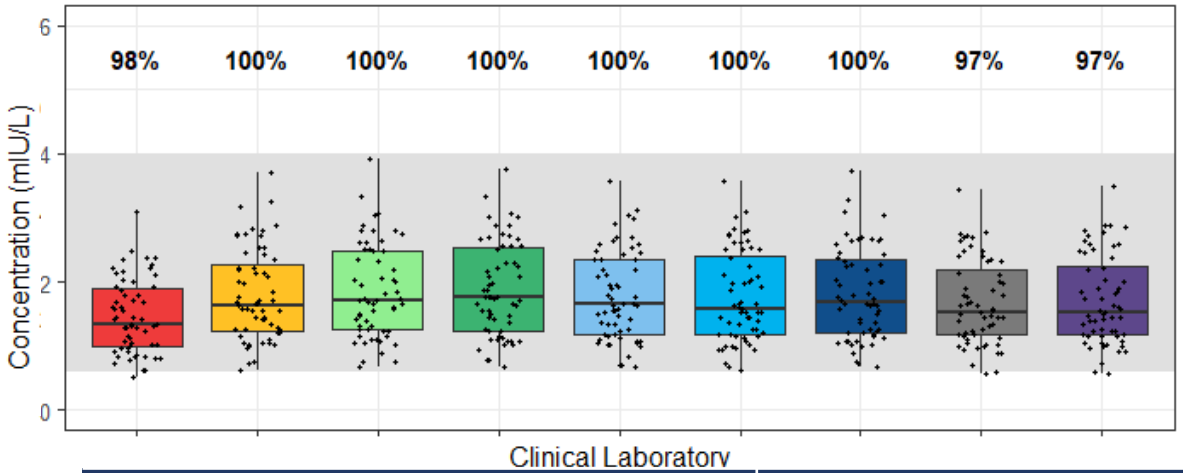
I

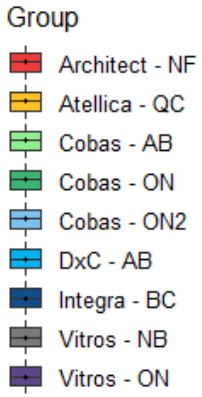
Direct and Indirect Canadian Data Supports Harmonization

Recommendation: 0.4-4.00 mIU/L

Thyroid 2014;24:1670-751. doi:10.1089/thy.2014.0028







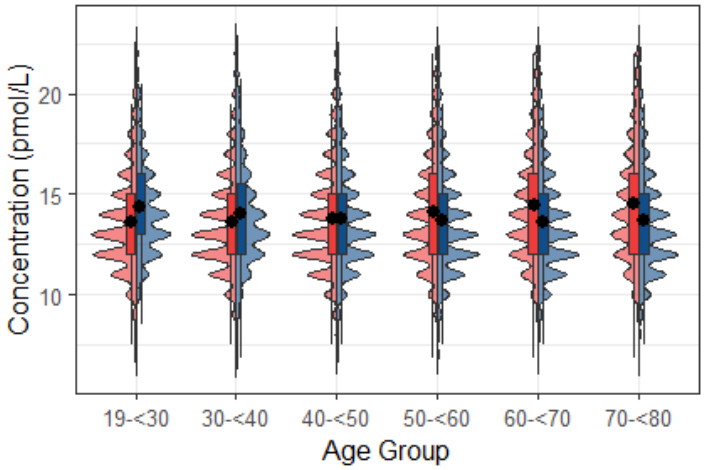
	Indirect Analysis of Provincial Data					Direct International Initiatives				hRI
	Advia – AB	Architect –BC	Architect – ON1	Cobas – ON2	All	AUSSIE	NHANES	UK	NORIP	
N	1121045	1648061	4207623	1688546	8665275					0.60-4.00 mIU/L
RI	0.59-4.48	0.55-4.14	0.54-4.05	0.68-5.26	0.56-4.20	0.34-3.40	0.50-3.60	0.60-4.40	0.45-4.12	

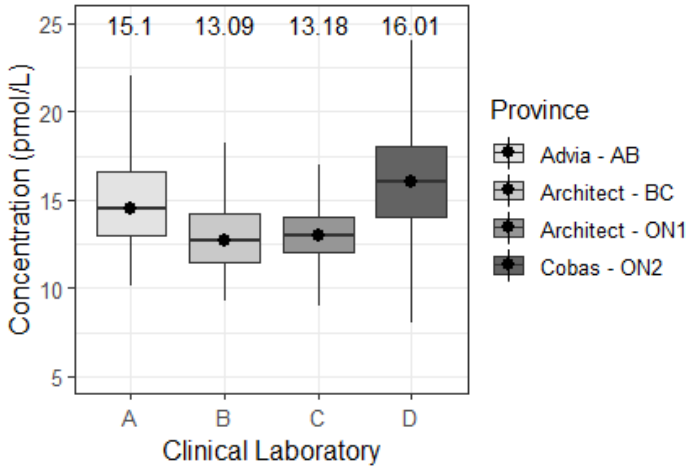
Result Summary:

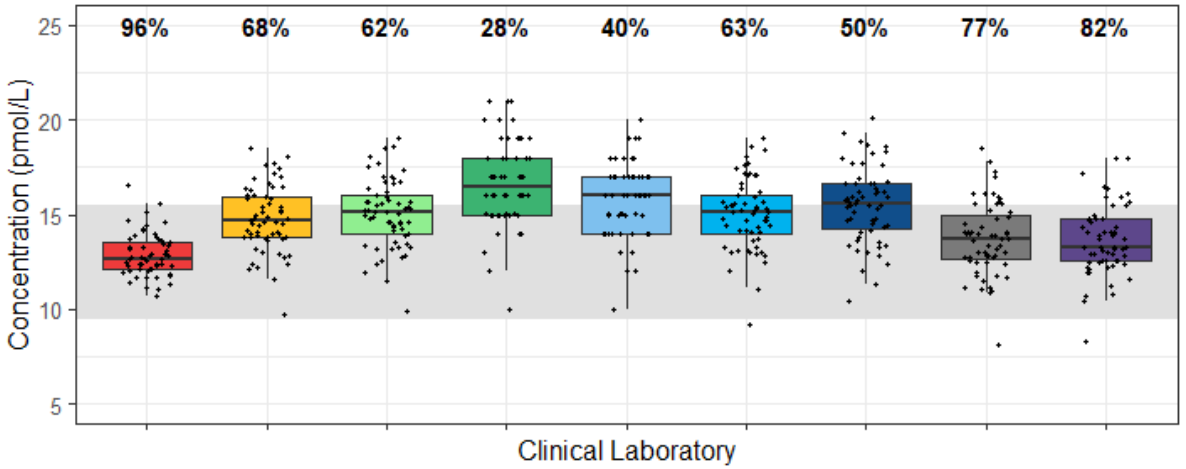
- Approximately **9 million results** evaluated
- No age/sex-specific differences observed
- Upper reference limits ranged from **4.05-5.26 mIU/L** across provincial community laboratories
- Recommended hRI verified in all nine Canadian Laboratories participating in cross-Canada verification program (*serum and plasma*)
- Results suggest excellent concordance between laboratories and acceptable analytical standardization of TSH for RI harmonization

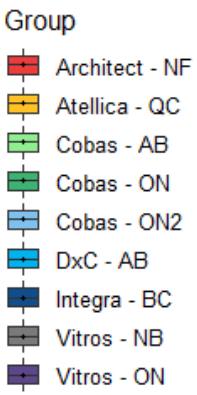
Free T4

Direct and Indirect Canadian Data do NOT Support Harmonization









	Indirect Analysis of Provincial Data					Direct International Initiatives			hRI
	Advia – AB	Cobas – BC	Cobas – ON1	Cobas – ON2	All	AUSSIE	NORIP	UK	
N	124713	190629	972585	376870	1664797				NONE
RI	10.4-19.2	9.6-16.4	9.7-16.8	10.9-20.8	9.7-15.5	10.7-17	10.9-16.9	11.8-19.2	

Result Summary:

- Approximately **1.6 million results** evaluated
- No age/sex-specific differences observed
- Upper reference limits ranged from **16.8-20.5 pmol/L** across provincial community laboratories
- Data suggests hRIs are not appropriate for free T4 test interpretation and manufacturer-specific results RIs are needed

Conclusions

- A **novel big data analytics** approach was undertaken to define preliminary hRIs for 16 analytes:
  - (1) extraction of data from community reference laboratories across Canada
  - (2) assessment of outliers
  - (3) statistical evaluation of age, sex, and center-specific differences
  - (4) derivation of preliminary hRIs using the TML method
  - (5) comparison of established hRIs to direct data in the healthy Canadian population.

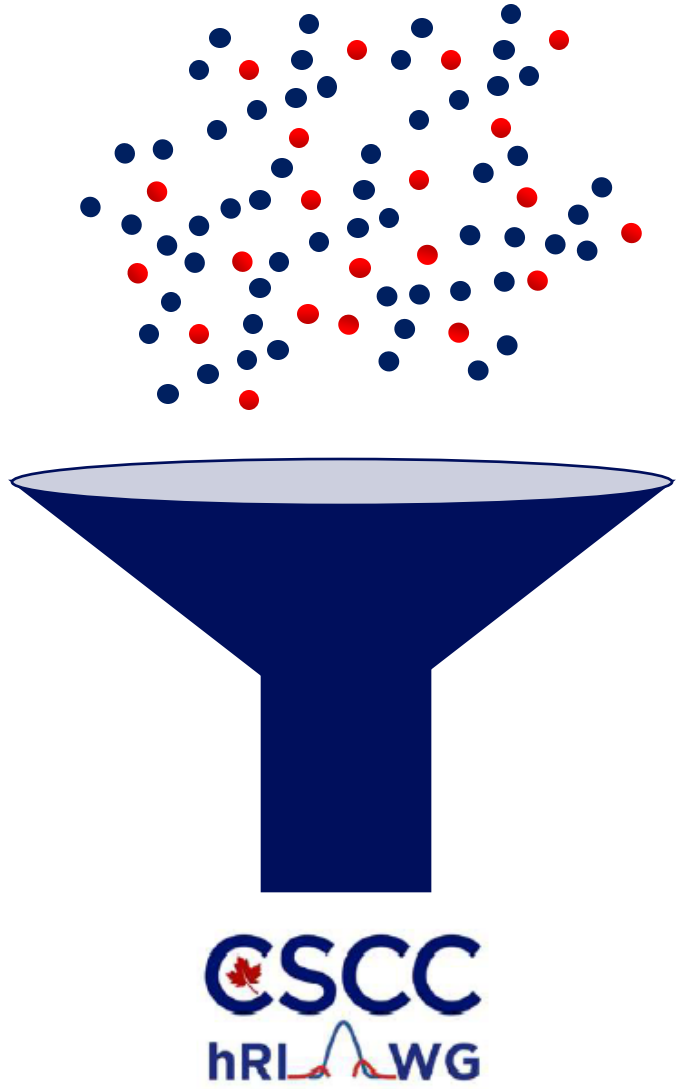
- Robustness of these data was assessed through a **Cross-Canada Verification Study where results supported implementation of these recommendations** (exceptions include: FT4)

- Showcases the power of big data and new statistical techniques to assist in addressing gaps in clinical service

How do we support implementation?

Path to Implementation

Dr. Christine Collier



Harmonization: Big Picture

Result interpretation may be affected by a variety of factors, several of which could be standardized or supported to optimize consistent patient care.

Pre-analytical variables (CVi)

- Patient variability – circadian and seasonal variation;
- Sample collection and handling variability
  - Fist closing/clenching; Plasma – invert 8x, order of draw; etc…

Analytical variables (CVa)

- Variation (imprecision); lots (reagents, calibrators), calibrations, within-day, between-day
- Bias: identical twins (mirrored instruments) are different to different extents
- 5 main manufacturers in Canada; less than 15 instrument platforms

Post-analytical variables

- **RIs – optimal: hRI**
- Critical value management, trending ability, monitoring, graphing; test specific identification (PSA-xx)

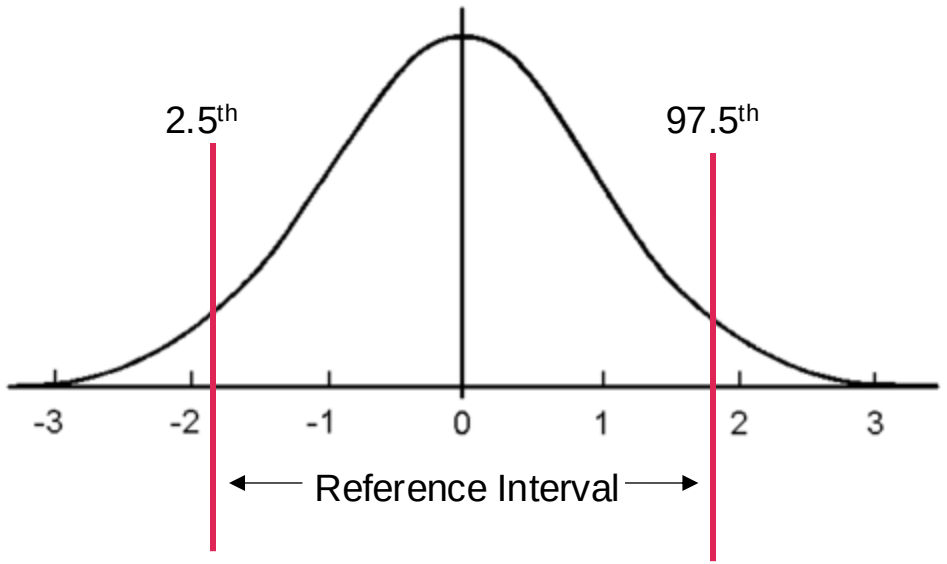
Discussion Point 1

Reference intervals currently reported by my laboratory are derived from:

A. Manufacturer package inserts
B. Publications or textbooks
C. Internal direct studies
D. Internal indirect studies
E. They were set when I started
F. Other

Current Practice for Determining RIs

1. Historical
2. Local volunteers
3. Publications
4. Textbooks
5. Manufacturer kits
6. Verification of published intervals
7. Data mining
8. Clinical judgement



Current Practice for Determining RIs

Stockholm Hierarchy

1. Clinical decision limit based on clinical outcome study
2. Other methods of demining reference intervals or clinical decision limits
    a) RIs derived from healthy populations (CALIPER, CHMS)
    b) Clinical decision limits based on clinician's opinion of disease
3. Published recommendations
    a) National or international expert bodies
    b) Expert local groups and/or individuals
4. Reference limits set by:
    a) Regulatory bodies
    b) Formal reference interval survey

5. Reference interval based on current state of art

Path to implementation: Barriers and Feedback

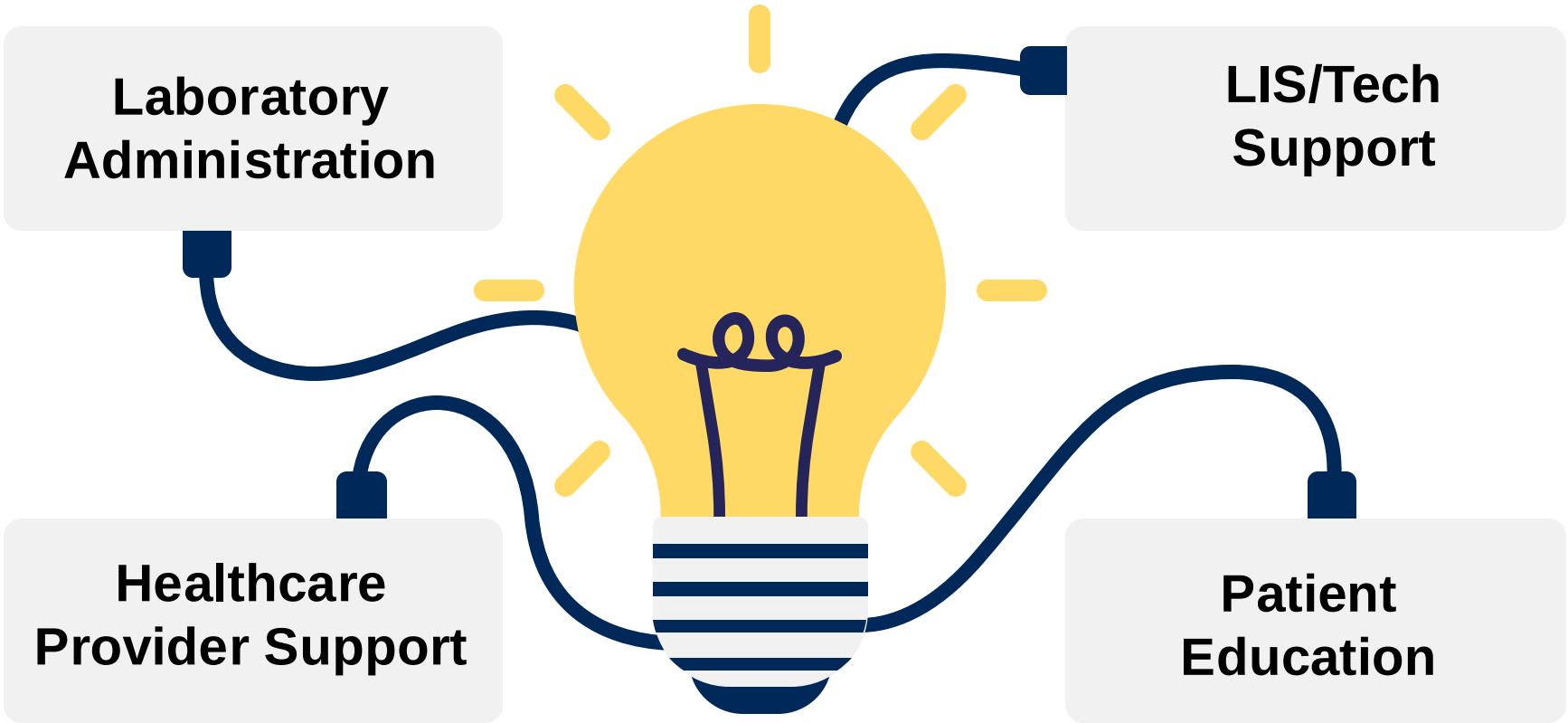
Goal of CSCC hRI WG is not only to establish evidence based harmonized reference intervals, but support their implementation

- Harmonization initiatives around the world have undertaken different approaches to support the implementation of proposed RIs, including:

  - *Assisting in completing verification studies*
  - *Using retrospective data to assess differential flagging rates*
  - *Working with representative societies to support implementation*

Facilitating Implementation

Aim: Develop tools and resources to support hRI implementation across key stakeholders



Facilitating Implementation

Aim: Develop tools and resources to support implementation across key stakeholders

1. Laboratory Administration

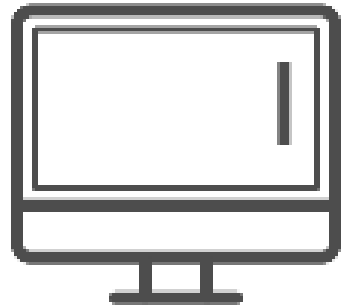
- Calculation of **FP and FN rates**, potentially for different patient cohorts to provide information to clinicians to support their implementation expectations and planning
- Example protocol on **reference interval verification** (potentially provide samples)
- **Educational resources** on how recommendations were derived and other key points (e.g. rationale age/sex bins)
- **New knowledge is acquired through current context and practice**
  - Change management; Science takes time; biases; systematic reviews
  - Stories/examples– how to explain innate result variation?
  - Effects of RI changes – analyte dependent; historical approach to RIs
  - What values are significant, or could be rounded? – analyte dependent

Facilitating Implementation

Aim: Develop tools and resources to support implementation across key stakeholders

2. LIS/Tech Support

- Limited number of systems, but processes may vary (eg. request process)
  - Example of LIS request(s)
  - Example protocol for testing implementations (table)
  - Units, decimals (1 decimal < 10; 0 decimals >20)
  - **Age**: use of “<” and “>/=”; infants; adults
  - Future: moving averages and variations, RCV flags

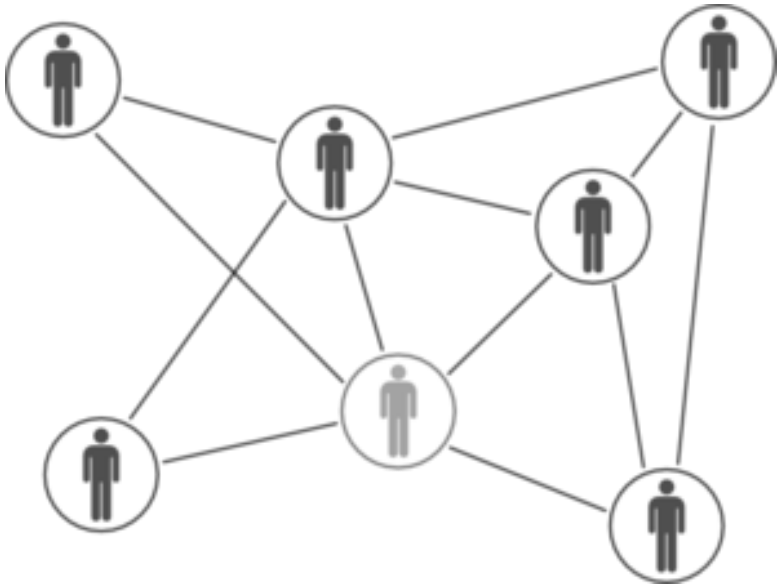


Facilitating Implementation

Aim: Develop tools and resources to support implementation across key stakeholders

3. Healthcare Provider Support and Education

- Announcement sheets with proposed changes and rational
- Expectations and need:
  - Ordering right test right, time constraints (batch, repeat testing)
  - Prevalence, false positives, false negatives
  - Repeat testing; for confirmation; reflex algorithms
  - MU, RU and RCVs



4. Patient Education

- Community engagement and advocacy

What other supports could be helpful?

Discussion Point 2

What do you see as the main barriers to implementation of harmonized reference intervals:

A. Scientific concern
B. Resources associated with verification
C. IT resources for LIS implementation
D. Other

Additional Discussion Points

- What to do for ages not covered?
- What might be the limitations of the current proposed RIs? (obesity, pre-diabetes and subclinical diseases not excluded; exclusion based on correlated panels vs single tests)
- What to consider for regions who have moved through this process recently?
- Effect and assessment, ongoing monitoring of Bias
- Regional differences in populations
- Personalized RIs – RCVs
- Data “mine” own lab patient data to verify the proposed intervals
- Stakeholders