Reference Interval Harmonization in Canada: An Update from the CSCC Working Group

Jennifer Taher

On behalf of the Canadian Harmonized Reference Interval (hRI) Working Group

Wednesday June 5th, 2019

Acknowledgements

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Annual Conference of the
Canadian Society of Clinical Chemists (CSCC)

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I have the following financial relationships to disclose:

- Honoraria from Abbott
  - Received for a project that will not be discussed today

AND

I will not discuss off label use and/or investigational use in my presentation.

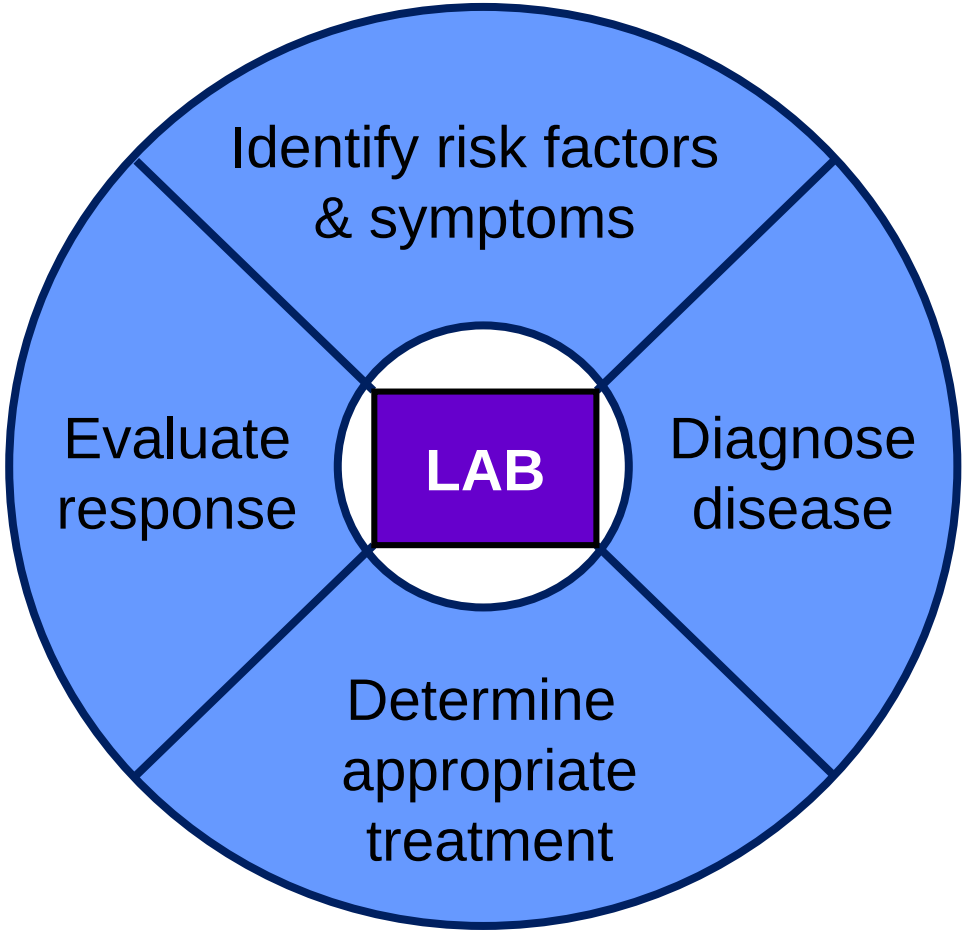
Outline

- Variation in test results and the impact on patient care and safety
- Lack of standardization and harmonization and major gaps in adult and pediatric reference intervals (RIs)
- International initiatives addressing harmonization using both direct and indirect methods
- Discuss the strategies for harmonized RIs being carried out by our CSCC hRI Working Group

Example: Alkaline Phosphatase (ALP) and Albumin

Central Role of Laboratory Medicine In Healthcare Delivery

Laboratory Medicine is part of a multi-disciplinary team at the centre of healthcare



The **quality** of the Clinical Laboratory Service is critically dependent on:

- Accurate/Precise Testing Process (validating methods/systems)
- Accurate interpretation of test results based on appropriate reference intervals or decision limits

Clinical Laboratory Testing & Interpretation – *Many Challenges*

- **Lack of Standardization or Harmonization of Assay Methodology**

  >>> Considerable variation in test results for the same test on the same patient

- **Major Gaps in Adult and Pediatric Reference Intervals and Appropriate Decision Limits**

  >>> Leading to inaccurate/erroneous interpretation

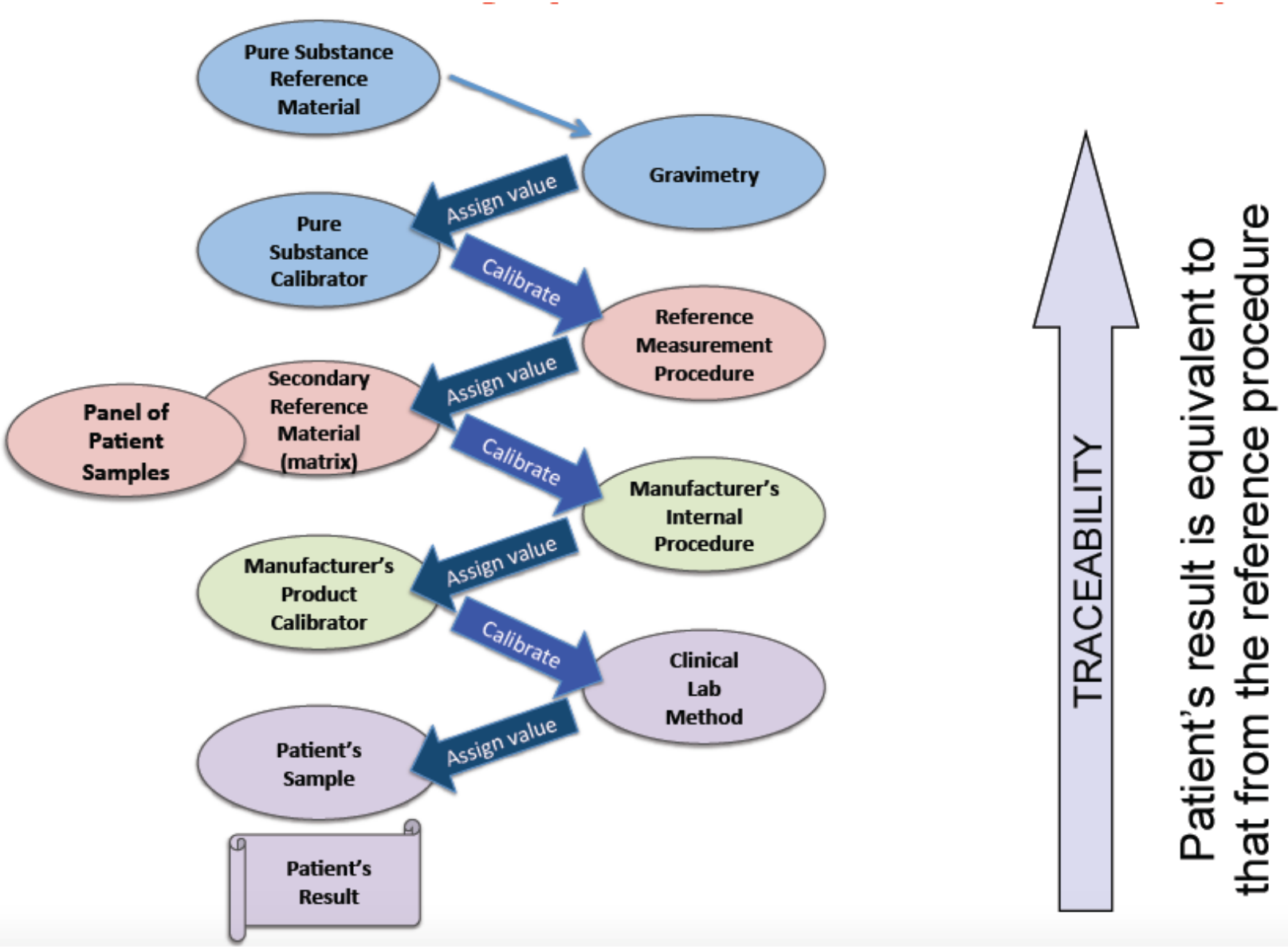
Should be regarded as a major source of laboratory error affecting patient care and patient safety

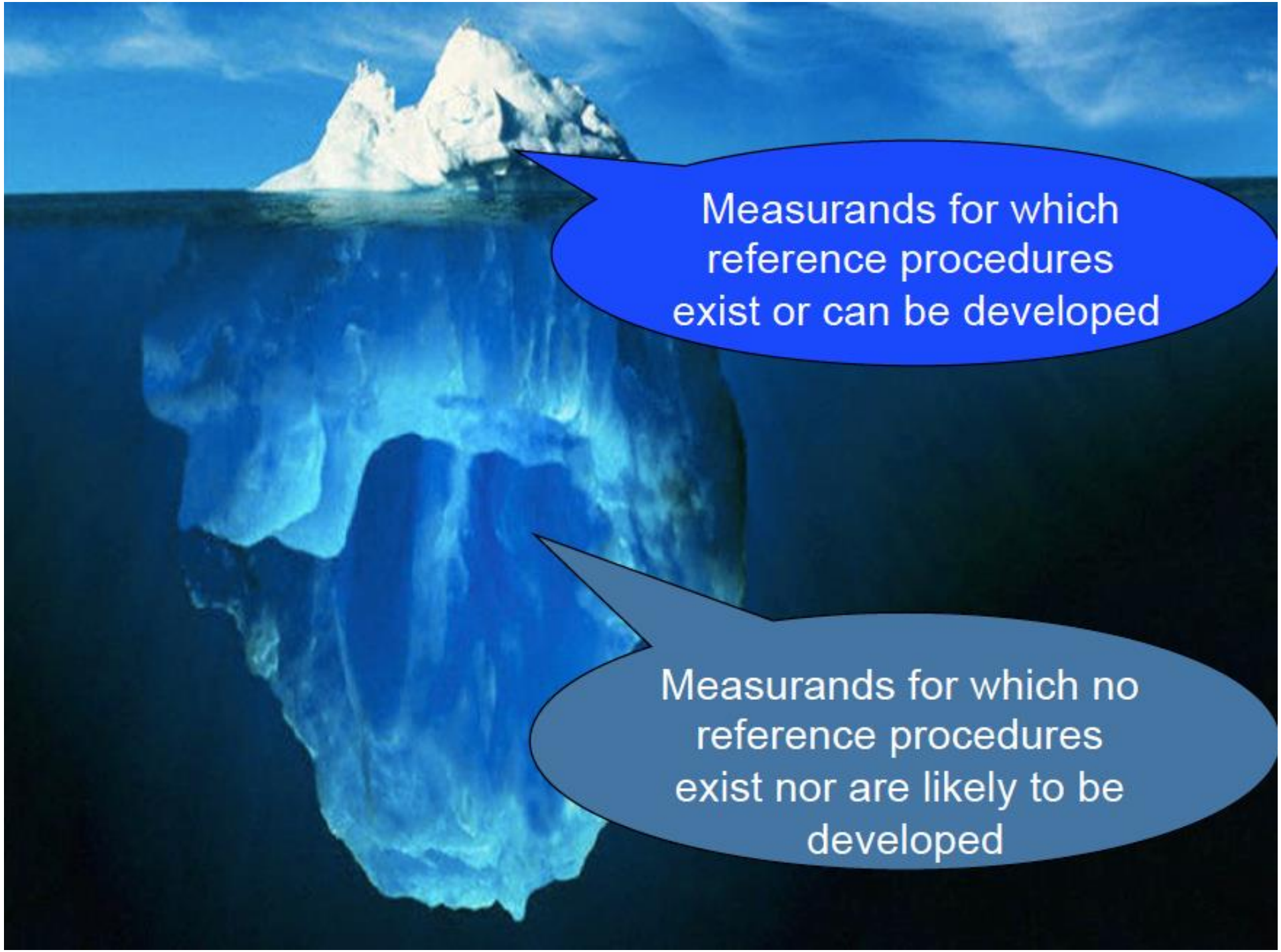
What Is The Solution?

- **Standardization:** achieving equivalent results by having calibration traceable to a reference system component

- **Harmonization:** achieving equivalent results among different measurement procedures

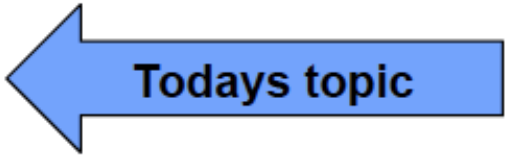
Standardization




Measurands for which reference procedures exist or can be developed
Measurands for which no reference procedures exist nor are likely to be developed

Harmonization

- Equivalent results among different measurement procedures for the same laboratory test
  - Nomenclature
  - Patient preparation
  - Specimen collection and handling
  - **Result value**
  - Reporting units
  - Interpretive information



Harmonization Initiative in Canada

Focus on Harmonization of Test Result Interpretation by Harmonizing Adult and Pediatric Reference Intervals

Reduce diagnostic errors and improve patient safety

Reference Interval Initiatives:
Towards Harmonization

1. **CHMS**
   - Canadian Health Measures Survey conducted by Statistics Canada
   - *Adeli et al. Clinical Chemistry 2015*
2. **Caliper**
   - Canadian Laboratory Initiative in Pediatric Reference Intervals
   - *Estey et al. Clinical Biochemistry 2013*
3. **AHRIA**
   - Australasian Harmonised Reference Intervals for Adults
   - *Tate et al. Clinical Biochemistry 2014*
4. **NORIP**
   - Nordic Reference Interval Project
   - *Hilsted et al. Scandinavian Journal of Clinical and Laboratory Investigation 2013*
5. **UK Path Harmony**
   - United Kingdom Pathology Harmony
   - *Berg, J. Clinica Chimica Act 2014*
6. **Aussie**
   - Aussie Normals study
   - *Koerbin et al. Chemical Pathology 2015*

CSCC Harmonization of Reference Interval (hRI) Working Group

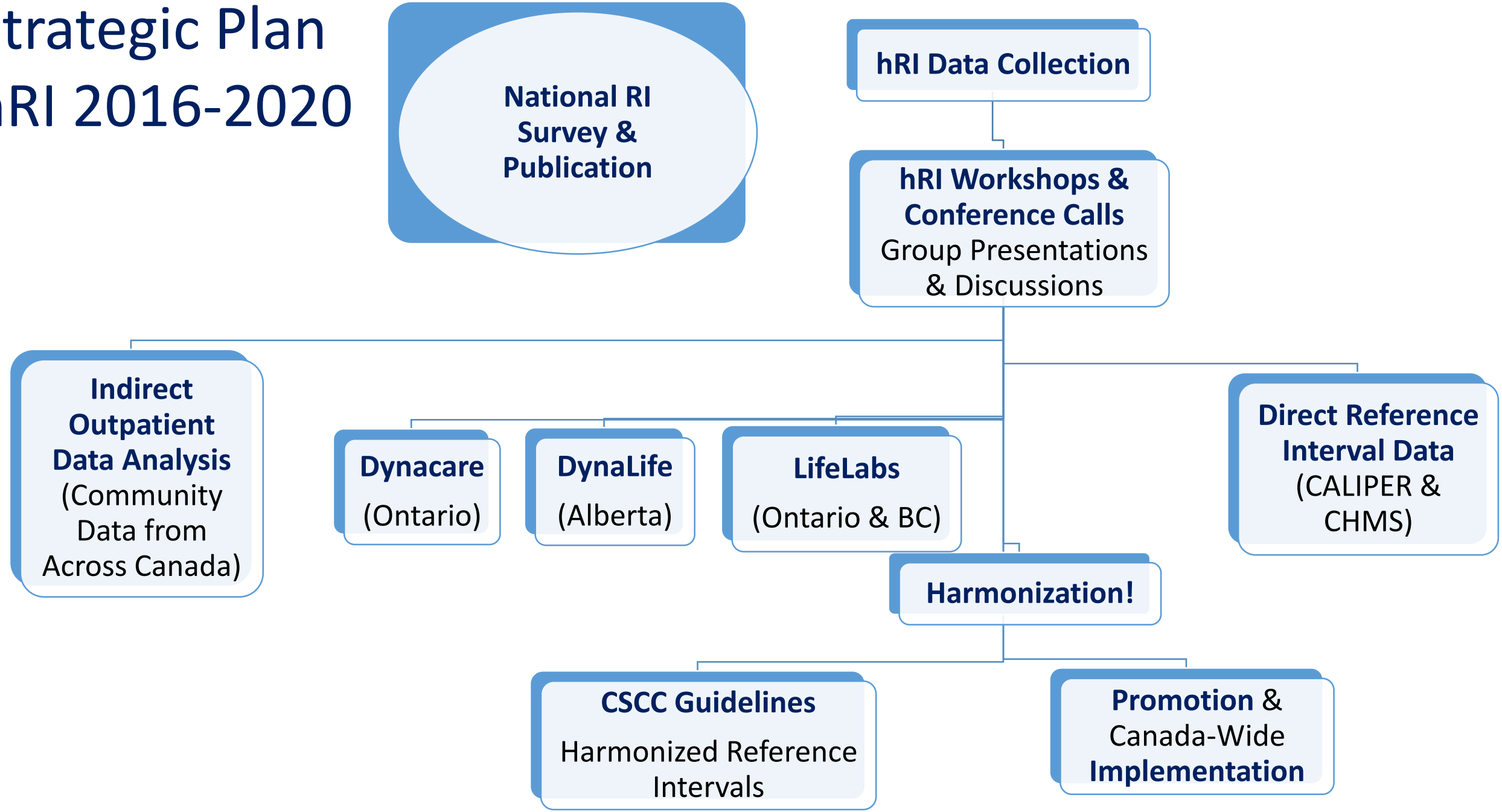
***Goal:* To establish evidence-based harmonized reference intervals and support their implementation in laboratories across the country**

Objective 1: Review adult and pediatric RIs currently in use in clinical laboratories across Canada

Objective 2: Assess the available evidence on RIs obtained in prospective studies of healthy populations

Objective 3: Develop appropriate recommendations and guidelines on the use of harmonized RIs across Canada

Strategic Plan hRI 2016-2020



Variation in RIs across Canada

Clinical Biochemistry 50 (2017) 925–935

Contents lists available at ScienceDirect

Clinical Biochemistry

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

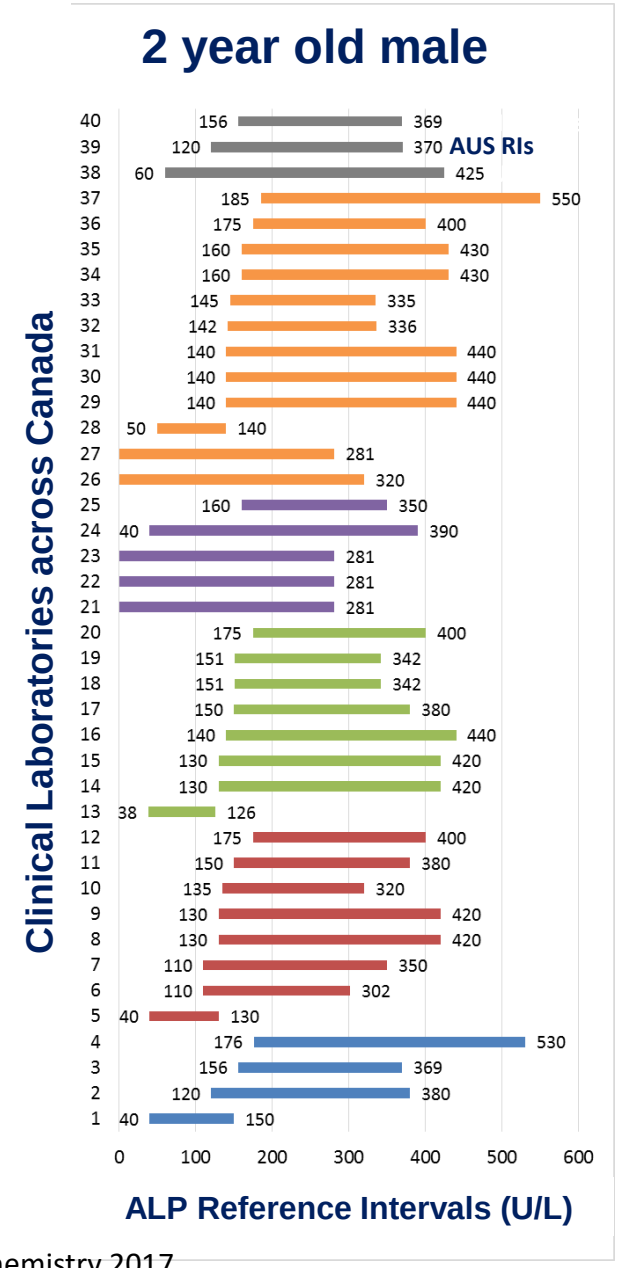
Analytical

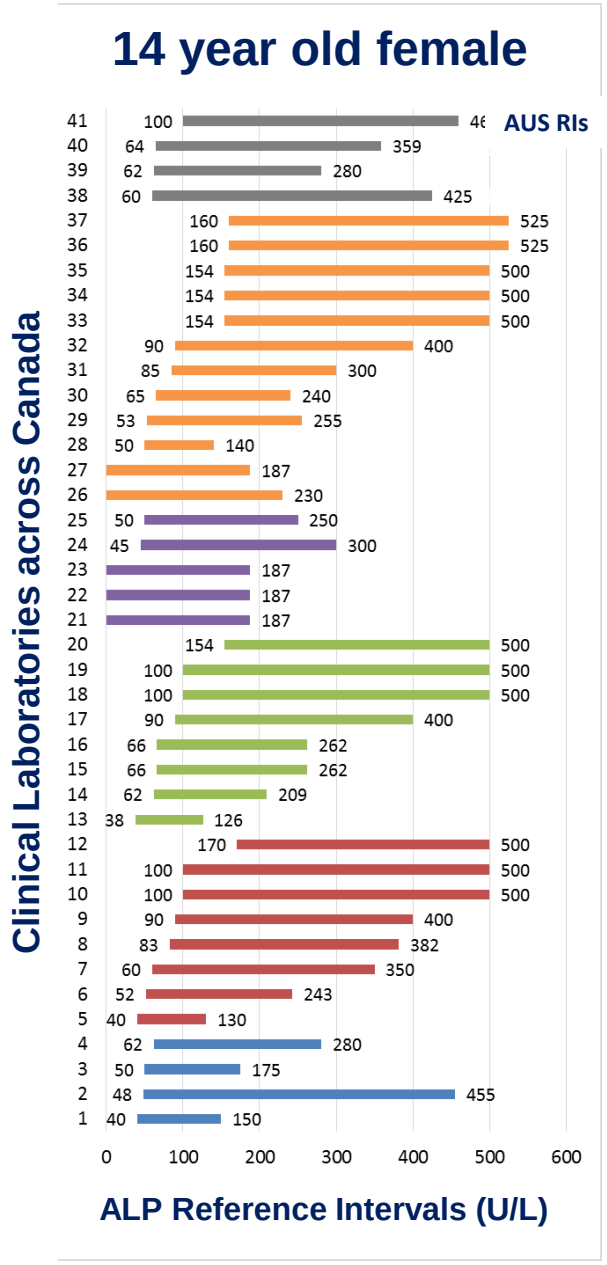
National Survey of Adult and Pediatric Reference Intervals in Clinical Laboratories across Canada: A Report of the CSCC Working Group on Reference Interval Harmonization

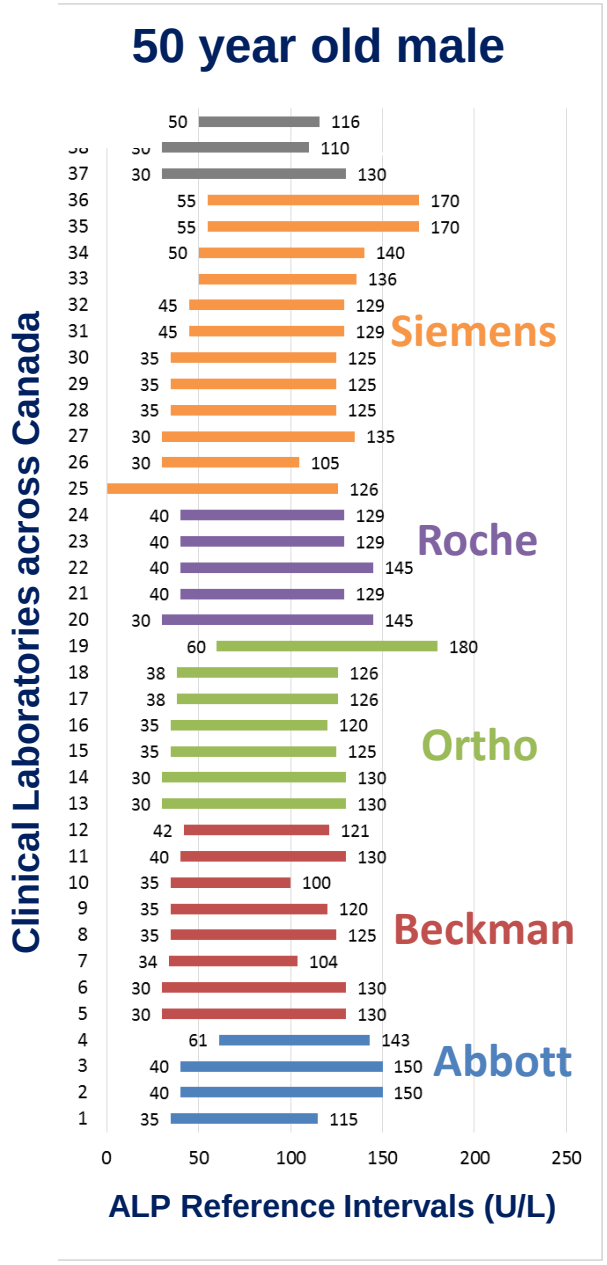
Khosrow Adeli[a,*], Victoria Higgins[a], David Seccombe[b], Christine P. Collier[c], Cynthia M. Balion[d], George Cembrowski[e], Allison A. Venner[f], Julie Shaw[g], on behalf of the CSCC Reference Interval Harmonization (hRI) Working Group

- **37 laboratories reported RIs for 7 analytes (ALT, ALP, calcium, creatinine, FT4, hemoglobin, sodium)**
- **40 laboratories measured 6 analytes (all except hemoglobin) in reference samples**
- **High variation in reported RIs even between laboratories using the same instrumentation**
- **RI variation was greater than test result variation for the majority of analytes**

ALP Reference Intervals







Adeli et al. Clinical Biochemistry 2017

Direct versus Indirect RI Studies

Direct

- Selection of healthy reference individuals using defined inclusion and exclusion criteria
  - E.g. volunteers recruited from the community

Indirect

- Uses patient data already collected and stored in a laboratory database
  - E.g. hospital laboratories or outpatient care settings

Direct RIs

PRO

- Recommended by CLSI
- Better representation of a true healthy population
- Minimal pre-analytical variation

CON

- Resource requirements (cost, time)
- Definition of “health” can vary between studies
- Difficult for pediatric and geriatric populations

Indirect RIs

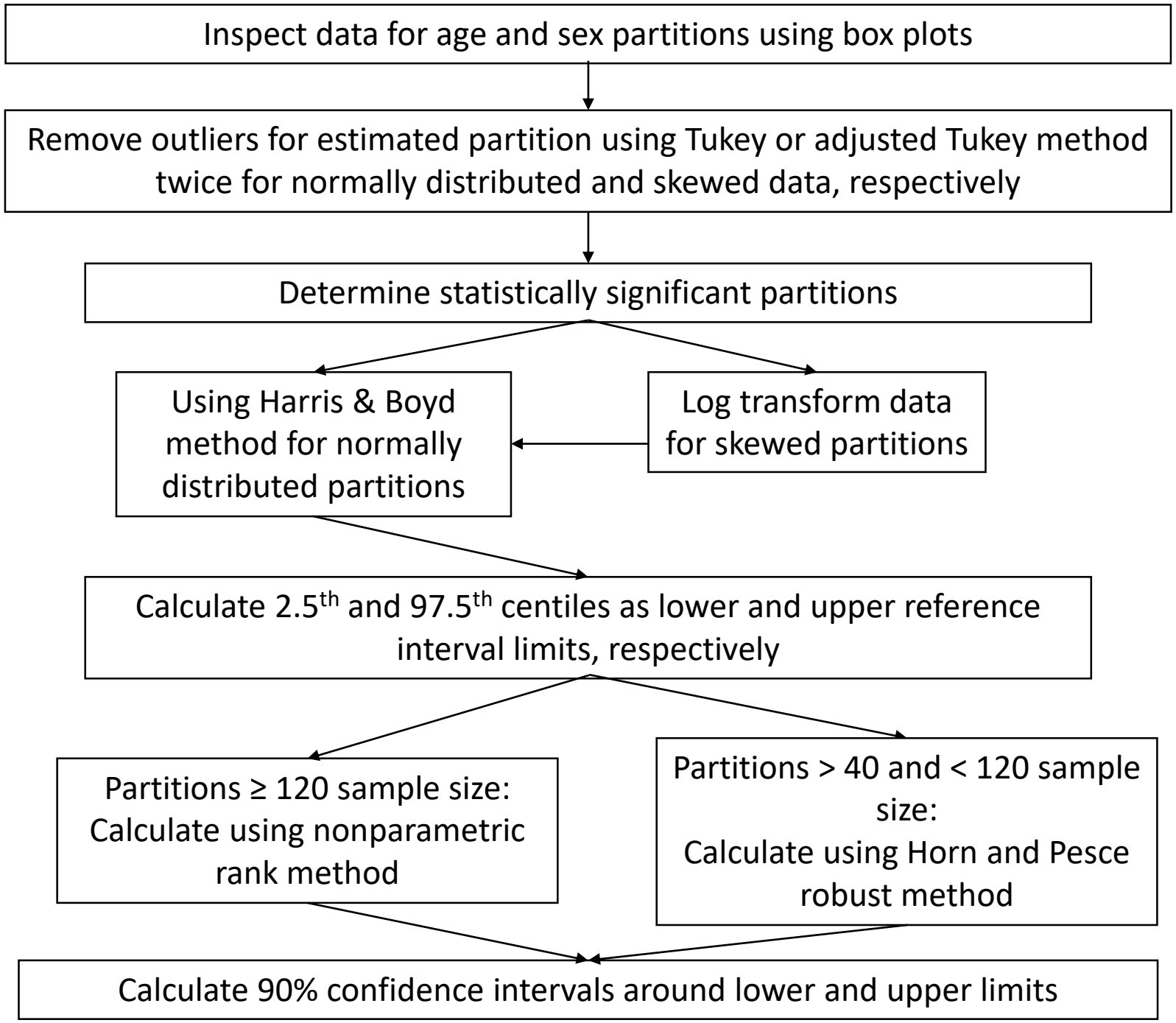
PRO

- Less resources required (time, cost)
- Analysis is directly targeting the local population
- Pre-analytical factors reflect those used in the local laboratory

CON

- Determination of healthy population/distribution relies on statistical methods
- Inclusion of unhealthy subjects may result in skewed or broader reference intervals
- Less control of pre-analytical specifications

Direct Statistical Method



Tahmasebi H, et al. eJIFCC 2017

Direct Reference Interval Databases in Healthy Canadian Children and Adults

Two robust, evidence-based reference interval databases established from the healthy Canadian population

- Pediatrics – **CALIPER** (1-<19 years)
- Pediatric, Adults, Geriatric – **CHMS** (3-<80 years)

CALIPER

'ARM' us with the knowledge to help others

A Comprehensive Database of Reference Intervals for over 170 Tests

Canadian Health Measures Survey

Statistics Canada Statistique Canada

The Canadian Health Measures Survey: CALIPER-CHMS Collaboration

The Canadian Health Measures Survey (CHMS) is a Canada-wide health information survey conducted by Statistics Canada

Pediatric, adult and geriatric reference intervals

Health Data Collection

Canada-wide health information survey

- In-home interview (general health information)
- Mobile examination centre (blood and urine samples, body measurements)

~12,000 Canadians aged 3-79y

Detailed exclusion criteria

- Included pregnancy, high blood pressure, diabetes, cancer, thyroid condition, hepatitis, etc.

Statistics Canada Statistique Canada

Adeli et al. Clinical Chemistry 2015

Indirect Reference Interval Establishment

There are many advantages to the establishment of direct reference intervals. However, it is very challenging to obtain a sufficient number of samples from healthy individuals

Indirect Reference Interval Establishment

- Use outpatient datasets across Canada
- Use statistical algorithms to define the distribution of the healthy population
- Subsequently define 2.5^{th} and 97.5^{th} percentile

Indirect Reference Interval Determination
Statistical Approaches

Hoffman 1963

Bhattacharya 1967

Naus et al. 1980

Martin et al. 1981

Baadenhuijsen et al. 1985

J. B. Hemel et al. 1985

Oosterhuis et al. 1990

Kairisto et al. 1995

Ferré-Masferrer et al. 1999

Ilcol et al. 2006

Arzideh et al 2007

Indirect Approach

We should employ statistical techniques with the abilities to:

1. Identify and separate the distribution of pathological and non-pathological subgroups with high reliability and accuracy and not in a subjective way.
2. Convert the distribution of only non-pathological subgroup to Gaussian.
3. Not rely on the distribution of pathological values.
4. Estimate the parameters of the distribution of pathological and non-pathological subgroups in an accurate way.
5. Evaluate whether the distribution fits well or not (goodness of fit).

Arzideh Method

Clin Chem Lab Med 2007;45(8):1033–1042 © 2007 by Walter de Gruyter • Berlin • New York. DOI 10.1515/CCLM.2007.249

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

An improved indirect approach for determining reference limits from intra-laboratory data bases exemplified by concentrations of electrolytes

Ein verbesserter indirekter Ansatz zur Bestimmung von Referenzgrenzen mittels intra-laboratorieller Datensätze am Beispiel von Elektrolyt-Konzentrationen

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

Farhad Arzideh[1], Gunnar Brandhorst[2], Eberhard Gurr[3], Wilhelm Hinsch[4], Torsten Hoff[5], Lennart Roggenbuck[6], Gregor Rothe[7], Gerhard Schumann[8], Bernd Wolters[9], Werner Wosniok[1] and Rainer Haeckel[7,*]

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

A plea for intra-laboratory reference limits. Part 1. General considerations and concepts for determination

Indirect determination of pediatric blood count reference intervals

Abstract

Background: Determination of pediatric reference intervals (RIs) for laboratory quantities, including hematological quantities, is complex. The measured quantities vary by age, and obtaining samples from healthy children is difficult. Many widely used RIs are derived from small sample numbers and are split into arbitrary discrete age

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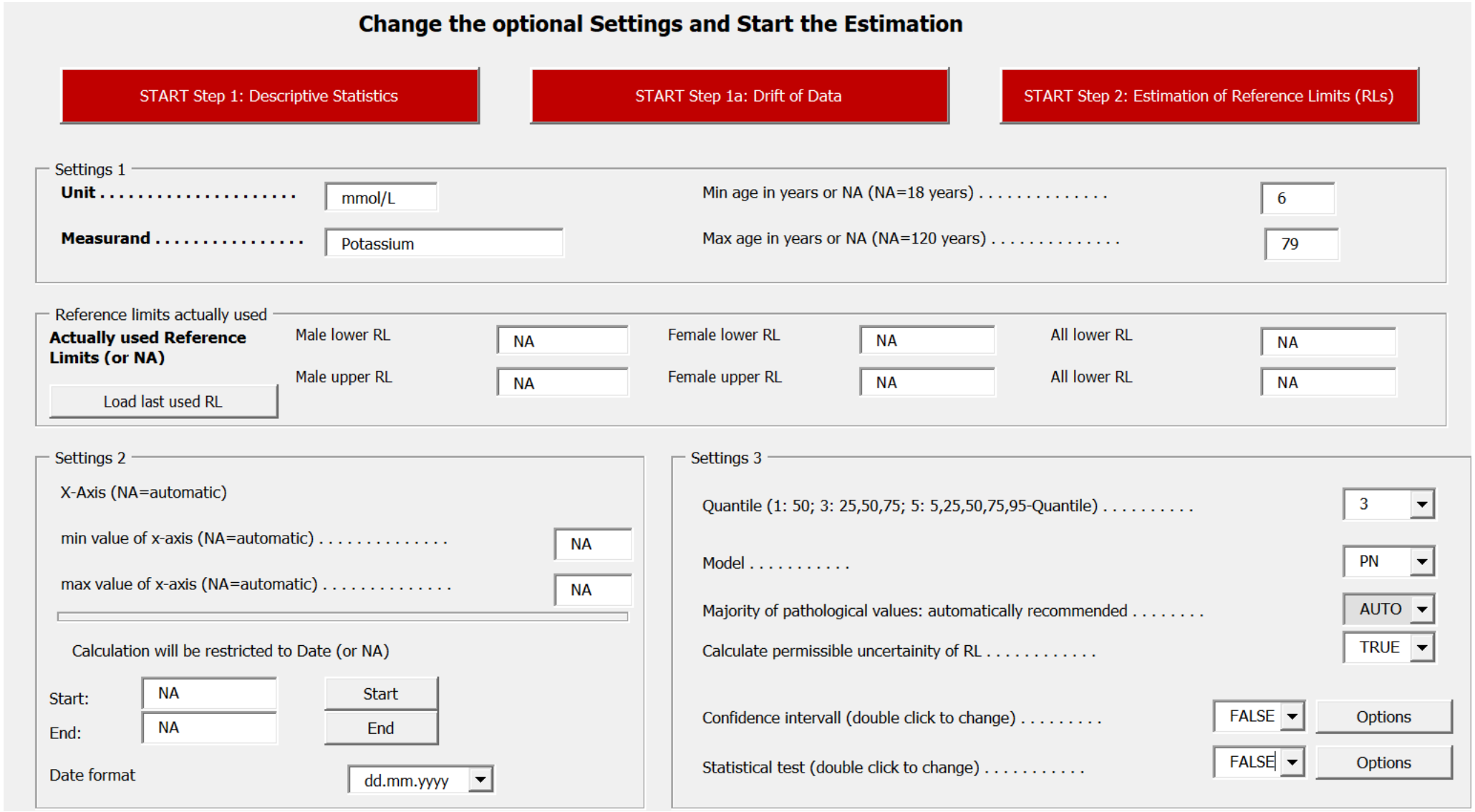
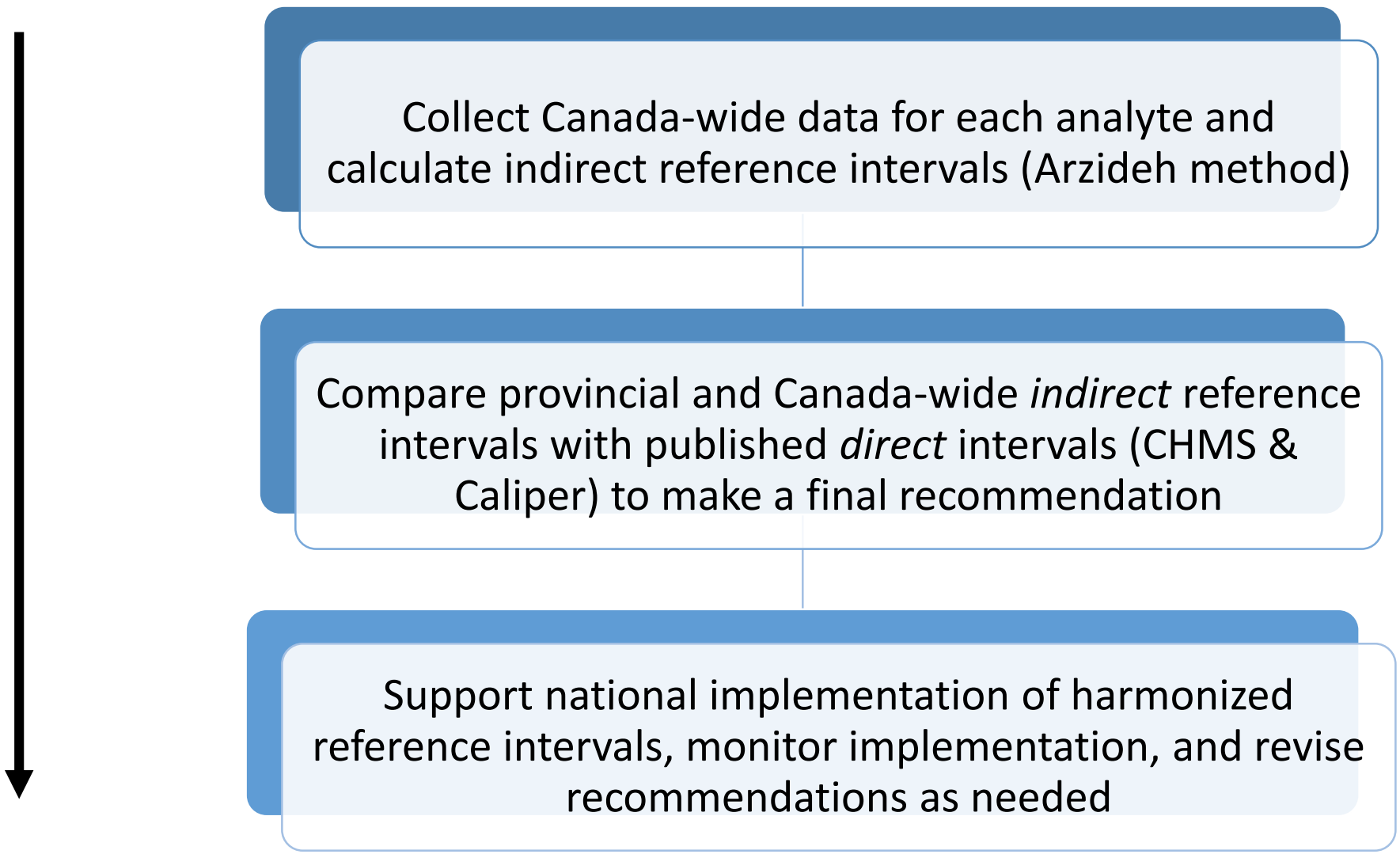
Reference Limit Estimator Software



CSCC hRI Approach



Assays Selected for RI Harmonization

Consider method bias, manufacturer traceability, biological variation and literature

Electrolytes

- Na, K, Cl, Mg, CO2

Renal

- Creatinine, Ca, Phosphate

Hepatic

- ALT, ALP, Alb, TP, TBIl, LDH

Endocrinology

- HbA1c, FT4, FT3, TSH, Glucose (fasting)

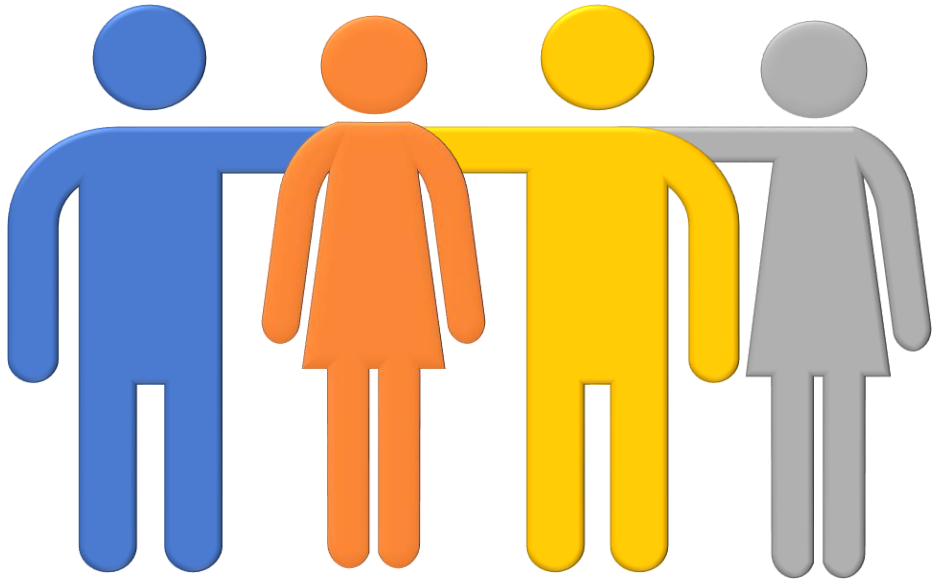
Lipids

- Cholesterol, HDLc, LDL, Non-HDLc, Triglycerides

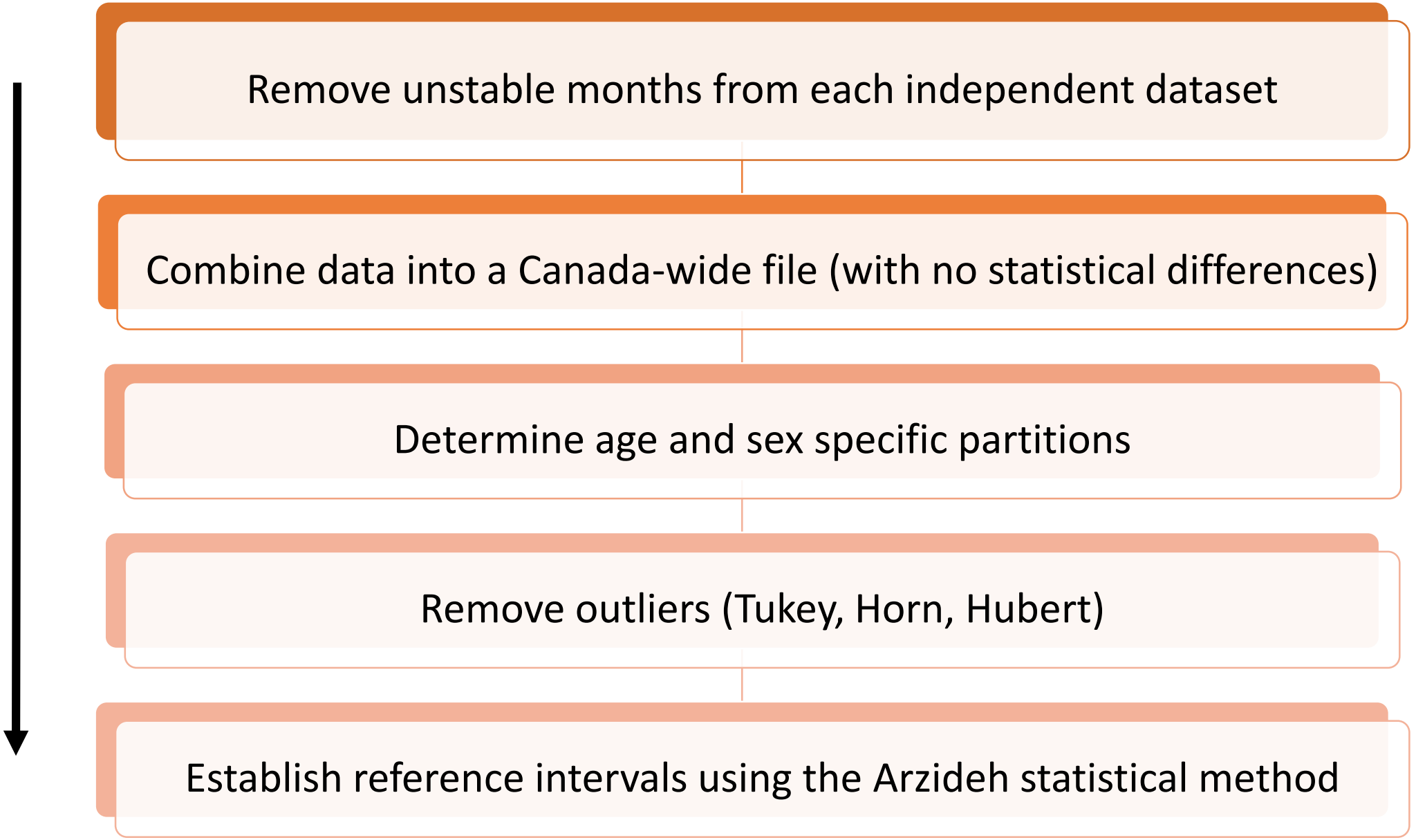
Data Collection – Canada Wide

Two years of data (Jan 2017-Dec 2018) with repeats (annually) removed across all age ranges

Alberta	British Columbia	Ontario	Ontario
DynaLIFE MEDICAL LABS	LifeLabs®	Dynacare®	LifeLabs®
Siemens ADVIA	Roche Cobas	Roche Modular Roche Cobas	Roche Cobas



Statistical Approach



Statistical Approach

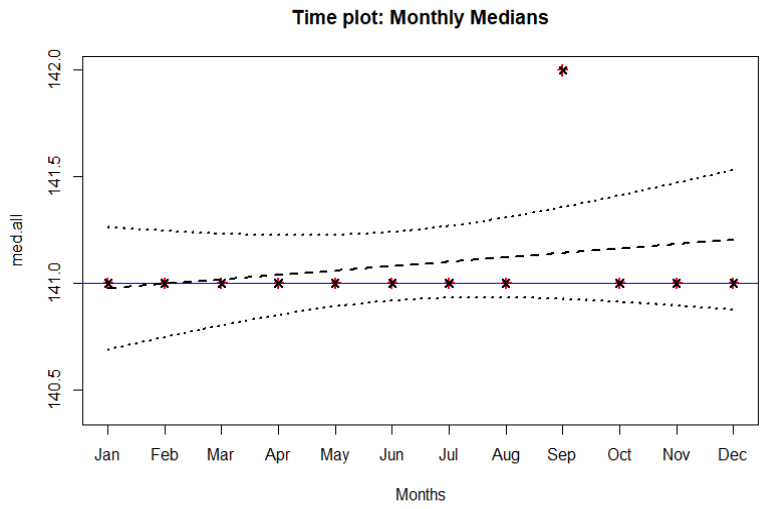
Remove unstable months from each data-set

Criteria Used: Total Allowable Error and Reference Change Value

Check Stability

Westgard
1/3rd TEA
½ RCV

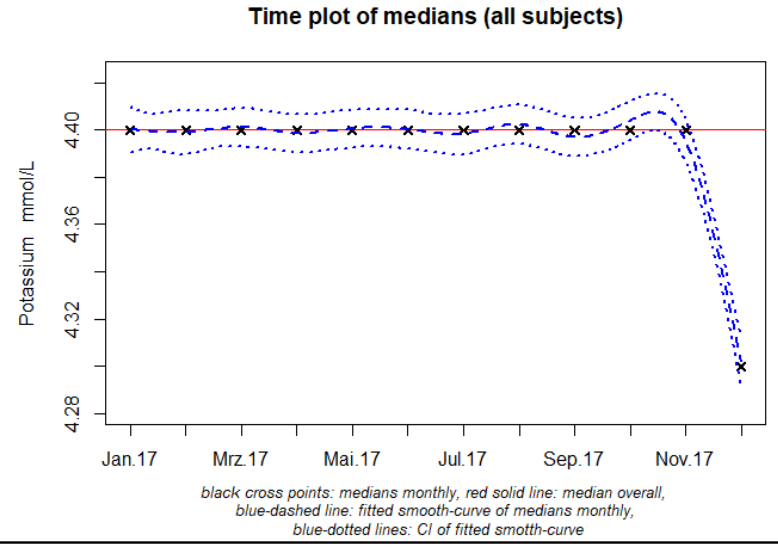
Example A:



Determine Instability:

- None

Example B:



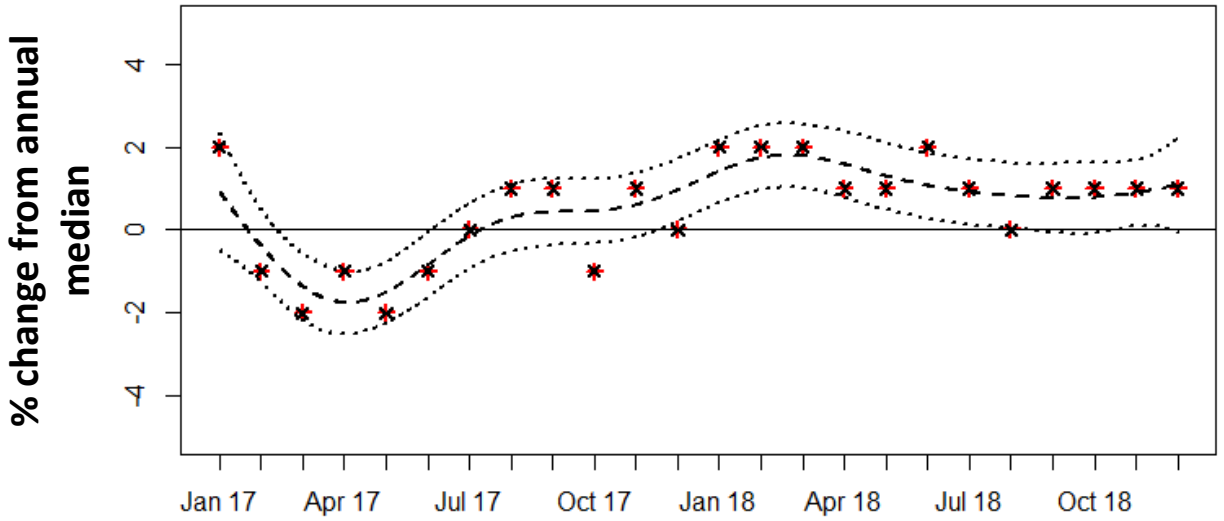
Determine Instability:

- December
- Compare % difference to TEA and RCV

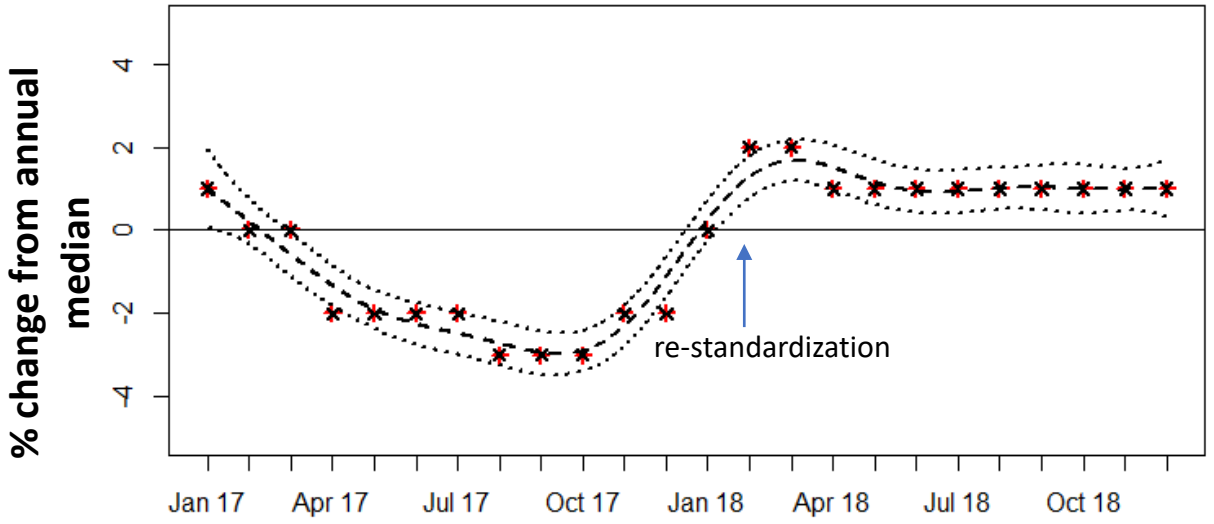
ALP Monthly Instability (%)

Westgard
1/3rd TEA (12%) = 4%
½ RCV (20 %) = 10%

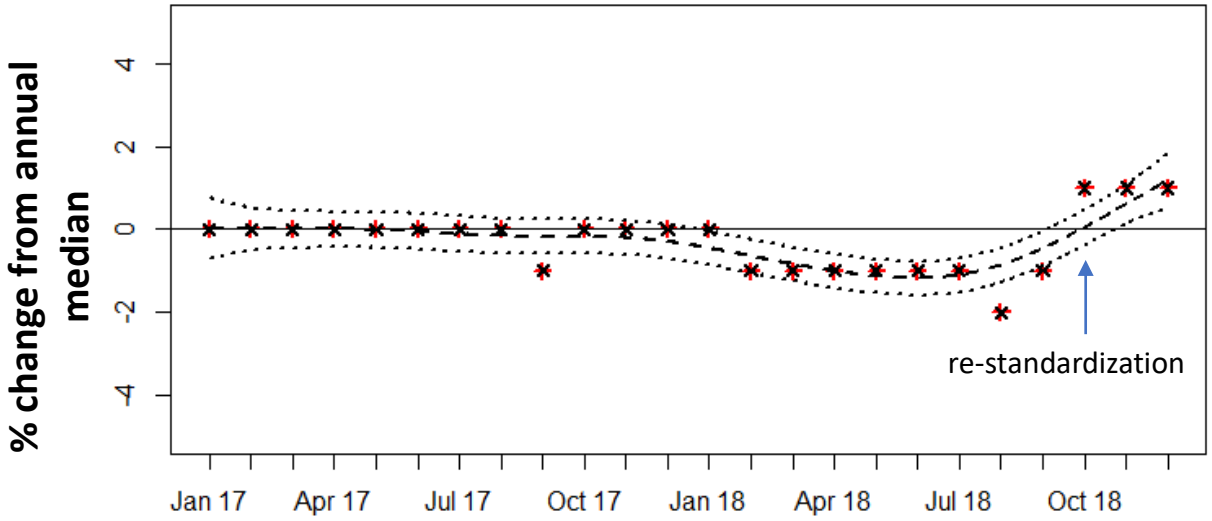
Alberta – DynaLIFE



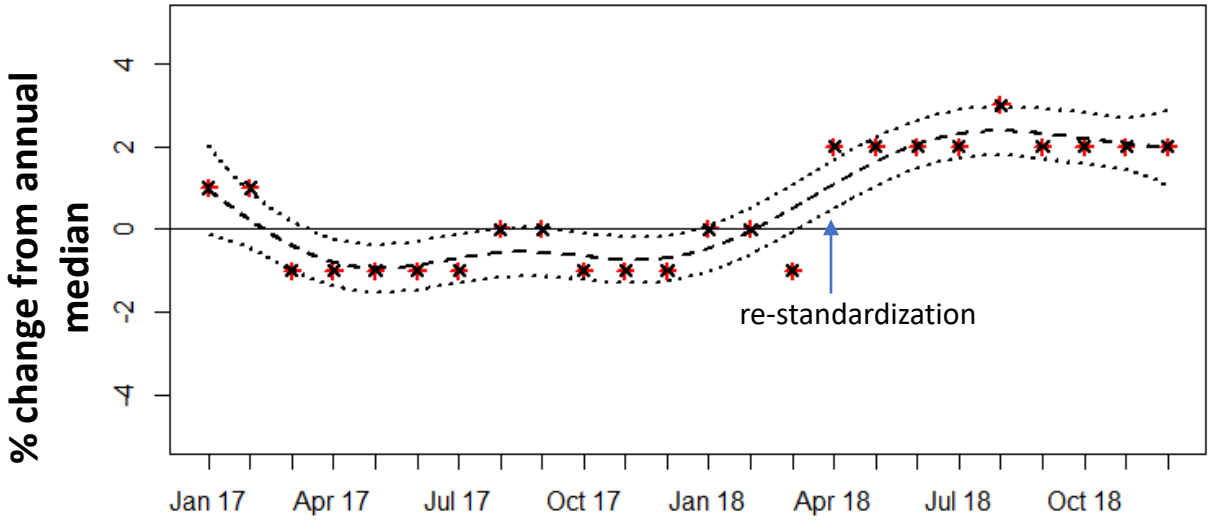
British Columbia - LifeLabs



Ontario - Dynacare



Ontario - LifeLabs



Statistical Approach

Combine data from each center into Canada-wide file

Statistical differences between centers confirmed using Harris & Boyd Method

Determine Provincial Differences

Method: Harris & Boyd

Example A:

Dataset (3-79y)	Differences?
Alberta	NO
British Columbia	NO
Ontario -LL	NO
Ontario - DC	NO
	NO
	NO
	NO

Harmonized Partitions
AB, BC & ON-LL, ON-DC

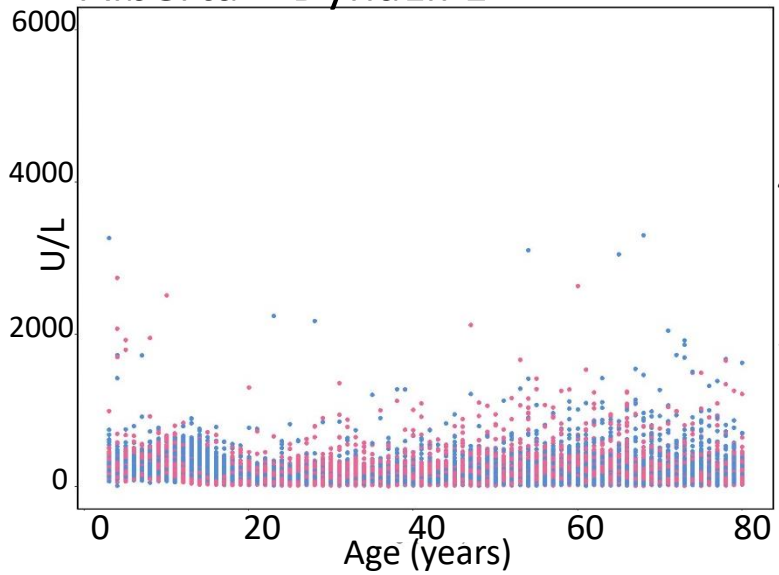
Example B:

Dataset (3-79y)	Differences?
Alberta	NO
British Columbia	NO
Ontario -LL	YES
Ontario - DC	YES
	NO
	YES
	YES

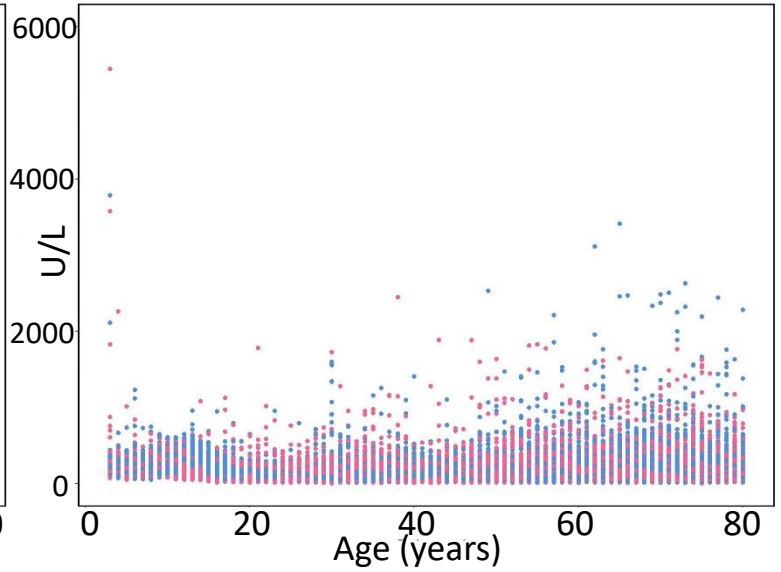
Harmonized Partitions
AB, BC, ON-LL

ALP: Dataset Differences

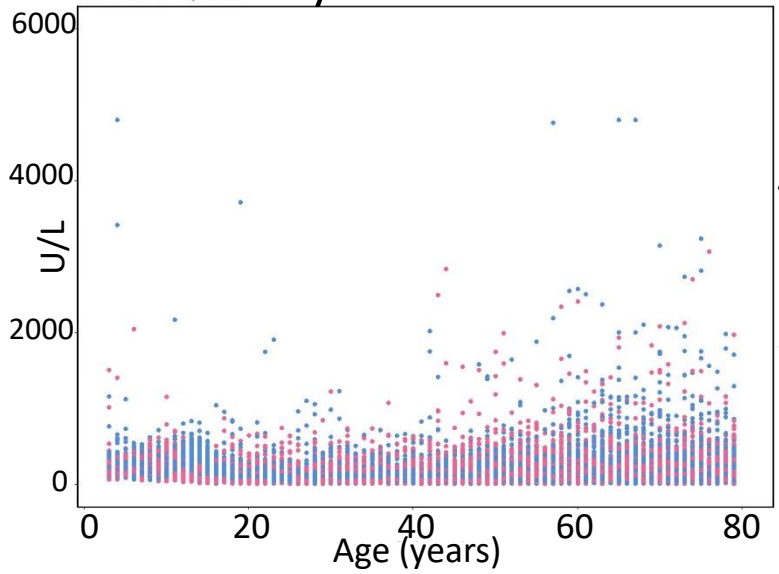
Alberta – DynaLIFE



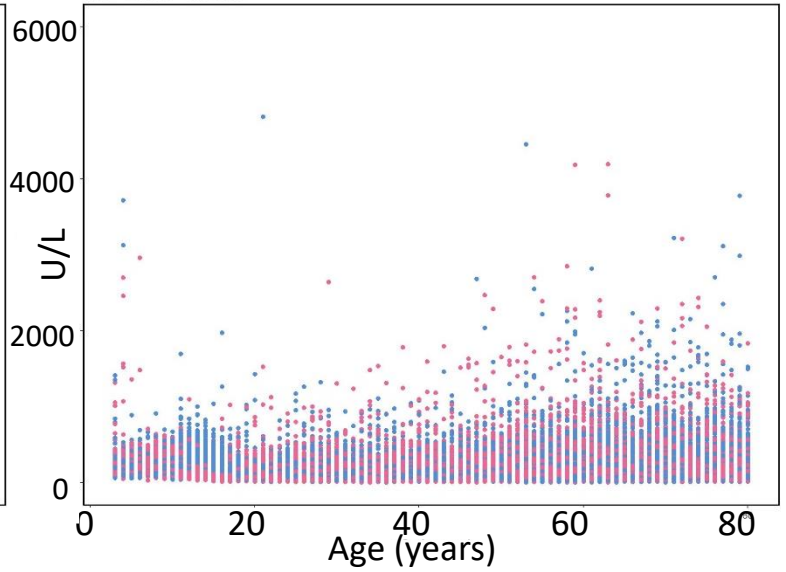
British Columbia - LifeLabs



Ontario - Dynacare

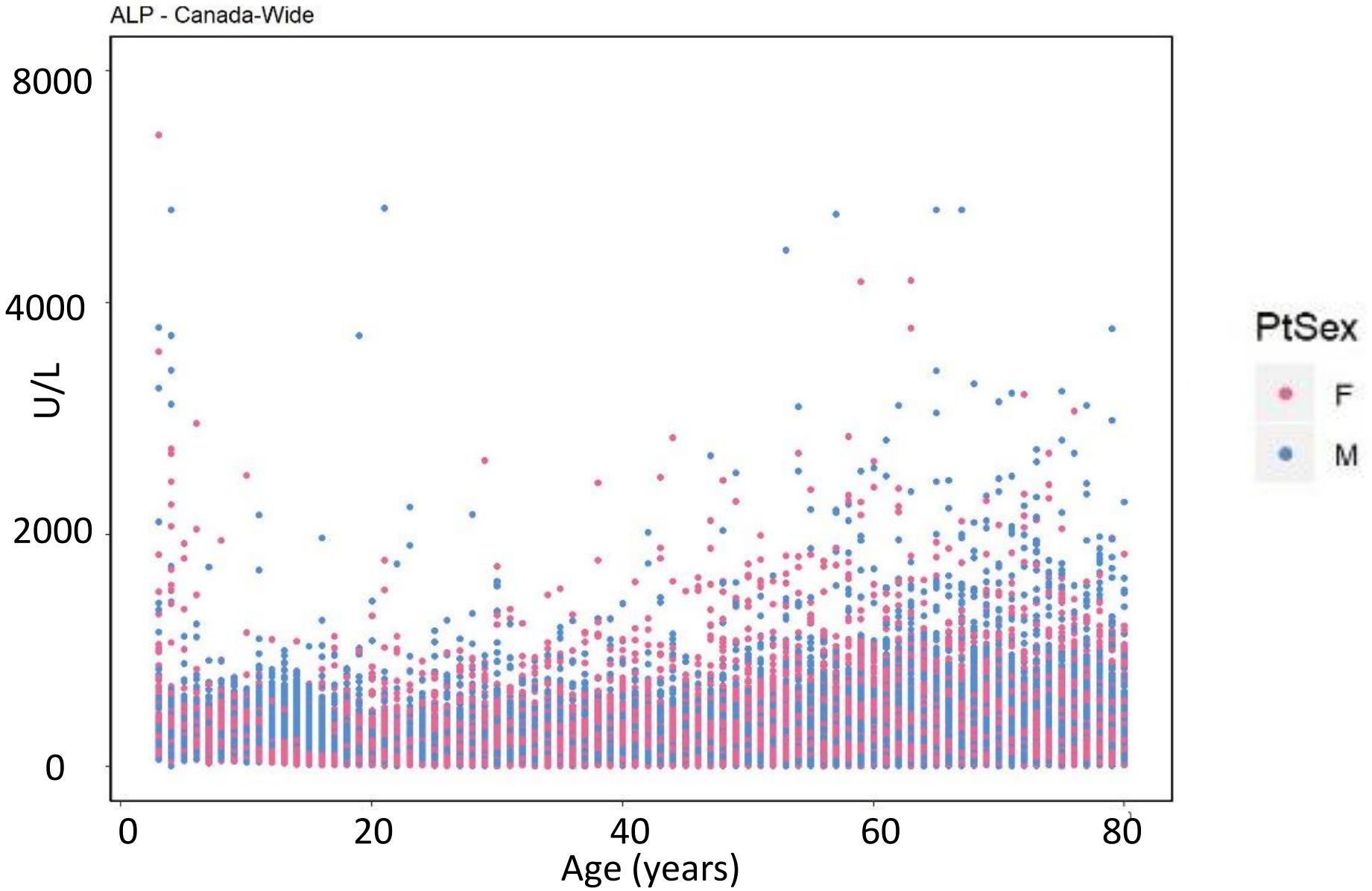


Ontario - LifeLabs



Province Comparison	Difference
Alberta DynaLife vs BC LifeLabs	No
Alberta DynaLife vs ON Dynacare	No
Alberta DynaLife vs ON LifeLabs	No
BC LifeLabs vs ON Dynacare	No
BC LifeLabs vs ON LifeLabs	No
ON Dynacare vs ON LifeLabs	No

Canada-Wide Dataset



Statistical Approach

Use Canada-wide file to determine age- and sex-specific partitions

Statistical differences confirmed using Harris & Boyd Method

Determine age/sex-specific differences

Method: Harris & Boyd

Example A:

Age Partition	Sex-specific differences?	Age-specific differences?
3-5 years	NO	NO
6-15 years	NO	NO
16-49 years	NO	NO
50-79 years	NO	NO

Harmonized Partitions
3-79 years

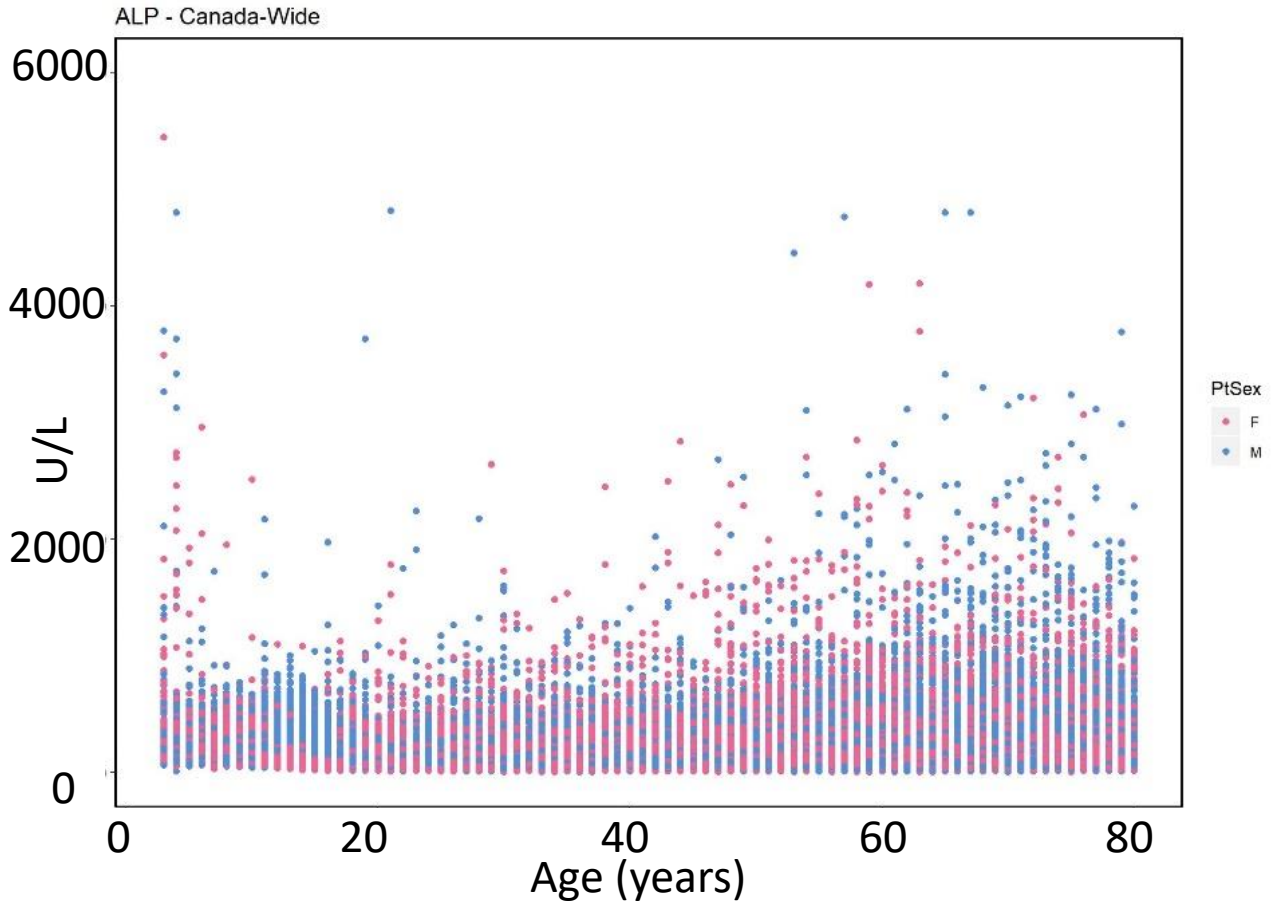
Example B:

Age Partition	Sex-specific differences?	Age-specific differences?
3-5 years	NO	YES
6-15 years	NO	NO
16-49 years	NO	NO
50-79 years	NO	NO

Harmonized Partitions
3-5 years
6-79 years

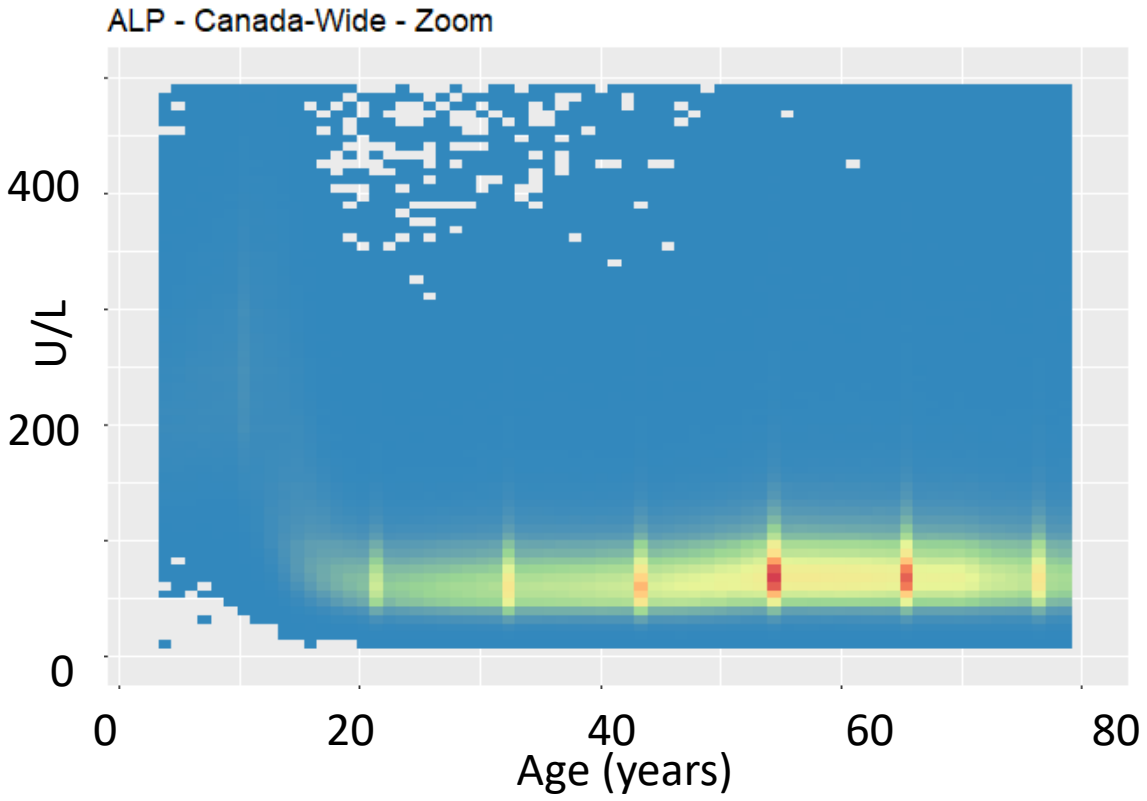
ALP: Partition Establishment

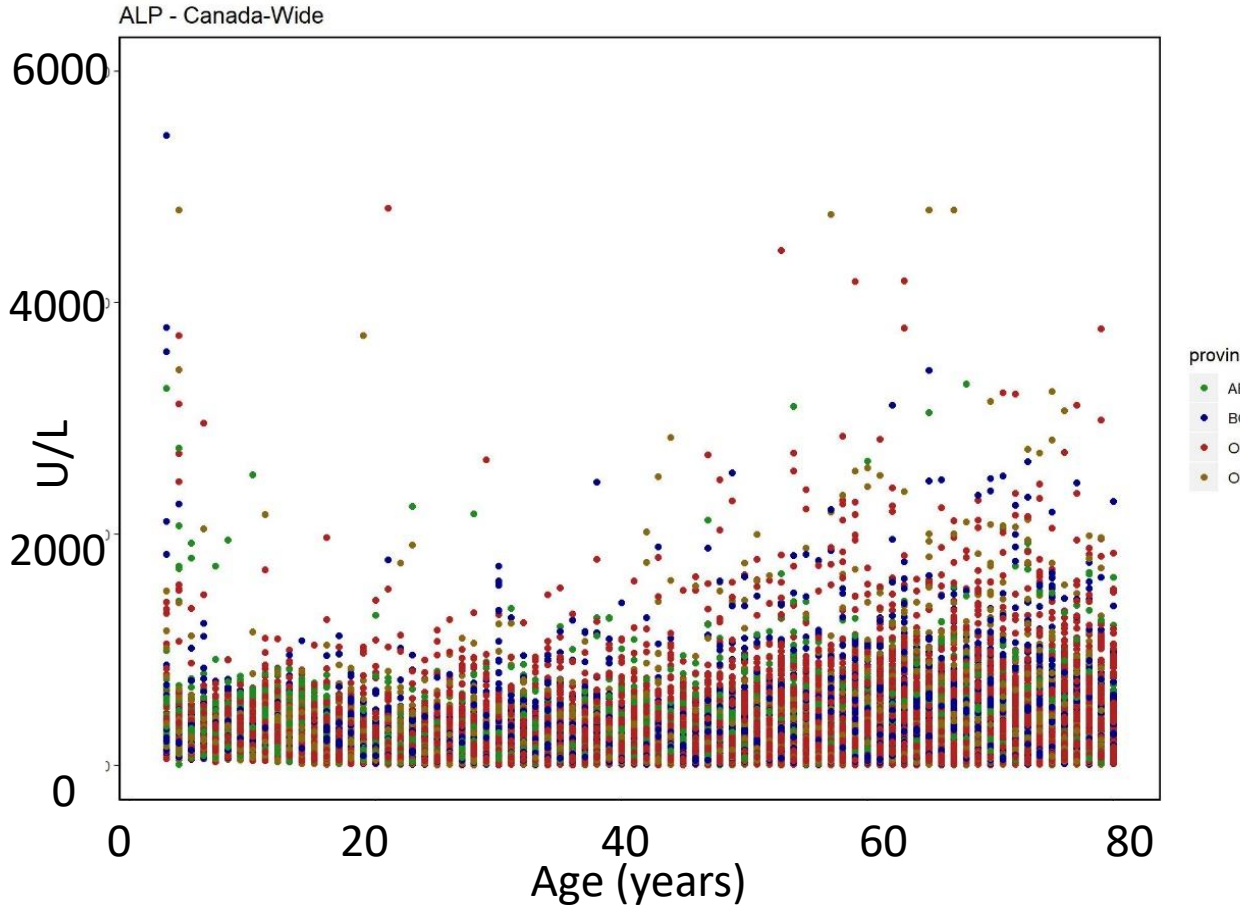
Partitions
3-10y
11-13y
14-17M/F
18-24y M/F
25-29 M/F
30-80



Consider Density of The Distribution

Create a density plot to help visually assess any age-specific differences





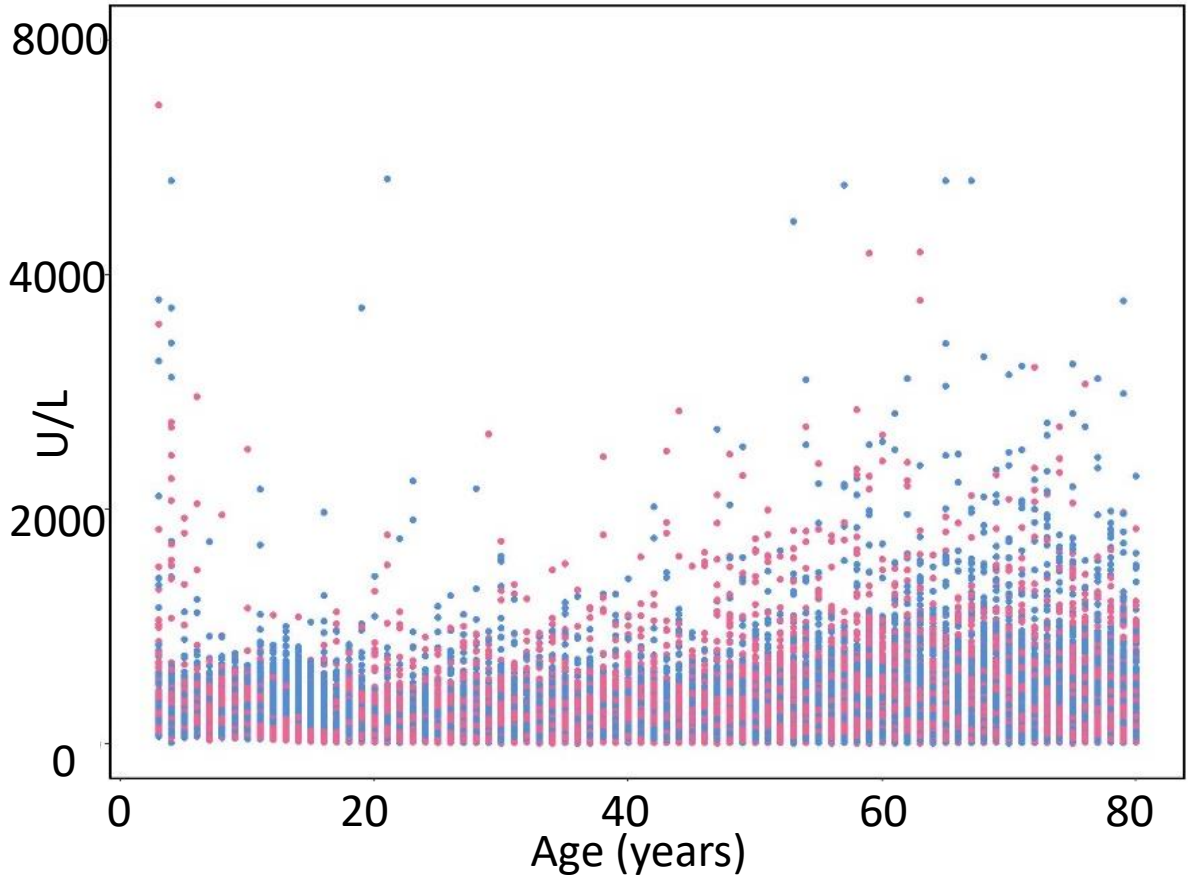
Statistical Approach

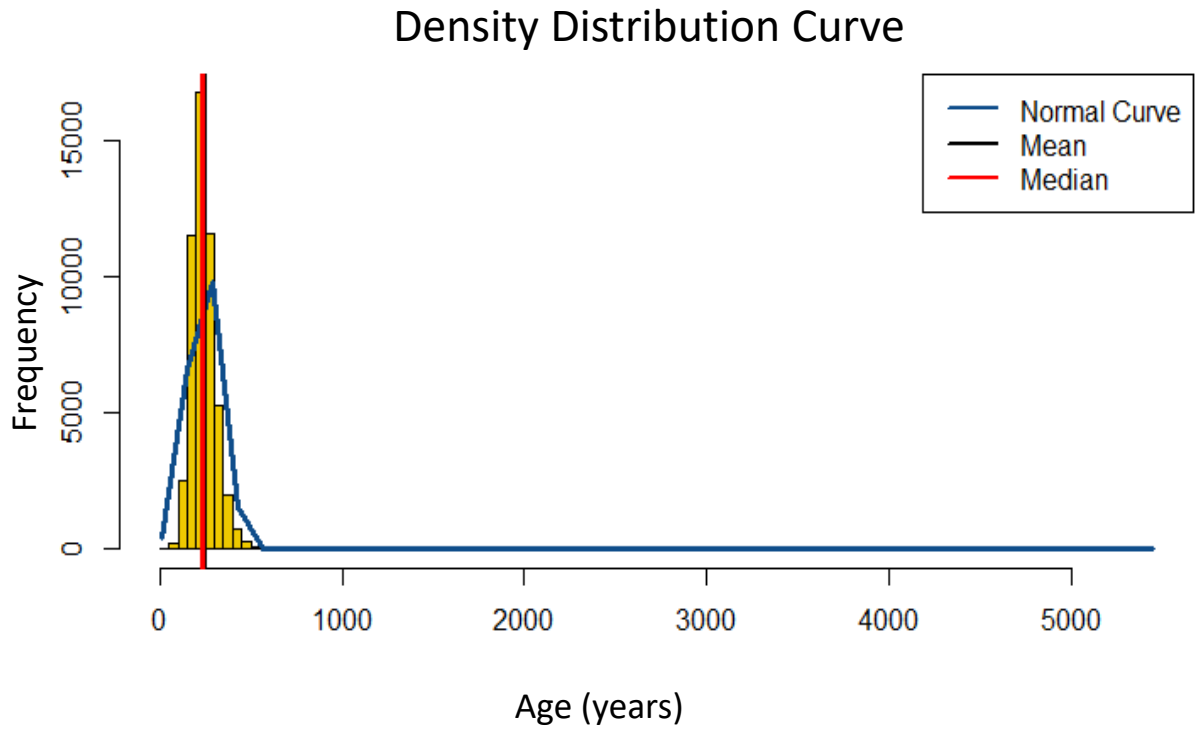
Remove outliers

Outliers are removed on a partition-specific basis for each dataset

ALP Outlier Removal

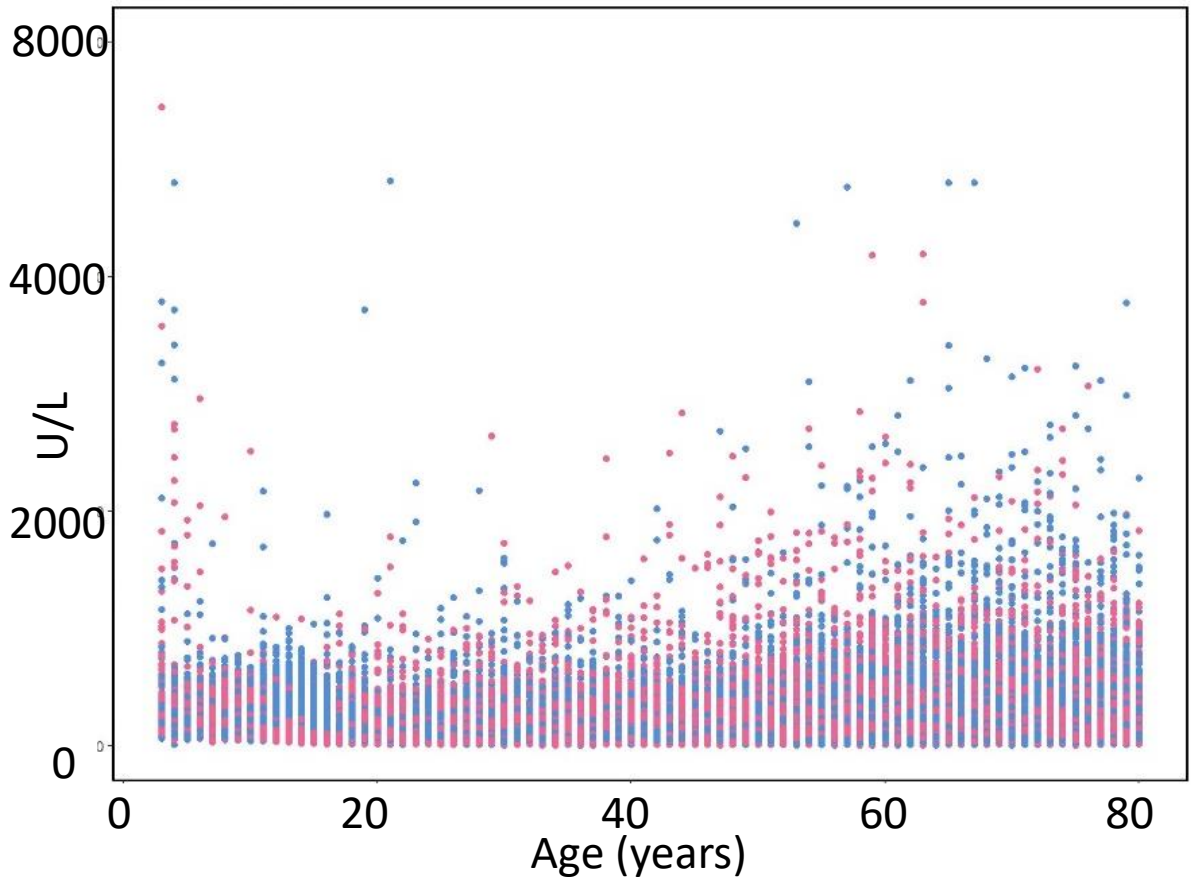
A. Tukey method: analytes with normal/Gaussian distribution – 1.5x IQR
B. Horn method: analytes with small skewness; recommended by CLSI
C. Hubert method: analytes with large skewness

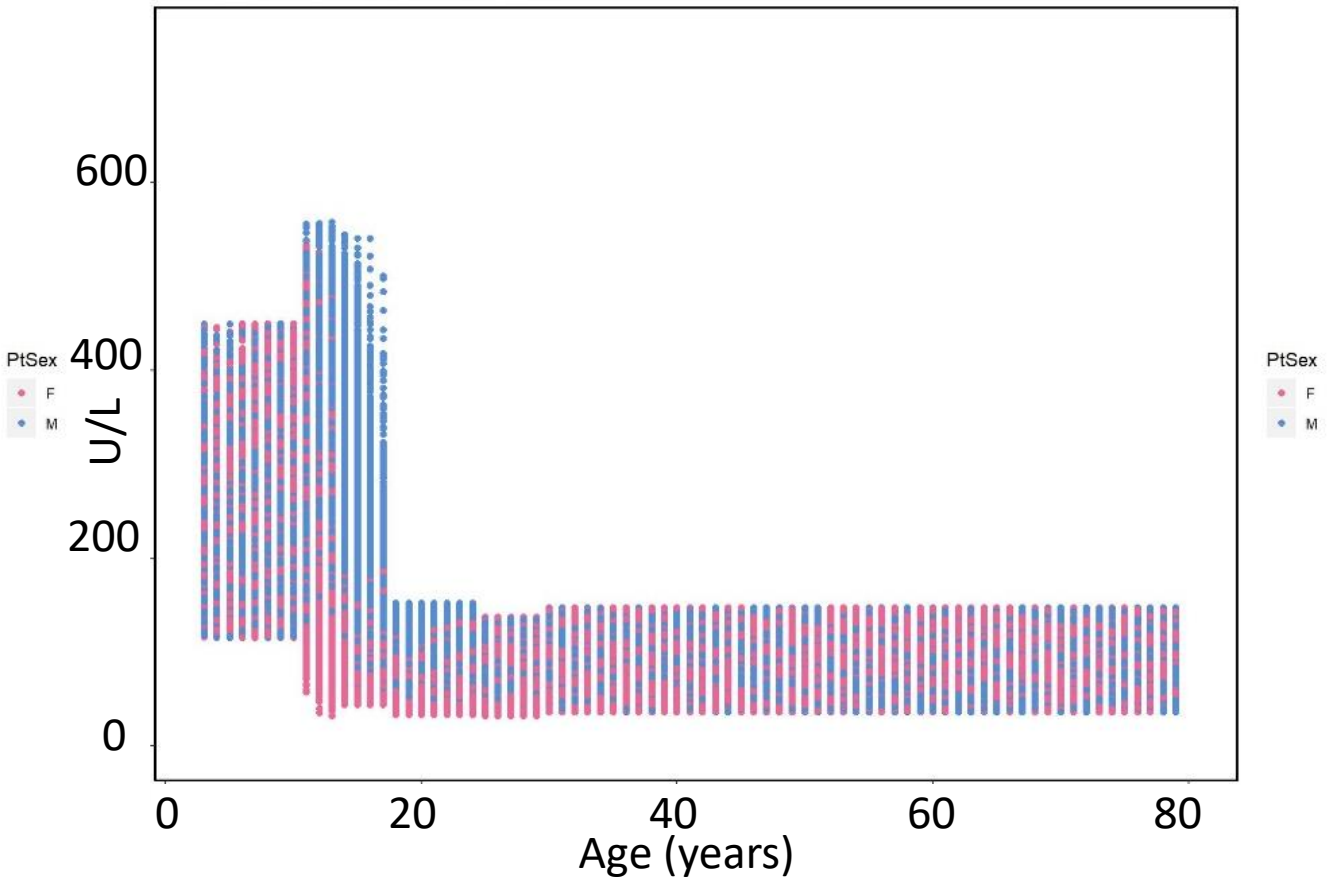




ALP Outlier-Removed Data

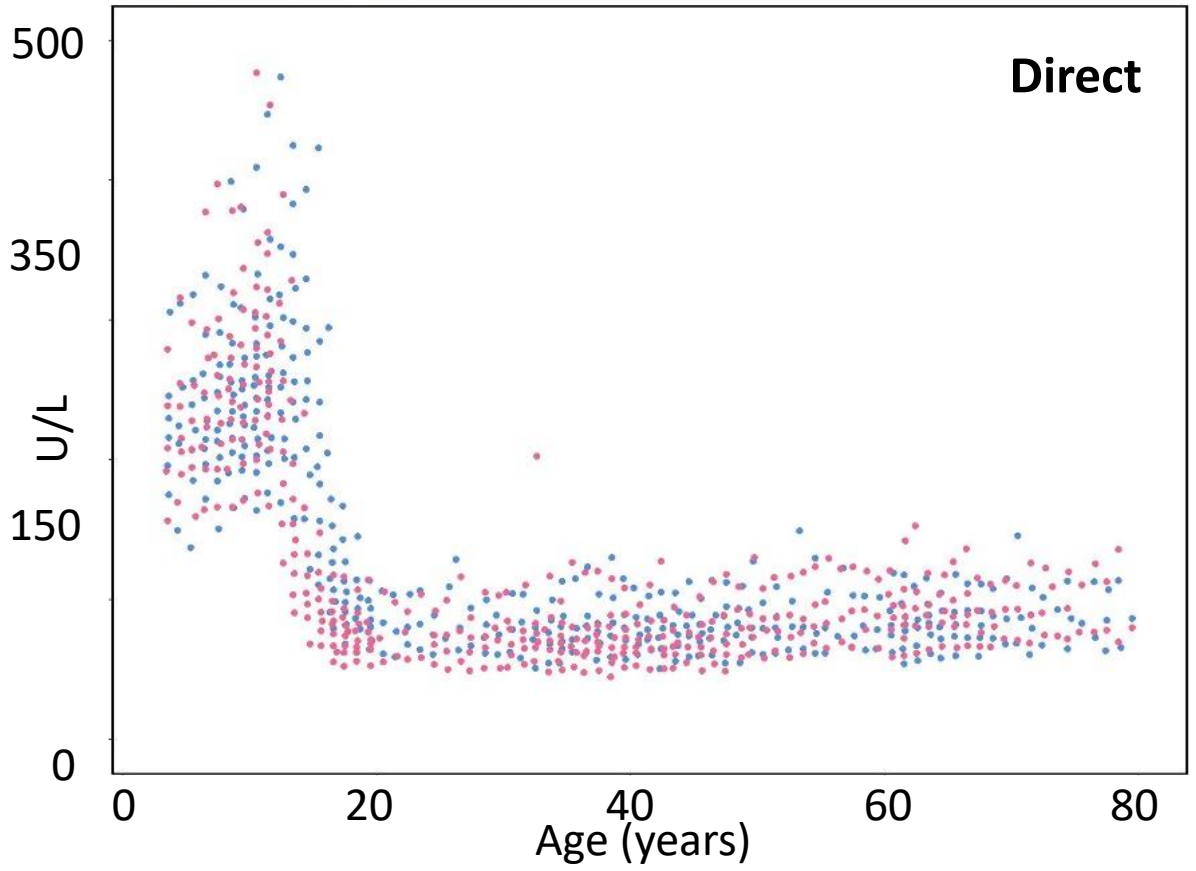
n before Outlier Removal: 5,209,913 | **Outlier Removal Method**: Hubert
 # Outliers Removed: 149,932 | **Proportion Removed: 3%**



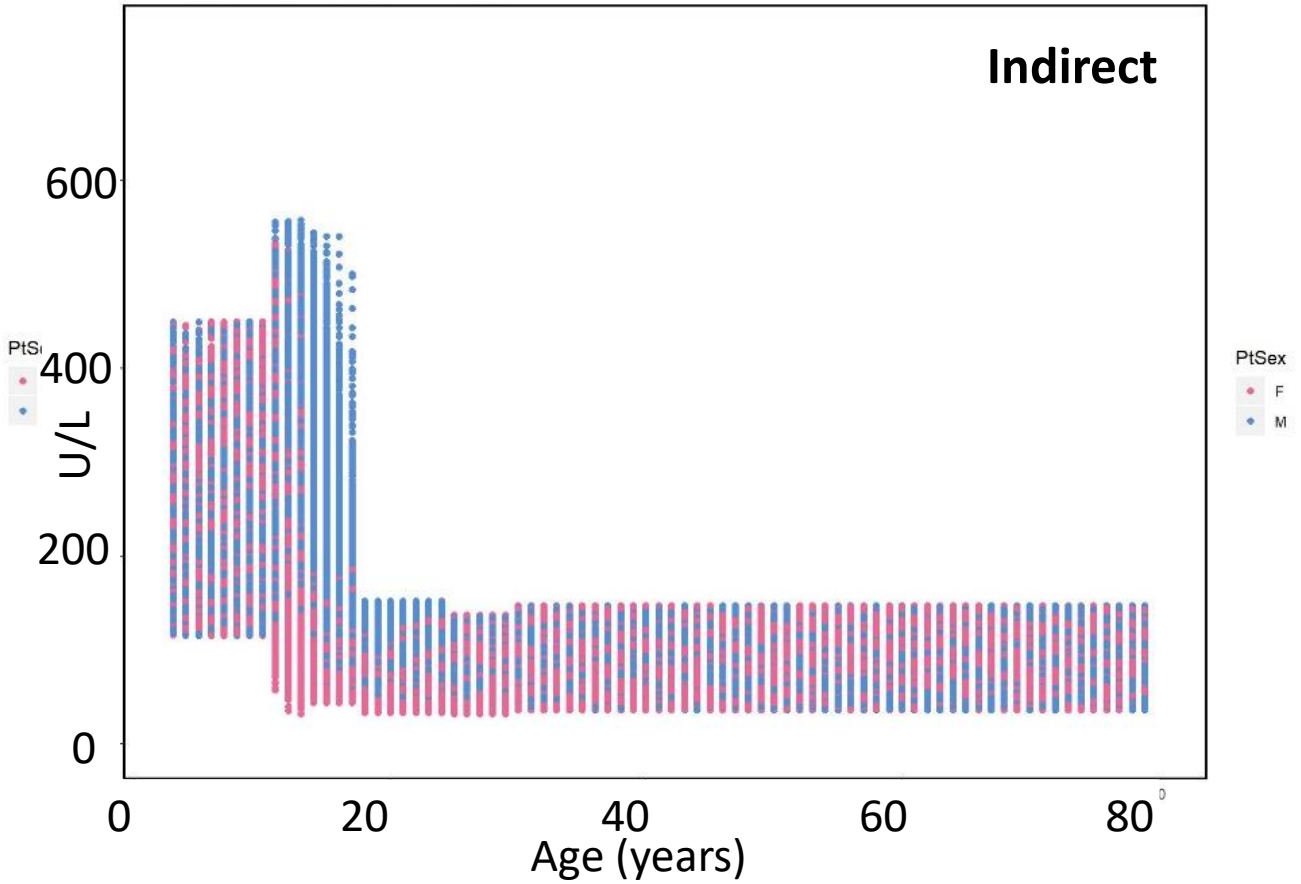


Comparison to CHMS

CHMS



hRI – Canada Wide



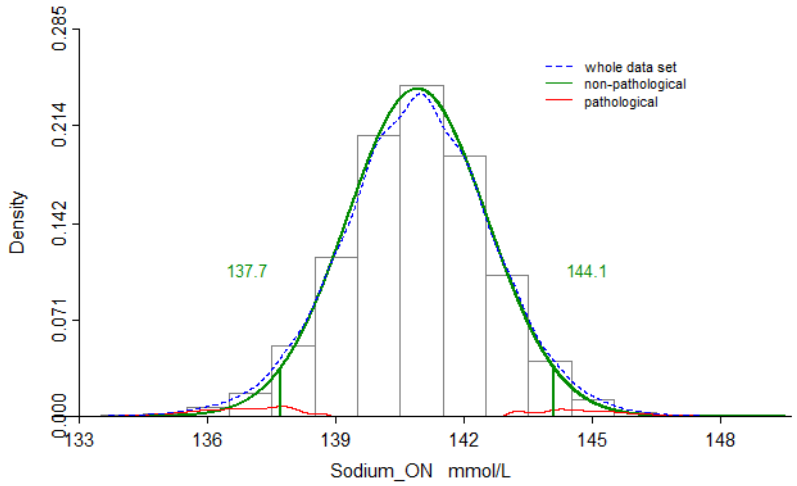
Statistical Approach

Reference Interval Determination

Reference Intervals Estimated Using the Arzideh Method

Confirm Assumptions

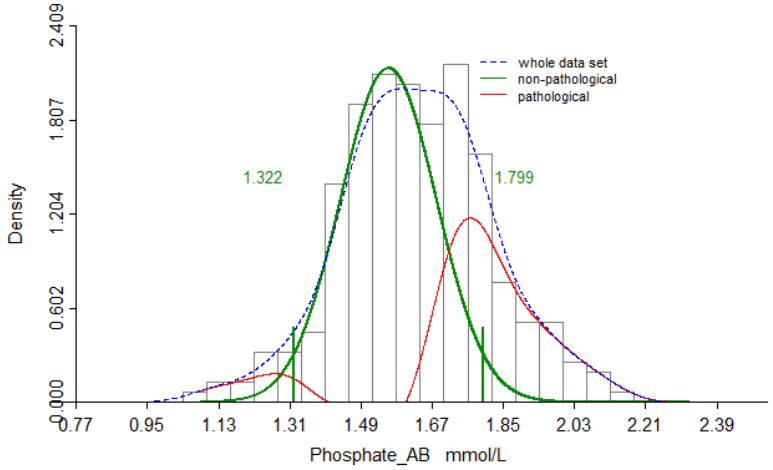
Example A:



Estimated distributions for non-pathological values (green curve), pathological values (red) and whole data (blue). Green lines (and given numers) indicate 2.5 and 97.5 percentiles of the estimated distribution for non-pathological values (RL).

- ✓ Non-pathological distribution is Gaussian.
- ✓ Total distribution is unimodal
- ✓ The overlap between pathological and non-pathological is partial
  - Pathological is <20%

Example B:



Estimated distributions for non-pathological values (green curve), pathological values (red) and whole data (blue). Green lines (and given numers) indicate 2.5 and 97.5 percentiles of the estimated distribution for non-pathological values (RL).

- ✓ Non-pathological distribution is Gaussian.
- X Total distribution is unimodal
- X The overlap between pathological and non-pathological is partial
  - Pathological is <20%

ALP – Canada-Wide: 3-10 years

Non-pathological: 99%

- ✓ Estimated distribution of NP is Gaussian
- ✓ Central part of distribution is unimodal
- ✓ Overlapping between pathological and non-pathological is partial

n: 50235

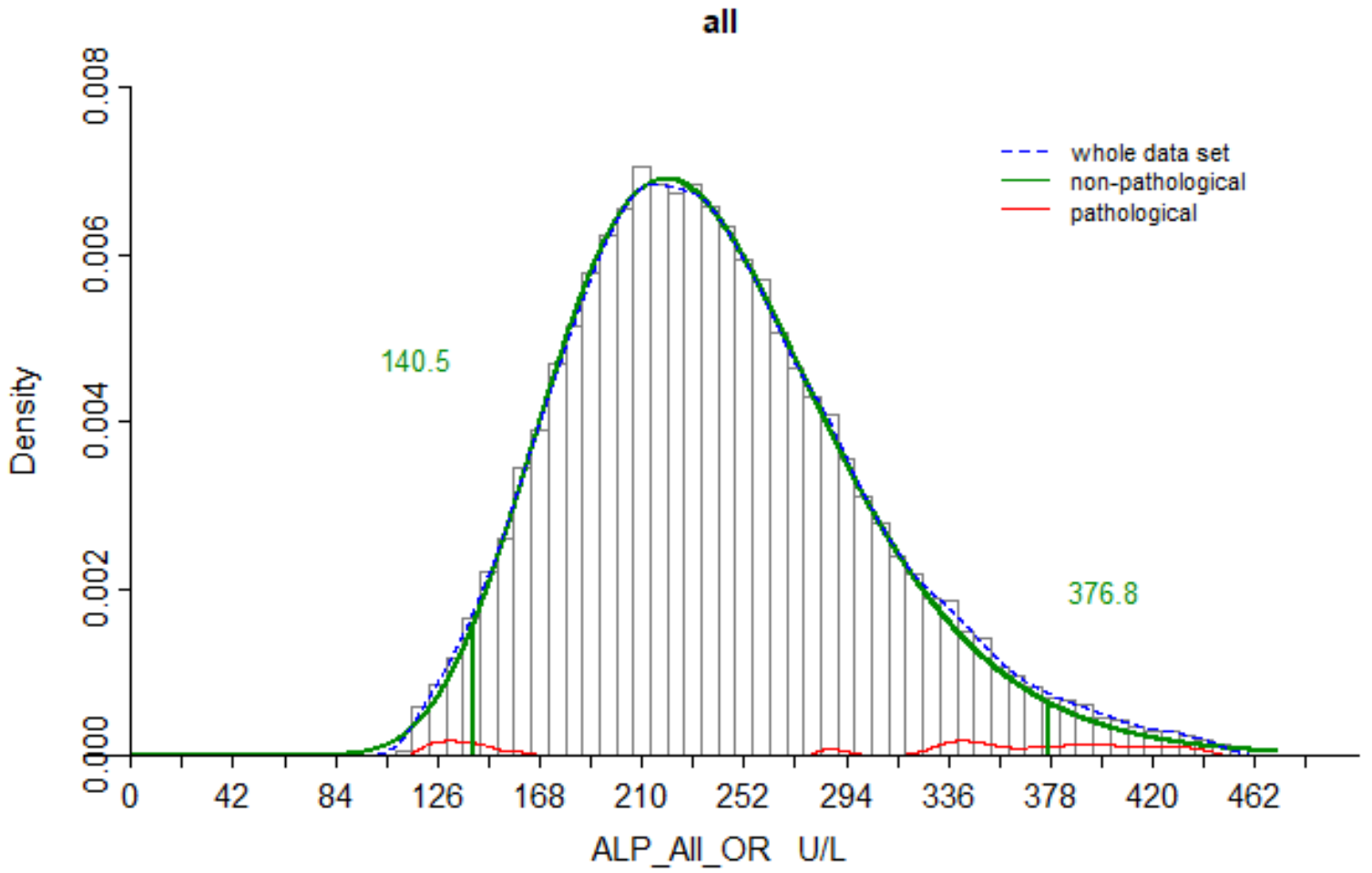
If <4000, report with caution

Reference Interval:

141-377 U/L

Confidence Interval:

L: (137, 143) **U:** (363, 385)



Estimated distributions for non-pathological values (green curve), pathological values (red) and whole data (blue). Green lines (and given numers) indicate 2.5 and 97.5 percentiles of the estimated distribution for non-pathological values (RL).

ALP – Alberta: 11-13 years (Female)

Non-pathological: 54%

✓ Estimated distribution of NP is Gaussian

X Central part of distribution is unimodal

X Overlapping between P and NP is partial

n: 2223

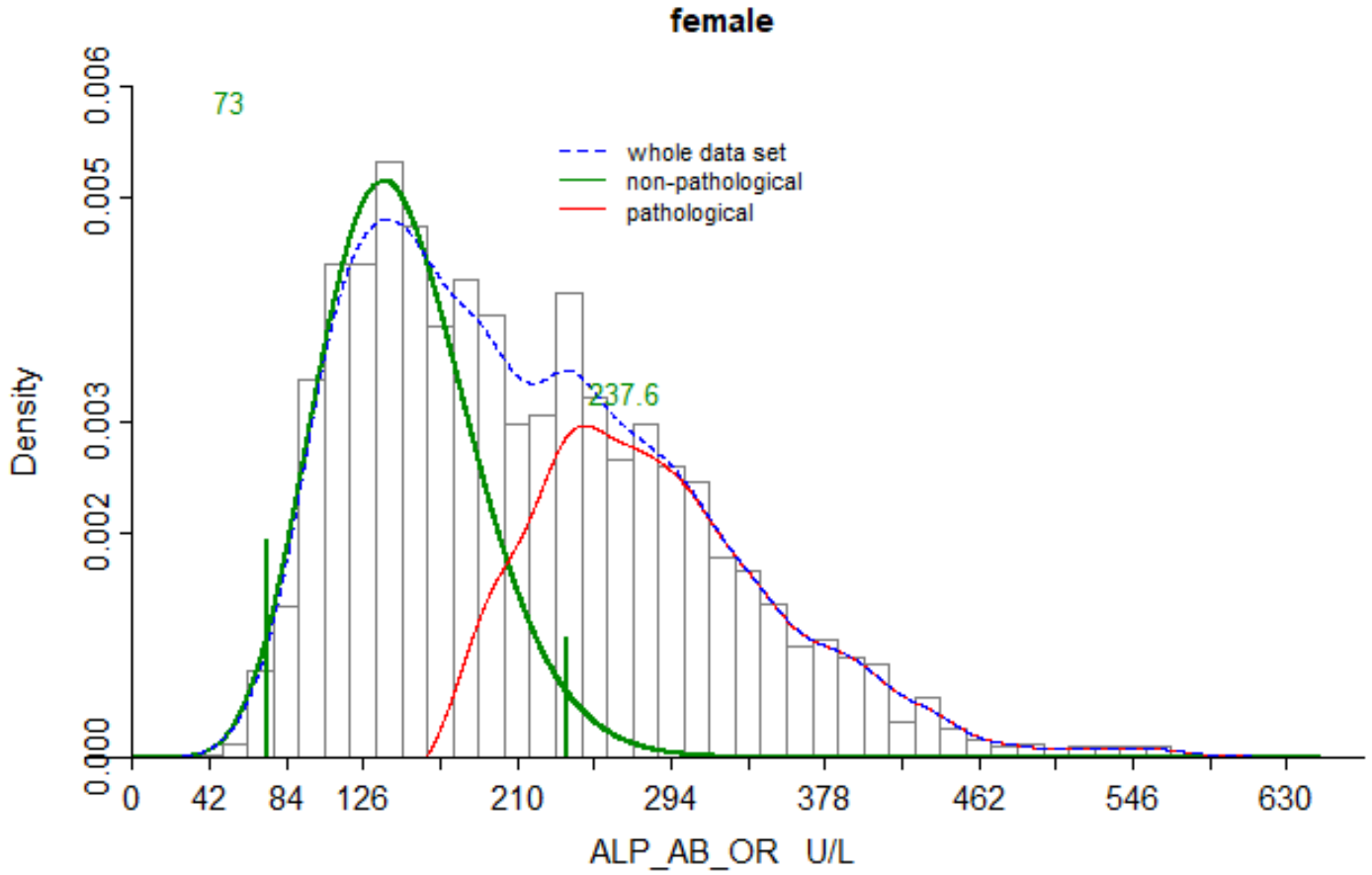
If <4000, report with caution

Reference Interval:

73.0-238 U/L

Confidence Interval:

Unable to estimate



Estimated distributions for non-pathological values (green curve), pathological values (red) and whole data (blue). Green lines (and given numers) indicate 2.5 and 97.5 percentiles of the estimated distribution for non-pathological values (RL).

Repeat for each partition: x9
Repeat for each dataset + Canada-Wide: x5

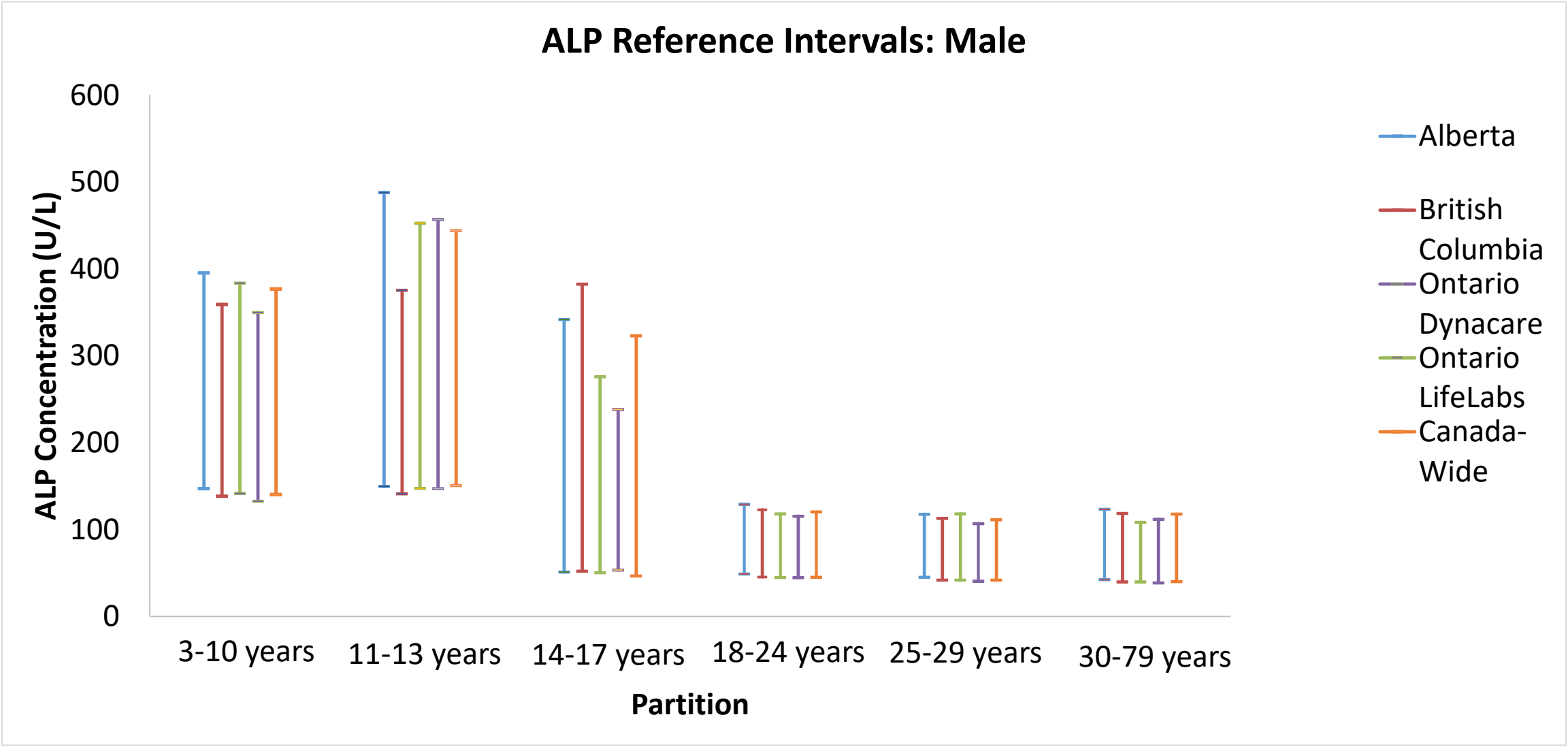
45 Calculations for ALP

Male and Female RI: Across Canada

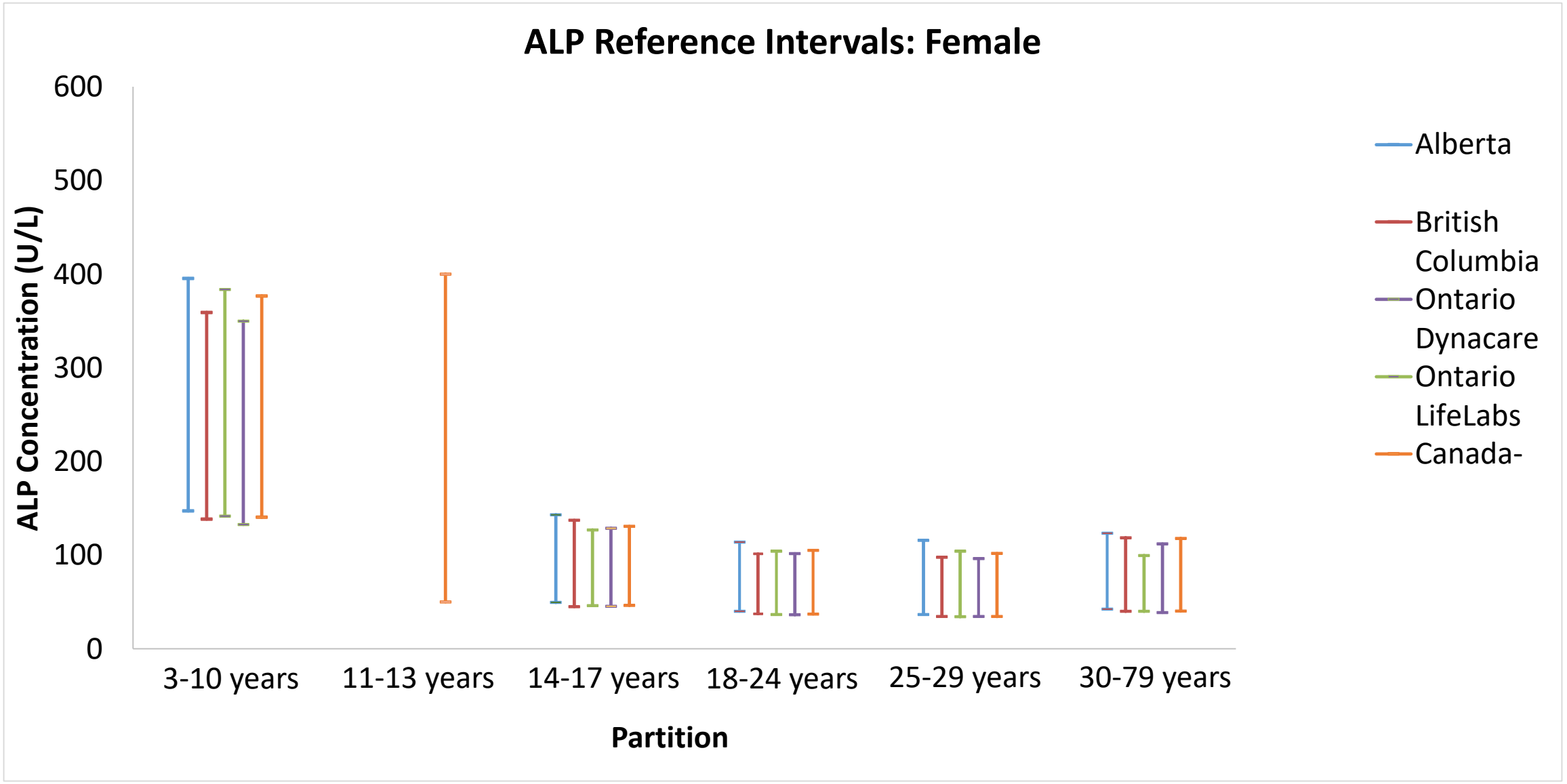
hRI Indirect Analysis	Alberta	British Columbia	Ontario - Dynacare	Ontario - LifeLabs	Canada-Wide
3-10y	147-395	138-359	133-350	142-384	141-376
11-13y*	150-488	141-375	14.3-457	148-453	151-444
14-17*	51.2-342	52.2-382	53.4-238	50.5-276	46.6-323
18-24y*	48.9-129	45.5-123	44.6-115	44.9-118	45.1-120
25-29*	45.1-118	41.9-113	40.6-107	42-109	41.9-111
30-80	42.5-123	40.0-119	38.7-112	40-116	40.2-118

hRI Indirect Analysis	Alberta	British Columbia	Ontario - Dynacare	Ontario - LifeLabs	Canada-Wide
3-10y	147-395	138-359	133-350	142-384	141-377
11-13y*	NA	NA	NA	NA	50.1-400
14-17*	49.5-143	45.0-137	45.4-129	46.1-127	46.3-131
18-24y*	40.0-114	37.4-101	36.3-102	36.7-104	37.0-105
25-29*	36.7-116	34.6-97.7	34.4-96.4	34.2-99.6	34.4-102
30-80	42.5-123	40.0-119	38.7-112	40-116	40.2-118

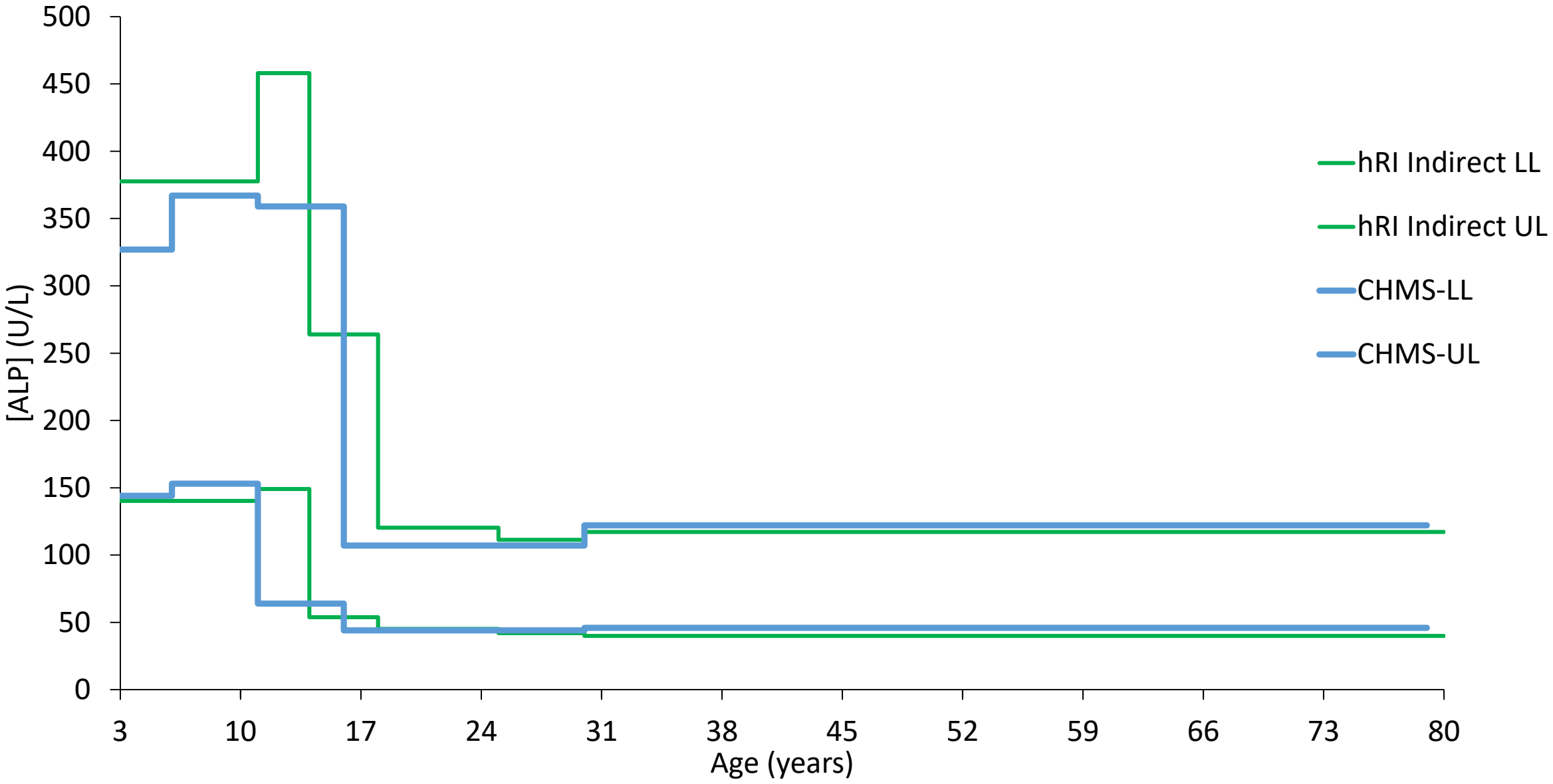
Male RI: Comparison Across Canada



Female RI: Comparison Across Canada



ALP Male RI: Canadian Direct vs Indirect Data

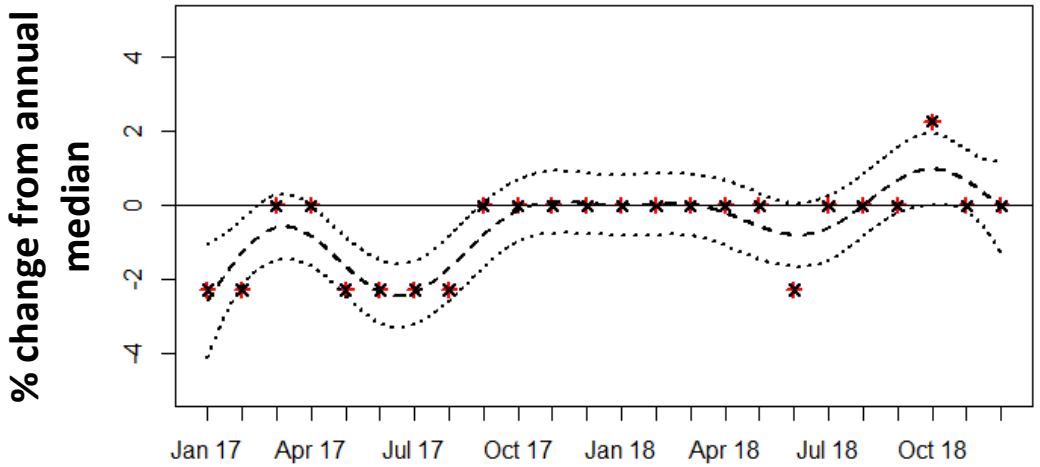


Albumin

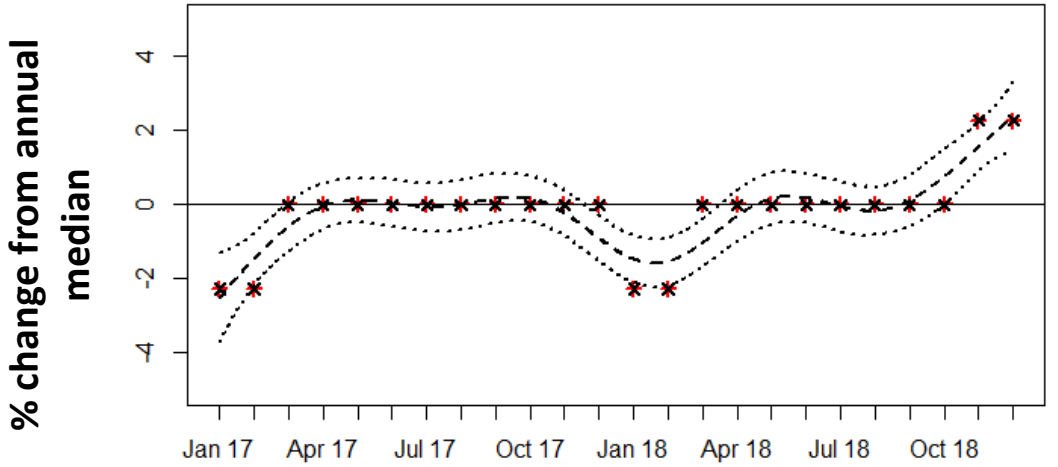
Albumin Monthly Instability

Westgard
1/3rd TEA (4%) = 1.3%
½ RCV (10 %) = 5%

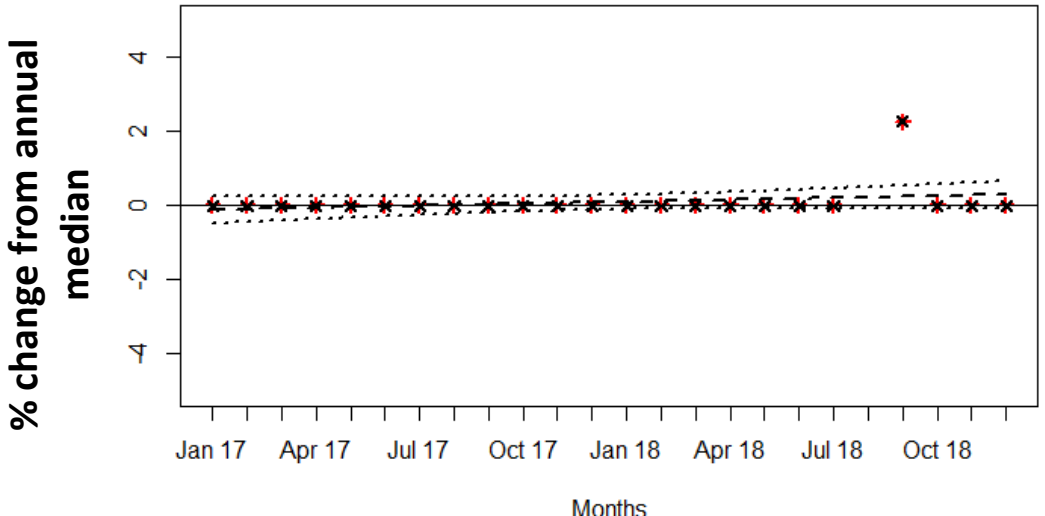
Alberta – DynaLIFE



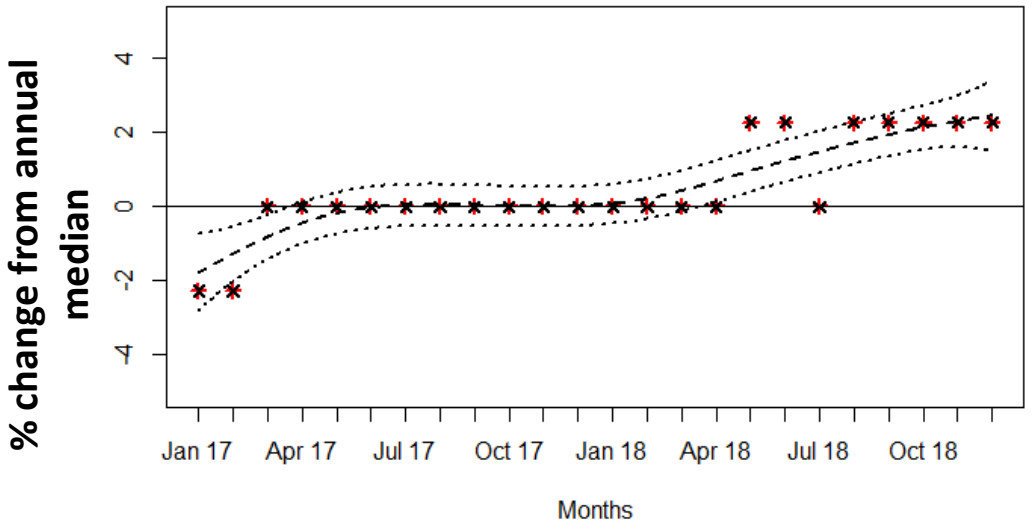
British Columbia - LifeLabs



Ontario - Dynacare

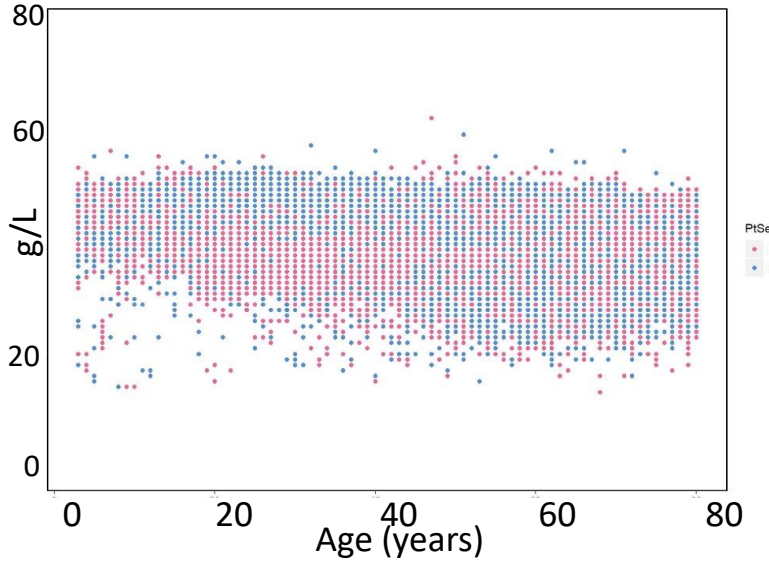


Ontario - LifeLabs

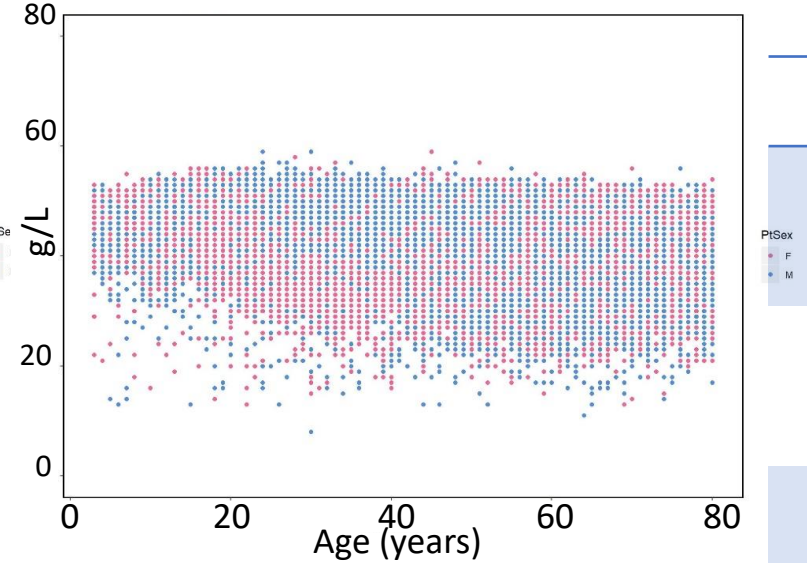


Albumin: Dataset Differences

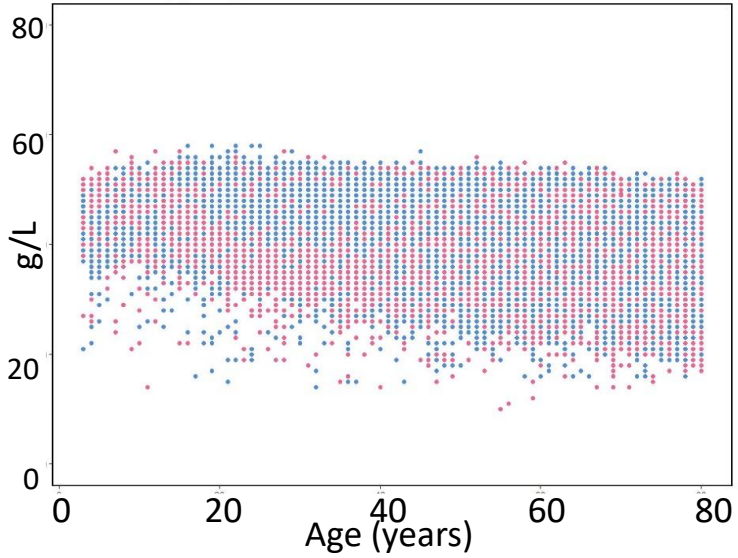
Alberta – DynaLIFE



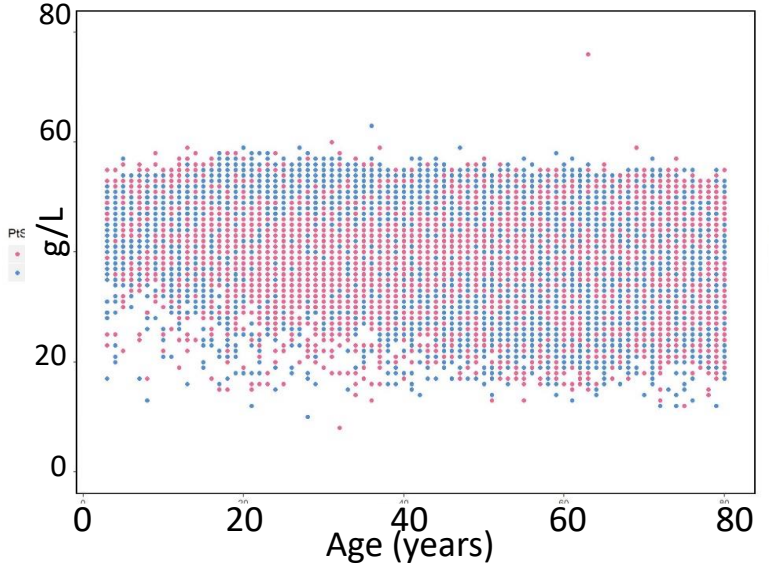
British Columbia - LifeLabs



Ontario - Dynacare

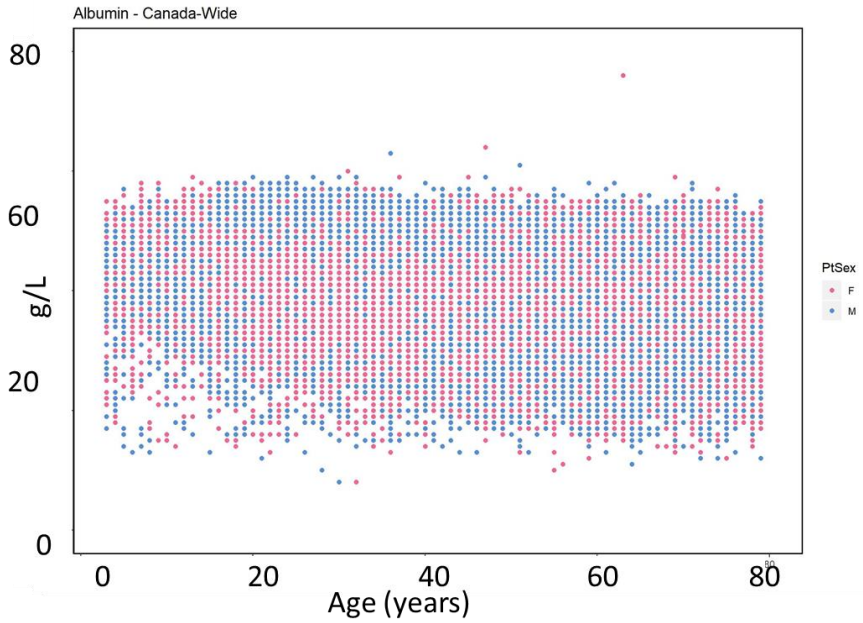


Ontario - LifeLabs

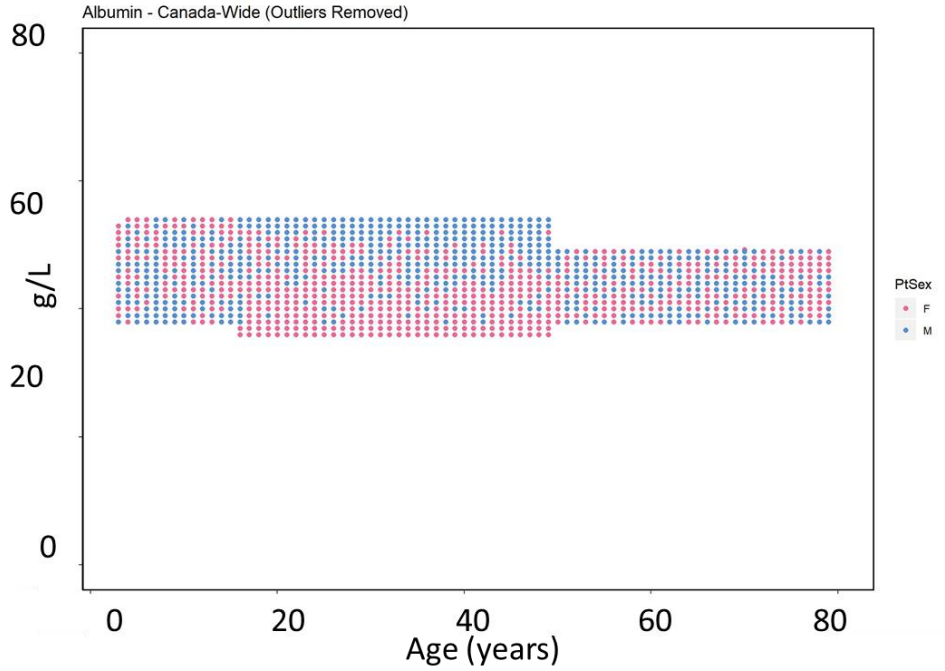


Province Comparison	Difference
Alberta DynaLife vs BC LifeLabs	No
Alberta DynaLife vs ON Dynacare	No
Alberta DynaLife vs ON LifeLabs	No
BC LifeLabs vs ON Dynacare	No
BC LifeLabs vs ON LifeLabs	No
ON Dynacare vs ON LifeLabs	No

Albumin: Partition Establishment And Outlier Removal



Partitions
3-15y
16-49y M/F
50-80y



Albumin: Canada-Wide 3-15yrs

Non-pathological: 98%

- ✓ Estimated distribution of NP is Gaussian
- ✓ Central part of distribution is unimodal
- ✓ Overlapping between pathological and non-pathological is partial

n: 73091

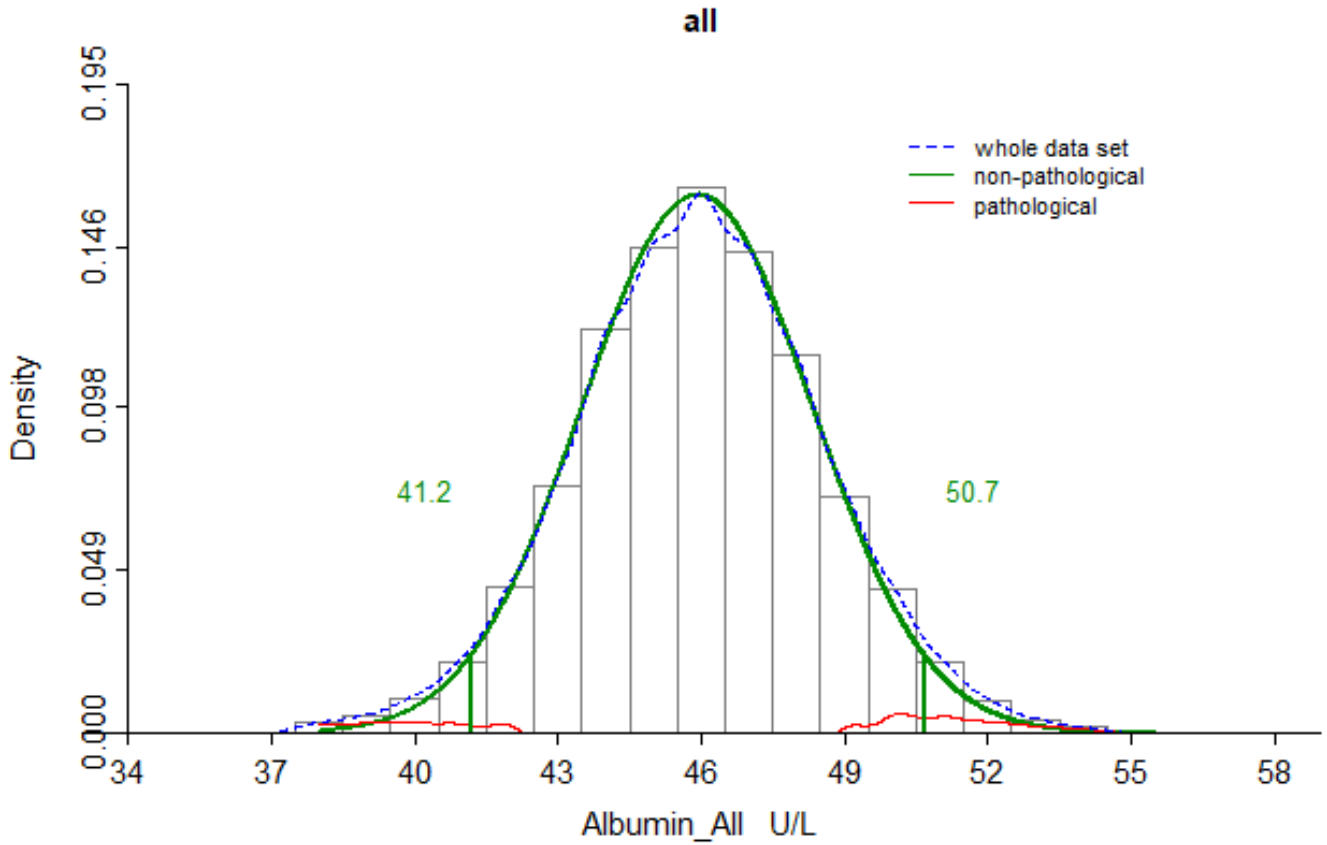
If <4000, report with caution

Reference Interval:

41.2-50.7 g/L

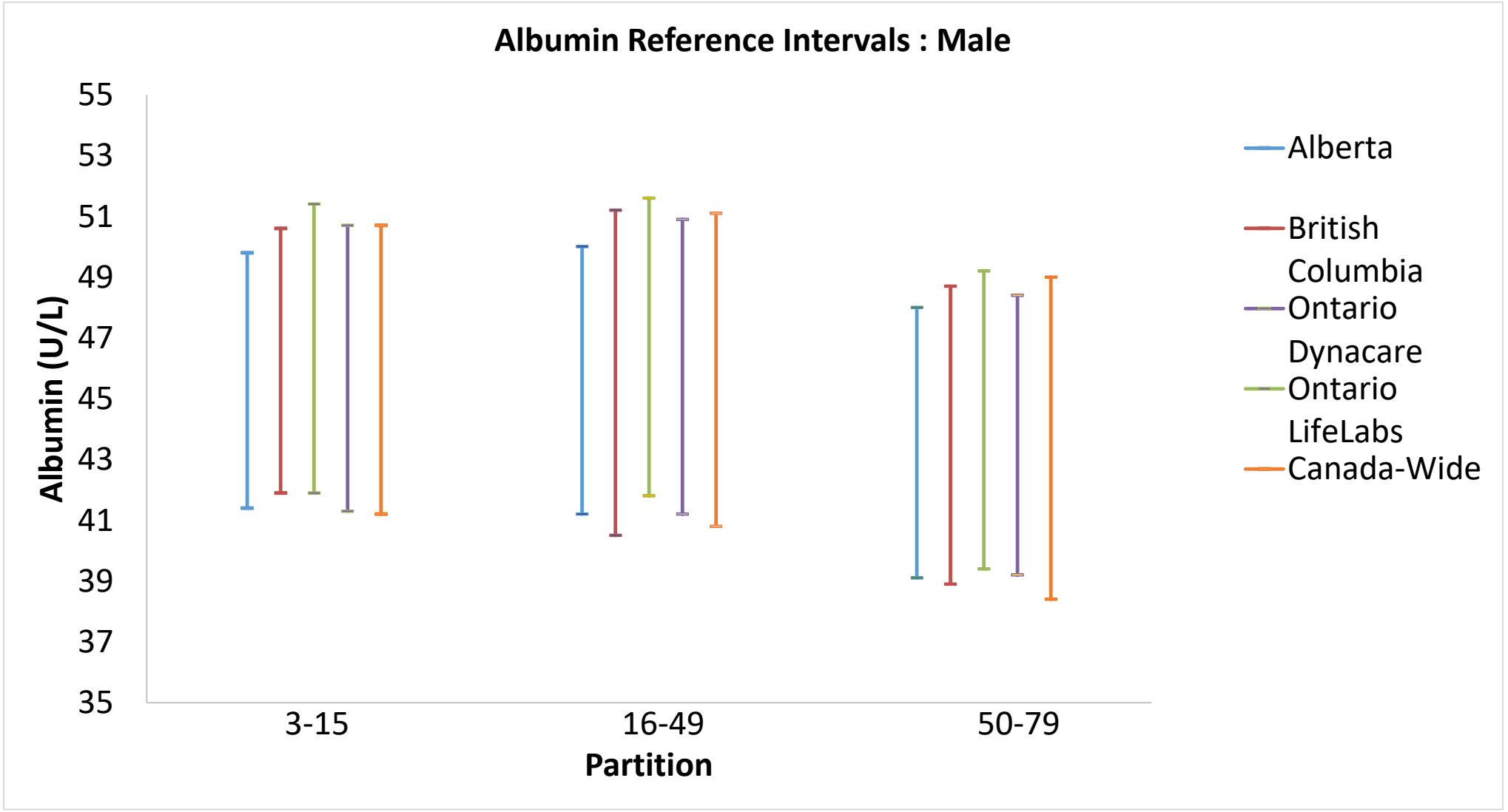
Confidence Interval:

L: (41.2-41.3) **U:** (50.6-50.7)

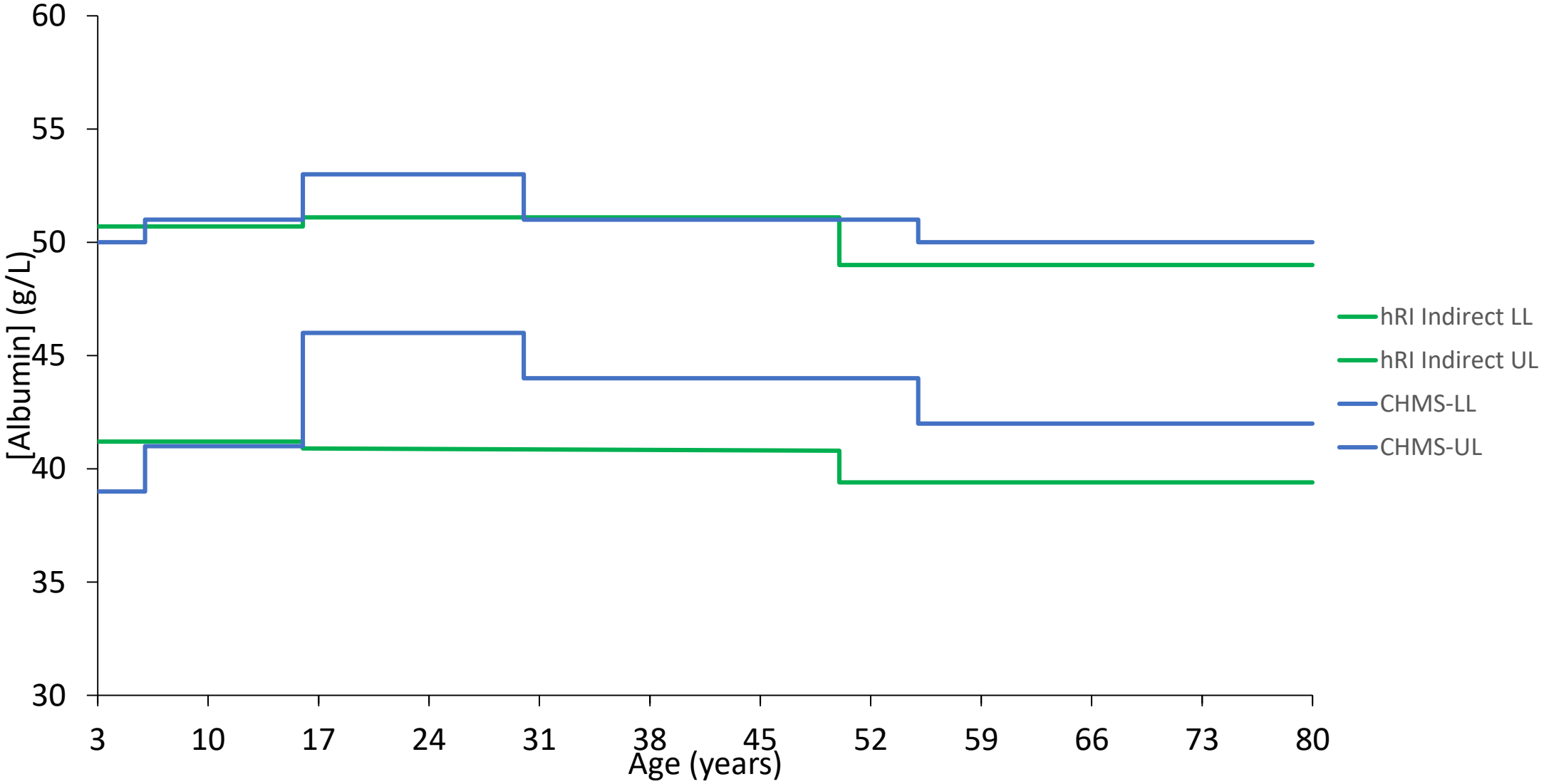


Estimated distributions for non-pathological values (green curve), pathological values (red) and whole data (blue). Green lines (and given numers) indicate 2.5 and 97.5 percentiles of the estimated distribution for non-pathological values (RL).

Male RI: Comparison Across Canada



Albumin Male RI: Canadian Direct vs Indirect Data

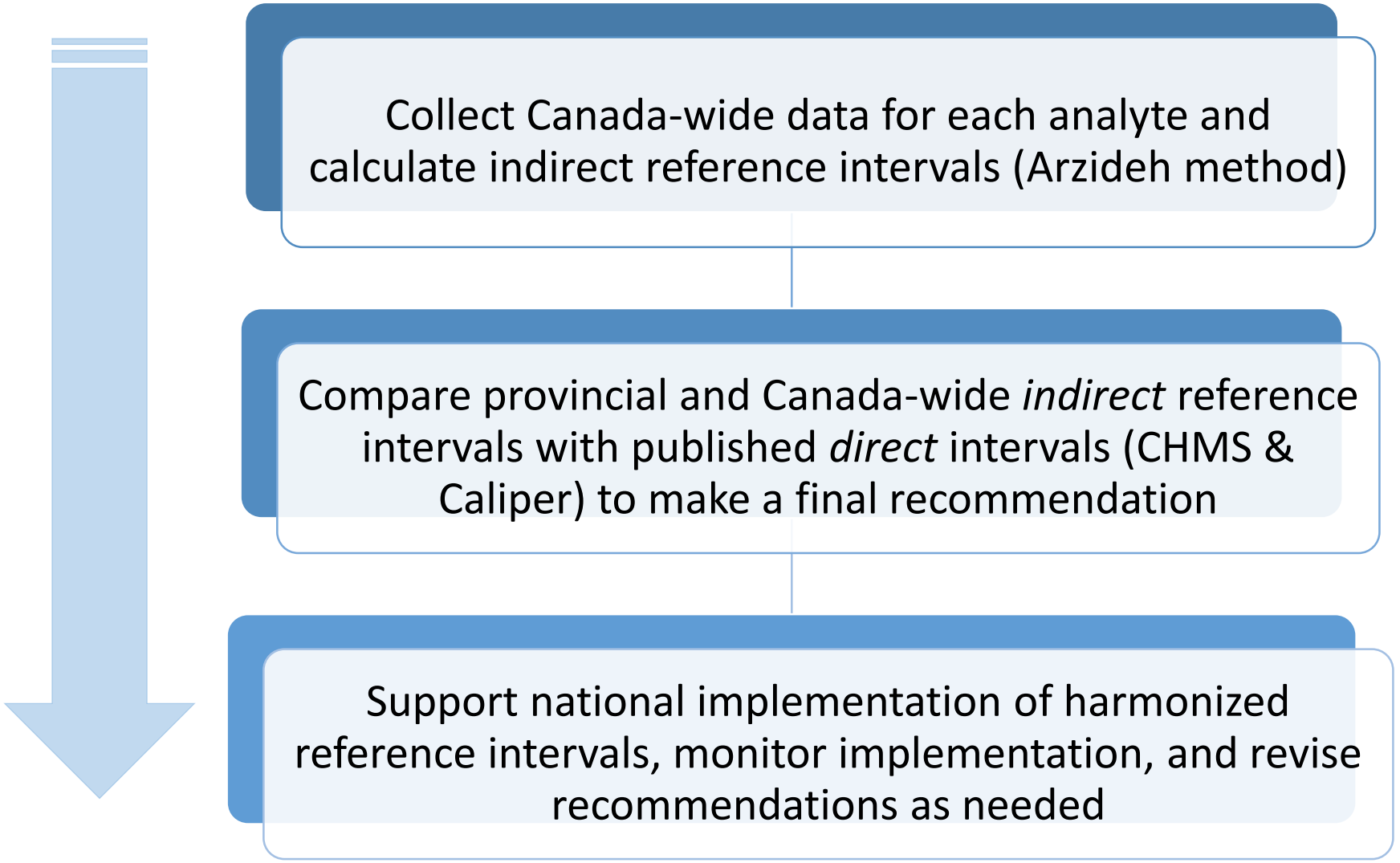


Indirect Analysis of Canada-Wide Datasets on Electrolytes

Electrolytes			Alberta		British Columbia		Ontario (Dynacare)		Ontario (LifeLabs)		Canada-Wide	
	Unit	Partition	n	RI	n	RI	n	RI	n	RI	n	RI
Bicarbonate	mmol/L	3-79	154144	22.7-31.8	95988	22.6-31.9	52687	21.5-30.9	158312	20.4-31.1	466785	21.8-31.6
Chloride*	mmol/L	3-79	178156	100.9-109.1	82934	96.4-107.1	649960	97.6-106.3	2330038	96.8-106.5	3223932	97.8-106.4
Magnesium	mmol/L	3-79	211038	0.725-0.988	130517	0.723-0.993	164991	0.744-1.01	438581	0.725-1.01	943956	0.729-1.00
Potassium	mmol/L	3-79	800397	3.73-5.05	1632009	3.82-5.23	1576108	3.84-5.18	4058977	3.78-5.09	8068310	3.8-5.13
Sodium	mmol/L	3-15	22746	137.3-143.6	25302	137.6-144.1	37634	138.6-144.8	61462	137.9-144.2	165593	137.7-144.3
		16-49 M	144582	136.4-144.0	223923	138.2-144.6	248510	138.0-145.6	550060	138.1-144.7	1151674	137.9-144.9
		16-49 F	189264	136.5-143.2	305561	137.3-143.7	337581	137.2-144.4	764870	137.2-143.8	1582037	137.1-144.0
		50-79	410956	137.5-144.7	1049863	138.1-145.3	903788	138.7-145.9	2537066	138.0-145.4	4897907	137.5-145.7

M, male; F, female; n, number of observations after removing the outliers; LRL, lower reference limit; URL, upper reference limit

CSCC hRI Strategic Plans



Acknowledgements

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Thank you!

*Any questions (**or additional datasets**) welcome!*

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