Accurate Reference Intervals and Decision Limits:

Critical to Clinical Decision Making

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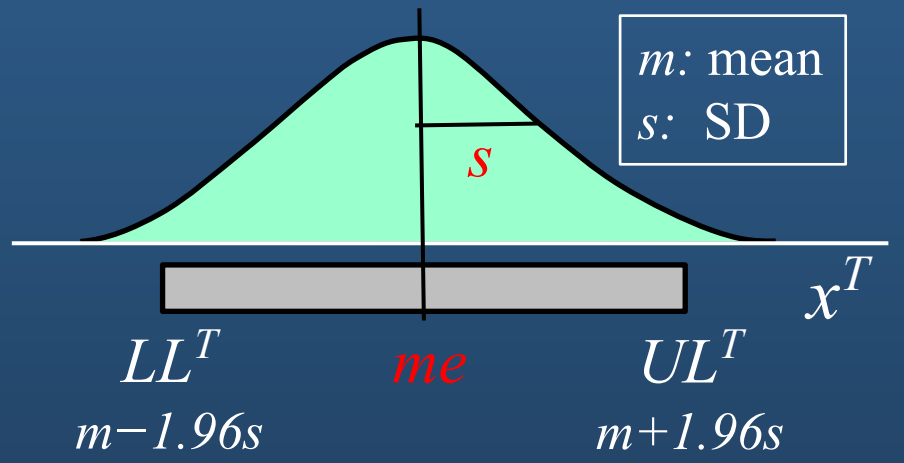
Khosrow Adeli PhD, FCACB, DABCC, FADLM

Head, Clinical Biochemistry, Pediatric Laboratory Medicine
Senior Scientist, Research Institute
The Hospital for Sick Children

Vice-Chair (Quality), Laboratory Medicine & Pathobiology,
University of Toronto

Past President, IFCC



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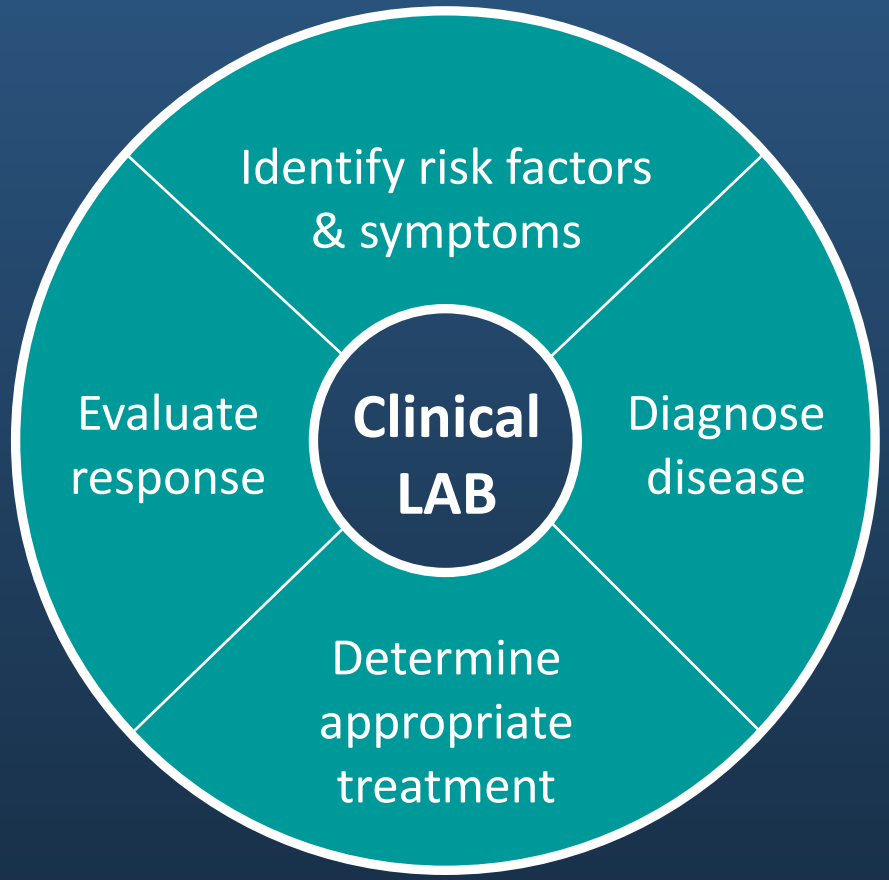
Critical Role of Laboratory Medicine in Healthcare Delivery

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



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

- Quality Lab Operations
  Accurate/Precise Testing Process (validated methods/systems)
- Appropriate Reference Intervals/Decision Limits
  Accurate interpretation of lab results based on appropriate reference intervals or clinical decision limits

Key to ensuring Postanalytical Quality of Laboratory Reports

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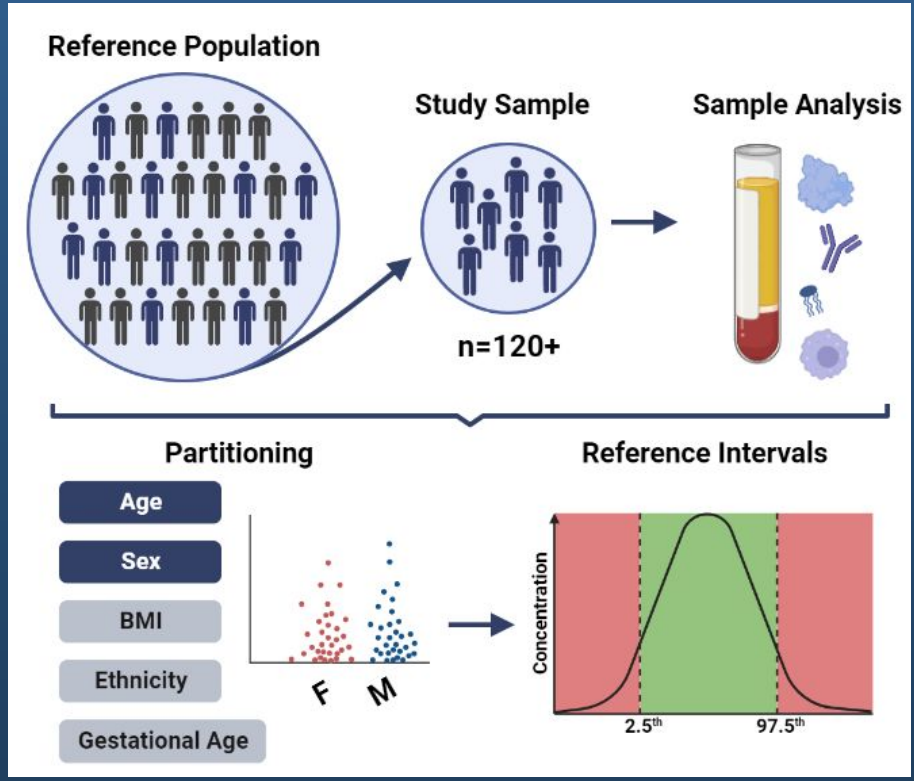
Reference Intervals: *Key Health Associated Benchmarks in Clinical Decision-Making*

- Health associated benchmarks, defined as the **2.5th** and **97.5th percentiles** in a healthy reference population
- Applied to flag abnormal results to prompt further follow-up of laboratory investigations

How do we define health and what covariates should be considered in reference interval establishment?

- *Age*
- *Sex*
- *Ethnicity*
- *Time of collection*
- *Fasting Status*
- *Analytical Methodology*



Key Resources:

CLSI and IFCC Guidelines

IFCC Taskforce on Global Reference Interval Database

IFCC Committee on Reference Intervals & Decision Limits

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Why Reference Intervals?

- Reference Intervals serve as health-associated benchmarks to accurately assess laboratory test results
- Patient Care (adult and pediatric) is highly dependent on the use and interpretation of medical lab tests
- Reference Intervals can be influenced by many covariates such as Age, Sex, Ethnicity, BMI
- Until recently, appropriately partitioned/up-to-date Reference Intervals have not been clearly defined in pediatric (and even adult) populations

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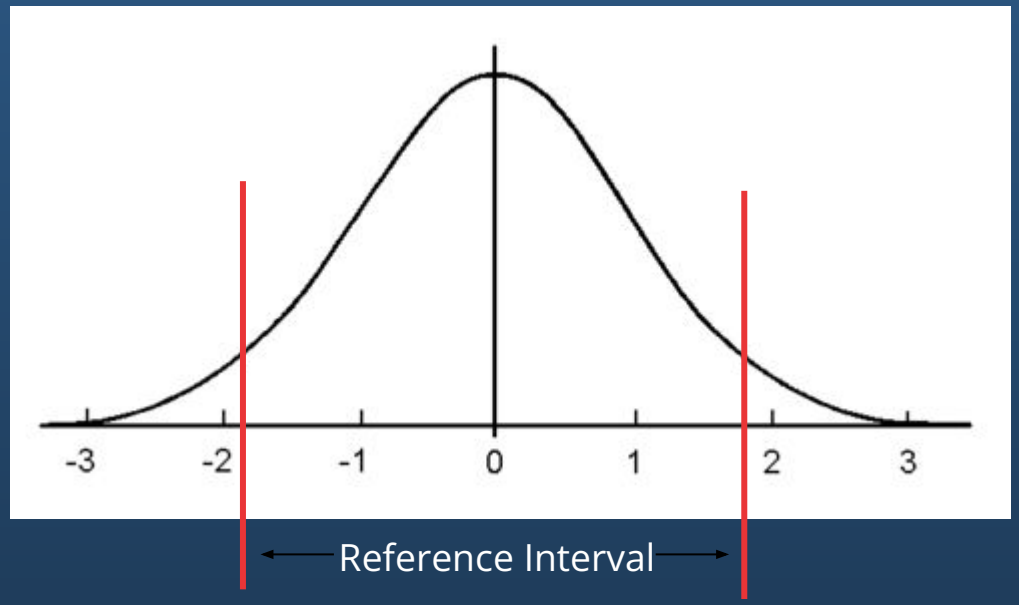
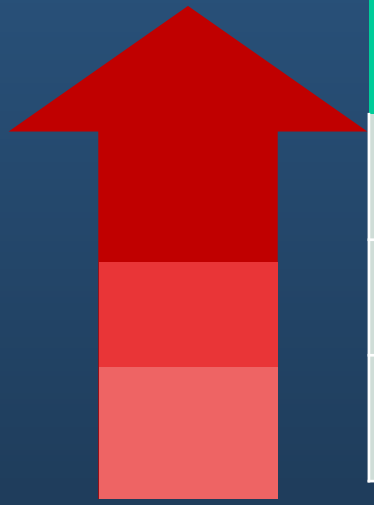
Population Reference Intervals vs Clinical Decision Limits

Reference Interval: health associated benchmarks used to assist in clinical decision-making (central 95% of result values obtained from a reference population).



Clinical Decision Limits: threshold values that indicate significant patient risk of clinical outcome or diagnosis of a specific disease.



	HbA1c (%)
Diabetes	≥6.5
Prediabetes	5.7 – 6.4
Normal	~ 5.7

- 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

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Reference Intervals vs Decision Limits

(Common Source of Error in Result Interpretation)

- **Clinical Decision Limits** determined based on clinical outcome studies are preferred to population reference intervals
- Decision Limits are available and must be used for some analytes (HbA1c, lipids, Vitamin D, Ferritin, and some others) based on latest clinical guidelines
- A common source of error is application of reference intervals in result interpretation when decision limits are more appropriate

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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

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Regulatory Requirement for Reference Intervals

- **DIRECTIVE 98/79/EC, In Vitro Diagnostics Directive (IVDD)** 8. Information supplied by the manufacturer 8.7. Where appropriate, the instructions for use must contain the following particulars:
  (l) the reference intervals for the quantities being determined, including a description of the appropriate reference population;

  ***A requirement for Manufacturers and Clinical Laboratories**

- **ISO 15189 Medical laboratories**

  Particular requirements for quality and competence

5.5.5 **Biological reference intervals shall be periodically reviewed**.

If the laboratory has reason to believe that a particular interval is no longer appropriate for the reference population, then an investigation shall be undertaken, followed, if necessary, by corrective action. A review of biological reference intervals shall also take place when the laboratory changes an examination procedure or pre-examination procedure, if appropriate.

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Establishment of Reference Intervals
(DIRECT & INDIRECT Methods)

1. The traditional method for establishing reference intervals, known as the **DIRECT approach**, is based on collecting samples from healthy individuals

2. **INDIRECT approach**, is to derive reference intervals by use of routine laboratory data stored in the laboratory information system – **(Big Data Approach)**

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Comparison of Direct & Indirect Methods

Statistical Methods for derivation of RIs for direct and indirect studies

DIRECT methods

a) Nonparametric method
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c) Parametric method without LAVE
d) Parametric calculation using Tukey's outlier detection method

INDIRECT methods

a) Hoffman method
b) Bhattacharya method
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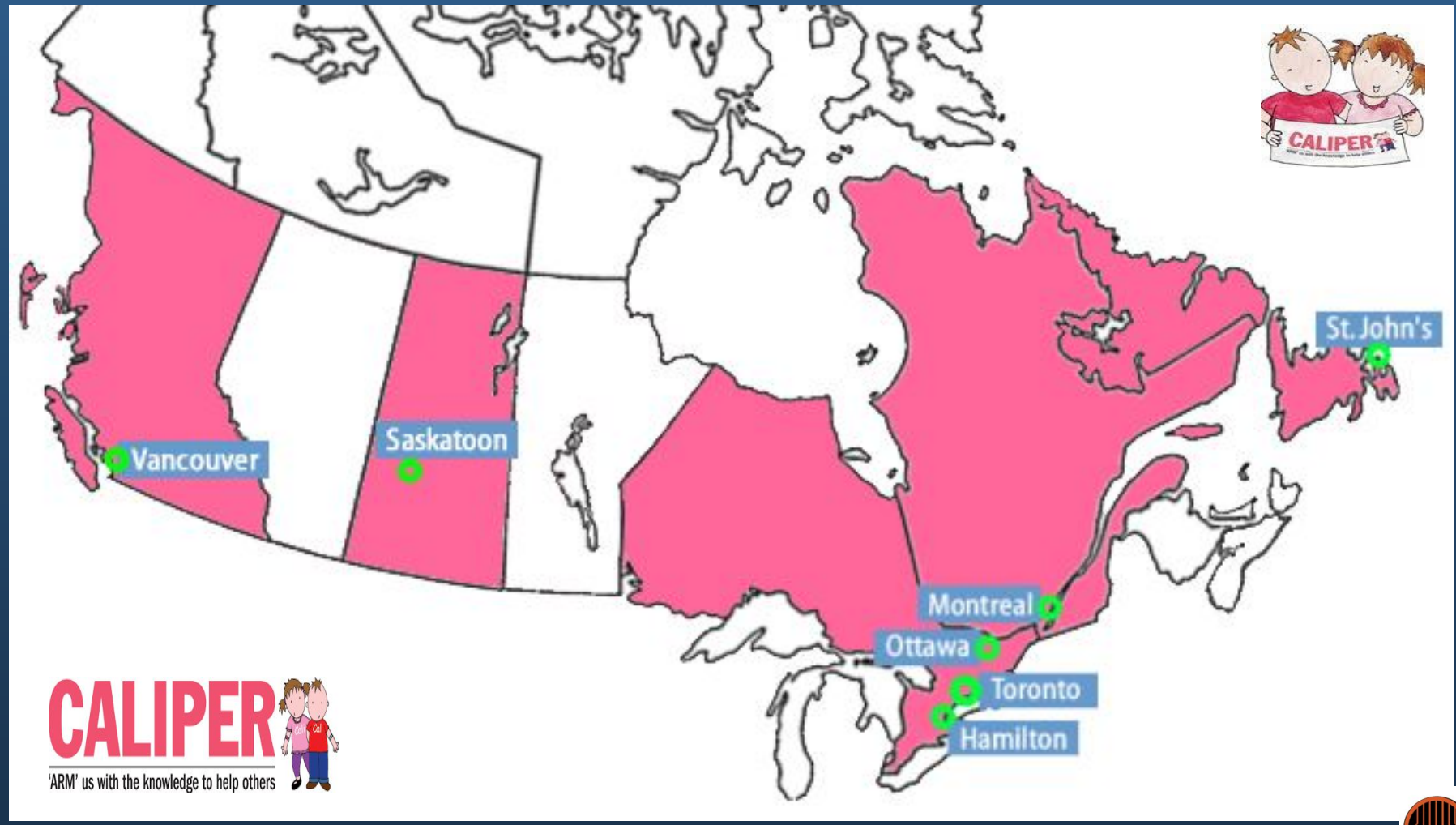
An Example of the DIRECT Approach

CALIPER Initiative in Canada



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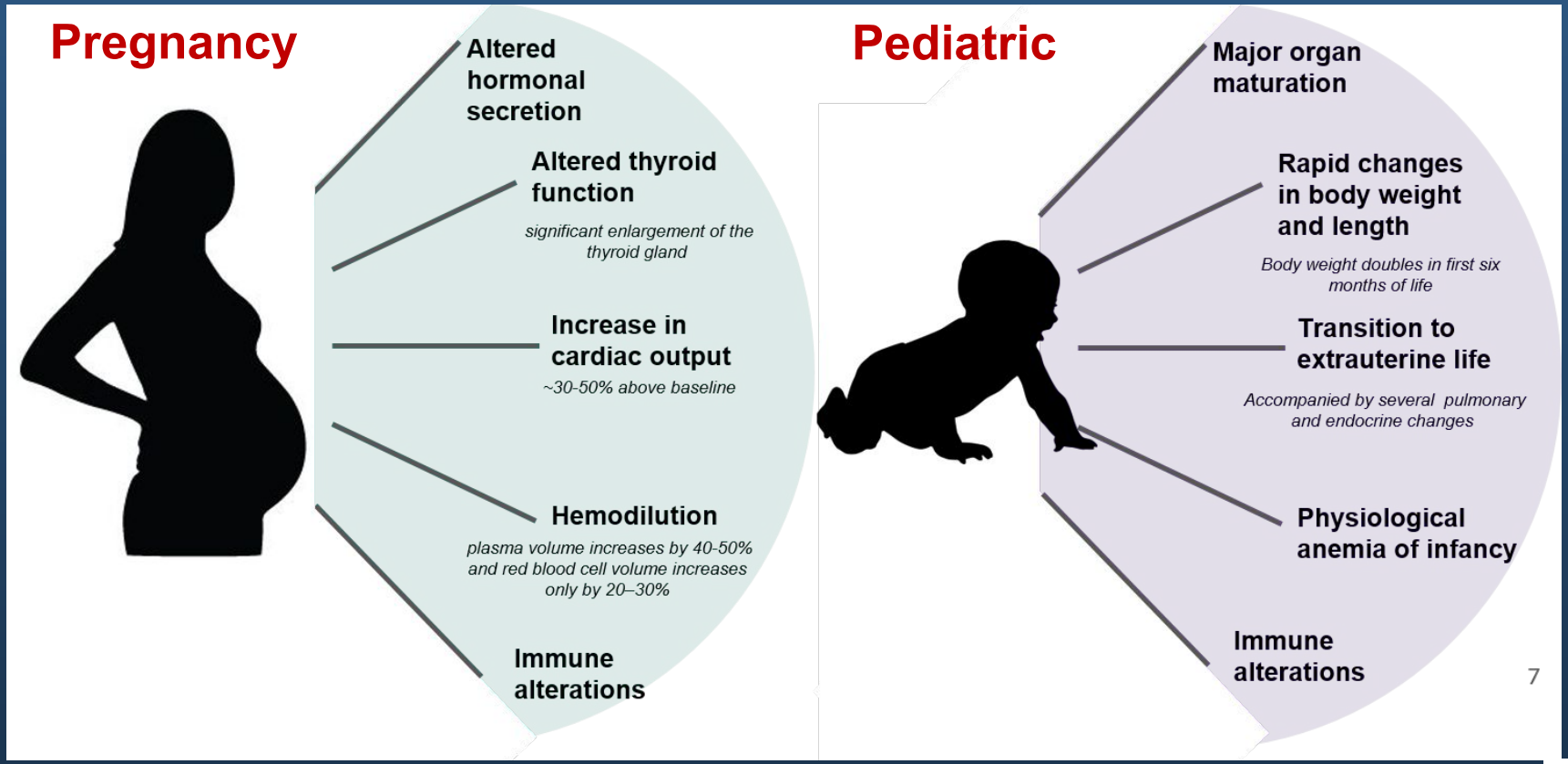
Reference Intervals

Major Gaps in Special Populations



Children are Not Small Adults

- **Body weight:**
  - Doubles by 6 months of age
  - Triples by the first birthday
- **Body length** increases by 50% during the first year
- **Major organ systems** grow and mature
- Important changes take place during **puberty**
  - Accelerated growth and sexual maturation occur

Pediatric Reference Intervals Need to reflect differences in:

- Development & physiologic function at different ages
- Key covariates including gender, age, BMI, sexual development (Tanner stage), and ethnicity

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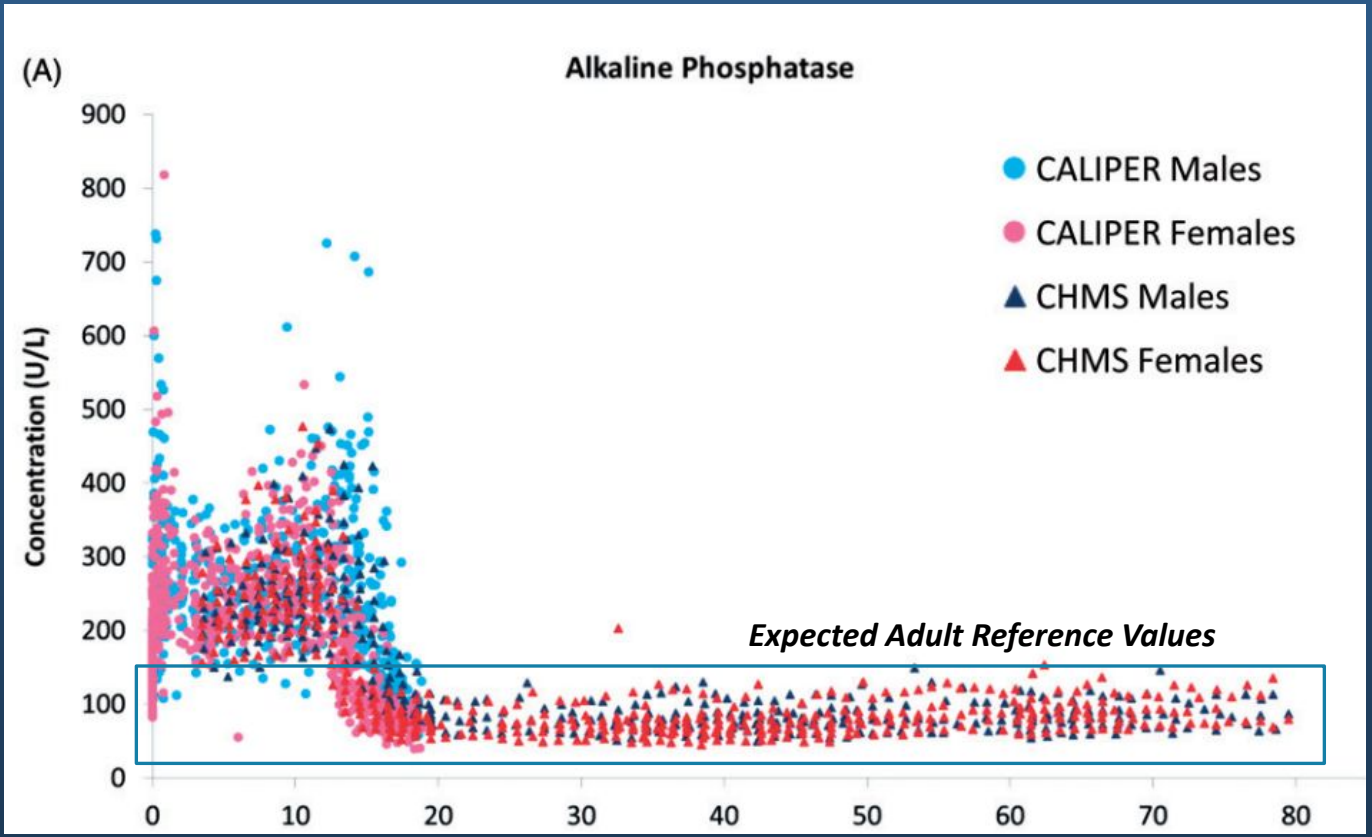
Shaw JL et al. Crit Rev Clin Lab Sci 2013; Adeli K et al. BMJ 2018

Clinical case example: *Alkaline Phosphatase*



Crit Rev Clin Lab Sci. 2017 Sep;54(6):358-413. doi: 10.1080/10408363.2017.1379945.

- Young children have significantly higher levels of ALP as compared to adults
- Application of an adult reference interval would result in flagging the majority of the pediatric population, resulting in:
  - *Unnecessary follow-up testing*
  - *Misinformed clinical decision making*

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Global Reference Interval 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 child x	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 analyte

Adapted from Tahmasebi et a

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PRINCE Study in China

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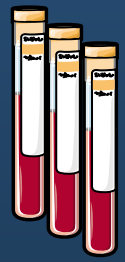
CALIPER: *A Canadian Initiative to Close the Evidence Gap in Pediatric Reference Intervals for Biomarkers of Health & Disease*

- **CALIPER (CANADIAN LABORATORY INITIATIVE ON PEDIATRIC REFERENCE INTERVALS):** A CIHR Funded Initiative (2010-present)

- **OBJECTIVE**: To establish ***a comprehensive database of healthy reference standards for biomarkers of health and disease in children and adolescents***.

- **STUDY COHORT**: >14500 Children and adolescents (birth to 18 years) recruited over the past decade through various **community-based programs** including:
  - *Schools*
  - *Community Centre*
  - *Art & Sport events and festivals*

- **STUDY BIOBANK**: Pediatric biobank of collected serum specimens (-80C)

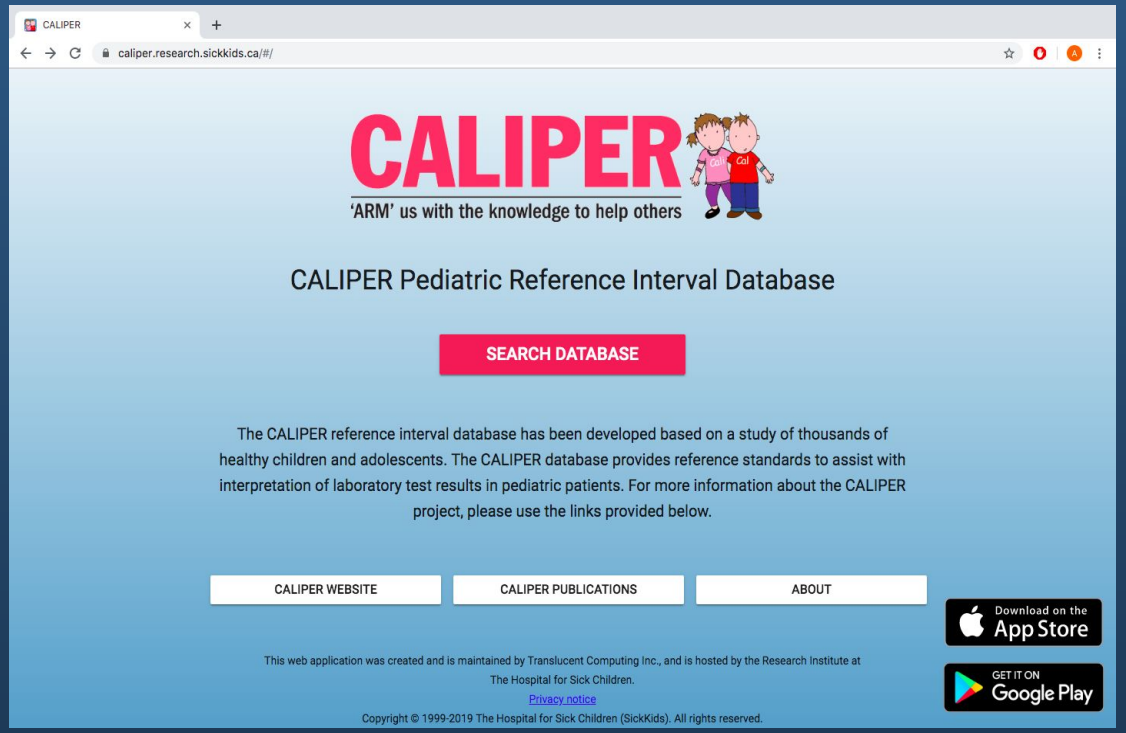


CALIPER
'ARM' us with the knowledge to help others

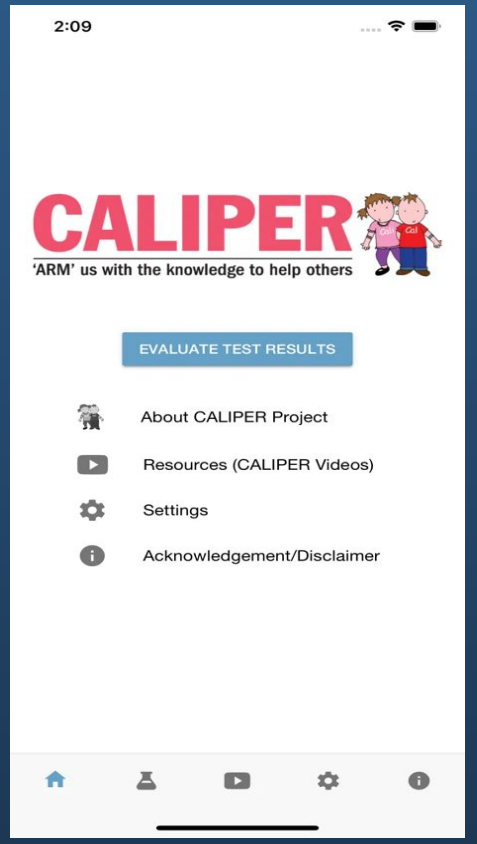
www.caliperdatabase.org

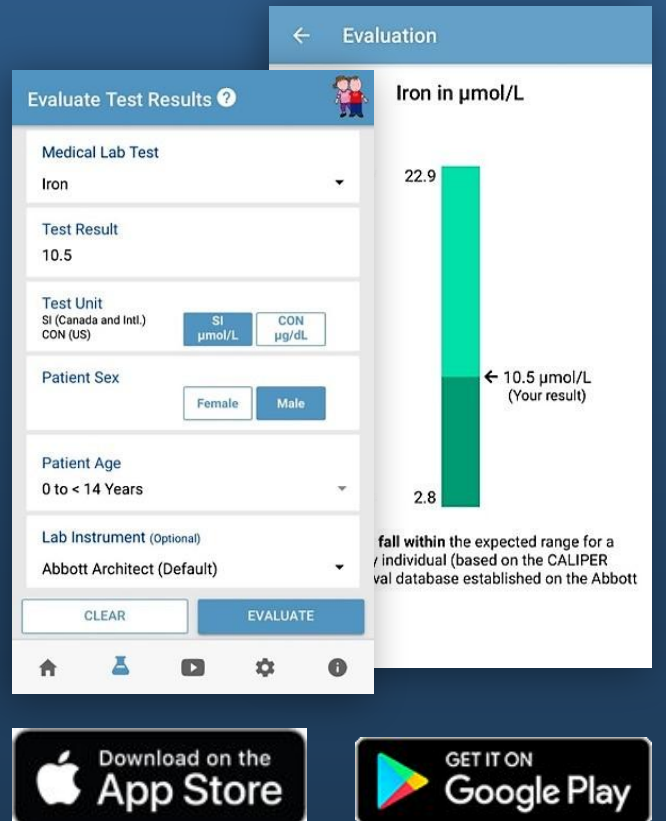
www.caliperproject.org

CALIPER Mobile & Web Apps



visit www.caliperdatabase.org for more details





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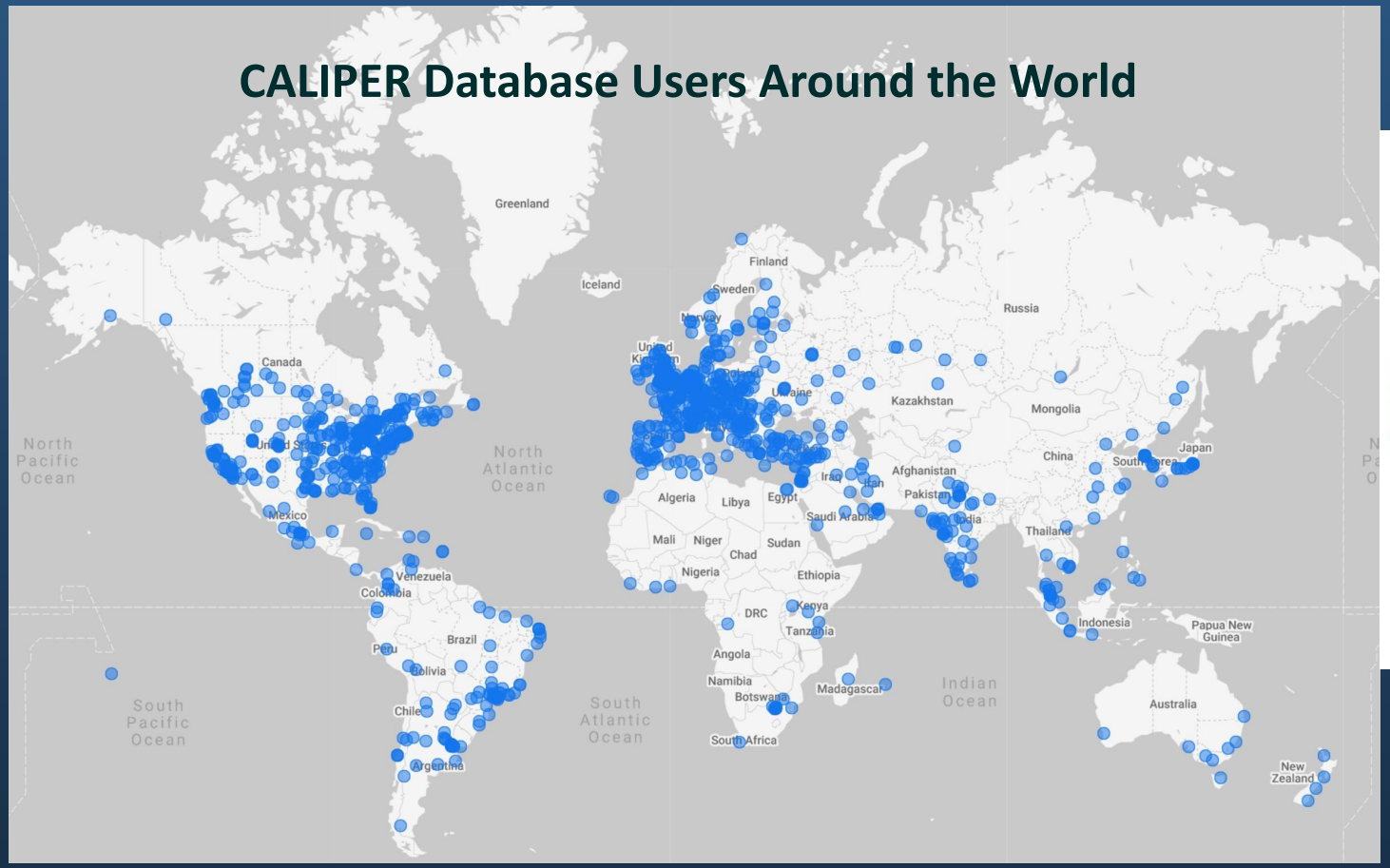
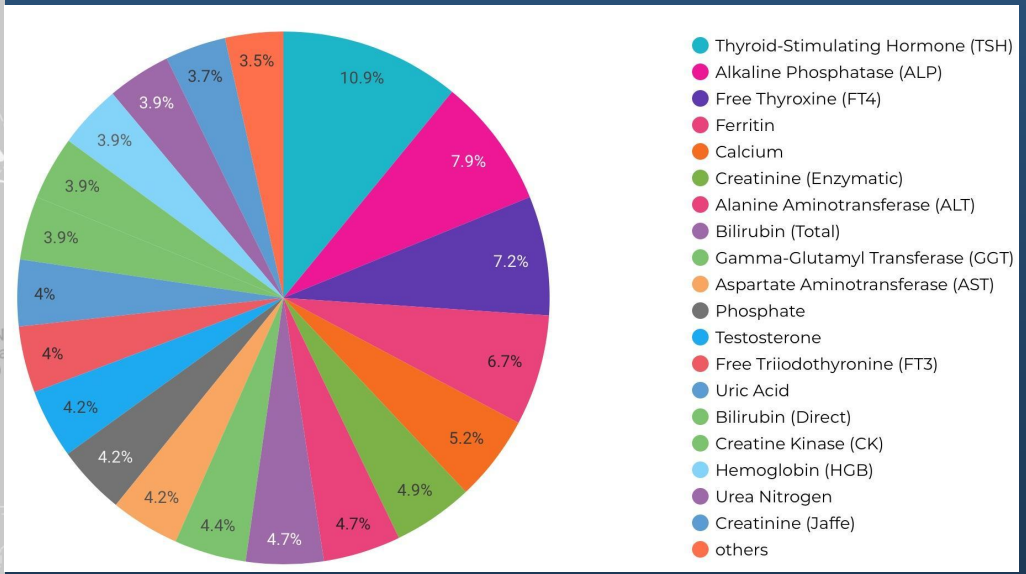
CALIPER Database: *A Global Resource*

Pediatric reference intervals available for over **200 laboratory biomarkers** on several analytical platforms based on data from thousands of healthy children and adolescents from birth to 18 years
CALIPER online database currently has over **57,000 registered users** from **3,650 institutions in 100 countries**



www.caliperdatabase.org



Most Popular Medical Lab Tests
Searched in CALIPER Datab

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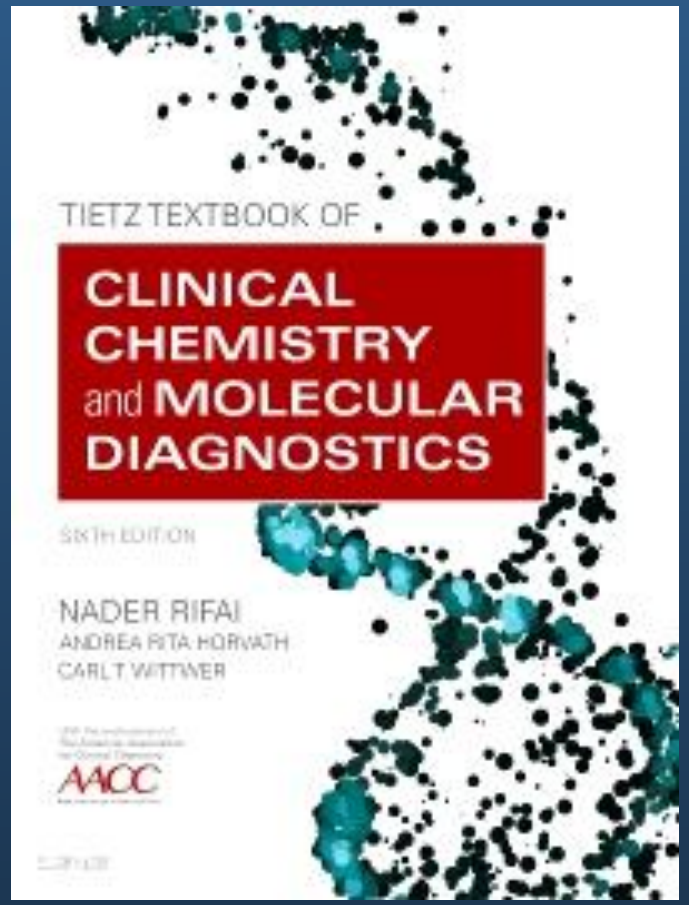
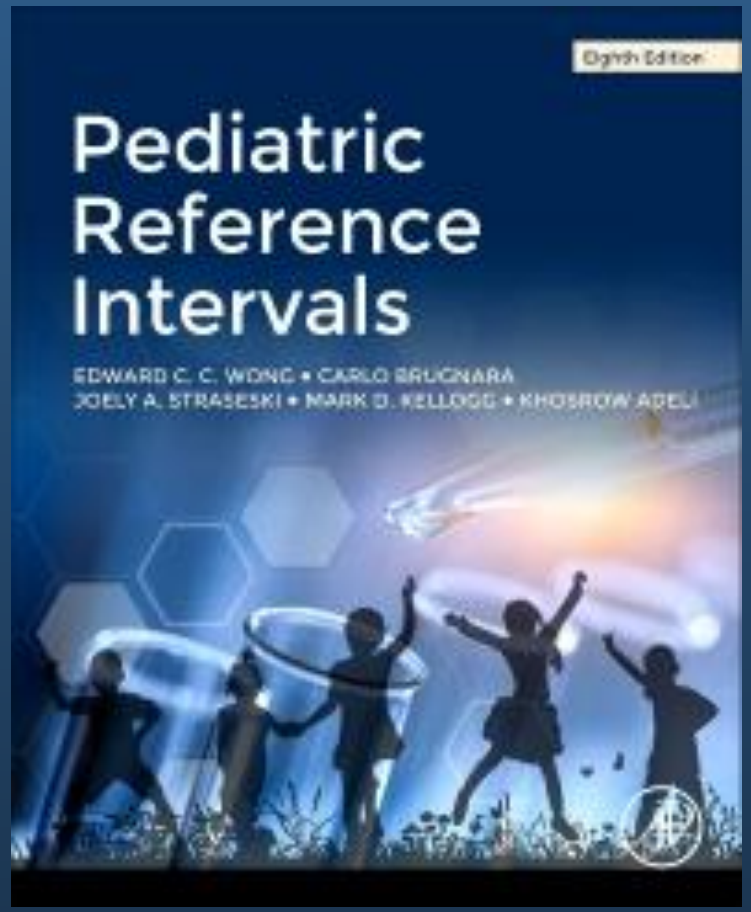
Reference Intervals – Publications/Resources





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Big Data Analytics:

Applications in Clinical Biochemistry

Indirect Reference Interval Studies in Canada:

Harnessing the Power of Big Data Analytics to Establish Reference Intervals

Clin Chem. 2023 Sep 1;69(9):991-1008.

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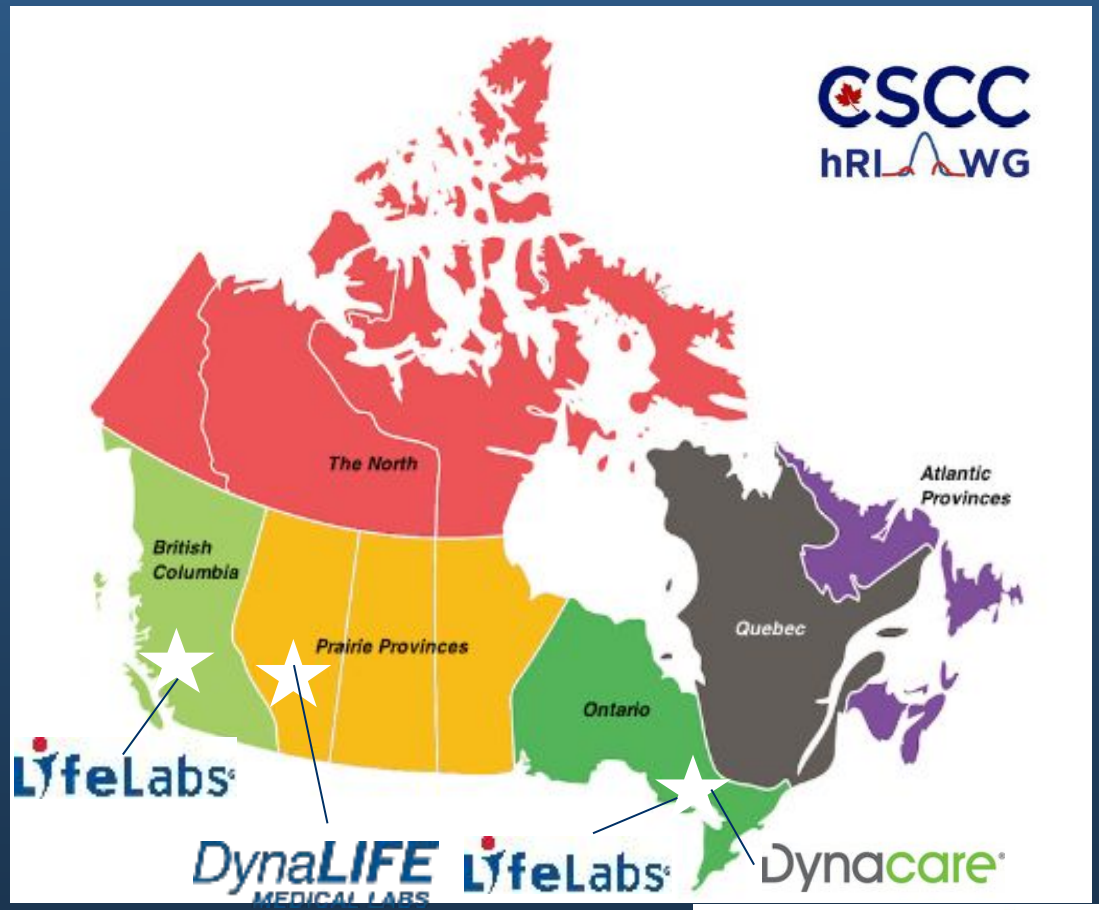
Indirect Study: Outpatient Data Collection from Across Canada

- 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



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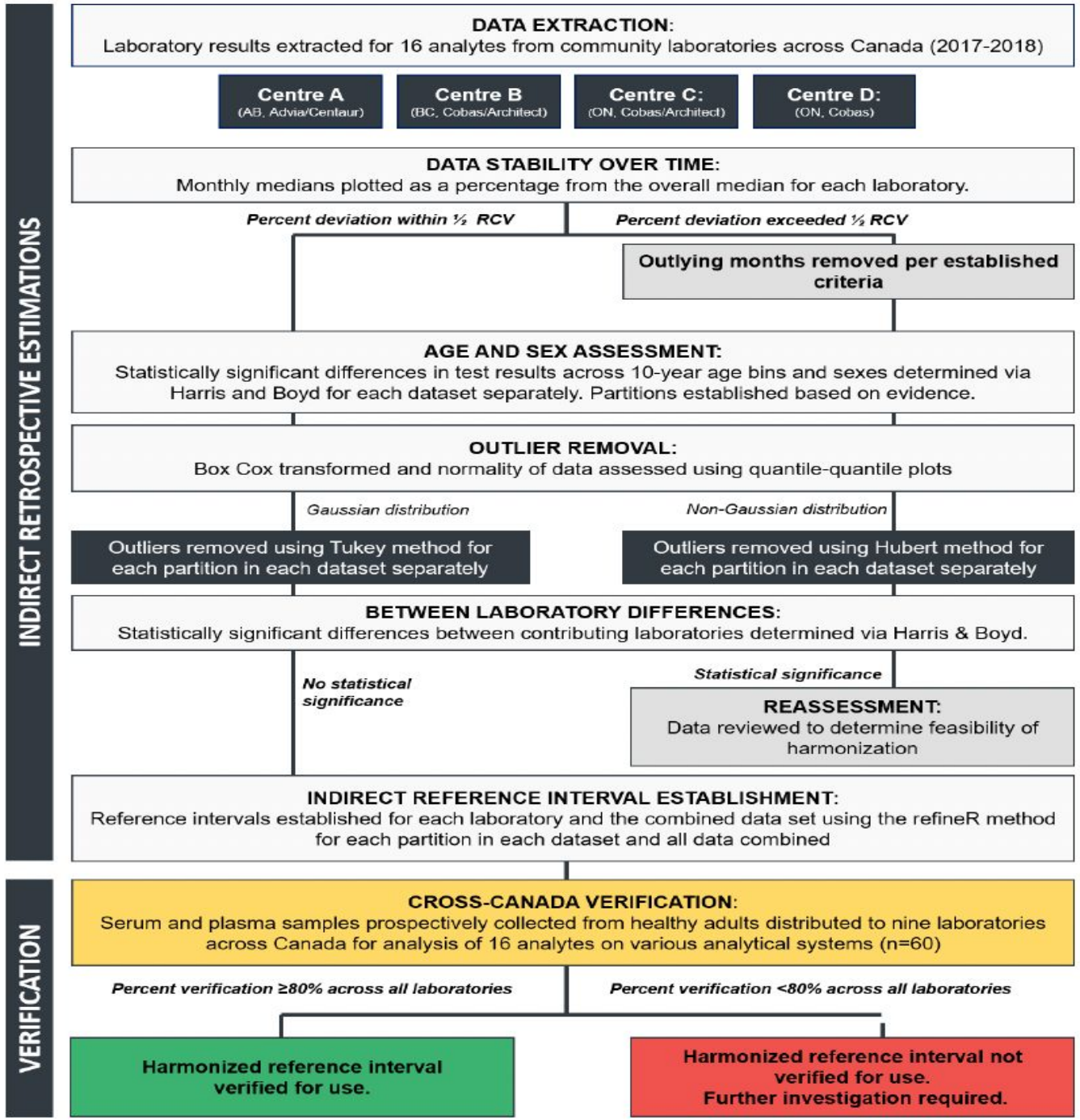
Big Data Analytics

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



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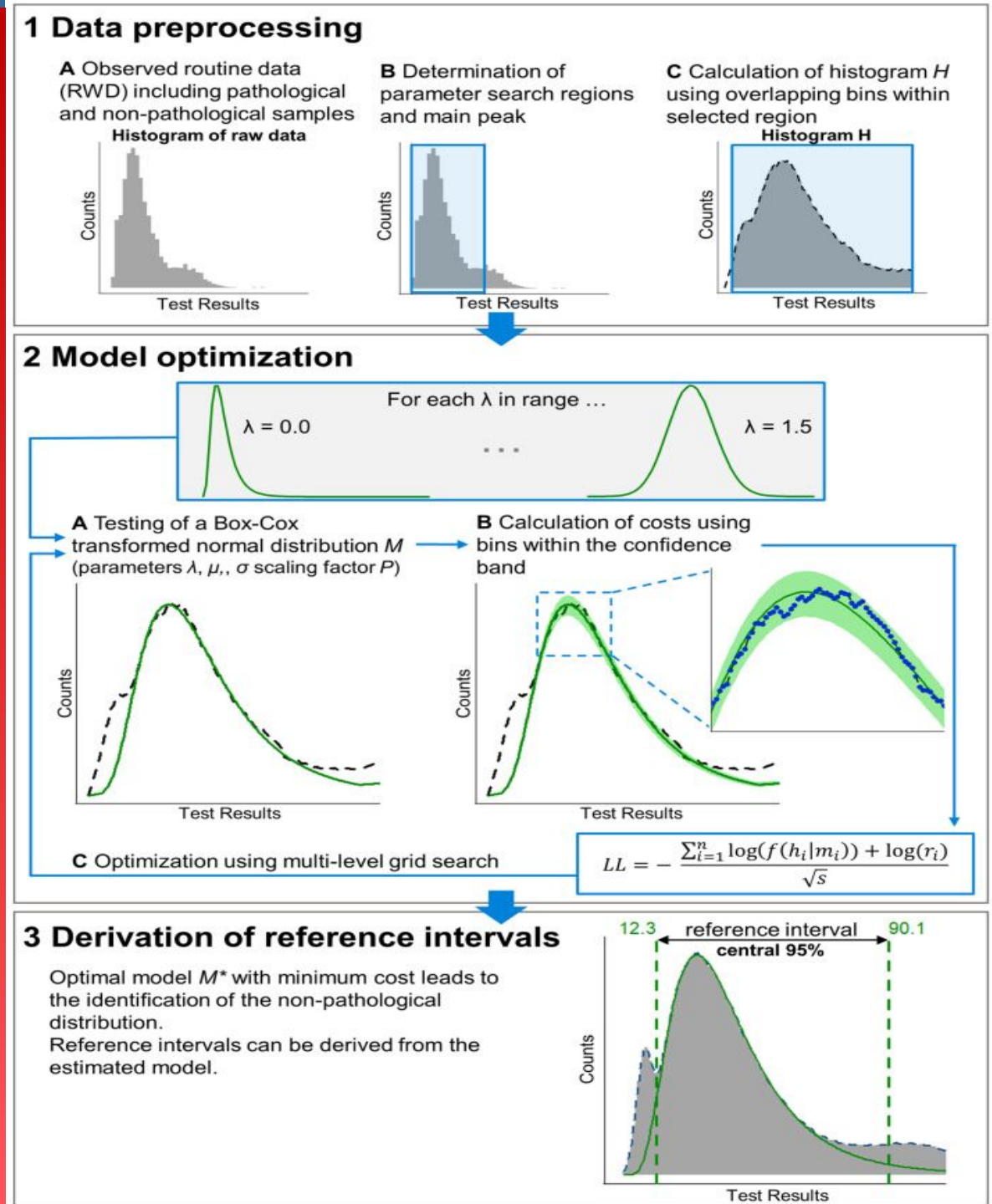
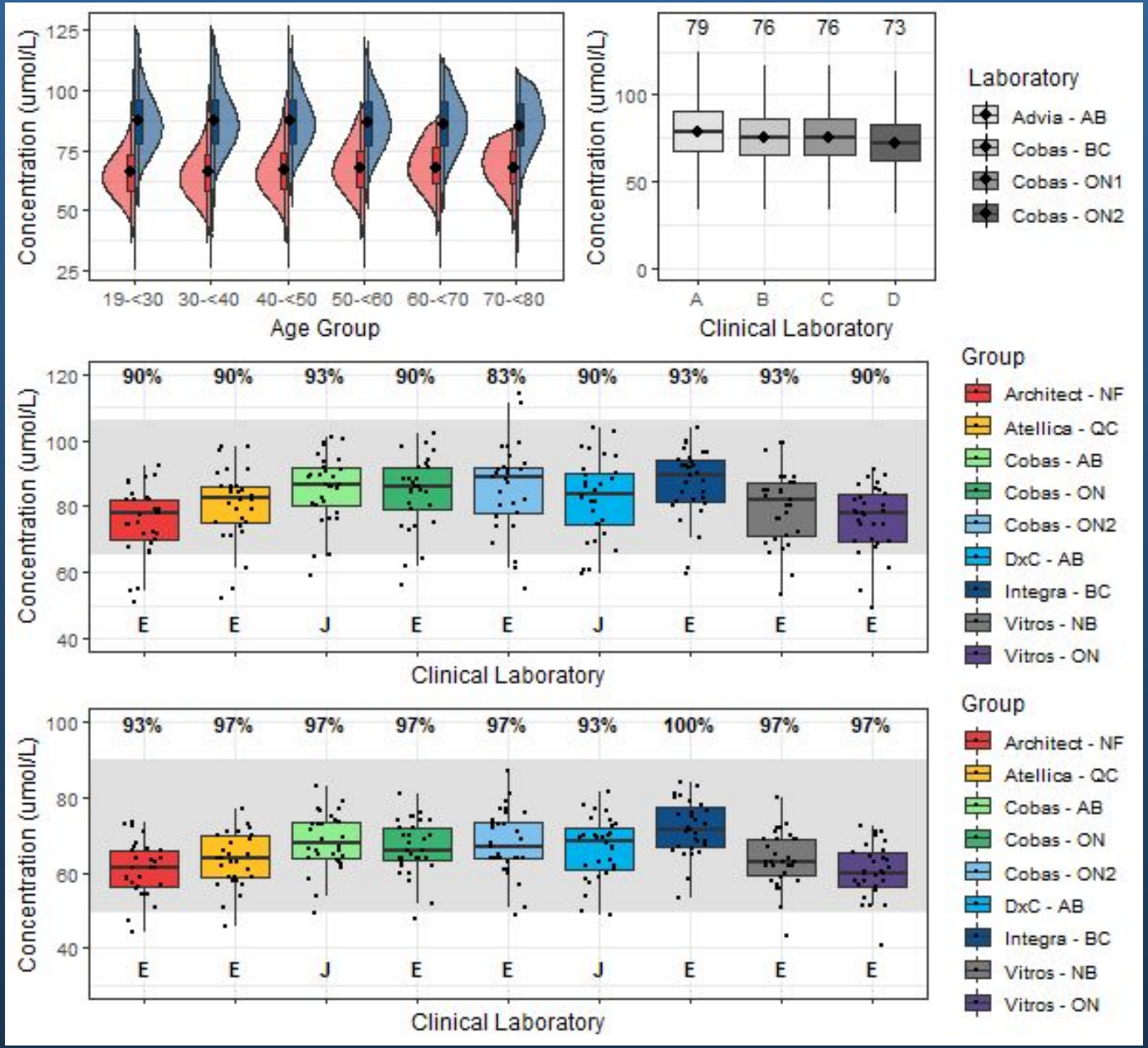
RefineR Algorithm

Ammer T, Schützenmeister A, Prokosch HU, Rauh M, Rank CM, Zierk J. refineR: a novel algorithm for reference interval estimation from real-world data. Scientific reports. 2021 Aug 6;11(1):1-7.



Creatinine



Further Data Analysis/Investigation Required

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**

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INDIRECT Big Analytics Approach

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

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dank je
thank you
gracias
tapadh leat
bedankt
hvala
mauruuru
dziękuję
mochchakkeram
go raibh maith agat
obrigado
sagolun
sukriya
kop khun krap
arigatō
takk
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