Modeling the Age-Stratified Impact of Diagnostic Assay Attributes

Meta-Analytic Lessons for Public Type 1 Diabetes Screening

Discussion Perspective

Presenter: David Seftel, M.D.

Clinician

Patient-focused medical practice

Researcher

Evidence-based investigation

Laboratory Director

CLIA-certified oversight

CAP Member

Quality assurance standards

Clinical Case Study: Pediatric Screening Event

This case illustrates the critical importance of comprehensive autoantibody testing and the challenges of assay discordance i n real-world screening scenarios. A young child participated in a community type 1 diabetes screening event that would ultimately lead to early intervention and disease-modifying therapy.

1

Initial ADAP Screening

Dried blood spot testing revealed both anti-insulin (AI) and GAD65 autoantibodies, indicating significant risk for type 1 diabetes progression

2

Quest Laboratory Confirmation

Follow-up confirmatory testing performed at Quest Diagnostics revealed anti-insulin antibodies using their standard methodology

3

ECL Confirmatory Testing

Additional electrochemiluminescence (ECL) testing confirmed GAD65 positivity, corroborating the initial ADAP findings

Clinical Significance: The presence of multiple autoantibodies indicated stage 2 type 1 diabetes with high risk of progression to symptomatic disease, warranting close monitoring and consideration of disease-modifying interventions.

What Would You Do?

When faced with discordant autoantibody results from different testing platforms, clinicians must make critical decisions abo ut next steps. Consider this scenario carefully—the choice impacts patient monitoring, family counseling, and potential therapeutic interventions.

1

Retest with ADAP

Repeat the original high-sensitivity DNA-barcoding assay to confirm initial findings and rule out potential false positives

2

Retest with Quest RSR and Radio Binding Assay

Utilize traditional radioimmunoassay methodology, considered a gold standard but with longer turnaround and different sensitivity profile

3

Put the Child on a Continuous Glucose Monitor

Initiate prospective glycemic surveillance to detect early dysglycemia and disease progression in real-time

4

Retest with ECL

Perform additional electrochemiluminescence testing to gather more data from an alternative platform technology

Clinical Decision and Outcome

The treating clinician made the decision to prioritize proactive monitoring over additional confirmatory testing. This choice reflects growing confidence in screening results when multiple antibodies are detected and the critical importance of early intervention.

Immediate Action: CGM Placement

The child was placed on a continuous glucose monitor to enable real-time detection of glycemic abnormalities and disease progres sion

1

Within One Month

Sustained dysglycemia was detected on continuous monitoring, confirming progression to stage 3 type 1 diabetes with symptomat ic hyperglycemia

2

Therapeutic Intervention

Teplizumab (Tzield) was promptly administered

Key Insight: Early detection through screening enabled disease-modifying treatment at an optimal window, potentially preserving beta cell function and delaying insulin dependence. This case underscores the clinical value of accurate, comprehensive autoantibody testing.

Comparative Analysis of the Sensitivity, Specificity, Concordance, and 5-Year Predictive Power of Diabetes-Related Autoantibody Assays FREE

Jeffrey P. Krischer; Sarah Muller; Lu You; Peter Achenbach; Elena Bazzigaluppi; Cristina Brigatti; Vito Lampasona;

Assay Discordance Remains a Significant Issue

Despite advances in autoantibody testing technology, substantial variation exists between different assay platforms. This dis cordance creates diagnostic uncertainty, complicates clinical decision-making, and may delay appropriate intervention for at-risk individuals.

diabetes

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Volume 74, Issue 9
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COMMENTARY | AUGUST 20 2025

Islet Autoantibody Testing as Type 1 Diabetes Early Detection Enters Mainstream Health Care FREE

The data presented above demonstrates concerning variability in autoantibody detection across different testing methodologies . These differences can result in missed diagnoses, conflicting results requiring additional testing, and confusion for both clinicians and families navigating screening results .

> "Inter-laboratory variability in autoantibody testing represents one of the most significant challenges facing type 1 diabetes s creening programs worldwide. Harmonization of assay performance is essential for reliable risk stratification."

Expert Recommendations: Krischer et al. and Gillespie

To help overcome some of the issues raised by discordance between platforms, the authors recommend that future confirmation or persistence samples are tested using the same platform. How will this pipeline be managed as type 1 diabetes early detection moves toward clinical care, where assay differences could affect clinical application? A

Standardization Imperative

All assays should demonstrate traceability to international reference standards and participate in ongoing proficiency testing through IASP

Comprehensive Validation

Analytical and clinical validation must include diverse age groups, ethnicities, and disease stages to ensure broad applicability

Transparency Requirements

Full disclosure of assay characteristics, limitations, and interference susceptibility enables appropriate clinical interpretation

Research Priorities for Diagnostic Excellence

Needed: Research into the Impact of Assay Architecture

The field requires systematic investigation into how fundamental design choices in autoantibody assays affect their performance characteristics. This research must examine key measures of accuracy and precision across different patient populations, with particular attention to age-related effects and steps to mitigate adverse impacts.

Assay Architecture Analysis

Comprehensive evaluation of how different detection methodologies—including radioimmunoassay, ELISA, ECL, and DNA barcoding—perform under varying conditions

Age-Stratified Performance

Investigation of sensitivity and specificity across pediatric, adolescent, and adult populations to identify age-dependent effects

Precision Metrics

Detailed assessment of intra-assay and inter-assay reproducibility to ensure consistent results across testing occasions and laboratories

Mitigation Strategies

Development and validation of approaches to minimize interference effects and optimize performance across diverse clinical scenarios

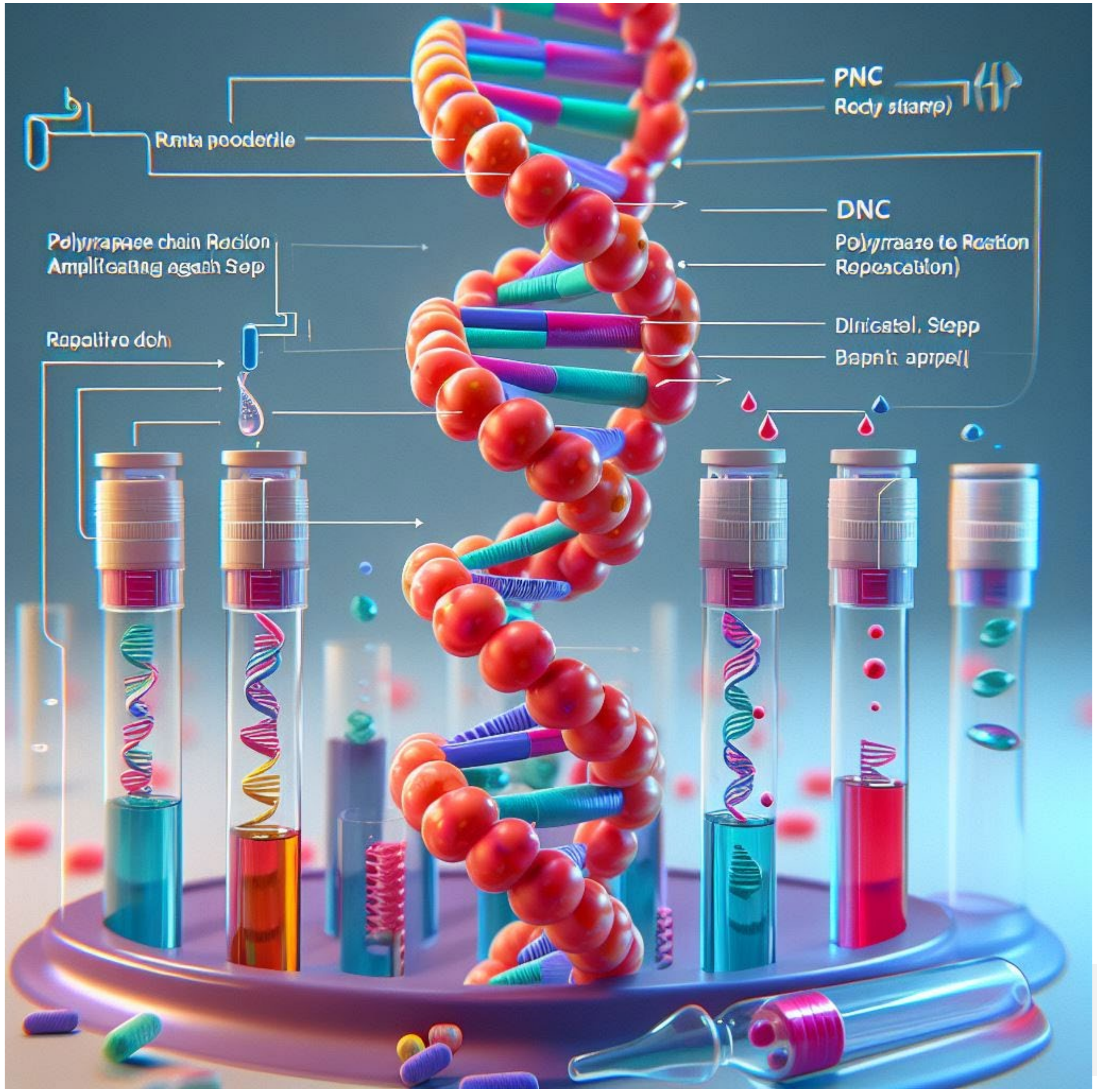
Three Classes of Conditions Affecting T1D Assay Performance

Understanding the broad spectrum of factors that influence autoantibody detection is essential for interpreting results and selecting appropriate testing methodologies. These conditions span pre-analytical, analytical, and biological domains.

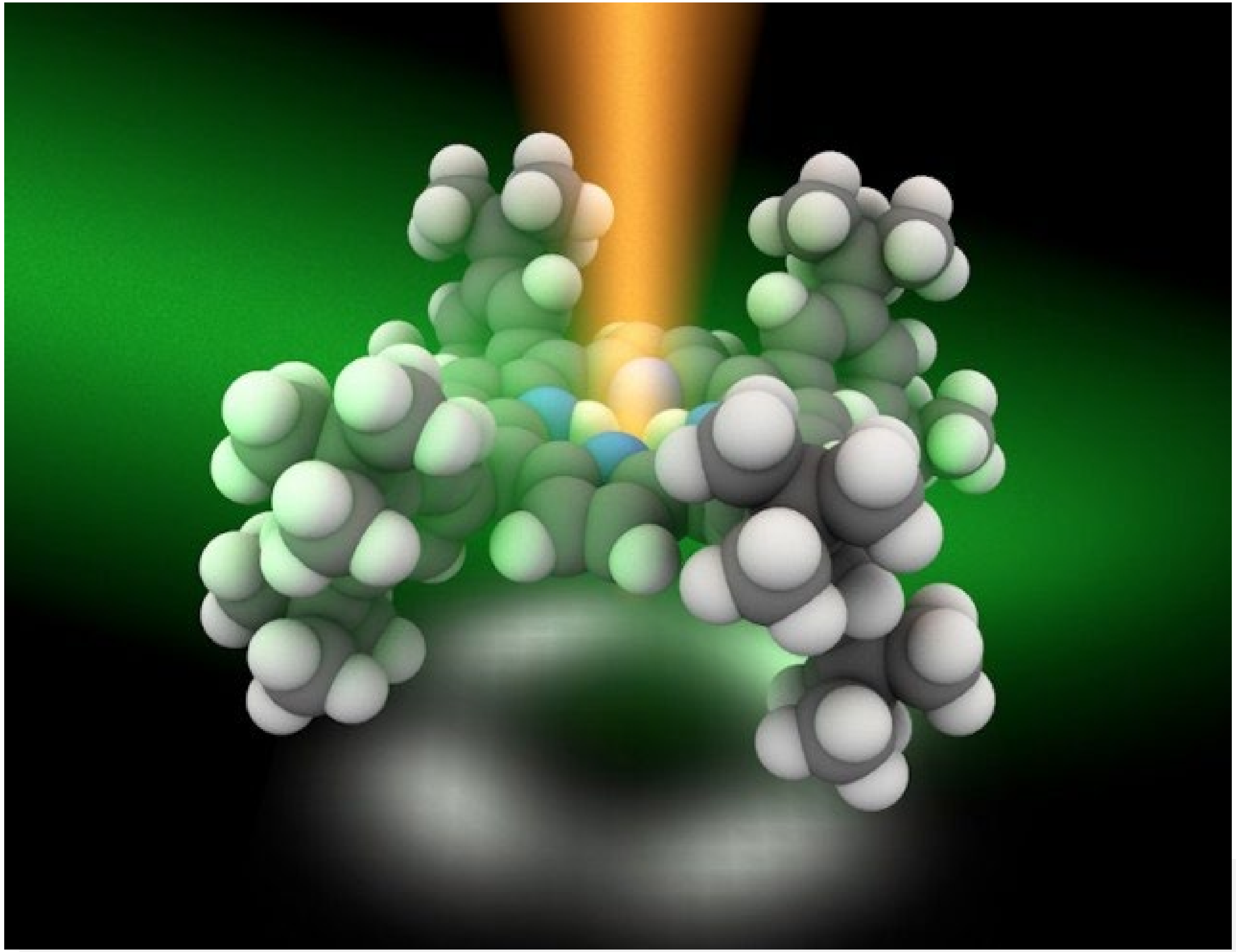
Class of Condition	Description	Impact on Sensitivity	Impact on Specificity	Examples/Notes
Pre-Analytical Variables	Conditions related to specimen collection, handling, and storage	Reduced sensitivity due to antibody degradation, insufficient sample volume, or sample matrix variation	Reduced specificity due to sample contamination, hemolysis, lipemia, or improper matrix effects	Sample volume variation; Sample transit temperature variation; Collection type (serum/plasma vs dried blood spots); Hemolysis, lipemia, icterus interfering substances
Analytical Factors	Assay design, reagent quality, interference from cross-reacting substances, and assay precision	False negatives from assay insensitivity or interference masking true signals	False positives from cross-reactivity, non-specific binding, or interference from endogenous/exogenous substances	Biotin interference common in streptavidin-based assays; Cross-reactivity to similar autoantibodies; Precision and detection limits; Assay architecture effects
Biological Variability	Patient-specific factors including autoantibody levels, affinity, disease stage, and genetic/environmental context	Low autoantibody titers or transient autoantibodies can reduce detection sensitivity	Autoantibody heterogeneity and non-pathogenic antibodies may decrease specificity	Early disease with low-titer autoantibodies; Different autoantibody profiles (GAD, IA-2, IAA, ZnT8); Persistence and affinity of autoantibodies influence predictive value; Age-related differences

Each class requires specific mitigation strategies. Pre-analytical variables demand rigorous standard operating procedures for sample collection and handling. Analytical factors necessitate thorough validation and interference testing. Biological variability requires comprehensive panels testing multiple autoantibodies and clinical correlation.

PCR (polymerase chain reaction)



is powerful enough to detect down to a **single** molecule



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For more than 44 years scientists have tried to use the incredible amplification power of PCR to successfully detect **antibodies**

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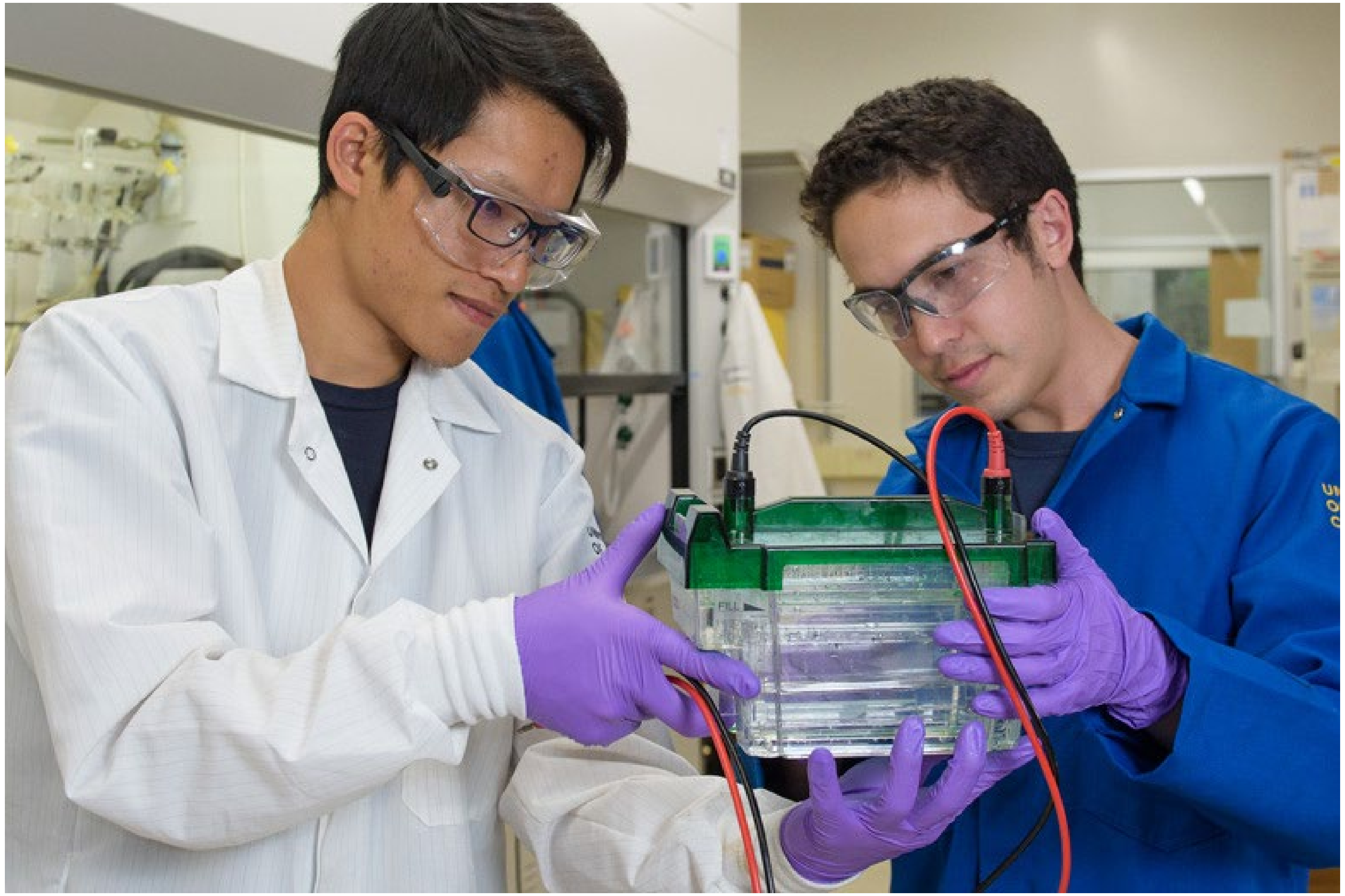
In 2015 three scientists from the University of California at Berkeley and Stanford succeeded

Prof Carolyn Bertozzi, PhD, Nobel Laureate 2022



Jason Tsai, PhD and Peter Robinson, PhD

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Prof. Bertozzi won both the Solvay and the Nobel Prize

Solvay Prize for Chemistry 2020

Nobel Prize for Chemistry 2022

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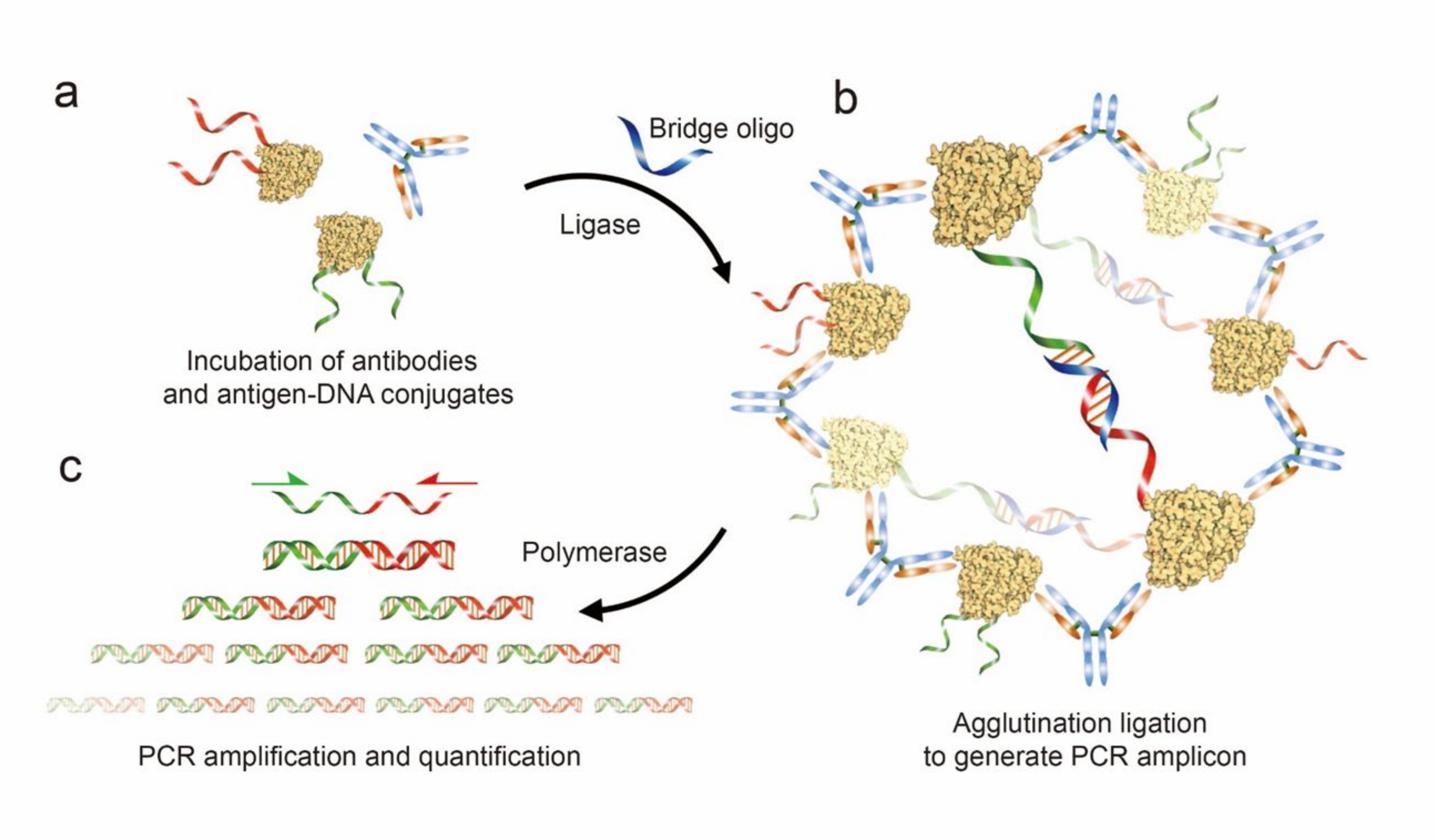
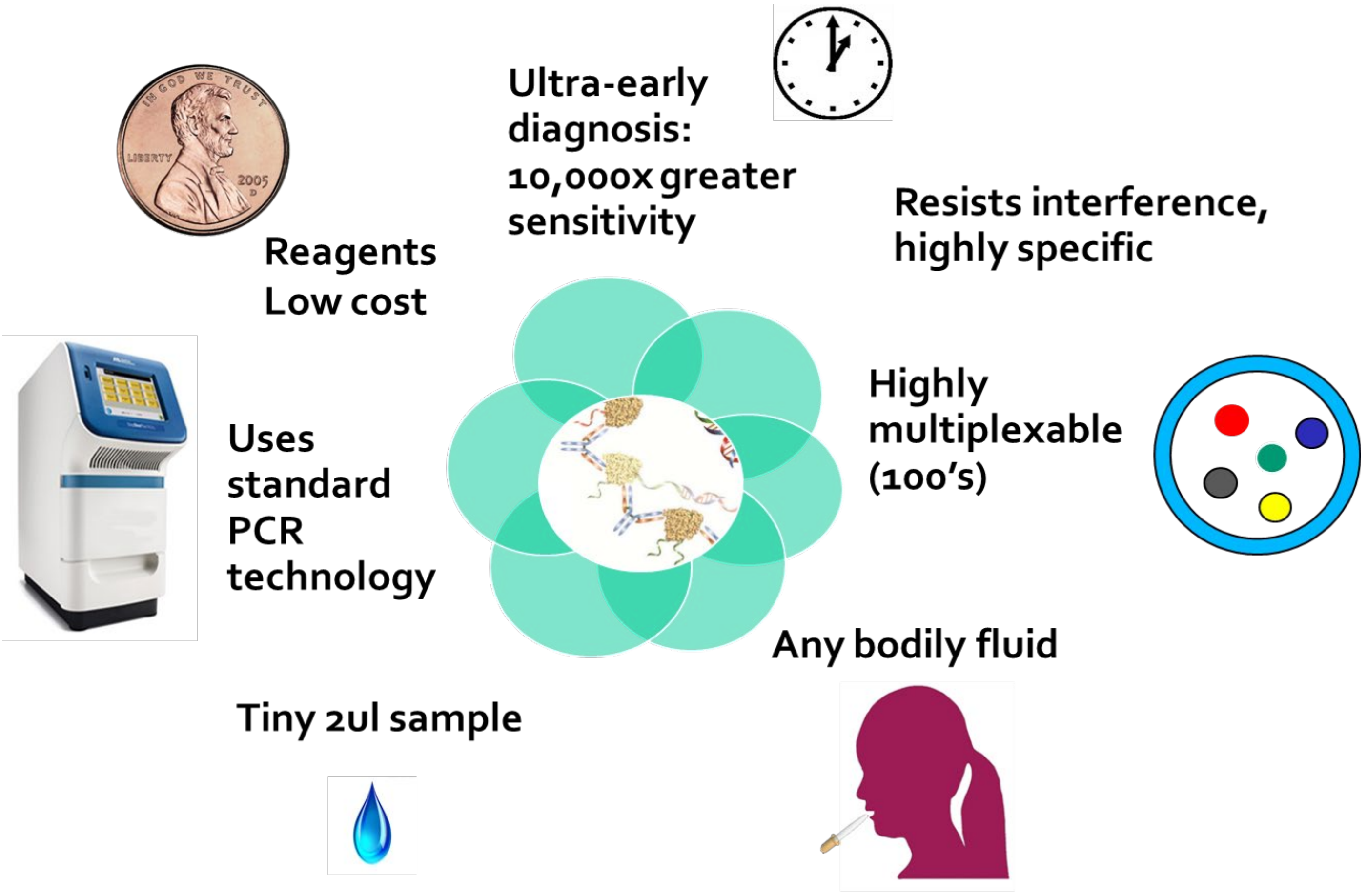
ADAP - Antibody Detection by Agglutination -PCR

Nobel prize winning foundational science powers Enable Biosciences patented DNA-barcoding technique for detecting autoantibodies/antibodies with unprecedented sensitivity and specificity in all sample matrices

Enable's Antibody Detection by Agglutination -PCR (ADAP) technology

A breakthrough patented DNA-barcoding, solution-phase homogenous assay with zeptomole sensitivity and ultra-specificity unlocks the amplification power of PCR for antibody detection





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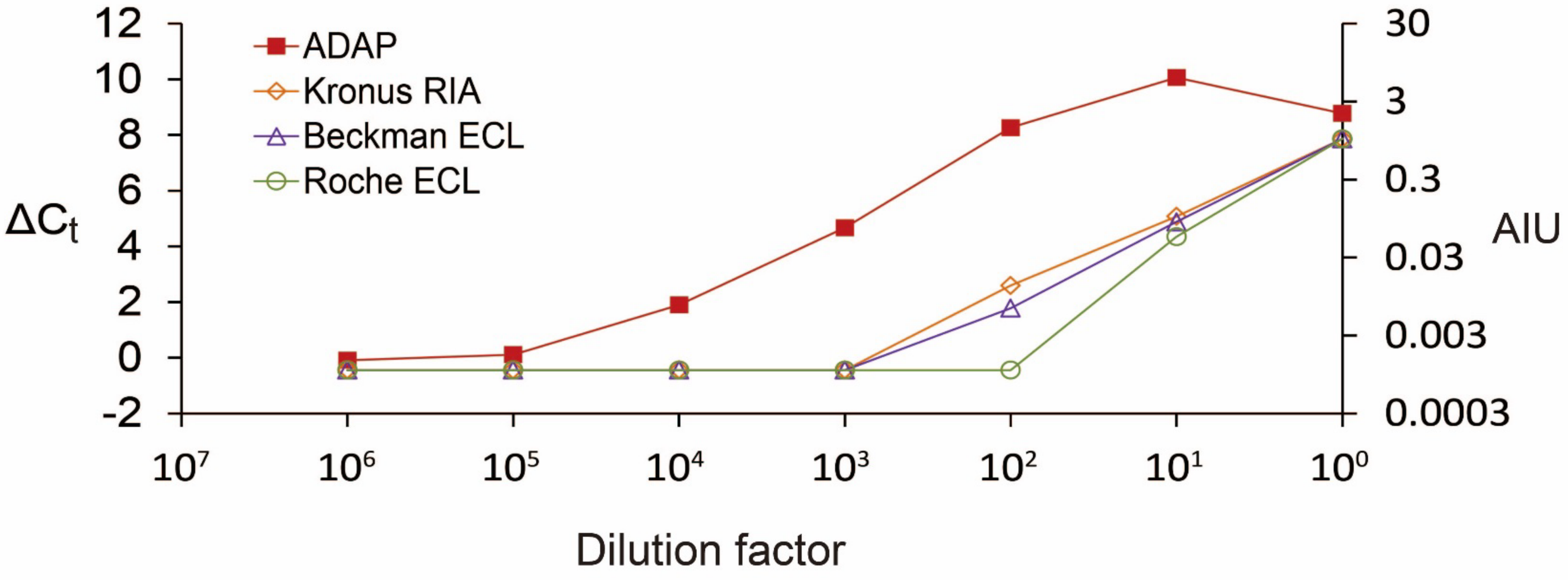
ACS Cent Sci. 2,139-147 (2016)

ADAP are more sensitive than traditional assays – enabling earlier detection – critical to successful treatment outcomes

https://pubs.acs.org/doi/full/10.1021/acscentsci.5b00340



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ADAP has over a decade's worth of peer-reviewed proficiency testing

PLOS ONE

OPEN ACCESS PEER-REVIEWED

RESEARCH ARTICLE

Sensitive detection of multiple islet autoantibodies in type 1 diabetes using small sample volumes by agglutination-PCR

Felipe de Jesus Cortez, David Gebhart, Peter V. Robinson, David Seftel, Narges Pourmandi, Jordan Owyoung, Carolyn R. Bertozzi, Darrell M. Wilson, David M. Maahs, Bruce A. Buckingham, John R. Mills, Matthew M. Roforth, Sean J. Pittock, [...], Cheng-ting Tsai [view all]

Published: November 13, 2020 • https://doi.org/10.1371/journal.pone.0242049

Islet cell Autoantibody Standardization Program (IASP) 2018			
	AS95 (Sensitivity at 95% specificity)		
	GAD Ab	IA-2 Ab	Insulin Ab
ADAP	88%	74%	66%
Reported Maximum	88%	74%	68%

The sensitivity at 95% specificity of ADAP is shown at the top, whereas the bottom values shows highest reported sensitivity among all participating laboratories worldwide using various testing methods. A total of 43 T1D, 7 high-risk relatives of T1D and 90 controls were analyzed with blinding.

https://doi.org/10.1371/journal.pone.0242049.t001

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ADAP maintains a record of excellence at IASP

Enable's patented ADAP testing was ranked first in the worldwide blinded assay performance program of the Immunology of Diabetes Society in 2018 and continued delivering top performance in 2020 and 2023

Clinical Chemistry 65:9
1141–1152 (2019)

Clinical Immunology

Islet Autoantibody Standardization Program 2018 Workshop: Interlaboratory Comparison of Glutamic Acid Decarboxylase Autoantibody Assay Performance

Vito Lampasona,[1*†] David L. Pittman,[2†] Alistair J. Williams,[3] Peter Achenbach,[4] Michael Schlosser,[5,6] Beena Akolkar,[7] William E. Winter,[2] and Participating Laboratories

BACKGROUND: The Islet Autoantibody Standardization Program (IASP) aims to improve the performance of immunoassays measuring type 1 diabetes (T1D)-associated autoantibodies and the concordance of results among laboratories. IASP organizes international interlaboratory assay comparison studies in which blinded serum samples are distributed to participating laboratories, followed by centralized collection and analysis of results, providing participants with an unbiased comparative assessment. In this report, we describe the results of glutamic acid decarboxylase autoantibody (GADA) assays presented in the IASP 2018 workshop.

ELISAs showed the best median pAUC95 (0.039; range, 0.036–0.041).

CONCLUSIONS: Several novel assay formats submitted to this study showed heterogeneous performance. In 2018, the majority of the best performing GADA immunoassays consisted of novel or established nonradioactive tests that proved on a par or superior to the radiobinding assay, the previous gold standard assay format for GADA measurement.

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https://academic.oup.com/clinchem/article/65/9/1141/5608493?searchresult=1

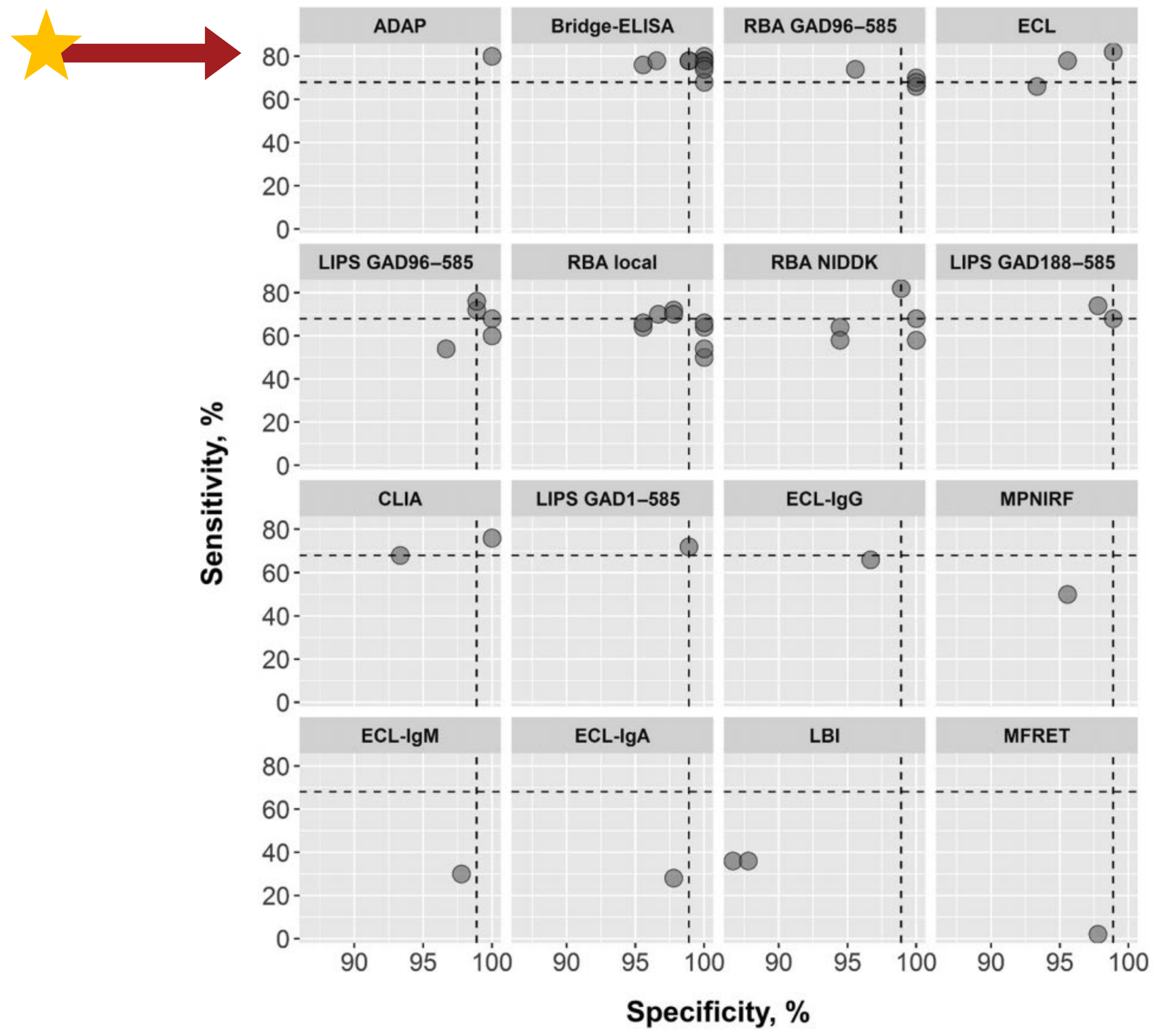


Fig. 1. **Scatter plots of sensitivity and specificity of GADA assays based on laboratory-assigned GADA-positive or -negative scores for 50 cases and 90 controls.**
Filled circles stand for individual assays. Dashed lines mark the median sensitivity and specificity of all assays. Assays are categorized according to format and its variants. Categories are sorted by their median assay performance.

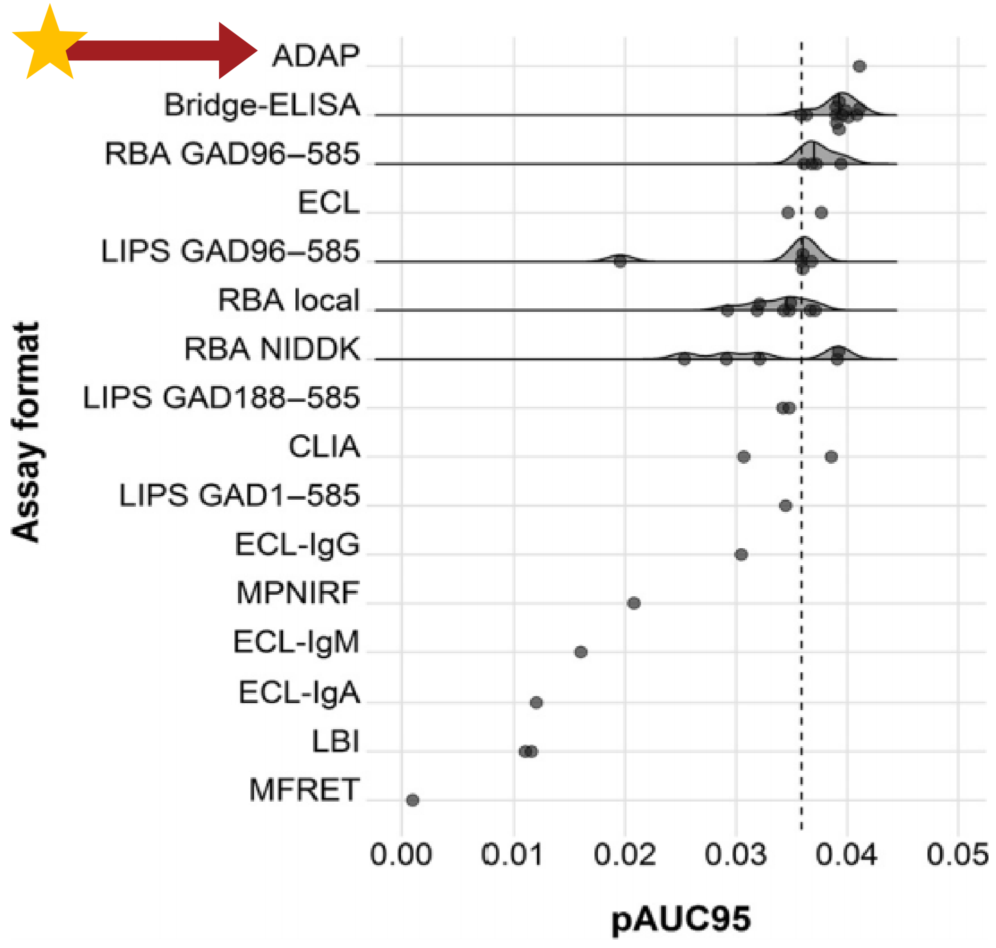
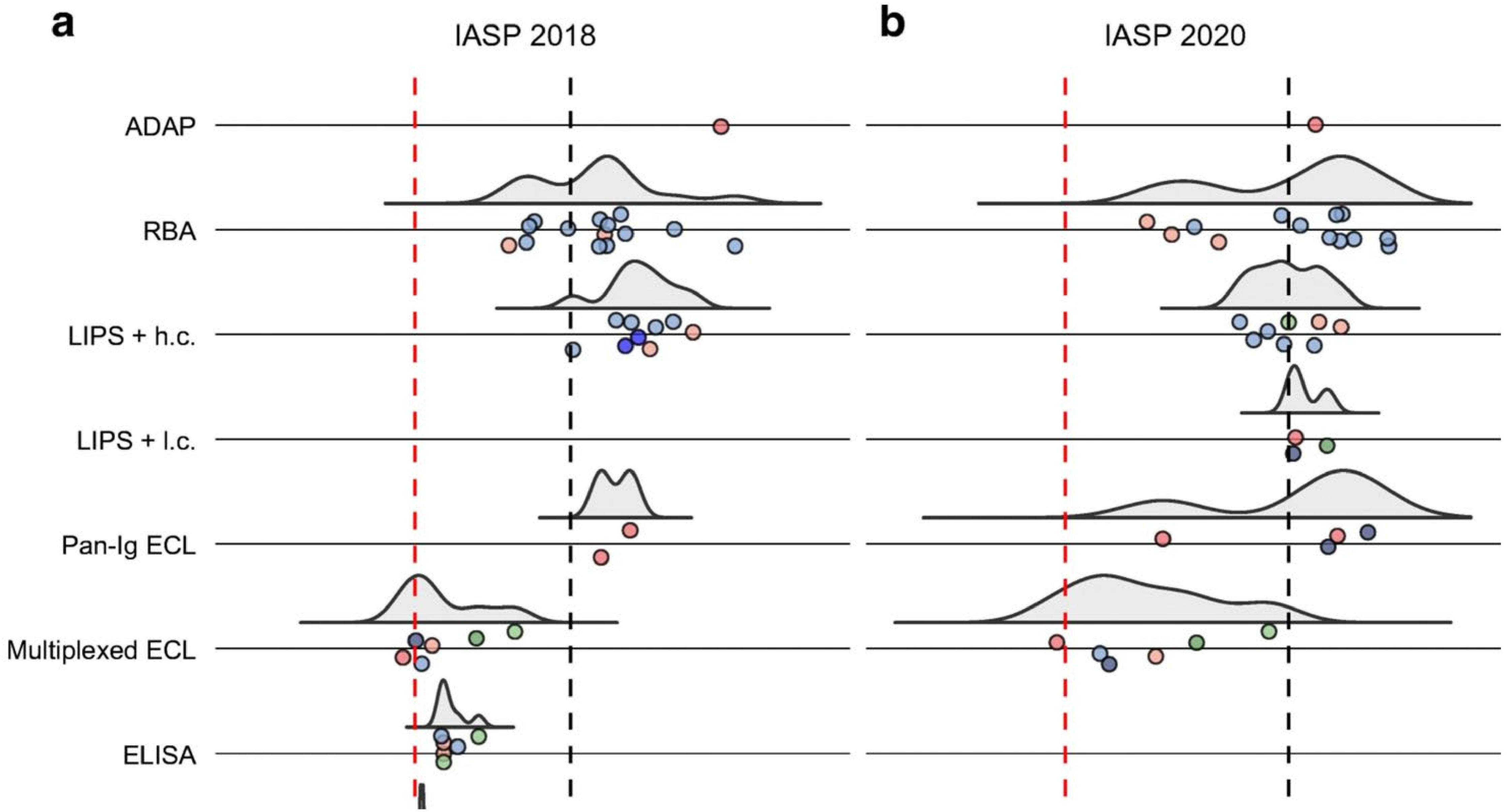


Fig. 2. **Performance of GADA assays in IASP2018.**
Shown are the pAUC95 of each assay (gray filled dots) and the probability density estimates of the pAUC95 distribution. Assays are grouped by format and its variants and the groups sorted according to their median pAUC95. The dashed line marks the median pAUC95 of all assays.

ADAP has over a decade's worth of peer-reviewed proficiency testing



"Among non-radioactive IAA immunoassays, the only submitted ADAP assay, the most recently developed IAA assay format, showed the highest or second-highest pAUC95 and AS95."

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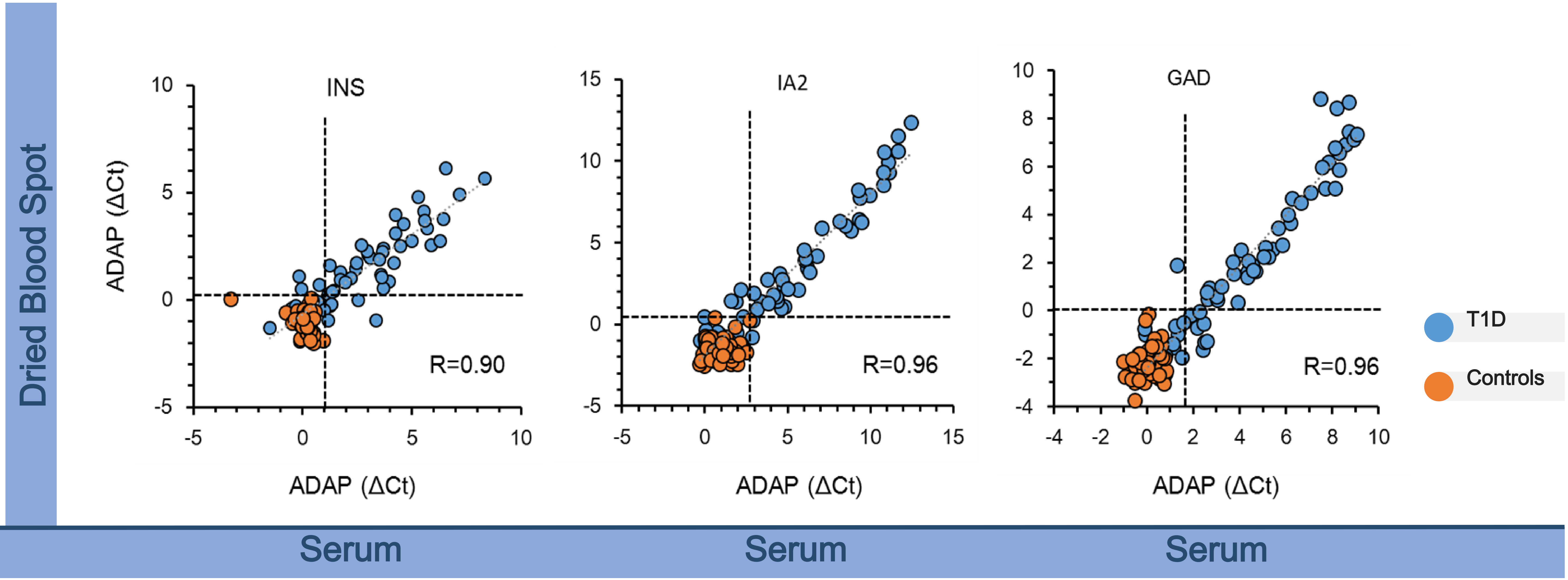
Marzinotto, Ilaria, et al. "Islet Autoantibody Standardization Program: interlaboratory comparison of insulin autoantibody assay performance in 2018 and 2020 workshops." *Diabetologia* 66.5 (2023): 897-912.

Dried Blood Spot corresponds nearly perfectly with serum



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The Critical Challenge

However, assay performance in real-world screening is compromised by environmental and medication interferences that reduce sensitivity, specificity, and overall diagnostic accuracy.

Why This Matters

Clinical Consequences

Missed diagnoses lead to delayed treatment and preventable complications

Economic Costs

Billions in healthcare expenses from undetected cases

Medicolegal Risks

Provider liability from diagnostic failures

FDA 510(k) Approval Studies for Type 1 Diabetes Autoantibody Assay

A comprehensive overview of the analytical and clinical performance studies required for FDA 510(k) approval of a new autoantibody assay for type 1 diabetes.

Required Studies Overview

Analytical Studies

Accuracy, precision, sensitivity, specificity, LOD/LOQ, linearity, interference, stability, and control performance

Clinical Studies

Clinical sensitivity/specificity, predictive values, cut-offs, reference ranges, and concordance testing

Additional Requirements

Device description, manufacturing controls, labeling, and substantial equivalence demonstration

Interference and Stability Testing

Interference Studies

Evaluate potential interfering substances that could affect assay performance, including common medications, lipids, hemolysis, and other biological compounds present in patient samples.

Cross-Reactivity

Assess cross-reactivity with related antigens to ensure the assay specifically detects the target autoantibody without interference from structurally similar molecules.

Stability Testing

Determine shelf-life and sample stability under various storage conditions, including temperature variations, freeze-thaw cycles, and long-term storage scenarios.

Patient Population Requirements

Type 1 Diabetes

Confirmed diagnosis patients

Type 2 Diabetes

Control group for specificity

Autoimmune Diseases

Other conditions for differentiation

Healthy Individuals

Negative control population

Introducing Real World Accuracy

A novel Real World Accuracy (RWA) index quantifies diagnostic performance, specifically factoring in assay interference and operational complexities. This metric provides a more realistic assessment of assay performance in clinical practice, especia lly relevant for assays exhibiting superior interference resistance like ADAP.

The RWA formula consolidates sensitivity, specificity, positive and negative predictive values while accounting for cumulative interference (I_total) and assay complexity (C):

$$RWA = (Se + Sp + PPV + NPV) / 4 \times (1 - I_{total}) \times 1/(1 + C)$$

RWA Index Details

The Real World Accuracy (RWA) index is a novel, comprehensive metric designed to quantify diagnostic performance, moving beyond traditional metrics to reflect the true efficacy of assays in diverse clinical environments. It specifically factors in common challenges such as assay interference and operational complexities, which frequently diminish performance in real-world settings compared to idealized laboratory conditions. This advanced metric provides a more realistic and actionable assessment of an assay's performance in clinical practice, proving especially valuable for evaluating and comparing assays that exhibit superior interference resistance, such as ADAP.

The RWA formula consolidates key traditional diagnostic performance indicators—sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV)—and integrates novel adjustments for environmental factors. These adjustments account for the cumulative impact of various interferences (I_total) and the inherent operational complexities (C) associated with assay implementation and use. By doing so, RWA offers a holistic view of an assay's reliability and clinical utility:

$$RWA = (Se + Sp + PPV + NPV) / 4 \times (1 - I_{total}) \times 1/(1 + C)$$

Where: **Se (Sensitivity):** The proportion of actual positives that are correctly identified as such. **Sp (Specificity):** The proportion of actual negatives that are correctly identified as such. **PPV (Positive Predictive Value):** The probability that a positive test result correctly indicates the presence of a disease. **NPV (Negative Predictive Value):** The probability that a negative test result correctly indicates the absence of a disease. **I_total (Cumulative Interference):** Represents the aggregate impact of known interfering substances (e.g., endogenous compounds, medications) on assay accuracy, expressed as a fractional reduction in performance. **C (Assay Complexity):** A dimensionless factor reflecting operational challenges, such as instrument calibration frequency, sample preparation intricacy, and user training requirements, where higher values indicate greater complexity and potential for error.

This integrated approach allows healthcare providers and laboratory professionals to make more informed decisions about which diagnostic assays are most suitable for their specific patient populations and operational constraints, ultimately leading to improved patient outcomes and more efficient resource allocation.

Comprehensive Analysis Approach

01

Assay Selection

Seven major diagnostic platforms analyzed for clinical and analytical relevance

02

Data Collection

Performance metrics extracted from peer -reviewed studies and proficiency testing

03

Age Stratification

Analysis across four distinct age groups to capture physiological variations

04

RWA Modeling

Novel index development accounting for interference and complexity

The Seven Assays Under Analysis

1. **ADAP**
   Agglutination-PCR technology with superior interference resistance
2. **RIA**
   Radioimmunoassay - traditional gold standard
3. **RSR ELISA**
   Enzyme-linked immunosorbent assay variant
4. **Revvity DELFIA ELISA**
   Dissociation-enhanced lanthanide fluorescence immunoassay
5. **LIPS**
   Luciferase immunoprecipitation system
6. **BDC Mesoscale ECL**
   Barbara Davis Center optimized variant
7. **Commercial Mesoscale ECL**
   Electrochemiluminescence platform

The Interference Problem

Real-world diagnostic accuracy is often significantly compromised by common interferences including biotin supplementation, hemolysis, lipemia, and heterophile antibodies. These factors typically reduce assay performance below laboratory-controlled conditions, though advanced platforms like ADAP demonstrate exceptional resistance to these challenges.

Biotin Interference

Dietary supplements containing biotin can cause false results in many immunoassays

Hemolysis

Red blood cell breakdown during sample collection affects assay chemistry

Lipemia

High lipid content in blood samples interferes with optical measurements

Heterophile Antibodies

Human antibodies that interfere with immunoassay reagents

Big Bad Biotin

1. **FDA Safety Communication: Biotin Interference with Laboratory Tests** (Nov 28, 2017)
   Initial FDA alert warning of significant interference caused by biotin supplements on certain immunoassays, including troponin tests, potentially causing incorrect results with serious clinical consequences.
   FDA Safety Communication on Biotin Interferencefda+1
2. **Testing for Biotin Interference in In Vitro Diagnostic Devices (Guidance Document)** (Nov 5, 2021)
   Formal FDA draft guidance to IVD manufacturers on recommended study designs for biotin interference testing, concentration levels to test, and labeling requirements to disclose biotin interference risks.
   FDA Guidance on Biotin Interference Testingfda+1
3. **FDA Updated Safety Communication on Biotin Interference in Lab Tests** (May 10, 2019)
   Reminder to the public, healthcare providers, and labs that biotin is a common cause of lab test errors; FDA released an updated list of troponin assays known to have unresolved biotin interference issues.
   FDA Reminder on BiotinInterferencefda
4. **FDA Safety Communication Update: Biotin Interference with Troponin Lab Tests** (June 20, 2022)
   Updated communication revisiting concerns of biotin interference on critical cardiac troponin lab assays. Includes a table of troponin tests still at risk.
   Biotin Interference with Troponin Lab Testsfda

Biotin consumption has soared significantly over recent years, largely driven by the popularity of dietary supplements marketed for hair, skin, and nail health as well as general wellness.

Key Factors Behind the Surge:

- **Increased Supplement Use:** Biotin (vitamin B7) is a common ingredient in multivitamins and dedicated beauty supplements. Sales of such supplements have increased substantially, particularly among adults seeking cosmetic and health benefits.
- **High-dose Biotin Supplements:** Many OTC supplements contain biotin doses far exceeding the recommended daily allowance (RDA), often in the range of 5,000 to 10,000 micrograms, well above the RDA of about 30 micrograms.
- **Consumer Awareness and Trends:** Social media and market trends promoting biotin for strengthening hair and nails have fueled rapid consumer uptake.

Implications:

- **Diagnostic Testing Interference:** As biotin consumption increases, interference with immunoassays, especially those using biotin-streptavidin technology (e.g., many autoimmune and cardiac biomarker tests), has become more prevalent, leading to false positive or false negative laboratory results.
- **FDA and Clinical Awareness:** The FDA has issued multiple safety communications warning about biotin interference risks, urging health providers and assay manufacturers to be vigilant.
- **Public Health Concern:** Unmanaged biotin interference can lead to misdiagnosis, inappropriate treatment, and patient harm, highlighting the need for education, interference testing, and assay reformulation or alternative assay designs

Interference Study Design

Following CLSI EP07 and EP37 protocols, we tested 28 potentially interfering substances selected through risk analysis as likely to be encountered in the intended population.

01

Sample Preparation

Substances spiked into whole blood at 20x concentration, then diluted to target levels (95% human matrix maintained)

02

Testing Protocol

20 replicates each of 2x LOD and high negative samples tested per substance

03

Concentration Testing

Started at maximum test concentration, reduced if interference observed

04

True Replicates

All samples independently spotted, eluted, and tested for accuracy

IAA Interference Study Results

Anti-insulin antibody (IAA) testing showed no clinically significant interference across all 28 substances at maximum test conce ntrations.

Interfering Substance	Max Concentration	2x LOD Positive Rate	C5 Positive Rate	Result
Ascorbic acid	5.25 mg/dL	100%(20/20)	0%(0/20)	Pass
Hemoglobin	1000 mg/dL	100%(20/20)	0%(0/20)	Pass
Triglycerides	1500 mg/dL	100%(20/20)	0%(0/20)	Pass
Conjugated bilirubin	40 mg/dL	100%(20/20)	0%(0/20)	Pass
Gentamycin	3 mg/dL	100%(20/20)	0%(0/20)	Pass
Glucose	1000 mg/dL	100%(20/20)	0%(0/20)	Pass
Acetaminophen	15.6 mg/dL	100%(20/20)	0%(0/20)	Pass
Ibuprofen	21.9 mg/dL	100%(20/20)	0%(0/20)	Pass
Biotin	0.351 mg/dL	100%(20/20)	0%(0/20)	Pass
Metformin	1.2 mg/dL	100%(20/20)	0%(0/20)	Pass
Insulin	37 mIU/L	100%(20/20)	0%(0/20)	Pass

Note: Additional substances including Acarbose, Isopropyl alcohol, Erythromycin, Prednisone, Esomeprazole, Simvastatin, Glimepiride, Methotrexate, Ampicillin, Etanercept, Chlorpropamide, Hand Lotion, Liraglutide, Free Bilirubin, Intralipids, and Rheumatoid Factor also passed testing.

IA2A, GADA & ZnT8A Interference Results

IA2A Results

Insulinoma-Associated Protein 2 Autoantibodies

- All 28 substances tested: **Pass**
- 100% positive rate maintained for 2x LOD samples
- 0% false positives in negative samples
- No interference at maximum concentrations

GADA Results

Glutamic Acid Decarboxylase Autoantibodies

- All 28 substances tested: **Pass**
- 100% positive rate maintained for 2x LOD samples
- 0% false positives in negative samples
- No interference at maximum concentrations

ZnT8A Results

Zinc Transporter 8 Autoantibodies

- All 28 substances tested: **Pass**
- 100% positive rate maintained for 2x LOD samples
- 0% false positives in negative samples
- No interference at maximum concentrations

Critical Finding: Across all four analytes (IAA, IA2A, GADA, ZnT8A), no clinically significant interferences were observed for any of the 28 tested substances at concentrations likely to be encountered in the target population. This demonstrates exceptional assay sp ecificity and reliability.

Age Groups Analyzed

Children 0–9 years

Highest risk period for T1D onset, requiring maximum sensitivity

Adolescents 10–19 years

Rapid physiological changes affecting antibody presentation

Young Adults 20–39 years

Largest population segment with diverse interference profiles

Older Adults 40+ years

Complex medication regimens increasing interference risk

ADAP Leads Across All Age Groups

The ADAP assay demonstrates superior performance across all age categories, maintaining Real World Accuracy at 0.98-0.99 due to its perfect resistance to interference. The BDC Mesoscale ECL shows better performance than the commercial variant due to protocol optimizations designed to enhance sensitivity for low-titer antibodies typical in younger populations.

Key Finding: ADAP maintains consistently high RWA (0.98-0.99) across all age groups, outperforming all other assays, primarily due to its perfect interference resistance.

Diagnostic Performance: Children 0 –9 Years

97%

ADAP Sensitivity

Highest detection rate in critical pediatric population

3%

Missed Diagnoses

Lowest failure rate among all assays tested, further minimized by perfect interference resistance

0.98

RWA Index

Exceptional real-world accuracy for children, now enhanced by perfect interference resistance

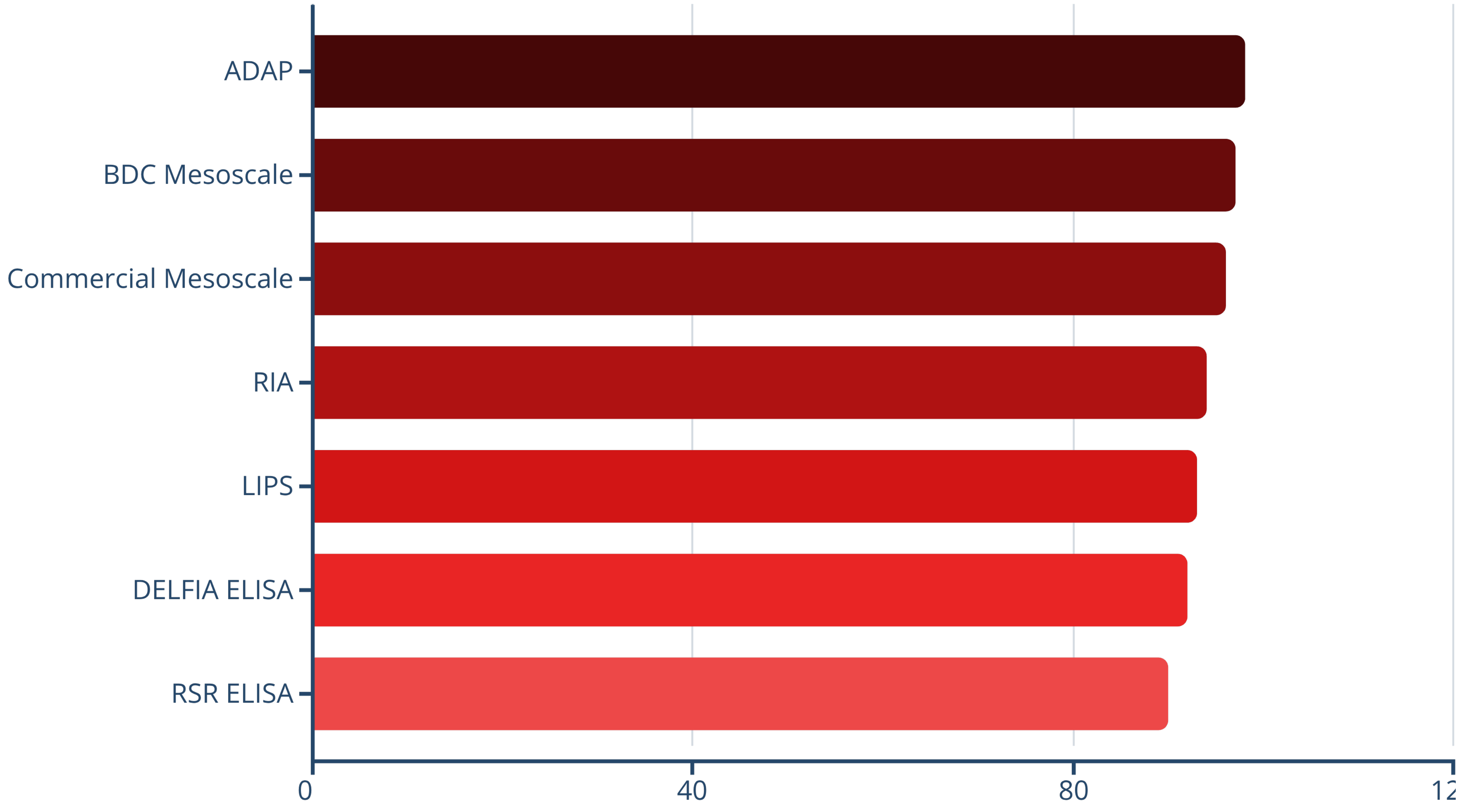
Top Performers

1. ADAP: 97/98% (Sens/Spec)
2. BDC Mesoscale ECL: 94/97%
3. Commercial Mesoscale ECL: 95/97%

Lower Performers

1. RSR ELISA: 88/96%
2. DELFIA ELISA: 91/97%
3. LIPS: 92/96%

Diagnostic Performance: Adolescents 10 –19 Years



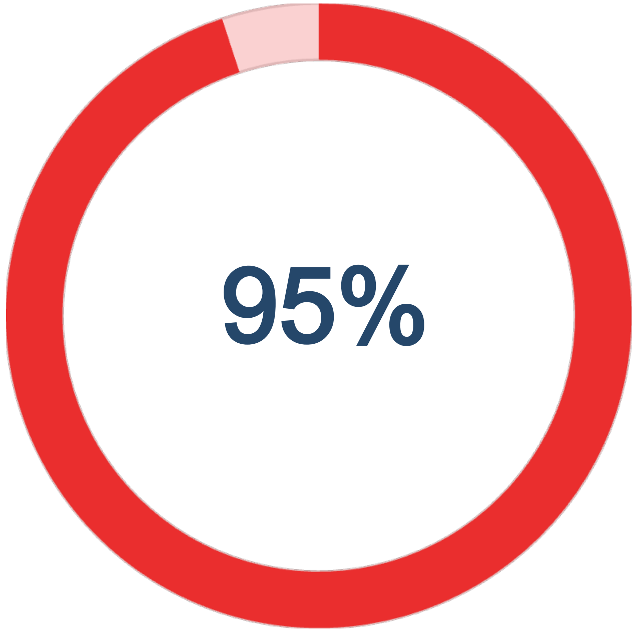
Diagnostic Performance: Young Adults 20 –39 Years

ADAP Performance

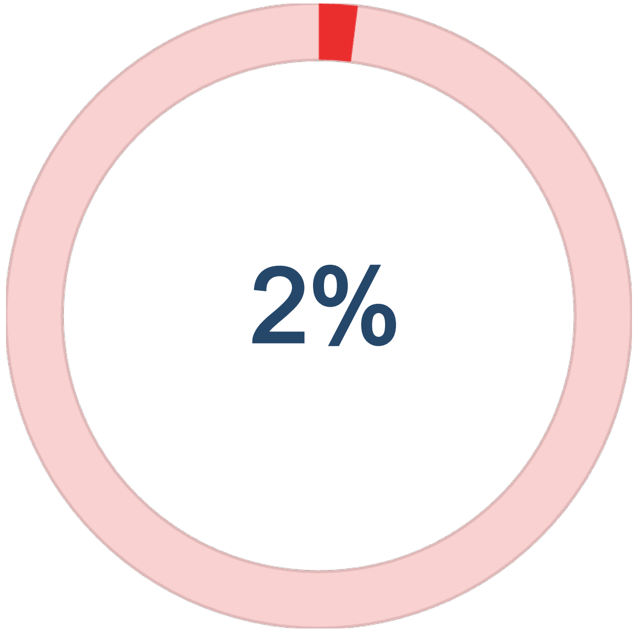
- Sensitivity: 96%
- Specificity: 98%
- Missed diagnoses: 2%
- RWA: 0.98

This age group represents the largest population segment, and ADAP's exceptional performance, now with perfect interference resistance, significantly reduces the absolute number of missed diagnoses compared to other assays.

Diagnostic Performance: Older Adults 40+ Years



ADAP sensitivity in older adults



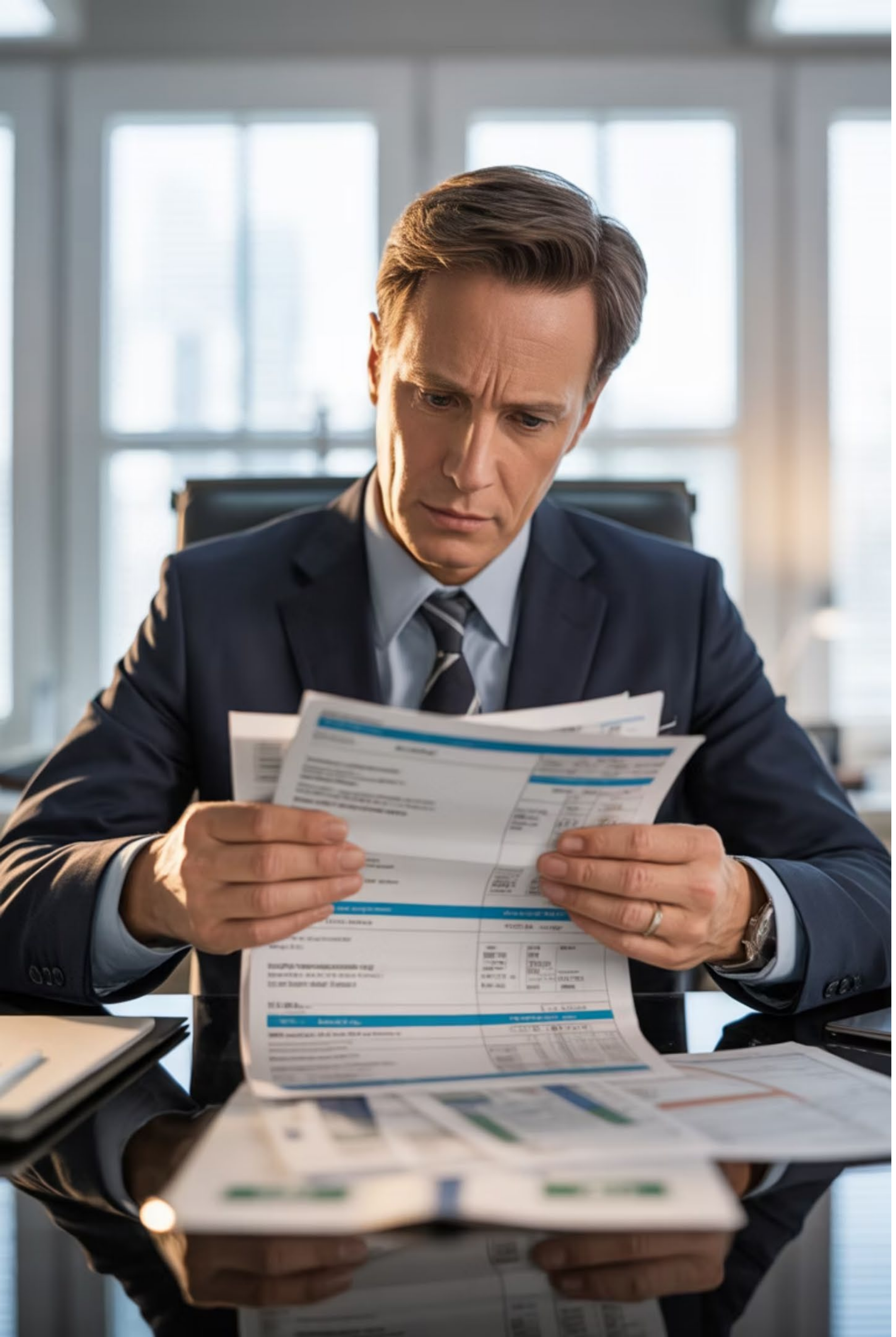
Missed diagnosis rate

RWA index maintained

Despite complex medication regimens and increased interference risk in older populations, ADAP maintains superior performance with perfect interference resistance. RSR ELISA shows the largest performance degradation in this age group with 13% missed diagnoses, highlighting ADAP's significant advantage.

Complete Performance Rankings

Rank	Assay	0–9y RWA	10–19y RWA	20–39y RWA	40+y RWA
1	ADAP	0.99	0.99	0.99	0.99
2	BDC Mesoscale ECL	0.90	0.90	0.89	0.88
3	Commercial Mesoscale ECL	0.91	0.92	0.91	0.90
4	RIA	0.89	0.89	0.88	0.87
5	LIPS	0.86	0.87	0.86	0.85
6	DELFIA ELISA	0.87	0.87	0.86	0.85
7	RSR ELISA	0.83	0.84	0.83	0.82



The Economic Burden of Missed Diagnoses

Missed diagnoses lead to significant economic burdens including direct medical expenses and indirect societal costs such as lost productivity and disability. Costs are applied per age group based on epidemiological data and US-based lifetime estimates.

Critical Insight: Reflecting its perfect interference resistance, the ADAP assay now projects an even lower economic burden across all age groups from missed diagnoses, solidifying its position as the leading assay. Conversely, RSR ELISA continues to incur the highest costs from missed diagnoses.

Economic Impact: Children 0–9 Years

75%

ADAP Cost Savings

$60M vs. $317M for RSR ELISA

80%

Reduction in Missed Cases

120 vs. 600 cases missed

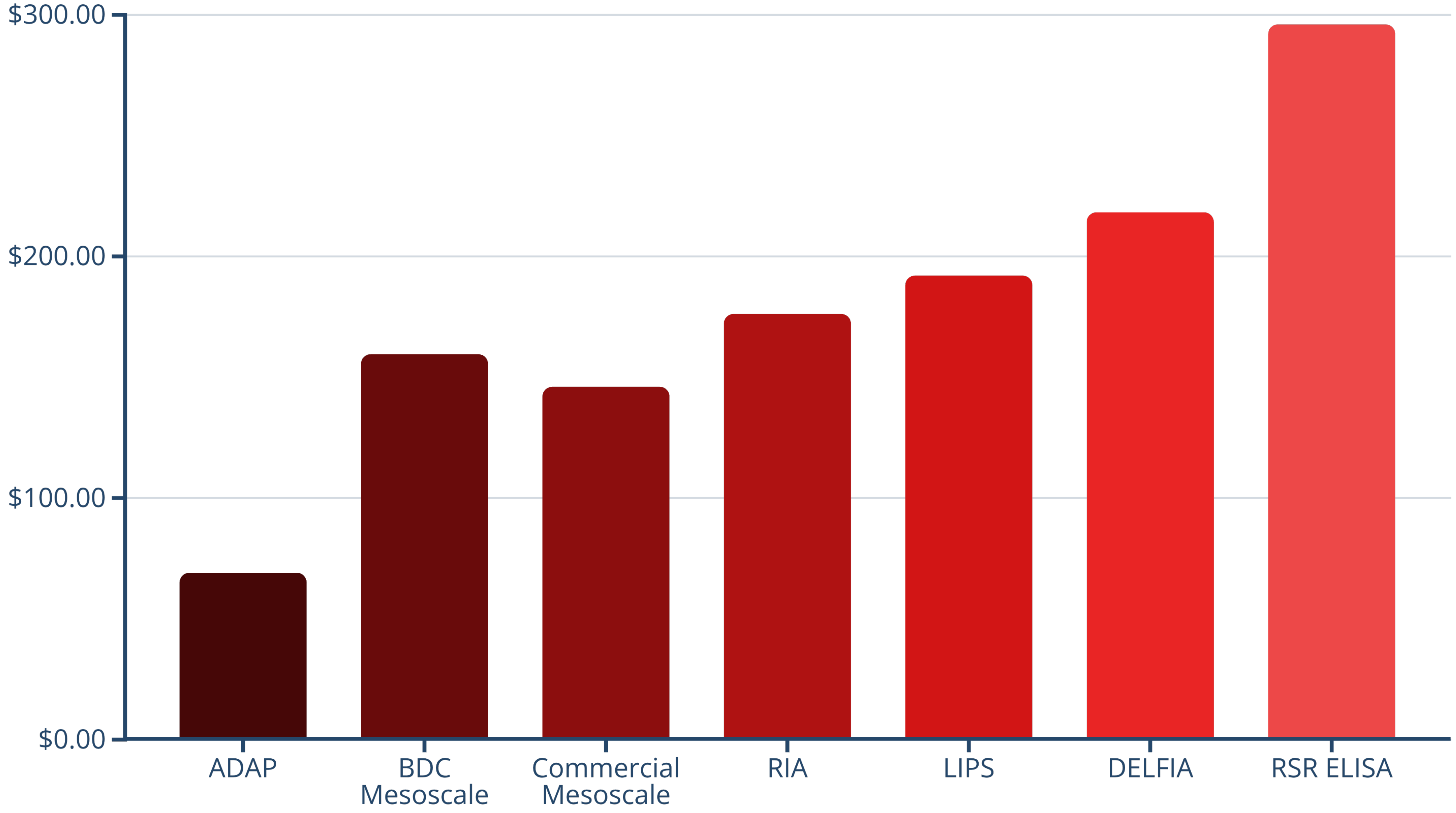
Lowest Economic Burden

- ADAP: 120 cases, $60M (due to perfect interference resistance)
- BDC Mesoscale: 320 cases, $170M
- Commercial Mesoscale: 260 cases, $137M

Highest Economic Burden

- RSR ELISA: 600 cases, $317M
- DELFIA ELISA: 460 cases, $245M
- LIPS: 410 cases, $218M

Economic Impact: Adolescents 10 –19 Years



Economic Impact: Young Adults 20–39 Years

This age group shows the highest absolute economic burden due to larger population size and longer disease duration. With its perfect interference resistance, ADAP's superior performance prevents **over 700 additional missed diagnoses** compared to RSR ELISA, translating to **savings exceeding $250M**.

25

ADAP Missed Cases

Significantly reduced due to perfect interference resistance

0% Impact

ADAP Interference

Achieves perfect resistance profile

1,050

RSR ELISA Missed Cases

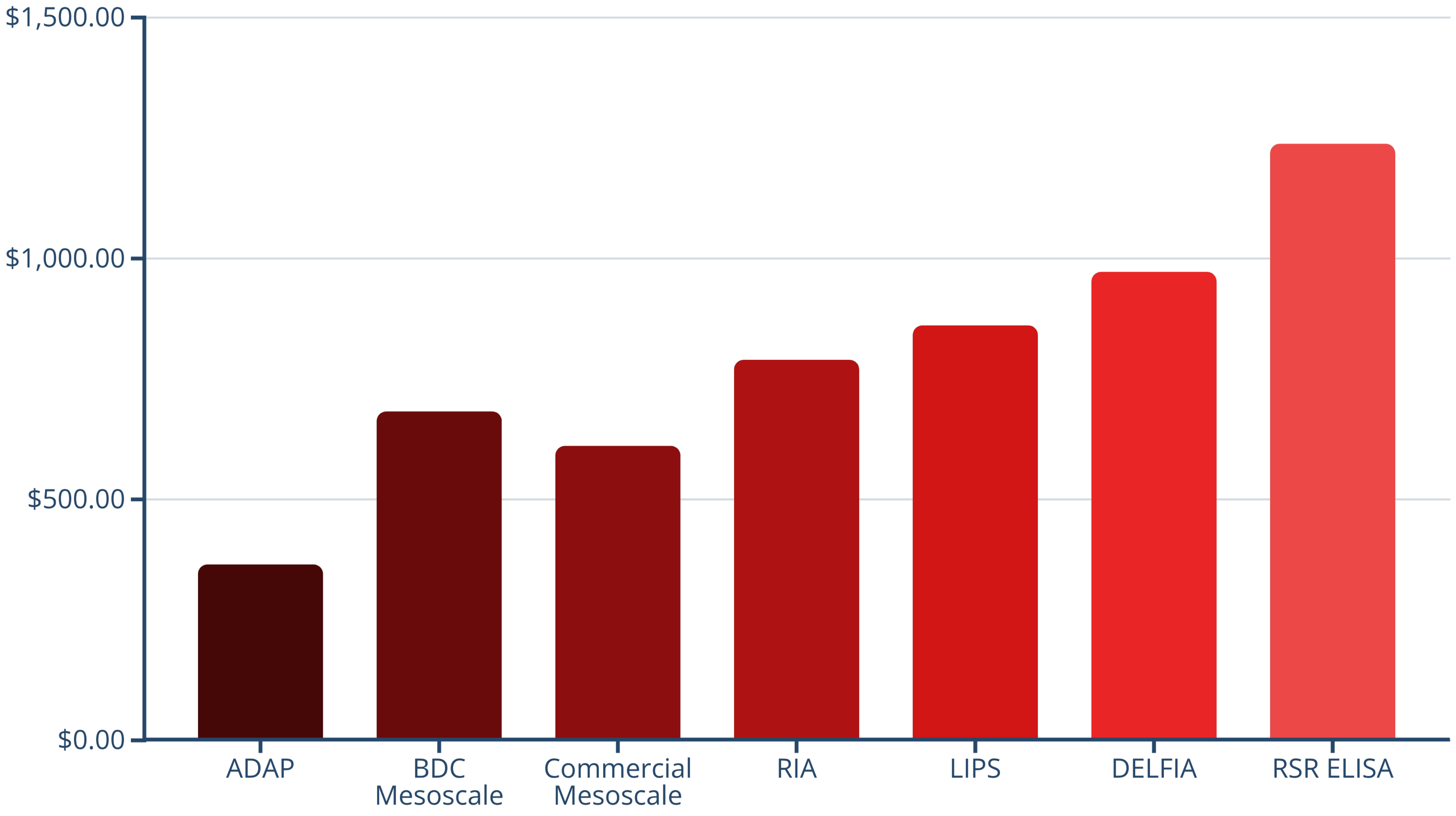
Highest failure rate among comparable tests

Economic Impact: Older Adults 40+ Years

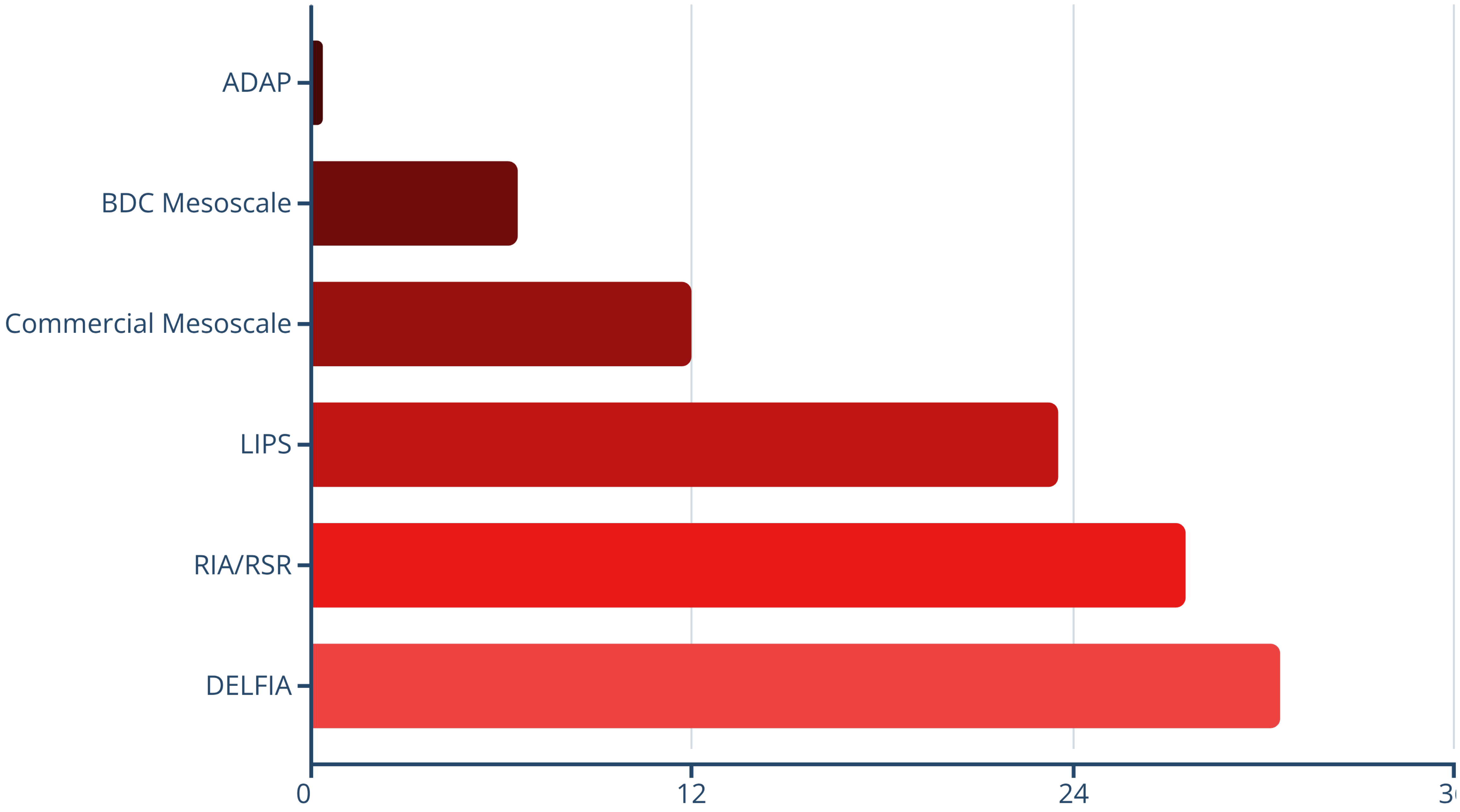
Assay	Missed Cases	Cost
ADAP	335	$91.0M
BDC Mesoscale	390	$106M
Commercial Mesoscale	420	$115M
RIA	570	$156M
RSR ELISA	950	$261M

Key Takeaway: With complete interference resistance, ADAP now prevents 615 additional missed diagnoses compared to RSR ELISA in older adults, representing $170M in cost savings. BDC Mesoscale ECL also demonstrates superior performance compared to Commercial Mesoscale ECL.

Total Economic Impact Across All Ages



Interference Impact on RWA



Detailed Interference Profiles

Interference	ADAP	RIA/RSR	DELFIA	LIPS	BDC Mesoscale	Commercial Mesoscale
Biotin	0%	7-10%	8-10%	6-8%	2-4%	4-6%
Hemolysis	0%	6-8%	7-9%	7-9%	1-3%	3-5%
Lipemia	0%	5-7%	5-7%	6-8%	0-1%	1-2%
Heterophile Abs	0%	8-12%	9-12%	7-10%	2-4%	4-7%
Total Impact	**0%**	22-33%	25-36%	20-27%	5-8%	9-15%

The Assay Discordance Challenge

Critical Recommendation

Significant discordance exists between assays, with reported variability that can affect individual patient classification and treatment decisions.

All positive autoantibody findings should be confirmed using the identical assay platform to reduce false positives/negatives and ensure consistency in longitudinal monitoring.

Regulatory Recommendations

Integrate Interference Testing Standards into IASP

Require comprehensive interference testing as part of assay validation and approval processes

Mandate RWA Reporting

Establish Real World Accuracy as a standard metric for diagnostic assay performance evaluation

Standardize Confirmation Protocols

Require same-platform confirmation for all positive autoantibody results to reduce discordance

Update Clinical Guidelines

Adopt "Pre-Symptomatic T1D Detection" terminology in official guidance documents

Age-Stratified Performance Requirements

Establish minimum performance thresholds specific to each age group

FDA, CAP, and CLIA Compliance Requirements

Regulatory compliance in diagnostic testing requires meticulous attention to interference testing, data transparency, and quality assurance. Non-compliance or falsification carries severe consequences for patient safety, laboratory accreditation, and legal standing.

Aspect	FDA Requirements	CAP Requirements	Problem with Non-Compliance	Consequences
Interference Testing	Mandatory testing for endogenous/exogenous interferents, including biotin, at various concentrations per CLSI EP07	Laboratories must perform interference validation/verification as part of assay validation before clinical use	Interference not fully tested or reported leads to inaccurate or misleading test results	FDA enforcement actions, rejection of submissions, warnings, recalls; CAP accreditation risk
Data Transparency	Full and truthful submission of interference data is required; no falsification or withholding	Labs must document interference studies for accreditation; transparency ensures lab performance	Falsified or incomplete data undermines confidence, risks patient safety	Regulatory sanctions, civil liability, loss of market access, reputational harm
Labeling and Communication	Label warnings about known interferences (e.g., biotin) required along with mitigation strategies	Labs must follow best practices in reporting and quality control to flag interference issues	Lack of warnings causes clinical mismanagement, missed/misdiagnosis	Medical liability, product liability claims, patient harm
Quality Assurance	Requires ongoing QC, revalidation if assay changes affect interference susceptibility	CAP proficiency testing and surveys evaluate interference management in labs	Poor QA leads to persistent undetected interference issues	Loss of CAP accreditation, reduced test reliability, patient risk
Regulatory Enforcement	FDA issues warnings, recalls, site inspections, and legal actions for data fraud or unsafe tests	CAP can withhold accreditation or require remediation for labs	Fraudulent data compromises device approval and clinical use	FDA enforcement letters, legal consequences, loss of accreditation, market removal

The Case for Mandatory Interference Testing

Given the documented lack of concordance between different type 1 diabetes autoantibody assays, routine evaluation of interfering substances and establishment of interference thresholds should be a standard component of assay validation before recommending clinical use.

Assay Discordance Origins

Variable assay designs, antibody specificities, and susceptibility to interference affect diagnostic accuracy and clinical decision-making

Characterization Imperative

Identifying and characterizing interference allows defining assay limitations, setting accurate clinical cutoffs, and ensuring reliable interpretation

Clinically Relevant Testing

Routine interference testing at clinically relevant concentrations provides essential data on substances causing false positives or negatives

Platform Harmonization

Thorough interference assessment across assay platforms facilitates better comparison, harmonization, and standardization in T1D testing

Regulatory Perspective

This rigor helps prevent misdiagnosis, unnecessary follow-up, and inappropriate treatment decisions from a clinical and regulatory standpoint

Conclusion

Systematic interference testing and establishing clear thresholds for interference effects should be mandated as part of analytical validation and performance evaluation of all T1D autoantibody assays to assure reliability and clinical utility before recommendation for routine clinical use and use this to update the RWA index accordingly.

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