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RESEARCH ARTICLE

HARMONIZATION OF ANTI-GAD AUTOANTIBODY ASSAYS: DEVELOPMENT AND VALIDATION OF A REFERENCE SYSTEM FOR A PROGRESSIVE METROLOGICAL APPROACH

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ABSTRACT

Measurement of anti-glutamic acid decarboxylase (anti-GAD) autoantibodies is a key biomarker for the diagnosis and classification of autoimmune diabetes, including type 1 diabetes (T1D) and latent autoimmune diabetes in adults (LADA). However, the lack of international harmonization across assay methods leads to substantial inter-laboratory heterogeneity, compromising diagnostic reliability, comparability of epidemiological studies, and patient eligibility for emerging immunomodulatory therapies. This study proposes a progressive metrological harmonization strategy based on the development of a robust reference system across five phases over 36 months: characterization of current variability (40 laboratories, 20 countries), development of commutable reference materials traceable to the WHO standard, multicenter analytical validation (target inter-lab CV <20%), harmonization of decision thresholds on 1,500 patients, and clinico-economic impact evaluation.

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INTRODUCTION

Diabetes mellitus is among the most prevalent chronic diseases worldwide, affecting more than 537 million adults in 2021 according to the International Diabetes Federation (IDF), with projections reaching 783 million by 2045 (1). Traditionally categorized into two main forms—type 1 diabetes (T1D) and type 2 diabetes (T2D)—this dichotomy is insufficient to reflect the heterogeneity observed in clinical practice. Latent autoimmune diabetes in adults (LADA) represents an intermediate form accounting for 5–12% of cases initially diagnosed as T2D in adults (2,3). It is characterized by the presence of autoantibodies against pancreatic beta-cell antigens, notably anti-glutamic acid decarboxylase (anti-GAD), and by a slower progression toward insulin dependence. Glutamic acid decarboxylase (GAD) is a 65 kDa enzyme mainly found in GABAergic neurons and pancreatic beta cells. Anti-GAD65 autoantibodies are the most frequently detected autoimmune biomarker in autoimmune diabetes and often persist for years or decades after diagnosis (4). Anti-GAD shows a diagnostic sensitivity of 70–80% in T1D and is often the only positive marker in LADA patients (5). The emergence of teplizumab, approved by the FDA in 2022, reinforces the need for reliable measurement to identify eligible patients (6). External quality assessment programs regularly report inter-laboratory coefficients of variation above 30% for identical samples, with qualitative discordance rates reaching 15–25% near decision thresholds (7,8). This situation has concerning clinical consequences: diagnostic errors, difficulties in longitudinal monitoring, and uncertainty in identifying candidates for clinical trials. The overall objective is to design, develop, and validate a metrological reference system enabling progressive harmonization and international traceability of anti-GAD measurements. Specific objectives include: (1) characterize inter-laboratory variability, (2) identify sources of variability, (3) develop traceable reference materials, (4) propose a calibration-transfer methodology, (5) establish harmonized decision thresholds, and (6) evaluate the impact of harmonization.

LITERATURE REVIEW AND STATE OF THE ART

Physiopathology of autoimmune diabetes: Autoimmune diabetes results from progressive and selective destruction of insulin-producing beta cells in pancreatic islets. This destruction involves both cellular immunity (cytotoxic CD8+ T cells, helper CD4+ T cells) and humoral immunity (autoantibodies). Mechanisms of tolerance breakdown include genetic factors (HLA class II alleles DRB1*03 and DRB1*04), environmental triggers (viral infections, nutritional factors), and immune regulation dysfunctions.

Historical evolution of assay methods: Anti-GAD assays have evolved substantially since the 1980s: radioimmunoprecipitation with iodine-125 (historical reference), ELISA methods (greater

automation, no radioactivity), indirect immunofluorescence, and electrochemiluminescence (ECL) with improved sensitivity (9). Each technological generation introduces new variability sources due to differences in antigen formats and detection protocols.

International evaluation programs: The Islet Autoantibody Standardization Program (IASP), coordinated by the Immunology of Diabetes Society (IDS), has organized regular workshops since 1996. Successive results show improvement but insufficient concordance: median sensitivity increased from 54% (1996) to 74% (2018), while specificity rose from 95% to 98% (10). The absence of a universal reference method perpetuates non-comparability.

Comparison with the state of the art: Our approach differs from prior initiatives by systematically integrating analytical, clinical, economic, and implementation dimensions into a coherent framework. Unlike studies focused solely on diagnostic evaluation, we propose a structured metrological strategy with commutable reference materials, inter-method calibration transfer, and impact validation in resource-limited settings.

PROBLEM STATEMENT AND HYPOTHESES

Core problem statement: How can a metrological reference system be developed and validated to enable progressive harmonization and international traceability of anti-GAD assays while accounting for technical, economic, and infrastructural constraints across diverse contexts, including resource-limited countries?

Working hypotheses

- **H1:** Inter-laboratory variability is mainly attributable to calibration heterogeneity rather than intrinsic analytical specificity differences.
- **H2:** Commutable secondary reference materials traceable to the WHO standard will reduce inter-lab CV to <20% for positive samples.
- **H3:** Harmonized decision thresholds based on robust clinical correlations will improve diagnostic and prognostic performance.
- **H4:** Implementing the harmonized system will increase inter-lab diagnostic agreement from 75–80% to >95%.

METHODOLOGY

Overall study design: This research uses a sequential mixed approach combining metrological development, analytical validation, and clinical evaluation. Five main phases span 36 months, as shown in Fig. 1.

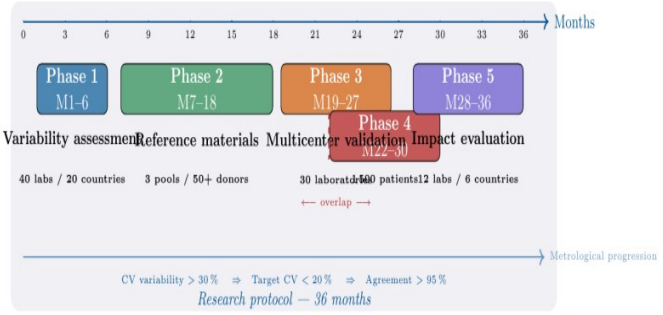

Fig. 1. Gantt chart of the five-phase research protocol (M1–M36). Phases 3 and 4 overlap during months 22–27. Metrological progression: baseline CV >30% → target CV <20% → agreement >95%

Table I. Phases of the research protocol

Phase	Period	Main Objective	N Participants
1	M1–6	Variability assessment	40 labs / 20 countries
2	M7–18	Reference materials dev.	3 pools / 50+ donors
3	M19–27	Multicenter validation	30 laboratories
4	M22–30	Threshold harmonization	1,500 patients
5	M28–36	Impact evaluation	12 labs / 6 countries

Phase 1: Assessment of current variability (months 1–6): Multicenter cross-sectional observational study involving 40 laboratories in 20 countries across five continents, selected to reflect analytical platform diversity (RIP: n=8; ELISA: n=20; ECL: n=12) and geographic contexts. Each laboratory triplicates measurement of a panel of 40 serum samples spanning a concentration gradient (10 negative, 10 weakly positive, 10 moderately positive, 10 strongly positive). Statistical analyses: inter- and intra-laboratory coefficients of variation, qualitative agreement (Cohen's kappa), Passing–Bablok regression, Bland–Altman plots, multi-factor ANOVA, and hierarchical modeling.

Phase 2: Development of reference materials (months 7–18): Three serum pools (low, medium, high) from characterized T1D patients (n ≥ 50 donors per pool). Value assignment by consensus among three candidate reference methods (standardized RIP, CDC ELISA, high-sensitivity ECL). Commutability study per CLSI EP30-A on 10 commercial analytical systems (15). Traceability to the WHO 1st International Standard for GAD Autoantibody (NIBSC code 97/550) (16). Fig. 2 shows the full metrological traceability chain. Accelerated stability (40°C × 7 d, 37°C × 30 d) and long-term stability (−20°C and −80°C over 24 months).

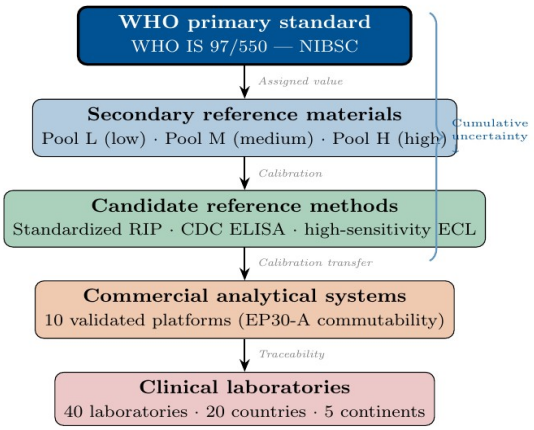

Fig. 2. Metrological traceability chain of the proposed reference system. Each level inherits the assigned value from the level above, ensuring international consistency

Phase 3: Multicenter analytical validation (months 19–27): Prospective interventional study in 30 laboratories that participated in Phase 1. Recalibration protocol using reference materials, followed by an evaluation panel of 50 patient samples. Primary endpoint: at least 40% reduction in inter-laboratory CV vs Phase 1. Secondary endpoints: qualitative agreement ≥95%, median deviation <15%, correlation r >0.95 between assigned and reported values.

Phase 4: Harmonization of decision thresholds (months 22–30): Retrospective multicenter cohort study including 1,500 patients: (1) recently diagnosed T1D (n=500), (2) suspected LADA (n=500), (3) first-degree relatives of T1D patients (n=500). Analyses: ROC curves for optimal thresholds; survival analyses (Kaplan–Meier, Cox regression) for time to insulin requirement as a function of anti-GAD titer.

Phase 5: Impact evaluation (months 28–36): Quasi-experimental before–after study in 12 laboratories across 6 countries (2 high-income, 2 middle-income, 2 low-income including Chad). Design: 6 months baseline + 6 months post-implementation. Endpoints: analytical performance (CV, discordance), clinical impact (time to diagnosis, T2D→LADA reclassification, identification of immunotherapy candidates), economic indicators (incremental cost per correctly diagnosed patient), and acceptability.

Ethical considerations: The protocol will be submitted to the ethics committees of the University of N'Djaména and partner institutions. Samples will come from approved biobanks with informed consent. Patient data will be fully anonymized and participating laboratories coded.

EXPECTED RESULTS

Anticipated scientific outputs

By the end of the study, the following major scientific results are expected:

- Comprehensive mapping of inter-laboratory variability and quantified identification of main sources.
- Validated commutable secondary reference materials (3 levels, WHO traceability, 24-month stability).
- Standardized calibration-transfer procedures across RIP, ELISA, and ECL, with documented conversion factors.
- Reduction of inter-laboratory CV to <20% (target: −40% vs baseline).
- Harmonized decision thresholds with 95% confidence intervals and contextual interpretation guidance.

Fig. 3 illustrates the expected reduction in coefficient of variation by analytical platform before and after harmonization.

Clinical implications: Harmonization will reduce misclassification between T2D and LADA: 5–12% of patients labeled T2D actually have LADA, and correct diagnosis substantially changes management (17). It will also support longitudinal monitoring under immunotherapy and fair identification of candidates for new therapies (teplizumab, baricitinib).

Epidemiological research: Harmonization will enable rigorous comparisons of T1D and LADA prevalence and incidence across countries. Meta-analyses pooling international cohorts will benefit from a common reference framework.

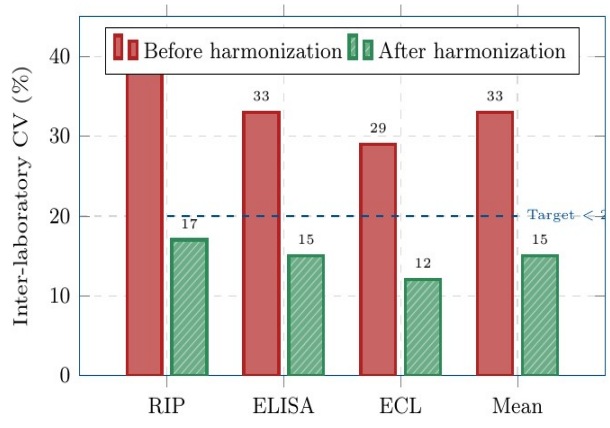

Fig. 3. Expected reduction in inter-laboratory CV by analytical platform. Dashed line = target <20%. Pre-harmonization values from EQA data (7,8); post-harmonization projections per Phase 3 objectives

Table II. Evaluation criteria for Phases 1 and 3

Indicator	Baseline (Ph. 1)	Target (Ph. 3)
Inter-lab CV (%)	> 30 %	< 20 %
CV reduction	—	≥ 40 %
Qualitative agreement	75–80 %	≥ 95 %
Median deviation	> 15 %	< 15 %
Correlation (r)	—	> 0.95

Relevance for resource-limited settings: Particular attention is paid to transferability of the reference system in resource-limited settings, including sub-Saharan Africa and Chad. Outputs will include barriers to implementation and pragmatic adaptation strategies: simplified QC, lower-cost methods with proper calibration, and regional reference networks (18).

Planned peer-reviewed outputs

Five publications are planned:

- *Methods paper* — reference material development (*Clinical Chemistry*, *J. Clin. Immunology*).
- *Results paper* — quantification of current variability (*Diabetes Care*, *Diabetologia*).
- *Implementation paper* (*Clin. Chemistry and Laboratory Medicine*).
- *Clinical paper* — harmonized thresholds (*Diabetes Care*, *Lancet Diabetes & Endocrinology*).
- *Synthesis paper* — implementation in limited settings (*BMJ Global Health*, *Lancet Global Health*).

PROSPECTIVE DISCUSSION

Strengths and originality: The work combines systematic metrological development with explicit clinical, economic, and implementation dimensions. The international scope, including often-underrepresented resource-limited contexts, is distinctive. Linking the reference system to clinically grounded thresholds ensures analytical harmonization translates into clinical utility.

Potential limitations and mitigation: Three main limitations: (i) Geographic representativeness — stratified recruitment via IDS and IFCC, with later open participation; (ii) Reference material stability — rigorous 24-month stability studies and multi-lot production; (iii) Implementation in low-resource settings — tiered strategies, online training, WHO and regional partnerships.

Future directions: Extensions include: (1) other islet autoantibodies (IA-2, ZnT8, IAA); (2) multiparametric prediction algorithms; (3) anti-GAD as a treatment-response biomarker (teplizumab, baricitinib); (4) applying the harmonization model to other autoimmune domains.

Policy implications: Recommendations will be directed to regulators (FDA, EMA, WHO) to encourage adoption in diagnostic device authorization. Including metrological harmonization criteria in public procurement could accelerate industrial uptake.

CONCLUSION

Key expected outcomes are reduced inter-laboratory variability (target CV <20%), improved qualitative agreement (≥95%), and harmonized decision thresholds to strengthen diagnostic reliability and international comparability. Anti-GAD measurement is essential for diagnosis, prognosis, and therapy selection in autoimmune diabetes; yet analytical heterogeneity and lack of harmonization undermine reliability with serious clinical

consequences. This doctoral research proposes a systematic five-phase framework: from characterizing current variability through reference materials, multicenter validation, harmonized thresholds, and impact assessment. Focus on transferability in resource-limited settings, especially sub-Saharan Africa, brings an equity perspective rarely addressed in biomedical metrology. Against rapid growth of immunomodulatory therapies and epidemiological transition in low- and middle-income countries, this project addresses an urgent need and should advance clinical immunology, biomedical metrology, and diabetology while providing practical tools for patients with autoimmune diabetes worldwide.

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