A low-volume rapid Luciferase Immunoprecipitation System assay utilising a novel antigen to detect autoantibodies to zinc transporter 8 in type 1 diabetes provides an alternative to conventional radioimmunoassay

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Introduction

Autoantibodies to zinc transporter 8 (ZnT8A) are one of the four major markers of islet autoimmunity used to identify high-risk individuals & are present in ~60-80% new-onset type 1 diabetes (1/2).

Routine biochemical assays that detect markers of islet autoimmunity:
Radioimmunoassays (RIAs) & enzyme-linked immunosorbent assays (ELISAs) test for autoantibodies directed to glutamic acid decarboxylase, islet antigen-2, insulin, & zinc transporter 8.

Historically used composite assay: Islet Cell antibody (ICA).

Why do we need new methods to detect islet autoimmunity?

Long-term target; To identify at-risk individuals in the general population.

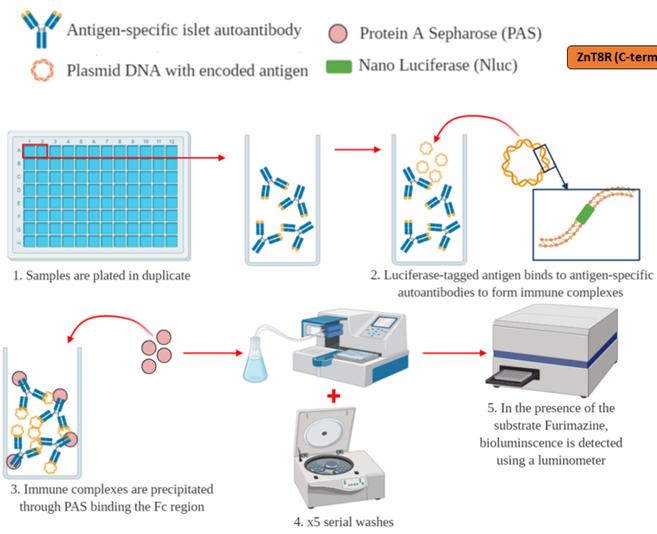
- ☐ cost-effective
- ☐ improved sensitivity
- ☐ non-radioactive for improved cost, safety, & environmental impact
- ☐ low sample volume for capillary sampling
- ☐ short-duration for high-throughput screening
- ☐ capacity for automation

Aim

To compare the performance of a novel optimised luciferase-based ZnT8 immunoassay (Nluc-ZnT8) with the "gold standard" radioimmunoassay (RIA-ZnT8).

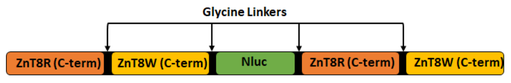
Methods

The LIPS Assay

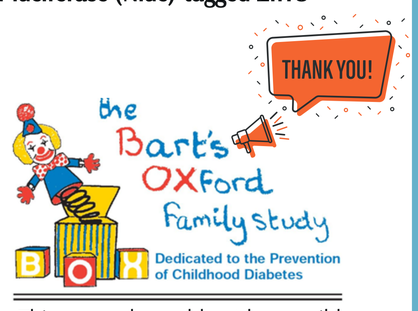


Luciferase Immunoprecipitation System (LIPS) Basic Method
Image created with BioRender.com

Nluc-ZnT8 Antigen



N-luciferase (Nluc)-tagged ZnT8

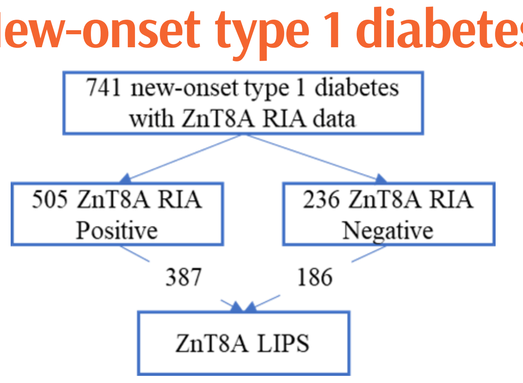


This research would not be possible without all the dedicated families of the BOX study est. 1985

Positivity threshold & units of ZnT8A

Positivity thresholds were set at the 97.5th percentile of 523 healthy schoolchildren (range 9.0-14years) in the RIA and Nluc-ZnT8 assays. Arbitrary units (AU) were derivedfrom a logarithmic standard curve.

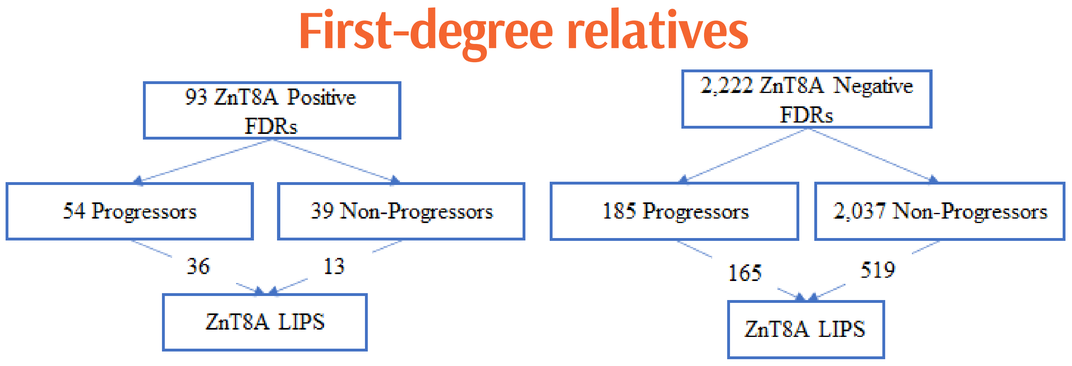
New-onset type 1 diabetes



Subjects with new-onset type 1 diabetes (sampled within 3 months of onset)

RIA-ZnT8 positive new-onset subjets were tested by Nluc-ZnT8 [n=573; 318 male; median diagnosis age 11.0 years (range 1-55)].

First-degree relatives



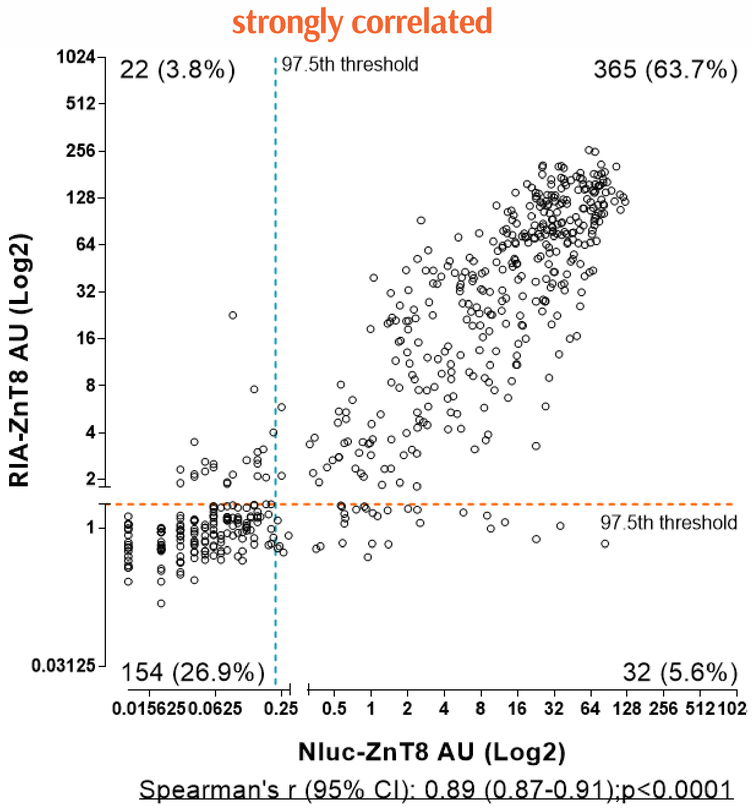
First-degree relatives of individuals with type 1 diabetes with long-term follow-up previously found positive or negative by RIA were randomly selected

RIA-ZnT8 positive relatives [n=49; 26 male; median age at sample 15.2 years (range 1.6-60.0); median follow-up 3.7years]. RIA ZnT8A negative relatives, [n=689; 361 male; median age at sample 34.3 years (range 1.3-66.0); median follow-up 16.5 years].

Results

New-onset type 1 diabetes

1) Levels of ZnT8A in RIA-ZnT8 & Nluc-ZnT8 are strongly correlated

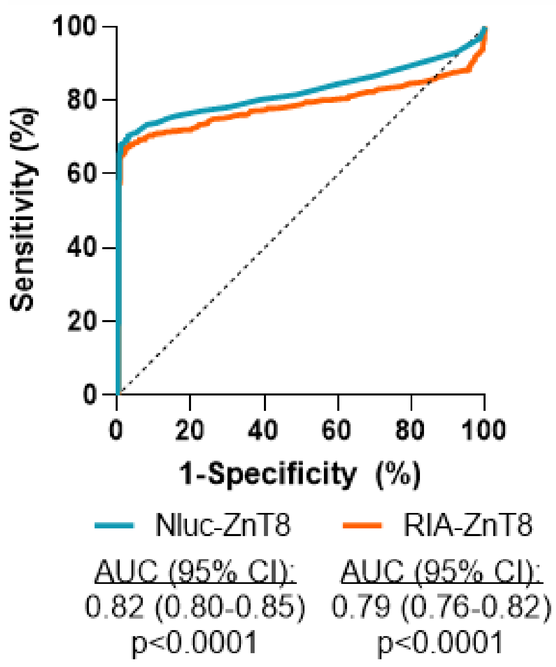


Spearman's r (95% CI): 0.89 (0.87-0.91);p<0.0001

Levels of ZnT8A determined by Nluc-ZnT8 & RIA

80% of discrepant samples had low-level ZnT8A (<3 AU) which may be explained by differences in assay detection limits & assay format.

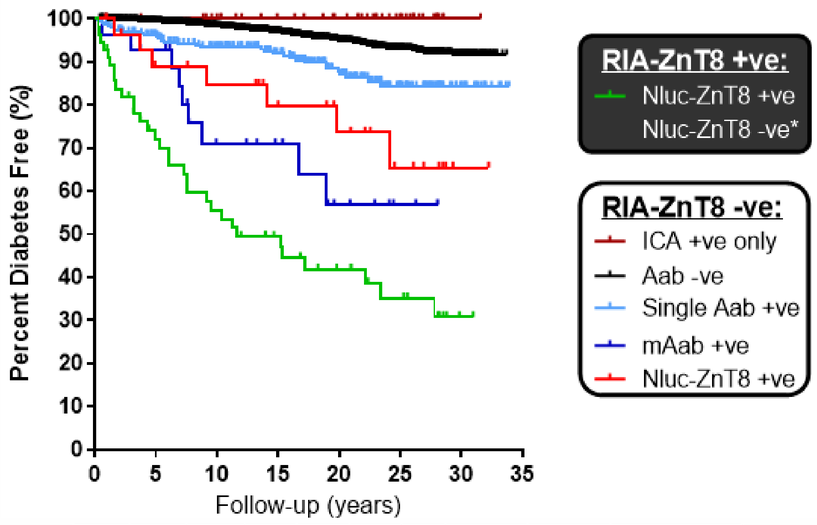
2) Nluc-ZnT8 has improved sensitivity compared with RIA-ZnT8



Receiver operating characteristic (ROC) analysis of Nluc-ZnT8 & RIA

Area under the curve (AUC)

First-degree relatives



	0	5	10	15	20	25	30	P value	20-yr diabetes risk (%)	Category
n	56	35	27	19	15	10	2	§	58.1	Nluc-ZnT8 +ve
n	4	4	4	4	2	1	1	*	*	Nluc-ZnT8 -ve
n	46	45	39	35	30	15	2	<0.0001	0.0	ICA +ve only
n	4287	3822	3379	2704	1679	877	167	<0.0001	5.1	Aab -ve
n	296	247	220	178	118	54	13	<0.0001	12.9	Single Aab +ve
n	28	25	15	11	7	3	1	NS	43.2	MAab +ve
n	30	24	21	16	13	8	2	0.0044	26.3	Nluc-ZnT8 +ve

* Only 2/4 positive by RIA but negative by Nluc-ZnT8 later progressed to disease & is not plotted for resolution
§ Reference category for Mantel-Cox test

Predicting type 1 diabetes risk in first-degree relatives

Kaplan-Meir survival curves to predict disease risk & the Mantal-Cox test to compare survival curves between categories. Samples tested by Nluc-ZnT8 and RIA by ZnT8A status (positive/negative) were compared to other major biochemical autoantibody markers determined by routine RIA or indirect immunofluorescence (ICA; historically used) to assess the long-term disease risk in relatives.

3) In relatives positive by both assays, the 20-year diabetes risk was 58%

4) Nluc-ZnT8 identified a subset of individuals with a 20-year diabetes risk of 26% that was not identified by RIA-ZnT8

5) Only 2/4 individuals found positive by RIA-ZnT8 but not by Nluc-ZnT8, progressed to disease after a 20-year follow-up*

Conclusion & Future Work

Advantages of Nluc-ZnT8 over RIA

- ✓ Cheaper with comparable consumables & methodology
- ✓ Offers improved sensitivity & can discrminate diabetes risk
- ✓ Non-radioactive - safer with less environmental impact
- ✓ Lower sample volume required ; 2ul vs 4ul
- ✓ Quicker; 1-day vs 2-day
- ✓ Automation possible - enhanced scalability

The Nluc-ZnT8 assay can replace RIA & will facilitate large-scale population screening

References:

(1) Wenzlau, J.M., et al., The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes. Proc Natl Acad Sci U S A, 2007. 104(43): p. 17040-5. (2) Achenbach, P., et al., Autoantibodies to zinc transporter 8 and SLC30A8 genotype stratify type 1 diabetes risk. Diabetologia, 2009. 52(9): p. 1881-8.

Acknowledgments

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