Validation and Clinical Application of a ddPCR Unmethylated Insulin cfDNA Methylation Assay

Marshall Jaroch, Patricia Barbalho, Jennifer Anderson, Robert Mons, Matthew Benson, Ioan Cucoranu, Pamela Clark, Kevan Herold, Clifford Morris
KIHEALTH, INC.

American Diabetes Association. 2026 SCIENTIFIC SESSIONS

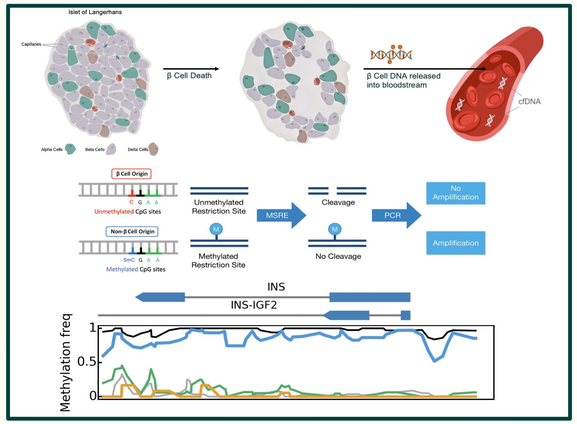
FINANCIAL DISCLOSURES

All authors are employees, consultants, advisors, or collaborators of Kihealth, Inc. Kihealth developed the ddPCR unmethylated INS cfDNA methylation assay evaluated in this study. The assay and associated data are proprietary to Kihealth, Inc. No external or federal funding was used for this poster.

BACKGROUND

APPROACH: Kihealth's multiplex ddPCR assay quantifies differentially unmethylated INS cfDNA across three CpG regions using methylation-sensitive restriction enzyme digestion and the Bio-Rad QX200 platform. The multi-site design supports strong analytical performance.

IMPACT: Standard biomarkers such as HbA1c, C-peptide, and autoantibodies do not directly measure beta-cell injury. Because beta cells contain unmethylated INS CpG regions while INS is largely methylated in non-beta-cell tissues, unmethylated INS cfDNA provides a non-invasive, quantitative readout for beta-cell death.



OBJECTIVES

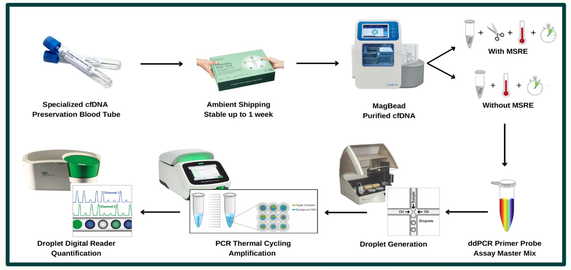
AIM 1: Analytically validate a multiplex ddPCR assay for quantifying differentially unmethylated INS cfDNA as a non-invasive biomarker of beta-cell injury.

AIM 2: Assess assay performance across three beta-cell-associated INS CpG regions

AIM 3: Evaluate clinical signal detection across disease state, treatment response, and beta-cell/islet-directed intervention datasets.

METHODOLOGY

ANALYTICAL: Peripheral blood specimens were processed for plasma or serum cfDNA extraction using an automated low-abundance cfDNA workflow, followed by QC and normalization. Differentially unmethylated INS cfDNA was determined by multiplex ddPCR. Parallel HpaII methylation-sensitive restriction enzyme and mock-treated reactions were performed for each sample. ddPCR used target-specific primer/probe sets and Poisson-based absolute quantification.



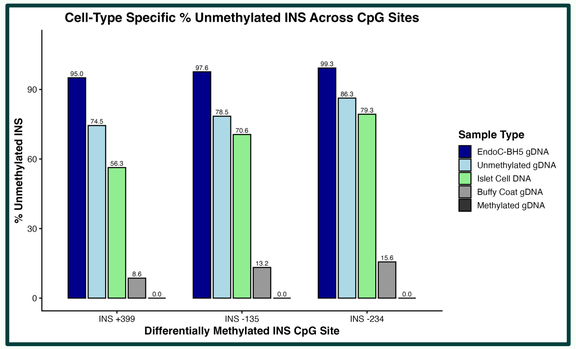
VALIDATION: Following CLIA guidelines used methylated /unmethylated human gDNA controls, mixed methylation standards, EndoC-βH5 beta-cell DNA, synthetic gBlocks, no-template controls, and donor-derived cfDNA. Studies assessed accuracy, precision, linearity, LOD, specificity, recovery, carryover, reference range and stability.

CLINICAL: Datasets included residual biobank longitudinal treatment samples and islet infusion recipient samples provided by Melena Bellin. Samples were analyzed across placebo and treated arms, pre-infusion baselines, 15-minute to 24-hour post-infusion timepoints, and follow-up through Day 28. All human specimen collection and analysis were conducted under Advarra IRB-approved protocol with written informed consent as required.

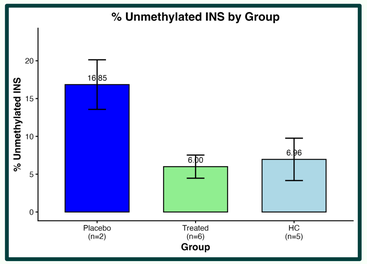
RESULTS

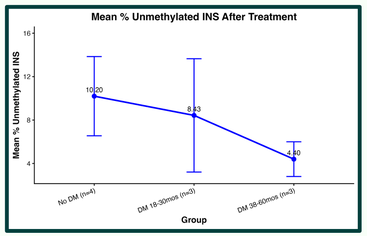
ASASY PERFORMANCE: Validated, reproducible, and low-abundance capable. Five-day accuracy and precision met acceptance criteria, all within 20%. Linearity was maintained from 2.0E+03 to 1.25 copies/µL, with average R^2 >0.9999 and an established LOD of 4.08 copies/µL.

The three-site INS methylation panel showed strong unmethylated signal in beta-cell DNA sources, including EndoC-BH5 and islet cell DNA, with minimal signal in fully methylated gDNA. This supports beta-cell specificity of the assay and demonstrates low expected leukocyte-derived background.

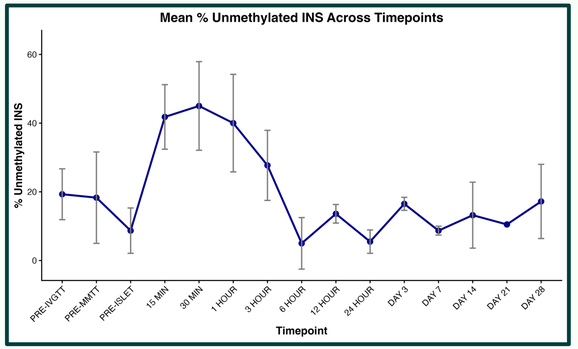


CLINICAL RESPONSE SIGNAL: Treatment response separation was clear. Mean % unmethylated INS cfDNA was higher in placebo than treated participants and healthy controls: Placebo 16.85 ± 3.28%, Treated 6.00 ± 1.53%, HC 6.96 ± 2.80%. Mean % unmethylated INS declined from 10.20 ± 3.65% in non-diabetic controls to 8.43 ± 5.22% at 18–30 months and 4.40 ± 1.60% at 38–60 months after treatment.





ISLET TRANSPLANT SIGNAL: Rapid beta-cell injury signal captured within minutes. Mean % unmethylated INS increased from 8.7 ± 6.6% pre-islet to a sharp early peak of 45.0 ± 12.9% at 30 minutes, then rapidly declined to 5.0 ± 7.5% at 6 hours and 5.5 ± 3.4% at 24 hours.



CONCLUSIONS

1. **CONFIRMED PERFORMANCE** of Multiplex ddPCR reliably quantified differentially unmethylated INS cfDNA with strong accuracy, precision, specificity, linearity, and low-abundance detection.
2. **STRENGTHENED BETA-CELL SIGNAL** of three site interrogation of INS reduces single-locus dependence and improved confidence in beta-cell-derived cfDNA detection.
3. **CAPTURED CLINICAL DYNAMICS** of unmethylated INS cfDNA changed coherently across treatment-response and beta-cell/islet infusion datasets, reflecting beta-cell injury, stress, and therapeutic effect.
4. **TRANSLATIONAL UTILITY** of the assay supports non-invasive beta-cell injury monitoring for clinical research, treatment-response assessment, and beta-cell-directed development.

REFERENCES

- Akirav EM, Lebastchi J, Galvan EM, Henegariu O, Akirav M, Ablamunits V, Lizardi PM, Herold KC. Detection of β cell death in diabetes using differentially methylated circulating DNA. *Proc Natl Acad Sci USA*. 2011;108:19018-19023. doi:10.1073/pnas.1111008108.
- Usmani-Brown S, Lebastchi J, Steck AK, Beam CA, Herold KC, et al. Analysis of β-cell death in type 1 diabetes by droplet digital PCR. *Endocrinology*. 2014;155(9):3694-3698. doi:10.1210/en.2014-1150.
- Herold KC, Usmani-Brown S, Ghazi T, Lebastchi J, Beam CA, et al. β cell death and dysfunction during type 1 diabetes development in at-risk individuals. *J Clin Invest*. 2015;125(3):1163-1173. doi:10.1172/JCI78142.
- Lebastchi J, Deng S, Lebastchi AH, Beshar I, Gitelman S, Willi S, et al. Immune therapy and β-cell death in type 1 diabetes. *Diabetes*. 2013;62(5):1676-1680. doi:10.2337/db12-1207.
- Speake C, Yleszcupidez A, Neiman D, Shemer R, Glaser B, Tersey SA, Usmani-Brown S, Clark P, Wilhelm JJ, Bellin MD, Herold KC, Mirmira RG, Dor Y, Evans-Molina C. Circulating unmethylated insulin DNA as a biomarker of human beta cell death: A multi-laboratory assay comparison. *J Clin Endocrinol Metab*. 2020;105(3):781-791. doi:10.1210/clinem/dgaa008.
- Bellin MD, Clark P, Usmani-Brown S, Dunn TB, Beilman GJ, Chinnakotla S, et al. Unmethylated insulin DNA is elevated after total pancreatectomy with islet autotransplantation: Assessment of a novel beta cell marker. *Am J Transplant*. 2017;17(4):1112-1118. doi:10.1111/ajt.14054.