Docket #: S24-512

Enhanced Calibration Method for CRISPR-Based Diagnostics

Stanford scientists have developed a novel calibration method to improve the accuracy and sensitivity of CRISPR-based diagnostic assays. This technology addresses a critical challenge in the field of molecular diagnostics by accounting for background signal and reducing false positive results in CRISPR-Cas systems that use fluorescent reporter molecules.

CRISPR-based diagnostics have gained significant attention for their potential in detecting specific nucleic acid sequences. However, these systems face limitations due to the low kinetic rates of CRISPR enzymes and associated low sensitivity of many CRISPR-based assays. The current invention provides a calibration technique including an appropriate control experiment and accurate treatment of background fluorescence. The method is also useful in accurate extraction of kinetic rate parameters. This approach significantly improves sensitivity of measurements and differentiation between positive and negative samples, especially at low target concentrations.

Stage of Development: Proof of concept - *in vitro* data

Applications

- Molecular diagnostics using CRISPR-Cas systems
- Nucleic acid detection in research and clinical settings
- Highly sensitive diagnostic tools for diseases
- Accurate evaluation of CRISPR enzyme orthologs for assay design

Advantages

- Applicable to most existing CRISPR-based diagnostic platforms

- Reduces false positive results
- Requires no additional reagents or significant protocol modifications
- Enables accurate quantification at low concentrations
- Enables accurate evaluation of assay-specific kinetic rate parameters

Publications

- Avaro, Alexandre S., Andrew D. Griffiths, and Juan G. Santiago. Degradation of Reporter Molecules Imposes a Fundamental Limit of Detection on CRISPR Diagnostics. *Analytical Chemistry* , 2025.

Innovators

- Alexandre Avaro
- Juan Santiago

Licensing Contact

Michael Spaid

Technology Licensing Associate 2

Email