

Towards a Metrological Validation of a Real-Time Impedance-Based System for Cellular Proliferation Analysis Using A549 Cells

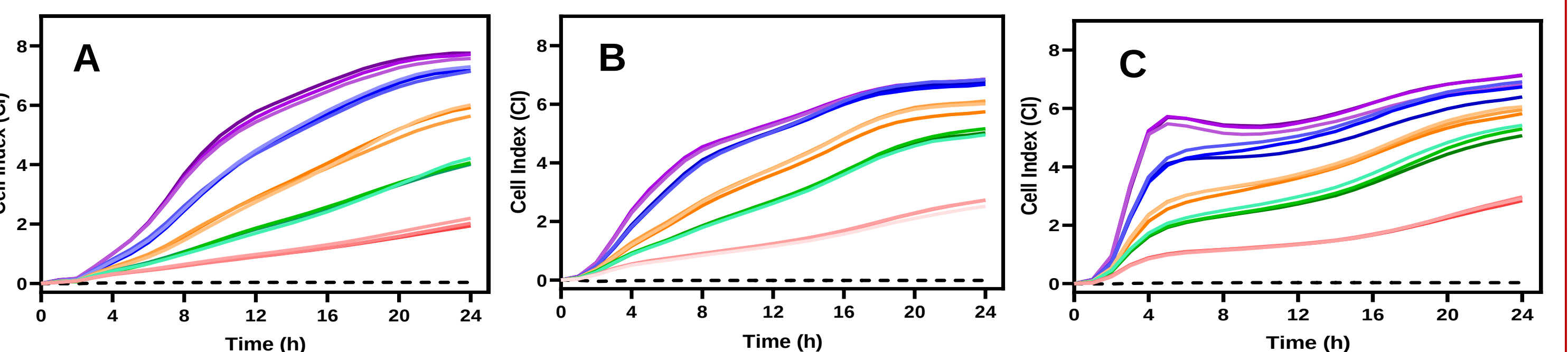
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RESULTS

Leveraging the **ISO 20391-2** standard for suspended cells, we expanded its applicability to adherent cell systems, ensuring compliance with metrological principles in cell-based assays. Prior to experimentation, operators conducted the ISO-mandated **pipetting integrity test**, achieving results within the predefined acceptance range. Subsequently, A549 cells were seeded into E-Plate 96 wells at controlled dilution fractions (DF) ranging **from 0.15 to 0.44**, ensuring precise initial conditions. Cell Index (CI) measurements were acquired at hourly intervals over a 24-hour period, providing real-time kinetic data on cellular adhesion, proliferation, and morphology.

Figure 1. Proliferation curve of A549 cells using different dilution fractions on separate days, performed by Operator A, with each DF conducted in triplicate. (A) Experiment 1; (B) Experiment 2; (C) Experiment. Dilution fraction color legend: 0.15 0.22 0.28 0.37 0.44 -- Culture medium control



For method validation, key performance metrics were assessed, including **trend analysis (R²)**, **proportionality index (PI)**, **precision (%CV)**, and **selectivity (t-test)**. Data analysis was conducted using the **COMET** tool to ensure robust statistical evaluation of method performance and reproducibility.

Figure 2. Graphical analyses provided by **COMET**. (A) Average value of the three experiments conducted for the coefficient of determination (R²). (B) Proportionality Index (PI) – calculated based on the absolute error of the smoothed stepped sum for each experiment.

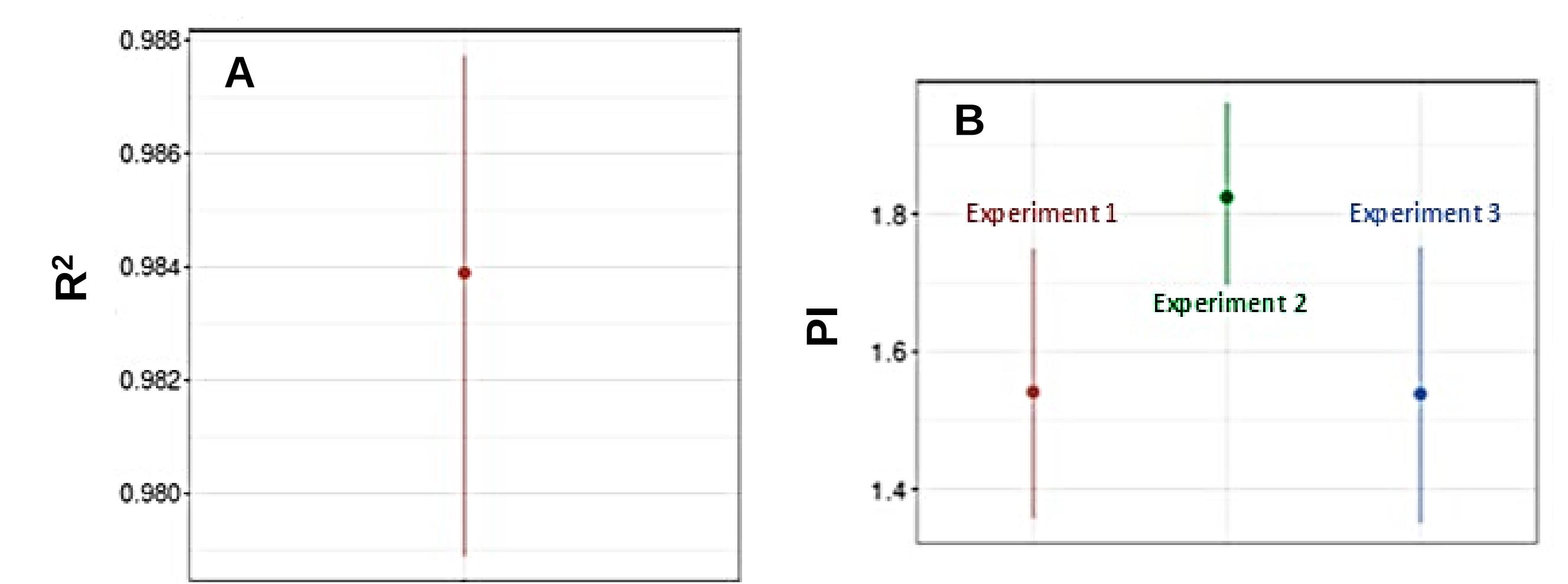


Figure 3. Coefficient of percentage variation for (A) precision, based on Operator A's experiments, and (B) intermediate precision, assessed across two different operators. Data are presented as mean ± 95% confidence intervals from independent experiments. Inset: Comparative analysis of the results.

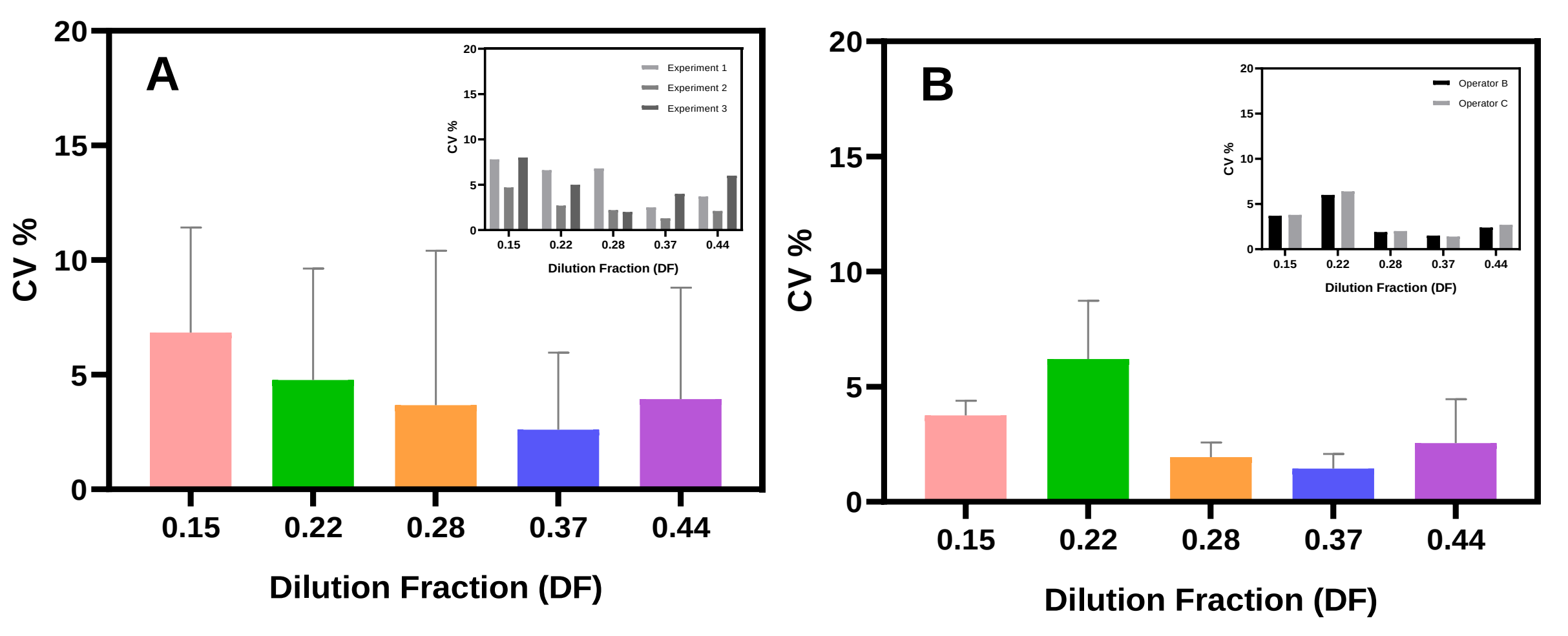


Figure 4. Proliferation curve of A549 cells under varying debris concentrations and respective controls. The medium control contains DMEM-high with FBS, the debris control consists of 100% lysate from 1×10⁴ cells/mL, and the cell control is debris-free. (A) Cell proliferation over time. (B) Average CI at 24 hours with a 95% confidence interval.

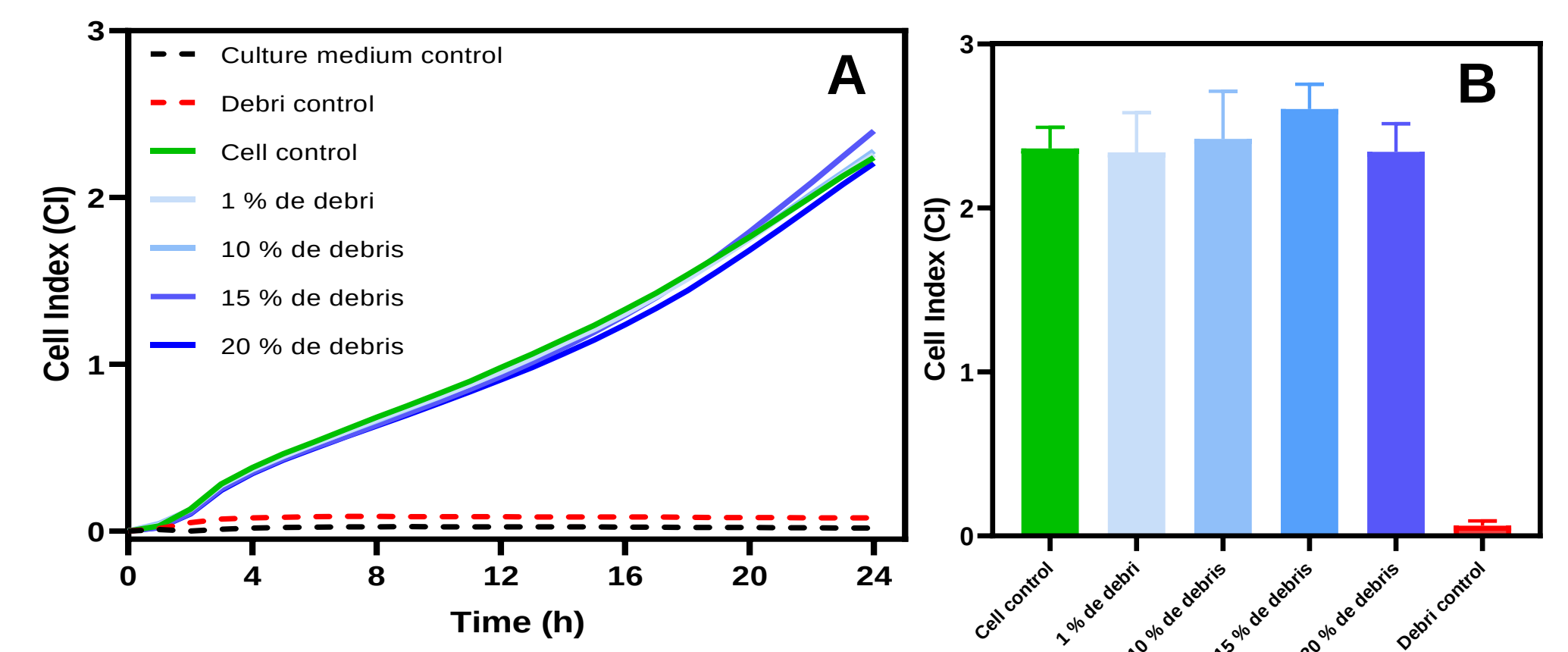


Table 1. Evaluation of experimental results and their respective acceptance criteria

Metrics	Acceptance criterion	Average result	Analytical Performance (Acceptable/Unacceptable)
Trend (coefficient of determination)	R ² ≥ 0.980	0.984	Acceptable
	PI ≤ 2.0	1.6	Acceptable
Precision	CV _{mean} ≤ 20 %	4.4 %	Acceptable
Intermediate Precision ¹	CV _{mean} ≤ 20 %	3.6 %	Acceptable
Method Selectivity	t < 2.01	t ₁ % - 0.067	Acceptable
		t ₁₀ % - 0.043	
		t ₁₅ % - 0.044	
		t ₂₀ % - 0.314	

¹ Average of three operators.

The results confirmed the method's reliability, precision, and selectivity, with trend analysis (R²) demonstrating high consistency, minimal variation in repeatability tests, and robust reproducibility across operators. Furthermore, selectivity tests indicated that cellular debris had no significant impact on CI measurements, reinforcing the method's robustness for real-time impedance-based cytotoxicity assessments.

CONCLUSION

We conclude that the method is well-suited for robust and reproducible cellular proliferation analysis. Ongoing efforts are directed toward refining uncertainty quantification to enhance reliability further and support its application in nanotoxicity studies. This validation highlights the critical role of metrological approaches in biological systems and contributes to advancing standardized methodologies for toxicity screening, especially nanomaterials, which already have evidence, for example, of interference with colorimetric methods.

REFERENCE

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Acknowledgements

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