Supplementary Information (SI) for Lab on a Chip.

Supplementary Material

Materials and Methods

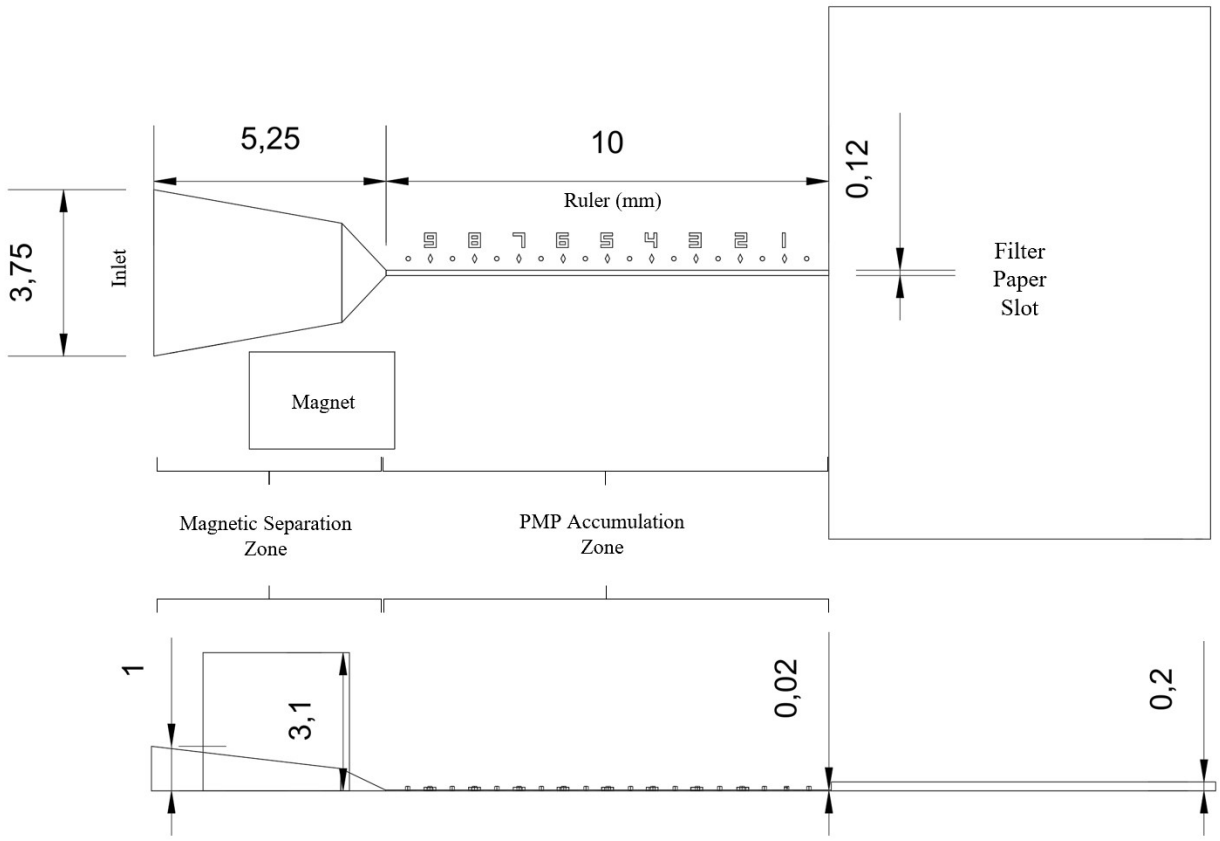

Fig. S1. Geometrical design of the microfluidic chip. Top view (Top) and side view (Bottom). Unit: mm.

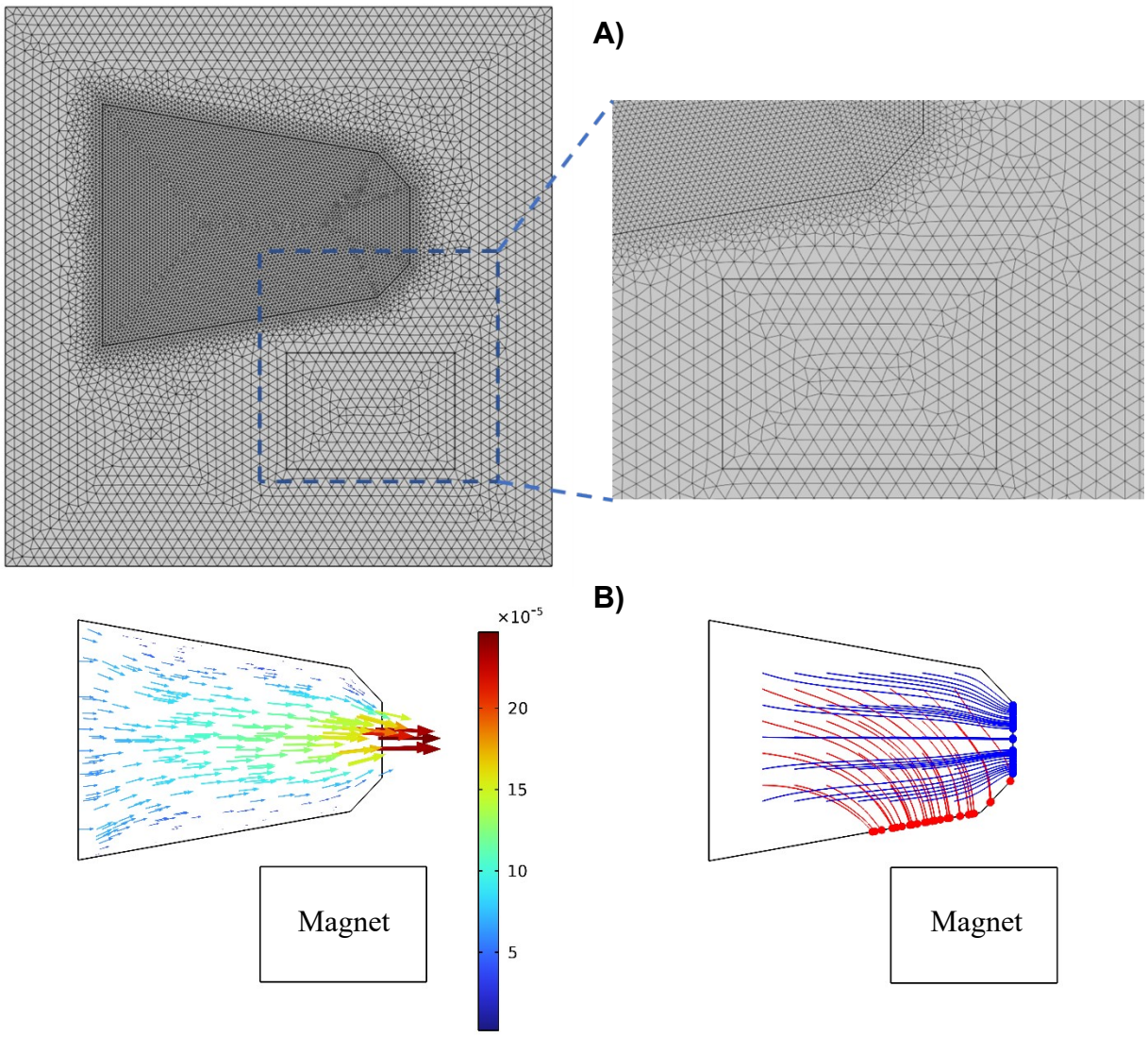

Fig. S2. Computational fluid dynamic simulation showing an effective magnetic separation in the inlet. (A) Mesh used in computational fluid dynamics. (B) Flow velocity fields shown by arrows with length and color indicating velocity magnitude. (C) Effective magnetic separation showing that MMPs (red) are efficiently deflected from the mainstream and attracted toward the sidewall under the magnetic force, while PMPs (blue) exhibit no response to the magnetic field and are carried along the streamlines to the outlet.

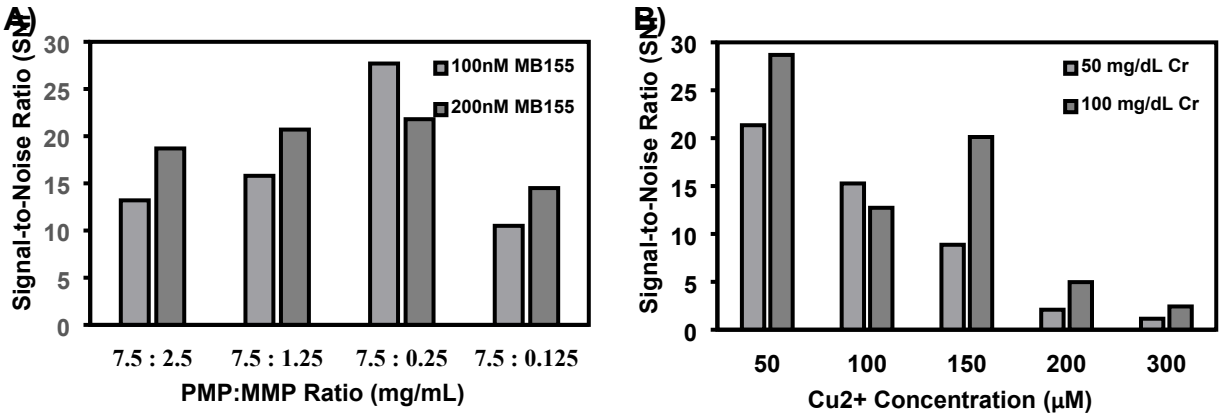

Fig. S3. Signal-to-noise ratio assessment. (A) Optimization of PMP:MMP ratio in Fig. 2B. (B) Optimization of Fenton's reaction using various Cu^{2+} concentrations in Fig. 2E.

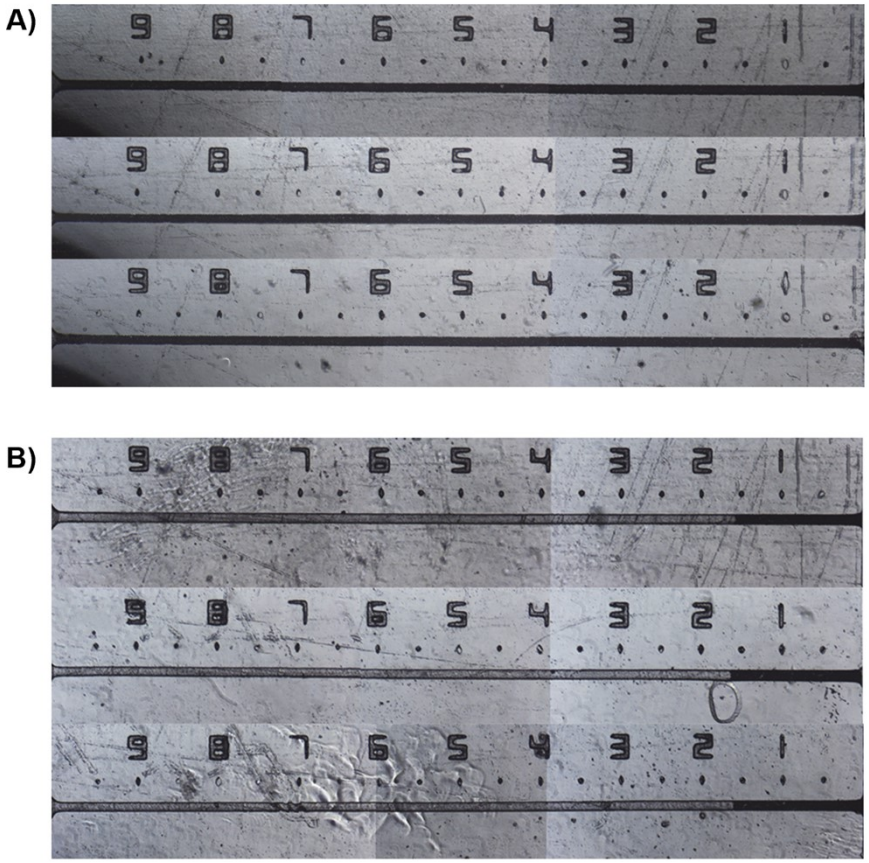

Fig. S4. Optical image of PMP accumulation demonstrating intra-batch and inter-batch precision. (A) PMP accumulation at 0 mg/dL creatinine on three chips cast from a single qualified master mold (intra-batch precision). (B) PMP accumulation at 100 mg/dL creatinine (ULOQ) on three chips cast on three independently 3D-printed master molds (inter batch precision). All six chips were measured on the same day, using the same reagent batch, and performed by the same operator.

Table S1. Normal physiological concentrations of major urinary components and their levels in the synthetic urine buffer used in this study.

Component	Normal Physiological Range (mg/24h)	Normal Physiological Range (mg/dL)*	Concentrations used in this our synthetic urine buffer (mg/dL)	Ref.
Creatinine	M: 955-2936	63.7-195.7* 20 – 320[1]	0-400	[1] [2]
	F: 601–1689	40.1-112.6* 20 – 275[1]		
Urea	10,000- 23,000	667-1533	1501	[3-5]
Uric Acid	<750	<50	16.811	[1]
Ammonia	255.45 – 272.48	17-18.2	36.077	[1]
L-Ascorbic Acid	<10-60	0.67-4	0	[6, 7]
Sodium	942 – 5228	62.8-348.5	68.969	[1]
Potassium	664.5-3010	44.3-200.7	117.295	[1]
Chloride	1418-7941	94.5-529.4	283.624	[1]

* : mg/dL range is calculated assuming of daily urine output of 1.5L [1]

Table S2. The sequence of oligonucleotides used in this work

Strand Name	Sequence
P1	5'-/biotin/CCCCTATCACG-3'
P2	5'-ATTAGCATTAA-/biotin/-3'
MB155	5'-TTAATGCTAATCGTGATAGGGG-3'

Table S3. Upper Limit of Quantification (ULOQ)

Nominal x (mg/dL)	Predicted x^ (mg/dL)	Accuracy% (Bias)	CV% (Statistical SDx)	ULOQ Status (Acc≤15% AND CV≤15%)
10	9.67	3.30	11.75	PASS
20	19.96	0.20	5.46	PASS
40	43.85	9.63	2.35	PASS
50	50.86	1.72	2.02	PASS
75	75.29	0.39	1.41	PASS
100 (ULOQ)	98.08	1.92	1.18	PASS
200	105.83	47.09	1.14	FAIL (Accuracy)
400	111.57	72.11	1.11	FAIL (Accuracy)

Linear Regression Equation

Using the linear regression model equation of $y = b_0 + b_1 x$; y represents the PMP accumulations length observed from the microfluidic device while x is the concentrations of urine creatinine. The equation constants b_0 and b_1 represents both the intercept and gradient of the regression model. To obtain the limit of detection, variances of the intercept s_{b_0}, the gradient s_{b_1}, as well as the regression $s_{\frac{y}{x}}$ needs to be considered and they can be obtained with the equations below:

$$\bar{x} = \sum_{i=1}^{n} \frac{x_i}{n} \quad (1)$$

$$\bar{y} = \sum_{i=1}^{n} \frac{y_i}{n} \quad (2)$$

$$b_0 = \bar{y} + b_1 \bar{x} \quad (3)$$

$$b_1 = \frac{\sum_{i=1}^{n} (x_i - \bar{x})}{\sum_{i=1}^{n} (x_i - \bar{x})^2} \quad (4)$$

$$s_{b_0}^2 = s_{\frac{y}{x}}^2 \left(\frac{1}{n} + \frac{\bar{x}^2}{\sum_{i=1}^{n} (x_i - \bar{x})^2} \right) \quad (5)$$

$$s_{b_1}^2 = \frac{s_{\frac{y}{x}}^2}{\sum_{i=1}^{n} (x_i - \bar{x})^2} \quad (6)$$

$$s_{\frac{y}{x}}^2 = \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{n-2} \quad (7)$$

In the equations above, $x_i \, and \, y_i$ indicates the individual *x* (creatinine concentrations) and *y* (PMP accumulation lengths) values obtained from experiments, and *n* Is the total number of data points which includes the repetition for the same concentration points which can be illustrated with the equation $n = \sum_{j=1}^{k} m_j$; where k is the number of measurements in the *x* axis (concentration levels) and m_j is the repetition at each concentration level. Therefore, we can express the calibration curve as:

$$y = b_0 + b_1 x \pm t_{(\alpha, n-2)} s_{\frac{y}{x}} \left(\frac{1}{m} + \frac{1}{n} + \frac{(x - \bar{x})^2}{\sum_{i=1}^{n}(x_i - \bar{x})^2}\right)^{\frac{1}{2}} \quad (8)$$

Where $t_{(\alpha, n-2)}$ is the critical value of t distribution where we opted for a confidence level of 90% in the two-tailed hypothesis setting (α = 0.05) where we selected the value of 1.645 and $\frac{1}{m}$ is the contribute of uncertainty of m replicates in the future observation.

Limit of Detection

For the limit of detection estimation x_D, a non-central t-distribution model chosen for this study can be described with the below equation:

$$x_D = \delta_{(\alpha,\beta,n-2)} \frac{s_{\frac{y}{x}}}{b_1} \left(1 + \frac{1}{n} + \frac{\bar{x}^2}{\sum_{i=1}^{n}(x_i - \bar{x})^2}\right)^{\frac{1}{2}}$$

where $\delta_{(\alpha,\beta,n-2)}$ is the non-centrality value of the non-central t-distribution which considers both type I (α, false-positive) and type II (β, false-negative) errors. The other variables in the equation

were calculated with the equations in the previous section. All of the LODs was determined based on the appropriate linear range evaluated by the R^2.

Upper Limit of Quantification (ULOQ)

The upper limit of quantification is defined as the highest concentration of an analyte that can be determined with acceptable accuracy and precision in a given analytical method. Unlike the Lower Limit of Quantification (LLOQ) or Limit of Detection (LOD), which have standard formulas based on the blank/noise signal and the standard deviation, the ULOQ is usually determined as the highest concentration standard in the calibration curve that still meets predefined acceptance criteria for accuracy and precision (e.g., Accuracy ≤ ±15% and Precision CV% ≤15%).

The Accuracy% quantifies the systematic error or bias between the value of the method vs the actual concentration of the standard. It is used to check whether the calibration model correctly translates to the measured signal (y) into the concentration (predicted at x) at the high end of the range. Which means that a high accuracy% (e.g., 47% at the 200 concentration) means that the linear model is not valid at that concentration because it is consistently reporting the wrong value. Similarly, the CV% test how much the results vary if you measure the exact same sample multiple times. If the CV% is high (e.g., 50%), it means that the measurement is unreliable and that you will get a different answer every time you run the sample.

The Accuracy or Bias percentage can be derived by the following formula:

$$Accuracy\% = \frac{|Average\ Predicted\ Concentration\ (\hat{x}) - Nominal\ Concentration\ (x)|}{Nominal\ Concentration\ (x)} \times 100$$

Where x is the known concentration of the standard, $\hat{x}$ is the average concentration back-calculated by the linear model from the measured responses.

The CV% equation is given by the following equation of

$$CV\% = \frac{SD_x}{\hat{x}} \times 100$$

Where CV% is the coefficient of variation, SD_x is the standard deviation of the predicted concentration, and $\hat{x}$ is the predicted concentration.

Furthermore, the SD_x can be derived by the following equation:

$$SD_x = \frac{S_y}{|b_1|}\sqrt{\left(\frac{1}{m}+\frac{1}{n}+\frac{(\hat{y}-\bar{\bar{y}})^2}{b_1^2\sum_{i=1}^{n}(x_i-\bar{x})^2}\right)}$$

Where S_y is the standard error of the estimate, b_1 is the slope of the linear regression line, m is the number of replicates for the measured sample at the ULOQ concentration, n is the total number of data points used in the entire calibration curve, $\hat{y}$ is the predicted response corresponding to the ULOQ measurement, $\bar{\bar{y}}$ is the average of all PMP accumulation length, and $\bar{x}$ is the average of the standard concentrations.

Power Analysis

Power analysis is the calculation commonly used to determine the minimum sample size needed for a research study. Power analysis calculation is derived from the following equation:

$$n \approx \frac{(z_{1-\propto/2} + z_{1-\beta})^2 \times s_d^2}{\delta^2}$$

To calculate the required minimum sample size, certain parameters need to be firstly determined

- Significance Level (α) : 0.05 (standard). Which makes $z_{1-\propto/2} = 1.96$
- Desired Power (1 – β) : usually set at 80% (0.80) or 90% (0.90).
    - For 80% power, $z_{1-\beta} = 0.84$
    - For 90% power, $z_{1-\beta} = 1.28$
- Expected Variability (s_d) : 9.84 mg/dL (based on the clinical data from this study)
- Target Bias (δ) : 10 mg/dL [8-10]

Based on the equation above, we can determine the required samples for different desired statistical power based on the table below

Desired Statistical Power	Required Samples (n)	Logic
80%	8	Minimum standard for research
90%	11	Stronger evidence
99.3% (This study)	21	>99% power

Bland-Altman Analysis

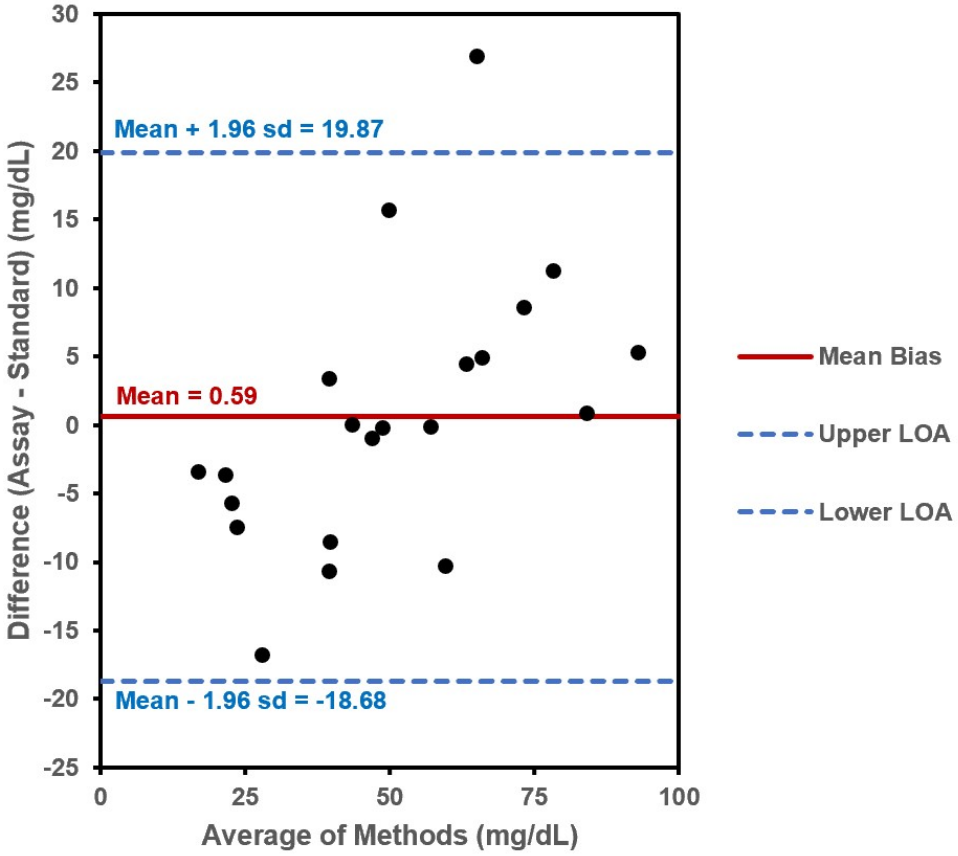

Fig. S5. Bland-Altman analysis of the microfluidic creatinine assay compared with the Jaffe Kinetic Method.

To evaluate the agreement between the developed microfluidic creatinine assay and the clinical gold standard (Jaffe's kinetic method). A Bland-Altman analysis was performed using 21 clinical urine samples. In this analysis, the difference between the concentrations measured by the two methods ($C_{microfluidic}$ - C_{Jaffe}) was plotted against the mean concentration of the two methods to identify any systematic bias or concentration-dependent errors.

As shown in Fig. S5, the analysis revealed a mean bias of 0.59 mg/dL, indicating that the microfluidic assay provides results consistent with the Jaffe method without significant over- or underestimation. The 95% limits of agreement (LOA), calculated as the mean bias ± 1.96 SD, ranged from -18.68 to 19.87 mg/dL. Statistically, 20 out of the 21 samples (95.2%) fell within these limits, which shows excellent agreement with the theoretical expectation for a 95% confidence interval. The single sample falling slightly outside the LoA is attributed to the complex nature of the CKD patient urine matrix, which may contain higher levels of interfering metabolites.

Overall, the mean bias remains well within the clinical target bias of 10 mg/dL [8], confirming that the microfluidic platform is a reliable and rigorous tool for point-of-care creatinine monitoring.

References

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