Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

Dry storage of multiple reagent types within a paper microfluidic device for phenylalanine monitoring

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Storage of colorimetric reagents mPMS and NBT

Differences between the mean signals of stored reagents and freshly-dried reagents are displayed. The difference in means was calculated as follows, $difference\ in\ means = (freshly\text{-}dried\ samples\ mean) - (stored\ samples\ mean)$, and then plotted for various storage times. The error bars display the 99% confidence intervals using the t-distribution. Degrees of freedom were estimated using the Welch-Satterthwaite approximation.

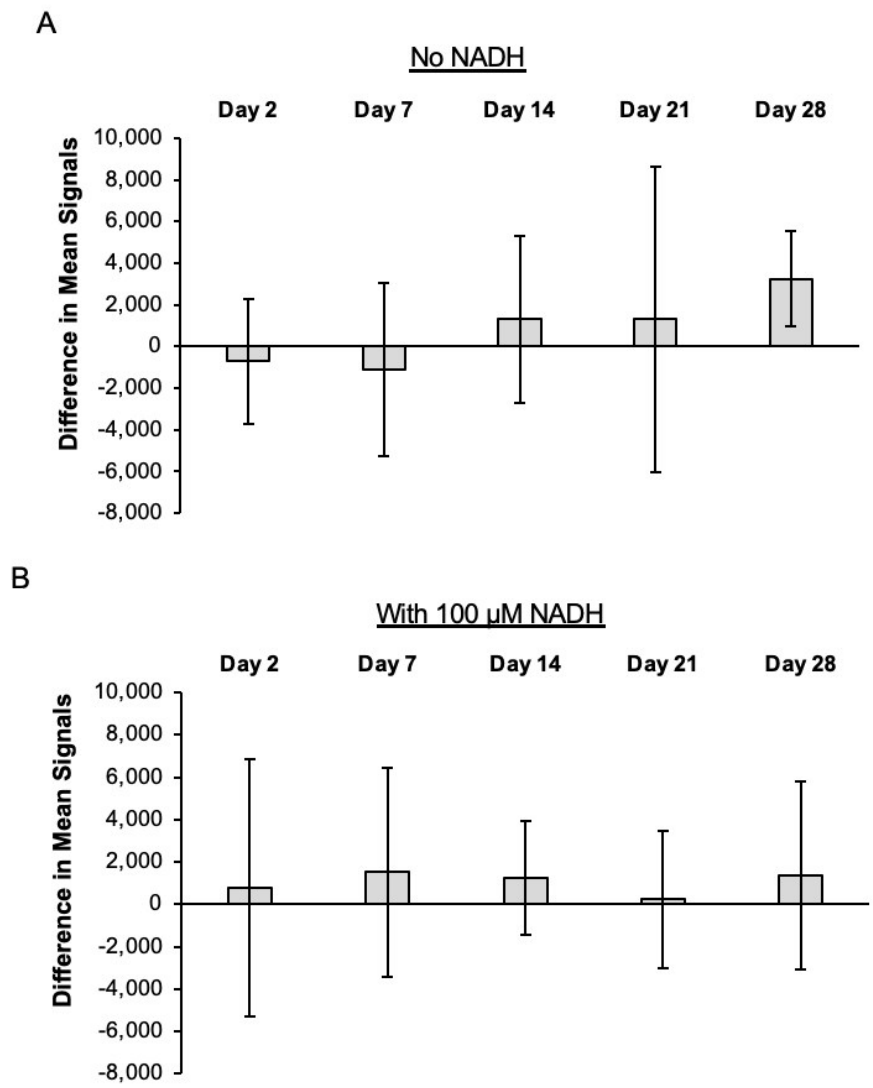

Figure S1: Difference in mean signals from stored colorimetric reagents and freshly-dried colorimetric reagents. A sample of DI water only (no NADH) was used to rehydrate the colorimetric reagents in (A), while a sample of 100 µm NADH was used to rehydrate the colorimetric reagents in (B). Error bars represent 99% confidence intervals ($N = 3$). As discussed in the article, by day 28, the mean signal from the stored colorimetric reagents with "No NADH" was significantly different ($\alpha = .01$) from the mean signal from the freshly-dried colorimetric reagents with "No NADH".

Comparison of PheDH activity from different source organisms

The effect of vacuum drying on the activity of two PheDHs, one from *Sporosarcina* sp. (Sigma-Aldrich) and another from *Thermoactinomyces intermedius* (Creative Enzymes), was investigated. Each of the enzymes was prepared in buffer (220 mM BTP pH 9.3) at 60 U/mL (using manufacturer specifications for U/mg of enzyme). Enzyme in buffer (10 μL) was added to Eppendorf tubes ($N = 5$), and the open tubes were dried in a vacuum dryer for 23 hours. Samples were then rehydrated in buffer (220 mM BTP pH 9.3). The enzyme (final concentration 0.5 U/mL) was added to a 384 well plate with Phe (final concentration 40 mg/dL) and the reaction was initiated with NAD^+ (final concentration 5 mM). The 340 nm absorbance signal at 10 minutes was recorded in a plate reader (Synergy-2, BioTek, Winooski, WA, USA), and the absorbance from wells with PheDH and NAD^+ only, was subtracted. The background-corrected absorbance signal was compared to the background-corrected absorbance signal of the analogous reaction using enzyme that had not been dried.

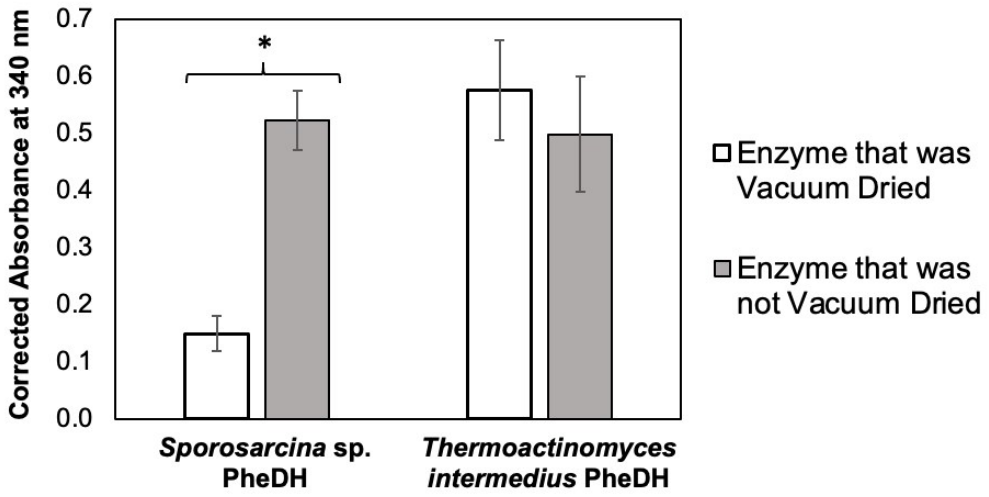

Figure S2: Comparison of the effect of vacuum drying PheDHs from different source organisms. Vacuum-dried PheDH from *Sporosarcina* sp. demonstrated a 71% reduction in activity compared to the corresponding *Sporosarcina* sp. PheDH that was not vacuum dried ($P = .0000046$). Vacuum-dried PheDH from *Thermoactinomyces intermedius* did not have a significantly different signal ($P = .2$) compared to the *Thermoactinomyces intermedius* PheDH that was not vacuum dried. Bars represent the background-corrected absorbance averages of replicates ($N = 5$) and error bars represent the standard deviation.

Stored PheDH enzyme activity over time

Differences between the mean signals of stored reagents and freshly-dried reagents are displayed. The difference in means was calculated as follows, $difference\ in\ means = (freshly\text{–}dried\ samples\ mean) - (stored\ samples\ mean)$, and then plotted for various storage times. The error bars display the 99% confidence intervals using the t-distribution. Degrees of freedom were estimated using the Welch-Satterthwaite approximation.

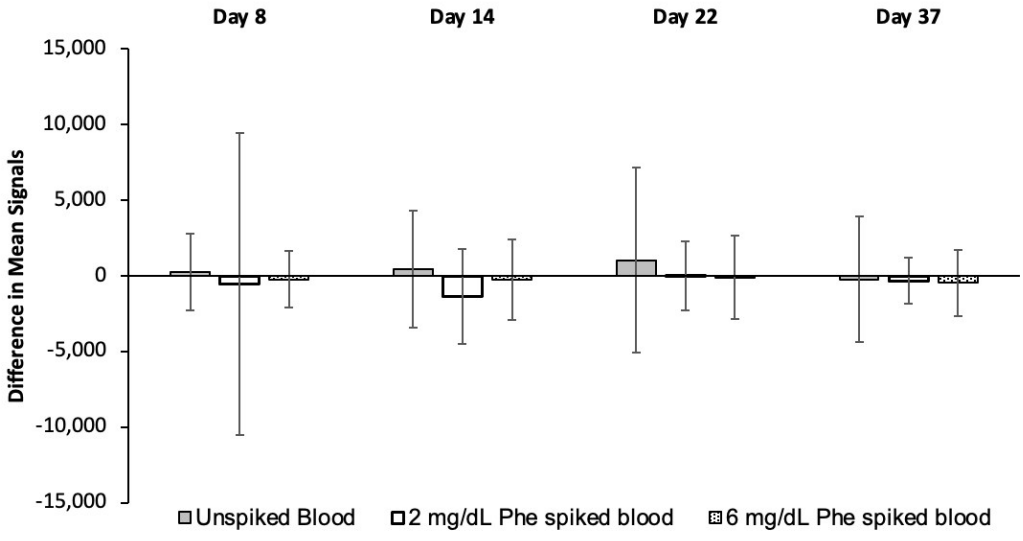

Figure S3: Difference in mean signals from stored PheDH and freshly-dried PheDH in full devices with all other reagents freshly dried. Devices were evaluated using samples of unspiked whole blood or whole blood spiked with 2 mg dL^{-1} or 6 mg dL^{-1} Phe after different storage times. Error bars represent 99% confidence intervals (N = 3 or 4).

Storage of coenzyme NAD$^+$

Differences between the mean signals of stored reagents and freshly-dried reagents are displayed. The difference in means was calculated as follows, $difference\ in\ means = (freshly\text{–}dried\ samples\ mean) - (stored\ samples\ mean)$, and then plotted for various storage times. The error bars display the 99% confidence intervals using the t-distribution. Degrees of freedom were estimated using the Welch-Satterthwaite approximation.

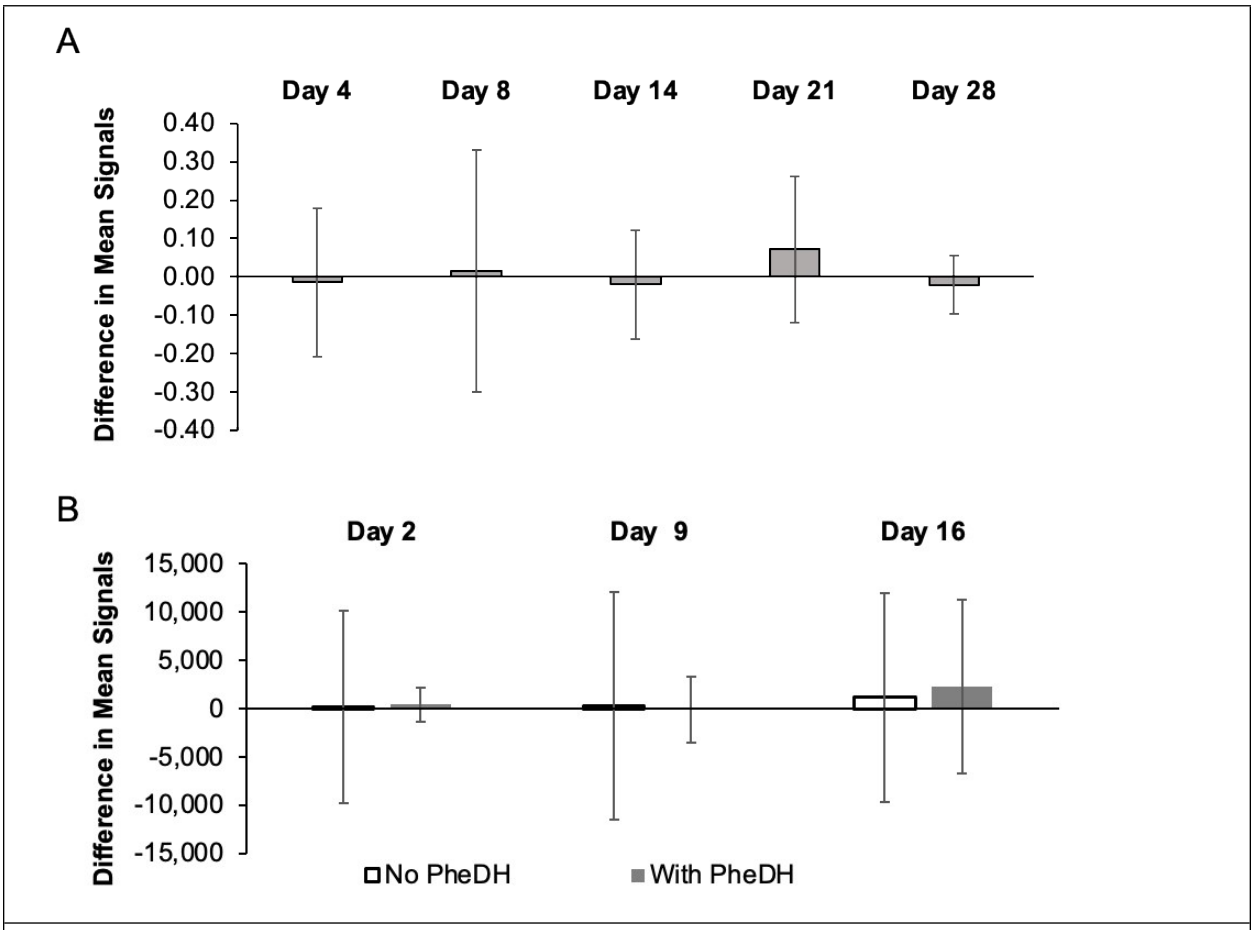

Figure S4: Difference in mean signals from stored NAD$^+$ and freshly-dried NAD$^+$. (A) NAD$^+$ stored in GR-PSM pads and rehydrated with water were evaluated using the enzymatic reaction (Equation 1) over a month. Error bars represent 99% confidence intervals (N = 4). (B) Full devices with pads stored with NAD$^+$ and all other reagents freshly dried were evaluated using samples of whole blood spiked with 6 mg dL^{-1} Phe after different storage times. Error bars represent 99% confidence intervals (N = 3).

Complete devices with all reagents stored dry

Differences between the mean signals of stored reagents and freshly-dried reagents are displayed. The difference in means was calculated as follows, $difference\ in\ means = (freshly\text{–}dried\ samples\ mean) - (stored\ samples\ mean)$, and then plotted for various storage times. The error bars display the 99% confidence intervals using the t-distribution. Degrees of freedom were estimated using the Welch-Satterthwaite approximation.

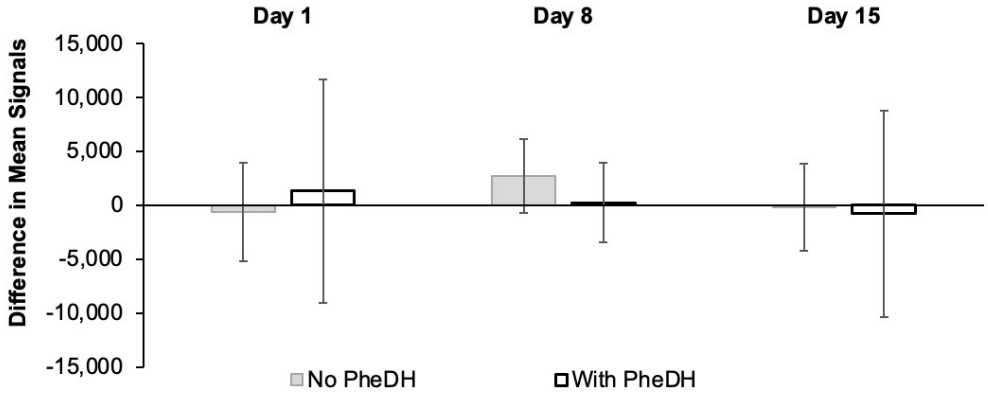

Figure S5: Difference in mean signals from pull-tab devices with stored reagents and devices with freshly-dried reagents. Devices were evaluated using samples of whole blood spiked with 6 mg dL^{-1} Phe after different storage times and compared to signals from devices with freshly dried reagents prepared on that day. Error bars represent 99% confidence intervals ($N = 3$).