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> Paper Test Card for Detection of Adulterated Milk Jamie L. Luther, Valentine Henry de Frahan, Marya Lieberman

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**Supplemental Figure S2.** ROC plot for sucrose test. 67 was the best threshold to categorize samples. Area under curve (AUC) = 0.945



**Supplemental Figure S3.** ROC plot for combination glucose-sucrose test. 63 was the best threshold to categorize samples after ImageJ quantification in the inverted channel. Area under curve (AUC) = 1.0



**Supplemental Figure S4.** ROC plot for combination glucose-sucrose test. A threshold of 69 from the inverted blue channel calibration curve was determined to not be a good threshold to categorize blind samples. Area under curve (AUC) = 1.0



**Supplemental Figure S5.** ROC plot for urease paper test. 40 was determined to be the best threshold to categorize samples. Area under curve (AUC) = 0.949



**Supplemental Figure S6.** Spoiled milk can be detected by the phenol red pH indicator (- urea). Spoiled milk spiked with 1000 mg/dL took 60 minutes to develop color (+ urea). If milk is spoiled, the test response may be delayed. Unspoiled milk included as a reference.



**Supplemental Figure S7**. ROC plot for starch test. Various thresholds were assessed for their ability to categorize blind samples. Area under curve (AUC) = 1.0



**Supplemental Figure S8.** Starch test stability over a 4 week period at 4C, 22C, or 37C. The mean intensity of plain 2% milk was subtracted from the mean intensity of a 1% potato starch milk solution. Each bar indicates the background subtracted mean intensity (n = 5). Dotted line is the mean intensity of a 1% (w/v) potato starch milk solution. Error bars = standard deviation



**Supplemental Figure S9.** Urea test stability over a 4 week period at 4C, 22C, or 37C. The mean intensity of plain 2% milk was subtracted from the mean intensity of a 70 mg/dL urea spiked milk solution. Each bar indicates the background subtracted mean intensity (n = 5). Dotted line is the mean intensity of a 70 mg/dL urea milk solution. Error bars = standard deviation



**Supplemental Figure S10**. Sucrose test stability after 4 week at 4C, 22C, or 37C. The mean intensity of plain 2% milk was subtracted from the mean intensity of a 4 mM sucrose spiked milk solution. Each bar indicates the background subtracted mean intensity (n = 5). Dotted line is the mean intensity of a 4 mM sucrose spiked milk solution. Error bars = standard deviation





**Supplemental Figure S11**. Glucose test stability after 4 weeks at 4C, 22C, or 37C. The mean intensity of plain 2% milk was subtracted from the mean intensity of a 4 mM glucose spiked milk solution. Each bar indicates the background subtracted mean intensity (n = 5). Dotted line is the mean intensity of a 4 mM glucose spiked milk solution. Error bars = standard deviation

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**Supplementary Figure S12**. Selectivity assay test strips developed with a. sodium bicarbonate, b. Tween 20 detergent, c. formalin, d. melamine, e. peroxide, and f. sodium chloride. A negative control (plain 2% milk) was run in parallel to milk solutions spiked with increasing concentrations of each adulterant. In each box from top to bottom: urea test strip, starch test strip, glucose test strip, sucrose test strip.

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**Supplementary Figure S13**. Selectivity assay quantification results. ImageJ was used to background subtract the mean intensity of a negative control (plain 2% milk) from samples of milk spiked with increasing concentrations of a. sodium bicarbonate, b. Tween detergent, c. formalin, d. melamine, e. peroxide, or f. sodium chloride. x-axis indicates test type, y-axis indicates mean intensity. Each bar represents a sample background subtracted mean intensity (n=1). Sample intensities were normalized to a positive control, which was 70 mg/dL urea milk solution (urea test), 1% (w/v) potato starch milk solution (starch test), 4 mM glucose milk solution (glucose test), or 4 mM sucrose milk solution (sucrose test).



**Supplemental Figure S14.** Light box setup. Picture a shows overall view of in-house light box. Picture b shows the inside of the light box. Light strips covered in paper are indicated. After test strip development, it is placed inside of the light box where indicated. The lid is then closed with the lights on. The camera phone lens is placed over the opening in the box lid as indicated in picture c, and an image is taken for analysis.