Stability of Folate and Hemoglobin in Dried Blood Spot Samples

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Introduction:

Dried Blood Spot Samples

•Dried blood spot samples are routinely collected from infants in the United States by spotting a small amount of capillary blood from the heel onto a specially designed filter paper card.

• This is done primarily to screen for treatable genetic diseases, including phenylketonuria. Leftover dried blood spot samples are often retained by state departments of health for several years. These spots offer a potential wealth of information for epidemiological research.

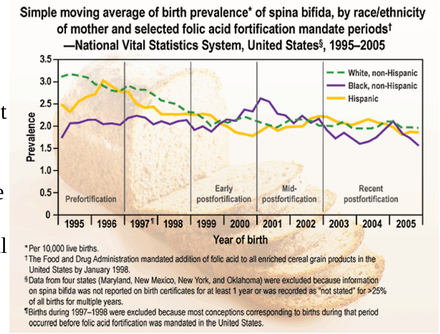
• Each state stores dried blood spot samples under different conditions of temperature and humidity. These factors are thought to influence the degradation of most metabolites of interest. Unlike DNA, which can be amplified by PCR to examine the content, the value in measuring many blood components including folate and hemoglobin is determining the concentration.

• Folate is known to protect against neural tube defects in infants. This is why folic acid supplementation is recommended for women of childbearing age, and many grain products are fortified with folate.

•Other effects of folate remain to be studied. It has been suggested that sufficient folate may protect against many other problems, and that excess folate might aggravate some conditions including cancer.

Folate is essential, but full effects are unknown

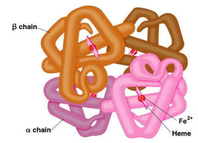
• Folate is a B vitamin that is essential for human life, and must be obtained from dietary sources since humans are unable to synthesize it. This graph from the CDC shows the decline in incidence of spina bifida, a neural tube defect, since the U.S. implemented grain fortification. However, excess folate may be harmful.



http://www.cdc.gov/Features/dsSpinaBifidaTrends/

Hemoglobin

Hemoglobin is a protein found in blood that coordinates with iron to transport oxygen throughout the body. Although infants and adults have slightly different forms of hemoglobin, they are similar and were both measured by spectrophotometry at 535nm in this experiment.



http://themedicalbiochemistrypage.org/hemoglobin-myoglobin.php

The hemoglobin-folate value is designed to standardize the folate concentration when the amount of blood in each sample varies. Hemoglobin is considered to be present at a roughly constant concentration in human blood, and thus useful for standardization.

Method:

•Healthy pregnant volunteers offered blood samples from their baby's umbilical cord to be spotted onto dried blood spot filter paper. This created four extra samples for research without drawing more blood from the baby.

•These dried blood spots were cut apart from each other and stored in four different conditions: -80 C, 4 C, room temperature, and a humid greenhouse. Samples were stored at the trial condition for nine months, then transferred to a -20 C freezer.

•Folate was measured using a microbiological assay.

•Hemoglobin was measured using a spectrophotometer set at the appropriate absorbance.

•Hemoglobin-folate values, which standardize the folate measurement for the amount of blood in each sample, were calculated from the two measured values.

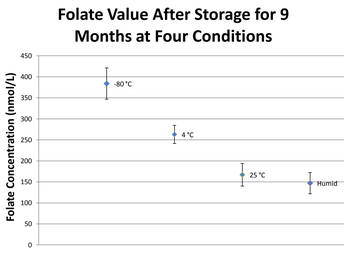
•We used a consistent sample size of saturated dried blood spot filter paper equivalent to about 12.5 microliters of whole blood. The concentration measurements assume that 12.5 microliters of whole blood was recovered from each filter paper sample.

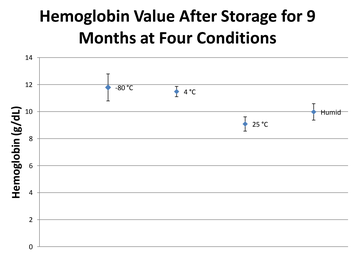
Results

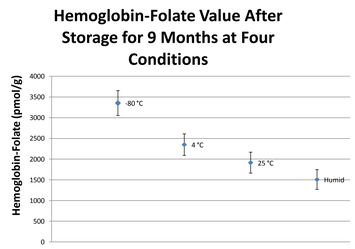
This experiment showed that folate values decreased significantly when dried blood spot samples were stored at warmer temperatures and with greater humidity. The folate values are shown with the 95% confidence intervals on the graph to the right.

The hemoglobin measurements were lower at room temperature and humid storage conditions than at -80°C and 4°C , but the differences between each set had overlapping confidence intervals. This experiment did not provide evidence that hemoglobin recovery is decreased by more humid storage conditions. The hemoglobin measurements are shown with the 95% confidence intervals on the graph to the right.

The hemoglobin-folate value was calculated from the measured hemoglobin and folate, and reflects a combination of the trends. It retains the general trend of the folate measurements, and is shown in the graph to the right.







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Rank Order

If the rank order is sufficiently retained despite differing storage conditions, then it would be possible to gain useful information about relative folate status between individuals whose samples were stored at the same condition. General estimating equation analysis of the rank orders showed that the folate rank order slope had a 95% confidence interval from -3 to 3. This indicates that in the sample of 50 individuals, 95% moved no more than 3 ranks up or down when measured at different conditions. The image to the right gives a visual depiction of the random mixing in rank between conditions. The shade of gray in each box represents the rank within its condition, and each row represents an individual.

The GEE analysis shows that the rank order is less well retained with the hemoglobin-folate data, with slope 95% confidence intervals from -3.9 to 3.9. The hemoglobin rank order was retained with the same calculated amount of mixing as the folate measurements.

-80 ° C	4 ° C	Ambient	Humid
140	51	25	43
152	192	95	57
159	124	115	116
185	143	96	39
237	159	107	35
246	233	189	28
246	170	93	54
254	187	111	115
260	174	102	94
274	177	105	119
275	223	109	118
280	245	129	69
293	179	104	115
312	261	148	79
315	190	156	137
322	307	151	120
329	173	166	177
336	244	238	187
348	244	142	172
358	227	114	69
359	164	72	58
361	280	146	107
364	269	151	142
368	188	91	63
368	226	175	160
373	235	162	166
374	317	216	71
378	237	140	122
378	211	136	158
384	352	223	262
408	194	136	82
410	377	270	226
416	295	144	161
417	269	211	198
442	327	300	211
444	490	278	293
452	115	124	120
458	328	208	193
462	377	269	244
466	381	206	198
470	367	240	195
475	315	167	129
480	296	169	198
481	321	171	125
571	374	185	276
580	337	211	279
585	335	215	219
655	311	220	262
656	412	278	185
847	657	340	328

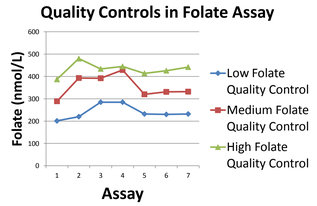
Sources of Variation

Each sample was allotted four wells for the microbiological folate assay at two different concentrations to provide verification of results on two different locations on the standard curve. There was variation between the results in each sample's four wells, but if the CV% of the variation between the wells exceeded 15, the sample was measured again.

	-80 °C	4 °C	Ambient	Humid
Average CV%	8.4	9.0	9.4	10.5

The variability in the folate concentration measurements of the controls between assays demonstrates that the microbiological assay has some variation based on factors associated with each assay.

The standard deviations of the quality control samples were 32, 50, and 29 nmol/L respectively, which is still small compared to the folate measurements in hundreds. The relatively constant standard deviation may explain the increased variability in samples with lower measurements.



Summary and Future Directions

This experiment demonstrated that folate in dried blood spot samples is sensitive to storage temperature and humidity. However, the retention of rank order indicates that samples stored at unfavorable conditions retain useful information about relative folate status that could be used for epidemiological research. Our results indicate that hemoglobin follows a different stability trend than folate with storage at varying conditions, which decreases the value of the hemoglobin-folate calculation when dealing with saturated dried blood spot filter paper of a consistent size.

Further experiments could confirm the trend in folate stability, and clarify the stability of hemoglobin at varying temperature and humidity. It would also be helpful to examine the amount of folate recovered after varying amounts of time at each storage condition., since samples are usually added to storage continuously as babies are born.