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A Solution for Ambient Storage of DNA

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Abstract

The need for a solution for room temperature storage of DNA is well known. Studies were conducted to assess stability of EBV DNA using Daykin Molecular Solutions GeneGuardDNA (Georgia). All laboratory testing was done at Prime Laboratories in Van Nuys California. The objective is to demonstrate sample stability of EBV DNA during shipping, storage, and multiple freeze/thaw cycles. To achieve the objectives, the study was divided into two parts; Part 1 to demonstrate stability during shipping and multiple freeze/thaw cycles. Samples were labeled as P1-1 (Positive sample 1, P1-2 first freeze/thaw, P1-3 second freeze/thaw, etc.). In Part 1 the standard deviation between positive samples ranged from 0.08 CTs to 0.25 CTs with no gradual deterioration detected with more freeze/thaw cycles). The average was 0.17 CT's which is within the instrument's precision range. In the past we have had no instance of repeating the same extracted sample more than two times. In Part 2 we demonstrated stability during storage. 185 archived clinical samples stored at room temperature from 2013 through 2017 were randomly chosen. DNA was extracted from these samples and EBV DNA assay testing was performed. The results from the new test were compared to the original results obtained for the samples. Concordance and p values were calculated to show the effects of long-term storage on the results of the EBV DNA assay. Pearson correlation coefficient between the original and recent assay results was 0.9564 with a p value of $p<0.001$ indicating statistically significant correlation between the two assay results. EBV DNA showed no deterioration of sample Ct signal was observed due to Freeze/Thaw cycles and the results were stable across multiple cycles for a period of 6 years at room temperature.

Keywords: DNA; Stability; Ambient Storage

Introduction

Over a billion specimens of research materials, including DNA, RNA, cells, clones, tissue, organ products, blood, buccal, mucosal, vaginal, and other swabs, are stored globally, many of which are both critical and often irreplaceable for scientific research. Researchers within these laboratories have assembled very large collections of biological samples from clinical and field studies, stored in laboratories within institutes, commercial organizations, and University systems. More than 40,000 of these facilities exist within the US alone. These specimens represent enormous scientific and financial value for the researcher and these organizations. The collection and storage costs per sample range from a few dollars to as high as $10,000, The overwhelming majority of samples are stored in cold temperature systems and are susceptible to loss or degradation due to a variety of well-known circumstances [1].

Several advantages have been ascribed to room-temperature sample storage over the current cold-temperature-based storage. Perhaps the most significant benefit is around increased sustainability of biological research when samples are transitioned from cold freezers to ambient room temperature. This process lowers energy consumption, reducing the carbon footprint (CO2 emission), lower the costs associated with equipment purchase and maintenance, lower material costs, and provide better utilization and optimization of lab space gained by retiring the cold freezers. A study by G. Jensen at Stanford University demonstrated in a Pilot Study demonstrated Stanford University could reduce its carbon footprint by eighteen thousand metric tons and save $16 million dollars in operating costs over the next ten years by transferring biological samples from frozen storage to room storage technology [2]. This equates to billions of dollars savings and millions of tons of carbon reduction globally and a dramatic reduction in risk of loss of critical Materials. DNA is particularly susceptible to degradation by hydrolytic and oxidative endogenous nucleases which if not prevented, break down DNA strands into fragments that may not be useful for PCR analysis [3-5]. While enzyme activity with the attendant DNA degradation may be limited by adjusting the ambient pH or salt concentration, cryopreservation is the preferred method of DNA protection. Deep freezing with dry ice or liquid nitrogen is often not convenient or feasible in an ambulatory setting such as in a physician's office or at a remote clinic and is expensive and not environmentally sustainable.

Therefore, a need exists for a simple method to preserve specimens, at ambient temperatures, during transportation and long-term storage for eventual recovery of high quality, intact DNA. We demonstrate that Gene Guard DNA (Daykin Molecular Systems) is a nucleic acid transport media that is suitable for the interim preservation of nucleic acid(s) in the field, during transport to the laboratory from an ambulatory or remote clinic, and for general research laboratory storage. The solution consists of a combination of compounds including TRIS, EDTA, and NaCl. The concentration of EDTA chelates all divalent cations rendering Type I nucleases nonfunctional. The high concentration of NaCl not only causes cell creation and membrane disruption but also stops Type II nuclease activity completely and facilitates dissociation of proteins. This combination of high EDTA and NaCl concentration virtually stops all nuclease activity. The Tris buffer stabilizes and maintains the pH of the solution preventing degradation of the DNA at acid pH and prevents the precipitation of EDTA and NaCl.

Material and Methods

Studies were performed to assess stability of EBV DNA using Daykin Molecular Solutions Gene Guard DNA (Georgia). All laboratory testing was done at Prime Laboratories in Van Nuys California.

DNA Stability

The objective is to demonstrate sample stability of EBV DNA during shipping, storage, and multiple freeze/thaw cycles. To achieve the objectives, the study was divided into two parts; Part 1 to demonstrate stability during shipping and multiple freeze/thaw cycles and Part 2 to demonstrate stability during storage.

Part 1: The samples were collected at the clinic and then preserved in the Gen Guard DNA during shipping, which ranged from 2 to 5 days. DNA extraction was performed 12 hours after receiving the samples, and the DNA was stored at 4oC. Five clinically positive samples were chosen for this study. After extraction the samples were each divided into 5 equal parts and they were subjected to up to 5 freeze/thaw cycles in the following manner: one part was run without freeze/thaw, another part with one cycle of freeze/thaw, the third part with 2 cycles of freeze/thaw, etc. All sample parts were run simultaneously. The standard deviation was calculated between each sample part to determine if there was a variation and signal drop due to freeze/thaw cycles.

Part 2: 185 archived clinical samples stored at room temperature from 2013 through 2017 were randomly chosen. DNA was extracted from these samples and EBV DNA assay testing was performed. The results from the new test were compared to the original results obtained for the samples. Concordance and p values were calculated to show the effects of long-term storage on the results of the EBV DNA assay.

Result

DNA Stability

i. **Part 1**

The following are the results obtained from each set of Freeze/Thawed samples: (Table 1)

Table 1: The following are the results obtained from each set of Freeze/Thawed samples.

Well	Sample	Reporter	Ct	STDEV	Well	Sample	Reporter	Ct	STDEV
A1	P1_1	FAM	23.8	0.14	A1	P1_1	VIC	26.9	0.04961
A3	P1_2	FAM	23.4		A3	P1_2	VIC	26.3	
A5	P1_3	FAM	23.5		A5	P1_3	VIC	26.5	
A7	P1_4	FAM	23.6		A7	P1_4	VIC	26.6	
A9	P1_5	FAM	23.5		A9	P1_5	VIC	26.5	
A11	P1_6	FAM	23.5		A11	P1_6	VIC	26.3	
B1	P2_1	FAM	23.8	0.13	B1	P2_1	VIC	24.9	0.140523
B3	P2_2	FAM	23.5		B3	P2_2	VIC	24.5	
B5	P2_3	FAM	23.4		B5	P2_3	VIC	24.6	
B7	P2_4	FAM	23.5		B7	P2_4	VIC	24.7	
B9	P2_5	FAM	23.6		B9	P2_5	VIC	24.6	
B11	P2_6	FAM	23.5		B11	P2_6	VIC	24.7	
C1	P3_1	FAM	20.2	0.08	C1	P3_1	VIC	23.6	0.068707
C3	P3_2	FAM	20.1		C3	P3_2	VIC	23.5	
C5	P3_3	FAM	20.1		C5	P3_3	VIC	23.5	
C7	P3_4	FAM	20.2		C7	P3_4	VIC	23.5	
C9	P3_5	FAM	20.1		C9	P3_5	VIC	23.6	
C11	P3_6	FAM	20.2		C11	P3_6	VIC	23.5	
D1	P4_1	FAM	21.7	0.4	D1	P4_1	VIC	22	0.31247
D3	P4_2	FAM	21.7		D3	P4_2	VIC	22.2	
D5	P4_3	FAM	21.4		D5	P4_3	VIC	22.2	
D7	P4_4	FAM	21.5		D7	P4_4	VIC	22.2	
D9	P4_5	FAM	21.3		D9	P4_5	VIC	22.2	
D11	P4_6	FAM	21.1		D11	P4_6	VIC	22.2	
E1	P5_1	FAM	23.5	0.25	E1	P5_1	VIC	23	0.072477
E3	P5_2	FAM	23		E3	P5_2	VIC	22.8	
E5	P5_3	FAM	23		E5	P5_3	VIC	22.9	
E7	P5_4	FAM	22.9		E7	P5_4	VIC	22.9	
E9	P5_5	FAM	22.9		E9	P5_5	VIC	22.9	
E11	P5_6	FAM	22.8		E11	P5_6	VIC	22.8	
AVERAGE				0.1					0.1

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Samples were labeled as P1-1 (Positive sample 1, P1-2 first freeze/thaw, P1-3 second freeze/thaw, etc.). The standard deviation between positive samples ranged from 0.08 CTs to 0.25 CT's with no gradual deterioration detected with more freeze/thaw cycles). The average was 0.17 CT's which is within the instrument's precision range. In the past we have had no instance of repeating the same extracted sample more than two times.

ii. Part 2

The following are the results obtained from the archived samples compared to the original sample results: (Table 2)

When concordance was calculated between the archived and recent results, it was shown that there was high concordance between them. The following chart summarizes the concordant samples for each category with the concordance calculation underneath the numbers. The highlighted diagonal results indicate the concordance values for the Abnormal samples (94.12), Normal samples (98.58) and Equivocal samples (70.0) (Figure 1). Pearson correlation coefficient between the original and recent assay results was 0.9564 with a p value of $p<0.001$ indicating statistically significant correlation between the two assay results.

Table 2: The following are the results obtained from each set of Freeze/Thawed samples.

Archived Results			Recent Results		
Sample	**Original EDL**	**Interpretation**	**Sample**	**EDL**	**Interpretation**
1	0.3	Normal	1	0	Normal
2	3	Abnormal	2	2.8	Abnormal
3	1.3	Normal	3	0.8	Normal
4	0.6	Normal	4	0.2	Normal
5	0	Normal	5	0	Normal
6	0	Normal	6	0	Normal
7	2.4	Equivocal	7	2.5	Equivocal
8	0.9	Normal	8	0.1	Normal
9	0.1	Normal	9	0	Normal
10	0	Normal	10	0	Normal
11	3	Abnormal	11	2.9	Abnormal
12	0	Normal	12	0	Normal
13	0.8	Normal	13	0.7	Normal
14	0.3	Normal	14	0.3	Normal
15	0	Normal	15	0	Normal
16	0.2	Normal	16	0	Normal
17	0	Normal	17	0	Normal
18	2.7	Abnormal	18	2.8	Abnormal
19	0	Normal	19	0	Normal
20	0.1	Normal	20	1.3	Normal
21	2.7	Abnormal	21	2.7	Abnormal
22	0	Normal	22	0	Normal
23	0.1	Normal	23	0	Normal
24	2.3	Equivocal	24	2.3	Equivocal
25	2.4	Equivocal	25	2.6	Equivocal
26	0	Normal	26	0	Normal
27	3.8	Abnormal	27	3.8	Abnormal
28	0	Normal	28	0	Normal
29	0	Normal	29	0	Normal
30	0.8	Normal	30	0.8	Normal

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31	0.2	Normal	31	0	Normal
32	0	Normal	32	0	Normal
33	0	Normal	33	0	Normal
34	0.4	Normal	34	0	Normal
35	0	Normal	35	0	Normal
36	0	Normal	36	0	Normal
37	2.5	Equivocal	37	2.4	Equivocal
38	0.7	Normal	38	0.4	Normal
39	0	Normal	39	0	Normal
40	1.2	Normal	40	0.8	Normal
41	1.8	Equivocal	41	1.9	Equivocal
42	2.4	Equivocal	42	2.8	Abnormal
43	0.1	Normal	43	0	Normal
44	1.2	Normal	44	1.1	Normal
45	1	Normal	45	1	Normal
46	0	Normal	46	0	Normal
47	2.4	Equivocal	47	2.5	Equivocal
48	0	Normal	48	0	Normal
49	0	Normal	49	0	Normal
50	0	Normal	50	0	Normal
51	0.2	Normal	51	0.5	Normal
52	0	Normal	52	0	Normal
53	0	Normal	53	0	Normal
54	0.9	Normal	54	1.1	Normal
55	0.7	Normal	55	0.4	Normal
56	1.3	Normal	56	0.3	Normal
58	0	Normal	58	0	Normal
59	0	Normal	59	0.2	Normal
60	2.9	Abnormal	60	3.5	Abnormal
61	3.9	Abnormal	61	2.5	Abnormal
62	0.1	Normal	62	0.1	Normal
63	0	Normal	63	0	Normal
64	0	Normal	64	0	Normal
65	0	Normal	65	0	Normal
66	3.9	Abnormal	66	4.1	Abnormal
67	0	Normal	67	0	Normal
68	0	Normal	68	0	Normal
69	0	Normal	69	0	Normal
70	0	Normal	70	0.4	Normal
71	2.8	Abnormal	71	0	Normal
72	0	Normal	72	0	Normal
73	2.3	Equivocal	73	2.7	Abnormal
74	0	Normal	74	0	Normal

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75	0	Normal	75	0	Normal
76	2.8	Abnormal	76	3.2	Abnormal
77	0	Normal	77	0	Normal
78	0	Normal	78	0	Normal
79	0	Normal	79	0.2	Normal
80	0	Normal	80	0	Normal
81	0	Normal	81	0.2	Normal
82	0	Normal	82	0	Normal
83	1	Normal	83	0	Normal
84	0.8	Normal	84	1.1	Normal
85	3	Abnormal	85	3.7	Abnormal
86	3.6	Abnormal	86	3.5	Abnormal
87	0	Normal	87	0	Normal
88	0	Normal	88	0	Normal
89	0.4	Normal	89	0.6	Normal
90	0	Normal	90	0	Normal
91	0	Normal	91	0	Normal
92	0.8	Normal	92	0.7	Normal
93	0.6	Normal	93	0.5	Normal
94	0	Normal	94	0	Normal
95	0.7	Normal	95	0.8	Normal
96	0.8	Normal	96	0.9	Normal
97	3.2	Abnormal	97	2.5	Abnorma
98	0	Normal	98	0	Normal
99	3.9	Abnormal	99	3.4	Abnormal
100	0.1	Normal	100	0	Normal
101	0.4	Normal	101	0.6	Normal
102	0	Normal	102	0	Normal
103	3.4	Abnormal	103	3.3	Abnormal
104	0	Normal	104	0	Normal
105	0	Normal	105	0	Normal
106	2.6	Equivocal	106	2.7	Abnormal
107	0.9	Normal	107	1	Normal
108	1.3	Normal	108	1.3	Normal
109	1.1	Normal	109	1.7	Equivocal
110	0.1	Normal	110	0.4	Normal
111	0.2	Normal	111	0	Normal
112	0.3	Normal	112	0	Normal
113	0.6	Normal	113	0.4	Normal
114	4	Abnormal	114	4.1	Abnormal
115	0	Normal	115	0	Normal
116	0	Normal	116	0	Normal
117	0	Normal	117	0	Normal

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118	0	Normal	118	0	Normal
119	0.6	Normal	119	0.5	Normal
120	3.9	Abnormal	120	3.7	Abnormal
121	0.5	Normal	121	0.1	Normal
122	0.7	Normal	122	0.4	Normal
123	3.4	Abnormal	123	3.1	Abnormal
124	3	Abnormal	124	1.9	Equivocal
125	0.4	Normal	125	0	Normal
126	4	Abnormal	126	4.4	Abnormal
127	0.6	Normal	127	0.3	Normal
128	0	Normal	128	0	Normal
129	0	Normal	129	0	Normal
130	0	Normal	130	0	Normal
131	2.7	Abnormal	131	2.5	Abnormal
132	0	Normal	132	0	Normal
133	3.1	Abnormal	133	3.3	Abnormal
134	0.1	Normal	134	0	Normal
135	0.8	Normal	135	0.7	Normal
136	0	Normal	136	0	Normal
137	3.2	Abnormal	137	3	Abnormal
138	0	Normal	138	0	Normal
139	0.1	Normal	139	0.1	Normal
140	0.1	Normal	140	0.3	Normal
141	0	Normal	141	0	Normal
142	2.9	Abnormal	142	3	Abnormal
143	0	Normal	143	0	Normal
144	1.3	Normal	144	1.1	Normal
145	0.2	Normal	145	0	Normal
146	0.8	Normal	146	0.4	Normal
147	0.7	Normal	147	0.5	Normal
148	1.6	Normal	148	1.3	Normal
149	0	Normal	149	0	Normal
150	0.4	Normal	150	0.2	Normal
151	3.5	Abnormal	151	4	Abnormal
152	0.4	Normal	152	0.7	Normal
153	3	Abnormal	153	3	Abnormal
154	0.2	Normal	154	0	Normal
155	1.4	Normal	155	0.9	Normal
156	1.2	Normal	156	0.5	Normal
157	1.3	Normal	157	1.3	Norm
158	3.5	Abnormal	158	3.5	Abnormal
159	0	Normal	159	0	Normal
160	0.3	Normal	160	0.4	Normal

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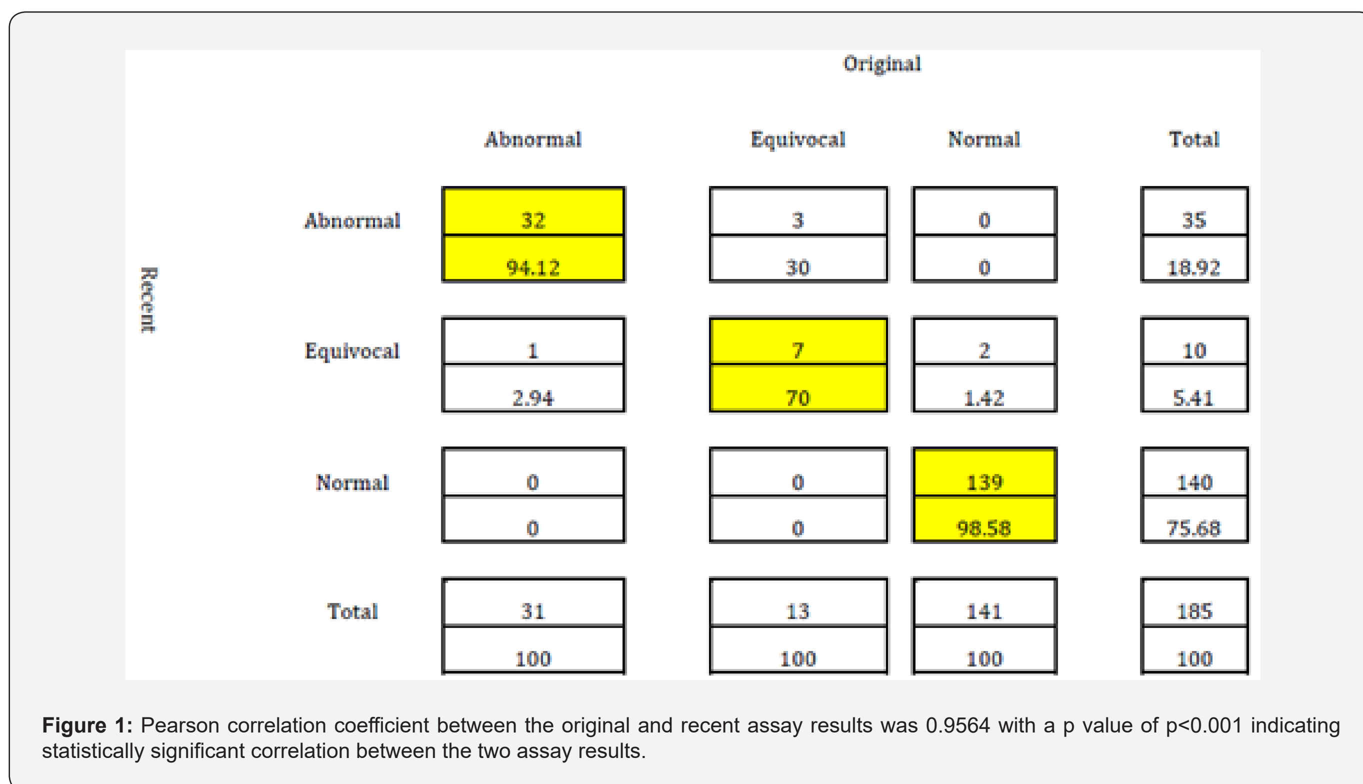
161	0	Normal	161	0	Normal
162	0	Normal	162	0	Normal
163	2.9	Abnormal	163	3	Abnormal
164	0.7	Normal	164	0.6	Normal
165	0.5	Normal	165	0.6	Normal
166	0	Normal	166	0	Normal
167	0.7	Normal	167	0.7	Normal
168	4.5	Abnormal	168	4.5	Abnormal
169	3.5	Abnormal	169	3.5	Abnormal
170	1.4	Normal	170	1.6	Normal
171	0.9	Normal	171	1.2	Normal
172	0.9	Normal	172	0.4	Normal
173	>5	Abnormal	173	4.6	Abnormal
174	1.6	Normal	174	1.7	Equivocal
175	3.8	Abnormal	175	3.8	Abnormal
176	0	Normal	176	0.3	Normal
177	0.5	Normal	177	0.1	Normal



Recent \ Original	Abnormal	Equivocal	Normal	Total
Abnormal	32 94.12	3 30	0 0	35 18.92
Equivocal	1 2.94	7 70	2 1.42	10 5.41
Normal	0 0	0 0	139 98.58	140 75.68
Total	31 100	13 100	141 100	185 100

Figure 1: Pearson correlation coefficient between the original and recent assay results was 0.9564 with a p value of $p<0.001$ indicating statistically significant correlation between the two assay results.

Discussion

A reliable and cost-effective system for DNA sample preservation invitro remains a significant challenge and opportunity for cost savings and environmental stewardship. Several methods exist commercially that are used to address these concerns. A new approach based on a formulation that uses mimicry of natural biological processes of DNA preservation has led to the development of novel synthetic formulations that have been shown to offer better stabilization, storage ability and shipping options for a wide range of nucleic acids. It mimics the natural biostability process of extremophile organisms in how they preserve their tissues and cells in a dry state for longer than

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100 years and are still able to resume their normal function upon hydration. The original observation of this natural phenomenon was made as far back as 275 years ago by Antony van Leeuwenhoek, a Dutch microbiologist, who first coined the term 'anhydrobiosis', or life without water, to describe this natural phenomenon. EBV DNA showed no deterioration of sample Ct signal was observed due to Freeze/Thaw cycles and the results were stable across multiple cycles for a period of 6 years at room temperature.

Conclusion

No significant deterioration of sample results was observed over long term storage of up to 6 years of the patient samples for the EBV DNA at room temperature.

Conflict of Interest

i. John Phillips is the Chief Medical Officer for Daykin Molecular Systems. He is an Associate Professor of Surgery at The University of Toronto and has extensive publications and experience in handling regulatory issues on launching medical devices in North America. Dr. Phillips was involved in the development and FDA approval of NPScreen which involves room temperature storage and transportation of cells for the molecular diagnosis of nasopharyngeal carcinoma.

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