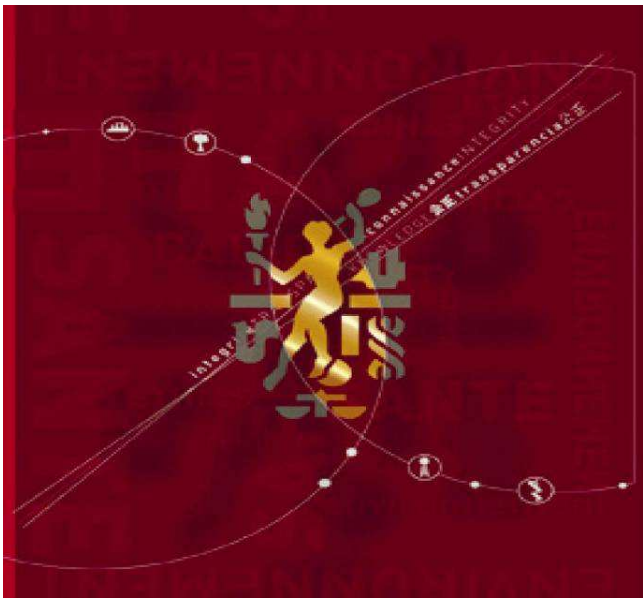

Product Solution

Engineering Services Group

Product Description: **SKUDO**

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Executive Summary

At the request of the client, the submitted samples were evaluated using Comet Assay to determine the ability of the Skudo product to reduce DNA damage in Jurkat (human T lymphocyte) cells caused by cell phone radiation.

Testing was performed on three different phones and included a baseline measurement without the Skudo product and a measurement with the Skudo product for each phone, as well as positive and negative control sets. The Etoposide treated positive controls demonstrated an increase in DNA damage, as evident by the increase in both Tail Moment and percent DNA in the tail. The percent DNA in the tail of all test samples is less than 10% indicative of a healthy cell population. Exposure to cell phone radiation for 1 hour without a Skudo sample applied resulted in a small increase in DNA damage relative to baseline measurements and the 1 hr control sample, however it is difficult to assess if the Skudo product is having a meaningful protective effect. A statistical analysis showed no statistical difference in the absence or presence of the Skudo product in iPhone group, and shows a marginal benefit to the BlackBerry group. In the Motorola phone group, there was a significant difference in the presence of the Skudo product relative to the Motorola phone alone; however, the Skudo product sample was also found to be significantly lower than the baseline control suggesting that the difference may be influenced by other experimental test factors[2,3]. The Blackberry and Motorola phones both have relatively high Specific Absorption Rate (SAR) values, and seemed to have stronger correlations than the iPhone. This suggests, but does not prove, that it is possible that the Skudo product can reduce risk of DNA damage from phones, and would provide a larger benefit to phones with higher Specific Absorption Rate (SAR) values.

Sample	Comets Scored	% DNA in the Tail	Difference of % DNA	Statistically Signficant (1-way Anova)
iPhone	303	3.46	-0.89	No
iPhone + Skudo	107	2.57		
Blackberry + Skudo	152	3.13	-1.71	No
BlackBerry	283	4.84		
Motorola	281	3.09	-1.30	Yes
Motorola + Skudo	222	1.79		
Positive Control (Combined Populations)	610	33.14	-29.82	Yes
Negative Control (Combined Populations)	403	3.32		

Based on the analysis performed, it is the opinion of BVCPS that the provided Skudo product may be able to provide a protective benefit, however this benefit has shown to be statistically significant on only 1 out of 3 tests as shown in the table above. It is also the opinion of BVCPS that further testing should be completed in order to prove the protective benefit with confidence.

This report is for informational purposes only.

Notes:

1: The provided white paper report from a previous study shows the baseline control samples had a tail intensity (similar to our %DNA in tail measurement) of about 1.8% and exposed samples had a tail intensity of about 8%, both within the healthy range. While mean values are about a 4 fold difference, when considering the standard deviation range, the difference is likely much smaller.

2: This differs from the finding of the University of Perugia white paper study where the presence of the Skudo product was reported to protect the cells from DNA damage induced by cell phone radiation. Also note that the University of Perugia test shows results with the Skudo product to be lower than the negative control sample, indicating experimental variances within that testing as well.

3: These differences could be due to experimental setup or number of comets being scored.

Figure 1 – Representative sample as received.

Background

The product was previously tested by a European lab, which tested one product sample on one cell phone using similar methods. This previous study appeared to have low cell population counts. In addition, it is unclear how the sample images were chosen for analysis. If a representative population was chosen it is possible that manually choosing sample images could influence the study. In this study, a larger cell population was utilized, and all viable cell images were included to eliminate the possibility of external influence.

Figure 2 shows the radiation exposure experimental set up from the previous study. By contrast, this study placed the cells in a tube which was fixed to the cellular device along the length of the device as seen in Figure 3, and placed on top so that gravity held the cells closer to the device than the previous study allowed. Further images of the test set up can be seen in Appendix A.

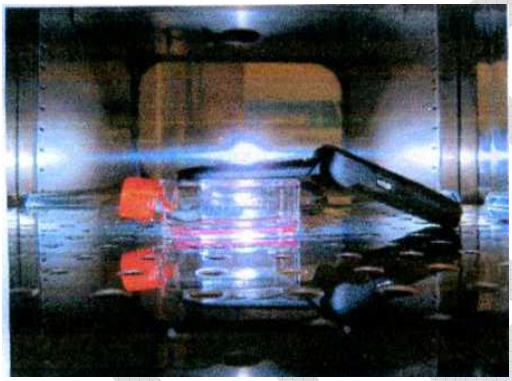


Figure 2 – Experimental set up from previous study

Figure 3 – Experimental set up from current study

Phones

Apple iPhone 3GS (SAR: 1.19 W/kg)
RIM BlackBerry 9000 (SAR: 1.51 W/Kg)
Motorola Atrix 4G (SAR: 1.47 W/Kg)

The radiation exposure standard for wireless devices employs a unit of measurement known as the Specific Absorption Rate, or SAR. The SAR limit set by the FCC/IC is 1.6W/kg*. The SAR limit recommended by The Council of the European Union and MIC is 2.0W/kg. The limit is set to minimize radiation exposure to consumers and any health risks that may be associated. These phones were chosen for their high SAR values and/or popularity.

Procedure

Cell Treatment:

1. 3 x 10^5 cell/ml Jurkat Cells in RPMI/10% FBS were seeded into 10 labeled 15 ml conical tubes from a stock at ~1x10^6 cells/ml prior to each treatment.
2. Sample treatment:
    a. Experimental Samples: The phone was placed in a zip lock bag to protect the phone from high humidity in the incubator. The polypropylene tube containing the cells was attached to the cell phone by rubber bands. The phone and tube were laid horizontal in 5% CO2 incubator at 37°C for 1 hr. Horizontal orientation allowed for exposure of cells along the length of the phone. Two treatment groups were performed simultaneously by placing two phones on opposite sides of the incubator to reduce radiation contamination between the two. Cell phone exposure was performed by placing a call between the phones which lasted for the duration of the 1 hr exposure. The length of each call was confirmed using the call log on the phone. When the Skudo device was installed to the phone it was placed on the phone's sim card.
    b. Negative Controls: Baseline control was processed immediately after seeding into the 15 ml conical. The control-1 hr was processed 1 hr after seeding (similar to the experimental samples).
    c. Positive Controls: Etoposide A: Cells were incubated in a 37°C, 5% CO_2 for 30 minutes with 2.5 µM Etoposide. Etoposide B: Jurkat cells were incubated in a 37°C, 5% CO_2 for 30 minutes with 5 µM Etoposide.
3. After treatment, 1 ml of the sample was collected and transferred to a labeled 1.7 ml tube and placed on ice.
4. The cells were pelleted by centrifugation @ 250 x g for 5 min @ 4°C.
5. The growth medium was removed.
6. The cells were suspended in 1 ml freeze medium (70% RPMI, 20% FBS, 10% DMSO). 50 µl aliquots were made and slowly frozen overnight at -80°C before transfer to liquid nitrogen for storage.

Alkaline Comet Assay:

1. Quickly, thaw samples and Alkaline Comet Control Cells (50 µl aliquots) at 37°C, and transfer immediately to ice.
2. Transfer 50 µl of each sample to a cold 1.7 ml tube, leave on ice
3. Add 500 µl of cold 1X PBS to each 50 µl aliquot, spin @ 250 x g for 5 min @ 4°C.
4. Remove 525 µl of PBS.
5. Add 300 µl of cold 1X PBS to pelleted samples and 75 µl of PBS to control cells (cell concentration ~2x10^5 cells/ml).
6. Add 30 µl cells to 300 µl of molten LMagarose (37°C).
7. Spread 30 µl of LMagarose/Cell mixture per well on 20 well slides.
    a. Samples and controls are run in triplicate.
8. Place slide @ 4°C for 20 minutes.
9. Transfer slide to Lysis Solution (pre-chilled to 4°C) for 30 minutes at 4°C.
10. Transfer slide to Unwinding Solution (200 mM NaOH/1mM EDTA) for 20 minutes at room temperature.
11. Electrophorese slides using the Trevigen Comet ES system using cold 200 mM NaOH/1 mM EDTA solution for 30 minutes at 21V.
12. Place slide twice in water for 5 minutes at room temperature.
13. Place slide in 70% Ethanol for 5 minutes at room temperature.
14. Dry slide on slide warmer (37-40 °C).
15. Add 50 µl/well of 1:30,000 SybrGold per well incubate for 30 minutes at room temperature in the dark.
16. Decant slide to remove SybrGold from slide.
17. Dip in water to quickly rinse.
18. Dry slide on slide warmer (37-40 °C).
19. Image using LOATS automated microscope and software analysis system.

Results

Table 1: Mean Tail Moment and %DNA in Tail ± Std Error

Sample	Comets Scored	%DNA in the Tail	%DNA SD	Tail Moment	TM SD
Negative Control	323	3.20	0.26	0.35	0.04
Positive Control: etoposide A	288	27.33	0.90	4.25	0.25
Positive Control: etoposide B	322	38.35	1.32	7.71	0.50
iPhone	303	3.46	0.33	0.45	0.06
BlackBerry	283	4.84	0.65	0.82	0.21
Motorola	281	3.09	0.26	0.27	0.03
iPhone + Skudo	107	2.57	0.46	0.22	0.06
Blackberry + Skudo	152	3.13	0.41	0.40	0.07
Motorola + Skudo	222	1.79	0.22	0.14	0.03
Negative Control 1 hr	80	3.82	0.60	0.39	0.07

Table 2: Summary of Statistic Significance between groups

Compared by phone with 1-way Anova/Tukey's test [95%]

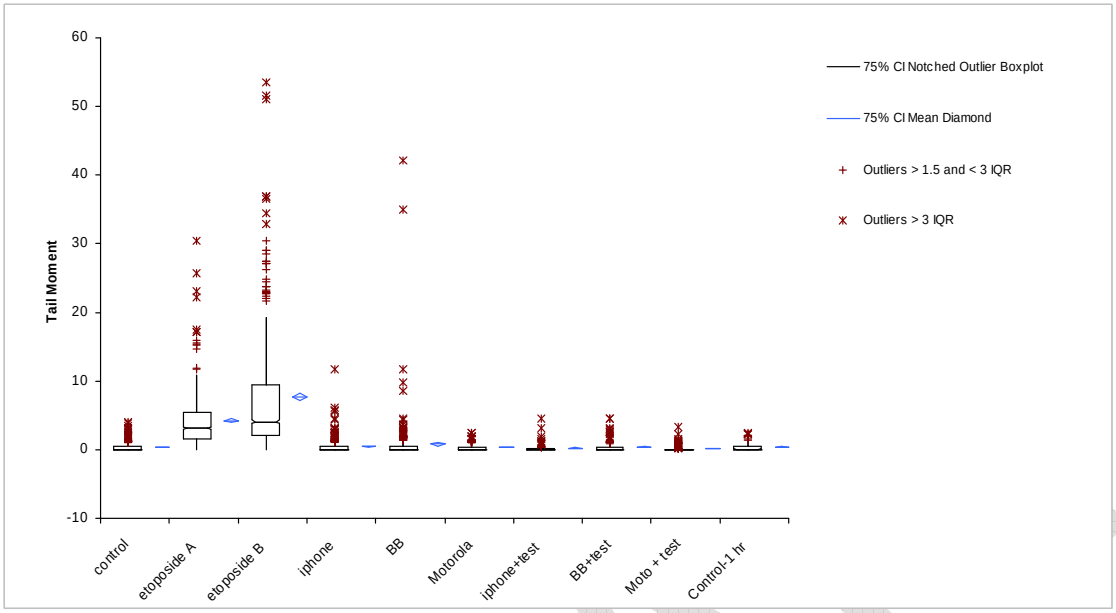
Sample	Control	iPhone	Blackberry	Motorola	Negative Control-1 hr
Negative Control	*N/A*	No	Yes*	No	No
iPhone + Skudo	No	No	*N/A*	*N/A*	No
Blackberry + Skudo	No	*N/A*	No	*N/A*	No
Motorola + Skudo	Yes*	*N/A*	*N/A*	Yes*	Yes*
Negative Control-1 hr	No	No	No	No	*N/A*

Yes – Significant difference found No – Significant difference not found

* - All values are within the healthy range between 1.7-5% DNA in the tail, therefore the statistical difference may possibly be due to inherent variability within the assay.

Positive results for statistical significance showed only on the phones with higher SAR values. This could indicate a link between SAR and effectiveness of the Skudo product. Phones with much higher SAR values would be more suitable to provide results beyond the inherent variability of the test, however such phones are not permitted for use in the US according to the Federal Communications Commission (FCC). Additionally, phones with lesser SAR values may have a benefit too marginal to measure accurately.

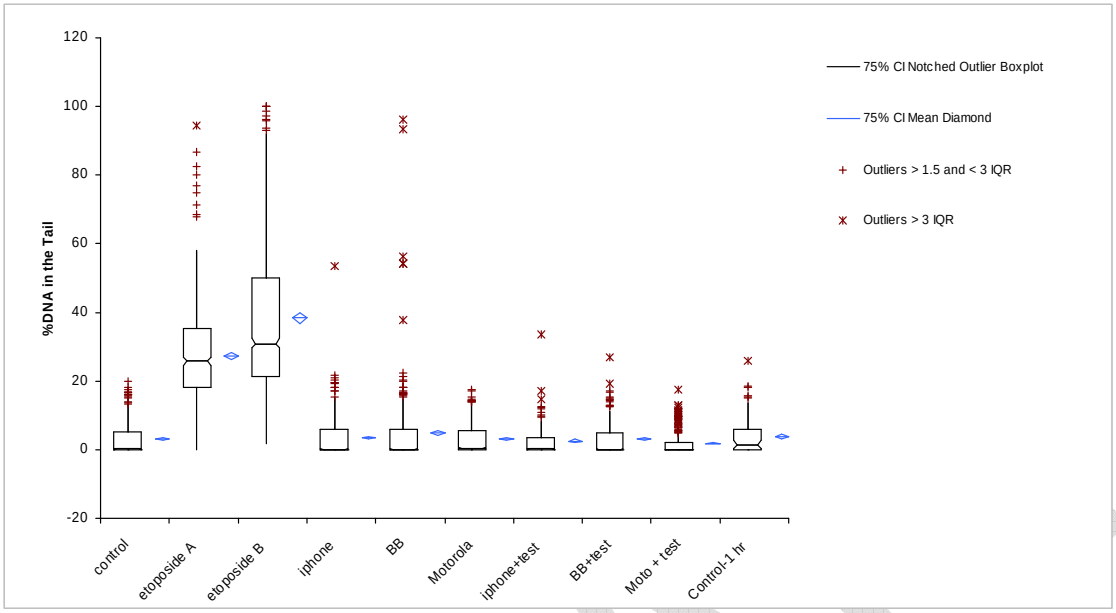
Tail Moment:



Tail Moment	n	Mean	75% CI		SE	SD
Negative Control	323	0.351	0.307	to 0.395	0.0381	0.6846
Positive Control: etoposide A	288	4.245	3.960	to 4.530	0.2474	4.1979
Positive Control: etoposide B	322	7.705	7.132	to 8.279	0.4974	8.9263
iPhone	303	0.447	0.374	to 0.520	0.0631	1.0992
BlackBerry	283	0.820	0.583	to 1.057	0.2053	3.4529
Motorola	281	0.275	0.243	to 0.307	0.0277	0.4651
iPhone + Skudo	107	0.223	0.155	to 0.292	0.0594	0.6143
Blackberry + Skudo	152	0.404	0.326	to 0.483	0.0679	0.8365
Motorola + Skudo	222	0.141	0.111	to 0.170	0.0255	0.3793
Negative Control 1 hr	80	0.387	0.305	to 0.470	0.0711	0.6356

Tail Moment	n	Min	Median	75% CI		Max
Negative Control	323	0.00	0.000	0.000	to 0.000	4.00
Positive Control: etoposide A	288	0.00	3.110	3.010	to 3.330	30.42
Positive Control: etoposide B	322	0.04	3.990	3.760	to 4.300	53.47
iPhone	303	0.00	0.000	0.000	to 0.000	11.68
BlackBerry	283	0.00	0.000	0.000	to 0.000	42.11
Motorola	281	0.00	0.000	0.000	to 0.010	2.44
iPhone + Skudo	107	0.00	0.000	0.000	to 0.000	4.53
Blackberry + Skudo	152	0.00	0.000	0.000	to 0.000	4.57
Motorola + Skudo	222	0.00	0.000	0.000	to 0.000	3.34
Negative Control 1 hr	80	0.00	0.055	0.000	to 0.100	2.49

%DNA in Tail:



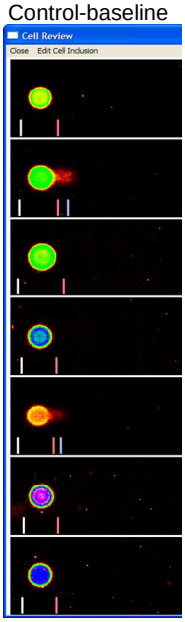
%DNA in the Tail	n	Mean	75% CI		SE	SD
Negative Control	323	3.200	2.901	to 3.499	0.2596	4.6651
Positive Control: etoposide A	288	27.329	26.297	to 28.361	0.8955	15.1977
Positive Control: etoposide B	322	38.352	36.827	to 39.877	1.3234	23.7474
iPhone	303	3.459	3.074	to 3.844	0.3341	5.8153
BlackBerry	283	4.844	4.100	to 5.588	0.6455	10.8590
Motorola	281	3.085	2.791	to 3.379	0.2553	4.2794
iPhone + Skudo	107	2.567	2.030	to 3.104	0.4642	4.8022
Blackberry + Skudo	152	3.131	2.658	to 3.604	0.4094	5.0480
Motorola + Skudo	222	1.792	1.534	to 2.050	0.2240	3.3382
Negative Control 1 hr	80	3.818	3.118	to 4.519	0.6045	5.4069

%DNA in the Tail	n	Min	Median	75% CI		Max
Negative Control	323	0.00	0.200	0.120	to 0.450	19.91
Positive Control: etoposide A	288	0.00	25.840	24.490	to 26.940	94.29
Positive Control: etoposide B	322	1.70	30.920	29.750	to 32.440	100.00
iPhone	303	0.00	0.100	0.050	to 0.220	53.43
BlackBerry	283	0.00	0.090	0.030	to 0.190	96.03
Motorola	281	0.00	0.240	0.160	to 0.760	17.45
iPhone + Skudo	107	0.00	0.150	0.100	to 0.260	33.59
Blackberry + Skudo	152	0.00	0.050	0.010	to 0.210	26.81
Motorola + Skudo	222	0.00	0.030	0.010	to 0.060	17.54
Negative Control 1 hr	80	0.00	1.350	0.140	to 2.610	25.88

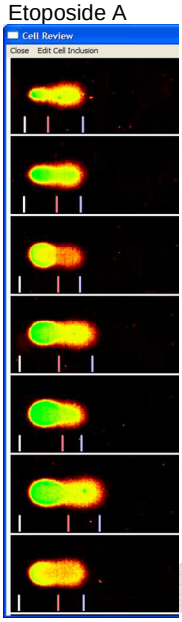
Controls: All Comet Control Cell populations were within stated parameters

Assay Images:
(LOATS System)
Below are sample images from the given population. These images may not represent the whole of the population and are only given as examples.

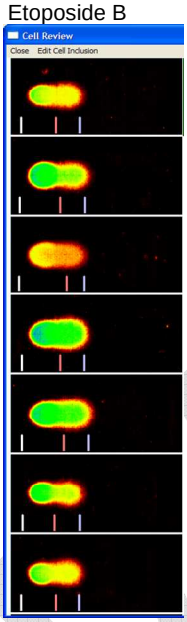
Control-baseline



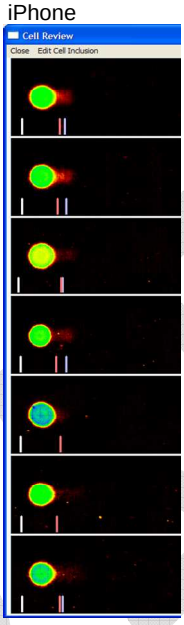
Etoposide A



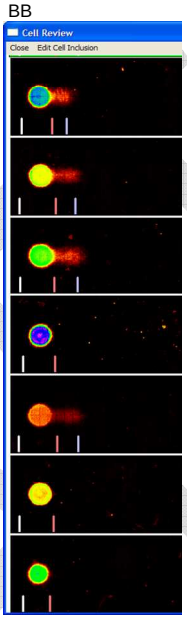
Etoposide B



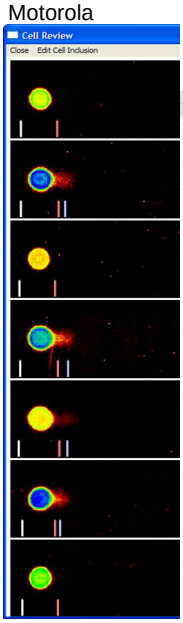
iPhone



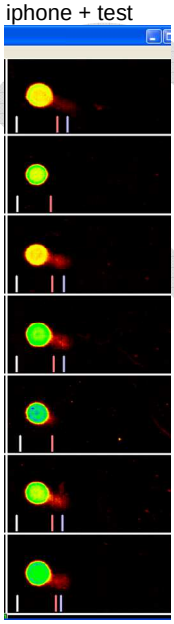
BB



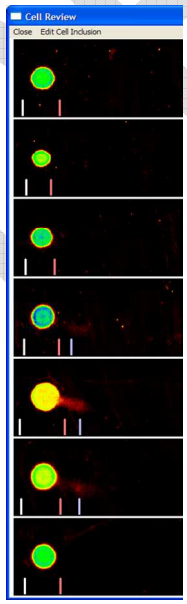
Motorola



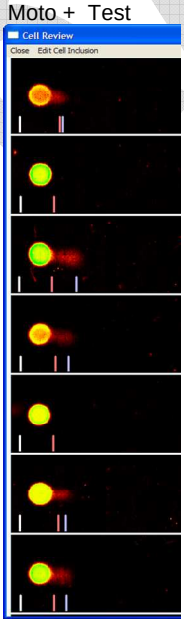
iphone + test



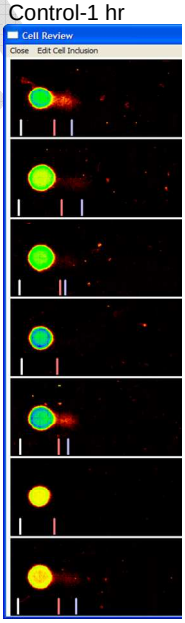
BB + Test



Moto + Test

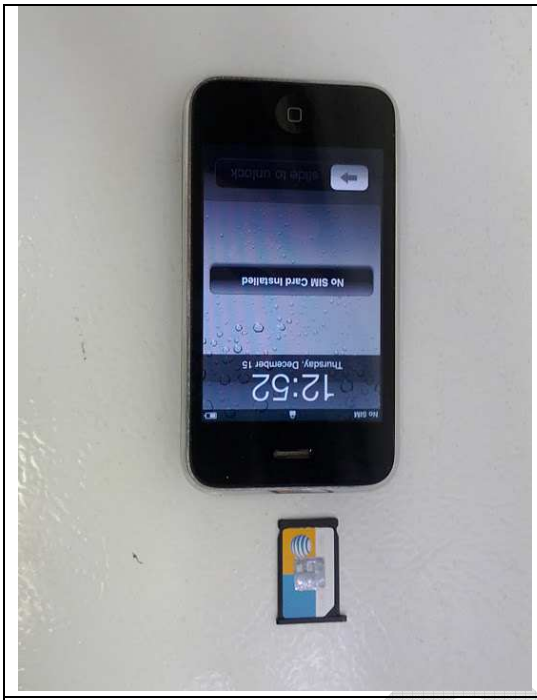


Control-1 hr



SIGNATURE BLOCK
Initials

Appendix A: Test Set Up Images

	
Apple iPhone 3GS with Skudo applied to SIM Card	Blackberry 9000 with Skudo applied to SIM Card
	
Motorola Atrix 4G with SIM Card installed	Samples as being removed from liquid nitrogen storage

	
Samples on slides being placed into Electrophoresis System	Samples under Electrophoresis process
	
Internal view of Electrophoresis System	Slides in a soak process