An Implementation of Electro-Conductive Ionic Properties of Plasmatic Bodily Fluids in a Novel Conductometric Based Analysis for the Advanced Detection of Leukemia Based Hematological Malignancies

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Introduction

The purpose of this biomedical study was to define a novel method of detecting hematological malignancies based on conductometric alterations within bloodstreams. The experiment measured the conductivity values of various ML3 leukemia cell concentrations, and compared them to conductivity readings from healthy leukocytes. Based on the electrical readings from these various cell samples, the research study was able to define significant data differences in conductivity between leukemia cells and normal cell samples. The data shows that Leukemia Cell samples produced conductivity values 2% less than that of healthy leukocytes. As will be further explained in the contents of this paper, Leukemia cells contain a malfunction hematopoiesis process, causing them to create electrolytic misbalances within the bloodstream. Blood Smearing images were taken at the Fred Hutchinson Cancer Research Center that shows the Leukemia Cell overflow in the bloodstream (Fig. 5b page. 9). Clinically, these data trends could eventually provide the foundations for a conductivity based leukemia detection device. Such a device could revolutionize the way blood cancer is detected in both rural and advanced regions. In stark comparison to current-day high cost blood cancer detection systems, such as flow-cytometers, cell morphological equipment, and related bone marrow data analyzers; this proposed conductivity based method of detecting blood cancer would function with decidedly lower costs, and with extreme portability. While not targeted for high accuracy, the conductivity based method could be compared to a “thermometer” for cancer and would detect leukemia at the earliest possible stage of the disease. In other words, the method would act as a low-cost, simple, and quick preliminary indicator for blood cancer, meant to signal individuals about potential blood-based malignancies.

Materials and Methods

The experimentation was conducted individually in the Fred Hutchinson Cancer Research Center, under the supervision of Dr. Valerie Morris. In order to test the hypothesis, the experiment used a sample of Canine Whole Blood in a 20 mL quantity. Along with the blood sample, a culture of (Myeloid Leukemia Line) ML3 Canine Leukemia Cells was used in order to test the effect of Leukemia on Conductivity. The following resource provides a brief description of the ML3 Cell Line, as well as related Myeloid Line Leukemia cells:

Myeloid leukemia lines (ML)*: Lines, ML1 (Kawakami et al., 1989, Leuk. Res. 13:709), as well as ML2 and ML3 (described in McSweeny et al., 1996, Blood 88:1992.) are myeloid leukemia lines with monocyte characteristics, that were generated from dogs after irradiation.*

Along with these blood tissue samples and Cell cultures, the experiment used a YSI-80 Conductivity Meter for all Conductivity readings. The conductivity meter took readings in MilliSiemens/Cm, which will further be referred to as MilliSiemens, or just mS. Below is a list of the major materials used for the experiment:

1	YSI-80 Conductivity Meter
2	20mL Whole Blood Sample Canine
3	24 mL ML3 Leukemia Cell Line Culture
4	40 mL 3 in. diameter Lab Test Tubes
5	Trypan Blue Dye
6	Hemacytometer Cell Counting Tool
7	PBS Dilution Solution
8	Bovine Media Solution
9	100 microliter, 10 microliter, 1 milliliter, and 10 milliliter Pipettes

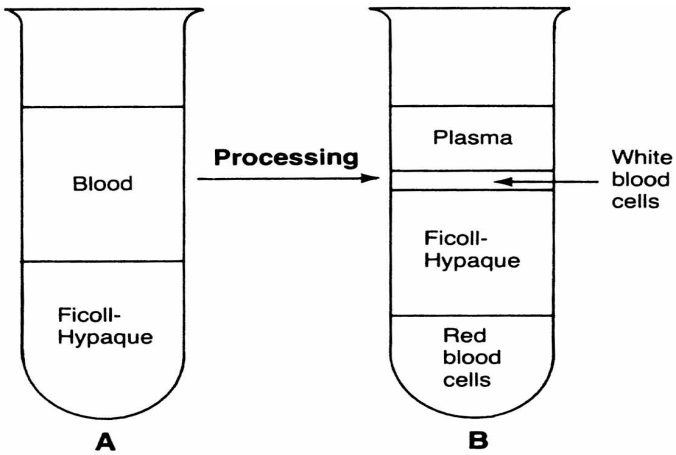
In brief, the experiment isolated healthy White Blood Cells from the Canine Blood Sample and concentrated these cells at 4 different concentration levels (0.125 million cells/ mL, 0.25 million, 0.5 million, and 1 million). The ML3 Culture was also concentrated at these exact same four concentration levels. Following these preparatory re-concentrations the major portion of the experiment took place with the sample Conductivity Readings in milliSiemens units. Below is a summary of methods:

1. Healthy Blood Sample Centrifuging
2. White Blood Cell Separation
3. White Blood Cell Counting
4. White Blood Cell Re-Concentrating to desired concentrations
5. ML3 Leukemia Cell Re-Concentrating to desired concentrations
6. Conductivity Testing in milliSiemens

1. Healthy Blood Sample Centrifuging:

The first step of the experimentation was to centrifuge the original canine blood sample in order to extract the White Blood Cell (WBCs) portion. The blood was diluted using PBS (Phosphate Buffered Saline solution) in a 1:1 concentration, and then suspended above Ficoll-Paque solution (polysucrose solution meant for cell separation). The entire solution of Ficoll and Blood dilution was then centrifuged for 30 minutes at 1500 RPM. Fig 1, below shows the process of centrifuging the blood sample.

Fig. 1 Ficoll Centrifuge Separation



As shown in part B of Fig. 1; the centrifuging causes the blood to separate into 4 distinct sections of cell type: Plasma, White blood Cells (WBCs), Ficoll-Hypaque, and Red blood cells (RBCs).

2. White Blood Cell Separation:

The next step was to extract the White Blood Cell layer from the rest of the centrifuged blood. This was done using a micropipette instrument that drew out the WBC layer. This layer was placed in a separate container and diluted in PBS once again into a 6 mL sample. The diluted WBC layer was re-spun in the Centrifuge at 1500 RPM for 10 Minutes.

3. White Blood Cell Counting:

The subsequent step was to derive the cell count of the 20 mL WBC cell sample, so that it could be appropriately re-concentrated into the 4 required concentrations of 0.125 million cells/mL, 0.25 million cells/mL, 0.5 million cells/mL, and 1 million cells/mL. The cell counts were derived by using a cell counting slide tool known as the Hemacytometer. The Hemacytometer is divided into 4 large outer square sections (see Fig. 2a Pg. 6) which are used for counting. In order to use the hemacytometer for cell counting, Protocol A (below) was used. For future reference; this method will be referred and used as **Protocol A**

Protocol A: Hemacytometer Cell Counting:

1. **Mix Cell Suspension thoroughly, and transfer 100 uL (microLiter) of solution into separate container**
2. **100 uL Cell Sample should be diluted in 1:1 ratio with Trypan Blue Dye solution**
3. **Mix dilution dyed sample and wait 5-15 minutes**

4. **Prepare Hemacytometer Microscope Slide by cleaning with Alcohol and Lint free cloth**
5. **Position Cover slip over both slide chambers**
6. **Draw sample of Cell suspension, and fill both chambers of Hemacytometer using micropipette**
7. **View Cell sample under microscope, and count Cells in each large square compartment (see Fig. 2a)**
8. **Cells dyed blue are considered "dead" or not viable, and clear/white cells are considered viable**
9. **Viewing the Cell under the Microscope, make a tally of all viable living cells in Hemacytometer Square 1, Square 2, Square 3, and Square 4 regions of the Hemacytometer Slide (see Fig. 2b). Derive the Viable Cells/ mL (Cell count value) by Multiplying the average Cells per large square by dilution factor (1:4) and 10^4**

Fig. 2a WBC Hemacytomer Values, below, shows the viable cell count values of the WBC sample layer.

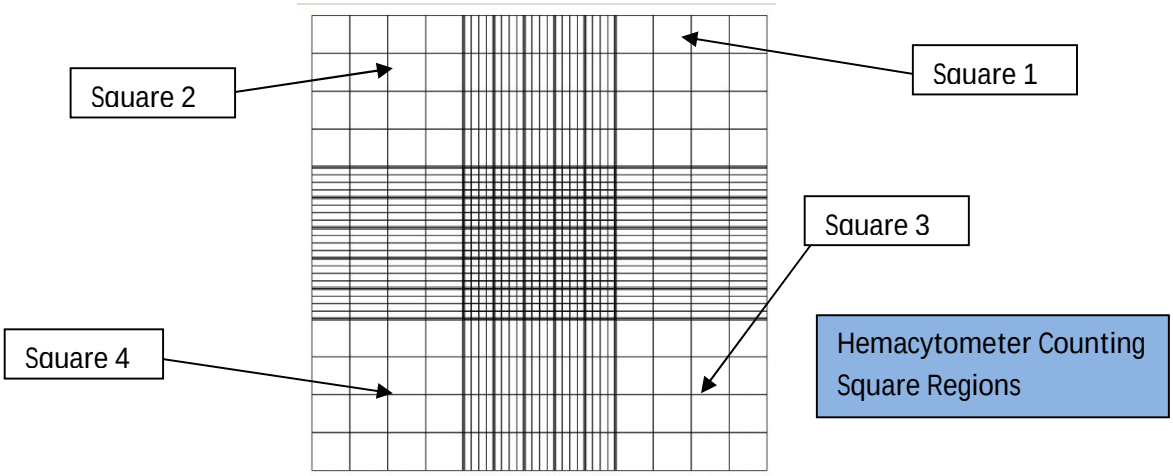
Fig. 2a WBC Hemacytometer Viable Cell Counts

Viable Count Hemacytometer **Square 1**	Viable Count Hemacytometer **Square 2**	Viable Count Hemacytometer **Square 3**	Viable Count Hemacytometer **Square 4**	Avg. Viable Count per Sq.	Viable Cell Count per mL
107	119	119	130	118.75	4.75 M*/mL

*Million

Figure 2b, below, shows an imaging of the hemacytometer slide cell counting tool under the microscope. The grids in the slide are used to denote the volume area, and each cell is counted visually.

Fig. 2b Hemacytometer Cell Counting Tool under Microscope



4. White Blood Cell Re-Concentrating to desired concentrations:

Once the viable WBC cells/ mL had been counted using the Hemacytometer:Protocol A, the next step was to re-concentrate the WBC Cell sample into the 4 desired concentrations of 0.125 million cells/ mL, 0.25 million cells/ mL, 0.5 million cells/mL, and 1 million cells/ mL. The sample re-concentration occurred so that four separate test groups could be created for the testing. The re-concentration of the samples was done by centrifuging the cells again for 10 min. at 1500 RPM, and then using the dilution change formula:

$$C_{1*} V_1 = C_{2*} V_2$$

In which C1 and V1 are the starting Concentration (cells/mL) and Volume(mL), and C2 and V2 are the desired concentration and volume. The calculation itself is rather trivial, but the result of the formula was that the original WBC sample of 4.75 million cells/ mL, was re-concentrated into the 4 **diluted** desired test group concentrations using PBS dilution solution. Therefore, the WBCs were

re-suspended into their calculated PBS dilution levels, and made into 4 test concentrations ready for the conductivity testing.

5. ML3 Cell Re-Concentrating to desired Concentrations:

The next procedure was to concentrate the ML3 Leukemia Cell Lines into these same 4 concentrations of 0.125 million cells/ mL, 0.25 million cells/ mL, 0.5 million cells/ mL, and 1 million cells/ mL. The re-concentration was completed so that four test groups were created equal to that of the WBCs. In order to complete this re-concentration for the ML3 line, a very similar procedure was followed.

First the ML3 culture was spun for 10 min. at 1500 RPM. Then the cell counts for the ML3 culture were derived using Protocol A: Hemacytometer Cell Counting. Fig. 3 ML3 Leukemia Hemacytometer Values, below, shows the viable cell count values of the ML3 Culture from the Hemacytometer.

Fig. 3 ML3 Leukemia Hemacytometer Viable Cell Counts

Viable Count **Square 1**	Viable Count **Square 2**	Viable Count **Square 3**	Viable Count **Square 4**	Avg. Viable Count per Sq.	Viable Cell Count per mL
68	62	74	80	71	1.42 M*/mL

*Million

Next it was required to re-concentrate the ML3 Cell line from 1.42 million cells/ mL into the 4 desired concentrations of 0.125 million cells/ mL, 0.25 million cells/ mL, 0.5 million cells/ mL, and 1 million cells/ mL. This was done using the exact same method as the WBCs in which the dilution change formula was applied:

$$C_{1*} V_1 = C_{2*} V_2$$

In the formula, C1 and V1 are the starting concentration (cells/mL) and volume (mL), and C2 and V2 are the desired concentration and volume. After the application of the formula (a simple concentration change) the ML3 concentration was able to be re-concentrated from 1.42 million cells/ mL into the 4 required concentrations of 0.125 million cells/ mL, 0.25 million cells/ mL, 0.5 million cells/ mL, and 1 million cells/mL. Therefore the ML3 samples were ready to begin conductivity testing as well.

For summary: At this point of time, after re-concentrating both WBCs and ML3s into the 4 desired test group concentrations, the experimentation was ready to begin conductivity testing. The conductivity test would be structured in 4 test groups. Each test group would have its own concentration level (0.125 million cells/ mL, 0.25 million cells/ mL, 0.5 million cells/ mL, and 1 million cell/mL), and would have the single variable of cell type- either ML3 or WBC. This was the reason why both WBCs and ML3s were re-concentrated into 4 equal concentrations, in order to allow for a constant cell concentration value in each of the four test groups, and only a variation in the cell type. Fig. 4 below (page 10) shows the four test groups constructed for the conductivity testing. Fig 5a and Fig 5b below show magnified Blood Smears of the normal WBCs Sample, and ML3 Sample. These smearing images were taken on 8/11/11at the Fred Hutchinson Cancer Research Center.

Fig. 5a WBCs Smearing 8/11/11

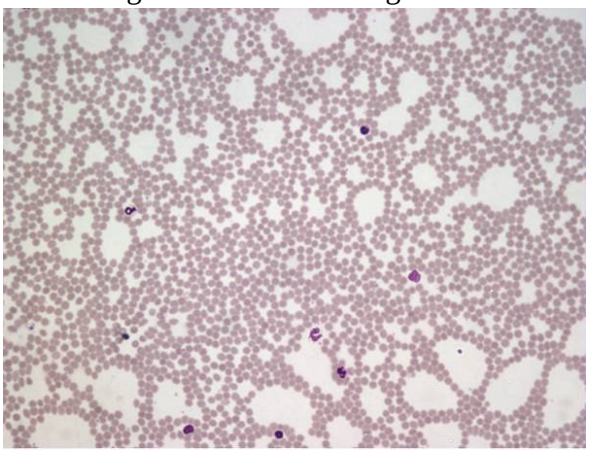


Fig 5b ML3s Smearing 8/11/11

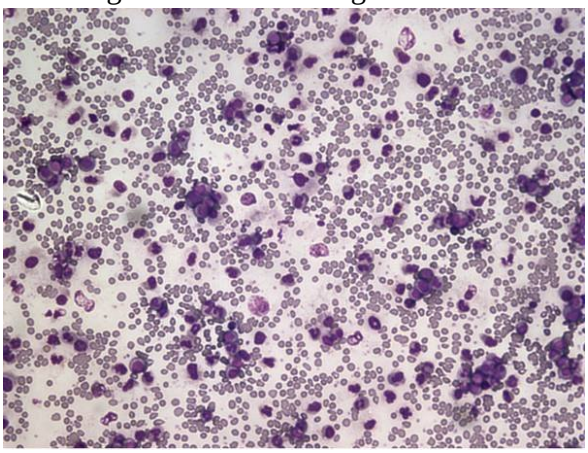


Fig. 4 Conductivity Testing Test Groups

Test Group	WBCs Cell Sample	ML3 Culture Sample
Group A	0.125 Million cells/ mL	0.125 Million cells/ mL
Group B	0.250 Million cells/ mL	0.250 Million cells/ mL
Group C	0.5 Million cells/ mL	0.5 Million cells/ mL
Group D	1 Million cells/ mL	1 Million cells/ mL

In each of these test groups, 5 trials were completed for both WBC concentrations and ML3 Concentrations.

6. Conductivity Testing:

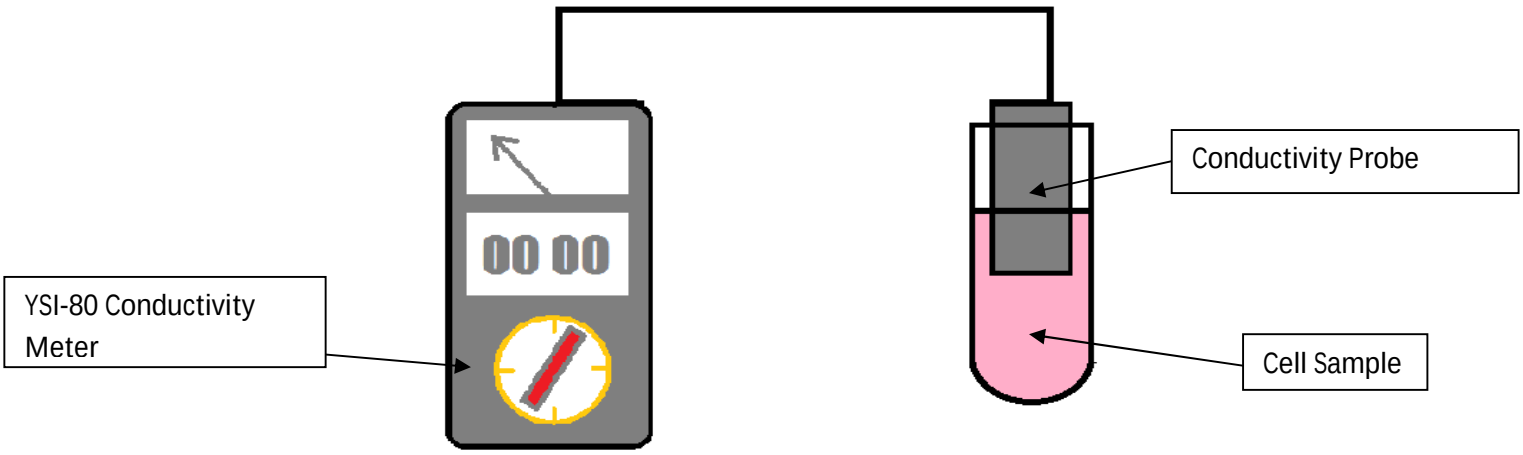
The consequent step was to begin the major portion of the experiment: the conductivity readings. As stated in the methods introduction, the experiment utilized a YSI- 80 lab grade conductivity meter in milliSiemens setting. It should be noted that any similar conductivity meter could have been used as well, as long as it met the accuracy requirements of 1-2 mS (milliSiemens) resolution. In order to derive the milliSiemens conductivity value of each test group, Protocol B (below) was used.

Protocol B Conductivity Testing:

1. **Draw 15 mL Sample of desired Test Group Cell concentration (ex. Group A ML3s) and place sample in Lab Test Tube Container**
2. **Start Conductivity Meter and set reading value to mS(MilliSiemens)**
3. **Submerge Conductivity Meter probe to a 5 inch level into Lab container**
4. **Allow milliSiemens Reading to steady for exactly 20 seconds, and then record data.**
5. **Clean Conductivity probe using Ethanol, and water, and allow drying for 10 min.**

6. **Complete steps 3 and 4, five times for accurate results in various trials**
7. **Complete above steps for all Test Groups, and appropriately record all results.**

Fig. 6 Conductivity Meter Set-Up



Following the conductivity testing using this protocol, the experimental section of the research had been completed. All readings had been recorded in all 4 samples for each of the 2 different cell types (healthy WBC or leukemic ML3s) in 5 trials each; for a total of 40 conductivity meter readings.

Results and Findings

The results of the given experimentation are best represented using the below tables and charts For Data Reference: Graph Group Concentration Key

Groups	Cell Concentration
Group A	0.125 Million Cells/milliliter
Group B	0.25 Million Cells/ milliliter
Group C	0.5 Million Cells/ milliliter
Group D	1 Million Cells/ milliliter

Table 1. ML3 Cell Conductivity Data in MilliSiemens (mS)

Sample	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Average
Sample A	13.9 mS	13.905 mS	13.89 mS	13.87 mS	13.88 mS	13.889 mS
Sample B	13.7 mS	13.69 mS	13.55 mS	13.53 mS	13.62 mS	13.618 mS
Sample C	13.55 mS	13.56 mS	12.94 mS	13.49 mS	13.52 mS	13.412 mS
Sample D	13.43 mS	13.65 mS	13.33 mS	13.32 mS	13.22 mS	13.39 mS

Graph 1. ML3 Cell Conductivity Data Averages in MilliSiemens (mS)

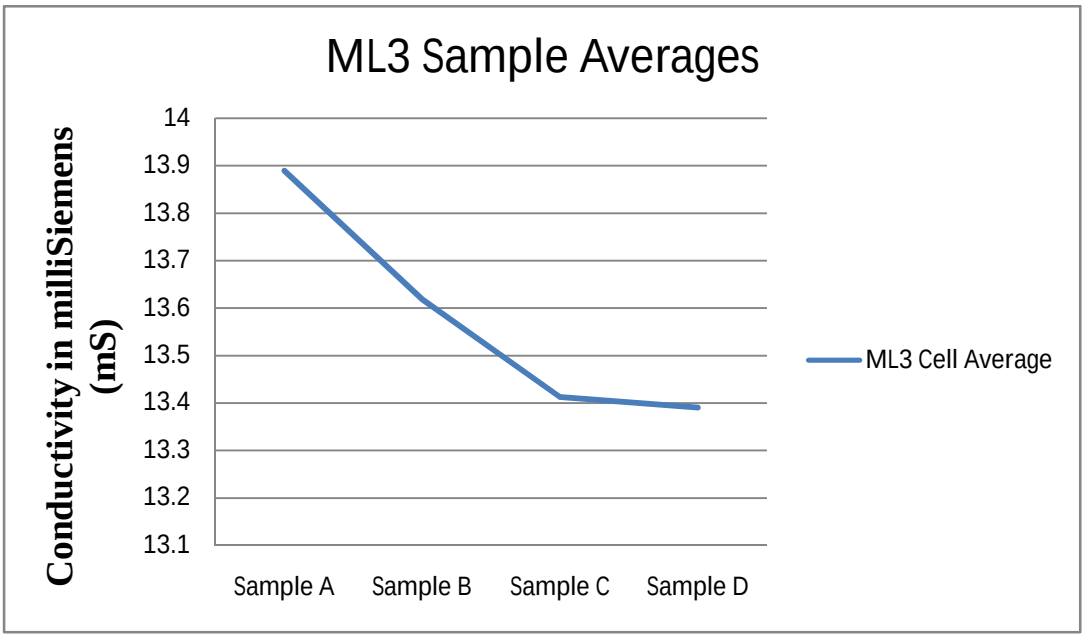
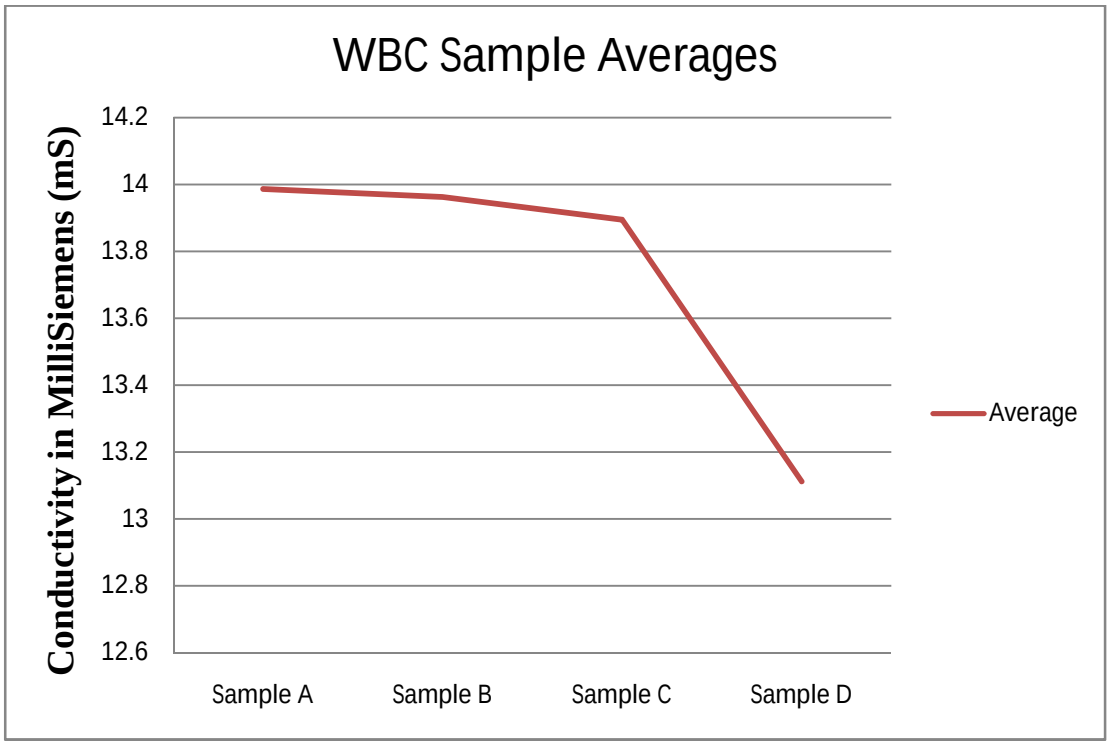


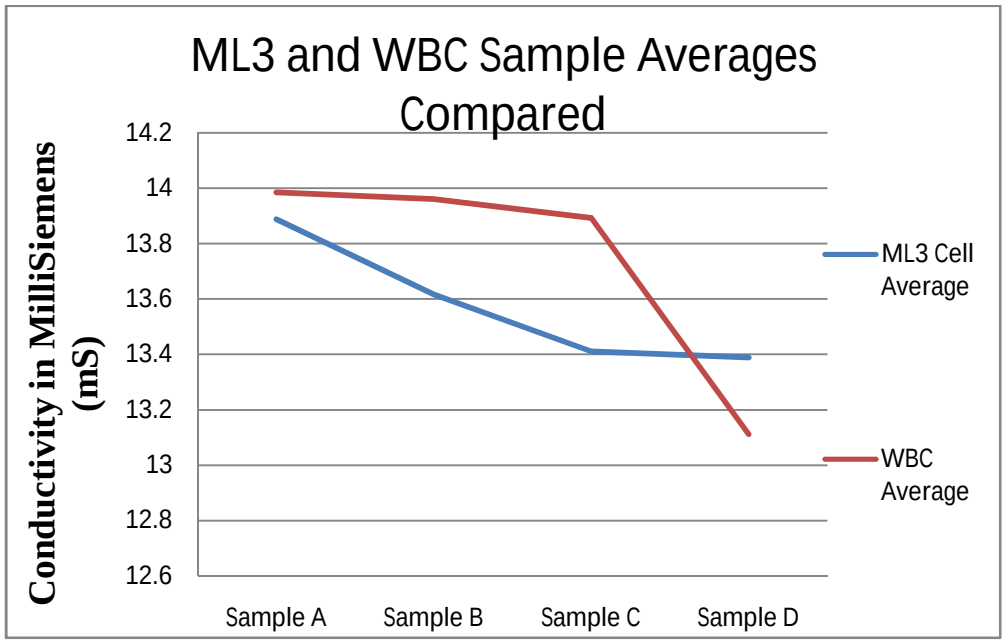
Table 2. WBC Conductivity Data in MilliSiemens (mS)

Sample	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Average
Sample A	13.99 mS	14.1 mS	13.96 mS	13.89 mS	13.99 mS	13.986 mS
Sample B	13.99 mS	14.1 mS	13.86 mS	13.89 mS	13.97 mS	13.962 mS
Sample C	13.96 mS	13.87 mS	13.87 mS	13.88 mS	13.89 mS	13.894 mS
Sample D	12.04 mS	13.65 mS	13.33 mS	13.32 mS	13.22 mS	13.112 mS

Graph 2. WBC Conductivity Average Data (mS)



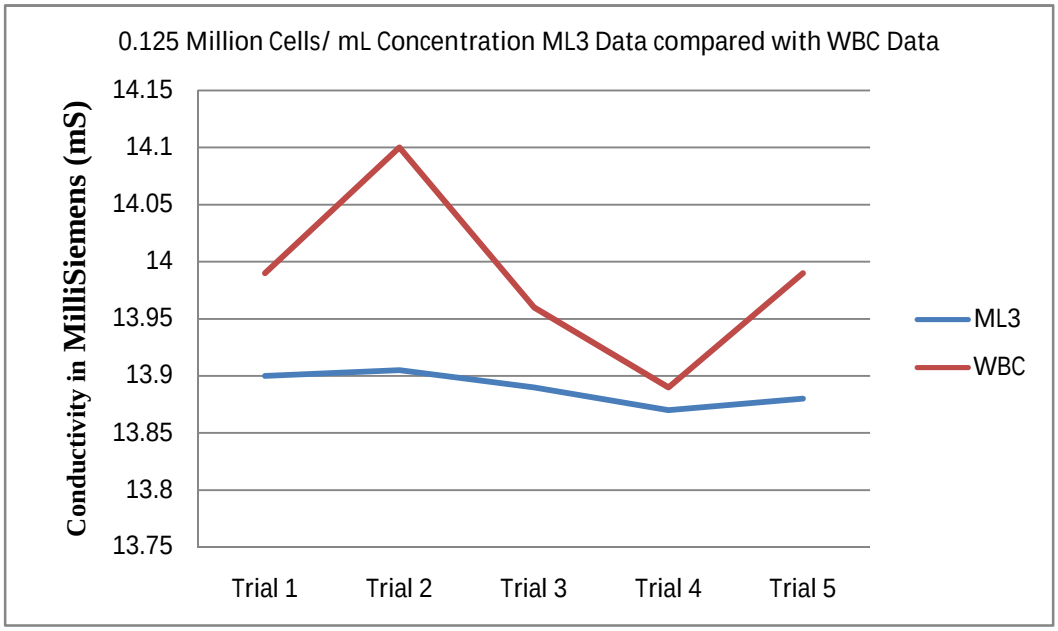
Graph 3. ML3 and WBC Sample Average Data Compared in MilliSiemens (mS)



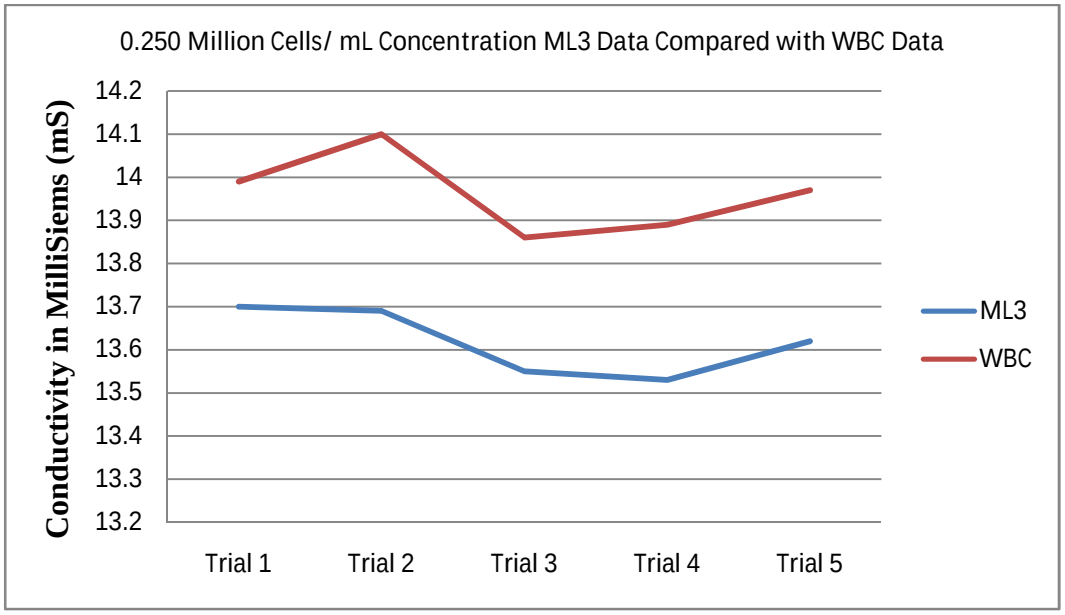
The above graph provides the best summary as to the entire results. It displays the averages for each Concentration Sample in both ML3 and WBC Test groups.

Individual Group Concentration Comparisons

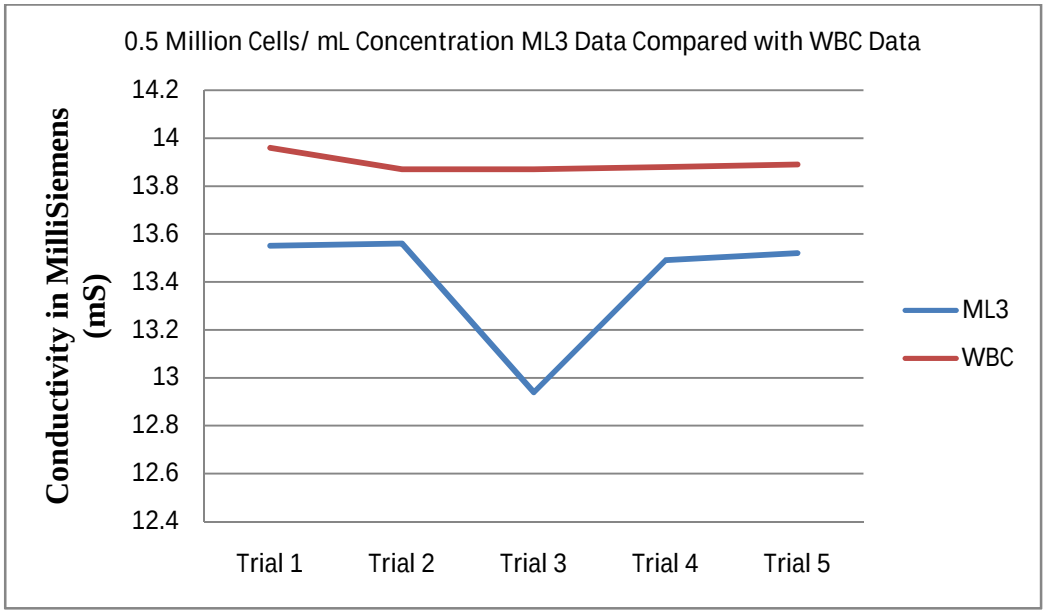
Graph 4. Group A Cell Data ML3 Compared to WBC D in MilliSiemens (mS)



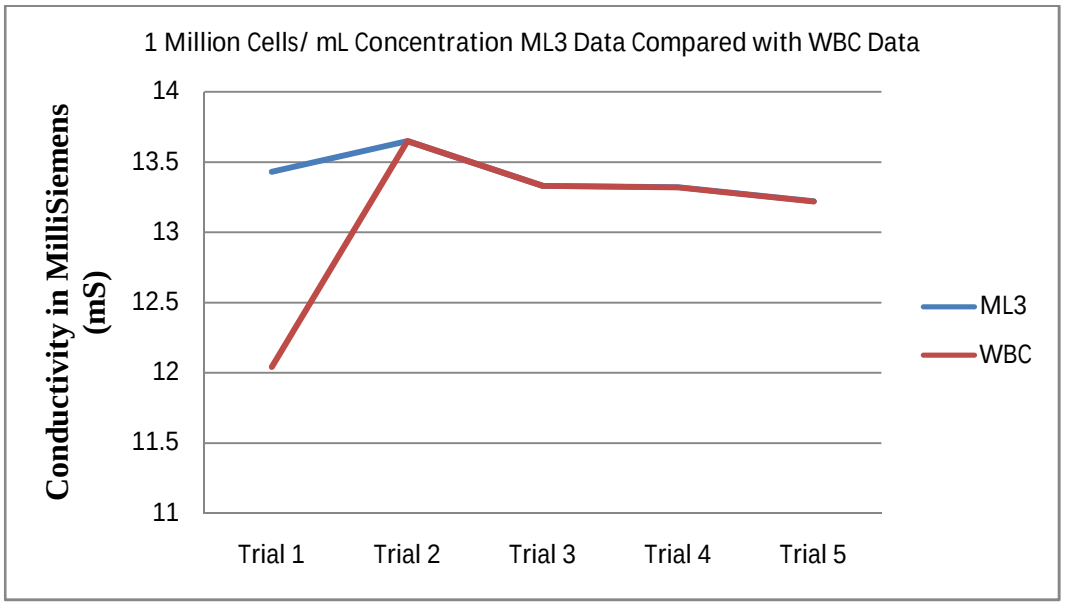
Graph 5. Group B Cell Data ML3 Compared to WBC Data in MilliSiemens (mS)



Graph 6. Group C Cell Data ML3 Compared to WBC Data in MilliSiemens (mS)



Graph 7. Group D Cell Data ML3 Compared to WBC Data in MilliSiemens (mS)



Discussion and Conclusion

In order to discuss the above shown results in a complete and thorough manner, this section will compose of two parts: First, the analysis of the results and data from each individual test group; and second, the illustration of the hypothesis, and theory behind the results from the experimentation.

Data Analysis

With the data from all test groups and corresponding trials, several significant trends should be noted. The first and most prevailing trend can be observed as the consistently lower MilliSiemens conductivity reading in nearly all leukemia cell samples. To represent the data section as a whole, Graph 3 provides the best understanding with averages from each sample and trial. In Graph 3, the ML3 Cell Sample maintained an average of 13.577 MilliSiemens throughout its samples; whereas the normal Leukocyte WBCs sample kept an average of 13.7385 MilliSiemens. This represents a near 2% drop in conductivity within ML3 Samples. It should be noted, however, that in Graph 3 the last average data set (Sample D) defied this trend, with higher ML3 Values. Furthermore, the

readings within each individual cell concentration group follow a similar trend: the ML3 concentrations maintain lower conductivity values throughout most trials. This individual sample data is a good indicator of the mostly steady lower data of ML3s. However, this trend in many cases can be seen as inconsistent, and the data does have some movement from the proposed trend. First of all, the exception on Graph 3 shows that in one case the conductivity of ML3 greatly exceeds that of the WBCs. Furthermore, in Graph 7 group D, the ML3 Conductivity and WBC conductivity do not differ at all in four of the five trials. Another major data analysis question is the seemingly random fluctuations between the conductivity values of each individual concentration. This fluctuation can be seen in Graphs 1, 2, and 3, specifically between Samples C and D. In WBC cells, this transition between the two concentration groups resulted in a sudden drop in conductivity, whereas in ML3 Cells, this caused a sudden leveling within the samples. Another issue is that of the sudden drop in conductivity in Trial 3 of Graph 6. Following the described drop, the conductivity values return to an average and normal level, suggesting experimental error, and an outlying piece of data. In order to begin developing the research further, it would be required to conduct more experimentation heavily on all these factors, and thoroughly answer all potential flaws in the theories and ideas in the hypothesis.

In a conclusion of the analysis, the data proposes several distinguishable trends that may hold clinical value in the future. At the same time, however, the data contains certain points that are not as consistent, and therefore requires much work before it can be further developed. All the points stated in this short data analysis will be entirely explained and further analyzed in the theoretical analysis below.

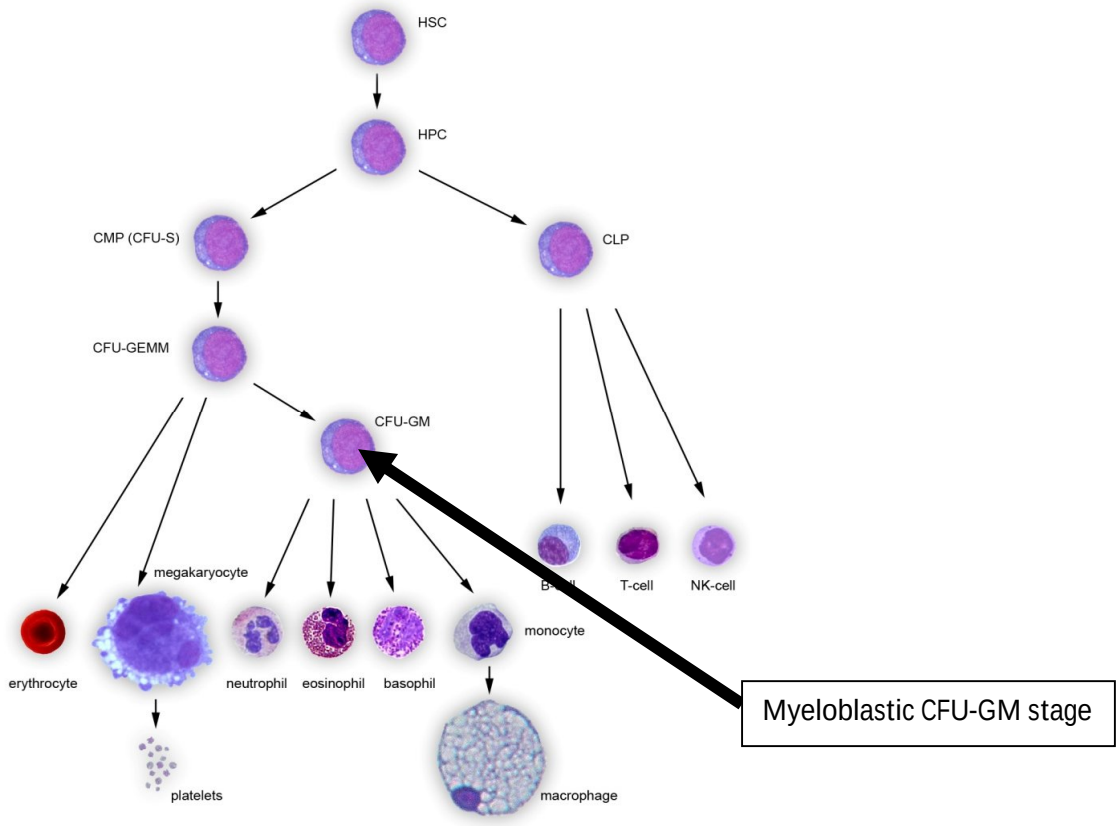
Theoretical Analysis

Following the data analysis there are two main points that must be theorized and explained:

- **Consistently Lower ML3 Cell Sample Conductivity Readings**
- **Seemingly Randomly Decreasing Values of Conductivity in higher Concentration Cell Samples**

First, the analysis of the ML3 Sample results: In order to fully define this conductivity difference in the Leukemia test group, it is imperative that the Leukemia Cell hematopoiesis procedure in comparison to that of a normal WBC. Fundamentally, leukemia cells are classified simply by their immaturity, or in other words their stall in an earlier stage of cellular development. Scientifically, a case of myeloid based Leukemia is paused in the CFU-GM (Myeloblast) stage of hematopoietic development. Myeloblasts, unlike mature and developed cells, are in a growth phase, and thus are larger and require more nutritional resources from the surrounding plasma. In a Leukemia state bloodstream, these Myeloblasts overflow the normal blood cell types, in order to create instability in the blood (see Fig. 5a compared to Fig. 5b page 9)Thus the bloodstream, composed of the nutrient/ion rich blood plasma, is depleted below normal levels in order to provide for the increased number of demanding Myeloblasts. In result, the ionic substances within the Leukemia bloodstream are reduced, thus causing a lowered conductivity value. This summarized theory explains the lowered Leukemia group conductivity values; and therefore I have named it the **Myeloblastic Absorption Theory.** The entire Myeloblastic Absorption Theory is explained in full detail on the next page. Fig. 7 below shows the Hematopoietic process diagrammatically.

Fig. 7 Hematopoietic Diagram

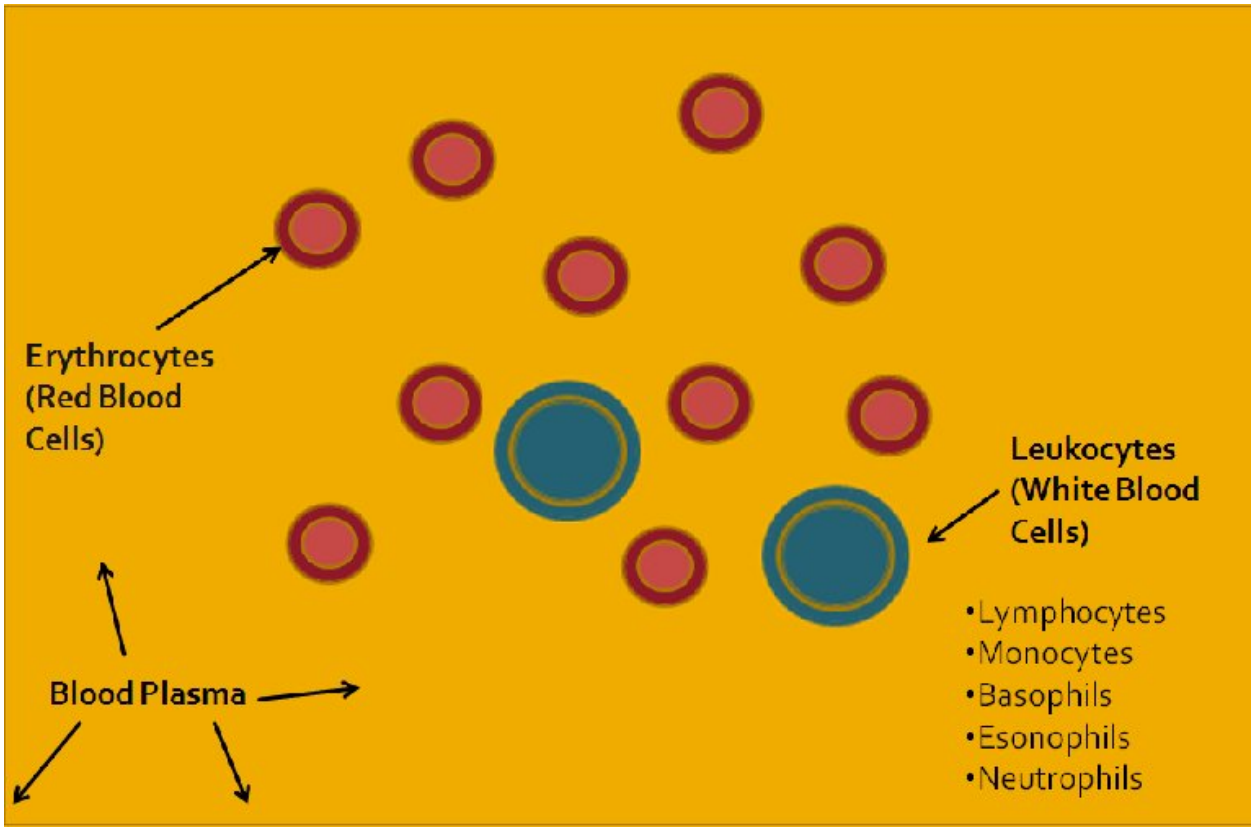


The diagram depicts the large and immature staged CFU-GM Myeloblast Cell in which Myeloid Leukemia cells are situated.

Myeloblastic Absorption Theory

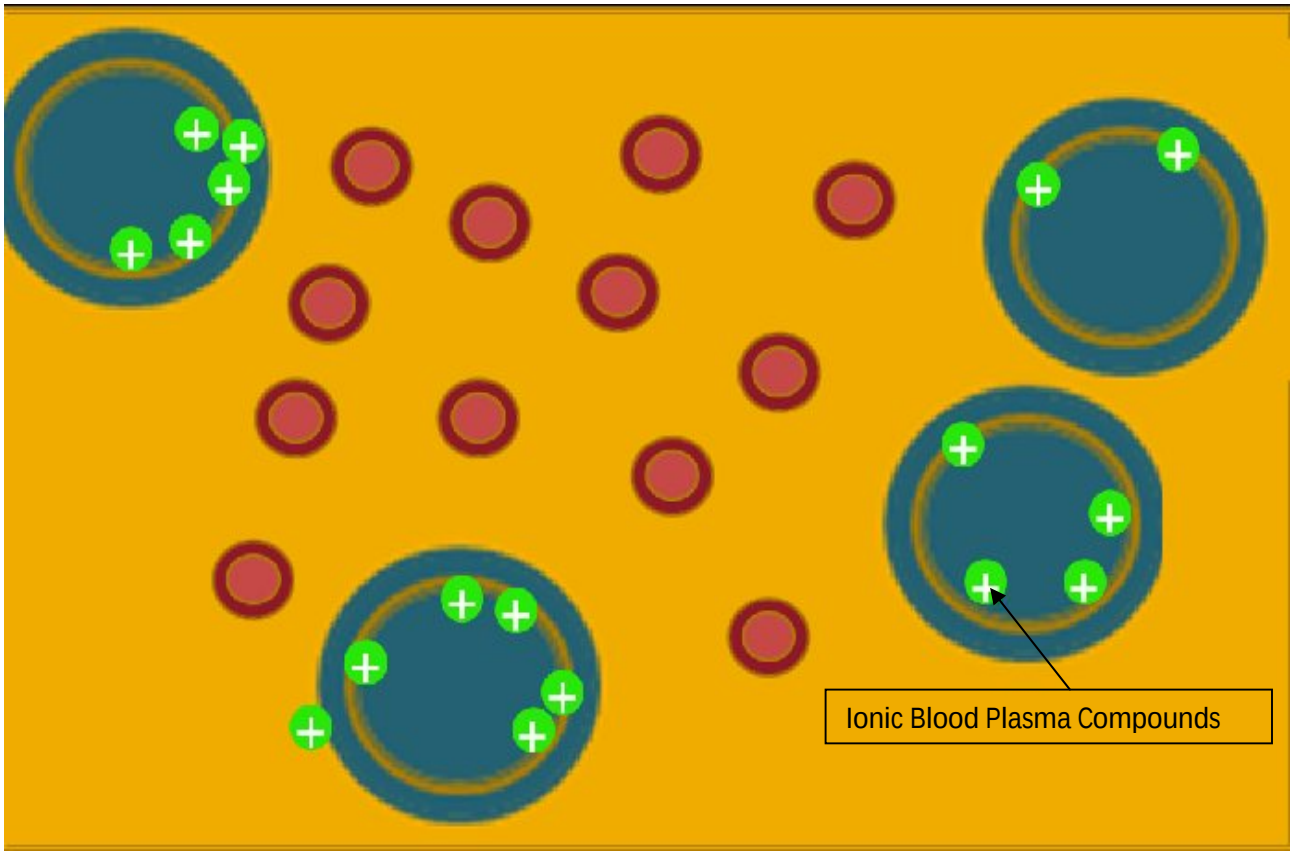
The Myeloblastic Absorption Theory explains the decrease in conductivity of Leukemia Blood Samples. First of all, all blood cells (Leukocytes and Erythrocytes) are suspended in an ionic solution known as the blood plasma. The blood cells absorb nutrients and ion from the plasma in a means of deriving their energy. Fig. 8 Below, shows the cells in a regular plasmatic blood stream.

Fig. 8 Regular Bloodstream



Unlike a regular bloodstream, however, Leukemia Cells (as shown in Fig. 7 on page 19) contain dysfunctional hematopoiesis maturing processes, and are therefore staged in an immature cell state. This immature stage is known as the CFU-GM stage, or the Myeloblast stage. Myeloblasts are much larger, and require more nutrients in order to sustain their cell bodies. Furthermore, they are considered to be in a growth stage (immature stage) and therefore require more ionic material to continue growth. Therefore, these immature Myeloblasts absorb an increased amount of Nutrients and ions from the blood plasma. Due to this increased depletion, the blood plasma loses its ionic compounds and salts at much quicker rates; thus keeping less and less nutrients and ions. This lack of ions is shown in Fig. 9 below.

Fig. 9 Myeloblast Stage of Leukemia



In Fig. 9, the Leukemia Cells are much larger and also absorb larger quantities of Nutrients from the Blood Plasma. This, as stated above, results in lowered ionic levels within the Bloodstream. Furthermore, due to the decreased number of ionic compounds, the blood stream obtains a lower conductivity value. Therefore, the conductivity readings from the immature staged Leukemia Cell groups contained lower conductivity values than that of regular blood samples.

In summary of this Myeloblastic Absorption Theory: Leukemia Cells, in most cases, are erroneous in the hematopoietic structure, and thus deplete the bloodstream of ionic nutrients, and in-turn reduce the conductivity of the blood sample.

To address the sudden conductivity drops in certain samples and concentrations, one must statistically examine the data. Generally, these sudden drops can be seen as outlier pieces of data, and therefore must be cautiously approached due to the nature of this specific experimentation. From a research based theoretical standpoint higher cell concentrations could cause an increased demand on the bloodstream, and thus cause a drop in the conductivity value (similar to the case of ML3 Cell drops). At the same time, the idea of experimental error could be brought forward. In both cases, further testing would be required to determine the exact nature of the cause.

In conclusion, the research conducted through this study can be of great potential value to the clinical medical field. This proposed conductivity based method of tracking blood cancer growth could revolutionize the way leukemia, and related Hematological Malignancies are detected in both rural and urban areas. As stated in the introduction, blood cancer detectors could become as cheap, simple and fast as a simple digital conductivity meter. The creation of such a device would be similar to the modern day thermometer: a low-cost, initial indicator driven device meant to signal a user of potential malignancy related illnesses. The research conducted in this study could increase Cancer survival rates by detecting blood cancer earlier, faster, and simpler. Rather than going through the lengthy process of visiting a physician, acknowledging symptoms, and scheduling blood test dates; the proposed conductivity test could be carried out within seconds by a user within the comfort of their home based environment. The conductivity blood test could be taken at synchronized time periods by an individual, and would become synonymous with taking one's temperature. Furthermore, the research could help in undeveloped areas, by providing a low-cost method of indentifying deadly sicknesses within blood streams. At the same time, however, it is very necessary to take note of the fact that the results are only trends, and much work is to be done before being declared conclusive. The several data inconsistencies serve as major limitations in the research, and the testing must be repeated for many trials in order to be considered clinical ready.

Acknowledgements

This experiment would not have been possible without the help of Dr. Valerie Morris of the Fred Hutchinson Cancer Research Institute, who took three days off from her busy schedule to train me completely in the lab, and help carry out the experimentation.

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From a research perspective, I would like to thank Dr. Ravinder Majeti from Stanford University for conversing over email and phone with me in order to help narrow down my research into a more feasible experiment that was best suited for the lab environment. Last but not least, I would like to thank my parents for taking me to Seattle (FHCRC) so that I could complete my research.

Annotated Bibliography References

"The Krebs Cycle." *The University of West Indies Department of Biological and Chemical Sciences* . University of West Indies, n.d. Web. 4 Nov. 2011.

This source was used in order to understand the complexities of the Krebs Cycle in a blood Cell's cellular respiration. I was able to determine how a regular White Blood cell differs in energy production from a Leukemia based Cell. The source was important in understanding the basics of hematology, along with some of the details regarding leukemia deformations.

"Regulation of the Glycolysis Process." Stanford Education Department of Hematology. Stanford University, n.d. Web 5 Nov. 2011

This Source was used in order to understand the Glycolysis Process of Cellular respiration. Similar to the Krebs Cycle resource, I used this source in order to gain an understanding of the cellular respiration of regular White Blood Cells in comparison to Leukemia Cell Lines. The source was important in the formulation of sections of my hypothesis along with my overall conclusion.

References and Questions answered by Dr. Valerie Morris from the Fred Hutchinson Cancer Research Center (Ficoll Protocl Sigma Aldrich and Hemaestosis Wikepedia Foundation Diagram)

The questions that Dr. Valerie Morris answered through email and in person were helpful to the research conducted in my project. Furthermore, she provided various resources for me in order to continue my research regarding the subject. Through the questions she answered, I was able to develop several components regarding my theory, and she was able to provide information specific to my research.

So, Chi Wai Eric, Dr. *Leukemia:Methods and Protocol*. N.p.: n.p., n.d. Print.

This resource was used in order to research the current day practices in diagnosing and treating Leukemia based diseases. Furthermore, I used the book to understand the genetic errors within a Leukemia Cell, and the potential effects on the blood stream. Overall the book provided a good summary of the subject, and introduced me to the topic in an in-depth format. This resource was also provided helpful diagrams related to Leukemia progression in a blood stream.

"Flexible energy storage devices based on nanocomposite paper." *PNAS*: n. pag. Abstract. *PNAS*. Web. 16 Jan. 2012. <http://pnas.org>.

This Scientific article regarded the use of nanotech devices in order to harness the electric potential within blood. The research paper was the first to give me the idea that Blood may carry measurable electric value within the bloodstream.

Erber, Wendy, Dr. *Diagnostic Techniques in Hematological Malignancies.* N.p.:n.p.Print.

This source was used in order to understand current methods of detecting Hematological Malignancies. The book provided detailed descriptions regarding the advancements in diagnostic devices for Leukemia. I was able to use certain information regarding the data and time taken to read blood tests, along with the analysis of blood for malignancies. Overall, the information was valuable to my research, and helped define my problem statement.

References and Questions answered by Dr. Ravi Mateji from Stanford Hematological Research Department

Dr. Majeti's answers to my research questions were helpful to my research. He was able to provide information specific to my project, and also helped with the explanation of Hematological Malignancies in further detail. The answers to my questions helped in the formulation of the hypothesis, and also the design of the experiment.

Oxford Handbook: Hematological Malignancies (various authors), Oxford Institute

The Oxford Handbook regarding Hematological Malignancies provided a wealth of knowledge relating to Hematological Malignancies. The book offered various diagrams, charts, and graphs relating to the progression and treatment of Leukemia and related Malginancies. Overall the book served as a good general resource regarding Leukemia, and I was able to use it well.

Kingfisher Encyclopedia Section 5.9, Multi-potential and Uni-Potential Stem Cells

The Encyclopedia Resource provided good information regarding the maturing process of stem cells. Furthermore, it explained the errors Leukemia cells contain regarding this process, and also defined several important terms relating to the subject.