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In-Vitro Biochemical Basis of Lactose Intolerance and the Catabolic Effects of *Lactobacillus acidophillus* on Commercial Milk Fed to *Mus musculus*

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Abstract

Lactose intolerance—the inability to digest lactose, which is mainly present in dairy products - is a very unpleasant reaction to the presence of glucose and lactose in the digestive system, characterized by cramping, bloating, nausea, flatulence and diarrhea. The deteriorating case of lactose intolerance worldwide apparently demanding an attention, this study is beneficial for the 65% of the population to experience a better digestion of lactose they acquire from the dairy products they take. This accordingly helps develop people's capability to be lactose tolerant, having their digestive system in good condition. Adding Lactobacillus Acidophilus to lactose products is also entitled in the production of lactase (a significant enzyme in the body system) hence, this is the lifeline of the majority. This allows people to achieve satisfaction and their maximum capacity of taking dairy products normally. This study apparently had a biochemical basis stating that regular milk contains no glucose while in the case of lactose- free milk, lactose has dissolved thus, has been evident to have glucose content. Although lactose has glucose and galactose, its glucose couldn't be read because of the chemical mixture of the monosaccharide sugars. Calculating the rate of the change in glucose level of the subjected mice, the observed potency of Lactobacillus A., being the main component of the milk amounted to the highest increase rate which was 65% and lactose-free milk resulted to 57% increase. Regular milk had 1% and the control group (water) had -17%. Three (3) treatments and 1 control were used in the experimentation; 3 males and 3 females per treatment. The blood glucose of each

mouse was tested by the method of tail pricking (with the use of Lancets) and was then measured by glucometer and glucose strips. The lactose when broken down gives an increased amount of glucose, paving way to lactose tolerance. The researcher highly recommends a long experimental period, replicates and more samples**.**

Research Plan

A. Problem Being Addressed

Lactose Intolerance has no cure since then and the growing population is experiencing this kind of problem

1. Babies aren't able to drink milk as the baby continues to drink milk until adolescence

B. Goals/ Expected Outcome/ Hypotheses

   The study will determine the effects of Lactobacillus Acidophilus in having Lactose-breaking properties

Hypotheses

1. Lactobacillus Acidophilus can help break lactose in such dairy products especially milk for lactose intolerant to intake, the mice that would be used will have a high glucose concentration
2. There would be a a big difference of glucose concentration in regular milk and lactose-free milk

Data Analysis

The measurement of glucose will be tested using glucose strips and glucometer and will be compared in different applications.

Introduction

Imagine not eating yogurts, ice cream, not drinking milk and other dairy products. Lactose Intolerant people are experiencing this kind of situation. Lactose intolerance is not a rare problem- it is in fact "normal" in the state of people carrying the digestive problem. Approximately 65% of the world's population are lactose intolerant. Lactose intolerance can lead to Abdominal bloating, stomach rumbling (burb orygmus), Abdominal pain or cramps, Diarrhea, Gas/ Platulence, Nausea and Vomiting.

A sugar which gives milk its sweetness, lactose which is a key constituent of breast milk, very essential that babies are capable of digesting it. Lactase an enzyme that is present in the baby's digestive tract, speeds up and catalyzes the breakdown of lactose into glucose and galactose. Unlike lactose, glucose and galactose are readily absorbed by the small intestine. One is able to digest lactose if they produce lactase, since lactase breaks down lactose, but people stop producing lactase between ages 2 to 5. Once the production of lactase stops, the undigested sugars end up in the colon, which is where they start fermentation. This is the main reason behind the uncomfortable digestive problems one gets when consuming milk or dairy products.

65 percent of the world's population is lactose intolerant. There was a genetic change in Northern Europe and in Mediterranean population that lead to lactose intolerance through the years until the present time. (American Journal, 1988) (United States Medicine Library, 2002)

This genetic change resulted in the maintenance of lactase production into adulthood. The current scientific consensus is that this mutation was advantageous and thus able to spread rapidly through the population of Europe.

For the past years, scientist and manufacturers invented a substance for lactose intolerant people for them to possibly drink milk without its side effects by having a product called as the lactose-free milk. The said inventors react the lactose chemically, altering its composition and converting it to molecules that the digestive system processes easily. Adding lactase to milk that splits lactose into its consistuent components, which are glucose and galactose.

Raising awareness about the biochemical basis of lactose intolerance of the range on how high or how low is the basis of lactose intolerant people, the concentration of glucose between lactose-free milk and regular milk. The next set will have the addition of lactase and be tested.

A long-life disease such as cystic fibrosis could be an effect of lactose intolerance. A body may also make less lactase if the small intestine is injured or digestive problems such as Crohn's or celiac disease.

Lactose intolerance is a common complication of diarrhoea in infants with malnutrition and a cause of treatment failure. A combination of nutritional injury and infectious insults in severe protein energy malnutrition reduces the capacity of the intestinal mucosa to produce lactase enzyme necessary for the digestion of lactose. (United States National Library Of Medicine, 2010)

Lactobacillus Acidophilus occurs in the animal and human gastrointestinal tract and mouth. It is species of gram positive bacteria. Lactobacillus A. can rduce the risk of coronary heart disease by 6-10 percent. (Journal of the American College Of Nutrition)

Lactobacillus Aa. has a big role in being able to prevent yeast infections and an ability to adhere the vaginal cells. It is part of vaginal microbiota.

It is also able to produce Vitamin K and lactase. (Mayo Clinic Article-LAcidophilus Copyright 2014)

It reduces intestinal pain by inducing u-opoid and cannabinoid receptrors in the intestines of animals but this effect has not been sufficiently shown in humans yet. (Animal Studies of NCFM)

The relation to gut- associated lymphoid tissue has been associated with good effects on the immune system such as antibody production, phagocytosis of Salmonella has been able to show the reduction of the symptoms of fever, cough and runny nose.

Statement of the Objectives

The study was conducted in effort to test the effect of Lactobacillus Acidophilus to be a component to break down lactose in lactose products. This study generally aimed to:

1. To determine the effects of Lactobacillus A. in blood glucose with the use of mice in taking milk with Lactobacillus A. bacteria

Specifically, it aimed to:

1. To find out the difference in glucose concentration between regular milk and lactose-free milk;
2. To have Lactobacillus Acidophilus effect in the presence of lactase enzyme to be used for lactose intolerant in dairy products;
3. To be able to compare of the glucose concentration of the control/treatments before and after added; and
4. To resolve the present economic status by stating its differences and capabilities.

Statement of the Hypotheses

Based on the foregoing objectives, the following statements are hypothesized:

1. Lactose-free milk is readily chemically combined with Lactobacillus Acidophilus and it has a high probability of breaking lactose faster than the milk that would be added with the said bacteria;

2. The glucose concentration of regular milk may be higher than of the lactose-free milk; and

3. Lactose free milk is much more affordable despite of its economic status than that of the regular milk added with *Lactobacillus Acidophilus*.

Significance of the Study

The deteriorating case of lactose intolerance worldwide apparently demanding an attention, this study is beneficial for the 65% of the population to experience a better digestion of lactose they acquire from the dairy products they take. This will accordingly help develop people's capability to be lactose tolerant, having their digestive system in good condition. Since, expectedly the mentioned problem has a chance to pave way to other worse illnesses with regards to enzymes in small intestines, adding Lactobacillus Acidophilus to lactose products which is also entitled in the production of lactase (a significant enzyme in the body system) is then the lifeline of the majority. This will allow people to achieve satisfaction and their maximum capacity of taking dairy products normally.

The significance of the study is most called for the case of newborn babies which already suffer from lactose intolerance. It would be so much abetment to discover a component of a regular milk which can be an intake substance of these unfortunate babies. Conducting a biochemical analysis of lactose decomposition done by the said bacteria raises awareness to people it concerns, provides a solution to the long-time dilemma and definitely contributes to the field of medicine.

Scope and Limitations

This study is beneficial for lactose intolerant in raising awareness about its biochemical basis and the effects of *Lactobacillus acidophilus* in lactose-diminishing properties. This study focused mainly on the glucose concentration of the mice after given the milk added with *Lactobacillus a.* and its comparison to lactose-free milk, negative control (water).

METHODOLOGY

Materials

The materials used in the study were the following: 24 mice, cage, pricker (lancets), glucometer, 75 glucose strips, water (2), regular milk, lactose free milk, lab coats, gloves, masks, spoon, beaker, centrifuge, *Lactobacillus acidophilus*, cotton, alcohol, syringe, boxes, wood shavings, tape, and scissors.

These were all gathered and prepared prior to the actual experimentation methods.

General Procedures

Mice are small rodents that are hard to grasp. Care should be taken to avoid getting bit or causing harm to the animal. One method of moving mice from one cage to another is by grasping the skin behind the neck with a pair of forceps. When using this method be careful not to grasp too hard. Restraining the mouse can be done by grasping the base of the tail with one hand and with the other grasp the loose skin behind its neck. Extra precaution should be taken to avoid getting bitten. When you have a firm grasp the tail may be secured in the same hand you have the scruff in to accomplish a one handed restraint. Protocol should be considered not to excite any of these animals. The job of restraining was made much easier by slow deliberate movements Noise should also be kept to a minimum. (Kenneth Scientific Corporation)

I. Preparation of the treatments

Three (3) treatments and 1 control were used in the experimentation; 3 males and 3 females per treatment. The glucose content was measured for the baseline values. Various experimental trials were conducted, to wit: Treatment 1(with Lactobacillus A.); Treatment 2 (with Lactose-free milk); Treatment 3 (with Regular milk) and; Control (with water).

Different amount of water and milk were mixed and were allowed to dissolve. (Table 1). Ten (10) ml of the solution were given to the mice of each treatment. The control group was provided with 10 mL of water.

Table 2. Preparation of the 3 treatments and 1 control

Treatment	Amount of milk	Amount of water (ml)	Amount of solution given to the mice (mL)
1	Regular milk with Lactobacillus A. (2 scoops)	50	10
2	Lactose- free milk (2 scoops)	60	10
3	Regular milk (2 scoops)	50	10
Control		10	10

A 30-minute testing period for each treatment was undergone by the mice. (Table 2).

Table 2. Testing Time

Time Given the Solution	**Time Tested**
Control group	1:00 - 1:30 PM

Treatment 1	3:30 - 4:00 PM
Treatment 2	2:00 - 2:30 P.M
Treatment 3	3:00 - 3:30 P.M

II. Blood Glucose Test

The blood glucose of each mouse was tested by the method of tail pricking (with the use of Lancets) and was then measured by glucometer and glucose strips. The tail of mouse was put in an empty box with a small hole in which the tail fits. Then, cotton with alcohol was applied to the tail and the tail pricking method was done. The tail was injected with the needle and the droplets of blood was dripped to the glucose strips inserted in the glucometer. Glucose level of the mouse (mg/dl) was then read.

Results were gathered and recorded.

III. Negative Control Procedure

Another test tube was filled with plain water and was labeled with*Negative Control.*Two (2) different strips were dipped into the positive control and into the negative control. Changes in color (as seen in the strips) were recorded and were compared to the key on the bottle to determine glucose concentration of the tested fluid. Having negative reaction for the water control, Four (4) tsp. of regular milk and 4 tsp. of lactose-free milk were poured into clean, separate containers. In order to find out the glucose concentration in the regular milk sample, the researcher dipped a strip into the milk sample. The same method was done in the lactose-free milk.

RESULTS

Table 3. Results on the blood glucose concentration

Table 3.1. TREATMENT 1 (in regular milk added with Lactobacillus Acidophilus)		
Treatment 1	Blood Glucose (1)	Blood Glucose (2)
41	81	114
83	99	78
68	167	197
97	97	102
103	100	73
56	195	153

Table 3.2. TREATMENT 2 (by the use of Lactose-free milk)		
Treatment 2	Blood Glucose (1)	Blood Glucose (2)
53	182	207

66	235	125
69	119	274
118	134	165
82	101	129
132	85	75

Table 3.3. TREATMENT 3 (by the use of regular milk)		
Treatment 3	Blood Glucose (1)	Blood Glucose (2)
69	89	98
70	89	60
203	127	162
84	187	110
133	104	169
101	95	81

Table 3.4. CONTROL (by the use of water)		
Control	Blood Glucose (1)	Blood Glucose (2)

114	86	99
120	101	90
142	133	80
52	53	62
65	65	84
100	69	100

Table 4. Group mean average of glucose test before the feeding (Column 1), first testing after the feeding (Column 2) and after 4 hours, another testing (Column 3)

Group number- average		
Before the feeding	After the feeding	
Control 1- 92.83	84.585.83	
Treatment 1- 74.67	108.17	119.5
Treatment 2- 86.67	142.33	161.67
Treatment 3-	106.67	113.33

Table 5.Results evaluated by percentage of increase or decrease of the average comparing to its blood glucose before the feeding has done

Group	Percenatge
Control Group	**-17%**
Treatment 1	**65%**
Treatment 2	**57%**
Treatment 3	**1%**

Table 6. The second feeding was done after 4 hours

Time Given the Solution	Time Tested
Control group	5:00 P.M - 5:30 PM
Treatment 1	7:00 P.M - 7:30 PM
Treatment 2	6:00 P.M - 6:30 P.M
Treatment 3	7:00 P.M - 7:30 P.M

DISCUSSION

The baseline values for the non-fasting blood glucose were initially collected before the introduction of milk treatments for each group to determine any increase or decrease in the blood glucose levels during the duration of the treatment. The control group (given only water) had a mean blood glucose level of -17%, treatment 1 mice (fed with regular milk with *Lactobacillus acidophilus*) had a mean blood glucose level of 65% mg/dl, treatment 2 (fed with lactose free milk) had a mean blood glucose of 57 % mg/dl, and treatment 3 (fed with regular milk) had an average blood glucose level of 1% mg/dl.

The mice were subjected to feed testing and the blood glucose levels were collected 30 minutes after each control/treatment. For replication purposes, the same method was done after 4 hours.

The regular milk which consists of lactose but its glucose content was not read since it is chemically combined with the galactose.On the other hand, lactose-free milk has lactase enzyme that breaks down lactose to glucose and galactose thus, it has high glucose concentrations.

Despite of that, the regular milk with Lactobacillus Acidophilus bacteria had a high increase rate of glucose level, indicating its potency, followed by the lactose-free milk then,the regular milk and lastly, the control group.

Adding a capsule of Lactobacillus Acidophilus is in fact, more cost-effective than the commercially produced lactose-free milk. In addition, Lactobacillus A. is an effective component having its lactase to help lactose intolerant digest dairy products.

Conclusion

The researchers concluded that Lactobacillus Acidophilus is an effective subject in breaking down lactose in dairy products.

Moreover,it was found out that there is glucose concentration in lactose-free milk and none in regular milk even if lactose is present due to its chemical property of being mixed up.Lactose-free milk's lactose is already broken down to glucose because of the presence of lactase.

Recommendation

The researcher recommends more samples, replicates and long-term experimental period.The researcher recommends the addition and further studies of investigating other possible health benefits of Lactobacillus acidophilus.

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To our parents, aunt and uncle, grandmother and grandfather, for the financial support, moral support.

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APPENDICES

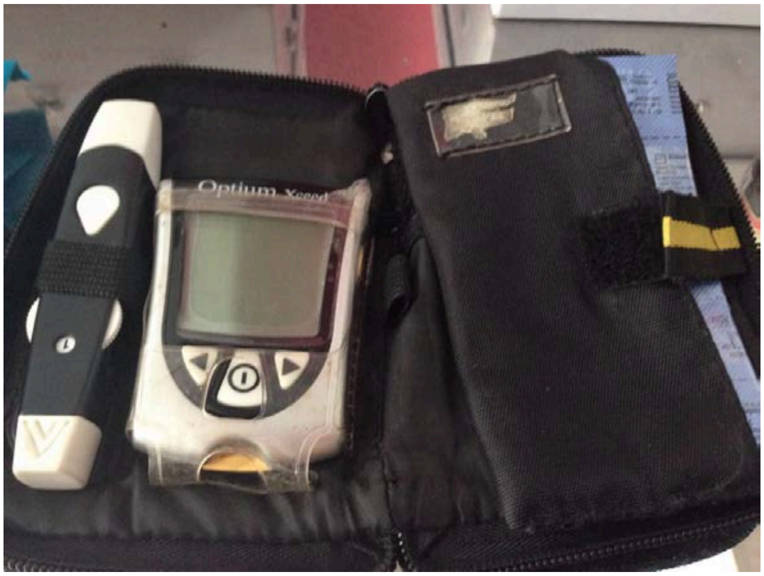
APPENDIX A


Glucose concentration in lactose-free milk

APPENDIX B


Glucose concentration in regular milk

APPENDIX C



Glucometer and Glucose Test Strips

APPENDIX D

Boxes; Treatment and Control with mice

APPENDIX E

Separation of male from female

APPENDIX F

APPENDIX G

6 mice in a treatment

APPENDIX H

Tail pricking of mice

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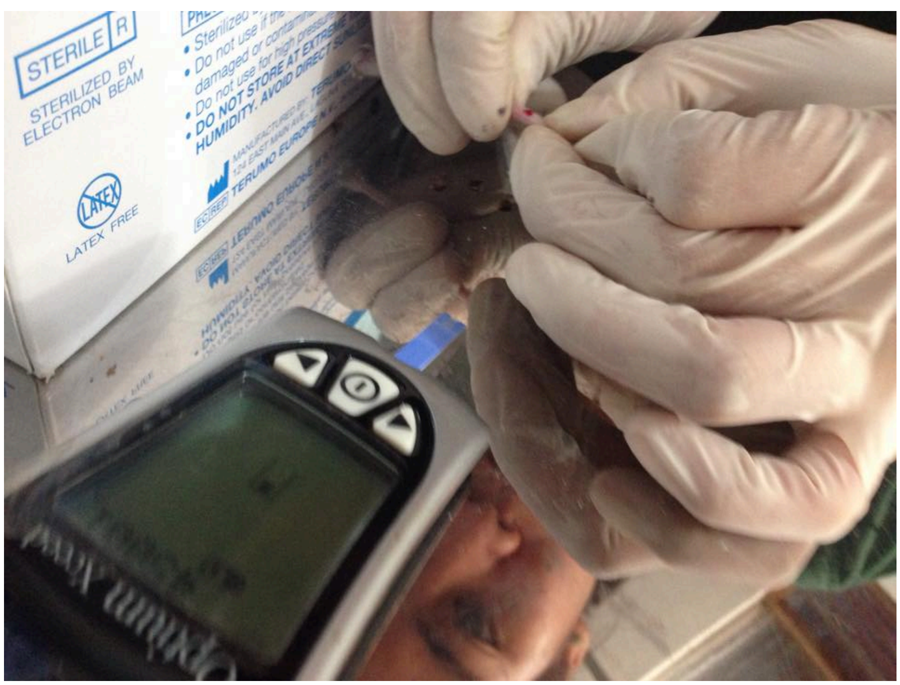
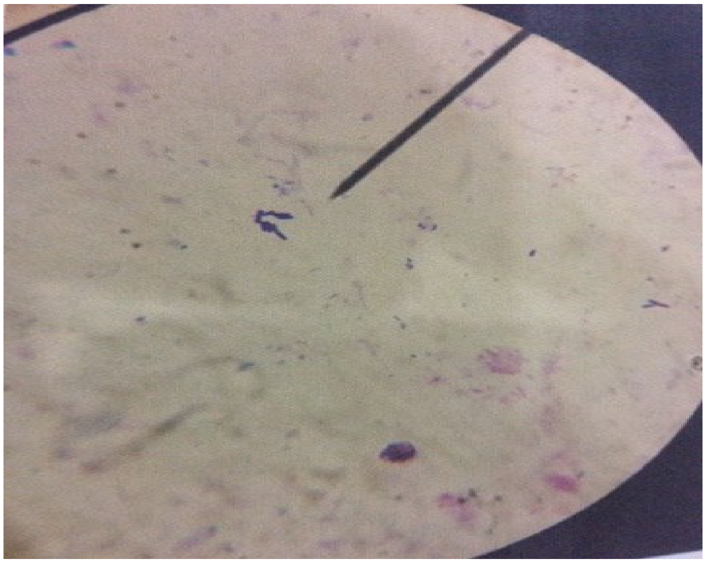
APPENDIX I

Tail pricking; blood testing in glucometer with the use of glucose strips

APPENDIX J

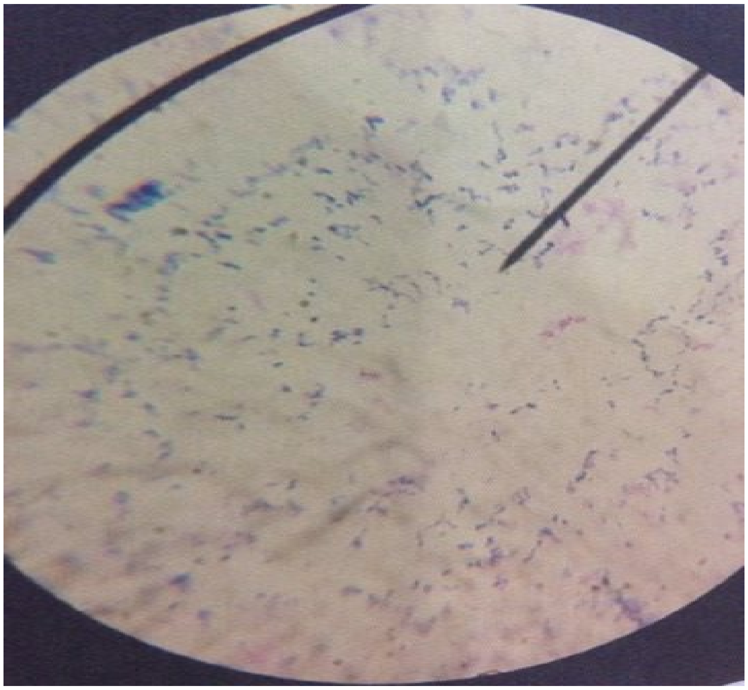


APPENDIX K



Lactobacillus acidophilus under microscope

APPENDIX M



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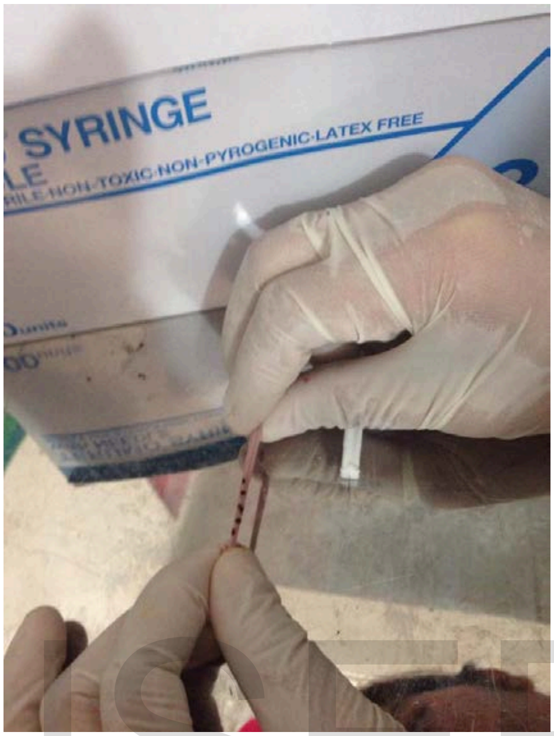
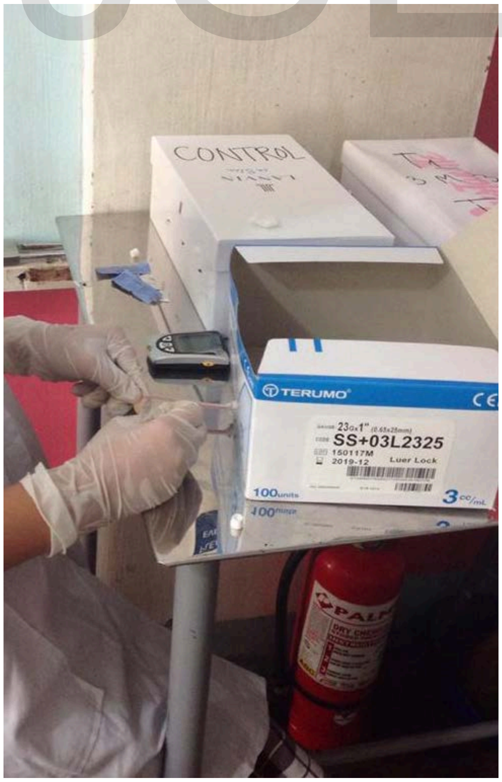
APPENDIX N

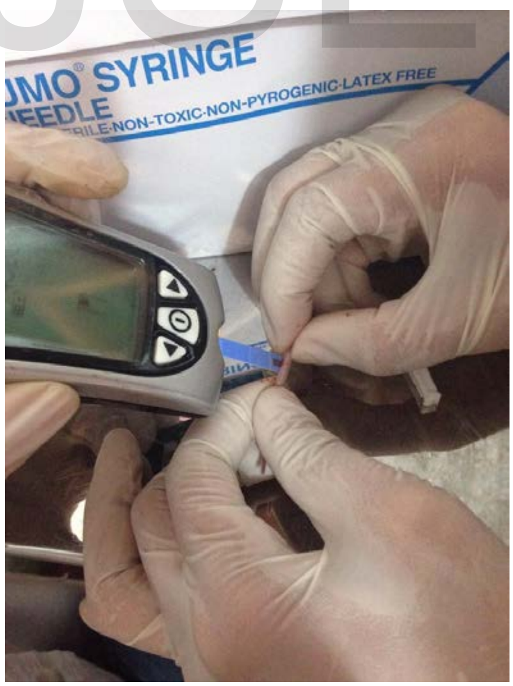
APPENDIX O

APPENDIX P

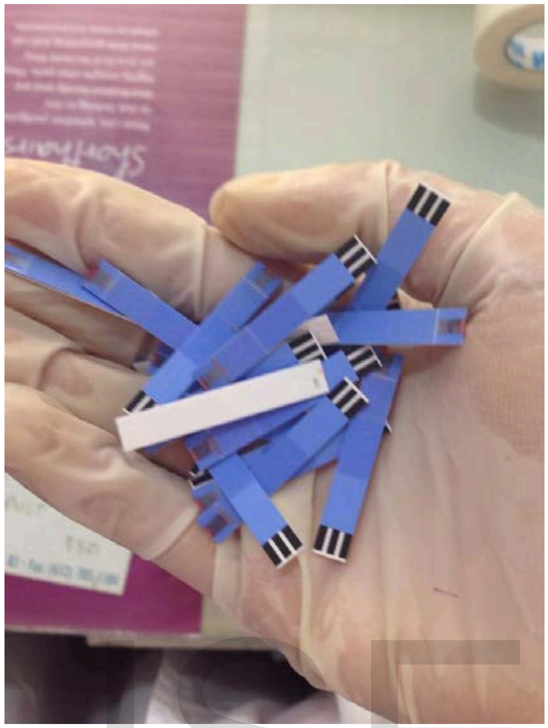

SYRINGE
NON-TOXIC-NON-PYROGENIC-LATEX FREE


CONTROL
TERUMO
SS+03L2325
150117M
2019-12
Luer Lock
100units
3cc/mL

APPENDIX Q



APPENDIX R



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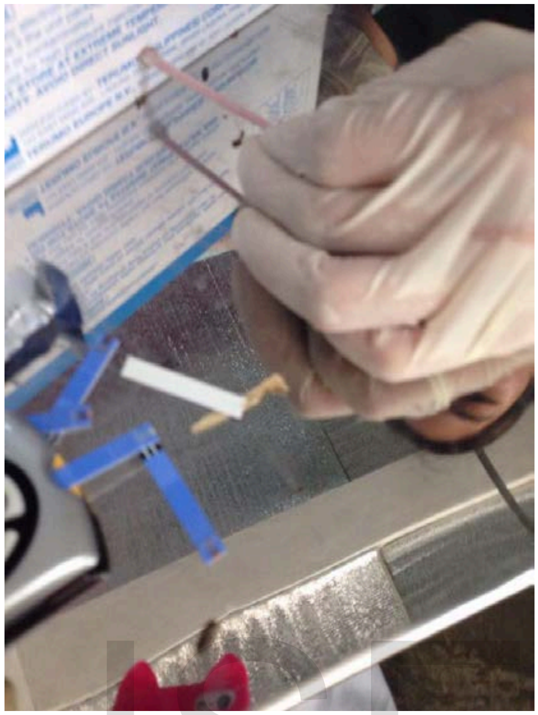
APPENDIX S



APPENDIX T

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APPENDIX U

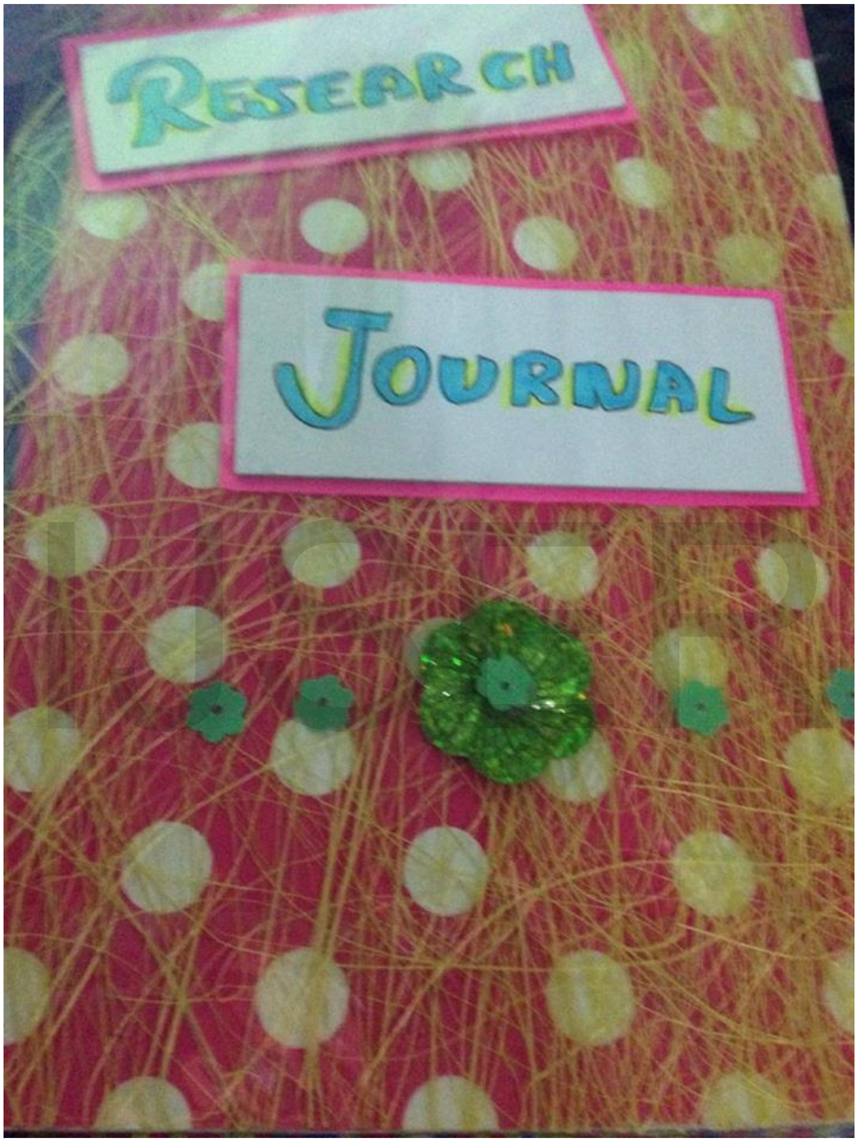
Milk used

APPENDIX V

Materials

APPENDIX W

Project Data Book



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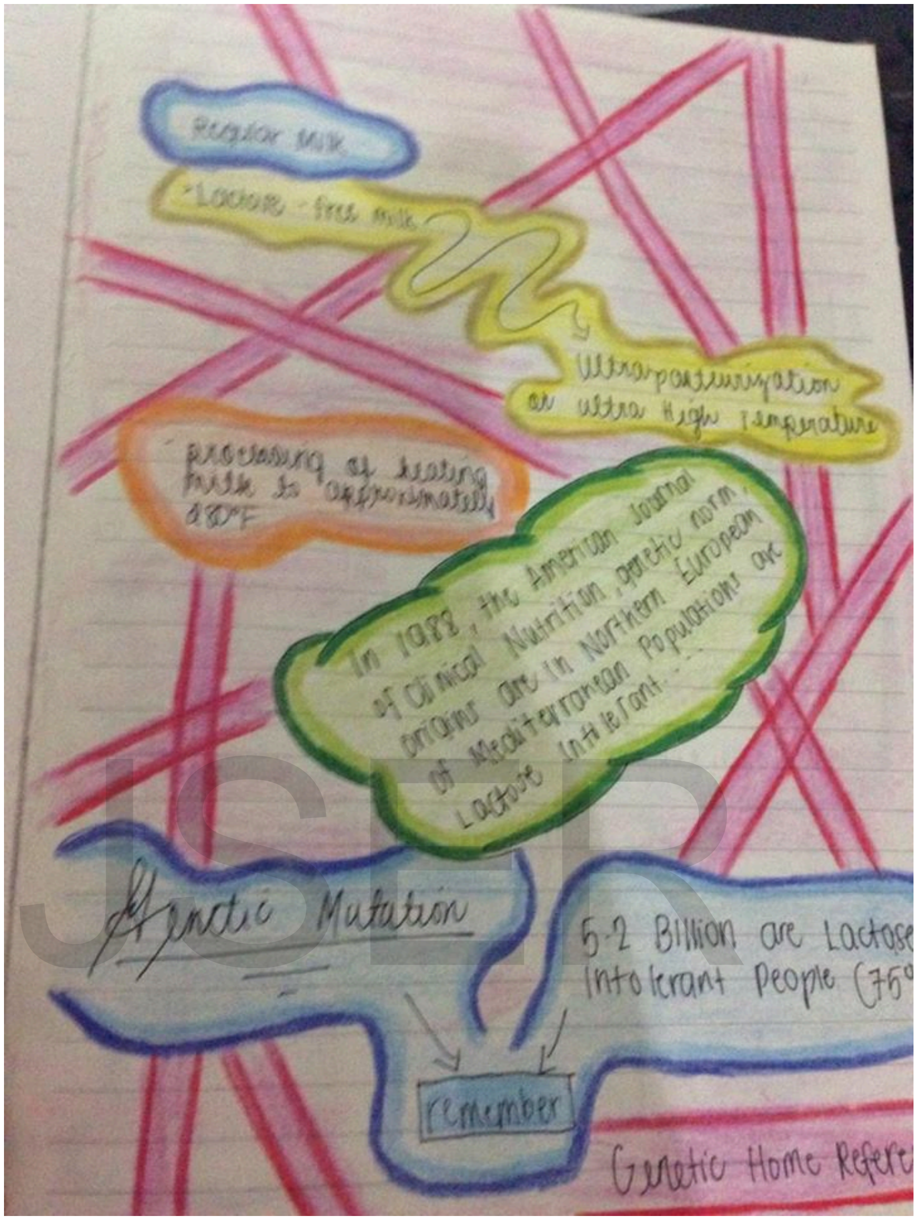

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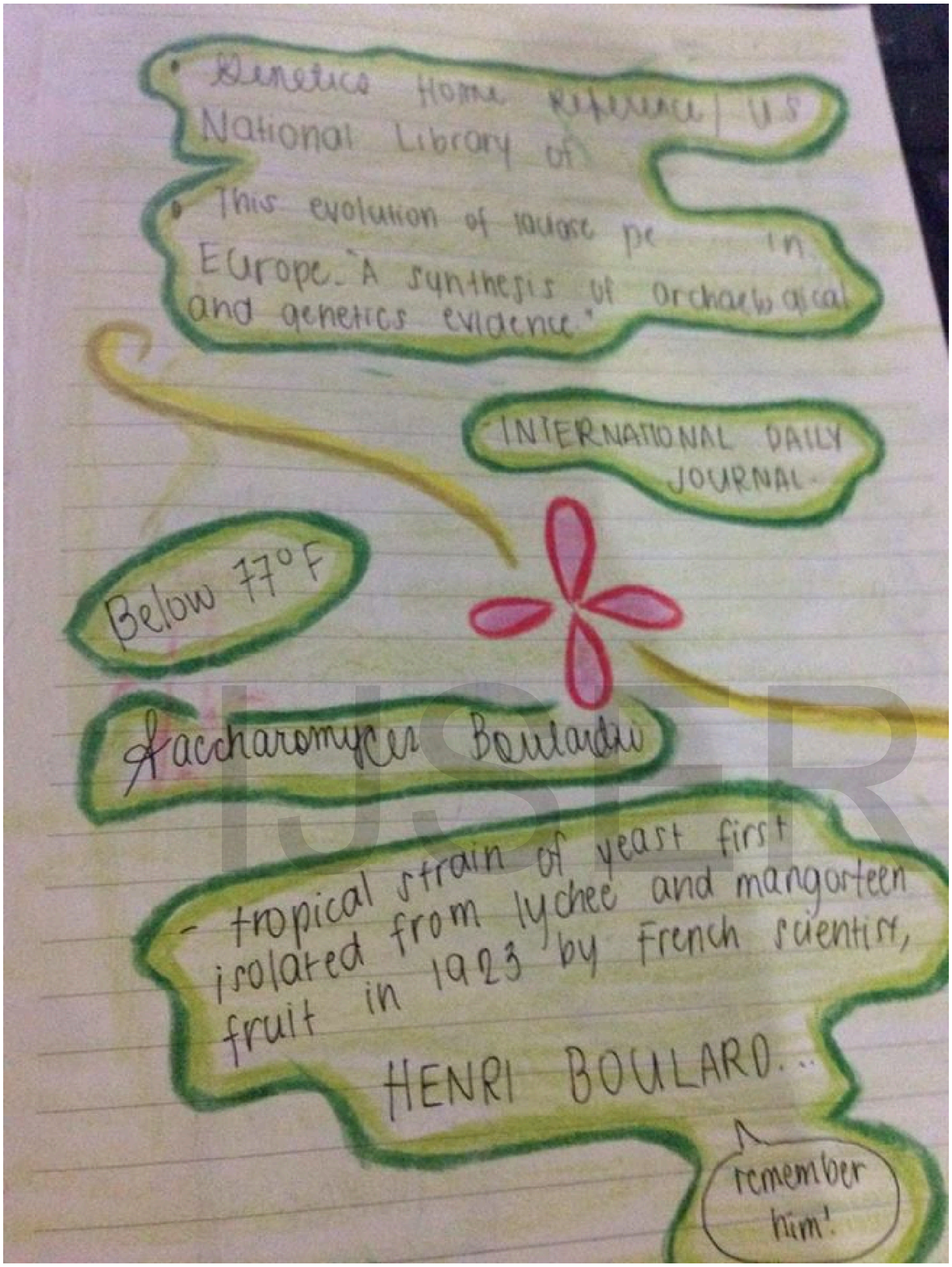

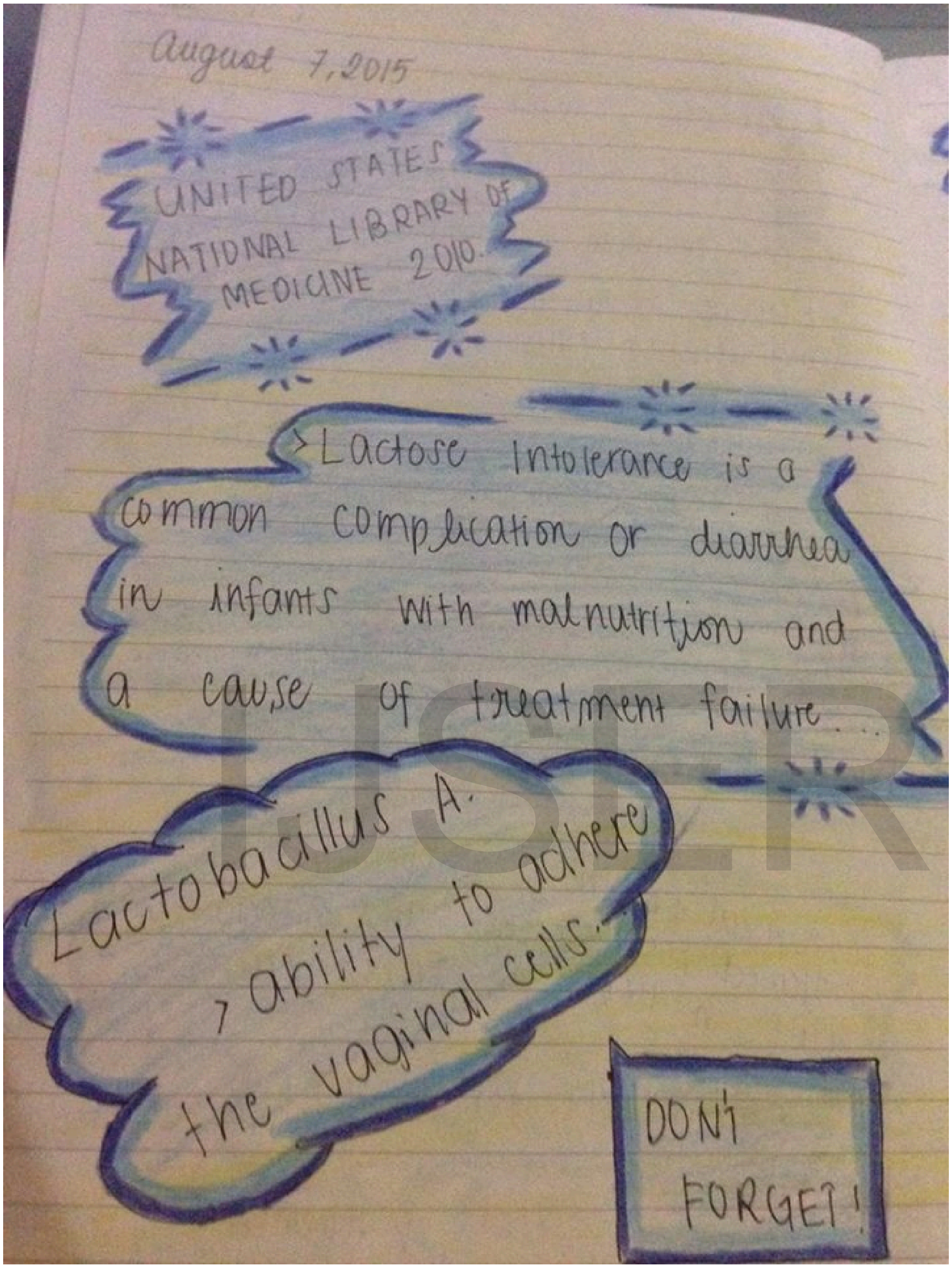
August 7, 2015
UNITED STATES NATIONAL LIBRARY OF MEDICINE 2010.
>Lactose Intolerance is a common complication or diarrhea in infants with malnutrition and a cause of treatment failure...
Lactobacillus A.
>ability to adhere the vaginal cells...
DON'T FORGET!

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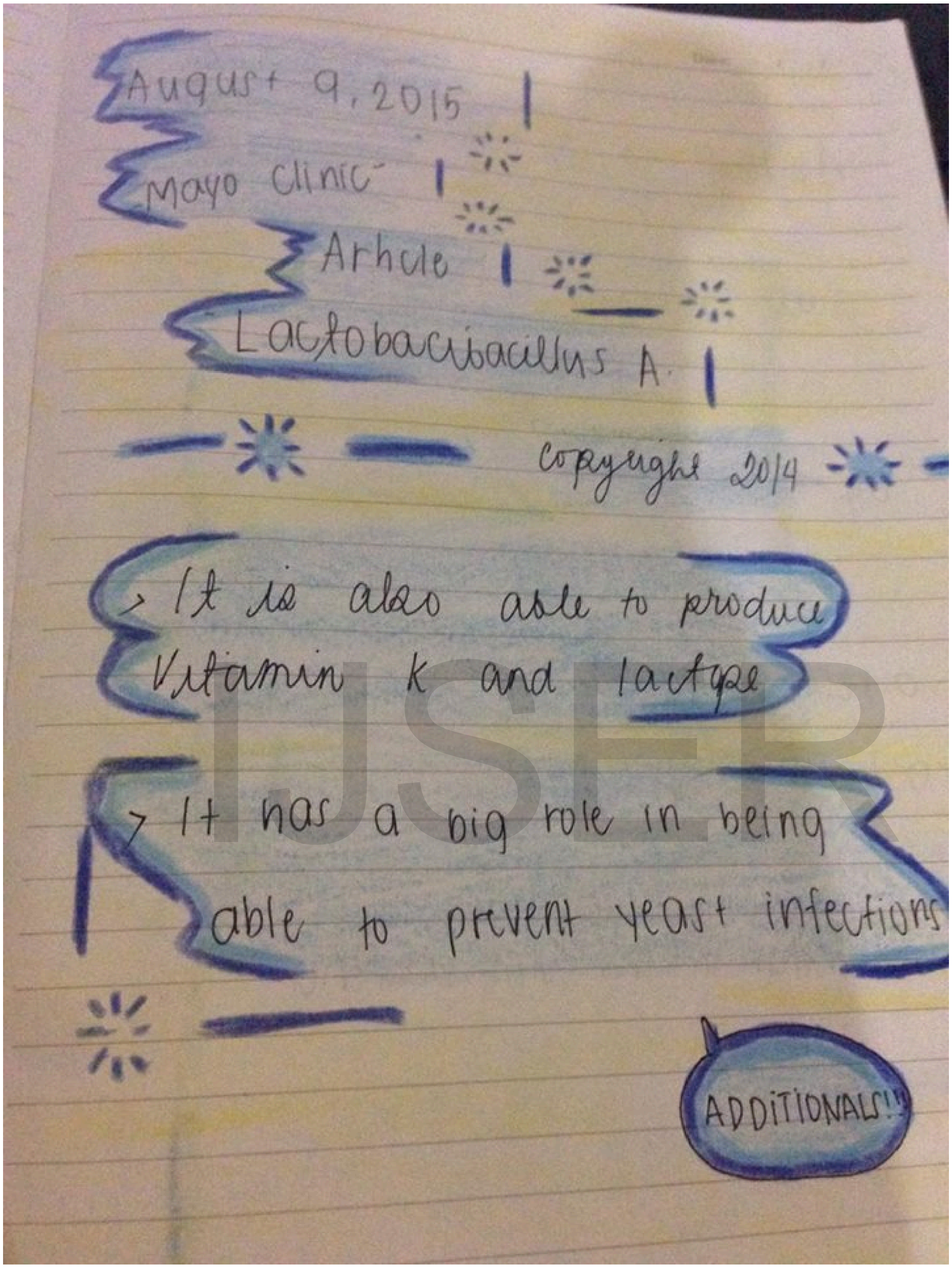

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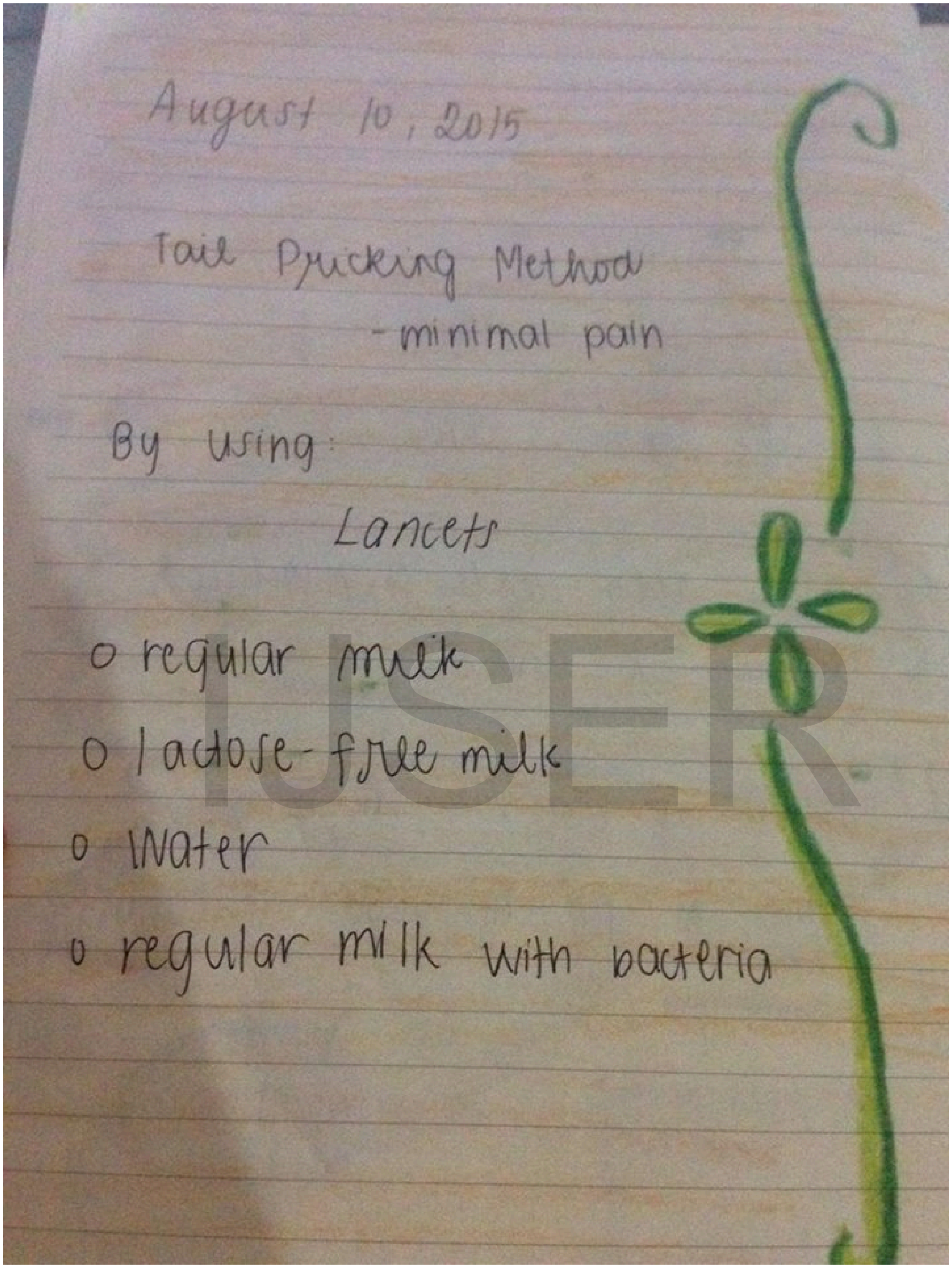

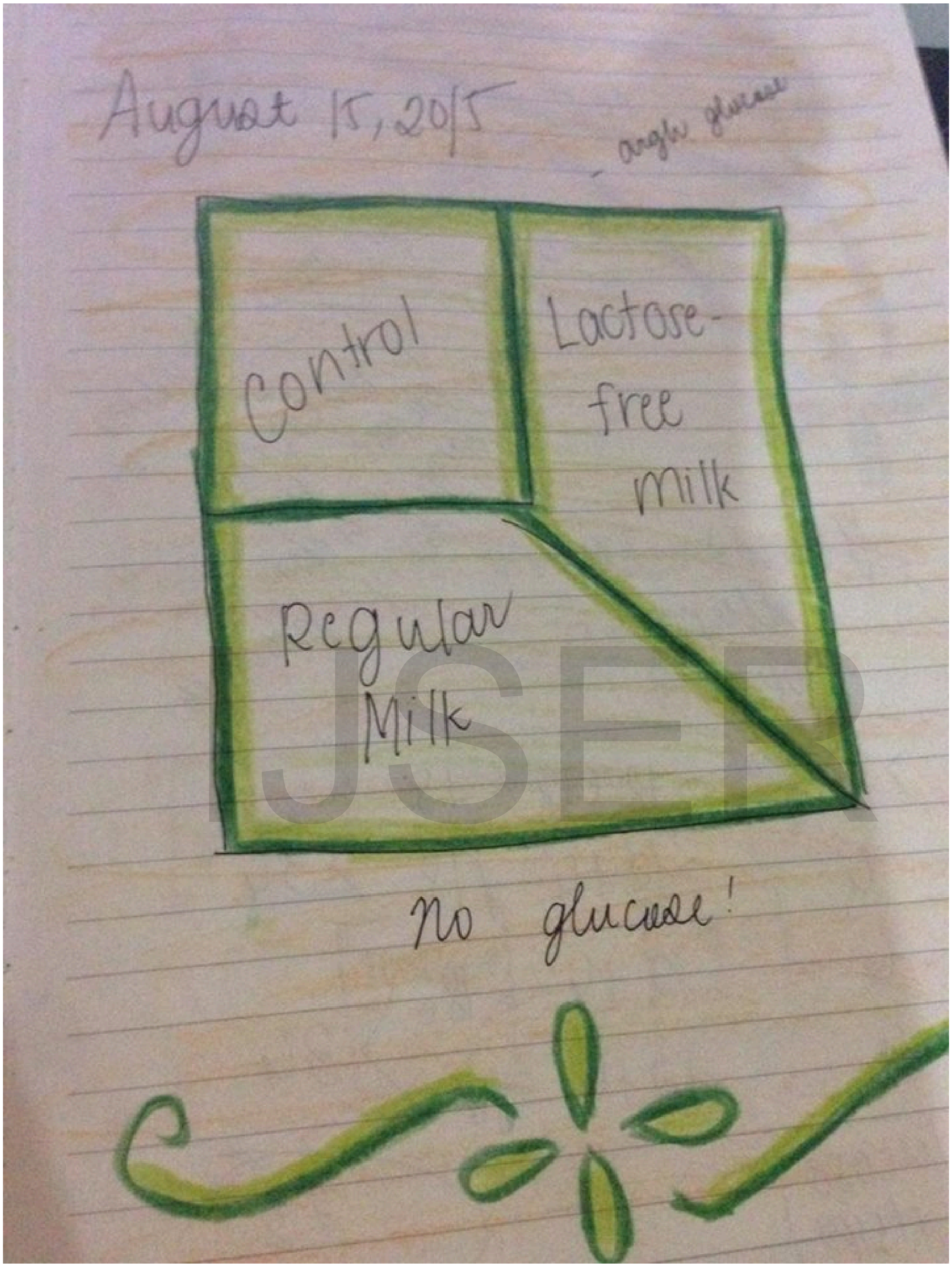
August 15, 2015
Control
Lactose-free milk
Regular Milk
No glucose!

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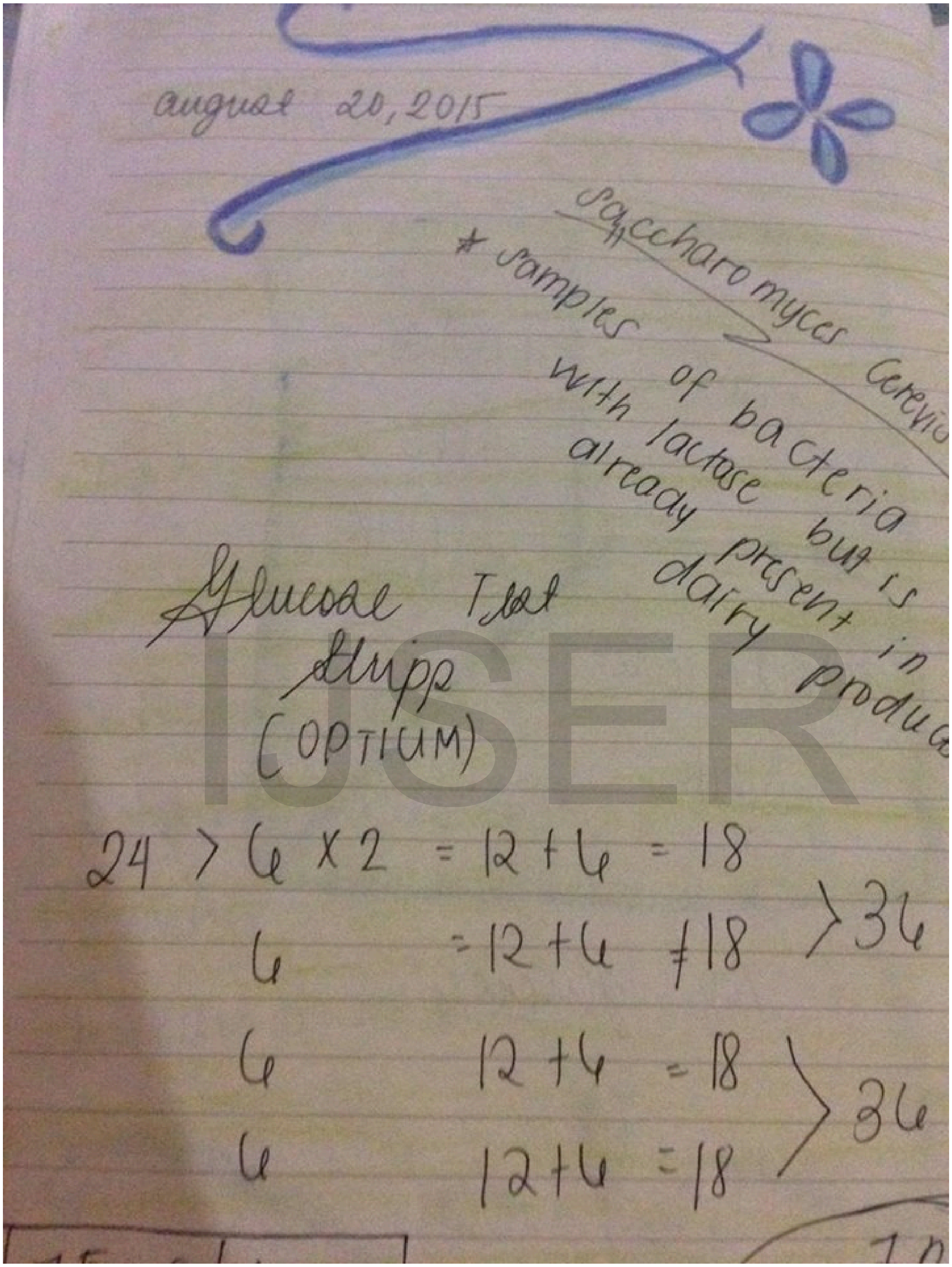

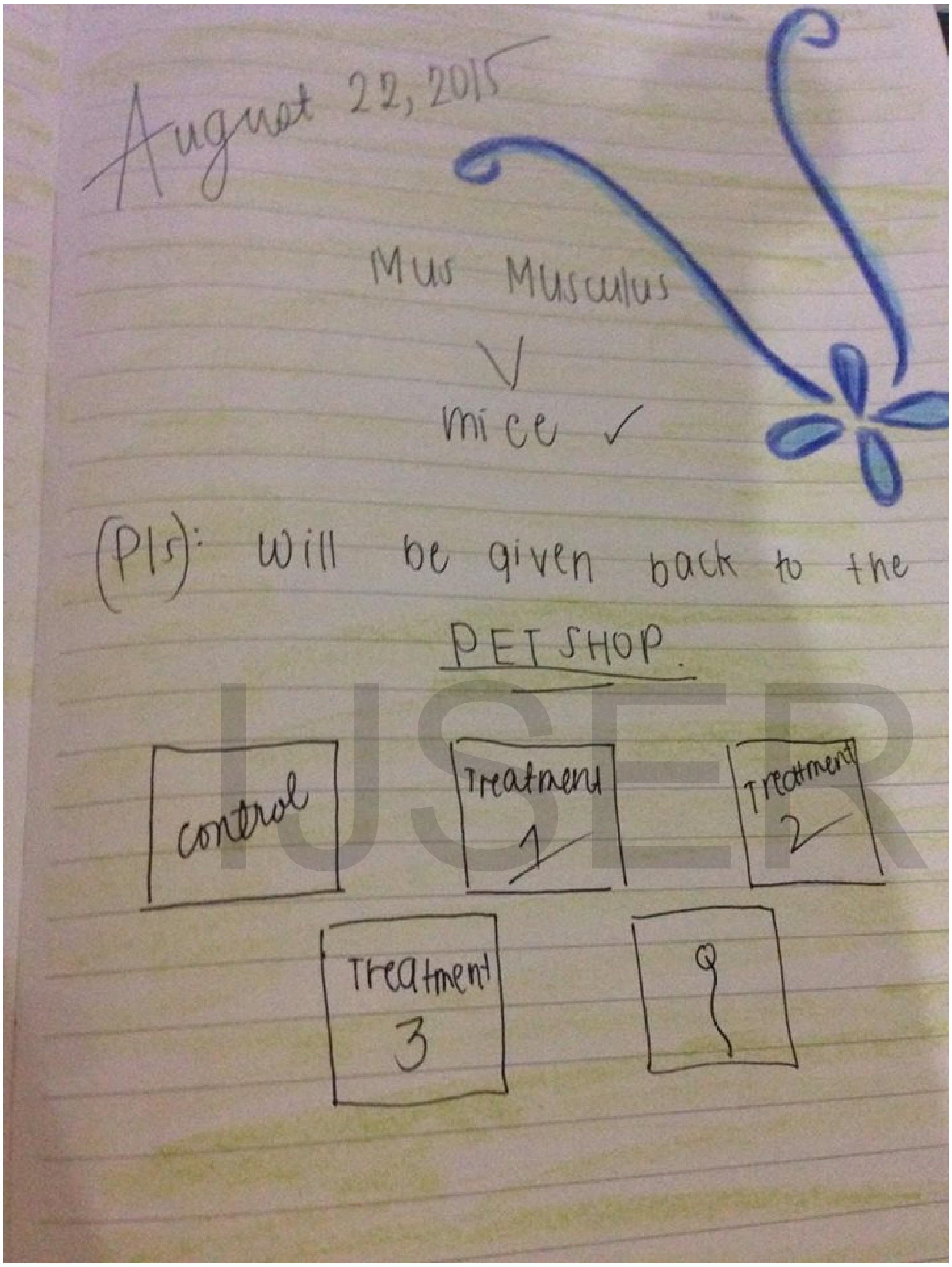
August 22, 2015
Mus Musculus
mice ✓
(Pls): will be given back to the
PET SHOP.
control
Treatment 1
Treatment 2
Treatment 3

Safety Laboratory Practices and Procedures

(National Institute Of Health)

Tip #1: Ask yourself, "What am I working with? What are the hazards?"

- Common hazards in the laboratory include: animal, biological, chemical, physical, and radiological. If there is an accident or emergency situation involving these hazards:
  - Seek immediate assistance. If you are splashed by any of these materials, use running water from an eyewash station or emergency shower for at least 15 minutes or until emergency assistance arrives and provides you with different instructions.
  - Report to your supervisor any accident, injury, or uncontrolled release of potentially hazardous materials - no matter how trivial the accident, injury, or release may appear.

Tip #2: Be prepared.

- Attend all required laboratory safety training prior to the start of your research assignment.
- Read all procedures and associated safety information prior to the start of an experiment.
- Perform only those experiments authorized by your supervisor.

- Follow all written and verbal instructions. Ask for assistance if you need guidance or help.
- Work under direct supervision at all times. Never work alone in the laboratory.
- Know the locations and operating procedures for all safety equipment. This includes the eyewash station and safety shower.
- Know the locations of the nearest fire alarms and at least two ways out of the building. Never use an elevator in emergencies.
- Be alert and proceed with caution at all times in the laboratory. Immediately notify the supervisor of any unsafe conditions.
- Know the proper emergency response procedures for accidents or injuries in the laboratory.

Tip #3: Prevent potential exposure.

- Conduct yourself in a responsible and professional manner at all times. No pranks. No practical jokes.
- Dress for work in the laboratory. Wear clothing and shoes that cover exposed skin and protect you from potential splashes. Tie back long hair, jewelry, or anything that may catch in equipment.

- Never eat food, drink beverages, chew gum, apply cosmetics (including lip balm), or handle contact lenses in the laboratory.
- Use a chemical fume hood or biosafety cabinet, as directed by your supervisor.
- Observe good housekeeping - keep aisles clear.
- Report damaged electrical equipment to the supervisor. Do not use damaged electrical equipment.
- Do not leave active experiments unattended. Never leave anything that is being heated or is visibly reacting unattended.

Tip #4: Protect yourself, others, your research, and the environment.

- Practice good personal hygiene. Wash your hands after removing gloves, before leaving the laboratory, and after handling a potentially hazardous material.
- While working in the laboratory, wear personal protective equipment - eye protection, gloves, laboratory coat - as directed by your supervisor.
- Properly segregate and dispose of all laboratory waste.

The study is still on-going and is to be improved with additional subjects and enhanced method.