

Teaching Through Practice

Building Concepts with Applied Simulations
and Universal Reagents

LABiEMol



Editors

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TEACHING THROUGH PRACTICE:
Building Concepts with Applied Simulations and Universal Reagents

RIO DE JANEIRO
TATIANE MILITÃO
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PREFACE

The use of simulation as a teaching strategy has gained importance in many educational contexts, especially in health-related disciplines. However, its application in basic sciences is still limited, particularly concerning the direct correlation with practical aspects related to health professions. This book is not just a guide; it is a starting point for using its principles, practical applications, and inspiring case studies, to equip learners and practitioners alike to champion innovation while honoring our collective responsibility to future generations when exploring practical classes.

This book addresses the development and implementation of practical classes that use pH indicators, which we have called universal reagents. These safe reagents can be used in a practical context and/or to facilitate the understanding of the relationship among fundamental knowledge from different basic areas, and various Biotechnology and Health themes as well as disease diagnoses, through laboratory simulations.

Each simulation employs a pH indicator extracted from red cabbage, highlighting its potential in connecting basic disciplines to clinical and/or professional applications. Also biological samples such as serum or plasma were substituted by apple vinegar, as this book provides a call to action using simple materials to simulate complex contexts.

This book is the result of a collaborative effort between Dr. Helena Carla Castro, from the Department of Cell and Molecular Biology at the Federal Fluminense University (UFF) in Niterói, Brazil, and Prof. Mansur Muhammad Dewu, from the Department of Microbiology and Biotechnology at the Federal University Dutse, in Dutse, Nigeria. Together, they organized this book during his doctoral studies in the Pathology Postgraduate Program at UFF in 2024. It is part of their work produced under the Extension Project called *The Human Project*, coordinated by Dr. Helena. This initiative aims to disseminate scientific knowledge across diverse settings, fostering learning and engagement with science in both academic and non-academic communities using and teaching safe and low-cost strategies.

Niterói, Rio de Janeiro, Brazil, December 22nd 2024

Helena Carla Castro
Mansur Dewu Muhammad

INTRODUCTION

In a world increasingly defined by the urgency of sustainability and innovation, the principles of green chemistry stand as a beacon of hope and responsibility. Green chemistry goes beyond minimizing harm. It envisions a future where science and sustainability are intrinsically linked. By fostering creativity, collaboration, and consciousness, it empowers scientists, educators, and industry leaders to design solutions that are safer, more efficient, and in harmony with our planet.

Similarly, simulation has emerged as an important tool in educational practices across diverse disciplines, particularly in health-related fields and clinical training. It allows the recreation of real-world scenarios in a controlled and risk-free environment, providing students with opportunities for practical and interactive learning. Studies have demonstrated the effectiveness of simulation in medical and nursing education, highlighting its impact on the development of technical skills and critical thinking [1-2]. However, its application in disciplines such as biochemistry is still underexplored.

Pathology, Biotechnology, Biochemistry, Immunology, and Oncology are fundamental basic disciplines in medical, health, and biological sciences, addressing complex biological human and animal processes. Concepts such as pH, antibody production, and vaccines are essential for understanding physiological homeostasis, being crucial in the diagnosis and management of various diseases [3].

In traditional educational environments, students often encounter different topics through theoretical classes and abstract discussions. This approach, however, can hinder the connection between theory and practice, limiting students' ability to relate the content they have learned in the classroom to the practical scenarios they will face in their careers.

The integration of applied simulation-based learning in basic disciplines presents a unique opportunity to bridge this gap. It allows students to better visualize and understand the role of learned concepts in professional practice, making learning more relevant and impactful [4].

The main purpose of this book is to present practical classes based on simulation that uses a natural and safe pH indicator, extracted from red cabbage.

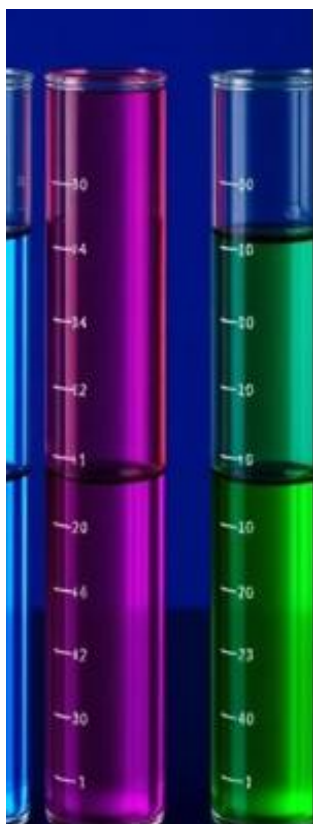
These classes were developed to demonstrate concepts from different areas in an applied context. The simulation involved the use of non-biological samples that will simulate body fluids, such as apple vinegar, lemon soda, water, bicarbonate, and milk of magnesia, decreasing the risks presented when using real biological samples.

In these simulations, students are challenged to identify these *patients*, *volunteers* approved “materials” (eg. Vaccines) and correlate the results with conditions associated with observed changes. These practical classes provide an engaging learning experience, allowing students to apply theoretical knowledge in a simulated practical scenario. At the same time, this book seeks to demonstrate the relevance of basic disciplines for establishing diagnosis for students from health areas, promoting a deeper understanding of how learned concepts directly relate to professional practice.

In this book, each chapter allows the discussion of a different topic of Pathology, Biotechnology, Immunology, Pharmaceutical, and Oncology areas, presenting the development, application, and evaluation of this simulation-based teaching strategy. The results of the simulation, including student feedback and the effectiveness of the approach in connecting theory and practice, should always be discussed at the end. Through this book, we hope to contribute to the investments on more studies on simulation-based learning and its application in the education of young people and adults at different educational levels, as it allows its use from kid to elderly students.

All practical classes include distinct orientations for students/participants and for monitors and professors to ensure the simulation environment aligns with the specific approach for each topic. For students, only the simulation environment is presented, whereas for monitors and professors, the entire class, including the preparation of the pH indicator and "biological samples," is reported. The final analysis of the results and the discussion are also stimulated, but not limited to, by some questions proposed in this manual to create an environment of thinking and learning simultaneously.

Please, feel free to reach out to the book's organizers with any questions about how to use the practical lessons (hcastro@id.uff.br). We are here to support you and ensure you make the most of this resource as well as we are also open to national and international collaborations.



CHAPTER I

APPLIED SIMULATION

Interdisciplinary Section (Biotechnology-Pathology)

Topic: *Understanding Vaccines And Their Medical Importance*

Version: *Monitors and Teachers/Professors*

Authors: *Sueli Braga,
Aldo Rodrigues,
Nadja Avila,
Leonardo Miceli,
Helena Carla Castro*

Logo of your Institution	Name of your Institution
	<p>Human Project: Applied Simulation Laboratory of Antibiotics, Biochemistry, Teaching, and Molecular Modeling Fluminense Federal University, Institute of Biology, Brazil</p>

Interdisciplinary Section (Biotechnology - Biochemistry)

Version: *Monitors and Teachers/Professors*

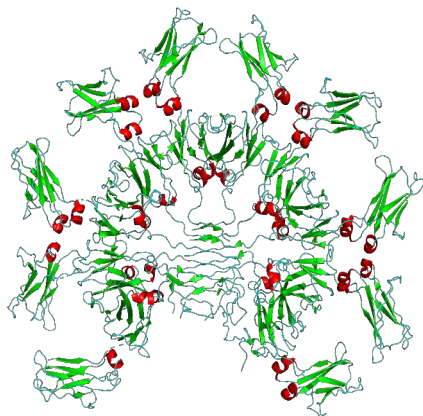
TOPIC: UNDERSTANDING VACCINES AND THEIR MEDICAL IMPORTANCE

1. Introduction

Vaccines are one of the most important strategies for preventing infectious diseases today. By activating the immune system to develop antibodies against specific pathogens, they work by presenting the body with components of the infectious agent, also called antigens (e.g. proteins, RNA, DNA, or inactivated viral particles). These molecules stimulate the immune system without inducing the disease, allowing the production of antibodies, which are proteins with defense activity that will protect the body through interaction with the immune cells of the vaccinated individual.

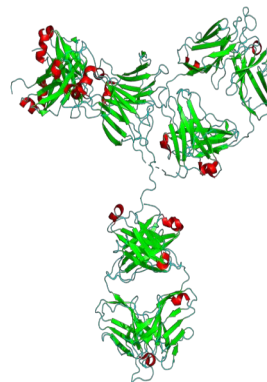
To verify the effectiveness of the vaccine, the production of antibodies is one of the main indicators of its protective efficiency. Among the evaluated antibodies, we have:

a) IgM, which indicates an initial immune response, being the first antibody produced after exposure to the antigen.



Immunoglobulin M (IgM)

b) IgG, an antibody related to immune memory and responsible for long-term protection. The neutralizing action of these antibodies in blocking pathogen infection is the main focus for evaluating the efficacy of various vaccines.



Immunoglobulin G (IgG)

Antibody tests, also known as serologies because they use blood serum, are used to evaluate the efficacy of the vaccine by measuring the levels of specific antibodies in the blood. These tests have various purposes, including: a) Confirmation of the Immune Response, by verifying the level of antibody production, b) Evaluation of the Duration of Protection, by monitoring the amount of antibodies over time and indicating the need for booster shots, and c) Analysis of clinical studies during vaccine development, where antibody tests help determine if a formulation is effective before being released for public use.

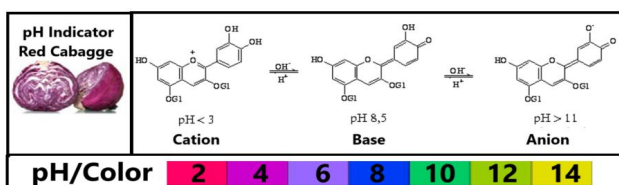
In this practice, each group of students will evaluate different hypotheses that will lead to the analysis of experimental vaccines against an unknown pathogen.

Therefore, it is important to perform a negative control test (Patient 1 Normal) where the serum does not cause a change in the reagent used, as well as a positive control test (Patient 2 Immunized) where the presence of the antibody is observed and the color change is guaranteed.

In this work, the substitution of the biological material with applied simulators will be using a different material for the preparation of the "volunteer samples". In this case, apple vinegar. The pH indicator will be prepared as a red cabbage solution. These reagents are easily accessible and easy to prepare with low cost and low risks.

Red cabbage can be used as an acid-base indicator because it contains molecules that changes color when in contact with acid or base. These molecules are anthocyanins, pigments belonging to the flavonoid group that are responsible for the wide variety of colors in fruits, leaves, and flowers.

The role of anthocyanin in the plant is to protect against ultraviolet light and prevent the production of free radicals. This substance is highly sensitive, and should be stored on a freezer or refrigerator until use.



2. Objective

Our purpose is to simulate the analysis of blood serum samples from volunteers for antibody production and the suitability of the four "vaccines" produced in the applied simulation proposal.

3. Reagents

-5 syringes of 5 ml or plastic Pasteur pipettes.

-5 recycled 6-well cell culture microplates or 14 glass tubes.

- 1 Red cabbage.

- 1 blender.

- 1 stainer.

- Water and apple vinegar



4. Preparation of Reagents

♦ pH Indicator:

Weigh 500g of chopped red cabbage and cook with 1L of water for 15 minutes. Then blend and strain through a sieve, storing in the freezer. Use 2 ml of the indicator in each well of the recycled cell culture microplate or glass tube.

♦ Patient Samples (Volume depends on the use of tube or plate)

Volunteer C1 (Normal): Negative Control

Normal: water (0.5 ml)

Volunteer C2 (Producing antibody): Positive Control

Altered: apple vinegar (0.5 ml)

Volunteer 1: Vaccine 1 (Antibody Production)

Altered: apple vinegar (0.5 ml)

Volunteer 2: Vaccine 2 (Need for Booster Shot)

Altered: Baking soda solution or milk of magnesia (0.5 ml)

Volunteer 3: Vaccine 3 (Not Immunized)

Normal: water (0.5 ml)

Volunteer 4: Vaccine 4 (Not Immunized)

Normal: water (0.5 ml)

5. Procedures

- ♦ Place 0.5 ml of each patient sample in each well of the plate respectively
- ♦ Transfer 1 ml of indicator/reagent;

RESULTS	
Unchanged color (purple)	Neutral
Bluish-green or green	Basic
Pink or Red	Ácidic

Result from C1 Volunteer: _____
 Result from C2 Volunteer: _____
 Result from 1st Volunteer: _____
 Result from 2nd Volunteer: _____
 Result from 3rd Volunteer: _____
 Result from 4th Volunteer: _____

6. Discussion Questions

6.1. Comment (briefly) on the results in relation to the patients analyzed

6.2. Define and compare the data found based on the age groups of the patients.

6.3. What type of test was performed: direct or indirect? Justify.

Colorimetric Reaction

INTRODUCTION

Colorimetric reactions provide high sensitivity and specificity, making them valuable tools for rapid and reliable diagnostics in various health contexts. This principle is widely used for clinical diagnostics, such as:

- HIV**: Detection of antibodies against the virus
- COVID-19**: Identification of antibodies or antigens of SARS-CoV-2.
- Autoimmune diseases**: Evaluation of autoantibodies.

A colorimetric reaction in a diagnostic test involving antigen and antibody can occur indirectly or directly:

A. Indirect Colorimetric Reaction

Immunoassays like ELISA (Enzyme-Linked Immunosorbent Assay) identify the specific interaction between an antigen (a molecule from a pathogen or disease marker) and an antibody linked to an enzyme. It generates a color change that indicates the presence of the antigen on the antibody in the sample by following these steps:

- *Plate Preparation*: A specific antigen or antibody is fixed on the surface of a microplate. The biological sample (such as blood or serum) is added, allowing the antigen to bind to the corresponding antibody if both are present.
- *Addition of Enzyme Conjugate*: A secondary antibody linked to an enzyme, such as horseradish peroxidase (HRP) or alkaline phosphatase, is added. This antibody binds to the complex formed between the antigen and the primary antibody.
- *Chromogenic Substrate*: After washing to remove unbound components, a specific substrate for the enzyme is added. When the enzyme interacts with the substrate, a chemical reaction occurs, resulting in the formation of a colored product.
- *Example of Chromogenic Substrate*: For HRP, the commonly used chromogenic substrate is TMB (3,3',5,5'-tetramethylbenzidine). In the presence of hydrogen peroxide, HRP catalyzes the oxidation of TMB, resulting in a blue color that can be turned yellow by adding sulfuric acid.
- *Color Detection*: The intensity of the generated color is proportional to the amount of antigen present

in the sample and can be measured by a spectrophotometer or visually, depending on the test.

B. Direct Colorimetric Reaction

There are direct colorimetric reactions where the binding between an antibody and an antigen results in a color change. These methods generally use antibodies conjugated to chromogenic molecules that change color upon binding to the target antigen. A classic example is the use of antibodies linked to colloidal gold particles, which generate a visible color change without the need for additional substrate or enzyme steps. These methodologies are useful in rapid diagnostics, especially in screening scenarios and medical emergencies.

Examples of Direct Colorimetric Reactions:

Rapid Tests Based on Colloidal Gold Particles:

- Widely used in rapid diagnostic tests, such as pregnancy tests or COVID-19 tests.
- The antibody is conjugated to gold nanoparticles that exhibit intense red coloration. When the antibody encounters the corresponding antigen, the gold particles accumulate, forming a visible colored line.

Use of Fluorophores or Chromophores Coupled to Antibodies:

Some antibodies can be directly conjugated to chromogenic compounds that change color in response to conformational changes during antigen binding. An example is compounds that change color or fluorescence due to a chemical environment or molecular proximity changes when the antibody binds to the antigen.

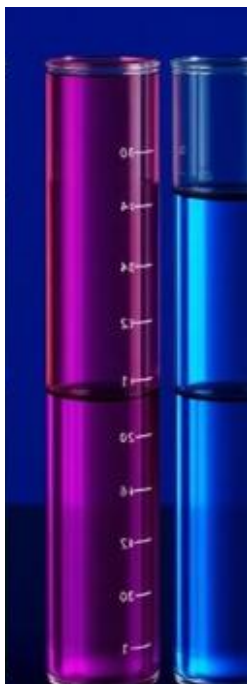
Advantages of Direct Reaction:

Fast: Eliminates intermediate steps, such as washing and adding substrates.

- **Practical**: Ideal for portable devices or field tests.
- **Reduced cost**: Lower technical and operational complexity

Limitations:

1. **Lower sensitivity**: Compared to enzymatic methods like ELISA.
2. **Dependent on high specificity**: To avoid false positives or negatives.



CHAPTER I

APPLIED SIMULATION

Interdisciplinary Section (Biotechnology-Pathology)

Topic: *Understanding Vaccines And Their Medical Importance*

Version: *Students/Participants*

Authors: *Sueli Braga,
Aldo Rodrigues,
Nadja Avila,
Leonardo Miceli,
Helena Carla Castro*

Logo of your Institution	Name of Institution
	<p>Human Project: Applied Simulation</p> <p>Laboratory of Antibiotics, Biochemistry, Teaching, and Molecular Modeling</p> <p>Fluminense Federal University, Institute of Biology, Brazil</p>

Interdisciplinary Section (Biotechnology-Biochemistry)

Version: *Students/Participants*

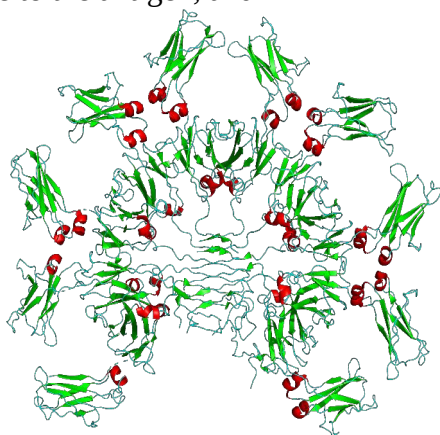
TOPIC: UNDERSTANDING VACCINES AND THEIR MEDICAL IMPORTANCE

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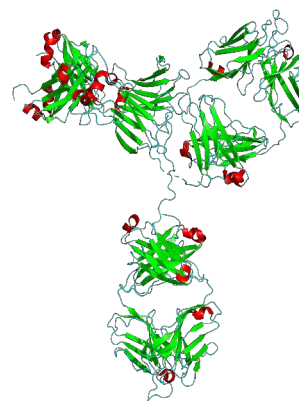
To verify the effectiveness of the vaccine, the production of antibodies is one of the main indicators of its protective efficiency. Among the evaluated antibodies, we have:

a) IgM, which indicates an initial immune response, being the first antibody produced after exposure to the antigen, and



Immunoglobulin M (IgM)

b) IgG, an antibody related to immune memory and responsible for long-term protection. The neutralizing action of these antibodies in blocking pathogen infection is the main focus for evaluating the efficacy of various vaccines.



Immunoglobulin G (IgG)

Antibody tests, also known as serologies because they use blood serum, are used to evaluate the efficacy of the vaccine by measuring the levels of specific antibodies in the blood. These tests have various purposes, including: a) Confirmation of the Immune Response, by verifying the level of antibody production, b) Evaluation of the Duration of Protection, by monitoring the amount of antibodies over time and indicating the need for booster shots, and c) Analysis of clinical studies during vaccine development, where antibody tests help determine if a formulation is effective before being released for public use.

In this practice, each group of students will evaluate different hypotheses that will lead

to the analysis of experimental vaccines against an unknown pathogen as they were hired by the National Health agency of the country, to do that evaluation. Therefore, it is important to perform a negative control test (Volunteer C1, Normal) where the serum does not cause a change in the reagent used, as well as a positive control test (Volunteer C2, Immunized) where the presence of the antibody is observed and the color change is guaranteed.

2. Objective

Our purpose is to analyze the blood serum samples of volunteers for antibody production and the suitability of the produced vaccines.

3. Reagents

-5 syringes/pipettes of 5ml or plastic Pasteur pipettes.

-5 recycled 6 or 12-well cell culture microplates or 14 glass tubes.

- Reagent with viral antigen marked with chromogenic substance.

- Volunteers serums.



4. Preparation of Reagents

- ♦ **Volunteers samples** (*Volume depends on the use of tube or plate*)
 - Label the tubes/plate corresponding to the sera.

Volunteer C1 (Normal): Negative Control

Volunteer C2 (Immunized): Positive Control

Volunteer 1: Vaccine 1 (Unknown)

Volunteer 2: Vaccine 2 (Unknown)

Volunteer 3: Vaccine 3 (Unknown)

Volunteer 4: Vaccine 4 (Unknown)

5. Procedures

- ♦ Place 0.5 ml of each Volunteer sample in each well of the plate respectively.
- ♦ Transfer 1 ml of the indicator;

- ♦ Mix and observe the color change of the liquid.

RESULTS	
Unchanged color (purple)	Neutral
Bluish-green or green	Basic
Pink or red	Ácidic

Result from C1 Volunteer: _____

Result from C2 Volunteer: _____

Result from 1st Volunteer: _____

Result from 2nd Volunteer: _____

Result from 3rd Volunteer: _____

Result from 4th Volunteer: _____.

6. Discussion Questions

6.1. Comment (succinctly) on the result relating to the analyzed vaccines.

6.2. Define and compare the data found for the two vaccines.

6.3. What type of test was performed: direct or indirect? Justify.

Colorimetric Reaction

INTRODUCTION

Colorimetric reactions provide high sensitivity and specificity, making them valuable tools for rapid and reliable diagnostics in various health contexts. This principle is widely used for clinical diagnostics, such as:

- HIV:** Detection of antibodies against the virus
- COVID-19:** Identification of antibodies or antigens of SARS-CoV-2.
- Autoimmune diseases: **Evaluation of autoantibodies.**

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Plate Preparation: A specific antigen or antibody is fixed on the surface of a microplate. The biological sample (such as blood or serum) is added, allowing the antigen to bind to the corresponding antibody if both are present.

Addition of Enzyme Conjugate: A secondary antibody linked to an enzyme, such as horseradish peroxidase (HRP) or alkaline phosphatase, is added. This antibody binds to the complex formed between the antigen and the primary antibody.

Chromogenic Substrate: After washing to remove unbound components, a specific substrate for the enzyme is added. When the enzyme interacts with the substrate, a chemical reaction occurs, resulting in the formation of a colored product.

Example of Chromogenic Substrate: For HRP, the commonly used chromogenic substrate is **TMB (3,3',5,5'-tetramethylbenzidine)**. In the presence of hydrogen peroxide, HRP catalyzes the oxidation of TMB, resulting in a blue color that can be turned yellow by adding sulfuric acid.

Color Detection: The intensity of the generated color is proportional to the amount of antigen present in the sample and can be measured by a spectrophotometer or visually, depending on the test.

B. Direct Colorimetric Reaction

There are direct colorimetric reactions where the binding between an antibody and an antigen results in a color change. These methods generally use antibodies conjugated to chromogenic molecules that change color upon binding to the target antigen. A classic example is the use of antibodies linked to colloidal gold particles, which generate a visible color change without the need for additional substrate or enzyme steps. These methodologies are useful in rapid diagnostics, especially in screening scenarios and medical emergencies.

Examples of Direct Colorimetric Reactions:

Rapid Tests Based on Colloidal Gold Particles:

- Widely used in rapid diagnostic tests, such as pregnancy tests or COVID-19 tests.
- The antibody is conjugated to gold nanoparticles that exhibit intense red coloration. When the antibody encounters the corresponding antigen, the gold particles accumulate, forming a visible colored line.

Use of Fluorophores or Chromophores Coupled to Antibodies:

Some antibodies can be directly conjugated to chromogenic compounds that change color in response to conformational changes during antigen binding.

An example is compounds that change color or fluorescence due to a chemical environment or molecular proximity changes when the antibody binds to the antigen.

Advantages of Direct Reaction:

Fast: Eliminates intermediate steps, such as washing and adding substrates.

- **Practical:** Ideal for portable devices or field tests.
- **Reduced cost:** Lower technical and operational complexity

Limitations:

Lower sensitivity: Compared to enzymatic methods like ELISA.

Dependent on high specificity: To avoid false positives or negatives.



CHAPTER II

APPLIED SIMULATION

Interdisciplinary Section (Pathology-Oncology)

Topic: *Prevention and Fight against Prostate Cancer.*

Version: *Monitors and Teachers/Professors*

Authors: *Helena Carla Castro
Nayra Cordeiro da Conceição,
Thais Dias,
Nathalia da Rosa Coelho Martins.
Leonardo Miceli
Aldo Rodrigues*

Logo of your Institution	Name of Institution
	Human Project: Applied Simulation Laboratory of Antibiotics, Biochemistry, Teaching, and Molecular Modeling Fluminense Federal University, Institute of Biology, Brazil

Interdisciplinary Section (Pathology/Oncology)
Version: *Monitors and Teachers/Professors*

TOPIC: THE PREVENTION AND FIGHT AGAINST PROSTATE CANCER

1. Introduction

Prostate cancer is one of the most common types of cancer among men, especially those over 50 years old. Early detection is essential for successful treatment, and the Prostate-Specific Antigen (PSA) is an important tool in this process. PSA is a protein produced mainly by prostate cells and is found in small amounts in the blood of healthy men. However, elevated PSA levels can indicate changes in the prostate, including infections, benign hyperplasia, or cancer.



Prostate-Specific Antigen (PSA)

Measuring PSA levels in the blood is a simple but highly relevant test. Generally, levels below 4 ng/mL are considered normal, although this reference varies according to age, family history, and other factors. Results between 4 and 10 ng/mL may indicate the need for additional tests, such as a digital rectal exam and biopsy. Values above 10 ng/mL increase the likelihood of malignancy.

An important point is that PSA is not exclusive to detecting cancer; other factors, such as prostatitis or benign prostate enlargement, can also elevate its levels. Additionally, recent procedures, such as catheter placement or ejacu-

lation, can temporarily influence the results, highlighting the importance of a detailed clinical evaluation.

Despite its limitations, PSA plays a fundamental role in the screening and monitoring of prostate cancer. It helps in identifying cases in early stages as well as in monitoring patients under treatment, being useful for evaluating therapeutic response and possible tumor recurrence. Combining PSA with other diagnostic methods improves accuracy, allowing for more effective and personalized interventions.

Regular PSA monitoring, along with periodic consultations with a specialist, is crucial for men belonging to risk groups, such as those with a family history of prostate cancer or African descent, due to higher genetic predisposition.

The main risk factors for developing prostate cancer include: age (significant increase in incidence and mortality in men over 60 years old) family history, (especially in first-degree relatives diagnosed before the age of 60), and lifestyle-related aspects (e.g. obesity and inadequate diet).

According to the World Health Organization (WHO), some measures can reduce the impact of these risk factors. These include adopting a balanced diet, regular physical exercise, maintaining a healthy weight, reducing alcohol consumption, and combating smoking and sedentary behavior.

In this practice, each group of students will evaluate different hypotheses leading to the diagnosis of patients analyzed. By detecting the presence of PSA in their serum using a reagent containing antibodies against PSA, They will perform two control tests:

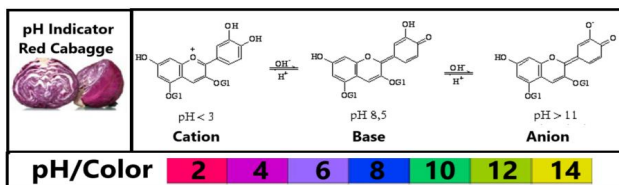
a) a negative control test (Patient 1 Normal) where the serum does not cause a change in the reagent used, and

b) a positive control test (Patient 2 with Prostate Cancer) where the presence of a color change is guaranteed.

The unique aspect of this work is the replacement of biological material with applied simulators. Since the substitution of these biological materials will be done using apple vinegar to prepare the "samples of volunteers". The pH indicator simulating the reagent formed by antibodies against PSA will be the solution of red cabbage, which is easily accessible as well as easy to prepare with low cost and low risks.

Red cabbage can be used as an acid-base indicator because it contains molecules that changes color when in contact with acid or base. These molecules are anthocyanins, pigments belonging to the flavonoid group that are responsible for the wide variety of colors in fruits, leaves, and flowers.

The role of anthocyanin in the plant is to protect against ultraviolet light and prevent the production of free radicals. This substance is highly sensitive, and should be stored on a freezer until use.



2. Objectives

Our purpose is to simulate the analysis of blood serum samples from patients searching for PSA production for the diagnosis of prostate cancer.

3. Reagents

-5 syringes of 5 ml or plastic Pasteur pipettes

-5 recycled 6-well cell culture microplates or 14 glass tubes.

- 1 red cabbage.

- 1 blender.

- 1 stainer

- Water, apple vinegar



4. Preparation of Reagents

◆ pH Indicator:

Weigh 500g of chopped red cabbage and cook with 1L of water for 15 minutes. Then blend in a blender and strain through a sieve, storing in the freezer. Use 1-2 ml of the indicator in each well of the recycled cell culture microplate or glass tube.

◆ Patient Samples : Volume depends on the material (tubes or plate) used in the test.

Patient 1 (Normal): Negative Control

Normal: water (0.5 ml)

Patient 2 (Prostate Cancer): Positive Control

Altered: apple vinegar (0.5 ml)

Patient 3 (Elderly → >60 years): Unknown (+)

Altered: apple vinegar (0.5 ml)

Patient 4 (Adult → 19-59 years): Unknown (-)

Normal: water (0.5 ml)

Patient 5 (*Young → A-J:15-17 years, J-J:18-24 years, J-Ad:25-29 years): Unknown (-)

Normal: water (0.5 ml)

*A=Adolescent, J=Young, Ad=Adult

5. Procedures

- ♦ Place 0.5 ml of each patient sample in each well of the plate respectively
- ♦ Transfer 1 ml of indicator/reagent;
- ♦ Mix and observe the color change of the liquid

RESULTS	
Unchanged color (purple)	Negative
Pink or Red	Positive

In the first patient: _____

In the second patient: _____

In the third patient: _____

In the fourth patient: _____

In the fifth patient: _____

6. Discussion Questions

6.1. Comment (briefly) on the results in relation to the patients analyzed

6.2. Define and compare the data found analyzing the age of the patients.

6.3. What type of test was performed: direct or indirect? Justify

Colorimetric Reaction

INTRODUCTION

Colorimetric reactions provide high sensitivity and specificity, making them valuable tools for rapid and reliable diagnostics in various health contexts. This principle is widely used for clinical diagnostics, such as:

- HIV**: Detection of antibodies against the virus
- COVID-19**: Identification of antibodies or antigens of SARS-CoV-2.
- Autoimmune diseases**: Evaluation of autoantibodies.

A colorimetric reaction in a diagnostic test involving antigen and antibody can occur indirectly or directly:

C. Indirect Colorimetric Reaction

Immunoassays like ELISA (Enzyme-Linked Immunosorbent Assay) identify the specific interaction between an antigen (a molecule from a pathogen or disease marker) and an antibody linked to an enzyme. It generates a color change that indicates the presence of the antigen on the antibody in the sample by following these steps:

- *Plate Preparation*: A specific antigen or antibody is fixed on the surface of a microplate. The biological sample (such as blood or serum) is added, allowing the antigen to bind to the corresponding antibody if both are present.
- *Addition of Enzyme Conjugate*: A secondary antibody linked to an enzyme, such as horseradish peroxidase (HRP) or alkaline phosphatase, is added. This antibody binds to the complex formed between the antigen and the primary antibody.
- *Chromogenic Substrate*: After washing to remove unbound components, a specific substrate for the enzyme is added. When the enzyme interacts with the substrate, a chemical reaction occurs, resulting in the formation of a colored product.
- *Example of Chromogenic Substrate*: For HRP, the commonly used chromogenic substrate is TMB (3,3',5,5'-tetramethylbenzidine). In the presence of hydrogen peroxide, HRP catalyzes the oxidation of TMB, resulting in a blue color that can be turned yellow by adding sulfuric acid.
- *Color Detection*: The intensity of the generated color is proportional to the amount of antigen

present in the sample and can be measured by a spectrophotometer or visually, depending on the test.

D. Direct Colorimetric Reaction

There are direct colorimetric reactions where the binding between an antibody and an antigen results in a color change. These methods generally use antibodies conjugated to chromogenic molecules that change color upon binding to the target antigen. A classic example is the use of antibodies linked to colloidal gold particles, which generate a visible color change without the need for additional substrate or enzyme steps. These methodologies are useful in rapid diagnostics, especially in screening scenarios and medical emergencies.

Examples of Direct Colorimetric Reactions:

Rapid Tests Based on Colloidal Gold Particles:

- Widely used in rapid diagnostic tests, such as pregnancy tests or COVID-19 tests.
- The antibody is conjugated to gold nanoparticles that exhibit intense red coloration. When the antibody encounters the corresponding antigen, the gold particles accumulate, forming a visible colored line.

Use of Fluorophores or Chromophores Coupled to Antibodies:

Some antibodies can be directly conjugated to chromogenic compounds that change color in response to conformational changes during antigen binding. An example is compounds that change color or fluorescence due to a chemical environment or molecular proximity changes when the antibody binds to the antigen.

Advantages of Direct Reaction:

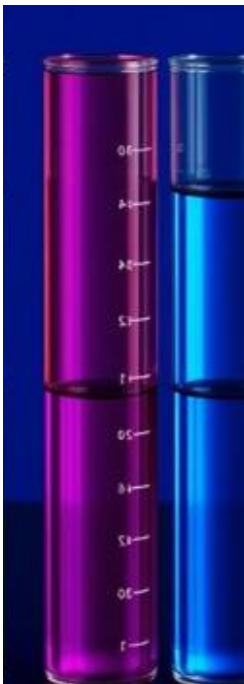
Fast: Eliminates intermediate steps, such as washing and adding substrates.

- Practical: Ideal for portable devices or field tests.
- Reduced cost: Lower technical and operational complexity

Limitations:

Lower sensitivity: Compared to enzymatic methods like ELISA.

Dependent on high specificity: To avoid false positives or negatives.



CHAPTER 2

APPLIED SIMULATION KIT

Interdisciplinary Section (Pathology-Oncology)

Topic: *Prevention and Fight against Prostate Cancer.*

Version: *Students/Participants*

Authors: *Helena Carla Castro
Nayra Cordeiro da Conceição,
Thais Dias,
Nathalia da Rosa Coelho Martins,
Leonardo Miceli,
Aldo Rodrigues.*

Logo of your Institution	Name of Institution
	<p>Human Project: Applied Simulation</p> <p>Laboratory of Antibiotics, Biochemistry, Teaching, and Molecular Modeling</p> <p>Fluminense Federal University, Institute of Biology, Brazil</p>

Interdisciplinary Section (Pathology/Oncology).
Version: *Monitors and Teachers/Professors*

TOPIC: THE PREVENTION AND FIGHT AGAINST PROSTATE CANCER

1. Introduction

Prostate cancer is one of the most common types of cancer among men, especially those over 50 years old. Early detection is essential for successful treatment, and the Prostate-Specific Antigen (PSA) is an important tool in this process. PSA is a protein produced mainly by prostate cells and is found in small amounts in the blood of healthy men. However, elevated PSA levels can indicate changes in the prostate, including infections, benign hyperplasia, or cancer.



Prostate-Specific Antigen (PSA)

Measuring PSA levels in the blood is a simple but highly relevant test. Generally, levels below 4 ng/mL are considered normal, although this reference varies according to age, family history, and other factors. Results between 4 and 10 ng/mL may indicate the need for additional tests, such as a digital rectal exam and biopsy. Values above 10 ng/mL increase the likelihood of malignancy.

An important point is that PSA is not exclusive to detecting cancer; other factors, such as prostatitis or benign prostate enlargement,

can also elevate its levels. Additionally, recent procedures, such as catheter placement or ejaculation, can temporarily influence the results, highlighting the importance of a detailed clinical evaluation.

Despite its limitations, PSA plays a fundamental role in the screening and monitoring of prostate cancer. It helps in identifying cases in early stages as well as in monitoring patients under treatment, being useful for evaluating therapeutic response and possible tumor recurrence. Combining PSA with other diagnostic methods improves accuracy, allowing for more effective and personalized interventions.

Regular PSA monitoring, along with periodic consultations with a specialist, is crucial for men belonging to risk groups, such as those with a family history of prostate cancer or African descent, due to higher genetic predisposition.

The main risk factors for developing prostate cancer include: age (significant increase in incidence and mortality in men over 60 years old) family history, (especially in first-degree relatives diagnosed before the age of 60), and lifestyle-related aspects (e.g. obesity and inadequate diet).

According to the World Health Organization (WHO), some measures can reduce the impact of these risk factors.

These factors include adopting a balanced diet, regular physical exercise, maintaining a healthy weight, reducing alcohol consumption, and combating smoking and sedentary behavior.

In this practice, each group of students will evaluate different hypotheses leading to the diagnosis of patients analyzed. By detecting the presence of PSA in their serum using a reagent containing antibodies against PSA, They will perform two control tests:

- a) a negative control test (Patient 1 Normal) where the serum does not cause a change in the reagent used, and
- b) a positive control test (Patient 2 with Prostate Cancer) where the presence of a color change is guaranteed.

The unique aspect of this work is the replacement of biological material with applied simulators. Since the substitution of these biological materials will be done using apple vinegar to prepare the "samples of volunteers". The pH indicator simulating the reagent formed by antibodies against PSA will be the solution of red cabbage, which is easily accessible as well as easy to prepare with low cost and low risks.

2. Objectives

Our purpose is to analyze the blood serum samples from patients searching for PSA production for the diagnosis of prostate cancer.

3. Reagents

- 5 syringes/pipettes of 5 ml or plastic Pasteur pipettes.
- 5 recycled 6-well cell culture microplates or 14 glass tubes.
- Reagent with antibodies marked with a chromogenic molecule.
- Patient serums.

4. Preparation of Reagents

♦ Patient Samples

- Label the Tubes/Plates corresponding to the patient sera.

Patient 1 (Normal): Negative Control

Patient 2 (Prostate Cancer): Positive Control

Patient 3 (Elderly → > 60 years): Unknown

Patient 4 (Adult → 29-59 years): Unknown

Patient 5 (*Young → A-J:15-17 years, J-J:18-24 years, J-Ad:25-29years): Unknown

*A=Adolescent, J=Young, Ad=Adult

5. Procedures

- ♦ Place 0.5 ml of each patient sample in each well of the plate respectively
- ♦ Transfer 1 ml of indicator/reagent;
- ♦ Mix and observe the color change of the liquid

RESULTS	
Unchanged color (purple)	Negative
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6. Discussion Questions

6.1. Comment (briefly) on the results in relation to the patients analyzed

6.2. Define and compare the data found based on the age of the patients.

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Colorimetric Reaction

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- **HIV:** Detection of antibodies against the virus
- **COVID-19:** Identification of antibodies or antigens of SARS-CoV-2.
- Autoimmune diseases: **Evaluation of autoantibodies.**

A colorimetric reaction in a diagnostic test involving antigen and antibody can occur indirectly or directly:

C. Indirect Colorimetric Reaction

Immunoassays like ELISA (Enzyme-Linked Immunosorbent Assay) identify the specific interaction between an antigen (a molecule from a pathogen or disease marker) and an antibody linked to an enzyme. It generates a color change that indicates the presence of the antigen on the antibody in the sample by following these steps:

Plate Preparation: A specific antigen or antibody is fixed on the surface of a microplate. The biological sample (such as blood or serum) is added, allowing the antigen to bind to the corresponding antibody if both are present.

Addition of Enzyme Conjugate: A secondary antibody linked to an enzyme, such as horseradish peroxidase (HRP) or alkaline phosphatase, is added. This antibody binds to the complex formed between the antigen and the primary antibody.

Chromogenic Substrate: After washing to remove unbound components, a specific substrate for the enzyme is added. When the enzyme interacts with the substrate, a chemical reaction occurs, resulting in the formation of a colored product.

Example of Chromogenic Substrate: For HRP, the commonly used chromogenic substrate is **TMB (3,3',5,5'-tetramethylbenzidine)**. In the presence of hydrogen peroxide, HRP catalyzes the oxidation of TMB, resulting in a blue color that can be turned yellow by adding sulfuric acid.

Color Detection: The intensity of the generated color is proportional to the amount of antigen present in the sample and can be measured by a spectrophotometer or visually, depending on the test.

D. Direct Colorimetric Reaction

There are direct colorimetric reactions where the binding between an antibody and an antigen results in a color change. These methods generally use antibodies conjugated to chromogenic molecules that change color upon binding to the target antigen. A classic example is the use of antibodies linked to colloidal gold particles, which generate a visible color change without the need for additional substrate or enzyme steps. These methodologies are useful in rapid diagnostics, especially in screening scenarios and medical emergencies.

Examples of Direct Colorimetric Reactions:

Rapid Tests Based on Colloidal Gold Particles:

- Widely used in rapid diagnostic tests, such as pregnancy tests or COVID-19 tests.
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Use of Fluorophores or Chromophores Coupled to Antibodies:

Some antibodies can be directly conjugated to chromogenic compounds that change color in response to conformational changes during antigen binding.

An example is compounds that change color or fluorescence due to a chemical environment or molecular proximity changes when the antibody binds to the antigen.

Advantages of Direct Reaction:

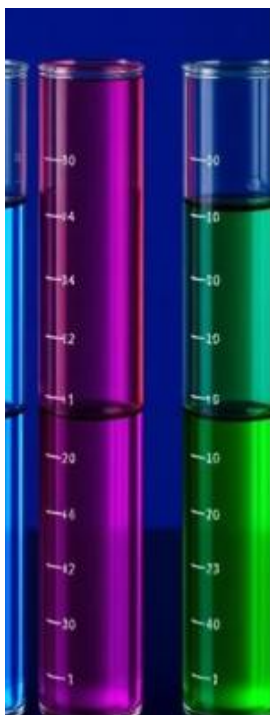
Fast: Eliminates intermediate steps, such as washing and adding substrates.

- **Practical:** Ideal for portable devices or field tests.
- **Reduced cost:** Lower technical and operational complexity

Limitations:

Lower sensitivity: Compared to enzymatic methods like ELISA.

Dependent on high specificity: To avoid false positives or negatives.



CHAPTER III

APPLIED SIMULATION

Interdisciplinary Section (Pathology/Immunology)

Topic: Identifying Allergies for Better Quality Of Life

Version: *Monitors and Teachers/Professors*

Authors: *Helena Carla Castro,
Marcelo Rodrigues,
Amanda Santos Antunes,
Leonardo Miceli
Aldo Rodrigues.*

<div>Logo of your Institution</div>	<div>Name of Institution</div>
	<div>Human Project: Applied Simulation</div> <div>Laboratory of Antibiotics, Biochemistry, Teaching, and Molecular Modeling</div> <div>Fluminense Federal University, Institute of Biology, Brazil</div>

Interdisciplinary Section (Pathology/Immunology)
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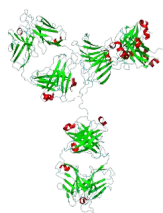
Topic: Identifying Allergies for Better Quality Of Life

1. Introduction

Allergy is an exaggerated immune response to molecules called allergens. Diagnosing allergies is crucial for the treatment and control of allergic reactions and often involves laboratory tests that assess the presence of specific antibodies.

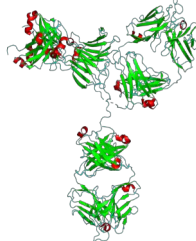
The most relevant antibodies for allergy process include:

- **IgE:** associated with immediate allergic reactions, such as those triggered by pollen, dust mites, or food.



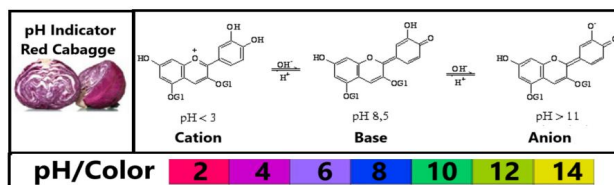
Immunoglobulin E (IgE)

- **IgG:** involved and used in studies for delayed reactions or evaluation of prolonged sensitization.



Immunoglobulin G (IgG)

This Allergy Diagnosis Simulation KitSA simulates the dynamics of immune responses using substitute reagents that reproduce changes in a controlled environment. This approach is not only economical but also sustainable and educational, employing natural pH indicators such as red cabbage extract.



Anthocyanins present in red cabbage are natural pigments that change color according to pH, making them useful for simulating chemical changes related to allergy diagnosis. These molecules also have antioxidant properties, which reinforces their role in protecting plants against environmental stresses.

2. Objective

Our purpose is to simulate the diagnosis of allergies by analyzing simulated samples, reproducing chemical reactions that mimic allergic responses, such as the release of inflammatory mediators.

3. Reagents and Materials

- 10 syringes of 5 mL or disposable Pasteur pipettes.
- 6 recycled well plates or 10 glass tubes.
- 1 red cabbage.
- 1 blender and stainer.
- Water, vinegar, lemon soda, milk of magnesia, and baking soda.
- Labels for identifying materials.

4. Preparation of Reagents

pH Indicator

- 1) Chop 500 g of red cabbage and add 1 L of water.
- 2) Cook for 15 minutes
- 3) Blend the mixture and strain using a fine sieve.
- 4) Store in a clean bottle, keeping it refrigerated.
- 5) Use 2 mL of the solution in each well or tube for the tests.

Patient samples

Each simulated patient will represent a specific condition of allergic response:

Patient	Normal (Control)	Altered (Allergic response)
Patient 1	Water (5 mL)	Vinegar (5 mL)
Patient 2	Water (5 mL), Bicarbonate (5 mL)	Soda (5 mL), Milk of Magnesia (5 mL)
Patient 3	Water (5 mL), Milk of Magnesia (5 mL)	Milk of Magnesia (5 mL), Soda (5 mL)
Patient 4	Soda (5 mL)	Vinegar (5 mL)

5. Experimental Procedure

1. Identify the wells or tubes with the conditions corresponding to each patient.
2. Add 5 mL of the prepared patient's sample to each container.

3. Add 2 mL of the pH indicator to each well or tube.
4. Mix gently
5. Observe and record the color changes using the following table:

Observed Color	pH Condition	Interpretation
Purple	Neutral	Normal response
Blue-green	Basic	Possible mild allergic reaction
Pink or red	Acidic	Intense allergic reaction

6. Results and Discussion

- Patient 1: _____
- Patient 2: _____
- Patient 3: _____
- Patient 4: _____

Questions To Discuss

1. Compare the results obtained for the different patients.
2. Relate the simulated conditions to the production of antibodies or the need for diagnostic reinforcement.

7. Conclusion

This experiment allows for an understanding of the basics of allergy diagnosis in a practical and interactive way. The use of natural reagents, such as red cabbage extract, offers an accessible and effective alternative for educational simulations of immunological processes.

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INTRODUCTION

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A colorimetric reaction in a diagnostic test involving antigen and antibody can occur indirectly or directly:

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Color Detection: The intensity of the generated color is proportional to the amount of antigen present in the sample and can be measured by a spectrophotometer or visually, depending on the test.

F. Direct Colorimetric Reaction

There are direct colorimetric reactions where the binding between an antibody and an antigen results in a color

change. These methods generally use antibodies conjugated to chromogenic molecules that change color upon binding to the target antigen. A classic example is the use of antibodies linked to colloidal gold particles, which generate a visible color change without the need for additional substrate or enzyme steps. These methodologies are useful in rapid diagnostics, especially in screening scenarios and medical emergencies.

Examples of Direct Colorimetric Reactions:

Rapid Tests Based on Colloidal Gold Particles:

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Advantages of Direct Reaction:

Fast: Eliminates intermediate steps, such as washing and adding substrates.

- **Practical:** Ideal for portable devices or field tests.
- **Reduced cost:** Lower technical and operational complexity

Limitations:

Lower sensitivity: Compared to enzymatic methods like ELISA.

Dependent on high specificity: To avoid false positives or negatives.



CHAPTER III

APPLIED SIMULATION

Interdisciplinary Section (Pathology/Immunology)

Topic: Identifying Allergies for Better Quality Of Life

Version: Students/Participants

Authors: *Helena Carla Castro,
Marcelo Rodrigues,
Amanda Santos Antunes,
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Logo of your Institution	Name of Institution
	<p>Human Project: Applied Simulation Laboratory of Antibiotics, Biochemistry, Teaching, and Molecular Modeling Fluminense Federal University, Institute of Biology, Brazil</p>

Interdisciplinary Section (Pathology/Biochemistry)
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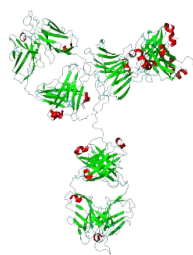
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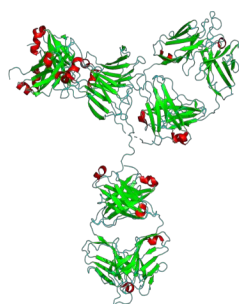
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The most relevant antibodies for allergy process include:

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- **IgG:** involved and used in studies for delayed reactions or evaluation of prolonged sensitization.



2. Objective

Our purpose is to help on the diagnosis of allergies by analyzing patients samples, by identifying allergic responses, such as the release of inflammatory mediators.

3. Reagents and Materials

- 10 syringes of 5 mL or disposable Pasteur pipettes.
- 6 recycled well plates or 10 glass tubes.
- Allergy reagent
- Samples from patients and controls (Negative and positive)
- Labels for identifying materials.

4. Preparation of Reagents

pH Indicator

a)ready to use, add 2 mL of the solution in each well or tube for the tests.

Patient samples

Each patient has a specific condition of allergic response:

Negative control (Normal) : 0.5 ml

Positive Control for Possible mild allergic reaction: 5ml

Positive Control for Intense allergic reaction 1: 0. 5ml

Patient 1 Unknown: 0.5 ml

Patient 2 Unknown: 0.5ml

Patient 3 Unknown: 0.5ml

Patient 4 Unknown: 0.5ml

5. Experimental Procedure

- a) Identify the wells or tubes with the conditions corresponding to each patient.
- b) Add 0.5 mL of the prepared patient's sample to each container.
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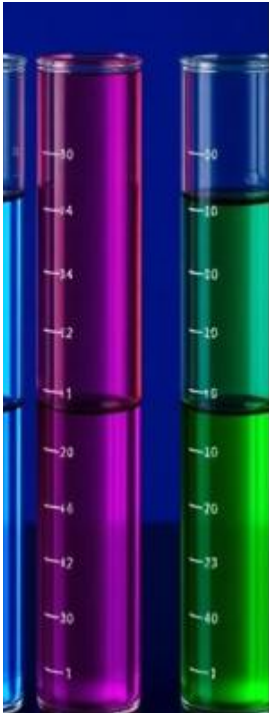
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CHAPTER IV

APPLIED SIMULATION

Interdisciplinary Section (Pathology/Biochemistry)

Topic: pH and Its Medical Importance

Version: *Monitors and Teachers/Professors*

Authors: *Mansur Dewu Muhammad,
Isabela Marinho Américo,
Luis Eduardo Cople Maia de Faria,
Helena Carla Castro.*

Logo of your Institution	Name of Institution
	<p>Human Project: Applied Simulation Laboratory of Antibiotics, Biochemistry, Teaching, and Molecular Modeling Fluminense Federal University, Institute of Biology, Brazil</p>

Interdisciplinary Section (Pathology/Biochemistry)

Version: *Monitors and Teachers/Professors*

TOPIC: pH AND ITS MEDICAL IMPORTANCE

1. Introduction

The knowledge about pH is of great importance for the human body. Since the maintenance of the homeostasis due to its role is standards is fundamental for the human and animal metabolism. Changes in pH can cause different problems including immune system depression, making the individual more susceptible to viral, bacterial, and fungal infections.

Many pathophysiological changes can occur as a consequence of pH alterations, which can be used for helping on the diagnosis of different diseases.

- In patients with untreated type 1 diabetes mellitus, there will be an accumulation of ketone bodies (ketoacidosis), which will consequently generate metabolic acidosis. This can be observed in the blood pH, considering the normal value of 7.4.
- Another disease where metabolic acidosis is observed is renal failure. It is caused by reduced acid excretion and, decreased reabsorption of HCO_3^- . In these patients, metabolic (pH of 7.1) can be observed in their blood, whereas their urine is basic due to the large elimination of HCO_3^- .
- A patient with primary hyperaldosteronism, including congenital adrenal hyperplasia, will experience renal acid loss. The overproduction of aldosterone, sodium reabsorption and potassium elimination in the urine will be favored which the sodium-potassium ATPase

pump compromise. This inhibits bicarbonate reabsorption in the proximal convoluted tubule. The bicarbonate in the tubular lumen will receive a proton and be eliminated in the form of carbonic acid. The significant acid elimination will result in metabolic alkalosis.

- The normal vaginal flora in women of reproductive age generally consists of *Lactobacillus* sp. This colonization maintains the vaginal pH within normal ranges, thus preventing the overgrowth of pathogenic bacteria and fungi. Some factors that can favor the establishment of a pathogenic process such as vaginal infection caused by *Candida* and use of antibiotics, alkaline vaginal pH, inadequate hygiene, and diabetes mellitus. This infection causes decrease in pH to values below 4.5.
- In a patient with acute pancreatitis caused by an obstruction due to a gallstone, there will be no release of pancreatic juice. Consequently, there will be no increase in pH, whereas an acidic pH can be detected in the duodenum. The exocrine pancreas, stimulated by cholecystokinin (CCK), will produce and secrete digestive enzymes and bicarbonate. Due to the large amount of bicarbonate, the pH of the pancreatic secretion is around 8, allowing the neutralization of stomach acid (HCl). This secretion is eliminated through the pancreatic duct, which is opened due to stimulation by secretin.

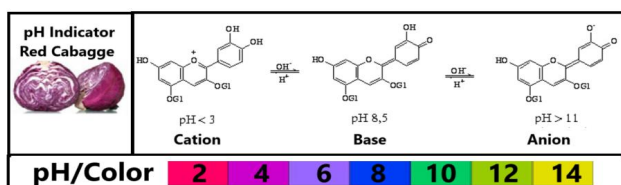
Cancer cells have a different metabolism compared to healthy cells. Due to their accelerated growth, they have a high rate of glycolysis followed by lactic fermentation in the cytosol. In healthy cells, glycolysis would be followed by the oxidation of pyruvate in the mitochondria. This effect in malignant cells is called the Warburg Effect.

It is important to note that the cell chooses the fermentation pathway even in the presence of oxygen. This pathway is extremely important to the need for accelerated growth. In fermentation, it is possible to produce NAD^+ , which will be restored and used again in glycolysis. The final product of fermentation is lactic acid, thus, in the area of the tumor, a decrease in pH can be observed.

In this practice, each group of students will evaluate different hypotheses that will lead to the diagnosis of different patients through the difference in pH found. Therefore, it is important to conduct a control test (Normal) that does not alter the pH indicator to observe the color change and positive controls (affected patients) to know what colors are characteristics of this assay.

In this work, there will be the replacement of biological material with didactic simulators. Different materials will be used for the preparation of patient samples whereas the pH indicator will be prepared using a red cabbage solution. These reagents are easily accessible and easy to prepare with low cost and low risks.

Red cabbage can be used as an acid-base indicator because it contains molecules that changes color when in contact with acid or base. These molecules are anthocyanins, pigments belonging to the flavonoid group that are responsible for the wide variety of colors in fruits, leaves, and flowers.



The role of anthocyanin in the plant is to protect against ultraviolet light and prevent the production of free radicals. This substance is highly sensitive, and should be stored on a freezer or refrigerator until use.

2. Objective

Our purpose is to analyze patient samples (urine and cells) simulators for pH variation.

3. Reagents

- 5 syringes of 5 ml or plastic Pasteur pipettes.
- 5 recycled 6 or 12-well cell culture microplates or 14 glass tubes
- 1 Red cabbage
- 1 Blender
- 1 Sieve
- Water, apple vinegar, lemon soda, milk of magnesia and sodium bicarbonate



4. Preparation of Reagents

◆ pH Indicator:

Weigh 500g of chopped red cabbage and cook with 1L of water for 15 minutes. Then blend in a blender and strain through a sieve, storing in the freezer. Use 1-2 ml of the indicator in each well of the recycled cell culture microplate or glass tube.

◆ Patient samples

Patient 1: Cancer

Negative control: Water (5ml)
Positive Control: apple vinegar (5ml)
Patient Unknown: apple vinegar (5ml)

Patient 2: Renal Insufficiency

Negative control: Water (5ml)
Positive Control: Bicarbonate solution (5ml)
Patient Unknown: Lemon soda (5ml)

Patient 3: Primary Hyperaldosteronism
Negative control: Water (5ml)
Positive Control: Milk of Magnesia (5ml)
Patient Unknown: Lemon soda (5ml)

Patient 4: Vaginal infection

Negative control: Lemon Soda (5ml)
Positive Control: apple vinegar (5ml)
Patient Unknown: Lemon soda

Patient 5 : Acute pancreatitis

Negative control: Bicarbonate (5ml)
Positive Control: apple vinegar (5ml)
Patient Unknown: apple vinegar (5ml)

5. Procedures

- ◆ Place 0.5 ml of each patient sample in each well of the plate, respectively.
- ◆ Transfer 1 ml of the indicator;
- ◆ Mix and observe the color of the liquid.

In the first patient: metabolic acidosis => acidic blood pH and basic urinary pH.

In the second patient: metabolic alkalosis => basic blood pH and acidic urinary pH.

In the third patient: vaginal infection by Candida => acidic vaginal secretion pH.

In the fourth patient: acute pancreatitis => acidic pH in intestinal secretion.

In the fifth patient: malignant tumor => acidic pH in a tissue solution.

6. Discussion Question

6.1. Comment (briefly) on the result relating it to the diseases addressed.

6.2. Define and differentiate (metabolically) the diseases addressed.

RESULTS	
Unchanged color (purple)	Neutral
Bluish-green or green	Basic
Pink or Red	Acidic

Colorimetric Reaction

INTRODUCTION

Colorimetric reactions provide high sensitivity and specificity, making them valuable tools for rapid and reliable diagnostics in various health contexts. This principle is widely used for clinical diagnostics, such as:

- HIV**: Detection of antibodies against the virus
- COVID-19**: Identification of antibodies or antigens of SARS-CoV-2.
- Autoimmune diseases**: Evaluation of autoantibodies.

A colorimetric reaction in a diagnostic test involving antigen and antibody can occur indirectly or directly:

E. Indirect Colorimetric Reaction

Immunoassays like ELISA (Enzyme-Linked Immunosorbent Assay) identify the specific interaction between an antigen (a molecule from a pathogen or disease marker) and an antibody linked to an enzyme. It generates a color change that indicates the presence of the antigen on the antibody in the sample by following these steps:

- *Plate Preparation*: A specific antigen or antibody is fixed on the surface of a microplate. The biological sample (such as blood or serum) is added, allowing the antigen to bind to the corresponding antibody if both are present.
- *Addition of Enzyme Conjugate*: A secondary antibody linked to an enzyme, such as horseradish peroxidase (HRP) or alkaline phosphatase, is added. This antibody binds to the complex formed between the antigen and the primary antibody.
- *Chromogenic Substrate*: After washing to remove unbound components, a specific substrate for the enzyme is added. When the enzyme interacts with the substrate, a chemical reaction occurs, resulting in the formation of a colored product.
- *Example of Chromogenic Substrate*: For HRP, the commonly used chromogenic substrate is TMB (3,3',5,5'-tetramethylbenzidine). In the presence of hydrogen peroxide, HRP catalyzes the oxidation of TMB, resulting in a blue color that can be turned yellow by adding sulfuric acid.
- *Color Detection*: The intensity of the generated color is proportional to the amount of antigen

present in the sample and can be measured by a spectrophotometer or visually, depending on the test.

F. Direct Colorimetric Reaction

There are direct colorimetric reactions where the binding between an antibody and an antigen results in a color change. These methods generally use antibodies conjugated to chromogenic molecules that change color upon binding to the target antigen. A classic example is the use of antibodies linked to colloidal gold particles, which generate a visible color change without the need for additional substrate or enzyme steps. These methodologies are useful in rapid diagnostics, especially in screening scenarios and medical emergencies.

Examples of Direct Colorimetric Reactions:

Rapid Tests Based on Colloidal Gold Particles:

- Widely used in rapid diagnostic tests, such as pregnancy tests or COVID-19 tests.
- The antibody is conjugated to gold nanoparticles that exhibit intense red coloration. When the antibody encounters the corresponding antigen, the gold particles accumulate, forming a visible colored line.

Use of Fluorophores or Chromophores Coupled to Antibodies:

Some antibodies can be directly conjugated to chromogenic compounds that change color in response to conformational changes during antigen binding. An example is compounds that change color or fluorescence due to a chemical environment or molecular proximity changes when the antibody binds to the antigen.

Advantages of Direct Reaction:

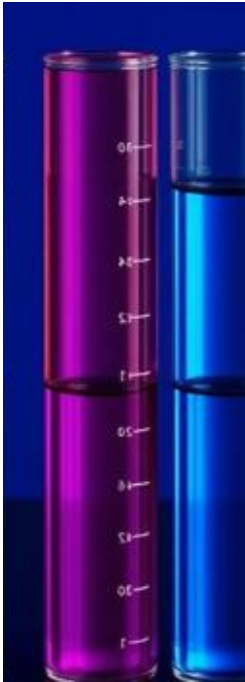
Fast: Eliminates intermediate steps, such as washing and adding substrates.

- Practical: Ideal for portable devices or field tests.
- Reduced cost: Lower technical and operational complexity

Limitations:

Lower sensitivity: Compared to enzymatic methods like ELISA.

Dependent on high specificity: To avoid false positives or negatives.



CHAPTER IV

APPLIED SIMULATION

Interdisciplinary Section (Pathology/Biochemistry)

Topic: pH and Its Medical Importance

Version: Students/Participants

Authors: *Mansur Dewu Muhammad,
Isabela Marinho Américo,
Luís Eduardo Cople Maia de Faria,
Helena Carla Castro*

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KitSA-LABiEMol (UFF-IB-GCM): Interdisciplinary Section (Pathology/Biochemistry)

Version: *Students/Participants*

TOPIC: pH AND ITS MEDICAL IMPORTANCE

1. Introduction

The knowledge about pH is of great importance for the human body. Since the maintenance of the homeostasis due to its role is standards is fundamental for the human and animal metabolism. Changes in pH can cause different problems including immune system depression, making the individual more susceptible to viral, bacterial, and fungal infections.

Many pathophysiological changes can occur as a consequence of pH alterations, which can be used for helping on the diagnosis of different diseases.

- f) In patients with untreated type 1 diabetes mellitus, there will be an accumulation of ketone bodies (ketoacidosis), which will consequently generate metabolic acidosis. This can be observed in the blood pH, considering the normal value of 7.4.
- g) Another disease where metabolic acidosis is observed is renal failure. It is caused by reduced acid excretion and, decreased reabsorption of HCO_3^- . In these patients, metabolic (pH of 7.1) can be observed in their blood, whereas their urine is basic due to the large elimination of HCO_3^- .
- h) A patient with primary hyperaldosteronism, including congenital adrenal hyperplasia, will experience renal acid loss. The overproduction of aldosterone, sodium reabsorption and potassium elimination in the urine will be favored which the sodium-potassium ATPase

pump compromise. This inhibits bicarbonate reabsorption in the proximal convoluted tubule. The bicarbonate in the tubular lumen will receive a proton and be eliminated in the form of carbonic acid. The significant acid elimination will result in metabolic alkalosis.

- i) The normal vaginal flora in women of reproductive age generally consists of *Lactobacillus* sp. This colonization maintains the vaginal pH within normal ranges, thus preventing the overgrowth of pathogenic bacteria and fungi. Some factors that can favor the establishment of a pathogenic process such as vaginal infection caused by *Candida* and use of antibiotics, alkaline vaginal pH, inadequate hygiene, and diabetes mellitus. This infection causes decrease in pH to values below 4.5.
- j) In a patient with acute pancreatitis caused by an obstruction due to a gallstone, there will be no release of pancreatic juice. Consequently, there will be no increase in pH, whereas an acidic pH can be detected in the duodenum. The exocrine pancreas, stimulated by cholecystokinin (CCK), will produce and secrete digestive enzymes and bicarbonate. Due to the large amount of bicarbonate, the pH of the pancreatic secretion is around 8, allowing the neutralization of stomach acid (HCl). This secretion is eliminated through the pancreatic duct, which is opened due to stimulation by secretin.

- k) Cancer cells have a different metabolism compared to healthy cells. Due to their accelerated growth, they have a high rate of glycolysis followed by lactic fermentation in the cytosol. In healthy cells, glycolysis would be followed by the oxidation of pyruvate in the mitochondria. This effect in malignant cells is called the Warburg Effect.

It is important to note that the cell chooses the fermentation pathway even in the presence of oxygen. This pathway is extremely important to the need for accelerated growth. In fermentation, it is possible to produce NAD^+ , which will be restored and used again in glycolysis. The final product of fermentation is lactic acid, thus, in the area of the tumor, a decrease in pH can be observed.

In this practice, each group of students will evaluate different hypotheses that will lead to the diagnosis of different patients through the difference in pH found. Therefore, it is important to conduct a control test (Normal) that does not alter the pH indicator to observe the color change and positive controls (affected patients) to know what colors are characteristics of this assay.

2. Objective

Our purpose is to analyze patient samples (urine and cells) for pH variation.

3. Reagents

- 5 syringes of 5 ml or plastic Pasteur pipettes.
- 5 recycled 6-well cell culture microplates or 14 glass tubes.
- pH indicator
- Patient serums and urine

4. Preparation of Reagents

- ♦ **pH Indicator:** ready for use.

♦ Patient Samples

- Label the Tubes/Plates corresponding to the serums

Patient 0 (Normal): Negative Control

Patient 1 (Prostate Cancer): Positive Control

Patient 2 (Renal Insufficiency): Positive Control

Patient 3 (Hyperaldosteronism): Positive Control

Patient 4 (Vaginal Infection): Positive Control

Patient 5 (Acute Pancreatitis): Positive Control

Patient 6: Unknown

Patient 7: Unknown

Patient 8: Unknown

Patient 9: Unknown

Patient 10: Unknown

5. Procedures

- ♦ Transfer 1 mL of the pH indicator;
- ♦ Place 0.5 ml of each patient samples (unknown) and the negative and positive controls in each marked well of the plate or tube, respectively;
- ♦ Mix and observe the color change of the liquid

RESULTS	
Unchanged color (purple)	Neutral
Bluish-green or green	Basic
Pink or Red	Acidic

Result from 6th patient: _____

Result from 7th patient: _____

Result from 8th patient: _____

Result from 9th patient: _____

Result from 10th patient: _____

6. Discussion Question

6.1. Comment (briefly) on the result relating it to the diseases addressed.

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present in the sample and can be measured by a spectrophotometer or visually, depending on the test.

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Advantages of Direct Reaction:

Fast: Eliminates intermediate steps, such as washing and adding substrates.

- Practical: Ideal for portable devices or field tests.
- Reduced cost: Lower technical and operational complexity

Limitations:

Lower sensitivity: Compared to enzymatic methods like ELISA.

To avoid false positives or negatives is dependent on high specificity.

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This book is the result of a collaborative effort between Dr. Helena Carla Castro, from the Department of Cell and Molecular Biology at the Federal Fluminense University (UFF) in Niterói, Brazil, and Prof. Mansur Muhammad Dewu, from the Department of Microbiology and Biotechnology at the Federal University Dutse, in Dutse, Nigeria. He organized this book together with her during his doctorate in Post-graduation program of Pathology of UFF in 2024.

This book is part of the work of these professors at the Extension Project called "The Human Project," coordinated by Dr. Helena. It has been 20 years since the project was created at UFF. It aims to disseminate scientific knowledge across diverse settings, fostering learning and engagement with science in academic and non-academic communities using safe and low cost strategies.