SECTION ONE

General Information

Equipment List

Ordering Information

Solution Preparation

Aliquots for Students

ELISA LAB - General Information

Goals of Lab Exercise

Learning Objectives

Time Requirements

Prep Time

The REAL Story

Alternative to the Actual Lab-

Many of the included sheets can be useful even if you decide to use a pre-packaged ELISA lab kit. Doing this would save considerable preparation time but would not provide as "real" an experience as the actual protocols provided here.

Equipment

Ordering Information

3,3' 5,5' Tetramethyl-Benzidine (TMB) Liquid substrate system:

1M Tris, pH 8.0

Rabbit Serum

Goat anti-rabbit IgG with Peroxidase

Microtubes

Microtube Storage Rack- Styrofoam

Microtube Storage Rack- Plastic

96 Well U-Bottom non-sterile flexible PVC Plates

Economy Micropipette: 5 l - 100 l

Micropipette Tips-0.5 l - 250 l

Micropipette Tips- 0.5 l - 250 l

Gelatin-

Handiwrap (or like product) and napkins

Solution Preparation

TBS

Aliquots for Students
(Per Station)

Tube Label	Tube Contents

NOT

YOU

SECTION TWO

Glossary of Terms

Colorimetric Reaction

Complementary

Enzyme

Immunoglobulins Igs

Mast Cell

Microliters / Micropipettes -

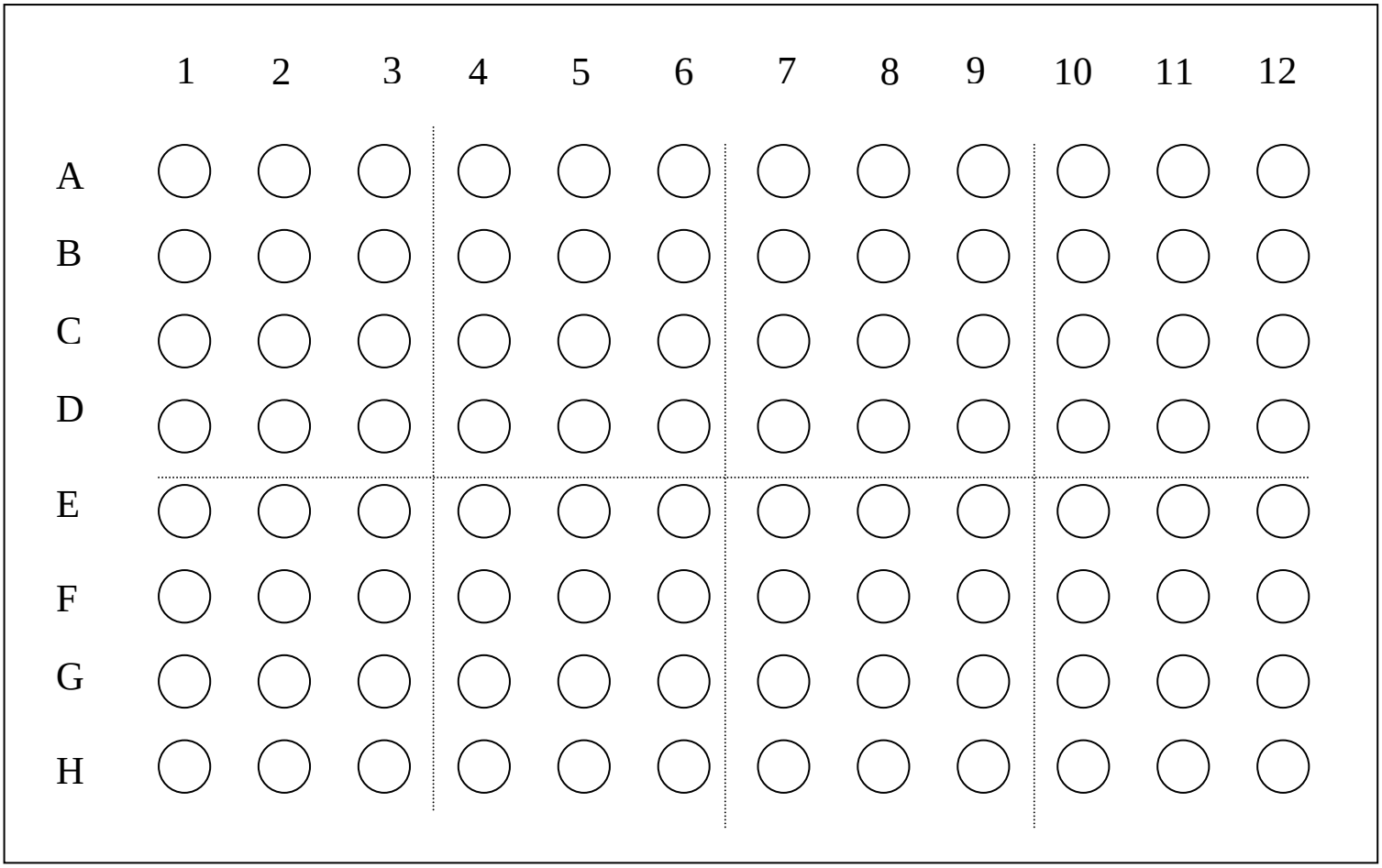
Serial Dilution-

Specificity

Spectrophotometer-

Substrate

96 Well Plate



SECTION THREE

Antibody Information

Antibody Questions

Antibody Questions with Answers

Asthma Information

ELISA in Asthma Research

ELISA / Asthma Questions

ELISA / Asthma Questions with Answers

ELISA and HIV

ELISA and HIV Questions

ELISA and HIV Questions with Answers

ANTIBODIES

Classes-

Antibody Questions (with answers)

Student answers to this will vary but the antibody shape should be a "Y" and the shape of the variable region of the "Y" should fit the antigen drawn.

Constant region

Variable region

Because the variable region of the antibody has a shape that will fit only one antigen, the antibody is specific for that antigen.

Immunoglobulins (Ig)

Immunoglobulin E (IgE)

parasitic worms

Immunoglobulin G (IgG)

Passive immunity

The child does not actively make his own antibodies.

ELISA IN USE

ELISA in Asthma Research-

not **not** **not**

not **not**

murine

ELISA and Asthma Research Questions (with answers)

Immunoglobulin E (IgE)

Airway Hyper-reactivity

HIV ELISA Questions

HIV ELISA Questions (with answers)

They do not have any of the symptoms of that disease.

A person exposed to HIV will have produced

SECTION FOUR

ELISA: Four Basic Steps

ELISA: Another Look

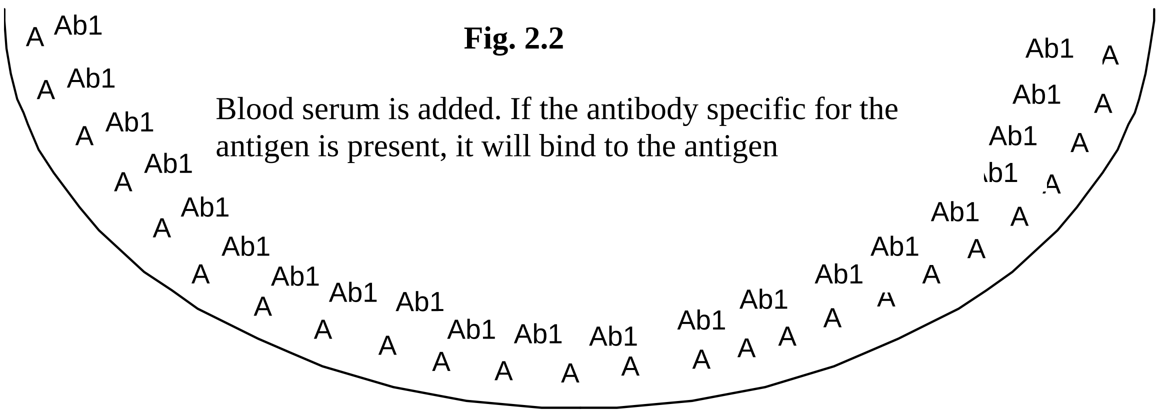
ELISA Questions

ELISA Questions with Answers

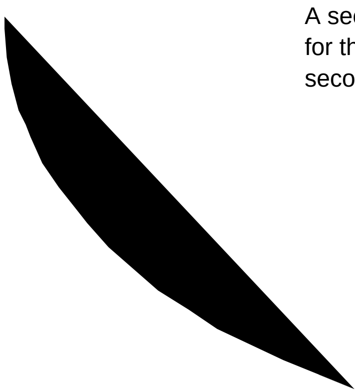
E L I S A

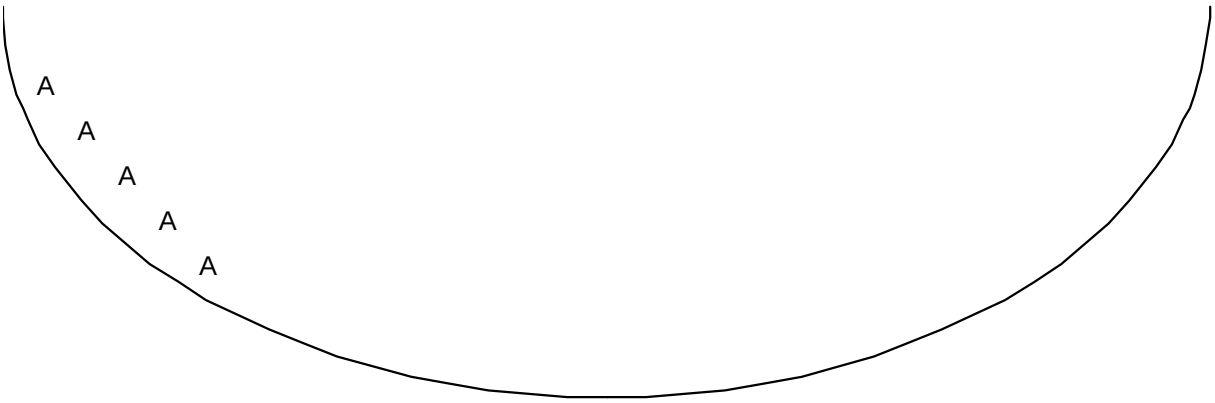
Fig. 2.2

Blood serum is added. If the antibody specific for the antigen is present, it will bind to the antigen



A second antibody (Ab2)is added. This antibody is specific for the first antibody and will bind to it. Attached to this second antibody is an enzyme (Enz)




A
A
A
A
A

ELISA: Another Look

Fig. 3.1

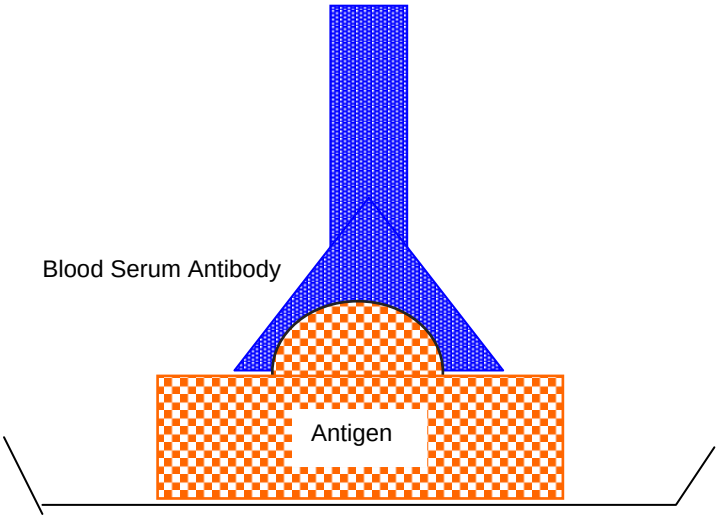
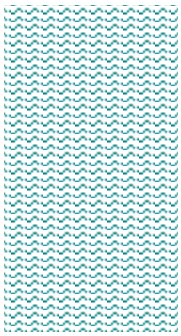


Fig.3.2



ELISA Questions

ELISA Questions

SECTION FIVE

Micropipette Information

Micropipette Practice

Serial Dilution Questions

Serial Dilution Questions with Answers

Small Volumes: Information and Questions

Micropipettes

Micropipette Practice

Serial Dilution Questions

Method #1

Note- Solution A was diluted to form Solution B which was then further diluted to form Solution C. Each solution is less concentrated than the one it was formed from. This procedure is called serial dilution.

Method #2-

Serial Dilution Questions (with answers)

Method #1

Solution C is 1% (0.01) of solution A

Method #2

The concentration of the new solution would be 1% (0.01) of Solution A.

One major difference is the amount of solution produced. In the first case, only 10 ml is produced. In the second case, 100 ml is produced. Also, using the first method, you will have some the original Solution A, some of Solution B and some of Solution C.

Small Volumes

Small Volumes (with answers)

2000 ml

10 ml **NO**

One ml **YES**

1000 l are in one ml.

One million l are in one liter.

One millionth (.000001)

0.1 ml (one tenth of a ml)

250 l

SECTION SIX

ELISA Protocol

ELISA Data Sheet and Questions

ELISA Data Sheet and Questions with Answers

Protocol Questions

Protocol Questions with Answers

Protocol Hints-

etc

STEP ONE-

STEP TWO-

Discard the micropipette tip.

STEP THREE-

Patient 1 (Tube D)

CHANGE THE MICROPIPETTE TIP

Patient 2 (Tube E)

CHANGE THE MICROPIPETTE TIP

Tube F

Discard the micropipette tip

STEP FOUR-

Tube G

Discard the micropipette tip

Tube H

Discard the micropipette tip

STEP FIVE-

Tube I

Discard the micropipette tip

STEP SIX-

Protocol Questions

Protocol Questions (with answers)

antigen **dilutes**

Blocks **washes**

The blocking agent attaches to any places in the well where the antigen did not attach. This prevents other proteins from attaching later on and affecting the accuracy of the results

There will be a definite color change in the positive control well. This provides a comparison to the test wells.

It was produced in some animal like a rabbit.

It has an enzyme attached to it.

The reaction between the enzyme and the substrate produces a color change.

A spectrophotometer can be used to measure the color intensity in each well.
A specific kind of spectrophotometer called a plate reader can be used to measure the color intensity in each well without removing the liquid from the wells.

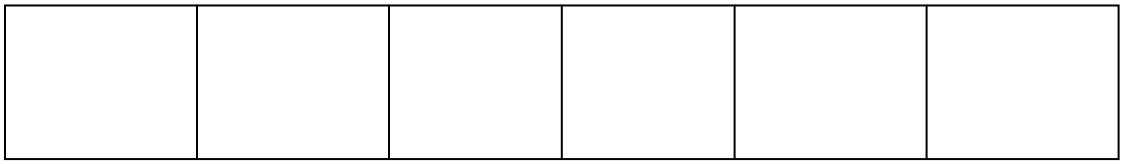
NO

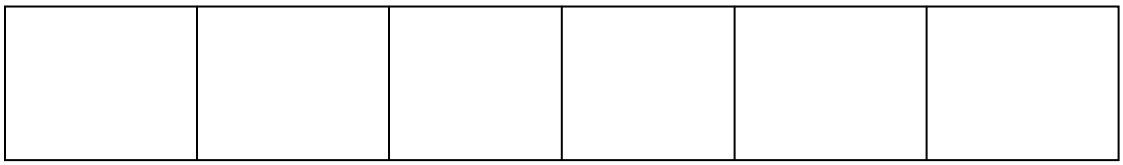
There was no antigen added to these wells.

Antibodies were added to this well in order to definitely produce a color change. This can be called a positive reaction.

compare

1 2 3 4





ELISA Data Sheet
(with answers)

Below are some websites of interest.

This is the Main Page for the Harvard University Outreach Program sponsored by the Department of Molecular and Cellular Biology and by the Howard Hughes Medical Institute.

http://outreach.mcb.harvard.edu/

This website has projects by participants in a summer program in immunology offered by Harvard University Outreach Program.

http://outreach.mcb.harvard.edu/teachermaterialsS04.shtml

This website lists other sites in immunology. It was put together by the Harvard University Outreach Program.

http://outreach.mcb.harvard.edu/summer04links.shtml

This website is "The Biology Project" produced by the University of Arizona. It covers several areas beyond immunology.

http://www.biology.arizona.edu/default.html

This website is for CityLab, a program sponsored by Boston University Medical School and the BU School of Education. It has several lab exercises of interest.

http://www.bumc.bu.edu/Dept/Home.aspx?DepartmentID=285