CAN THO UNIVERSITY

BIOTECHNOLOGY RESEARCH AND DEVELOPMENT INSTITUTE

LABORATORY REPORT

PRACTICAL TRAINING IN INDUSTRY (BT480C)

SOME BASIC TESTS IN HUMAN (HIV, HBV, HCV, BIOCHEMICAL TESTS, INTESTINAL MICROFLORA, *STREPTOCOCCUS* SP.) AND FOOD (PESTICIDES, FORMALDEHYDE, COLORING AGENTS, BORIC ACID)

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Can Tho, November 2012

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In addition to our teachers, we would like to thank Lieutenant-Colonel (Dr.) Phan Van Vinh, Leader of the Military Region 9 Preventative Medicine Center, for support good conditions for us to study. Despite being busy, he usually inquired us about our practical works, what works we need to focus on, etc.

We are deeply grateful to our supervisor, Lieutenant Phan Truong Giang and aslo thanks to Lieutenant Pham Ngoc Trang and Lieutenant Tran Dinh Duc, Lab technicians of the Military Region 9 Preventative Medicine Center, for their patience, enthusiasm, motivation, and immense knowledge. Their wide knowledge and the logical teaching ways have been of great value for us. Besides, their understanding, encouraging and personal guidance has been easy and comfortable for us to study new techniques, lab works and safety requirements when we directly do some experiments with dangerous virus samples such as HIV or HBV. We wish to express our warm and sincere thanks to their detailed and constructive criticism and excellent advice during the preparation of this report.

Last but not the least, during this work we have collaborated with our friends, Nguyen Thi Huynh Nhu, Nguyen Huu Tuong and Mach Tu Uyen. We wish to extend our warmest thanks to all those who have helped us with our works in Military Region 9 Preventative Medicine Center.

Can Tho, Vietnam, August 2012

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CHAPTER 1. INTRODUCTION OF MILITARY REGION 9 PREVENTATIVE MEDICINE CENTER

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Preventive medicine was established as a specialty to gather physicians working in illness prevention and public health, to incorporate teaching about these topics into medical school curricula, and to advance opportunities for training in the specialty. Preventive medicine physicians are "uniquely trained in both clinical medicine and public health. They have the skills needed to understand and reduce the risks of disease, disability, and death in individuals and in population groups". The core disciplines of public health are biostatistics, epidemiology, health policy and administration, health behavior, and environmental health. Board certification can be obtained in public health and general preventive medicine or in the subspecialties of aerospace medicine, medical toxicology, or occupational medicine.

Many infectious diseases controlled during the 20^{th} century through vaccines and antibiotics (tuberculosis, pertussis, and measles) are exhibiting resurgence and being joined by emerging conditions such as HIV/AIDS, severe acute respiratory syndrome (SARS), and the potential for pandemic avian influenza. The threats of man-made and natural disasters such as biological warfare and weather-related events focus attention on how medicine manages to care for large numbers of people who have been injured or don't have access to uncontaminated food and drinking water. These conditions have in common their effects upon large populations rather than on one patient with a given condition.

Figure 1. Military Region 9 Preventative Medicine Center

1. History

Military Region 9 Preventative Medicine Center is a part of Medical Service Corps. Therefore, to understand the history of this center we need to follow that of Medical Service Corps. However, according to Lieutenant-Colonel (Dr.) Phan Van Vinh, this project of Medical Service Corps history is still in the stage of completion.

Located in the centre of Mekong Delta, Military Region 9 Preventative Medicine Center plays an important role for many army or civil activities. This location is useful not only for this center to complete their sanitation and epidemic prevention functions *(below)* but also for relative activities such as health checking for workers in some industrial zone in Can Tho City or vaccinating and biochemical – physical testing for the locals.

Besides, there are some rooms (Figure 2) for medical examination and testing, we practiced in 3 laboratories:

- Microorganisms laboratory
- Blood analysis laboratory
- Parasite analysis laboratory

Besides 3 laboratories above, the centre also has another laboratory (water analysis laboratory).

Equipments: there is some equipment we used during practical time (Figure 3).

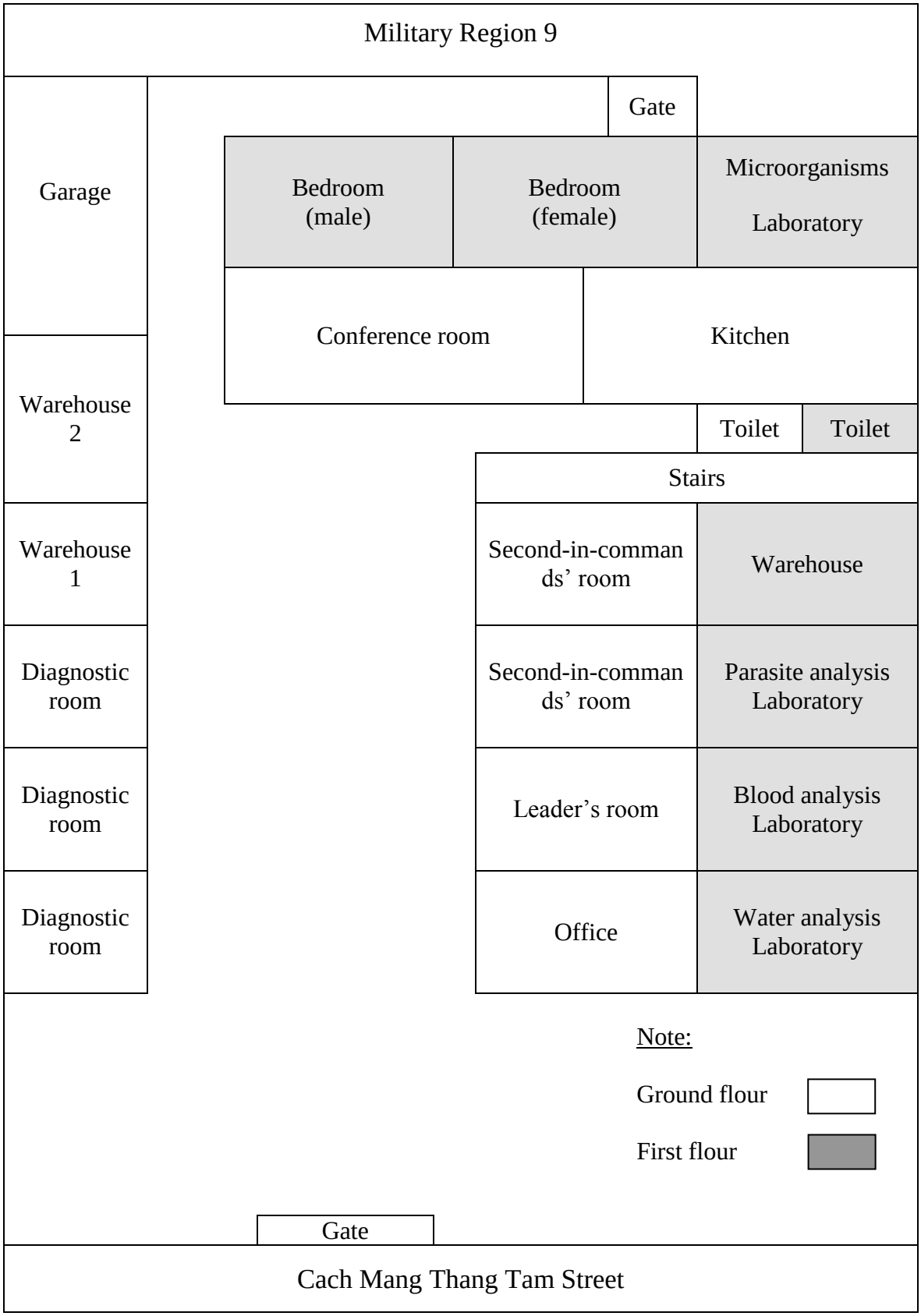

Figure 2. Diagram of Military Region 9 Preventative Medicine Center

Figure 3. Equipment in Military Region 9 Preventative Medicine Center

Some equipment include Blood analysis Lab (a), Microorganisms Lab (b), Grant Shaking Incubator ES20 (c), *Biohit Microplate Washer* (d), *A Bacteriological incubator* (e), *Anthos* 2010 *Microplate Reader UK* (f), *Tomy autoclave SS-325 (left) Steriliser SFE 400 (right) (g).*

2. The Sanitation and epidemic prevention functions in military

The epidemic prevention team is established to actively prevent toxic environmental factors, infectious diseases in military and contribute to health and working improvement of soldiers.

The sanitation and epidemic prevention activities

– Military practice and exercises hygiene: ensuring the health and physical development for soldiers to complete the training program without any accidents and increase the soldiers' ability to suffer different changes of the training environment.

– Military working hygiene: maintaining the environmental and personal sanitation; health management, hygiene educational propaganda in military to help soldiers stay healthy, working ability improvement.

– Outdoor hygiene: personal and environmental hygiene during operating.

– Water hygiene: maintaining the environmental and personal sanitation related to water that uses for life activities, eating etc…

– Nutritional hygiene and food safety hygiene.

– Barack hygiene.

– Propaganda and education sanitation and epidemic prevention.

– Reconnaissance about hygiene and epidemic.

– Preventive vaccination: using vaccine to increase immune systems of soldiers and prevent some infectious diseases.

– Sterilize; detect insect and intermediate animal diseases: using mechanical, physical, biological and chemical methods to limit and annihilate insect animals that are considered to be intermediates for diseases spreading.

– Control infectious disease.

– Prevent HIV and AIDS infection in military.

3. Treatment and prevention mission

3.1 Purposes and contents

– Check and control the health and diseases of soldiers

– Detect the diseases in different conditions; combine to the local health center to minimize the ratio of fatality, invalid; rehabilitate the highest fighting power, work and training of wounded soldiers and sick soldiers.

3.2 Activities

– Citizen soldier recruitment: health examination of soldiers before and after enlisting.

– Soldier health control: management of soldiers' health profiles, doing reexamination if there is any suspect expression of soldiers.

– First aid and treatment: education and control to prevent any accident can occur during practice time.

– Treat and rehabilitate: support good conditions for treatments and rehabilitation.

– Medical Appraisement.

4. Preventive Medicine Center Structure

There are 23 members:

– Lieutenant-Colonel (Dr.) Phan Van Vinh and two second-in-commands (Leaders of the Military Region 9 Preventative Medicine Center).

– Six doctors, 3 BSc of Laboratory Medicine and 11 physicians.

CHAPTER 2. PRACTICAL CONTENTS

1. Biochemical Test of Human Serum

1.1 General Information

There are many kinds of biochemical test; depend on the facilities and the cost of test, people use suitable method with low price, reliable results and saving time. However, clinical diagnostic should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

Figure 4. BioSystem BT-330 machine

In the world, rapid serum tests are more favorable because of its advantages such as time saving, reliable results. The differences can be seen that what kinds of test (producers) use. For example, we used tests of Biosystems Reagents and Instruments during practised time while other laboratory uses tests of Sekisui, Biovision, MyLabYogi, etc.

At this laboratory, we use tests of Biosystems Reagents and Instruments. Collected blood samples were centrifuged to get the serum. Then, correspond to what kind of test, the appropriate chemicals of test was used. After determining the absorbance of control - serum samples and calculating the actual value of test that were done by Biosystems machine, the result was gotten. To conclude the value was normal or abnormal, we needed to compare this result with the standard of Ministry of Health of Vietnam. For examples, AST/GOT value, glucose and cholesterol are normal if the results are 0-40 U/l, 4.4-6.1 mmol/l and 3.0-5.2 mmol/l, respectively.

To be more detailed, each step for testing the concentration of Aspartate aminotransferase and Cholesterol oxidase are followed these requirements:

1.2 Aspartate Aminotransferase (AST/GOT)

1.2.1 Diagnostic Characteristics

The aminotransferase catalyze the formation of glutamic acid from 2-oxoglutarate by transfer of amino acid group. AST is found in highest concentration in the liver and heart muscle but it is also abundant in skeletal muscle, kidney and pancreas.

The important role of AST/GOT testing (*http://webmd.com/digestive-disorders/aspartate-aminotransferase-ast*) is to:

- Check for liver damage.
- Help identify liver disease, especially hepatitis and cirrhosis. Liver disease may produce symptoms such as pain in the upper abdomen, nausea, vomiting, and sometimes jaundice.
- Check on the success of treatment for liver disease.
- Find out whether jaundice was caused by a blood disorder or liver disease.
- Keep track of the effects of cholesterol-lowering medicines and other medicines that can damage the liver.

1.2.2 Principle of Methods

Aspartate aminotransferase (AST or GOT) catalyzes the transfer of the amino group from aspartate to 2-oxoglutarate, form oxalacetate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm, by means of the malate dehydrogenase (MDH) coupled reaction.

$$\text{Aspartate + 2-oxoglutarate} \xrightarrow{AST} \text{Oxalacetate + Glutamate}$$

$$\text{Oxalacetate + NADH + } H^+ \xrightarrow{MDH} \text{Malate + } NAD^+$$

1.2.3 Contents & Compositions

A. Reagent: Tris 121 mmol/L, L-aspartate 362 mmol/L, malate dehydrogenase >460 U/L, lactase dehydrogenase >660 U/L, Sodium hydroxide 255 mmol/L, pH 7.8.

B. Reagent: NADH 1.3 mmol/L, 2-oxoglutarate 75 mmol/L, sodium hydroxide 148 mmol/L, Sodium azide 9.5 g/L.

C. Auxiliary reagent: Pyridoxal phosphate 10 mmol/L, 5 mL.

1.2.4 Storage

Store at 2-8^{o}C.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

- Reagents: presence of the particulate material, turbidity, absorbance of the blank lower than 1.1 at 340 nm (1 cm cuvette).

1.2.5 Reagents Preparation

Working reagent: Pour the content of the Reagent B into the Reagent A bottle. Mix gently. Other volume can be prepared in the proportion: 4 mL Reagent A + 1 mL Reagent B. Stable for 2 moths at 2-8^{o}C.

Working reagent with Pyridoxal phosphate: Mix as follows: 10 mL of the Working Reagent + 0.1 mL Reagent C. Stable for 6 days at 2-8^{o}C.

Sample collection

Collect the sample.

Aspartate aminotransferase in serum is stable for 7 days at 2-8^{o}C.

1.2.6 Procedure

- Bring the Working Reagent and instrument to reaction temperature (Table 1).

- Pipette into a cuvette.

Table 1. Proportion of used sample volume in 37^{o}C and 30^{o}C

Reaction temperature	**37^{o}C**	**30^{o}C**
Working reagent	1.0 mL	1.0 mL
Sample	50 µL	100 µL

- Mix and insert cuvette into the photometer. Start the stopwatch.

- After 1 min, record initial absorbance and at 1 minute intervals there after for 3 minutes.

- Calculate the different between consecutive absorbance and the average absorbance difference per minute (ΔA/min).

Calculations

The AST/GOT concentration in the sample is calculated using the formula:

$$\Delta A/\min = \frac{Vt * 10^6}{\varepsilon * l * VS} = U/L$$

The molar absorbance (ε) of NADH nm is 6300, the lightpath is 1 cm, the total reaction volume (Vt) is 1.05 at 37°C and 1.1 30°C, the sample volume (Vs) is 0.05 at 37°C and 0.1 at 30°C, and 1 U/L are 0.166 μkat/L. The following formulas are deduced for the calculation of the catalytic concentration:

Table 2. Formula for calculation of the AST/GOT content on 37°C and 30°C

	37°C	**30°C**
ΔA/min	x 3333 = U/L	x 1746 = U/L
	x 55.55 = μkat/L	x 29.1 = μkat/L

The calculations are programmed in Biosystems Reagents and Instruments machine. We waited the machine and got the final results.

1.3 Cholesterol Oxidase/Peroxidase

1.3.1 Diagnostic Characteristics

Cholesterol is an essential body fat (lipid). It is necessary for building cell membranes and for making several essential hormones. Too much cholesterol can cause it to build up on the walls of blood vessels and allow clots to develop.

Cholesterol is a steroid of high molecular weight and possesses the cyclopentanophenanthrene skeleton. Dietary cholesterol is partially absorbed and it is also synthesized by the liver and other tissues. Cholesterol is transported in plasma by lipoproteins. It is excreted unchanged into bile or after transformation to bile acids.

Actually, people are recommended that they should test their cholesterol level at least once every five years because increased total cholesterol values are associated with a progressive escalating risk of atherosclerosis and coronary artery disease.

1.3.2 Princible of Methods

Free and esterified cholesterol in the sample originates, by means of the couple reactions described below, a coloured complex that can be measured by spectrophotometry.

$$\text{Cholesterol ester} + H_2O \xrightarrow{chol.esterase} \text{Cholesterol} + \text{fatty acid}$$

$$\text{Cholesterol} + \frac{1}{2}O_2 + H_2O \xrightarrow{chol.oxidase} \text{Cholestenone} + H_2O_2$$

$$2\ H_2O_2 + \text{4-Aminoantipyrine} + \text{Phenol} \xrightarrow{peroxidase} \text{Quinoneimine} + 4\ H_2O_2$$

1.3.3 Contents & Compositions

A. Reagent: Pipes 35 mmol/L, sodium cholate 0.5 mmol/L, phenol 28 mmol/L, cholesterol esterase > 0.2 U/mL, cholesterol oxidase >0.1 U/mL, peroxidase > 0.8 U/mL, 4-aminoantipyrine 0.5 mmol/L, pH 7.0.

S. Cholesterol Sandard. Cholesterol 200 mg/dL (5.18 mmol/L). Aqueous primary standard.

1.3.4 Storage

Store at 2-8^{o}C.

Reagents and standard are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

- Reagents: presence of the particulate material, turbidity, absorbance of the blank over 0.200 at 550 nm (1 cm cuvette).

1.3.5 Reagents Preparation

Reagent and Standard are provided ready to use.

Sample collection

Collect the sample: plasma or serum collected by standard procedures.

Cholesterol is stable for 7 days at 2-8^{o}C. Heparin, EDTA, oxalate and fluoride may be used as anticoagulants.

1.3.6 Procedure

- Bring the Working Reagent and instrument to reaction temperature.

- Pipette into a cuvette.

Table 3. Volume of each used component

	Blank	Standard	Sample
Cholesterol Standard (S)	-	10 µL	-
Sample	-	-	10 µL
Reagents	1.0 mL	1.0 mL	1.0 mL

- Mix thoroughly and incubate the tubes for 10 min at room temperature (16-25^{o}C) or for 5 min at 37^{o}C.

- Measure the absorbance (A) of the Standard and Sample at 500 nm against the Blank. The color is stable for at least 2 hours.

Calculations

The cholesterol concentration in the sample is calculated using the following general formula:

$$\frac{A_{Sample}}{A_{Standard}} * C_{Standard} = C_{Sample}$$

If the Cholesterol Standard provide has been used to calibrate:

$$\frac{A_{Sample}}{A_{Standard}}$$ x200 if the unit is mg/dL cholesterol

(x5.18 if the unit is mmol/L cholesterol)

The calculations are programmed in Biosystems Reagents and Instruments machine. We waited the machine and got the final results.

1.3 Interpretations of the results

After calculations, tested samples of Cholesterol oxidase/Peroxidase aspartate aminotransferase (AST/GOT) were read on a paper to get the results.

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Độc Lập - Tự Do - Hạnh Phúc

PHIẾU XÉT NGHIỆM

Họ và tên: ..... Tuổi .....
Địa chỉ: .....
Loại mẫu: Phân ☐☐ Máu ☒☐ Nước tiểu ☐☐
Ngày giờ lấy mẫu: .....

Xét nghiệm	Giá trị bình thường	Kết quả
☐ Glucose	4,4 - 6,1 mmol/l	
☐ Ure	2,5 - 7,5 mmol/l	
☐ Creatinin	44 - 110 umol/l	
☐ AST/SGOT	0 - 40 U/l	
☐ ALT / SGPT	0 - 42 U/l	
☐ Cholesterol	3,0 - 5,2 mmol/l	
☐ Triglycerit	< 0,5 - 1,7 mmol/l	
☐ HDL	> 1,1 - 2,3 mmol/l	
☐ LDL	< 3,9 mmol/l	

Figure 5. Standard values for serum analysis

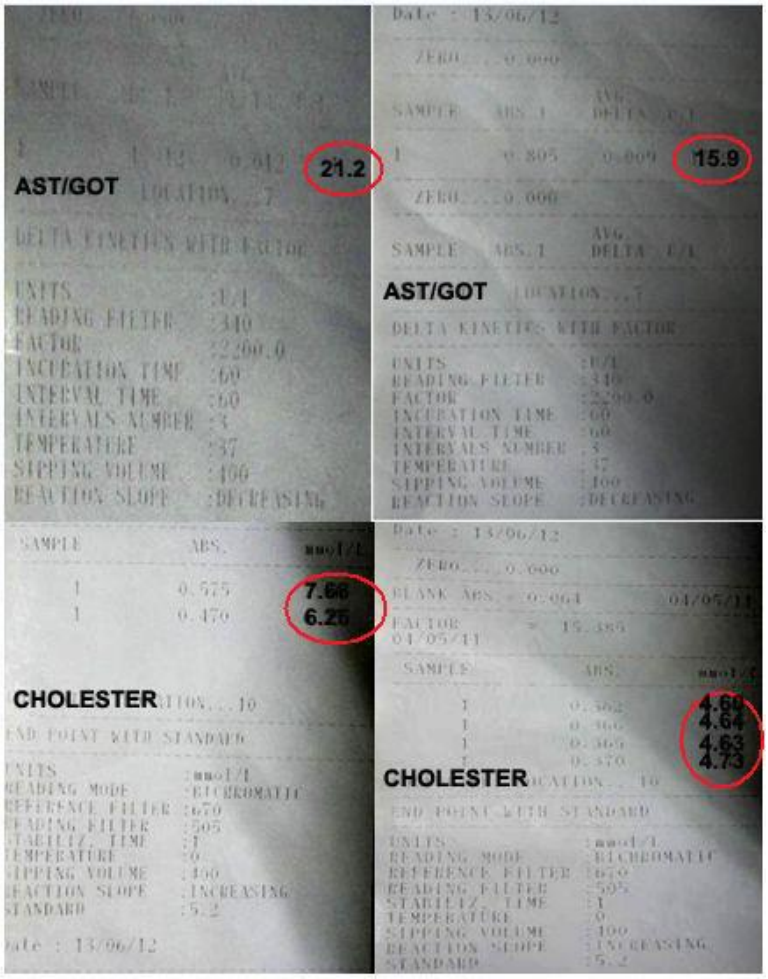


Figure 6. AST/GOT and Cholesterol value in the printed results

Standard value: 0-40 mmol/l

→ 2 samples had normal AST/GOT values.

Standard value: 3-5.2 mmol/l

→ Two first samples had high cholesterol value.

→ Therefore, 4 normal cholesterol values.

→ Recommendations were given for the first two patients.

In the total of 8 biochemical tests, there were no abnormal in liver, partially, AST/GOT; however, in the case of cholesterol tests, 2/3 cases we tested had high level of cholesterol. It was significant thing that, recommendations for each abnormal case with high cholesterol level were given and the government need to improve the locals'

life and hold some conferences to guide them how to lower their cholesterol by themselves.

1.4 Discussion

There are many benefits and drawbacks of serum test (AST/GOT and Cholesterol Oxidase/Peroxidase test). These include:

- Aspartate Aminotransferase (AST/GOT)

Advantages: besides low price, reliable results and saving time, the AST test is more effective than the alanine aminotransferase (ALT) test for detecting liver damage caused by alcohol abuse. The AST ratio may sometimes help determine if liver damage is related to alcohol dependence.

Disadvantages: This only one way to test concentration of AST/GOT. We shouldn't find the final results with a single test because the results can be affected by many factors:

- Taking medicines. Talk with your doctor about all the prescription and nonprescription medicines you are taking. You may be instructed to stop taking your medicines for several days before the test.
- Taking large doses of vitamin A.
- Taking some herbs and natural products, such as echinacea and valerian.
- Injury to a muscle.
- Recent cardiac catheterization or surgery.

- Cholesterol Oxidase/Peroxidase test

Advantages: low price, reliable results and saving time.

Disadvantages: This only one way to test concentration of cholesterol oxidase/peroxidase. We shouldn't find the final results with a single test because this result can exactly determine when other exams are done:

- Perform a full physical exam, discussing patients' medical history, checking heart rate, listening to heartbeat, and taking blood pressure.

- If your cholesterol is found to be high, especially if the patients have other risk factors for heart disease, the doctor will recommend some further tests to get various treatment options to lower the cholesterol.

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

2. Human Immunodeficiency Virus (HIV) test

2.1 Introduction of HIV

According to World Health Organization (WHO) (*http://who.int/topics/hiv_aids/en/*), the human immunodeficiency virus (HIV) is a retrovirus that infects cells of the immune system, destroying or impairing their function.

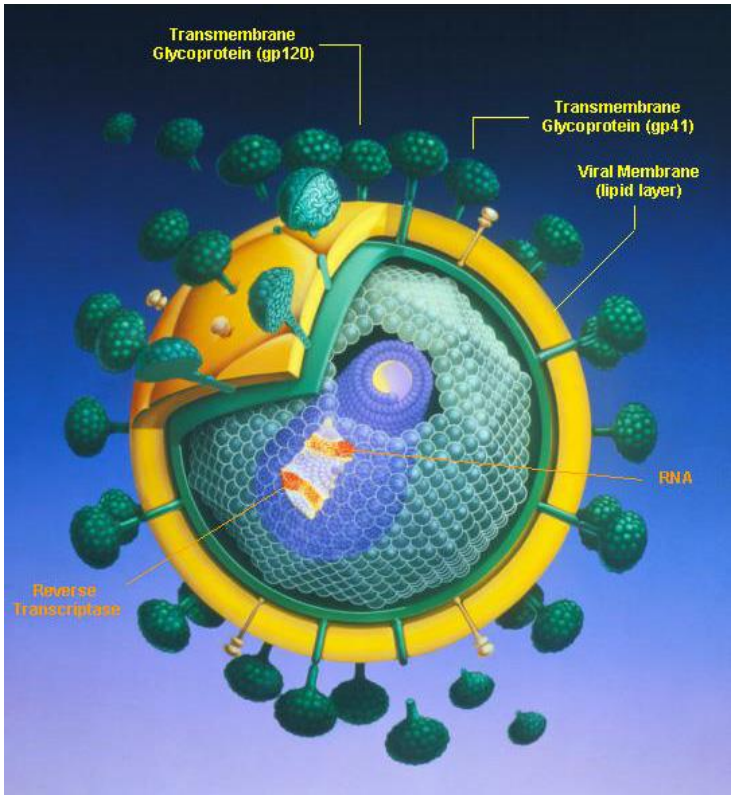


Figure 7. Human immunodeficiency virus (HIV) *(http://davidpratt.info/health)*

Many HIV-positive people do not have symptoms of HIV infection. The most advanced stage of HIV infection is acquired immunodeficiency syndrome (AIDS). Often people only begin to feel sick when they progress toward AIDS (Acquired Immunodeficiency Syndrome). Sometimes people living with HIV go through periods of being sick and then feel fine (*http://aids.gov/hiv-aids-basics/hiv-aids-101/signs-and-symptoms/*).

While the virus itself can sometimes cause people to feel sick, most of the severe symptoms and illnesses of HIV disease come from the opportunistic infections that attack a damaged immune system.

HIV is transmitted through unprotected sexual intercourse (anal or vaginal), transfusion of contaminated blood, sharing of contaminated needles, and between a mother and her infant during pregnancy, childbirth and breastfeeding.

In the world, because it is a dangerous disease, many projects for finding out a good way to detect the presence of HIV in the hosts (human) to have effective treatments for each individuals and have effective ways to prevent HIV spread to the community. For instance, to detect HIV presence, we can use many ways such as (*http://www.niaid.nih.gov/topics/hivaids/understanding/treatment/pages/default.aspx*):

– **CD4 count** (CD4 cells are a type of white blood cell that's specifically targeted and destroyed by HIV. A healthy person's CD4 count can vary from 500 to more than 1,000. Even if a person has no symptoms, HIV infection progresses to AIDS when his or her CD4 count becomes less than 200.

– **Viral load**. This test measures the amount of virus in your blood. Studies have shown that people with higher viral loads generally fare more poorly than do those with a lower viral load.

– **Drug resistance**: This blood test determines whether the strain of HIV you have will be resistant to certain anti-HIV medications and the ones that may work better.

In HIV treatment, scientists still focused on finding new and more effective therapies, drug classes, and antiretroviral drug combinations that can extend and improve the quality of life for people living with HIV/AIDS. Besides some recent new discoveries but until now, these do not apply on human, some remarkable achievements can be listed like Drug Resistance, Drugs that Fight HIV or Highly Active Anti-Retroviral Therapy (or HAART), etc.

In Military Region 9 Preventative Medicine Center, we used three tests for HIV determination such as SERODIA-HIV1/2 MIX, Determine® HIV-1/2 and Genscreen™ HIV-1/2 Version 2. Advantages/disadvantages, principles, procedures and other detailed information of each test are introduced.

2.2 Human Immunodeficiency Virus Types 1 and 2 (HIV-1 and HIV-2) Antibody Test

Human Immunodeficiency Virus Types 1 and 2 (HIV-1 and HIV-2) Antibody Test is known as particle-agglutination Test for the detection of antibodies to human immunodeficiency viruses type 1 and/or type 2 (HIV-1/HIV-2).

2.2.1 Intended use

SERODIA-HIV1/2 MIX is intended to serve as a tool for the detection of antibodies to HIV-1 and/or HIV-2 and as an acid in the diagnosis of infection by HIV-1 or HIV-2. The test is ideally suited for screening of blood donors and high risks populations. The test may be performed on either plasma or serum specimens.

2.2.2 Assay principle

SERODIA-HIV1/2 MIX is an *in vitro* diagnostic test for detection of antibodies to HIV-1 and/or HIV-2 which is manufactured using gelatin particle, sensitized with recombinant HIV-1 antigens (HIV-1/gp 41 and HIV-1/p 24) and HIV-2 antigen (HIV-2/gp 36). The SERODIA-HIV1/2 MIX (particle agglutination) test is based on the principle that sensitized particles are agglutinated by the presence of antibodies to HIV-1 and/or HIV-2 in human serum/plasma.

2.2.3 Contents of the SERODIA-HIV1/2 Mix Kit

All the reagents included in the kit are intended for *in vitro* diagnostic use.

A. Reconstituting Solution (liquid) – For use in the reconstitution of Sensitized Particles and Control Particles. This reagent contains 0.1% (w/v) of sodium azide as preservative.

Figure 8. SERODIA-HIV1/2 Mix Kit

B. Sample Diluent (Liquid) – For use in diluting test specimens. This reagent contains 0.1% (w/v) of sodium azide as preservative.

C. Sensitized Particles (Lyophilized) – Lyophilized preparation of gelatin particles sensitized with recombinant HIV-1 antigens (gp 41 and p 24) and HIV-2 antigen (gp-36), reconstituted by adding the prescribed quantity of Reconstituting Solution. The reconstituted reagent contains 1% of gelatin particles sensitized with recombinant HIV-1/2 antigens and 0.1% (w/v) of sodium azide as preservative.

D. Control Particles (Lyophilized) – Reconstituted by adding prescribed quantity of Reconstituting solution. Reconstituted Particles contains 1% of gelatin particles sensitized with *E. coli* extract and 0.1% (w/v) sodium azide as preservative.

E. Positive control (Liquid) – Liquid preparation containing HIV-1 mouse monoclonal antibodies and HIV-2 mouse monoclonal antibodies. The control gives a 1:128 (± 1 dilution) antibody titer at the final solution when tested according to the Positive Control Test Procedure. This reagent contains 0.1% (w/v) of sodium azide as preservative.

The 2 dropper (25µl) included in the kit are designed for the sole purpose of dispensing the reconstituted Sensitized Particles or Control Particles.

All reagents contain normal rabbit serum.

Reconstitution of reagents

Note: before use, allow the reagents to reach room temperature (15-30^{o}C).

a. Reagents ready to use:

Reagent A: Reconstituting Solution

Reagent B: Sample Dilutent

Reagent E: Positive Control

b. Reagents to reconstitute:

Reagent C: Sensitized particles

To reconstitute with the following volume of reconstituting solution (Reagent A): 0.6 mL for the 100 tests kit.

Reagent D: Control particles

To reconstitute with the following volume of reconstitution solution (Reagent A): 1 mL for the 100 tests kit.

The suspension of sensitized particles and of control particles must be homogenized by gently shaking and inverting just before distribution.

2.2.4 Sample collection

Collect a blood sample according to the current particles.

Extract the serum or plasma from the clot or red cells as soon as possible in order to avoid hemolysis. Extensive hemolysis may affect test performance. Samples with aggregates should be clarified by centrifugation prior testing. Suspended fibrin particles or aggregates may yield falsely positive results.

Figure 9. Sample preparation for SERODIA-HIV1/2 Mix Kit

Collected sample (a) was centrifuged in Hettich machine (b) to get serum (c)

Serum and plasma specimens should be stored at 2-8°C if the test is to be run within 7 days of collection. If testing is delayed more than 7 days, the specimen should be frozen (-20°C or colder).

2.2.5 Assay Procedure

Preliminary remarks:

Specimens that are found positive with SERODIA-HIV1/2 MIX quantitative test should be re-tested in duplicate. If either are both repeated tests are positive or intermediate, then the specimens should be tested using the semi-quantitative procedure.

When performing the semi-quantitative procedure, it is not unusual to see specimens with titers well beyond well # 12. Additional dilution of these specimens may be necessary, prior to dispensing them into microplate, for end-point determination in the semi-quantitative test.

Preparatory work:

1. Take your SERODIA kit out of the refrigerator and allow all components of the kit to reach the ambient temperature.

2. In the meantime check if there are enough reconstituted Sensitized and Unsensitized Particles (SPs & USPs). If not, carry out the reconstitution procedure. Both particles must be reconstituted at least 30 minutes prior to the test.

Figure 10. Opening SERODIA kit and check the components of the kit

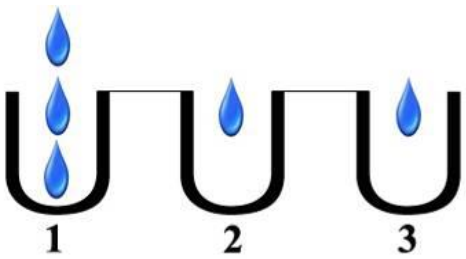
3. Fill the heading of the SERODIA worksheet and write down the lab. numbers/bag numbers of the samples to be examined. In this way, a position on the worksheet and consequently on the microtiter plate is allocated to each sample to be examined.

4. Partition and label the microtiter plate in accordance with the SERODIA worksheet. For each serum sample, 4 wells on the microtiter plate (referred to here in after as wells 1, 2. 3 and 4) are allocated. Always include Serum Diluent Control = Reagent Control (RC) and Positive Control (PC) for both HIV 1 and HIV 2. Only U bottom shaped nicrotiter plates may be used for SERODIA HIV.

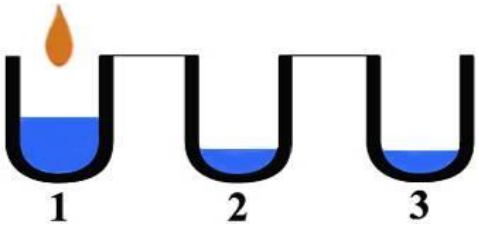
5. New microtiter plates must be rinsed in distilled water and air-dried before the test procedure. The microtiter plates mustn't be reused.

Test Procedure: **Quantitative assay method**

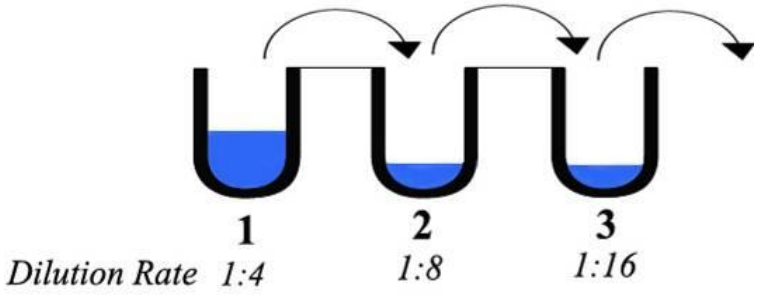
1. Place 75 µL (3 drops of 25µL) of Sample Diluent (B) in well # 1 of a microplate and 25 µL each (1 drop of 25 µL) into wells # 2 and # 3.



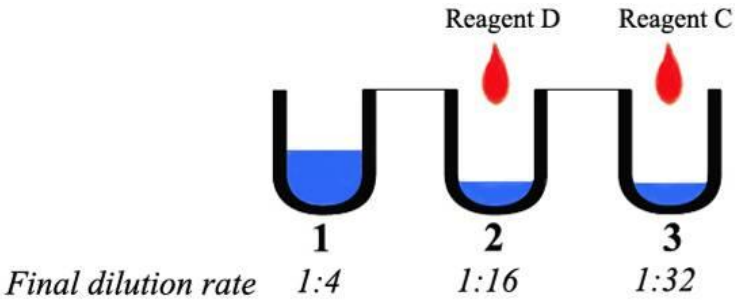
2. Using a micropipette, add 25 µL of serum/plasma specimen into well # 1.



Mix the contents of well # 1 by filling and discharging the micropipette 5 or 6 times. Then, using a micropipette, transfer 25 µL of the diluted solution from well # 1 into well # 2. Mix the contents of well # 2 in the same manner as described above and transfer 25 µL into well # 3. Following the same procedure, mix the contents of well # 3 and then discard 25 µL of solution remaining in the pipette after mixing.



3. Using the one of droppers supplied in the kit, place 25 µL (1 drop) of reconstituted Control Particles in to well # 2. Using the other dropper, place 25 µL (1 drop) of reconstituted Sensitized particles into well # 3.



4. Mix the content of the wells by rotating the plate with hand on a flat 5 to 6 times surface or using a rotary mixer.

5. Cover a plate and place it on a vibration-free surface. Allow it to stand at the room temperature (15-30^{o}C) for 2 hours.

6. Read and interpret the patterns.

2.2.6 Result test of samples

A: control; B3: positive control

- Negative: C3, D3, E3

- Positive: F3, G3, H3

Table 4. Results of Serodia HIV-1/2 test

Order	Sample	Result
A	NIHE – HIV 1210 – 01	-
B3	NIHE – HIV 1210 – 01	+
C3	NIHE – HIV 1210 – 01	-
D3	NIHE – HIV 1210 – 01	-
E3	NIHE – HIV 1210 – 01	-
F3	NIHE – HIV 1210 – 01	+
G3	NIHE – HIV 1210 – 01	+
H3	NIHE – HIV 1210 – 01	-

("+": means positive; "-" means negative)

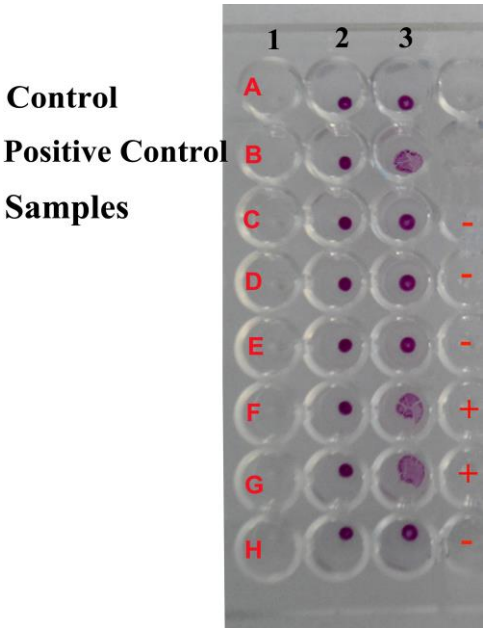


Figure 11. Results of SERODIA-HIV1/2 kit

2.2.7 Discussion

Advantage: the Kit is designed for the sole purpose of detecting HIV related antibodies in serum/plasma specimens. The results are easy to evaluate.

Disadvantages:

- If the test is negative, this means the tested sample does not contain HIV antibodies. However, this cannot exclude the possibility to an exposure to an infection of HIV.

- Other clinical symptoms and information are useful for clinical diagnosis. If the test is positive, specimen should be confirmed and re-tested at different time intervals and the result compared.

- Vibrations (like caused by the centrifugal machine) may affect the result quality.

- Because of the requirement of this kit, using microplates other that "U" shaped microplates can prevent the agglutination.

2.3 Genscreen HIV – 1/2 version 2

2.3.1 Introduction

There are two types of Genscreen HIV-1/2 version, depends on the number of used tests, we selected the suitable types to ensure the economical aspect.

72278 Genscreen HIV-1/2 Version 2, 96 tests

72279 Genscreen HIV-1/2 Version 2, 480 tests

In this centre, we used the genscreen^{TM} HIV-1/2 version 2 kit is one of three ways to determine the presence of HIV in the sample.

2.3.2 Principle of the Genscreen^{TM} HIV-1/2 Version 2 Kit

The kit is an enzyme immunoassay base on the principle of the two-step sandwich technique for the detection of the various antibodies with HIV-1 and/or HIV-2 virus in human serum or plasma.

Figure 12. GenscreenTM HIV-1/2 Version 2 Kit

The principle of the kit is based upon the use of a solid phase coated with purified antigens and of an antigens - peroxidase conjugate; particularly, gp160 and p25 recombinant protein of HIV-1 and a peptide mimicking the immunodominant epitopes of the HIV-2 envelope protein (antigens) coated with peptides mimicking the immudominant epitopes of the HIV-1 and HIV-2 envelope glycoproteins and nucleocapsid recombinant protein (peroxidase conjugate).

The assay produce includes the following reaction steps:

1. Firstly, pipette the serum samples and control sera into microplate wells. HIV-1 and/ or HIV-2 antibodies, if any, bind to the antigens immobilized on the solid phase.

Observe color changes of samples deposition; it is validated if the purple change to blue (SDP = Sample Deposition Proof).

2. Add peroxidase labeled purified HIV-1 and HIV-2 antigens into microplate wells. They bind in turn to the IgG, IgM or IgA, captured on the solid phase, from the patent samples.

3. After incubation, the presence of the enzyme immobilized on the complexes is shown.

4. Stop the reaction. Based on the result of the absorbances determination at 450/620-700 nm.

Figure 13. Anthos 2010 Microplate Reader UK

Warnings and precautions: Be careful that these chemical reagent and infectious agents could be present in waste washing fluids, and any apparatus (pipette tips etc.). Therefore, these must be suitably decontaminated and handled in accordance with Good Laboratory Practice. Another remarkable thing is that it contains heat inactivated (+56°C for 60 minutes) human sera known to be reactive for anti-HIV-1.

Calculation

After absorbances determination at 450/620-700 nm, the OD (optical density) values were used to calculate to get the final conclusion:

$$\text{ODR4} = \frac{\text{OD(B1)} + \text{OD(C1)} + \text{OD(C2)}}{3}$$

And threshold value (CO) is equal to 1/10*ODR4: $CO = \frac{ODR4}{10}$

The results are valid if these required values are satisfied:

ODR4 > 0.8 ODR3 < 0.7*CO ODR5/ODR4 ≥ 1.3

The conclusions for the samples base on the results of comparison between CO and OD values:

- Positive if OD ≥ CO
- Negative if OD < CO

2.3.3 Compositions

Table 5. Compositions of GenscreenTM HIV-1/2 Version 2 Kit

LABEL		NATURE OF THE REAGENT
R1	Microplate	Microplaque 12 strips of 8 wells coated with purified HIV-1 and HIV-2 antigens
R2	Concentrated Washing Solution (20X)	Concentrated Washing Solution (20X) Tris NaCl Buffer pH 7,4 Preservative: ProClin^{TM} 300 (0,04%)
R3	Negative Control	Negative Control Heat inactivate human plasma negative for HBs antigens, HIV antigens, anti HIV-1, anti HIV-2 and anti HCV antibodies Preservative: Sodium azide < 0.1%
R4	Cuff – off Control	Cuff – off Control Heat inactivate human plasma positive for anti HIV antibodies, negative for HBs antigens and anti HCV antibodies Preservative: Sodium azide < 0.1%
R5	Positive Control	Positive Control Heat inactivate human plasma positive for anti HIV antibodies, negative for HBs antigens and anti HCV antibodies Preservative: Sodium azide < 0.1%
R6	Sample Diluent	Sample Diluent Calf serum solution (Tris buffer with 0.1% chloroform and ProClin^{TM} 300)
R7a	Conjugate	Conjugate Lyophilised peroxidase labelled purified HIV-1 and HIV-2 antigens
R7b	Conjugate Diluent	Conjugate Diluent Skimmed milk solution (Tris buffer with 0.1% chloroform and ProClin^{TM} 300)

R8	Peroxidase Substrate Buffer	Peroxidase Substrate Buffer Sodium citrate and sodium acetate solution pH 4.0 containing H_2O_2 (0.015%) and DMSO (4%)
R9	Chromogen	Chromogen Solution containing tetramethylbenzidine (TMB)
R10	Stopping Solution	Stopping Solution 1N sulfuric acid solution

2.3.4 Handling and storage conditions *(followed by the producer's guide)*

– Avoid contact with skin and eyes.

– Reagents are to be kept at 2-8ºC upon receipt.

– Reagents may be stored at 2-8ºC until use by date.

– Reagents should be divided into measured sub-aliquots of one use and stored below -20ºC to avoid freeze/thaw cycles.

– When thawed for use, store at 2-8ºC. Once thawed, use within one month and do not refreeze.

– Ensure all containers are properly sealed to avoid drying out of reagent.

– Avoid microbial contamination of this product as this may alter product performance.

– Avoid excessively high temperatures or humidity.

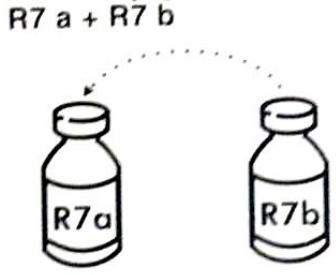
2.3.5 Assay procedure

Strictly follow the proposed procedure: Use the negative, positive and cut-off controls for each series of determinations to validate the test results.

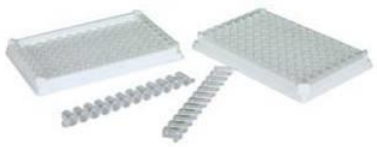
1. Carefully establish the sample distribution and identification plan

2. Prepare the dilute washing solution (R2) in distilled water (1:20) for 3 lines

3. Prepare the conjugate solution (R7a+R7b) (use within 1 month at 2-8^{o}C)

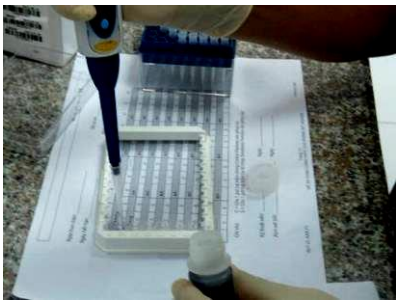


4. Take the carrier tray and the strips (R1) out of the protective pouch



(Well number = sample number + 05)

5. Add 25 µL of Sample Diluent (R6) in all wells



Add 75 µL of Samples/Controls:

- A1: Negative control (R3)
- B1, C1, D1: Cut-off control (R4)
- E1: Positive control (R5)
- F1, G1, H1: Patient 1, 2, 3

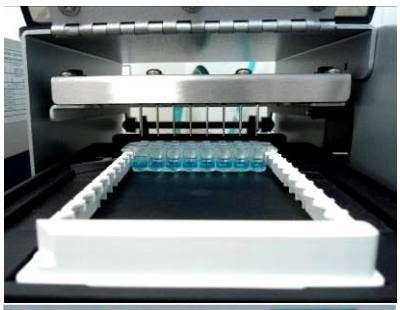
6. Incubate at 37^{o}C ± 1^{o}C, 1 hour ± 5 min

7. Wash x5 times with 1X Washing Solution

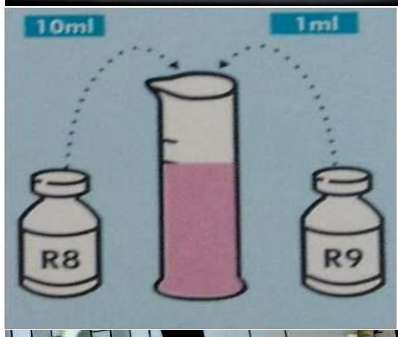
8. Add 100 µL of prepared conjugate solution (R7a+R7b)

9. Incubate at room temperature (18-30^{o}C), 30±5 min

10. Wash x5 times with 1X Washing Solution

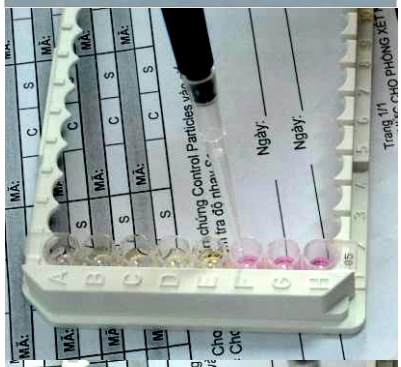


11. Prepare the development solution (R8:R9=10:1)



12. Add 80 µL of development solution

13. Incubate at room temperature, in the dark for 30 ± 5 min



14. Add 100 µL of Stop Solution (1N H_2SO_4) (R10)

15. Wait at least 4 min after adding Stop solution and within 30 min of stop reaction

Read plate at 450/620 – 700nm

Notes: There are two people for doing the test, including one technician and the other for checking the procedure of technician.

2.3.6 Results

The results were showed on screen and conclusion was gotten based on the comparison between the determined value and standard value.

Table 6. Result of Genscreen HIV-1/2 test

Order	Sample	Calculated value	Conclusion
1	NIHE – HIV 1210 – 01	0.204	Negative
2	NIHE – HIV 1210 – 01	26.007	Positive
3	NIHE – HIV 1210 – 01	0.211	Negative
4	NIHE – HIV 1210 – 01	0.197	Negative
5	NIHE – HIV 1210 – 01	0.218	Negative
6	NIHE – HIV 1210 – 01	25.556	Positive
7	NIHE – HIV 1210 – 01	26.042	Positive
8	NIHE – HIV 1210 – 01	0.275	Negative

2.3.7 Discussion

This is one of effective ways to detect the presence of HIV-1/2 by using ELISA method. After the test was done, we can recognize its benefits and drawbacks.

Advantages: Using a small amount of whole blood, serum or plasma can generate highly accurate results.

Disadvantages: high technique, strict following the procedure is required. The results can be affected if any mistakes occur.

2.4 Rapid HIV-1/2 Test (*Using the Abbott Determine HIV-1/2 Test*)

2.4.1 Introduction

Determine® HIV-1/2 is an easy-to-use, rapid (15-minute) test for HIV antibodies developed by Abbott. A small amount of whole blood, serum or plasma collected is used to get the accurate result of HIV antibodies presence.

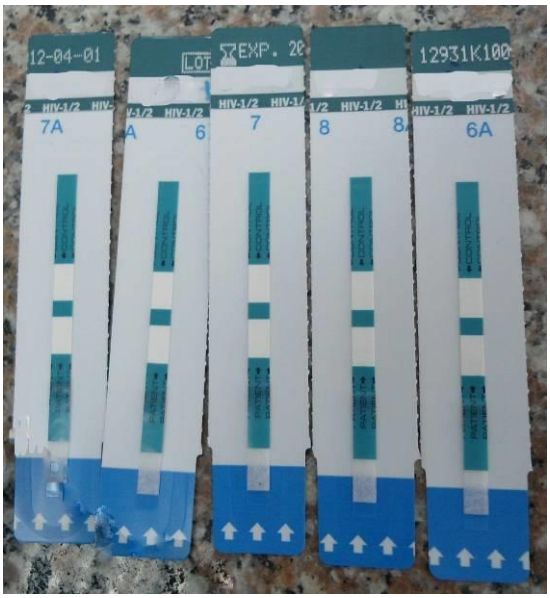


Figure 14. Abbott Determine HIV-1/2 test strip

The Abbott Determine HIV-1/2 Test is used *in vitro* conditions and intended as an aid to detect antibodies rapidly to HIV-1/2 from the infected individuals. This is a visually read, qualitative immunoassay for the detection of antibodies to HIV-1 and HIV-2 and in human serum, plasma or whole blood.

Storage: The Test must be stored at 2-30°C until expiration date.

2.4.2 Summary and Explanation of the Test

HIV-1/2 is virus that can lead to AIDS (Acquired Immunodeficiency Syndrome) in infected individuals. The person susceptible to opportunistic infections and some malignancies was reduced under the depletion of helper T-cells caused by virus. In AIDS period, there are significant changes in the population of T-cell lymphocytes. The test is based on the fact that when the AIDS virus presence on the bodies, this will make the hosts elicits the production of specific antibodies to either HIV-1 or HIV-2.

2.4.3 Biological Principles of the Procedure

Determine HIV-1/2 is an immuno-chromatographic test for the qualitative detection of antibodies to HIV-1 and HIV-2. This test works based on the principle that antigen-selenium colloid in the test that will become red if antibodies to HIV-1/2 are present.

According to Determine HIV-1/2 guide, the sample is added to the sample pad. As the sample will migrate through the conjugate pad, it reconstitutes and mixes with the selenium colloid-antigen conjugate. This mixture continues to migrate through the solid phase to the immobilized recombinant antigens and synthetic peptides at the patient window site.

The results will be read after 15-20 min included following cases:

If antibodies to HIV-1 and/or HIV-2 are present in the sample, the antibodies bind to the antigen-selenium colloid and to the antigen at the patient window, forming a red line at the patient window site.

If antibodies to HIV-1 and/or HIV-2 are absent, the antigen-selenium colloid flows past the patient window, and no red line is formed at the patient window site.

To insure assay validity, a procedural control bar is incorporated.

2.4.4 Collection of sample

Human serum, plasma, and whole blood collected by venipuncture should be collected aseptically in such a way as to avoid hemolysis.

Specimen Storage: The samples can be stored at 2 – 8^{o}C if the test is performed within 7 days or they may be deep-frozen at -20^{o}C.

2.4.5 Test Procedure

To test the presence of HIV antibodies, we did the following steps:

1. Remove the protective foil cover from each test. Check the expiration date. Do not use expired kits. Label with the appropriate patient/client identification.

2. For serum or plasma samples:

- Transfer 2 drops of serum, plasma or venipuncture whole blood 50 µL of sample to the specimen pad of the test strip (marked by the arrow symbol).

- Wait a minimum of 15 mins (up to 60 mins) and read result on worksheet.

Figure 15. Determine HIV-1/2 procedure

In the Figure 15, collected blood sample (a) was centrifuged (b) to get the serum (c). After that, transfer serum, to the specimen pad of the test strip (d), Wait a minimum of 15 mins (up to 60 mins) and read result on worksheet (e).

2.4.6 Interpretation of Results

Based on above cases we can interpret and conclude the results:

Positive (Two Bars) (Figure 16a)

Red bars appear in both the control window (labeled "Control") and the patient window (labeled "Patient") of the strip.

Negative (One Bar) (Figure 16b)

One red bar appears in the control window of the strip (labeled "Control"), and no red bar appears in the patient window of the strip (labeled "Patient").

Invalid (No Bar) (Figure 16c)

If there is no red bar in the control window of the strip, and even if a red bar appears in the patient window of the strip, the result is invalid and should be repeated.

Figure 16. Results on worksheet

Figure 16a. Positive

Figure 16b. Negative

Figure 16c. Invalid

2.4.7 Result

According to Figure 15e, the tests only had one red bar appears in the control window of the strip, and no red bar appears in the patient window of the strip. Therefore, we concluded that five samples were negative with HIV-1/2 by using Determine HIV-1/2 test.

Continue to test the samples used in Serodia and Genscreen HIV-1/2 tests to compare the result of Determine test to the others.

2.4.8 Discussion

Advantages: easy-to-use, rapid (15-minute) test for HIV antibodies developed by Abbott. Using a small amount of whole blood, serum or plasma collected by a finger prick, any hospital or remote setting can generate highly accurate results regardless of its access to laboratory equipment or electricity.

Disadvantages: No test provides absolute assurance that a sample does not contain low levels of antibodies to HIV such as those present at a very early stage of infection. Therefore a negative result at any time does not preclude the possibility of exposure to or infection with HIV. Positive specimens should be retested using another method and the results should be considered in light of the overall clinical evaluation before a diagnosis is made.

To insure assay validity, a procedural control is incorporated in the device and is labeled "Control". If the control bar does not turn red by assay completion, the test result is invalid and the sample should be retested.

2.5. Final conclusion of HIV tests

In sum, the combination of results of three HIV-1/2 tests (Serodia, Genscreen, Determine) of these samples, the final results and conclusion were shown on Table 7.

Table 7. Final conclusions base on Serodia, Genscreen and Determine HIV test

Order	Sample	Serodia	Genscreen	Determine	Final conclusion
1	NIHE - HIV 1210 - 01	-	-	-	Negative
2	NIHE - HIV 1210 - 01	+	+	+	Positive
3	NIHE - HIV 1210 - 01	-	-	-	Negative
4	NIHE - HIV 1210 - 01	-	-	-	Negative
5	NIHE - HIV 1210 - 01	-	-	-	Negative
6	NIHE - HIV 1210 - 01	+	+	+	Positive
7	NIHE - HIV 1210 - 01	+	+	+	Positive
8	NIHE - HIV 1210 - 01	-	-	-	Negative

("+": means positive; "-" means negative)

Because these samples were only used for testing of quality and skills of technicians; these are positive effects if the governments widen the areas, objectives for HIV test to have a suitable solution for each unlucky infected case in treatments and prevention of HIV in the community.

There is a variety of HIV tests (showed on 2.1) to determine the presence of this virus in the host; however, depend on the conditions of each countries, we can choose the suitable test. The result is combination of results of different tests. Positive specimens should be re-tested using another method and the results should be considered in light of the overall clinical evaluation before a diagnosis is made.

– **Recommendations:** these are effective ways with highly reliable results. Therefore, this has been a required test for the citizen soldier recruitment and soldier health control. Moreover, these tests are also for HIV tests for the locals.

Cautions for HIV test:

Appropriate safety practices should be used for materials that contain or are suspected of containing infectious agents. These precautions include, but are not limited to the following:

- Wear gloves when handling specimens or reagents.
- Clean and disinfect all spills of specimens or reagents using a tuberculocidal disinfectant, such as 0.5% sodium hypochlorite, or other suitable disinfectant.
- Decontaminate and dispose of all specimens, reagents, and other potentially contaminated materials in accordance with local regulations.
- Kit components are stable until expiration date when handled and stored as directed. Do not use kit components beyond expiration date.

3. Rapid Hepatitis B, C Test

Antibody to Hepatitis B Surface Antigen (HBsAb/HBsAg) and Hepatitis C Antibody

3.1 Introduction

Viral hepatitis is a systemic disease primarily involving the liver. Most cases of acute viral hepatitis are caused Hepatitis B virus (HBV) or Hepatitis C virus.

- Hepatitis B

(*http://webmd.com/hepatitis/hepb-guide/hepatitis-b-topic-overview*):

- Hepatitis B virus causes a major health problem by liver infection. According to WHO (World Health Organization), it is considered to be the most serious type of viral hepatitis. Worldwide, an estimated two billion people have been infected with the hepatitis B virus and more than 240 million have chronic (long-term) liver infections. (*http://who.int/mediacentre/factsheets/fs204/en/*).

- Symptoms: most of HBV infected people have no symptoms. If it does, some remarkable symptoms will be recognized like tired feeling, mild fever, headache, anorexia, vomiting, belly pain, diarrhea or constipation, muscle aches and joint pain, skin rash…

- Diagnosis: A simple blood test. The doctor also may be able to tell if a person has had the vaccine to prevent the virus. In the case of liver damage, some other ways, especially liver biopsy (taking a tiny sample of your liver for testing), are used to detect exactly the present of HBV to get the appropriate treatment.

- Treatments: In most cases, hepatitis B goes away on its own. You can relieve your symptoms at home by resting, eating healthy foods, drinking plenty of water, and avoiding alcohol and drugs. Also, find out from your doctor what medicines and herbal products to avoid, because some can make liver damage caused by hepatitis B worse.

- Prevention: A vaccine against hepatitis B has been available since 1982. Hepatitis B vaccine is 95% effective in preventing infection and its chronic consequences, and is the first vaccine against a major human cancer.

- Hepatitis C

(*http://webmd.com/hepatitis/hepc-guide/hepatitis-c-topic-overview?page=2*):

- Hepatitis C is a viral disease that leads to swelling (inflammation) of the liver.
- Symptoms: Feeling very tired, joint pain, belly pain, itchy skin, sore muscles, dark urine, yellowish eyes and skin (jaundice). Most people go on to develop chronic hepatitis C but still don't have symptoms.
- Diagnosis: A simple blood test. In the case of liver damage, a liver biopsy (taking a tiny sample of your liver for testing) is required. In a simple way, people can buy Home Access Hepatitis C Check to test themselves. Besides, Anti-HCV antibody or HCV RIBA tests (to detect HCV antibodies) and HCV genetic material (RNA) testing (to select suitable treatments) are used.
- Treatments: suitable treatments are used that depend on how damaged your liver is, how much virus in the liver, and what type of hepatitis C infect. Doing exercise and eat healthy foods are recommended.
- Until now, there is no vaccine for hepatitis C.

3.2 Test kit

QuickTest™ HBsAb and QuickTest™ HBsAg Strip and hepatitis C virus whole blood/serum/plasma Test were used to determine the presence of hepatitis virus B/C.

This test is a rapid chromatographic immunoassay for the qualitative detection of Hepatitis B Surface Antigen (HBsAb/HBsAg) and Hepatitis C Antibody in whole blood, serum or plasma. For professional *in vitro* diagnostic use only.

In this laboratory, we just used the Quick test to detect the presence of Hepatitis B Surface Antigen and Hepatitis C antibody:

- Antigens (HBsAg test) are markers made by bacteria or viruses. So the presence of HBV antigens means that the virus is in the body. HBsAg test: The test utilizes a combination of monoclonal and polyclonal antibodies to selectively detect elevated levels of HBsAg in whole blood, serum or plasma.

- Antibodies (HBsAb and Hepatitis C Antibody) are proteins produced by the body to fight infection. The presence of HBV/HCV antibodies means that you have been exposed to the Hepatitis B/C virus at some time. HBsAb test: Vaccination against HBV was introduced to control the morbidity and mortality associated with the virus. The minimum standard titer of HBsAb is 10 mIU/mL for protective immunity to HBV.

In the case of people vaccinated HBV within 2 years since vaccinating, firstly, we should test HBsAb to make sure them have the antibody to HBV. If the result is negative, this means they don't respond the vaccine. Then we should continue to test HBsAg to check their body that is infected by HBV or not.

In HCV test, we just check the infection of HCV into a body. Positive result means that people need to use some other treatments for HCV. Quick tests are helpful not only to detect Hepatitis C virus but also to screen people to prevent the spread of this virus.

Storage

The kit can be stored at room temperature or refrigerated (2-30°C) but cannot be frozen. The test strip is stable through the expiration date printed on the sealed pouch. The test strip must remain in the sealed pouch until use.

Quality Control

A procedural control is included in the test. A colored line appearing in the control region (C) is the internal procedural control. It confirms sufficient specimen volume and correct procedural technique.

Control standards are not supplied with this kit; however, it is recommended that a positive control (containing 10 ng/mL HBsAg) and a negative control (containing 0 ng/mL HBsAg) be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

Precautions

- For professional *in vitro* diagnostic use only. Do not use after expiration date.
- Handle all specimens as if they contain infectious agents.

– Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are being tested.

– Humidity and temperature can adversely affect results.

3.3 Principle

The main differences between QuickTest™ HBsAb and QuickTest™ HBsAg Strip are the QuickTest™ HBsAb Strip is a qualitative, lateral flow immunoassay for the detection of HBsAb while The QuickTest™ HBsAg Strip test is a qualitative, solid phase, two-site sandwich immunoassay for the detection of HBsAg in whole blood, serum or plasma. However, the principle of these strips is the same:

The membrane is pre-coated with HBsAg (or anti-HBsAg antibodies in HBsAb test) on the test line region of the strip. During testing, the whole blood, serum or plasma specimen reacts with the particle coated with HBsAg (or anti-HBsAg antibodies). The mixture migrates upward on the membrane chromatographically by capillary action to react with HBsAg (or anti-HBsAg antibodies) on the membrane and generate a colored line. The presence of this colored line in the test region indicates a positive result, while its absence indicates a negative result.

The principle of Hepatitis C Virus Whole Blood/Serum/Plasma Test is similar to that of The QuickTest™ HBsAg Strip.

3.4 Interpretation of Results

The result interpretation is the same with that of Abbott Determine HIV-1/2 Test (Figure 16):

– Positive (two bars): Red bars appear in both the control window (labeled “Control”) and the patient window (labeled “Patient”) of the strip.

– Negative (one bar): One red bar appears in the control window of the strip (labeled “Control”), and no red bar appears in the patient window of the strip (labeled “Patient”).

– Invalid (no bar): If there is no red bar in the control window of the strip, and even if a red bar appears in the patient window of the strip, the result is invalid and should be repeated.

3.5 Test Procedure

1. Collect the sample. Serum and plasma specimens may be stored at 2-8°C for up to 3 days. For long term storage, specimens should be kept below -20°C.

2. Remove the protective foil cover from each test. Check the expiration date. Do not use expired kits. Label with the appropriate patient/client identification.

3. For serum or plasma samples:

a. Apply 50 µL of sample (precision pipette) to the sample pad (marked by the arrow symbol). In the simple way, if only one test are done, we dip directly the sample pad to serum (supernatant after centrifuge) but keep this sample pad not to contact with the sediment.

b. Wait a minimum of 15 minutes and read result on worksheet.

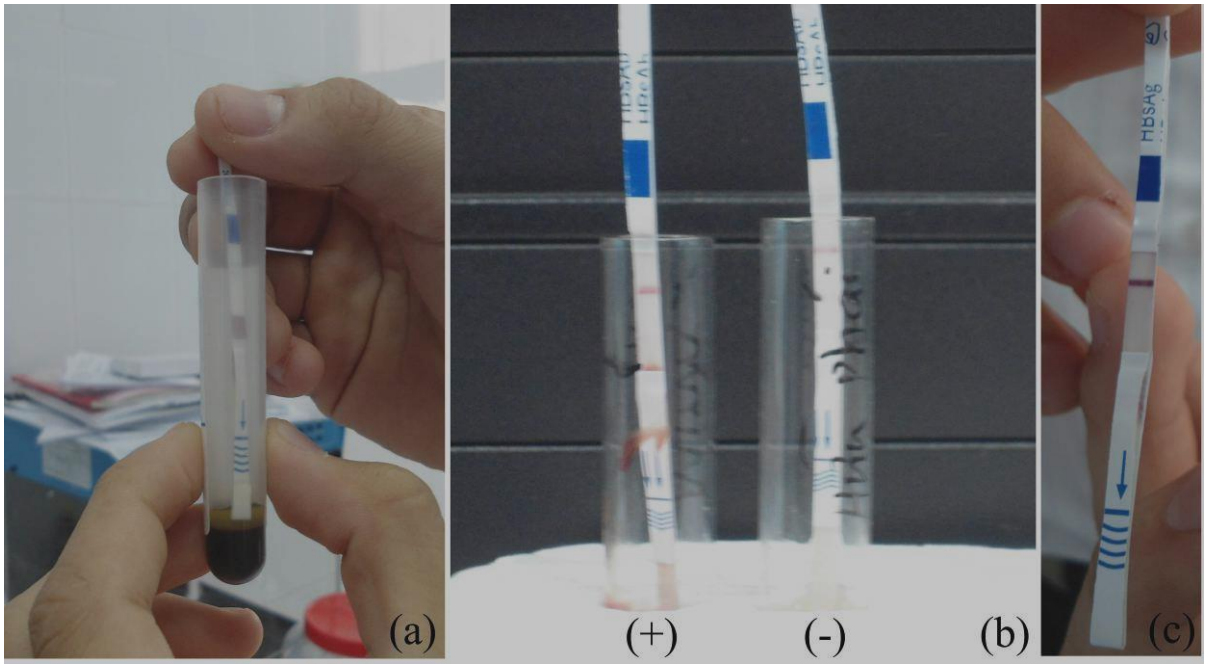


Figure 17. Rapid HBV, HCV test procedure

After dipping the sample pad to serum (a), the results were showed on Figure 14b and c. In Figure 14, "+" symbol means positive and "-" symbol means negative.

3.6 Results

Three samples were negative on HbsAg and four negative samples on Hepatitis C Antibody test (no antigen means there was no infection), one positive sample on HbsAb test (responding to vaccination).

Depend on the results; we need to get more suitable tests for finding out an effective way for the patients.

These sample tests usually use for testing the result after vaccinating (HBV). If the result of HbsAb is positive, this means that the serum contains antibodies of HBV or success in vaccinating.

3.7 Discussion

Advantages: short time (1-3 min), reliable results.

Disadvantages: the test kit has some following limitations:

- The test is for *in vitro* diagnostic use only. The test should be used for the detection of HBsAg in whole blood, serum or plasma specimen.

- The test will only indicate the presence of HBsAg in the specimen and should not be used as the sole criteria for the diagnosis of Hepatitis B viral infection.

- As with all diagnostic tests, all results must be considered with other clinical information available to the physician.

- The test cannot detect less than 1 ng/mL of HBsAg in specimens. If the test result is negative and clinical symptoms persist, additional follow-up testing using other clinical methods is suggested. A negative result at any time does not preclude the possibility of Hepatitis B infection.

4. Testing of Microbes in Human Intestinal Microflora

June 25 – July 1, 2012, we had a chance to go to the CATFISH Company (Cantho Import – Export Fisheries Limited Company at Tranoc Industrial & Export Processing Zone) for testing of microbes in gut flora of workers.

The objective of this testing is detect whether pathogenic microorganisms present in workers or not. Because products of CATFISH Company are used for food so they have to follow the U.S/EU standards of Food Safety Standards. Therefore, testing for workers contained pathogenic microbes is necessary in this case.

Figure 18. Cantho Import – Export Fisheries Limited Company

Addrress: Lot 4, Tranoc Industrial & Export Processing Zone, Cantho city, Vietnam

At this time, we had to prepare the 2 media, including MacConkey agar (MAC) and Cary-Blair Transport Medium for microbial tests.

4.1 Media introduction

MacConkey agar (MAC) is a both selective and differential medium. MAC is designed based on the bile salt-neutral red-lactose agar of MacConkey to isolate and differentiate enterics based on their ability to ferment lactose (*austincc.edu/microbugz/macconkey_agar.php*).

Figure 19. MacConkey agar (MAC)

Normally, MAC components include bile salts, crystal violet and lactose.

- Bile salts and the dye crystal violet: inhibit the growth of gram-positive bacteria and select for gram-negative bacteria.

- Lactose is used to allow differentiation of gram-negative bacteria based on their ability to ferment lactose. Organisms which ferment lactose produce acid end-products which react with the pH indicator that turns red at a pH below 6.8 and is colorless at any pH greater than 6.8.

- In the lactose positive case, *E. coli, Klebsiella* and *Enterobacter* can be considered while in the opposite case, this is *Samonella, Shigella* or *Psedomonas*.

The original MacConkey medium was used to differentiate strains of *Salmonella typhosa* from members of the coliform group. Formula modifications improved growth of *Shigella* and *Salmonella* strains, especially *E. coli* O157:H7. These modifications include the addition of 0.5% sodium chloride, decreased agar content, altered bile salts, and neutral red concentrations.

Cary-Blair Transport Medium

- Sodium Chloride 5.00 g/l
- Disodium Phosphate 1.10 g/l
- Sodium Thioglycollate 1.50 g/l
- Calcium Chloride 0.09 g/l
- Agar 5.50 g/l
- Final pH 8.4 ± 0.2 at 25ºC

Cary-Blair Medium is recommended to collect and transport fecal and rectal samples, maintaining viability of *Salmonella* and *Shigella* in fecal samples. This medium has a low oxidation/reduction potential, which assures bacterial survival for long periods of time.

The medium has a low nutrient content and a phosphate buffer, together with the Sodium thioglycollate, that inhibit the massive growth of strains such as *Escherichia coli* and *Klebsiella aerogenes*. Agar is the solidifying agent.

The survival of bacteria in a transport medium depends on various factors such as bacteria type and concentration in the specimen, transport medium formulation, and

transport temperature and duration. Optimal growth and typical morphology can only be expected if direct inoculation and appropriate cultivation are followed (*condalab.com/pdf/1530.pdf*).

4.2 Procedure

4.2.1 Media preparations

Nowadays, the centre used the modified media of MacConKey with some changes in the components:

- Peptic digest of animal tissue: 20.0 gm/lit.
- Agar: 20.0 gm/lit.
- Lactose: 10.0 gm/lit.
- Sodium taurocholate (bile salts): 5.0 gm/lit.
- Neutral Red: 0.04 gm/lit.
- pH at 25°C : 7.4 ± 0.2
- Storage: Store between 8-25°C

The medium is selective medium for *Escherichia coli*, *Enterobacter aerogenes*, *Shigella flexneri*, *Proteus vulgaris*, *Enterococcus faecalis*, *Salmonella typhi*, *S. paratyphi*, *S. enteritidis*, *Staphylococcus aureus*… (*http://srlchem.com/products/product_details/productId/3583/*).

Directions:

1. Add 55.0 gm powder to distilled/deionized water.

2. Bring volume to 1.0 liter and mix thoroughly.

3. Gently heat and bring to boiling.

4. Autoclave at 15 psi pressure at 121°C for 15 minutes.

Figure 20. Color of Macconkey medium

4.2.2 Principle of sample collection

It is just a principle for stool collection from the anus. Normally, in this company because this is a tender subject, we just guided the workers how to collect by themselves. In an opposite way, in the army health check, technicians are required to collect the samples directly. And the principle consists of some primary steps:

- For male patients or if a gynecologic table with stirrups is not available, lateral recumbency is more commonly used. Here the patient is lying on his side, with his knees drawn up toward his chest.

- The Dacron swab is inserted about 5-6 cm into the anal canal past the anal verge, into the rectal vault. This is done without direct visualization of the anal canal. Firm lateral pressure is applied to the swab handle as it is rotated and slowly withdrawn from the anal canal, inscribing a cone-shaped arc. Make sure that the head of the swab contains patient's stool.

- Transfer the Dacron swab into a vial containing transport medium (Cary-Blair medium).

- Transfer the sample to the laboratory to do next steps for detecting.

Figure 21. Transferred medium (Cary-Blair medium)

After transferring the sample into prepared MacConKey medium, inoculate at 37^{o}C for 24 hours. Observe the changes of color of the medium.

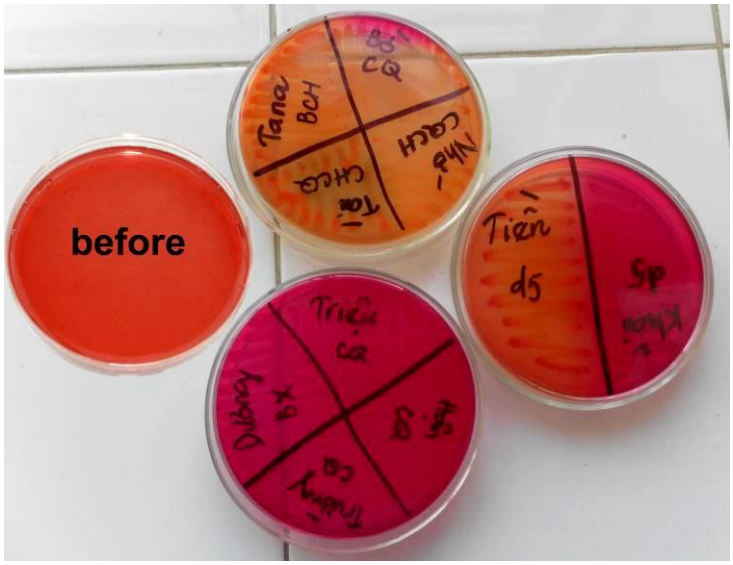


Figure 22. Color changes in Macconkey medium after incubation

4.2.3 Results

There were many color changes in all collected samples with different color after incubation.

Conclusion: all of the samples contained microbes that can ferment lactose and changes pH of the medium to make the changes of color (colorless). The difference in colors depended on the lactose fermentation of the isolated strains. The pink color, in the samples of Trieu, Duong, Truong, Han and Khai, was generated when lactose had been converted into maltose.

This is just representative samples for the test. In the fact that in total 126 samples collected in CATFISH, there were 64.62% (126/195) that positive lactose fermentation while the remaining percentage (35.71% - 43 cases) with no fecal sample or containing no microbes with non-lactose fermentation. Two workers did not send back the vial containing transport medium (Cary-Blair medium). The reasons for these could be that because of a tender thing, therefore, workers did not collect fecal samples by themselves; they just send back an original vial.

Table 8. Results of microflora tests in CATFISH

	Positive	**Negative**	**Other**
Case	126	71	2 *(did not send the vial back)*
Percentage	64.62%	36.41%	1.03%

(195 in total)

4.2.4 Discussion

Advantages: specific selective medium was used; therefore any change on color of medium we can get a reliable conclusion.

Disadvantages: we cannot identify the strains of microbes present in samples based on the color changes, to be more exactly in identification of this microbe, we have to use other methods (sequencing…).

5. *Streptococcus* sp. test

5.1 Introduction

Streptococci are Gram-positive, facultatively anaerobic, nonmotile, catalase - negative cocci without forming spore. According to the Lancefield classification, *Streptococcus* can be classified into group A (*S. pyogenes* Rosenbach) is the most foodborne streptococcal disease), whereas group C (*S. equi*), group D (*S. faecalis*, *S. bovis*), and group G (*S. intermedius*, *S. anginosus*) cause rare foodborne outbreaks (Cyrus, 2008).

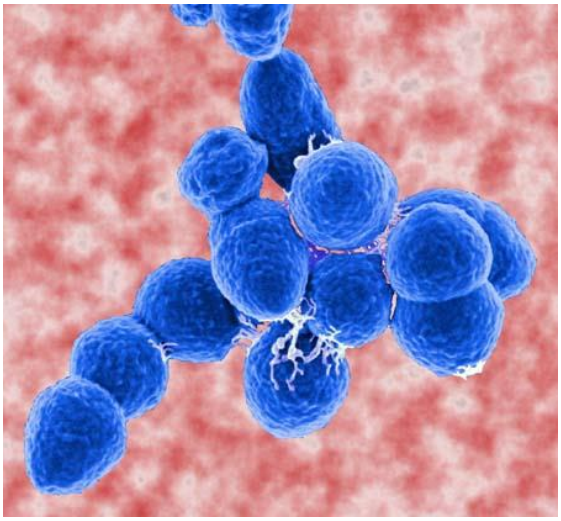


Figure 23. *Streptococcus* sp. *(http://sitemaker.umich.edu/mc13/bacterial_meningitis_causative_organism)*

Toxins

Cyrus (2008) suggested that there are more than 15 species of *Streptococcus* genus. Lancefield Group A class (*S. pyogenes*) is most important as a cause of foodborne illness. They possess a hyaluronic acid containing an outer capsule with fimbrial M proteins. Depends on which M proteins that present in the capsule, the symptoms in the patients are different. Strains with M proteins 1, 3, 5, 6, 14, 18, 19, 24, and 29 are generally associated with throat infections and rheumatic fever, whereas M proteins 2, 49, 57, 59, 60, and 61 are usually associated with skin infections (Bessen et al., 1989).

M proteins mediate the major virulence determinant of group A streptococci including lipoteichoic acid (LTA), protein F, fibronectin - binding proteins, serum opacity factor, fibrinogen/laminin/vitronectin/galactose and collagen - binding protein, glyceraldehyde - 3 - phosphate dehydrogenase, (Cunningham, 2000).

Dose Response: Colony counts of group A *Streptococcus* in implicated foods range from 10^5 to 10^7 CFU/gm.

Clinical Response

After infection, the group A *Streptococcus* needs about 1-3 days for symptoms develop in the host. A brief prodrome of muscle aches, nausea, and fatigue may precede throat symptoms.

In addition, some remarkable symptoms are confined primarily to sore throat, odynophagia, fever, pharyngeal erythema, tonsillar enlargement, and sub-mandibular lymphadenopathy, whereas cough and other respiratory symptoms usually accompany sore throat secondary to airborne streptococcal disease. Foodborne streptococcal gastroenteritis is relatively rare, and pharyngitis is the typical presentation of foodborne streptococcal disease (Elsea et al., 1971).

Diagnostic Testing

The most common method for analysis is throat culture that uses to confirm streptococcal pharyngitis. Rapid antigen screens are 95% specific, but only 70 – 90% sensitive. Negative antigen screens should be followed by routine culture in suspected cases (Gerber and Shulman, 2004).

The medium are used for identification of Group A *Streptococcus* is 5% of sheep/rabbit blood agar. After incubation in anaerobic conditions that enhance streptolysin activity, Group A *Streptococcus* produces a zone of β- hemolysis in the presence of trimethoprim - sulfamethoxazole, which inhibits the growth of normal flora (Tolliver et al., 1987).

To get more reliable result in identification of *Streptococcus*, the molecule method (PCR) is more favour to use. This method detects both live and dead strains of *Streptococcus*, and PCR for this bacterium yields 99% specificity and 100% sensitivity (Beall et al., 1996).

In some modern laboratory, to detect the presence of *Streptococcus* sp. in the hosts, subtyping procedures are useful for tracking individual strains responsible for outbreaks of *Streptococcus*. Analytical methods for subtyping include phenotypic sub-typing (biogrouping, serotyping, phage typing, esterase typing) and genotypic subtyping (pulsed - field gel electrophoresis, multilocus enzyme electrophoresis, ribotyping, PCR - based subtyping techniques, amplified fragment length

polymorphism, PCR - restriction fragment length polymorphism, repetitive element PCR, DNA sequencing - based subtyping techniques such as multilocus sequence typing). These subtyping techniques are reserved for the investigation of epidemics. Rapid DNA hybridization is an alternative technique of correlating T - antigen agglutination factors and M protein factors in human specimens to those in food specimens (Kaufhold et al., 1994).

Throat or skin lesion specimens obtained from asymptomatic human carriers (e.g., food handlers) are tested by similar methodologies to identify potential sources of an outbreak.

5.2 Principle

Depend on facilities of laboratory and identification time, we used which effective method is. In this Preventive Medicine Center of Military Zone 9, we just used simple analytical methods but reliable results for detecting of *Streptococcus*. This method based on the feature that on blood agar, the species exhibit various degrees of haemolysis, which can be used as an early step in identifying clinical isolates.

Sample collection: pus, blood…

Haemolysis on blood agar:

Table 9. Three types of haemolysis on blood agar

α-haemolysis - partial lysis of the red blood cells surrounding a colony causing a greenish discolouration of the medium.	
β-haemolysis - complete lysis of the red blood cells surrounding a colony causing a clearing of the blood from the medium.	
γ-haemolysis or non-haemolytic - no colour change or clearing of the medium.	*(Source: http://faculty.ccbcmd.edu/courses/bio141/labmanua/lab14/abg_asm.html)*

Gram staining: technique used to differentiate two large groups of bacteria based on their different cell wall constituents. The Gram stain procedure distinguishes between Gram positive (violet) and Gram negative (red) groups.

- Reagents:

  - Crystal violet (primary stain)
  - Iodine solution (mordant that fixes crystal violet to cell wall)
  - Decolorizer (e.g. ethanol)
  - Safranin (secondary stain)
  - Water (preferably in a squirt bottle)

- Steps for Gram staining:

  - Make a slide of cell sample to be stained (Heat fix the sample).
  - Add the primary stain (crystal violet) to the sample/slide and incubate for 1 minute. Rinse slide with a gentle stream of water for a maximum of 5 seconds to remove unbound crystal violet.

- Add Gram's iodine for 1 minute - this is a mordant, or an agent that fixes the crystal violet to the bacterial cell wall.
- Rinse sample/slide with acetone or alcohol for approximately 3 seconds and rinse with a gentle stream of water. The alcohol will decolorize the sample if it is Gram negative, removing the crystal violet. However, if the alcohol remains on the sample for too long, it may also decolorize Gram positive cells.
- Add the secondary stain, safranin, to the slide and incubate for 1 minute. Wash with a gentle stream of water for a maximum of 5 seconds. Observe the color under the light microscope.

Catalase is an enzyme that decomposes hydrogen peroxide into oxygen and water. Hydrogen peroxide forms as one of the oxidative end products of aerobic carbohydrate metabolism. The catalase test is commonly used to differentiate streptococci (negative) for staphylococci (positive).

- Steps of catalase test:

- With loop or applicator stick, transfer cells from the center of a well-isolated colony to a glass slide.
- Add 1-2 drops of the 3% Hydrogen peroxide to the bacterial cells.

- Interpretation the result:

- Positive: rapid, appearance of sustained gas bubbles
- Negative: No gas bubble production

5.3 Procedure

This procedure shares many advantages as well as disadvantages.

- **Advantages**: simple analytical methods but reliable results for detecting of *Streptococcus.*

- **Disadvantages:** this procedure includes many steps (incubation, Gram staining, catalyse reaction…) therefore, it takes a long time to detect while molecular methods are more favored.

Sample collection: pus, blood… the following procedure was used for analysis of blood to identification of *Streptococcus* sp.

After spreading on the blood agar or chocolate agar plate, incubation at 37^{o}C for 24 hours.

Observation the morphology of colony in the plate and under the microscope.

Identification:

- Gram staining to check whether bacteria is Gram-positive or not (violet).

- Catalase test based on the haemolysis on blood agar: *Streptococcus* sp. with negative result. If positive, we concluded that this bacterium is not *Streptococcus* sp. and we selected the suitable method for identification of the microbes.

- To detect the presence of *Streptococcus* we needed to follow the below diagram with medium and test used such as:

- CAMP test: The CAMP test is used to identify *Streptococcus agalactiae (*group B) (CAMP positive) and to differentiate it from *Streptococcus pyogenes* (group A) (CAMP negative) and non-group B *Streptococcus* (CAMP negative). Principle of the test is the β-lysin produced by β-hemolytic *Staphylococcus aureus* acts synergistically with the CAMP factor produced by both β-hemolytic and nonhemolytic *Streptococcus agalactiae* (group B). This synergistic reaction results in an enhanced and very visible zone of hemolysis in the region between the two cultures. The synergistic zone is not observed in group A, C, and G *Streptococcus* (*http://microbelibrary.org/component/resource/laboratory-test/3086-camp-test-protocols*).

- PYR disks are mainly used for the presumptive identification of group A β -hemolytic streptococci and the presumptive identification of *Enterococcus* species. This method is based on the fact that organisms possessing the enzyme L-pyrrolidonyl arylamidase can hydrolyze the disk substrate L-pyrrolidonyl-β-naphthylamide to release L-pyrrolidone and β-naphthylamide. Visual detection can be achieved by the addition of PYR reagent. The active ingredient, p-dimethylaminocinnamaldehyde, combines with the end-product β-naphthylamide to form a red Schiff base. A positive reaction is a yellow to red color change (*http://prep4usmle.com/forum/thread/8434/*).

+ PYR (Bacitracin test): is reasonably accurate in identifying clinical isolates of beta haemolytic streptococci.

+ PYR (6.5% NaCl) is used to presumptive identification of enterococci and group D streptococci.

- Bile Esculin Agar (BEA) is a selective differential agar used to isolate and identify members of the genus *Enterococcus*. BEA is used primarily to differentiate *Enterococcus* from *Streptococcus*. Members of the genus Enterococcus are capable of growing in the presence of 4% bile and hydrolyzing esculin to glucose and esculetin. Esculetin combines with ferric ions to produce a black complex.

- Detecting of *Streptococcus* followed in the below diagram:

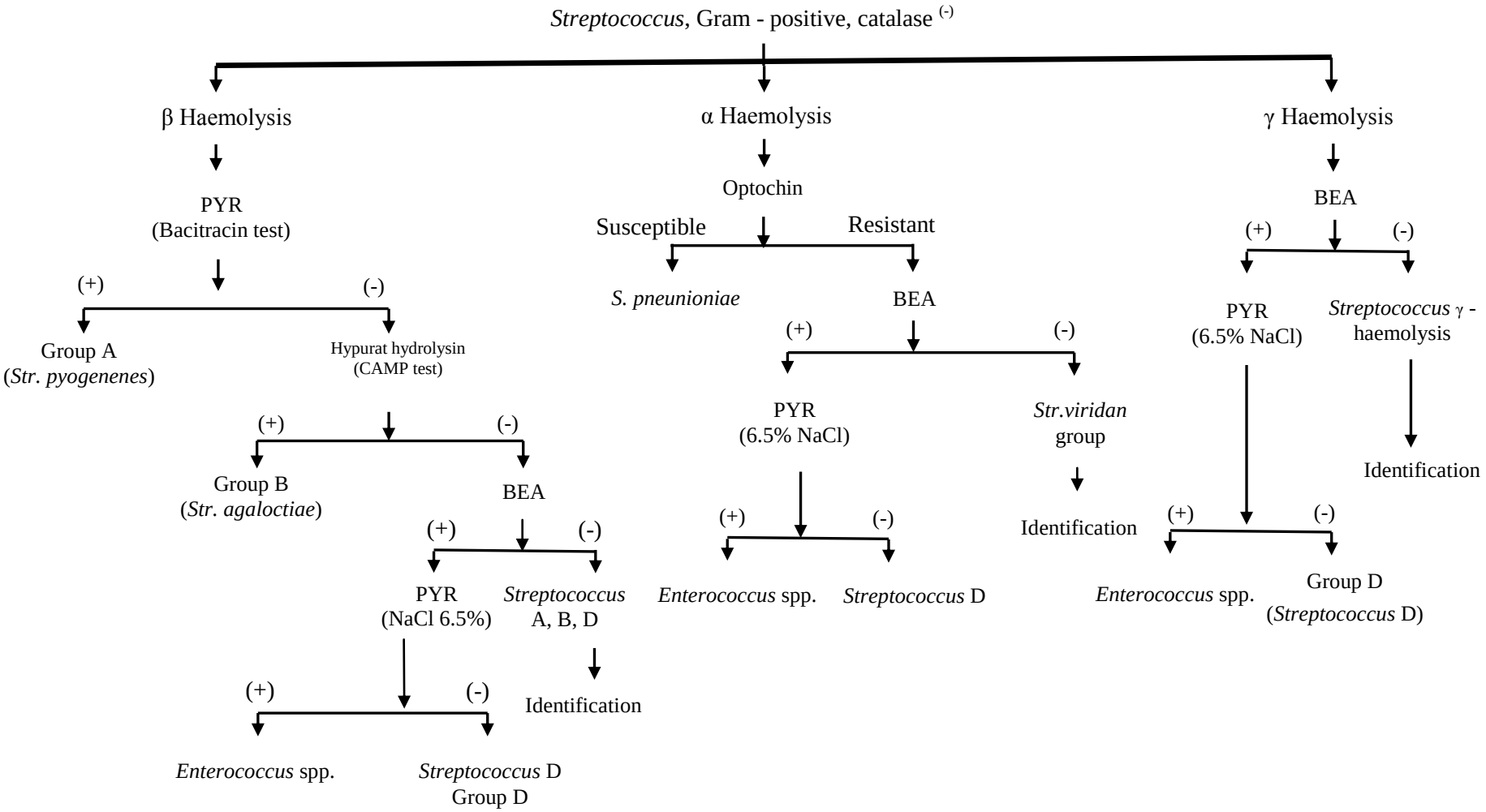


Figure 24. Diagram of *Streptococcus* sp. detection

(In Figure 24, "+" symbol means positive and "-" symbol means negative)

In the Military Region 9 Preventative Medicine Center, we only found out *Streptocccus pyogenenes* base on the Figure 24 (identification base on the features on Haemolysis and PYR - Bacitracin test).

Table 10. Detecting of *Streptococcus pyogenenes*

Order	Steps description	Image
1	This was a strain with β-Haemolysis	
2	Continue to use PYR - Bacitracin test	
3	Positive in PYR - Bacitracin test with color change	
	Conclusion	*Streptocccus pyogenenes*

In the total 5 samples for *Streptococcus* sp. test, we only identified one sample that was positive with *Streptocccus pyogenenes* test while four remaining samples, we had to follow other procedure because 3 samples were Gram negative and the other was positive with Gram staining but it was also positive in catalase reaction.

This is an effective method for detection of *Streptococcus* sp. that is popular in many hospitals.

Comparison with other modern techniques (showed on 5.1), this is a suitable tests for detection of *Streptococcus* sp. because it has given reliable results and taken a cheaper

cost. With different kinds of sample, we have to follow the appropriate method to detect *Streptococcus* sp. For example, to test *Streptococcus* sp. in food, Pasteur Institute Ho Chi Minh City used PCR technique while other Preventative Medicine Center uses Tryptose agar plates containing 1.5% agar and 0.04% of sodium azide and incubation for 12-14 hours at 35°C.

6. Rapid food test kits

6.1 Test Kit for Pesticides in Food (Phosphate and Carbamate Group)

6.1.1 Introduction

Phosphate and carbamate group

Pesticide is a broad term that includes insecticides, herbicides, fungicides, growth regulators, and other materials or products that kill snails and slugs (molluscicides). These can use as agents to control, prevent, kill, suppress, or repel organisms being considered to be harmful. Normally, depend on the uses of pesticides, its component can be different but carbamate and phosphate group present in all of Pesticides products.

– Organophosphate - Organophosphate insecticides inhibit the cholinesterase enzyme. This enzyme is used in nerve function. A number of insecticides in this family are used to control a wide range of landscape insect pests. These products tend to have a short persistence in the soil. These include phorate, malathion, diazinon, and dimethoate.

– Carbamate - The carbamate group includes insecticides, fungicides, and herbicides. Most have a short persistence in the environment. Like organophosphate, carbamate insecticides inhibit cholinesterase. Carbamate have moderate to high toxicity. Common examples include aldicarb, carbaryl, carbofuran, methomyl, and pirimicarb.

Carbamate and phosphate are two of agents used in cholinesterase inactivation because cholinesterase is one of many important enzymes needed for the proper functioning of the nervous systems of humans, other vertebrates, and insects.

Health Impact: Pesticides can cause a variety of adverse health effects.

– These effects can range from simple irritation of the skin and eyes to more severe effects such as affecting the nervous system, mimicking hormones causing reproductive problems, and also causing cancer.

– Obtaining high concentration of Pesticides can cause dizziness, vomiting, dyspnea, and cardiac arrest.

– Strong evidence also exists for other negative outcomes from Pesticides exposure including birth defects, fetal death, and neuro developmental disorder.

Pesticides are poisonous agents limited for agricultural use only. Because its reverse effects if being overused, many recommendations are noted. However, in the actual fact, Pesticides residue over standard is still found in many kinds of food. The reasons for this could be that farmers do not conform to which producers strongly recommend like concentration, harvested time after spraying… Therefore, it is essential to have a rapid test to classify which products are safe or not.

In the world, there are many recommendations to limit the pesticides use but it is not effective in developing countries because farmers usually overuse the concentration of pesticides and do not care about the warnings of producers. Consequently, to protect the consumers from pesticides, there are more and more kinds of test that can quickly determine the presence of phosphate and carbamate in food or not. Because its benefits, people tend to use the rapid kit rather than other modern techniques like a photothermal biosensor (Pogacnik and Franko, 2003) or gas chromatograph/nitrogen phosphorus detector (Sherma and Shafik, 1975).

6.1.2 Test kit

In this Preventive Medicine Centre, we used the rapid test VPR10 (the product of Ministry of Public Security) to detect the presence of Pesticides residue (phosphate, carbamate groups) in food, with fast and reliable result.

Figure 25. The rapid pesticides test VPR10

Target Sample: Peas, cabbage, tomatoes, carrots, lettuce, grapes, strawberries, pears, apples and cherries.

Storage: room temperature (for the kit) and 4^{o}C (reagents).

Based on the colours of the paper (colour indicator), we can conclude that:

- Negative if the colour is white
- Positive if the colour is blue.

In Figure 26, "+" symbol means positive and "-" symbol means negative.

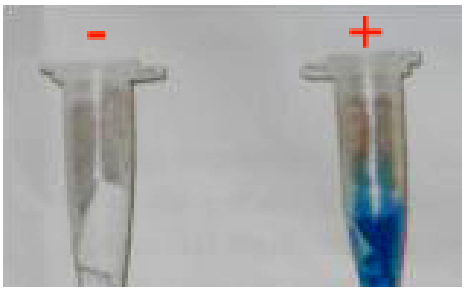


Figure 26. Result interpretation of pesticides test

6.1.3 Procedure

1. Spoon chopped food 10 grams (2/3 of the volume of plastic bag).

Figure 27. Mix the spoon chopped food with activating agent

2. Dilute the activating reagent on 10mL of distilled water before mixing the solution with the sample on the plastic bag in 3 minutes.
3. Add all of extract reagent in the tube, close cap vigorously shake, and leave for 2 minutes.
4. Collect the solution on the bag (only liquid) and transfer this onto a column in which the solution was divided into 2 separated layers.

Figure 28. Collection of the lower layer of the solution on the column

5. Continue to collect the lower layer of the solution on the column. Spread the lower layer solution on the Petri disk for natural evaporation occurred.
6. Open the reagent bag to get CV1 and CV2 reagent.
7. After the solution on Petri disk evaporated completely, a piece of silk-cotton with a syringe was used to collect the remained components on the surface of the disk.

Figure 29. Collection of the remained components on the surface of the disk

8. Transfer the collected solution (nearly 0.2 mL) into CV1 column, mix carefully and wait 30 minutes.
9. Dissolve all the components on CV2 column by using 0.1 mL mixing solution. Then, transfer CV2 into CV1 column. Shake carefully and wait 5 minutes.

10. A colour indicator (colour paper) was added into CV1 column.

Read the result after 5 minutes.

Figure 30. Result of the pesticides test (salad and green bean)

6.1.4 Result of the test

Base on the color of test, we had the positive result with perticides.

Conclusion: two samples (salad and green bean) had high levels of pesticides.

6.1.4 Discussion

Normally, many laboratories use rapid test for detection of pesticides' presence. The difference of Military Region 9 Preventative Medicine Center and other testing center is which kinds of test they use to get high profit but reliable results. Benefits and drawbacks of the test are:

Advantages: save time, reliable result. This is one of worldwide effective way to detect the food that contents phosphate or carbamate that presents in many kinds of pesticides.

Disadvantage: When disinfectant contacts your skin, wash out with clean water.

6.2 Formaldehyde test

6.2.1 Introduction

Formaldehyde in food

Formaldehyde, CH_2O, is an organic compound commonly called formalin or formol. Formalin is an aqueous solution that contains around 37% of formaldehyde.

According to the Agency for toxic substances and disease registry (*http://atsdr.cdc.gov/mmg/mmg.asp?id=216&tid=39*), formaldehyde is highly toxic to all animals. In human, formaldehyde is toxic, allergenic, carcinogenic and other symptoms such as:

– Formaldehyde inhaled may cause headaches and difficulty breathing.

– In dangerous fact that ingestion of as little as 30 mL of a solution containing 37% formaldehyde has been reported to cause death in an adult human. Water solution of formaldehyde ingestion can cause severe injury to the upper gastrointestinal tract with nausea, vomiting, pain, bleeding, and perforation.

– Formaldehyde solutions may cause transient discomfort and irritation or more severe effects (corneal opacification and loss of vision) depend on the concentration. At concentrations above 0.1 ppm in air formaldehyde can irritate the eyes and mucous membranes, resulting in watery eyes.

Because the formaldehyde in formalin is responsible for its disinfectant properties, and helps prevent growth of bacteria in fish. The strong substance of formaldehyde kills bacteria by dehydrating them, causing the normal fluid in the cells to coagulate, or become rigid. Consequently, formaldehyde can be found naturally or sprayed in food including fruits and vegetables, meats, fish and other dried and preserved foods.

To lessen the impact of formaldehyde toxicity, consumers need to determine which the really fresh foods are or not. Besides other formaldehyde analytical techniques like miniaturised electrophoretic method (Zhang et al., 2011) or high-performance liquid chromatography (Li et al., 2007), the rapid kit for the presence of formaldehyde is useful to identify the reliable food premises and retailers.

6.2.2 Test kit

In this Preventive Medicine Centre, we used the rapid test FT04 (the product of Ministry of Public Security) to detect the presence of formaldehyde in food.

Target sample: seafood, fresh meats or fishes, rice vermicelli.

Figure 31. The rapid formaldehyde test FT04

Storage: avoid direct sunlight.

Based on the colors of the solution after adding reagent, we can conclude:

- Positive if the color is pinkish-orange (light or dark color depends on the formaldehyde concentration).

- Negative if the color is yellow.

In Figure 32, "+" symbol means positive and "-" symbol means negative.

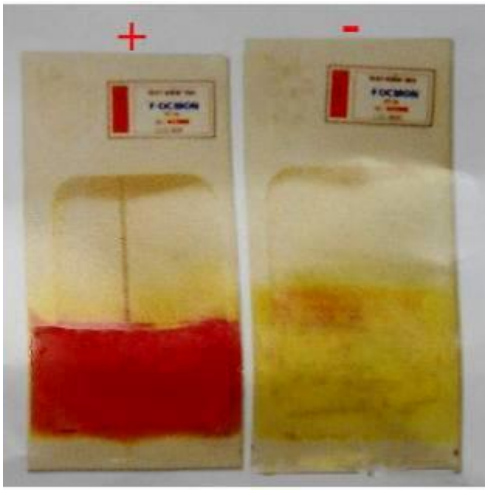


Figure 32. Result interpretation of the formaldehyde test

6.2.3 Procedure

1. Sample preparations:

   – Seafood: collect the solution after steeping with distilled water.

   – Others: chopped food 2 grams.

Open the bag, mix the chopped sample with 3mL distilled water (about level 2 of plastic bag).

Figure 33. One kind of sea fishes

2. Crush the colored ampoule, shake carefully to make the solution on bag become yellow.

3. Crush the non-colored ampoule, shake carefully and observe the color change of the solution.

4. Read the result.

Figure 34. Result interpretion of formaldehyde test

6.2.4 Result of the test

As observation, the color of test changed to reddish-orange. Therefore, the test was positive with formaldehyde.

Conclusion: this product contents a remarkable amount of formaldehyde.

6.2.5 Discussion

Normally, many laboratories use rapid test for presence of formaldehyde. The difference of Military Region 9 Preventative Medicine Center and other testing center is which kinds of test they use to get high profit but reliable results. Its advantages and disadvantages are:

Advantages: save time, reliable result. This is one of effective way to presence of formaldehyde in food.

Disadvantage: the result may be affected by the color of original sample.

6.3 Quick coloring agents test

6.3.1 Introduction

Coloring agents in food

Color is an important ingredient in deception. Food coloring, or color additive, is any dye, pigment or substance that imparts color when it is added to food or drink. Food coloring is also, as in essence, into natural and artificial synthesis in two ways. Besides, synthetic food coloring, natural pigment of the pigment is extracted directly from the plant and animal tissue.

Coloring agents have been used more and more popular because the purpose of making processed foods look more appetizing and improvements of appearance; particularly, coloring agents can offset color loss (due to exposure to air, light, moisture and storage), correct natural variations in color or enhance color, provide visual appeal to nutritious foods and color to foods that would otherwise be colorless.

Figure 35. Food coloring

(http://anorganicwife.com/2011/05/food-dye-problem.html)

However, if we overuse coloring agents, there are many effects like (*http://livestrong.com/article/459633-adverse-effects-of-artificial-food-coloring/*):

– Allergic response: Certain artificial food colorings can cause adverse effects in people who have sensitivities to them.

– Carcinogenic potential

– Hyperactivity: artificial food colorings that may increase hyperactivity children.

Because of economical aspects, people overuse the coloring agent, especially artificial food coloring agents. Therefore, some standard for coloring agent present in

foods have been established. For examples, artificial synthetic edible pigments cannot be added to meat, fish and its products, in a variety of spices, milk and dairy products, fruit and baby biscuits cannot add artificial food coloring. In some fruity drink can be controlled using, but through the food does not exceed the limit of 0.05-0.1 g/kg.

In addition, there are many ways to detect the coloring agents in food such as HPLC or combination with mass spectrometry method; however using rapid test is more favored to quickly determine the presence of coloring agents is essential to protect safety of consumers.

6.3.2 Test kit

The kit is used to determine quickly food coloring agents.

Figure 36. The rapid coloring agent test CT04

In this Preventive Medicine Centre, we used the rapid test CT04 (the product of Ministry of Public Security) to detect the presence of alkaline food coloring agents.

Target Sample: candies, cakes, soft-drinking…

Storage: avoid direct sunlight.

Read the result of this test based on the colours of the solution after adding reagent:

- Positive if CT3 is coloured.

- Negative if the colour of CT3 is non-coloured.

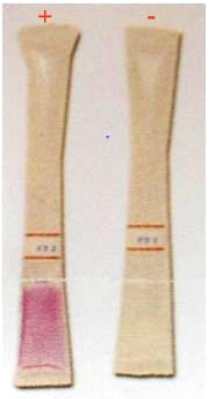


Figure 37. Result interpretation of coloring agent test

In Figure 37, "+" symbol means positive and "-" symbol means negative.

6.3.3 Procedure

1. Sample preparations:

   – Liquid: 3 mL.

   – Others: chopped food 2 grams.

   Open the bag CT04, collect the sample into the bag and shake slightly in 2 minutes.

2. Transfer the solution on CT2 column into the bag, shake slightly in 2 minutes before waiting for the solution divided into 2 separated layers.

3. Use the CT3 column to collect the solution on the bag. When the height of solution on CT3 column was about 1cm, stop and wait 1 minute to read the result.

Figure 38. Transfer the solution on CT2 column into the bag

6.3.4 Result of the test

Result: negative

Conclusion: this product contents no or contents but lower minimum amount of coloring agent that the kit can recognize.

Figure 39. Result of coloring agent test

6.3.5 Discussion

Advantages: short time (1-3 min(s)), reliable result.

Disadvantage: the result may be affected by the colour of original sample.

Normally, many laboratories use rapid test for detection of coloring agent. The difference of Military Region 9 Preventative Medicine Center and other testing center is which kinds of test they use to get high profit but reliable results.

6.4 Boric acid test

6.4.1 Introduction

Boric acid and borates in food

Borax, also known as Boric Acid, is a colorless, salt-like substance that may also be found as a white powder.

Boric acid can be found in pesticides, herbicides… because its effects in nervous systems of insects, growth inhibition of fungi by preventing the production of reproductive spores, interrupting the plant's photosynthetic pathway. Therefore, boric acid is widely used in manufacturing, fertilizers, pesticides and pharmaceuticals. However, in some cultures, it is used in food preparation as a firming agent, meat rub, preservative, or tenderizer.

According to National Pesticide Information Center (*npic.orst.edu/factsheets/borictech.html*), after consumption 2 hours, commonly reported symptoms include nausea, vomiting (often with blue-green coloration), abdominal pain and diarrhea (which may contain blood or have a blue-green color):

– Other less commonly reported symptoms include headaches, lethargy, weakness, restlessness, tremors, unconsciousness, respiratory depression, kidney failure, shock and death.

– Large oral exposures have resulted in an intense red skin rash within 24 hours of exposure, followed by skin loss in the affected area 1-2 days after the skin coloration first appears. These skin rashes typically affect the face, palms, soles, buttocks and scrotum.

– Some evidence showed that boric acid is also many negative effects to the skin, respiratory, reproductive systems… and cause many dangerous diseases like cancer, unfertile…

In the world, there are many ways to detect boric acid in food such as HPLC, gas chromatography-mass spectrometry or inductively coupled plasma mass spectrometry

method; however using rapid test is more favoured to quickly determine the presence of boric acid is essential to protect safety of consumers.

6.4.2 Test kit

The test paper allows the quick and easy detection of boric acid and borates.

In this Preventive Medicine Centre, we used the rapid test BK04 (the product of Ministry of Public Security).

Target Sample: fish, meat, rice cake …

Storage: avoid direct sunlight.

Figure 40. The rapid boric acid (borax) test

Read the result of this test based on the colors of the strip:

- Positive if the strip has two different colors between control and dipped part of the strip (slight yellow to reddish-brown).

- Negative if the colors of two parts of the strip are the same.

6.4.3 Procedure

1. Sample preparations:

   – Two samples were Vietnamese preserved mustard cabbage and meat.

   – Liquid: mix 10 grams of chopped food with 20 drops of buffer. The mixture was mashed to the fragment and waited for 5-10 mins to get 1 mL of solution.

Figure 41. Two samples for boric acid detection (meat and mustard cabbage)

– Continue to add 20 drops of buffer.

2. Add 1 drop of buffer into control part of the strip test (at the middle of the strip test).
3. Dip the below part of the strip in to prepared solution. Wait 15-20 mins to allow time for the strip to dry and change color.

6.4.4 Result of the test

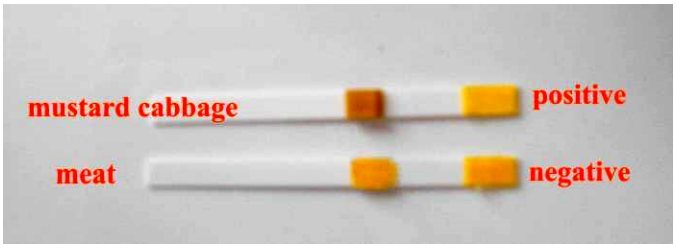


Figure 42. Results of boric acid test

Results:

- Vietnamese preserved mustard cabbage: positive
- Meat: negative

6.4.5 Discussion

Normally, many laboratories use rapid test for detection of coloring agent. The difference of Military Region 9 Preventative Medicine Center and other testing center is which kinds of test they use to get high profit but reliable results. Benefits and drawbacks of the test are:

Advantages: short time (1-3 min), reliable result.

Disadvantage: the time for color change of the strip depends on the concentration of boric acid and borates present in the samples.

7. Laboratory Safety Requirements in Military Region 9 Preventative Medicine Center

1. Wear laboratory coats or other protective clothing at all times in areas where dangerous materials are used.
2. Wear diposable gloves at all times while handling radioactive materials.
3. Either after each procedure or before leaving any area, monitor your hands for contamination in low-background area.
4. Do not store food, drink, or personal effects in areas where radioactive material is stored or used.
5. Fill a pipette by using a pipette bulb or mechanical pipette only; never pipette by mouth.

CHAPTER 3. CONCLUSIONS AND SUGGESTIONS

1. Conclusions

In this subject, it was a good opportunity for us to not only review what we learned in class but also obtain some new information and many processes in Military Region 9 Preventative Medicine Center, including:

- General information about Military Region 9 Preventative Medicine Centre
- Biochemical Test of Human Serum:
    + Aspartate Aminotransferase (AST/GOT)
    + Cholesterol Oxidase/Peroxidase
- Human Immunodeficiency Virus (HIV) test:
    + HIV-1/2 Antibody Test (SERODIA HIV-1/2 test)
    + Genscreen HIV – 1/2 Version 2
    + Rapid HIV-1/2 Test (Using the Abbott Determine HIV-1/2 Test)
- Rapid Hepatitis B, C Test
- Testing of Microbes in Human gut flora
- *Streptococcus* species testing
- Rapid food test kit:
    + Test Kit for Pesticides in Food (Phosphate and Carbamate Group)
    + Formaldehyde test
    + Quick coloring agent test
    + Boric acid test

Normally, there are many tests for an object; each process shares some benefits and drawbacks. Therefore, convenient ways are more favored because they are suitable with the facilities of centre and characteristics of tests. In this centre, we used tests with saving time, easy-to-use features but getting reliable results.

In addition to these processes, during practical time, we were asked to follow laboratory safety requirements. These help us avoid many dangerous sources such as HIV, HBV, HIV or other toxics during doing tests.

In the Military Region 9 Preventative Medicine Center, we had worked in discipline of army. It is great because this trains us to be familiar with working styles in the future.

2. Suggestions

Comparison results tested in Military Region 9 Preventative Medicine Center with that from other laboratories has some limitations. Firstly, because of its trading aspects in laboratory, we do not compare completely what we did with that in other laboratories. Another remarkable thing is results with name of methods used are showed; technicians in other laboratory just list results in simple way to help non-professional customers understand easily, therefore, in some cases, detailed processes are still not clear.

Annually, technicians in the Military Region 9 Preventative Medicine Center have a conference with foreign experts for education of new techniques. This event was held on August 2012 while our practical time was from 2nd June to 14th July - This was information we got during practical time. Therefore, we should make a contact before time selecting for practice to choose practical time with more effective and useful things.

Another important thing, we hope that practical time was more flexible than 6 weeks. Students can practice in their convenient time but they still encourage duration for practice with agreement of the company or institute. This is a got chance for them to get new and interesting things at convenient time for themselves.

In addition, increasing in the number of introduced company and institute and making agreements to the practical places with various aspects in biotechnology are necessary. Position, working regulations and other factors need to be considered carefully.

Leader and other staffs in Military Region 9 Preventative Medicine Center are willing to give many chances for other students who want to practice there. They will show and give students new techniques with their patience, enthusiasm, motivation, and knowledge.

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