CANTHO UNIVERSITY

LABORATORY REPORT

PRACTICAL TRAINING IN INDUSTRY (BT480C)

Some Basic Tests in Human (HIV, HBV, HCV, Biochemical Tests, Intestinal Microflora and *Streptococcus* sp.) and Food (Formaldehyde, Boric acid, Coloring agents and Pesticides)

TEACHERS:

Dr. Nguyen Van Thanh

MSc. Tran Vu Phuong

SUPERVISOR:

Lt. Phan Truong Giang

GROUP 15:

Lam Thi Thuy Huynh (3083408)

Tran Nguyen Nhat Khoa (3082524)

(Military Region 9 Preventative Medicine Center)

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1. Introduction of Military Region 9 Preventative Medicine Center

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1. Introduction of Military Region 9 Preventative Medicine Center

Address: 91 Cach Mang Thang Tam Street, An Thoi ward, Binh Thuy, Can Tho

Phone: (+84)710 3884 952

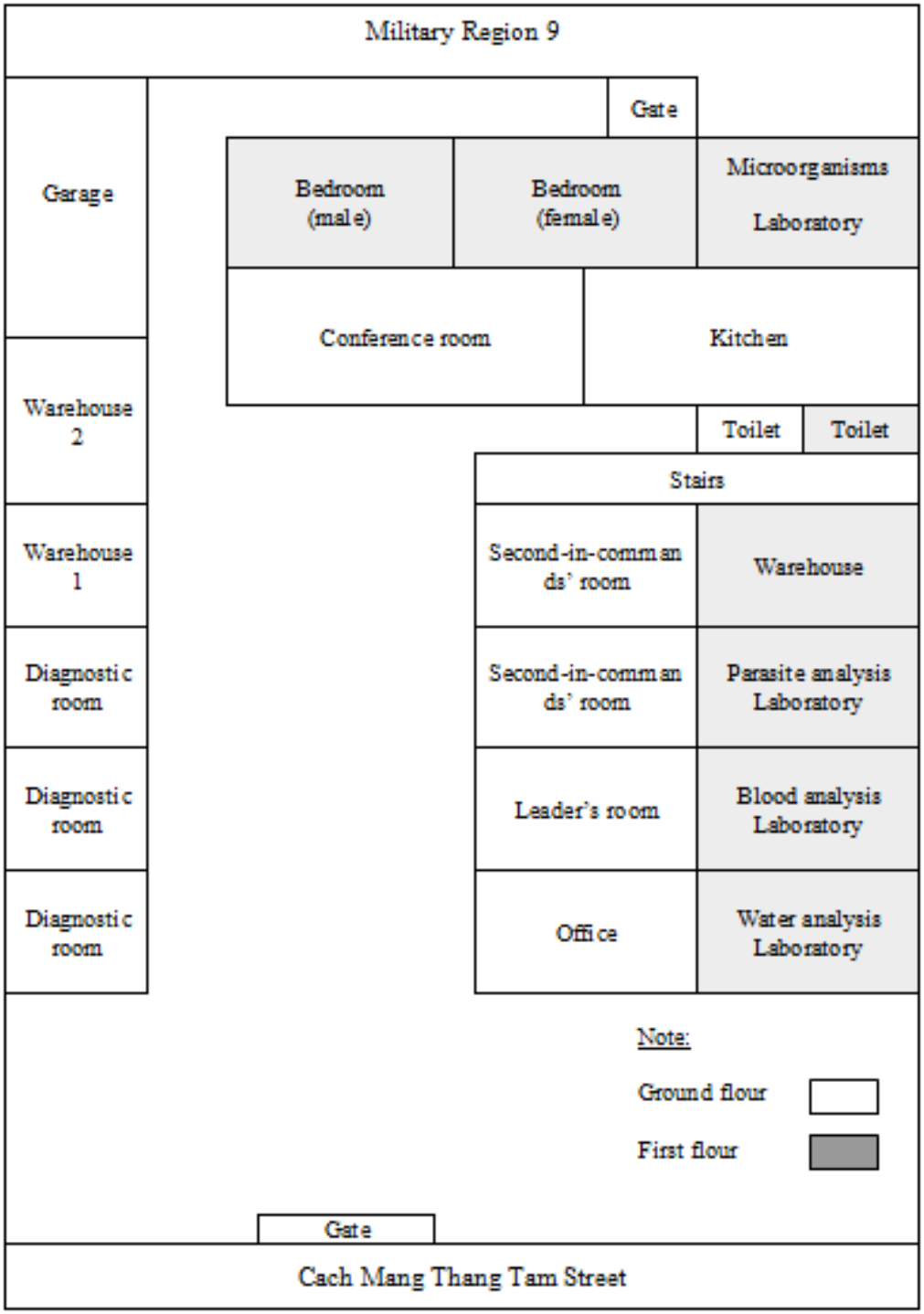
1. Introduction of Military Region 9 Preventative Medicine Center

- **History**
  - A part of Medical Service Corps
  - The project of Medical Service Corps history is still in the stage of completion.
- **Location**: the centre of Mekong Delta

  →The sanitation and epidemic prevention functions

  →Health checking or vaccinating and biochemical – physical testing

- 4 labs
- 3 diagnostic rooms
- 23 members:
  - Lt.Col.Dr Phan Van Vinh
  - 2 second-in-commands
  - 6 doctors, 3 Bachelors of Laboratory Medicine and 11 physicians



1. Introduction of Military Region 9 Preventative Medicine Center

- **The Sanitation and epidemic prevention functions in military**
  - Military practice and exercises hygiene
  - Military working hygiene
  - Outdoor hygiene
  - Water hygiene
  - Nutritional hygiene and food safety hygiene
  - Barack hygiene
  - Propaganda and education sanitation and epidemic prevention
  - Reconnaissance about hygiene and epidemic
  - Preventive vaccination
  - Sterilize; detect insect and intermediate animal diseases
  - Control infectious disease
  - Prevent HIV and AIDS infection in military

1. Introduction of Military Region 9 Preventative Medicine Center

- **Treatment and prevention mission**
  - **Purposes and contents**
    - Check and control the health and diseases of soldiers
    - Detect the diseases in different conditions
  - **Activities**
    - Citizen soldier recruitment
    - Soldier health control
    - First aid and treatment
    - Treat and rehabilitate
    - Medical Appraisement

2. Practical contents

2.1 Biochemical Test of Human Serum

Tests of Biosystems Reagents and Instruments

- **Aspartate Aminotransferase (AST/GOT)**
  - The aminotransferase catalyzes the formation of glutamic acid from 2-oxoglutarate.
  - AST/GOT testing to identify:
    - Liver damage
    - Help identify liver disease
    - Check on the success of treatment for liver disease

→ **AST/GOT test**

2.1 Biochemical Test of Human Serum

Aspartate Aminotransferase (AST/GOT) test

- **Principle**

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

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

→The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm.

- **Compositions:**

  – Reagent A: 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.

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

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

2.1 Biochemical Test of Human Serum

Aspartate Aminotransferase (AST/GOT) test

- **Reagents Preparation**

  4 mL Reagent A + 1 mL Reagent B + 0.05 mL Reagent C

- **Procedure**
  - Pipette sample and reagent into cuvette:
    - At 30°C: 1.0 mL of reagent + 100 µL sample
    - At 37°C: 1.0 mL of reagent + 50 µL sample
  - Mix and insert cuvette into the photometer.
  - Wait 4 mins
  - Get the result for conclusion

2.1 Biochemical Test of Human Serum

Aspartate Aminotransferase (AST/GOT) test

- Standard value: 0-40 mmol/l

→ 2 samples had normal AST/GOT values.

2.1 Biochemical Test of Human Serum

Aspartate Aminotransferase (AST/GOT) test

- **Advantages:** low price, reliable results and saving time
- **Disadvantages:** the results can be affected by many factors:
  - Taking medicines. 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

2.1 Biochemical Test of Human Serum

Tests of Biosystems Reagents and Instruments

- **Cholesterol Oxidase/Peroxidase**

  – Cholesterol is an essential body fat (lipid). Too much cholesterol can allow clots to develop.

  – AST/GOT testing to control the cholesterol level in the body because increased total cholesterol values are associated with a progressive escalating risk of atherosclerosis and coronary artery disease.

→ **Cholesterol Oxidase/Peroxidase test**

2.1 Biochemical Test of Human Serum

Cholesterol Oxidase/Peroxidase test

- **Principle**

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

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

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

→A coloured complex that can be measured at 550 nm.

- **Compositions:**

  – Reagent A: 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.

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

2.1 Biochemical Test of Human Serum

Cholesterol Oxidase/Peroxidase test

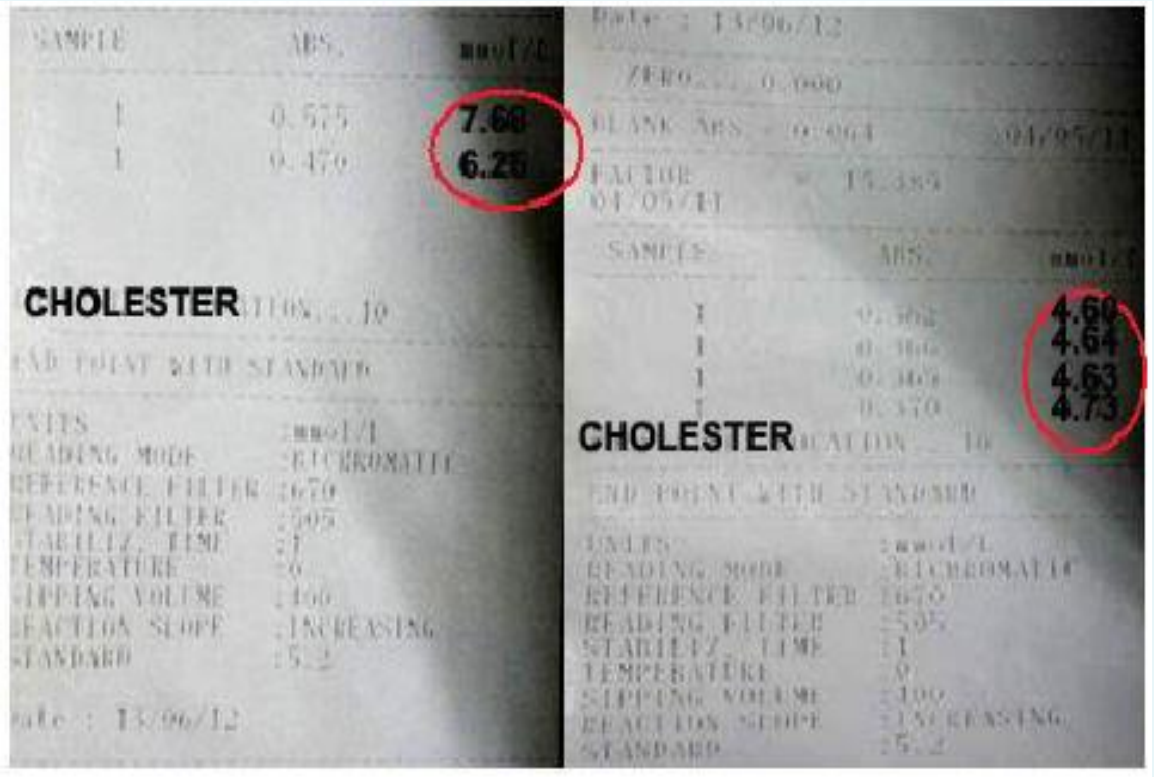
- **Procedure**
  - Pipette sample and reagent into cuvette with the ratio:

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

  - Mix and incubate the tubes for 10 min at room temperature (16-25^{o}C) or for 5 min at 37^{o}C
  - Insert cuvette into the photometer
  - Get the result for conclusion

2.1 Biochemical Test of Human Serum

Cholesterol Oxidase/Peroxidase test



- Standard value: 3-5.2 mmol/l

→ Two first samples had high cholesterol value.

→ 4 normal cholesterol values.

☞ Recommendations were given for the first two patients.

2.1 Biochemical Test of Human Serum

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, checking heart rate, listening to heartbeat, and taking blood pressure
  - Discuss patients' medical history

2.2 Human Immunodeficiency Virus (HIV) test

• HIV is a retrovirus that infects cells of the immune system, destroying or impairing their function.

• Many HIV-positive people do not have symptoms of HIV infection. The most advanced stage of HIV infection is acquired immunodeficiency syndrome (AIDS).

→HIV test → Effective treatments

→ Prevent HIV spread to the community

2.2 Human Immunodeficiency Virus (HIV) test

- HIV-1/2 Antibody Test (Serodia Mix)
- Genscreen HIV – 1/2 version 2
- Rapid HIV-1/2 Test

2.2 Human Immunodeficiency Virus (HIV) test

HIV-1/2 Antibody Test (Serodia Mix)

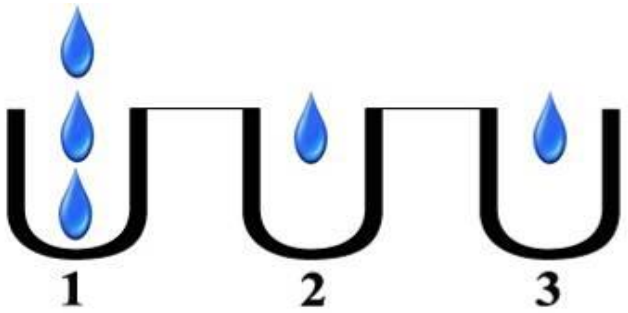
- **Principle**: gelatin particle, sensitized with recombinant HIV-1 antigens and HIV-2 antigen, are agglutinated by the presence of antibodies to HIV-1 and/or HIV-2 in human serum/plasma.
- **Compositions:** Reconstituting Solution **A**; Sample Diluent **B**; Sensitized Particles **C**; Control Particles **D**; Positive control **E**.
- **Preparation:**
  - Centrifuge collected blood to get serum
  - Preparation work: microtiter plate, allow all components of the kit to reach the ambient temperature…

2.2 Human Immunodeficiency Virus (HIV) test

HIV-1/2 Antibody Test (Serodia Mix)

- **Test Procedure**

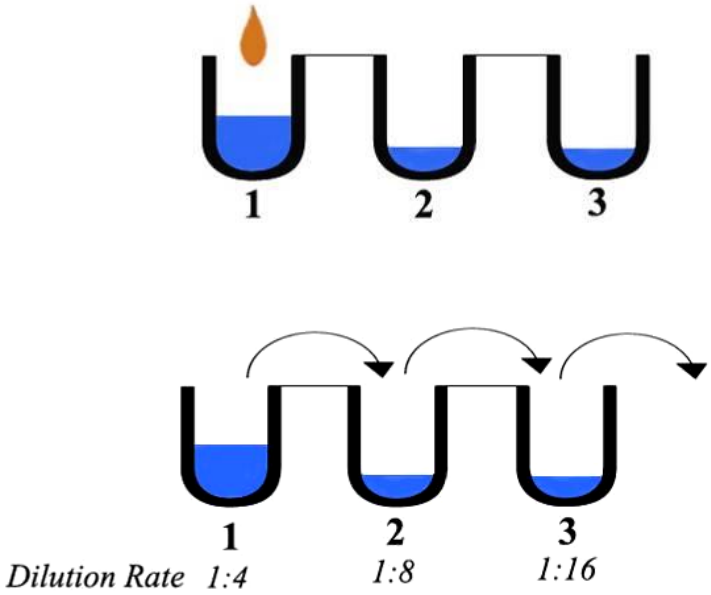
  – 75 µL (3 drops of 25µL) of Sample Diluent (B) in well # 1 of a microplate and 25 µL each (1 drop of 25 µL) into wells # 2 and # 3:



2.2 Human Immunodeficiency Virus (HIV) test

HIV-1/2 Antibody Test (Serodia Mix)

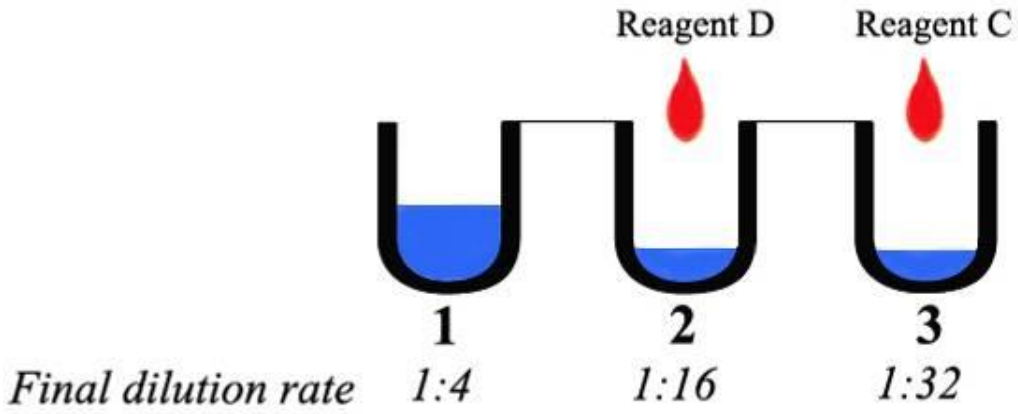
- **Test Procedure**
  - –Add 25 µL of serum/plasma specimen into well #1
  - –Mix the contents of well #1
  - –Transfer 25 µL of the diluted solution from well #1 into well #2
  - –Mix the contents of well #2
  - –Transfer 25 µL of the diluted solution from well #2 into well #3



2.2 Human Immunodeficiency Virus (HIV) test

HIV-1/2 Antibody Test (Serodia Mix)

- **Test Procedure**
  - Place 25 µL (1 drop) of reconstituted Control Particles D in to well # 2. Using the other dropper, place 25 µL (1 drop) of reconstituted Sensitized particles C into well # 3



2.2 Human Immunodeficiency Virus (HIV) test

HIV-1/2 Antibody Test (Serodia Mix)

- **Test Procedure**
  - –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
  - – Incubation at the room temperature (15-30^{o}C) for 2 hours
  - –Read and interpret the patterns

2.2 Human Immunodeficiency Virus (HIV) test

HIV-1/2 Antibody Test (Serodia Mix)

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

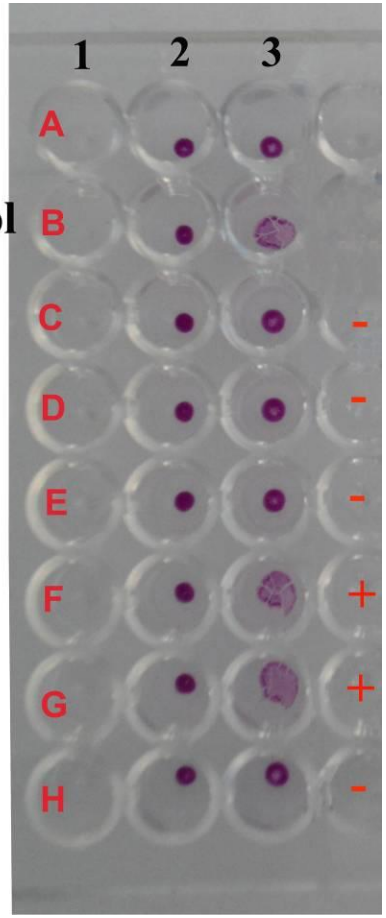
Order	Sample	Result
A	NIHE – HIV 1210 – 01	-
B3	NIHE – HIV 1210 – 01	+
C3	NIHE – HIV 1210 – 01	-
D3	NIHE – HIV 1210 – 01	-
E3	NIHE – HIV 1210 – 01	-
F3	NIHE – HIV 1210 – 01	+
G3	NIHE – HIV 1210 – 01	+
H3	NIHE – HIV 1210 – 01	-

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

Control

Positive Control

Samples



2.2 Human Immunodeficiency Virus (HIV) test

HIV-1/2 Antibody Test (Serodia Mix)

- **Advantage**: easy-to-evaluate kit for detecting HIV related antibodies in serum/plasma specimens.
- **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.
  - 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.

2.2 Human Immunodeficiency Virus (HIV) test

Genscreen HIV – 1/2 version 2

- **Principle**: based upon the use of a solid phase coated with purified antigens and of an antigens - peroxidase conjugate.

- **Compositions:** Microplate **R1**, Concentrated Washing Solution (20X) **R2**, Negative Control **R3**, Cuff – off Control **R4**, Positive Control **R5**, Sample Diluent **R6**, Conjugate **R7a**, Conjugate Diluent **R7b**, Peroxidase Substrate Buffer **R8**, Chromogen **R9**, Stopping Solution **R10**.

2.2 Human Immunodeficiency Virus (HIV) test

Genscreen HIV – 1/2 version 2

- **Procedure:**

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).

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

2.2 Human Immunodeficiency Virus (HIV) test

Genscreen HIV – 1/2 version 2

- **Procedure:**

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

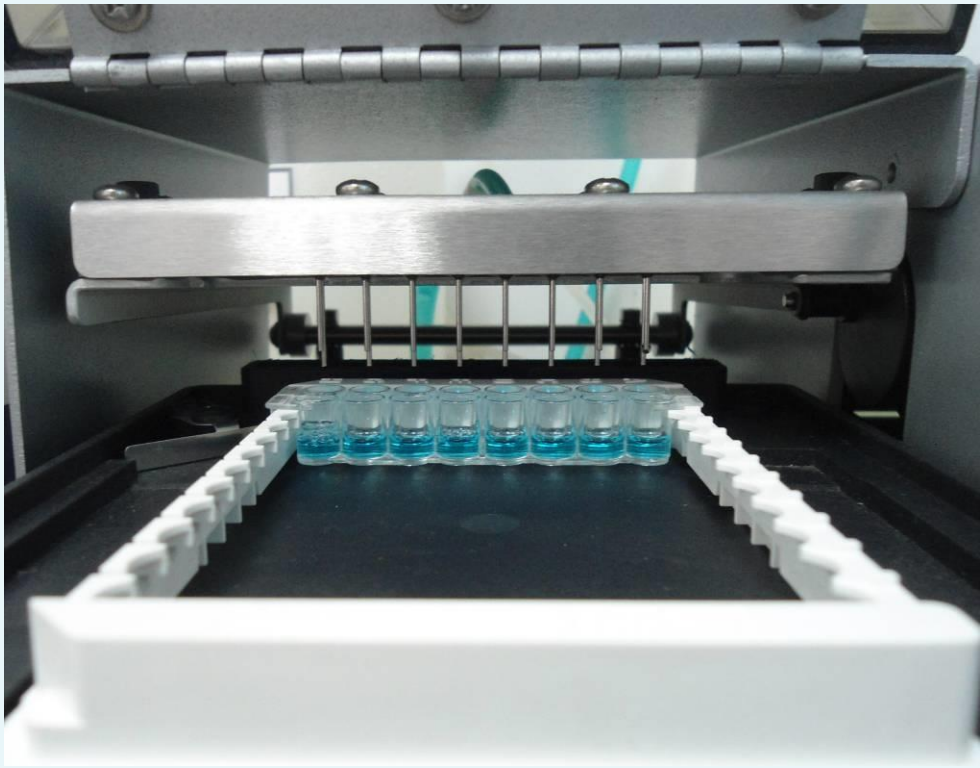
6. Incubate at 37°C ± 1°C, 1 hour ± 5 min

7. Wash x5 times with 1X Washing Solution

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

9. Incubate at 37°C ± 1°C, 1 hour ± 5 min

10. Wash x5 times with 1X Washing Solution



2.2 Human Immunodeficiency Virus (HIV) test

Genscreen HIV – 1/2 version 2

- **Procedure:**

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

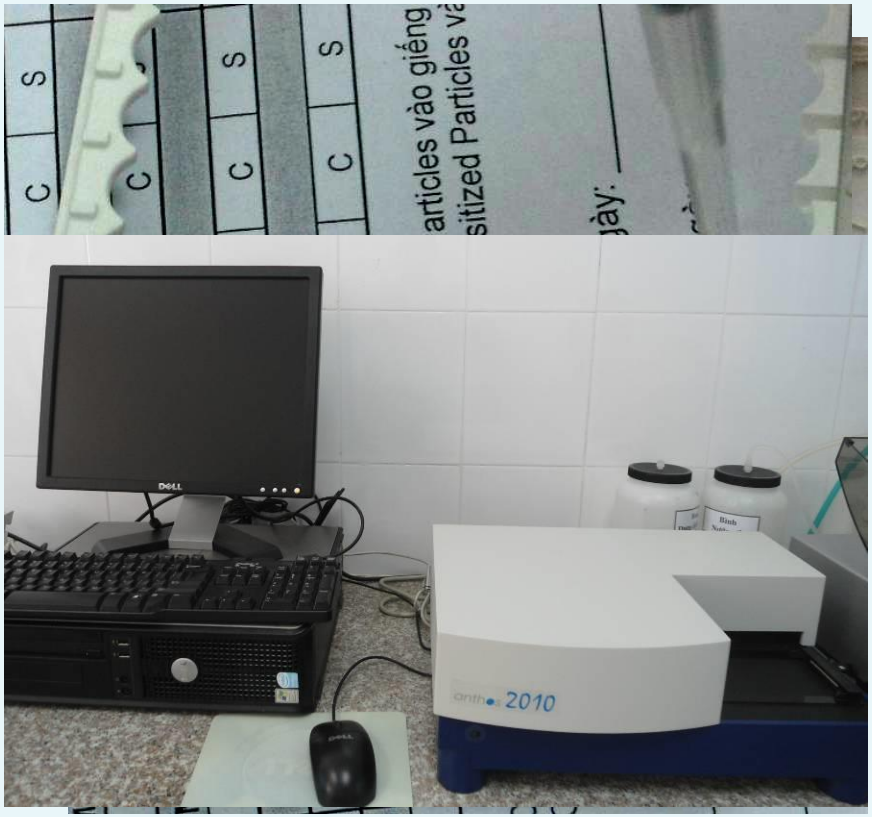
12. Add 80 µL of development solution

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



2.2 Human Immunodeficiency Virus (HIV) test

Genscreen HIV – 1/2 version 2

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

2.2 Human Immunodeficiency Virus (HIV) test

Genscreen HIV – 1/2 version 2

• **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.

2.2 Human Immunodeficiency Virus (HIV) test

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

- **Principle**: 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.

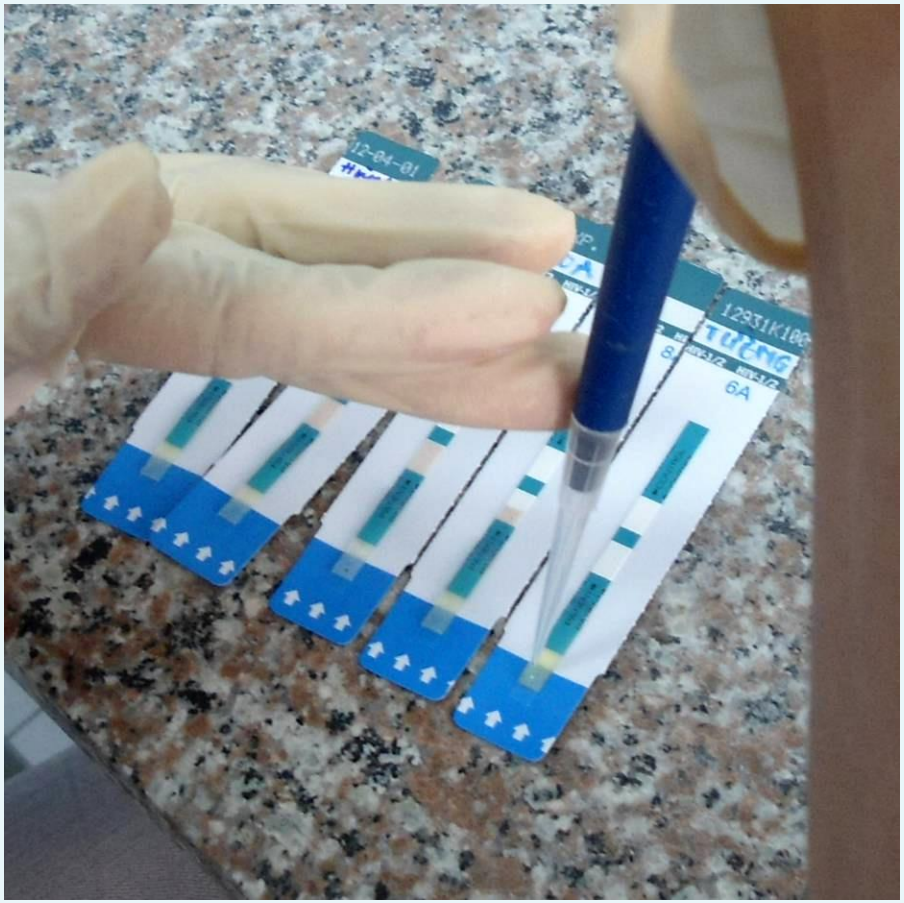
2.2 Human Immunodeficiency Virus (HIV) test

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

- **Procedure**:

- Transfer 2 drops of serum, plasma or venipuncture whole blood 50 µL of sample to the specimen pad of the test strip

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



2.2 Human Immunodeficiency Virus (HIV) test

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

→ Negative

2.2 Human Immunodeficiency Virus (HIV) test

Dertermine HIV – 1/2 version 2

- **Advantages:**
  - Easy-to-use, rapid (15-min) test for HIV antibodies
  - Using a small amount of whole blood, serum or plasma
  - Highly accurate results
- **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.

2.2 Human Immunodeficiency Virus (HIV) test

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

No	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)

2.2 Human Immunodeficiency Virus (HIV) test

- There is a variety of HIV tests 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.

2.3 Rapid Hepatitis B, C Test

2.3 Rapid Hepatitis B, C Test

Introduction

- **Hepatitis B:**
  - Symptoms: most of HBV infected people have no symptoms.
  - Diagnosis: blood test; In the case of liver damage, some other ways, especially liver biopsy.
- **Hepatitis C:**
  - Symptoms: 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.

→ **Using the rapid test for hepatitis B, C**

2.3 Rapid Hepatitis B, C Test

Introduction

• **Antigens** (HBsAg test) are markers made by bacteria or viruses. So the presence of HBV antigens means that the virus is in the body.

→ **Rapid test base on the principle**: The membrane is pre-coated with HBsAg on the test line region of the strip. During testing, the whole blood, serum or plasma specimen reacts with the particle coated with HBsAg. The mixture migrates upward on the membrane chromatographically by capillary action to react with HBsAg on the membrane and generate a colored line.

2.3 Rapid Hepatitis B, C Test

Introduction

• **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.

→ **The principle of the Rapid test** is the same with that of HBsAg test, the difference is HBsAg antigens that is replaced by anti-HBsAg antibodies.

2.3 Rapid Hepatitis B, C Test

Introduction

Status	HBV test		HCV test
	HBsAg	HBsAb	
Infected status	+ive	-	+ive
Healthy status	-ive	+/-ive	-ive

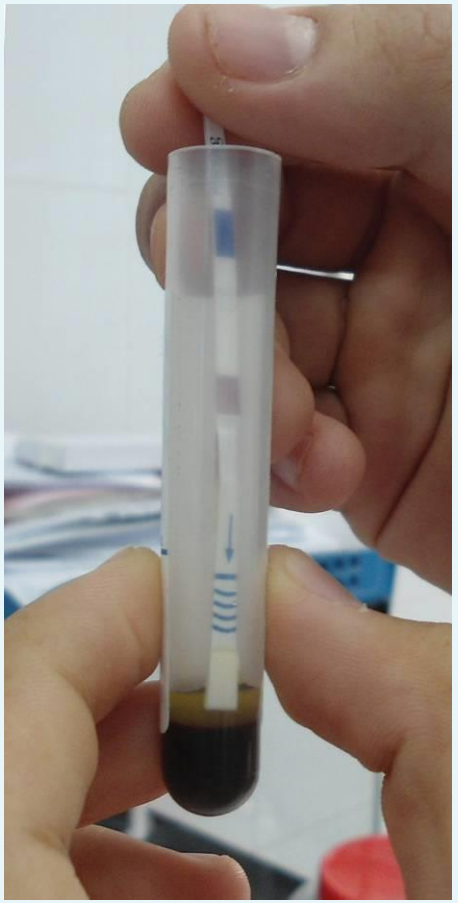
("+ive" means positive; "-ive" means negative)

2.3 Rapid Hepatitis B, C Test

- **Procedure:**

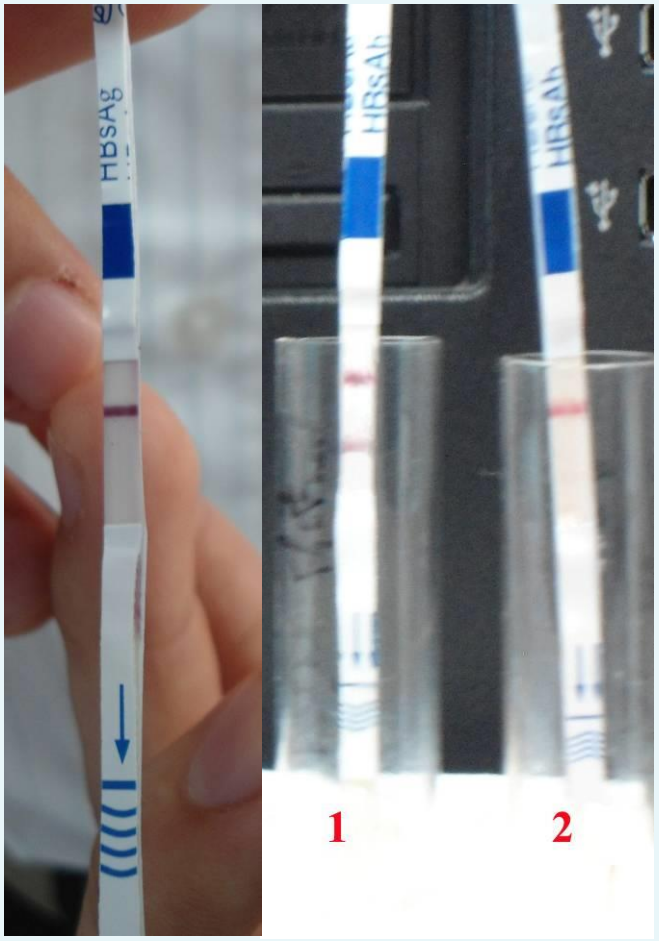
  – Dip directly the sample pad to serum (supernatant after centrifuge)

  – Wait a minimum of 15 minutes and read result on worksheet



2.3 Rapid Hepatitis B, C Test

- **Result:**
  - HBsAg: negative
  - HBsAb:
    - +1: positive
    - +2: negative



2.3 Rapid Hepatitis B, C Test

- **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 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.

2.3 Rapid Hepatitis B, C Test

- 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.

2.4 Testing of Microbes in Human gut flora

2.4 Testing of Microbes in Human gut flora

Cantho Import – Export Fisheries Limited Company (CATFISH)

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

☞ ***The objective of this testing is detect whether pathogenic microorganisms present in workers or not.***

2.4 Testing of Microbes in Human gut flora

- **Preparations:**
  - –MacConkey agar (MAC): use to isolate and differentiate enterics based on their ability to ferment lactose.
    - •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 gm/lit.
      - –Neutral Red: 0.04 gm/lit.
      - –pH at 25˚C : 7.4 ± 0.2
      - –Storage: 8-25˚C

2.4 Testing of Microbes in Human gut flora

- **Preparations:**
  - –Cary-Blair Transport Medium: This medium has a low oxidation/reduction potential, which assures bacterial survival for long periods of time.
    - •Components:

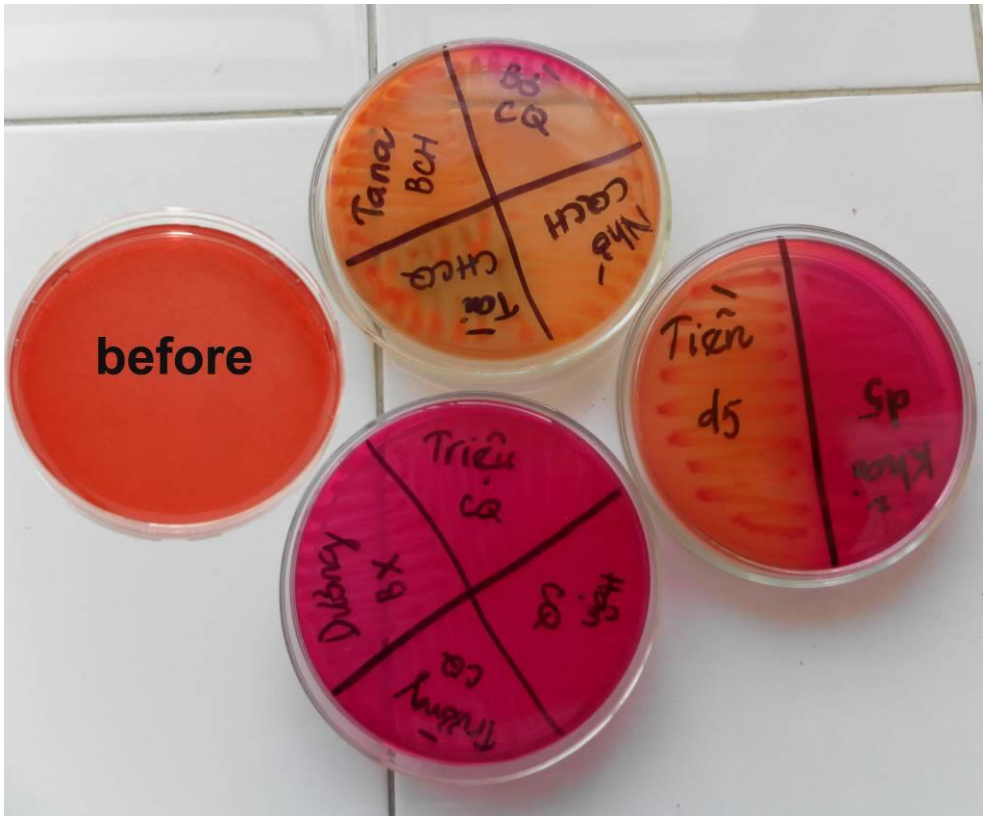
–Sodium Chloride	5.00 g/l
–Disodium Phosphate	1.10 g/l
–Sodium Thioglycollate	1.50 g/l
–Calcium Chloride	0.09 g/l
–Agar	5.50 g/l
–Final pH	8.4 (25ºC)

2.4 Testing of Microbes in Human gut flora

- **Procedure:**
  - Collect fecal sample
  - 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
  - After transferring the sample into prepared MacConKey medium, inoculate at 37^{o}C for 24h
  - Observe the changes of color of the medium

2.4 Testing of Microbes in Human gut flora

Results



- There were many color changes in all collected samples with different color after incubation.
- All of the samples contained microbes that can ferment lactose and changes pH of the medium to make the changes of color (colorless).
- The pink color was generated when lactose had been converted into maltose.

2.4 Testing of Microbes in Human gut flora

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)

2.4 Testing of Microbes in Human gut flora

- 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 (Table 8).
- Two workers did not send back the vial containing transport medium (Cary-Blair medium) (Table 8).

→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.

2.4 Testing of Microbes in Human gut flora

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%

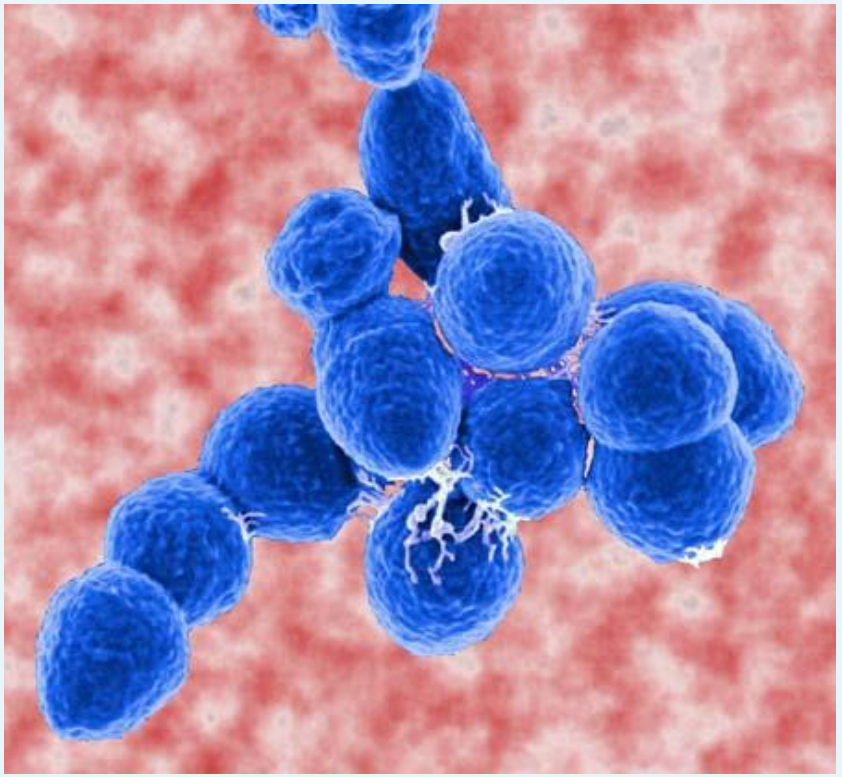
(195 in total)

2.5 *Streptococcus* sp. test

2.5 *Streptococcus* sp. test

Introduction

- Streptococci are Gram-positive, facultatively anaerobic, nonmotile, catalase - negative cocci without forming spore.
- Cause muscle aches, nausea, and fatigue … after consumption.



***Streptococcus* sp.**

(http://sitemaker.umich.edu/mc13/bacterial_meningitis_causative_organism)

2.5 *Streptococcus* sp. test

Introduction

- **Diagnostic Testing:**
  - Throat culture, haemolysis features, the molecule methods.
  - Analytical methods for subtyping include phenotypic sub-typing (biogrouping, serotyping, phage typing, esterase typing) and genotypic subtyping.
  - Rapid DNA hybridization…

☞ ***Used simple analytical methods base on the haemolysis characteristics***

Table 8. 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)*

2.5 *Streptococcus* sp. test

Procedure

- **Sample collection**: pus, blood…
  - Spread on the blood agar plate
  - Incubate at 37^{o}C for 24 hours
  - Observe the morphology of colony in the plate and under the microscope
- **Identification**:
  - Gram staining (Gram-positive (blue/violet))
  - Catalase test: negative

2.5 *Streptococcus* sp. test

Procedure

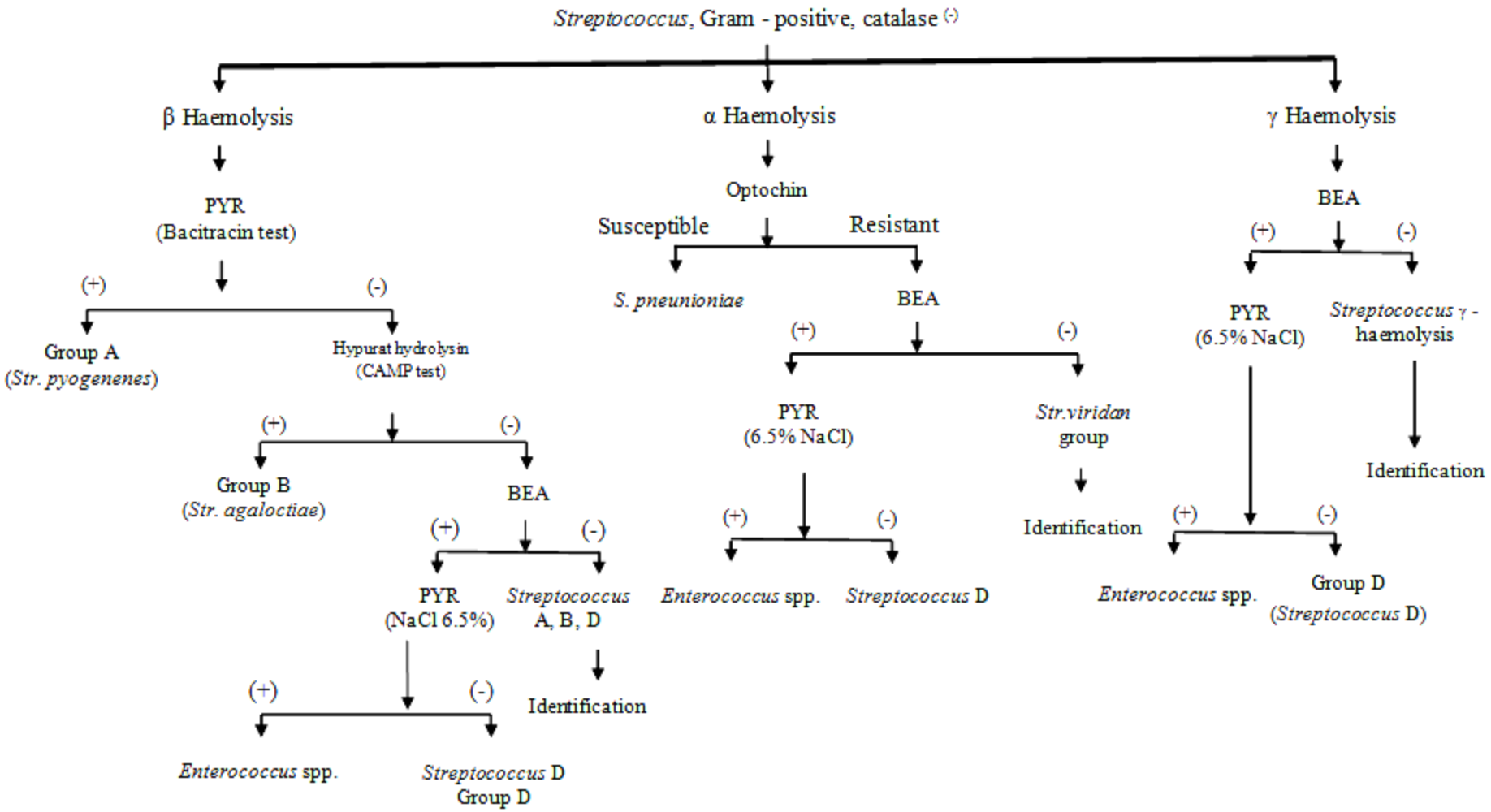
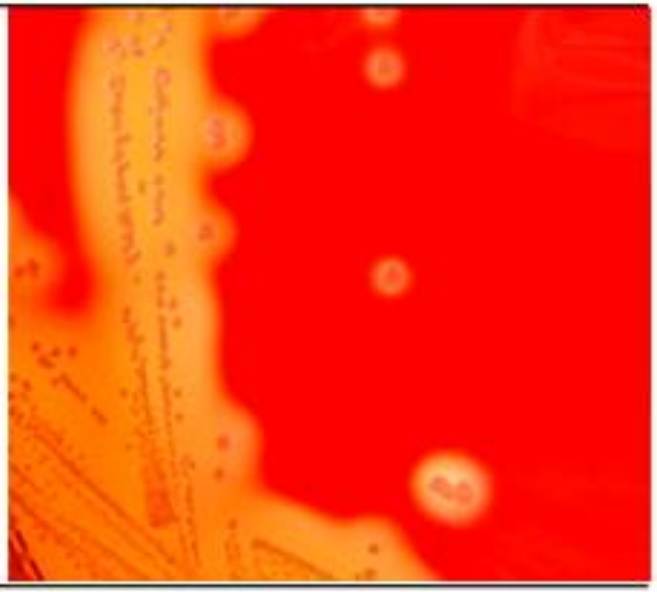


Diagram of *Streptococcus* sp. detection

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*

2.5 *Streptococcus* sp. test

Discussion

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

- **Disadvantages:** this procedure includes many steps (incubation, Gram staining, catalase test…); therefore, it takes a long time to detect.

2.5 *Streptococcus* sp. test

Discussion

- 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.

2.5 *Streptococcus* sp. test

Discussion

- 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.
  - *Streptococcus* sp. test 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.

2.6 Rapid food test kits

- Pesticides Test Kit (Phosphate and Carbamate Group)
- Formaldehyde test
- Quick coloring agents test
- Boric acid test

2.6 Rapid food test kits

Pesticides Test Kit (Phosphate and Carbamate Group)

- **Organophosphate** and **Carbamate** (insecticides, fungicides and herbicides) are used to inhibit the cholinesterase enzyme (nerve function) to control a wide range of landscape insect pests.
- **Health effects:**
  - Nervous system, mimicking hormones causing reproductive problems and causing cancer
  - Birth defects, fetal death, and neuro developmental disorder
- **Detection**: a photothermal biosensor or gas chromatograph/nitrogen phosphorus detector.

☞ **Rapid test (VPR10)**

2.6 Rapid food test kits

Pesticides Test Kit (Phosphate and Carbamate Group)

Procedure:

- Spoon chopped food (10g).
- Mix the activating reagent on 10mL of distilled water with the sample on the plastic bag in 3 mins.
- Add all of extract reagent in the tube, close cap vigorously shake, and leave for 2 mins.

2.6 Rapid food test kits

Pesticides Test Kit (Phosphate and Carbamate Group)

Procedure:

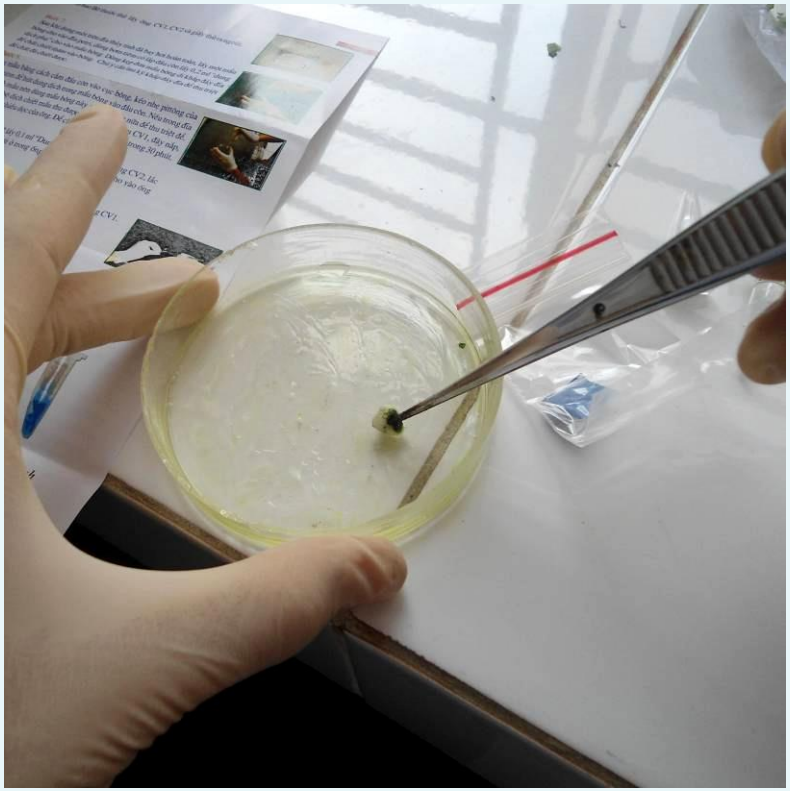
- Collect the solution on the bag and transfer this onto a column in which the solution was divided into 2 separated layers.
- Collect the lower layer of the solution on the column before natural evaporation on Petri disk.

2.6 Rapid food test kits

Pesticides Test Kit (Phosphate and Carbamate Group)

Procedure:

- Collect the remained components on the surface of the disk by a piece of silk-cotton.
- Transfer the collected solution into CV1 column, mix carefully and wait 30 mins.
- Dissolve all the components on CV2 column by using 0.1 mL mixing solution. Then, transfer CV2 into CV1 column.
- A colour indicator (colour paper) was added into CV1 column.
- Read the result after 5 minutes.



2.6 Rapid food test kits

Pesticides Test Kit (Phosphate and Carbamate Group)

Result:

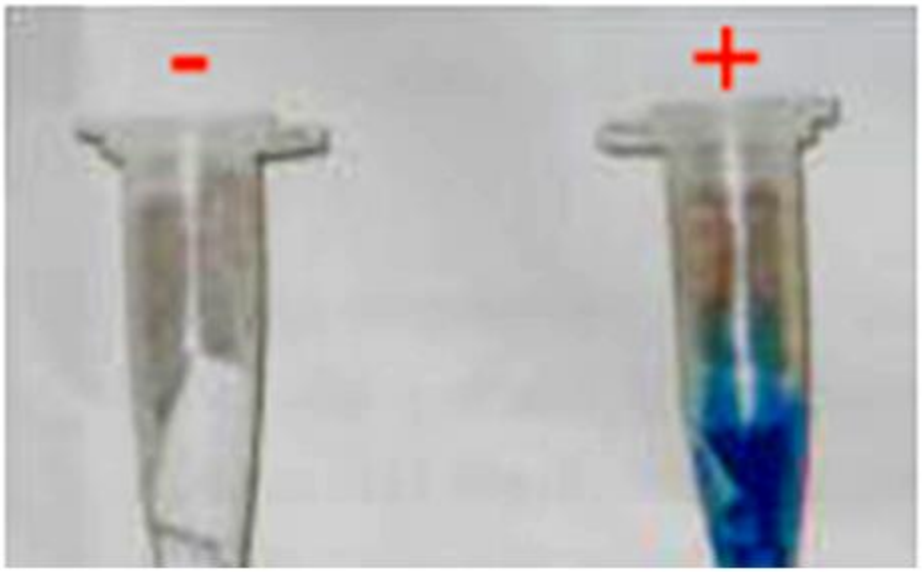


Figure 26. Result interpretation of pesticides test

☞ *Two samples (green bean and salad) had high levels of pesticides.*

2.6 Rapid food test kits

Pesticides Test Kit (Phosphate and Carbamate Group)

- **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:** because of its toxicity of used chemicals, if disinfectant contacts your skin, wash out with clean water.

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2.6 Rapid food test kits

Formaldehyde test

- **Formaldehyde** is used to kill bacteria.
- **Health effects:** In human, formaldehyde is toxic, allergenic, carcinogenic and other symptoms.
- **Detection**: miniaturised electrophoretic method, high-performance liquid chromatography.

☞ **Rapid test (FT04)**

2.6 Rapid food test kits

Formaldehyde test

Procedure:

- Open the bag, mix the chopped sample with 3mL distilled water.
- Crush the colored ampoule, shake carefully to make the solution on bag become yellow.
- Crush the non-colored ampoule, shake carefully and observe the color change of the solution.
- Read the result.

Figure 33. One kind of sea fishes

2.6 Rapid food test kits

Formaldehyde test

- As observation, the color of test changed to reddish-orange.

→ **Positive** with formaldehyde

Formaldehyde test:

- **Advantages:** save time, reliable result.
- **Disadvantage:** the result may be affected by the color of original sample.

2.6 Rapid food test kits

Quick coloring agents test

- **Coloring agents** is used for the purpose of making processed foods look more appetizing and improvements of appearance.
- **Health effects:** allergic response, carcinogenic potential, hyperactivity.
- **Detection**: HPLC or HPLC combination with multiple scales method.

☞ **Rapid test (CT04)**

2.6 Rapid food test kits

Quick coloring agents test

Procedure:

- Collect the chopped/liquid sample into the bag CT04 and shake slightly in 2 mins.
- Transfer the solution on CT2 column into the bag, shake slightly in 2 mins.
- 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 min to read the result.

2.6 Rapid food test kits

Quick coloring agents test

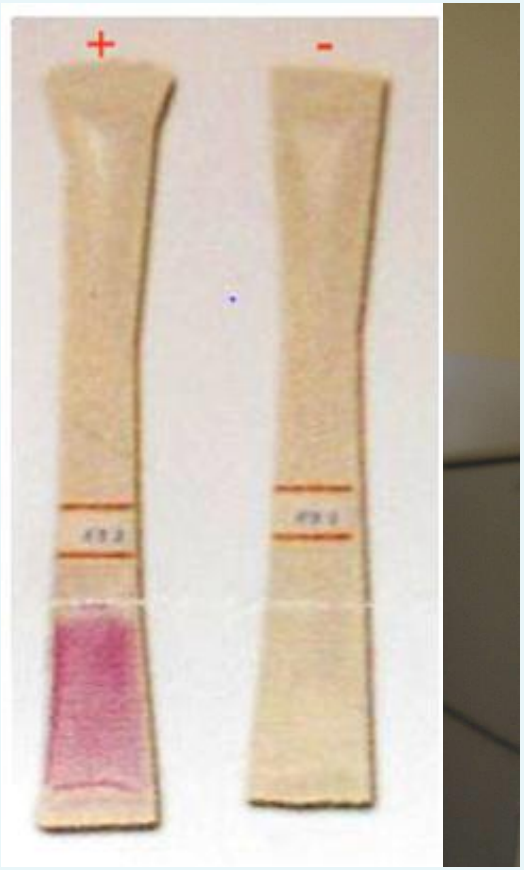
Result:

- Negative

→ This product contents no or contents but lower minimum amount of coloring agent that the kit can recognize. The original color might affect to the final result.

Discussion:

- **Advantages**: short time (1-3 min(s)), reliable result.
- **Disadvantage**: the result may be affected by the color of original sample.



2.6 Rapid food test kits

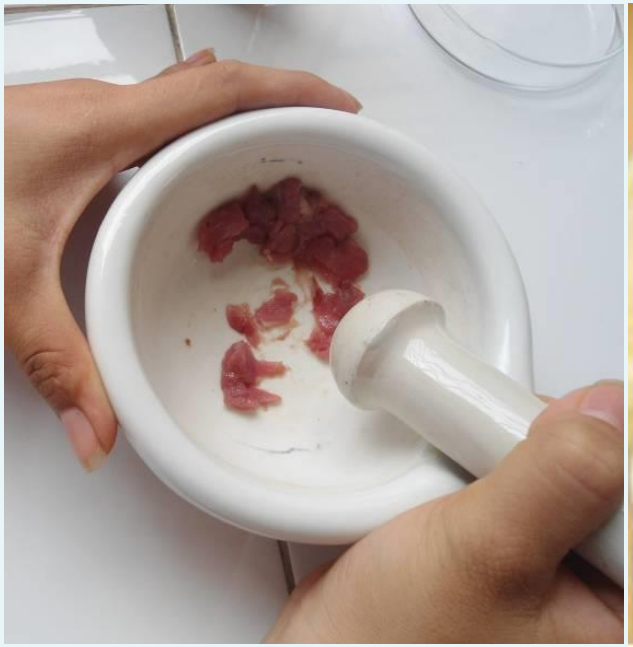
Boric acid test

- **Boric acid:** effects in nervous systems of insects, fungal growth inhibition by preventing the production of reproductive spores, interrupting the plant's photosynthetic pathway.
- **Health effects:** nausea, vomiting, abdominal pain diarrhea and leading to cancer, unfertile...
- **Detection**: HPLC, gas chromatography-mass spectrometry or inductively coupled plasma mass spectrometry method.

☞ **Rapid test (BK04)**

2.6 Rapid food test kits

Boric acid test



Samples: meat and preserved mustard cabbage

2.6 Rapid food test kits

Boric acid test

Procedure:

- Mix 10 grams of chopped food with 20 drops of buffer. The mixture was mashed to get 1 mL of solution
- Continue to add 20 drops of buffer.
- Add 1 drop of buffer into control part of the strip test (at the middle of the strip test).
- 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.

2.6 Rapid food test kits

Boric acid test

- **Result:**
  - **Positive: mustard cabbage**
  - **Negative: meat**

- **Discussion:**
  - **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.

Figure 42. Results of boric acid test

2.7 Laboratory Safety Requirements

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.

3. Conclusions and Suggestions

3. Conclusions and Suggestions

1. Conclusions

- Review what we learned in class and obtained some new information and many processes.
- Depend on the conditions of laboratory to use suitable methods.
- Follow the laboratory safety requirements.
- Work in discipline of army.

3. Conclusions and Suggestions

2. Suggestions

- Because of economic aspects, comparison the results from different labs has had some limitations.
- We should make a contact before time selecting for practice to choose practical time with more effective and useful things (conference with foreign experts).
- Practical time should be more flexible than consecutive 6 weeks.
- Increasing in the number of introduced company and institute with various aspects in biotechnology is essential.

Thanks for your attention!

REFERENCES

http://faculty.ccbcmd.edu/courses/bio141/labmanua/lab14/abg_asm.html *(Sept 23rd, 2012)*

http://sitemaker.umich.edu/mc13/bacterial_meningitis_causative_organism *(Aug 05th, 2012)*