NATIONAL
LAB-BASED SURVEILLANCE SYSTEM

for

INFECTIOUS DISEASES
IN MALAYSIA

Ministry of Health
Malaysia

FIELD GUIDELINES FOR LABORATORY-BASED SURVEILLANCE

1. *Salmonella spp.*
2. *Salmonella typhi & paratyphi*
3. *Haemophilus influenzae*
4. *Neiserria meningitidis*

Coordinated by:

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Ministry of Health
MALAYSIA

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ACKNOWLEDGMENT

The Editorial Board would like to express sincere gratitude to these officers for their contribution in producing the guidelines in 2003 / 2004.

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This manual is a guide for implementation of
national laboratory-based surveillance for infectious diseases.

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1.0 INTRODUCTION

The National Laboratory-based Surveillance workshop was organized by Disease Control Division, MOH, on 1 – 4 July 2002, to discuss the implementation of pathogens surveillance system. In the workshop, an emphasis was placed on what reports from laboratories could add value to surveillance, control and prevention of diseases. A prioritizing process was developed that was a modification of the Canadian process to give priority to diseases under surveillance.

Surveillance by laboratory reports could provide valuable additions to current systems by either expanding the diseases under surveillance or by improving the quality and use of existing notification/surveillance data. The group developed a final short list of initial organisms to include in surveillance, although these are not ranked (Appendix 1).

2.0 OBJECTIVES OF LABORATORY-BASED SURVEILLANCE

2.1 To strengthen the existing laboratory-based surveillance.
2.2 To determine the baseline and monitor the circulating pathogenic agents in the country.
2.3 To detect emerging pathogen strains.
2.4 To detect impending outbreaks and outbreaks of infectious disease.
2.5 To determine clonal strain identification by sero-typing, phage typing and molecular methods (PCR, PFGE, etc.)

3.0 METHODOLOGY

3.1 Selection of Hospital Laboratories

All diagnostic microbiological laboratories (government including universities and private) that have culture facilities.

3.2 Isolates

1. All isolates from human specimens. For *Haemophilus influenzae* the surveillance includes all isolates (both type b and non-type b) from invasive infections (isolates from cerebrospinal fluid and blood) only.

3.3 Procedure for Laboratory-based surveillance

This procedure to be used by all microbiology laboratories of public and private hospitals.

3.3.1 Reference laboratory:

1. **Institute for Medical Research.**

2. **Public Health Laboratory, Ipoh** (For hospitals in Perlis, Perak, Kedah, Pulau Pinang to send ***Salmonella spp*** **isolates to PHL Ipoh).**

3. Identified laboratories either regional or national of non-MOH (to be decide later).

3.3.2 Responsibility:

The person in charge of hospital microbiology laboratory is responsible for notifying the Surveillance Section of Disease Control Division MOH of any positive result, using the notification form (*Labsurv/1.2005 Pind 1*). At the same time, the isolates should be sent together with a copy of the notification form to the reference laboratory for typing.

The reference laboratory is responsible for typing the isolates and reporting the result to the Surveillance Section, MOH as soon as possible.

The Surveillance Section is responsible for collating and analyzing the data on a weekly basis and to produce a report to be sent to all relevant departments / institutions at regular interval.

3.3.3 Procedure:

1. The microbiology laboratory:

a) Processes all the clinical samples for bacteriological investigation using the standard protocols.

b) Identifies all significant isolates to genus/species level depending on the capability of the laboratory.

c) Notifies the Surveillance Section once the strain is identified using the laboratory-based surveillance notification form (*Labsurv/1.2005*).

d) Makes a copy of the above form and sends it to the reference laboratory together with the isolate on the same day as the notification is made to Surveillance Section. The cultures should be packaged according to the standard guidelines (Appendix 2).

2. The reference laboratory:

a) Performs the typing on the isolate and reports to the Surveillance Section, MOH and the requesting hospital laboratory.

b) Perform further tests e.g. pulse field gel electrophoresis (PFGE) in the event of an outbreak

3. Surveillance Section:

a) Analyses the data and produces a weekly report.

b) Initiate an investigation upon suspicion of an outbreak, if needed.

3.3.4 Related documents:

a. Laboratory SOP (appendix 3)

b. Laboratory-based surveillance notification form (*Labsurv/1.2005 Pind 1*) (appendix 4)

3.4. NOTIFICATION OF RESULT

3.4.1 All positive results for isolates should be notified, by email or through fax to Surveillance Section MOH using the Laboratory Notification Form (*Labsur/1.2005 Pind 1*).

Fax no: 03-8888 6271
e-mail: survelan@dph.gov.my

All isolates should be sent to IMR or reference laboratories as soon as possible for further serotyping and phage typing. These laboratories will notify Surveillance Section as soon as possible when the serotyping and the phage typing results are available. This process is summarized in Appendix 5.

3.4.2 For hospitals with web-based notification shall use the system. These hospitals are:

Hospital Sultanah Aminah, JB
Hospital Queen Elizabeth, KK, Sabah
Hospital Umum Kuching, Sarawak
Hospital Pulau Pinang.
Hospital Kuala Terengganu.

The variable for notification is as in the notification form (*Labsur/1.2005 Pind 1*).

3.4.3 For Hospital Kuala Lumpur and Hospital Kota Bharu notification is done through Laboratory Information System (LIS). Whatever information in the system is accepted and will be used for analysis.

4.0 SURVEILLANCE SYSTEM INDICATORS

4.1 Process indicators

a) Percentage of isolate positive notified to the system and sent to IMR for serotyping (target: 100 %)
b) All positive isolates and serotyping are notified (target: 100 %)

4.2. Surveillance quality indicators

a) Number of outbreaks and impending outbreaks detected through this system.
b) Report produce regularly every quarter of the year (target: 100%)

5.0 DATA ANALYSIS

Data will be analyzed by using Statistical Analysis software on a weekly basis to see the

a. Trend of isolates according to their serotypes.
b. Demographic and geographic distribution of isolates.

Feedback is a crucial component of any surveillance system. This will reinforce the laboratory staff commitment to participate in the surveillance system and also creates an awareness of disease prevention and control.

Feedback in the pathogens laboratory-based surveillance system will be through weekly bulletins.

6.0. CONTACT INFORMATION

Surveillance Section **Disease Control Division** **Level 4, Block E10, Presint 1** **PUTRAJAYA** Tel:03-8883 4278 Fax: 03-8888 5271 E-mail: survelan@dph.gov.my	**Bacteriology Unit** **Institute for Medical Research** **Jalan Pahang** **50588 KUALA LUMPUR** Tel: 03-26986033 Fax: 03-26924949 E-mail: rohani@imr.gov.my

National Public Health Laboratory **Lot 1853 Kg Melayu Sungai Buluh** **43000 Sungai Buluh** **SELANGOR** Tel: 03-51565109 Fax: 03-61402249 E-mail: mkak1@tm.net.my	**Regional Public Health Laboratory** **Jalan Jelapang** **30020 Ipoh** **PERAK** Tel: 05-5287829 Fax: 05-5287836 E-mail: normahmkai@yahoo.com

Appendix 1

List of organisms for laboratory surveillance as suggested at Lab-based Surveillance Workshop in Langkawi from 1st until 4th July, 2002.

VIRAL

- Poliovirus
- Rabies virus
- JE virus
- Nipah virus
- Herpes simplex virus
- Enterovirus
- Dengue virus
- Yellow Fever virus
- Measles virus
- Hepatitis A, B, C & E virus.
- HIV
- Ebola virus
- Rubella virus
- Mumps virus
- Rotavirus
- Adenovirus 40 & 41
- SSR virus

BACTERIAL

- *Neisseria meningitidis*
- *Salmonella typhi*
- *Leptospira spp*
- *Vibrio cholerae*
- *Legionella spp*
- *E. coli* O157 H7
- *Haemophilus influenzae* type B
- *Salmonella spp*
- *Campylobacter spp*
- *Corynebacterium diptheria*
- *Streptococcus pneumoniae*
- *Neisseria gonorrhoea*
- *Clostridium tetani*
- *Bordetella pertussis*
- *Treponema pallidum*
- *Haemophilus ducreyi*
- *Bacillus anthracis*
- *Yersinia pestis*
- *Francisella tularensis* (tularemia)
- *Pseudomonas pseudomallei* (melioidosis)
- *Rickettsia tsutsugamushi*

PARASITE

- *Toxoplasma gondii*
- *Entamoeba histolytica*

Final list of organisms for initial laboratory surveillance, started in October 2003.

Organism	Reason for inclusion in laboratory surveillance
Haemophilus influenza type b	Not currently notifiable & vaccine has been introduced since 2004.
Salmonella typhi	Added value to notifications system by increasing speed & completeness of typing.
Salmonella sp	Not individually notifiable, completeness & timeliness of typing is needed to identify clusters for epidemiological investigation.
Neisseria meningitides	Not currently notifiable & vaccine type implications.
Vibrio cholerae	Added value. Send for molecular study in the event of outbreak.
*Leptospira**	It is endemic in Malaysia. Monitoring of the common serogroup infected human being is very important.

* It was dropped in May 2004 because the test is done on serum specimen, not the isolate as for other pathogens.

Appendix 2

STANDARD GUIDELINE FOR PACKAGING OF LABORATORY-BASED SURVEILLANCE ON SALMONELLA SPECIES SEROTYPE

Protocol :

Transportation of *Salmonella* species isolates to reference laboratories

1. For isolate from human, sub-culture the isolates on an appropriate media:
   - Salmonellae isolates are sub-cultured in nutrient agar slant, incubated overnight in aerobic condition.
   - *H. influenzae* and *N. meningitidis* isolates are inoculated onto chocolate agar slant (in screw-capped bottle/tubes), incubated overnight in CO_2 and fully top-up with sterile paraffin after growth is obtained.

   Incubate at 35 ^{0}C – 37 ^{0}C for 18 – 24 hours.

2. Label the Bijou bottle with the name of participating laboratory, type of specimen, and date of isolation.

3. Fill in the Laboratory-based Surveillance notification form as required.

4. Wrap the Bijou bottle with a piece of cotton or soft tissue paper and pack in a box.

5. Samples should be packaged in three layers (see diagram in figure 1**):**

PACKAGING PROCEDURES

i. a primary watertight non breakable container containing the sample
   - it must be firmly capped and the cap should then be sealed with parafilm, adhesive cloth or zinc oxide tape (not cellulose tape)
   - the container must then be cocooned in absorbent material
   - several primary containers may be packed in one secondary container

ii. a secondary watertight non breakable container enclosing enough absorptive material between it and the primary container to absorb all of the fluid in the specimen in case of leakage
   - it must be firmly capped and sealed in the same way as the primary container

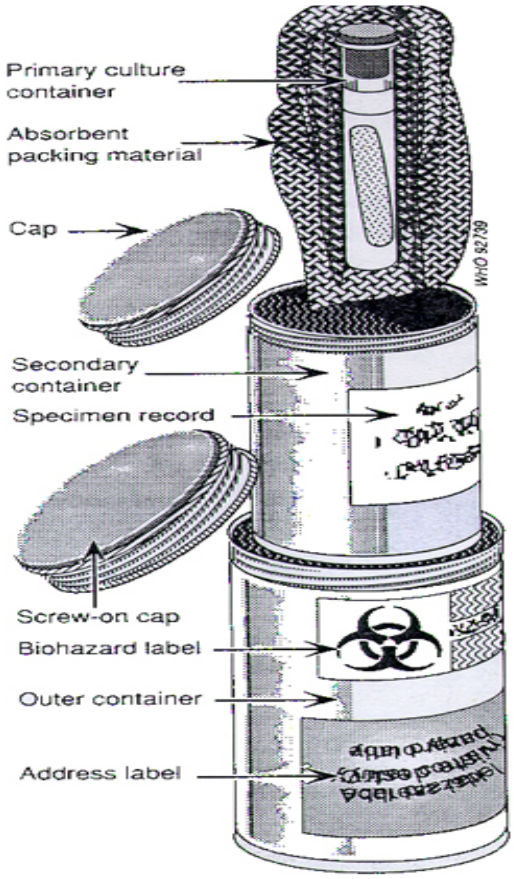
- the secondary container must then be packed firmly with absorbent material into the outer container
- several secondary containers may be packed in one outer container

iii. an outer container which is intended to protect the secondary package from outside influence, such as physical damage and water, during transportation
- absorbent, shockproof packing between the secondary and outer containers
- the lid is again sealed with tape

5. Send the pack of isolates to reference laboratories by ordinary post or by courier service.

PACKING INFECTIOUS SUBSTANCES FOR THE POST

Figure 1: Packing infectious substance for the post



Appendix 3

STANDARD OPERATING PROCEDURE (SOP) FOR LABORATORIES WORK

1.0 Isolation and identification of *Salmonella spp.*

1.1 Isolation

Salmonella grows on MacConkey as pale yellow (non lactose fermenting) colonies, 2-3 mm in diameter, moist, circular and smooth convex surface.

On *Salmonella - Shigella* agar they are colourless with black center. They grow rapidly at temperature of 35 - 37 °C.

1.2 Identification

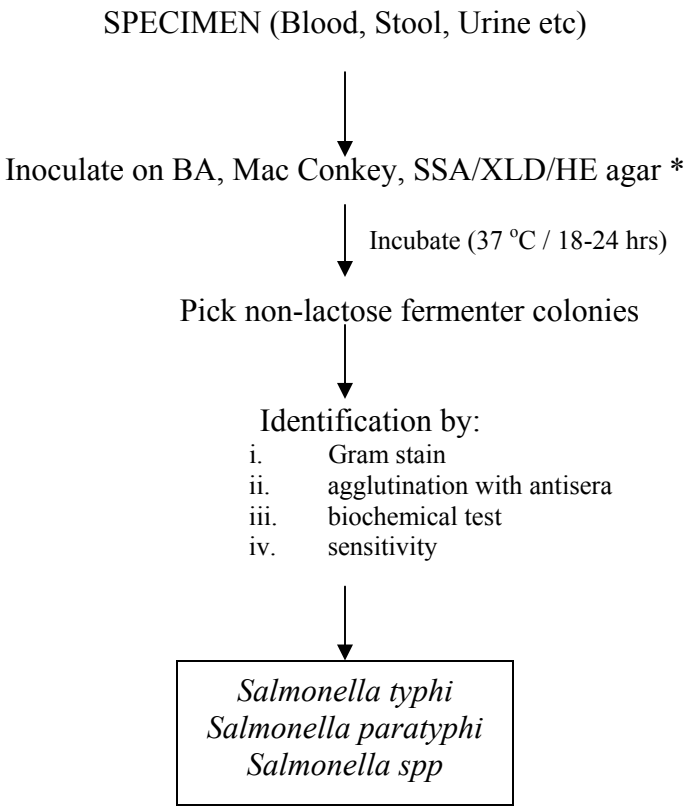
1.2.1 Morphology and biochemical tests

The organisms belonging to genus *Salmonella* are gram negative, non acid fast, non-capsulated and non-sporing bacilli measuring approximately 2-4 µm X 0.6 µm. Almost all species are motile. Catalase is positive and oxidase negative. It reduces nitrate to nitrite.

1.2.2 Slide agglutination test

- Use polyvalent 'Vi', 'O' and polyvalent 'H' antisera against salmonellae.
- Place one drop each of normal saline at 3 different site on a clean glass slide.
- A loopful of suspect colony of *Salmonella* is emulsified in three sites.
- Place one drop of antisera.
- Observe for visible agglutination.
- If positive agglutination for polyvalent 'Vi', 'O' and 'H', it confirm *Salmonella typhi.*
- If positive agglutination for polyvalent 'O' and 'H', it confirm *Salmonella spp.*
- Proceed to biomedical test for confirmation of identification.

1.2.3 Flow chart



* BA – Blood Agar
SSA - Salmonella-Shigella Agar
XLD -
HE - Hektoin Enteric Agar

2.0 Isolation and identification of *Haemophilus influenzae**

2.1 Isolation

The colonies on enriched Chocolate agar is small, 1 - 2 mm in diameter, convex with entire edge, semi opaque, smooth, moist, pearly with a characteristic mousy odour. It grows better in a humid atmosphere with added 5 - 10 % CO_2 at 35 - 37 °C.

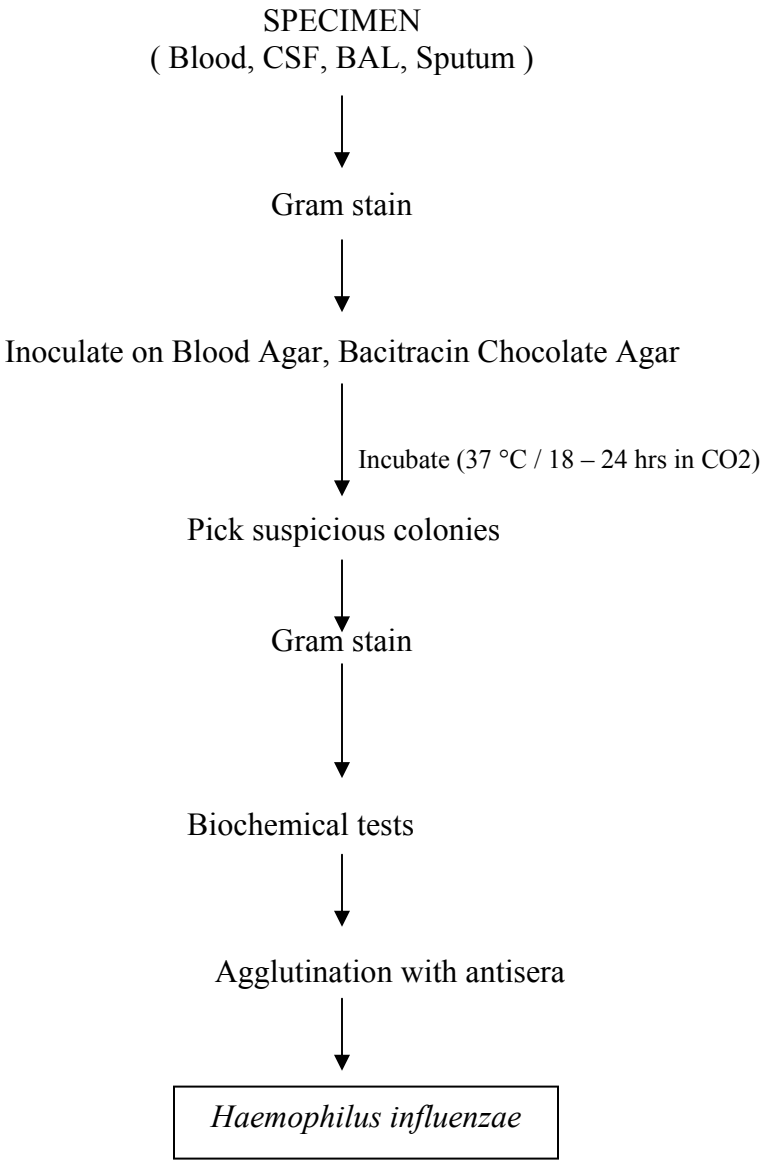
2.2 Identification

2.2.1 Morphology and biochemical tests

The organisms are small, gram negative, coccobacilli to filamentous rods and as the culture ages the become pleomorphic. They are non acid fast, non motile and non

sporing. It ferments glucose but not sucrose or lactose. It requires both 'X' and 'V' factors. On blood agar, they will grow as satellite colonies around a staphylococcal streak.

2.2.2 Processing



2.2.3 Serotyping of *Haemophilus influenzae*

Various techniques are available to serotype *H. influenzae*. One of them is outlined below.

- A coagglutination culture confirmation test (Phadebact *Haemophilus* test) can be use to identify and serotype *H. influenzae* type b.
- This kit contain a vial of staphylococcal cells sensitized with type b antisera (test reagent) and a second vial of staphylococcal cells sensitized with type a, c, d, e and f antisera (control reagent).
- Colonies from the growth media are mixed with each of the 2 reagent on a cardboard slide.
- After mixing, rock slide for 30 - 60 seconds.
- Visible agglutination of the mixture with type b reagent but not control reagent, identifies the isolate as type b.
- A positive reaction in the control reagent indicates that the organism belong to the capsular type a, c, d, e or f.

3.0 Isolation and identification of *Neisseria meningitidis**

3.1 Isolation

The colonies on Chocolate agar is medium to large and mucoid (encapsulated strains). It also grow on selective media such as Modified Thayer Martin agar. It grows in a humid atmosphere with added 5 – 10 % CO_2 at 35 – 37 °C.

3.2 Identification

3.2.1 Morphology and biochemical tests

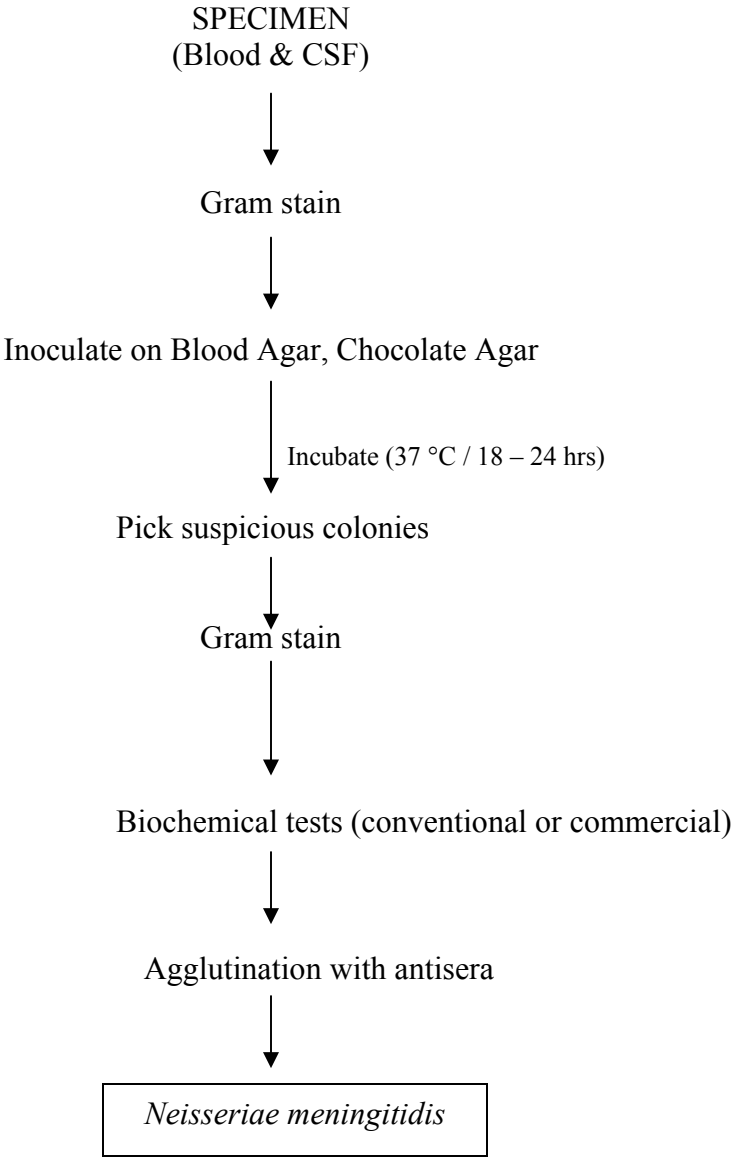
The organisms are gram negative non motile diplococcus approximately 0.8 µm in diameter. Individual cocci are kidney shaped. In direct smears from clinical specimen the organisms are usually found both extracellularly and intracellularly in polymorphonuclear leucocytes.

Positives oxidase reaction. Acid production from glucose and maltose using CTA medium containing these carbohydrates. Do not degrade sucrose or lactose.

3.2.2 Meningococcal grouping

Meningococci are subdivided into serological groups depending on the presence of either capsular or cell wall antigens. Serological identification to differentiate groups A, C, D, X - Z and W135 is done for epidemiological and diagnostic purposes. Agglutination is the most routinely used method and the agglutinating sera can be obtained commercially.

3.2.3 Processing



Note:

***** Please consult appropriate reference laboratories for additional information regarding specific bacterial identification procedures.

LABORATORY-BASED SURVEILLANCE
NOTIFICATION FORM
DISEASE CONTROL DIVISION
MINISTRY OF HEALTH MALAYSIA
TEL: 03-26946601 FAX: 03-26946404
Email: survelan@dph.gov.my

1. Reporting Laboratory: …….. 2. Lab. No: ……
3. Patient Name: ……..
4. Patient's
Address: ……. District:……
5. IC No: ……. 6. RN No: ……
7. Age: …….. yr …… mth 8. Sex: ☐ Male ☐ Female
9. Race: ☐ Malay ☐ Chinese
☐ Indian ☐ Other (please state): ……
10. Case seen at: ……. 12. Warded ☐ Yes ☐ No
13. If warded, which ward ☐ Medical ☐ Paediatric ☐ Surgical ☐ O & G
Others, please state: ……..
14. Specimen type (please tick the relevant box) Date specimen taken
☐ Stool
☐ Blood
☐ Urine
☐ CSF
☐ Sputum
☐ Other (please state):……
15. Source of specimen (please tick the relevant box)
☐ Case
☐ Contact
☐ Food handler
☐ Other (please state): ……
☐ Unknown

16. Isolate	Hospital Laboratories	Reference laboratories
	☐ Salmonella spp	☐ Serotype: …… Chroramphenicol: Sensitive ☐ Resistant ☐
	☐ Salmonella typhi	☐ Phage type Chroramphenicol: Sensitive ☐ Resistant ☐
	☐ Salmonella paratyphi	☐ Serotype: ……
	☐ Haemophilus influenzae	
	☐ Nesseirae meningitides	
	☐ Vibrio cholerae Serotype: …… Biotype: ……...	☐ Vibrio cholerae Serotype: …… Biotype: ……... Tetracycline: Sensitive ☐ Resistant ☐
	☐ Leptospira titre:	Serogroup _____
Reporting Officer	Signature: …… Name: …….. Designation …… Date (1) ……	Signature: …… Name: …….. Designation …… Date (2) ……

All information must be filled.

Note: Send this form to: 1) Surveillance Section, Disease Control Division, MOH through fax, e-mail or web-based notification
2) Reference Laboratory together with the isolate.

EXPLANATORY NOTES
ON FILLING NOTIFICATION FORM
Labsurv/1.2005/pind 1

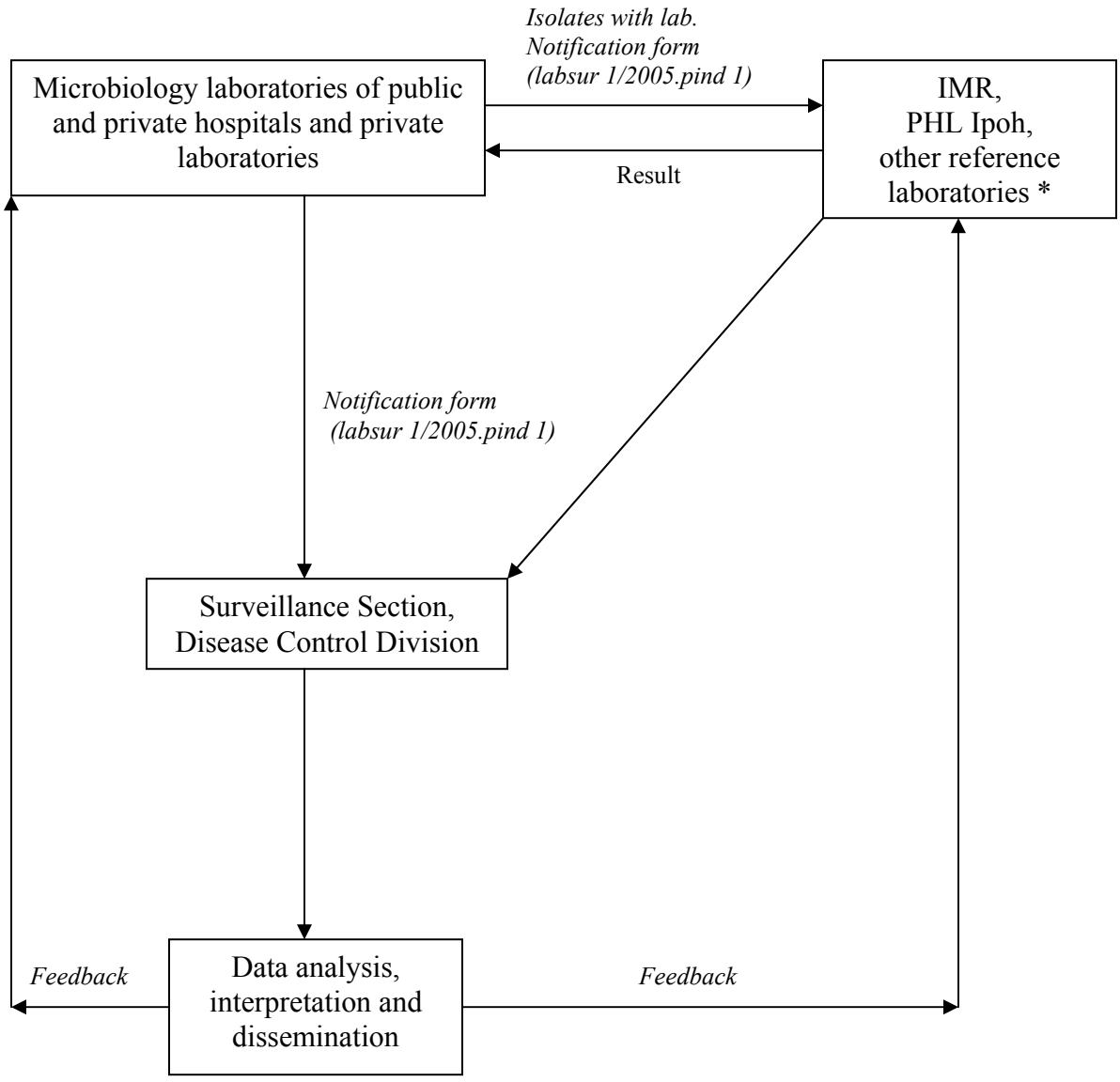
NO	VARIABLE	EXPLANATION
1.	Reporting laboratory	The laboratory that isolate the pathogens.
2.	Lab. No.	The laboratory number of the specimen tested. This is necessary for counter check specimen if the IC or the RN is not available.
3.	Patient's name	As written in request form.
4.	Patient address	As written in request form. Fill at least the district for crude mapping of cases.
5.	I/C no	New identity card number. If not available, please provide the RN number. It is a reference to data puncher when the serotyping result comes back from IMR.
6.	Age, sex and race	As written in request form.
7.	Case seen at	The facility where the patient seek treatment. For active case detection e.g. in cholera or typhoid outbreak or food poisoning, please state the requesting District Health Office.
8.	Warded	Whether patient was treated as outpatient (No) or warded (Yes).
9.	Type of ward	If patient is warded please tick the type of ward he / she is admitted; whether it is medical, paediatric, surgical, gyanecological ward or others (please state).
10.	Specimen type	As written in request form.
11	Date specimen taken	Is **very important** for □eningit, as a clue indicating specimen from an outbreak or isolated cases. It is also used to look at the trend over epid week and predicting outbreak. The best date is the onset date of symptoms; however it is usually not available at the laboratory request form.
12.	Source of specimen	As written in request form. Unknown source if the information is unavailable. **Case** is a person infected with *Salmonella sp* or *Salmonella typhi / paratyphi* or *Haemophilus influenzae* or *Neiserria □eningitides* and having symptom of the disease. **Contact** is a person that has an associated or linked with an infected person that might provide an opportunity to acquire the infective

		pathogen.

NO	VARIABLE	EXPLANATION
13.	Isolate	The pathogens isolated from the clinical specimen and serotyped by the reference laboratories.
14.	Reporting officer	The reporting officer of the hospital laboratories and reference laboratories.
15.	Date (1)	Date of the isolates send to the reference laboratories or entered to the web-based notification system.
16.	Date (1)	Date of the result sends to Disease Control Division, MOH or entered to the web-based notification system.

Appendix 5

The flow of isolates and notification



**** future other reference laboratories.***

Appendix 6

Contact number of laboratories with microbiological test facilities.

HOSPITAL	PHONE NO.	FAX NO.	e-MAIL ADDRESS