KINGDOM OF CAMBODIA

NATION RELIGION KING

MINISTRY OF HEALTH

Standard Operation Procedure

For

Cambodia Laboratory-based AMR Surveillance System

November 2017

Preface

Antimicrobial Resistance (AMR) is a growing global public health concern. It needs to be addressed by a multi-sectoral, multi-disciplinary approach called 'One Health AMR Approach'. Cambodian Ministry of Health (MOH) and Ministry of Agriculture, Forestry and Fisheries (MAFF) and other relevantministries and agencies developed the National Policies and Strategic Plans to combat AMR. One of the core objectives of the policies and strategic plan is to strengthen the AMR surveillance in Cambodia.

Currently, Cambodia has 17 government and partner laboratories that can perform microbiology, Initial Identification (ID) and Anti-microbial Susceptibility Testing (AST). Cambodia also registered with the Global AMR Surveillance System (GLASS). A Protocol for Cambodia Laboratory-Based AMR Surveillance System was drafted and preceded for MOH endorsement.

Eight sentinel sites were selected to start the AMR surveillance. The system will be integrated with the Cambodia Laboratory Information System (CamLIS) and the CCDC website.

The AMR surveillance data will be very useful to guide the development of the National Treatment Protocol for bacterial infections to support the clinicians to select the appropriate antibiotics and to strengthen the rational use of the medicine at the health facilities. This data is expected to share with the international community, especially the GLASS every year.

The protocol was jointly developed by the Ministry of Health and its partners. The Ministry of Health would like to highly appreciate the technical and financial support of the US- CDC and KOICA, WHO, FAO, Pasteur Institute, DMDP, NAMRU-II, AHC-University of Oxford.

Phnom Penh, 6th November, 2017

Prof. ENG HUOT
SECRETARY OF STATE

Table of Contents

List of Abbreviations

AET	Applied Epidemiology Training
AMR	Antimicrobial Resistance
AST	Antibiotic Sensitivity Test
CAMLIS	Cambodia Laboratory Information System
CCDC	Cambodia Communicable Diseases Control
CAI	Community-associated infections
CRF	Case Report Form
CSF	Cerebral Spinal Fluid
DMDP	Diagnostic Microbiology Development Program
GLASS	Global Antimicrobial Resistance Surveillance System
HAI	Healthcare-associated infection
IPC	Infection Prevention and Control
KOICA	Korea International Cooperation Agency
MIC	Minimum Inhibition Concentration
NIPH	National Institute of Public Health
NPHL	National Public Health Laboratory
MDRO	Multi-Drug Resistant Organism
MoH	Ministry of Health
PMRS	Patient Management and Registration System
TWG	Technical Working Group
WHO	World Health Organization
US CDC	United States Centers of Disease Control and Prevention

ACKNOWLEDGEMENTS

On behalf of the Ministry of Health, we wish to acknowledge the following contributors for their invaluable support for development and finalization of the Standard Operation Procedure of Cambodia Laboratory based Antimicrobial Resistance Surveillance System.

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Others partner as US CDC, KOIKA, WHO, DMDP, NAMRU-2, Members of AMR Technical Working Group, and Other Medical Laboratory Services.

I. BACKGROUND

1. Introduction

Establishing an antimicrobial resistance (AMR) surveillance system is essential for monitoring national and global trends, quickly detecting outbreaks and new resistance mechanisms, as well as guiding implementation of effective prevention and control measures. The World Health Organization (WHO) is developing a Global Antimicrobial Resistance Surveillance System (GLASS) with the goal of collecting standardized and comparable AMR data to be analyzed and shared among countries. GLASS combines patient, laboratory, and epidemiological surveillance data to enhance understanding of the burden of AMR. Given the challenges of collecting these data, gradual implementation is encouraged. In early 2017, with technical support from the Diagnostic Microbiologic Development Program (DMDP) and one-year financial support from WHO, the Ministry of Health's (MoH) AMR Technical Working Group (TWG) established a pilot laboratory-based AMR surveillance system for blood specimens in 14 sentinel sites. With additional funding from the Korea International Cooperation Agency (KOICA) and technical assistance from partners including WHO, DMDP, US-CDC, Angkor Hospital for Children, and Center of Hope, the AMR TWG will expand the surveillance system to include additional specimen types, clinical variables, and denominators for a more accurate estimate of the AMR burden in Cambodia and to generate more reliable data to submit to WHO GLASS.

2. Objectives

- Describe the burden of AMR in Cambodia by selected indicators
- Contribute standardized AMR data to WHO (GLASS) for global AMR reporting
- Provide reliable data to inform national and international interventions for AMR containment
- Detect emerging resistance patterns and potential spread
- Assess the impact of AMR prevention and control interventions
- Describe the mechanisms of Antimicrobial Resistance

II. SURVEILLANCE METHODS

1. Sentinel sites

The Cambodia Communicable Diseases Control (CCDC) will recruit eight sentinel sites, six public (i.e., Siem Reap Provincial Referral Hospital, Battambang Provincial Referral Hospital, Takeo Provincial Referral Hospital, Kampong Cham Provincial Referral Hospital, National Pediatric Hospital, and Calmette Hospital) and two NGO (Angkor Hospital for Children and Sihanouk Hospital Center of Hope) laboratories to participate in AMR Surveillance. The sites were selected based on the following criteria:

- Commitment by hospital leadership and essential staff (e.g., physicians and laboratory staff)
- Commitment to high quality AMR data
- Availability of laboratory information system (CAMLIS or others), Patient Management Recording System (PMRS), information technology (IT) and Applied Epidemiology Training (AET) support.
- Location that is geographically representative
- Availability of transportation system

AMR WG will expand the surveillance system to other sites when appropriate.

2. Levels of AMR surveillance system

Due to capacity differences in reporting AMR clinical and laboratory and data and based on lessons learned from the pilot phase, national AMR surveillance system will be implemented in two different tiers.

- Tier 1 (basic level)

  Siem Reap Provincial Referral Hospital, Kampong Cham Provincial Referral Hospital, Calmette Hospital, and National Pediatric Hospital will automatically extract a line-list of priority pathogens among priority specimens (Blood culture and CSF) from their laboratory information systems and submit to a web-based national AMR database on a daily basis.

  Variables for each pathogen include patient demographic information (i.e., age and sex), date of admission, date of specimen collection, diagnosis at time of specimen collection

(if available), pathogen identifications (up to species level if available), and susceptibility results (with zone diameter or minimum inhibition concentration [MIC] if available). See example 1 for a model of line-list. Please see in Annex 2 for CRF Tier 1.

- Tier 2 (intermediate level)

  Following good performance during the pilot phase and with more resources available, Battambang Provincial Referral Hospital, Takeo Provincial Referral Hospital, Angkor Hospital for Children, and Sihanouk Hospital Center of Hope were selected by the AMR TWG to implement a more complex AMR surveillance system. These four laboratories will automatically extract a line-list of priority pathogens among priority specimens (Blood culture and CSF) to the web-based national AMR database on a daily basis. Monthly Epi forms will be sent to NIPH with isolates and will capture total Blood and CSF culture and total patient admissions by age group.

  Variables for each specimen include:

  - Hospital name/code
  - Specimen type
  - Patient's hospital ID
  - Diagnosis on lab request form
  - Culture results (Positive and Negative)
  - Pathogens
  - Age
  - Gender
  - Hospital admission date
  - Specimen collection Date
  - Received antibiotics (yes/no /unknown)
  - Susceptibility results
  - Resistance mechanism, if known
  - The patient referred from other Hospital/ facility

  Note: Hospital admission and specimen collection dates will be used to determine healthcare-associated infection (HAI) versus community-associated infections (CAI) during data analyses.

  In addition to the information on specimens tested, the hospitals will also report monthly numbers of hospital admissions and discharges. These numbers will be used as denominators to calculate infection rates. Please see in Annex 3 for CRF Tier 2.

3. Specimen types and priority pathogens

Specimen	Laboratory case definition	Priority pathogens
Blood	Isolation of pathogen from blood	*E. coli* *K. pneumoniae* *Acinetobacter* spp. *S. aureus* *S. pneumoniae* *Salmonella* spp. *B. pseudomallei*
CSF	Isolation of pathogen from CSF	*Any bacterial pathogen*

- Specimens collected from outpatients must not be reported to the system
- Specimens collected from admitted patients must be reported regardless of the source of infection, healthcare- or community-associated infection. De-duplicate isolates from the same patients: if several cultures are collected from one patient during the same hospital admission, only one result per surveyed specimen type and pathogen should be reported for each patient. For example, if two blood cultures from the same patient yield growth of *E. coli*, only the first should be included in the report regardless of AST results. If growth of *E. coli* is detected in one culture and *K. pneumoniae* in the other, both results should be reported. Please see Annex 4, Specimen Collection.

4. Data sources and format

Data will be extracted from laboratory information system (CAMLIS web-based or others) and uploaded into a web-based national AMR database, located on the MoH-CDC website. The national AMR database will be developed by WHO and MoH-CDC IT specialists. Designated laboratory staffs are responsible for entering data into CAMLIS or other laboratory information system used in the hospitals and extracting (auto-extraction) and uploading into the national AMR database. MoH-AMR focal persons will be responsible for generating the national AMR report, with support from AET residents and relevant partners (i.e., US CDC, WHO, and DMDP).

Please see the Example of model data sheet. EPI data sheet is complete by surveillance sentinel site of Tier 2 in weekly basis (ideally Wednesday). Please see the Annex 1.

5. Quality control assurance

- **External Quality Assurance Scheme**

  Participating laboratories have been participating with the EQAS for Microbiology provided by Paramedical Training Center, New Zealand (PPTC) three times per year for the last 5 years. The average result is 94% accurate (range: 75 to 100%).

- **Confirmatory testing at the National Public Health Laboratory (NPHL)**

  To continue monitor quality of pathogen identifications and susceptibility testing (AST) done at the participating laboratories and to strengthen capacity of NPHL in microbiology, isolates from all sentinel sites (except Angkor Hospital for Children and Sihanouk Hospital Center of Hope which will send only the data) will be sent to NPHL for re-identifications, repeated AST, and storage.

  Pathogen identification and AST results from the participating laboratories and NPHL during the first year will be evaluated to determine the proportion of discordant results and define quality of data. Depending on the findings of this evaluation, participating laboratories might require to send fewer isolates (e.g., 50% of isolates recovered) to NPHL for confirmatory testing. AMR TWG will reassess the necessity of confirmatory testing at the end of year 1.

- **Special considerations for discordant results**

  Once results on pathogen identifications and AST are available at NPHL, NPHL's staff must cross check the results with those submitted from the participating laboratories and notify the staff of the participating laboratories of any discordant results for immediate correction in the laboratory information systems and in the national web-based database. Results from NPHL should be used as the gold standard. In a rare circumstance, NPHL could send isolates to NAMRU2 or Institute Pasteur du Cambodge for additional testing. However, it is recommended that NPHL staff consult with the AMR TWG before sending the isolates.

6. Isolates referral system

Participating laboratories (except AHC and SHCH) will ship Isolates and data to NPHL once a month, every first Wednesday of the month (except S. pneumonia should refer as soon the isolates is ready), following the National Referral Laboratory (NPHL) specimen transport SOP with a safety concept (i.e., 1st Ice Box Container, 2nd Screw Cap Container, and 3rd Rack support the media transport tube of isolates) (See Annex 2). All isolates must be sent in a container with individual case report forms completed by designated staff of the participating laboratories. To ensure there is not a delay in receiving the isolates, containers should be labeled with the date of transport (referral), name and contact information of senders and receivers. NPHL is responsible for operating costs, through with Local Transport Company (Company name and contact will provide to sentinel site according to the contract), using CoAg funds. Please see Annex 5, Isolate Referral Form.

7. Mechanisms of Antimicrobial Resistance

The mechanism of antimicrobial resistance study could be one of the topic may be possible to perform. NPHL will look for the mechanism of resistance to specific type of antibiotic which shown as a pattern of phenotyping resistance. The result outcome is data contribute to scientific description for the pathogen resistance to antibiotic used.

III. DATA MANAGEMENT, REPORTING, USAGE, AND OWNERSHIP

The hospital management assigns one lab focal point and two alternatives to ensure that the AMR data is uploaded on monthly basis. The AMR data needs to share with IPC's committee and local AET and RRT on daily basis using mobile applications (Telegram, Messenger, WhatApps, or Line) for timely actions.

The data management team, under the leadership of the MoH AMR focal point, is responsible for monitoring data submission to the national AMR database, analyzing, and generating a report for national and international stakeholders. Data from the surveillance system will be analyzed domestically to define the scope and magnitude of antimicrobial resistance in Cambodia, and will be submitted to WHO GLASS to support the development of international strategies to combat AMR.

Facility-level data (sentinel site data) will be used to improve and implement infection control measures to contain outbreaks or reduce AMR prevalence at individual facilities. To achieve this objective, each facility will share a line list of positive blood and CSF cultures with the hospital IPC committee daily. The IPC committee will review the line list and implement appropriation interventions, as needed. The committee will report the status of the interventions and outcomes to hospital leadership on a monthly basis.

The MoH AMR focal points and Cambodia AMR TWG leads may consult with subject matter experts (SMEs) from the Division of Healthcare Quality Promotion (DHQP) CDC Atlanta, on data analysis and monitoring and evaluation as needed.

MoH AMR TWG has the ownership of the national AMR data. The participating hospitals have the ownership of their facility-level data and will be able to use them for presentations or publications with a courtesy approval of the MoH AMR TWG. The partners involving in this surveillance including the University of Health Sciences could request for ad-hoc accesses to the database or permission to review or use data for training purposes from the chair of MoH AMR TWG.

IV. DATA ANALYSIS PLAN

Data management and analysis will be conducted by the MoH's Department of Communicable Diseases and Control in collaboration with relevant partners who are in AMR TWG.

Note: For simplicity, blood specimens and *K. pneumoniae* are used in the proposed example calculations below. The same calculations will apply for all specimen types. Where appropriate, the analyses must be stratified by priority pathogens, age, gender, community-acquired infections (i.e., date of sample collection < 2 calendar days after admission), and healthcare-associated infections (i.e. date of sample collection >= 2 calendar days after admission).

The following calculations will be performed quarterly and annually:

- Total number of patients with blood, and CSF (from sterile sites only) collected.
- Total number of isolates stratified by specimen types.

- Proportion of positive blood isolates with *K. pneumoniae* resistant to Carbapenem over all *K. pneumoniae* isolates cultured from blood; compute the same calculations for other pathogens, antibiotics, and specimen types.

- Proportion of patients with positive blood cultures over patients with blood samples (positive + negative), stratified by priority pathogens; compute the same calculations for other specimen types

- Proportion of patients with MDRO (i.e., resistant to at least one antibiotic in at least 3 antibiotic classes) recovered from blood over patients with blood samples (positive + negative), stratified by priority pathogens; compute the same calculations for other specimen types

- Proportion of patient with carbapenem-resistant *K. pneumoniae* isolate recovered from blood over patients with blood samples (positive + negative); compute the same calculations for other pathogen-antibiotic combinations and other specimen types

- Pathogen distribution among isolates recovered from blood, and CSF stratified by age group and gender

- Frequency of sampling per 10,000 admissions for each specimen type (number of patients with blood samples/total number of admissions for the same time period x 10,000; compute the same calculations for other specimen types)

- Frequency of patients with positive cultures per 10,000 admissions for each specimen type (number of patients with positive blood cultures/total number of admissions for the same time period x 10,000, stratified by priority pathogens; compute the same calculations for other specimen types)

- Frequency of patients with MDRO per 10,000 admissions (number of patients with positive blood culture for MDRO/total number of admissions for the same time period x 10,000, stratified by priority pathogens); compute the same calculations for other specimen types

- Frequency of pathogen-antibiotic susceptibility combination (e.g., Carbapenem-resistant *K. pneumoniae*) in each specimen type (e.g., blood) per 10,000 admissions (number of patients with carbapenem-resistant *K. pneumoniae* isolates recovered from blood / total number of admissions x 10,000); compute the same calculations for other specimen types and pathogen-antibiotic combinations

See the example in data shells.

V. OUTBREAK INVESTIGATION AND RESPONSE

1. National level:

- Decision to initiate investigation and response of a suspected outbreak will be based on a risk assessment and taken within 48 hours of the receipt of the AMR data report from the Focal Person of the Sentinel Lab Sites of the National Hospitals and Centre of Hope.
- The decision will be taken by the National AET Outbreak Management Team and Outbreak Communication of the CDC Department in collaboration with Department of Hospital Services (Infection Prevention and Control), National Institute of Public Health (National Public Health Laboratory) and the IPC's Committee of the National Hospital Sentinel Sites and relevant partners.
- In case of a suspected or probable animal source of the outbreak, the General Directorate of Animal Health and Production (GDAHP) is immediately notified by the National AET Outbreak Management Team.

2. Provincial level:

- Once the report of the AMR data from the Focal Person of the Hospital Sentinel Lab Sites is received, the Provincial AET Graduate and/or Rapid Response Teams (RRT) in collaboration with Provincial Hospital Infection Prevention and Control Committee (IPC's Committee) and Hospital RRT decide to initiate an investigation and response.

VI. ETHICAL CONSIDERATION AND REVIEW

This national AMR surveillance system is a public health surveillance activity. Individual patient consent is therefore not required and individual patient or their families will not be contacted. Every reasonable effort will be made to protect patient privacy. Electronic and physical security

measures will be taken to protect personal health information. Electronic data will be stored in a database on a secure server that can only be accessed using an encrypted password.

VII. PERSONNEL AND RESPONSIBILITIES

1. **Principle investigator**

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2. **Project manager**

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   Dr. SOK SRUN, Director of Department Hospital Services, Ministry of Health

3. **Cambodia subject matter experts**

   - Dr. KRANG SIDONN, Vice-Chief of Bureau of Prevention and Control, CDC Department and Cambodia National AMR Focal Point
   - Dr. CHAU DARAPHEAK, Director of National Public Health Laboratory, NIPH, Ministry of Health
   - Dr. SAU SOKUNNA, Deputy Director of Department Hospital Services, Ministry of Health

VIII. EXAMPLE OF DATA SHEET

Example 1: Line-List

			Patient information						Results			
No.	Hospital name	Specimen types	Patient ID	In vs out patients	Age	Gender	Admission date	Date sample collected	Cultures	Pathogens	AST	Resistance mechanism if known
1		Blood	1	Inpatient	30	M	4/12/2017	4/15/2017	Positive	E. coli		
2		Urine	1	Inpatient	30	M	4/12/2017	4/15/2017	Negative	Nothing		
3		Stools	1	Inpatient	30	M	4/12/2017	4/16/2017	Positive	E. coli		
4		Blood	2	Inpatient	62	F	4/7/2017	4/13/2017	Positive	A. baumannii		
5		Urine	2	Inpatient	62	F	4/7/2017	4/13/2017	Negative	Nothing		
6		Blood	3	Inpatient	55	F	4/2/2017	4/8/2017	Positive	E. coli		
7		Blood	4	Inpatient	60	M	4/1/2017	4/5/2017	Negative	Nothing		
8		Vaginal	5	Inpatient	35	F	4/10/2017	4/15/2017	Negative	Nothing		
9		Urine	6	Inpatient	70	F	4/3/2017	4/5/2017	Positive	E. coli		
10		Stools	6	Inpatient	70	F	4/3/2017	4/6/2017	Negative	Nothing		
11		Blood	7	Inpatient	20	M	4/11/2017	4/15/2017	Positive	A. baumannii		
12		Vaginal	8	Outpatient	25	F	NA	4/6/2017	Positive	N. gonorrheae		
13		Urine	9	Inpatient	80	F	4/8/2017	4/12/2017	Negative	Nothing		
14		Stools	10	Inpatient	2	M	4/19/2017	4/26/2017	Positive	Shigella spp.		

Example 2: Data shells and graphs showing how data will be presented

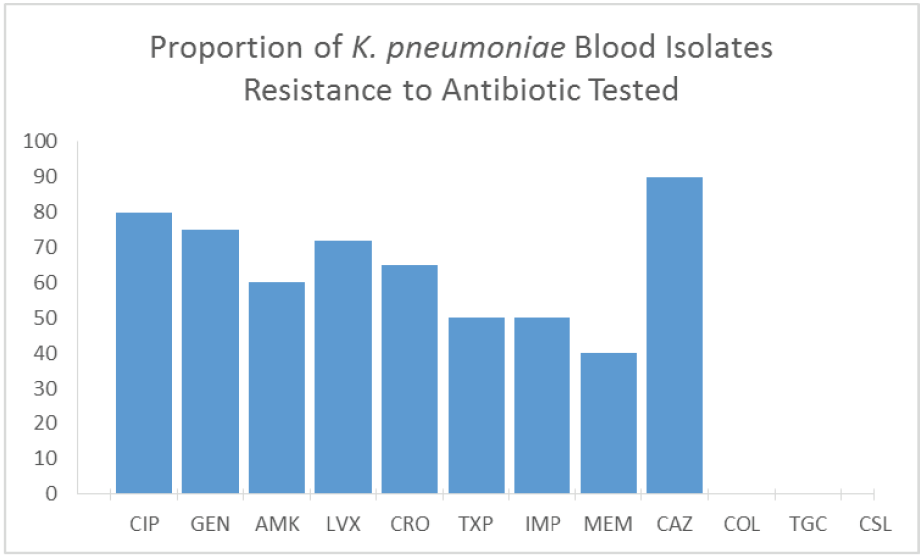
1. Distribution of specimen types among patients with samples collected

Patients with samples collected	**N (%)**
Blood	aa (bb)
CSF	aa (bb)

2. Distribution of specimen types among positive cultures

Patients with positive cultures	**N (%)**
Blood	aa (bb)
CSF	

3. Proportion of resistant blood isolates



4. Distribution of priority pathogens among positive **blood** and **CSF** cultures

Priority Pathogens	**% Positive Cultures**
Blood	
E. coli	
K. pneumoniae	
A. baumannii	
S. aureus	
S. pneumoniae	
Salmonella spp.	
B. pseudomallei	
CSF	

S. agalactiae
H. influenzae
S. pneumonie
S. suis
N. meningitidis
Listeria monocytogenes
Others

5. Distribution of priority pathogens among positive **blood** cultures stratified by gender

Priority Pathogens	**% Positive Cultures**		
	Male	**Female**	**Total**
E. coli			
K. pneumoniae			
A. baumannii			
S. aureus			
S. pneumoniae			
Salmonella spp.			
B. pseudomallei			

6. Distribution of priority pathogens among positive **blood** cultures stratified by age groups

Priority Pathogens	**% Positive Cultures**					
	<1	**1 – 4**	**5 – 14**	**15 – 65**	**>65**	**Total**
E. coli						
K. pneumoniae						
Acinetobacter spp						
S. aureus						
S. pneumoniae						
Salmonella spp.						

7. Proportion of **MDR *E. coli*** over all *E. coli* isolates stratified by specimen type

Specimen Type	**% MDRO**
Blood	50
CSF	50

MDR E.coli: Muti-drug resistance *E.coli*

8. Number of patients with blood MDRO over 10,000 patients with blood samples collected stratified by priority pathogens

Priority Pathogens	**Number MDRO per 10,000 blood samples**
E. coli	
K. pneumoniae	
A. baumannii	
S. aureus	
S. pneumoniae	
Salmonella spp.	
Total	

9. Number of inpatients with samples collected over 10,000 admissions stratified by specimen type

Specimen Type	**Number inpatients with samples collected per 10,000 admissions**
Blood	
CSF	
Total	

10. Number of inpatients with positive cultures over 10,000 admissions stratified by specimen type and priority pathogens

Specimen Type	**Number inpatients with positive cultures per 10,000 admissions**
Blood	
- *E. coli*	

- *K. pneumoniae*
- *A. baumannii*
- *S. aureus*
- *S. pneumoniae*
- *Salmonella* spp.
CSF
- *S. agalactiae*
- *H. influenzae*
- *S. pneumonie*
- *S. suis*
- *N. meningitidis*
- *Listeria monocytogenes*
Total

11. Number of inpatients with blood MDRO over 10,000 admissions stratified by priority pathogens

Priority Pathogens	**Number MDRO per 10,000 admissions**
E. coli	
K. pneumoniae	
A. baumannii	
S. aureus	
S. pneumoniae	
Salmonella spp.	
Total	

IX. ANNEXS

Annex 1: EPI Form

Annex 2: CRF Tier 1

Annex 3: CRF Tier 2

Annex 4: Specimen Collection, SOP

Annex 5: Isolate Transportation SOP

Annex 6: Isolate Transportation Form

Annex 7: Local Transport Company

Annex 8: Flow and Job Aid

The annexes are in the Khmer Version of the Lab-Based AMR Sentinel Surveillance SOP.

Epidemiology Form (ANNEX 1)

AMR Sentinel Sites Tier 2: ☐ AHC ☐ BTB ☐ TKO ☐ SCH

Monthly: _____ (From: _____/ _____/ _20___ to _____/ _____/ _20____)

Number of admitted inpatient to Hospital																		
Gender	All ages		0-28 days		29 days-11 months		1-4 years		5-14 years		15-24 years		25-49 years		50-64 years		≥65 years	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
IPD																		
Blood																		
Blood positive																		
CSF																		
CSF positive																		

ANNEX 2

AMR-CRF TIER I **ID#: SRH-1709-000**

Hospital/Laboratory Request Form

☐ **SRH** ☐ **KCH** ☐ **NPH** ☐ **CAL**

Hospital Service/ ward:……
Hospital/Laboratory contact: ……

1. Age:…… BoD: …….Days ……Months ……..Years
2. Sex: ☐ Male ☐ Female
3. Patient's hospital ID#:……..
4. Date of admission: ……..… Time:……
5. Lab ID#: …….....
6. Specimen type: ……
7. Date of specimen collection: ……...
8. Diagnosis: ……. ……..

ANNEX 3

AMR-CRF TIER II **ID#: AHC-1709-000**

Hospital/Laboratory Request Form

☐ **AHC** ☐ **BTB** ☐ **TKO** ☐ **SCH**

Hospital Service/ ward:……

Hospital/Laboratory contact: ……

1. Age:…… BoD: …….Days ……Months ……..Years
2. Sex: ☐ Male ☐ Female
3. Patient's hospital ID#:……..
4. Date of admission: ……..… Time:……
5. Lab ID#: …….....
6. Specimen type: ……
7. Date of specimen collection: ……...
8. Diagnosis: ……. ……..

SPECIMEN COLLECTION – Blood culture (ANNEX 4a)

1. Objective

To provide instructions on how to perform aseptic blood culture collection ensuring appropriate volume is collected and avoiding contamination of specimen.

2. Responsibility

All phlebotomists (may include nurses, doctors, laboratory staff).

3. Principle

Standard precautions should be observed when drawing blood. The phlebotomist should perform appropriate hand hygiene procedure both pre and post blood collection to avoid possible cross contamination.

4. Phlebotomy Procedure (for blood culture)

- Staff collecting blood will explain the process of blood collection and ask consent from the patient to perform blood collection.
- Staff will prepare the material for specimen collection.
- Label blood culture bottle with patient name, gender, ID number, patient date of birth, site of collection (e.g. left arm), date and time of collection.
- Staff perform hand hygiene
- Staff position the patient
- Put on tourniquet then palpate and select a vein that feels easy to collect blood from. Veins that can be felt are more reliable than those that are seen but not felt well.
- Disinfect the skin using 70% alcohol wipe. Use a circular motion to clean the site first then move outwards to skin beyond the proposed puncture site. If the alcohol wipe is dirty, repeat the procedure until the skin is clean. Discard the alcohol wipe and leave to dry for 1 minute. Clean the area again with betadine 10% (povidone iodine) and leave to dry for 2 minutes.
- Then remove the lid from blood culture bottle and disinfect the rubber stopper using povidone iodine. Leave to dry. It is important to do this prior to organizing needle and syringe to allow time for the povidone to dry (2 minutes).

- Carefully organize the sterile needle and syringe. If syringe comes with incorrect needle size, remove and replace with an appropriate sized needle:
    - Children (≤ 14 years old): using a 23G needle and a 5ml syringe, collect 2-5 ml of blood for a 50ml blood culture bottle
    - Adult (> 14 years old): using a 21G needle and a 10ml syringe collect 10 ml of blood from two different sites for a 100ml blood culture bottle. If the doctor has ordered other tests, use a larger syringe and collect up to 20 mls.
- Without touching the proposed puncture site, carefully insert needle through the skin and into the vein and collect the required amount. If the vein is not entered, you must not use the same needle to re puncture the skin. Replace with a sterile needle.
- Inject total required blood into blood culture bottle and gently invert and mix. If blood is also required for other tests, place blood into tubes only **after** placing in blood culture bottles. Discard the needle and syringe immediately into the sharps container.
- For adults, blood should be collected from 2 sites – 10 mls from each site so repeat the procedure after finding an appropriate vein. Do not take blood from an IV catheter and avoid taking blood proximal to an IV line (may dilute the blood – take blood from another site or distal to the IV catheter). It may be necessary to take blood from the same arm or a leg vein.
- Perform hand hygiene
- Send the blood culture bottles to laboratory along with the microbiology request form that has been completed (signed, date, time). Do not refrigerate.

Important note for blood culture collection:

- If possible, collect blood cultures before antibiotic treatment is given to the patient, however do not delay treatment of severely ill patient. If the patient is already receiving antibiotics, take blood culture specimen immediately before the next prescribed dose of antibiotic.
- If the patient has an IV running, draw blood from the opposite arm, other site (leg) or distal to the IV catheter site should a suitable vein be found.
- Blood culture specimen should be requested by the doctors according to the indications agreed to by the hospital.

- The clinician must complete all sections of the request form including clinical syndrome, relevant co-morbidities and whether patient is already on antibiotics. The phlebotomist must sign, date and include time of collection on the request form

Blood culture bottles

- Blood culture bottles have either 50ml (paediatric) or 100 ml (adult) broth

SPECIMEN COLLECTION – CSF culture (ANNEX 4b)

1. Objective

To provide instructions on how to perform aseptic CSF culture collection ensuring appropriate volume is collected and avoiding contamination of specimen.

2. Responsibility

The collection of CSF is an invasive procedure and should only be performed by experienced personnel under aseptic conditions.

3. Principle

It is important to adhere to proper biosafety guidelines while handling potentially infectious clinical specimens in order to maintain a safe working environment for patients, health care workers, and laboratorians. Infection may be transmitted from patient to staff and from staff to patient during the procedures described below.

4. Preparing for Lumbar puncture

If possible, three tubes (1 ml each) of CSF should be collected for microbiology, chemistry, and cytology. If only one tube of CSF is available, it should be given to the microbiology laboratory. Because the presence of blood can affect cultures of CSF, if more than one tube of CSF is collected from a patient, the first tube collected (which could contain contaminating blood from the lumbar puncture) should not be the tube sent to the microbiology laboratory.

1. Skin disinfectant: 70% alcohol swab and povidone-iodine
    - Alcohol with concentrations greater than 70% should not be used because the increased concentrations result in decreased bactericidal activity. Do not use alcohol with glycerol added to it.
2. Sterile gloves
    - Be sure to check the expiration date.
3. Sterile gauze
4. Surgical mask
5. Adhesive bandage
6. Lumbar puncture needle
    - 22 gauge/89 mm for adults
    - 23 gauge/64 mm for children
7. Sterile screw-cap tubes
8. Syringe and needle

9. Transport container

5. Lumbar puncture Procedure (for blood culture)

Follow all appropriate biosafety precautions.

1. Gather all materials from the CSF collection kit and a puncture-resistant autoclavable container for used needles.

2. Wear surgical mask and sterile latex or nitrile gloves that are impermeable to liquids and change gloves between every patient.

3. Label the collection tubes with appropriate information: patient's name, date and time of specimen collection, and Unique Identification Number. Be sure this number matches the number on both the request and report forms.

4. Ensure that the patient is kept motionless during the lumbar puncture procedure, either sitting up or lying on the side, with his or her back arched forward so that the head almost touches the knees in order to separate the lumbar vertebrae during the procedure (Figure 2).

5. Disinfect the skin along a line drawn between the crests of the two ilia with 70% alcohol and povidone-iodine to clean the surface and remove debris and oils. Allow to dry completely.

6. Position the spinal needle between the 2 vertebral spines at the L4-L5 level and introduce into the skin with the bevel of the needle facing up.
Accurate placement of the needle is rewarded by a flow of fluid, which normally is clear and colorless.

7. Remove CSF (1 ml minimum, 3-4 ml if possible) and collect into sterile screw-cap tubes. If 3-4 ml CSF is available, use 3 separate tubes and place approximately 1ml into each tube.

8. Withdraw the needle and cover the insertion site with an adhesive bandage. Discard the needle in a puncture-resistant, autoclavable discard container.

9. Remove mask and gloves and discard in an autoclavable container.

10. Wash hands with antibacterial soap and water immediately after removing gloves.

AMR Surveillance System Bacterial Isolate Packaging and Transport SOP (ANNEX 5)

1. Objective

This document describes how to pack, label and prepare documents for transport of bacterial isolates to laboratories within Cambodia for the Cambodia Communicable Disease Control (CCDC) AMR Surveillance System. This ensures isolates are packed and transported in appropriate packaging using the triple packaging systems. It will provide the highest level of safety for transportation.

2. Responsibility

Laboratory personnel

Transportation personnel

3. Principle

The key principles of packaging infectious substances are:

1. To protect the environment
2. To protect the handlers and couriers
3. To protect the sample

Classification of infectious substances

Dangerous goods are classified and assigned UN numbers.

Infectious substances are classified in Division 6.2.

UN2814: Category A infectious substance affecting humans: an infectious substance in a form capable of causing permanent disability or life-threatening or fatal disease in otherwise healthy humans or animals when exposure occurs

Examples: Burkholderia pseudomallei

UN2900: Category A infectious substance affecting animals

UN3373: Category B infectious substance not included in Category A

Examples: Salmonella sp., Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Acinetobacter, Haemophilus influenzae, Streptococcus pneumoniae, Streptococcus agalactiae (Group B) Neisseria meningitidis, Listeria monocytogenes, Streptococcus suis.

There are no national transport regulations in Cambodia.

Most pathogens transported under the AMR Surveillance System are classified as-

UN3373: Category B infectious substance not included in Category A

4. Materials

1. Primary container sealed with parafilm.

   Examples: Plastic screw capped TSA tubes (glass tubes should NOT be used) OR agar plates.

2. Leak-proof secondary container

   Examples: Screw cap container used for Microbiology EQA in Cambodia. See Figure1. Right: is a strong, leak proof screw capped container.

   **Attention: Several primary containers may be placed in one secondary container.

Figure 1. Strong leak proof screw capped Secondary container

3. Strong outer package. A large container can hold several Secondary containers if required.

   Examples: Large plastic ice box that can be secured with a padlock See Figure 2.

Figure 2. Large plastic icebox Strong leak proof screw capped Secondary container

4. Absorbent material to be placed between primary and secondary container.

   Examples: Paper towel or cotton wool.

5. Labels: Hazard and or handling labels

6. Documents: Specimen Shipping List and Referral Request Form

5. Reagent

None

6. Standard and control

Not required

7. Sample

Bacterial Isolates

8. Procedure

Isolates are packaged by using **the** triple packaging system.

8.1.1. Isolates are inoculated into a media vial or on agar plates

Make sure that the vial or plate is secure by wrapping with parafilm.

This is the primary receptacle.

8.1.2. Put the isolate vial or plate in a secure screw cap container (secondary container).

Put absorbent material on the bottom of this container to absorb all fluid in case of breakage.

A Specimen shipping list (F-MCU-045): Is a contents list that records all isolates present in the package. This is placed between the second and third container.

8.1.3. Put the secure secondary container in a strong outer package.

9. Reporting results:

N/A

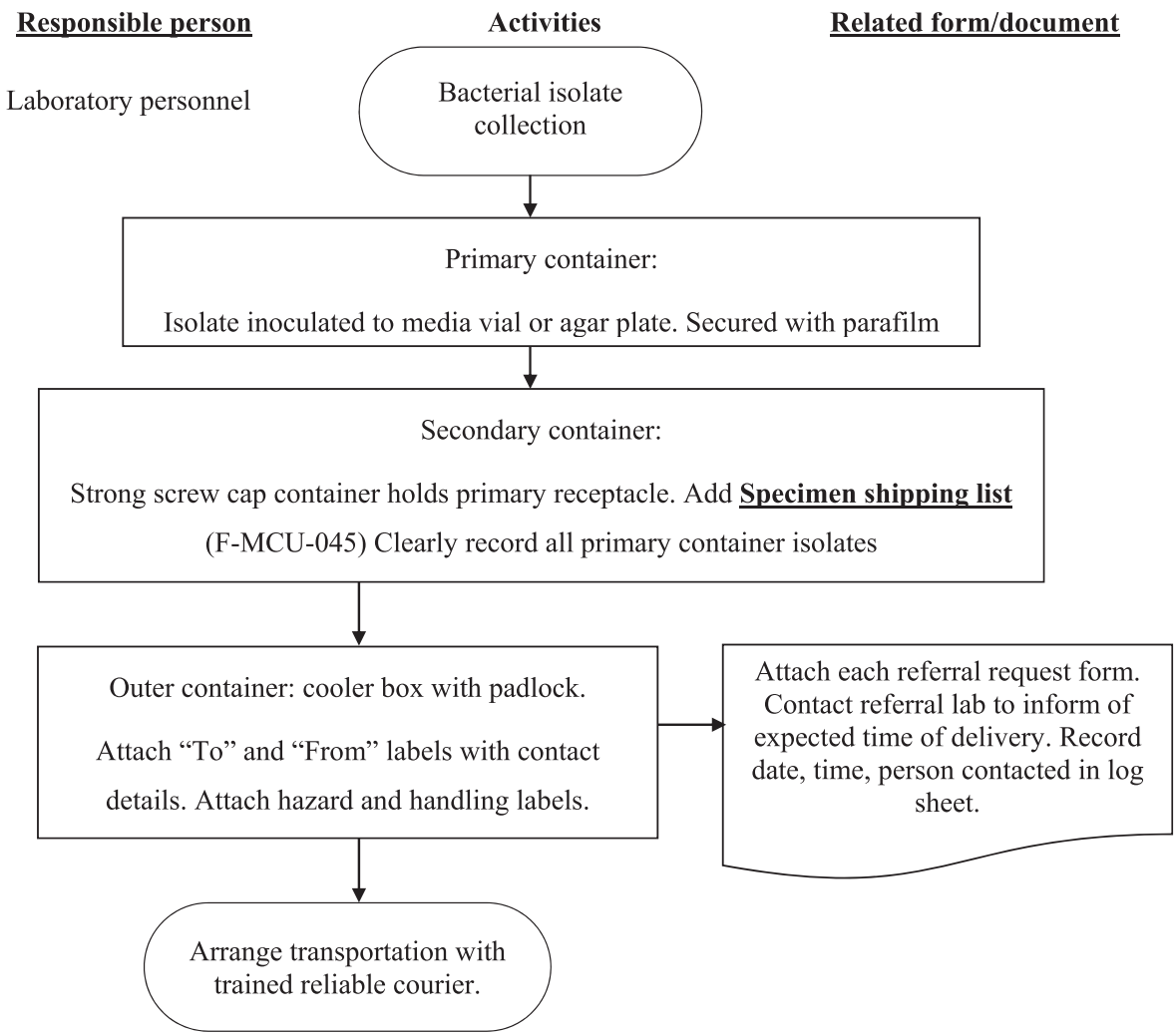
10. Normal Reference Range

N/A

11. References

- IATA infectious substances shipping guideline 10th edition, January 2009.
- Guideline on regulations for the transport of infectious substances 2009-2010, World Health Organization.
- DMDP SOP, version 2, document code 018

12. Flow chart



13. Safety Precaution

Use required PPE when preparing and packaging isolates: lab coat, gloves.

14. Supplementary notes

a. Packaging:

To ensure isolate, environment and handler protection the triple packaging system is used. Where materials are not available, use similar materials and ensure triple packaging system is followed.

As far as possible, Pack according to Figure 3. with the packaging containers described under **Materials** (see above).

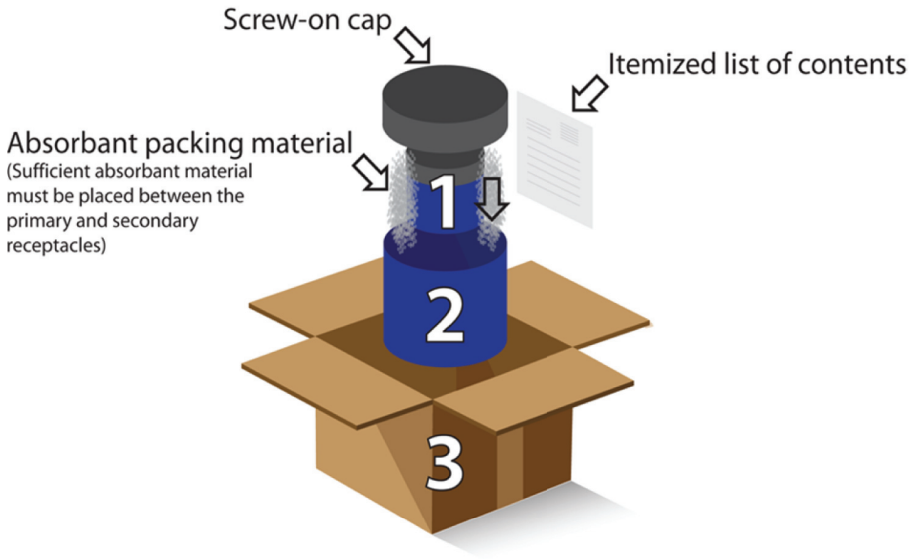


Figure 3. Example of a triple packaging system

Source:
https://web3.unt.edu/riskman/index.php?section=onlinetraining&group=hazmattransportation&module=11

b. Labeling and Marking:

There are 2 types of labelling

1. Hazard labels

Hazard labels indicate the classification of the infectious substance being transported. See Figure 4.

Figure 4. Hazard labelling for **UN3373:** Category B infectious substance not included in Category A

2. **Handling labels**

   See Figure 5. for example, of labels that should be attached to the outer container according to the type of specimen being transported and special handling requirements. These labels can be printed from ANNEXE 2. and attached to the outer container with tape.

Figure 5. Orientation arrow label

c. Sender and Receiver details

For all shipments include clearly marked on the outer packaging:

1. Sender name, address and telephone number
2. Receiver name, address and telephone number

d. Documentation: Every shipment must include the following documents

1. **Specimen shipping list** (Refer to the shipping list in the last page): must be placed between the secondary and outer container and list the complete contents of the shipment.
2. **Referral request form**: Clearly record detail of the AMR Surveillance bacterial isolate. One form should be completed for each isolate

e. Communication: Clear communication between sender, transporter and receiver is essential.

1. The **sender** will always inform the receiver beforehand by **phone** about the moment the shipment is leaving his laboratory and the expected date and time of arrival at the receivers laboratory. It should be confirmed that there is informed staff available for receiving the shipment.
2. The **receiver** will always confirm the arrival of the shipment to the sender by e-mail or phone.
3. All communication should **be marked clearly** on the Referral request form (F-MCU-044)

NATIONAL AMR SURVEILLANCE	Document code : F-MCU-045	Prepared date: 25/Oct/17	Revision No:00
			Issued date: 28/Oct /17
TITLE: SPECIMEN SHIPPING LIST			Revised date: N/A

Ship to:_____

Shipped Date:_____

N^{o}	Patient Name	Patient ID	Specimen Type	Date collection	Test request	Remark
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
Total						

Prepared by:_____

Prepared date:_____

Approved by:_____

Approved date:_____

Picked up by:_____

Picked up date:_____

Received by:_____

Received date: _____

Cambodia-Antimicrobial Resistance Surveillance Form: Isolate Referral Form (ANNEX 6)

Patient ID: _____ **Lab ID:** _____

Specimen and Isolate Information

☐ **Blood**

1. Number of blood culture bottles collected _____
2. Number of blood culture bottles positive _____
3. Date specimen collected_____
4. Date positivity first detected _____
5. Isolate Identification _____

☐ **CSF**

1. CSF White Blood Cell Count _____
2. CSF Gram stain _____
3. Date specimen collected_____
4. Date culture positive _____
5. Isolate Identification _____

6. What method did you use for AST? ☐ Disc Diffusion ☐ Minimal Inhibitory Concentration (MIC) ☐ Other _____
7. What standard for your AST method was used? ☐ CLSI ☐ EUCAST ☐ CA-SFM Year of standard's last update? _____

Antibiotic Susceptibility Testing (AST) Results

mm-zone diameter **µg/mL**-MIC
R-Resistant, **I**-Intermediate, **S**-Susceptible**:** Interpretation

Antibiotics	Disc code	µg	mm or µg/mL	R/I/S	Antibiotics	Disc code	µg	mm or µg/mL	R/I/S
Ampicillin	AM	10			Ciprofloxacin	CIP	5		
Amoxicillin/ Clavulanic acid	AMC	20/10			Perfloxacin (*Salmonella*) Surrogate for fluoroquinolones e.g. CIP	PF	5		
Cefazolin	CZ	30			Azithromycin (*Salmonella Typhi*)	AZM	15		
Ceftriaxone	CRO	30			Trimethoprim/ Sulfamethoxazole	SXT	1.25/ 23.75		
Cefotaxime #	CTX	30			Penicillin	P	10		
Ceftazidime #	CAZ	30			Cefoxitin (*Staphylococcus*) Surrogate for Oxacillin	FOX	30		
Ceftazidime + Clavulanate #	CAZ/CLA	30/10			Oxacillin (*Staphylococcus*)				
Cefotaxime + Clavulanate #	CTX/CLA	30/10			Vancomycin MIC (*Staphylococcus*)	V			
Cefepime	FEP	30			Tetracycline	TE	30		
Imipenem	IMP	10			Erythromycin	E	15		
Meropenem	MEM	10			Clindamycin	CC	2		
Gentamicin	GM	10			Penicillin MIC (*Streptococcus*)	P			
Amikacin	AN	30			Ceftriaxone MIC (*Streptococcus*)	CRO			
Chloramphenicol	C	30			Vancomycin (*Streptococcus*)	VA	30		
Other					Other				

Salmonella serotyping: ☐ Polyvalent O Positive ☐ Other _____

ESBL detected? ☐ YES ☐ NO, ESBL Detection Method: ☐ 'Keyhole' effect ☐ Clavulanate combination disc testing
If Clavulanate Combination disc testing was performed, please ensure zone sizes are recorded for # antibiotics
ICR (Inducible Clindamycin Resistance) detected? ☐ YES (D-test Positive) ☐ NO (D-test Negative)
Is isolate Resistant or Intermediate to Imipenem or Meropenem? ☐ YES ☐ NO
Did you test for **Carbapenamase**? ☐ YES ☐ NO
Detection method: ☐ mCIM (modified Carbapenem Inactivation Method) ☐ Other ……What was the result?……

Completed by: _____ Date of Referral: _____Contact Number: _____

ANNEX 7

KERRY EXPRESSS, CAMBODIA

Head Office: # 38, Street 86T (Near Wat Samrong Andeth)

Sankat Phnom Penh Thmei, Khan Sen Sok,

Phnom Penh, Cambodia

Tel: 023 231 232 (Receptionist)

Tel: 081 555 199 (Sale service)

EMS – EXPRESS MAIL SERVICE, CAMBODIA

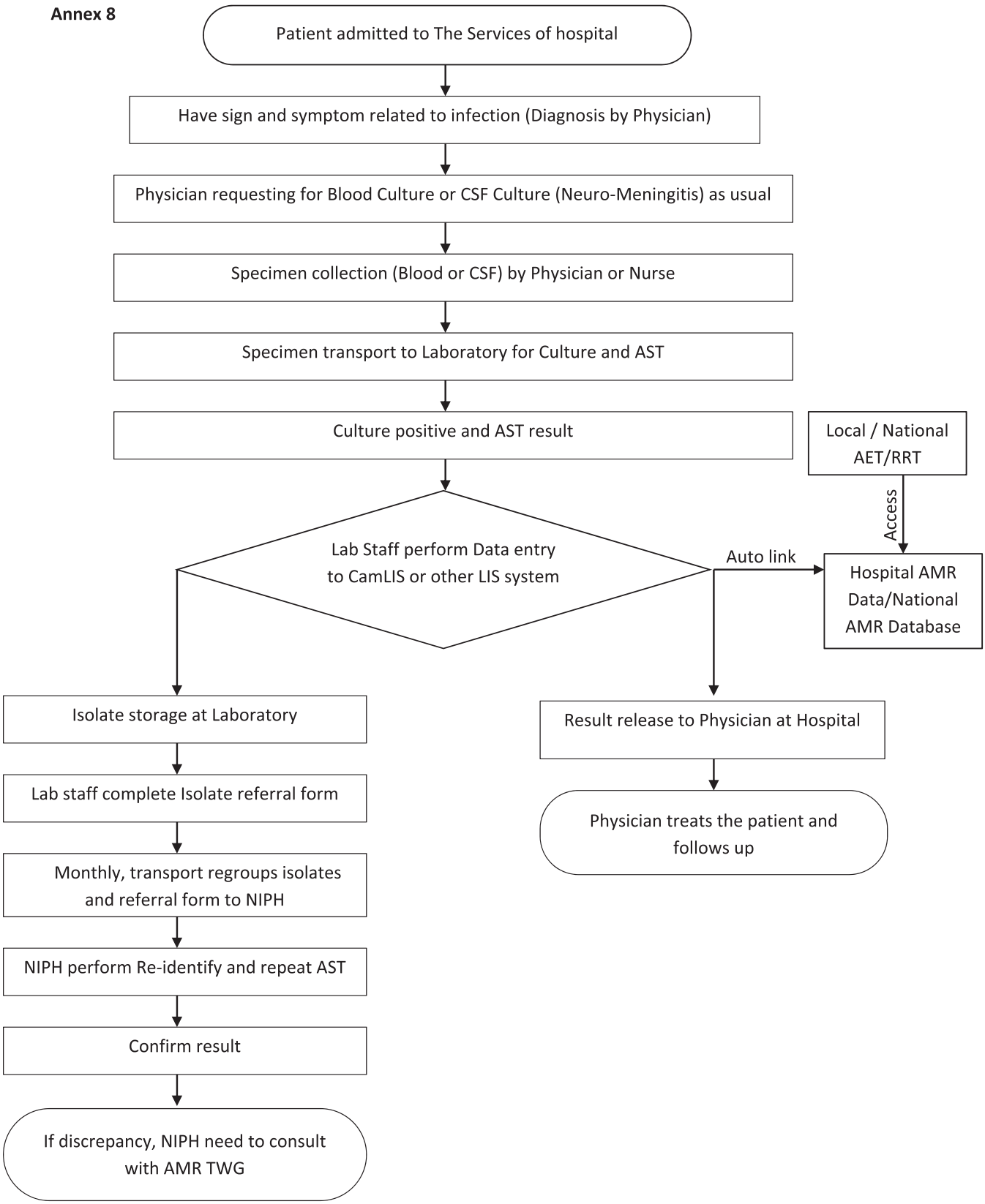
No. 486BC, Street. Mao Tse Toung Blvd (245)

Phnom Penh, Cambodia

Tel: +855 23 88 87 74

Mobile: +855 88 87 11 839

Annex 8


Patient admitted to The Services of hospital
Have sign and symptom related to infection (Diagnosis by Physician)
Physician requesting for Blood Culture or CSF Culture (Neuro-Meningitis) as usual
Specimen collection (Blood or CSF) by Physician or Nurse
Specimen transport to Laboratory for Culture and AST
Culture positive and AST result
Local / National AET/RRT
Access
Lab Staff perform Data entry to CamLIS or other LIS system
Auto link
Hospital AMR Data/National AMR Database
Isolate storage at Laboratory
Lab staff complete Isolate referral form
Monthly, transport regroups isolates and referral form to NIPH
NIPH perform Re-identify and repeat AST
Confirm result
If discrepancy, NIPH need to consult with AMR TWG
Result release to Physician at Hospital
Physician treats the patient and follows up