

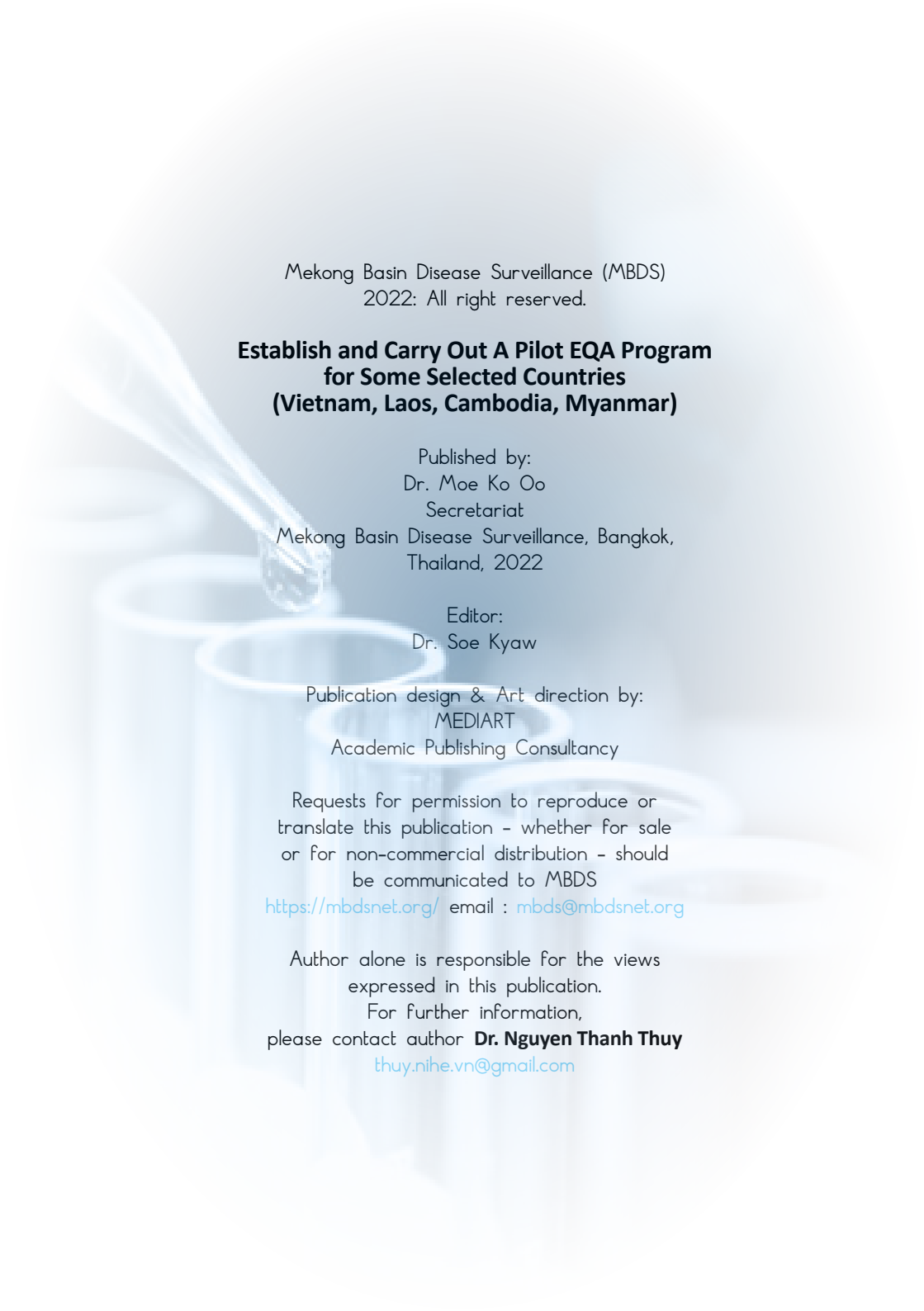


NATIONAL INSTITUTE OF HYGIENE AND EPIDEMIOLOGY
MINISTRY OF HEALTH, VIETNAM

ESTABLISH AND CARRY OUT A PILOT EQA PROGRAM FOR SELECTED COUNTRIES



SUPPORTED BY
CANADA'S WEAPONS THREAT REDUCTION PROGRAM &
MEKONG BASIN DISEASE SURVEILLANCE (MBDS)



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**Establish and Carry Out A Pilot EQA Program
for Some Selected Countries
(Vietnam, Laos, Cambodia, Myanmar)**

Published by:
Dr. Moe Ko Oo
Secretariat

Mekong Basin Disease Surveillance, Bangkok,
Thailand, 2022

Editor:
Dr. Soe Kyaw

Publication design & Art direction by:
MEDIART
Academic Publishing Consultancy

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ACKNOWLEDGMENTS

Report on “The Establish and Carry Out a Pilot EQA program for Some Selected Countries (Viet Nam, Laos, Cambodia, Myanmar)” was jointly developed by the Mekong Basin Disease Surveillance (MBDS) Foundation and National Institute of Hygiene and Epidemiology (NIHE) – Vietnam team lead by (Dr. Nguyen Dong Tu, Dr. Nguyen Thanh Thuy; Dr. Tran Dieu Linh, Dr. Trinh Quynh Mai, MSc. Le Thanh Huong, Msc. Vu Thi Mai Hien, Msc. Dang Thi Kieu Oanh).

This document provides an overview of the manufacturing process, provides EQA panels for the identification of enteropathogenic bacteria in the first pilot, the testing techniques for enteric pathogens are practices in medical facilities as well as the importance of quality assurance.

MBDS would like to express its gratitude and appreciation to several regional, national, and subnational stakeholders, who participated and contributed significantly in this first pilot EQA program. The contribution of Ministry of Health (Cambodia), Ministry of Health (Lao P.D.R), Ministry of Health and Sports (Myanmar), Ministry of Health (Vietnam), and respective EQA country focal points has been invaluable in supporting the pilot EQA activities in CLMV countries.

The tremendous support and assistance from the Canada’s Weapons Threat Reduction Program is also gratefully acknowledged.

Dr. Moe Ko Oo

Secretariat

Mekong Basin Disease Surveillance (MBDS)

ABBREVIATIONS

ASEAN	Association of Southeast Asian Nations
ATCC	American Type Culture Collection
CDC	Centre for Disease Prevention and Control
CLMV	Cambodia, Lao PDR, Myanmar, and Viet Nam
COVID-19	Coronavirus Disease 2019
EQA	External quality assessment
ISO	International Organization for Standardization
MALDI-TOF	Matrix-assisted laser desorption/ionization-time of flight
MBDS	Mekong Basin Disease Surveillance
MOH	Ministry of Health
NIHE	National Institute of Hygiene and Epidemiology
PMC	Preventive Medicine Centers
/QĐ-	Decision
WHO	World Health Organization

PREFACE

Establishment or strengthening laboratory quality assurance system will allow laboratories to improve the reliability and reproductivity of the laboratory results. External quality assessment scheme (EQAS) is necessary to ensure comparability of results among laboratories. Therefore, EQAS is valuable tools in the quality improvement process. They provide objective evidence of laboratory competence for customers, accrediting bodies, and regulatory agencies, and serve as a unique source of information that is not obtainable in the other ways.

This report is the result of the first pilot phase of the development of an external control program for the Identification of Pathogenic Enterobacteriaceae. Due to the complicated situation of the Covid-19 epidemic, this pilot phase is only conducted at medical facilities in Vietnam. The EQA panels was produced by the National Institute of Hygene and Epidemiology, provided to medical facilities, collected results of participating facilities and conducted data analysis. This pilot phase is a premise to implement expanded EQA programs for medical facilities in other countries in the region in the future. The results also can be used as references for continuous improvement plan and advocacy purposes, especially for developing countries.

MBDS Foundation Secretariat

Mekong Basin Disease Surveillance Foundation

EXECUTIVE SUMMARY

External Quality Assessment Scheme (EQAS) strengthening is able to improve the reliability and reproductivity of laboratory result. Testing capacity not only plays an important role in disease diagnosis, but also in disease surveillance and response systems, especially in responding to public health emergencies. The results provided by this report are expected to be used as a baseline information in establishing and maintaining EQA network in the region, to strengthen laboratory capacity (including accuracy, reliability and timeliness) for effective disease control and public health.

An EQA program to identify enteric pathogens was provided to 15 medical facilities in Vietnam. There were several types of hospitals and medical facilities that have participated including CDCs/ PMCs, public hospitals and private hospitals. An EQA panel consisting of 5 coded samples was provided to the participating facilities. They conduct tests on EQA panel using routine laboratory methods such as isolation cultures, automated equipment, or a combination of different measures. The results of the medical facilities are sent to the National Institute of Hygiene and Epidemiology for analysis and evaluation.

All participants completed EQA result reports with enough information and valid for data analysis. The results show that only about 53.3% of medical facilities have correct results in 3/5 samples or more. This shows that the quality of testing in medical facilities needs to be better controlled and this Pilot Program is a premise to do so.

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1. INTRODUCTION

1.1. BACKGROUND

Medical laboratory services are a critical component of national health systems and are central to disease diagnosis, treatment, prevention, surveillance, outbreak investigations and biosecurity. For the global health security, quality of laboratory testing including accuracy, reliability and timeliness is very important for effective disease control and public health.

WHO defines External Quality Assessment (EQA) as a system for objectively checking a laboratory's performance using an external agency or facility. EQA participation is associated with improved laboratory performance over time and is a requirement for accreditation. Laboratory professionals should routinely perform quality control testing to guarantee that test methods and equipment perform according to established standards. Laboratory professionals are required to participate in EQA/ proficiency testing programs in order to demonstrate that acceptable systems are in place and that specimens are collected and processed appropriately.

According to the need assessment report for EQA in Mekong Region, the capacity to test for pathogenic microorganisms of the assessed facilities from participating countries are well functioning, reflected in a wide variety of agents handled, as well as high technology application capabilities. However, many laboratories in ASEAN Member States are having resource limitation to take

part in EQA programs. The facilities have EQA program needs to improve the quality of staff and the quality of testing. Therefore, there is a need to establish an EQA network in the region in order to strengthen laboratory capacity and increase the number of laboratories that can provide accurate test results. EQA schemes are also valuable for early detection of laboratory errors and identification of underlying problems facing peripheral laboratories.

Within the framework of the project “Establishment of an EQA network among Southeast Asia countries to strengthen the quality of medical testing in disease control and response as well as public health care” funded by Canada’s Weapons Threat Reduction Program and Mekong Basin Disease Surveillance, the activity on “Establish Carry out a pilot EQA Program for some selected countries” is a prerequisite activity for the development of EQA programs in the future.

1.2. OBJECTIVES

To establish and maintain a pilot EQA network in selected ASEAN member States.

1.3. INFORMATION ON EXTERNAL QUALITY ASSESSMENT PROGRAM PROVIDER

The National Institute of Hygiene and Epidemiology (hereinafter referred to as the Institute or NIHE), the national institute in the field of preventive

medicine belonging to Ministry of Health, was founded according to the Decision no. 291/QĐ-BYT dated March 25th, 1961 by Minister of Health. In 2009, the Ministry of Health issued regulations on the organization and operations of the Institute under the Decision no. 4344/QĐ-BYT dated November 10th, 2009 by Minister of Health. NIHE has been selected to perform additional functions as the nation's public health reference laboratory and will create an EQA network and provide EQA schemes nation- and region-wide for strengthening Southeast

1.4. INFORMATION ON EXTERNAL QUALITY ASSESSMENT PROGRAM

External Quality Assessment Program (hereinafter referred to as EQA program) was founded pursuant to Decision no. 576/QĐ-VSDTTU dated May 27th, 2016 and implemented pursuant to Decision no. 1442/QĐ-VSDTTU dated October 24th, 2016 by Director of NIHE.

EQA scheme for Identification of pathogenic enterobacteria was established in 2019 with technical and financial supports from the Project for “Establishment of an EQA network among Southeast Asia countries to strengthen the quality of medical testing in disease control and response as well as public health care” funded by Canada’s Weapons Threat Reduction Program. This is the pilot round of External Testing for this program.

2. PARTICIPANTS AND CONFIDENTIALITY

As originally planned, the medical facilities participating in the external inspection program are laboratories of selected countries including: Vietnam, Laos, Cambodia, Myanmar. However, due to the complicated situation of the Covid-19 epidemic in Southeast Asia, the participation of countries is very difficult, so the pilot EQA program can only be implemented in Vietnam.

According to the need assessment report for EQA in Mekong Region, CDCs/ PMCs have capacity to conduct isolation/ culture, serology, and molecular biology techniques. CDCs/ PMCs have quite well invested in physical facilities as well as have sufficient essential equipment to perform isolation culture/ serology/ molecular biology testing techniques. As the key facilities in epidemic prevention and control, assessment results also showed that the availability and engagement of CDCs/PMCs in the epidemic prevention are mainly at level 4 and 5, of which the engagement in terms of reported information and the availability of budget for epidemic prevention was assessed at level 5, and the other factors are assessed at level 4. Level 1 reflects the lowest availability and least engagement in epidemic prevention and control activities and 5 represents the highest availability and greatest engagement in epidemic prevention and control.

Therefore, participants are laboratories in health facilities belonging to Preventive Medicine or Medical Service system in Vietnam. Hospitals and preventive medicine centers (CDCs/ PMCs) are suitable for this first pilot phase of the development of an external control program for the Identification of Pathogenic Enterobacteriaceae. In total, there are 15 government and private

organizations registered to enroll in this EQA round (Figure 1). Of the 15 medical facilities participating in the EQA program, 6/15 (40%) have met ISO standards and 14/15 (90%) have participated in at least 1 EQA program.

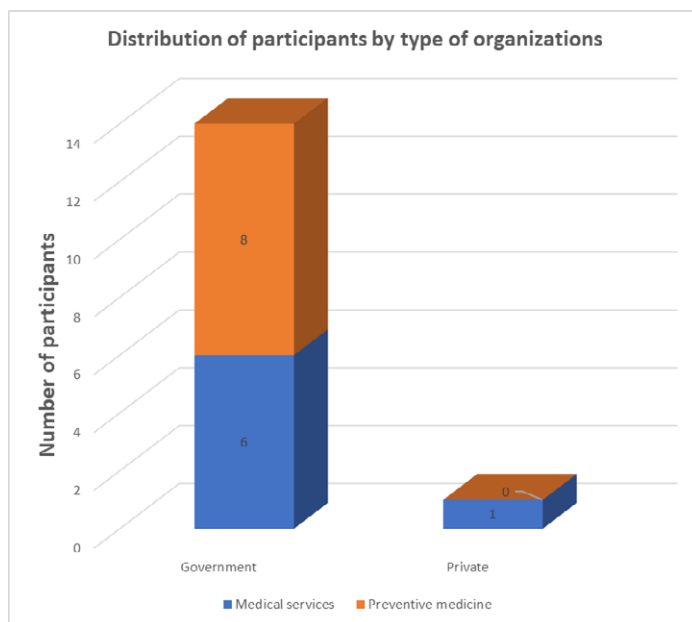


Figure 1. Distribution of participants by type of organizations

Because main purpose of this EQA scheme is to improve testing quality, EQA Program provide each participant an unique, confidential ID instead of real names of participants to use throughout the program (coded as NEXX). Only Director of EQA Program, coordinator and participants know their IDs. Information of participants will be provided for regulatory agencies only if having approval of Director of EQA program. In this case EQA Program will notify participants.

3. TIMEFRAME AND METHODS

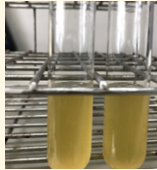
3.1. TIMEFRAME

3.2. PANEL PREPARATION, TESTING AND DISTRIBUTION

Samples used in the program are ATCC strains purchased from suppliers that will be tested and transferred and stored at -80°C. After being removed from the deep freezer, the Bacterial Strains were cultured in a suitable medium, redefining the biochemistry of the bacterial strains. The laboratory produces freeze-dried samples.

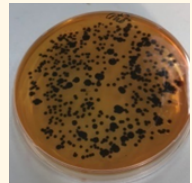
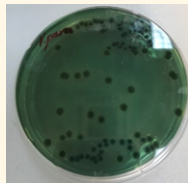
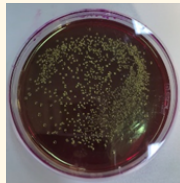
STEP 1

Bacteria were
cultured in
LB broth



STEP 2

Bacterial isolation
culture



STEP 3

Check the biochemical
properties of bacteria



STEP 4

Panel sample
production



STEP 5
Freeze-dried
samples



STEP 6
Labelling



STEP 7
Packing



Figure 2. Pictures of external control panel production

After preparation, samples will be aliquoted into different tubes. These tubes will be tested for homogeneity to ensure all aliquot tubes are same before distribution. EQA panels will be distributed to all participants at ambient temperature. For checking the stability of samples during transportation, two (02) participants including most remote ones were selected to give one extra panel per participants to send back to NIHE.

All participants are required to send their feedback on status of EQA panels when receiving and EQA Program will send replaced panels if panels were damaged during transportation.

3.3. REFERENCE RESULTS (ASSIGNED VALUES)

EQA panels provided by NIHE EQA program have following characteristics:

- Panel code: DV08-NIHE-VKND-2001
- Sample code: VK20-01, VK20-03, VK20-05, VK20-06, VK20-07
- Number of sample per panel: 5;
- Nature: Mixed sample of pathogenic and non-pathogenic enterobacteria;
- Transportation condition: ambient temperature;
- Storage condition: room temperature;

Enterobacteria pathogens were selected from the beginning of the Project. These are common enterobacteria found in human specimens. Results of EQA panels tested by NIHE using methods of culturing bacteria (Table 1). EQA pan-

Table 1. Characteristics of EQA panels (reference results)

No	Sample code	Results
1	VK20- 01	<i>V. parahaemolyticus</i> ATCC ® 17802 TM <i>P. aeruginosa</i> ATCC ® 27853 TM
2	VK20- 03	<i>Shi. flexneri</i> ATCC ® 12022 TM <i>E. coli</i> ATCC ® 25922 TM
3	VK20- 05	<i>Sal. enteritidis</i> ATCC ® 13076 TM <i>C. freundii</i> ATCC ® 43864 TM
4	VK20- 06	<i>Sal. typhimurium</i> ATCC ® 14028 TM <i>P. mirabilis</i> NCTC 11938
5	VK20- 07	<i>E. coli</i> ATCC ® 25922 TM Non-agglutinating Cholera vibrios (NAG)

3.4. REQUIREMENTS AND DEADLINES

Program requested all participants to put EQA panels in room temperature right after they received.

Deadline for:

- Sending feedback on EQA panel status upon receipt: 30/12/2020.
- Testing EQA panels: no later than 05/01/2021
- Sending EQA result reports:
 - Optional: electronic version via email: no later than 30/01/2021;
 - Mandatory: hard copies no later than 10/02/2021 (based on the date of sending on postal stamp if sending by post).

3.5. PERFORMANCE EVALUATION

Results of participants will be analyzed and evaluated based on:

-The agreement between results of participants and reference results of EQA program;

For each EQA sample, the participants will be scored:

- 0 point: if their results are incorrect;
- 2 point: if their results are correct (Identify the main pathogen in panel). For example: 01 participant whose results are correct for 4 out of 5 samples will be scored as 8/10 points.

4. RESULTS OF EQA PROGRAM

4.1. PARTICIPANT VALIDITY FOR ANALYZED DATA

Participants will be considered to include in data analysis and performance evaluation if the following criteria are met:

- EQA result reports filled with required information;
- Testing was done before the defined deadline of EQA program;
- Hard copies of EQA result reports are sent to the EQA program before the closing date of the round (based on the sending date on the envelope);
- There is no evidence of collusion, frauds on EQA results.

Data of invalid participants will not be analyzed.

In summary, all 15 participants passed the validity assessment (no exclusion) therefore all their results were submitted to data analysis.

4.2. CONDITIONS OF EQA PANELS UPON RECEIPT

100% (15/15) participating laboratories received EQA panels in good conditions (no leak, no broken, appropriate number of samples and attached information).

4.3. METHODS/KITS USED

Participants used Bacterial isolation culture method (n=8), automatic identification equipment (n=6) or both methods (n=1) (Table 2).

Table 2. List of testing methods used by participants

No	Methods	Name of equipment	Quantity and percentage of participants used
1	Isolation culture	N/A	08 (53.3%)
2	Automatic identification equipment	Vitek 2	03 (20%)
		MALDI-TOF +VITEK	02 (13.3%)
		PHEONIX 100	01 (6.7%)
3	Isolation culture + Automatic identification equipment	VITEK 2	01 (6.7%)
Total			15 (100%)

Table 2 shows that out of 15 participating health facilities, 8/15 facilities use the culture method, accounting for 53.3%, and 6/15 (40%) use the automatic analysis equipment and 1/15 facilities using a combination of both methods, accounting for 6.7% participants used.

4.4. RESULTS OF PARTICIPANTS

No	Participant ID	Method/ equipment's used	Results					Number of correct results	Total score
			VK20- 01	VK20- 03	VK20- 05	VK20- 06	VK20- 07		
			<i>V. parahaemolyticus</i> <i>P. aeruginosa</i>	<i>Shi. Flexneri</i> <i>E. coli</i>	<i>Sal. Enteritidis</i> <i>C. freundii</i>	<i>Sal. Typhimurium</i> <i>P. mirabilis</i>	<i>E. coli</i> / <i>Non-agglutinating</i> <i>Cholera vibrios</i> (NAG)		
1.	NE02	Isolation culture	<i>Salmonella</i>	<i>Shi. flexneri</i> <i>E. coli</i> tam giá II	<i>Salmonella</i> nhóm T	<i>Salmonella</i> nhóm T <i>E. coli</i> tam giá II	<i>V. cholerae</i> O1 serotype Ogawa <i>E. coli</i> tam giá II	4/5	8/10
2.	NE03	Isolation culture	<i>E. coli</i>	<i>E. coli</i> gây bệnh, <i>Shigella</i>	Not detected	<i>Salmonella</i>	<i>E. coli</i> gây bệnh	3/5	6/10
3.	NE08	Isolation culture	<i>Salmonella</i>	<i>E. coli</i> , <i>Shigella flexneri</i>	<i>Proteus</i> , <i>Salmonella</i>	<i>E. coli</i> , <i>Salmonella</i>	<i>E. coli</i> <i>V. cholerae</i>	4/5	8/10
4.	NE09	Isolation culture	<i>E. coli</i> nhóm I	<i>Shi. flexneri</i>	<i>Sal. Typhi</i>	<i>Proteus</i>	<i>V. Cholerae- Ogawa</i>	2/5	4/10
5.	NE11	Isolation culture	<i>E. coli</i> nhóm II	<i>E. coli</i> nhóm II	<i>Salmonella</i> nhóm O9	<i>P. mitabilis</i>	<i>V. cholerae</i> O1 – Ogawa.	1/5	2/10
6.	NE13	VITEK II	<i>Salmonella</i> group	<i>Shigella</i> group	<i>C. freundii</i>	<i>E. coli</i>	<i>E. coli</i>	2/5	4/10
7.	NE28	VITEK II	<i>Salmonella</i> group	<i>E. coli</i>	<i>Salmonella</i> group	<i>Salmonella</i> group	<i>E. coli</i>	3/5	6/10
8.	NE37	VITEK II	<i>E. coli</i>	<i>E. coli</i> , <i>Salmonella spp</i>	<i>Salmonella spp.</i> <i>C. freundii</i>	<i>E. coli</i>	<i>E. coli</i>	2/5	4/10
9.	NE38	01,05, 06, 07: Maldi-ToF (MS), 02, 07: Vitek 2	<i>Sal. Enteritica</i>	<i>E. coli</i> <i>Shigella sp. (loại trừ Shigella sonnei)</i>	<i>Sal. enteritica</i>	<i>Sal. enteritica</i> <i>P. mirabilis</i>	<i>E. coli</i> <i>V. cholerae</i>	4/5	8/10
10.	NE53	Isolation culture	<i>Salmonella</i>	<i>Shigella</i>	<i>Salmonella</i>	<i>Salmonella</i>	<i>E. coli</i>	4/5	8/10
11.	NE57	Isolation culture	<i>Salmonella</i>	<i>E. coli</i>	<i>Proteus</i>	<i>Shingella</i>	<i>Vibrio</i> <i>E. coli</i>	1/5	2/10
12.	NE64	Phoenix 100	<i>E. coli</i> <i>V. parahaemolyticus</i>	<i>E. coli</i> <i>Shi. boydii</i>	<i>Salmonella</i> species <i>C. freundii</i>	<i>Salmonella</i> species	<i>E. coli</i>	5/5	10/10
13.	NE67	Isolation culture	<i>Salmonella</i>	<i>Shigella</i> <i>E. coli</i>	<i>Salmonella</i>	<i>Salmonella</i>	<i>Vibrio cholerae</i> <i>E. coli</i>	4/5	8/10
14.	NE73	01, 03,07 : vitek, 05: Vitek, serum agglutination 06: Isolation culture	<i>E. coli</i>	<i>E. coli</i>	<i>Salmonella</i> group D	<i>P. mitabilis</i>	<i>E. coli</i>	2/5	4/10
15.	NE83	Vitek® 2 và Vitek® MS	<i>Sal. enterica</i>	not detected	<i>Sal. enterica</i>	<i>Sal. enterica</i>	not detected	2/5	4/10
Number of correct results			1/15	9/15	12/15	9/15	12/15		

There are 8/15 (53.3%) participating medical facilities with 3/5 results matching the program's answers or more, equivalent to a score of 6/10 or more. There was only one unit with all the answers matching the results, scoring 10/10. Have 46.7% of the participating units scored less than 6/10 points.

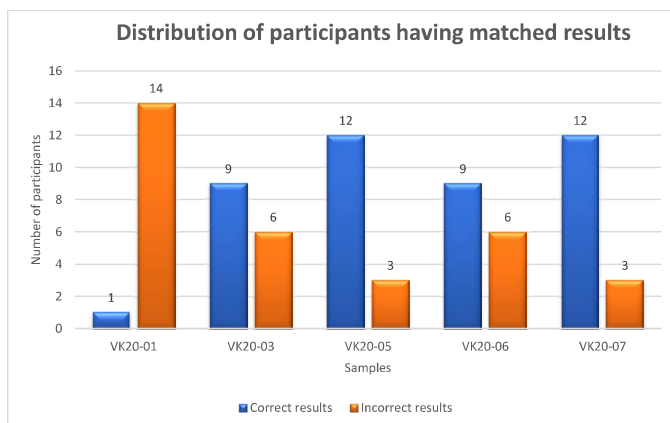


Figure 3. Distribution of participants having correct results

The chart above shows that with samples VK20-05 and VK20-07, there were many medical facilities with the most correct answers accounting for 12/15 facilities, sample VK-03 and VK20-06 both have the number of participating facilities with correct answers of 9/15 medical facilities. Sample VK20-01 had the lowest rate of correct results (only 1/15 facilities).

5. DISCUSSIONS

There are only 15 medical facilities participating in the EQA program, however there are many methods/ equipments used to perform the test of EQA samples including isolation culture method, Vitek2 machine, Maldi-tof machine, Phoenix machine. In which, 8/15 (53.3%) medical facilities use the method considered as the gold standard, which is the isolation culture method. The rest is to use automated methods. There are 2 facilities that use a combination of 2 different methods. The results showed that the unit using the Phoenix 100 gave accurate results of 5/5 samples, accounting for 100%. There was no discernible difference in test results between different methods/ equipment.

The EQA results also showed that the VK20-01 sample was the only one medical facility that gave correct test results. This is likely to be the most difficult sample in the distributed EQA sample panel. This shows that the quality of test results depends not only on the method selected by laboratories but also on chemicals, biological products, and testing techniques of medical staffs. Currently, laboratories still have some difficulties such as: human resources, training. One of the difficulties is that the participation in EQA programs to ensure the quality of testing is not regular and not diversified. Therefore, building an EQA network to provide more external control programs with different agents and techniques for laboratories is an important issue in improving the quality of testing for medical facilities. In addition, in Vietnam, there is no agency or organization capable of evaluating ISO 17043 for external audit program providers. This assessment must be done by experts from other countries (eg Thailand) so this is also a difficulty to consider when building an EQA network in Vietnam.

6. LIMITATIONS

Due to the complicated situation of the Covid-19 epidemic, there are only a few medical facilities participating in the external inspection program, especially only 15 medical facilities in Vietnam participate, there are no medical facilities of other countries in Southeast Asia. This leads to the sample size being not large enough and not representative of Southeast Asian countries.

7. CONCLUSIONS

- 15/15 (100%) participants completed EQA result reports with enough information and valid for data analysis.
- 1/15 (6.67%) of the participants showed correct results of all 5 samples in EQA panels comparing to EQA Program's results.
- Among participants presented wrong results, there is 2 participants (NE 11 and NE57) submitted only 1 out of 5 correct results.
- Among the EQA sample, sample VK20-01 had the lowest rate of correct results (only 1/15 facilities).
- The culture method is still considered the gold standard in bacterial diagnostic testing. However, the results show that the use of automated testing equipment also gives accurate results (eg phoenix: 100% correct results), so a combination of methods can be used to support each other in bacteriological testing.

8. RECOMMENDATIONS



- To avoid cross-contamination during handling, open the lid of the lyophilized vial, disinfect the vial cap with alcohol before opening. Sample vial openers need to be disinfected prior to opening and these activities should be performed in a biosafety cabinet.
- Unsatisfactory samples need to be conducted to find the cause and take corrective action.
- Participate in regular EQA programs to ensure test quality.