

**Project Completion Report**  
on  
**‘Establish Aquatic Animal Disease Diagnostic  
Laboratory Project’**



*An intervention, funded by the USAID under Feed the Future Bangladesh Aquaculture Activity project (FtF BAA) implemented by WorldFish (PLA: PLA12168, AG10423, BUS: BU11146, AEC: G008) in partnership with FISHTECH (BD) LIMITED.*

**04 April, 2021**



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## Acknowledgements

It is wished to express the profound sense of gratitude and sincere appreciation to all the concerned of USAID, FtF BAA, WorldFish for their great supports to establish the modern molecular laboratory (first Real Time PCR-based laboratory in Bangladesh) dedicated to the aquatic animal health, which is such a pragmatic and pressing intervention for the development of the aquaculture sector of Bangladesh.

It is also expressed the deepest sense of indebtedness and praise to the Management of Fishtech (BD) Limited especially Mr. Md. Ataul Karim Bhuiyan, Managing Director and Mr. Mohammed Tarique Sarker, Director (R&D) for initiating such constructive and well-timed steps for the improvement of the fisheries industries of Bangladesh by establishing a well-equipped sophisticated laboratory in Khulna, the Southern part of Bangladesh.

It is noteworthy that the successful execution of the project is accomplished by the continuous encouragements and proper coordination throughout the project implementation timeline by few of the personnel, especially Mr. Md. Nurul Karim Bhuiyan, Senior Manager, FtF BAA; Mr. Mohammad Hasnal Alam, Market System Specialist, FtF BAA, Mr. Rafiul Hasan Chowdhury, DGM, Fishtech (BD) Limited and Mr. Mohammad Sohel Miah, Manager (R&D), Fishtech (BD) Limited.

Moreover, it is commended to the two experts for their scholastic guidance, valuable suggestions and overall supervision to establish the laboratory in practical. Dr. Saengchan Senapin, Consultant, Fishtech Laboratory, Principal Researcher of National Center for Genetic Engineering and Biotechnology (BIOTEC), Pathum Thani, Thailand and Center of Excellence (Centex) for Shrimp Molecular Biology and Biotechnology, Mahidol University, Bangkok, Thailand; and Dr. Md. Mer Mosharraf Hossain, Co-Ordinator, Fishtech Laboratory, Associate Professor, Department of Fisheries and Marine Bioscience, Jashore University of Science and Technology, Bangladesh.

-The PoC, Subgrantee

## Executive Summary

Fishtech Laboratory is the first Real-Time PCR-based molecular laboratory in Bangladesh which is specialized in diagnosing a wide range of disease-causing pathogens of fish and shrimp as well as in providing testing facilities of many water and soil parameters of the culture ponds. There are three major units of the laboratory (i) Molecular Unit (detects 11 number of viruses & bacteria) (ii) Bacteriological Unit (provides 5 types of bacteriological tests) and (iii) Water & Soil Unit (determines 20 types of soil & water quality parameters) which are compulsory for the sustainable fish & shrimp farming. The aim of the laboratory is to ensure the problem identification & solution optimization with preventive or treatment measures to keeping apart from the devastating effects of the diseases and other constraints as well as avoiding the inappropriate and misuse of harmful antibiotics and chemicals with a cost effective and ecofriendly approach to the farmers. Fishtech Laboratory is ensuring access to the all scales of farmers (small scale farmers, commercial farmers, hatcheries, nurseries etc.) and customized services which are the pressing needs for their farms with a reasonable price ensuring the global standards. Emphasizing to boost up the small-scale farmers of Bangladesh especially in Khulna and Barishal division, Fishtech (BD) Limited is providing few portable testing facilities (water pH & ammonia) door to door to the farmers with its technical team (more than 150 people) for ensuring the productivity of their ponds. Likewise, the small-scale farmers are availing a special concessional charge for the laboratory-based tests. The laboratory is established in Khulna prioritizing as a farming area of both the fin fishes and export potential shrimps.

Fishtech (Bd) Limited established the laboratory through partnership with USAID funded Feed the Future Bangladesh Aquaculture Activity (BAA) project implemented by WorldFish during October 2019 to February 2021. As per the proposed and approved activities for establishing and marketing of the laboratory, 100% activities are well executed to make it in proper function to ensure the services. This Aquatic Animal Health laboratory is established by the consultation of an international laboratory expert Dr. Saengchan Senapin, Principal Researcher, Center of Excellence for Shrimp Molecular Biology and Biotechnology, Mahidol University, Bangkok, Thailand. The Coordinator of Laboratory is Dr. Mer Md. Mosharraf Hossain, Associate Professor, Fisheries and Marine Bioscience Department, Jashore University of Science & Technology, Jashore. The technicians of the laboratory are fisheries graduated and well trained by the Consultant and the Coordinator. After starting the laboratory in functioning, the commercial shrimp farmers are taking laboratory services. They are sending the water & soil samples through LSP (local service provider) and the Fishtech technical or marketing team by the proper guidance of the laboratory technicians. Notably, Fishtech technical team and few local youths were trained up on how to collect and preserve the samples using the laboratory equipment & materials and how to deliver the samples to the laboratory with proper cautions to avoid any kinds of contamination. A number of commissions paying LSPs are being increased day by day over the adjacent districts of Khulna & Barishal division. Earlier, many customers communication meeting, seminar and workshop were arranged and facebook page,

website and other promotional materials are being used to make aware the relevant stakeholders.

The Laboratory is also conducting researches & experiments in association with few Public Universities where Fisheries & aquaculture is studied. For example, Fisheries and Marine Resource Technology Discipline Laboratory of Khulna University, Fisheries and Marine Bioscience Department Laboratory of Jashore University of Science. The Fishtech Laboratory also keeps collaboration with the Quality Control Laboratory under the Department of Fisheries (DoF, GoB), Khulna and the Laboratory under Bangladesh Fisheries Research Institute (BFRI), Bagerhat.

In fine, enormous gratitude to the WorldFish for initiating such well-timed pragmatic intervention with the sustainable market system approach by enabling the private limited company in collaboration with Fishtech (BD) Limited which is one of the most responsible, reputed and market leading organizations in the aquaculture industries of Bangladesh. Fishtech is determined to increase customized laboratory facilities and services all over the country by creating a network among the customers, agents, service providers and other beneficiaries. Also continue research and experiments in relationship with national & international laboratories for finding out possible outcomes as much as we can for the development of the aquaculture sector of Bangladesh.

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### 1.1. Project Background:

Fisheries and aquaculture play an important role in the socio-economy of Bangladesh by providing 60% of animal protein to the population, generating employment opportunities and livelihoods (involved 18 million people to this sector) and supports to export earnings of 500 million USD per year for the country (DoF, 2020). However, with the advancement of the culture intensification and higher fish production in the farms, the disease occurrence has become a common threat to the fish farmers for the all-cultured fish and shrimp species. The economic impacts on aquaculture production due to disease outbreaks is alarming in fin fish and shrimp farming. A relevant report (Faruk, 2004) showed that average loss 344 USD per hectare of fish farming due to diseases incidence in Bangladesh. Shrimp farming is drastically affected by different severe disease-causing pathogens such as white spot syndrome virus (WSSV), *Vibrio parahaemolyticus* (causes AHPND), Enterocytozoon Hepatopenaei (EHP) and also so many pathogens. Likewise, in case of tilapia farming, severe mortality occurred due to the prevalence of Tilapia Lake Virus, *Streptococcus agalactiae*, *S. inae*, etc. Epizootic ulcerative syndrome (EUS) and so many diseases are caused in finfish (Indian major carps and other catfishes farming). A list of concerned bacterial, viral, fungal and parasitic diseases/infectious pathogens is shown in the draft National Fish Health Management Strategy of Bangladesh (NFHMSB) (Annex 1.1). The productivity rate in shrimp/ prawn farming is surprisingly very low, (0.504 MT/hectare per year only). Yield and productivity loss demotivate aquaculture entrepreneurs in terms of shifting businesses and switching culture practices. So, FISHTECH has given emphasis to reduce-

- Losses due to disease outbreaks to improving aquaculture production and profitability to the fish farmers, hatchery owners and other stakeholders.

### 1.2 Key components of the Proposed Laboratory

The name of the proposed laboratory will be 'Fishtech Laboratory'. The theme of the laboratory will be "a modern molecular laboratory for fish and shrimp health". There will be three major units for diagnosing any kind of problems of fish and shrimp farming. These are:

- (i) Water and soil quality testing unit: Physico-chemical parameters will be tested.
- (ii) Microbiological unit: Bacterial loads, CFU counting, plankton will be studied.
- (iii) Molecular unit: Viral and Bacterial molecular identification will be conducted.

### 1.3 Goal & objective of the proposed concept

The goal is to enable fish farmers, hatcheries, nurseries and other stakeholders in the aquaculture to increase the productivity and profitability by providing disease diagnostic services through establishing a well-equipped disease diagnostic laboratory for fish and shrimp.

FISHTECH is intended to set up a laboratory with the following specific objectives:

- to minimize the disease risks to the fish farmers to maximize their productivity and profitability;
- to ensure the disease free broodstock of the hatcheries to supply quality seeds to the farmers; and
- to establish a business model centering the fish disease diagnostic laboratory with the other stakeholders.

## 2.1 Approved Project Overview

1) Project Title: <b>Establish Aquatic Animal Disease Diagnostic Laboratory</b>			
2) Sub-grantee Name and Address: <b>FishTech (BD) Limited House# 02, Road# 07, Sector# 05, Uttara, Dhaka-1230</b>		3) Sub-grant agreement Title: <b>Bangladesh Aquaculture Activity (BAA)</b> Implemented by: WorldFish House# 2/B, Road# 04, Block# B, Banani, Dhaka-1213	
4) Sub-grant Period: <b>17 October 2019 to 28 February 2020</b>			
5) <b>PLA: PLA12168</b>	6) <b>AG10423</b>	7) <b>BUS: BU11146</b>	8) <b>AEC: G008</b>
		5) Sub-grant Amount: <b>BDT 11,928,896 (US Equivalent: USD142,095)</b> <b>WorldFish Contribution: BDT 7,608,877 (US Equivalent: USD90,636)</b> <b>Grantee Contribution: BDT 4,320,019 (US Equivalent: USD51,459)</b>	
9) <b>Sub-grant No Cost Extension-1 (01 October 2020 to 30 November 2020)</b>		First No cost Extension of the sub-grant agreement period to allow field operations that slowed down due to the effect of COVID-19.	
10) <b>Sub-grant No Cost Extension-2 (01 December 2020 to 28 February 2021)</b>		Second No cost Extension of the sub-grant agreement period to allow field operations that slowed down due to the effect of COVID-19.	



Activities	Measurable Unit	Total Target	Time Line																
			Oct-19	Nov-19	Dec-19	Jan-20	Feb-20	Mar-20	Apr-20	May-20	Jun-20	Jul-20	Aug-20	Sep-20	Oct-20	Nov-20	Dec-20	Jan-21	Feb-21
Training Module Preparation by the consultant	Unit	1															1		
Design the Laboratory by the consultant	Unit	1											1						
Conduct 10 days training for laboratory technicians conducted by Intl' consultant through online	Training	1															1		
Lab Visit & Protocol Optimization by the consultant	Visit	1																1	
Regional Promotional Event for existing clients youth and women farmers of FISHTECH	Event	4																2	2
Capacity building Workshop to the FISHTECH technical team (60 members= 30 participants *2 events)	Training	2																	2
Website development & update	Website	1																1	
Social media and mass media develop and update	No Budget	1																1	
Awareness building workshop for using quality seeds	Event	1																	1
Accreditation and certification by an International organization	Certificate	1																	1
Design/printing and distribution of promotional	Number	1000																	1,000

materials (Leaflet=1,000)																		
Design/printing and distribution of promotional materials (brochure 1,000 pcs)	Number	<b>1000</b>														1,000		
Advertisement on lab facilities to the national and local newspaper, local cable operator's channels		<b>1</b>														1		
Popular Article on Lab Facilities	Publication	<b>1</b>																1
Youth development program	Person	<b>1</b>																1
Project monitoring and recording (Soft) copy	Dossiers	<b>1</b>																1
Monthly Progress Report	Monthly	<b>17</b>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Monthly Financial Report	Monthly	<b>17</b>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
VAT Coupon Report	Monthly	<b>17</b>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Project Completion Report/Final Report	Programmatic	<b>1</b>																1

### 2.3 Approved budget summary

Summary Budget		Budget Ration		Revised Budget (BDT)		
SL#	Major Activity	% of WorldFish	% of Grantee	Total cost BDT	WorldFish	Sub-Grantee
01	Direct Labor (Personnel)	58%	42%	2,046,242	1,191,992	854,250
02	Supplies, Furniture & Equipment's	20%	80%	931,643	188,325	743,318
03	Travel and Per diem	81%	19%	231,861	187,146	44,715
04	Other Direct Cost	60%	40%	158,637	95,100	63,537
05	Activity/Program Costs - Services, training, workshops, products	59%	41%	10,011,020	5,941,356	4,069,664
	<b>Grand Total (BDT)</b>	<b>57%</b>	<b>43%</b>	<b>13,379,403</b>	<b>7,603,919</b>	<b>5,775,484</b>
	<b>Grand Total (USD)</b>	<b>57%</b>	<b>43%</b>	<b>159,478</b>	<b>90,636</b>	<b>68,842</b>

### 3. Accomplished Project Activities in details

#### 3.1 Agreement Signing Ceremony

The agreement signing ceremony held on 17<sup>th</sup> October 2019 at the WorldFish Office (House# 2/B, Road# 04, Block# B, Banani, Dhaka-1213) in the presence of the high officials from the both parties.



Plate-1: Agreement Signing. Signatories are: Jon Thiele, Chief of Party, FtF BAA, WorldFish & Mr. Md. Ataul Karim Bhuiyan, Managing Director, Fishtech (BD) Limited.



Plate-2: Exchange of signed copy of the Agreement in the presence of the high officials of FtF BAA, WorldFish & Fishtech (BD) Limited.



### 3.2 Staff recruitment

The staffs (LOE 100%) are recruited as per the HR policy of Fishtech (BD) Limited which follows the US compliance in terms of staff recruitment. The recruitment procedures were in brief as: Advertisement to the popular online portal of Bangladesh (www.bdjobs.com) and collection of the candidate's CV > Primary screening of the CVs and shortlisted the eligible candidates as per the ToR > Called for the written & Viva test > Candidates were selected based on their written, viva and experiences as per the HR policy of Fishtech (BD) Limited with a negotiated salary. Finally, the candidates selected are:

1. Mr. Md. Ariful Haque Rupom, Laboratory Technician, Fishtech Laboratory
2. Mrs. Habiba Islam, Jr. Laboratory Technician, Fishtech Laboratory
3. Mr. Md. Abdus Sabur, Admin & Accounts, Fishtech Laboratory



Plate-3: Interview board was held on November 2019 in the presence of the board members from the both organization FtF BAA, WorldFish & Fishtech (BD) Limited.

Notably, for the position of Laboratory Consultant (contractual) and Laboratory Coordinator (LOE100%), there was no competent candidates as per ToR. So, further job circular published and communicated to the national and international experts/professional in this field through their email. Following the similar aforementioned steps, finally two experts are selected:

1. Dr. Saengchan Senapin, Consultant, Fishtech Laboratory (contractual), Principal Researcher of National Center for Genetic Engineering and Biotechnology (BIOTEC), Pathum Thani, Thailand and Center of Excellence (Centex) for Shrimp Molecular Biology and Biotechnology, Mahidol University, Bangkok, Thailand; and
2. Dr. Md. Mer Mosharraf Hossain, Co-Ordinator, Fishtech Laboratory (contractual), Associate Professor, Department of Fisheries and Marine Bioscience, Jashore University of Science and Technology, Bangladesh.

In addition, the staffs for the project (LOE 5% to 20%) from the Sub grantee are also nominated by the Fishtech Management and they are also included accordingly.

### 3.3 Project Orientation Meeting to the Staffs and Views sharing

The recruited staffs were oriented with the project Goals & objectives, Project background, Proposed activities, target beneficiaries and future outcomes following the market system approach.



Plate-4: The staffs are being oriented on their job responsibilities. The speaker Mr. Mahammad Sohel Miah, Manager (R&D), Fishtech (BD) Limited.

After conducting the Orientation meeting, Views sharing meeting was held among the Fishtech staffs, FtF, BAA, WorldFish staffs and few local stakeholders.



Plate-5: Views sharing meeting held about the project aims and objectives among the Fishtech staffs, FtF, BAA, WorldFish staffs and few local stakeholders.

### 3.3 Procurement Committee formation

A procurement committee was formed for purchasing the laboratory apparatus, chemicals and consumable items ensuring the product price & quality for establishing the laboratory.


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Date: 07.07.2020  
Ref: 02020000173/FBD

**Committee for the Purchase of Lab Apparatus and Chemicals**  
Of the project  
*'Establish Aquatic Animal Disease Diagnostic Laboratory'*

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 (Md. Ataul Karim Bhuiyan)  
 Managing Director  
 FISHTECH (BD) LIMITED



Table-1: List of the Procurement Committee

The committee conducts several meetings through online (due to the COVID19 pandemic). Took necessary steps for procuring the items accordingly.

### 3.4 Meeting of the Procurement Committee with the Consultant & Coordinator

The consultant of the Laboratory prepared and shared the list of apparatus, chemicals and consumables. The consultant also made shared the laboratory design and the contents of training to the technicians for building up their capacity.

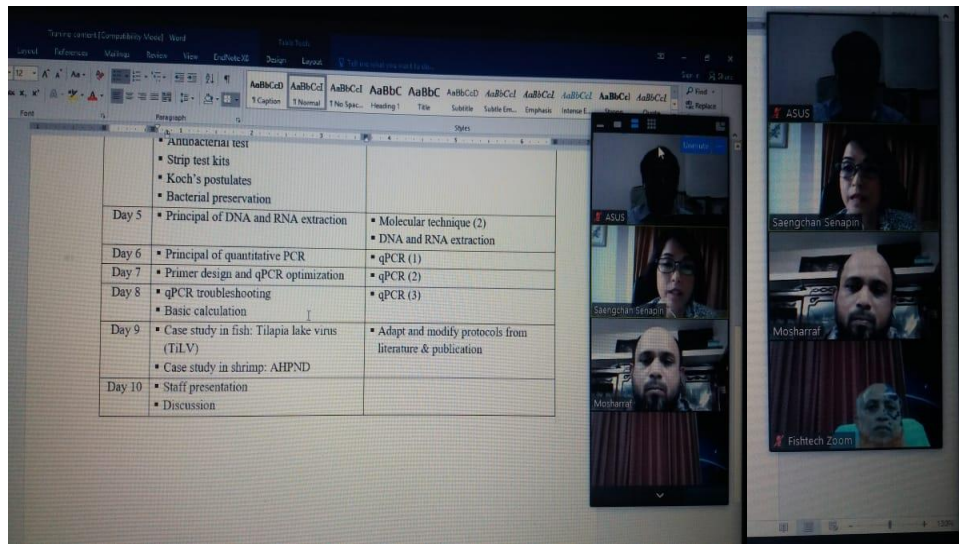


Plate-6: Virtual Meeting among the Consultant, Coordinator & the Procurement Committee: Discussing apparatus-chemical purchase following the US Compliance & the Training outlines in July, 2020

### 3.5 Establish the Laboratory

The consultant of the laboratory provided the Design of the Laboratory (Figure-1) along with the list of apparatus, chemical & consumables (Annex 1.2). The procurement committee maintain every steps of procurement. Such as i) Took approval from the procurement committee > ii) Sent RFQ to the vendors > iii) Collected quotations > iv) Made a bid evaluation summary > v) Sent copy of Award or purchase order to the vendors who are already negotiated based on their offered price and quality of the products > vi) Preserved the copy of invoices with challan > vii) Preserved other payments documents > and viii) Made a work completion report with photographs of the Laboratory.

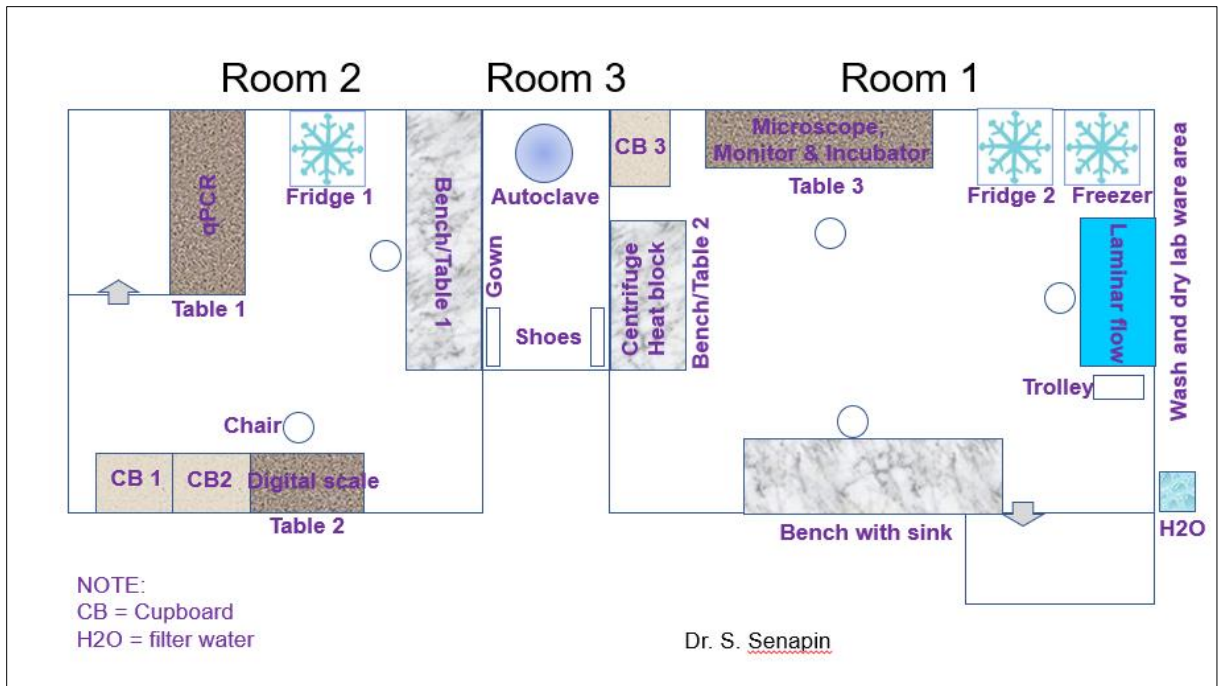


Figure-1: Design of the Fishtech Laboratory

### 3.5.1 Apparatus delivery to the Fishtech Laboratory at Khulna, Bangladesh

The awarded vendors supplied the apparatus, glass wares and few consumables to the Fishtech Laboratory on October 2020.



Plate-6: Delivered the apparatus to the Fishtech Laboratory



Plate-7: Shifting the apparatus to the Fishtech Laboratory



Plate-8: Apparatus safely reached to the Fishtech Laboratory



Plate-9: Scrutinizing the Apparatus as per the PO by the Coordinator of the Fishtech Laboratory



Plate-10: Scrutinizing the Installation & application of the RT- PCR Machine by the Coordinator of the Fishtech Laboratory



Plate-11: Organized Equipment's in Fishtech Laboratory



Plate-12: Neat & clean ensuring 100% Bio-safety of Fishtech Laboratory



### 3.6 Training to the Laboratory Technician (10 days) by the Consultant

Ten days long fundamental training given to the Laboratory Technicians. The training conducted by the Consultant and also monitored by the Coordinator at the Fishtech Laboratory. Duration: (~9.00-17.00 BD time).

#### Training Schedule and Contents

Date	Lecture/Discussion	Laboratory
Day 0 20.01.2021	<ul style="list-style-type: none"> <li>▪ Introducing Centex Shrimp</li> <li>▪ Briefing the workshop content</li> </ul>	
Day 1 21.01.2021	<ul style="list-style-type: none"> <li>▪ Discussion on laboratory workflow settings (SS)</li> <li>▪ Diagnosis in aquaculture (SS)</li> <li>▪ Introduction to aquatic animal health (DH)</li> <li>▪ <i>Brief lab</i> (SS)</li> </ul>	<ul style="list-style-type: none"> <li>▪ TSA &amp; TCBS agar plate preparation</li> </ul>
Day 2 22.01.2021	<ul style="list-style-type: none"> <li>▪ Fish &amp; shrimp anatomy, necropsy and sample collection (DH)</li> <li>▪ Tilapia &amp; catfish diseases I (DH)</li> <li>▪ <i>Brief lab</i> (SS, DH)</li> </ul>	<ul style="list-style-type: none"> <li>▪ Fish &amp; shrimp necropsy</li> <li>▪ Sample collection</li> <li>▪ Wet mount</li> <li>▪ Bacterial isolation</li> </ul>
Day 3 23.01.2021	<ul style="list-style-type: none"> <li>▪ Tilapia &amp; catfish diseases II (DH)</li> <li>▪ <i>Brief lab</i> (SS, DH)</li> </ul>	<ul style="list-style-type: none"> <li>▪ Gram staining</li> <li>▪ Bacterial culture</li> </ul>
Day 4 25.01.2021	<ul style="list-style-type: none"> <li>▪ Microbiological techniques (SS)</li> <li>▪ Bacteria identification techniques (SS)</li> <li>▪ Shrimp diseases: WSSV, EHP, AHPND, Vibrio (SS)</li> <li>▪ <i>Brief lab</i> (SS)</li> </ul>	<ul style="list-style-type: none"> <li>▪ Bacterial preservation</li> <li>▪ DNA extraction from bacteria</li> </ul>
Day 5 26.01.2021	<ul style="list-style-type: none"> <li>▪ Principle of DNA and RNA extraction (SS)</li> <li>▪ DNA/RNA quantification</li> <li>▪ <i>Brief lab</i> (SS)</li> </ul>	<ul style="list-style-type: none"> <li>▪ DNA and RNA extraction from fish and shrimp</li> </ul>
Day 6 27.01.2021	<ul style="list-style-type: none"> <li>▪ Principle of quantitative PCR (SS)</li> <li>▪ qPCR machine operation and data analysis (SS)</li> <li>▪ <i>Brief lab</i> (SS)</li> </ul>	<ul style="list-style-type: none"> <li>▪ qPCR (1)</li> </ul>
Day 7 28.01.2021	<ul style="list-style-type: none"> <li>▪ Primer design and qPCR optimization (SS)</li> <li>▪ qPCR troubleshooting (SS)</li> <li>▪ Basic calculation (SS)</li> <li>▪ <i>Brief lab</i> (SS)</li> </ul>	<ul style="list-style-type: none"> <li>▪ qPCR (2)</li> </ul>
Day 8 30.01.2021	<ul style="list-style-type: none"> <li>▪ OIE-listed diseases of fish and shrimp (SS)</li> <li>▪ Antibody based diagnosis (SS)</li> <li>▪ <i>Brief lab</i> (SS)</li> </ul>	<ul style="list-style-type: none"> <li>▪ qPCR (3)</li> </ul>
Day 9 31.01.2021	<ul style="list-style-type: none"> <li>▪ Case study in fish: Tilapia lake virus (DH)</li> <li>▪ Case studies of tilapia red egg disease and AHPND in shrimp (SS)</li> </ul>	<ul style="list-style-type: none"> <li>▪ Lab discussion</li> </ul>
Day 10 02.02.2021	<ul style="list-style-type: none"> <li>▪ Staff's presentation</li> <li>▪ Discussion</li> </ul>	

Workshop staff	Affiliation
Dr. Saengchan Senapin (SS) senapin2010@gmail.com	National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA) & Fish Health Platform, Center of Excellence for Shrimp Molecular Biology and Biotechnology (Centex Shrimp), Faculty of Science, Mahidol University, Bangkok, Thailand
Dr. Ha Thanh Dong (DH) hadongntu@gmail.com	Faculty of Science and Technology, Suan Sunandha Rajabhat University, Bangkok, Thailand
Participants	Affiliation
Mrs. Habiba Islam	FISHTECH LABORATORY
Mr. Shariar Kabir Shourov	FISHTECH LABORATORY
Mr. Sudhin Kumar Biswash	FISHTECH LABORATORY

*Saengchan Senapin*

**(Dr. Saengchan Senapin)**  
Consultant  
Fishtech Laboratory

N.B.: Training activities and training report shown in the Annex-1.3



Plate-13: Training activities in the Fishtech Laboratory



Plate-14: Training activities in the Fishtech Laboratory

### 3.7 Training to the Fishtech Technical Team

Two training conducted to develop the capacity to the Fishtech Technical Services Officers who are already involved in the market for ensuring the technical guidance to the customers particularly from the ZOI of FtF BAA. The main issue of the training was 'Sample Collection, Preservation and Delivery Procedures'. The training conducted by the Laboratory Technician Mrs. Habiba Islam and Dr. Tayabur Rahman.



Plate-15: Training to Technical Staffs of Fishtech (BD) Limited on Sample collection, Preservation & Delivery Procedures of Fish and Shrimp Farm

### 3.8 Training to the Local Youth

One training conducted to develop the capacity to the Local Youths. The main issue of the training was 'Sample Collection, Preservation and Delivery Procedures'. The training conducted by the Laboratory Technician Mrs. Habiba Islam at the Fishtech Laboratory.



Plate-16: Youth Development Training to the Local Youth on Sample collection, Preservation & Delivery Procedures of Fish and Shrimp Farm

### 3.9 Laboratory visited

Several team from Fishtech (BD) Limited and WorldFish visited the progress of the laboratory activities at Khulna.



Plate-17: Country Finance Manager of WorldFish (Mrs. Tahmina Begum) Visits the Fishtech Laboratory in January 2021



Plate-18: High official of Fishtech Honorable DGM and Manager (Accounts) Visit the Fishtech Laboratory in November 2020

### 3.10 Grand Opening of the Laboratory

A WorldFish-led partnership to increase fish and shrimp disease research and technical service for sustained aquaculture growth and livelihoods of small-scale farmers in southern districts in Bangladesh. WorldFish Bangladesh joined their hands with Fishtech (BD) Limited to a grand opening and ribbon-cutting ceremony to celebrating the newest the 'Aquatic Animal Disease Diagnostic Laboratory', opening on 11 February, 2021.



Plate-19: High officials of Fishtech, DoF and Khulna University present during the Grand Opening Ceremony of Fishtech Laboratory on 11 February 2021



Plate-20: High officials of Fishtech, DoF and Khulna University (Pro-vice Chancellor, Professor Dr. Mosummath Hosna Ara) present during the Grand Opening Ceremony of Fishtech Laboratory on 11 February 2021

**3.10.1 There are few news coverages on the Grand Opening of Fishtech Laboratory:**

Financial Express: <https://www.thefinancialexpress.com.bd/home/worldfish-bangladesh-opens-first-ever-aquatic-animal-disease-diagnostic-laboratory-1613054465?amp=true>

The World News: <https://www.google.com/amp/s/theworldnews.net/amp/bd-news/maacher-rog-gbessnnaayy-phishttek-lyaabrettri-r-yaatraa-shuru?fbclid=IwAR3RItiIrWZ3HoL9zzqJhwmhS5ENLUiVkrZly0DGEzf9sIWojvtHqEf0sE>

The Business Standard

(English): <https://www.google.com/amp/s/tbsnews.net/economy/agriculture/laboratory-launched-boost-fish-production-khulna-200332%3famp?fbclid=IwAR1CkKDr2V31qqKuxLZLNZpZFqUzEH8OBbwNT3anL0uSX997xuf70SIGlh4>

BanglaTribune: <https://banglatribune.com/666486/%E0%A6%AE%E0%A6%BE%E0%A6%B%E0%A7%87%E0%A6%B0-%E0%A6%B0%E0%A7%8B%E0%A6%97-%E0%A6%97%E0%A6%AC%E0%A7%87%E0%A6%B7%E0%A6%A3%E0%A6%BE%E0%A7%9F-%E0%A6%AB%E0%A6%BF%E0%A6%B6%E0%A6%9F%E0%A7%87%E0%A6%95-%E0%A6%B2%E0%A7%8D%E0%A6%AF%E0%A6%BE%E0%A6%AC%E0%A6%B0%E0%A7%87%E0%A6%9F%E0%A6%B0%E0%A6%BF%27%E0%A6%B0-%E0%A6%AF%E0%A6%BE%E0%A6%A4%E0%A7%8D%E0%A6%B0%E0%A6%BE-%E0%A6%B6%E0%A7%81%E0%A6%B0%E0%A7%81?fbclid=IwAR1CkKDr2V31qqKuxLZLNZpZFqUzEH8OBbwNT3anL0uSX997xuf70SIGlh4>

Agrinews24: <http://www.agrinews24.com/%E0%A6%96%E0%A7%81%E0%A6%B2%E0%A6%A8%E0%A6%BE%E0%A7%9F-%E0%A6%A6%E0%A7%87%E0%A6%B6%E0%A7%87%E0%A6%B0-%E0%A6%AA%E0%A7%8D%E0%A6%B0%E0%A6%A5%E0%A6%AE-%E0%A6%B8%E0%A6%B0%E0%A7%8D%E0%A6%AC%E0%A6%BE/>

Agrilife24.com:

<http://agrillife24.com/2021/2018-02-24-11-10-23/4658-fishtech-11fb.html>

### 3.11.1 Collaboration with other Laboratories:

It is important to keep collaboration with few laboratories of the same field for the proficiency test (PT) issues. PT is kind of cross-checking test for confirming the efficacy of the instrument and results. In this regard, Fishtech Laboratory signed a Memorandum of Understanding (MoU) with the Laboratory of the Fisheries and Marine Resource Technology (FMRT) Discipline, Khulna University during the inaugural of the laboratory.



Plate-21: MoU Signing between Fishtech Laboratory & FMRT, Khulna University

### 3.12 Accreditation by the Bangladesh Accreditation Board (BAB):

Accreditation procedures of the Fishtech Laboratory is under process, which requires at least 3-4 months for completing all the procedures. After finishing the formal procedures, the laboratory will avail the accreditation certificate.



## 4. Promotional activities

### 4.1 Regional Stakeholders Seminar

Three Regional Stakeholders Seminar conducted at Khulna, Shatkhira and Jashore inviting the target beneficiaries, for example fish and shrimp farmers, hatchery owners, dealer and other stakeholders. Average attendees were 40 in those events.



Plate-22: Seminar at Khulna



Plate-23: Seminar at Shatkhira



Plate-24: Attendees in the Seminar at Khulna



Plate-25: Seminar at Jashore

### 4.2 Promotional materials (brochure & leaflet)

Two categories of promotional materials developed and distributed to the customers.

The brochure is 12 pages (the contents are the introduction, offered three categories of services, details of the technical, about the Fishtech (BD) Limited etc.).

The leaflet (3 foldings) in Bangla and English versions are printed (the contents are the introduction, offered three categories of services and their prices).

Establish Aquatic Animal Disease Diagnostic Laboratory Project



Plate-26: The cover page of the brochure

Plate-27: English & Bangla version of the leaflet



#### 4.4 Advertisement to the local daily newspaper:

The following advertisement published on the Daily Janmobhumi newspaper in Khulna.

**মাছ ও চিংড়ির রোগ নির্ণয়ে দেশের প্রথম আর টি-পিসিআর ভিত্তিক  
'ফিসটেক ল্যাবরেটরী' এর সেবা সমূহঃ**



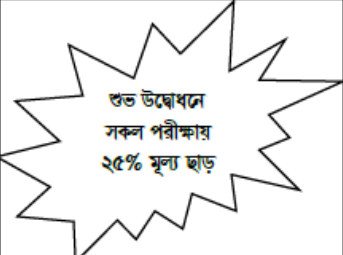
মাছ ও চিংড়ির নির্ণয়কৃত রোগ সমূহঃ	ব্যাকটেরিওলজিক্যাল পরীক্ষা সমূহঃ	পুকুরের পানির পরীক্ষা সমূহঃ
১) চিংড়ির হোয়াইট স্পট (WSSV)	১) Water Vibrio plate count	১) আয়রন (Fe++)
২) চিংড়ির এএইচপিএনডি (EMS)	২) Soil Vibrio plate count	২) পটাশিয়াম (K+)
৩) চিংড়ির ইএইচপি (EHP)	৩) Bacterial load in water column	৩) ক্যালসিয়াম (Ca++)
৪) তেলাপিয়া লেক আইরাস (TiLV)	৪) Plankton abundance tests	৪) ম্যাগনেসিয়াম (Mg++)
৫) তেলাপিয়ার স্ট্রেপ্টোকক্কাস <i>Streptococcus agalactiae</i>	৫) Plankton Profile study	৫) সোডিয়াম (Na+)
৬) তেলাপিয়ার স্ট্রেপ্টোকক্কাস <i>Streptococcus iniae</i>	<b>পুকুরের মাটির পরীক্ষা সমূহঃ</b>	৬) টোটাল হার্ডনেস
৭) <i>Flavobacterium columnare</i>	১) মাটির সোডিয়াম (Na+)	৭) নাইট্রাইট
৮) <i>Pseudomonas</i>	২) মাটির ক্যালসিয়াম (Ca++)	৮) টোটাল অ্যামোনিয়া নাইট্রোজেন
৯) <i>Edwardsiella</i>	৩) মাটির ম্যাগনেসিয়াম (Mg++)	৯) অ্যামোনিয়া
১০) <i>Aeromonas</i>	৪) মাটির পটাশিয়াম (K+)	১০) দ্রবীভূত অক্সিজেন
১১) <i>Vibrio</i>		১১) লবনাচ্ছতা
		১২) পি-এইচ
		১৩) কার্বন-ডাই-অক্সাইড
		১৪) অ্যাকফসিনিটি
		 <p>সর্ব উল্লেখনে সকল পরীক্ষায় ২৫% মূল্য ছাড়</p>
<p>কিছু বিস্তারিত তথ্যের জন্য যোগাযোগ করুন : +৮৮০১৯৫৮৫১১৫৪৯ +৮৮০১৯২৬৯৯০৫১৬ এবং +৮৮০১৯২৬৯৯০১৭১</p>		

Table-2: Advertisement to the local newspaper

#### 4.5 Popular article publish:

A popular article published titled as 'Potentiality of Fishtech Laboratory for Disease Diagnosis' written by Dr. Md. Mer Mosharraf Hossain, Associate Professor, Fisheries and Marine Bioscience department, Jashore University of Science and Technology.

<https://news.fishtechlaboratory.com/category/articles/>

## 5. Coordination Meetings

### 5.1 Bi-monthly Coordination Meeting

One bi-monthly coordination meeting was held focusing the activity progress and the next activities to be accomplished in time.

### 5.2 Final Coordination Meeting

One final coordination meeting was held virtually among the staffs of WorldFish and Fishtech (BD) Limited. Where the activities performed are shown and discussed on the future progress of the Laboratory.

## 6. Project outcomes:

### 6.1. Target beneficiaries:

No.	Target beneficiary groups	Reached the no. of target beneficiaries	Total no. target for 6 months	Remarks
1.	Small scale farmers	320	2500	
2.	Commercial shrimp & fish farmers	15	250	
3.	Shrimp Hatcheries	1	10	
4.	Tilapia & fin fish Hatcheries	1	15	
5.	Researcher/students	7	200	
6.	Women farmers	0	25	
7.	Local Youths	3	3	
	<b>Total</b>	<b>347</b>	<b>3003</b>	

## 6.2. Business outcomes for Fishtech (BD) Limited:

### 6.2.1 Return from Laboratory tests:

No.	Types of tests	Quantity	Unit price (tk)	Total price (tk)
1.	Microbiological	30	170	5,100
2.	Water & soil	26	150	3,900
3.	PCR	5	2,625	13,125
	<b>Total</b>			<b>30,150.00</b>

### 6.2.2 Sales increased of other segments of Fishtech (BD) Limited:

#### 6.2.2.1 Sales increased of Fish seeds:

Fishtech (BD) Limited has a sister organization Fishtech Hatchery Limited. The hatchery produces and sales Shrimp seed, Prawn seed and Tilapia seed from its three different hatcheries. Fishtech ensured the brood fishes and the produced baby fishes from its hatcheries are quite free from any kinds of diseases for all of the hatcheries. Which gives a difference to the seeds of Fishtech Hatchery than those of others. As per the data from the sales department, the total sales volume of March 2021 is 30% higher than the previous years. It is also observed that there is an increasing demand trend among the conscious farmers for the laboratory tested seeds.

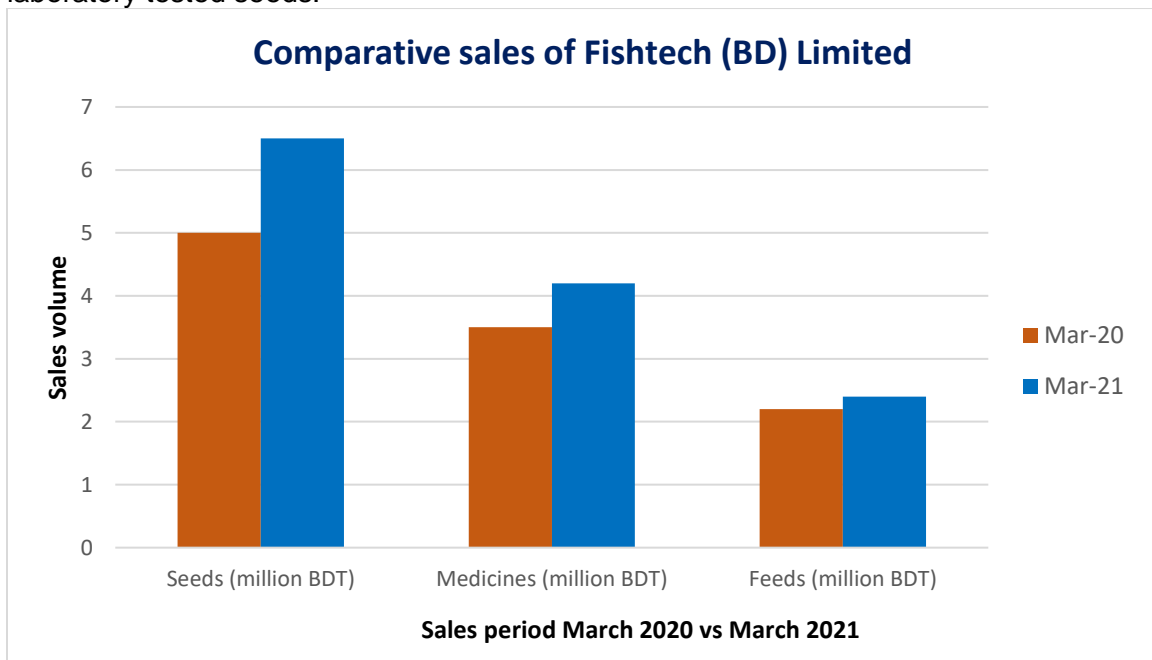


Figure 03: Comparative sales of Fishtech (BD) Limited (March 2020 vs March 2021)

**6.2.2.2 Sales increased of Fish medicine:**

Aqua medicine sales are being increased of Fishtech (BD) Limited day by day in the Southern part of Bangladesh as well as all over the country. Around 20% sales increased in the aqua medicine segment of Fishtech during the last month in compared to the last year.

Remarkably, Fishtech is making lot of laboratory trials for increasing efficacy of their probiotics. For example, Fishtech has got an outstanding finding on their one of the popular probiotics GASONEX+Y (ammonia reducer products). Recently, the R&D Department of Fishtech conducted few experiments on their GASONEX+Y. They founded that if they apply the product using the Activation Method (activate the probiotics with Sugar for 24 hours (as a source of organic carbon), the number of probiotic colonies are massively increased). Notably, the probiotic was being used through Direct Methods (no activation before application) to the farmers ponds. As a result, the efficacy of the GASONEX+Y has been increased, which made the sales persons of the company very confident on their own product. Before, setting the laboratory, the staffs had no chance to make any experiment for enhancing the efficiency.



Plate-28: Experiment materials of GASONEX+Y in Fishtech Laboratory



Plate-28: Sample preparation and incubation in Fishtech Laboratory



Plate-28: Colony observation (less colonies)

Plate-28: Colony observation (uncountable colonies)

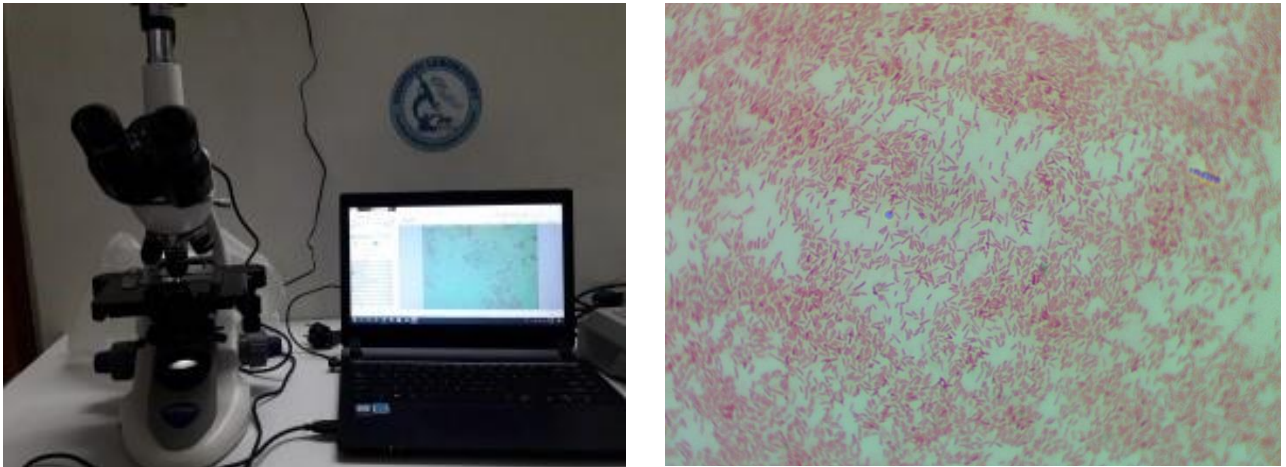


Plate-28: Microscopic observation of the colonies in Fishtech Laboratory

#### 6.2.2.3 Sales increased of Fish feeds:

Sales volume is also getting boost up in the feed segment as well by 04%. Hopefully it will be explored greatly during the pick season (April to December).

#### 6.2.2.4 Branding:

Over all the Brand Image of Fishtech (BD) Limited is being extended.

### 7. Future Plan

- Increase the customer involvement by ensuring their benefits due to laboratory tests (750 PCR tests in 2021 and 1000 PCR tests in 2022).
- Enhance the product efficacy experiment of Fishtech (BD) Limited (GASONEX+Y, other tow probiotics Viva soil, Ecotoxnil; Pond sanitizer POLGARD+).
- Increase customized laboratory facilities and services all over the country (heavy metal tests, feed proximate tests)
- Continue research in relationship with national & international laboratories
- Ensure the more training to the technicians.

### 8. Conclusion

The aquatic animal health laboratory will be a great initiative for the development of the aquaculture sector of Bangladesh, which denotes the institutionalization and enhance the opportunity to many ways.



## Annex

### Annex 1.1: List of fish pathogens/ diseases of concern

Prepare the list pathogens/ diseases of concern is a major goal the National Fish Health Management Strategy of Bangladesh (NFHMSB). The list has been prepared in consideration of cultured and traded species, the economic impact of diseases, the threat of exotic diseases and diseases present in neighboring countries in view of shared water sheds and land borders, as well as existing regional and world organization for animal health (OIE) disease lists (OIE, 2018).

Table 1.1: List of fish pathogens/ diseases of concern Justification (susceptible species)	Included in NFHMSB	
<b>1. FISH</b>		
<b>1.1 OIE listed Disease/pathogen</b>		
EUS / <i>Aphanomyces invadans</i>	Snakehead, carp	√
Red sea bream iridoviral disease	Seabass	√
Spring viraemia of carp	Chinese and Indian carp	√
<b>1.2 Non OIE listed Disease/ pathogen</b>		
<b>1.2.1 Viral diseases</b>		
Tilapia Lake Virus (TiLV)	Tilapia	√
Viral encephalopathy and retinopathy	Asian seabass	√
Tilapia larvae encephalitis by herpes virus	Tilapia	√
Spinning Tilapia by iridovirus	Tilapia	√
Carp edema virus disease	Gold fish, common carp	√
<b>1.2.2 Bacterial diseases</b>		
Enteric septicaemia of catfish ( <i>Edwardsilla ichtaluri</i> )	Pangasius	√
Dropsy	Carp	√
Bacillary Necrosis of Pangasius (BNP)	Pangasius	√
Tail and fin rot ( <i>Pseudomonas fluorescens</i> )	Carp, Tilapia	√
Gill rot disease / Branchiomycosis	Common carp	√
<i>Aeromonas hydrophila</i>	FW Fish	√
Edwardsiellosis ( <i>Edwardsiella tarda</i> )	Pangasius, walking catfish	√
<i>Mycobacterium</i>	FW Fish (Zoonotic)	√
Streptococcosis ( <i>Streptococcus</i> )	Tilapia	√

<i>Enterococcus faecalis</i>	Tilapia, catfish	
<i>Flavobacterium</i>	Tilapia, Climbing perch	√
<b>1.2.3 Parasitic disease</b>		

Justification (susceptible species)	Included in NFHMSB	
<i>Lerneasis (Lernaea sp)</i>	Carp, Tilapia	√
<i>Argulosis (Argulus sp)</i>	Carp, Tilapia	√
<i>Trichodina</i>	Carp, Tilapia	√
<i>Myxobolus sp</i>	FW Fish	√
<i>Gyrodactylus sp</i>	FW fish	√
<i>Dactylogyrus sp</i>	FW fish	√
<i>White spot disease (Ichthyophthirius multifiliis)</i>	Carp	√
Isopod infestation	Tilapia, <i>Puntius sp</i>	√

**1.2.4 Fungal disease**

Saprolegniasis ( <i>Saprolegnia sp</i> )	FW Fish	
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**2. CRUSTACEANS**
**2.1 OIE listed Disease/ pathogen**

Acute hepatopancreatic necrosis disease (AHPND)	Shrimp	√
<i>Hepatobacter penaei</i> / necrotising hepatopancreatitis (NHP)	Shrimp	√
Infectious hypodermal and haematopoietic necrosis virus (IHHNV)	Shrimp	√
Infectious myonecrosis virus (IMNV)	Shrimp	√
<i>Macrobrachium rosenbergii</i> nodavirus (MrNV) (white tail disease) and extra small virus (XSV)	Prawn	√
Taura syndrome virus (TSV)	Shrimp	√
White spot disease (WSD) /WSSV	Shrimp	√
Yellow head virus genotype 1 (YHV)	Shrimp	√

**2.2 Non OIE listed Disease/ pathogen**
**2.2.1 Viral diseases**

Monodon Baculo virus (MBV)	Shrimp	√
Monodon slow growth syndrome (MSGs)	Shrimp	√
Shrimp hemocyte iridescent virus (SHIV)	Shrimp	√

Hepatopancreatic parvo virus (HPV)	Shrimp	√
Baculovirus penaei (BP)		√
Baculoviral Midgut gland Necrosis (BMN)	Shrimp	√
<i>Macrobrachium rosenbergii</i> Taihu Virus (MrTV)	Prawn	√
Covert Mortality nodavirus (CMNV)	Shrimp	√
<b>2.2.2 Bacterial disease</b>		
Vibriosis ( <i>Vibrio harveyi</i> , <i>V. parahaemolyticus</i> )	Shrimp	√
<i>Spiroplasma eriocheiris</i>	Prawn/ Crab	√
Black gill disease	Shrimp/ prawn	
<b>2.2.3 Parasitic disease</b>		
Hepatopancreatic microsporidiosis ( <i>Enterocytozoon hepatopenaei</i> ) (HPM-EHP)	Shrimp	√
Isopod infestation	Shrimp, Prawn	√
<i>Zoothamnium</i>	Shrimp, Prawn	√

## Annex 1.2

<b>Table 1.2.1 List of Equipment</b>			
<b>No.</b>	<b>Items</b>	<b>Brand</b>	<b>Amount</b>
1	BIORAD CFX Connect Real-Time PCR Detection System & computer	BIORAD	1
2	Microscope and monitor	Olympus or Leica	1
3	Biosafety Cabinet Class II	Esco or any	1
4	Electric Bunsen Burner		1
5	Gas		1
6	2 door-fridge		2
7	Freezer (-20°C)		1
8	Autoclave		1
9	Centrifuge		1
10	Mini centrifuge		1
11	Heat block		1
12	Pipette 2 ul	Gilson or Eppendorf	2
13	Pipette 20 ul	Gilson or Eppendorf	2
14	Pipette 200 ul	Gilson or Eppendorf	2
15	Pipette 1000 ul	Gilson or Eppendorf	2
16	Digital weighing scale		1
17	Lab incubator		1

No.	Items	Brand	Amount
1	Table		3
2	Bench/Table		2
3	Chair		5
4	Bench with sink		1
5	Cupboard		3
6	Wash and dry for lab ware set		1
7	Water filter		1
8	Water tank		2
9	Shoes		6
10	Gown hanger		1
11	Gown		2
12	Rack for freezer		6
13	Cardboard boxex		30
14	Trolley		1
15	Bin		5
16	Power extension cord		4

No.	Items	Brand	Amount
1	Glass bottle 100 ml		2 to 5
2	Glass bottle 250 ml		2 to 5
3	Glass bottle 500 ml		2 to 5
4	Glass bottle 1000 ml		2 to 5
5	Glass beaker 200 ml		2 to 5
6	Glass beaker 500 ml		2 to 5
7	Glass beaker 1000 ml		2 to 5
8	Glass Erlenmeyer flask 500 ml		2 to 5
9	Glass Erlenmeyer flask 1000 ml		2 to 5
10	Tip 2 ul		10 boxes
11	Tip 20 ul		10 boxes
12	Tip 200 ul		10 boxes
13	Tip 1000 ul		10 boxes
14	Sterile 0.1 ml PCR strip tubes & lids	BIORAD	200-500 pieces
15	Sterile 1.5 ml tubes		1000 pieces
16	Racks for 1.5 ml tubes		5-10 racks
17	Sterile 15 ml tubes		100 tubes
18	Sterile 50 ml tubes		100 tubes
19	Plastic water & alcohol bottles		6
20	Alcohol Bunsen		2

## Establish Aquatic Animal Disease Diagnostic Laboratory Project

21	Plastic spray bottles	6
22	Stainless steel loop	10
23	Stainless steel needles	10
24	Glass slides	5-10 boxes
25	Cover slips	5-10 boxes
26	Sterile Petri dish, 90 mm diameter	200 plates
27	Plastic dropper, 3 ml	50
28	Syringe 1 ml	30 to 50
29	Syringe 3 ml	30 to 50
30	Needle 23G	30 to 50
31	Needle 26G	30 to 50
32	Plastic cylinders 100 ml	1
33	Plastic cylinders 250 ml	1
34	Plastic cylinders 500 ml	1
35	Plastic cylinders 1000 ml	1
36	Tissue homogenizer	50-100
37	Surgery set	2
38	Stainless steel tray	2
39	Lab notebook	5 to 10
40	Permanent pen	10
41	Timer	2
42	Calculator	2
43	Stationary	2 sets
44	Cotton	1 pack
45	Filter cloth	1 piece (2 meter)
46	Aluminum foil	2-3 packs
47	Tissue paper	20 boxes
48	Autoclave tape	1 role
49	Hand towel	5
50	PP plastic bags	2-3 packs
51	Rubber bands	1 pack
52	Zip loack bags	2-3 packs
53	Black garbage bags	2-3 packs
54	Face masks	5 boxes
55	Latex glove	5 boxes each size
56	Cloth glove	1 pair
57	Clear tape and stand	2
58	Plastic basket	2
59	Scissors	2
60	Ice box	2
61	Salinity meter	1
62	Plastic containers	3 to 5
63	Air pump set	2 to 3

64	Cleaning brushes & sponge		1 set
65	Glass rod (15 & 20 cm)		1 set
66	Lab thermometer (-2-100 °C)		1

<b>Table 1.2.4 List of Chemicals and Reagents</b>			
<b>No</b>	<b>Items</b>	<b>Brand</b>	<b>Amount</b>
1	Probe based PCR reagents for detection of WSSV, AHPND, EHP	TBI*	
2	Probe based RT-PCR reagent for detection of TiLV	TBI*	
3	SYBR based PCR reagent for confirmation of bacteria	TBI*	
4	Probes and primers for WSSV	TBI*	
5	Probes and primers for AHPND	TBI*	
6	Probes and primers for EHP	TBI*	
7	Probes and primers for TiLV	TBI*	
8	Primers for Streptococcus agalactiae	TBI*	
9	Primers for Streptococcus iniae	TBI*	
10	Primers for Flavobacterium columare	TBI*	
11	Primers for Pseudomonas	TBI*	
12	Primers for Edwardsiella	TBI*	
13	Primers for Vibrio	TBI*	
14	Primers for Aeromonas	TBI*	
15	Positive controls	Consultant	
16	DNA extraction kit (Qiagen DNeasy Blood & Tissue Kit)	Qiagen	2x50 reactions
17	RNA extraction kit (Qiagen RNeasy Mini Kit)	Qiagen	2x50 reactions
18	Gram stain kits		2
19	10X TE buffer		2x500 ml
20	10X PBS buffer		2x500 ml
21	PCR grade water		5x500 ml
22	Tryptic Soy Broth (TSB)		2 to 3
23	Tryptic Soy Agar (TSA)		2 to 3
24	Thiosulfate-citrate-bile salts-sucrose agar (TCBS)		2 to 3
25	Cytophaga agar		2 to 3
26	Streptococcus agalactiae agar M1257 HiMedia		2 to 3
27	Bacto Agar		2 to 3
28	Sodium Chloride		0.5-1 kg
29	95% Ethyl Alcohol, molecular grade		4-5 bottles
30	95% Ethyl Alcohol, lab grade		2-3 bottles
31	Clove oil		1-2 bottles
32	Paraformaldehyde (37-40% Formalin)		1-2 bottles
33	Aquarium salt		5 kg

Establish Aquatic Animal Disease Diagnostic Laboratory Project

34	Bleach		2-3 bottles
35	Washing detergent		2-3 bottles
36	Liquid hand soap		2-3 bottles
	*TBI=to be informed		

**Annex 1.3:**

Training Completion report as follows:

**Report on  
10 Days Training**



Prepared By-  
**Fishtech Laboratory**

**Lab work on Training at a glance**

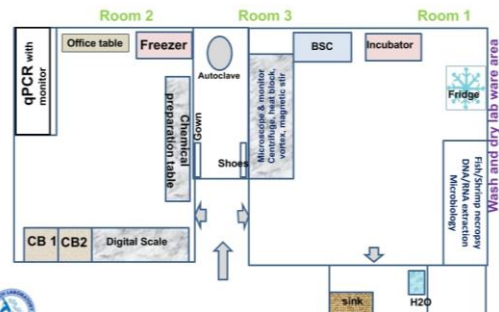
1. Laboratory workflow settings
2. TSA agar plate preparation
3. Fish & shrimp necropsy
4. Sample collection & preservation
5. Wet mount
6. Bacterial isolation
7. Gram staining
8. Bacterial culture
9. Rapid identification on Tilapia, Catfish & Shrimp diseases
10. Bacterial preservation
11. DNA extraction from bacteria
12. DNA and RNA extraction from fish and shrimp
13. qPCR
14. Software analysis



**Lab picture**



**Fishtech Lab Work Flow**



**Day 1 (21 January 2021)**

**TSA agar plate preparation for Bacterial Isolation**

**Useful equipments:**

1. Digital scale
2. Autoclave
3. Biosafety cabinet

**Useful chemicals:**

1. TSA agar powder
2. De mineralized water
3. NaCl
4. 70% ethanol as sanitizer
5. 95% ethanol as fuel

**Useful materials:**

1. Calculator
2. Measuring papers
3. Measuring cylinder
4. Spoon
5. Glass beaker
6. Glass rod
7. Aluminum fuel
8. Autoclave tape
9. Permanent marker
10. Heat prove hand gloves
11. Tissue

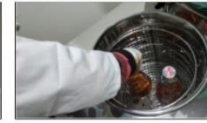


**Day 1 (21 January 2021)**

**TSA agar plate preparation for Bacterial Isolation**

- ✓ 40g TSA agar powder with 1000ml distilled water.
- ✓ 30g TSA agar powder with 750ml distilled water.

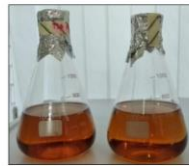
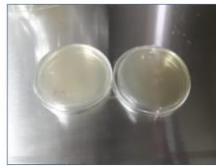
1. 30 g TSA + 750ml distilled water.
2. 30 g TSA + 15g NaCl + 750ml distilled water.



**Day 1 (21 January 2021)**

**TSA agar plate preparation for Bacterial Isolation**

1. Stir With a glass rod.
2. Autoclave at 121°C for 15 min.
3. Cooled to 45°C.
4. Stir gently and pour into sterile petri dish.
5. Air dry in Biosafety cabinet and stored at 16°C



**Day 2 (22 January 2021)**

**Fish and shrimp necropsy and Bacterial Isolation**



**Tilapia**  
 Total Length: 7.2"  
 Standard length: 6"  
 Width: 2.7"  
 External Symptoms: Scale loss, red coloration on skin, Deform.

**Shrimp**  
 Length: 6"  
 External Symptoms: No



Sample condition: Live fish and Moribund shrimp  
 Source: Gollamari fish market, khulna.



**Day 2 (22 January 2021)**

**Air dry agar media in biosafety cabinet before use**



**Day 2 (22 January 2021)**

**Air dry agar media in biosafety cabinet before use**





**Day 2 (22 January 2021)**

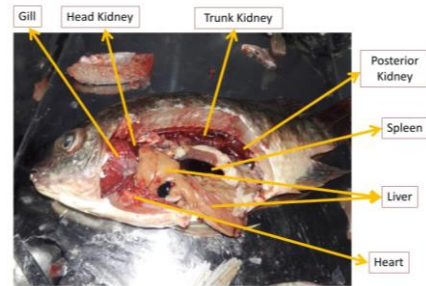
**Sample collection and preservation From tilapia**

S.N.	Target Organ	Bacteria isolation on TSA agar media	Freeze at - 20°C	Preserved in 95% ethanol at - 20°C
01	Spleen	Tilapia 1 & 2	Tilapia 1 & 2	Tilapia 1 & 2
02	Kidney	Tilapia 1 & 2	Tilapia 1 & 2	Tilapia 1 & 2
03	Liver	Tilapia 1 & 2	Tilapia 1 & 2	Tilapia 1 & 2



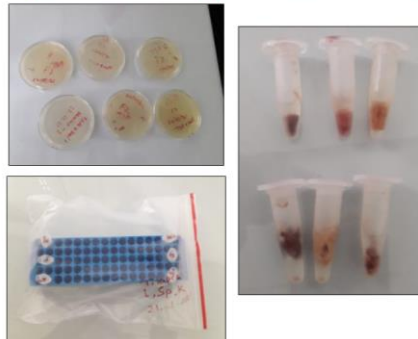
**Day 2 (22 January 2021)**

**Fish (Tilapia) necropsy**



**Day 2 (22 January 2021)**

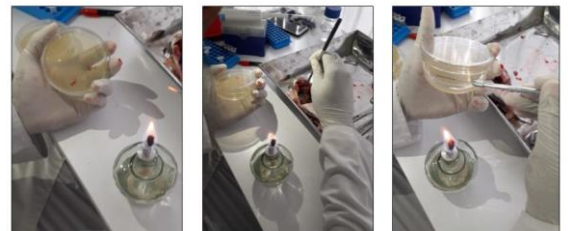
**Sample collection and preservation From tilapia**



**Day 2 (22 January 2021)**

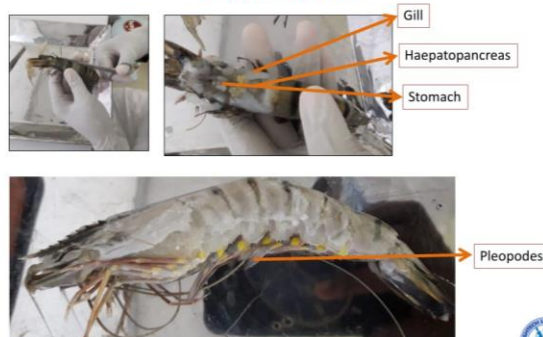
**Sample collection and preservation From tilapia**

**Bacteria Isolation From Tilapia**



**Day 2 (22 January 2021)**

**Shrimp necropsy**



**Day 2 (22 January 2021)**

**Sample collection and preservation From Shrimp**

S.N.	Target Organ	Bacteria isolation on TSA+NaCl agar media	Freeze at - 20°C	Preserved in 95% ethanol at - 20°C
01	Haepatopancreas	Shrimp 1 & 2	Shrimp 1 & 2	Shrimp 1 & 2
02	Gill	Shrimp 1 & 2	Shrimp 1 & 2	Shrimp 1 & 2
03	Stomach	Shrimp 2	Shrimp 2	Shrimp 2
04	Pleopods	Shrimp 1 & 2	Shrimp 1 & 2	Shrimp 1 & 2



**Day 2 (22 January 2021)**

**Incubate Agar Media at 30°C**




**Day 2 (22 January 2021)**

**Sample collection and preservation From Shrimp**



**Bacteria Isolation From Shrimp**




**Day 3 (23 January 2021)**

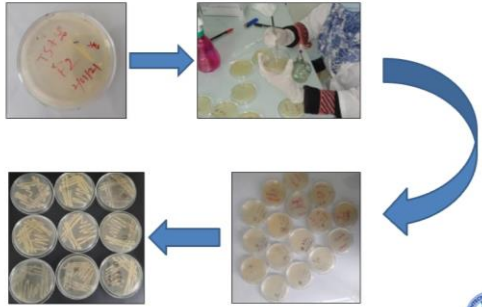

**Selection of Bacterial DNA extraction**

Target Organ
Tilapia 1 Liver (A)
Tilapia 1 spleen (C)
Shrimp 2 gill (F)
Shrimp 2 stomach (I)

**Day 3 (23 January 2021)**

**Observe bacteria plate and Subculture of Bacteria**





**Day 3 (23 January 2021)**

**DNA extraction from Fish and Shrimp Tissue**

Target organ	Tilapia1 Liver	Tilapia1 spleen	Shrimp2 Gill	Shrimp2 Pleopod
20mg sample	180µl Digestion buffer	20µl Proteinase K	55°C (2hr)	13,000rpm 3 min
vortex	200µl Lysis/Binding Buffer	RT for 2min	vortex	20 µl RNase A
200µl 96-100% ethanol	vortex	Transfer into spin column	10,000rpm 1min	discard flow-through
50µl Elution Buffer	spine column in a 1.5ml tube	10,000rpm 1min	500 µl Wash Buffer 2	10,000rpm 1min
10,000rpm for 1min	The tube contains purified genomic DNA	Stored at -20°C		

( Note: Ethanol preserved sample need to wash with 500µl distilled water)



**Day 4 (25 January 2021)**

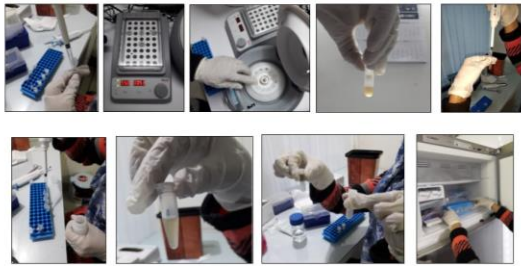
**Gram staining**

- Glass slide
- 1 Drop of water
- Fix Bacteria dry
- 1 drop crystal violet 1min
- 1 drop gram's iodine wash
- Decolorizer 5 sec
- Safranin 1min
- Wash
- Dry with tissue



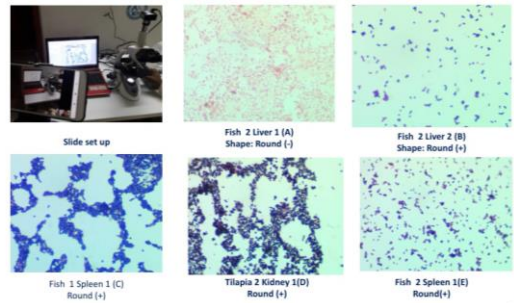

**Day 3 (23 January 2021)**

**DNA extraction from Fish and Shrimp Tissue**



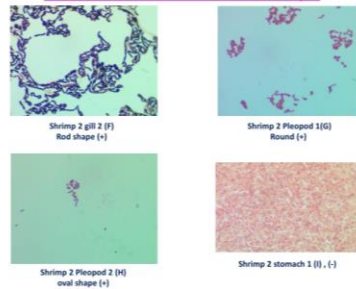
**Day 4 (25 January 2021)**

**Observe slide under microscope**



**Day 4 (25 January 2021)**

**Observe slide under microscope**



**Day 5 (26 January 2021)**

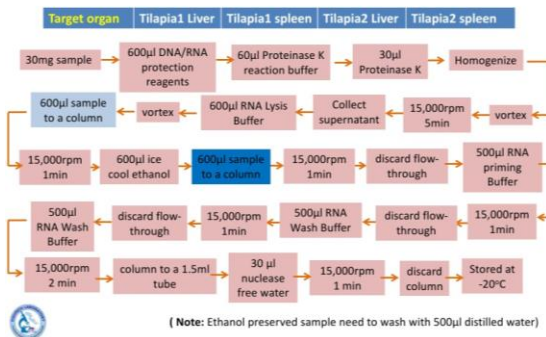
**DNA extraction from Bacteria**

- 500µl distile water in 1.5ml tube
- Suspended 5-10 colonies bacteria
- Centrifuge 5min at 10,000rpm
- Add 150µl molecular grade water
- Mix and incubate at 95°C for 10min
- Cool at ice for 5min
- Centrifuge 5min at 10,000rpm
- Collect supernatant and store at -20°C



**Day 5 (26 January 2021)**

**RNA extraction from Tissue**



**Day 5 (26 January 2021)**

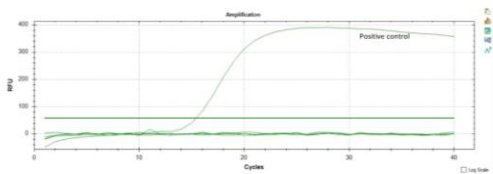
**RNA extraction from Fish and Shrimp Tissue**



**Day 7 (28 January 2021)**

*Streptococcus agalactiae* qPCR

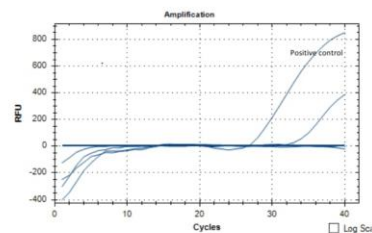
Sample | Tilapia1 liver | Tilapia1 spleen | Bacteria C



**Day 6 (27 January 2021)**

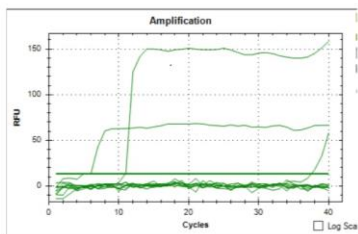
WSSV qPCR

Sample | Shrimp2 pleopod | Shrimp2 gill



**Day 9 (31 January 2021)**

*F. columnare* & *E. tarda* qPCR



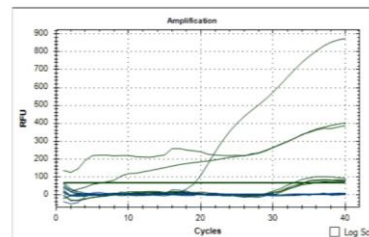
Sample:  
Bacteria A ET  
Bacteria A FV  
Bacteria I et  
Bacteria I FV (+)  
Liver et  
Liver FV  
Spleen et  
Spleen FV



**Day 8 (30 January 2021)**

*Vibrio sp.* qPCR

Sample | Bacteria A | Bacteria I | Shrimp 2 | shrimp 2 | Tilapia 1 | Tilapia1  
          | Bacteria A ET | Bacteria I FV (+) | gill | pleopod | liver | spleen



**Rapid Detection of Disease**



Flavobacterium  
Streptococcus sp.  
TILV  
Dactylogyus  
Ambiphrya sp.  
Trichodina  
Argulus  
Saprolegnia

- 1. Macroscopic Examination:**
  - Abnormal Behaviors
  - External Sign
  - Internal sign by Necropsy
- 2. Rapid Microscopic Examination:**
  - Wet mount
  - Smear tissue/blood & rapid staining



WSSV  
EHP  
AHPND  
DIV1  
YHV  
IMNV



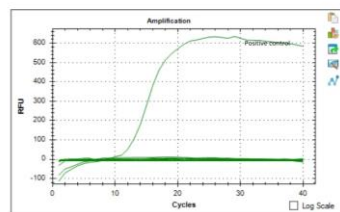
Bacillary Necrosis, Hemorrhagic Septicemia, Columnaris, Monogenean infection



**Day 9 (31 January 2021)**

AHPND qPCR

Sample | Shrimp2 gill | Shrimp2 pleopod | Bacteria A | Bacteria I



Day 10 (02 February 2021)

## Red egg disease in Tilapia

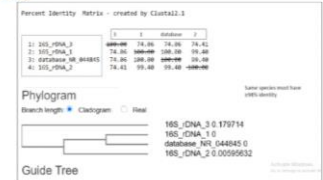
Causative agent: *Habellia chejuensis*

Day 10 (02 February 2021)

## Bacterial Classification and species identification by phenotypic and molecular approaches

Necessary software and website:

1. Blast (Identification)
2. MEGA (comparing)
3. Cluster Omega (comparing)
4. Primer blast (Primer identification)
5. NCBI (searching)



## Acknowledgement

We are expressing our heartfelt gratitude to

1. Dr Saengchan Senapin
2. Dr Ha Thanh Dong
3. Ariful Islam (Fishtech BD Limited)
4. World Fish & USAID

Overall, the authority of Fishtech BD Limited

