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USER-FRIENDLY AQUAPATHO KIT FOR RAPID DETECTION OF *VIBRIO* BACTERIA IN BIOFLOC BASED AQUACULTURE SYSTEMS

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Abstract

Vibrio infections are a major impediment to the sustainable expansion of the aquaculture industry worldwide. Early detection of *vibriosis* in aquaculture systems can protect not only fishes but also save economy. A novel Aquapatho kit was engineered for the specific detection of *Vibrio* bacteria in water samples, utilizing a selective medium that enhances the growth of *Vibrio* species while inhibiting other bacterial growth. This selective medium facilitates the identification of active *Vibrio* bacteria by inducing a color change to red/orange. The kit's efficacy was initially tested using two *Vibrio* species, *Vibrio cholerae* and *Vibrio parahaemolyticus*, along with two negative control bacteria, *Escherichia coli* and *Bacillus cereus*. Subsequent validation was conducted on water samples from aquaculture ponds and biofloc-based aquaculture farms. The kit effectively quantified *Vibrio* concentrations for both tested species in the aquaculture environments, while the negative controls did not elicit a response. Thus, this kit demonstrates high specificity and reliability for detecting *Vibrio* bacteria in aquaculture settings.

Keywords: Pathogens, Aquapatho kit, Biofloc pond

Introduction

A primary challenge in aquaculture is the prevalence of diseases induced by both infectious agents and non-infectious factors. Viral and bacterial infections are major causes of productivity losses and economic setbacks. Within the realm of harmful bacteria, vibrio species are particularly common, widely distributed and frequently liable for the spread of diseases in aquaculture systems (Gao *et al.*, 2017). *Vibrio* infections are a major impediment to the sustainable expansion of the aquaculture industry worldwide. As a result, these infections are the focus of research community to develop methods for preventing illness (Sanches-Fernandes *et al.*, 2022). Vibrio species are a common source of significant mortality in farmed fish and shellfish during their early larval stages. During these stages, host organisms are being prone to illness, however, they are also susceptible to

preventive measures approaches to therapy, such as giving vaccinations and medicines. The Vibrio genus comprises asporogenous, Gram-negative, rod-shaped bacteria that are either straight or exhibit a singular, rigid curvature (García-Bernal *et al.*, 2018). These organisms are motile due to a single polar flagellum. They can ferment glucose without gas production. Additionally, most Vibrio species produce both catalase and oxidase enzymes (Kaysner et al., 2004). Within the *Vibrio* genus, *V. parahaemolyticus*, *V. cholerae*, and *V. vulnificus* are prominently identified as pathogens affecting humans (Bonnin-Jusserand et al., 2019). *Vibrio cholerae*, the etiological agent of cholera, is primarily delivered indirectly via contaminated water sources. In addition to their prevalence in potable water, *vibrios* constitute a substantial component of microbial communities across various ecosystems, encompassing marine environments, estuarine habitats, and aquaculture facilities. Moreover, several species within this genus exhibit pathogenicity towards animals under aquaculture cultivation, underscoring their significance in both environmental and economic contexts (Chatterjee et al., 2012; Haldar et al., 2011). Irrespective of the developmental stage of the host, *Vibrio* infections can manifest abruptly, resulting in the potential decimation of every individual inside a particular aquatic system. The increasing demand for aquaculture products has sparked a resurgence of interest in innovative aquaculture systems (Stankus, 2021). To protect the environment and preserve natural resources while encouraging the aquaculture sector's sustainability, any expansion must occur in a manner that prioritizes sustainability. In pursuit of this goal, environmentally friendly industrial methods, like biofloc technology, have emerged as promising solutions, garnering significant attention from aquaculturists globally (Bossier and Ekasari, 2017).

Biofloc technology (BFT) represents an economically viable and sustainable approach to fish cultivation, leveraging fish and shrimp waste that is high in nitrogen combined with leftover feed, to generate a nutrient-rich protein diet (Xu *et al.*, 2016; Huang *et al.*, 2022; Khanjani *et al.*, 2022). Biofloc technology (BFT) culture systems rely on bioflocs, which are intricate microbial communities comprising rotifers, grazing macroinvertebrates, algae, fungi, protozoa, and detritus (Baiduk *et al.*, 2023; Minaz *et al.*, 2023) These bioflocs perform multifaceted roles, functioning as saprophytes, grazers of algae, and pathogen regulators, alongside facilitating nitrification processes and serving as hosts for floc-farming organisms (Nor Azman Kasan *et al.*, 2018; Nageswari *et al.*, 2022).

In a well-functioning biofloc technology (BFT) system, every component species need to remain prevented within the column of water, actively fulfilling their respective roles and maintaining sustainable interactions with other microorganisms (Kumar *et al.*, 2021). The degradation and recycling of chemical waste within biofloc systems are significantly enhanced due to the existence of autotrophic and chemophototrophic microorganisms. Notable members of the heterotrophic beneficial microbial community in biofloc include *Bacillus sp., Acinetobacter sp., Sphingomonas sp., Pseudomonas sp., Rhodopseudomonas sp., Micrococcus sp., Nitrosomonas sp., Nitrospira sp., Nitrobacter sp., Cellulomonas sp.*, and yeast. The thriving growth and overall health of cultivated organisms are contingent upon the microbial aggregates within the floc, which serve as vital nutrient providers within the system (Kumar *et al.*, 2019).

However, certain microorganisms pose challenges to biofloc technology, with *Vibrio sp.* being among the most prevalent. This bacterium has been recognized as a primary culprit for significant economic losses in shrimp culture, particularly within biofloc systems (Baker-Austin *et al.*, 2018; Tepaamorndech *et al.*, 2020). Effective management of the C:N ratio has been demonstrated to be effective in reducing acute hepatopancreatic necrosis disease (AHPND), a condition caused by Vibrio parahaemolyticus in biofloc systems (Hostins *et al.*, 2019). Several *Vibrio* diseases exhibit distinct symptoms discernible from others. Shrimps commonly manifest bacterial septicemia attributed to Trichomonas anguillarium, Parahaemolyticus, and Alginolyticus diocleclei. Necrosis can also be caused by Pseudomonas sp., Aeromonas sp., and Flavobacterium sp. Vibrio sp. is frequently identified as the pathogen in conjunction with necrosis.

Pathogenic microorganisms become active in the BFT system when conditions conducive to their proliferation are established. The diversity and abundance of microorganisms, along with

fluctuations in their populations over time, in biofloc technology aquaculture systems are influenced by various factors. A comprehensive understanding of these factors is essential for farmers, as it enables them to implement effective management strategies and foster the optimal health of their aquatic livestock (Emerenciano *et al.*, 2022). This aspect presents a significant challenge for both farmers and researchers in biofloc technology (BFT), as achieving a balance within the system has proven elusive. Maintaining this equilibrium is crucial to ensure the optimum probiotic bacterial performance and to control ammonia and other potentially hazardous intermediate products within reasonable bounds (Ju *et al.*, 2008). The efficacy of biofloc technology hinges upon the meticulous maintenance of water quality parameters and the recognition of the pivotal role played by microorganisms in the system (Huang *et al.*, 2022). Considering the multifaceted roles attributed to microorganisms in numerous research studies, specific genera emerge as fundamental within the context of biofloc technology (BFT), owing to their ability to fulfill multiple functions essential for effective system operation. While the significance of other microorganisms should not be overlooked, the overlapping roles performed by these so-called fundamental genera can notably compensate for the absence of other microorganisms within the BFT environment. Furthermore, their absence within the floc matrix may signify a dysfunctional BFT system, potentially compromising various critical processes including nutrients from organic deposits to support further biosynthesis within the BFT system, as well as nutrient synthesis, detoxification, breakdown and sedimentation of organic materials.A highly effective BFT is demonstrated by the existence of a robust population of Bacillus sp., Lecane sp., and Pseudomonas sp. in the microbial community. An important characteristic of such effective BFTs is the prevalence of these important microbial species. But it's important to be aware of the possible dangers connected to some genera, such Enterobacter and Vibrio species, which can become dangerous pathogens in poor water circumstances capable of thriving within nutrient-rich BFT systems. Specifically, conditions characterized by low oxygen levels and elevated temperatures can facilitate rapid bacterial proliferation, thereby increasing the likelihood of fish diseases within the BFT environment (Akange *et al.*, 2024). To mitigate potential repercussions, it is imperative to closely monitor microbes posing a threat to biofloc integrity under adverse water conditions, as their proliferation can result in diminished growth rates and disease outbreaks. Given the significant epidemiological ramifications of *Vibrio spp.* in both public health and aquaculture, there exists a pressing imperative to develop a rapid and portable detection kit. Such a kit would facilitate straightforward testing of water samples, thereby pre-empting disease outbreaks that could lead to widespread transmission and considerable economic losses. However, the application of this technique is hindered by the requirement for sophisticated laboratory facilities, which are often unavailable at shrimp or fish farms. Various detection methods for Vibrio spp. exist, encompassing genetic, molecular, cultural, and antibody-based tests (Ramamurthy *et al.*, 2020). While these methods offer rapid results, they are often costly and necessitate trained personnel for their administration. Conversely, traditional methods, though commonly employed, are characterized by slow processing times and complexity. Moreover, none of these techniques are conducive to onsite testing without access to microbiological laboratories and skilled personnel.

This study would evaluate the efficacy of the Aquapatho kit will also be designed by using Presence/Absence media for screening pathogens *vibrio cholera, vibrio parahemolyticus*. This kit used to monitor the microbes of concern to manage the bio flocculated ponds for timely maintenance. The proposed kit for microbial analysis at aquaculture level is inexpensive, simple and “Do yourself” type which reduce the need of sophisticated instrument, laboratory or skill to perform.

The sample is introduced to this specially designed kit medium, which inhibits the growth of other bacterial species. Consequently, it facilitates the detection of Vibrio in the sample by causing a color change to orange or red.

MATERIALS AND METHODS

Preparation of Presence/Absence Aquapatho kit

Rapid detection kit for pathogenic microbes in the aquaculture pond water called the "Aquapatho kit" was developed in the Microbial Biotechnology and Aquatoxicology laboratory of Zoology department, GC Women University, Faisalabad. The comprehensive step-by-step process for making and using the Aquapatho kit is illustrated in Figure 1. Analytical grade reagents of (Sigma-Aldrich) were used in the preparation of medium for the Aquapatho kit. Components of the Aquapatho kit are proteins, carbon source, vitamin B complex, and additional vital nutrients for growth. Gram positive bacteria and coliforms are inhibited by sodium citrate in this kit. Additionally, sodium thiosulphate is a good source of sulfur. A fermentable carbohydrate called sucrose is given to aid in the metabolism of pathogens. In a sterile capped bottle, 2mL of autoclaved medium was poured and labelled as the 'Aquapatho kit'.

Figure 1: Systematic diagram of Aquapatho kit

The composition of the Aquapatho kit medium is shown in Table 1. The medium was prepared with slide modifications by this method proposed by (Baird et al., 2017). For all microbiology investigations, thiosulfate citrate bile salts sucrose (TCBS) Agar, nutrient broth, and nutrient agar were utilized. The *vibrio* kit was tested and evaluated using *Escherichia coli*, *Bacillus cereus*, *Vibrio cholerae*, and *Vibrio parahaemolyticus* (collected from pondwater and identified in our laboratory).

Table 1: Composition of selective growth medium for *Vibrio* spp.

Ingredients	g/L
Peptone	24.000
Sucrose	5.000
Sodium thiosulphate	1.000
Sodium citrate	5.000
Bile salt	1.000
Sodium Chloride	5.000
Indicator mix	0.018
pH at 25 °C	8.6±0.2

The kit consists of a 15ml glass vial containing 2ml of detector reagent. To utilize the kit, 10ml of water sample is added to the glass vial, thoroughly mixed, and then incubated at room temperature or 37°C in an incubator. The color change is observed for a duration of one hour. Notably, *Vibrio species*, being predominantly carbohydrate fermenters, typically induce a red or orange color change.The prepared medium initially appears as a dark bluish-green clear solution within the glass vials, with a pH ranging from 8.0 to 8.6. Upon detection, the color transitions to reddish orange as show in Figure 2. This kit was employed in water samples obtained during an experiment involving carp larvae, aimed at assessing the theoretical risk of pathogen evolution within the biofloc system to mitigate pathogen proliferation.

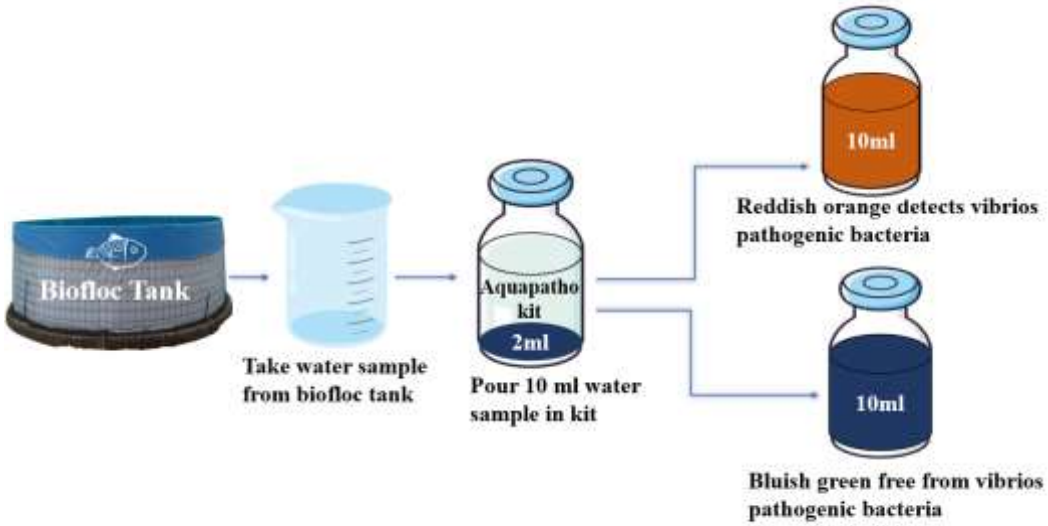

Figure 2: Diagrammatic representation of Aquapatho kit

Investigating Selective Growth Medium for Vibrio Species Detection

To assess the efficacy two *Vibrio* species, namely *V. cholerae* and *V. parahaemolyticus*, were grown in selective growth medium, and initially chosen for evaluation. These were utilized as positive controls, while *E. coli* served as the negative control. Water samples extracted from biofloc tanks were subjected to analysis using the Aquapatho kit. The concentration of the prepared medium (2 ml/2000 µL) was standardized across varying density of cells (10^{-2} – 10^{-8} CFU/mL) at intervals of 10, 15, 30, and 60 minutes for all selected bacteria.

Evaluation of the Aquapatho kit's efficacy within biofloc tanks through sample collection and subsequent analysis

To assess the accuracy of the detection kit, water samples were initially collected from biofloc tanks. These samples were then spread onto thiosulfate citrate bile salts sucrose agar (TCBS) plates and nutrient agar plates, respectively, to measure the concentration of Vibrio and the overall bacterial load. Furthermore, a different set of samples was made using different dilutions for comparison purposes. These dilutions were also spread onto nutrient agar and TCBS plates, and colony counting was performed using an Interscience Scan 1200 automatic colony counter to determine the overall load of bacteria. Following that, each mixture and distinct dilution was added to the vibrio detection kit, which included 2ml of selective growth media.

The inoculated kits were incubated at 37°C for 15 minutes. Subsequently, the water color change was then monitored on a regular basis within the kit. All experiments were carried out in triplicate to assure the accuracy as well as reliability of the data.

Assessment of the Aquapatho kit's performance within aquaculture farms through systematic evaluation and analysis

The application of the Aquapatho kit was assessed across various aquaculture farms characterized by disparate environmental conditions and geographically distant locations. Water samples were collected from aquaculture ponds situated along Satiana Road in Faisalabad. These ponds employed an intensive culture method, with a stocking density of $100/m^2$, and routinely utilized diverse chemical additives to enhance the productivity. Polypropylene bottles were used to collect samples for physicochemical parameter analysis, while sterile screw cap vials were employed for microbiological analysis. Physicochemical parameters like salinity, digital pH meter (Innotech), digital TDS meter (Milwaukee), and total dissolved solids, in that order. Water samples were spread out onto thiosulfate citrate bile salts sucrose (TCBS) agar plates in order to calculate the overall *Vibrio* count.

Subsequently, 10 milliliters of each water sample were collected in sterile transparent containers containing 2 milliliters of selective growth medium. The samples were then incubated for 45 minutes at 37°C, and the color change in the water was periodically monitored. All experiments were conducted in triplicates to ensure result reliability and accuracy.

Results and Discussion

Optimization of Medium Concentration for Aquapatho Kit

The Vibrio detection kit operates on the principle that a specific medium within a glass vial, when added to a sample of water, facilitates the *Vibrio spp.* growth while inhibiting the proliferation of other non-specific bacteria. This selective medium induces a color change to red/orange in the presence of active *Vibrio* species. The medium is formulated with proteose peptone or yeast extract to provide critical growth nutrients, vitamin B complex, or nitrogenous substances. To further suppress gram-positive bacteria and coliforms, sodium citrate and a derivative of bile salt are added (Howard, 1994). Sodium thiosulphate is used as a sulphur source, and sucrose is added as a fermentable carbohydrate for *Vibrio* metabolism.

Medium Concentration Optimization

Initial experiments were conducted to determine the optimal medium concentration for the detection kit by testing a range of medium volumes (100 µL to 2000 µL) with varying cell densities of the control bacteria, both positive and negative. The results indicated that volume of medium between 100 µL and 800 µL resulted in a color change for negative control bacteria across all bacterial concentrations (Table 2). This lack of specificity rendered these medium volumes unsuitable for the detection kit.

In contrast, a 2000 µL medium volume produced inconsistent results. At higher bacterial concentrations, the color change was delayed, while at lower concentrations, the color change occurred more rapidly compared to other medium volumes tested. The 1000 µL medium volume was identified as the optimal concentration, demonstrating negative control bacteria show delay or no color change, and the results are consistent across different bacterial concentrations.

Incubation Time Optimization

To optimize incubation time, durations of 15, 45, and 60 minutes were evaluated. An incubation period of 45 minutes was selected as it effectively inhibited the growth of non-specific bacteria without compromising the consistency of results. Shorter incubation periods (e.g., 15 minutes) were insufficient to inhibit non-specific bacterial growth, while longer periods (e.g., 60 minutes) led to inconsistent results due to the exponential *Vibrio spp.* growth, which affected the timing of the color change.

Table 2: The effect of a certain medium concentration on the vibrio detection kit's color change for different density of cells of negative and positive control bacteria

Bacteria	Media conc. (µL)	Time (h) required for the relevant bacterial load (CFU/mL) to develop color						
		10^2	10^3	10^4	10^5	10^6	10^7	10^8
Vibrio parahaemolyticus	100	15:30	11:30	7:35	4:20	3:15	1:45	00:45
	200	15:35	11:25	7:20	4:20	3:15	1:45	00:45
	300	15:00	12:00	6:15	5:10	2:00	1:15	00:50
	400	14:15	11:00	6:20	5:15	2:25	1:30	00:50
	500	15:15	10:55	6:15	4:30	2:30	2:00	00:40
	800	13:10	10:43	5:40	4:20	2:25	1:45	00:45
	1000	12:30	10:15	5:30	4:10	2:15	1:25	00:45
	2000	10:00	9:00	5:25	3:45	2:10	1:05	00:45
Vibrio Cholera	100	14:35	11:25	7:20	3:20	3:15	1:45	00:45
	200	15:00	12:00	6:15	5:10	2:00	1:15	00:50
	300	13:15	11:00	6:20	5:15	2:25	1:30	00:50
	400	15:15	10:55	6:15	4:30	2:30	2:00	00:40
	500	13:10	10:43	5:40	4:20	2:25	1:45	00:45
	800	11:30	10:15	5:30	4:10	2:15	1:25	00:45
	1000	10:00	9:00	5:25	3:45	2:10	1:05	00:45
	2000	9:35	11:25	7:20	4:20	3:15	1:45	00:45
Escherichia coli	100	-	-	-	-	-	-	-
	200	-	-	-	-	-	-	-
	300	-	-	-	-	-	-	-
	400	-	-	-	-	-	-	-
	500	-	-	-	-	-	-	-
	800	-	-	-	-	-	-	-
	1000	-	-	-	-	-	-	-
	2000	-	-	-	-	-	-	-
Bacillus cereus	100	-	-	-	-	-	-	-
	200	-	-	-	-	-	-	-
	300	-	-	-	-	-	-	-
	400	-	-	-	-	-	-	-
	500	-	-	-	-	-	-	-
	800	-	-	-	-	-	-	-
	1000	-	-	-	-	-	-	-
	2000	-	-	-	-	-	-	-

(–): No colour development

Experiments demonstrated that for *Vibrio parahemolyticus* and *Vibrio cholera*, the rate of color development increased with an incubation time of 15 to 45 minutes. Extending the incubation time to 60 minutes did not significantly enhance or alter the rate of color development across various bacterial loads. In contrast, *Escherichia coli* and *Bacillus cereus* exhibited no color development regardless of the bacterial load or incubation duration, indicating the medium's effective selectivity. Given these observations, an incubation period of 45 minutes was deemed optimal. This duration maximized the color development rate for *Vibrio spp.* while preventing any color change for *E. coli* and *B. cereus*, thereby ensuring the kit's selectivity and effectiveness. Based on previous optimization studies, a medium concentration of 1000 µL was selected for its consistency and specificity. The results showed that this concentration provided reliable detection of *Vibrio spp.* without inducing color changes in negative control bacteria.

Final Kit Configuration

The finalized configuration for the *Vibrio* detection kit consists of a 1000 µL medium concentration with an incubation time of 45 minutes. This setup has proven to be sensitive and selective for *Vibrio spp.*, as shown in Table 3, which highlights the kit's ability to induce color changes in the presence of Vibrio spp. while showing delayed or no color change for negative control bacteria. Figure 3 illustrates the color change process, with positive control bacteria inducing a noticeable color

change, whereas negative control bacteria did not. The experiments were repeated in triplicates, and the time required for color change was consistent across trials.

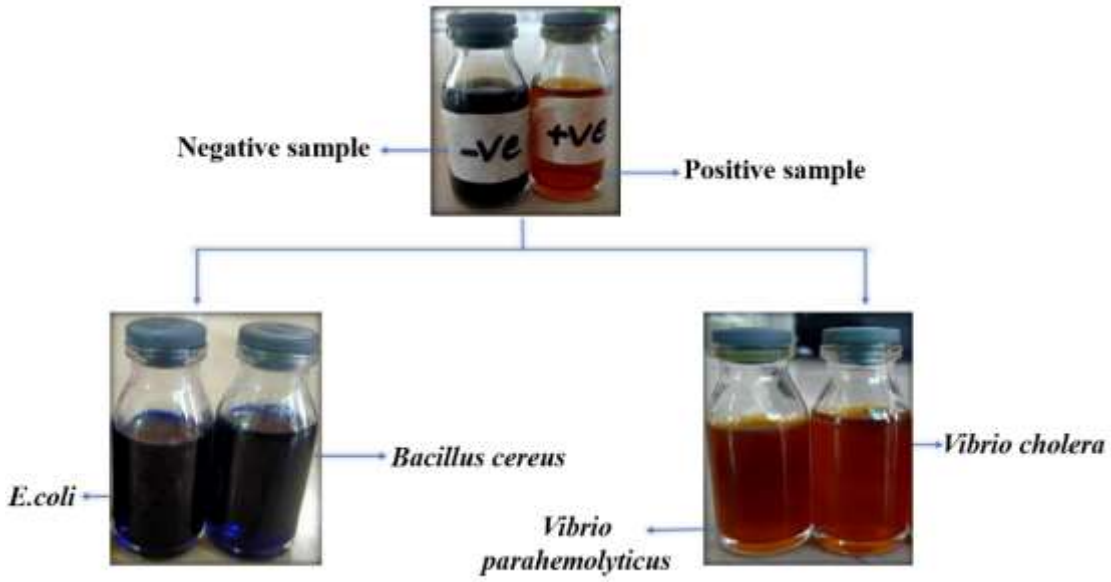

Figure 3: Images of Aquapatho kit; tested for various negative and positive control microorganisms

In conclusion, the Vibrio detection kit, with a medium concentration of 1000 µL and an incubation time of 45 minutes, is highly effective for detecting *Vibrio spp.* in water sources. The kit's sensitivity and selectivity make it a reliable tool for monitoring water quality.

Table 3: Impact of incubation period in color change on Aquapatho kit for different density of cells of negative and positive control bacteria

Bacteria	Incubation Time	Time taken (in h) for colour development by corresponding bacterial load (CFU/mL)						
		10^2	10^3	10^4	10^5	10^6	10^7	10^8
Vibrio parahaemolyticus	10 min	7:30	6:35	6:15	5:10	3:15	1:00	00:45
	15 min	7:10	6:25	5:20	4:20	3:00	1:15	00:45
	30 min	6:40	5:05	4:15	3:10	2:00	1:05	00:50
	60 min	5:15	5:00	4:20	3:15	2:25	1:30	00:45
Vibrio Cholera	10 min	6:35	5:25	4:25	3:20	2:15	1:05	00:45
	15 min	5:25	5:10	4:15	3:05	2:00	1:15	00:50
	30 min	4:15	4:00	3:20	3:15	2:25	1:10	00:45
	60 min	4:10	4:55	3:15	3:30	2:10	0:55	00:45
Escherichia coli	10 min	-	-	-	-	-	-	-
	15 min	-	-	-	-	-	-	-
	30 min	-	-	-	-	-	-	-
	60 min	-	-	-	-	-	-	-
Bacillus cereus	10 min	-	-	-	-	-	-	-
	15 min	-	-	-	-	-	-	-
	30 min	-	-	-	-	-	-	-
	60 min	-	-	-	-	-	-	-

(–): No colour development

Evaluation of the Effect of Non-Vibrio Microbes on the Sensitivity of the Aquapatho Kit in Biofloc Tanks

Mixed microbial cultures are frequently utilized in Biofloc ponds to enhance fish innate immunity, improve growth rates, and specifically reduce the prevalence of harmful *Vibrio spp.* (Martínez Cruz *et al.*, 2012). To determine whether non-*Vibrio* microbes in Biofloc ponds affect the sensitivity of

the *Vibrio* detection kit, water samples were collected from two different Biofloc tanks containing mixed microbial cultures. The presence of *Vibrio spp.* in these tanks was assessed to evaluate the kit's performance in the presence of other microbes. The results indicated that the presence of mixed microbial cultures did not interfere with the color change induced by the kit when *Vibrio spp.* were present. As demonstrated in Figure 3, the kit consistently changed color in the presence of *Vibrio spp.*, confirming its specificity and reliability. The experiment was conducted in triplicates, and a consistent trend was observed in all repetitions. Therefore, the *Vibrio* detection kit is reliable for detecting *Vibrio* contamination in aquaculture pond water, even in the presence of diverse microbial communities.

Aquapatho Kit Utilization in Two Distinct Aquaculture Farms

Field evaluation of the *Vibrio* detection kit is essential to confirm its effectiveness under diverse and fluctuating environmental conditions. The kit successfully detected *Vibrio spp.* in ponds with high salinity at an aquaculture farm. The detection was accurate when compared to a standard color chart for *Vibrio* concentrations ranging from 10^{-2} to 10^{-3} CFU/mL.

Pond 1 exhibited high water hardness and contained dead fish, necessitating the addition of hydrofloc and zeolite to improve water quality. Pond 2 also received hydrofloc and disinfectants to manage water quality. Despite these interventions, the kit accurately estimated the *Vibrio* concentration in both ponds. The consistency of these results demonstrates the reliability of the Vibrio detection kit in diverse aquaculture environments as dissussed in (Kumar *et al.*, 2021). The control sample and water from a freshwater reservoir exhibited no Vibrio colonies on TCBS plates, and the kit showed no color change when testing these samples. This result indicates that the kit does not produce false positive results. Additionally, it was confirmed that the kit functions effectively at room temperature, allowing for direct onsite application. The kit can be standardized for specific salinity and temperature conditions of particular fields, enabling direct distribution to farmers. Rapid color change compared to the standard time indicates an increase in *Vibrio* load, allowing farmers to take immediate action.

The study further demonstrated that various additives do not influence the working mechanism of the *Vibrio* detection kit. Its effective field application eliminates the need for sample transportation to distant laboratories, which often affects the final results.

Conclusion

A unique Aquapatho kit was developed with easy operating mechanism. It is low cost, easy to use, and does not require skilled personnel for operation. The kit uses only 800 µL of selective medium to inhibit non-specific bacteria within 15 minutes. It can detect even low concentrations (10^{-2} CFU/mL) of *Vibrio* within 6–7 hours. This kit provides a basis for developing other specific bacterial detection kits, which would be useful for the early detection of harmful bacterial pathogens, particularly *Vibrio spp.*, in water sources. The kit is ideal for onsite operation and visual result interpretation, making it suitable for use by aquaculture farmers without specialized training.

Competing Interest Declaration

There are no competing interests regarding the publication of this paper. The work has carried out independently, and there are no conflicts of interest to disclose.

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