**Editorial comments:**

• Your manuscript has been modified by your editor, please maintain the current formatting throughout the manuscript. **Please use the updated manuscript located in your Editorial Manager account (under “File Inventory”) for all subsequent revisions**.

The instruction was followed.

• Please take this opportunity to thoroughly proofread your manuscript to ensure that there are no spelling or grammatical errors. Your JoVE editor will not copy-edit your manuscript and any errors in your submitted revision may be present in the published version.

The manuscript was revised and proofread.

• JoVE reference format requires that DOIs are included, when available, for all references listed in the article. This is helpful for readers to locate the included references and obtain more information. Please note that often DOIs are not listed with PubMed abstracts and as such, may not be properly included when citing directly from PubMed. In these cases, please manually include DOIs in reference information.

Reference section is revised an include DOIs whenever possible.

•Formatting:

-Please use “hr” as the abbreviation for “hour(s)”.

The format was revised as suggested.

-All centrifuge speeds should be listed in terms of centrifugal g force rather than rpm.

The manuscript was revised to use RCF instead of RPM.

•Grammar: “bromthymol” should be “bromothymol”.

According to PubChem, bromthymol blue and bromthymol blue are MeSH synonyms. The term was revised as suggested.

•Additional detail is required:

-What is the composition of the chromogenic agar?

The composition of the agar is now included in the Discussion.

-3.4 – Is any equipment used to analyze the colonies?

Equipment is not required to analyze the colonies. Colony morphology observed by naked eyes under ambient light is sufficient to differentiate *Vibrio parahaemolyticus* from other species. It is an advantage of this chromogenic medium because it allows the user to rapidly differentiate *Vibrio* species without the need of expensive equipment and sophisticated analyses. The medium is designed to be used as a screening tool primarily and therefore cost per test must be comparable to existing screening methods. Our study shows that the chromogenic medium demonstrate better sensitivity and specificity.

-6.2.1, 6.3.1 – How much oyster homogenate is used?

These steps follow 6.1.2. Therefore 500 g diluted oyster homogenate was used for Steps 6.2.1 and 6.3.1. The amount of the oyster homogenate is now clarified in these steps.

•Results: Figure 1 and Figure 2 are cited incorrectly in the results text.

We acknowledge this error. Thanks for pointing it out. The figures are now cited correctly in the results.

**Reviewers' comments:**

**Reviewer #1:**

***Editorial Note: Please note that this reviewer has raised some significant concerns regarding your method and your manuscript.***

*Manuscript Summary:*

This paper is about a more sensitive and specific chromogenic agar medium for the isolation and differentiation of Vibrio species.

(1)The object of this study is to evaluate the ability of a newly formulated chromogenic medium to differentiate V. parahaemolyticus from other species. Authors used 54 microbial strains including Vibrio, E. coli, Pseudomonas and so on (Table 1). However, ATCC standard strain is not included in the experimental strains, all microbial strains are isolated bacteria. Such a design is not scientific because the stability of the isolated strains is not as good as the standard strains.

Thanks for the comments. ATCC strains were used in this study along with environmental and clinical isolates primarily provided by FDA and CDC. Since the new medium is developed with a purpose to test environmental samples, we feel that it is important to include environmental isolates to determine its effectiveness. The purity of all ATCC strains and isolates was checked periodically by streaking for colony isolation. In addition, PCR with species-specific primers was done to confirm the identity of the isolates. The identity of the isolates were determined by multiple laboratories, such as CDC and FDA. All strains and isolates were subjected to standard operating procedure, including frozen stock preparation. All strains and isolates were subcultured at least twice prior to each experiment. This is done to ensure all cells are not in injured state which may cause aberrant results.

In addition, the source of experimental strains and their serotypes are not clear in Table 1.

The Table is revised to include source of strains. Serotypes info, which is relatively more important for *V. cholerae* and *V. parahaemolyticus*, is also included.

(2)Academic rigor of the paper is poor. There are several writing mistakes in the paper. Example 1: in Abstract, authors tell us V. parahaemolyticus was incubated at 37 oC for 24-96 h. But in the part of Protocol, the culture temperature of V. parahaemolyticus is 35 oC (lines 209-210; lines 239-240).

Thanks for the comments. We had a digital thermometer placed in the incubator throughout the experiments which monitored the temperature fluctuation due to door opening and closing. The minimum and maximum temp recorded by the digital thermometer was 35 and 37oC respectively. We revised the manuscript to show the range of incubation temp.

Example 2: according to the description of lines 409-413, Fig.1 should be the result of TCBS, Fig.2 is the results of new chromogenic medium. However, Figure legends show the opposite description (lines 462-468).

We acknowledge this error. The Figures are now cited correctly in the results.

In addition, single colonies in Figure 1 and 2 are seldom, which is not conducive to isolate the target bacteria.

The streak plate technique was used to observe isolated colonies. It is common to observe isolated colonies in the last section. Differentiation of *Vibrio* species on TCBS or the chromogenic medium is based on the morphology of isolated colonies. Color of the colony is the most important attribute in the differentiation decision for both media. Figures 1 and 2 show the color of the cultures as well as isolated colonies. These are representative images from four replicate experiments.

*Major Concerns:*

N/A

*Minor Concerns:*

N/A

*Additional Comments to Authors:*

N/A

**Reviewer #2:**

*Manuscript Summary:*

The manuscript described the development of a more sensitive and specific chromogenic agar for isolation and differentiation of Vibrio species. The manuscript is well written.

*Major Concerns:*

A major concern for the writing is the insufficient information provided for the new chromogenic agar. It is unclear what is the component of this agar, including the pH and NaCl concentration. I think authors need to provide this information and highlight the detail of how different is this new agar (the significance of these differences) with the existing TCBS or existing Chrom agar available in the market.

Information about the exact amount and composition of commercially available chromogenic agar media is considered proprietary information by the companies. A brief comparison between our medium with a commercial medium is now included in the Discussion. This study highlights the procedure to evaluate a newly developed growth medium before commercialization. The general ingredients such as carbon and nitrogen source, minerals are similar to other complex media. We discussed levels of pH and salt must be tested to select for *Vibrio* species effectively. The role of chromogenic substrate to differentiate different *Vibrio* species is also emphasized in the manuscript.

*Minor Concerns:*

These references should be cited in this work:-

1) Letchumanan et al., 2014. doi: 10.3389/fmicb.2014.00705

This reference is a review paper that summarizes the pathogenesis, prevalence and advance molecular identification techniques of *Vibrio parahaemolyticus*. We do not believe this review paper adds to the current manuscript as the pertinent information is similar to the review and book chapter written by our group previously. Therefore we do not wish to include this reference in our manuscript.

2) Raghunanth et al., 2015. doi: 10.3389/fmicb.2014.00805

This is a review article summarizing the roles of thermostable direct hemolysin (TDH) and TDH-related hemolysin (TRH) in *Vibrio parahaemolyticus*. Although this is an interesting review article, we do not believe it is relevant to our manuscript. The review article stresses the distribution of *tdh* and *trh* genes in samples collected from different locations using PCR based methods. Our manuscript focuses on the detection of all *V. parahaemolyticus* using culture-depending method which is often the first step carried out by environmental labs. Thereafter, the isolates can be further analyzed such as the presence of *tdh* or *trh*. Owing to the irrelevancy, we do not wish to include this reference in our manuscript.

3) Haendiges et al., 2015. doi: 10.3389/fmicb.2015.00125

This study compared the whole genome sequences between 34 *Vibrio parahaemolyticus* strains, which were isolated from clinical cases in the state of Maryland, with the genomes of 17 strains obtained from US and 15 international strains. The authors had a specific study aim, which was to determine the feasibility of whole genome sequencing as a tool to improve public health surveillance. They built phylogenetic trees to determine relatedness of the strains. They concluded that the Maryland strains were highly diverse yet genetically distinct from other strains. While it is an interesting study about *V. parahaemolyticus*, we do not believe it is relevant to our manuscript. Therefore we do not wish to include this reference.

*Additional Comments to Authors:*

The method section should be simplify to ease the readers' understanding.

The method section was not simplified due to the instruction given by the publisher as follows:

“**Editorial recommendation**: Please keep JoVE’s protocol requirements in mind as you address the above comment - the protocol must contain sufficient details in order to enable users to accurately replicate your technique. We recommend NOT removing any details from the protocol text.”

**Reviewer #3:**

*Manuscript Summary:*

The title of the manuscript is to vague,because the most important species you vocalize much to V. parahamolyticus. Also, this medium destine to Foodborne infections Vibrio (it should be appear in the title)

The title was revised to be more specific.

the results (sensitivity, specificity) of this manuscript should be discussed and compared with the results found in other studies

More previous studies are now included to discuss and compare the results.

we don't see any author in the discussion part.

More previous studies are now included in the discussion.

*Major Concerns:*

N/A

*Minor Concerns:*

N/A

*Additional Comments to Authors:*

N/A

[**Editorial recommendation**: Please keep JoVE’s protocol requirements in mind as you address the above comment - the protocol must contain sufficient details in order to enable users to accurately replicate your technique. We recommend NOT removing any details from the protocol text.]