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R. Eddabra, Y. Piemont, J. M. Scheftel. Evaluation of a new chromogenic medium, chromID™ Vibrio, for the isolation and presumptive identification of and from human clinical specimens. *European Journal of Clinical Microbiology and Infectious Diseases*, 2011, 30 (6), pp.733-737. 10.1007/s10096-010-1145-2 . hal-00687022

**HAL Id: hal-00687022**

**<https://hal.science/hal-00687022>**

Submitted on 12 Apr 2012

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**Evaluation of a new chromogenic medium, chromID™ Vibrio, for the isolation and presumptive identification of *Vibrio cholerae* and *Vibrio parahaemolyticus* from human clinical specimens**

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## ABSTRACT

The aim of this study was to evaluate the performance of the ChromID Vibrio medium for the detection of *Vibrio cholerae* and *Vibrio parahaemolyticus* in stool and swab specimens in comparison with TCBS medium. A total of 96 samples including 30 fresh stool, 32 stool and 34 swab specimens coming from routine laboratory were tested. All samples were seeded on both media: the TCBS medium and the ChromID Vibrio directly and after enrichment step on alkaline peptone water. Of 96 samples studied, 34 were positive to *V. cholerae* and 30 were positive to *V. parahaemolyticus*. The sensitivity for isolation of *V. cholerae* in fresh stool specimens was identical for both media: 78.5%, 100% before and after enrichment respectively. However, positive test with ChromID concluded immediately to presence of *V. cholerae*. In the case of artificial contaminations, sensitivity of ChromID Vibrio was more important than TCBS after enrichment for *V. cholerae* and for *V. parahaemolyticus* before and after enrichment. In fresh stool specimens the specificity of ChromID Vibrio for screening *V. cholerae* was significantly higher than TCBS (100%, 100% compared to 50%, 50% before and after enrichment, respectively), and important for *V. parahaemolyticus* (100% ChromID Vibrio; 93.33% TCBS).

**Keywords:** Chromogenic media, *V. cholerae*, *V. parahaemolyticus*

## INTRODUCTION

In recent years, members belonging to the genus *Vibrio* of the family *Vibrionaceae* have acquired increasing importance because of the association of several of its members with human disease (Farmer et al., 1985). The most feared of the *Vibrio* species is *Vibrio cholerae*, the etiological agent of epidemic cholera, which causes watery diarrhea, similarly, *Vibrio*

*parahaemolyticus* is a marine bacterium that occurs naturally in coastal waters worldwide and is a cause of gastroenteritis associated with the consumption of seafood (Grimes, 1991, Blanco-Abad et al. 2009). Due to the outbreak of these diseases, it is often necessary to detect these bacteria in patients, or in environments such as food and water.

Several protocols have been developed to detect *Vibrio cholerae* and *V. parahaemolyticus* in different samples. The most widely used method for routine clinical analyses includes a two-step protocol in which a selective enrichment with alkaline peptone water (APW) or salt polymyxin broth (SPB) is used, followed by a culture on agar plate, characteristically thiosulfate citrate bile salts sucrose (TCBS) agar (Donovan and van Netten, 1995). However, the method is very labor-intensive and time-consuming. The thiosulfate-citrate-bile salts-sucrose agar (TCBS) it uses may not differentiate *V. cholerae* from some strains of *V. alginolyticus* or *V. fluvialis* and *V. parahaemolyticus* from *V. vulnificus* or *V. mimicus*.

The introduction of fluorogenic or chromogenic substrates directly into the agar to reveal species-specific enzyme activity allows better discrimination of bacteria, as well as rapid identification directly on the primary plates (Manafi, 1996). In general, chromogenic media demonstrate superior sensitivity and specificity compared to conventional selective media (Su et al. 2005, Duan and Yi-Cheng Su. 2005). Recently, a new chromogenic medium: ChromID *Vibrio* (BioMerieux) was developed for differentiating *V. parahaemolyticus* and *V. Cholerae* from other *Vibrio* species based on the formation of pink colonies by *V. parahaemolyticus* and blue green colonies by *V. Cholerae* on the medium.

The aim of this study was to:

- Evaluate the performance of ChromID *Vibrio* with commonly used TCBS for their specificities and sensitivities of detecting *V. parahaemolyticus* (Vp) and *V. cholerae* (Vc) in human clinical specimens,

- Describe color and morphology of *Vibrio vulnificus* (Vv) and *Vibrio alginolyticus* (Va) only in ChromID Vibrio.

## MATERIALS AND METHODS

### Media and reagents

ChromID Vibrio, a proprietary product, was provided as pre-poured plates for evaluation by bioMérieux (Marcy l'Etoile, France). As indicated by the manufacturer, ChromID Vibrio plates were stored at 4°C in a cold room and used within 4 weeks. Columbia agar was purchased as commercially prepared plates (Marcy l'Etoile, France). TCBS medium was obtained in bottles from Bio-Rad (Marne-la-coquette, France) and poured in plates in laboratory.

### Samples and inoculation of media

The protocol used in this study follows the recommendations of the NHS method (« Investigation of faecal specimens for bacterial pathogens », BSOP 30) for detection of *V. parahaemolyticus* and *V. cholerae* in human clinical specimens.

The study was conducted on:

- 30 natural samples, including 28 were repatriated from the Pasteur Institute in Abidjan (frozen samples) and 2 were from the routine laboratory. All these samples were taken for research in *Vibrio* part of the routine laboratories.

- 66 clinical specimens (34 rectal swabs and 62 stool specimens), that have been subject to artificial contamination, including 22 contamination at *V. cholerae* (Vc), 32 at *V. parahaemolyticus* (Vp), 6 at *V. alginolyticus* (Va) and 6 at *V. vulnificus* (Vv), (bacterial strains were provided by bioMérieux and the Laboratory). Eleven strains (1 Va, 1 Vv, 1 Vc and 8 Vp) strains were used 2 times, and each specimens was single (from different patients).

These contaminations were carried out on stools and rectal swabs from the routine laboratory, but not destined for a search for *Vibrio*.

*Vibrio* strains were stored frozen at 160°C; for the present study, they were thawed, seeded on blood agar, and incubated overnight at 37°C. For testing their growth characteristics on chromogenic media and on TCBS, bacteria were suspended in sterile saline to match a 2 McFarland standard and then diluted 100× (d1) before to added stool (2g/ 2mL d1) or swab (0.5mL/0.5mL d1).

Artificial and natural contaminated clinical specimens were plated directly and after enrichment (for 5 to 8 h in Alkaline Peptone Water (APW) (Bio-Rad)), into ChromID *Vibrio* and TCBS by the semi-quantitative three loop technique using a calibrated loop (10 µl).

#### **Recognition and confirmation of *Vibrio* species**

After incubation for 24 h at 37 °C, plates were evaluated for qualitative recovery, the color and size of colonies. Presumptive *V. cholerae* and *V. parahaemolyticus* colonies of each morphological type, from each agar plate were subcultured to Colombia blood agar plates (BioMerieux). Blood agar was incubated for 24 h at 37°C.

The identity of presumptive *Vibrio* species are subjected to a battery of biochemical tests (oxidase test, gram) and confirmed using Vitek 2 and/or ID32E biochemical strips (BioMerieux, Craponne, France) according to the manufacturer's instructions.

In addition, identification of certain isolates that are not confirmed by Vitek2 and ID32E as *V. cholerae* or *V. parahaemolyticus*, were confirmed genotypically using polymerase chain reaction (PCR).

At the conclusion of the study, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were determined for each medium at 24h.

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## RESULTS AND DISCUSSION

### Identification study

We first evaluated the colonial appearance of *Vibrio* isolates on ChromID Vibrio and TCBS media. All isolates that are identified by Vitek 2 and/or IDE32E of *V. cholerae* and *V. parahaemolyticus* isolates are blue green (Positive) or pink colony on ChromID Vibrio and yellow or blue-green colony on TCBS, which were usually 2 to 3 mm in diameter after 24 h of incubation (Fig. 1).

Color and morphology of *Vibrio vulnificus* (Vv) and *Vibrio alginolyticus* (Va) was described only in ChromID Vibrio (fig. 2)

### Sensitivity and specificity for *V. cholerae*

Natural specimens: In total, 14 (46.66%) of 30 natural specimens screened were positive for *V. cholerae* in both media used in the study. Results summarized in Table 1.

The sensitivity of ChromID Vibrio and TCBS was identical for both 78.57% and 100% before and after enrichment respectively, whereas the sensitivity of ChromID Vibrio higher than for TCBS media, it was 100%, 50% before and after enrichment for ChromID Vibrio and TCBS respectively (Table 1).

Artificially contaminated specimens: the sensitivity was similar (85%) for both media but it is more important before enrichment then after enrichment. The sensitivity for ChromID Vibrio (75%) is more then TCBS media (60%) after enrichment. We concluded that enrichment does not increase the sensitivity, may be due to specimens used for artificial contamination that become from patient subject to antibiotic treatment, and the enrichment could promote the growth of resistant bacteria to detriment of *Vibrio* species and could explain the fact that there are fewer positive samples after enrichment in artificially contaminated samples.

### Sensitivity and specificity for *V. parahaemolyticus* in artificial contaminated specimens



The sensitivity and the specificity for ChromID *Vibrio* are similar before and after enrichment (86.67% for sensitivity, 100% for specificity) and are important for ChromID *Vibrio* then TCBS (80% for sensitivity similar before and after enrichment, 93.33% for specificity similar before and after enrichment) ([Table 1](#)).

On TCBS, 11 give right results for suspected *Vc* after direct seeding and 9 which led to different result. The rate of unnecessary confirmation performed on TCBS is 45% and drops to 36.36% after enrichment. For ChromID, whatever the methodology used (direct seeding or after enrichment), all confirmations performed on characteristic colonies on ChromID *Vibrio* led to results of suspected *V. cholerae*.

The same analysis could not be made for the species of *Vp*, because no case of *Vp* has been detected in natural samples. However, no false positive to *VP* has been identified on ChromID while 2 false positive appear on TCB. Thus, two unnecessary confirmations were made on the TCBS.

Finally, the descriptive study conducted on the species *V. alginolyticus* showed that this species is distinguished from other *Vibrio* by its appearance beige drape over the ChromID. Species of *V. vulnificus* and *V. cholerae* can not be distinguished without confirmation.

Isolation and identification of pathogenic microorganism is one of the most important aspects of clinical microbiology. *V. cholera*, *V. parahaemolyticus*, *V. vulnificus* and *V. alginolyticus* are pathogens that cause food borne disease, generally contagion by polluted water and vegetable ([Jak et al. 2002](#)). Conventionally TCBS culture originally developed for isolation of pathogenic vibrios ([Kobayashi et al. 1963](#)) have poor sensitivity in natural specimens. The inclusion of a chromogenic agar such as ChromID *Vibrio* enhanced the isolation of presumptive colonies of *V. cholerae* and *V. parahaemolyticus*, mainly because of the easy identification of color colonies, which with the reduction in the presence of competitive flora on plates, results in improved sensitivity of plate culture methods.

165 In conclusion, ChromID Vibrio has a sensitivity equivalent to TCBS medium, appears to be  
166 two times more specific and no confirmation has been made unnecessary.  
167 The enrichment on natural specimens increase the sensitivity, the specificity and selectivity.  
168 The ChromID Vibrio responds well to the goal of isolation of *Vibrio* species from clinical  
169 specimens with presence of characteristic colonies to differentiate *V. cholerae* and *V.*  
170 *parahaemolyticus*.

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*Bacteriol.* **18**:Table 1: Sensitivity and specificity analysis of chromID Vibrio and TCBS for *V. cholerae* in natural and artificially contaminated specimens

	Direct		After enrichment	
	ChromID Vibrio	TCBS	ChromID Vibrio	TCBS
Natural specimens (n=30)	11 <sup>a</sup> , 3 <sup>b</sup> , 16 <sup>c</sup>	11 <sup>a</sup> , 3 <sup>b</sup> , 8 <sup>c</sup> , 8 <sup>d</sup>	14 <sup>a</sup> , 16 <sup>c</sup>	14 <sup>a</sup> , 8 <sup>c</sup> , 8 <sup>d</sup>
<b>Test sensitivity (%)</b>	<b>78.57</b>	<b>78.57</b>	<b>100</b>	<b>100</b>
<b>Test specificity (%)</b>	<b>100</b>	<b>50</b>	<b>100</b>	<b>50</b>
Artificially contaminated specimens (n=22)	17 <sup>a</sup> , 3 <sup>b</sup>	17 <sup>a</sup> , 3 <sup>b</sup>	15 <sup>a</sup> , 5 <sup>b</sup>	12 <sup>a</sup> , 8 <sup>b</sup>
<b>Test sensitivity (%)</b>	<b>85</b>	<b>85</b>	<b>75</b>	<b>60</b>

Table 2: Sensitivity and specificity analysis of chromID Vibrio and TCBS for *V. parahaemolyticus* in artificially contaminated specimens

	Direct		After enrichment	
	ChromID Vibrio	TCBS	ChromID Vibrio	TCBS
Artificially contaminated specimens (n=32)	26 <sup>a</sup> , 30 <sup>c</sup>	24 <sup>a</sup> , 28 <sup>c</sup>	26 <sup>a</sup> , 30 <sup>c</sup> , 2 <sup>d</sup>	24 <sup>a</sup> , 28 <sup>c</sup> , 2 <sup>d</sup>
<b>Test specificity (%)</b>	<b>100</b>	<b>93.33</b>	<b>100</b>	<b>93.33</b>
<b>Test sensitivity (%)</b>	<b>86.67</b>	<b>80</b>	<b>86.67</b>	<b>80</b>

<sup>a</sup> A true positive (TP) defined as a blue-green or pink colony on chromID Vibrio or yellow or blue-green colony on TCBS that was identified as Vc or Vp by VITEK 2.

<sup>b</sup> A false negative (FN) defined as an isolate that was confirmed as a Vibrio on 1 medium but did not grown on another medium.

<sup>c</sup> A true negative (TN) defined as the lack of a typically colored colony.

<sup>d</sup> A false positive (FP) defined as an isolate that exhibited typical coloration on the respective medium but was not identified as Vibrio by VITEK 2 or confirmed by PCR.

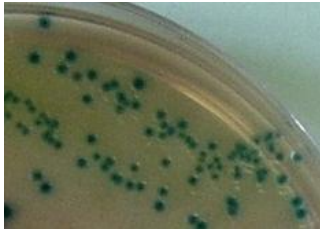
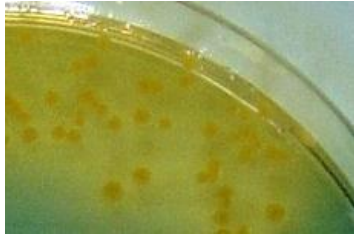

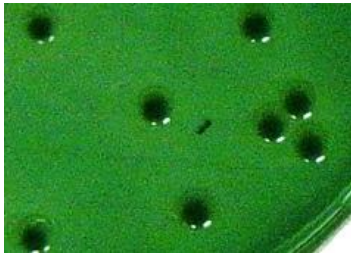
Organism	ChromID Vibrio	TCBS
<i>V. cholerae</i>	 <p>Blue green</p>	 <p>Yellow</p>
<i>V. parahaemolyticus</i>	 <p>Pink</p>	 <p>Green</p>

Fig.1. Colonial appearance of *V. cholerae* and *V. parahaemolyticus* on ChromID Vibrio and TCBS media after 24h incubation at 37°C

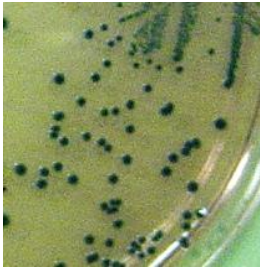
<i>Vibrio vulnificus</i>	<i>Vibrio alginolyticus</i>
 <p>Blue</p>	 <p>beige</p>

Fig.2. Colonial appearance of *V. vulnificus* and *V. alginolyticus* on ChromID Vibrio