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

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

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3 19 The aim of this study was to evaluate the performance of the ChromID Vibrio medium for the
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5 20 detection of *Vibrio cholerae* and *Vibrio parahaemolyticus* in stool and swab specimens in
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7 21 comparison with TCBS medium. A total of 96 samples including 30 fresh stool, 32 stool and
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9 22 34 swab specimens coming from routine laboratory were tested. All samples were seeded on
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11 23 both media: the TCBS medium and the ChromID Vibrio directly and after enrichment step on
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13 24 alkaline peptone water. Of 96 samples studied, 34 were positive to *V. cholerae* and 30 were
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15 25 positive to *V. parahaemolyticus*. The sensitivity for isolation of *V. cholerae* in fresh stool
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17 26 specimens was identical for both media: 78.5%, 100% before and after enrichment
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19 27 respectively. However, positive test with ChromID concluded immediately to presence of *V.*
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21 28 *cholerae*. In the case of artificial contaminations, sensitivity of ChromID Vibrio was more
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23 29 important than TCBS after enrichment for *V. cholerae* and for *V. parahaemolyticus* before
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25 30 and after enrichment. In fresh stool specimens the specificity of ChromID Vibrio for
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27 31 screening *V. cholerae* was significantly higher than TCBS (100%, 100% compared to 50%,
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29 32 50% before and after enrichment, respectively), and important for *V. parahaemolyticus* (100%
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31 33 ChromID Vibrio; 93.33% TCBS).
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43 35 **Keywords:** Chromogenic media, *V. cholerae*, *V. parahaemolyticus*
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49 37 **INTRODUCTION**
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51 38 In recent years, members belonging to the genus *Vibrio* of the family *Vibrionaceae* have
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53 39 acquired increasing importance because of the association of several of its members with
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55 40 human disease (Farmer et al., 1985). The most feared of the *Vibrio* species is *Vibrio cholerae*,
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57 41 the etiological agent of epidemic cholera, which causes watery diarrhea, similarly, *Vibrio*
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42 *parahaemolyticus* is a marine bacterium that occurs naturally in coastal waters worldwide and
43 is a cause of gastroenteritis associated with the consumption of seafood (Grimes, 1991,
44 Blanco-Abad et al. 2009). Due to the outbreak of these diseases, it is often necessary to detect
45 these bacteria in patients, or in environments such as food and water.

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

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

62 The aim of this study was to:

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

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

68

69 **MATERIALS AND METHODS**

70 **Media and reagents**

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

77 **Samples and inoculation of media**

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

81 The study was conducted on:

82 • 30 natural samples, including 28 were repatriated from the Pasteur Institute in Abidjan
83 (frozen samples) and 2 were from the routine laboratory. All these samples were taken for
84 research in *Vibrio* part of the routine laboratories.

85 • 66 clinical specimens (34 rectal swabs and 62 stool specimens), that have been subject to
86 artificial contamination, including 22 contamination at *V. cholerae* (Vc), 32 at *V.*
87 *parahaemolyticus* (Vp), 6 at *V. alginolyticus* (Va) and 6 at *V. vulnificus* (Vv), (bacterial
88 strains were provided by bioMérieux and the Laboratory). Eleven strains (1 Va, 1 Vv, 1 Vc
89 and 8 Vp) strains were used 2 times, and each specimens was single (from different patients).

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

92 *Vibrio* strains were stored frozen at 160°C; for the present study, they were thawed, seeded on
93 blood agar, and incubated overnight at 37°C. For testing their growth characteristics on
94 chromogenic media and on TCBS, bacteria were suspended in sterile saline to match a 2
95 McFarland standard and then diluted 100× (d1) before to added stool (2g/ 2mL d1) or swab
96 (0.5mL/0.5mL d1).

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

100 **Recognition and confirmation of *Vibrio* species**

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

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

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

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

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115 **RESULTS AND DISCUSSION**

116 **Identification study**

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

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

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

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

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

132 Artificially contaminated specimens: the sensitivity was similar (85%) for both media but it is
133 more important before enrichment then after enrichment. The sensitivity for ChromID Vibrio
134 (75%) is more then TCBS media (60%) after enrichment. We concluded that enrichment does
135 not increase the sensitivity, may be due to specimens used for artificial contamination that
136 become from patient subject to antibiotic treatment, and the enrichment could promote the
137 growth of resistant bacteria to detriment of *Vibrio* species and could explain the fact that there
138 are fewer positive samples after enrichment in artificially contaminated samples.

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

140 The sensitivity and the specificity for ChromID *Vibrio* are similar before and after enrichment
141 (86.67% for sensitivity, 100% for specificity) and are important for ChromID *Vibrio* then
142 TCBS (80% for sensitivity similar before and after enrichment, 93.33% for specificity similar
143 before and after enrichment) (Table 1).

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

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

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

156 Isolation and identification of pathogenic microorganism is one of the most important aspects
157 of clinical microbiology. *V. cholera*, *V. parahaemolyticus*, *V. vulnificus* and *V. alginolyticus*
158 are pathogens that cause food borne disease, generally contagion by polluted water and
159 vegetable (Jak et al. 2002). Conventionally TCBS culture originally developed for isolation of
160 pathogenic vibrios (Kobayashi et al. 1963) have poor sensitivity in natural specimens. The
161 inclusion of a chromogenic agar such as ChromID *Vibrio* enhanced the isolation of
162 presumptive colonies of *V. cholerae* and *V. parahaemolyticus*, mainly because of the easy
163 identification of color colonies, which with the reduction in the presence of competitive flora
164 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
1
2 166 two times more specific and no confirmation has been made unnecessary.
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5 167 The enrichment on natural specimens increase the sensitivity, the specificity and selectivity.
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8 168 The ChromID Vibrio responds well to the goal of isolation of *Vibrio* species from clinical
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10 169 specimens with presence of characteristic colonies to differentiate *V. cholerae* and *V.*
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12 170 *parahaemolyticus*.
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55 196 medium for vibrio group on a modified Nakanishi's medium (TCBS agar medium), *Jpn. J.*

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

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

199
 200 Table 2: Sensitivity and specificity analysis of chromID Vibrio and TCBS for *V.*
 201 *parahaemolyticus* in artificially contaminated specimens

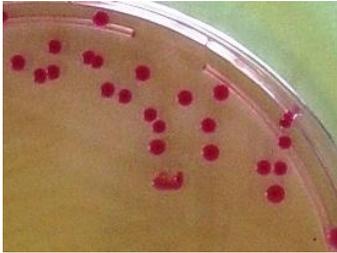
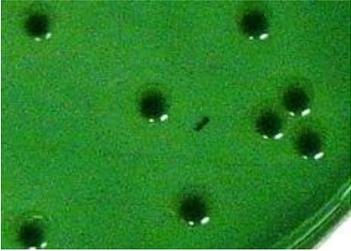
	Direct		After enrichment	
	ChromID Vibrio	TCBS	ChromID Vibrio	TCBS
Artificially contaminated specimens (n=32)	26 ^a , 30 ^c	24 ^a , 28 ^c	26 ^a , 30 ^c , 2 ^d	24 ^a , 28 ^c , 2 ^d
Test specificity (%)	100	93.33	100	93.33
Test sensitivity (%)	86.67	80	86.67	80

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

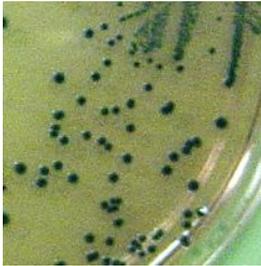
205 ^b A false negative (FN) defined as an isolate that was confirmed as a Vibrio on 1 medium but
 206 did not grown on another medium.

207 ^c A true negative (TN) defined as the lack of a typically colored colony.

208 ^d A false positive (FP) defined as an isolate that exhibited typical coloration on the respective
 209 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>

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

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

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