

## Clinical Laboratory Evaluation of a Reverse CAMP Test for Presumptive Identification of *Clostridium perfringens*

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Ninety-six percent of *Clostridium perfringens* isolates from clinical specimens were reverse CAMP test positive, whereas several other *Clostridium* species tested were reverse CAMP test negative. *C. perfringens* was detected by direct inoculation of clinical specimens to reverse CAMP plates, and the reverse CAMP procedure provided reliable presumptive identification of this organism.

*Clostridium perfringens*, the most important of the histotoxic species commonly isolated from clinical specimens, is routinely identified in clinical laboratories by the Nagler reaction (5), but other lecithinase-producing *Clostridium* species also give a positive reaction (4, 6). Environmental isolates of *C. perfringens* have been identified by a reverse CAMP test (2, 3), but such isolates may differ significantly from clinical isolates (1). Thus, the reverse CAMP test was evaluated to determine its efficacy in the clinical microbiology laboratory for routine presumptive identification of *C. perfringens* in clinical specimens.

*C. perfringens* and other *Clostridium* species isolated from 140 clinical specimens of various body sites, including blood (35 specimens), abdomen (28), wounds (18), rectum (13), genitals (3), biliary tract (3), lung (2), eye (1), cerebrospinal fluid (2), and other sources (35), were submitted to three hospitals in Winnipeg, Canada. Cultures were identified by standard laboratory procedures and were inoculated at right angles to within 1 to 2 mm of a  $\beta$ -hemolytic group B *Streptococcus* streak on sheep blood agar plates. Plates were incubated anaerobically and read after 18 to 24 h. A positive reverse CAMP test showed a "bow-tie" or "reverse arrow" pattern of hemolysis at the junction of the two cultures. Thirty-three clinical specimens, consisting of swabs or aspirates from patients with wounds (7 specimens), abdominal sepsis (14), or other sources (12) in which anaerobic infection was likely, described as "direct inocula", were inoculated as received in the clinical laboratory to reverse CAMP plates on blood agar (BA), BA with vitamin K, or BA with phenylethyl alcohol. The direct inoculum specimens and 20 cooked meat broths inoculated with clinical specimens were examined for *C. perfringens* and other bacteria as part of routine laboratory work-up

and provided controls for the reverse CAMP test. Different personnel were involved in both procedures, and results of the reverse CAMP test were checked independently against the final report sent out by the clinical microbiology laboratory.

Of 98 *C. perfringens* isolates tested, 94 (96%) were reverse CAMP test positive, 1 gave an intermediate reaction ( $\pm$ ), and 3 others were reverse CAMP test negative (Table 1). One isolate of *C. butyricum* was reverse CAMP test positive, and intermediate reactions were observed in three other species (Table 1). Of the 33 direct inocula tested, 5 were reverse CAMP test positive, 1 was intermediate, and the others were reverse CAMP test negative. Of the 20 cooked meat broths inoculated with clinical specimens, 7 were reverse CAMP test positive; the others were reverse CAMP test negative. *C. perfringens* was not isolated in the clinical laboratory by routine processing of clinical specimens for *C. perfringens* and other bacteria from any of the reverse CAMP test-negative specimens, but was isolated from all reverse CAMP test-positive specimens of both the direct inocula and the cooked meat broths. In an attempt to determine some of the factors influencing the reverse CAMP test, undiluted *C. perfringens* type A antitoxin (Wellcome Reagents, Beckenham, England), used in the Nagler reaction (5), was applied to reverse CAMP plates with an inoculating loop at the junction of the *C. perfringens* isolate and the  $\beta$ -hemolytic *Streptococcus* isolate. The antitoxin prevented formation of the bow-tie pattern of hemolysis (indicative of a positive test) in 30 of 30 *C. perfringens* isolates examined and inhibited the hemolysis of the *C. perfringens* isolates but not that of the *Streptococcus* isolate. "Free" hemolysin or lecithinase (or both) not complexed to antibody was apparently required to give a positive reverse CAMP

TABLE 1. Reverse CAMP test reactions in *Clostridium* species from clinical sources

<i>Clostridium</i> organism	No. of strains tested	Reverse CAMP test reaction		
		Positive	Intermediate ( $\pm$ )	Negative
<i>C. perfringens</i>	98	94	1	3
<i>C. butyricum</i>	6	1	— <sup>a</sup>	5
<i>C. sphenoides</i>	5	—	—	5
<i>C. bifermentans</i>	4	—	1	3
<i>C. clostridiiforme</i>	4	—	1	3
<i>C. sordellii</i>	3	—	—	3
<i>C. ramosum</i>	3	—	—	3
<i>C. botulinum</i>	2	—	—	2
<i>C. tetani</i>	2	—	—	2
<i>C. difficile</i>	2	—	—	2
<i>C. tertium</i>	1	—	—	1
<i>C. sporogenes</i>	1	—	—	1
<i>C. innocuum</i>	1	—	—	1
<i>C. haemolyticum</i>	1	—	—	1
<i>C. paraputrificum</i>	1	—	—	1
<i>Clostridium</i> species	6	—	1	5

<sup>a</sup> —, No reaction.

test, since the antibody preparation used here inhibits *C. perfringens* lecithinase as detected by the Nagler reaction (5).

When the more common clinical isolates of *Clostridium* species were tested, the reverse CAMP test reaction was highly specific for *C. perfringens* and improved the identification procedures available for the organism. The single isolate of *C. butyricum* which gave a positive reverse CAMP test reaction raised the possibility of false-positive reactions, but *C. butyricum* is not frequently encountered in clinical specimens (6). Clinical specimens as received in the laboratory and cooked meat broths inoculated with

clinical specimens were applied directly to reverse CAMP plates, thus eliminating the customary isolation on BA before testing by the Nagler reaction and significantly reducing the time taken to identify the organism. Numerous isolates were tested on a single reverse CAMP plate, thus lowering the cost of identifying an isolate encountered frequently in the clinical laboratory. Intermediate reactions were apparently infrequent and were readily differentiated from true-positive reactions. The results presented in this report indicate that the reverse CAMP test can be used in clinical microbiology laboratories for the rapid presumptive identification of *C. perfringens* in clinical specimens.

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