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Rapid, Economical Diagnosis of Enteric Fever by a Blood Clot Culture Coagglutination Procedure

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Coagglutination tests with *Salmonella* A, D, Vi, and polyvalent antiserum-sensitized staphylococcal cells were compared with conventional culture methods for detecting salmonellae in ox bile cultures of blood clots from enteric fever patients. The coagglutination tests appeared equally as effective as conventional subculture methods for detecting positive cultures (95% agreement). In addition, the coagglutination method yielded earlier results at reduced cost.

Blood culturing in broth is generally recognized as the best procedure for definitive diagnosis of acute enteric fever. In conventional systems, salmonellae in the broth culture are detected by isolation on agar plates followed by identification with biochemical and serological tests. These systems require a minimum of 3 days for completion and use a variety of culture media, thus becoming relatively costly. Cost and time factors are important considerations, especially in developing countries where enteric fever diagnosis is frequently needed.

Selective blood culturing in ox bile has been demonstrated a useful, inexpensive alternative to whole blood culturing in broth (1, 4). Furthermore, blood clot culturing has been demonstrated clearly more efficient than whole blood culturing for isolating salmonellae (7).

This report describes a diagnostic system which used a blood clot culture coagglutination (COAG) test (2) for early detection and identification of *Salmonella* antigens.

Blood specimens were obtained from clinically suspect enteric fever patients at the Alexandria Fever Hospital in Alexandria, Egypt. Clots obtained from 5 to 10 ml of blood were placed in 15 ml of 10% oxgall (Difco Laboratories) containing 100 U of streptokinase (Varidase; Lederle Laboratories). These cultures were incubated at 37°C. At intervals of 1, 3, and 7 days, subcultures were placed on MacConkey agar plates. When these subcultures were done, a 0.5-ml sample was also taken for COAG tests.

Isolates from the MacConkey agar plates were identified by a conventional biochemical system which uses TSI Agar (BBL Microbiology Systems) and other differential, tubed media. Bacterial suspensions were made from the TSI Agar slant, and bacteria were serologically identified by slide agglutination.

COAG tests were performed on microscope slides. Loopfuls of ox bile blood clot cultures were mixed with equal amounts of each COAG reagent: 5% stabilized staphylococcal cells sensitized with *Salmonella* A, D, Vi, and polyvalent antisera (2). Slides were rocked back and forth and observed for 2 min for grossly visible clumping. If spontaneous agglutination or cross-reactions occurred, culture samples were placed in a boiling water bath for 1 min and then retested. This procedure eliminated all nonspecific reactions that could have been caused by heat-labile blood components.

Results comparing conventional culture detection with COAG test detection of salmonellae are shown in Table 1. For the 39 positive cultures, agreement between the two detection methods was noted in 37; however, one *Salmonella typhi* culture was detected by conventional means only, and one *S. paratyphi* A was detected by the COAG test only. These results indicated that conventional and COAG methods were equally as effective in detecting positive *Salmonella* blood clot cultures.

Early diagnosis of enteric fever can be an important factor in suggesting appropriate, effective therapy. In one study, after 1 day of incubation of whole blood ox bile cultures, COAG tests detected 22% positives (5). In the present study, COAG tests detected >60% positives after 1 day of incubation of blood clot cultures (Table 1). The difference may be that culturing only blood clots instead of whole blood reduces or eliminates inhibitory blood compo-

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TABLE 1. Comparison of COAG and conventional culture methods for the detection of salmonellae in ox bile blood clot cultures

Culture isolate	Day[a] of sampling	No. of positive samples identified by:	
		Conventional subculturing	COAG test
S. typhi	1	24	23
	3	11	11
	7	2	2
S. paratyphi A	1	1	1
	3	0	0
	7	0	1

[a] Two more days were required for identification by conventional subculturing.

nents in the culture system. These results tend to confirm previous experience (7). Furthermore, the COAG test detection system offers same-day detection, in contrast to conventional culture detection methods, which require an additional 48 h.

Some blood serum components are known to cause nonspecific agglutination of COAG reagents, often necessitating elaborate pretest specimen treatments such as lengthy heating, absorption with staphylococcal cells, and specimen dilution (3, 5). These procedures were not required with the blood clot culture COAG system. Some cultures exhibited cross-reactions, but 1 min of boiling the culture sample eliminated these.

In many developing countries, local customs restrict the availability of adequate amounts of blood specimens for culturing, although limited-size blood samples are frequently available for Widal tests. Clots are usually discarded from these serology specimens. If blood clot culturing is used, these precious blood specimens can be used in diagnostic serology as well as in diagnostic culturing.

The cost of preparing and using COAG reagents is relatively low (6). COAG tests also save money by eliminating the need for secondary culture media and by reducing technician waiting time. Thus, in countries where *Salmonella* enteric fevers are endemic, rapid and economical diagnostic results can be significant.

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