teriological findings. Both the direct and indirect techniques were evaluated and found to be comparable. The specificity and sensitivity of the technique have been studied. It is our conclusion that this method may be a useful one to add to the existing methods for diagnosing cholera.

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Experience in Darkfield Examination of Stools from Diarrheal Patients[1]

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Darkfield examination of rectal swabs or stool material from acute diarrheal cases permits the rapid recognition of organisms with characteristic motility or shape. The application of this technique to the identification of *Vibrio cholerae* has been described in the Bulletin of the World Health Organization (*1*). The present paper reports the results obtained on a larger patient group by technicians trained in our laboratory.

Methods

For the identification of *Vibrio cholerae:*

(1) A freshly obtained rectal swab is immersed (before drying) in 0.3–0.5 ml. of broth.
(2) A drop of this suspension is placed under a coverslip on a clean slide.
(3) This preparation is examined under darkfield illumination (or phase illumination, if available).
(4) If no organisms with rapid motility are seen, examination of the liquid stool itself may prove positive. In any event, the rectal swab should be incubated in the broth for 6–18 hours at 37° C. and reexamined.
(5) If organisms with the characteristic rapid motility of the cholera vibrio are seen, two coverslip preparations are set up, one after mixing with a drop of anti-Inaba serum, and the other with a drop of anti-Ogawa serum. It is essential that no preservative is present in the antisera used.
(6) These preparations are examined in the darkfield. If the original rectal swab contained *Vibrio cholerae,* vibrios of the specific serotype will be immobilized by the homologous antiserum. If the characteristic motility is not affected by either serum, the organism is presumed to be a noncholera vibrio or some other rapidly motile species.

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When the small Cooke-McArthur microscope [2] (McArthur, 1945) is used, the microscope, antisera, slides, and cover slips, together with match sticks which can be used as applicators, can be carried in a coat pocket to the site of an outbreak, wherever this is. These comprise the essentials for the specific diagnosis of cholera.

Fluorescent antibody-staining was carried out as described by Finkelstein and La Brec (2), using labeled antisera provided by Dr. R. A. Finkelstein.

Results

With the darkfield technique identification of the infecting serotype of *Vibrio cholerae* was made within 2–5 minutes after seeing the patient, in 49.5 percent of admissions during the period February to December 1964 (table 1). When the microscopic examination was performed after overnight incubation, the etiological agent was recognized in 84.8 percent of those examined (table 2). Included as positive cases are some who were positive on darkfield examination but negative bacteriologically, who had a clinical course typical of cholera, associated with a significant rise in agglutinating antibodies for live *Vibrio cholerae*.

Table 1.—Darkfield recognition of *Vibrio cholerae* rectal swab of positive cases, February–December 1964

Direct examination:	
Correctly identified	162
Vibrios positive, but too few to type	67
Missed by darkfield	98
Total	327
Percent identified	49.5

Table 2.—Darkfield recognition of *Vibrio cholerae* rectal swab of positive cases, February–December 1964

After overnight incubation:	
Correctly identified	273
Vibrios positive, no type	48
Total	322
Percent identified	84.8

Five hundred and sixty-eight cases negative bacteriologically and serologically were examined; two of these were reported by direct darkfield examination to contain *Vibrio cholerae*. Of 757 negative cases examined after overnight incubation, only 1 was falsely reported positive for *Vibrio cholerae* (table 3).

Table 3.—Darkfield recognition of *Vibrio cholerae* rectal swab of negative cases, February–December 1964

Direct examination:	
Reported negative	566
False-positive *V. cholerae*	2
After overnight incubation:	
Reported negative	756
False-positive *V. cholerae*	1

In addition, darkfield examination permits the visualization of all formed elements within the stool. Flagellates are not infrequently seen. Most interesting are the spirochetal forms resembling *Borrelia,* which are rapidly moving, and sometimes are present in numbers estimated to reach 10^8 or 10^9 per ml. Rectal swabs were examined by the senior author from 65 consecutive cases of acute diarrhea; 70 percent were vibrio-positive and 70 percent were positive for spirochetes; in 55 percent both vibrios and spirochetes were present (table 4). Thus, acute diarrheal disease was noted with vibrios alone, with spirochetes alone, and with both together.

Table 4.—Observation of vibrios and spirochetes in 65 consecutive cases of diarrheal disease

Vibrio cholerae	Spirochetes	Number	Percent
Negative	Negative	7	11
Negative	Positive	13	20
Positive	Positive	33	51
Positive	Negative	12	18
Total		65	100

In view of the relationship of these forms to vibrios suggested by Koch (5), it was especially interesting to note the lack of effect of specific anticholera sera on the motility of these spirochetes: in preparations in which the vibrios were completely immobilized, spirochetes continued to be active for long periods of time. Attempts were made to stain the spirochetes with fluorescent-labeled antibodies against *Vibrio cholerae*. Although the antiserum contained antibodies directed against group and specific antigens of *Vibrio cholerae,* no fluorescence of the spirochetes was noted, while sharp fluorescent staining of the accompanying vibrios occurred.

Discussion

Examination of rectal swabs or stool by darkfield microscopy provides information not available by routine culture

[2] Available from Vickers Instruments Ltd., 226 Purley Way, Croydon, Surrey.

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