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Use of Filter Paper as a Transport Medium for Laboratory Diagnosis of Cholera under Field Conditions[▽]

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Confirmation of a cholera epidemic is based on bacteriological identification of the agent and requires the sending of samples to a culture laboratory, often in countries with limited resources. Comparison of the use of filter paper with the use of Cary-Blair reference medium for stool transport showed that this simple transport medium is appropriate for the recovery of *Vibrio cholerae*.

Cholera outbreak confirmation and isolate characterization are critical to set up an appropriate response. However, cholera outbreaks frequently occur in remote areas with limited access to adequate microbiology laboratories. Samples need to be sent to reference laboratories which are sometimes located outside the country, with shipment times reaching a few days. The only recommended transport medium for *Vibrio cholerae* according to the World Health Organization (WHO) is Cary-Blair transport medium (3, 4). Stuart's (9) and Amie's (1) transport media are inferior to Cary-Blair medium for transport of *V. cholerae*, and alkaline peptone water (APW) is recommended for use only when Cary-Blair medium is not available and subculture can be done within 6 h of collection (4).

The use of strips of blotting paper in sealed plastic envelopes for stool transportation for cholera testing was first developed in the late 1960s, following the satisfactory use of this alternative mode of transportation for the isolation of *Salmonella* and *Shigella* spp. (2, 8). Despite demonstrations of its efficiency, this mode of transportation has not been added to the list of WHO-recommended transport media for the isolation of *V. cholerae*, possibly due to the difficulties involved in packing the samples in plastic envelopes and ensuring that they remain sufficiently moist during shipment. In the early 1990s, the Institut Pasteur and the nongovernmental medical organization Médecins Sans Frontières (MSF) improved this alternative mode of transportation by standardizing the packaging (7) and modifying the procedure used. This simple method has now been used by MSF for over 20 years and has made it possible to recover and to characterize samples from various locations around the world.

We compared this improved filter paper transport method with the method of using Cary-Blair medium for the recovery of *V. cholerae* from stool samples. The findings reported here

relate to a substudy in the evaluation of a rapid test for the diagnosis of cholera during an outbreak in Lubumbashi, Democratic Republic of Congo (DRC), which will be published elsewhere. The study took place in two Ministry of Health cholera treatment centers (CTC) supported by MSF in Lubumbashi. Ethical approval was obtained from the Ethics Committee of the Ecole de Santé Publique, Kinshasa, DRC, and the Comité de Protection des Personnes, Ile de France XI, France.

Patients presenting to the CTC were included in the study if they were over 5 years of age, if they had acute watery diarrhea with or without vomiting, and if they or their guardian signed written informed consent. Exclusion criteria were ingestion of antibiotics in the previous 7 days and absence of stool during the observation period. The sample size required for McNemar statistical comparison was initially estimated as 148 specimens based on the following hypotheses and parameters: 10% difference in culture results, 20% discordant pairs, an alpha risk of 5%, and study power of 80%.

Samples were prepared in Cary-Blair medium according to the manufacturer's recommendations (Copan Diagnostics, Italy), i.e., the swab was dipped into the nonchlorinated liquid stool specimen and immersed in the transport medium. For transportation on filter paper, a blotting paper disc (commercially available nonimpregnated paper discs [6.35 mm in diameter, not sterilized]; Bio-Rad, Marnes-la-Coquette, France) was dipped into the nonchlorinated liquid stool and placed in a screw-cap microtube. A few drops (2 or 3, around 200 μ l) of normal saline solution (0.9% NaCl) were added to stop the sample from drying out, as our experience in practice and published data (2, 8) showed that the maintenance of moisture is essential for the recovery of strains (Fig. 1). The tube was hermetically closed with Parafilm. Both transport media were stored at room temperature and sent weekly to the Institut Pasteur, Paris, France, following International Air Transport Association (IATA) regulations for infectious substances. The liquid supernatant from the filter paper tube or the swab from the Cary-Blair medium was plated directly on appropriate medium. The paper disc or the swab was transferred directly to

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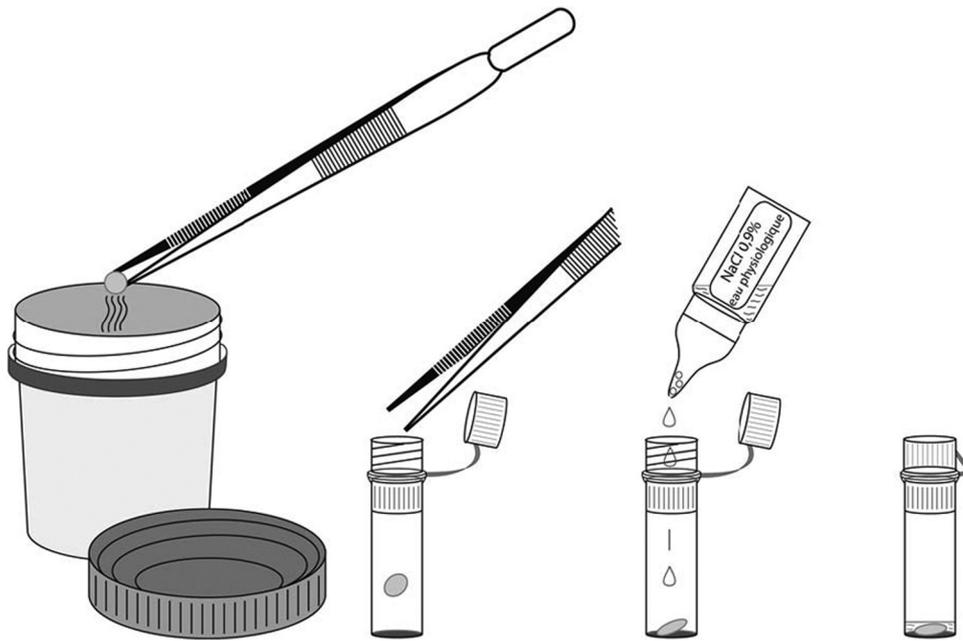


FIG. 1. Device/method used by MSF for the transport to the laboratory of stool specimens for research of *Vibrio cholerae*. Filter paper was standardized by using commercialized nonimpregnated discs (Bio-Rad, Marnes-la-Coquette, France). (Copyright Institut Pasteur.)

hyperalkaline peptone water for enrichment. Isolation of choleraenic vibrios (*Vibrio cholerae* serogroup O1 or O139) was performed by culture following enrichment steps according to standard methods (5).

The study started on 2 March 2008 and ended on 2 May 2008. In total, 296 patients were included in the general study (i.e., the evaluation of a rapid diagnostic test), 234 of whom were included in the substudy comparing the filter paper and Cary-Blair medium methods for stool transport. The patients included in this substudy had a median age of 29 years (interquartile range [IQR], 19 to 40 years), and the ratio of males to females was 1.3; these values were similar to those for the patients in the general study. Dehydration was assessed on the basis of clinical signs at admission according to WHO guidelines (10) and was considered severe in 33 (14%) of the patients included, moderate in 54 patients (23%), and absent in 147 patients (63%). Among the first 34 samples sent both on filter paper and in Cary-Blair medium, 20 tested positive for *V. cholerae* O1 when cultured from filter paper, while only 10 tested positive when cultured from Cary-Blair medium. This difference was unexpected, and we thought that it might be an anomaly. We therefore decided to exclude these 34 samples

from the study and to use a new, single batch of Cary-Blair medium for subsequent comparisons. This resulted in a final sample size of 200 for the comparison of filter paper and Cary-Blair medium (Table 1). The median delay between sample collection and culture in Paris was 13 days (range, 7 to 17 days) for both Cary-Blair medium and filter paper. There was a good correlation, with a kappa value of 0.76 (95% confidence interval [CI], 0.62 to 0.89), and no statistically significant difference (McNemar's exact χ^2 test P value = 0.10) between the two transport media. The proportion of positive samples was still high even when culture was performed more than 2 weeks after collection, with no statistically significant differences in proportions of positive samples between transport media (Table 2). These results show that the filter paper method is as effective as use of the recommended Cary-Blair medium for specimen transport for culture in field conditions, confirming the results obtained during earlier studies (6, 8).

We added a small volume of saline to the specimen. This was

TABLE 1. Comparison of culture results of specimens transported on Cary-Blair medium or filter paper, Lubumbashi, Democratic Republic of Congo, 2008 ($n = 200$)

Result for specimens on filter paper	No. of specimens from Cary-Blair medium with indicated result		
	Positive	Negative	Total
Positive	74	8	82
Negative	16	102	118
Total	90	110	200

TABLE 2. Proportion of positive specimens by culture using Cary-Blair medium or filter paper for transport according to the duration between sample collection and culture, Lubumbashi, Democratic Republic of Congo, 2008 ($n = 150$)

Delay (in days) from sample collection to culture (no. of specimens) ^a	Specimens on:				McNemar's test P value
	Cary-Blair medium		Filter paper		
	No.	%	No.	%	
7-10 (18)	7	39	7	39	1
11-14 (67)	22	33	21	31	1
>14 (65)	38	58	33	51	0.226

^a To facilitate the comparison, the analysis was restricted to the samples with the same delays for culture performed from Cary-Blair medium or filter paper.

not done in previous studies and was found to be sufficient to prevent dehydration problems, even for shipment periods of up to 17 days. Moreover, the filter paper method has several advantages over use of Cary-Blair medium. Filter paper and normal saline are easy to find and inexpensive and can be stored for long periods at room temperature, in contrast to Cary-Blair medium, which requires storage at a temperature less than 25°C or even refrigeration (2 to 8°C), according to the manufacturer, and has a relatively short shelf life. In addition, as observed in this study, there appears to be performance variation between batches of Cary-Blair medium, which may lead to false-negative culture results. Although the high batch-to-batch consistency of filter paper has yet to be proven, the simplicity of the method and medium might make it less susceptible to variations and better adapted to field conditions than the more sophisticated Cary-Blair medium.

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REFERENCES

1. Amies, C. R. 1967. A modified formula for the preparation of Stuart's transport medium. *Can. J. Public Health* **58**:296–300.
2. Barua, D., and C. Z. Gomez. 1967. Blotting-paper strips for transportation of cholera stools. *Bull. World Health Organ.* **37**:798.
3. Cary, S. G., and E. B. Blair. 1964. New transport medium for shipment of clinical specimens. *J. Bacteriol.* **88**:96–98.
4. Centers for Disease Control and Prevention. 1999. Laboratory methods for the diagnosis of epidemic dysentery and cholera. WHO/CDS/CSR/EDC/99.8. Centers for Disease Control and Prevention, Atlanta, GA.
5. Dodin, A., and J. M. Fournier. 1992. Diagnosis of the cholera vibrio (*Vibrio cholerae* O1), p. 59–82. In Institut Pasteur (ed.), Laboratory methods for the diagnosis of cholera vibrio and other vibrios. Institut Pasteur, Paris, France.
6. Estrada, S., E. Jaramillo, M. Gaviria, and S. Ospina. 1994. Comparative study of two transport systems for *Vibrio cholerae*. *Trop. Doct.* **24**:35–36.
7. Fournier, J. M., and M. L. Quilici. 2007. Cholera. *Presse Med.* **36**:727–739.
8. Huq, M. L., and M. M. Rahaman. 1983. Blotting paper strip for the transport of stool specimens for laboratory diagnosis of cholera. *Indian J. Med. Res.* **78**:765–768.
9. Stuart, R. D. 1959. Transport medium for specimens in public health bacteriology. *Public Health Rep.* **74**:431–438.
10. World Health Organization. 1993. Guidelines for cholera control. World Health Organization, Geneva, Switzerland.