

Comparative analysis of transport media for isolating *Shigella*

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ABSTRACT

Transport media for *Shigella* include buffered glycerol saline (BGS), and Cary-Blair (CB). However being a liquid medium BGS may leak or spill during transport and thus may cause contamination. The other concern is the 30% concentration of glycerol in the BGS which may be inhibitory to some susceptible *Shigella* species. This study was conducted to determine the best and safe transport media for *Shigella*. Rectal swab samples were obtained from 289 dysenteric patients and transported to the laboratory in Cary-Blair (CB) transport medium, standard buffered glycerol saline (BGS), BGS with the addition of 0.5% agar (BGS-A), and BGS with the addition of 0.5% agar and reduced glycerol to 15% (BGS-M). Recovery rates between CB, BGS, BGS-A and BGS-M and their combinations were compared. The overall prevalence of *Shigella* recovered from any of the four tubes was 24.9% (72/289). CB and BGS-M recovered *Shigella* in 54 out of 289 patients (18.7%), CB and BGS-A in 50 (17.3%), and CB and BGS in 49 (17.0%), while CB, BGS, BGS-A, and BGS-M alone gave positive *Shigella* in 30 (10.4%), 29 (10.0%), 34 (11.8%) and 46 (15.9%), respectively. This study suggests that a minor modification to the BGS raised the recovery rate of *Shigella*.

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Univ Med 2008; 27: 51-6.

Keywords: Transport media, *Shigella*, isolation

INTRODUCTION

Shigellosis occurs both in epidemic and endemic forms in children and adults and remains a major public health problem in developing countries, causing high morbidity and mortality, particularly in children.^(1,2) Specific

diagnosis of *Shigella* infection depends on the isolation and identification of the organism from microbiological cultures of stool or rectal swab sample. It is important to laboratory examination that care should be taken to collect suitable fecal specimen, to use appropriate transport medium, and to ensure that specimen is transported to laboratory in a timely manner.

Shigellae are fastidious microorganisms and survive poorly in the environment. They remain viable for a limited time outside the human body, therefore, specimens for culture should be processed as soon as possible after collection.⁽³⁾ Delays of specimen process can result in substantial under-detection of the organism since it will reduce the number of viable *Shigella*.⁽⁴⁾ If a delay is anticipated between collection and processing of specimens, transport medium should be used. Transport medium is most commonly used for specimens collected for enteric pathogens analysis, and many media have been developed for this purpose.⁽⁵⁾ For many years transport media commonly used for *Shigella*-containing stool include buffered glycerol saline (BGS) and Cary-Blair (CB).⁽⁶⁻⁹⁾ BGS has been widely used without undergone any major changes since it was developed and later perfected by Sachs in 1939.⁽¹⁰⁾ Although CB is probably the best overall transport medium for diarrheal stool or rectal swab samples,⁽⁷⁾ for the recovery of *Shigella*, BGS was reported to be a better medium than CB.^(3,11,12) One disadvantage of BGS, however, is that being a liquid medium it may leak or spill during transport and thus may cause environmental contamination with bacterial pathogens of the specimen being transported. The other concern is the 30%

concentration of glycerol in the BGS which may be inhibitory to some susceptible *Shigella* species. A literature search failed to find a transport medium for *Shigella* which has a smaller concentration of glycerol other than the BGS. Recent publications on BGS and other transport media were also scarce.

To determine the best and safe transport media for *Shigella*, we evaluated CB medium, standard traditional BGS as described and modified by Sachs,⁽¹⁰⁾ and two modified BGS media, which were (i) BGS with the addition of 0,5% agar without any other changes (BGS-A), and (ii) BGS with the addition of 0,5% agar and reduced glycerol content to a final concentration of 15% (BGS-M).

METHODS

Preparation of transport media

CB (Becton Dickinson, Sparks, MD) was prepared according to the direction provided by the manufacturer. Standard BGS (BGS) was prepared from the formula according to the method described elsewhere.^(13,14) For BGS-A, a 0.5% agar was added to the formula of standard BGS, and for BGS-M a 0.5% agar was added to the formula of standard BGS and the glycerol content was reduced to a final concentration of 15% (Table 1).

Table 1. Formula of buffered glycerol saline (BGS) and its modifications

Ingredient	Standard BGS (BGS)	BGS + 0.5% agar (BGS-A)	BGS + 0.5% agar with 15% glycerol (BGS-M)
NaCl	4.2 g	4.2 g	4.2 g
K ₂ HPO ₄	3.1 g	3.1 g	3.1 g
KH ₂ PO ₄	1.0 g	1.0 g	1.0 g
Phend red	0.003 g	0.003 g	0.003 g
Glycerol	300 mL	300 mL	150 mL
Distilled water	700 mL	700 mL	850 mL
Agar	0.0 g	5.0 g	5.0 g

Collection and transportation of specimens

From September 2005 through November 2007, a *Shigella* surveillance was conducted in Jakarta, involving community health center. Consenting patients of all age groups presenting with dysenteric diarrhea (stool with blood and/or mucus) were invited to participate. Four rectal swabs were collected from each patient and each swab was placed in CB, BGS, BGS-A and BGS-M, respectively. Swab samples in transport media were held in the refrigerator (2^o to 8^oC) until the end of the normal working day, at which they were transported in a cool-box to the microbiology laboratory of Trisakti Medical Faculty. The swabs were processed immediately.

Bacteriological procedure

Swab specimen in transport medium was inoculated directly onto the surface of MacConkey (MAC) agar (DIFCO, Becton Dickinson, Sparks, MD) and xylose lysine desoxycholate (XLD) agar plate (DIFCO, Becton Dickinson, Sparks, MD). Plates were incubated at 37^oC, aerobically, for 20-24 hours. Three suspicious colonies (non-lactose fermenting) from each culture media were picked and identified by conventional biochemical test.⁽¹⁵⁾ Serological confirmation of isolates was performed by slide agglutination

method employing group-specific *Shigella* antisera (DIFCO, Becton Dickinson, Sparks, MD). Rectal swab specimen in a particular transport medium was considered negative for *Shigella* when there were no growth on both MAC and XLD.

Statistical analysis

Chi-square test to compare the proportions of positive samples were used to determine the significant differences in isolation rates for individual and combined transport media. A probability value $p < 0.05$ was considered statistically significant.

RESULTS

A total of 289 rectal swab samples from patients presenting with dysenteric diarrhea were obtained and processed. *Shigella* was recovered from 72 (24.9%) of the rectal swab samples. The proportional distribution of *Shigella* species was presented in Table 2. *Shigella flexneri* was the most prevalent subgroup isolated with a rate of 17.3% (50/289), followed by *S. sonnei* with 7.3% (21/289) and *S. boydii*, with 0.3% (1/289). No *S. dysenteriae* was isolated. Both *S. flexneri* and *S. sonnei* comprised 98.6% (71/72) of all *Shigella* isolated in this study.

Table 2. Isolation of *Shigella* spp. from rectal swab samples (n=289) in 4 transport media

Transport medium/ Combination*	Number (%) <i>Shigella</i> isolates				Total
	<i>S. flexneri</i>	<i>S. sonnei</i>	<i>S. boydii</i>	<i>S. dysenteriae</i>	
CB	20 (6.9)	9 (3.1)	1 (0.3)	0	30 (10.4)
BGS	22 (7.6)	7 (2.4)	0	0	29 (10.0)
BGS-A	22 (7.6)	12 (4.2)	0	0	34 (11.8)
BGS-M	31 (10.7)	15 (5.2)	0	0	46 (15.9)
CB+BGS	34 (11.8)	14 (4.8)	1 (0.3)	0	49 (17.0)
CB+BGS-A	33 (11.4)	16 (5.5)	1 (0.3)	0	50 (17.3)
CB+BGS-M	38 (13.1)	15 (5.2)	1 (0.3)	0	54 (18.7)
CB+BGS/A/M	50 (17.3)	21 (7.3)	1 (0.3)	0	72 (24.9)

* CB=Cary-Blair; BGS=buffered glycerol saline; BGS-A= buffered glycerol saline + agar; BGS-M= buffered glycerol saline + agar and reduced glycerol

In relation to the media used for transportation of the specimen, *Shigella flexneri* was recovered from 10.7% (31/289) rectal swab samples preserved and transported in BGS-M, followed by those in BGS and BGS-A each with 7.6% isolation rate, and in CB with 6.9%. *Shigella sonnei* was isolated from 9 (3.1%) rectal swab samples preserved and transported in CB which was almost similar to that in BGS (2.4%), while BGS-A and BGS-M yielded 4.2% and 5.2% *S. sonnei* isolates, respectively. For both *S. sonnei* and *S. flexneri*, BGS-M with 15.9% (46/289) isolation rate provided the highest result compared to the other three transport media. For the isolation of *S. flexneri* the combination of CB+BGS-M (13.1%) was slightly better than those of CB+BGS (11.8%) and CB+BGS-A (11.4%), whereas for *S. sonnei* it was CB+BGS-A (5.5%) which gave higher isolation rate.

Overall isolation rate of *Shigella* from specimens preserved and transported in CB was 10.4%, in BGS was 10.0%, in BGS-A was 11.8% and in BGS-M was 15.9%. Compared to CB and BGS, BGS-M was significantly superior ($p < 0.05$) in the total recovery of *Shigella* species, but it was not significantly different than BGS-A ($p > 0.05$). The combination of CB and BGS-M provided 18.7% isolation rate of *Shigella*, whereas the combination of CB and BGS, CB and BGS-A yielded 17.0% and 17.3%, respectively. The combination of CB and BGS-M demonstrated a slightly better isolation rate for *Shigella*, however, it did not differ significantly from other combinations of transport media ($p > 0.05$).

DISCUSSION

For over five decades since Sachs⁽¹⁰⁾ made a modification on the BGS from its original formulation this medium has been widely used without undergone any changes for preservation

and transportation of *Shigella* species in stool or rectal swab specimen. For recovery of *Shigella*, the efficiency of BGS was reportedly superior than CB.^(3,11,12)

Very few studies evaluated this medium were available since approximately over 20 years ago.^(3,12) Maybe because BGS was considered to be the best and convenient transport medium for *Shigella* in stool that further evaluations were not required. Study conducted in 1997 by Bonten et al⁽¹¹⁾ was the most recent on BGS which involved fecal flora of non-*Shigella*. They compared the recovery of bacteria from stool specimens or rectal swabs that had been frozen using three preservatives including CB and BGS and concluded that BGS performed better than CB.

Being a liquid medium BGS is more likely to spill or leak during transport and the specimen may contaminate the environment with enteric pathogens, however, there has been no attempt to minimize the risk. By the addition of 0.5% agar which made the medium semisolid, the problem of spillage and contamination has been able to be avoided. Thus, buffered glycerol saline with the addition of 0.5% agar (BGS-A) is a safer transport medium than the standard formula. Our data shows that 0.5% agar in the standard BGS also increased slightly the yield of *Shigella* species in the stool or rectal swab specimen from 10.0% (in BGS) to 11.8% (in BGS-A). It appears that agar not only served as a solidifying agent but increased the preserving capacity of the medium for *Shigella* as well.

The standard BGS contains glycerol at 30% concentration. BGS was a better medium to CB for preservation and transportation of *Shigella* as reported by several investigators.^(6-8,15) According to Bonten et al⁽¹¹⁾ the stabilizing effects of glycerol in BGS explained the superiority of this medium to CB. Our study shows that the total isolation rates of *Shigella*

from the rectal swab specimens preserved in the standard BGS was no difference than that obtained from CB. The isolation rate, however, increased significantly from 10.0% to 15.9.0% ($p < 0.05$) when the content of glycerol in the BGS was reduced to a final concentration of 15% in addition to the incorporation of agar (0.5%) such as that in the BGS-M (Table 2). It appears that the relatively high concentration of glycerol (30%) in the standard BGS was inhibitory to some susceptible *Shigella* species. The inhibitory effect of 30% glycerol was reported on *Vibrio parahaemolyticus* and to a lesser degree on *Salmonella* as well.⁽¹⁶⁾ In the long-term storage of bacterial isolates, glycerol was also used and added into the broth medium, namely trypticase soy broth, but in a lower concentration than that in the transport medium. In this maintenance medium the concentration of glycerol was between 15% to 20%.^(9,17,18) The presence of glycerol as a preservative in a storage medium was associated with organism survival. Glycerol protects the intracellular environment of the organism.⁽¹⁷⁾ Compared to CB, the isolation rate of *Shigella* also increased significantly ($p < 0.05$) when using BGS-M as transport medium for fecal or rectal swab specimen.

In most studies of diarrheal diseases we usually employed two transport media, BGS specially for *Shigella* and CB for the isolation of other enteric pathogens. Specimen in BGS and CB were both processed for *Shigella*. Our data show that the combination of CB and BGS-M was better than other combinations although the differences were minimum.

CONCLUSION

The use of CB and BGS may underestimate the true *Shigella* prevalence, whereas a minor modification to the BGS, namely the BGS-M, raised the recovery rate of *Shigella*.

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