

Comparison of agar based media for primary isolation of *Helicobacter pylori*

C Y Hachem, J E Clarridge, D G Evans, D Y Graham

Abstract

Aims—To determine the best medium for the primary isolation of *Helicobacter pylori*.

Methods—Sixty six gastric mucosal biopsy specimens frozen in 1 ml Cysteine Albimi media with 20% glycerol from 22 histologically proven *H pylori* infected patients were cultured on brain heart infusion agar (BHIA) with 7% fresh whole defibrinated horse blood, egg yolk agar (EYA), Columbia blood agar-cyclodextrin agar (CBA-Cd), and commercial trypticase soy agar (TSA) supplemented with 5% sheep blood.

Results—Successful primary isolation of *H pylori* was 96% with BHIA, 78% with TSA, 64% for EYA, and 32% with CBA-Cd. Colonies appeared earlier on BHIA (4.7 ± 0.1 days, 5.3 ± 0.4 days, 5.3 ± 0.4 days, and 7.1 ± 0.9 days for BHIA, TSA, EYA, and CBA-Cd) and there were more colonies on BHIA than on CBA-Cd, EYA or TSA (599 ± 88 , 104 ± 66 , 260 ± 107 , and 358 ± 89 , respectively).

Conclusions—Success of a medium for passage of isolates apparently does not reliably predict usefulness for primary isolation. Freshly made BHIA with 7% horse blood medium is recommended for primary isolation. However, the easily obtainable TSA media would be the best alternative for routine clinical laboratories with no access to BHIA.

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Keywords: *Helicobacter pylori*, culture media, isolation, clinical trial.

Helicobacter pylori is a Gram negative, spiral shaped, microaerobic bacterium that causes gastritis and peptic ulcer disease. Isolation of *H pylori* from gastric mucosal biopsy specimens is important to confirm *H pylori* as the cause of gastritis and is a prerequisite for further studies of the organism such as drug susceptibility testing, analysis of putative virulence factors, or other comparative studies. Many hospital and research laboratories have found it difficult to culture *H pylori* from gastric mucosal biopsy specimens.^{1,2} A number of complex media containing serum, blood, blood derivatives, egg yolk, or cyclodextrins have been described for the culture of *H pylori*.^{1,3-6} The best media for the primary isolation of *H pylori* as well as the suitability of new media containing egg yolk⁶ or cyclodextrins⁴ for primary isolation is unknown.

Multicentre trials of antimicrobial therapy for *H pylori* infection often use central laboratories for culture and histological confirmation of *H pylori* infection. Popular and new media have not been directly compared for the primary isolation of *H pylori*. We evaluated four media, including both commercially prepared, blood based agars as well as the newer egg yolk and cyclodextrin containing media, for the primary isolation of *H pylori* from frozen gastric mucosal biopsy specimens.

Methods

Media tested included those prepared in our laboratory: brain heart infusion agar (BHIA) (Difco, Detroit, Michigan, USA) with 7% fresh whole defibrinated horse blood as non-selective and selective media (10 mg/l nalidixic acid, 5 mg/l trimethoprim, 3 mg/l vancomycin, 2 mg/l amphotericin B) (Sigma, St Louis, Missouri, USA); egg yolk agar (EYA) consisting of Columbia agar 39 g/l, 10% egg yolk emulsion and 1% Isovitalex (Difco)⁶; Columbia blood agar-cyclodextrin (CBA-Cd), 44 g/l, (Difco) containing 1% B-cyclodextrin supplemented with a mixture of nalidixic acid, trimethoprim, vancomycin, and amphotericin B as noted above⁴; and commercial trypticase soy agar (TSA) supplemented with 5% sheep blood (BBL Microbiology Systems, Cockeysville, Maryland, USA).

BIOPSY SPECIMENS

Three gastric mucosal biopsy specimens for culture were collected from 26 patients, two from the antrum and one from the corpus. Separately obtained biopsy specimens were examined histologically using the Genta stain⁷ and were scored as *H pylori* positive or negative. Histology results were used as the reference for *H pylori* status. Primary isolation of *H pylori* was attempted from the biopsy specimens shipped by overnight mail to the laboratory frozen on dry ice in 1 ml Cysteine Albimi media (which contains casamino acids, peptone, yeast extract, L-cysteine, and dextrose) with 20% glycerol. Upon receipt, the frozen specimens were stored at -70°C . Immediately before culture, each biopsy specimen was thawed to room temperature and processed by grinding between two frosted sterile microscope slides. A suspension was made with 0.5 ml Cysteine Albimi broth media and 60 μl of the suspension was streaked for isolation onto the different agar plates.

Plates were incubated in 12% CO_2 , 96 to 100% humidity at 37°C for up to 14 days.

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Comparison of the different media for the primary isolation of *H pylori*

Patient No.	BHIA		CBA-Cd		EYA		TSA	
	No. of colonies	Day*	No. of colonies	Day*	No. of colonies	Day*	No. of colonies	Day*
1	>1000	4	0	-	100	4	>1000	5
2	>1000	4	50	10	50	4	200	4
3	200	5	0	-	0	-	50	5
4	>1000	5	0	-	80	4	200	5
5	0	-	0	-	0	-	0	-
6	>1000	4	15	7	>1000	4	200	5
7	>1000	4	60	7	>1000	7	1000	5
8	200	5	0	-	15	8	0	-
9	100	5	5	11	0	-	0	-
10	>1000	5	0	-	16	8	200	5
11	200	6	0	-	5	10	200	6
12	>1000	4	0	-	150	4	>1000	5
13	>1000	4	0	-	80	4	200	4
14	>1000	4	500	4	>1000	4	>1000	4
15	200	6	0	-	0	-	50	11
16	500	5	0	-	0	-	100	7
17	150	6	50	6	0	-	0	-
18	500	4	0	-	100	4	200	5
19	>1000	5	0	-	0	-	200	5
20	40	5	0	-	6	5	0	-
21	200	5	50	5	50	5	200	5
22	300	5	0	-	0	-	100	5

* Days to first appearance of colonies.

Plates were first checked for growth on day 3 and daily thereafter. Typical small, round colonies positive for catalase, oxidase and urease with typical morphology on Gram staining were regarded as *H pylori*. Growth, number of colonies, and days to appearance on the different agar media were recorded. Infection was scored as present if any of the biopsy sites were positive. In practice, biopsy specimens were either all positive or all negative.

TRIAL OF ISOLATION STRATEGY

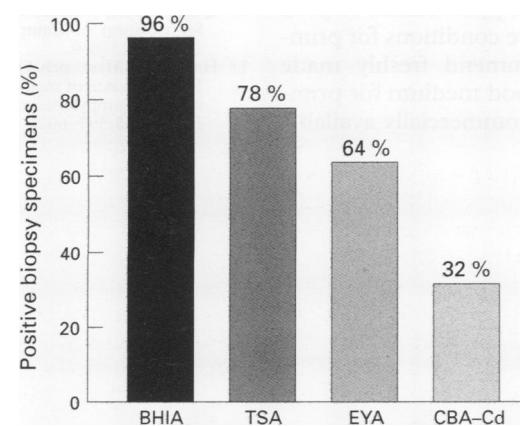
Frozen gastric mucosal biopsy specimens from 253 consecutive patients with duodenal ulcer disease were treated and cultured as described earlier. The results for each patient on culture of two antral and one corpus biopsy specimen, and on histological examination of two antral biopsy specimens were compared.

Results

Seventy eight gastric mucosal biopsy specimens from 26 patients were evaluated on each medium. Twenty two patients were infected with *H pylori*; four were not. The time from collection to culture ranged from two to seven days. *H pylori* was isolated from 96% of positive

cases, confirmed on histology, and from none of the *H pylori* negative cases. Although primary isolation of *H pylori* was possible with all of the agar based media tested in this study, the usefulness of these media varied (table). Primary isolation of *H pylori* was most successful with BHIA, followed by TSA, EYA, and least with CBA-Cd (figure) ($p < 0.05$ for BHIA compared with EYA or CBA-Cd and TSA *v* CBA-Cd). *H pylori* colonies were also larger on BHIA than any other medium and appeared earlier (4.7 ± 0.1 days, 5.3 ± 0.4 days, 5.3 ± 0.4 days, and 7.1 ± 0.9 days for BHIA, TSA, EYA, and CBA-Cd, respectively) ($p < 0.05$ for BHIA compared with TSA or CBA-Cd). Despite comparing the mean number of colonies (for our estimations, 1000 was used for >1000), there were significantly ($p < 0.05$) more colonies present on BHIA than on CBA-Cd, EYA or TSA (599 ± 88 , 104 ± 66 , 260 ± 107 and 358 ± 89 , respectively) (table).

The results of the small study were confirmed using 759 gastric mucosal biopsy specimens obtained under the typical conditions of a multicentre clinical trial (frozen specimens, stored and overnight mail). The delay between sample collection and isolation ranged from two to 30 days. *H pylori* was isolated from 474 (93.3%) of the 508 histologically positive cases using freshly prepared BHIA.



The success of the different media for the primary isolation of *H pylori* from frozen gastric mucosal biopsy specimens.

Discussion

H pylori is both microaerophilic and fastidious requiring a nutrient rich media and an atmosphere enriched in CO_2 . *H pylori* was first isolated from gastric antral mucosa using non-selective culture medium consisting of blood or chocolate agar in conjunction with Skirrow's selective medium. In the initial study specimens were processed within one hour of collection and incubated at $37^\circ C$ using an atmosphere consisting of 25% air, 7.5% H_2 , 7.5% CO_2 and 60% N_2 .⁸ The two main requirements for successful isolation of *H pylori* are use of fresh media made with fresh blood and maintenance of adequate humidity throughout incubation. Although the procedure and the culture con-

ditions originally described by Marshall *et al*⁸ remain successful, variations have been described with respect to maintenance conditions, time and procedure for tissue processing, composition of the culture medium, and atmospheric conditions. Marshall *et al* originally used brain heart infusion chocolate agar with 7% horse blood. Since then, blood based media, either selective or non-selective chocolate media, prepared with fresh blood, have remained the choice of most investigators. Brucella agar,^{9,10} brain heart infusion,^{9,11-13} containing either 5 or 10% sheep blood^{10,11} or 7% horse blood,^{8,11,14} egg yolk emulsion,⁶ cyclodextrins,⁴ and modified Thayer-Martin medium^{15,16} have also been used successfully. Haemoglobin or haemin is not an absolute requirement for growth of *H pylori* as GC agar containing either 1% starch or 0.2% charcoal, egg yolk emulsion, and cyclodextrins has been reported to be effective.^{4,6,9} The addition of tetrazolium salts to agar results in the *H pylori* colonies acquiring a characteristic golden colour and may facilitate their identification.¹⁷

In this study, BHIA with 7% fresh horse blood proved to be the best and most sensitive medium of those tested for the primary isolation of *H pylori* from frozen gastric mucosal biopsy specimens. We were unable to confirm recent reports of the potential usefulness of media containing cyclodextrins.⁴ We used 12% CO₂ instead of 5% O₂, 10% CO₂ and 85% N₂ in the original reports regarding the usefulness of egg yolk medium or media containing cyclodextrins.^{4,6} Other factors that differed between this and those studies were that the study of cyclodextrin containing medium did not use a standard to confirm the true rate of *H pylori* infection among the cases evaluated and attempted to isolate *H pylori* from fresh gastric mucosal biopsy specimens only.⁴ In addition, egg yolk media for isolation of *H pylori* were described for use with laboratory adapted isolates.⁶ It appears notably less useful for primary isolation; success of a media for passage of isolates apparently does not reliably predict usefulness for primary isolation.^{1,18} It is unknown whether any of these differences may have, in part, been responsible for the relatively poor results we obtained with cyclodextrin or egg yolk containing media.

Part of the problem perceived by many laboratories in isolating *H pylori* relates to poor choice of media or culture conditions for primary isolation. We recommend freshly made BHIA with 7% horse blood medium for primary isolation. Although commercially available

TSA was less sensitive in detecting *H pylori*, it was better than egg yolk emulsion or cyclodextrin media. Because of its commercial availability, it might be a reasonable choice for primary *H pylori* isolation in clinical laboratories.

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