JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1991, p. 819–821
0095-1137/91/040819-03$02.00/0

Vol. 29, No. 4

Egg Yolk Emulsion Agar, a New Medium for the Cultivation of *Helicobacter pylori*

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Received 10 August 1990/Accepted 15 January 1991

We developed a new agar, egg yolk emulsion (EYE) agar, for cultivation of *Helicobacter pylori*. EYE agar contains Columbia agar base (Oxoid), 10% EYE (Oxoid), 1% IsoVitaleX (BBL), and 40 mg of triphenyltetrazolium chloride (Sigma) per liter. We compared EYE agar with the following agars: (i) brain heart infusion agar–7% horse blood–1% IsoVitaleX (GDW agar; C. S. Goodwin, E. D. Blincow, J. R. Warren, T. E. Waters, C. R. Sanderson, and L. Easton, J. Clin. Pathol. 38:1127–1131, 1985), (ii) brain heart infusion agar–10% horse serum–0.2% charcoal–1% yeast extract–40 mg of triphenyltetrazolium chloride per liter (GLU agar; Y. Glupczynski, M. Labbe, and F. Thiabaumont, p. 3–6, *in* F. Megraud and H. Lamouliatte, ed., *Gastroduodenal Pathology and Campylobacter pylori*, 1989), (iii) Columbia agar with 7% lysed horse blood (D&M agar; J. C. Dent and C. A. M. McNulty, Eur. J. Clin. Microbiol. Infect. Dis. 7:555–558, 1988), and (iv) brain heart infusion agar–10% EYE–1% IsoVitaleX (BHIE agar). *H. pylori* CFU counts, expressed as average percentages of maximum growth, were as follows: EYE agar, 96; GDW agar, 76; BHIE agar, 57; D&M agar, 52; and GLU agar, 23. Colony counts for EYE agar were significantly higher than for GDW agar ($P = 0.027$), BHIE agar ($P = 0.005$), D&M agar ($P = 0.0001$), and GLU agar ($P < 0.0001$). EYE agar also had higher CFU counts than two commercial chocolate media; the EYE agar count was 80%, versus 33% for BBL chocolate medium and 63% for Remel chocolate medium.

Helicobacter pylori, formerly *Campylobacter pylori* (5), is a fastidious slow-growing organism that requires rich culture media for adequate growth. One of the first media used for this organism was brain heart infusion agar mixed with 7% defibrinated horse blood and 1% IsoVitaleX (BBL Microbiology Systems, Cockeysville, Md.) (6). Some investigators have favored Columbia agar with lysed horse blood (2). In 1988, Glupczynski et al. reported the use of a charcoal medium with brain heart infusion agar and horse serum (4). Recently, we discovered that *H. pylori* can grow in chicken eggs after inoculation of the egg yolk. This prompted a search for a new type of medium for *H. pylori*. We report the results of this search and our comparison of a new agar formulation with other types of *H. pylori* media.

We first investigated a suitable agar formulation containing egg yolk. When we compared Trypticase soy agar, Mueller-Hinton agar, and brucella agar in combination with egg yolk in concentrations from 2.5 to 20%, brucella agar was superior to the other two. Ten percent egg yolk had higher growth than 2.5 and 5% egg yolk, but an increase to 20% did not result in any further improvement. The addition of 10% fetal bovine serum to media containing 10% egg yolk did not increase the growth compared with egg yolk alone. Addition of 1% IsoVitaleX increased the average density of growth from 2.6 to 3.4 on the 0-to-4 scale of Buck and Smith (1). In a comparison between brucella agar and brain heart infusion agar, both with 10% egg yolk and 1% IsoVitaleX, the brain heart infusion agar had slightly better growth. When we performed colony counts on identical inocula of 16 strains of *H. pylori*, brain heart infusion agar had an average CFU count 13% higher than that of brucella agar after 7 days of incubation. In the current experiments, we included this brain heart infusion formulation for an additional comparison with the egg yolk emulsion (EYE) formula with Columbia agar described in this article.

Five different media were compared. The formula of Goodwin et al. (GDW agar) contained brain heart infusion agar, 7% whole defibrinated horse blood, and 1% IsoVitaleX (6). The medium of Glupczynski et al. (GLU agar) contained brain heart infusion agar, 10% horse serum, 0.2% charcoal, 1% yeast extract, and 40 mg of triphenyltetrazolium chloride per liter (4). Columbia blood agar as described by Dent and McNulty (D&M agar) consisted of Columbia agar and 7% lysed horse blood (2). EYE agar consisted of Columbia agar, 10% EYE, 1% IsoVitaleX, and 40 mg of triphenyltetrazolium chloride per liter. Brain heart infusion egg (BHIE) agar was made from brain heart infusion agar with 10% EYE and 1% IsoVitaleX. For all media, the brain heart infusion agar and EYE were manufactured by Oxoid USA Inc. (Columbia, Md.), yeast extract was manufactured by Difco Laboratories (Detroit, Mich.), and triphenyltetrazolium chloride and activated charcoal were manufactured by Sigma Chemical Co. (St. Louis, Mo.). Horse blood and horse serum were acquired from Colorado Serum Co. (Denver, Colo.). All media were made within 24 h of the others, and stored under identical conditions at 4°C. No plates were more than 2 weeks old at the time of use. In a second experiment, we compared EYE agar with two commercial chocolate media (one manufactured by BBL and the other manufactured by Remel, Lenexa, Kans.).

Twelve strains of *H. pylori* were used in the first experiment, and 14 were used in the second. One of the strains was an Australian reference strain (NCTC 11637), and the others were American clinical isolates. The American strains had originally been isolated on a medium consisting of brucella agar with 10% bovine blood and 1% IsoVitaleX. The Australian strain had been isolated on a chocolate agar (7). All strains were first grown in a liquid medium consisting of brucella broth with 10% bovine serum and 1% IsoVitaleX.

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TABLE 1. CFU of *H. pylori* on five media after 7 days of microaerophilic incubation

Agar	Reference	% of maximum CFU[a]			P^b
		Avg	Range	SD	
EYE	This study	95.7	67–100	9.9	1.000
GDW	6	76.1	23–100	26.8	0.026
BHIE	This study	56.8	<1–100	42.5	0.005
D&M	2	51.8	1–94	31.0	0.0001
GLU	4	23.3	<1–80	25.7	<0.0001

[a] Colony counts given as percentages of maximum growth for each strain.
[b] Comparison with EYE agar by two-sample analysis *t* test.

They were incubated in CampyPak jars for 3 to 5 days until turbid. Serial 10-fold dilutions were made up to 10^{-7} of the original concentration in broth. Ten microliters from each dilution was placed on each plate and streaked to form a circular area with a diameter of 0.5 in. (ca. 1.3 cm). All plates to be compared for each strain were always incubated in the same CampyPak jars.

The numbers of colonies on the plates were counted on days 2, 3, 4, 5, and 7. When several dilutions on the same plate had countable colonies, calculations were performed for the dilution showing between 10 and 99 CFU. After 7 days, we calculated the maximum growth for each strain. The maximum growth was defined as the highest CFU count seen for each strain on any of the media. We expressed all other colony counts as percentages of this maximum growth. Statistical analysis of data was performed with Statgraphics 3.0 (Graphics Software Systems, Inc., Rockville, Md.).

Table 1 shows colony counts of *H. pylori* on five different media after 7 days of incubation. Of the 12 strains used, 9 had maximum growth (100%) on EYE agar, 2 had maximum growth on GDW agar, and 1 had maximum growth on BHIE agar. The average percentage of maximum growth for EYE agar was 96. This was significantly higher than the 76% for GDW agar ($P = 0.027$) and 57% for BHIE agar ($P = 0.005$). The lower counts found for the D&M and GLU media were even more significant ($P = 0.0001$ and $P < 0.0001$, respectively). EYE agar also had the fastest growth of the five media. On each day, EYE agar had the highest CFU counts (Fig. 1). It reached 25 and 50% of maximum growth an average of 8 h faster than the second-best agar and 16 and 48 h faster, respectively, than the third-best agar. In the second experiment, the average percentage of maximum growth was 80% for EYE agar, compared with only 33% for BBL chocolate agar and 63% for the Remel medium. All 14 tested strains grew on EYE agar, while 2 failed to grow on Remel agar and 4 did not grow on the BBL medium. Colonies were easy to spot on the EYE agar, being red against a pale yellow background. The size of the colonies was always larger on the EYE media. After 7 days of incubation the average colony size (mean diameter ± standard deviation, in millimeters) was 2.5 ± 0.7 for EYE agar. In falling order, the values for the other media (in millimeters) were as follows: D&M, 1.6 ± 0.5; Remel, 1.4 ± 0.3; GDW, 1.2 ± 0.3; BHIE, 1.2 ± 0.4; BBL, 1.1 ± 0.4; and GLU, 0.8 ± 0.3.

Media containing egg yolk have been used in the past to cultivate *Bacillus subtilis*, *Fusobacterium necrophorum*, and some *Clostridium* species (3, 8). We have demonstrated that egg yolk also can support the growth of *H. pylori*. In our first experiment, the EYE agar had significantly higher colony counts and produced growth more rapidly than both the BHIE agar and three commonly used media for *H. pylori*. In

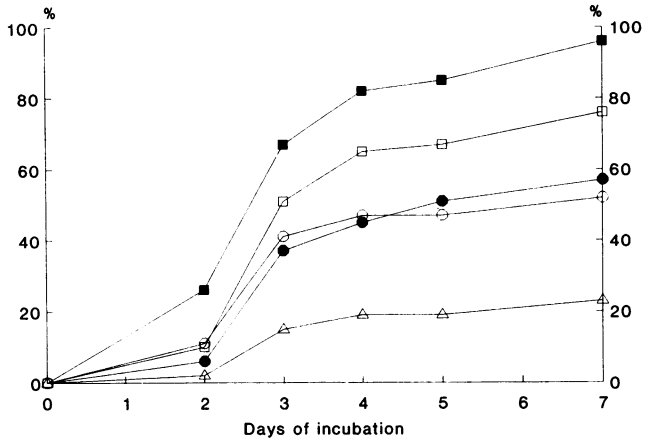

FIG. 1. Average CFU of *H. pylori* on five media during 7 days of incubation. Colony counts are given as percentages of maximum growth at the end of incubation. Media shown are EYE agar (black boxes), GDW agar (open boxes), BHIE agar (black circles), D&M agar (open circles), and GLU agar (triangles).

our second experiment, it was better than two common commercial chocolate media. Since all strains used in these experiments had been originally isolated and subcultured on blood-containing media, no selection favoring egg yolk media had taken place. The red color induced by the triphenyltetrazolium chloride made the colonies easy to spot on the EYE agar, even early in the incubation. This color change is not unique to *H. pylori*, but contaminants were easily identified by their lack of the golden hue originally described by Queiroz et al. (9). *H. pylori* always had red colonies that also showed this characteristic golden shine when viewed at an angle to a light source. We have now started using the EYE medium for clinical specimens at St. Louis University. Preliminary results suggest that the addition of selective antibiotics (Table 2) does not interfere with the performance of the medium. Charcoal-based media such as GLU medium often bind antibiotics, particularly amphotericin B, and lead to increased levels of contaminants. We have not seen any inhibition of antibiotics by the egg yolk. A prospective study is under way to compare EYE agar with chocolate agars and blood-containing media for primary isolation of *H. pylori*.

The knowledge that *H. pylori* can grow in pure egg yolk raises some interesting epidemiologic questions. We know that other pathogenic organisms, such as *Salmonella* species, can infect and survive in chicken eggs (10, 11). No environmental source for *H. pylori* has been found. It would

TABLE 2. Contents of EYE agar for *H. pylori*

Ingredient	Amt
Columbia agar	39 g
EYE	100 ml
IsoVitaleX	10 ml
Triphenyltetrazolium chloride	40 mg
Distilled water	890 ml
Optional antibiotics	
Cefsulodin	5 mg
Trimethoprim	5 mg
Vancomycin	6 mg
Amphotericin B	6 mg

be of interest to include chicken eggs in future epidemiologic investigations of potential vectors of infection.

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