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Comparison of CHROMagar Salmonella Medium and Xylose-Lysine-Desoxycholate and Salmonella-Shigella Agars for Isolation of *Salmonella* Strains from Stool Samples

Susan Maddocks,* Tom Olma, and Sharon Chen

Centre for Infectious Diseases and Microbiology Laboratory Services, Institute of Clinical Pathology and Medical Research, Westmead Hospital, Westmead, New South Wales, Australia

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The growth and appearance of 115 stock *Salmonella* isolates on a new formulation of CHROMagar Salmonella (CAS) medium were compared to those on xylose-lysine-desoxycholate agar (XLD), Salmonella-Shigella agar (SS), and Hektoen enteric agar (HEA) media. CAS medium was then compared prospectively to XLD and SS for the detection and presumptive identification of *Salmonella* strains in 500 consecutive clinical stool samples. All stock *Salmonella* isolates produced typical mauve colonies on CAS medium. Nine *Salmonella* strains were isolated from clinical specimens. The sensitivities for the detection of salmonellae after primary plating on CAS medium and the combination of XLD and SS after enrichment were 100%. The specificity for the detection of salmonellae after primary plating on CAS medium (83%) was significantly ($P < 0.0001$) higher than that after primary plating on the combination of SS and XLD media (55%) (a 28% difference in rates; 95% confidence interval, 23.0 to 34%). Twenty-nine non-*Salmonella* organisms produced mauve colonies on CAS medium, including 17 *Candida* spp. (59%) and 8 *Pseudomonas* spp. (28%). These were easily excluded as salmonellae by colony morphology, microscopic examination of a wet preparation, or oxidase testing. One biochemically inert *Escherichia coli* isolate required further identification to differentiate it from *Salmonella* spp. The use of plating on CAS medium demonstrated high levels of sensitivity and specificity and reduced the time to final identification of *Salmonella* spp., resulting in substantial cost savings. It can be recommended for use for the primary isolation of *Salmonella* spp. from stool specimens. Other media (e.g., XLD) are required to detect *Shigella* spp. concurrently.

Infections due to *Salmonella* spp., including *Salmonella enterica* serovar Typhi, continue to be a major global health problem. While direct detection of *Salmonella* isolates in stool specimens by PCR is possible, this technique is not readily available for routine use in most clinical laboratories (13). Isolation of the organism by stool culture remains the most reliable method for detection, allowing precise identification of the bacteria and antimicrobial susceptibility testing, both of which are critical for disease control. A variety of selective media which rely on visualization of simple biochemical features such as the nonfermentation of lactose and the production of hydrogen sulfide to identify *Salmonella* spp. in stool specimens have been developed for this purpose. The specificities of such media are poor, however, and time-consuming complementary testing is required to exclude as significant colonies of organisms with similar biochemical features.

Recently, media allowing the detection of *Salmonella* spp. by the incorporation of chromogenic substrates have been introduced (2, 8, 10). Compared to conventional selective media such as Hektoen enteric agar (HEA), chromogenic media had higher specificities but lower sensitivities, with more false-negative results (2, 8, 11, 12). One particular formulation of chromogenic medium, Rambach agar, was highly specific for salmonellae but failed to detect *S. enterica* serovar Typhi (10). As a result, many of these earlier media cannot be recommended for use for the primary plating of stool specimens to screen for *Salmonella* spp. (2, 8, 11, 12). CHROMagar Salmonella (CAS) is a newer selective chromogenic medium and purports to detect salmonellae as mauve colonies at 18 to 24 h of incubation, with other members of the family *Enterobacteriaceae* appearing as blue or uncolored colonies (CHROMagar Salmonella Product Information; CHROmagar Microbiology, Paris, France). The sensitivity of an early formulation of CAS medium for the detection of *Salmonella* spp. was similar to that of HEA on primary plating and after enrichment in broth culture (O. Gaillot, C. Maruejouls, P. Di Camillo, N. Fortineau, R. Courcol, and C. Savage, Abstr. 98th Gen. Meet. Am. Soc. Microbiol., abstr. C-445, p. 205, 1998). False-positive results were, however, reported with *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, and *Candida* spp.; the proportion of false-positive results caused by *P. aeruginosa* was subsequently reduced by the addition of the antimicrobial agent cefsoludin into the medium (4). A more recent formulation of CAS medium aiming to reduce these specificity issues is now available. Experience with this formulation as a screen for *Salmonella* isolates is limited, and its utility in the routine examination of stool specimens has not been defined.

In this study, we initially compared the appearances of known stock cultures of *Salmonella*, *Shigella*, other enteric organisms, and *Candida* spp. on CAS medium with those on Salmonella-Shigella agar (SS), xylose-lysine-desoxycholate agar (XLD), and HEA (enteric media commonly used in Australia). In the second phase of the study we assessed the sen-

* Corresponding author. Present address: Centre for Virus Research, Westmead Millenium Institute, Westmead Hospital, Hawkesbury Rd., Westmead 2145, Australia. Phone: 61-2-9845 9113. Fax: 61-2-9845 9103. E-mail: susan_maddocks@wmi.usyd.edu.au.

TABLE 1. Distributions of *S. enterica* serovars from stock isolates

Serogroup	Serovar	No. of isolates
A	Paratyphi A	4
B	Typhimurium	64
B	Birkenhead	8
C1	Virchow	9
C1	Infantis	5
C2	Bovis morbificans	7
D	Enteritidis	3
D	Typhi	15

sitivity and specificity of CAS medium using 500 consecutive clinical stool samples, comparing it with our standard laboratory protocol of plating on SS and XLD with enrichment in selenite broth. We also compared the work flow and cost-benefit differences between these two methods.

MATERIALS AND METHODS

Culture media. CAS medium was provided by Dutec Diagnostics, Croydon, New South Wales, Australia. The medium was supplied in preweighed batches of white powder sufficient to provide 250 ml of medium and was prepared according to the instructions of the manufacturer. Powdered CAS was added to distilled water and dispersed by slow rotation. Once the CAS was dissolved, the mixture was brought to a boil with regular stirring for approximately 2 to 3 min until complete fusion of the agar grains was observed. The medium was cooled to 48°C, gently stirred to homogeneity, and poured into sterile 9-cm-diameter petri dishes to set and dry. The plates were stored in the dark at room temperature and were used within a week. SS, XLD, and HEA media were prepared from commercially available SS powder (57 g/liter of water; Oxoid Australia, Heidelberg, Victoria, Australia), XLD medium powder (53 g/liter of water; Oxoid Australia), and HEA powder (76 g/liter of water; Oxoid Australia). These media were stored at 4°C prior to use. Selenite enrichment broth was prepared from commercially available selenite broth powder (23 g/liter of water; Difco, Becton-Dickinson, North Ryde, New South Wales, Australia).

Stock isolates. For the initial phase of the study, 115 stock *Salmonella* isolates representing eight serovars that had previously been isolated from human stool samples were obtained from the culture collection of the Enteric Reference Laboratory (ERL) (Table 1). This laboratory is integrated within the Centre for Infectious Diseases and Microbiology Laboratory Services (CIDMLS) at Westmead Hospital and is the enteric reference laboratory for the state of New South Wales. The *Salmonella* serovars selected, other than those causing enteric fever, are representative of those causing infections in New South Wales. Eighty-four other enteric isolates consisting of *Shigella sonnei* ($n = 9$ isolates), *Shigella flexneri* ($n = 5$), *Yersinia enterocolitica* ($n = 3$), *Citrobacter freundii* ($n = 9$), *Hafnia alvei* ($n = 2$), *Plesiomonas shigelloides* ($n = 1$), *Aeromonas* spp. ($n = 2$), *Proteus* spp. ($n = 5$), *Providencia* sp. ($n = 1$), *Shewanella* spp. ($n = 2$), *Enterococcus* spp. ($n = 4$), *P. aeruginosa* ($n = 10$), *Escherichia coli* ($n = 6$), *Candida* spp. ($n = 15$), and *Staphylococcus aureus* ($n = 10$) including five isolates of methicillin-resistant *S. aureus* (MRSA) were also obtained from among the isolates stored at CIDMLS. All isolates were plated onto horse blood agar and incubated overnight in air at 37°C to ensure purity. Suspensions were prepared from freshly grown colonies in sterile saline solution and were adjusted to a turbidity equivalent to that of a 0.5 McFarland standard suspension. Five hundred microliters of each suspension was inoculated onto CAS, XLD, SS, and HEA media with a Steer's replicator. The plates were incubated overnight in air at 37°C and were examined on the following morning and after 48 h of incubation.

Clinical samples. In the second phase of the study, 500 consecutive stool samples were collected from both hospitalized and nonhospitalized patients with diarrhea. Stool samples were cultured directly onto CAS, XLD, and SS plates; and the plates were examined after incubation overnight in air at 37°C. Following enrichment in selenite broth, an aliquot was subcultured onto SS and XLD media at 18 to 24 h as part of the laboratory's routine practice. All plates were examined after a further 24 h of incubation under conditions identical to those described above. Enrichment was not performed when CAS medium was used.

Presumptive identification. In the first part of the study, the identities of the isolates used were known. Colony color on CAS medium was noted for the different *Salmonella* spp., and their colonial appearances on the different medium types were compared. In the second phase of the study, colonies suspected of being *Salmonella* spp. were defined as follows: on CAS, mauve colonies; on SS, transparent colonies with or without black centers; and on XLD, transparent colonies with or without black centers. In all instances the colonial appearances of the isolates were evaluated after incubation for 18 to 48 h. All plates were inspected by the same investigator (S.M.).

Confirmatory tests and final identification. Colonies suspicious of being *Salmonella* spp. on SS and XLD media were processed in the following manner. An oxidase test (Kovács indirect filter paper procedure) (7) was initially performed with all colonies suspected of being *Salmonella* spp. Oxidase-negative isolates were inoculated onto a urea slope and incubated at 37°C for 24 h. Oxidase-negative, urease-negative isolates were then subjected to a panel of biochemical tests to screen for *Salmonella* and other enteric pathogens. This panel comprises overnight incubation or culture (in air at 37°C) in 1% glucose peptone water, *o*-nitrophenyl-β-D-galactopyranoside, lysine iron agar, Kligler's iron agar, Sims agar, and MacConkey agar. A possible *Salmonella* sp. identified by this screen (and, in our laboratory, any presumptive *Shigella*, *Yersinia*, *Aeromonas*, or *Plesiomonas* sp.) was then referred to ERL for extended confirmatory biochemical and serological testing by the Kauffman-White scheme (6).

Colonies suspicious of being *Salmonella* on CAS medium were processed in the following manner. Microscopic examination of a wet preparation and an oxidase test (see above) were performed with all mauve colonies to exclude *Candida* and *Pseudomonas* spp., respectively. Isolates that were oxidase negative and wet preparation negative were then identified with the Vitek identification system by inoculating a Gram-Negative Identification card (Biomerieux, Baulkham Hills, New South Wales, Australia). Isolates identified as *Salmonella* spp. with the Vitek system or those that were unable to be definitively identified were referred to ERL for confirmatory testing and/or further testing.

RESULTS

Evaluation of appearance of stock *Salmonella* isolates on different selective media. All (100%) stock *Salmonella* isolates, including those of serovars Typhi and Paratyphi A, produced mauve colonies on CAS medium after 24 h of incubation. On XLD, SS, and HEA media, all non-serovar Typhi and Paratyphi salmonellae appeared as characteristic black colonies, reflecting H_2S production. *S. enterica* serovar Typhi isolates appeared as transparent colonies on SS and HEA media and as transparent colonies surrounded by yellow halos on XLD medium. Serovar Paratyphi A isolates produced transparent colonies on all three of the other media evaluated, XLD, SS, and HEA. Gram-negative isolates other than *Salmonella* spp. that appeared as transparent and/or black colonies on these media were *Y. enterocolitica*, *H. alvei*, and *Shigella*, *Plesiomonas*, *Morganella*, *Providencia*, and *Shewanella* spp.; *C. freundii* and *Proteus* spp. (H_2S only); *Aeromonas* spp. (SS medium only); and *P. aeruginosa* (XLD medium only). *Enterococcus faecalis* and *Enterococcus faecium* also appeared as transparent colonies on SS and HEA media.

Conversely, isolates other than *Salmonella* spp. that appeared as mauve or mauve-like colonies on CAS medium were *Aeromonas*, *Morganella*, *Providencia*, and *Candida* species and 1 of 10 isolates of *P. aeruginosa*. Notably, all *E. coli* isolates tested appeared as distinct blue colonies.

Performance of media in detection of *Salmonella* in stool specimens. In the second phase of the study, the performance of CAS medium as a primary plating medium for routine laboratory use was evaluated and compared with the performance of the laboratory's current protocol for the isolation of enteric pathogens. Nine *Salmonella* spp. were isolated from 500 consecutive stool specimens over a period of 3 months (Fig. 1). The distribution of the serovars identified is shown in Table 2. All nine isolates were detected on CAS medium within 48 h of incubation without enrichment. Six of nine

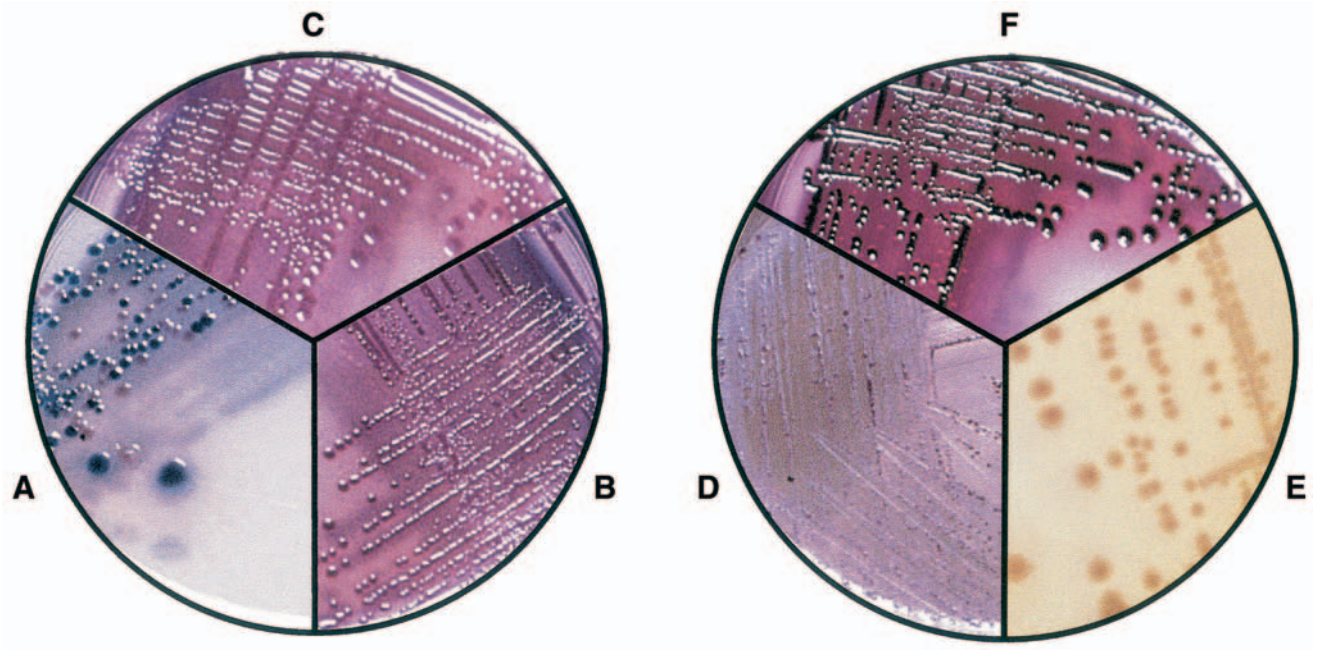

FIG. 1. (A) Appearance of a clinical stool specimen on CAS medium, with *Salmonella* spp. appearing as mauve colonies amid other enteric organisms, which appear as blue or colorless colonies. (B) Appearance of a pure culture of *S. enterica* serovar Typhi on CAS medium. (C) Appearance of a pure culture of inactive *E. coli* on CAS medium. (D) Appearance of a pure culture of *Candida* spp. on CAS medium. (E) Appearance of a pure culture of *Pseudomonas* spp. on CAS medium. (F) Appearance of a pure culture of *S. marcescens* on CAS medium.

(67%) isolates appeared as typical mauve colonies within 24 h of incubation; three (33%) isolates required 48 h to develop the mauve coloration, appearing initially as colorless colonies, and comprised a single isolate each of serovars Typhi, Typhimurium, and Virchow. The combination of SS and XLD detected six of the nine *Salmonella* spp. on primary plating; however, the three serovar Typhi isolates were detected only after enrichment.

The overall sensitivities of both methods (with either CAS medium alone without an enrichment technique or the combination of SS and XLD with enrichment) were 100% (Table 3). The specificity of CAS medium, however, was substantially higher (83%) compared with that of the SS and XLD combination (specificity, 55%) (Table 4).

One hundred sixty-nine isolates (34%) were considered potential pathogens on either SS or XLD but not on CAS medium. All required a period of 24 to 72 h of additional biochemical and serological testing prior to their exclusion as enteric pathogens. Conversely, 29 isolates (6%) were detected as potential pathogens on CAS medium but not on SS or XLD agar. Seventeen of the 29 (59%) isolates were *Candida* spp.; these were small (diameter, <2 mm) mauve-purple colonies which were easily distinguishable from *Salmonella* spp. on the basis of their colonial morphologies (Fig. 1D) and could also be readily excluded as *Salmonella* by microscopic examination of a wet preparation. Eight of the 29 isolates were identified as *Pseudomonas* spp. and 1 isolate was identified as an *Aeromonas* sp. (all appeared as mauve colonies, but they had prominent, clear opaque halos and could easily be excluded by the oxidase test; Fig. 1E), and 2 isolates were identified as *Proteus* spp. (both isolates appeared as mauve colonies, but with a clear halo). In contrast, none of the *Salmonella* colonies were associated with surrounding halos. Only one isolate required additional biochemical testing to definitively identify it as a biochemically inactive *E. coli* isolate.

The majority of the 57 isolates that appeared as potential pathogens on both CAS medium and the combination of SS and XLD were able to be excluded as nonenteric pathogens by rapid and simple biochemical tests comprising microscopic examination of a wet preparation of the isolates and the oxidase and urease tests. Eighteen isolates appearing as mauve-purple colonies on CAS medium and as transparent or black-centered

TABLE 2. Distributions of *S. enterica* serovars isolated from clinical specimens

Serogroup	Serovar	No. of isolates
B	Typhimurium	4
B	Reading	1
C1	Virchow	1
D	Typhi	3

TABLE 3. Sensitivity of CAS medium compared with those of the combination SS and XLD on primary plating and after enrichment

Medium	No. of isolates with true-positive results	No. of isolates with false-negative results	Sensitivity (%)
CAS (48 h)	9	0	100
SS and XLD with primary plating	6	3	67
SS and XLD with enrichment	9	0	100

TABLE 4. Specificity of CAS medium compared with that of the combination of SS and XLD

Medium	No. of isolates with true-negative results	No. of isolates with false-positive results	Specificity (%)
CAS	414	77	83
SS and XLD	274	217	55

colonies on SS and XLD were not excluded as salmonellae by simple biochemical tests and either were identified as *Salmonella* spp. by the Vitek system or were inconclusively identified by the Vitek system. These 18 isolates underwent further biochemical testing for definitive identification. Of these organisms, nine were confirmed to be salmonellae and nine were biochemically inactive *E. coli* isolates (Fig. 1C). One isolate appeared as clear colonies on SS medium but was excluded as a *Salmonella* sp. by routine follow-up testing. This isolate was bright purple on CAS medium and was identified as a *Serratia marcescens* isolate (Fig. 1F).

The use of simple biochemical tests, as described above, with all mauve and mauve-like isolates on CAS medium therefore improved the specificity to 96% and reduced the time to identification to 48 to 72 h, compared with the up to 96 h required by our current laboratory practice.

DISCUSSION

This study demonstrates the utility of CAS medium as a reliable and time-saving method for the detection and presumptive identification of *Salmonella* spp. in clinical stool specimens and is the first to evaluate the new formulation of CAS medium for this purpose. Importantly, distinguishing the mauve colonies of *Salmonella* spp. from the blue or uncolored colonies of other enteric flora was simple, and the procedure was easily adapted into the laboratory's routine practice. We observed fewer false-positive colonies on CAS medium than on SS and XLD, and the great majority could be ruled out as *Salmonella* spp. with a minimum number of rapid and inexpensive tests.

The number of *Salmonella* strains isolated from the 500 consecutive stool specimens was relatively low (positivity rate, 1.8%). This is consistent with the prevalence of enteric *Salmonella* infections in Australia (9).

The initial phase of the study demonstrated the ability of a wide variety of salmonellae of human origin to grow adequately on CAS agar and demonstrated that all salmonellae tested, including serovars Typhi and Paratyphi A, could be detected as typical mauve colonies. This contrasts with the findings obtained with some other formulations of chromogenic *Salmonella*-specific media such as Rambach agar (3, 5, 11), modified semisolid Rappaport-Vassiliadis medium (1, 12), and novobiocin-brilliant-green-glycerol-lactose medium (3, 11), all of which have previously been reported to be unable to detect serovars Typhi and Paratyphi A. This has important implications for both clinicians and microbiologists, given the significant morbidity and public health implications of these agents of enteric fever.

The reduced sensitivity of the combination of SS and XLD media without enrichment for primary plating of stool specimens was expected, as it is widely accepted that, with traditional culture methods, an enrichment technique increases the yield of *Salmonella* spp. from stool specimens. This is especially true for the detection of *S. enterica* serovar Typhi stool carriage, a clinical scenario not addressed in this study. Although the incorporation of an enrichment procedure following primary plating onto CAS medium was not performed in the study, we observed a sensitivity of 100%, suggesting that enrichment may not be necessary. The number of *Salmonella* spp. isolated from stool specimens, however, was small. One previous study noted that enrichment improved the sensitivity of the first-available formulation of CAS medium, as well as that of HEA, when they were used as primary plating media (4). Studies with larger numbers of clinical stool specimens are thus required to adequately assess the need for enrichment with the newer CAS medium.

In this study, CAS medium was shown to be more specific than the combination of SS and XLD with enrichment, despite the presence of false-positive colonies including those of *P. aeruginosa*, *Candida albicans*, and *Aeromonas* spp. We were able to exclude the majority of these isolates as pathogens by performing rapid oxidase tests, microscopic examination of a wet preparation of the isolate, and urease tests. With experience, the ability to distinguish the mauve color of *Salmonella,* the purple color of *Serratia* spp., the small colonial size of *Candida*, and the colonial morphology of certain non-*Salmonella* enteric organisms (large clear or opaque colonies with a mauve center) can be developed by bench technicians, further improving the specificity of CAS medium as a primary plating medium. Interestingly, biochemically inactive strains of *E. coli* from stool specimens also produced mauve colonies on CAS medium, a phenomenon not observed in the initial phase of our study or reported by other investigators with CAS medium.

The manufacturers of CAS medium suggest that 18 h of incubation is adequate for the detection of *Salmonella* spp. Three of the nine *Salmonella* isolates recovered from stool specimens in the study required greater than 24 h of incubation to develop the typical mauve coloration. These colonies may have been dismissed as nonenteric pathogens if the plates had been discarded and/or not reexamined after 18 h of incubation. We therefore recommend that stool specimens cultured onto CAS medium be incubated for 48 h and examined on the first and second days following inoculation before the plates are discarded.

Importantly, the use of CAS medium reduced the time to identification of *Salmonella* isolates by 24 to 48 h. While the incorporation of chromogenic substrates into selective media has a significant impact on cost (CAS medium, \$A1.04 [\$A1 = US\$0.56 in 2002 Australian dollars] per plate; SS or XLD, \$A0.63 per plate), we found this to be offset by the substantially reduced costs related to technician time and the use of other consumables associated with the detection and identification of *Salmonella* spp. on traditional agars. We estimate a 26% reduction in overall costs (\$A1,746.50/month for SS and XLD, compared with \$A1,297.15/month for CAS medium) and the saving of half of a full-time bench technician position.

In conclusion, the high degrees of sensitivity and specificity of CAS medium make this medium a reliable addition to the array of media available for the isolation of *Salmonella* spp. It can be recommended for use for the primary isolation of *Sal-*

monella spp. from clinical stool specimens and has the added advantage that it reduces the time to identification of the causative agent. Further studies are required to evaluate the appearances of more *Salmonella* serovars on CAS medium and to assess the need for enrichment techniques. Other media (for example, XLD) should be used concurrently to evaluate specimens for the presence of *Shigella* spp.

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