Evaluation of a Novel Chromogenic Medium as a Replacement for MacConkey Agar and Hektoen Enteric Agar

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Revised Abstract

Several selective and differential media are available for the isolation of Salmonella and Shigella spp., but these media do not distinguish enteric pathogens from non-pathogenic, non-lactose-fermenting organisms. Isolation on these media require excessive colony picking to screen for possible pathogens, most of which turn out to be false-positive normal flora, such as Proteus, Providencia, Morganella, and Citrobacter spp. While chromogenic media formulations are available for Salmonella spp., none previously existed for Shigella spp.

To alleviate these labor-intense workups, Hardy Diagnostics' novel chromogenic media, HardyCHROM[™] SS, allows for the selective isolation and differentiation of Salmonella and Shigella spp. from non-pathogenic enteric bacteria (both lactose and non-lactose-fermenting organisms).

The Intermountain Central Laboratory processed 400 stool specimens by plating on HardyCHROM[™] SS (HC SS), MacConkey Agar (MAC), and Hektoen Enteric Agar (HE), in parallel. Suspect colonies growing on HC SS were taken directly to a MicroScan[®] panel for confirmation, while suspect colonies growing on MAC and HE were first screened using TSI and LIA slants; those giving expected results for Salmonella or Shigella were taken to a MicroScan® panel for confirmation.

All three agar types recovered five Salmonella isolates, with HC SS and MAC also recovering a Shigella isolate missed by HE. Further, HC SS recovered an additional Salmonella isolate that was missed by both MAC and HE. The number of pickings from the primary plate were reduced with HC SS (32 pickings) when compared to MAC (59) and HE (53). Time to results was also decreased by one day when using HC SS (since TSI and LIA slants were not necessary). Based on these findings, HardyCHROM[™] SS, utilizing a novel, patented chromogenic substrate (under exclusive license), can be employed as a replacement for MAC and HE agars, as well as TSI and LIA screens from these media. HC SS yields better recovery, results in less pickings and subsequent work-ups, and reduces the time to results by one day.

Introduction

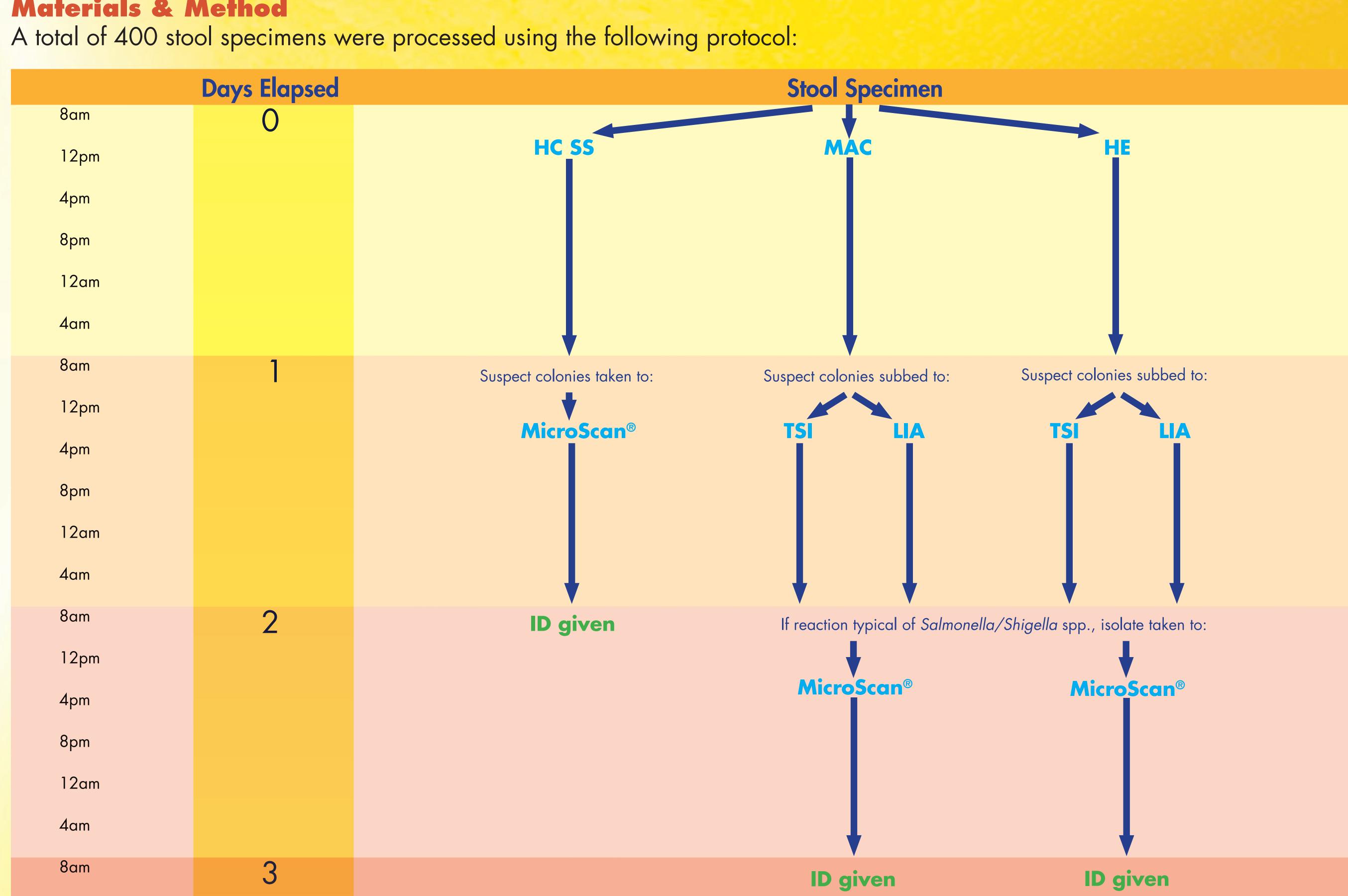
Salmonella and Shigella spp. continue to be a major cause of disease and illness due to food-borne and water-borne infection.⁽¹⁻⁶⁾ Many formulations of culture media (such as HE, SS, and XLD) have been developed to isolate and differentiate Salmonella and Shigella spp. from non-pathogenic enteric bacteria.^(4,6,7) Most formulations rely on carbohydrate fermentation, pH indicators, and an indicator system for the detection of hydrogen sulfide.⁽⁷⁾ These media are made selective by the addition of bile salts and can also differentiate between Salmonella, Shigella, and lactose-fermenting organisms. However, colonies of non-lactose-fermenting organisms that are non-pathogenic can appear similar in appearance to Salmonella and Shigella and must be subjected to further testing by using Triple Sugar Iron (TSI) Agar, Lysine Iron Agar (LIA), or Kligler Iron Agar (KIA).^(4,6-9) Screening of primary plates or secondary plates inoculated from enrichment broths often requires the inoculation of large numbers of secondary screening tubes and/or the use of costly automated identification systems.

> The use of chromogenic substrates (chromogens) in media formulations has increased greatly in the last several years. Chromogens, when broken down by specific bacterial enzymes, will result in colored colonies. Previously, chromogenic formulations were available for Salmonella spp., but not for Shigella spp.^(5-7,10,11)

> > HardyCHROM[™] SS allows for the selective isolation and differentiation of both Salmonella and Shigella spp. from nonpathogenic enteric bacteria (both lactose and non-lactosefermenting organisms). Differentiation of Salmonella and Shigella spp. from non-pathogenic bacteria is accomplished by three mechanisms: chromogenic reactions, carbohydrate fermentation, and hydrogen sulfide production. HardyCHROM[™] SS provides better differentiation of colonies obtained from clinical samples and enrichment procedures, resulting in less secondary screening of isolates and less false-positive results.

Salmonella enterica (ATCC® 14028) colonies growing on HardyCHROM[™] SS. Incubated aerobically for 24 hours at 35 °C.

Materials & Method



Suspect colonies from HC SS included turquoise and colorless colonies, with or without black centers. Suspect colonies from MAC included all colorless colonies. Suspect colonies from HE included green to blue-green colonies, with or without black centers. TSI reactions typical of Salmonella and Shigella species were red slant/yellow butt/gas positive/H₂S positive and red slant/yellow butt/gas negative/H₂S negative, respectively. LIA reactions typical of Salmonella and Shigella species were purple slant/purple butt/H₂S positive and purple slant/yellow butt/H₂S negative, respectively.

All plates and slants were incubated at 35°C overnight before interpreting results. MicroScan® panels were incubated overnight before identifications were determined.

Doculto

Primary plating medium	Number of suspect isolates subbed to secondary screening (TSI/LIA)	Number of suspect isolates taken to confirmation testing (MicroScan®)	Number of Salmonella spp. confirmed by MicroScan®	Number of Shigella spp. confirmed by MicroScan®	Number of days from primary plating to confirmed identification
HC SS	0	32	6	1	2
MAC	59	6	5	1	3
HE	53	5	5	0	3



Conclusion

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• HardyCHROM[™] SS yielded better recovery than MacConkey Agar, which missed one Salmonella isolate, and Hektoen Enteric Agar, which missed one Salmonella isolate and one Shigella isolate.

• Use of HardyCHROM[™] SS resulted in a reduction of technician labor (fewer colony pickings and work-ups) compared to MAC and HE. MAC required picking 59 suspect colonies and subbing to 59 TSI slants, 59 LIA slants, and 6 MicroScan[®] panels. HE required picking 53 suspect colonies and subbing to 53 TSI slants, 53 LIA slants, and 5 MicroScan[®] panels. HC SS only required picking 32 suspect colonies and subbing to 32 MicroScan[®] panels. • Use of HardyCHROM[™] SS reduced the turn-around-time (TAT) from three days (using MAC/HE, TSI/LIA, MicroScan[®]) to two days (using HC SS, MicroScan[®]). This one day reduction in time to identify and report results in faster patient treatment and less time spent in hospitalization (savings for both patients and hospitals).

• While there was a slight cost increase during this study (due to the increased number of MicroScan[®] panels used with the HC SS method), this cost is offset by the reduction of wasted time doing work-ups, decreased technician frustration, and the one day faster TAT. Additionally, as labs become more familiar with the colony colors on HardyCHROMTM SS, the number of suspect isolates taken to confirmation testing will be greatly reduced, resulting in overall cost savings. • Based on these findings, HardyCHROM[™] SS can be employed as a replacement for MacConkey Agar and Hektoen Enteric Agar, as well as TSI and LIA screens from these media. HardyCHROM[™] SS is a reliable and economical method for the selective isolation and differentiation of Salmonella and Shigella spp. from non-pathogenic enteric bacteria.

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