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Evaluation of a Novel Chromogenic Medium for the Isolation and Differentiation of *Salmonella* and *Shigella* spp.

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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 exist 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 Microbial Diseases Laboratory, California Department of Public Health, inoculated a total of 80 enteric isolates using HardyCHROM™ SS in order to evaluate the ability of this medium to accurately recover and differentiate *Salmonella* and *Shigella* spp.

HardyCHROM™ SS yielded 100% recovery and 100% appropriate colony colors for all of the *Salmonella* and *Shigella* spp. tested. Some low H_2S-producing *Salmonella* strains appeared turquoise as expected. Based on these findings, HardyCHROM™ SS, utilizing a novel, patented chromogenic substrate (under exclusive license), can be employed as a cost effective, reliable method for the selective isolation and differentiation of *Salmonella* and *Shigella* spp. from non-pathogenic enteric bacteria in stool cultures.

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 non-pathogenic enteric bacteria (both lactose and non-lactose-fermenting 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.

Materials & Methods

All of the strains tested were retrieved from the Microbial Diseases Laboratory, California Department of Public Health.

- 80 total strains were tested
- 2 genera were tested (*Salmonella* and *Shigella*)
- 16 different species, serotypes, or groups were tested

Suspension Preparation and Inoculation

- Isolates were grown in tryptone or brain heart infusion broth for approximately four hours in a water bath
- A loopful of each suspension was used to streak HardyCHROM™ SS plates for isolation
- Plates were incubated at 35 °C for 18-20 hours

Results

Genus	Species, Serotype, or Group	Number of Isolates Tested	Number of Isolates Positive for Growth on HardyCHROM™ SS	Number of Isolates Yielding Expected Color Reaction on HardyCHROM™ SS	% Isolates Positive (Growth)	% Isolates Positive (Color)
Salmonella	*enterica* subsp. salamae (Group II)	3	3	3	100%	100%
Salmonella	*enterica* subsp. arizonae (Group IIIa)	3	3	3	100%	100%
Salmonella	*enterica* subsp. diarizonae (Group IIIb)	2	2	2	100%	100%
Salmonella	*enterica* subsp. houtenae (Group IV)	3	3	3	100%	100%
Salmonella	serotype Typhi	5	5	5	100%	100%
Salmonella	serotype Paratyphi A	2	2	2	100%	100%
Salmonella	serotype Heidelberg	1	1	1	100%	100%
Salmonella	spp. (serotype not determined)	19	19	19	100%	100%
Salmonella	group B	1	1	1	100%	100%
Salmonella	group C2	1	1	1	100%	100%
Salmonella	group D	1	1	1	100%	100%
Shigella	*sonnei*	13	13	13	100%	100%
Shigella	*flexneri*	12	12	12	100%	100%
Shigella	*boydii*	8	8	8	100%	100%
Shigella	*dysenteriae*	4	4	4	100%	100%
Shigella	provisional serotype	2	2	2	100%	100%
Total		**80**	**80**	**80**	**100%**	**100%**

Conclusion

- HardyCHROM™ SS yielded 100% recovery of *Salmonella* and *Shigella* spp. tested
- 100% of *Salmonella* spp. yielded the expected color reactions (turquoise, with or without black centers)
- 100% of *Shigella* spp. yielded the expected color reactions (turquoise for *Shigella sonnei, flexneri,* and *boydii;* turquoise or colorless for *Shigella dysenteriae*)
- Based on these findings, HardyCHROM™ SS can be employed as a cost effective, reliable method for the selective isolation and differentiation of *Salmonella* and *Shigella* spp. from non-pathogenic enteric bacteria

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

Shigella sonnei (ATCC® 9290) colonies growing on HardyCHROM™ SS. Incubated aerobically for 24 hours at 35 °C.

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