Decreased Time to Reporting of Stool Culture Results Using HardyCHROM SS Agar Combined with MALDI-TOF-MS

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Abstract

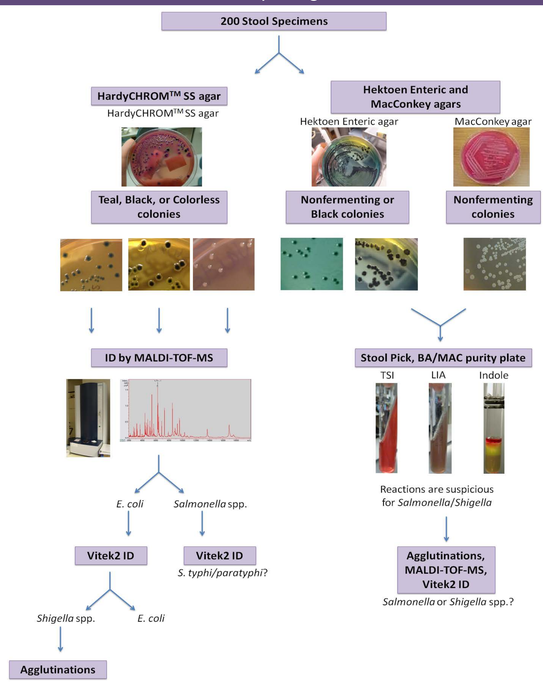
Introduction: *Salmonella* and *Shigella* species are important causes of bacterial gastroenteritis worldwide. Stool culture remains the mainstay of diagnosis, with media such as Hektoen Enteric (HE) and MacConkey (MAC) agars being frequently employed. Because of the abundance of non-fermenting enteric bacteria in normal fecal flora, stool cultures generally require labor-intensive picking of colonies to secondary media for further screening. With the intention of optimizing stool culture work-flow in our laboratory, we evaluated an approach of combining HardyCHROM SS (HC-SS) chromogenic agar with organism identification by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS).

Methods: 200 stool specimens received for culture were plated on HC-SS, HE, and MAC agars in parallel. Non-fermenting colonies growing on MAC and HE agars were further screened using TSI and LIA slants, as well as the tube indole test. Organisms suspicious for *Salmonella* or *Shigella* were then confirmed by agglutination, Vitek, and MS. Well-isolated colonies suspicious for *Salmonella* or *Shigella* were identified directly from HC-SS by MALDI-TOF-MS, or identified after additional subculture if warranted. Colonies identified as *E. coli* by MS were also subjected to testing using the Vitek 2 to rule out *Shigella*.

Results: All three media types identified one case of *Salmonella*, with HC-SS and HE agars also identifying a *Shigella* case missed by MAC. In addition, HC-SS recovered one *Salmonella* case and one *Shigella* case missed by both HE and MAC agars. Use of HC-SS agar was associated with a 55% reduction in the number of colonies requiring additional work-up. MALDI-TOF-MS was demonstrated to be suitable for organism identification directly from HC-SS agar.

Conclusions: HC-SS agar is a viable and more sensitive alternative to HE and MAC agars. Additional benefits associated with utilization of the media included improved turnaround time for reporting of results as well as decreased costs associated with further work-up of suspicious colonies. The performance of MALDI-TOF-MS for organism identification directly from colonies growing on HC-SS agar further improves the turnaround time associated with the use of HC-SS agar.

Study Design



All media and slants were incubated at 35°C in ambient air for 18-24 hours before analysis. Vitek2 identifications were performed according to manufacturer's instructions with Vitek2 GN ID cards (bioMérieux, France). The Bruker MALDI Biotyper (Bruker Daltonics, Germany) was used according to the manufacturer's recommendations using whole colonies spotted onto ground steel target plates and overlaid with matrix. A score threshold of ≥ 2.0 was considered valid to the species level, score values <1.7 were considered invalid.

Results

I. HC-SS agar recovered 100% of all Salmonella and Shigella isolates tested

Organism	Number of Isolates Tested	Percent of Isolates Positive for Growth on HC-SS	Percent of Isolates Yielding Expected Color Reaction on HC-SS
Salmonella spp.	16	100%	100%
Shigella spp.	3	100%	100%
Shigella flexneri	4	100%	100%
Shigella sonnei	4	100%	100%

All isolates tested grew on HC-SS and produced the expected color reactions. *Salmonella* species tested included *S. enterica* and *S. typhi*. *Shigella* species tested included *S. flexneri* and *S. sonnei*. However, *Shigella dysenteriae* was not tested although this organism is expected to yield colorless colonies on HC-SS agar per data provided by the manufacturer.

II. MALDI-TOF-MS correctly identified all Salmonella isolates and normal enteric flora directly from HC-SS agar

Organism	Number of Isolates Tested	MALDI-TOF-MS Identification	Mean Score Value from colonies taken from HC-SS	Mean Score Value from Colonies Taken from SBA
Salmonella spp.	5	*Salmonella* spp.	2.257	2.418
Salmonella typhi	1	*Salmonella* spp.	2.411	2.425
Salmonella enterica	10	*Salmonella* spp.	2.258	2.372
Citrobacter spp.	15	*Citrobacter* spp.	2.263	2.347
Morganella morganii	8	*Morganella morganii*	2.443	2.577
Proteus mirabilis	5	*Proteus mirabilis*	2.418	2.504
Hafnia alvei	2	*Hafnia alvei*	2.619	2.44
Providencia spp.	2	*Providencia* spp.	2.203	2.268

III. All Shigella isolates were incorrectly identified as E. coli by MALDI-TOF-MS

Organism	Number of Isolates Tested	MALDI-TOF-MS Identification	Mean Score Value from colonies taken from HC-SS	Mean Score Value from Colonies Taken from SBA
Shigella spp.	3	*E. coli*	2.246	2.293
Shigella flexneri	4	*E. coli*	2.182	2.182
Shigella sonnei	4	*E. coli*	2.322	2.343

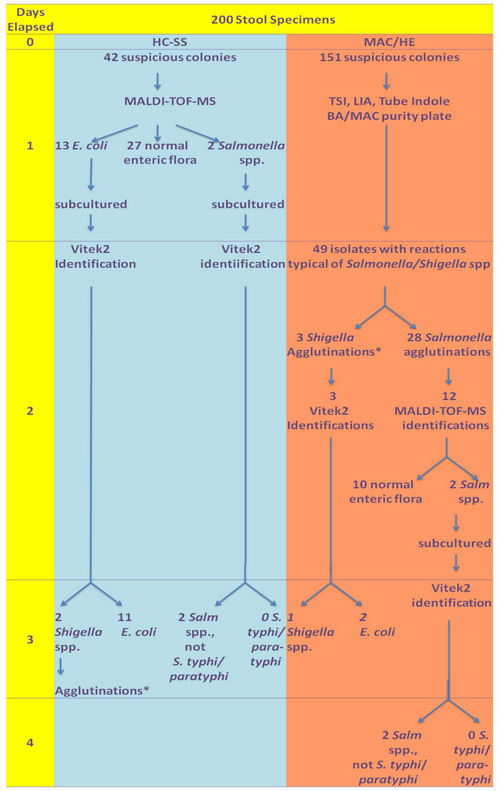
There was no significant difference in accuracy between colonies taken directly from HC-SS agar and Sheep Blood agars (SBA). As observed in previously published studies, MALDI-TOF-MS incorrectly identified all *Shigella* isolates as *E. coli*.

IV. HC-SS agar is superior to MAC and HE as a stool screening media

Media	Number of suspicious colonies	Number of isolates confirmed as *Salmonella* spp.	Number of isolates confirmed as *Shigella* spp.
HC-SS	42	2	2
MAC	74	1	0
HE	77	1	1

HC-SS agar demonstrated superior performance over HE and MAC agars. The sensitivity and specificity of HC-SS agar were 100% and 83.76% compared with a combined sensitivity and specificity for MAC and HE agars of 50% and 69.75%, respectively.

V. HC-SS agar combined with MALDI-TOF-MS improves turnaround time for stool cultures



*For each isolate in which *Shigella* agglutinations were indicated according to our algorithm, a total of 5 agglutinations were performed: *Shigella* A, B, C, D, and *Alkalescens-Dispar.*

- Implementation of the HC-SS/MALDI-TOF-MS method decreased the turnaround time for identification and reporting of *Salmonella* spp. by 1 day, potentially reporting out a reliable *Salmonella* spp. ID 24 hours after inoculation.
- Although turnaround time for the identification and reporting of *Shigella* spp. did not change, 27 of the 42 suspicious colonies (64%) were identified by MALDI-TOF-MS as normal enteric flora, thus shortening the turnaround time for reporting of a negative result by up to 2 days.

Results

VI. HC-SS agar combined with MALDI-TOF-MS decreases costs associated with stool culture

Expenses for 200 stool cultures (Including labor costs/Tech time)	HC-SS	MAC/HE
Selective media	$360	$98
Stool Picks (TSI, LIA, Tube Indole)	N/A	$1,407.32
Agglutinations (Difco™ Antiserum)	$155.80	$669.94
MALDI-TOF-MS Identifications	$396.90	$113.40
Vitek2 Identifications	$296.85	$98.95
Total	$1,209.55	$2,387.61

- Based on these 200 stool cultures, the HC-SS/MALDI-TOF-MS method decreased costs associated with stool cultures by 49.34%. Projected savings over a year would be $9,878.03

Summary & Conclusions

- HC-SS agar successfully recovered all *Salmonella* and *Shigella* isolates tested and yielded the expected color reactions in all cases. Furthermore, HC-SS agar demonstrated improved sensitivity and specificity over MAC and HE agars.
- MALDI-TOF-MS was able to correctly identify organisms directly from HC-SS agar without the need for subculture to non-chromogenic nutrient agar. No differences in accuracy for identification by MALDI-TOF-MS were observed between colonies taken directly from HC-SS agar compared with thosetaken from SBA.
- All *Salmonella* species tested directly from HC-SS agar were correctly identified to genus level by MALDI-TOF-MS. However, identification to the species-level was problematic most likely due to the poor representation of this organism in the Bruker Biptyper database. As expected, MALDI-TOF incorrectly identified all *Shigella* isolates tested as *E. coli.* Thus, additional identification methods must be pursued on all non-fermenting isolates yielding an identification of "*E. coli*" by MALDI-TOF-MS.
- HC-SS agar combined with MALDI-TOF-MS improved the turnaround time for the reporting of negative results by up to 2 days, we well as reducing the costs associated with stool cultures by almost 50%.

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