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HardyCHROM™ Group A Strep: a Chromogenic Culture Medium for the Detection of *Streptococcus pyogenes*

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Revised Abstract

Introduction: It is estimated that acute pharyngitis from strep throat causes approximately 5.2 million outpatient visits and 2.8 million antibiotic prescriptions annually in the United States.[1] Globally, the burden of strep throat is approximately 288.6 million cases among children alone.[2] Testing for *Streptococcus pyogenes*, or group A streptococci (GAS), via throat culture may be performed to confirm a negative rapid antigen test direct from specimen, particularly in children and adolescents. Throat culture, the gold standard for GAS detection, is performed by inoculating throat swabs to TSA with 5% sheep's blood (blood agar) and observing for beta hemolysis after incubation. Additional biochemical tests are subsequently performed for confirmation. The selective and differential HardyCHROM™ Group A Strep agar (HC GAS) was formulated to culture and isolate GAS based on colony color. *S. pyogenes* colonies appear light orange to red, while non-target microorganism growth appears white, blue, or the growth is inhibited. The ability to differentiate by colony color enhances the detection of GAS in the presence of normal upper respiratory flora or other species that are beta-hemolytic on blood agar. HC GAS performance was evaluated in three studies: analytical reactivity, cross reactivity, and a contrived specimen study.

Methods: For the analytical reactivity study, *S. pyogenes* (*n* = 49) were streaked for isolation with a 10µL loop at the limit of detection (LoD) ($1.5x10^3$ CFU/mL) to HC GAS. To evaluate potential cross reactivity, non-target organisms (*n* = 48) were tested at high concentrations ($1.5x10^6$-10^8 CFU/mL) and streaked for isolation with a 10µL loop to HC GAS. Additionally, 20 pre-screened, GAS-negative throat cultures were spiked with *S. pyogenes* isolates (*n* = 20) at the LoD and streaked for isolation onto HC GAS. In all studies, HC GAS plates were incubated at 35°C in 5% CO_2 for 24 hours and the growth and color was recorded. Non-selective media was inoculated in parallel as a control.

Results: For the analytical reactivity study, all 49 (100%) *S. pyogenes* were recovered at the LoD with expected color reactions after 24 hours of incubation. Non-target bacterial species (*n* = 46) and fungal species (*n* = 2) evaluated in the cross reactivity study were white (*n* = 11), blue (*n* = 13), or did not grow on HC GAS (*n* = 24). All 20 (100%) *S. pyogenes* strains were recovered from the contrived throat cultures when inoculated to HC GAS at the LoD.

Conclusions: Whether a microbiologist is scanning plates or a laboratory uses automated systems to interpret chromogenic reactions on media, utilization of HardyCHROM™ Group A Strep agar will allow for enhanced visual detection of *S. pyogenes* by colony color.

Introduction

Bacterial strep throat is a common bacterial infection resulting in inflammation in the throat that requires antibiotic intervention to alleviate. The causative agent of bacterial strep throat is *S. pyogenes*, which is identified by large beta-hemolytic zones on blood agar followed by a confirmatory test such as Lancefield latex agglutination. Identification can be missed due to overgrowth of non-pathogenic flora or weak hemolysis. To remedy this problem, HardyCHROM™ GAS was formulated with selective agents to inhibit a wider range of background microflora and a sensitive chromogen mixture to increase detection of *S. pyogenes*.

Methods

Analytical Reactivity Study: After culturing on blood agar, suspensions of *S. pyogenes* (*n* = 49) equivalent to a 0.5 McFarland ($1.5x10^8$ CFU/mL) were prepared. Suspensions were diluted to the LoD ($1.5x10^3$ CFU/mL) and streaked for isolation onto HC GAS and blood agar using a 10µL loop. The strains were sourced from a variety of culture collections (ATCC, LSI, IHMA, NCIMB, and BEI Resources) and four clinical isolates were included in the evaluation.

Cross Reactivity Study: 48 phylogenetically similar organisms or organisms found in the upper respiratory tract were streaked for isolation onto HC GAS and an appropriate non-selective medium at high concentrations ($1.5x10^8$ CFU/mL for bacteria and $1.5x10^6$ CFU/mL for yeasts and filamentous fungi) with a 10µL loop. Of the 48 organisms tested, 46 were bacterial and 2 were fungal (1 mold and 1 yeast).

Contrived Specimen Study: 20 negative pre-screened throat specimens (Eswabs™) were collected from Marian Medical Hospital in Santa Maria, California. Group A strep strains (*n* = 20) were diluted and inoculated into unique specimens to obtain a final concentration of $1.5x10^3$ CFU/mL (LoD) in the specimen matrix. The spiked specimens were vortexed and streaked onto HC GAS with a 10µL loop. For all analytical studies performed, inoculated HC GAS plates were incubated at 35°C and 5% CO_2 for 24 hours.

Results

Analytical Reactivity: All strains of *S. pyogenes* (*n* = 49) were recovered at the LoD from HC GAS. Each strain was recovered after 24 hours and was easily identifiable by the colonies' orange pigmentation.

Cross Reactivity: Of the 48 organisms evaluated, 24/48 (50%) organisms were completely inhibited (Table 1), 10/48 (20.8%) were partially inhibited (Table 2), and 14/48 (29.2%) exhibited luxuriant growth (Table 2). Of the 24 organisms that grew, 11 species had white colored growth and 13 species had blue colored growth (Table 2). The majority of the non-target organisms that grew were *Streptococcus* species (*n* = 19) and all resulted in blue or white growth (Table 2).

Contrived Specimen Study: All inoculated plates (100%) demonstrated recovery of target *S. pyogenes* by exhibiting orange colonies on the medium at the LoD (Table 3).

Table 1. Species Inhibited on HardyCHROM™ GAS (*n* = 24)

Organisms Inhibited		
Aggregatibacter aphrophilus	*Arcanobacterium haemolyticum*	*Aspergillus brasiliensis*
Bordetella pertussis	*Candida albicans*	*Corynebacterium diphtheriae*
Corynebacterium pseudodiphtheriticum	*Escherichia coli*	*Haemophilus influenzae*
Haemophilus haemolyticus	*Haemophilus parahaemolyticus*	*Haemophilus parainfluenzae*
Lactobacillus fermentum	*Moraxella catarrhalis*	*Neisseria gonnorheae*
Neisseria lactamica	*Neisseria meningitidis*	*Neisseria sicca*
Rothia mucilaginosa	*Staphylococcus aureus*	Methicillin-resistant *Staphylococcus aureus*
Staphylococcus epidermidis	*Streptococcus anginosus*	*Streptococcus constellatus*

Results

Table 2. Non-target Species Recovered (*n* = 24) on HardyCHROM™ GAS

Source	Strain	Non-target organism	HardyCHROM™ GAS Quadrant Growth	Color
Clinical	001	*Corynebacterium striatum*	1+	White
ATCC	29212	*Enterococcus faecalis*	1+	Blue
ATCC	700221	*Enterococcus faecium*	4+	Blue
HDX	5083	*Lactobacillus casei*	4+	Blue
ATCC	7830	*Lactobacillus leichmannii*	1+	Blue
ATCC	12386	*Streptococcus agalactiae*	4+	Blue
Clinical	002	*Streptococcus canis*	2+	Blue
ATCC	43078	*Streptococcus dysgalactiae*	4+	White
ATCC	12388	*Streptococcus dysgalactiae* subsp. *equisimilis*	1+	Blue
ATCC	700400	*Streptococcus equi* subsp. *zooepidemicus*	1+	White
ATCC	49147	*Streptococcus gallolyticus*	4+	Blue
Clinical	003	*Streptococcus gordonii*	4+	Blue
Clinical	004	*Streptococcus intermedius*	1+	White
Clinical	005	*Streptococcus lutetiensis*	4+	Blue
ATCC	35668	*Streptococcus mutans*	1+	White
ATCC	6249	*Streptococcus oralis*	4+	White
Clinical	006	*Streptococcus parasanguinis*	4+	White
ATCC	6305	*Streptococcus pneumoniae*	4+	White
ATCC	13419	*Streptococcus salivarius*	4+	Blue
Clinical	007	*Streptococcus sanguinis*	4+	Blue
BEI	HM-1063	*Streptococcus sobrinus*	2+	White
ATCC	19258	*Streptococcus thermophilus*	4+	White
ATCC	700407	*Streptococcus uberis*	4+	Blue
BEI	HM-561	*Streptococcus vestibularis*	1+	White

Table 3. Recovery of *S. pyogenes* (*n* = 20) from Contrived Specimens on HardyCHROM™ GAS

Specimen	Spiked Strain	HardyCHROM™ GAS Result	Color
1	19615	Positive	Orange
2	12384	Positive	Orange
3	1285434	Positive	Orange
4	13285	Positive	Orange
5	1156026	Positive	Orange
6	1453421	Positive	Orange
7	1191547	Positive	Orange
8	1112608	Positive	Orange
9	LS4304	Positive	Orange
10	LS4337	Positive	Orange
11	1146837	Positive	Orange
12	1117021	Positive	Orange
13	128426	Positive	Orange
14	1200286	Positive	Orange
15	1200270	Positive	Orange
16	1305246	Positive	Orange
17	1305174	Positive	Orange
18	1285446	Positive	Orange
19	1305247	Positive	Orange
20	1451090	Positive	Orange

Conclusions

- HardyCHROM™ Group A Strep is a sensitive and specific agar medium that can detect *S. pyogenes* in 24 hours as orange colonies.
- During analytical reactivity testing, 49/49 (100%) of *S. pyogenes* were recovered at the LoD.
- Other beta-hemolytic *Streptococcus* species evaluated did not cross react on the medium and this could reduce the time spent working up non-target beta-hemolytic growth from blood agar plates.
- HardyCHROM™ Group A Strep was able to recover 100% of the Group A Strep from spiked throat specimens at the LoD.

References:

(1) 1. Lewnard JA, King LM, Fleming-Dutra KE, Link-Gelles R, Van Beneden CA. Incidence of pharyngitis, sinusitis, acute otitis media, and outpatient antibiotic prescribing preventable by vaccination against group A *Streptococcus* in the United States. Clin Infect Dis. 2021;73(1):e47–e58.

(2) 2. Miller, K. The global burden of sore throat and group A *Streptococcus* pharyngitis: a Systematic Review and Meta-Analysis. eClinicalMedicine, Part of The Lancet. Volume 48, 101458. May 20, 2022.