Research Summary Report
Strep B Carrot Broth™ Transport Swab Inoculation Study

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Objective:

The Copan WASP® (Walk Away Specimen Processor) is becoming more and more prevalent in clinical microbiology laboratories. Hardy Diagnostics offers a version of its Carrot Broth™ (Cat# Z144BX) which is designed to be compatible with WASP® systems. One problem that has been reported while using the WASP® with Carrot Broth™ was that the specimen collection swab that is left in the tube sometimes interferes with the WASP's ability to further process the samples using the WASP® inoculator. Therefore, a different inoculation and incubation procedure for Carrot Broth™, when used with the WASP®, was suggested as a solution to this problem.

This study was conducted to determine the recovery of GBS (Group B Streptococcus) and reactivity of Carrot Broth™ inoculated with transport swabs which are immediately discarded after seeding, instead of remaining in Carrot Broth™ during incubation.

Experimental Design:

35 Carrot Broth™ tubes were prepared for use by placing a single reagent tile into each tube aseptically.

For this study, five strains of GBS were used in mixed culture dilutions with other natural human flora. The five strains tested were a strongly hemolytic strain of GBS (ATCC# 12386), a non-hemolytic strain of GBS (ATCC# 13813), a weakly hemolytic clinical strain of GBS (QOVH1), and two clinical strains of GBS with average hemolytic activity (MS4, MS14). The strains selected to represent natural human anovaginal flora were *Lactobacillus, Enterobacter faecalis, Candida albicans, and Escherichia coli.*

0.5 McFarland dilutions of each organism were made and diluted ten-fold until a concentration of approximately 10^4CFU/mL was achieved. 1mL of each 10^4CFU/mL dilution of GBS was mixed with 1mL of the 10^4CFU/mL dilutions of *E. faecalis, E. coli, C. albicans*, and 6mL of the 10^4CFU/mL dilution of *Lactobacillus.* This yielded five mixtures, one for each GBS, with concentrations of approximately 10^3CFU/mL of each organism except for *Lactobacillus*, which was at a concentration of approximately $6x10^3$CFU/mL.

15 Amies Gel Rayon Tipped Transport Swabs and 15 Stuarts Liquid Rayon Tipped Transport Swabs, three for each mixed culture of GBS, were dipped into the prepared mixed cultures and swirled for approximately three seconds. An estimated 100μL of suspension was soaked in each swab, corresponding to an average of 100 CFU's of GBS. Each swab was placed into their corresponding transport media for 30 minutes at room temperature.

Five Nylon Tipped Flocked Amies Liquid Transport Swabs, one for each mixed culture of GBS, were dipped into the prepared mixed cultures and swirled for approximately three seconds. An estimated 100µL of suspension was collected in each swab, corresponding to an average of 100 CFU's of GBS. Each swab was placed into their corresponding transport media for 30 minutes at room temperature.

The following conditions were setup as described below, using the inoculum as prepared above:

Transport Swab Dipped and Disposed

Five of the Amies Gel inoculated transport swabs and five of the Stuarts Liquid inoculated transport swabs (one for each GBS strain) were dipped into Carrot Broth™, swirled at the bottom of the tube for three seconds and squeezed against the side of the tube to elute the inoculum. Swabs were then discarded and the Carrot Broths were incubated at 35-37°C for 18 hours.

Transport Swab Vortexed and Disposed

Five of the Amies Gel inoculated transport swabs and five of the Stuarts Liquid inoculated transport swabs (one for each GBS strain) were placed into Carrot Broth™, shafts were broken, capped, and vortexed for three seconds to elute the inoculum. Swabs were then discarded using sterile forceps and the Carrot Broths were incubated at 35-37°C for 18 hours.

Flocked Swab Control

The five inoculated flocked transport swabs were vortexed for three seconds to elute the swab's contents into the transport media. 30µL of the transport media was pipetted directly into Carrot Broth™. The Carrot Broths were inverted three times to mix and were incubated at 35-37°C for 18 hours.

Transport Swab Control

Five of the Amies Gel inoculated transport swabs and five of the Stuarts Liquid inoculated transport swabs (one for each GBS strain) were placed into Carrot Broth™, capped, and the Carrot Broths were incubated at 35-37°C for 18 hours.

After incubation, Carrot Broth™ tubes which showed the characteristic orange coloration were recorded and discarded. Any Carrot Broth™ tubes that showed no coloration were subcultured to a GBS Detect™ (Cat# A300) plate to look for enhanced hemolysis and β-hemolytic colonies were confirmed as GBS by StrepPRO™ Latex Agglutination (Cat# PL030HD). This was predicted as necessary for non-hemolytic GBS strain ATCC# 13813.

Results:

All four hemolytic strains of GBS produced the characteristic orange positive reaction in Carrot Broth™, regardless of transport media or inoculation method used. All Carrot Broths inoculated with the non-hemolytic strain of GBS failed to produce the characteristic orange positive reaction in Carrot Broth™, as predicted, but produced enhanced beta hemolysis on GBS Detect™ and a positive GBS latex agglutination reaction, regardless of transport media or inoculation method used. Table 1 summarizes the results.

Table 1 – Carrot Broth™ Transport Swab Incubation Study

AG = Amies Gel LS = Liquid Stuarts	**Swirled Swab** Carrot Broth Reaction	**Swirled Swab** A300/Latex (if used)	**Vortexed Swab** Carrot Broth Reaction	**Vortexed Swab** A300/Latex (if used)	**Undiscarded Swab** Carrot Broth Reaction	**Undiscarded Swab** A300/Latex (if used)
ATCC# 12386 AG	+	N/A	+	N/A	+	N/A
ATCC# 12386 LS	+	N/A	+	N/A	+	N/A
ATCC# 13813 AG	-	+	-	+	-	+
ATCC# 13813 LS	-	+	-	+	-	+
QOVH-1 AG	+	N/A	+	N/A	+	N/A
QOVH-1 LS	+	N/A	+	N/A	+	N/A
Clinical Isolate 1 AG	+	N/A	+	N/A	+	N/A
Clinical Isolate 1 LS	+	N/A	+	N/A	+	N/A
Clinical Isolate 2 AG	+	N/A	+	N/A	+	N/A
Clinical Isolate 2 LS	+	N/A	+	N/A	+	N/A

	30µL of Transport Media from Flocked Swab Carrot Broth Reaction	**30µL of Media from Flocked Swab** A300/Latex (if used)
ATCC# 12386	+	N/A
ATCC# 13813	-	+
QOVH-1	+	N/A
Clinical Isolate 1	+	N/A
Clinical Isolate 2	+	N/A

Conclusions:

It is the opinion of the Hardy Diagnostics research and development team that Carrot Broth™ is effective when used with swabs that are discarded after inoculation instead of remaining in the Carrot Broth™ tube during incubation as the IFU currently recommends. This allows users to swirl their transport swab in Carrot Broth™ for three seconds, then discard the transport swab so it does not interfere with the proper functioning of the WASP®.

Another acceptable inoculation procedure would be to use 30µL of the transport media to inoculate Carrot Broth™ when a flocked swab is used.

These alternative procedures were effective in our study down to a total of approximately 100 CFUs of GBS in mixed culture per transport swab.