



Evaluation of Optimal Storage Temperature, Time and Transport Medium for the Detection of Group B Streptococcus in StrepB Carrot Broth



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Abstract

Background: Group B streptococcus (GBS) is the leading infectious cause of morbidity and mortality among newborns. In 2010, the Centers for Disease Control and Prevention (CDC) guidelines included GBS identification by StrepB Carrot Broth (CB). However, limited data are available to determine the optimal storage and transport medium (TM). We sought to compare recovery of GBS in CB when ESwab and Amies TM are stored for up to 144 h at 4°C, 21°C and 24°C.

Methods: 50 isolates of GBS were evaluated. 100 µL aliquots of 10³ CFU/mL suspensions were inoculated onto Amies and ESwab TM and stored at 4°C, 21°C or 24°C for up to 144 h. At 24 h intervals, Amies and ESwab TM were inoculated into CB and incubated at 35°C for 24 h. Results were quantified as 0, 1+, 2+, 3+, and 4+. The difference in GBS survival were compared.

Results: For Amies TM at 4°C, GBS recovery was 100% at 96 h and 84% at 144 h. Recovery of GBS at 144 h decreased significantly to 34% and 54% when Amies TM were stored at 21°C and 24°C, respectively (P = 0.0001). For ESwab TM at 4°C, GBS recovery was 100% up to 120 h and declined to 88% at 144 h. In contrast, 100% of GBS in ESwab TM stored for 144 h were recovered at 21°C and 24°C. At 21°C and 24°C, 0/50 GBS were recovered at 3+/4+ using Amies TM whereas 43/50 was recovered at 3+/4+ using ESwab TM (P = 0.0001).

Conclusion: Optimal specimen collection and TM are essential for identification of GBS. Results from this study support the use of CB to facilitate detection of GBS. Furthermore, optimal recovery is observed at 4°C for Amies TM and 21°C/24°C for ESwab TM. Overall, this study demonstrates that ESwab TM is superior to Amies TM for recovery of GBS, particularly when stored at 21°C and 24°C.

Introduction

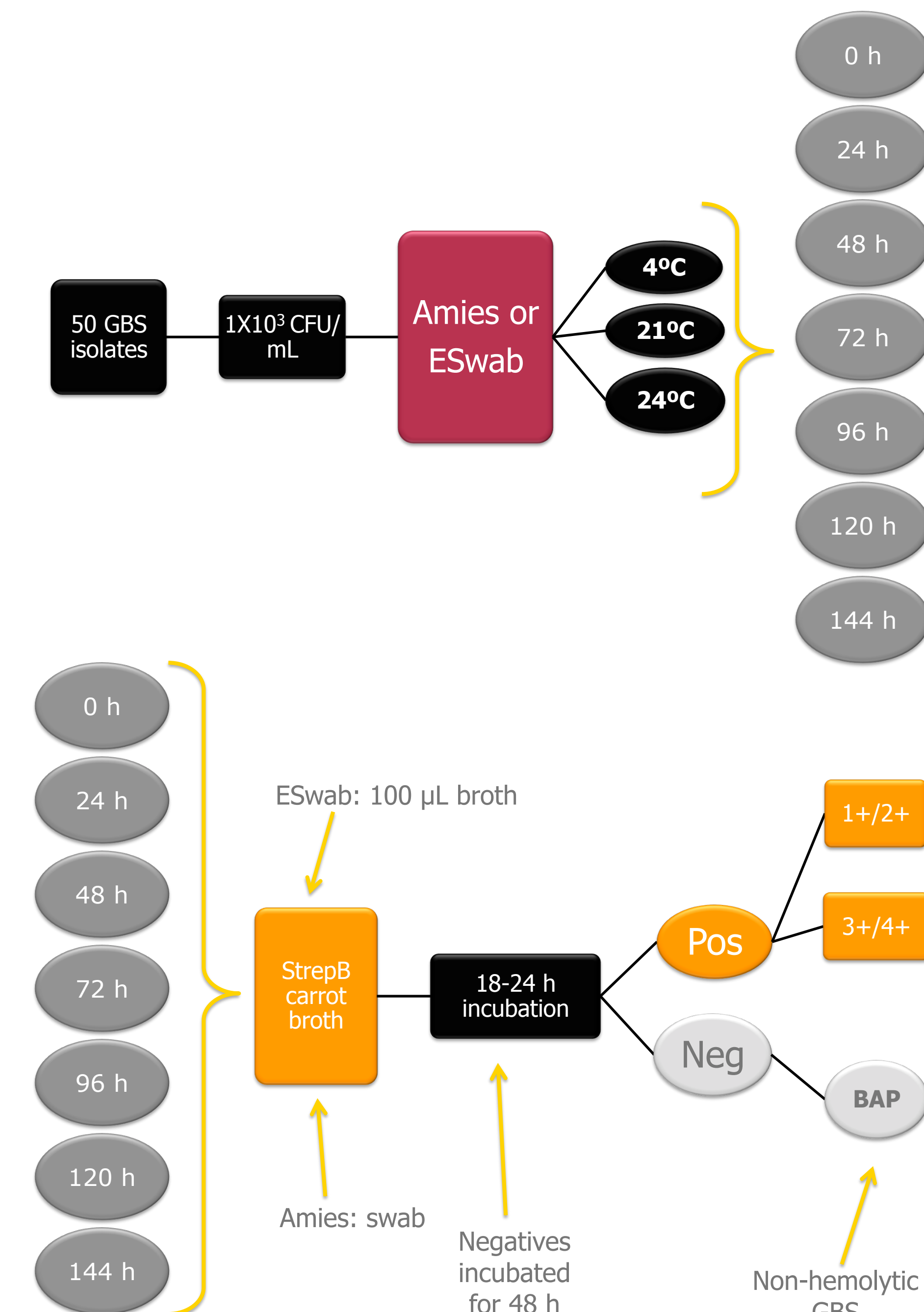
- Despite the implementation of universal screening to identify candidates for intrapartum antibiotic prophylaxis, GBS remains the leading infectious cause of early neonatal morbidity and mortality in the US.
- Maternal colonization with GBS in the genitourinary or gastrointestinal tract is the primary risk factor with 10-35% of pregnant women reported to be carriers of GBS. 61.4% of infants with GBS disease are born to women who are GBS screen negative. Therefore, optimal detection of GBS colonization is paramount to initiate appropriate prophylaxis.

- Optimal confirmation of GBS colonization is facilitated by the appropriate collection and transport of the clinical specimen to the laboratory. Specimens in transit for long durations may compromise the integrity of the specimen. CDC guidelines recommend the transport of swabs in a non-nutritive medium, such as Amies transport medium for up to 4 days at room or refrigeration temperature. However, limited data is available to support this recommendation.

Goals of this study:

- To evaluate the recovery of GBS from StrepB carrot broth at three different temperature settings for up to 6 days using Amies swab, a non-nutritive transport medium, and ESwab, a nylon-tipped flocked swab designed to optimize recovery of microorganisms. StrepB carrot broth is a chromogenic enrichment broth that can directly detect hemolytic GBS isolates.
- To determine the degree of pigmentation of StrepB carrot broth when Amies and ESwab transport medium were stored at various times and temperatures.

Figure 1. Experimental Design



Methods

Data:

Comparative Study Design (Figure 1):

- 50 isolates of hemolytic GBS previously recovered from vaginorectal swab were included in the study and a concentration of 1x10³ CFU/mL was prepared for each isolate
- 100 µL aliquots of the suspension were inoculated onto Amies and ESwab transport media and stored at 4°C, 21°C or 24°C for up to 144 h
- At each 24 h interval, Amies swab and 100 µL of ESwab transport media were inoculated into StrepB carrot broth and incubated at 35°C and 5% CO₂ for 24 h
- Results were recorded as 0+, 1/2+, and 3+/4+ with the degree of pigmentation correlating with the recovery of β-hemolytic GBS
- Negative broths were further subcultured onto Columbia sheep blood agar and incubated for 18-24 hours. Colonies resembling GBS were confirmed by Prolex™ Streptococcal Grouping latex kit (Prolab Diagnostics, Austin, Texas, USA) for rapid antigen detection

Data Analysis:

- Two-tailed Fisher's exact test (Graphpad Prism 5, La Jolla, CA, USA) was used to evaluate statistical significant difference in GBS survival when time, temperature and collection swabs were compared.

Results

- Recovery of GBS from Amies transport medium was optimal at 4°C with 100% GBS recovery at 96h which decreased to 84% at 144h. In contrast, GBS recovery decreased significantly when Amies was stored at 21°C and 24°C. Recovery was 98% up to 72 h, followed by a significant decline in recovery ranging from 34%-54% at 144h (P=0.0001) (Figure 2).
- For storage of ESwab transport medium at 4°C, GBS recovery was 100% up to 120h and declined to 88% at 144h. Recovery of GBS significantly improved at 21°C and 24°C; 100% of GBS was recovered when swabs were stored at 21°C and 24°C for the entire 144 h duration (P=0.0001) (Figure 2).
- The difference in degree of pigmentation of positive StrepB carrot broth was also determined between transport media.
 - At 4°C, GBS recovery was similar for both transport media with 42/50 and 44/50 isolates recovered at 144 h, respectively.
 - Of the isolates that were positive for GBS at 144 h, 4 each were at high quantities (3+/4+) and 38 to 40 were at low quantities (1+/2+) (Figure 3a)
- The degree of pigmentation at 21°C and 24°C were very similar. Results obtained at 21°C are described (Figure 3b):
 - At 21°C, both transport media yielded full recovery of all 50 isolates up to 48 h but 45/50 were recovered at high quantities (3+/4+) for ESwab and only 8/50 were recovered at high quantities for Amies.
 - At 144h and 21°C, 0 isolates were recovered at high quantities, 27 at low and 23 GBS isolates failed to recover from Amies transport media. In contrast, storage in ESwab yielded 100% recovery at 144 h; 43 at high quantities and 7 at low quantities.
- At 96 h, significant differences were found between ESwabs that were stored at 4°C, 21°C and 24°C. At 4°C, 41/50 isolates were recovered at low quantities and 9/50 isolates were recovered at high quantities. At 21°C, 6/50 were recovered at low and 44/50 were recovered at high. Similarly, at 24°C, 5/50 were recovered at low and 45/50 were recovered at high (Figure 4).

Figure 2. GBS Recovery in Amies and ESwab Transport Medium

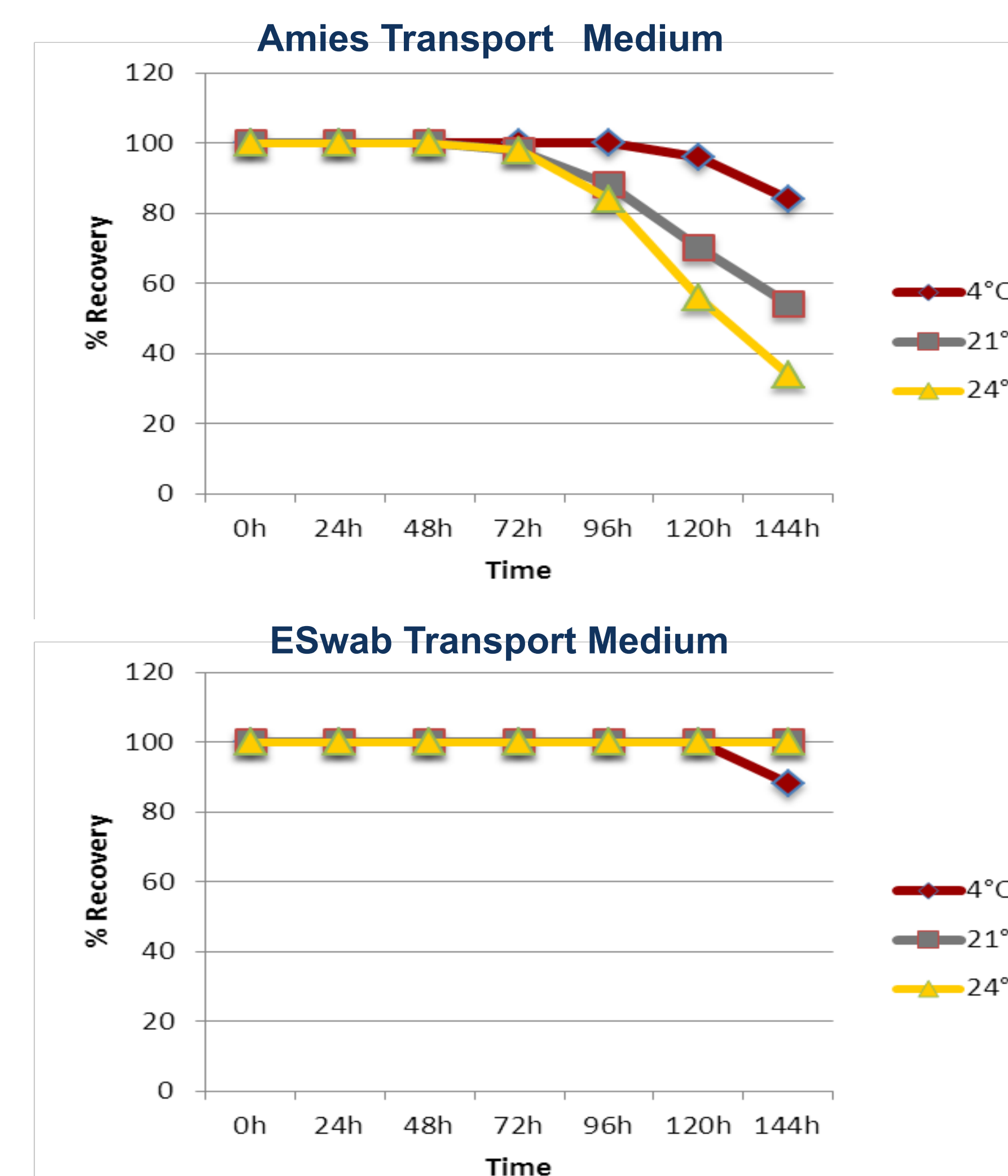


Figure 3. Degree of Pigmentation After Inoculation of StrepB Carrot Broth with Amies or ESwab

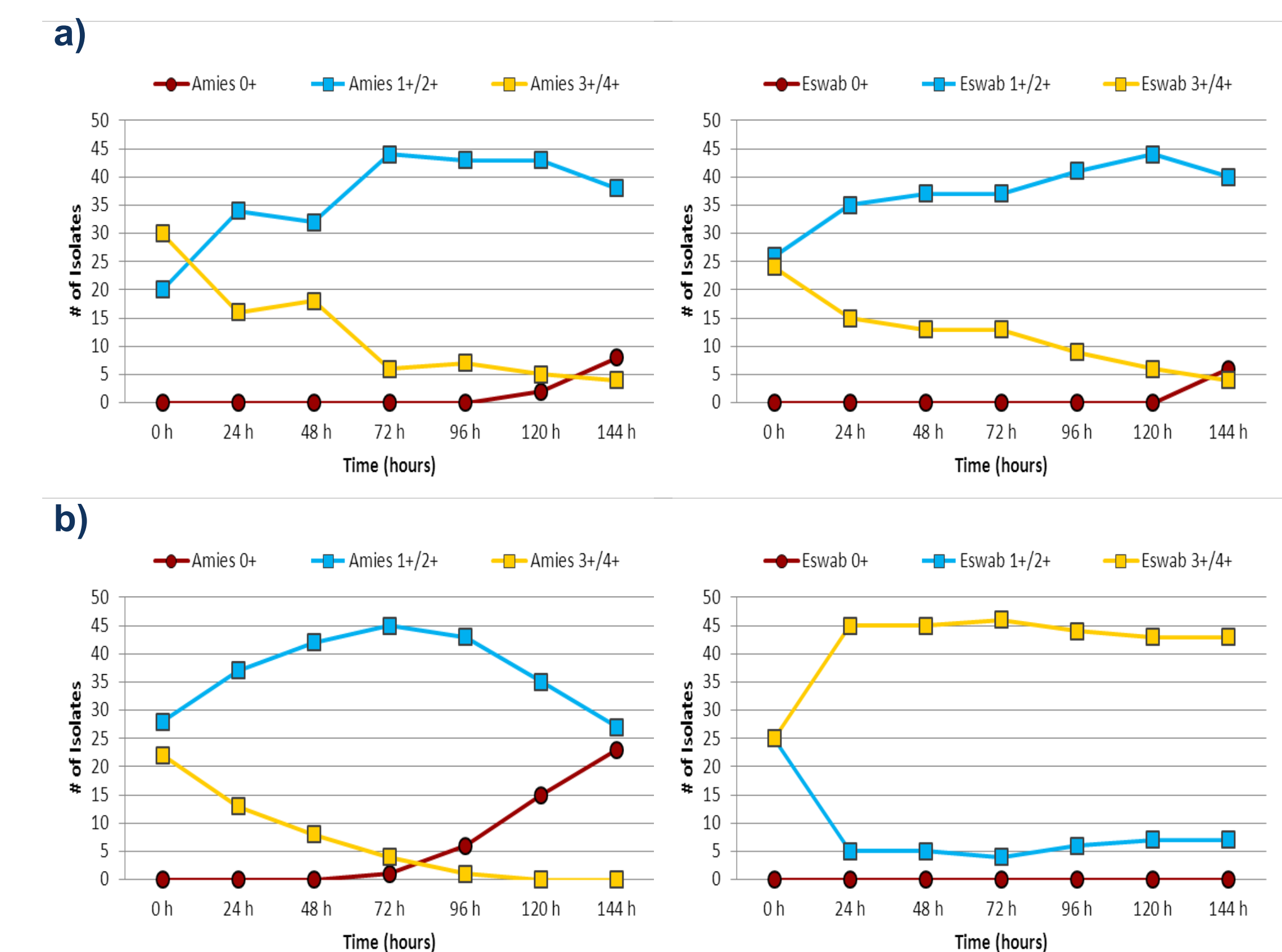
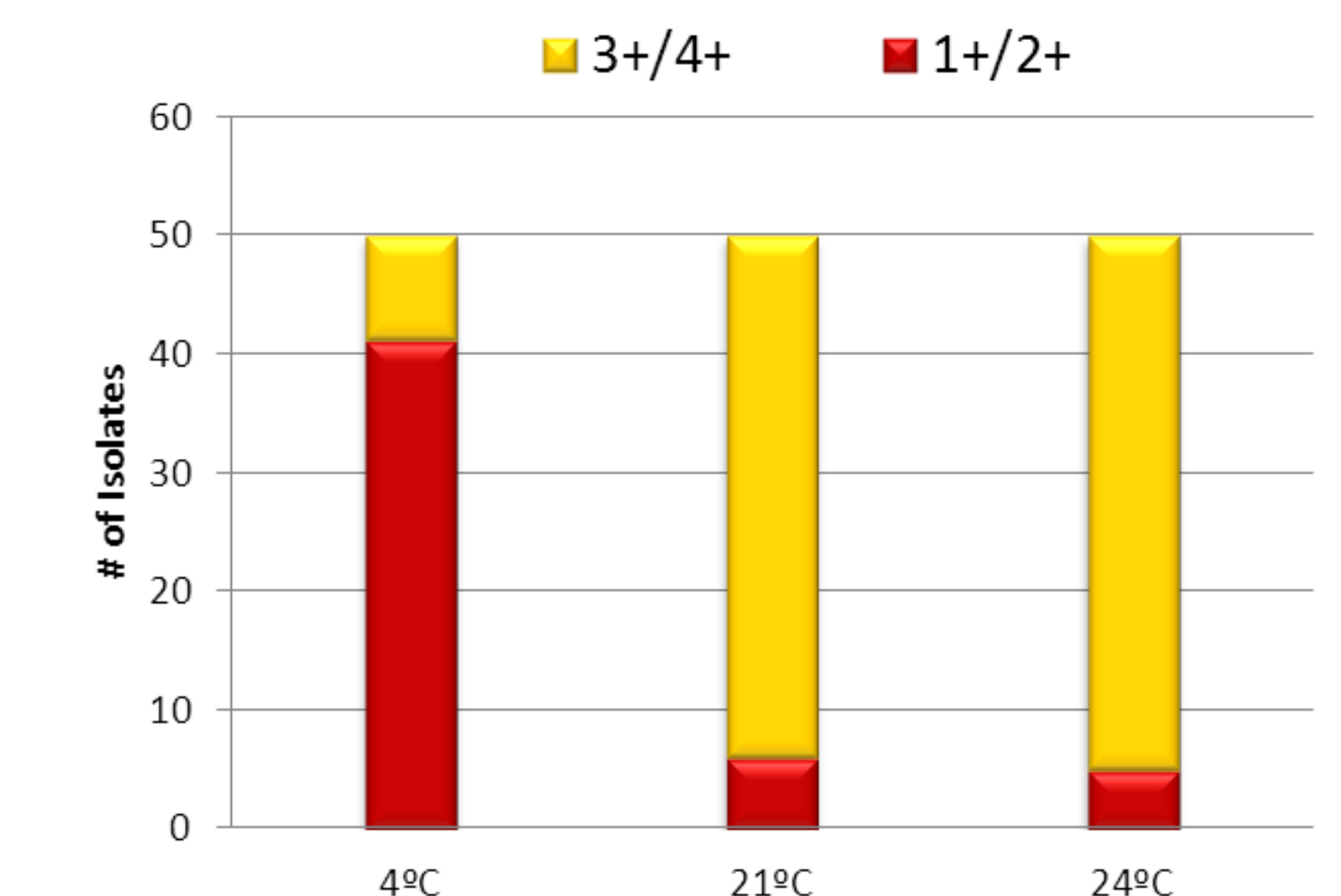


Figure 4. Degree of Pigmentation of StrepB Carrot Broth After 96 h of Inoculation with ESwab



Conclusion

- GBS recovery was optimal at 4°C for storage in Amies transport medium, and thus vaginorectal swabs stored in this medium should be refrigerated if delayed.
- ESwab transport medium is superior to Amies in the preservation of GBS in StrepB carrot broth, particularly at RT conditions where full GBS recovery was demonstrated for up to 6 days
- Chromogenic enrichment broths, such as StrepB carrot broth, may be used as an enrichment and detection broth for GBS
- CDC may consider expanding recommendations to include alternate transport media in addition to non-nutritive medium

Acknowledgement

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