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Survival of vaginal microorganisms in three commercially available transport systems

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ABSTRACT

Transport systems are used to collect and maintain the viability of microorganisms. Two Amies media based transport systems, BD CultureSwab™ MaxV(+) Amies Medium without Charcoal (MaxV(+)) and Fisherfinest® with Amies gel Transport Medium without charcoal (Fisherfinest®) were compared to a Cary-Blair media based transport system, Starswab® Anaerobic Transport System (Starswab®), for their capacity to maintain the viability of 17 clinical microorganisms commonly isolated from the vagina (*Lactobacillus crispatus, L. jensenii*, *L. iners*, group B streptococci, *Candida albicans*, *Escherichia coli*, *Enterococcus faecalis, Atopobium vaginae*, *Peptoniphilus harei, Mycoplasma hominis*, *Gardnerella vaginalis*, *Dialister microaerophilus*, *Mobiluncus curtisii*, *Prevotella amnii, P. timonensis*, *P. bivia*, and *Porphyromonas uenonis*). Single swabs containing mixtures of up to five different species were inoculated in triplicate and held at 4 °C and room temperature for 24, 48, 72, and 96 h (h). At each time point, swabs were eluted into a sterile salt solution, serially diluted, inoculated onto selected media, and incubated. Each colony type was quantified and identified. A change in sample stability was reported as a ≥1 log increase or decrease in microorganism density from baseline. Overall, the viability of fastidious anaerobes was maintained better at 4 °C than room temperature. At 4 °C all three transport systems maintained the viability and prevented replication of *C. albicans*, *E. faecalis*, GBS, and *E. coli*. Microorganisms having a ≥1 log decrease in less than 24 h at 4 °C included *A. vaginae, G. vaginalis*, and *P. uenonis* in Starswab®, *L. iners, A. vaginae*, and *P. amnii* in MaxV(+), and *A. vaginae, G. vaginalis, P. bivia*, and *P. amnii* in Fisherfinest®. At 48 h at 4 °C, a ≥1 log decrease in concentration density was observed for *P. harei and P. amnii* in Starswab®, *G. vaginalis, P. bivia and P. uenonis* in MaxV(+), and *L. iners, P. harei, P. timonensis*, and *P. uenonis* in Fisherfinest®. Overall, at 4 °C the viability and stability of vaginal microorganisms was maintained better in the Cary-Blair based transport system (Starswab®) than in the two Amies based transport systems.

1. Introduction

Cultivation-dependent identification of vaginal microbiota has an important role in our understanding of the vaginal microbiome. While cultivation-independent methods allow for identification of noncultivable microorganisms, isolation of these microorganisms through cultivation-dependent identification offers information on phenotypes, insight to multiple microbial interactions, and opportunity for experimental manipulations [1]. The sensitivity of cultivation-dependent identification of vaginal microbiota can be limited by loss of viability during transport. With the use of centralized laboratories, shipping times can exceed 24 h (h) and transport conditions can vary. To ensure sample integrity, preservation of fastidious anaerobes is required during transport because mucosal sites are colonized by commensal bacteria and yeast, which replicate rapidly at room temperature.

Semisolid nonnutritive transport media were first developed to ensure the viability of microorganisms and prevent replication during transport [2]. The two most commonly used transport media are both derivations of Stuart's media; Cary-Blair and Amies [3,4]. Cary-Blair media was developed primarily for the transport of fecal specimens, while Amies media has been widely used for the

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transport of swab specimens from multiple body sites [3,4]. Studies comparing transport systems have varied in their recommendation of an optimal transport system and temperature. Citron et al. compared the Port-A-Cul® transport system, based on a Cary-Blair based media, and the Copan Venturi Transystem, which contains an Amies medium, and reported that both systems could be used for shipping clinical specimens at room temperature within 24 h [5]. However, in a comparison of Starplex Starswab II and the Copan Vi-Pak Amies Agar Gel with the Port-A-Cul® transport system it was stated that the Port-A-Cul® was an Amies-based gel [6]. Although they reported that none of the tested Amies transport systems were ideal, the Copan Vi-Pak was recommended for shipment of clinical specimens at room temperature within 24 h [6]. Rishmawi et al. reported that MaxV(+) maintained viability of three aerobic fastidious microorganisms and it was able to maintain the concentration density of group B *Streptococcus* (GBS), but not *Escherichia coli* in short shipping times of 24 h or less at room temperature [7].

There have been few evaluations of transport systems for the cultivation of fastidious anaerobes from vaginal samples. Stoner et al. compared the Port-A-Cul® with the Copan transport system [8]. Stoner et al. reported that transport of swab samples at 4 °C is one approach to ensuring viability of anaerobes while preventing replication of facultative bacteria and yeast for shipments over 24 h and both transport systems were adequate for transport at 4 °C [8].

The objective of our study was to perform a contemporary evaluation of the capacity of three commercially available transport systems; the Cary-Blair media based Starswab® Anaerobic Transport System (Starswab®; Starplex Scientific, Etobicoke, Ontario) and two Amies media based transport systems, BD CultureSwab™ MaxV(+) Amies Medium without Charcoal (MaxV(+); manufactured by Copan Diagnostics Inc., Murrieta, CA for Becton Dickinson and Co., Sparks, MD), and Fisherfinest® with Amies gel Transport Medium without charcoal (Fisherfinest®; Fisher Scientific, Pittsburgh, PA) to maintain the integrity of 17 anaerobic and aerobic microorganisms commonly isolated from the vagina over 96 h at 4 °C and room temperature.

2. Methods

All microorganisms used in this study were isolated from vaginal swab samples and stored frozen in litmus milk at ≤ −70 °C until further testing. Clinical wild type (WT) isolates were previously identified using phenotypic and genotypic methods. To evaluate the capacity of three transport systems, 17 clinical WT species were used in five different mixtures at the following densities: aerobic bacteria and yeast mixture 1 contained *Lactobacillus crispatus* (10^5 cfu/mL), GBS (10^3 cfu/mL), and *Candida albicans* (10^2 cfu/mL); aerobic bacteria and yeast mixture 2 contained *L. jensenii* (10^5 cfu/mL), *E. coli* (10^4 cfu/mL), and *Enterococcus faecalis* (10^4 cfu/mL); bacterial vaginosis associated bacteria (BVAB) mixture 1 contained *L. iners* (10^4 cfu/mL), *Atopobium vaginae* (10^5 cfu/mL), *Peptoniphilus harei* (10^3 cfu/mL), *Prevotella amnii* (10^4 cfu/mL), and *Mycoplasma hominis* (10^5 cfu/mL); BVAB mixture 2 included *Gardnerella vaginalis* (10^6 cfu/mL), *Dialister microaerophilus* (10^4 cfu/mL), and *Mobiluncus curtisii* (10^4 cfu/mL); BVAB mixture 3 included *P. timonensis* (10^5 cfu/mL), *P. bivia* (10^5 cfu/mL), and *Porphyromonas uenonis* (10^5 cfu/mL). The microorganism densities used within each mixture were approximately mirrored based on previous findings [9]. Microorganisms in each mixture were combined based on phenotype and colony morphology for easy identification during quantification.

Individual microorganism suspensions were prepared and serially diluted as previously described with the exception of using brain heart infusion broth (Becton Dickinson) for the initial suspension medium [8]. The swabs provided within the Starswab®, MaxV(+), and Fisherfinest® systems were inoculated with 100-μL of each mixture. Triplicates of each transport system were stored at room temperature or 4 °C for 24 h, 48 h, 72 h, or 96 h. Following the designated storage times, serial 1:10 dilutions were made from each swab and 100-μL of each dilution was subsequently used to inoculate Columbia CNA agar with 5% sheep blood (CNA; Becton Dickinson), MacConkey II agar (MAC; Becton Dickinson), Sabdex (Sabouraud Dextrose) Agar with Chloramphenicol (SAB; Hardy Diagnostics, Santa Maria, CA), and Rogosa (ROG; prepared in house) for the aerobe/yeast mixtures. A plates and M broth (both prepared in house), ROG, Brucella blood agar with hemin and vitamin K (BRU; Hardy Diagnostics), and human blood tween bilayer medium (HBT; Becton Dickinson) were inoculated for cultivation of the BVAB mixtures as previously described [8]. All plates were streaked for isolation. CNA, MAC, SAB, A plate, and M broth were incubated at 36–37 °C in 5–7% CO_2 for 48 h. ROG, BRU, and HBT agar plates were incubated anaerobically at 36–37 °C for 4–7 days.

A change in sample stability was reported when a ≥1 log decrease (loss of viability) or increase (replication) in microorganism density was observed. American Type Culture Collection (ATCC) strains of each of the microorganisms except for *P. amnii, D. microaerophilus*, and *P. timonensis* were evaluated with the WT strains in parallel. Similar results were observed for all organisms, therefore only the results from the evaluation of the WT strains are presented.

Statistical analyses were performed using Stata statistical software release 14.2 (Stata Corp., College Station, TX), and statistical tests were evaluated at the two-sided 0.05 significance level. Linear regression models were used to evaluate the effects of transport media and time on the geometric mean concentrations of microorganisms at each transport temperature.

3. Results

The three transporters evaluated differed in their capacity to maintain the viability of microorganisms at room temperature. Starswab® maintained the viability of all organisms at room temperature through 96 h with the exception of *P. amnii* to 48 h, and *G. vaginalis* and *P. uenonis* to 72 h (Table 1). MaxV(+) maintained viability of all organisms through 96 h with the exception of *L. iners* and *M. hominis* to 72 h, *G. vaginalis* to 48 h, and all three *Prevotella* species and *P. uenonis* to 24 h (Table 1). Fisherfinest® maintained the viability of all organisms through 96 h with the exception of

Table 1
A comparison of Starswab®, MaxV(+), and Fisherfinest® transport systems for maintenance of viability at room temperature* and 4 °C up to 96 h.

Microorganism	Time (h) viability sustained					
	Starswab®		MaxV(+)		Fisherfinest®	
	RT	4 °C	RT	4 °C	RT	4 °C
Lactobacillus crispatus	96	96	96	96	96	96
Lactobacillus jensenii	96	96	96	96	96	96
Lactobacillus iners	96	96	72	96	48	96
Atopobium vaginae	96	96	96	96	24	96
Peptoniphilus harei	96	96	96	96	96	96
Prevotella amnii	48	96	24	72	<24	24
Mycoplasma hominis	96	96	72	96	96	96
Gardnerella vaginalis	72	96	48	96	48	96
Dialister microaerophilus	96	96	96	96	24	96
Mobiluncus curtisii	96	96	96	96	96	96
Prevotella timonensis	96	96	24	96	<24	96
Prevotella bivia	96	96	24	96	<24	24
Porphyromonas uenonis	72	96	24	96	24	96

* 22–26 °C