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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 hours (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.

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Keywords

Transport systems; fastidious anaerobes; vaginal microbiota

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 hours (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 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.

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* (10⁵ cfu/mL), GBS (10³ cfu/mL), and *Candida albicans* (10² cfu/mL); aerobic bacteria and yeast mixture 2 contained *L. jensenii* (10⁵ cfu/mL), *E. coli* (10⁴ cfu/mL), and *Enterococcus faecalis* (10⁴ cfu/mL); bacterial vaginosis associated bacteria (BVAB) mixture 1 contained *L. iners* (10⁴ cfu/mL), *Atopobium vaginae* (10⁵ cfu/mL), *Peptoniphilus harei* (10³ cfu/mL), *Prevotella amnii* (10⁴ cfu/mL), and *Mycoplasma hominis* (10⁵ cfu/mL); BVAB mixture 2 included *Gardnerella vaginalis* (10⁶ cfu/mL), *Dialister microaerophilus* (10⁴ cfu/mL), and *Mobiluncus curtisii* (10⁴ cfu/mL); BVAB mixture 3 included *P. timonensis* (10⁵ cfu/mL), *P. bivia* (10⁵ cfu/mL), and *Porphyromonas uenonis* (10⁵ 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% CO₂ 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.

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 *L. iners* and *G. vaginalis* to 48 h, *A. vaginae*, *D. microaerophilus*, and *P. uenonis* to 24 h, and did not maintain the viability of all three *Prevotella* species for even 24 h (Table 1). By contrast, when the transport media were held at 4°C, viability of all microorganisms was maintained through for 96 h in Starswab®, 72 h in MaxV(+) and 24 h in Fisherfinest® transport (Table 1).

Commensal aerobes and yeast can replicate in transport media at room temperature [8], and this replication can mask the presence of fastidious microorganisms. Only the Starswab® transport system maintained the concentration density of *C. albicans* for 96 h at room temperature (Fig. 1a). None of the three transport systems maintained the density of GBS, *E. coli*, and *E. faecalis* within one log of the original concentration when held for 24 h at room temperature (Fig. 1b, 1c, 1d). Overall, the lowest increase in concentration density at room temperature was observed in the Starswab® transport system which only allowed a 1–2 log increase in GBS and *E. faecalis* (Fig. 1b and 1d), and a 2–3 log increase in *E. coli* over 24 h (Fig. 1c). *E. faecalis* and *E. coli* had a 2–3 and 5 log increase, respectively in both Amies transport systems at 24 h (Fig. 1c and 1d). Replication was limited to < 1 log increase in all three transport systems at 4°C for *C. albicans*, GBS, *E. coli*, and *E. faecalis* through 96 h (data not shown).

Although all microorganisms were assessed over 96 h, it is acknowledged that clinical specimen transport generally occurs within 48 h to a central laboratory for processing. To simulate typical transport conditions, we evaluated how well the three transport media maintained the concentration density of microbiota associated with vaginal health of bacterial vaginosis for 48 h at room temperature (Fig. 2). The concentration densities of *L. iners* and *A. vaginae* were decreased by 2 logs in Starswab® and MaxV(+), however a 4 log decrease in *L. iners* and no viability of *A. vaginae* was observed at 48 h for the Fisherfinest® transport system (Fig. 2a and 2b). The concentration density of *P. amnii* decreased < 2 logs in Starswab® at 48 h, whereas it was nonviable after 48 h in MaxV(+) and less than 24 h in Fisherfinest® (Fig. 2c). Swabs transported in the Fisherfinest® transport system had a greater than 5 log decrease in *G. vaginalis*, *D. microaerophilus*, *P.*

timonensis, *P. bivia* and *P. uenonis* at 48 h when held at room temperature (Fig. 2d–2h). At room temperature, Starswab® maintained the concentration densities of *P. harei*, *M. curtisii*, *M. hominis*, *L. jensenii*, and *L. crispatus* (data not shown). MaxV(+) and Fisherfinest® maintained the concentration densities of these five microorganisms with the exceptions of *P. harei* at 24 h in Fisherfinest®, *M. hominis* at 48 h and *L. jensenii* at 24 h in MaxV(+) (data not shown).

The viability of all microorganisms was maintained better when all three transport media were held at 4°C (Table 2). Overall, the Starswab® transporter best maintained the concentration of *Lactobacillus* species, *G. vaginalis* and anaerobes at 4°C through 96 h when compared to the MaxV(+) and Fisherfinest® transporters. Although *G. vaginalis* and *P. uenonis* were the only microorganisms nonviable in all three transport systems by 96 h at room temperature (Fig. 2d and 2h), when held at 4°C through 96 h, viability was maintained to ≥ 2 log decrease in Starswab® and MaxV(+), and < 3 log and < 4 log decrease in Fisherfinest® for *G. vaginalis* and *P. uenonis*, respectively (Table 2). All three *Lactobacillus* species evaluated were maintained for 48 h in all three media, with the exception of *L. iners* in MaxV(+) at 24 h and Fisherfinest® at 48 h (Table 2). *A. vaginae* decreased by > 1 log in all three transport systems within 48 h, while *M. curtisii*, *M. hominis* and *D. microaerophilus* were stable in all three transporters for 48 h at 4°C (Table 2). *P. harei* was maintained through 48 h in MaxV(+), but had a > 2 log decrease in Starswab® and Fisherfinest® (Table 2). For the *Prevotella* species evaluated, the Starswab® transporter maintained integrity at 4°C for 24 h, whereas, samples in MaxV(+) transporters showed > 1 log decrease in *P. amnii* at 24 h, and samples in Fisherfinest® transporter had a > 1 log decrease in both *P. amnii* and *P. bivia* at 24 h (Table 2).

Discussion

The present study demonstrated that none of the transport media evaluated maintained sample stability, defined by ≥ 1 log change in density, at room temperature for 24 h. At 4°C, however, differences in the capacity of the three transport systems to maintain viability and prevent decreases in density for some vaginal microbiota were revealed. While room temperature transport may be appropriate for some types of specimens, vaginal swab samples should be transported under refrigeration to protect sample integrity, both preventing replication of facultative microbiota and to prevent decreased viability of *Lactobacillus* species, *G. vaginalis* and anaerobes.

Stoner et al. previously reported that the optimal transport temperature for samples containing vaginal microbiota is 4°C, and the present evaluation confirms those findings using different transport media [8]. The manufacturer's instructions supplied with the Starswab®, MaxV(+), and Fisherfinest® transporters recommend that specimens containing fastidious anaerobes be processed for culture within 24 h [10–12]. Although none of the package inserts from the three transport systems recommend transport at 4°C, the results of the present study suggest that significant loss of viability of fastidious anaerobes will occur within 24 h and replication of commensal aerobes will also occur without sample refrigeration. At 4°C a decrease in viability was observed in all transporters at 24 h for *A. vaginae* and at 48 h for *G. vaginalis*, *P. amnii*, and *P. uenonis*, highlighting the need to

minimize transport times for these microorganisms (Table 2). Combined, these data suggest that for the microorganisms present in vaginal swab samples, transport at 4°C within 24 h in the S tarswab® transport system yielded the best results.

This study compared three transport systems using a unique group of clinical isolates from vaginal samples. Although the data were not presented here, ATCC strains of 14 of the 17 microorganisms were evaluated in parallel with the wild type strains and similar results were obtained with respect to preservation of viability. These data suggest that the loss of viability during storage in these transport systems was not unique to recent clinical isolates.

Tyrrell et al. reported that the eSwab transport system maintained the integrity of fastidious anaerobes comparably with the Anaerobic Transport Medium system [13]. They evaluated ATCC strains of anaerobes, none of which were evaluated in the present study [13]. Nys et al. reported that at room temperature and 4°C the eSwab transport system maintained integrity of ATCC strains of *E. coli*, GBS, and *C. albicans* as well, if not better than Copan Venturi Transystem through 48 h [14]. Nys et al. also reported replication of *E. coli* and *C. albicans* in both transport systems at room temperature for 48 h, and a decrease in replication at 4°C [14].

Differences in media composition play a role in the viability or replication of microorganisms during transport. Stuart's transport media, with a pH of 7.4, was originally developed to maintain the viability of *Neisseria gonorrhoeae*, but was shown to also maintain *Trichomonas vaginalis*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *S. pyogenes*, and *Corynebacterium diphtheriae* [2]. Cary-Blair media was developed for shipments of fecal specimens, and has a pH of 8.4 [3]. Amies media utilizes phosphate buffer, salts and charcoal, at a pH of 7.2–7.4, to prevent proliferation and maintain survival of microorganisms [4]. One limitation of this study was the use of mixtures of microorganisms that may not mimic the diversity of microorganisms found in clinical specimens. Unknown microorganism interactions within the mixtures used, could impact viability within the mixtures in ways that are not understood.

In the present study, one glass and two plastic transport tubes were evaluated. Citron et al. noted that the use of plastic transport tubes, which allow the diffusion of oxygen into the sample, may affect the survival of obligate anaerobes [5]. Since both MaxV(+) and Fisherfinest® are packaged in plastic rather than glass tubes, the diffusion of oxygen into the transport media may have accounted for the decreased viability of obligate anaerobes like *P. amnii* and *P. bivia* in our study. The data generated from the present study suggest that cultivation based studies of the microbiota could be adversely impacted by longer transport times, ambient temperature during transport, and glass versus plastic transport containers. Studies evaluating transport media for their capacity to maintain a specific range of microbiota may be beneficial to ensure adequate growth in a clinical specimen.

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Highlights

- Vaginal microorganisms have variable viability in transport media.
- Ideal transport temperature for vaginal microorganisms is 4°C for 48 hours.
- Starswab® preserved the viability of anaerobes better than Amies-based transport systems.

Figure 1.

A comparison of concentration density over 96 hours at room temperature of microorganisms held in Starswab®, MaxV(+) and Fisherfinest® transport systems. Microorganisms shown are known to overgrow when stored at room temperature. P values of the following microorganisms indicate a significant increase in concentration density in MaxV(+) and Fisherfinest® in comparison to the Starswab® transport system. P values from linear regression models evaluating changes in concentrations of microorganisms held in MaxV(+) and Fisherfinest® transport systems compared to those held in the Starswab® transport system over 96 h. All models were adjusted for time.

Figure 2.

A comparison of concentration density over 96 hours at room temperature of microorganisms held in Starswab®, MaxV(+) and Fisherfinest® transport systems. A decrease in viability was shown at room temperature for the microorganisms below. P values from six of the eight following microorganisms indicate a significant decrease in concentration density in MaxV(+) and Fisherfinest® when compared to the Starswab® transport system. P values from linear regression models evaluating changes in concentrations of microorganisms held in MaxV(+) and Fisherfinest® transport systems compared to those held in the Starswab® transport system over 96 h. All models were adjusted for time.

Table 1

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

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

* 22–26°C

Table 2

A species comparison of mean log change (CFU) from 0 h in Starswab®, MaxV(+), and Fisherfinest® transport systems when held at 4°C.

Microorganism	Mean Log Change (CFU)				P-value*
	24 h	48 h	72 h	96 h	
<i>Lactobacillus crispatus</i>					
Starswab®	0.1	0.0	-0.1	-0.1	Referent
MaxV(+)	-0.2	-0.7	-0.6	-0.9	<0.001
Fisherfinest®	-0.6	-0.2	-0.4	-0.5	0.009
<i>Lactobacillus jensenii</i>					
Starswab®	-0.3	-0.3	-0.2	-0.1	Referent
MaxV(+)	-0.9	-0.8	-0.9	-1.0	<0.001
Fisherfinest®	0.4	0.1	0.0	-0.2	<0.001
<i>Lactobacillus iners</i>					
Starswab®	-0.6	-0.9	-1.3	-1.3	Referent
MaxV(+)	-1.3	-1.3	-1.9	-1.9	<0.001
Fisherfinest®	-0.6	-1.2	-1.4	-1.5	0.25
<i>Atopobium vaginae</i>					
Starswab®	-2.1	-2.0	-2.2	-2.1	Referent
MaxV(+)	-1.2	-1.4	-1.3	-1.5	0.25
Fisherfinest®	-1.0	-2.0	-2.6	-3.0	0.59
<i>Peptoniphilus hareii</i>					
Starswab®	-0.7	-1.5	-1.4	-1.4	Referent
MaxV(+)	0.0	-0.4	-0.3	-0.4	<0.001
Fisherfinest®	-0.6	-1.2	-1.6	-1.9	0.66
<i>Prevotella amnii</i>					
Starswab®	-0.7	-1.2	-2.6	-2.6	Referent
MaxV(+)	-1.7	-2.3	-2.4	-3.1	0.03
Fisherfinest®	-2.3	-3.0	-3.0	-3.0	0.001
<i>Mycoplasma hominis</i>					
Starswab®	-0.3	-0.3	-1.0	-0.3	Referent

Microorganism	Mean Log Change (CFU)				P-value*
	24 h	48 h	72 h	96 h	
MaxV(+)	-0.1	-0.7	-0.2	-0.4	0.67
Fisherfinest®	-0.2	-0.4	-0.5	-0.4	0.54
<i>Gardnerella vaginalis</i>					
Starswab®	-1.2	-1.2	-1.5	-1.6	Referent
MaxV(+)	-0.9	-1.0	-1.2	-1.3	0.08
Fisherfinest®	-1.7	-2.2	-2.4	-3.2	0.89
<i>Dialister microaerophilus</i>					
Starswab®	-0.3	-0.3	-0.1	-0.3	Referent
MaxV(+)	-0.1	-0.1	-0.1	-0.4	<0.001
Fisherfinest®	-0.3	-0.7	-1.0	-1.6	<0.001
<i>Mobiluncus curtisi</i>					
Starswab®	-0.1	-0.3	-0.1	-0.4	Referent
MaxV(+)	-0.1	-0.1	-0.3	-0.3	0.003
Fisherfinest®	-0.2	-0.7	-0.5	-0.6	<0.001
<i>Prevotella timonensis</i>					
Starswab®	0.0	0.0	-0.6	-0.4	Referent
MaxV(+)	-0.1	-0.2	-0.6	-1.1	<0.001
Fisherfinest®	-0.6	-1.2	-1.8	-2.4	<0.001
<i>Prevotella bivia</i>					
Starswab®	-0.6	-0.4	-0.6	-0.7	Referent
MaxV(+)	-0.3	-1.0	-1.8	-2.8	0.001
Fisherfinest®	-2.1	-5.3	-5.3	-5.3	<0.001
<i>Porphyromonas uenonis</i>					
Starswab®	-1.0	-1.0	-1.8	-1.6	Referent
MaxV(+)	-0.5	-1.2	-2.0	-1.8	<0.001
Fisherfinest®	-0.8	-2.1	-3.0	-3.9	<0.001

*P values from linear regression models evaluating changes in concentrations of microorganisms held in MaxV(+) and Fisherfinest® transport systems compared to those held in the Starswab® transport system over 96 h. All models were adjusted for time.