Equine Veterinary Journal

Equine Veterinary Journal ISSN 0425-1644
DOI: 10.1111/evj.12476

Comparison of disinfectant efficacy when using high-volume directed mist application of accelerated hydrogen peroxide and peroxymonosulfate disinfectants in a large animal hospital

N. T. SAKLOU, B. A. BURGESS†, D. C. VAN METRE, K. J. HORNIG, P. S. MORLEY and S. R. BYERS*

Department of Clinical Sciences, Colorado State University, Fort Collins, USA
†Department of Population Health Sciences, Virginia Tech, Blacksburg, USA.

*Correspondence email: stacey.byers@colostate.edu; Received: 22.12.14; Accepted: 17.06.15

This study was presented in part at the 94th Annual Meeting of the Conference of Research Workers in Animal Diseases, Chicago, 2013; and the American College of Veterinary Internal Medicine Forum, Nashville, Tennessee, 2014.

Summary

Reasons for performing study: Effective decontamination of animal holding environments is critical for providing high quality patient care and maintaining a safe working environment. Disinfection of animal holding environments is a significant challenge during times of epidemic disease.
Objectives: The purpose of this study was to evaluate the disinfectant efficacy of 3 strategies for high-volume directed mist application of accelerated hydrogen peroxide and peroxymonosulfate disinfectants; 4.25% accelerated hydrogen peroxide (Accel®; AHP) at a 1:16 dilution and single and double applications of 2% peroxymonosulfate solution (Virkon-S®; VIR-1 and VIR-2) for decontamination of a large animal hospital environment.
Study design: Experiment.
Methods: After cleaning and disinfection of the hospital environment, transparencies experimentally contaminated with known concentrations of *Staphylococcus aureus*, *Salmonella enterica* and *Pseudomonas aeruginosa* were placed on vertical surfaces. Disinfectant solution was applied by directed mist application and, after 30 min of contact time, transparencies were collected and individually placed into tubes containing 10 ml Dey-Engley broth. The process was repeated for each disinfectant. Tenfold dilutions of each sample were plated onto tryptic soy blood agar with 5% sheep blood. Bacterial counts from transparencies exposed to disinfectants were compared with counts from control transparencies (unexposed to disinfectants) to evaluate reduction in colony forming units.
Results: The least squares mean reduction (log_{10}) in colony forming units (CFUs) for *S. aureus* and *P. aeruginosa* was 1.5–2.5 logs and approximately 0.8–1.0 logs for *S. enterica*. Reductions were generally largest for VIR-2 and smallest for AHP, although these differences were not all statistically significant and the magnitude of differences may not be clinically relevant.
Conclusions: For the organisms evaluated, all 3 disinfectants applied as a directed mist were effective at reducing CFUs in a veterinary hospital environment. Effective disinfection using this method of application is dependent on adequate cleaning prior to application, and use of adequate volumes of disinfectant.

Keywords: horse; biosecurity; cleaning; disinfection; infection control

Introduction

Veterinary infection control is critical to providing high quality animal care as well as maintaining a safe working environment for personnel. Of 38 American Veterinary Medical Association accredited veterinary teaching hospitals, 82% reported the occurrence of at least one epidemic of disease in patients during the previous 5 years, and 50% reported the occurrence of zoonotic disease among personnel in the previous 2 years [1]. During times of epidemic disease it is common to find significant environmental contamination [2–4]. This contamination most likely originates from infected animals (as opposed to other sources, such as contaminated feed and water), as indicated by recovery of *Salmonella* isolates from the hospital environment and animals at the Colorado State University Veterinary Teaching Hospital (CSU-VTH) with the same phenotype (serotype and antimicrobial susceptibility) during the same month [5]. Additionally, persistence of pathogens in the environment has been demonstrated after times of epidemic disease [4,6].

The infection control programme at the CSU-VTH employs periodic environmental disinfection using high-volume directed mist application of disinfectant. Directed mist application of disinfectants can be an effective method to reduce the environmental burden of microorganisms, particularly in areas that are not easily cleaned through scrubbing with detergents and copious amounts of water, such as overhead ductwork and electrical conduits [7,8]. We have previously demonstrated the efficacy of a 4% peroxymonosulfate disinfectant solution (Virkon® S)[a], applied as a directed mist, in reducing counts of target bacterial organisms in a hospital setting [7,8], but this concentration is higher than the manufacturer's label recommendations of a 2% maximum concentration.

Recently, accelerated hydrogen peroxide disinfectants have been marketed in the USA and Canada for use in environmental disinfection of veterinary hospitals (e.g. Accel®)[b]. We hypothesised that common peroxygen disinfectants, such as Virkon® and Accel®, will have similar efficacies for reducing bacterial contamination on surfaces in a veterinary hospital environment. Specifically, the purpose of this study was to compare the efficacy of 2 disinfectant solutions: a 4.25% accelerated hydrogen peroxide (Accel®) at a 1:16 dilution, and single and double applications of 2% peroxymonosulfate solution (Virkon® S) for decontamination of a veterinary hospital environment.

Materials and methods

Study overview

High-volume, directed mist application of different disinfectants (4.25% accelerated hydrogen peroxide solution diluted 1:16 [AHP], 2% peroxymonosulfate solution applied once (VIR-1), and 2% peroxymonosulfate applied twice [VIR-2]), were compared in the Livestock Hospital at the CSU-VTH. Experimentally contaminated surfaces were placed throughout the hospital and collected after each disinfectant application, in turn. Disinfectant efficacy was evaluated by determining the percentage reduction in colony forming units (CFUs) for *Pseudomonas*

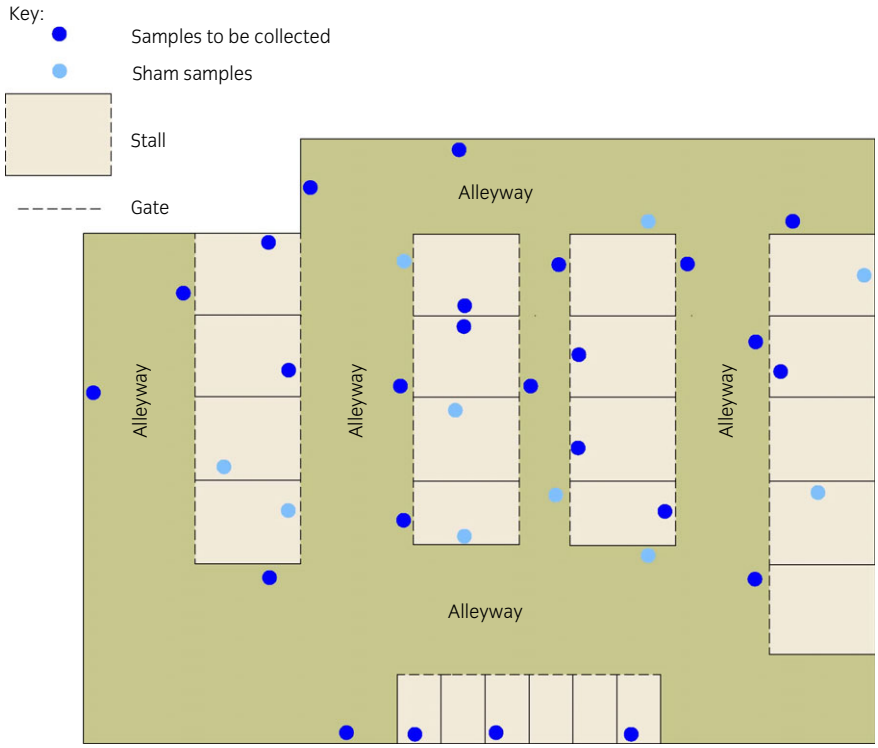

Fig 1: Transparency locations in the Livestock Hospital at the Colorado State University Veterinary Teaching Hospital. Twenty-five sampling locations were randomly selected from 35 pre-selected sampling locations distributed throughout the hospital; the remaining 10 locations were used as 'sham' locations to aid in the blinding of study personnel.

aeruginosa, *Salmonella enterica* and *Staphylococcus aureus* before and after application of disinfectants.

Bacterial inoculates

Stock solutions of each indicator bacterium were created by individually inoculating 5 ml of tryptic soy broth[c] with reference strains of *S. aureus* (ATCC 29213)[d], *S. enterica* (ATCC 13311)[d], or *P. aeruginosa* (ATCC 27853)[d] which were incubated at 37°C for 12 h. Tenfold dilutions of these broth cultures were plated onto tryptic soy agar with 5% sheep blood (TSA)[e]. Through quantification of CFUs of serial dilutions, the bacterial concentrations of broth cultures were estimated to be 1.54×10^7 CFU/ml for *S. aureus*, 1.10×10^7 CFU/ml for *S. enterica* and 1.54×10^7 CFU/ml for *P. aeruginosa*.

Transparencies

A total of 285 polyester transparencies[f], 3 cm × 4 cm, pre-labelled with numbered ID codes, were disinfected with 70% ethanol, and allowed to dry. Once dry, one side of each transparency was inoculated with a known concentration of *S. aureus*, *S. enterica* or *P. aeruginosa* (90 transparencies per indicator organism; one organism per transparency). The inoculated transparencies were allowed to air dry at 21°C for 14 h in a laminar flow biological safety cabinet. Fifteen additional pre-labelled and disinfected transparencies were used as uninoculated controls. Approximately 48 h before transparency placement in the Livestock Hospital, all hospital surfaces were cleaned and disinfected according to normal infection control standard operating procedures [9]. Briefly, all surfaces were thoroughly scrubbed with detergent, disinfected with a hypochlorite solution, rinsed with water and disinfected with a quaternary ammonium disinfectant solution, which was allowed to dry in place.

Thirty-five sampling locations distributed throughout the hospital were preselected for transparency placement (8 transparencies per location with and without indicator organisms) on vertical hospital surfaces (i.e. walls and gates), and sampling was randomly assigned, using a random number generator, to occur at either 0.914 m (3 ft; low) or 1.524 m (5 ft; high) from the floor (Fig 1). A 12 inch adhesive strip (Velcro[g]) was placed at each sampling location to allow placement of 8 transparencies (test sites). Using a random number generator, 25 of 35 sampling locations were randomly selected for placement of transparencies contaminated with each of the indicator organisms (i.e. one transparency per organism) along with uncontaminated (control) transparencies to aid in the blinding of cleaning and laboratory personnel. The order of placement of the contaminated transparencies was randomly assigned to the 8 test sites at each sampling location. All transparencies placed at the remaining 10 sampling locations were uncontaminated, for additional blinding of cleaning personnel. Additionally, 5 uninoculated transparencies, fastened to a wall within the Livestock Hospital in an area not exposed to disinfectant misting, were used as negative controls. Five inoculated transparencies of each organism were maintained in the laboratory in a laminar flow biological safety cabinet as positive controls. Transparencies, including negative controls, were replaced with new transparencies before each application of disinfectant. To minimise cross-contamination, transparencies for each indicator organism were placed/replaced by one researcher per indicator organism while wearing gloves.

Directed mist disinfection

Three application strategies of different disinfectant solutions were evaluated in this study: AHP, VIR-1, VIR-2. Disinfectants were mixed according to manufacturer's recommendations. Briefly, to prepare the AHP solution, 1.18 l (40 ounces [US]) of the 4.25% AHP concentrate were added to every 19 l (5 gallons [US]) of water used (1:16 dilution). To prepare the VIR-1 and VIR-2 (2% peroxymonosulfate) solution, 380 g of peroxygen compound was added to every 19 l of water used. Directed mist application of disinfectant was accomplished using a commercially available backpack mist blower (Solo450)[h], which, according to

manufacturer specifications, produced aerosol particles of approximately 100–200 μm in diameter and distributed the disinfectant solutions at an average of 0.99 l/min. Output from the mist blower can be dispersed over >9 m (>30 ft) from the nozzle, with an output diameter of >4 m (>13 ft). To cover all of the surface area in the Livestock Hospital (approximately 1297 m^2 [13,961 ft^2], which included all floors, walls, ceilings, stalls and other surfaces), 45.4 l (12 gal; 35 ml/m^2) were distributed during an approximately 30 min time period, with a 30 min additional contact time. All surfaces remained wet during the contact time and this contact time was observed after each disinfectant application. Personnel applying the disinfectants were not informed of plans for placement of transparencies, were blinded to the location of the transparencies, and were also blinded to the disinfectant being used during each application. Thirty minutes after disinfectant application, all inoculated transparencies were collected (see sample processing below). Surfaces were allowed to dry and the process was repeated for each of the solutions being evaluated.

Safety precautions

Safety precautions used during preparation and application of disinfectant solutions were reviewed and approved by university occupational safety personnel. Briefly, to prevent accidental exposure to aerosolised disinfectants, all animals were removed from the area being disinfected, all doors were closed with access limited to authorised personnel, and ventilation systems were inactivated during application. A second individual always accompanied the person applying disinfectant with the backpack mister, serving as a 'spotter' and assisting with moving equipment when necessary; a third person remained outside of the disinfection area, but within the Livestock Hospital, to assist with oversight and communication. Gloves and N95 respirators were worn while mixing disinfectant solutions. Personal protective equipment worn by the person applying disinfectant and the spotter included powered air purifying respirators[i], rubber gloves, disposable water-resistant coveralls with hoods, and calf-height rubber boots.

Sample processing

Thirty minutes after finishing disinfectant application, all inoculated and control transparencies were collected and placed in sterile tubes with 10 ml of Dey-Engley broth[e] (disinfectant neutralising broth) and transported to the laboratory for processing. Samples were vortexed for 3 s and then 6 tenfold dilutions were made using buffered peptone water[j] ; 100 μl of each dilution was plated on TSA for enumeration and incubated at 37°C for 24 h. Colonies were visually enumerated on each plate at 24 and 48 h. Limits for detection and accurate enumeration were assumed to be 25–50 CFUs on a plate [10]. For each plate, CFUs were multiplied by the dilution factor to estimate CFUs in the original solution (reported as CFU/ml). Numerical ID codes were used throughout laboratory processing and all personnel were blinded to which transparencies were contaminated with which agent.

Data analysis

Data were recorded in a spreadsheet, validated and explored using descriptive statistics. CFUs were log transformed to meet the assumptions of the parametric analysis. Log reduction was determined using positive control samples as a baseline count of CFUs per plate. Multivariable linear regression was used to evaluate disinfectant efficacy for each individual indicator organism using log CFUs as the outcome variable. Independent variables included in the model were determined *a priori* as factors of interest or potential confounding variables and were therefore included in the model regardless of P value. The independent variables evaluated included disinfectant (AHP, VIR-1, VIR-2), sampling location, test site (1–8) at each sampling location, and height of placement (high or low). Least squares mean (LSMean) bacterial reduction and 95% confidence intervals were derived from linear regression models.

Results

Overall, reductions in average CFUs (log_{10}) for contaminants were detected after all disinfectant applications. Reductions in LSMean CFUs (log_{10}) on inoculated transparencies ranged from 0.8 to 2.5 logs, and varied among indicator organisms and disinfection application (Table 1). The LSMean reduction (log_{10}) in CFUs for *S. aureus* and *P. aeruginosa* was 1.5–2.5 logs and approximately 0.8–1.0 logs for *S. enterica*. Reductions were generally largest for VIR-2 and smallest for AHP, although these differences were not all statistically significant and the magnitude of differences may not be clinically relevant.

The CFUs after disinfectant exposure were below the limit of quantification (<25 CFUs) for 53 of 75 (71%) transparencies for *S. enterica*, 44 of 75 (59%) transparencies for *P. aeruginosa* and 45 of 75 (60%) transparencies for *S. aureus* and thus, for purposes of analyses, were assigned bacterial counts of 25 CFU/ml. The CFUs were greater than the limit of quantification (>50 CFUs) for 6 of 75 (8%) transparencies for *S. enterica*, 12 of 75 (16%) for *P. aeruginosa* and 8 of 75 (11%) for *S. aureus* and thus, for purposes of analyses, were assigned bacterial counts of 50 CFU.

The CFUs of the positive controls were less than the limit of quantification for 1 of 15 (7%) transparencies for *S. enterica,* 3 of 15 (20%) transparencies for *P. aeruginosa* and 3 of 15 (20%) transparencies for

TABLE 1: Mean reduction in log_{10} CFUs/ml after directed mist disinfectant application

Organism	Disinfectant	n	LSMeans of log_{10} CFU/ml	95% CI	Reduction of log_{10} CFU/ml	95% CI	Percentage reduction
S. enterica	AHP	25	3.75	3.59–3.91	0.75[a]	0.58–0.91	82
	VIR-1	25	3.70	3.54–3.86	0.80[a]	0.64–0.96	84
	VIR-2	25	3.53	3.37–3.69	0.97[a]	0.81–1.13	89
	Positive control	15	4.50		Reference		
P. aeruginosa	AHP	25	4.28	4.01–4.54	2.06[a]	1.80–2.33	99
	VIR-1	25	4.15	3.89–4.42	2.19[a]	1.92–2.45	99
	VIR-2	25	3.55	3.29–3.81	2.79[b]	2.53–3.05	100
	Positive control	15	6.34		Reference		
S. aureus	AHP	25	4.56	4.25–4.88	1.54[a]	1.23–1.86	97
	VIR-1	25	4.17	3.86–4.48	1.94[a]	1.62–2.24	99
	VIR-2	25	3.59	3.29–3.91	2.51[b]	2.19–2.82	100
	Positive control	15	6.11		Reference		

AHP = accelerated hydrogen peroxide; CFU = colony forming units; CI = confidence interval; n = number of sampling locations; Positive control = transparencies that were contaminated with the same stock solutions but were not exposed to disinfectant; VIR-1 = peroxymonosulfate applied once; VIR-2 = peroxymonosulfate applied twice.

Different superscripts indicate a statistically significant difference (P<0.05) in reduction between disinfectant solutions within each indicator organism group.

S. aureus and thus, for purposes of analyses, were assigned bacterial counts of 25 CFUs. The CFUs of the positive controls were greater than the limit of quantification for 10 of 15 (67%) transparencies for *S. enterica,* 4 of 15 (27%) transparencies for *P. aeruginosa* and 5 of 15 (33%) transparencies for *S. aureus* and thus, were assigned bacterial counts of 50 CFUs.

There was no observable growth for the negative control transparencies. The effect of transparency placement height was not associated with significant differences in LSMean reductions (log_{10}) in bacterial counts ($P = 0.9$).

Discussion

These results suggest that the 3 disinfectant applications used in this study (AHP, VIR-1 and VIR-2) reduced viable indicator bacteria on vertical surfaces in a veterinary hospital environment. Although the average reduction differed slightly between the disinfectants, it is questionable that these differences (0.2–1.0 log, depending upon the organism) would be biologically relevant (Table 1). Greater differences were observed among the 3 indicator organisms, smaller reductions being consistently seen for *S. enterica*, and the greatest reductions for *P. aeruginosa*, which reiterates the importance of considering target organisms when selecting disinfection protocols used in veterinary hospitals.

The goal of this high-volume directed mist application is to achieve efficient and thorough coverage of all potentially contaminated surfaces, and motorised mist blowers are suitable for this purpose. We have used this type of disinfection at the CSU-VTH on a regularly scheduled basis with the intention of minimising potential build-up of environmental contaminants in the hospital environment [9], as well as to aid in mitigation of recognised outbreaks of healthcare-associated infections [3,11]. In our experience, this type of application is less labour- and time-intensive than other application methods, particularly in hard-to-reach areas [2].

The reductions demonstrated in this research are markedly smaller than those shown in a previous study using similar methods in the same hospital environment [8]. In the previous study, directed misting using the same model of backpack mister to dispense 4% peroxymonosulfate solution resulted in approximately 6 log reductions in *S. enterica* and *S. aureus* [8]. However, in addition to the stronger concentration of disinfectant (4% in the previous study vs. 2% in this study), a much larger volume of disinfectant was also applied in the same hospital space (170 l for the previous study vs. 45.4 l for VIR-1 and 90.8 l for VIR-2). Thus, in attempts to reliably achieve higher reductions we would recommend dispensing greater volumes onto the same surface area (i.e. >35 ml/m^2), perhaps even 2–3 times this volume given previous results.

While results of this study demonstrate reductions in contamination using all 3 disinfectant applications, a few caveats should be considered when interpreting these findings. First, the polyester transparencies used in this trial are an ideal surface for disinfection because of their smooth, nonporous surface, in contrast to surfaces frequently found in large animal hospitals that can impede the disinfection process (e.g. surfaces with cracks or crevices, or even unsealed concrete blocks or wood). Imperfections in surfaces found in the hospital environment can decrease the effectiveness of the cleaning and disinfection process, creating a potential environmental reservoir for contamination [12]. Second, it is important to note that effective removal of dirt and organic material through cleaning, the environmental temperature, and the contact time can all have a great impact on disinfection efficacy, and methods used in this research should have allowed optimal disinfection results, which might not be achieved in other circumstances.

This study indicates that applying a disinfectant solution as a directed mist, in conjunction with rigorous cleaning, may be an effective method for decreasing environmental contamination in a veterinary hospital. Despite this, infection control programmes should be tailored to each facility taking into account their physical and operational limits, and practitioners and facility managers should select a disinfectant and application modality based on their specific needs and target organism(s).

Authors' declaration of interests

No competing interests have been declared.

Ethical animal research

Not applicable.

Sources of funding

This study was supported by an Animal Health and Disease grant from the College Research Council, College of Veterinary Medicine and Biomedical Sciences, Colorado State University.

Authorship

Study conceptualisation was performed by P.S. Morley, B.A. Burgess and D.C. Van Metre. Project supervision was provided by P.S. Morley and S.R. Byers. B.A. Burgess was responsible for laboratory procedures and was assisted by N.T. Saklou and K.J. Hornig. N.T. Saklou was the primary author of the manuscript, assisted by B.A. Burgess, P.S. Morley and S.R. Byers; all authors had the opportunity to comment on drafts and approved the final version.

Manufacturers' addresses

[a]Antec International, a DuPont Company, Wilmington, Delaware, USA.
[b]Virox™ Technologies, Oakville, Ontario, Canada.
[c]BD Diagnostic Systems, Sparks, Maryland, USA.
[d]Thermo Scientific-Remel, Lenexa, Kansas, USA.
[e]Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA.
[f]Apollo, Frisco, Texas, USA.
[g]Velcro USA Inc., Manchester, New Hampshire, USA.
[h]Newport News, Virginia, USA.
[i]3M Personal Safety Division, St. Paul, Minnesota, USA.
[j]Becton Dickinson and Company, Cockeysville, Maryland, USA.

References

1. Benedict, K.M., Morley, P.S. and Van Metre, D.C. (2008) Characteristics of biosecurity and infection control programs at veterinary teaching hospitals. *J. Am. Vet. Med. Assoc.* **233**, 767-773.
2. Tillotson, K., Savage, C.J., Salman, M.D., Gentry-Weeks, C.R., Rice, D., Fedorka-Cray, P.J., Hendrickson, D.A., Jones, R.L., Nelson, W. and Traub-Dargatz, J.L. (1997) Outbreak of *Salmonella infantis* infection in a large animal veterinary teaching hospital. *J. Am. Vet. Med. Assoc.* **211**, 1554-1557.
3. Steneroden, K.K., Van Metre, D.C., Jackson, C. and Morley, P.S. (2010) Detection and control of a nosocomial outbreak caused by *Salmonella* Newport at a large animal hospital. *J. Vet. Intern. Med.* **24**, 606-616.
4. Dallap Schaer, B.L., Aceto, H. and Rankin, S.C. (2010) Outbreak of salmonellosis caused by *Salmonella enterica* serovar Newport MDR-AmpC in a large animal veterinary teaching hospital. *J. Vet. Intern. Med.* **24**, 1138-1146.
5. Burgess, B.A., Morley, P.S. and Hyatt, D.R. (2004) Environmental surveillance for *Salmonella enterica* in a veterinary teaching hospital. *J. Am. Vet. Med. Assoc.* **225**, 1344-1348.
6. Dunowska, M., Morley, P.S., Traub-Dargatz, J.L., Davis, M.A., Patterson, G., Frye, J.G., Hyatt, D.R. and Dargatz, D.A. (2007) Comparison of *Salmonella enterica* serotype Infantis isolates from a veterinary teaching hospital. *J. Appl. Microbiol.* **102**, 1527-1536.
7. Dunowska, M., Morley, P.S. and Hyatt, D.R. (2005) The effect of Virkon®S fogging on survival of *Salmonella enterica* and *Staphylococcus aureus* on surfaces in a veterinary teaching hospital. *Vet. Microbiol.* **105**, 281-289.
8. Patterson, G., Morley, P.S., Blehm, K.D., Lee, D.E. and Dunowska, M. (2005) Efficacy of directed misting application of a peroxygen disinfectant

for environmental decontamination of a veterinary hospital. *J. Am. Vet. Med. Assoc.* **227**, 597-602.
9. Morley, P.S., Burgess, B.A. and Van Metre, D.A. (2013) *Infection Control and Biosecurity Standard Operating Procedures*, James L. Voss Veterinary Teaching Hospital, Colorado State University, Fort Collins, USA. Available at http://csuvets.colostate.edu/biosecurity/biosecurity_sop.pdf
10. Sutton, S. (2011) Accuracy of plate counts. *J. Validation Technol.* **3**, 42-46.
11. Goehring, L.S., Landolt, G.A. and Morley, P.S. (2010) Detection and management of an outbreak of equine herpesvirus type 1 infection and associated neurological disease in a veterinary teaching hospital. *J. Vet. Intern. Med.* **24**, 1176-1183.
12. Alinovi, C.A., Ward, M.P., Couetil, L.L. and Wu, C.C. (2003) Detection of *Salmonella* organisms and assessment of a protocol for removal of contamination in horse stalls at a veterinary teaching hospital. *J. Am. Vet. Med. Assoc.* **223**, 1640-1644.