


Standard Article

J Vet Intern Med 2018;32:469–473

Quality Control of Compounded Crystalloid Fluids for Intravenous Delivery to Horses

C.B. Magnusson, K.P. Poulsen , J.A. Budde, F.A. Hartmann, N.A. Aulik, S.M. Raabis, A.S.D. Moreira, B.J. Darien, and S.F. Peek

Background: Periodic lack of availability and high cost of commercially produced isotonic fluids for intravenous (IV) use in horses have increasingly led to use of home-made or commercially compound fluids by veterinarians. Data regarding the quality control and safety of compounded fluids would be of benefit to equine veterinarians.

Objectives: To compare electrolyte concentrations, sterility, and endotoxin contamination of commercially available fluids to 2 forms of compounded isotonic crystalloid fluids intended for IV use in horses.

Methods: Prospective study. Two methods of preparing compounded crystalloids formulated to replicate commercial Plasma-Lyte A (Abbott, Chicago, IL) were compared. One formulation was prepared by a hand-mixed method involving chlorinated drinking water commonly employed by equine practitioners, and the other was prepared by means of ingredients obtained from a commercial compounding pharmacy. The variables for comparison were electrolyte concentrations, sterility, and presence of endotoxin contamination.

Results: Electrolyte concentrations were consistent within each product but different between types of fluids ($P < 0.0001$). Hand-mixed fluids had significantly more bacterial contamination compared to commercial Plasma-Lyte A ($P = 0.0014$). One of the hand-mixed fluid samples had detectable endotoxin contamination.

Conclusions and Clinical Importance: Chlorinated drinking water is not an acceptable source of water to compound isotonic fluids for IV administration. Equine practitioners should be aware of this risk and obtain the informed consent of their clients.

Key words: Electrolytes; Endotoxin; Equine; Sterility.

Intravenous (IV) fluid therapy is commonly indicated in both medical and surgical treatment of horses. Unfortunately, it carries a substantial cost (often hundreds of dollars per day), which can limit patient management options. Further complicating the high cost of IV fluid therapy for horses is variability in supply of commercially available 3- and 5-L isotonic crystalloid fluid products. The most recent instance of a supply issue was the nationwide fluid shortage in 2015, as reported by the Food and Drug Administration's Center of Veterinary Medicine.¹ The short- and long-term impacts of such shortages include restrictive product

Abbreviations:

BSC	biological safety cabinet
EU	endotoxin unit
IV	intravenous
MALDI-TOF	matrix-assisted laser desorption ionization-time of flight
PSI	pounds per square inch
USP	United States Pharmacopeia

allocations and high fluid costs that may persist for extended periods of time.

The inconsistent availability and high cost of crystalloid fluids during shortages have prompted equine veterinarians to devise alternative methods for IV fluid therapy as demand remains constant for medical and surgical veterinary care of horses. Historically, production and use of home-made or commercially available compounded IV fluids have not been considered ideal as a result of clinical experiences with hypersensitivity reactions, or “jug reactions,” in horses. Although these adverse reactions are well known among equine veterinarians, very little published information is available on the subject. One study, with limited data, suggested substantial risk for endotoxin contamination of reverse osmosis water and plastic containers used to mix and store fluids when compounded fluids are used.²

Clinical susceptibility to endotoxin in horses is widely accepted by equine practitioners and has been described in specific experimental scenarios.³ Low concentrations of endotoxin in IV fluids can cause clinical signs that include pyrexia, sweating, diarrhea, and leukopenia.^{4,5} Equine patients often receive large volumes of crystalloid fluids IV during surgery to maintain blood pressure or to replace large deficits during the medical treatment

From the Department of Medical Sciences, (Magnusson, Poulsen, Raabis, Moreira, Darien, Peek); Wisconsin Veterinary Diagnostic Laboratory, (Poulsen, Aulik); UW Veterinary Care Pharmacy, (Budde); Clinical Pathology Laboratory, School of Veterinary Medicine/University of Wisconsin-Madison, Madison, WI (Hartmann).

This study was performed at UW Veterinary Care and the Wisconsin Veterinary Diagnostic Laboratory, University of Wisconsin, Madison, Wisconsin.

This study was funded by internal funding mechanisms from UW Veterinary Care and the Wisconsin Veterinary Diagnostic Laboratory.

Corresponding author: K.P. Poulsen, Wisconsin Veterinary Diagnostic Laboratory and Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI 53706; e-mail: keith.poulsen@wvdl.wisc.edu

Submitted April 7, 2017; Revised September 1, 2017; Accepted September 27, 2017.

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DOI: 10.1111/jvim.14864

of dehydration caused by colitis or other illnesses. This highlights the importance of maintaining sterility and knowing if, and to what extent, endotoxin is likely to be a contaminant of a particular IV fluid source.⁵ Because clinicians use electrolyte concentrations in crystalloid IV fluids as starting points to correct electrolyte derangements and acid-base disorders in patients, being able to confidently rely on electrolyte concentrations in IV fluids used therapeutically is a crucial factor for patient management.

In times of shortage and when economic restrictions for patient care cause veterinarians to seek alternatives to expensive conventional commercial sources, equine practitioners increasingly seek to use compounded fluids using powdered electrolyte products hand-mixed in 20-L chlorinated drinking water containers.⁶ The commercial IV fluid shortage in 2015 prompted us to develop protocols to compound isotonic crystalloid fluids for IV use in horses at our teaching hospital with quality and sterility controls. The objective our study was to examine and compare differences in commercial IV fluids to compounded fluids using our methodology and the hand-mixed method. Our null hypothesis was that electrolyte concentration, sterility, and endotoxin contamination of the 3 different types of fluids would not be significantly different. The goal of the study was to provide large animal, especially equine, veterinarians with more information on the quality and sterility of compounded IV fluids.

Materials and Methods

Fluid Preparation

The formulation used for producing compounded fluids was developed by the UW Veterinary Care Pharmacy at the University of Wisconsin-Madison. The water source used for compounded fluids was steam distilled, followed by ultraviolet irradiation and nanofiltration^a. A 9-L (L) concentrated electrolyte stock solution was first prepared as described below in Table 1. The ingredients^b and water were mixed in a 10-L plastic carboy^c until all powder was in solution. Personal protective equipment was not utilized during the preparation of the concentrate because of the sterilization steps used. Aliquots of 300 mL of the concentrate solution were filtered with a sterile 0.2- μ m filter unit^b in a biological safety cabinet^d (BSC) located in the pharmacy clean room, which is designed and certified for compounding pharmaceuticals. To make

Table 1. Electrolyte concentrations of commercial Plasma-Lyte A, compounded fluids, and hand-mixed fluids. Concentrations of sodium, chloride, and potassium in compounded fluids were significantly different than commercial had hand-mixed fluids ($P < 0.05$). Data are expressed as mean (standard deviation).

Electrolyte	Commercial Plasma-Lyte A	Compounded Fluids	Hand-Mixed Fluids
Sodium (mmol/L)	130.9 (0.99)	151.4 (12.14)	133.6 (1.58)
Potassium (mmol/L)	4.7 (0)	5.2 (0.37)	4.8 (0.08)
Chloride (mmol/L)	94 (0)	107.6 (9.66)	96.8 (1.48)

10-L carboys of compounded IV fluids, filtered concentrate aliquots were added to an unsterilized 10-L plastic carboy, also in the clean room, containing 9.7 L of distilled water from the same source as used to make the concentrate solution. Prepared carboys were batch autoclaved^c using a heat cycle that maintained an internal temperature of the carboy fluids of 120°C at 19.0 pounds per square inch (psi) by gravity cycle for 5 hours. Individual, internal digital thermometers were used to ensure appropriate water temperature and hold time in the autoclave. Carboys then were stored, sealed, in the pharmacy for future use.

Hand-mixed fluids were prepared using powered chemical grade electrolyte packets mixed in 19 L of chlorinated drinking water, a method commonly used in equine private practice.⁶ The chlorinated water used in this study was provided by a private firm that delivers water, stored in reusable 20-L plastic jugs, intended for human consumption. The electrolytes for these fluids were a prepackaged electrolyte powder^f and included sodium chloride (99.9 g), sodium gluconate (95.4 g), sodium acetate (69.9 g), potassium chloride (7 g), and magnesium chloride (5.7 g). Contents of each electrolyte packet were added to a single jug of water that then was rolled on the ground or shaken until all powder was dissolved. Hand-mixed fluids were prepared as needed just prior to use.

Both compounded and hand-mixed fluids were formulated to contain 526 mg sodium chloride, 502 mg sodium gluconate, 368 mg sodium acetate, 37 mg potassium chloride, and 30 mg magnesium chloride per 100 mL to replicate the electrolyte concentrations of commercial Plasma-Lyte A. One-liter bags of Plasma-Lyte A were used as a control group.

Electrolyte Concentration Determination

Samples were collected from commercial ($n = 8$), compounded ($n = 8$), and hand-mixed ($n = 8$) fluids in the pharmacy clean room BSC and stored in conical test tubes at -20°C until later analysis. Concentrations of strong ions (sodium, chloride, and potassium) were measured with an automated chemistry analyzer^g at the UW Veterinary Care Clinical Pathology Laboratory at the University of Wisconsin-Madison.

Sterility Testing

Sterility of fluids was evaluated as described by the United States Pharmacopeia (USP) Section 37, Microbiological and Sterility Tests, requiring evaluation of 10% of each unit volume.⁷ This was a qualitative test only as dictated by USP. One liter of each compounded fluid carboy (10 L, total volume; $n = 8$), 2 L of each hand-mixed jug (20 L, total volume; $n = 8$), and 100 mL of commercial bag (1 L, total volume; $n = 8$) of fluids were aseptically filtered through a sterile 0.45- μ m-membrane filter with a sterile filtration apparatus under vacuum in a BSC. The whole membrane was aseptically removed and placed into thioglycolate broth enriched with vitamin K and hemin^h and incubated at 36°C in 5% CO₂ for 7 days. Broth cultures were observed daily for macroscopic evidence of microbial growth. At the first sign of microbial growth, broth cultures were subcultured to 2 trypticase soy agar plates supplemented with 5% sheep blood^h and incubated both aerobically (36°C in 5% CO₂) and in an anaerobic chamberⁱ using a mixed gas of 5% hydrogen, 5% carbon dioxide, and 90% nitrogen at 36°C. All colony types were identified to the genus and species level (when possible) by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry.^j

Endotoxin Concentration Determination

Endotoxin contamination was evaluated by the Wisconsin Veterinary Diagnostic Laboratory^k using the E-Toxate assay^l

according to the manufacturer's instructions. The included endotoxin standard stock solution was reconstituted to obtain a 4,000 endotoxin unit (EU)/mL concentration. The stock solution was then used to prepare 9 serial dilutions. Ten milliliter of aliquots from each fluid solution were collected at the time of preparation (or breaking the manufacturer seal on commercial fluids) and stored at -80°C until later batch analysis. According to manufacturer instructions, 0.1 mL of the aliquots was tested. All assays were conducted in triplicate and compared to intra-assay standard positive and negative controls according to manufacturer protocol.

Statistical Analysis

Based on pilot electrolyte concentration data, 8 samples per fluid type were required to achieve 80% power and significance of $P < 0.05$. The data were normally distributed, as determined by the D'Agostino-Pearson omnibus normality test, and parametric methods were used for one-way analysis of variance with multiple comparisons. Commercial fluids were considered as controls for multiple comparisons with Dunnett's post-test. Fischer's exact test was used to compare bacterial sterility between compounded and hand-mixed fluids to control, commercially available fluids. Commercially available statistical software^m was used to perform statistical tests. For all statistical comparisons, a P value of <0.05 was considered significant.

Results

Electrolyte concentrations and consistency in compounded and hand-mixed fluids

The electrolyte concentrations were consistent within all 3 fluid types. The electrolyte concentrations of commercial and hand-mixed fluids were not different ($P = 0.661$). Compounded fluids varied significantly from both other fluid types in sodium, potassium, and chloride concentrations ($P < 0.0001$, $P = 0.0002$, and $P = 0.0002$) for each of the 3 electrolytes, respectively (Fig 1).

Sterility of compounded and hand-mixed fluids

Microbial growth was not detected in any of the commercial fluid samples. Therefore, no difference was found in sterility between compounded and commercial fluids ($P = 1.00$), but 1 of the 8 compounded fluid samples grew *Bacillus megaterium*. Hand-mixed fluids had significantly more contamination compared to commercial fluids ($P = 0.0014$) with 7 of the 8 samples contaminated with different bacterial species including *Rhodococcus sp.*, *Delftia acidovorans*, *Neisseria sp.*, and *Cupriavidus sp.* (Table 2).

Endotoxin contamination in compounded and hand-mixed fluids

All commercial and compounded fluid samples were negative for endotoxin. Therefore, no difference was found in endotoxin contamination between hand-mixed and commercial fluids ($P = 1.00$) but 1 of the 8 hand-mixed fluids was positive for endotoxin at an estimated concentration of 0.03 EU/mL.

Table 2. Bacterial contamination of commercial Plasma-Lyte A, compounded fluids, and hand-mixed fluids.

Sample	Plasma-Lyte A	Compounded Fluids	Hand-Mixed Fluids
1	NG*	NG	<i>Rhodococcus sp</i>
2	NG	NG	NG
3	NG	NG	<i>Delftia acidovorans</i>
4	NG	NG	<i>Neisseria sp</i>
5	NG	NG	<i>Cupriavidus sp</i> <i>Non-spore-forming Aerobic gram-positive Rod</i>
6	NG	NG	<i>Cupriavidus sp</i>
7	NG	<i>Bacillus megaterium</i>	<i>Cupriavidus sp</i> <i>Delftia acidovorans</i>
8	NG	NG	<i>Cupriavidus sp</i>

Bacterial species cultured from each sample of compounded and hand-mixed fluids.

*NG is no growth.

Discussion

Our results indicate that compounded IV fluids, whether reconstituted in an approved clean room or hand-mixed, are not equivalent to commercially available IV fluids and thus we reject our null hypothesis. Differences in bacterial sterility were perhaps the most important finding of our study with respect to immediate clinical implementation of compounded products. It was reassuring to find that all commercial IV fluid samples tested were sterile. However, potential future shortages of commercially prepared IV solutions could make our observation that noncommercially prepared IV fluids, particularly the hand-mixed ones, were contaminated, troublesome. Sterility in the compounded fluids was maintained in all but 1 carboy tested, which grew *Bacillus megaterium*, a nonpathogenic, gram-positive bacteria commonly found in a variety of environments.⁸ It is unknown at what point in preparation of the fluids the contamination occurred, but we hypothesize that it was after autoclave sterilization during collection of the sample for bacterial culture. Future study is warranted to identify critical control points for avoidance of bacterial contamination when preparing compounded fluids using our protocol, or those used in other clinics.

The sterility of hand-mixed fluids was poor, and 7 of the 8 samples were contaminated with a variety of bacterial species. The only gram-positive bacterium found was a *Rhodococcus sp.*, from a single sample. The remaining 6 contaminated samples grew multiple gram-negative bacteria including *Delftia acidovorans*, *Neisseria sp.*, and *Cupriavidus sp.* The most likely source of bacterial contamination of the fluids was the water used, which is not sterile or indicated for IV use, but processed to be safe for human oral consumption. It is unlikely that the chemical grade electrolyte packets were contaminated at source because they were from a quality-controlled commercial source and are not suitable media to support bacterial survival.

The finding that the majority of hand-mixed fluids were contaminated with gram-negative organisms is important because their presence in a solution is more likely to result in higher concentrations of endotoxin.⁹ We did find EU in one of the hand-mixed fluid preparations that also grew a gram-negative bacterium (*Cupriavidus sp.*). However, the endotoxin at a concentration of 0.03 EU/mL was low and, based on limited available published reports, unlikely to cause clinical signs of endotoxemia. The threshold concentration of endotoxin to cause clinical signs of endotoxemia in horses is unknown however and likely varies considerably among individuals. The threshold established in the USP Section 85 standard for EU in IV fluids for humans and domestic animals is 5 EU/kg/h.¹⁰ Experimental models have used 300 EU/kg to cause clinical signs in the horse.¹¹ To put our study findings into perspective, the EU concentration found in 1 sample in our study would have delivered a total of 300 EU to a horse in 10 L, delivered over 5–10 hours, depending on fluid administration rate. Assessment of the clinical relevance of the single instance of 0.03 EU/mL detected in the hand-mixed fluids is difficult because of a lack of available published reports. For context, if it is assumed that a 450-kg horse receives IV fluids at 2 L/h, using the threshold dosage of 5 EU/kg/h, an IV fluid product can contain at maximum, 1.13 EU/mL before exceeding the USP section 85 standard. This is substantially higher than the 0.03 EU/mL concentration we detected in the sample.

Electrolyte concentrations in hand-mixed fluids were equivalent to those of commercial Plasma-Lyte A. However, those in compounded fluids prepared by the protocol we developed were different. We do not believe that the differences are clinically relevant, but additional studies are required to confirm this conclusion. Because of the ongoing fluid shortage at the time of our study, compounded fluids prepared by the protocol we developed were being used for patients in the large animal hospital at UW Veterinary Care. The data presented

here represents multiple batches of concentrated electrolyte stock solutions that were used to prepare the carboys. We think that the variation in electrolyte concentration of the compounded fluids was due to water loss in the autoclave sterilization process, resulting in differences among batches. Another source of variability also could have been lack of precision in volume measurement in the 10-L carboys. The initial 9.7 L of water in each carboy, to which filtered concentrate was added, had to be estimated based on the 1-L graduations on the carboy. This measurement lacked precision and may have affected the final volume, consequently slightly altering electrolyte concentration in the final product.

Based on our study, compounded and hand-mixed IV fluids have limitations for use compared to commercially prepared IV fluids and may not be perfect substitutions in times of shortage or economic limitation. We observed more frequent sterility with our method of compounding fluids compared to hand-mixed fluids, but electrolyte concentrations varied. Consistent electrolyte concentrations were obtained with hand-mixed fluids, but nearly all preparations had microbial contamination and 1 preparation had detectable endotoxin present. Therefore, use of hand-mixed preparations should be considered a risk to patient safety by veterinarians using potable drinking water as a base for IV solutions when compounding IV fluids for horses. Further study is warranted to consider shelf life of compounded fluids as an important control point for potential introduction of either bacteria or endotoxin to patients through IV fluid delivery systems because storage in conducive environments may permit proliferation of gram-negative bacteria and a more substantial endotoxin load. Awareness of the increased likelihood of bacterial and endotoxin contamination when compounded fluids are used, particularly if hand-mixed, may help practitioners with selection of certain types of cases for noncommercial fluid use. Further study is required to investigate any correlation of clinical signs and administration of these fluids IV. Higher-risk patients, such as those already

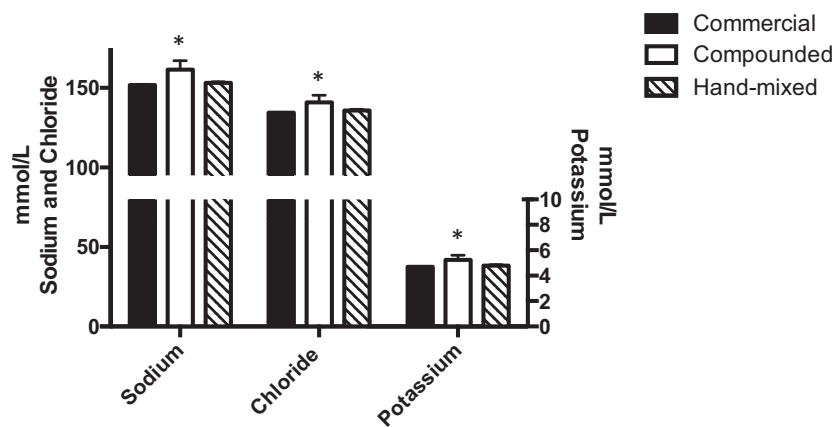


Fig 1. Electrolyte concentrations of commercial Plasma-Lyte A, compounded fluids, and hand-mixed fluids. Concentrations of sodium, chloride (left y-axis), and potassium (right y-axis) in compounded fluids were significantly different than commercial had hand-mixed fluids (* $P < 0.05$). Error bars are mean \pm SD and $n = 8$.

showing signs of endotoxemia (e.g. postoperative colic and enterocolitis patients) may be poorer candidates for such fluids by comparison with more stable patients where volume expansion is the major clinical goal (e.g. impaction colics).

Footnotes

- ^a ModuLab – Continental Water Systems, San Antonio, TX
^b Spectrum Chemical Manufacturing Corp., New Brunswick, NJ
^c Nalgene – Thermo Fisher Scientific, Waltham, MA
^d The Baker Company, Sanford, ME
^e Consolidated Sterilizer, Boston, MA
^f Hemo-Dial – Edlaw Pharmaceuticals, Farmingdale, NY
^g Vitros 5,1 FS Johnson & Johnson, New Brunswick, NJ
^h Remel, Lenexa, KS
ⁱ Bactron, Shellab, Sheldon Manufacturing, Incorporation, Cornelius, OR
^j Bruker Daltonics, Bremen, Germany
^k Madison, WI
^l Limulus Ameboyte Lysate – Sigma, St. Louis, MO
^m GraphPad Prism 6.0, La Jolla, CA
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Acknowledgments

The authors acknowledge Mr. Nicholas Keuler from the University of Wisconsin-Madison College of Agriculture and Life Sciences Statistical Counseling Service for his aid in statistical methods design.

Conflict of Interest Declaration: Authors declare no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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