Plasma transfusions have been advocated for the early treatment of trauma-induced coagulopathy in dogs,1 and > 90% of veterinary teaching hospitals and private referral hospitals have FFP on hand.2 However, because FFP must be maintained frozen from the time of its production and then thawed over 30 to 45 minutes under precisely controlled conditions (ie, at a specific temperature for a specific time) prior to administration, its immediate usefulness can be limited.3 Rather, having plasma immediately available (ie, already thawed) for emergent situations (eg, shock) is necessary and becoming commonplace in human medicine.4 However, the need for refrigeration of FFP-T and its short shelf life present challenges for maintaining adequate inventory.

ABBREVIATIONS
aPTT Activated partial thromboplastin time
AT Antithrombin
FDP Freeze-dried plasma
FFP Fresh frozen plasma
FFP-T Fresh frozen plasma—thawed
LP Liquid plasma
MA Maximum amplitude
PT Prothrombin time
R-time Reaction time

Acute traumatic coagulopathy is a trauma-induced, endogenous dysfunction of hemostasis that occurs in 25% to 33% of severely injured people and often requires immediate plasma transfusion to mitigate.5–7 Acute traumatic coagulopathy can lead to excessive bleeding, be exacerbated by crystalloid fluid administration, and be associated with a 4-fold increase in risk of death.5,7 Signs of acute traumatic coagulopathy have also been identified in severely injured dogs.8,9 Plasma transfusion reverses coagulation abnormalities in animals with experimentally induced acute traumatic coagulopathy10 and decreases the risk of death for people requiring massive transfusions of blood products.11,12

One alternative to FFP is LP. Liquid plasma is prepared from whole blood as usual but is then stored refrigerated rather than frozen. Veterinarians may desire to store plasma as LP so it can be immediately administered for hemostatic resuscitation, similar to its use by physicians. Additionally, unique to veterinarians is the treatment of small patients that may not require a full unit of plasma, such that plasma remains and could be administered to another patient, if the plasma (coagulation factors, etc) is stable. Although a study13 of human LP and FFP revealed that the hemo-

Effects of refrigerated storage on hemostatic stability of four canine plasma products

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OBJECTIVE
To assess clotting times, coagulation factor activities, sterility, and thromboelastographic parameters of liquid plasma (LP), thawed fresh frozen plasma (FFP-T), and 2 novel formulations of freeze-dried plasma (FDP) stored refrigerated over 35 days.

SAMPLE
6 units of canine LP and FFP-T from a commercial animal blood bank and 5 units each of 2 formulations of canine FDP.

PROCEDURES
Prothrombin time; activated partial thromboplastin time; activities of coagulation factors II, V, VII, VIII, IX, X, XI, and XII; and thromboelastographic parameters were determined for each product on days 0 (baseline), 3, 7, 14, 21, 28, and 35. For each day, a sample of each product was also submitted for aerobic bacterial culture.

RESULTS
Small changes in coagulation factor activities and mild increased time to initial clot formation in LP and FFP-T were noted over the 35-day storage period. Activities of factor VIII in FDP1 and factor XII in FDP2 were < 50% at baseline but varied throughout. Compared with FFP-T, time to initial clot formation was increased and clot strength was preserved or increased for the FDPs throughout the study. One FDP had decreased pH, compared with other products. No plasma product yielded bacterial growth.

CONCLUSIONS AND CLINICAL RELEVANCE
Liquid plasma and FFP-T would be reasonable to use when stored refrigerated for up to 35 days. Both FDP products showed variability in coagulation factor activities. Studies investigating the usefulness of these plasma products (FDPs) in dogs and the variable days of refrigerated storage (all products) are warranted. (Am J Vet Res 2020;81:964–972)
static profiles of LP were superior and the majority of coagulation factors and inhibitors retained much of their activities for longer than FFP-T, studies of canine plasma showed variable results. One study of the activities of coagulation factors in canine LP stored refrigerated for 14 days indicated that despite losses of their activities, coagulation test results remained within reference intervals and similar to FFP-T. In a second study, PT and aPTT results for LP after 7 days of storage at 2°C and after refreezing for 30 days at −30°C were similar and only minimally affected. A third study, however, showed that LP stored at 4°C for 48 to 72 hours had decreased activity of factors VIII, IX, and XI, leading to prolonged aPTT.

A second alternative is FDP. Freeze-dried (lyophilized) plasma is prepared by freezing pooled or individual donor plasma under a vacuum and removing the ice by sublimation. Millions of doses were successfully used during World War II, but FDPs were largely abandoned because of disease transmission until the 1990s, when technologies became available to detect and kill potential pathogens in donor plasma. These technologies enabled safe FDP production in many countries. Freeze-dried plasma may be advantageous over FFP and LP because FDP can be stored at room temperature (approx 20°C to 22°C) for up to 2 years and reconstituted with sterile water in < 5 minutes, increasing the speed that a plasma transfusion can be initiated. Many militaries have used human FDP (produced in France and Germany) in recent combat operations and have shown it to be superior to the standard of care, demonstrating the benefit of FDP in trauma resuscitation. Canine FDP, however, is not yet commercially available but has been manufactured for investigation. The hemostatic properties of canine plasma products—LP, FFP-T, and FDP—initially and over days when stored refrigerated have not yet been compared in a single study to determine their suitability for transfusion.

The objective of the study presented here was to compare hemostatic and biochemical parameters of canine LP, FFP-T, and FDP (2 formulations) when stored refrigerated for up to 35 days. Our hypothesis was that changes to the hemostatic and biochemical variables among these canine plasma products would not significantly differ.

Materials and Methods

Plasma

Six units of canine FFP and LP were purchased from a commercial canine blood bank. After collection of whole blood from a donor dog, plasma was separated from the RBCs, and then each plasma unit was divided into two 120-mL aliquots. One aliquot (FFP) was stored at −20°C, and the other (LP) was stored at 4°C, per the blood bank’s standard protocols. The products were then shipped with dry ice (FFP) and ice packs (LP) separately overnight to the US Army Institute of Surgical Research. Each LP sample was first analyzed < 24 hours after collection. The units of FFP were warmed to 37°C with a commercial plasma thawer per manufacturer’s instructions prior to analysis.

Five units each of canine FDP from 2 sources (FDP1 and FDP2) were obtained. The manufacturing processes for each differed; the process for FDP1 involved pooling several units of canine plasma before lyophilization and for FDP2 involved lyophilization of an individual dog’s plasma. The 5 units of FDP1 were from the same lot. Each unit of FDP was reconstituted per each manufacturer’s instructions. Briefly, a specified volume of sterile water was added to each bag of FDP. The bags were then gently agitated by hand until the lyophilized plasma was fully dissolved (< 5 minutes).

Procedure

Five milliliters were aseptically withdrawn from each bag of LP, FFP-T, and FDP with a needle and syringe through a sampling site coupler and placed into 2 sterile, preservative-free plastic tubes for various analyses. The first analysis was on day 0 (baseline), the day after whole blood collection from the donor dog for LP, the day the FFP was thawed, and the day the FDPs were reconstituted. Analyses were also performed on days 3, 7, 14, 21, 28, and 35 and included clotting times, coagulation factor activities, biochemical and blood gas analyses, and aerobic bacterial culture. After baseline analysis, all plasma products were stored at 4°C in a monitored and alarmed refrigerator.

Prothrombin time, aPTT, fibrinogen concentration, and activities of AT and coagulation factors II, V, VII, VIII, IX, X, XI, and XII were determined with an automated commercial coagulation analyzer and associated reagents, as previously reported. Because the coagulation analyzer was calibrated for use with people, AT and coagulation factor activities in FFP-T at baseline were set at 100% and AT and factor activities in LP and FDPs were reported as percentage activity of FFP-T. Factors with activities > 50% were considered acceptable on the basis of a previous study of canine refrigerated plasma (LP). Thromboelastography was conducted in duplicate with kaolin activator, and R-time (time to initial clot formation), α angle (measure of the rate of clot formation), and MA (related to maximum clot strength) were the parameters of interest. Biochemical analyses were performed with a handheld point-of-care analyzer. Blood gas analyses for LP, FFP-T, and FDP2 were performed with the same analyzer, but blood gas analysis for FDP1 was performed with a benchtop analyzer because pH values were below the limit of detection for the handheld analyzer. For aerobic bacterial culture of each sample, blood and MacConkey agar plates were inoculated and incubated at 37°C. Trained laboratory personnel examined the plates for bacterial growth at 24 and 48 hours after inoculation.
Statistical analysis

All data were analyzed with a commercial statistical software package. Data determined to be normally distributed with a Shapiro-Wilk test were reported as mean ± SD. Repeated-measures ANOVA was used to determine intra- and intergroup differences for LP and FFP-T, with a Sidak test for multiple comparisons among selected pairs. The Dunnett multiple comparisons test was used to compare FDP1 and FDP2 with FFPT, with repeated-measures analysis performed over storage duration. Values of \( P < 0.05 \) were considered significant.

Results

LP versus donor-matched FFP-T

Within groups for LP and FFP-T, PT significantly increased from baseline (LP: mean ± SD, 8.94 ± 0.42 seconds; FFP-T: 8.71 ± 0.53 seconds) to day 35 (LP: 9.25 ± 0.53 seconds; FFP-T: 9.01 ± 0.52 seconds), but the differences were small, and results were within the reference interval (5.9 to 9.3 seconds; Figure 1). Likewise, significant but small differences in PT were noted between LP and FFP-T on days 14, 28, and 35. Within groups, aPTT for LP was significantly increased between baseline (mean ± SD, 16.12 ± 0.84 seconds) and day 35 (18.26 ± 1.57 seconds), but not for FFP-T (baseline, 16.33 ± 0.97 seconds; day 35, 15.57 ± 1.13 seconds). Activated partial thromboplastin time was only significantly different between LP and FFP-T on day 35, but aPTT, as for PT, was within the reference interval (9.9 to 20.4 seconds) for both plasma types on all days. Fibrinogen concentrations were significantly less by day 14 for LP and day 21 for FFP-T, compared with baseline, but decreases were small, and concentrations did not significantly differ between LP and FFP-T. Fibrinogen concentrations could not be determined on day 7 for LP because assay reagents were not available.

Compared with the factor activities observed for FFPT at baseline (considered 100% activity for each factor), factor II activity for LP and FFP-T was ≥ 95%. Activities of AT and factors V, VII, IX, and X were > 50% throughout the study, despite significant intra- and
intergroup differences on some days. No significant intra- and intergroup differences in factor VIII activity were noted. Factor XI and factor XII activities decreased over the study period, but only factor XI activity in FFP-T on day 7 was < 50% (mean ± SD, 49.90 ± 11.38%).

Within each group, thromboelastography revealed a significant increase in R-time between baseline and day 7 (LP: mean ± SD, 5.21 ± 0.86 minutes vs 5.86 ± 1.04 minutes; FFP-T: 4.12 ± 0.57 minutes vs 5.71 ± 0.70 minutes; Figure 2). Alpha angle did not significantly change over the study period. Maximum amplitude for FFP-T was significantly increased from baseline (mean ± SD, 14.41 ± 5.48 mm) on days 14 (15.39 ± 5.31 mm), 28 (15.74 ± 5.35 mm), and 35 (16.67 ± 5.18 mm). No significant differences in R-time, α angle, and MA were noted between LP and FFP-T.

Sodium, potassium, chloride, glucose, BUN, and creatinine concentrations infrequently differed within each group and did not significantly differ between groups (Supplementary Table S1, available at: avmajournals.avma.org/doi/suppl/10.2460/ajvr.81.12.964). However, pH within each group significantly increased, compared with baseline, beginning on day 14 (LP: mean ± SD, 7.43 ± 0.05 [day 14] vs 7.37 ± 0.05 [baseline]; FFP-T: 7.50 ± 0.04 vs 7.35 ± 0.04); also, the pH of FFP-T was significantly greater than the pH of LP on day 14 (Table 1). Correspondingly, base excess also significantly increased beginning on day 14 for FFP-T and day 21 for LP; however, lactate concentrations did not significantly differ within groups throughout the study. Partial pressure of CO₂ for LP and FFP-T was significantly decreased on days 21 and 14, respectively, compared with baseline. Partial pressure of CO₂ for LP was significantly greater than for FFP-T on day 14.

**FFP-T versus FDPs**

Compared with PT for FFP-T, PT for FDP1 was significantly increased and exceeded the upper limit of the reference interval on all days, including baseline (mean ± SD, 8.71 ± 0.53 seconds vs 10.95 ± 0.15 seconds; Figure 3). Prothrombin time for FDP2 was increased, compared with FFP-T, beginning on day 3 (10.13 ± 0.42 seconds vs 8.95 ± 0.52 seconds) and also exceeded the upper limit of the reference interval. Significant prolongation of aPTT was noted for FDP1, compared with FFP-T, on all days except for day 7 and exceeded the upper limit of the reference interval on days 3 and 14, while prolongation of aPTT for FDP2 was only significant and exceeded the reference interval on days 21 and 35. Fibrinogen concentrations in FDP1 and FDP2 were significantly decreased beginning on days 21 and 3, respectively, compared with baseline.

Antithrombin activity was significantly less in FDP2 than in FFP-T for all days except days 7 and 35 (Figure 3). Antithrombin activity was significantly less in FDP1 on day 14 (85.52 ± 0.35%) versus baseline (99.11 ± 6.49%) and versus FFP-T (108.08 ± 3.51%). However, AT activities were ≥ 64% for all plasma types on all days. Factor II activity was lower in both FDPs, compared with FFP-T, on all days, although activity was > 50%. A significant loss of factor V activity was observed for FDP1 (54% decrease) and FDP2 (35% decrease) between baseline and day 35. Both FDPs had activities of factors V, VIII, XI, and XII that were < 50% prior to day 35.

Thromboelastography revealed higher R-time for FDP1 and FDP2, compared with FFP-T, on all days (Figure 4). The difference was greatest for FDP1 between baseline and day 35 (maximum increase, 7.41 seconds), and that difference was greater than the largest difference for FFP-T (2.83 seconds), which was between baseline and day 28. Alpha angle for FDP1 was significantly less than for FFP-T on all days except for day 7. Maximum amplitude within groups FFP-T and FDP1 significantly differed on several days from respective baseline MA but did not differ between groups.

Sodium, potassium, chloride, glucose, BUN, and creatinine concentrations frequently did not significantly differ within groups throughout the study, but significant yet small differences were observed between FDP1
The objective of the study presented here was to compare select hemostatic and biochemical variables of canine LP, FFP-T, and FDP when stored refrigerated for up to 35 days. Fresh frozen plasma (thawed) was chosen as the comparative plasma type because it is the most common canine plasma product available for transfusions. On the basis of the results, we suggest that LP and FFP-T would be reasonable to use when stored refrigerated (4°C) for up to 35 days.

Refrigerated storage of LP and FFP-T (after initial thaw) for up to 35 days is recommended because PT and aPTT were maintained within their reference intervals, activities of coagulation factors (except factor XI on day 7 for FFP-T) exceeded the desired minimum activity of 50%, and thromboelastography results were minimally affected. Small, significant differences were noted for PT and aPTT plus fibrinogen concentration within and between LP and FFP-T throughout the study, but those differences were unlikely to be clinically important. A previous study of human LP and FFP-T revealed that the hemostatic potential of LP was significantly better than FFP-T, mainly because of higher procoagulant platelet microparticles in LP. Because PT and aPTT for LP (vs FFP-T) were significantly increased throughout the study period, residual platelet microparticles in canine LP may have been less numerous than in human LP.

Furthermore, small differences in coagulation factor activities within and between LP and FFP-T were noted throughout the study, but activities were frequently >70%, and only factor XI activity in FFP-T was slightly <50% on day 7. However, because factor XI activity was >50% on day 14 and thereafter, the day 7 measurements may have been anomalous. Thromboelastography for LP and FFP-T revealed a significant increase of R-time, no significant increase of α angle, and a significant increase in MA for FFP-T throughout the study period, residual platelet microparticles in canine LP may have been less numerous than in human LP.
refrigerated plasma samples in the previous study\textsuperscript{11} were shipped frozen to a reference laboratory and then stored frozen for later batch analysis. Therefore, before analysis, LP was frozen and thawed. Similarly, FFP samples were thawed on 1 day and then refrozen for shipment and storage until batch analysis (ie, 2 freeze-thaw cycles). In the present study, plasma samples were analyzed at an on-site laboratory and assayed on the day of sampling rather than batch analyzed. However, Yaxley et al\textsuperscript{22} reported that a single freeze-thaw cycle of FFP had no significant impact on coagulation factor activities (albeit without examining the impact of long-term storage after a freeze-thaw cycle).

The recommended length of time for refrigerated storage of FDPs is unclear. Acknowledging that numerous variables were measured in this study and that their relative importance varies, we propose FDP1 and FDP2 would be suitable substitutes for FFP on the day they are reconstituted through 14 days when stored refrigerated. Although this proposal is subjective, it is based on the following: PT and aPTT were within their reference intervals or only minimally increased on day 14; activities of coagulation factors V (FDP1), VIII (FDP2), XI (FDP2), and XII (FDP2) did not meet the desired minimum activity of 50% on day 21; and R-time for FDP1 was significantly increased from baseline on day 21, whereas MA was at least maintained throughout the study. Because no aerobic bacterial growth was noted for any plasma type, culture results did not factor in the above recommendations.

Prothrombin time for FDP1 exceeded the upper limit of the reference interval on all days and was 143% of the upper limit of the reference interval on day 35 and for FDP2 exceeded the reference interval on days 3, 21, and 35 and was 138% of the upper limit of the reference interval on day 35. Activated partial thromboplastin time was within the reference interval at baseline for FDP1 and FDP2 and thereafter was more variable than PT. Coincidentally, aPTT for both FDPs also exceeded, albeit minimally, the upper limit of the reference interval on several days, with 103% and 97% on day 14 and 96% and 103% on day 21 for FDP1 and FDP2, respectively. The reason for the disparity in PT and aPTT was unclear. Prothrombin time evaluates the extrinsic and common coagulation pathways, and aPTT evaluates the

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure3.png}
\caption{Mean ± SEM coagulation parameters for FFP-T (light gray bars), FDP1 (dark gray bars), and FDP2 (black bars), with FFP-T harvested from the dogs in Figure 1. †Within a day, value significantly (\(P < 0.05\)) differs from that for FFP-T. See Figure 1 for remainder of key.}
\end{figure}
intrinsic and common pathways. Yet factor VII activity was > 50% for both FDPs throughout the study period. Because neither PT nor aPTT exceeded 150% of the upper limit of the reference interval, the clinical significance of these findings is likely minimal. Lyophilization is a biochemically gentle process but has been shown to alter PT and aPTT in some situations. Therefore, lyophilization plus the specific formulations of these FDPs may have affected PT and aPTT in the present study. Fibrinogen concentrations only mildly decreased over time for the FDPs.

At baseline, activities of factors II, V, VIII, and XI in FDP1 and factors V and XII in FDP2 were significantly lower than FFP-T. Importantly, however, only activities of factor VIII in FDP1 and factor XII in FDP2 were < 50% (FDP1, 45%; FDP2, 49%). Lower factor VIII activity in FDP1 at baseline was similar to the activity noted on day 3, but then activity was > 50% on days 7, 14, and 21, likely because of increased sample variance on those days, with 2 samples that had a 2- to 3-fold increase versus the prior testing day. One study showed that factor VIII activity increased over 4 months in canine plasma that was stored frozen, a phenomenon that could have occurred more rapidly in refrigerated, previously frozen plasma and FDPs. The clinical importance of low factor VIII activity seen with FDP1 is unknown, but factor VIII activity in FDPs may be important to consider when treating small animals with factor VIII deficiencies (eg, hemophilia A). The clinical importance of low factor XII activity in FDP2 is unknown, but factor XII deficiencies do not lead to bleeding diatheses in people.

Thromboelastography revealed significantly increased R-time for FDP1 and FDP2, compared with FFP-T, throughout the study. Furthermore, R-time increased beginning on day 21 for FDP1 and day 14 for FDP2 (significantly increased except for day 21). Alpha angle between FFP-T and FDP2 did not significantly differ throughout the study, but the difference in alpha angle between FFP-T and FDP1 on all days, except for day 7, indicated decreased rate of clot formation in FDP1, which may have been related to its low pH. A small but significant increase in MA for FDP1 was noted at baseline and days 3, 7, and 35, and MA for FDP2 was not significantly different on all days. Overall, the FDPs (vs FFP-T) took more time to form an initial clot, but the clots formed by factors in these FDPs were as strong as those formed by factors in FFP-T.

An important finding from the blood gas analysis was the markedly low pH of FDP1 throughout the study. Concurrently, bicarbonate concentrations were decreased and lactate concentrations were increased. The pH of FDP1 was not compared with the pH of FFP-T because pH was determined with different analyzers; yet pH of FDP1 was below the limit of detection for the handheld analyzer, which then necessitated the use of the benchtop analyzer. Therefore, the difference is suspected to be relevant. An acidic environment can reduce activities of tenase (factor X) and prothrombinase (factor II) complexes by 50% to 70%. Therefore, the low pH of FDP1 may have been responsible for the lower than expected factor activity on some days, compared with FFP-T. Because of these pH results, the manufacturer of FDP1 adjusted its manufacturing process, such that the reconstituted product is now expected to have a pH of 7.2. Factor activities are expected to increase at this nearly physiologic pH. An increase in pH with a corresponding decrease in Pco2, best observed with LP, FFP-T, and FDP2, was predictable. This is most likely because of diffusion of CO2 from the plasma and a subsequent increase in pH, a phenomenon that has been previously reported.

If canine FDPs that were to be used immediately (not stored) were subject to the same regulatory requirements as human plasma, factor VIII activity in FDP1 would preclude FDP1 from use in Australia and the United Kingdom, which both require factor VIII activity of > 70%, and in Canada, which requires factor VIII activity of > 52%. We are unaware of any similar guidelines for acceptable factor XII activity in the United States. In the United States, no
specific factor activity is required for LP or thawed plasma (FFP-T that is then refrigerated for > 24 hours and used within 4 days). The American Association of Blood Banks notes that factor activity in human FDPs is variable and decreases over time; therefore, they are indicated for immediate use after reconstitution for emergent bleeding patients or as part of the initial treatment of patients undergoing massive transfusion. However, because small dogs may not require the entire reconstituted unit of FDP and any remaining plasma could theoretically be administered to another dog, hemostatic properties of refrigerated, reconstituted FDPs were evaluated.

Despite the aforementioned country-specific guidelines for factor activity in human plasma, a factor activity threshold of 30% is often cited as sufficient to prevent spontaneous bleeding and minimize surgical bleeding. If < 30% factor activity translated into decreased efficacy for the correction of bleeding abnormalities, then refrigerated FDP1 should not be used > 28 days after reconstitution because of loss of factor VIII activity and refrigerated FDP2 should not be used > 21 days after reconstitution because of loss of factor XI and factor XII activities. In contrast, refrigerated FFP-T could be used until 35 days after thawing.

There were several limitations in the present study. The number of each plasma type was small (6 units each of LP and FFP-T; 5 units each of FDP1 and FDP2); therefore, results may not be representative. Platelet count was not determined; however, residual platelets and platelet microparticles may have played a role in the hemostatic properties of LP. The samples of FDP1 were from the same batch, so its hemostatic parameters were only minimally variable; therefore, type II error is possible. Because the pH of FDP1 was low, a benchtop analyzer was used, and results, including lactate concentrations, generated with this analyzer could not be compared with those for FFP-T obtained with a handheld analyzer. Because the formulation of FDP1 has changed (pH, 7.2) per the manufacturer, results for this new formulation may be different.

On the basis of the results, LP and FFP-T stored refrigerated retained sufficient hemostatic capacity for up to 35 days, and reconstituted FDP1 and FDP2 are proposed to retain sufficient hemostatic capacity for up to 14 days in refrigerated storage, although factor activities were more variable than for LP and FFP-T. The present study yielded promising findings regarding the long-term refrigerated storage of LP, FFP-T, and FDPs.

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Footnotes

b. DH8, Helmer Scientific, Noblesville, Ind.
c. StablePlas, Bodevet, Rockville, Md.
d. Mantel Technologies, Fort Collins, Colo.
e. Fenwall Inc, Lake Zurich, Ill.
f. GDM-49, True Manufacturing Co, O’Fallon, Mo.
g. STA R Max, Stago Inc, Parsippany, NJ.
h. TEG 5000, Haemonetics Corp, Braintree, Mass.
i. Chem 8, Abbott, Princeton, NJ.
j. iSTAT, Abbott, Princeton, NJ.
k. CG4, Abbott, Princeton, NJ.
l. Rapidpoint 500, Siemens Medical Solutions USA Inc, Malvern, Pa.
m. Prism 8, GraphPad Software, San Diego, Calif.

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