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**METHODS TO OBTAIN BLOOD SAMPLES PERIODICALLY DURING
EXERCISE RESEARCH STUDIES WHILE SUBJECTS ARE IMMersed
IN WATER OR OTHERWISE INACCESSIBLE**

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The experiments reported herein were conducted according to the principles set forth in the current edition of the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This technical report has been reviewed by the NMRI scientific and public affairs staff and is approved for publication. It is releasable to the National Technical Information Service where it will be available to the general public, including foreign nations.

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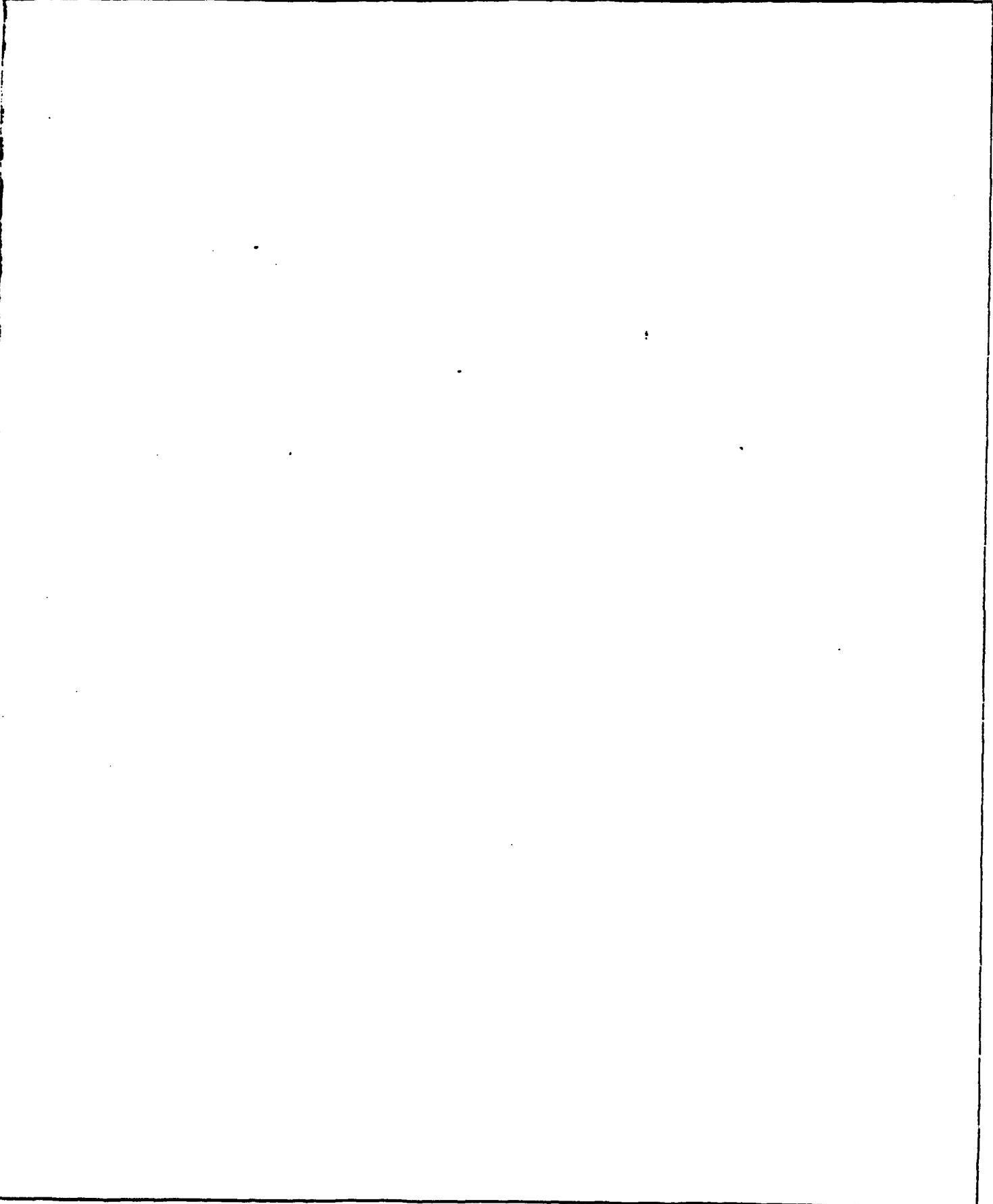


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The techniques described were used only during the conduct of research that was approved by the Committee for the Protection of Human Subjects of the Naval Medical Research Institute.

BACKGROUND

Evaluating metabolic changes during exercise studies requires a method to obtain blood samples periodically without interrupting the subject's work. In some studies, the task is more difficult because the subject is relatively inaccessible (e.g., immersed in water, exercising on a treadmill or other exercise equipment, or wearing protective clothing). We describe a method to obtain periodic blood samples during exercise studies by use of an indwelling intravenous catheter. We have used this method successfully in several studies with subjects performing exercise while immersed in water.

METHODS

The components of the system are illustrated schematically in Figure 1. An 18-gauge intravenous catheter is connected by variable lengths of extension tubing to a three-way stopcock. The stopcock allows access to obtain samples with a syringe and to allow infusion of a heparinized solution. The heparinized solution replaces the volume of fluid withdrawn and prevents clotting.

The following is the step-by-step process for establishing the intravenous line and obtaining samples. Steps we have found especially useful to facilitate the process and to avoid complications are presented in more detail. Appendix A is a list of supplies used in the process.

A. Prepare Equipment

1. Prepare solution: normal saline containing heparin, one unit per ml of solution
 - a. Into a container of normal saline, add heparin (supplied as 1000 units

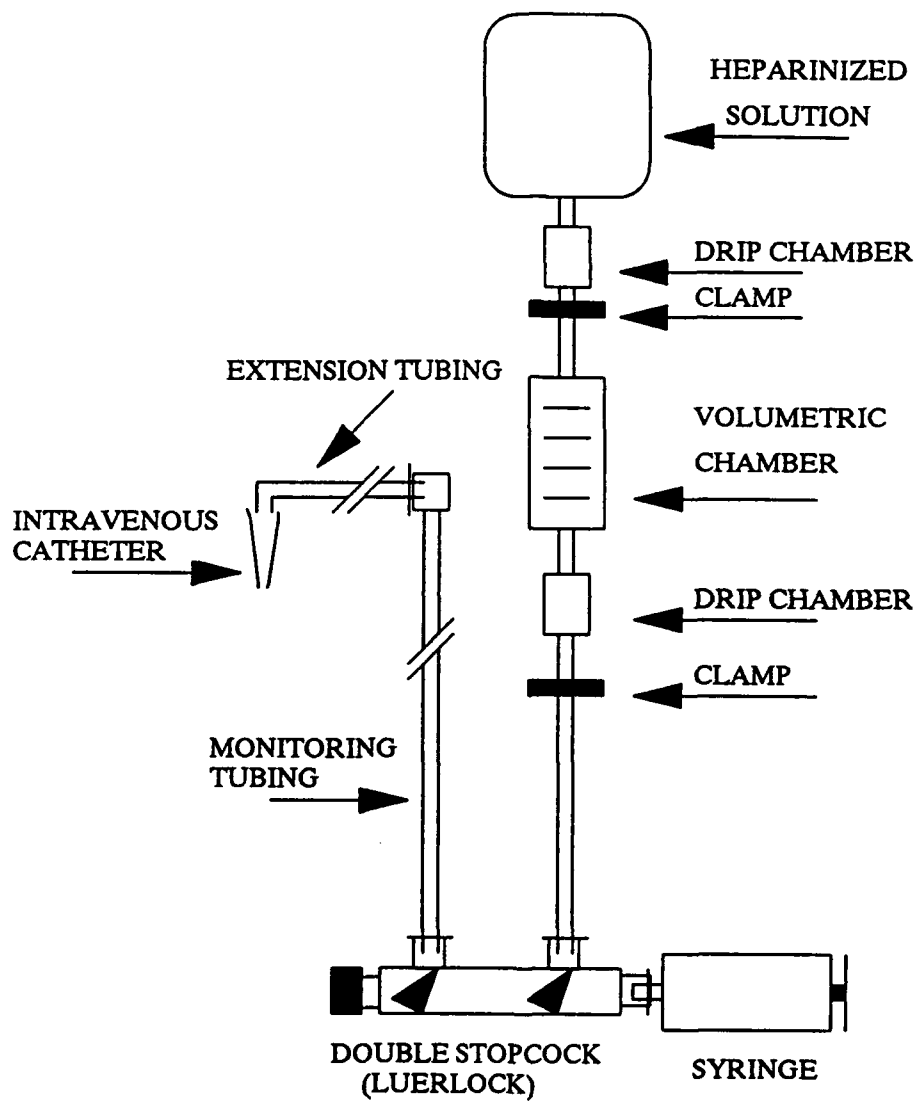


FIGURE 1. Schematic of equipment assembly for indwelling intravenous catheter to obtain blood samples.

per ml); draw-up aliquot with 1-ml tuberculin syringe

b. Label solution with date and strength of solution

2. Connect volume-control tubing apparatus to container of solution
3. Attach supply to proximal side-arm of double stopcock with luer-lock fittings
4. Attach extension tubing with luer-lock connections to distal end or sidearm of stopcock. Length required will depend upon conditions of study
5. Fill measuring chambers and lines with solution and remove air bubbles
6. Have tubing available to connect to intravenous catheter

B. Establish Intravenous Line

Insertion into the antecubital vein is preferred. If subject will be wearing a shirt during exercise, it should not be worn until after intravenous line is established. If a shirt is worn, the rolled-up sleeve occasionally acts as a tourniquet to hamper the process of inserting the intravenous catheter.

1. Insert 18-gauge plastic catheter
 - a. Shave hair
 - b. Prep with alcohol or iodine
 - c. Use local anesthetic with 2% Lidocaine Hcl (no epinephrine) and 18-gauge sterile needle to incise skin for insertion of catheter for maximum comfort of subjects
 - d. Insert intravenous catheter
2. *Connect tubing*
3. Secure locally with adhesive tape applied sticky-side up and looped across

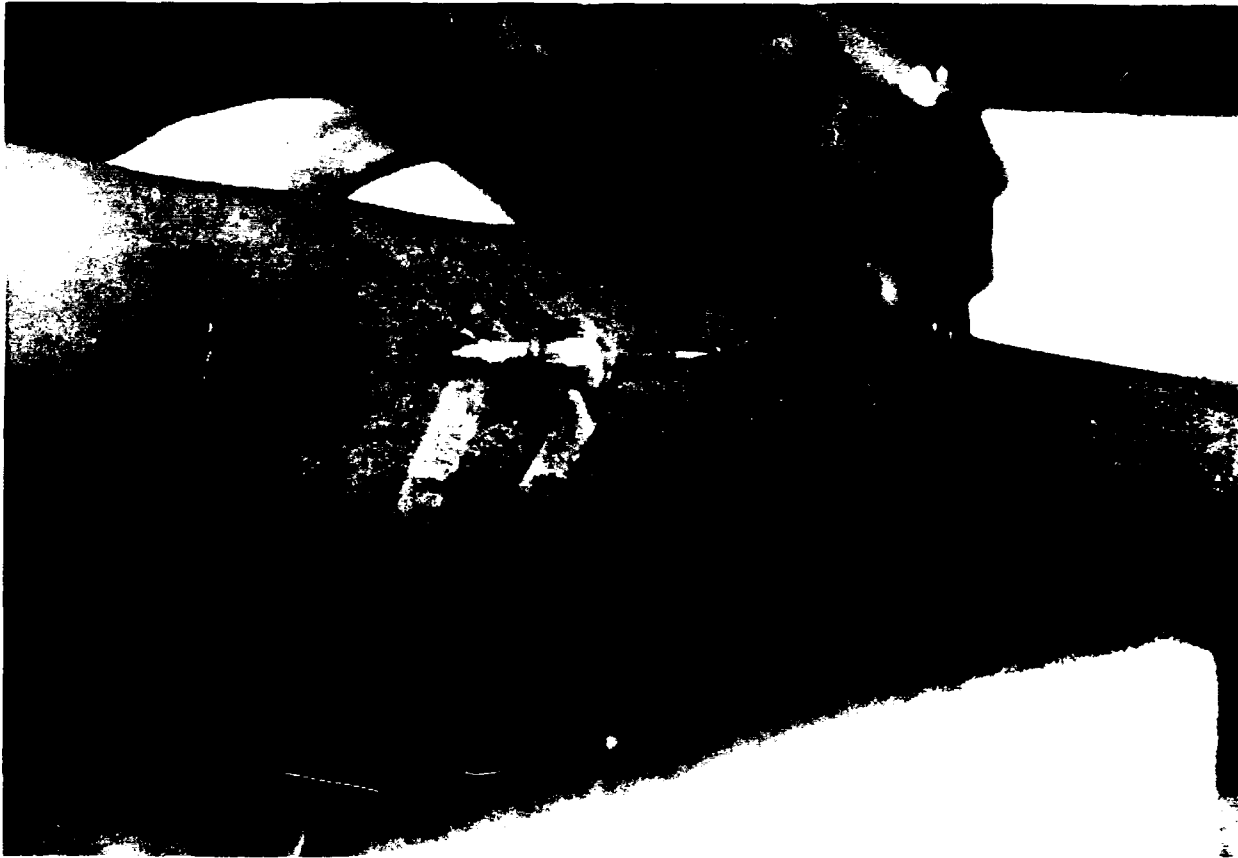


FIGURE 2. Extension tubing attached to intravenous catheter, and both taped to arm.



FIGURE 3. Elastic wrap around extension tubing and catheter and loop secured in extension tubing to prevent accidental dislodgement.

catheter and tubing; use one piece for catheter and second piece for end of tubing where it connects to catheter (Figure 2).

4. Apply collodion at break in skin.
5. Place tape flat over catheter but not encircling the limb
6. Wrap with ace bandage snugly but not excessively tight around the tubing and catheter. After one layer of wrapping, create loop of tubing and continue wrapping such that pulling on tubing will NOT be in a direction to cause tubing to disconnect from catheter or catheter to be pulled out of vein (Figure 3).
7. If subject is wearing a shirt, bring solution and tubing assembly through sleeve. Apparatus could exit through neck opening, but this adds additional loops, which increases the chance of occlusion and increases the length of extension tubing needed.

C. Obtain Blood Samples

1. Clear line of infusion solution
 - a. Fill volumetric chamber with amount of solution that will be infused after sampling (amount of sample plus 2 ml)
 - b. If necessary, apply tourniquet to arm (blood pressure cuff works well)
 - c. Attach 10-ml syringe to stopcock assembly
 - d. Turn stopcocks so that:
 - 1) Proximal stopcock is open line-to-syringe and closed to bottle of solution
 - 2) Distal stopcock is open to line

- e. Withdraw with syringe until blood enters syringe (there is clear demarcation of blood-solution interface); withdraw until about 2 ml of blood has been drawn into syringe; line is now considered "clear"
 - f. Turn proximal stopcock to a **diagonally** closed position to prevent loss of blood and to prevent any solution from entering stopcock from bottle. (When the stopcock is closed **diagonally**, there is no flow in any direction. No solution can get into the holes of the stopcock to cause dilution of samples that will be obtained later.)
 - g. Remove syringe
2. Obtain blood sample(s)
- a. Attach syringe to be used for sampling (prefer to use syringes that can be used for centrifuging without need to transfer to another tube)
 - b. Turn proximal stopcock open to line-to-syringe and closed to bottle of solution, and withdraw sample.
 - c. Turn proximal stopcock to a **diagonally** closed position to prevent loss of blood and to prevent any solution from entering stopcock from bottle; remove sample.
 - f. Repeat steps a. through c. as often as needed to obtain the various samples needed
3. Clear line to prevent clotting
- a. When last sample has been withdrawn,

1) LOOSEN OR REMOVE TOURNIQUET

- 2) turn proximal stopcock so that solution flows into line and vein.
- b. When replacement volume of solution has been infused, turn stopcocks to a diagonal position so that no additional infusion will occur and blood will not return into line. **INFUSION MUST BE WATCHED TO INSURE THAT, AS SOON AS INFUSION IS COMPLETE, LINE IS OCCLUDED (STOPCOCKS IN DIAGONAL POSITION) SO THAT BLOOD DOES NOT RETURN INTO LINE AND AIR FROM DRIP CHAMBERS CANNOT ENTER THE LINE.**

DISCUSSION

With these methods, we have been able to obtain samples from subjects performing heavy exercise on cycle ergometers and from other subjects who were immersed for up to 3 h in water at 18 °C and working on an underwater ergometer. Hemorrhage, infection or other complications did not occur. The only failure was an occasional inability to obtain a blood sample, due to inadequate blood flow. This problem was rare when a large antecubital vein was used. The items we consider most important to the process described in this paper are discussed below.

1. Heparin

We decided to use heparinized saline to avoid clotting while the line is not being used. We did not evaluate whether non-heparinized saline (or other solution) would work as well. We have had no problems with hemorrhage nor failure of the line due to clotting. In addition, we have been able to obtain serum samples because the blood

sample does clot when drawn into syringes without anticoagulant. It may be necessary to use a different anticoagulant or no anticoagulant if heparin might be expected to interfere with biochemical analyses, e.g., free fatty acids.

2. Tubing with volume control

This method is used to ensure that the amount of solution infused after samples are withdrawn is carefully measured to equal only the amount needed for replacement. We decided that it would be inappropriate to use syringes or pumps to infuse the solution because the intravenous catheter is not visible during the research study. It would therefore be possible to create infiltration or other problems more easily if the infusion solution were returned under pressure greater than provided by gravity flow. If the solution does not infuse by gravity, we either stop using the catheter or, if possible, we unwrap all occlusive dressings so that we can observe whether the catheter is intact and functional.

3. Luer-lock connections

In most of our studies, the connections are not easily seen. Luer-lock connections are used to minimize the possibility of complications from being unable to see that tubing has become dislodged or disconnected.

4. Antecubital vein

Many forearm veins tend to collapse while the subject is immersed in cold water. We have been most successful in obtaining samples by using the larger antecubital vein rather than other forearm veins. When drawing samples, it may be necessary to have the subject extend the forearm to adequately straighten the tubing so that samples can be

obtained.

5. 18-gauge catheter

This size catheter is about the smallest that can be used and still obtain adequate blood flow to withdraw samples. Larger catheters can be used, but there is more discomfort to the subject and greater risk that scar tissue will develop at the catheter site.

6. Collodion

Most of our studies are with immersed subjects. The collodion is used as added protection against foreign matter entering the skin around the intravenous catheter. We did not evaluate whether the collodion is necessary, but we have had no incidence of infection or other complications related to its use.

APPENDIX A

SUPPLIES USED FOR INTRAVENOUS CATHETERIZATION AND BLOOD SAMPLING

1. 0.9% Sodium Chloride Injection USP, single dose container, 500 or 250 ml:
Generic
2. Heparin 1000 units per ml: Generic
3. Tuberculin syringe, 1.0 cc: Generic
4. 72-inch, 100 ml Volu-Trole I.V. Set: Cutter Laboratories Biological Inc.,
Berkeley, CA 94710
5. Double stopcock with male Luer Lock: Cobe Corporation, Lakewood, CO
80215
6. Disposable Pressure Monitoring Lines: Medi-Trace Corporation, Buffalo, NY
7. Sterile Extension Tubing, 30-inch: Churchill Corp., Horcham, PA 19044
8. 18 GA, 2-INCH Angiocath: The Deseret Medical Company (Parke Davis)
Sandy, Utah 84070
9. Safety razor, disposable: Generic
10. Alcohol or iodine prep pads: Generic
11. Lidocaine HCl, 2%: Generic
12. Collodion site prep: Astra Pharmaceutical Inc., Westborough, MA 01581
13. Tape, waterproof, 1/2-inch and 2-inch sizes: Generic
14. Elastic wrap, 2-inch: Generic
15. 10-cc disposable hypodermic syringe: Generic

16. Safety-Monovette Blood Collection Tubes/Syringe varying by volume and type
of anticoagulant or other reagent: Sarstedt Inc., P.O. Box 4090, Princeton, NJ
08540