J of Nuclear Medicine Technology, first published online September 15, 2016 as doi:10.2967/jnmt.116.177535

The Necessity of Using Heparin in an UltraTag RBC Kit when Tagging Blood for a Nuclear Medicine Study

Sarah R. Pigmon[1], Kara D. Weatherman[2], Nicholas C. Brehl[1], Cybil J. Nielsen[1]

Author Note:

Sarah R. Pigmon, Department of Radiologic and Imaging Sciences, Indiana University School of Medicine. Correspondence concerning this article should be addressed to Sarah R. Pigmon, 541 Clinical Drive Indianapolis, IN 46202-5111. Contact: pigmons@umail.iu.edu and/or (812) 212-9745.

1. Indiana University School of Medicine
2. Purdue University College of Pharmacy

Financial Support

Supplies donated by Mallinckrodt Pharmaceuticals, Radiopharmacy of Indianapolis, Indiana University School of Medicine- Department of Radiology and Imaging Sciences and Department of Pathology and Laboratory Medicine, Purdue University College of Pharmacy, and Riley Hospital for Children at Indiana University Health.

Word Count = 3.119

Abstract

The purpose of this research is to evaluate the necessity of using heparin in UltraTag RBC kits used for nuclear medicine studies. **Methods:** Non-heparinized blood (n=15) and heparinized blood (n=15) were added into UltraTag RBC kits. The samples were evaluated for macroscopic blood clots and microscopic platelet clumping. Control groups with heparin (n=15) and control groups without heparin (n=15) were used to help evaluate the effectiveness of the anticoagulant properties within the UltraTag RBC kits (sodium citrate) and to evaluate if that played a role in preventing blood clots/clumps. To detect macroscopic clotting the wooden applicator stick method was used. To detect microscopic platelet clumping blood smears were evaluated using a light microscope. The number of macroscopic clots and microscopic platelet clumping were compared between the two individual samples. Fisher's Exact Test was used to evaluate the significance of the data. **Results:** Macroscopically for the UltraTag RBC kits, two of fifteen UltraTag RBC Non-heparinized vials clotted and zero of fifteen UltraTag RBC Heparin vials clotted. Macroscopically for the control group two of fifteen Control Non-heparinized tubes clotted and zero of fifteen Control Heparin tubes clotted. Microscopically for the Ultra-Tag RBC kits three of the fifteen UltraTag RBC Non-heparinized vials clumped and three of the fifteen UltraTag RBC Heparin vials clumped. Microscopically for the control group fifteen of fifteen Control Non-heparinized tubes clumped and ten of the fifteen Control Heparin tubes clumped. **Conclusion:** When heparin isn't used, Ultra-Tag RBC kits are more likely to form macroscopic clots. Heparin should always be used when making an Ultra-Tag RBC kit for nuclear medicine studies.

Keywords: UltraTag, macroscopic clots, microscopic platelet clumping, heparin

Introduction

In nuclear medicine some studies require blood to be withdrawn from the patient, radiolabeled via an UltraTag Red Blood Cell (RBC) kit and then injected back into the patient for imaging. Per package insert instructions, an anticoagulant such as heparin or Acid Citrate Dextrose (ACD) is added to the syringe prior to withdrawing the blood from the patient to prevent blood clots from forming during the radiolabeling process. However, it has been noted that in some institutions, technologists are preparing the radiolabeled kit without the use of anticoagulants when drawing the patient blood samples. Rationale for this deviation from package insert instruction includes enhanced documentation and other institutional specific issues required in order to acquire and use anticoagulants, recurrent difficulties with heparin drug shortages, recognition of the presence of components with anticoagulant properties in the UltraTag RBC kit *(1)* and the fairly rapid readministration of the radiolabeled product. This research was done to evaluate the necessity of using an anticoagulant such as heparin when radiolabeling blood using UltraTag RBC kits to prevent the formation of clots during the labeling process.

The recommended amount of heparin is 10-15 units per mL of blood (*1*). When tagging blood heparin is an anticoagulant that reduces the chances of blood clotting (*2*). The body also has its own fibrinolytic system responsible for lysing clots. This fibrinolytic system was discovered in the beginning of the 20th century by Niewiarowski, who showed that the end products of fibrinolysis inhibit the process of coagulation (*3*). The UltraTag RBC kit also contains sodium citrate. Sodium citrate is another common anticoagulant used in vitro (*4*). Despite the presence of sodium citrate in the kit and the body's own fibrinolytic system, the

package insert states that the syringe used to draw the blood must contain heparin before radiolabeling the blood in the UltraTag RBC kit.

Common nuclear medicine studies that use UltraTag™ RBC kits are Multi Gated Acquisition studies and Gastrointestinal Bleed studies. An indication for a Multi Gated Acquisition study is to evaluate left ventricular function at baseline before chemotherapy (*5*). An indication for a Gastrointestinal Bleed study is to detect the presence and site of an acute Gastrointestinal bleed (*5*). While it is important to prevent blood clots for the health of the patient, it is also important for the accuracy of the study. If the radiolabeled blood containing a blood clot was injected into the patient the blood clot could potentially get caught in a vein blocking the rest of the radiolabeled blood from getting to the appropriate area.

In this study we evaluated clots in two ways: macroscopic clots and microscopic platelet clumping. Macroscopic clots are visible to the naked eye. They appear as large mucous-like threads in the specimen. These mucous-like threads are formed by the aggregation and accumulation of platelets and the formation of fibrin from fibrinogen. One of the most common side effects from a macroscopic blood clot is an acute pulmonary embolism. Pulmonary emboli account for 50,000 deaths annually (*6*). Microscopic platelet clumping can only be seen with a microscope. They are referred to as clumping and not clots because microscopically only aggregated platelets are visualized. There is no macroscopic sign of mucous threads or fibrin formation. Both clotting and clumping could potentially lead to pulmonary embolism, stroke, and deep vein thrombosis *(7)*.

The purpose of this research was to evaluate the necessity of using heparin when preparing UltraTag RBC kits.

Materials and Methods

Subjects. This research study included 15 volunteer subjects. Institutional Review Board (IRB) approved this study and all subjects signed a written informed consent. Health Insurance Portability and Accountability Act guidelines were followed. There were no exclusion criteria regarding the volunteer subjects. A flyer was made and sent out to the undergraduate radiologic and imaging science students at the university requesting volunteers to participate in this study. The volunteer subjects were picked on a first come first serve basis in regards to responding to the flyer. Of the 15 volunteers 12 were students, two were nuclear medicine technologists, and one was a nuclear pharmacist.

Supplies. The supplies, for each volunteer subject, included: two UltraTag RBC kits, 40 units of unfractionated heparin solution 74 megabecquerels (MBq) of Technetium-99m Sodium Pertechnetate, eight microscope slides, eight wooden applicator sticks, one IV starter kit with a 20 gauge needle, two SmartSite vented vial access devices, two BD Falcon Round-Bottom Tubes with lids, eleven 3mL syringes with an attaching 20 gauge needle, a Dose Calibrator, a lead shield, a Geiger Mueller survey meter, Nikon Eclipse Ni-U microscope, Hema-Tek I 1000, Wright-Giemsa Pack, and pliers.

Procedures. A 20-gauge IV was started on each volunteer. Four samples of blood were drawn and labelled as follows. The first was UltraTag RBC Heparin: Three mL of blood was drawn into a syringe containing 30 units (0.3 mL) of heparin. The second was UltraTag Non-heparin: Three mL of blood was drawn into an empty syringe. The third was Control Heparin: One mL of blood was drawn into a syringe containing 10 units (0.1 mL) of heparin. The fourth was Control Non-heparinized: One mL of blood was drawn into an empty syringe. The heparin

used had a concentration of 100 units per mL solution. Ten units of heparin per mL of blood was used.

The two controls were done to determine if the sodium citrate contained within the UltraTag RBC kits imparted sufficient anticoagulant properties to prevent blood clots / clumps. The blood from the two UltraTag syringes were each added in a separate UltraTag vial (heparin and non-hepranized). The blood from the two control groups were each added into a separate empty tube (heparin and non-hepranized). The UltraTag RBC Heparin vial and UltraTag RBC Non-heparinized vial were radiolabeled following the package insert guidelines, using approximately 37 MBq of Technetium-99m Sodium Pertechnetate.

All four samples of blood were evaluated for macroscopic clots and microscopic platelet clumping. To evaluate for macroscopic clots the wooden applicator stick method was used. Two wooden sticks were held together like chop sticks and swirled around in the UltraTag RBC kits and Control tubes. The wooden sticks were then gently pulled up the side of the UltraTag RBC kits and Control tubes and once the ends of the wooden sticks were visible they were analyzed for macroscopic clots. If a macroscopic clot was present then a *yes* was recorded on the data sheet. If a macroscopic clot was not present then a *no* was recorded on the data sheet. Figure 1 shows an example of a negative macroscopic clot and a positive macroscopic clot. To evaluate for microscopic platelet clumping a blood smear was prepared. A drop of blood was placed onto a microscope slide and then another microscope slide was placed directly on top of the drop of blood and the slides were pulled across each other making a thin smear. Eight microscope slides were labeled for the blood smearing method; two for each of the four samples. Two slides were labeled H_V for the UltraTag RBC Heparin vial, two slides were labeled H_T for the Control Heparin tube, two slides were labeled X_V for the UltraTag RBC Non-heparinized vial, and two

slides were labeled X_T for the Control Non-heparinized tube. Blood smears were prepared using a similar methodology for UltraTag RBC Heparin vial, UltraTag RBC Non-heparinized vial, Control Heparin tube, and Control Non-heparinized tube and the blood smearing method was implemented. This entire procedure was repeated for all 15 subjects.

The slides were stained and analyzed by a medical laboratory scientist. The slides were stained with a Wright-Giemsa Pack using a Hema-Tek I 1000. The slides were evaluated by the medical laboratory scientist using a Nikon Eclipse Ni-U microscope. Each slide was analyzed by looking at 20 fields of view at 500x oil magnification. The fields of view were chosen at random to provide a fair representation of the slide. If the slide showed an area of platelet clumping then a *yes* would be recorded on the data sheet and if the slide showed no platelet clumping then a *no* would be recorded on the data sheet. Figure 2 shows an example of negative microscopic platelet clumping and positive microscopic platelet clumping.

Statistical Tests. To evaluate the data, Fisher's Exact Test was used. A Fisher's Exact test is useful with contingency tables of a small sample size *(8).* A p-value of less than 0.05 represents statistical significance and allowed rejection of the null hypothesis. The null hypothesis was that there is no difference in the prevalence of blood clotting/clumping when heparin is used compared to when heparin is not used in an UltraTag RBC kit. The alternate hypothesis was that there is a difference in the prevalence of blood clotting/clumping when heparin is used compared to when heparin is not used in an UltraTag RBC kits.

Results

Subjects. Of the 15 volunteer subjects three were male and twelve were female. Nine volunteer subjects ranged in ages of 18-25 years old and six volunteer subjects ranged in ages of 26-65 years old.

Outcome. The UltraTag RBC kits with heparin had zero macroscopic clots and three had microscopic platelet clumping. The non-heparinized UltraTag kits had two macroscopic clots and three had microscopic platelet clumping. The control group with heparin had zero macroscopic clots and ten had microscopic platelet clumping. The control group without heparin had two had macroscopic clots and ten microscopic platelet clumping.

For the UltraTag RBC kits, macroscopically, the p-value was 0.48 and microscopically the p-value was 1.0. For the control group, macroscopically the p-value was 0.98. In each of these cases there is not enough evidence to reject the null hypothesis. Statistically, there is no difference macroscopically and microscopically between the UltraTag RBC Heparin vials and the UltraTag RBC Non-heparinized vials and there is no difference macroscopically between the Control Heparin tubes and Control Non-heparinized tubes.

Microscopically for the control group the p-value was 0.04. The null hypothesis is rejected in favor of the alternate hypothesis. There was a statistically significant difference in microscopic platelet clumping for control group between heparinized and non-heparinized samples. The results of the Fisher's Exact Test are given in Table 1.

Incidental findings. The raw data is seen in Table 2.. Three volunteers (4, 9 and 13) had microscopic platelet clumping present in the heparinized UltraTag kit that was not seen in the non-heparinized UltraTag RBC kit.

Discussion

If macroscopic clots are seen, the microscopic clumps must also be present. The reverse is not true; you can have microscopic clumps but not have macroscopic clots. If macroscopic clots were present, then microscopic clumps were automatically recorded as *yes.* When comparing the control group with the UltraTag RBC group it appears that the anticoagulant

properties within the UltraTag RBC kits (sodium citrate) are effective at preventing microscopic platelet clumping but not effective enough to stop macroscopic clots. Although not statistically significant, it appears that heparin is necessary to stop macroscopic clots from forming due to two clots forming in the absence of heparin compared to zero with heparin (volunteer 1 and 14, Table 2). There is a statistically significant difference in microscopic platelet clumping in the control group. A larger sample size could get statistically significant results regarding the other samples.

There were three volunteers (4, 9 and 13) where microscopic clumping was identified with heparin and not identified without heparin. This may be due to two things. First, the sampling of each of the samples was small. There may have been clumping in the without heparin sample and it was not identified on the small sample that was tested. Second, the amount of heparin used in the study was small (due to the small volume of blood). If a greater amount of heparin was used, this discrepancy may not have appeared.

Although the data did not show statistically significant results it is still clinically significant. Ultimately injecting a macroscopic clot could cause detrimental side effects to the patient and effect the accuracy of the study. Further research should be done microscopically taking more samples of blood from the UltraTag RBC kits to test for microscopic clumping.

A limitation to the study is the time frame during which the blood was drawn and when it was exposed to the sodium citrate in the UltraTag RBC kit. Clotting is a time dependent action. This is a mute point with the heparin kits because the heparin was already in the syringe when the blood was drawn. Additional limitations to this study include a small sample size, as well as the age range and sex of the volunteers were not fixed to one group or restricted under a more controlled criteria. Additional research controlling these limitations could be useful.

Conclusion

Ultra-Tag RBC kits can produce macroscopic blood clots without the use of an anticoagulant such as heparin during the blood collection process, despite the presence of kit components with some anticoagulant properties. . Heparin should always be used when making an Ultra-Tag RBC kit for nuclear medicine studies.

Acknowledgments

A special thanks to Dennis J. Ernst MT(ASCP), NCPT(NCCT) and Center for Phlebotomy Education for the education and guidance on blood clots. Mallinckrodt Pharmaceuticals for the generous donation of 30 UltraTa RBC kits. Radiopharmacy of Indianapolis for the donation of 1,100 MBq of Technetium-99m Sodium Pertechnetate. IU School of Medicine Department of Radiology and Imaging Sciences for the syringes, needles, IV starter kits, and gloves. IU School of Medicine Department of Pathology and Laboratory Medicine for the wooden sticks and the use of the Nikon Eclipse Ni-U microscope, Hema-Tek I 1000, and Wright-Giemsa Stain. Purdue College of Pharmacy for the heparin and microscope slides. Riley Hospital for Children at IU Health for the use of the Dose Calibrator, lead shield, a Geiger Mueller survey meter, and hot lab.

References

1. Mallinckrodt. UltraTag RBC kit for the preparation of Technetium Tc 99m- Labeled Red Blood Cells. *Mallinckrodt Inc.* 2012.

2. Cosmi B, Palareti G. Old and new heparins. *Thrombosis Research.* 2012;129(3):388-391.

3. Reiner AP, Bell WR. The fibrinolytic system in man. *Critical Reviews in Oncology-Hematology.* 1984; 2(1):33-81.

4. Ramsay DM, Robertson EP, MacArthur E. Comparison of anticoagulants for the preservation of prothrombin time specimens. *Journal of Clinical Pathology.*1977; 30(8):766-769.

5. Farrell M, Mantel E. Quick-Reference Protocol Manual for Nuclear Medicine Technologists 2014.

6. Yoo HH, Queluz TH, El Dib R. Anticoagulant treatment for subsegmental pulmonary embolism. *Cochrane Database of Systematic Reviews.* 2014; 4:CD010222.

7. Fritsma G., Thrombotic disorders and laboratory assessment. In Keohane E, Smith L, Walenga J. (Eds), *Rodak's Hematology: Clinical Principles and Applications 5th ed.* St. Louis, MO: Elsevier; 2016.

8. Petrie A, Sabin C. *Medical Statistics at a Glance 3rd ed.*. Hoboken, NJ: Blackwell Publishing; 2009:69.

A B

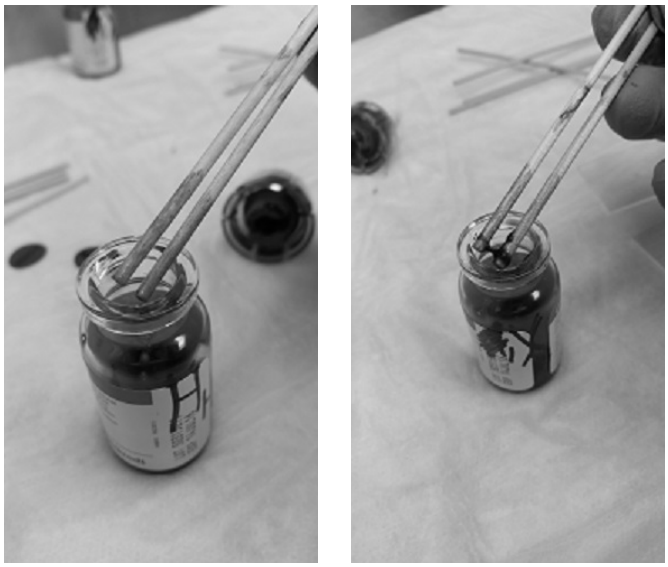

Figure 1: Image A shows an example of no macroscopic clot. Image B shoes an example of a macroscopic clot

A B

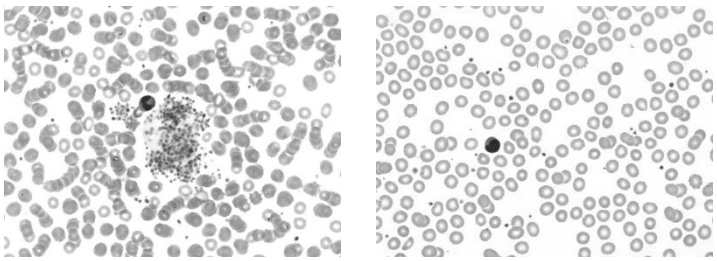

Figure 2: Image A shows an example of no microscopic platelet clumping. Image B shoes an example of microscopic platelet clumping.

		With Heparin	Without Heparin	P-value
UltraTag	Macroscopic Clotting	0/15 (0%)	2/15 (13%)	0.48
	Microscopic Clumping	3/15 (20%)	3/15 (20%)	P = 1.0
Control Group	Macroscopic Clotting	0/15 (0%)	2/15 (13%)	P = 0.48
	Microscopic Clumping	10/15 (67%)	15/15 (100%)	P = 0.04

Table 1: Comparison of positive results with and without heparin for the UltraTag group and the control group

Volunteer	UltraTag with heparin	UtraTag without heparin	Control with heparin	Control without heparin
1	-	M and u	u	u
2	-	-	-	u
3	-	-	-	u
4	u	-	-	u
5	-	-	-	M and u
6	-	-	u	u
7	-	-	u	u
8	-	-	u	u
9	u	-	-	u
10	-	-	u	u
11	-	u	u	u
12	-	-	u	M and u
13	u	-	u	u
14	-	M and u	u	u
15	-	-	u	u

u = microscopic platelet clumping M = macroscopic clotting

Table 2: Clotting and clumping results by volunteer