

Summary Report: Western Pond Turtle blood sampling portion of Environmental Contaminants Program project entitled "Assessment of Impacts to Fish and Wildlife From Pesticide Use On the Western Oregon Refuge Complex".

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Acknowledgments:

I would like to recognize that this project would not have been successful without the help of certain key individuals who helped me greatly in the execution of turtle capture and blood sampling. Many thanks to Bruce Bury and Larry Gangle, USGS-BRD, Dr. Julianne Vicstrom, Willamette Veterinary Clinic, Dr. Rob Bildfell (professor), Dr. Ursula Beckhart (professor), Katie Samuelson (student) and Terry Warren (student), OSU College of Veterinary Medicine.

I. Introduction:

The Western Oregon Refuge Complex consists of 11 National Wildlife Refuges (NWR's). Three Willamette Valley Refuges, William L. Finley, Ankeny, and Baskett Slough, were primarily established to provide wintering habitat for Canada Geese (*Branta canadensis*), especially dusky Canada Geese (*B. c. occidentalis*). In order to provide high quality, palatable forage for geese, a large percentage of the refuge lands are cultivated. Crops established on the refuge include annual ryegrass, perennial ryegrass, fescue, and a grass/clover (pasture) mix. Grass crops on the refuges must be of equal or better quality than adjacent private lands, in order to attract geese and reduce off-refuge crop depredation. Lush, dense stands of grass, provide excellent forage throughout the winter because these crops will grow back several times after being browsed. Farming of refuge crops is accomplished through cooperative agreements with local farmers. Pesticide use is necessary for the certification of grass seed produced, thereby making the refuge farming program attractive to local farmers. In addition, each of the valley refuges exists in a mosaic of croplands, dairies and timberlands, where agricultural chemicals are commonly applied. The potential for chemical contaminants to enter refuge waterways is of concern because aquatic communities and species that inhabit them may be harmed by exposure to such contaminants.

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This report was prepared as one facet of the current research project entitled "Assessment of Impacts to Fish and Wildlife From Pesticide Use on the Western Oregon Refuge Complex". This research project was designed to investigate pesticide impacts on aquatic communities on refuge lands. The study is scheduled to be completed in 4 phases, including; (1) collection of background information, (2) collection and analysis of water samples, (3) performance of in-situ fish bioassay, and (4) collection and analysis of blood hormone levels in common carp and western pond turtles

As part of phase (4) of the above research project, a trapping effort to capture Western Pond Turtles (WPT) was conducted at William L. Finley NWR, and at a reference site along the South Umpqua River. This trapping project was a capture and release effort designed to obtain blood samples from non-breeding adult WPT's. Blood samples will be analyzed for blood hormone levels in order to assess potential impacts from environmental contaminants (endocrine disrupting chemicals) in the aquatic environment. This report is a summary of trapping and sampling of western pond turtles during the summer, July 27 through October 2, of 1998.

II. Objective:

The objective of *this portion of the research* was to capture and draw blood samples from 20 (10 males and 10 females) non-breeding, adult Western Pond Turtles at W. L. Finley NWR and 20 (10 males and 10 females) from a control site along the South Umpqua River. The blood samples are to be analyzed for blood hormone levels.

III. Methods:

Turtles were captured using traps and by hand. Two types of traps were used to capture WPT's. See *Appendix A* for description and diagram of traps. Traps were placed along creek and marsh banks in a perpendicular fashion with an entrance pointed toward deeper water. Traps were baited with dead salmon and steelhead smolts, sardines-packed-in-oil, or both. Bait was positioned at the trap rear, sardine cans were partially opened to allow oil to spread over the water surface and bait scent to move with the current. Traps were checked daily and new bait was added as needed. Turtles were captured by hand by snorkeling through pools where turtles had been observed basking. In some cases, when the water was extremely turbid and had low visibility, turtles were captured by "muddling", which consisted of searching for turtles by feeling along habitat substrate, such as wood debris.

Upon capture, turtles were transported to a central location for blood sampling and data recording. A volume of 1 cc of whole blood was drawn from the jugular vein of WPT's. This blood was placed in ice, then centrifuged and blood plasma removed and placed on dry ice. The samples were transferred to ultracold freezer (-80° F) for storage, until analysis. This sampling procedure followed guidelines provided by the USFWS, Oregon State Office, contaminants division. See *Appendix B* for blood sampling procedure. Following the blood drawing, turtles were aged, sexed, weighed, and measured. Individual turtles were given permanent marks by filing notches into the marginal scutes of the carapace using the numbering system described by Holland, 1991. All turtles were released at the point of capture, as soon as possible after they were processed.

IV. Results:

A total of 21 western pond turtles were captured at W. L. Finley NWR. From these, 18 blood samples were taken; 11 from males, 6 from females, and 1 from a juvenile of undetermined sex. The other turtles were not sampled because (2) they were too small (juveniles), or (1) the observer was unable to draw the blood sample. See Table 1 for data summary of turtle captures from W. L. Finley NWR.

A total of 39 turtles were captured from the control site along the South Umpqua River. From these, 26 blood samples were drawn; 16 from males, 8 from females, and 2 from juveniles of undetermined sex. The other turtles were not sampled because they were too small (juveniles). See Table 2 for data summary of turtle captures from the South Umpqua River.

Samples are currently stored in ultracold freezer (-80 °) and will be shipped and analyzed for estrogen/testosterone levels and for the presence of vitellogenin. Methods for analysis will follow those of the University of Florida's BEECS Reproductive Analysis Core Facility.

V. Conclusions:

The initial research plan indicated that a total of 20 samples (10 from males and 10 from females) from each site would be desired to conduct analysis and to draw comparisons between sites. Although this number of samples was not collected, all of the samples will be analyzed. This will allow for investigation and determination if further sampling should be conducted.

Table 1: Western Pond Turtle Trapping and Blood Sampling Record

Refuge Contaminants investigation - Western Pond Turtle Trapping and Blood Sampling Record 1998																		
W. L. Finley National Wildlife Refuge Experimental site, Benton County, Oregon																		
date	location	Code (ID#)	Sex	Weight?	Class	Age	C length	C Max width	C Width at 6 th scute**	Height (shell)	Plastron length	Plastron length***	Blood	Blood Volume (cc)	Time captured	Time released	Comments	previous captures
7/30/98	Cabell Marsh	✓4828 M		590	AD		154.4	128.4					Y	1	11:10	18:00	taken to Willamette Vet clinic	10/04/93, 10/11/92
8/20/98	Muddy Creek	✓4830 M		790	AD	18+	168	142	137	62.5	148.5	142	Y	1	10:30	14:00	blood taken on wet ice to Henny lab to centrifuge and put in freezer	5/13/93
9/3/98	Greenberry Oxbow	✓4834 F		960	AD		178	150	144	72	169	162	Y	1	13:00	15:10	blood taken on wet ice to Henny lab to centrifuge and put in freezer	9/4/93
7/29/98	Cabell Marsh	✓4841 M		1010	AD		199.62	158.38					Y	1	14:20	19:00	taken to Willamette Vet clinic	9/30/94
8/27/98	Greenberry Oxbow	✓4842 F		370	AD	9	132.8	108.5	106.8	50	127	121.2	Y	1	13:00	15:40	blood taken on wet ice to Henny lab to centrifuge and put in freezer	4/25/95
7/30/98	Cabell Marsh	✓4848 F		830	AD		173.7	141.8					Y	0.7	11:25	18:05	taken to Willamette Vet clinic	7/13/95
8/1/98	Muddy Creek	✓4852 M		750	AD	7	178.7	137.8	130	59	158.5	150	Y	0.7	13:00	15:35	forgot to heparinize needles/sample hemolized	8/4/95
7/29/98	Cabell marsh	✓4853 M		835	AD		171.92	148.18					Y	1	14:20	19:00	taken to Willamette Vet clinic	
7/30/98	Cabell marsh	✓4854 M		615	AD		165.52	131.17					Y	1	11:10	18:00	taken to Willamette Vet clinic	
8/1/98	Muddy Creek	✓4855 F		>1000	AD	15	187.9	159.3					Y	1	13:20	15:35		
8/12/98	Muddy Creek	✓4856 M		890	AD	12	188.8	147.5	138.2	64.5	168.8	159.8	Y	1	10:15	12:30		
8/18/98	Cabell marsh (snorkel)	✓4857 ?		400	JUV	6	145	117	116	50.5	142	136	Y	1	10:30	13:30	blood taken on wet ice to Henny lab to centrifuge and put in freezer	
8/18/98	Beaver Pond (snorkel)	4858 M		1710	AD	16+	218.5	173	166.5	86	192.5	124.5	Y	1	11:00	13:30	blood taken on wet ice to Henny lab to centrifuge and put in freezer	
8/19/98	Muddy Creek	✓4859 F		775	AD	16+	164	135	130	98	151	145	Y	1	9:00	13:30	blood taken on wet ice to Henny lab to centrifuge and put in freezer	
8/19/98	Muddy at Bruce Rd.	✓4860 F		800	AD	16+	172	146	142	64	168.5	162	Y	0.5	10:00	14:00	blood taken on wet ice to Henny lab to centrifuge and put in freezer	
8/27/98	Muddy Creek	4861 ?		230	JUV		119.2	99.9	97.3	43.7	112.1	109.5	N		11:55	15:30	too small to draw blood	
8/28/98	Muddy Creek	✓4862 M		920	AD		181.9	150.2	140.8	63.3	161.5	150.7	Y	0.7	12:30	14:45	blood taken on wet ice to Henny lab to centrifuge and put in freezer	
8/28/98	Muddy Creek	4863 M		920	AD		177.1	150.5	144.3	57.1	157.5	147.8	N		12:30	14:45	unable to draw blood	
8/28/98	Muddy Creek	4864 ?		340	JUV		134.8	108.6	103.3	44.2	127.7	121.5	N		12:30	14:45	too small to draw blood	
9/2/98	Greenberry Oxbow	✓4865 M		980	AD		184.5	148	147	65	169	160	Y	1	13:00	15:30	blood taken on wet ice to Henny lab to centrifuge and put in freezer	
9/2/98	Greenberry Oxbow	✓4866 M		895	AD		180	153.5	147	62	158	148	Y	1	13:00	15:30	blood taken on wet ice to Henny lab to centrifuge and put in freezer	
* all weights are measured in grams																		
** Carapace width is the straight line distance between the 6 th scute on either side, measured at the bottom of the sixth scute																		
*** the shorter plastron length is the length measured from the inside of the notch at the midline of the plastron																		

Table 2: Western Pond Turtle Trapping and Blood Sampling Record

Refuge Contaminants Investigation - Western Pond Turtle Trapping and Blood Sampling Record 1998																
South Umpqua River Reference Site - Douglas County, Oregon																
Observers: Larry Gangle, USGS-BRD, Jeremy Buck, USFWS-OSO, Heidi Brunkal, USFWS W. L. Finley NWR																
Katie Samuelson, USFWS Volunteer was present 08/12/98-08/13/98																
date	location	Code (ID#)	sex	Weight*	Class	age	C length	C Max width	C Width at 6th scute**	Height (shell)	Plastron length	Plastron length**	Blood	Blood Volume (cc)	Time captured****	Time released*****
8/12/98	S.U. @ Dumont Crk.	2414	F	570	AD	16+	152		117	59	147	141	Y	1	18:30	(08/13/98) 12:00
8/12/98	S.U. @ Dumont Crk.	1752	F	790	AD	16+	181		130	64	171	163	Y	1	18:30	(08/13/98) 12:00
8/12/98	S.U. @ Dumont Crk.	3106	F	535	AD	13	154		119	54	145	139	Y	1	18:30	(08/13/98) 12:00
8/12/98	S.U. @ Dumont Crk.	7227	M	655	AD	16+	162		124	58	143	136	Y	1	18:30	(08/13/98) 12:00
8/12/98	S.U. @ Dumont Crk.	828	M	410	AD	12	144		109	47	134	129	Y	1	18:30	(08/13/98) 12:00
8/12/98	S.U. @ Dumont Crk.	5569	F	625	AD	16+	171		129	63	163	157	Y	1	18:30	(08/13/98) 12:00
8/12/98	S.U. @ Dumont Crk.	8317	M	305	AD	10	129		103	44	120	116	Y	1	18:30	(08/13/98) 12:00
8/12/98	S.U. @ Dumont Crk.	606	F	165	JUV	6	102		87	35	96	93	N		18:30	(08/13/98) 12:00
8/12/98	S.U. @ Dumont Crk.	510	F	650	AD	16+	162		124	60	159	153	Y	1	18:30	(08/13/98) 12:00
8/12/98	S.U. @ Dumont Crk.	7221	M	680	AD	16+	162		127	53	152	148	Y	1	18:30	(08/13/98) 12:00
8/12/98	S.U. @ Dumont Crk.	7000	F	205	JUV	8	112		93	41	104	102	N		18:30	(08/13/98) 12:00
8/12/98	S.U. @ Dumont Crk.	2908	F	400	AD	15	131		104	47	130	124	Y	1	18:30	(08/13/98) 12:00
8/12/98	S.U. @ Dumont Crk.	1760	M	400	AD	9	142		110	58	133	129	Y	1	18:30	(08/13/98) 12:00
8/12/98	S.U. @ Dumont Crk.	9806	M	660	AD	16+	166		128	53	156	146	Y	1	18:30	(08/13/98) 12:00
8/12/98	S.U. @ Dumont Crk.	1761	F	105	JUV	4	85		73	30	82	81	N		18:30	(08/13/98) 12:00
8/12/98	S.U. @ Dumont Crk.	1762	F	110	JUV	4	88		74	32	81	79	N		18:30	(08/13/98) 12:00
8/13/98	Carmine Lake	1766	F	210	JUV	5	111		85	40	104	102	Y	0.5	8:30	11:00
8/13/98	Carmine Lake	504	F	225	JUV	4	114		88	42	112	109	N		8:30	11:00
8/13/98	Carmine Lake	1764	F	190	JUV	5	110		87	37	104	102	Y	0.5	8:30	11:00
8/13/98	Carmine Lake	1765	F	175	JUV	5	109		86	37	101	100	N		8:30	11:00
9/16/98	S.U. @ Three C Rock	1768	F	157	JUV	5	102	87	86	35	95.5	93	N		14:30	15:30
9/16/98	S.U. @ Three C Rock	1769 (a)	M	820	AD	16+	182	137	133	59	164.5	155	Y	1	14:30	15:30
9/16/98	S.U. @ Three C Rock	1770	M	755	AD	16+	173	138	131.5	56	158	150	Y	1	14:30	15:30
9/16/98	S.U. @ Three C Rock	1771	F	240	JUV	6	115	96.5	93	41.5	117.5	104.5	N		14:30	15:30
9/16/98	S.U. @ Jackson Crk.	1772	M	795	AD	16+	176	137	131	59	160.5	151.5	Y	1	16:00	17:45
9/16/98	S.U. @ Jackson Crk.	1773	M	655	AD	14	165	131	124	57	152.5	146.5	Y	1	16:00	17:45
9/16/98	S.U. @ Jackson Crk.	1774	M	495	AD	14	152.5	122.5	117	49	139.5	134	Y	1	16:00	17:45
9/17/98	S.U. @ Dumont Crk.	7230	F	385	AD	11	133.5	119.5	114	48	130	125	Y	1	11:00	14:30
9/17/98	S.U. @ Dumont Crk.	7281	M	600	AD	16+	164.5	126.5	120	54	149	144	Y	1	11:00	14:30
9/17/98	S.U. @ Dumont Crk.	7215 (b)	F	565	AD	15	150	124	117	55	142	136	Y	1	11:00	14:30

Table 2: Western Pond Turtle Trapping and Blood Sampling Record

Refuge Contaminants Investigation - Western Pond Turtle Trapping and Blood Sampling Record 1998																
South Umpqua River Reference Site - Douglas County, Oregon																
Observers: Larry Gangle, USGS-BRD, Jeremy Buck, USFWS-OSO, Heidi Brunkal, USFWS W. L. Finley NWR																
Katie Samuelson, USFWS Volunteer was present 08/12/98-08/13/98																
date	location	code (ID#)	sex	weight	class	age	C. length	C. Max width	C. Width at 6th scute**	Height (shell)	Plastron length	Plastron length***	Blood	Blood Volume (cc)	Time captured****	Time released****
9/17/98	S.U. @ Dumont Crk.	7260	M	618	AD	16+	160.5	132.5	124	53	152	145.5	Y	1	11:00	14:30
9/17/98	S.U. @ Dumont Crk.	7218	M	615	AD	16+	166	132.5	124	54	155	148.5	Y	1	11:00	14:30
9/17/98	S.U. @ Dumont Crk.	1775 (c)	M	358	AD	12	139	114	108.5	45	132	127.5	Y	1	11:00	14:30
9/17/98	S.U. @ Dumont Crk.	1776	M	330	AD	9	132.5	117	102	47	124	119.5	N		11:00	14:30
9/17/98	S.U. @ Dumont Crk.	1777	?	200	JUV	8	113	91	87.5	39	107	104.5	N		11:00	14:30
9/18/98	Jackson Crk @ Beaver Crk.	1778	?	170	JUV	7	105.5	90	84.5	38	100	98	N		10:00	12:00
9/18/98	Jackson Crk @ Beaver Crk.	1779	M	685	AD	16+	168	134	129	56.5	157	150.5	Y	1	10:00	12:00
a = 13 total marginal scutes																
b = very large crack in 200 scute																
c = has some female characters, plastron not very concave																
* all weights are measured in grams																
** Carapace width is the straight line distance between the 6th scute on either side, measured at the bottom of the sixth scute																
*** the shorter plastron length is the length measured from the inside of the notch at the midline of the plastron																
**** ALL TIMES are ESTIMATED (time was not recorded with the original data)																
All turtles from the Umpqua River Site were captured by snorkeling, EXCEPT those captured at Carmine Lake.																
Carmine Lake site used Applegarth traps baited with oil packed sardines.																

APPENDIX A:

During the late summer of 1998, July 27 through October 2, a trapping effort to capture Western Pond Turtles (WPT) was conducted at William L. Finley NWR. This trapping project was a capture and release effort designed to assess the potential effects of environmental contaminants (endocrine disrupting chemicals) on non-breeding adult WPT's.

During this project, 2 types of traps were used. The following is a description of each type of trap. The OSO trap used was a rigid, rectangular trap. These traps were covered netting, had only 1 entrance and did not require the use of floats because the closed end was tall enough to be left partially out of the water. The Applegarth trap is a collapsible trap consisting of 5 hoops covered by netting and held open with 2 detachable plastic tubes placed outside the netting and fixed to the end hoops. These plastic tubes serve as supports for expanding the nets and as floatation devices to allow for an air space in which captured turtles can breathe. Entrances are located on each end; however, 1 entrance was closed to minimize opportunity of escape.

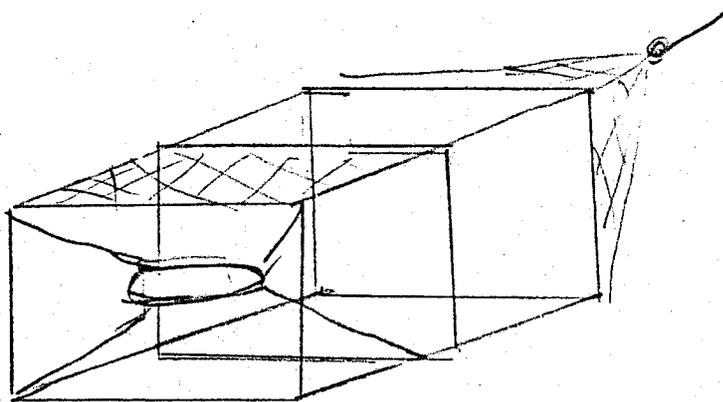
1. The OSO (USFWS Oregon State Field Office) Trap

These traps were supplied by the OSO from past research projects. These traps are constructed of steel rectangular frames, and have a wide mesh net (1") that covers the external frame and the net also creates the funnel entry for the trap.

The trap dimensions are approx 3" wide, 2.5" tall, and 4" long. The steel frame is constructed of 3 rectangular pieces, one on each end and one in the middle of the length of the trap. The frame is supported by two wooden 1 x 2 "struts", one on each side, that have hooks on the ends which connect to the trap frame. The entrance funnel is at one end of the trap, while the other end is gathered netting, and usually has a rope to tie the trap securely to an anchor point.

We placed these traps in fairly shallow areas so that the trap could not become submerged, and so that there would always be air in the trap for any captured WPT. The traps were baited with hatchery salmon smolts (dead) or sardines. The bait was hung toward the rear of the trap, in a "bait cage" constructed of window screen material.

Diagram:



2. The "Applegarth" trap (Created by John Applegarth, BLM Eugene)

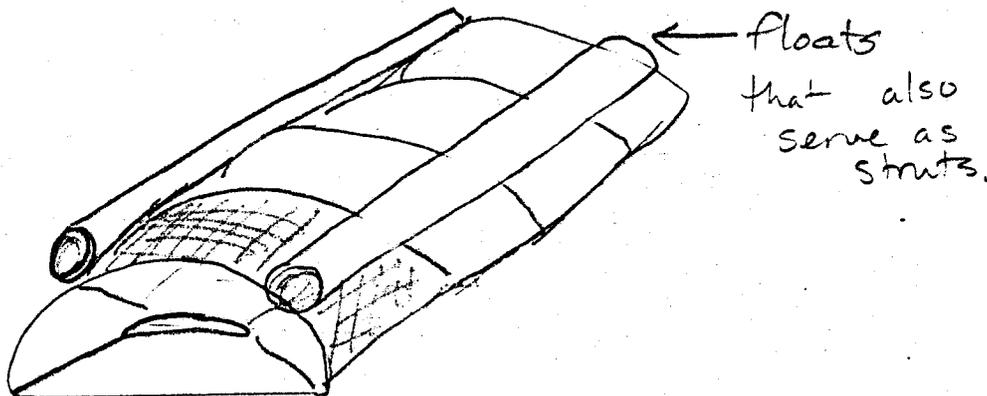
The Applegarth trap is the standard WPT trap used by field biologists in the Willamette Valley. They were originally built by John Applegarth, but he no longer builds them, however Army Corps of Engineers, Fern Ridge in Eugene also knows how to build these traps.

The Applegarth trap is a lighter weight trap, covered with finer ($\frac{1}{4}$ ") mesh netting. The frame is 5 pieces of aluminum (or another lightweight alloy) which form a half-circle shape, flat across the bottom, and rounded over the top. These traps are approx. 2.5" wide, 2" tall and 3.5" in length.

These traps are supported with PVC plastic struts which support the frame and also serve as floats so that the traps are never fully submerged, and trapped WPT always have air.

The majority of these traps were deployed in and along Muddy Creek. They are also baited with hatchery salmon smolts (dead) or sardines.

Diagram:



APPENDIX B: Turtle Blood Collection Methods

Remember:

- Wear gloves while handling turtle (use new gloves for each turtle)*
- Keep heparin chilled*
- Make sure turtle is kept cool during handling and transport*
- Disinfect buckets and other turtle handling equipment between captures*

Once turtle is removed from trap, perform the tasks of marking, weighing, measuring, and sexing the specimen and record appropriate information on the data sheet.

Assemble a 25 gauge needle to a syringe. The syringes are of Luer-lok variety which means the needle twists on to the syringe in a clockwise direction. The cap pulls straight off. To ensure that the needle does not come loose while capping or uncapping the syringe, twist the cap off with a slight clockwise rotation.

Heparinize the needle and syringe by uncapping the needle and drawing approximately 0.2 ml of the heparin solution into the syringe, inverting it (needle up), and withdrawing the plunger to its full extent. Move the plunger up and down the full length of the syringe a couple of times to insure a complete coating. Dispense the heparin back into its container but leave a visible meniscus in the syringe (< 0.1 ml) and the hub of the needle.

Note: Heparin is a powerful anti-coagulant -- be careful!

While holding the turtle securely, position the animal so that a puncture can be made into the jugular vein in the neck. If repeated attempts to draw blood result in several withdrawals of the needle, the likelihood of a clot forming in the needle is increased. If a clot does form, withdraw the needle from the turtle and expel the clot with slight pressure on the plunger of the syringe.

Fill the syringe with 1 ml of blood, if possible. Record the appropriate information on the data sheet, be sure to include any problems encountered.

Using pliers, remove the needle from the syringe, crush it, and put it in the sharps container for later disposal. Uncap the Vacutainer and GENTLY expelled the blood from the syringe. Recap the Vacutainer and GENTLY invert the tube several times to mix the wafer and blood. Place the Vacutainer in a tube rack cradled in wet ice within a small cooler and chill until processed. Using large pliers, crush the syringe and dispose in the garbage (NOT the sharps container).

Place the chilled Vacutainers in the centrifuge without the caps. Make sure the rotor is balanced by spacing the tubes evenly or using a tube filled with water. Close the lid, making sure it is completely latched as otherwise the centrifuge will not spin