

Comparison of Ocular Tissue Weights (Volumes) and Tissue Collection Techniques in Commonly Used Preclinical Animal Species

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Purpose

Ocular pharmacokinetic and distribution studies are key elements for the development of ophthalmic drugs and are used to correlate drug levels in preclinical species with human dosages and exposures. The purpose of this study was to compare ocular tissue weights for five commonly used preclinical species and two less common species, using two tissue excision collection techniques.

Methods

Standard ocular tissues were collected from the eye globes of five males and five females of each species: pigmented Dutch Belted (DB) rabbits, beagle dogs, Gottingen minipigs and cynomolgus monkeys; from 7 male and 5 female albino New Zealand White (NZW) rabbits; and from five male Yorkshire domestic pigs and five male purpose-bred hounds. All animals used were non-naïve from Covance stock colonies. Animals were euthanized by overdose of the appropriate anesthetic and the eye globes were enucleated and trimmed of extraneous tissue prior to tissue collection.

Two tissue excision techniques were used; a collection from nonfrozen (fresh) eyes (OS eyes) and a collection from frozen eyes (OD eyes, following flash freezing in liquid nitrogen for 15-20 seconds). The aqueous humor was collected prior to freezing. The procedures followed involve a specific collection sequence with frequent changing of instruments and rinsing of instruments in appropriate solvents to prevent or minimize any cross contamination as shown in Figure 1.

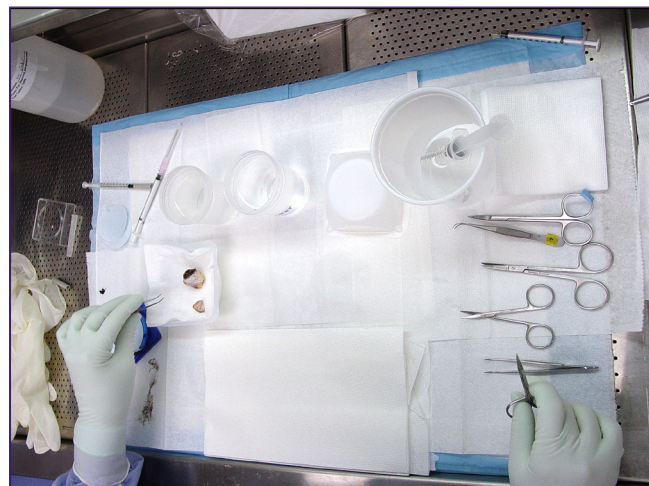


Figure 1. Bench layout for ocular tissue collection.

Table 1. Representative Weights for Ocular Tissues Collected by Using a Fresh (Nonfrozen) Eye Collection Technique

Species (Strain)	Approximate Age	Body Weight (kg)	Matrix and Weight (g)										Percent Collected
			Whole Eye	Aqueous Humor	Cornea	Lens	Iris-Ciliary Body	Vitreous Humor	Retina	Choroid-RPE	Sclera		
Albino Rabbit (New Zealand White)													
Male Mean	6.5 mo	3.07	2.93	0.293	0.0841	0.448	0.0839	1.17	0.0371	0.0273	0.269	82.5	
SD	2.6	0.493	0.304	0.0373	0.00952	0.0652	0.0241	0.148	0.00440	0.00394	0.0211	2.75	
Female Mean	12.8 mo	2.94	3.43	0.323	0.0952	0.556	0.0933	1.34	0.0489	0.0298	0.301	81.2	
SD	4.5	0.427	0.269	0.0299	0.0121	0.0639	0.0107	0.167	0.00421	0.00443	0.0342	2.25	
Mean Male and Female	9.2 mo	3.34	3.14	0.306	0.0887	0.493	0.0878	1.24	0.0420	0.0284	0.282	82.0	
SD	4.7	0.559	0.380	0.0363	0.0116	0.0829	0.0196	0.172	0.00504	0.00416	0.0308	2.54	
Pigmented Rabbit (Dutch Belted)													
Male Mean	9.3 mo	2.31	2.98	0.315	0.0745	0.477	0.0834	1.16	0.0403	0.0221	0.201	79.3	
SD	4.3	0.182	0.201	0.0291	0.00870	0.0630	0.0191	0.147	0.00388	0.00211	0.0223	4.24	
Female Mean	13.5 mo	1.56	2.97	0.310	0.0839	0.497	0.0891	1.09	0.0405	0.0216	0.191	78.3	
SD	0	0.221	0.0931	0.0189	0.00689	0.0189	0.00446	0.0988	0.00589	0.00223	0.0153	4.89	
Mean Male and Female	11.4 mo	1.93	2.97	0.313	0.0792	0.487	0.0863	1.12	0.0404	0.0218	0.196	78.8	
SD	3.6	0.234	0.148	0.0233	0.00883	0.0450	0.0134	0.123	0.00470	0.00206	0.0189	4.35	
Monkey (Cynomolgus)													
Male Mean	3.7 yr	3.22	3.09	0.116	0.0424	0.0999	0.0775	1.49	0.0725	0.0648	0.206	70.3	
SD	0.21	0.444	0.322	0.0194	0.00316	0.00861	0.0217	0.169	0.0148	0.0207	0.0240	4.65	
Female Mean	3.9 yr	2.98	3.13	0.142	0.0449	0.101	0.0798	1.57	0.0734	0.0610	0.210	73.3	
SD	0.11	0.319	0.374	0.0230	0.00506	0.00809	0.00782	0.122	0.0255	0.0161	0.0382	4.06	
Mean Male and Female	3.8 yr	3.10	3.11	0.129	0.0436	0.100	0.0786	1.53	0.0730	0.0629	0.208	71.8	
SD	0.19	0.386	0.330	0.0244	0.00419	0.00788	0.0154	0.146	0.0196	0.0176	0.0302	4.40	
Swine (Gottingen Minipig)													
Male Mean	5.6 mo	11.9	4.14	0.210	0.122	0.301	0.093	1.43	0.0825	0.0430	0.517	67.6	
SD	1.8	1.02	0.737	0.0471	0.0132	0.0506	0.0210	0.342	0.0190	0.00715	0.0615	2.84	
Female Mean	7.0 mo	12.8	4.28	0.233	0.128	0.325	0.099	1.62	0.0834	0.0469	0.554	72.4	
SD	1.6	1.45	0.453	0.0378	0.0118	0.0519	0.0146	0.131	0.01705	0.00819	0.0640	2.80	
Mean Male and Female	6.3 mo	12.4	4.21	0.221	0.125	0.313	0.096	1.52	0.0829	0.0450	0.535	70.0	
SD	1.8	1.28	0.582	0.0420	0.0122	0.0500	0.0173	0.264	0.0170	0.00753	0.0623	3.67	
Swine (Domestic - Yorkshire)													
Male Mean	3.5 mo	35.48	5.29	0.229	0.122	0.288	0.131	1.72	0.152	0.0698	1	70.5	
SD	0	2.09	0.349	0.011	0.006	0.01	0.004	0.251	0.0247	0.01	0.0733	6.45	
Canine (Beagle)													
Male Mean	11.2 mo	5.86	5.54	0.808	0.150	0.503	0.188	1.90	0.0756	0.109	0.626	78.5	
SD	4.2	0.321	0.394	0.177	0.0177	0.0424	0.0314	0.118	0.0134	0.0326	0.0508	2.26	
Female Mean	12.5 mo	9.06	5.39	0.804	0.140	0.503	0.212	2.00	0.0767	0.0705	0.602	81.7	
SD	3.3	0.428	0.183	0.0364	0.0218	0.0547	0.0260	0.0796	0.00801	0.00427	0.0300	2.91	
Mean Male and Female	11.9 mo	7.46	5.47	0.806	0.142	0.503	0.200	1.95	0.0761	0.0896	0.614	80.1	
SD	3.6	0.518	0.300	0.120	0.0188	0.0462	0.0299	0.109	0.0104	0.0297	0.0413	3.00	
Canine (Hound)													
Male Mean	10.6 mo	27.70	6.55	1.06	0.141	0.539	0.234	1.95	0.0913	0.106	0.928	76.94	
SD	0.5	0.327	0.162	0.263000	0.0128	0.0183	0.0434	0.4	0.0426	0.0221	0.0477	6.73	

Mean and SD for 5 animals/gender for all species except Albino rabbits where mean and SD is for 7 males and for 5 females. mo = month yr = year

Results

Aqueous and vitreous humor, cornea, lens, iris-ciliary body (ICB), retina, choroid-with retinal pigmented epithelium (RPE) and sclera were collected. Results are summarized in Tables 1 and 2 for fresh and frozen eye techniques, respectively. The combined weights of these tissues represent means of 67.6 to 82.5% and 82.0 to 89.9% of the total eye weight, respectively, for fresh and frozen collections. Gender differences were minimal with either technique. Tissue recovery levels were consistently higher using the frozen collection technique, especially for the vitreous humor. For the fresh collection, the vitreous humor weight represented approximately 30-37%, 37-40%, 33-36% and 50% of the total eye weight for dogs, rabbits, pigs and monkeys, respectively. For the frozen collection, the vitreous humor weight represented approximately 40%, 44%, 53-56% and 65%, of the total eye weight for dogs, rabbits, pigs and monkeys respectively. The mean combined male and female vitreous humor weights using the frozen collection were 1.41, 1.31, 2.22, 2.34 and 2.04 g for NZW and DB rabbits, beagle dogs, minipigs and monkeys, respectively. Mean vitreous humor weights, using the frozen collection, for the larger Yorkshire pigs and canine hounds were 2.78 and 2.57 g, respectively.

Table 2. Representative Weights for Ocular Tissues Collected by Using a Frozen Eye Collection Technique

Species (Strain)	Approximate Age	Body Weight (kg)	Matrix and Weight (g)										Percent Collected
			Whole Eye	Aqueous Humor	Cornea	Lens	Iris-Ciliary Body	Vitreous Humor	Retina	Choroid-RPE	Sclera		
Albino Rabbit (New Zealand White)													
Male Mean	6.5 mo	3.07	2.94	0.288	0.0807	0.457	0.0807	1.33	0.0280	0.0291	0.266	87.4	
SD	2.6	0.493	0.305	0.0440	0.00515	0.0685	0.00924	0.0687	0.00551	0.00669	0.0194	0.0390	
Female Mean	12.8 mo	3.72	3.40	0.293	0.0841	0.448	0.0839	1.17	0.0433	0.0273	0.269	86.6	
SD	4.5	0.427	0.327	0.0373	0.00952	0.0652	0.0241	0.148	0.0044	0.00394	0.0211	1.5400	
Mean Male and Female	9.2 mo	3.34	3.13	0.301	0.0848	0.503	0.0887	1.41	0.0287	0.0268	0.276	87.1	
SD	4.7	0.559	0.382	0.0451	0.0119	0.0878	0.0132	0.128	0.0101	0.00634	0.0260	0.0305	
Pigmented Rabbit (Dutch Belted)													
Male Mean	9.3 mo	2.31	2.98	0.313	0.0715	0.458	0.0769	1.36	0.0255	0.0260	0.198	84.8	
SD	4.3	0.182	0.195	0.0276	0.00979	0.0691	0.00735	0.0689	0.00524	0.00477	0.0141	1.41	
Female Mean	13.5 mo	1.56	2.97	0.309	0.0808	0.507	0.0858	1.26	0.0271	0.0246	0.193	83.7	
SD	0	0.221	0.0941	0.0179	0.00501	0.0196	0.00400	0.0431	0.00473	0.00280	0.0103	1.11	
Mean Male and Female	11.4 mo	1.93	2.98	0.311	0.0762	0.482	0.0813	1.31	0.0263	0.0253	0.195	84.3	
SD	3.6	0.234	0.144	0.0221	0.00881	0.0544	0.00728	0.0761	0.00478	0.00376	0.0119	1.33	
Monkey (Cynomolgus)													
Male Mean	3.7 yr	3.22	3.10	0.116	0.0403	0.109	0.0839	2.06	0.0481	0.0686	0.212	88.4	
SD	0.21	0.444	0.318	0.0204	0.00323	0.0118	0.00929	0.172	0.0106	0.0127	0.0305	2.61	
Female Mean	3.9 yr	2.98	3.13	0.135	0.0439	0.104	0.0786	2.01	0.0465	0.0679	0.202	86.2	
SD	0.11	0.319	0.310	0.0174	0.00320	0.00421	0.0106	0.223	0.00661	0.0159	0.0233	5.33	
Mean Male and Female	3.8 yr	3.10	3.12	0.126	0.0421	0.106	0.0812	2.04	0.0473	0.0682	0.207	87.3	
SD	0.19	0.386	0.296	0.0206	0.00356	0.00890	0.00979	0.189	0.00836	0.0136	0.0262	4.12	
Swine (Gottingen Minipig)													
Male Mean	5.6 mo	11.9	4.13	0.215	0.111	0.304	0.124	2.28	0.0619	0.0411	0.499	88.0	
SD	1.8	1.02	0.727	0.0487	0.0146	0.0548	0.0198	0.450	0.0152	0.00491	0.0666	1.0	
Female Mean	7.0 mo	12.8	4.24	0.229	0.113	0.330	0.122	2.41	0.0624	0.0424	0.507	89.9	
SD	1.6	1.45	0.503	0.0379	0.0123	0.0636	0.0157	0.286	0.00623	0.00545	0.0473	0.9	
Mean Male and Female	6.3 mo	12.4	4.19	0.222	0.112	0.317	0.123	2.34	0.0622	0.0417	0.503	88.9	
SD	1.8	1.28	0.592	0.0417	0.0128	0.0577	0.0169	0.362	0.0109	0.00493	0.0546	1.3	
Swine (Domestic - Yorkshire)													
Male Mean	3.5 mo	35.45	5.28	0.239	0.113	0.291	0.095	2.78	0.121	0.0676	1.01	89.27	
SD	0	2.09	0.361	0.014	0.004	0.0102	0.014	0.308	0.0256	0.0113	0.0816	1.93	
Canine (Beagle)													
Male Mean	11.2 mo	5.86	5.55	0.821	0.138	0.508	0.145	2.14	0.0570	0.129	0.617	82.0	
SD	4.2	0.321	0.418	0.175	0.0199	0.0380	0.0449	0.312	0.00892	0.0281	0.0591	0.0615	
Female Mean	12.5 mo	9.06	5.37	0.726	0.140	0.497	0.144	2.29	0.0502	0.111	0.583	84.5	
SD	3.3	0.428	0.134	0.0599	0.0233	0.0505	0.0329	0.181	0.00443	0.0160	0.0322	0.0270	
Mean Male and Female	11.9 mo	7.46	5.46	0.773	0.139	0.502	0.144	2.22	0.0536	0.120	0.600	83.3	
SD	3.6	0.518	0.307	0.133	0.0204	0.0425	0.0371	0.253	0.00754	0.0236	0.0483	0.0466	
Canine (Hound)													
Male Mean	10.6 mo	27.70	6.56	1.18	0.143	0.518	0.125	2.57	0.0799	0.201	0.908	87.2	
SD	0.5	0.327	0.183	0.226	0.00769	0.0165	0.0166	0.163	0.00912	0.025	0.065	2.5	

Mean and SD for 5 animals/gender for all species except Albino rabbits where mean and SD is for 7 males and for 5 females. mo = month yr = year

Conclusions