Protocol for injection of the Guam Coconut Rhinoceros beetle genotype (*Oryctes rhinoceros*) *w*ith nudivirus (OrNV)

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This was a first attempt at injection of adult beetles with a virus isolate (Dug42) obtained AgResearch, New Zealand. Adults have an extremely hard exoskeleton and according to the description of a previous laboratory injection protocol, which relied on finding a point on the ventral surface at the junction of the hind leg and the thoracic to inject, proved to be problematic. While holding the adult dorsal side down by hand, the beetle tends to flip-over while simultaneously attempting to move forward. There is no available source of CO_2 in our lab to anesthetize them, so the next best thing would have been to put them on ice or in a refrigerator for a short time (i.e. 5 min) before an attempt at inject. However, we tried the more difficult, expedient approach by not immobilizing the beetle and injected 20 µl of virus sample into each beetle with a sterile 30-gauge BD syringe. Another problem was that the small needle used for injection was too flexible, easily bent, and this probably caused some of the individual beetles not to receive the complete virus dose.

For the injection experiments, adult beetles were divided into three groups of 10 individuals with each group receiving a different treatment (sterile-activated virus, heat-inactivated virus, or filtered water as a control). Adult weights were measured just prior to injection. After treatment each beetle was transferred to a glass mason jar filled with moist, commercially blended steer manure and soil. Daily measurements of adult weight were taken until either the beetle showed possible viral symptoms or death from some other cause (e.g. bacteria).

Results

After two weeks post-injection none of the beetles showed signs of viral infection. However, there were some deaths during the experiment, but it was attributed to some other unknown cause. Table 1 shows the adult weights.

Day I.D.	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Sterile virus														
1597	3.085	2.876	2.792	2.773	2.750	2.738	2.846		2.748		2.696			2.616
0865	2.510	2.649	2.664	2.305	2.338	2.263	2.401		2.272		2.315			2.293
0946	3.794	3.234	3.121	3.281	3.484	3.191	3.253		3.132		2.984			2.975
0819	5.387	4.945	4.815	4.805	5.075	4.655	4.957		4.648		4.677			4.607
3226	2.590	2.846	2.711	2.526	2.505	2.451	2.416		2.361		2.334			2.345
1369	3.578	3.354	3.273	3.276	dead									
0636	5.055	4.867	4.487	4.382	4.507	4.267	4.426		4.513		4.485			4.398
1423	3.353	3.352	3.254	2.932	3.084	2.761	3.000		2.832		2.754			2.726
1328	3.848	dead												
Heat-inactivated virus														
0267	4.770	5.129	4.721	4.384	4.292	4.258	4.245		4.197		4.167			4.213
0750	4.106	4.392	4.421	3.911	4.224	3.897	3.862		3.905		3.953			3.810
0259	3.954	3.903	3.505	3.485	3.755	3.430	3.506		3.535		3.382			3.314
1111	3.690	4.059	3.639	3.504	3.864	3.564	3.599		3.497		3.456			3.432
0087	3.643	3.750	3.782	3.468	3.671	3.510	3.502		3.403		3.518			3.410
1221	4.240	4.646	4.367	4.265	4.124	3.954	3.833		3.730		3.685			3.734
1841	5.800	5.139	5.666	5.126	5.059	5.009	5.119		5.077		4.931			5.019
1364	3.161	3.142	3.110	dead										
1618	3.949	3.673	3.666	3.615	3.573	3.567	nd		3.513		3.737			3.788
Control														
0941	3.473	dead												
0473	3.980	3.920	3.918	dead										
1202	3.550	4.079	3.917	3.877	3.850	3.677	3.606		3.600		3.577			3.507
0397	4.551	4.981	4.879	4.690	4.996	4.646	4.844		4.755		4.611			4.599
1288	4.250	4.063	3.988	3.848	3.809	3.717	3.691		3.612		3.526			3.447
0421	4.690	5.804	5.402	5.277	4.802	4.783	4.745		4.631		4.569			4.436
0898	5.177	5.146	5.136	5.128	5.130	5.143	5.098		5.060		5.066			5.025
3232	4.362	4.228	4.781	4.455	4.362	4.246	4.123		4.035		3.972			3.900
3235	1.750	1.725	1.728	1.727	1.712	1.707	1.682		1.641		1.607			1.580
0628	2.916	2.836	2.837	2.785	2.768	2.767	2.861		2.760		2.836			2.760

Table 1. Adult beetle weight (g)