Text S1

Additional trial of different PrepMan Ultra and Chelex resin extraction methods

PrepMan Ultra extraction trials

Using the same standards described in section "Comparison of spin column and non-spin column extraction method", we extracted standard samples (18 samples for each plus 3 negative controls) using two different protocols using PrepMan Ultra, one extraction method using beads and a cell homogenization step (PrepMan with beads), and one method without the beads (PrepMan Alone). Specifically, for the PrepMan alone methods we added 50 µL of PrepMan Ultra to each sample, samples were incubated at 95 °C to lyse the cells for 10 min, then the supernatant was then collected and stored at –20 °C. For the PrepMan Ultra method including the cell homogenization step (PrepMan with beads), 50 µL of PrepMan Ultra and 30–40 mg of 0.5 mm silica beads was added to each sample, samples were homogenized using a cell homogenizer for 2 min at 1400 oscillations per sec, incubated at 95 °C to lyse the cells for 10 min, then the supernatant was then collected and stored at –20 °C.

Samples were analyzed vis qPCR as described in section "Analysis of the samples using qPCR". All template DNA was diluted 6:100 in molecular grade water, and all samples were analyzed via qPCR in triplicate. A reaction well was considered positive if > 2 *Bd* DNA copies amplified. A sample was considered positive if one reaction well was positive, with the quantity of *Bd* determined as an average of all reaction wells (as described in the main document). Negative samples were categorized as having a *Bd* load of 0.

Chelex resin extraction trial

Using the same standards described in section "Comparison of spin column and non-spin column extraction method", we extracted standard samples (18 samples for each plus 3 negative controls) using two different protocols using Chelex extraction methods, one with Chelex alone (Chelex Alone), and one with a proteinase K step added to the extraction (Chelex with PK). For the Chelex extraction method a 5% Chelex slurry in molecular grade water was made, and 200 µL of well mixed slurry was added to each sample. The samples were vortexed and incubated at 95 °C to lyse the cells for 20 min, then the supernatant was then collected and stored at –20 °C. For the Chelex with PK extraction, 200 µL of well mixed 5% Chelex slurry and 3 µL of Proteinase K (Qiagen #19131) was added to each sample. Samples were incubated at 56 °C to for 60 min while intermittently vortexing the samples, then incubated at 95 °C for 15 min. The supernatant was then collected and stored at –20 °C. For each extraction method, three negative control swab sample (no *Bd* added) was extracted alongside the *Bd*-containing samples. qPCR was performed exactly the same as for the PrepMan extraction.

Dilution of Chelex extracted template DNA has not been trialed to our knowledge in the literature. Here we compared 6:100 dilution in DNase free water, and 1:5 dilution of the Chelex and Proteinase K extracted samples.

Statistical analysis

To compare the efficacy of the two PrepMan extraction methods (PrepMan Alone, and PrepMan with beads), we used a linear model (lm). In this model the DNA copies ($\log_{10}$ transformed) per whole sample was the dependent variable, the fixed effects were zoospores added ($\log_{10}$ transformed) to the sample, extraction method and the interaction between zoospores added and extraction method. Because there were no positive samples below $\log_{10}$

Zoospores = 1, we only included 1–4 in the analysis to maintain normal distribution of the residuals for linear regression analysis.

To compare the efficacy of the two Chelex extraction methods (Chelex, and Chelex with Proteinase K), a lm was performed like for the PrepMan extraction method. Similarly, an lm was performed to comparing the Chelex DNA template dilution.

Results

When we compared the extraction efficacy using the two different PrepMan extraction methods, we found there was no differences in qPCR results between the two (lm: Log_{10} Zoospores added, $F_{1,20} = 141.935$, $p < 0.001$; Method, $F_{1,20} = 0.021$, $p = 0.887$, Zoospores added * Method, $F_{1,20} = 0.047$, $p = 0.83$; Fig S1a). This analysis indicates that PrepMan Ultra extraction can be performed with or without the cell homogenization/bead beating step for effective extraction of *Bd* zoospores. Because of this equivocal result, we chose to include the PrepMan extraction with the bead and cell homogenization step for our methods analysis, because that is the method often reported in the literature.

When we compared the extraction efficacy using the two different Chelex resin extraction methods, we found that including proteinase K did not affect the amount of *Bd* detected in the sample (lm: Log_{10} Zoospores added, $F_{1,20} = 219.302$, $p < 0.001$; Method, $F_{1,20} = 3.137$, $p = 0.092$, Zoospores added * Method, $F_{1,20} = 0.2079$, $p = 0.653$; Fig S1b). While there is no statistical difference between Chelex extractions with and without Proteinase K, we chose to include the extraction with proteinase K in the manuscript because that method has been used in *Bd* studies published in the literature.

When we compared the qPCR results for different dilutions of template DNA, we found that higher dilution actually resulted in higher *Bd* DNA quantities (lm: Log_{10} Zoospores added, $F_{1,20} = 236.634$, $p < 0.001$; Method, $F_{1,20} = 11.725$, $p = 0.003$, Zoospores added * Method, $F_{1,20} = 2.143$, $p = 0.159$; Fig S2). This higher efficiency at higher dilution is likely due to inhibitors present in the Chelex resin, although no internal positive controls were added to the samples, therefore we did not test that explicitly. With high potential for inhibitors due to the Chelex extraction method, it is important to test the extraction efficacy on field samples prior to use in the field and we recommend that all qPCR reactions be performed with 6:100 dilution of DNA template if the Chelex resin extraction is conducted.

Threshold of detection for all extraction methods

Statistical analysis

Threshold detection analysis follows manuscript section 2.4.1, where we ran several linear regressions on the data from each extraction method (Prepman Ultra, Chelex Resin, IBI gMAX mini extraction kit and Zymo Quick DNA miniprep). We used the whole data set for each extraction, the lower half and the upper half of the data set and compared R^2 values and the standard deviation of R^2 value using bootstrapping as described in the main text. We considered the best model to be the one with the highest R^2 value that the standard deviation did not overlap with the R^2 values and standard deviations of the other models.

For the Prepman Ultra and the Chelex resin analysis we combined all data from the extraction trials described here in Text S1 (Chelex resin with and without Protinase K; Prepman Ultra with and without a cell homogenization step) because they were shown to be equivocal in results. We analyzed the data as a whole (100, 1000, 10000 zoospores added), fewer zoospores added (100 and 1000 zoospores added) and more zoospores added (1000 and 10000 zoospores added).

For the IBI gMAX mini extraction kit and Zymo Quick DNA miniprep extraction kits we analyzed the data as a whole (100, 1000, 10000 zoospores added), fewer zoospores added (10 and 100 zoospores added) and more zoospores added (1000 and 10000 zoospores added). Because the sample size for these analyses was smaller, we only performed bootstrapping analysis for the R^2 values for the whole data set analysis.

Results

The accuracy of DNA copies detected under Prepman Ultra extraction methods did not increase with higher loads added to the extraction, indicating that if *Bd* DNA was detected in the well, the quantity reported by qPCR was accurate (Fig S3a) (lm, whole data set: $F_{1,16} = 49.92$, $p < 0.001$, $R^2 = 0.742$, bootstrapping sd = 0.226; from 100–1000 zoospores added: $F_{1,10} = 14.57$, $p = 0.003$, $R^2 = 0.552$, bootstrapping sd = 0.104; from 1000–10000 zoospores added: $F_{1,10} = 23$, $p < 0.001$, $R^2 = 0.667$, bootstrapping sd = 0.203).

Similarly, the accuracy of DNA copies detected under Chelex extraction methods did not increase with higher loads Fig S3b; lm, whole data set: $F_{1,16} = 70.55$, $p < 0.001$, $R^2 = 0.804$, bootstrapping sd = 0.239; from 100–1000 zoospores added: $F_{1,10} = 38.15$, $p < 0.001$, $R^2 = 0.772$, bootstrapping sd = 0.419; from 1000–10000 zoospores added: $F_{1,10} = 36.50$, $p < 0.001$, $R^2 = 0.763$, bootstrapping sd = 0.154).

The accuracy of DNA copies detected in both the IBI gMAX mini extraction kit and Zymo Quick DNA miniprep extraction kits mirrored what was found in the Qiagen DNeasy Blood and Tissue extraction results (Fig 1), where accuracy in quantity detected is low when few zoospores are present in the sample (Fig S3c,d). The R^2 value was lowest when low loads were added to the sample (10–100 zoospores) for both extraction methods. While it is possible to detect *Bd* at low loads (under 100 zoospores within the sample), the load estimation is accurate only at higher loads (100 zoospores or more within the sample). (IBI gMAX mini extraction kit: lm, full data set: $F_{1,10} = 154.30$, $p < 0.001$, $R^2 = 0.933$, bootstrapping sd = 0.140; from 10–100 zoospores added: $F_{1,4} = 35.690$, $p = 0.004$, $R^2 = 0.874$; from 1000–10000 zoospores added: $F_{1,4} = 360.7$, $p < 0.001$, $R^2 = 0.986$; Zymo Quick DNA miniprep extraction kit, lm, full data set: $F_{1,10} = 196.2$, $p < 0.001$, $R^2 = 0.947$, bootstrapping sd = 0.132; from 10–100 zoospores added: $F_{1,4} = 81.30$, $p = 0.001$, $R^2 = 0.941$; from 1000–10000 zoospores added: $F_{1,4} = 201.4$, $p < 0.001$, $R^2 = 0.976$).

Supplemental Figures S1-S3

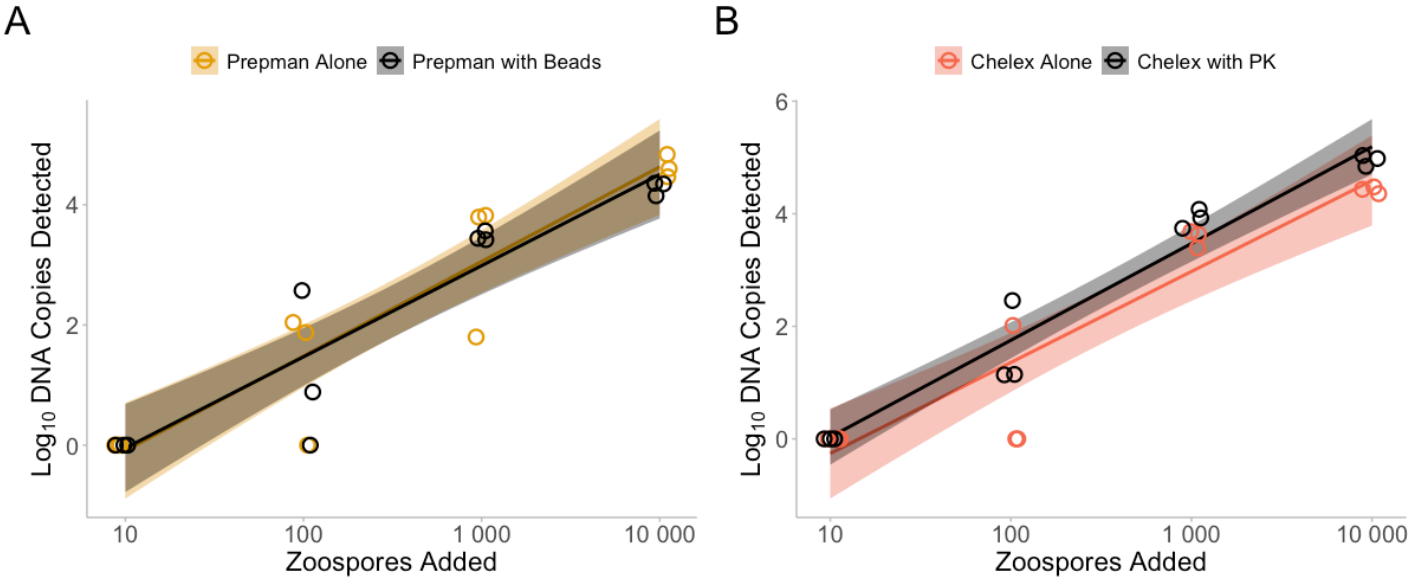

Figure S1. Comparison of extraction efficacy using two variations of A) PrepMan Ultra and B) Chelex Resin extraction methods. A) Prepman Ultra extraction was conducted using beads and a cell homogenizer (PrepMan with Beads), and without (PrepMan Alone). B) Chelex resin extraction was conducted using proteinase K (Chelex with PK), and without (Chelex Alone). Each point represents an individual sample (averaged across triplicate qPCR reactions), the lines represent the smoothed conditional means, and shaded area represent standard error of each extraction method.

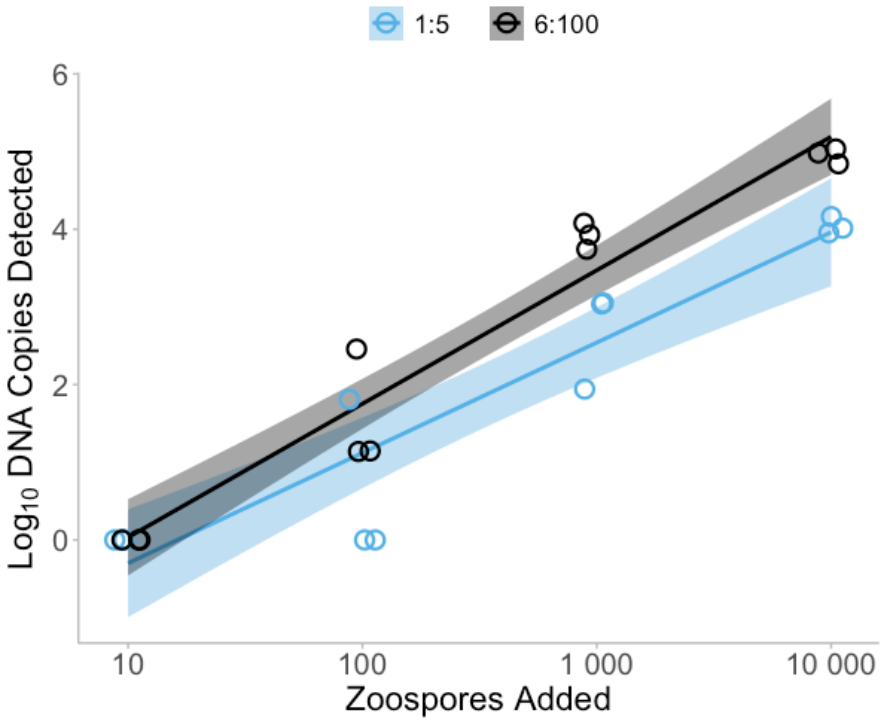

Figure S2. Comparison of DNA template dilution prior to qPCR for the Chelex with Proteinase K extraction method. The DNA template was diluted 6:100 and 1:5 in molecular grade water. Each point represents an individual sample (averaged across triplicate qPCR reactions), the lines represent the smoothed conditional means, and shaded area represent standard error of each extraction method.

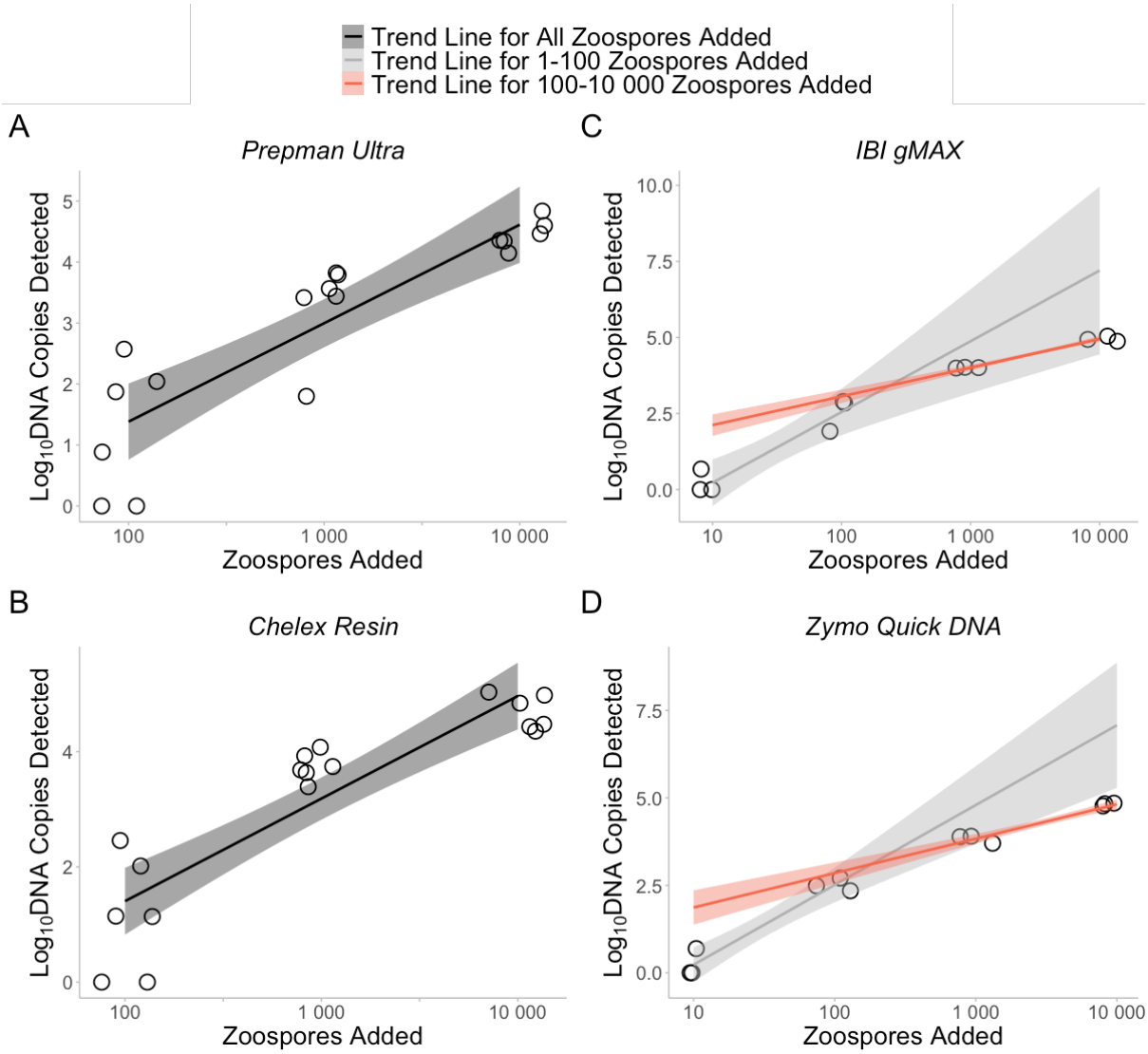

Fig S3. Threshold detection for all extraction methods. A) Prepman Ultra and B) Chelex resin, where detection accuracy is similar for different concentrations of zoospores added to the extraction, indicating that as long as the DNA is detected via qPCR, the quantitative results will be accurate. The black line represents the smoothed conditional means of the whole data set, and shaded area represent standard error of the extraction method. C) IBI gMAX mini extraction kit and D) Zymo Quick DNA miniprep extraction kits, where detection accuracy is worse at low loads. The two trend lines represent the trend lines for low numbers of *Bd* zoospores added to the samples (10–100 zoospores added, grey line), and high numbers of *Bd* zoospores added to the samples (1000–10000 zoospores added, pink line). Both trend lines have been extrapolated to the whole range of data. The lines represent the smoothed conditional means, and shaded area represent standard error of the extraction method.

Supplementary Tables S1-S3

Table S1| Summary of the results from the three cultured *Bd* extraction trials: Qiagen extraction threshold detection, non-spin column trial, spin column trial. Total number of qPCR reaction wells for each dilution is 9: each dilution was extracted in 3 samples, and each sample was analyzed in 3 qPCR reaction wells.

Dilution	*Bd* zoospores added (log)	*Bd* DNA quantity (log_{10})	Number of samples +ve	Number of wells +ve
Threshold experiment				
A	Negative Control	0	0	0
	–1	0	0	0
	0	0.86	2	3
	1	1.05	3	4
	2	3.48	3	9
	3	4.61	3	9
	4	5.57	3	9
B	Negative Control	0	0	0
	–1	0	0	0
	0	0.24	1	1
	1	1.13	2	4
	2	3.42	3	9
	3	4.32	3	9
	4	5.25	3	9
Non-spin column extraction				
Chelex	Negative Control	0	0	0
	–1	0	0	0
	0	0	0	0
	1	0	0	0
	2	1.58	3	4
	3	3.91	3	9
	4	4.95	3	9
Prepman	Negative Control	0	0	0
	–1	0	0	0
	0	0	0	0
	1	0	0	0
	2	1.26	3	5
	3	2.88	3	9
	4	3.76	3	9
Qiagen	Negative Control	0	0	0
	–1	0	0	0
	0	0	0	0
	1	0	0	0
	2	0.86	2	2
	3	3.47	3	9
	4	4.28	3	9
Spin column extraction				
IBI	Negative Control	0	0	0
	–1	0	0	0
	0	0	0	0
	1	0.22	1	1
	2	2.55	3	8
	3	4.01	3	9
	4	4.95	3	9
Zymo	Negative Control	0	0	0
	–1	0	0	0
	0	0	0	0
	1	0.23	1	1
	2	2.51	3	9

Dilution	*Bd* zoospores added (log)	*Bd* DNA quantity (log_{10})	Number of samples +ve	Number of wells +ve
	3	3.83	3	9
	4	4.82	3	9
Qiagen	Negative Control	0	0	0
	–1	0	0	0
	0	0	0	0
	1	0.28	1	1
	2	2.66	3	9
	3	4.90	3	9
	4	5.57	3	9

Table S2| Summary of results from the comparison of Prepman and Qiagen extracted field swabs. Each individual was doubly swabbed, and the swab was randomly assigned to either Qiagen or Prepman extraction. for each extracted swab, it was analyzed in triplicated using qPCR. Number of wells +ve refers to the number of triplicate reaction wells that returned a *Bd* positive result. qPCR reaction wells were considered positive if there were at least 2 DNA copies present in the reaction well. Sample *Bd* quantity is an average of all reaction wells (3 in total) for that sample.

ID	Species	Prepman extraction		Qiagen extraction	
		Bd quantity (log_{10})	Number of wells +ve	*Bd* quantity (log_{10})	Number of wells +ve
1	*Rana clamitans*	3.490	3	2.996	3
2	*Rana clamitans*		0	1.839	2
3	*Rana clamitans*		0	1.865	2
4	*Rana clamitans*		0	1.857	2
5	*Rana clamitans*		0	3.351	3
6	*Rana clamitans*		0	2.497	3
7	*Rana clamitans*		0	3.484	3
8	*Rana clamitans*		0	3.044	3
9	*Rana clamitans*	3.448	3	3.165	3
10	*Rana clamitans*	0.880	1	3.110	3
11	*Rana clamitans*		0	3.265	3
12	*Rana clamitans*		0	3.059	3
13	*Rana clamitans*		0	2.942	3
14	*Rana clamitans*	4.287	3	3.969	3
15	*Rana clamitans*		0	3.000	3
16	*Rana clamitans*	2.969	3	3.152	3
17	*Rana clamitans*	4.537	3	3.669	3
18	*Rana clamitans*		0	3.165	3
19	*Rana clamitans*		0	3.260	3
20	*Rana clamitans*	4.212	3	3.647	3
22	*Rana clamitans*	3.507	3	3.520	3
23	*Rana clamitans*		0	4.055	3
24	*Rana clamitans*		0	3.733	3
25	*Rana clamitans*		0	3.386	3
26	*Rana clamitans*	3.425	3	3.670	3
27	*Rana clamitans*		0	3.690	3
28	*Rana clamitans*	3.557	3	3.481	3
29	*Rana clamitans*	3.509	3	6.093	3
30	*Rana clamitans*		0	3.398	3
31	*Rana clamitans*	1.948	2	3.532	3
32	*Rana clamitans*		0	3.155	3
33	*Rana clamitans*		0	3.128	3
34	*Rana clamitans*		0	3.266	3
35	*Rana catesbeiana*	4.986	3	3.347	3
36	*Rana catesbeiana*	3.713	3	4.288	3
37	*Rana catesbeiana*	4.612	3	3.865	3
38	*Rana catesbeiana*	4.815	3	3.854	3
39	*Rana catesbeiana*	2.686	3	3.243	3

Table S3| Summary of results from the comparison of Zymo and Qiagen extracted field swabs. Each individual was swabbed with 6 swabs, and the swabs were was randomly assigned to either Qiagen or Zymo extraction. For each extracted swab, it was analyzed in triplicated using qPCR. Number of wells + refers to the number of triplicate reaction wells that returned a *Bd* positive result (a total of 9 wells were analyzed per individual – 3 samples extracted per individual per extraction method, and each sample was analyzed in 3 qPCR reaction wells), and number of samples + refers to the number of swabs for which at least 2 reaction wells were positive.

ID	Species	Zymo extraction			Qiagen extraction		
		Bd quantity (log_{10})	Number of samples +ve	Number of wells +ve	*Bd* quantity (log_{10})	Number of samples +ve	Number of wells +ve
1	*Rana sphenocephala*	4.858	3	9	5.230	3	9
2	*Rana catesbeiana*	5.058	3	9	4.970	3	9
3	*Rana catesbeiana*	4.615	3	9	4.639	3	9
4	*Rana catesbeiana*	4.143	3	9	4.650	3	9
5	*Rana catesbeiana*	3.299	3	9	3.443	3	9
6	*Rana catesbeiana*	3.357	3	9	3.769	3	9
7	*Rana catesbeiana*	1.288	2	5	0.773	2	3
8	*Rana catesbeiana*	0.880	1	3	0.823	2	3
9	*Rana catesbeiana*	0.282	1	1	0.941	1	3
10	*Rana catesbeiana*	0.243	1	1	0.871	1	3
11	*Rana catesbeiana*	0	0	0	0.535	1	2
12	*Rana catesbeiana*	0	0	0	0	0	0
13	*Rana catesbeiana*	0	0	0	0	0	0
14	*Rana catesbeiana*	0	0	0	0	0	0