02.07.2017

Preliminary Study of *Babesia gibsoni*

1. Background

Biogal approached the Vector Borne Laboratory at North Carolina University to participate in a small study relating to *Babesia gibsoni*. The laboratory supplied 40 freshly prepared DNA samples (100 µl each) extracted from whole blood samples present in their archives. According to the protocol, the sample group consisted of 20 positive, 10 negative for all VBD and 10 positive for a VBD other than *B. gibsoni*. The DNA was transferred to Biogal following Real Time PCR analysis and sequencing of the amplicons. The samples were sent from NC 23.06.17 and arrived at Biogal on the 29th of June. They were maintained at 4^0C until released and arrived at Biogal in a Styrofoam container containing several cooling bags in good condition.

2. Method:

Each sample was tested once using standard PCRun protocol for DNA samples.

Product	Lot #
PCRun Babesia gibsoni	160927
PCRun Babesia canis	170125
PCRun Anaplasma platys	160913
PCRun Ehrlichia canis	1619301
PCRun Buffer	161207
Ustar	20170118-32
MGW	1534354

3. Results

Run # 170703AA	PDQ# 18															
Well	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
SampleID	IPRL BG1	IPRL BG2	IPRL BG3	IPRL BG4	IPRL BG5	IPRL BG6	IPRL BG7	IPRL BG8	IPRL BG9	IPRL BG10	IPRL BG11	IPRL BG12	IPRL BG13	IPRL BG14	Ctr+	Ctr-
Tmax	0 *	22	0	12	0	0	17	15	0	0	0	11	0	20	13	0
Pmax	1227	3668	56	3129	520	1047	4486	2148	8.4	119	230	2924	579	5539	7767	0
Ustar	+		-		-	-			-	-	-		-			

Run # 170703AA	PDQ# 27															
Well	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
SampleID	IPRL BG15	IPRL BG16	IPRL BG17	IPRL BG18	IPRL BG19	IPRL BG20	IPRL BG21	IPRL BG22	IPRL BG23	IPRL BG24	IPRL BG25	IPRL BG26	IPRL BG27	IPRL BG28	Ctr+	Ctr-
Tmax	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	0
Pmax	3	48	57	91	89	73	197	353	130	881	45	291	47	24	2540	805
Ustar	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-

Run # 170703AA	PDQ#05															
Well	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
SampleID	IPRL BG29	IPRL BG30	IPRL BG31	IPRL BG32	IPRL BG33	IPRL BG34	IPRL BG35	IPRL BG36	IPRL BG37	IPRL BG38	IPRL BG39	IPRL BG40	Ctr+	Ctr-		
Tmax	14	15	17	17	15	12	16	13	15	19	15	16	12	0		
Pmax	1559	3035	2562	1582	1275	1773	2406	3130	2338	1544	2207	2866	4062	130		
Ustar																

Babesia gibsoni samples repeated

Run # 170704 AC	PDQ# 5															
Well	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
SampleID	IPRL BG1	IPRL BG2	IPRL BG5	IPRL BG6	IPRL BG15	IPRL BG16	IPRL BG17	IPRL BG18	IPRL BG19	IPRL BG20	IPRL BG21	IPRL BG22	Ctr+	Ctr-		
Tmax	25	20	0	0	0	0	0	0	0	0	0	0	13	25		
Pmax	1364	3317	36	310	0	2	49	186	0	13	0	282	5615	1364		
Ustar																

Run # 17075AA								
Well	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
SampleID	IPRL BG23	IPRL BG24	IPRL BG25	IPRL BG26	IPRL BG27	IPRL BG28	Ctrl -	Ctrl +
Tmax	0	0	0	0	0	0	0	12
Pmax	137	442	445	382	0	121	401	5669
Ustar								

Ehrlichia canis

Run # 170704AB	PDQ# 18															
Well	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
SampleID	IPRL BG1	IPRL BG2	IPRL BG3	IPRL BG4	IPRL BG5	IPRL BG6	IPRL BG7	IPRL BG8	IPRL BG9	IPRL BG10	IPRL BG11	IPRL BG12	IPRL BG13	IPRL BG14	Ctr+	Ctr-
Tmax	0	0	0	0	0	0	0	68	0	0	0	0	0	0	16	0
Pmax	0	268	161	0	14	486	124	2100	0	59	172	0	81	0	5381	4
Ustar								-								

Run # 170704AB	PDQ# 27															
Well	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
SampleID	IPRL BG15	IPRL BG16	IPRL BG17	IPRL BG18	IPRL BG19	IPRL BG20	IPRL BG21	IPRL BG22	IPRL BG23	IPRL BG24	IPRL BG25	IPRL BG26	IPRL BG27	IPRL BG28	Ctr+	Ctr-
Tmax	0	0	0	0	0	0	0	0	0	0	32	0	36	0	16	0
Pmax	61	0	0	0	2	609	0	80	0	320	2040	116	1341	0	4146	0
Ustar						-										

Run # 170704AB	PDQ#05															
Well	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
SampleID	IPRL BG29	IPRL BG30	IPRL BG31	IPRL BG32	IPRL BG33	IPRL BG34	IPRL BG35	IPRL BG36	IPRL BG37	IPRL BG38	IPRL BG39	IPRL BG40	Ctr+	Ctr-		
Tmax	0	0	0	0	0	0	0	0	0	0	0	0	16	0		
Pmax	0	0	0	0	0	0	0	0	0	526	0	67	3123	75		
Ustar										-						

Anaplasma platys

Run # 170704AA	PDQ# 18															
Well	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
SampleID	IPRL BG1	IPRL BG2	IPRL BG3	IPRL BG4	IPRL BG5	IPRL BG6	IPRL BG7	IPRL BG8	IPRL BG9	IPRL BG10	IPRL BG11	IPRL BG12	IPRL BG13	IPRL BG14	Ctr+	Ctr-
Tmax	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	0
Pmax	513	487	421	184	223	720	421	427	336	409	562	590	723	215	4478	501
Ustar																

Run # 170704AA	PDQ# 27															
Well	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
SampleID	IPRL BG15	IPRL BG16	IPRL BG17	IPRL BG18	IPRL BG19	IPRL BG20	IPRL BG21	IPRL BG22	IPRL BG23	IPRL BG24	IPRL BG25	IPRL BG26	IPRL BG27	IPRL BG28	Ctr+	Ctr-
Tmax	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	0
Pmax	375	248	319	333	244	106	335	544	552	641	316	388	255	191	4424	340
Ustar																

Run # 170704AA	PDQ#05															
Well	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
SampleID	IPRL BG29	IPRL BG30	IPRL BG31	IPRL BG32	IPRL BG33	IPRL BG34	IPRL BG35	IPRL BG36	IPRL BG37	IPRL BG38	IPRL BG39	IPRL BG40	Ctr+	Ctr-		
Tmax	0	0	0	0	0	0	0	0	0	0	0	0	16	0		
Pmax	236	168	244	191	173	0	47	89	199	674	126	344	2720	391		
Ustar										-						

Babesia canis

Run # 170703AB	PDQ# 18															
Well	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
SampleID	IPRL BG1	IPRL BG2	IPRL BG3	IPRL BG4	IPRL BG5	IPRL BG6	IPRL BG7	IPRL BG8	IPRL BG9	IPRL BG10	IPRL BG11	IPRL BG12	IPRL BG13	IPRL BG14	Ctr+	Ctr-
Tmax	0	0	0	0	0	0	0	0	0	0	0	0	0	0	41	0
Pmax	0	488	634	65	208	809	349	371	118	486	442	0	377	0	5958	231
Ustar																

Run # 170703AB	PDQ# 27															
Well	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
SampleID	IPRL BG15	IPRL BG16	IPRL BG17	IPRL BG18	IPRL BG19	IPRL BG20	IPRL BG21	IPRL BG22	IPRL BG23	IPRL BG24	IPRL BG25	IPRL BG26	IPRL BG27	IPRL BG28	Ctr+	Ctr-
Tmax	0	0	0	0	0	0	0	0	0	0	0	0	0	0	35	0
Pmax	171	162	116	32	226	16	274	336	212	611	159	346	76	13	2032	276
Ustar																

Run # 17073AB	PDQ#05															
Well	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
SampleID	IPRL BG29	IPRL BG30	IPRL BG31	IPRL BG32	IPRL BG33	IPRL BG34	IPRL BG35	IPRL BG36	IPRL BG37	IPRL BG38	IPRL BG39	IPRL BG40	Ctr+	Ctr-		
Tmax	0	0	0	0	0	0	0	0	0	0	0	0	41	0		
Pmax	83	89	133	153	128	133	0	103	0	616	50	54	4136	174		
Ustar																

All the negative tests samples were repeated using the standard PCRun protocol.

Results

Summary

1. All of the samples remained negative which would imply that they are truly negative.

All PCRun Reader results can be examined at the following site.

J:\PCRUN\Feild studies\US\NCSU\Babesia gibsoni preliminary\Results Raw Data

Summary

1. Nineteen samples out of 40 were positive for *Babesia gibsoni*. The expected number was 20. The negative samples were repeated and none were found to be positive for *B. gibsoni.*
2. Two samples were positive for *E. canis*. Both after 30 min (32 and 36 min). One late positive was recorded at 68 min. Two additional very late positives (after 70 min) appeared in the Ehrlichia samples.
3. No *A. platys* or *B. canis* were observed.

7.7.2017

Results were received from NCSU. The following table contains all of the results. Following the table is a list of comments by Riccardo.

	BIOGAL (time to peak)				VBDDL (Ct values)					
Sample Code	B. gibsoni	E. canis	A. platys	B. canis	BAB sp.	BART sp.	ANAP sp.	EHRL sp.	MYCO sp.	RICK sp.
IPRL-BG1	25	-	-	-	Bgibs27.0	-	-	-	-	-
IPRL-BG2	22	-	-	-	Bgibs25.3	-	-	-	-	-
IPRL-BG3	-	-	-	-	Bgibs24.6	-	-	-	-	-
IPRL-BG4	12	-	-	-	Bgibs26.5	-	-	-	-	-
IPRL-BG5	-	-	-	-	Bcan42.2	-	-	-	-	-
IPRL-BG6	-	-	-	-	-	-	Aphago	-	-	-
IPRL-BG7	17	-	-	-	Bgibs24.3	-	-	-	-	-
IPRL-BG8	15	-	-	-	Bgibs24.6	-	-	-	-	-
IPRL-BG9	-	-	-	-	Bcan37.6	-	-	-	-	-
IPRL-BG10	-	-	-	-	-	-	Aphago	-	-	-
IPRL-BG11	-	-	-	-	-	-	Aphago	-	-	-
IPRL-BG12	11	-	-	-	Bgibs26.6	-	-	-	-	-
IPRL-BG13	-	-	-	-	-	-	Aphago	-	-	-
IPRL-BG14	20	-	-	-	Bgibs27.3	-	-	-	-	-
IPRL-BG15	-	-	-	-	-	-	-	-	-	-
IPRL-BG16	-	-	-	-	-	-	-	-	-	-
IPRL-BG17	-	-	-	-	-	-	-	-	-	-
IPRL-BG18	-	-	-	-	-	-	-	-	-	-
IPRL-BG19	-	-	-	-	-	-	-	-	-	-
IPRL-BG20	-	-	-	-	-	-	-	-	-	-
IPRL-BG21	-	-	-	-	-	-	-	-	-	-
IPRL-BG22	-	-	-	-	-	-	-	-	-	-
IPRL-BG23	-	-	-	-	-	-	-	-	-	-
IPRL-BG24	-	-	-	-	-	-	-	-	-	-
IPRL-BG25	-	32	-	-	-	-	-	Ecan	-	-
IPRL-BG26	-	-	-	-	-	-	-	Ecan	-	-
IPRL-BG27	-	35	-	-	-	-	-	Ecan	-	-
IPRL-BG28	-	-	-	-	-	-	-	Ecan	-	-
IPRL-BG29	14	-	-	-	Bgibs28.2	-	-	-	-	-
IPRL-BG30	15	-	-	-	Bgibs29.4	-	-	-	-	-
IPRL-BG31	17	-	-	-	Bgibs28.2	-	-	-	-	-
IPRL-BG32	17	-	-	-	Bgibs27.2	-	-	-	-	-
IPRL-BG33	15	-	-	-	Bgibs27.9	-	-	-	-	-
IPRL-BG34	12	-	-	-	Bgibs26.7	-	-	-	-	-
IPRL-BG35	16	-	-	-	Bgibs30.1	-	-	-	-	-
IPRL-BG36	13	-	-	-	Bgibs30.0	-	-	-	-	-
IPRL-BG37	15	-	-	-	Bgibs30.1	-	-	-	-	-
IPRL-BG38	19	-	-	-	Bgibs28.4	-	-	-	-	-
IPRL-BG39	15	-	-	-	Bgibs29.0	-	-	-	-	-
IPRL-BG40	16	-	-	-	Bgibs27.6	-	-	-	-	-

- **Ct values are noted along Sp. detected (Babesia) for reference.**
- **Samples positive for B. gibsoni were all Asian genotype.**
- **Anaplasma and Ehrlichia PCR were ran using conventional PCR**
- **Bartonella, Mycoplasma, and Rickettsia were ran using real-time PCR.**
- **Samples positive for E. canis had good chromatograms (high intensity peaks) except for sample IPRL-BG26 that had a relatively low intensity chromatogram (but clean and good for reading).**

Comparative summary of NSU results and Biogal Results

	Results							
	B. gibsoni		E. canis		A. platys		B. canis	
Sample Code	PCRun	NCSU	PCRun	NCSU	PCRun	NCSU	PCRun	NCSU
IPRL-BG1	25	27	-		-		-	
IPRL-BG2	22	25.3	-		-		-	
IPRL-BG3	-	24.6	-		-		-	
IPRL-BG4	12	26.5	-		-		-	
IPRL-BG5	-		-		-		-	42.2
IPRL-BG6	-		-		-	Aphago	-	
IPRL-BG7	17	24.3	-		-		-	
IPRL-BG8	15	24.6	-		-		-	
IPRL-BG9	-		-		-		-	37.6
IPRL-BG10	-		-		-	Aphago	-	
IPRL-BG11	-		-		-	Aphago	-	
IPRL-BG12	11	26.6	-		-		-	
IPRL-BG13	-		-		-	Aphago	-	
IPRL-BG14	20	27.3	-		-		-	
IPRL-BG15	-		-		-		-	
IPRL-BG16	-		-		-		-	
IPRL-BG17	-		-		-		-	
IPRL-BG18	-		-		-		-	
IPRL-BG19	-		-		-		-	
IPRL-BG20	-		-		-		-	
IPRL-BG21	-		-		-		-	
IPRL-BG22	-		-		-		-	
IPRL-BG23	-		-		-		-	
IPRL-BG24	-		-		-		-	
IPRL-BG25	-		32	Ecanis	-		-	
IPRL-BG26	-		-	Ecanis	-		-	
IPRL-BG27	-		35	Ecanis	-		-	
IPRL-BG28	-		-	Ecanis	-		-	
IPRL-BG29	14	28.2	-		-		-	
IPRL-BG30	15	29.4	-		-		-	
IPRL-BG31	17	28.2	-		-		-	
IPRL-BG32	17	27.2	-		-		-	
IPRL-BG33	15	27.9	-		-		-	
IPRL-BG34	12	26.7	-		-		-	
IPRL-BG35	16	30.1	-		-		-	
IPRL-BG36	13	30.0	-		-		-	
IPRL-BG37	15	30.1	-		-		-	
IPRL-BG38	19	28.4	-		-		-	
IPRL-BG39	15	29.0	-		-		-	
IPRL-BG40	16	27.6	-		-		-	
Pos Ctrl 1	13		15		15		40	
Pos Ctrl 2	14		15		14		33	
Pos Ctrl 3	12		16		15		40	
Neg Ctrl 1	-		-		-		-	

Neg Ctrl 2	-		-		-		-	
Neg Ctrl 3	-		-		-		-	

Summary

1. Nineteen samples of *B. gibsoni* were positive by PCRun. Sample BG-3 which had a relative low Cq (24.6) was not identified by PCRun.
2. The two samples that were positive by RT for *B. canis* (BG5 and BG9) had very late Cqs (42.2 and 37.6) were not identified by PCRun.
3. Four samples were determined to be *E. canis* by RT (BG25-28,) but only two were identified by PCrun (BG25 and 27). Both PCRun amplicons gave relatively late TTP readings (32 and 35).

Calculation of sensitivity, specificity and accuracy

Sensitivity = 95%

Specificity = 100%

Accuracy = 97.6%