SRAS Final Report: Penside Detection of Anti-Kunjin Virus Antibodies in Farmed Saltwater Crocodiles Using the ChimerDx Lateral Flow Test Platform

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Abstract

The endemic Australian strain of West Nile virus, Kunjin virus (WNV_{KUNV}), causes economically significant skin lesions ('pix') in farmed saltwater crocodiles (*Crocodylus porosus*). Rapid, field-deployable serosurveillance tools are critical for monitoring WNV_{KUNV} exposure in crocodile farm populations, yet no penside diagnostic currently exists for this purpose. Here we report the first field evaluation of the ChimerDx lateral flow test (LFT) platform for the detection of anti-WNV_{KUNV} IgY antibodies in farmed crocodile blood samples, conducted at a crocodile farm in the Northern Territory, over four consecutive days (21–24 April 2026). Testing was performed on 88 animals in triplicate using the Lumos Diagnostics LeeLu portable reader with positive and negative serum controls included for each batch of samples tested. Sample cohorts included harvest-sized crocodiles (2-3 years) and euthanised hatchlings with developmental defects. A side-by-side comparison was also performed, evaluating the in-house developed 5H4 anti-crocodile IgY monoclonal antibody (mAb) against a commercially sourced anti-alligator polyclonal antibody (pAb). All 82 crocodiles tested negative for antibodies to WNV_{KUNV}, consistent with expected seronegative status in farm-raised animals without prior flavivirus exposure. Three of five euthanised hatchlings returned positive results, with two further hatchlings equivocal, attributable to maternally transferred IgY. Positive control serum performance was consistent throughout, with capture line values of 0.22–0.50 peak above background, contrasting clearly with the uniformly near-zero values in harvest sized cohorts. The 5H4 mAb gold conjugate used as the LFT detector, substantially outperformed the anti-alligator pAb conjugate, which produced elevated non-specific background and discordant results on samples that were clearly negative with the mAb. These results demonstrate the suitability of the ChimerDx LFT for penside WNV_{KUNV} serosurveillance in commercial crocodile farm settings under ambient tropical field conditions.

Introduction

Kunjin virus (WNV_{KUNV}) is the Australian variant of West Nile virus (WNV), a mosquito-transmitted flavivirus that circulates endemically in northern Australia. While WNV_{KUNV} rarely causes severe clinical disease in humans or horses [1], it poses a significant economic threat to the saltwater crocodile (Crocodylus porosus) farming industry, where infection is associated with characteristic cutaneous lesions known as 'pix' that permanently devalue crocodile hides [2, 3]. The role of WNV_{KUNV} and related flaviviruses in crocodile farm disease has been the subject of active research, and seroprevalence surveys in Northern Territory (NT) farms have demonstrated widespread exposure among adult farmed crocodiles [4]. Despite this, no rapid field diagnostic currently exists to support penside serosurveillance on crocodile farms.

The ChimerDx platform is a chimeric insect-specific virus (ISV)-based antigen technology developed at the University of Queensland for the detection and characterisation of flavivirus-specific antibodies in vertebrate sera [5]. The platform exploits chimeric virus particles that present authentic flavivirus envelope antigens on a replication-incompetent ISV backbone, generating highly specific antigens suitable for lateral flow test (LFT) applications. Previous work has demonstrated the utility of ChimerDx LFTs for the detection of anti-WNV/WNV_{KUNV} antibodies in crocodile serum under laboratory conditions [5].

The present study describes the first deployment of the ChimerDx WNV_{KUNV} LFT in a field setting, conducted penside at a commercial saltwater crocodile farm in the NT during April 2026. The

objectives of this evaluation were to: (1) assess the performance of the ChimerDx LFT across diverse crocodile cohorts under ambient tropical field conditions; (2) determine the serostatus of farm animals across several pens; (3) evaluate the utility of euthanised hatchlings with developmental defects as biological low-positive controls by virtue of maternally transferred IgY; and (4) compare the performance of an in-house anti-crocodile mAb conjugate against a commercially sourced anti-alligator pAb conjugate.

Methods

Study Site and Animals

Testing was conducted at a crocodile farm in the NT, Australia, over four consecutive days (21–24 April 2026). Ambient temperatures ranged from 22–25°C across testing days. Sampled cohorts included: (i) 82 harvest sized crocodiles (2-3 years of age); and (ii) six euthanised hatchlings with developmental defects (extended unabsorbed yolk sac) from four wild-harvested clutches. A total of 88 field samples were collected, with blood drawn from the dorsal cervical sinus after euthanasia. Known positive control sera from two previously confirmed WNV_{KUNV} seropositive crocodiles (samples 22 and 25) and negative control sera from two flavivirus seronegative animals (samples 1 and 2) were included in each testing session.

ChimerDx Lateral Flow Test

The ChimerDx WNV_{KUNV} LFT strip is based on a chimeric Binjari virus/WNV_{KUNV} (BinJV/WNV_{KUNV}) antigen applied at the capture line, with an anti-mouse IgG antibody (or anti-goat IgG for the conjugate comparison) at the control line. Detection is mediated by crocodile IgY antibodies in the sample binding to the chimeric antigen, captured by a gold nanoparticle-conjugated anti-crocodile monoclonal antibody (5H4). Two conjugate formats were evaluated: (i) the 5H4 anti-crocodile mAb (raised in mouse); and (ii) an anti-alligator polyclonal antibody (pAb, raised in goat). Each sample was run in triplicate. Strips were read using the Lumos Diagnostics LeeLu portable strip reader, which generates a pixel-intensity scan profile and reports a peak-above-background value for each line.

Blocking ELISA confirmation assessment

A blocking ELISA was performed to assess the presence of West Nile virus (WNV_{KUNV} strain) antibodies in the sera as demonstrated by Habarugira et.al [3]. Round-bottom 96-well plates were coated overnight at 4°C with 50 µL/well of WNV_{KUNV} cell lysate (batch CA130, F3S3R2B20) diluted 1/400 in coating buffer, with no-antigen control wells receiving coating buffer alone. Following three washes with PBST, plates were blocked with 150 µL/well of blocking buffer for 2 hours at room temperature. Test sera and controls were pre-diluted 1/5 in blocking buffer in a separate 96-well plate and incubated overnight at 4°C, with a 1/200 diluted positive crocodile control included on each plate. Diluted sera (50 µL/well) were then added in duplicate and incubated at 28°C for 1 hour, after which 50 µL/well of either anti-flavivirus E protein monoclonal antibody 6B6C-1 (1/800) or WNV_{KUNV}-specific anti-NS1 mAb 3.1112G (1/1000), diluted in blocking buffer, was added directly to each well without prior removal of the sera. After a further 1-hour incubation at 28°C, the sera–antibody mixture was removed and plates were washed four times with PBST before addition of 50 µL/well of pre-adsorbed goat anti-mouse HRP conjugate (1/3000) for 1 hour at 28°C. Plates were washed six times with PBST and developed with 100 µL/well of ABTS substrate solution in the dark at room temperature, then read at 405 nm.

Classification Thresholds and Consensus

Capture line peak-above-background values were classified as: Negative (< 0.0125), Equivocal (0.0125–0.0500), or Positive (> 0.0500). Consensus results across triplicates were determined by majority classification (≥ 2 of 3 replicates). Triplicates where no single classification accounted for ≥ 2 replicates were flagged as discordant (*). Strips where the control line was absent from the reader analysis region were excluded as failed reads.

Results

Control Performance

Positive and negative controls were run in triplicate at the start of each session and repeated mid-session on 21 and 22 April. Both positive control samples produced strong, consistent capture line signals across all sessions (range: 0.22–0.50 above background; Figure 1), confirming adequate assay sensitivity. Negative controls produced capture line values within the negative threshold (< 0.0125), confirming specificity and absence of non-specific binding under field conditions. Control line peaks were consistently in the range 0.60–0.78 above background for all valid strips, confirming adequate conjugate flow and strip performance. One strip failure was recorded on 23 April (negative control, replicate 3, run counter 185), attributable to incorrect strip placement; the replicate was excluded and the remaining two returned valid negative results.

Field Sample Results — anti-crocodile IgY mAb 5H4 Conjugate

Results for all 88 field samples tested with the mAb 5H4 conjugate are presented in Appendix 1.

All 82 harvest-sized crocodiles returned negative consensus results. Capture line values were consistently at or below background (range: −0.026 to +0.015), with no samples crossing the equivocal threshold. Triplicate consistency was high across this cohort, with inter-replicate variation attributable to normal sampling and strip-to-strip variability. Two animals returned equivocal consensus results (Sample 22: capture line values 0.0144, 0.0326, 0.0025 and Sample 32: capture line values 0.0010, 0.0366, 0.0164). Both values remain well below the positive threshold. Three further harvest-sized animals (samples 7, 33 and 35) showed one discordant replicate each but returned negative consensus results overall.

Six euthanised hatchlings from four clutches were tested across 22–23 April. Three returned positive consensus results (samples 55-57), with capture line values ranging from 0.046 to 0.129. Three returned equivocal results (samples 53,54 and 58), with consistent signals across replicates in the range 0.031–0.048. No hatchlings returned negative results. The positive signal in hatchlings was markedly lower than in the adult positive controls (0.046–0.129 vs. 0.222–0.500), consistent with maternally transferred antibody at reduced titre relative to active seroconversion (Habarugira et al. 2025).

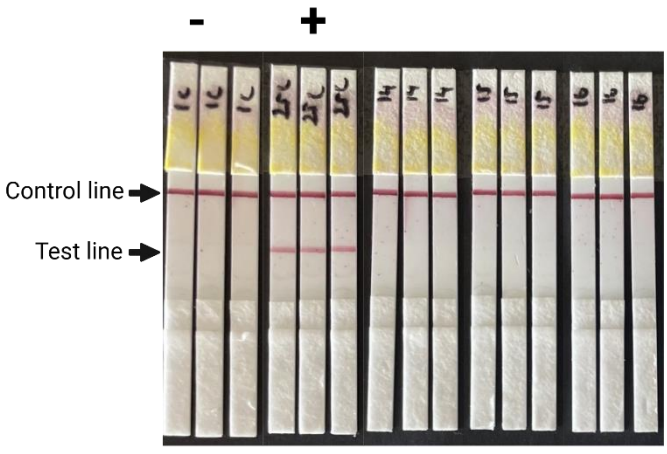


Figure 1. *Lateral flow assay (LFA) positive and negative controls for crocodile IgY detection across field deployment sessions. Representative LFA strips showing positive and negative control results run at the start of each session during field deployment. Strips are arranged in groups corresponding to individual sessions/dates as labelled. Arrows (left) denote the positions of the control line (upper) and capture line (lower). Positive control strips (exemplified by the second strip group) display both a visible control line and a distinct capture line, confirming adequate assay sensitivity. All remaining strips display a single control line only, consistent with negative control results and confirming assay specificity under field conditions. Yellow colouration at the sample pad end reflects normal conjugate pad appearance and is not indicative of strip failure. All displayed strips returned valid results.*

A subset of 16 crocodile sera was selected from the penside LFT screening cohort for confirmatory analysis by blocking ELISA (bELISA), including samples spanning the range of LFT capture line signal values as well as those flagged for further investigation (Appendix 1). Assay validity was confirmed by the positive controls (positive crocodile serum and positive horse serum) which returned inhibition values of 90.7% and 88.7%, respectively, while both negative controls returned values below the 30% inhibition threshold. Of the 16 samples tested, two (samples 55 and 56) had returned positive LFT results during penside screening. Both were confirmed positive by bELISA with 6B6C-1 (91.2% and 96.3% inhibition, respectively) and 3.1112G (38.4% and 84.5% inhibition, respectively), demonstrating full concordance between platforms for LFT-positive samples. Among the 14 LFT-negative samples, five exceeded the 30% inhibition threshold in bELISA (samples 36, 54, 57, 58 and 64), although sample 64 fell marginally below the 6B6C-1 threshold.

Notably, the hatchling samples from three of the four clutches (2, 3 and 4) accounted for most discordant results. These animals were deformed hatchlings, and elevated inhibition values likely reflect high levels of maternally derived antibody rather than active infection. One additional sample, (53), returned an elevated 6B6C-1 inhibition value (69.9%) but was considered unreliable due to severe haemolysis noted at time of testing. The remaining nine of the 16 samples (22, 24, 43, 62, 71, 74, 79, 87 and 88) were concordantly negative across both platforms. Overall, bELISA confirmed LFT positivity in all flagged samples, while additionally identifying a subset of LFT-negative animals with detectable WNV_{KUNV}-specific antibody inhibition above threshold, suggesting the bELISA has higher sensitivity in samples with lower antibody concentrations.

Conjugate Comparison — 24 April

Five samples (84-88) were tested in parallel with both the 5H4 mAb conjugate and the anti-alligator pAb conjugate (as demonstrated by Johnston et.al) [5], on 24 April. Results are presented in Table 1.

The 5H4 mAb conjugate returned unambiguous negative results across all five samples, with capture line values tightly clustered near zero (mean range: −0.005 to +0.007). The anti-alligator pAb conjugate produced elevated and variable background across all five samples. One sample (84) returned a discordant result with one replicate reaching 0.063 (above the positive threshold), and sample 87 returned an equivocal consensus (0.0356, 0.0313, −0.0057). All five samples were clearly negative with the mAb conjugate.

Table 1. *Side-by-side conjugate comparison on the same five samples. Standard = 5H4 anti-crocodile mAb (anti-mouse control line); alternative = anti-alligator pAb (anti-goat control line).*

Pen ID	Conjugate	Result	Capture Line Values	Notes
DC 27	5H4 anti-crocodile mAb	Negative	−0.0016, −0.0016, −0.0046	Clean negative
DC 28	5H4 anti-crocodile mAb	Negative	0.0010, 0.0073, −0.0018	Clean negative
DC 29	5H4 anti-crocodile mAb	Negative	−0.0029, 0.0013, 0.0002	Clean negative
DC 30	5H4 anti-crocodile mAb	Negative	−0.0001, −0.0017, 0.0032	Clean negative
DC 31	5H4 anti-crocodile mAb	Negative	−0.0051, −0.0049, 0	Clean negative
DC 27	Anti-alligator pAb	Discordant	0.0105, 0.0630, 0.0261	High replicate variance; one false positive
DC 28	Anti-alligator pAb	Negative	0.0135, 0.0056, 0.0003	Higher background than standard
DC 29	Anti-alligator pAb	Equivocal	0.0356, 0.0313, −0.0057	Elevated background in 2/3 reps
DC 30	Anti-alligator pAb	Negative	−0.0005, 0.0263, −0.0004	One elevated replicate
DC 31	Anti-alligator pAb	Negative	0.0183, −0.0095, −0.0009	Noisy across reps

Discussion

This study represents the first field evaluation of the ChimerDx LFT for penside WNV_{KUNV} serosurveillance in a commercial saltwater crocodile farm setting. Across four days of testing under ambient NT field conditions (22–25°C, 86-90% RH), the assay performed consistently and reliably, correctly classifying 82 harvest-sized animals as seronegative. These results are consistent with the expected seroprevalence pattern in unexposed crocodiles that have not had prior flavivirus exposure [6], providing evidence of field validity.

The detection of antibody signal in euthanised hatchlings is a particularly informative finding. As these animals were physically deformed and euthanised immediately after hatching without environmental exposure, any seroreactivity is necessarily attributable to maternally transferred IgY deposited in the egg yolk, as has been documented for anti-WNV_{KUNV} antibodies in *C. porosus* hatchlings [4, 7]. The consistent positive or equivocal signals across all five hatchlings, from multiple clutches, confirms that the wild dams of these animals carried anti-WNV_{KUNV} antibodies at the time of egg formation. Importantly, the hatchling signals (0.046–0.129) were clearly distinguishable from both the adult positive controls (0.222–0.500) and negative controls (< 0.015), demonstrating that the assay can resolve low-titre maternally transferred antibody from background noise at the point of care. This has practical implications for on-farm surveillance design, as it suggests that hatchling testing could serve as a sentinel for the immune status of breeding females without requiring invasive sampling of adults. In addition, it will inform the timing of vaccine schedules to coincide with when maternal antibodies have dissipated.

One disadvantage of the farm chosen to conduct this study on is that they have been implementing bird and mosquito control since 2020. A serology study conducted later in 2020 showed that the number of infected crocodiles had already been reduced to 4.5% {Habarugira, 2022 #1534}. As such, the lack of positive results in the harvest-sized crocodiles was not surprising although was disappointing for the purposes of this validation. Regardless, two harvest sized crocodiles (samples 22 and 23) returned equivocal consensus results, with values in the range 0.014–0.037. These results sit in the grey zone between negative and positive and could reflect low-level non-specific signal, sub-threshold seroconversion, or waning antibody from a prior exposure. Repeat testing of these animals is recommended to establish whether signal is persistent or transient.

The bELISA results broadly support the diagnostic utility of the LFT platform for penside WNV_{KUNV} surveillance in crocodilians, with full concordance observed between the two assays for LFT-positive samples. The discordant bELISA positivity observed in a subset of LFT-negative animals warrants consideration. For hatchlings in three of the four clutches, the high inhibition values across both monoclonal antibodies are most consistent with elevated maternally derived IgY rather than active seroconversion, a conclusion supported by the comparably high inhibition observed in the unabsorbed yolks. For samples 56 and 84, the discordant results may instead reflect sub-threshold antibody concentrations that fall below the LFT's limit of detection but remain detectable under the more analytically sensitive competitive format of the bELISA. Taken together, these data suggest that while the LFT performs well as a rapid screening tool, bELISA confirmation adds meaningful diagnostic resolution in cohorts where maternal antibody interference or low-titre responses are anticipated, for second pass (laboratory borne) analysis.

The conjugate comparison revealed a clear performance differential between the two detection antibodies evaluated. The 5H4 anti-crocodile mAb produced consistently clean, near-zero background on all five comparison samples, while the anti-alligator pAb generated substantially higher and more variable non-specific signal, resulting in one discordant false-positive and one equivocal result on samples that were unambiguously negative with the mAb. This elevated background is likely attributable to cross-reactivity of the polyclonal anti-alligator antibody with non-target crocodile IgY subclasses or serum proteins. The 5H4 mAb conjugate is therefore recommended for continued use in field deployments. Further optimisation of the anti-alligator pAb concentration, blocking buffer composition, and conjugation conditions may improve its specificity if an alternative conjugate is required.

Overall, this field evaluation demonstrates that the ChimerDx LFT platform is suitable for penside WNV_{KUNV} serosurveillance in crocodile farm settings. The assay is rapid, requires no cold chain or laboratory infrastructure, and is operable by farm staff with minimal training using the LeeLu portable reader. These characteristics make it well suited for integration into routine farm biosurveillance programmes in the Northern Territory, where WNV_{KUNV}-related pix lesions pose an ongoing economic risk to the industry. Although this testing was limited to WNV_{KUNV} as this is the endemic strain in Australia, the test has been designed to be effective on other WNV strains.

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Appendix 1

Table 1. *Field sample LFT results — standard conjugate (5H4 anti-crocodile mAb, anti-mouse control line). * Discordant triplicate; consensus determined by majority (≥ 2/3 replicates). *Capture line peak-above-background values: Negative (< 0.0125), Equivocal (0.0125–0.0500), or Positive (> 0.0500). **bELISA %inhibition >30% positive, <30% negative. Samples 1-26 were collected on 21st April, samples 27-53 on 22nd April, samples 54-83 on the 23rd April and samples 84-88 on the 24th April.*

Sample ID	Cohort	Result	Capture Line Values	6B6C-1 bELISA % inhibition	3.1112G bELISA % inhibition	Notes
1	Harvest-sized crocodile	Negative	−0.0015, −0.0086, −0.0115	N/A	N/A	
2	Harvest-sized crocodile	Negative	−0.0091, −0.0048, −0.0018	N/A	N/A	
3	Harvest-sized crocodile	Negative	−0.0034, −0.0114, −0.0116	N/A	N/A	
4	Harvest-sized crocodile	Negative	−0.0086, −0.0192, −0.0069	N/A	N/A	
5	Harvest-sized crocodile	Negative	−0.0092, −0.0013, −0.0051	N/A	N/A	
6	Harvest-sized crocodile	Negative	−0.0123, −0.0107, −0.0185	N/A	N/A	
7	Harvest-sized crocodile	Negative*	0.0040, −0.0006, 0.1001	N/A	N/A	One discordant replicate; 2/3 negative
8	Harvest-sized crocodile	Negative	−0.0058, −0.0123, −0.0052	N/A	N/A	
9	Harvest-sized crocodile	Negative	−0.0031, −0.0012, −0.0008	N/A	N/A	
10	Harvest-sized crocodile	Negative	−0.0082, −0.0074, −0.0056	N/A	N/A	
11	Harvest-sized crocodile	Negative	−0.0073, −0.0177, −0.0017	N/A	N/A	
12	Harvest-sized crocodile	Negative	−0.0188, −0.0259, −0.0114	N/A	N/A	
13	Harvest-sized crocodile	Negative	−0.0181, −0.0078, −0.0080	N/A	N/A	
14	Harvest-sized crocodile	Negative	−0.0071, −0.0140, −0.0113	N/A	N/A	
15	Harvest-sized crocodile	Negative	0.0046, 0.0001, −0.0105	N/A	N/A	
16	Harvest-sized crocodile	Negative	−0.0101, −0.0043, −0.0003	N/A	N/A	

Sample ID	Cohort	Result	Capture Line Values	6B6C-1 bELISA % inhibition	3.1112G bELISA % inhibition	Notes
17	Harvest-sized crocodile	Negative	−0.0085, −0.0180, −0.0106	N/A	N/A	
18	Harvest-sized crocodile	Negative	−0.0054, 0.0123, −0.0099	N/A	N/A	
19	Harvest-sized crocodile	Negative	−0.0035, 0.0047, −0.0095	N/A	N/A	
20	Harvest-sized crocodile	Negative	−0.0049, −0.0114, −0.0093	N/A	N/A	
21	Harvest-sized crocodile	Negative	0.0066, −0.0016, −0.0069	N/A	N/A	
22	Harvest-sized crocodile	Equivocal*	0.0144, 0.0326, 0.0025	-6.31	-17.84	Mixed replicates; 1 equivocal, 1 near threshold
23	Harvest-sized crocodile	Negative	−0.0064, −0.0043, 0.0116	N/A	N/A	
24	Harvest-sized crocodile	Negative	0.0015, −0.0025, 0.0009	-7.60	-5.49	
25	Harvest-sized crocodile	Negative	−0.0361, −0.0038, −0.0021	N/A	N/A	
26	Harvest-sized crocodile	Negative	−0.0051, −0.0016, −0.0198	N/A	N/A	
27	Harvest-sized crocodile	Negative	0.0162, 0.0108, −0.0027	N/A	N/A	
28	Harvest-sized crocodile	Negative	−0.0102, 0.0088, −0.0057	N/A	N/A	
29	Harvest-sized crocodile	Negative	0.0112, 0, −0.0086	N/A	N/A	
30	Harvest-sized crocodile	Negative	−0.0048, −0.0012, −0.0016	N/A	N/A	
31	Harvest-sized crocodile	Negative	−0.0099, −0.0033, −0.0054	N/A	N/A	
32	Harvest-sized crocodile	Equivocal*	0.0010, 0.0366, 0.0164	N/A	N/A	Two replicates above equivocal threshold
33	Harvest-sized crocodile	Negative*	−0.0042, 0.0263, −0.0170	N/A	N/A	One discordant replicate
34	Harvest-sized crocodile	Negative	0.0021, −0.0011, −0.0117	N/A	N/A	
35	Harvest-sized crocodile	Negative*	0.0234, −0.0125, 0.0085	N/A	N/A	One replicate near threshold
36	Harvest-sized crocodile	Negative	0.0063, 0.0139, 0.0031	32.04	37.94	
37	Harvest-sized crocodile	Negative	−0.0002, −0.0050, −0.0061	N/A	N/A	
38	Harvest-sized crocodile	Negative	0.0019, 0.0112, 0.0018	N/A	N/A	

Sample ID	Cohort	Result	Capture Line Values	6B6C-1 bELISA % inhibition	3.1112G bELISA % inhibition	Notes
39	Harvest-sized crocodile	Negative	−0.0064, −0.0005	N/A	N/A	Two replicates only
40	Harvest-sized crocodile	Negative	−0.0025, −0.0086, −0.0031	N/A	N/A	
41	Harvest-sized crocodile	Negative	0.0068, −0.0026, −0.0059	N/A	N/A	
42	Harvest-sized crocodile	Negative	0.0042, 0.0102, −0.0010	N/A	N/A	
43	Harvest-sized crocodile	Negative	−0.0028, −0.0028, 0.0011	3.51	-12.19	
44	Harvest-sized crocodile	Negative	−0.0003, −0.0030, −0.0027	N/A	N/A	
45	Harvest-sized crocodile	Negative	−0.0121, −0.0151, −0.0116	N/A	N/A	
46	Harvest-sized crocodile	Negative	0.0013, 0.0010, −0.0027	N/A	N/A	
47	Harvest-sized crocodile	Negative	−0.0093, −0.0075, −0.0086	N/A	N/A	
48	Harvest-sized crocodile	Negative	−0.0058, −0.0017, −0.0087	N/A	N/A	
49	Harvest-sized crocodile	Negative	−0.0046, 0.0037, −0.0076	N/A	N/A	
50	Harvest-sized crocodile	Negative	−0.0044, 0.0003, −0.0003	N/A	N/A	
51	Harvest-sized crocodile	Negative	−0.0009, −0.0032, −0.0059	N/A	N/A	
52	Harvest-sized crocodile	Negative	−0.0060, −0.0109, −0.0069	N/A	N/A	
53	Hatchling, euthanised	Equivocal	0.0458, 0.0478, 0.0312	69.89	-6.94	Consistent low-positive; maternal antibody
54	Hatchling, euthanised	Equivocal	0.0479, 0.0455, 0.0407	83.37	44.97	Consistent; maternal antibody
55	Hatchling, euthanised	**Positive**	0.0972, 0.1073, 0.1087	91.18	38.43	Consistent positive; maternal antibody
56	Hatchling, euthanised	**Positive**	0.1185, 0.1289, 0.1107	69.34	84.53	Consistent positive; maternal antibody
57	Hatchling, euthanised	**Positive***	0.0574, 0.0462, 0.0630	81.00	46.10	2/3 positive, 1 equivocal; maternal antibody
58	Hatchling, euthanised	Equivocal	0.0373, 0.0386, 0.0424	79.00	44.48	Consistent equivocal; maternal antibody
59	Harvest-sized crocodile	Negative	−0.0023, −0.0046, −0.0009	N/A	N/A	

Sample ID	Cohort	Result	Capture Line Values	6B6C-1 bELISA % inhibition	3.1112G bELISA % inhibition	Notes
60	Harvest-sized crocodile	Negative	−0.0070, 0.0047, −0.0059	N/A	N/A	
61	Harvest-sized crocodile	Negative	−0.0084, 0.0022, 0.0099	N/A	N/A	
62	Harvest-sized crocodile	Negative	−0.0015, −0.0035, 0	1.00	6.86	
63	Harvest-sized crocodile	Negative	−0.0091, −0.0014, −0.0068	N/A	N/A	
64	Harvest-sized crocodile	Negative	−0.0081, −0.0086, −0.0045	24.87	42.22	
65	Harvest-sized crocodile	Negative	−0.0021, −0.0010, −0.0005	N/A	N/A	
66	Harvest-sized crocodile	Negative	−0.0079, 0.0020, −0.0116	N/A	N/A	
67	Harvest-sized crocodile	Negative	−0.0033, −0.0036, −0.0021	N/A	N/A	
68	Harvest-sized crocodile	Negative	0.0007, −0.0017, −0.0028	N/A	N/A	
69	Harvest-sized crocodile	Negative	−0.0093, −0.0095, −0.0112	N/A	N/A	
70	Harvest-sized crocodile	Negative	−0.0119, −0.0052, −0.0149	N/A	N/A	
71	Harvest-sized crocodile	Negative	−0.0069, −0.0041, −0.0019	-5.95	1.37	
72	Harvest-sized crocodile	Negative	−0.0101, 0.0027, −0.0135	N/A	N/A	
73	Harvest-sized crocodile	Negative	0.0127, −0.0031, 0.0016	N/A	N/A	
74	Harvest-sized crocodile	Negative	0.0107, −0.0071, −0.0025	-3.44	-1.29	
75	Harvest-sized crocodile	Negative	0.0002, −0.0016, 0.0030	N/A	N/A	
76	Harvest-sized crocodile	Negative	−0.0001, −0.0018, −0.0043	N/A	N/A	
77	Harvest-sized crocodile	Negative	0.0011, 0.0002, 0.0093	N/A	N/A	
78	Harvest-sized crocodile	Negative	0.0033, −0.0060, −0.0011	N/A	N/A	
79	Harvest-sized crocodile	Negative	0.0025, 0.0026, 0.0014	3.87	0.32	
80	Harvest-sized crocodile	Negative	0.0001, 0.0005, 0.0021	N/A	N/A	
81	Harvest-sized crocodile	Negative	−0.0062, −0.0024, −0.0045	N/A	N/A	

Sample ID	Cohort	Result	Capture Line Values	6B6C-1 bELISA % inhibition	3.1112G bELISA % inhibition	Notes
82	Harvest-sized crocodile	Negative	−0.0014, 0.0014, −0.0052	N/A	N/A	
83	Harvest-sized crocodile	Negative	0.0002, 0.0003, 0.0007	N/A	N/A	
84	Harvest-sized crocodile	Negative	−0.0016, −0.0016, −0.0046	N/A	N/A	
85	Harvest-sized crocodile	Negative	0.0010, 0.0073, −0.0018	N/A	N/A	
86	Harvest-sized crocodile	Negative	−0.0029, 0.0013, 0.0002	N/A	N/A	
87	Harvest-sized crocodile	Negative	−0.0001, −0.0017, 0.0032	N/A	N/A	
88	Harvest-sized crocodile	Negative	−0.0051, −0.0049, 0	-7.53	-6.94	