

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE ONLY TEMPLATE**

A. 510(k) Number:

k041068

B. Purpose for Submission:

New device clearance

C. Analyte:

West Nile Virus IgG Antibody

D. Type of Test:

Qualitative, ELISA

E. Applicant:

PANBIO Limited

F. Proprietary and Established Names:

West Nile Virus IgG Indirect ELISA

G. Regulatory Information:

a) Regulation section:

West Nile Virus, serological reagents (21 CFR 866.3940).

b) Classification:

Class II

Product Code:

NOP

c) Panel:

83 Microbiology

H. Intended Use:

a) Intended use(s):

The PANBIO West Nile Virus IgG Indirect ELISA is for the qualitative presumptive detection of IgG antibodies to West Nile virus in serum. In conjunction with the PANBIO West Nile Virus IgM Capture ELISA, this test is intended as an aid in the clinical laboratory diagnosis of West Nile virus infection in patients with clinical symptoms consistent with encephalitis / meningitis. Positive results must be confirmed by plaque reduction neutralization test (PRNT), or by using the current Centers for Disease Control and Prevention (CDC) guidelines for diagnosis of this disease.

Assay performance characteristics have not been established for testing cord blood, neonate, prenatal screening, general population screening without symptoms of meningioencephalitis or automated instruments

b) Indication(s) for use:

The PANBIO West Nile Virus IgG Indirect ELISA is for the laboratory diagnosis of West Nile Virus infection in patients with clinical symptoms consistent with meningitis/encephalitis

c) Special condition for use statement(s):

The device is for prescription use only

d) Special instrument Requirements:

NA

I. Device Description:

Indirect IgG ELISA

J. Substantial Equivalence Information:

a) Predicate device name(s):

Focus Technologies West Nile Virus IgG ELISA

b) Predicate K number(s):

K031953

Comparison with predicate:

Similarities		
Item	Device	Predicate
Same indications for use. Same target population. Same ELISA methodology	PanBio West Nile Virus IgG Indirect ELISA (K041068) Test persons having symptoms of meningioencephalitis IgG Indirect ELISA	Focus West Nile Virus IgG ELISA (K031953) Test persons having symptoms of meningioencephalitis IgG Indirect ELISA
Differences		
Item	Device	Predicate
Assay Procedure	PanBio West Nile Virus IgG Indirect ELISA (K041068) Does not use Background Subtraction method in the Assay Procedure	Focus West Nile Virus IgG ELISA (K031953) Does use Background Subtraction method in the Assay Procedure

K. Standard/Guidance Document Referenced (if applicable):

Class II Special Controls Guidance Document: Serological Reagents for the Laboratory Diagnosis of West Nile Virus. October 30, 2003

L. Test Principle:

Specific antibodies combine with purified and inactivated WNV antigen coated on the polystyrene surface of the microwell test strips (assay plate). Residual serum is removed from the assay plate by washing. HRP-conjugated anti-human IgG monoclonal antibody (Mab) is added to the assay plate. After incubation, the microwells are washed and a colorless substrate system, tetramethylbenzidine/hydrogen peroxide (TMB/H₂O₂), is added. The substrate is hydrolyzed by the HRP, if present, and the chromogen changes to a blue color. After stopping the reaction with acid, the TMB becomes yellow. Color development is indicative of the presence of WNV antibodies in the test sample.

M. Performance Characteristics (if/when applicable):

Analytical performance:

a) Precision/Reproducibility:

The reproducibility of the PANBIO West Nile Virus IgG Indirect ELISA was determined by testing 8 sera 3 times each on 3 different assays at one Australian study site and two study sites in the USA. Within-run, between day, between site and total precision were estimated by Analysis of Variance (ANOVA) Type II. The results are presented in the Table below.

PANBIO West Nile Virus IgG Indirect ELISA

Sample	n	*Mean	Within		Between Day		Between Site		Total	
			*SD	CV	*SD	CV	*SD	CV	*SD	CV
Reactive	27	5.83	0.25	4.3%	0.00	0.0%	0.00	0.0%	0.24	4.2%
Negative	27	0.19	0.02	8.8%	0.00	0.0%	0.08	45.5%	0.07	38.8%
#1	27	2.24	0.16	7.0%	0.12	5.1%	1.08	48.3%	0.92	41.0%
#2	27	2.94	0.20	6.9%	0.16	5.5%	0.13	4.3%	0.26	9.0%
#3	27	4.07	0.32	7.9%	0.00	0.0%	0.52	12.8%	0.53	13.1%
#4	27	1.48	0.11	7.7%	0.11	7.6%	0.08	5.6%	0.16	11.0%
#5	27	1.53	0.06	4.2%	0.06	3.9%	0.18	11.9%	0.17	11.2%
#6	27	1.23	0.09	7.4%	0.03	2.3%	0.16	13.3%	0.17	13.5%
#7	27	0.82	0.05	6.2%	0.02	1.9%	0.13	16.0%	0.12	14.8%
#8	27	0.84	0.07	7.9%	0.00	0.0%	0.12	14.7%	0.12	14.4%

All values are calculated from Ratios *

SD = Standard Deviation; CV = Coefficient of Variation (%)

a. Linearity/assay reportable range:

NA

b. Traceability, Stability, Expected values (controls, calibrators, or method):

NA

c. *Detection limit:*

NA

d. *Analytical specificity:*

This study consisted of a panel of 314 specimens from patients with confirmed diseases other than WNV. The purpose of this study was to establish the analytical specificity of the PANBIO West Nile Virus IgG Indirect ELISA through the analysis of specimens from patients with diseases that have the potential for cross-reactivity. Each of the specimens included in the study was characterized with respect to disease state prior to analysis of the specimens with the PANBIO West Nile Virus IgG Indirect ELISA. The Table below provides a summary of specimens in the disease panel.

Disease State	Total Specimens ^a	PANBIO IgG ELISA Results		
		Pos	Eqv	Pos and Eqv
<i>Dengue virus</i>	15	14	0	14/15
<i>St. Louis encephalitis</i>	35	25	3	28/35
<i>Japanese encephalitis</i>	3	3	0	3/3
<i>La Crosse encephalitis</i>	26	2	0	2/26
<i>California encephalitis</i>	11	0	1	1/11
<i>Eastern Equine encephalitis</i>	1	0	0	0/1
<i>Varicella-Zoster virus</i>	15	0	0	0/15
<i>Cytomegalovirus</i>	48	7	0	7/48
<i>Epstein-Barr virus</i>	40	7	0	7/40
<i>Enterovirus</i>	15	1	0	1/15
<i>Ross River virus</i>	39	10	1	11/39 ^b
<i>Barmah Forest virus</i>	36	10	3	13/36 ^c
<i>Rheumatoid Factor</i>	15	4	0	4/15
<i>Anti-Nuclear Antibody</i>	15	2	0	2/15

^a Sample testing was conducted at PANBIO (Site 5) except as follows:

Site 1: Testing on 25 Saint Louis encephalitis, 9 California encephalitis and 1 Eastern Equine encephalitis specimens was conducted at a state health laboratory in Louisiana, USA.

Site 2: Testing on 5 Dengue virus, 10 Saint Louis encephalitis and 2 California encephalitis specimens was conducted at a private reference laboratory in Utah, USA.

Site 3: Testing on 26 La Crosse encephalitis specimens was conducted at a hospital laboratory in Ohio, USA.

Site 6: Testing on 25 Epstein-Barr virus and 33 Cytomegalovirus specimens was conducted at a private research laboratory in Maryland, USA.

^b 11 of 11 samples that were reactive on PANBIO West Nile virus IgG Indirect ELISA were confirmed flavivirus positive by Western blot.

^c 9 of 13 samples that were reactive on PANBIO West Nile virus IgG Indirect ELISA were confirmed flavivirus positive by Western blot.

Caution: Cross-reactivity has been noted with the PANBIO West Nile Virus IgG assay in specimens containing antibody to cytomegalovirus (CMV), Epstein-Barr Virus (EBV) and rheumatoid factor (RF). Reactive results must be reported with a caution statement regarding possible cross-reactivity with CMV, EBV and RF.

a. Assay cut-off:

The cut-off was determined using a total of 379 characterized positive and 593 characterized negative specimens from three external clinical trials conducted within the USA. Specimens were characterized by immunofluorescence assay (IFA). The cut-off was determined by two-graph receiver operating characteristic analysis (TG-ROC).

b. Comparison studies:

a. Method comparison with predicate device:

The PanBio Indirect IgG ELISA was compared to reference assays: The plaque-reduction neutralization test (PRNT) and the WN IgG IFA ASR

b. Matrix comparison:

NA

c. Clinical studies:

a. Clinical sensitivity:

NA

b. Clinical specificity:

NA

c. Other clinical supportive data (when a and b are not applicable):

PERFORMANCE CHARACTERISTICS

Study Site 1:

Three hundred (300) retrospective sera from individuals of various ages and both genders were tested at a state health laboratory in Louisiana, USA. The sera include samples from the following groups: 100 samples characterized as positive for WN virus by PRNT and 200 randomly selected normal specimens from routine laboratory testing not known to have a flavivirus related illness. These samples were masked and tested on the PANBIO West Nile Virus IgG Indirect ELISA and the results were compared to the clinical and serological characterization of the samples to determine performance of the assay. The data is summarized in Table 1.

Table 1 – Study Site 1

PANBIO West Nile Virus IgG Indirect ELISA Reactivity with Endemic Normal and WNV PRNT Confirmed Specimens

Specimen Characterisation	PANBIO IgG ELISA Results			
	Pos	Eqv ^a	Neg	Total
Endemic normal specimens (randomly selected) ^b	18 ^d	1	181	200
West Nile virus Positive^c (PRNT confirmed)	79	0	21 ^e	100
Total	97	1	202	300

^a Retesting of equivocal was not conducted as samples were unavailable.

^b Randomly selected normal specimens from routine laboratory testing from 2002-2003. Not known to have a flavivirus related illness. Further tested by WNV IgG IFA.

^c West Nile virus PRNT positive. Collected in 2002. Further tested by WNV IgG IFA ASR.

^d Nine of the 18 endemic specimens that were positive by PANBIO ELISA were also positive by IgG IFA ASR. Refer to note below.

^e Ten of the 21 PRNT confirmed positive specimens that were negative by PANBIO ELISA were negative by IgG IFA and positive by PANBIO WNV IgM Capture ELISA.

Endemic normal specimens

95% CI*

Negative presumptive agreement = 181/200 90.5% 85.6 – 94.2%

West Nile virus positive specimens

Serological sensitivity (PRNT) = 79/100 79.0% 69.7 – 86.5%

*CI = Confidence

Study Site 2:

Three hundred and twenty-five (325) retrospective sera from individuals of various ages and both genders were tested at a private reference laboratory in Utah, USA. The serum panel was comprised of 166 samples that were characterized positive and 159 samples that were characterized negative for WNV by IFA slides (ASR). Thirty-four of these samples were from patients with clinical symptoms consistent with encephalitis / meningitis, of which 32 were characterized positive and 2 were characterized negative for WNV by IFA. The samples were not masked and were tested by the PANBIO West Nile

Virus IgG Indirect ELISA. Assay performance was determined by comparing PANBIO West Nile Virus IgG Indirect ELISA results with the clinical and serological characterization of the samples. The data is summarized in Table 2 and Table 3.

Table 2 – Study Site 2

PANBIO West Nile Virus IgG Indirect ELISA Reactivity with Encephalitis / Meningitis Patients

Specimen Characterization	PANBIO IgG ELISA Results			
	Pos	Eqv ^a	Neg	Total
Encephalitis/ meningitis patients (IgG IFA positive)	26	2	4	32
Encephalitis/meningitis patients (IgG IFA negative)	0	0	2	2
Total	26	2	6	34

^a Retesting of equivocal was not conducted as cut-off was modified following clinical trials.

Encephalitic symptoms (IgG IFA positive) 95% CI*

Positive Presumptive

Agreement = 26/32 = 81.3% 63.6 – 92.8%

WNV (presumptive by (IgG IFA negative)

Negative Presumptive

Agreement = 2/2 =100.0% 15.8 – 100.0%

*CI = Confidence

Table 3 – Study Site 2

**PANBIO West Nile Virus IgG Indirect ELISA Reactivity with WNV IFA
Characterized Specimens**

Specimen Characterization	PANBIO IgG ELISA Results			
	Pos	Eqv ^a	Neg	Total
WNV positive (IgG IFA positive)	146	5	15	166
WNV negative (IgG IFA negative)	15	4	140	159
Total	161	9	155	325

^a Retesting of equivocal was not conducted as cut-off was modified following clinical trials.

WNV IFA positive (presumptive)	95% CI*		
Positive Presumptive Agreement = 286/325	= 88.0%	84.5 – 91.5%	
Negative Presumptive Agreement = 140/159	= 88.1%	83.0 – 93.1%	

*CI = Confidence interval

d. Clinical cut-off:

NA

e. Expected values/Reference range:

Expected Values

A total of 203 normal prospective sera collected during 2002 from patients of various ages in Ohio and Maryland, USA, were assayed on the PANBIO West Nile Virus IgG Indirect ELISA. The distribution of females was 51% (103/203), and males 49% (100/203). The data in Table 1 illustrates the prevalence of IgG antibodies in different age groups when using the PANBIO West Nile Virus IgG Indirect ELISA.

Prevalence of West Nile virus by Age

Age	Total	Equivocal ^a	Positive	Prevalence ^e	Flavivirus Reactive ^b
0 – 9	29	0	4	13.8%	1
10 – 19	24	1	3	16.7%	0
20 – 29	25	0	4	16.0%	2
30 – 39	25	0	7	28.0%	4
40 – 49	25	0	3	12.0%	2
50 – 59	25	0	3	12.0%	2
60 – 69	25	0	3	12.0%	2
≥ 70	25	0	3	12.0%	1
Total	203	1	30	15.3%	14

^a Retesting of equivocal samples was not conducted as cut-off was modified following clinical trials.

^b Number of West Nile virus reactive samples that were positive by Dengue IFA ASR

N. Conclusion:

The submitted material in this premarket notification is complete and supports a substantial equivalence decision.