Design Verification

DENGUE IgG ELISA

Content

1. Assay Principle

HUMAN Dengue IgG ELISA is an indirect antibody colorimetric enzyme immunoassay. It is intended for the qualitative determination of immunoglobulin G class antibodies to dengue virus (anti-DENV IgG) in human serum and plasma. Specific dengue virus antigen (DENV-2) is coated on the microtiter wells. Anti-DENV antibodies, if present in patient's specimen, bind to immobilized DENV Ag. Subsequently, bound IgG antibodies are identified by addition of enzyme-labeled anti-human-IgG antibodies. The enzyme activity of the bound complex is determined photometrically by the turnover of H_2O_2/TMB substrate.

2. Sensitivity and Specificity

85 patient samples were assayed in parallel with HUMAN Dengue IgG ELISA, ref. 51240, and a reference CE-marked anti-DENV IgG ELISA (PanBio Dengue IgG Indirect ELISA, ref. 01PE30). Three lots of HUMAN Dengue IgG ELISA were used throughout the study (107G/K095, 107G/K026 and 107G/K035). The assays were performed according to the instruction for use. Each run included all kit controls (negative, positive, cut-off).

Acceptance criterion: ≥ 90% method agreement

Results:

		PanBio Dengue IgG ELISA, ref. 01PE30		
		Positive	Negative	Total
HUMAN Dengue IgG ELISA, ref. 51240	Positive	**45**	0	45
	Negative	0	**40**	40
	Total	**45**	**40**	85

Agreement between methods: 100.0% (95% CI: 96.0 % to 100.0%)
Agreement positive results (sensitivity): 100.0% (95% CI: 92.1% to 100.0%)
Agreement negative results (specificity): 100.0% (95% CI: 91.2% to 100.0%)

Excluded from the evaluation were samples classified as borderline by any of the two assays.

Conclusion:
Based on the 85 samples classified unequivocally as negative or positive, HUMAN Dengue IgG ELISA features a sensitivity and specificity of 100.0% (95% CI: 92.1% to 100.0%) and 100.0% (95% CI: 91.2% to 100.0%), respectively. The overall agreement between the HUMAN and the reference assay is 100.0% (95% CI: 95.8% to 100.0%).

3. Cross-reactivity

A total of 57 samples known to contain potentially cross-reacting IgG antibodies were evaluated with HUMAN Dengue IgG ELISA (Lot 107G/K035). The assay was performed according to the instructions for use. Each run included all kit controls (negative, positive, cut-off).

Acceptance criterion:
Potentially cross-reactive if false-positive results appear, or if there is scientific evidence for cross-reactivity of analogous anti-DENV IgG assays.

Results:

Potentially cross-reacting agent	Positive samples	Positive/equivocal with HUMAN Dengue IgG ELISA
anti-HSV 1 IgG or anti-HSV 2 IgG	6	0/0
anti-Rubella IgG	7	0/0
anti-VZV IgG	7	0/0
anti-EBV IgG	9	0/0
anti-TBE IgG	10	1/0
anti-RSV IgG	6	0/0
anti-Parvovirus IgG	6	0/0
anti-CMV IgM	6	0/0

Conclusion:
HUMAN Dengue IgG ELISA didn't show cross reactivity with IgG antibodies to variety of viral pathogens. Only one pooled sample reactive to TBE was found to be positive. This one positive result doesn't indicate a general cross-reactivity with IgG antibodies to TBE. Positive reactions of specimens containing IgG to other flaviviruses cannot be excluded either.

4. Potentially Interfering Substances

Effects of endogenous blood-borne substances known to occasionally interfere with diagnostic assays at elevated concentrations were examined with HUMAN Dengue IgG ELISA (Lot 107G/K026). For this purpose, two specimens positive and one specimen negative for anti-DENV IgG were spiked with concentrated bilirubin, hemoglobin, or triglycerides to borderline or pathological levels. Non-spiked and spiked samples were evaluated as per the instructions for use.

Acceptance criterion:
Deviation of sample OD with interfering substance ≤ 20% from sample without interfering substance.

Results:
Mean OD of non-spiked and spiked samples with endogenous potentially interfering substances.

Potentially interfering substance	OD			Relative to non-spiked sample		
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
None	1.68	1.20	0.46	100.0%	100.0%	100.0%
Bilirubin 0.5 mg/ml	1.80	1.10	0.49	107.1%	91.7%	106.5%
Hemoglobin 4.0 mg/ml	1.69	1.14	0.46	100.6%	95.0%	100.0%
Triglycerides 30.0 mg/ml	1.73	1.07	0.48	103.0%	89.2%	104.3%

Conclusion:
All results meet the acceptance criteria. Bilirubin, hemoglobin, and triglycerides do not interfere with the HUMAN Dengue IgG ELISA at the tested borderline or pathological concentrations.

5. Precision

Within-run precision (intra-assay precision, repeatability) of HUMAN Dengue IgG ELISA was determined with one assay lot and 12 samples with different levels of anti-DENV IgG measured at 20 replicates per run. The assay was performed according to the instructions for use. Each run included all kit controls (negative, positive, cut-off).
Within-laboratory precision (inter-assay precision, reproducibility) of HUMAN Dengue IgG ELISA was determined with one lot and 5 samples (3 patient samples, the cut-off, and positive controls) evaluated in duplicate in 20 independent runs. The assay was performed according to the instructions for use. Each run included all kit controls (negative, positive, cut-off).
The lot-to-lot variability of the assay was assessed by testing 3 samples, 6 measurement each utilizing 3 different lots of Human Dengue IgG ELISA.

Acceptance criteria

Within-run variability	CV ≤ 10.0 %
Within-laboratory variability	CV ≤ 15.0 %
Lot-to-lot variability	CV ≤ 15.0 %

Results:

Within-run variability

Sample	1	2	3	4	5	6	7	8	9	10	11	12
Mean OD	0.411	0.393	0.383	0.873	0.958	0.990	1.039	1.077	1.279	1.559	1.472	1.480
CV%	5.8	5.8	3.6	3.9	3.1	2.1	2.2	2.4	4.1	5.1	5.1	4.0
Mean CV%	3.9											
Status (<10%)	passed											

Within-laboratory variability

Sample	**Mean OD**	**SD**	**%CV**	**Status (<15%)**
1	0.933	0.11	11.7	passed
2	0.537	0.07	13.1	passed
3	1.210	0.11	8.8	passed
Positive control	1.177	0.11	9.4	passed
Cut-off control	1.146	0.10	9.0	passed

Lot-to-lot variability

Sample	**mean OD Lot 1**	**mean OD Lot 2**	**mean OD Lot 3**	**mean OD (Lots 1-3)**	**Lot-to-lot variability %**	**Status (<15%)**
1	2.419	2.358	2.238	2.338	4.5	passed
2	2.302	2.066	2.135	2.168	6.4	passed
3	2.390	2.274	2.396	2.353	3.7	passed

Conclusion:

The coefficients of variation for all assessed precision types satisfy the acceptance criteria. This has been shown with samples that cover a broad range of positive signals. The data allow to conclude that the assay exhibits a very good precision. The product meets the requirements for batch homogeneity.

6. Validated Sample Types

To evaluate the equivalence of serum and plasma-types samples in HUMAN Dengue IgG ELISA, samples were diluted either in EDTA, Li-heparin, citrate plasma or human serum. All samples were assayed according to the instructions for use.

Acceptance criteria:

OD recovery range for plasma samples compared to the corresponding serum samples is 100% ± 20%.
Diagnosis must not change

Results:

	Serum		Li-Heparin			EDTA			Citrate		
Sample	OD	Diagnosis	OD	Diagnosis	Recovery %	OD	Diagnosis	Recovery %	OD	Diagnosis	Recovery %
1	2.022	Positive	1.982	Positive	98.0	2.029	Positive	100.3	2.062	Positive	102.0
2	2.097	Positive	2.043	Positive	97.4	1.975	Positive	94.2	1.777	Positive	84.7
3	0.909	Positive	0.890	Positive	96.7	0.918	Positive	101.0	0.917	Positive	100.9
4	0.939	Positive	0.879	Positive	93.6	0.886	Positive	94.4	0.913	Positive	97.2
5	1.858	Positive	1.880	Positive	99.7	1.873	Positive	100.8	1.995	Positive	107.4
6	1.435	Positive	1.365	Positive	95.1	1.355	Positive	94.4	1.238	Positive	86.3
7	0.891	Positive	0.869	Positive	97.5	0.901	Positive	101.1	0.910	Positive	102.1
8	1.801	Positive	1.732	Positive	96.2	1.658	Positive	92.1	1.643	Positive	91.2
9	2.037	Positive	2.000	Positive	98.2	2.004	Positive	98.4	2.111	Positive	103.6
10	0.952	Positive	0.920	Positive	96.6	0.890	Positive	93.5	0.873	Positive	91.7
Status			**Passed**			**Passed**			**Passed**		

Conclusion:
HUMAN Dengue IgG ELISA is compatible with samples based on the serum, EDTA-, Li-heparin- and citrate plasma matrices. Sample matrix has no effect on the performance of the assay.

7. Standardization

No international standard or other certified reference material with assigned unitage of biological activity of human IgG antibodies to dengue virus suitable for standardization of HUMAN Dengue IgG is available. Therefore, the standardization of the method has been done using 39 proficiency testing samples provided by INSTAND. The 39 samples were run utilizing 3 batches of HUMAN Dengue IgM ELISA (107G/K095, 107G/K026 and 107G/K035). The results (Positive/Negative) were compared to the expected results of the tested samples.

Acceptance criteria: results in accordance with the expected results of the proficiency testing samples.

Conclusion: all the results (39 samples) obtained with HUMAN Dengue IgG ELISA were in accordance with the expected results of the proficiency testing samples.

8. Stability:

Real-Time Stability:
The purpose of the study is to analyse the real time stability of the HUMAN Dengue IgG ELISA kit.
Method:
Three batches (107G/K076, 107G/K031, 107G/K056-2) were used throughout the study. The assay was performed according to the instruction for use. Kits of each lot were used to diagnose 3 patient samples in 3, 6, 9, 12 and 18 (24 for Lot1) months after production date and compare it to day 0. Kits were stored at 2-8°C and samples at -20°C.

Acceptance criteria: Recovery of OD is ± 40% and diagnosis must not change compared to day 0.

Results:

	Lot 1		OD Value	Acceptance Range		Status
	Controls			from	to	
	Negative control		0.022	0.000	0.200	passed
	Cut-Off control		0.415	0.350	0.850	passed
	Positive control		1.074	0.650	3.000	passed
Lot 1	Day 0		24 months		Recovery	Status
Samples	OD	Diagnosis	OD	Diagnosis	%	
Sample 1	1.65	positive	1.250	positive	75.76	passed
Sample 2	1.25	positive	1.173	positive	98.49	passed
Sample 3	1.52	positive	1.310	positive	89.12	passed

	Lot 2		OD Value	Acceptance Range		Status
	Controls			from	to	
	Negative control		0.077	0.000	0.200	passed
	Cut-Off control		0.505	0.350	0.850	passed
	Positive control		1.223	0.650	3.000	passed
Lot 2	Day 0		18 months		Recovery	Status
Samples	OD	Diagnosis	OD	Diagnosis	%	
Sample 1	1.163	positive	1.194	positive	102.7	passed
Sample 2	1.071	positive	1.106	positive	103.3	passed
Sample 3	1.147	positive	1.292	positive	112.6	passed

	Lot 3		OD Value	Acceptance Range		Status
	Controls			from	to	
	Negative control		0.100	0.000	0.200	passed
	Cut-Off control		0.535	0.350	0.850	passed
	Positive control		1.076	0.650	3.000	passed
Lot 3	Day 0		18 months		Recovery	Status
Samples	OD	Diagnosis	OD	Diagnosis	%	
Sample 1	1.060	positive	0.975	positive	92.0	passed
Sample 2	0.706	positive	0.645	positive	91.4	passed
Sample 3	0.322	positive	0.256	positive	79.5	passed

Conclusion:
Storage for 18 months at 2-8°C neither affect the results obtained for the three samples and controls nor are the specifications lost. The HUMAN Dengue ELISA IgG fulfilled all specifications and is given a shelf life of 18 months.

Open vial stability
One HUMAN Dengue ELISA IgG Kit was used for the estimation of open vial stability. Three samples and kit controls (Negative, positive and cut-off controls) were evaluated at day 0 (all kit vials were opened) and reevaluated in 1, 2, 4 and 8 weeks of storage at 2 - 8 °C. Samples were stored at -20 °C. The assay was performed according to the IFU.

Acceptance criteria: Diagnosis must not change compared to day 0. The recovery for ODs should be found between 80 % - 120 %.

Results:

Controls/Samples	Day 0		8 weeks storage 2 - 8 °C (Open Vials)		Recovery %	Status
	OD	Diagnosis	OD	Diagnosis		
Negative control	0.021	negative	0.017	negative	80.95	passed
Cut-Off control	0.590	equivocal	0.590	equivocal	100.00	passed
Positive control	1.058	Positive	0.887	Positive	83.83	passed
Sample 1	1.099	Positive	1.129	Positive	102.72	passed
Sample 2	1.122	Positive	1.098	Positive	97.86	passed
Sample 3	1.625	Positive	1.751	Positive	107.75	passed

Conclusion:
Open kit stability could be demonstrated for all samples (controls) for 8 weeks at 2-8°C storage.