in vitro Diagnostic Medical Device Technical File

DENGUE COMBO RAPID TEST CASSETTE

RAPG-DEC-001

The signature below certifies that this document has been reviewed and accepted for its accuracy.

	Signature	Position	Date
Approved by		Quality Manager	06/07/2022

1 BACKGROUND

Dengue is a flavivirus, transmitted by Aedesaegypti and Aedesalbopictus mosquitoes. It is widely distributed throughout the tropical and subtropical areas of the world,[1]and causes up to 100 million infections annually.[2] Classic Dengue infection is characterized by a sudden onset of fever, intense headache, myalgia, arthralgia and rash.

The Dengue Combo Rapid Test Cassette consists of two independent tests contained in the same plastic housing.

One is a Dengue IgG/IgM rapid test that utilises a combination of Dengue antigen coated coloured particles for the detection of IgG and IgM Dengue antibodies in human whole blood, serum, or plasma. Primary Dengue infection causes IgM antibodies to increase to a detectable level in 3 to 5 days after the onset of fever. IgM antibodies generally persist for 30 to 90 days.[3] Most Dengue patients in endemic regions have secondary infections,[4] resulting in high levels of specific IgG antibodies prior to or simultaneous with IgM response.[5] Therefore, the detection of specific anti-Dengue IgM and IgG antibodies can also help to distinguish between primary and secondary infections.

The other test is a Dengue NS1 rapid test that utilises a combination of Dengue antibodies coated coloured particles for the detection of Dengue NS1 antigen in human whole blood, serum, or plasma.

NS1 is one of 7 Dengue Virus non-structural proteins which are thought to be involved in viral replication. NS1 exists as a monomer in its immature form but is rapidly processed in the endoplasmic reticulum to form a stable dimer. A small amount of NS1 remains associated with intracellular organelles where it is thought to be involved in viral replication. The rest of NS1 is found either associated with the plasma membrane or secreted as a soluble hexadimer. NS1 is essential for viral viability but its precise biological function is unknown. Antibodies raised in response to NS1 in viral infection can cross react with cell surface antigens on epithelial cells and platelets and this has been implicated in the development of Dengue Hemorrhagic fever.

1.1 Test Principle

The Dengue IgG/IgM test component is a qualitative membrane-based immunoassay for the detection of Dengue antibodies in whole blood, serum, or plasma. This test consists of two components, an IgG component and an IgM component. In the IgG component, anti-human IgG is coated in IgG test line region. During testing, the specimen reacts with Dengue antigen-coated particles in the test cassette. The mixture then migrates upward on the membrane chromatographically by capillary action and reacts with the anti-human IgG in IgG test line region. If the specimen contains IgG antibodies to Dengue, a coloured line will appear in IgG test line region. In the IgM component, anti-human IgM is coated in IgM test line region. During testing, the specimen reacts with anti-human IgM. Dengue IgM antibodies, if present in the specimen, reacts with the anti-human IgM and the Dengue antigen-coated particles in the test cassette, and this complex is captured by the anti-human IgM, forming a coloured line in IgM test line region. Therefore, if the specimen contains Dengue IgG antibodies, a coloured line will

appear in IgG test line region. If the specimen contains Dengue IgM antibodies, a coloured line will appear in IgM test line region. If the specimen does not contain Dengue antibodies, no coloured line will appear in either of the test line regions, indicating a negative result. To serve as a procedural control, a coloured line will always appear in the control line region indicating that the proper volume of specimen has been added and membrane wicking has occurred.

The Dengue NS1 test component is a qualitative membrane-based immunoassay for the detection of Dengue NS1 antigen in whole blood, serum, or plasma. During testing, the specimen reacts with Dengue antibody-conjugate in the test cassette. The colloidal gold-antibody conjugate will bind to any Dengue antigen in the specimen sample. As the reaction mixture moves across the membrane, the Dengue NS1 antibody on the membrane will bind to any antibody-antigen complex formed, causing a red line to form at the test line region of the test membrane. The intensity of the lines will vary depending upon the amount of antigen present in the sample. The appearance of pink line in the test region should be considered as positive result.

1.2 Illustrations

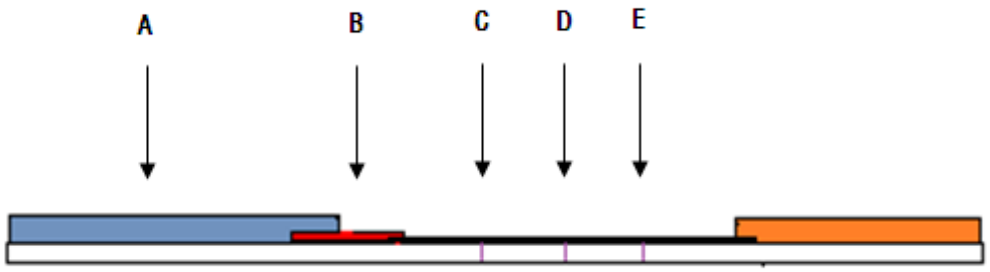

Figure 1: Dengue IgG/IgM Test Principle

As shown in illustration above, the specimen (A) migrates via capillary action along the membrane to react with the gold conjugate (B). Dengue IgG and/or IgM present in the specimen binds to the conjugate, forming a coloured antibody-Dengue antigen complex. The mouse anti-human IgM and mouse anti-human IgG immobilised in the test zones (C and D) of the membrane captures any coloured complexes that have formed. The formation of visible coloured line(s) in the test region indicates a positive result. The absence of any coloured line in the test zones suggests a negative result. In the control zone (E) of the membrane, immobilised reagents capture coloured conjugate regardless of test specimen composition. The resulting visible coloured band acts as the control line.

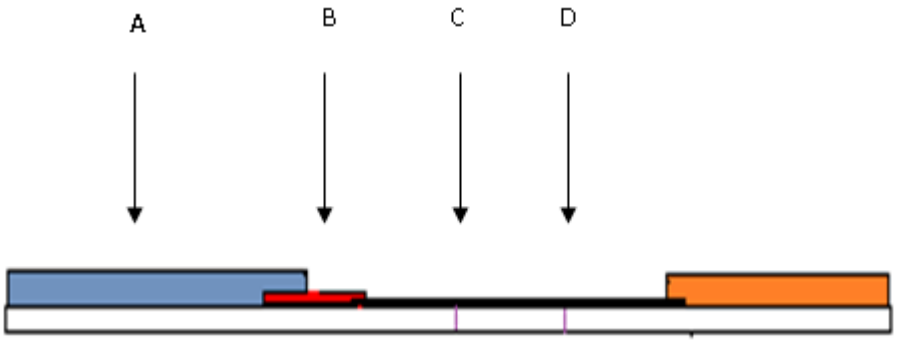

Figure 2: NS1 Test Principle

As shown in illustration above, the specimen (A) migrates via capillary action along the membrane to react with the coloured conjugate (B). Any Dengue NS1 antigen present in the specimen binds to the conjugate, forming a coloured antibody-antigen complex. The mouse anti-Dengue NS1 antibody in the test zone (C) of the membrane captures any coloured complexes that have formed. The formation of a visible coloured line in the test region indicates a positive result. The absence of a coloured line in the test zones suggests a negative result. In the control zone (D) of the membrane, immobilised reagents capture coloured conjugate regardless of test specimen composition. The resulting visible coloured band acts as the control line.

1.3 Storage

Store the test at 2-30°C. Freezing must be avoided.

1.4 Stability

The test is stable through to the expiration date printed on the sealed pouch. The test must remain in the sealed pouch until use. DO NOT FREEZE. Do not use beyond the expiration date.

1.5 Description of Test Methods

Allow the test, specimen, buffer and/or controls to reach room temperature (15-30°C) prior to testing.

1. Bring the pouch to room temperature before opening it. Remove the test cassette from the sealed pouch and use it within 1 hour.
2. Place the cassette on a clean and level surface.

➢ For **Serum or Plasma specimens**:

For NS1:

- Hold the dropper vertically and transfer 3 drops of serum or plasma (approximately 75µl) to the specimen area, and start the timer. See illustration below.

For IgG/IgM:

To use a dropper: Hold the dropper vertically, draw the specimen up to the Fill Line (approximately 5 µl), and transfer the specimen to the specimen well of the test cassette, then add 1 drop of buffer (approximately 40µl) and start the timer. Avoid trapping air bubbles in the specimen well.

To use a micropipette: Pipette and dispense 5 µl of whole blood to the specimen well of the test cassette, then add 1 drop of buffer (approximately 40 µl) and start the timer.

➢ For **Whole Blood(Venipuncture/Fingerstick)** specimen:

For NS1:

- To use a dropper: Hold the dropper vertically and transfer 3 drops of serum or plasma (approximately 75 µL) to the specimen area, and start the timer. See illustration below.
- To use a capillary tube: Fill the capillary tube and transfer approximately 75 µL of fingerstick whole blood specimen to the specimen area of test cassette, then add 1 drop of buffer (approximately 40 µL) and start the timer. See illustration below.
- To use hanging drops: Allow 3 hanging drops of fingerstick whole blood specimen (approximately 75 µL) to fall into the specimen area of test cassette, then add 1 drop of buffer (approximately 40 µL) and start the timer. See illustration below.

For IgG/IgM:

- To use a dropper: Hold the dropper vertically, draw the specimen above 1cm above the Fill Line, and transfer 1 drop of whole blood (approximately 10 µl) to the specimen well of the test cassette, then add 1 drop of buffer (approximately 40 ul) and start the timer. See illustration below.
- To use a micropipette: Pipette and dispense 10 µl of whole blood to the specimen well of the test cassette, then add 1 drop of buffer (approximately 40 µl) and start the timer. See illustration below.

1.6 Composition of Product

A)Goat anti-human IgG	B) Goat anti-human IgM
C) Mouse anti-Dengue NS1-1	D) Mouse anti-Dengue NS1-2
E) Mouse anti-Dengue NS1-3	F) Mouse anti-Dengue NS1-4
G) Dengue antigen-1	H) Dengue antigen-2
I) Dengue antigen-3	J) Pouch
K) NC Membrane	L) Label pad
M) Absorbant pad	N) Sample pad
O) Mouse IgG	P) Goat anti-mouse IgG

1.7 Manufacturing Procedure

Dengue IgG/IgM

- Coat the gold conjugated Dengue antigen-1/2/3on the label pad.
- Use the sprayer to dispense mouse anti-human IgG, mouse anti-human IgM, and goat anti-mouse IgG onto the membrane.
- Assemble the membrane, label pad, absorbent pad and sample pad on the plastic backing.
- Use the cutter to cut the plastic backing into strips of selected size.
- Test the cassette according to the QC procedure and release the finished product.

Dengue NS1

- Coat the gold conjugated Dengue NS1-4 on the label pad.
- Use the sprayer to dispense mouse anti-Dengue NS1-1/2/3 and goat anti-mouse IgG onto the membrane.
- Assemble the membrane, label pad, absorbent pad and sample pad on the plastic backing.
- Use the cutter to cut the plastic backing into strips of selected size.
- Test the cassette according to the QC procedure and release the finished product

2 PERFORMANCE CHARACTERISTICS

2.1 Sample Correlation

The Dengue Combo Rapid Test Cassette has passed a seroconversion panel and has been compared against a leading commercial Dengue NS1 ELISA test using clinical obtained from a population of symptomatic and asymptomatic individuals. Clinical performance of the Dengue IgG/IgM were confirmed by a leading commercial Dengue ELISA test for IgG and IgM.

Dengue Primary Infection for IgM/IgG test results

Method			**EIA**		
Dengue IgG/IgM Rapid Test Cassette	Result		Positive		Negative
			IgM	IgG	
	Positive	IgM	20	0	0
		IgG	4	0	0
	Negative		0	0	0
Relative Sensitivity			83.3%	/	/

Dengue Secondary Infection for IgM/IgG test results

Method			**EIA**		
Dengue IgG/IgM Rapid Test Cassette	Result		Positive		Negative
			IgM	IgG	
	Positive	IgM	46	1	0
		IgG	18	63	0
	Negative		0	0	0
Relative Sensitivity			71.9%	98.4%	/

Non-Dengue Infection for IgG/IgM Test Results

Method			**EIA**		
Dengue IgG/IgM Rapid Test Cassette	Result		Positive		Negative
			IgM	IgG	
	Positive	IgM	0	0	1
		IgG	0	0	3
	Negative		0	0	429
Relative Sensitivity			/	/	99.1%

Relative Sensitivity: (20+63)/ (24+64) =94.3% (95%CI*: 87.2%~98.1%)
Relative Specificity: 429/433=99.1% (95%CI*: 97.7%~99.7%)
Accuracy: (20+63+429)/ (24+62+433) =98.3% (95%CI*: 96.7%~99.2%). *Confidence Interval

Dengue NS1

Method		**ELISA**		**Total Result**
Dengue NS1 Rapid Test Cassette	Results	Positive	Negative	
	Positive	137	8	145
	Negative	6	200	206
Total Result		143	208	351

Relative sensitivity: 95.8% (95%CI*: 91.1%~98.4%)
Relative specificity: 96.1% (95%CI*: 92.6%~98.4%)
Accuracy: 96.0%(95%CI*:93.4%~97.8%) *Confidence Intervals

2.2 Interfering Substances

Analytes were spiked into negative serum pools (ELISA confirmed), Dengue IgM positive, Dengue IgG positive serum specimens, and Dengue NS1 positive serum (ELISA confirmed) at the concentrations listed. The specimens were tested on the Dengue Combo test in triplicate with visual interpretations occurring at 10 minutes after specimen application.

Table: Interfering Substances Results

Analyte	Conc.	Lot: DEC14060001-T											
		Negative			IgM Positive			IgG Positive			NS1 Positive		
Ascorbic acid	20mg/ml	-	-	-	+	+	+	+	+	+	+	+	+
Hemoglobin	1000mg/dl	-	-	-	+	+	+	+	+	+	+	+	+
Gentistic acid	20mg/dl	-	-	-	+	+	+	+	+	+	+	+	+
Oxalic acid	60mg/dl	-	-	-	+	+	+	+	+	+	+	+	+
Bilirubin	1000mg/dl	-	-	-	+	+	+	+	+	+	+	+	+
Uric acid	20mg/ml	-	-	-	+	+	+	+	+	+	+	+	+
Acetoaminophen	20mg/dl	-	-	-	+	+	+	+	+	+	+	+	+
Aspirin	20mg/dl	-	-	-	+	+	+	+	+	+	+	+	+
Methanol	10%	-	-	-	+	+	+	+	+	+	+	+	+
Creatine	200mg/dl	-	-	-	+	+	+	+	+	+	+	+	+
Albumin	2000mg/dl	-	-	-	+	+	+	+	+	+	+	+	+
Caffeine	20mg/dl	-	-	-	+	+	+	+	+	+	+	+	+
Analyte	**Conc.**	**Lot: DEC14060002-T**											
		Negative			**IgM Positive**			**IgG Positive**			**NS1 Positive**		
Ascorbic acid	20mg/ml	-	-	-	+	+	+	+	+	+	+	+	+
Hemoglobin	1000mg/dl	-	-	-	+	+	+	+	+	+	+	+	+
Gentistic acid	20mg/dl	-	-	-	+	+	+	+	+	+	+	+	+
Oxalic acid	60mg/dl	-	-	-	+	+	+	+	+	+	+	+	+
Bilirubin	1000mg/dl	-	-	-	+	+	+	+	+	+	+	+	+
Uric acid	20mg/ml	-	-	-	+	+	+	+	+	+	+	+	+
Acetoaminophen	20mg/dl	-	-	-	+	+	+	+	+	+	+	+	+
Aspirin	20mg/dl	-	-	-	+	+	+	+	+	+	+	+	+
Methanol	10%	-	-	-	+	+	+	+	+	+	+	+	+

Creatine	200mg/dl	-	-	-	+	+	+	+	+	+	+	+	+
Albumin	2000mg/dl	-	-	-	+	+	+	+	+	+	+	+	+
Caffeine	20mg/dl	-	-	-	+	+	+	+	+	+	+	+	+
Analyte	**Conc.**	**Lot: DEC14060003-T**											
		Negative			**IgM Positive**			**IgG Positive**			**NS1 Positive**		
Ascorbic acid	20mg/ml	-	-	-	+	+	+	+	+	+	+	+	+
Hemoglobin	1000mg/dl	-	-	-	+	+	+	+	+	+	+	+	+
Gentistic acid	20mg/dl	-	-	-	+	+	+	+	+	+	+	+	+
Oxalic acid	60mg/dl	-	-	-	+	+	+	+	+	+	+	+	+
Bilirubin	1000mg/dl	-	-	-	+	+	+	+	+	+	+	+	+
Uric acid	20mg/ml	-	-	-	+	+	+	+	+	+	+	+	+
Acetoaminophen	20mg/dl	-	-	-	+	+	+	+	+	+	+	+	+
Aspirin	20mg/dl	-	-	-	+	+	+	+	+	+	+	+	+
Methanol	10%	-	-	-	+	+	+	+	+	+	+	+	+
Creatine	200mg/dl	-	-	-	+	+	+	+	+	+	+	+	+
Albumin	2000mg/dl	-	-	-	+	+	+	+	+	+	+	+	+
Caffeine	20mg/dl	-	-	-	+	+	+	+	+	+	+	+	+

Conclusion: No substances showed any interference with the combo test. There were no obvious differences among the 3 lots of products at 10 minutes.

2.3 Cross Reactivity

HAV+, HIV+, HBV+, HCV+, HEV+, Syphilis+, HAMA+, RF+, MONO+, CMV+, Rubella+, TOXO+ confounding specimens as confirmed by ELISA and clinical diagnosis were tested with the Dengue Combo Rapid Test Cassette, with results read at 10 minutes after specimen application**.**

Table: Cross Reactivity

Confounder Specimens	**Lot no.**								
	DEC14060001-T			**DEC14060002-T**			**DEC14060003-T**		
	10mins			**10mins**			**10mins**		
HAV+	-	-	-	-	-	-	-	-	-
HIV+	-	-	-	-	-	-	-	-	-
HBV+	-	-	-	-	-	-	-	-	-
HCV+	-	-	-	-	-	-	-	-	-
HEV+	-	-	-	-	-	-	-	-	-
Syphilis+	-	-	-	-	-	-	-	-	-
HAMA+	-	-	-	-	-	-	-	-	-
RF+	-	-	-	-	-	-	-	-	-
MONO+	-	-	-	-	-	-	-	-	-
Rubella+	-	-	-	-	-	-	-	-	-

TOXO+	-	-	-	-	-	-	-	-	-
CMV+	-	-	-	-	-	-	-	-	-

Conclusion: There was no cross-reaction with the confounder specimens above at 10 minutes.

2.4 Between Day Reproducibility

Negative, IgG middle positive, IgM middle positive, IgG high positive, IgM high positive, Dengue NS1 middle positive and high positive serum and plasma specimens were run individually on ten separate days using the same lots of combo cassettes. Results were read visually as positive or negative at 10 minutes after specimen application.

Table: Between Day Reproducibility Result

Lot: DEC14060001-T

Day			1	2	3	4	5	6	7	8	9	10
Negative	Serum	IgG	-	-	-	-	-	-	-	-	-	-
		IgM	-	-	-	-	-	-	-	-	-	-
	Plasma	IgG	-	-	-	-	-	-	-	-	-	-
		IgM	-	-	-	-	-	-	-	-	-	-
IgG middle positive	Serum	IgG	+	+	+	+	+	+	+	+	+	+
		IgM	-	-	-	-	-	-	-	-	-	-
	Plasma	IgG	+	+	+	+	+	+	+	+	+	+
		IgM	-	-	-	-	-	-	-	-	-	-
IgM middle positive	Serum	IgG	-	-	-	-	-	-	-	-	-	-
		IgM	+	+	+	+	+	+	+	+	+	+
	Plasma	IgG	-	-	-	-	-	-	-	-	-	-
		IgM	+	+	+	+	+	+	+	+	+	+
IgG high positive	Serum	IgG	+	+	+	+	+	+	+	+	+	+
		IgM	-	-	-	-	-	-	-	-	-	-
	Plasma	IgG	+	+	+	+	+	+	+	+	+	+
		IgM	-	-	-	-	-	-	-	-	-	-
IgM high positive	Serum	IgG	-	-	-	-	-	-	-	-	-	-
		IgM	+	+	+	+	+	+	+	+	+	+
	Plasma	IgG	-	-	-	-	-	-	-	-	-	-
		IgM	+	+	+	+	+	+	+	+	+	+
Day			1	2	3	4	5	6	7	8	9	10
Negative	Serum	NS1	-	-	-	-	-	-	-	-	-	-
	Plasma	NS1	-	-	-	-	-	-	-	-	-	-
Dengue	Serum	NS1	+	+	+	+	+	+	+	+	+	+

NS1 middle positive	Plasma	NS1	+	+	+	+	+	+	+	+	+	+
Dengue NS1high positive	Serum	NS1	+	+	+	+	+	+	+	+	+	+
	Plasma	NS1	+	+	+	+	+	+	+	+	+	+

Lot: DEC14060002-T

Day			1	2	3	4	5	6	7	8	9	10
Negative	Serum	IgG	-	-	-	-	-	-	-	-	-	-
		IgM	-	-	-	-	-	-	-	-	-	-
	Plasma	IgG	-	-	-	-	-	-	-	-	-	-
		IgM	-	-	-	-	-	-	-	-	-	-
IgG middle positive	Serum	IgG	+	+	+	+	+	+	+	+	+	+
		IgM	-	-	-	-	-	-	-	-	-	-
	Plasma	IgG	+	+	+	+	+	+	+	+	+	+
		IgM	-	-	-	-	-	-	-	-	-	-
IgM middle positive	Serum	IgG	-	-	-	-	-	-	-	-	-	-
		IgM	+	+	+	+	+	+	+	+	+	+
	Plasma	IgG	-	-	-	-	-	-	-	-	-	-
		IgM	+	+	+	+	+	+	+	+	+	+
IgG high positive	Serum	IgG	+	+	+	+	+	+	+	+	+	+
		IgM	-	-	-	-	-	-	-	-	-	-
	Plasma	IgG	+	+	+	+	+	+	+	+	+	+
		IgM	-	-	-	-	-	-	-	-	-	-
IgM high positive	Serum	IgG	-	-	-	-	-	-	-	-	-	-
		IgM	+	+	+	+	+	+	+	+	+	+
	Plasma	IgG	-	-	-	-	-	-	-	-	-	-
		IgM	+	+	+	+	+	+	+	+	+	+
Day			1	2	3	4	5	6	7	8	9	10
Negative	Serum	NS1	-	-	-	-	-	-	-	-	-	-
	Plasma	NS1	-	-	-	-	-	-	-	-	-	-
Dengue NS1 middle positive	Serum	NS1	+	+	+	+	+	+	+	+	+	+
	Plasma	NS1	+	+	+	+	+	+	+	+	+	+
Dengue NS1high positive	Serum	NS1	+	+	+	+	+	+	+	+	+	+
	Plasma	NS1	+	+	+	+	+	+	+	+	+	+

Lot: DEC14060003-T

Day			1	2	3	4	5	6	7	8	9	10
Negative	Serum	IgG	-	-	-	-	-	-	-	-	-	-
		IgM	-	-	-	-	-	-	-	-	-	-

	Plasma	IgG	-	-	-	-	-	-	-	-	-	-
		IgM	-	-	-	-	-	-	-	-	-	-
IgG middle positive	Serum	IgG	+	+	+	+	+	+	+	+	+	+
		IgM	-	-	-	-	-	-	-	-	-	-
	Plasma	IgG	+	+	+	+	+	+	+	+	+	+
		IgM	-	-	-	-	-	-	-	-	-	-
IgM middle positive	Serum	IgG	-	-	-	-	-	-	-	-	-	-
		IgM	+	+	+	+	+	+	+	+	+	+
	Plasma	IgG	-	-	-	-	-	-	-	-	-	-
		IgM	+	+	+	+	+	+	+	+	+	+
IgG high positive	Serum	IgG	+	+	+	+	+	+	+	+	+	+
		IgM	-	-	-	-	-	-	-	-	-	-
	Plasma	IgG	+	+	+	+	+	+	+	+	+	+
		IgM	-	-	-	-	-	-	-	-	-	-
IgM high positive	Serum	IgG	-	-	-	-	-	-	-	-	-	-
		IgM	+	+	+	+	+	+	+	+	+	+
	Plasma	IgG	-	-	-	-	-	-	-	-	-	-
		IgM	+	+	+	+	+	+	+	+	+	+
Day			1	2	3	4	5	6	7	8	9	10
Negative	Serum	NS1	-	-	-	-	-	-	-	-	-	-
	Plasma	NS1	-	-	-	-	-	-	-	-	-	-
Dengue NS1 middle positive	Serum	NS1	+	+	+	+	+	+	+	+	+	+
	Plasma	NS1	+	+	+	+	+	+	+	+	+	+
Dengue NS1high positive	Serum	NS1	+	+	+	+	+	+	+	+	+	+
	Plasma	NS1	+	+	+	+	+	+	+	+	+	+

Conclusion: Test results for both serum and plasma were consistent over the ten day period.

2.5 Between Lot Reproducibility

Negative, IgG middle positive, IgM middle positive, IgG high positive, IgM high positive, NS1 middle positive and high positive specimens (serum and plasma) were run in replicates of ten on three different lots of combo test. Results were read as positive or negative at 10 minutes after specimen application. The results below have been presented with the IgG/IgM and NS1 separated.

Table: Between Lot Reproducibility Results (Serum)

Lot: DEC14060001-T						
Specimens	Negative		IgG middle positive		IgM middle positive	
	IgG	IgM	IgG	IgM	IgG	IgM

1	-	-	+	-	-	+
2	-	-	+	-	-	+
3	-	-	+	-	-	+
4	-	-	+	-	-	+
5	-	-	+	-	-	+
6	-	-	+	-	-	+
7	-	-	+	-	-	+
8	-	-	+	-	-	+
9	-	-	+	-	-	+
10	-	-	+	-	-	+
Lot: DEC14060002-T						
Specimens	Negative		IgG middle positive		IgM middle positive	
	IgG	IgM	IgG	IgM	IgG	IgM
1	-	-	+	-	-	+
2	-	-	+	-	-	+
3	-	-	+	-	-	+
4	-	-	+	-	-	+
5	-	-	+	-	-	+
6	-	-	+	-	-	+
7	-	-	+	-	-	+
8	-	-	+	-	-	+
9	-	-	+	-	-	+
10	-	-	+	-	-	+
Lot: DEC14060003-T						
Specimens	Negative		IgG middle positive		IgM middle positive	
	IgG	IgM	IgG	IgM	IgG	IgM
1	-	-	+	-	-	+
2	-	-	+	-	-	+
3	-	-	+	-	-	+
4	-	-	+	-	-	+
5	-	-	+	-	-	+
6	-	-	+	-	-	+
7	-	-	+	-	-	+
8	-	-	+	-	-	+
9	-	-	+	-	-	+
10	-	-	+	-	-	+

Lot: DEC14060001-T				
Specimens	IgG high positive		IgM high positive	
	IgG	IgM	IgG	IgM
1	+	-	+	-
2	+	-	+	-
3	+	-	+	-
4	+	-	+	-
5	+	-	+	-
6	+	-	+	-
7	+	-	+	-
8	+	-	+	-
9	+	-	+	-
10	+	-	+	-
Lot: DEC14060002-T				
Specimens	IgG high positive		IgM high positive	
	IgG	IgM	IgG	IgM
1	+	-	+	-
2	+	-	+	-
3	+	-	+	-
4	+	-	+	-
5	+	-	+	-
6	+	-	+	-
7	+	-	+	-
8	+	-	+	-
9	+	-	+	-
10	+	-	+	-
Lot: DEC14060003-T				
Specimens	IgG high positive		IgM high positive	
	IgG	IgM	IgG	IgM
1	+	-	+	-
2	+	-	+	-
3	+	-	+	-
4	+	-	+	-
5	+	-	+	-
6	+	-	+	-
7	+	-	+	-
8	+	-	+	-
9	+	-	+	-
10	+	-	+	-

Table: Between Lot Reproducibility Results (Plasma)

Lot: DEC14060001-T						
Specimens	Negative		IgG middle positive		IgM middle positive	
	IgG	IgM	IgG	IgM	IgG	IgM
1	-	-	+	-	-	+
2	-	-	+	-	-	+
3	-	-	+	-	-	+
4	-	-	+	-	-	+
5	-	-	+	-	-	+
6	-	-	+	-	-	+
7	-	-	+	-	-	+
8	-	-	+	-	-	+
9	-	-	+	-	-	+
10	-	-	+	-	-	+
Lot: DEC14060002-T						
Specimens	Negative		IgG middle positive		IgM middle positive	
	IgG	IgM	IgG	IgM	IgG	IgM
1	-	-	+	-	-	+
2	-	-	+	-	-	+
3	-	-	+	-	-	+
4	-	-	+	-	-	+
5	-	-	+	-	-	+
6	-	-	+	-	-	+
7	-	-	+	-	-	+
8	-	-	+	-	-	+
9	-	-	+	-	-	+
10	-	-	+	-	-	+
Lot: DEC14060003-T						
Specimens	Negative		IgG middle positive		IgM middle positive	
	IgG	IgM	IgG	IgM	IgG	IgM
1	-	-	+	-	-	+
2	-	-	+	-	-	+
3	-	-	+	-	-	+
4	-	-	+	-	-	+
5	-	-	+	-	-	+
6	-	-	+	-	-	+

7	-	-	+	-	-	+
8	-	-	+	-	-	+
9	-	-	+	-	-	+
10	-	-	+	-	-	+

Lot: DEC14060001-T				
Specimens	IgG high positive		IgM high positive	
	IgG	IgM	IgG	IgM
1	+	-	+	-
2	+	-	+	-
3	+	-	+	-
4	+	-	+	-
5	+	-	+	-
6	+	-	+	-
7	+	-	+	-
8	+	-	+	-
9	+	-	+	-
10	+	-	+	-
Lot: DEC14060002-T				
Specimens	IgG high positive		IgM high positive	
	IgG	IgM	IgG	IgM
1	+	-	+	-
2	+	-	+	-
3	+	-	+	-
4	+	-	+	-
5	+	-	+	-
6	+	-	+	-
7	+	-	+	-
8	+	-	+	-
9	+	-	+	-
10	+	-	+	-
Lot: DEC14060003-T				
Specimens	IgG high positive		IgM high positive	
	IgG	IgM	IgG	IgM
1	+	-	+	-
2	+	-	+	-
3	+	-	+	-
4	+	-	+	-
5	+	-	+	-
6	+	-	+	-

7	+	-	+	-
8	+	-	+	-
9	+	-	+	-
10	+	-	+	-

Table: Between Lot Reproducibility Results (Serum)

Lot	Day	Negative	NS1 Low Positive	NS1 Medium Positive	NS1 High Positive
DEC14060001-T	1	-	+	+	+
	2	-	+	+	+
	3	-	+	+	+
	4	-	+	+	+
	5	-	+	+	+
	6	-	+	+	+
	7	-	+	+	+
	8	-	+	+	+
	9	-	+	+	+
	10	-	+	+	+
DEC14060002-T	1	-	+	+	+
	2	-	+	+	+
	3	-	+	+	+
	4	-	+	+	+
	5	-	+	+	+
	6	-	+	+	+
	7	-	+	+	+
	8	-	+	+	+
	9	-	+	+	+
	10	-	+	+	+
DEC14060003-T	1	-	+	+	+
	2	-	+	+	+
	3	-	+	+	+
	4	-	+	+	+
	5	-	+	+	+
	6	-	+	+	+
	7	-	+	+	+
	8	-	+	+	+
	9	-	+	+	+
	10	-	+	+	+

Table: Between Lot Reproducibility Results (Plasma)

Lot	Day	Negative	NS1 Low Positive	NS1 Medium Positive	NS1 High Positive

DEC14060001-T	1	-	+	+	+
	2	-	+	+	+
	3	-	+	+	+
	4	-	+	+	+
	5	-	+	+	+
	6	-	+	+	+
	7	-	+	+	+
	8	-	+	+	+
	9	-	+	+	+
	10	-	+	+	+
DEC14060002-T	1	-	+	+	+
	2	-	+	+	+
	3	-	+	+	+
	4	-	+	+	+
	5	-	+	+	+
	6	-	+	+	+
	7	-	+	+	+
	8	-	+	+	+
	9	-	+	+	+
	10	-	+	+	+
DEC14060003-T	1	-	+	+	+
	2	-	+	+	+
	3	-	+	+	+
	4	-	+	+	+
	5	-	+	+	+
	6	-	+	+	+
	7	-	+	+	+
	8	-	+	+	+
	9	-	+	+	+
	10	-	+	+	+

Conclusion: Test results for both serum and plasma were consistent between the three lots of combo test cassettes.

2.6 Accelerated Stability Study

Accelerated Stability of the Dengue Combo Rapid Test Cassette was evaluated using samples from three different batches. These were placed in an incubator with the temperature calibrated at 45°C and 55°C. Relative humidity (RH) calibrated at about 60%. A series of stability tests were performed at 0, 7, 14, 21, 28, 35, 42, 56, 77, 84 days for 45°C. At 55°C, the same tests were performed 0, 7, 14, 21, 28, 35, 42 days according to Arrhenius Plot. Test cassettes were assayed using negative, middle positive IgG, IgM, and NS1 standard specimens. Testing at each specific time interval consisted of 3 replicates for each specimen. The tests were performed according to

the package insert and results were read at 10 minutes.

Arrhenius Formula:

In K=-Ea/RT + In A

"K" mean Rate constant

"A" mean Arrhenius constant

"Ea" mean Activation energy

"R" mean Gas constant

"T" mean Temperature in Kelvin

Table: Timeline for Accelerated Stability Study

Day / Temp.	0day	7days	14 days	21 days	28 days	35 days	42 days	56 days	77 days	84 days
45°C	√	√	√	√	√	√	√	√	√	√
55°C	√	√	√	√	√	√	√	×	×	×

Table: 45°C Accelerated Stability Summary

Day	Specimen	RAPG-DEC-001								
		DEC14060001-T			DEC14060002-T			DEC14060003-T		
0	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+
	NS1 Positive	+	+	+	+	+	+	+	+	+
7	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+
	NS1 Positive	+	+	+	+	+	+	+	+	+
14	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+
	NS1 Positive	+	+	+	+	+	+	+	+	+
21	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+
	NS1 Positive	+	+	+	+	+	+	+	+	+
28	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+
	NS1 Positive	+	+	+	+	+	+	+	+	+
35	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+

	NS1 Positive	+	+	+	+	+	+	+	+	+
42	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+
	NS1 Positive	+	+	+	+	+	+	+	+	+
56	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+
	NS1 Positive	+	+	+	+	+	+	+	+	+
77	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+
	NS1 Positive	+	+	+	+	+	+	+	+	+
84	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+
	NS1 Positive	+	+	+	+	+	+	+	+	+

Table: 45°C Accelerated Stability Summary

Day	Specimen	RAPG-DEC-001								
		DEC14060001-T			DEC14060002-T			DEC14060003-T		
0	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+
	NS1 Positive	+	+	+	+	+	+	+	+	+
7	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+
	NS1 Positive	+	+	+	+	+	+	+	+	+
14	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+
	NS1 Positive	+	+	+	+	+	+	+	+	+
21	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+
	NS1 Positive	+	+	+	+	+	+	+	+	+
28	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+
	NS1 Positive	+	+	+	+	+	+	+	+	+
35	Negative	-	-	-	-	-	-	-	-	-

	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+
	NS1 Positive	+	+	+	+	+	+	+	+	+
42	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+
	NS1 Positive	+	+	+	+	+	+	+	+	+

Conclusion: The Dengue Combo Rapid Test Cassette was stable at 45°C for 84 days and at 55°C for 42 days. This data was plotted on an Arrhenius Plot and the shelf life of this product was determined to be at least 24 months from the date of manufacture.

2.7 Real-Time Stability Study

Real-time stability of the Dengue Combo Rapid Test Cassette was evaluated using three different transfer lots of test kit. These were placed as packed in a regular storage area, where the temperature varies from 12°C up to 26°C depending on the time of year. Each batch of test kit was in month-long intervals for the first 6 months, then every 3 months thereafter up until the 27^{th} month.

During testing, the devices were assayed in triplicate using negative, and medium positive IgG, IgM and NS1 specimens. Testing was performed according to the prescribed test procedure with results read at 10 minutes.

Table: Real-time stability study results

Month	**Specimen**	**Lot no.**								
		DEC14060001-T			DEC14060002-T			DEC14060003-T		
0	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+
	NS1 Positive	+	+	+	+	+	+	+	+	+
1	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+
	NS1 Positive	+	+	+	+	+	+	+	+	+
2	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+
	NS1 Positive	+	+	+	+	+	+	+	+	+

3	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+
	NS1 Positive	+	+	+	+	+	+	+	+	+
4	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+
	NS1 Positive	+	+	+	+	+	+	+	+	+
5	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+
	NS1 Positive	+	+	+	+	+	+	+	+	+
6	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+
	NS1 Positive	+	+	+	+	+	+	+	+	+
7	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+
	NS1 Positive	+	+	+	+	+	+	+	+	+
8	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+
	NS1 Positive	+	+	+	+	+	+	+	+	+
9	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+
	NS1 Positive	+	+	+	+	+	+	+	+	+
12	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+
	NS1 Positive	+	+	+	+	+	+	+	+	+
15	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+
	NS1 Positive	+	+	+	+	+	+	+	+	+
18	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+

	IgM Positive	+	+	+	+	+	+	+	+	+
	NS1 Positive	+	+	+	+	+	+	+	+	+
21	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+
	NS1 Positive	+	+	+	+	+	+	+	+	+
24	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+
	NS1 Positive	+	+	+	+	+	+	+	+	+
27	Negative	-	-	-	-	-	-	-	-	-
	IgG Positive	+	+	+	+	+	+	+	+	+
	IgM Positive	+	+	+	+	+	+	+	+	+
	NS1 Positive	+	+	+	+	+	+	+	+	+

Conclusion This study successfully validated the initial projected shelf-life of 24 months derived from the accelerated stability study. The Dengue Combo Rapid Test Cassette was stable for at least 24 months from the date of manufacture when stored at the prescribed storage conditions.

2.8 Transport Stability Study

The stability of the Dengue Combo Rapid Test Cassette under simulated transportation conditions was evaluated using one lot of test. The tests were placed in a refrigerator with a monitored temperature range of 2 - 8°C, and an incubator calibrated at 45°C. These temperatures were chosen to represent the extremes of temperature likely to be encountered in transport. It is already known that freezing the tests causes serious deterioration. Stability tests will be performed at 0, 7, 14, 21 and 28 days. This represents the longest reasonable time that the tests could be in transit, taking into account delay. Test cassettes were assayed using negative, and medium positive IgG, IgM and NS1 specimens. Testing at each specific time interval consisted of 3 replicates for each specimen. The tests were performed according to the package insert. Results are presented in Table below.

Table: 2-8°C Transport Stability Results

Day	**Specimen**	**RAPG-DEC-001**		
		DEC14060002-T		
0	Negative	-	-	-
	IgG Positive	+	+	+
	IgM Positive	+	+	+
	NS1 Positive	+	+	+

7	Negative	-	-	-
	IgG Positive	+	+	+
	IgM Positive	+	+	+
	NS1 Positive	+	+	+
14	Negative	-	-	-
	IgG Positive	+	+	+
	IgM Positive	+	+	+
	NS1 Positive	+	+	+
21	Negative	-	-	-
	IgG Positive	+	+	+
	IgM Positive	+	+	+
	NS1 Positive	+	+	+
28	Negative	-	-	-
	IgG Positive	+	+	+
	IgM Positive	+	+	+
	NS1 Positive	+	+	+

Table: 45°C Transport Stability Results

Day	**Specimen**	**RAPG-DEC-001**		
		DEC14060002-T		
0	Negative	-	-	-
	IgG Positive	+	+	+
	IgM Positive	+	+	+
	NS1 Positive	+	+	+
7	Negative	-	-	-
	IgG Positive	+	+	+
	IgM Positive	+	+	+
	NS1 Positive	+	+	+
14	Negative	-	-	-
	IgG Positive	+	+	+
	IgM Positive	+	+	+
	NS1 Positive	+	+	+
21	Negative	-	-	-
	IgG Positive	+	+	+
	IgM Positive	+	+	+
	NS1 Positive	+	+	+
28	Negative	-	-	-
	IgG Positive	+	+	+

	IgM Positive	+	+	+
	NS1 Positive	+	+	+

Conclusion:

The Dengue Combo Rapid Test Cassette was stable for at least 28 days in simulated transport conditions of between 2 - 45°C.

3 RISK ASSESSMENT

Process step / component	Cause and effect of failure	S	O	D	RPN before	Preventative measure(s)	S	O	D	RPN after
Storage of kit outside of prescribed environmental conditions	Degradation of the components of the kits due to storage at incorrect temperature may lead to erroneous test results. Such degradation is not immediately obvious.	3	4	7	**84**	Label on the kit box as well as product instructions should clearly state the storage conditions. Lack of control line will indicate if test has degraded due to poor storage.	3	2	3	**18**
Use of kit past expiration date	Degradation of the components of the kit past their expiration date may lead to erroneous test results.	3	3	3	**27**	Label on the kit box and components clearly stating expiration date.	3	2	2	**12**
Reuse of kit	Test cassettes must not be reused.	5	3	2	**30**	"Do not re-use" symbol on kit box.	5	2	2	**20**
Use by unskilled operator	The kit is not for self testing and must be used by a trained laboratory technician. Misuse can cause erroneous test results.	5	3	3	**45**	Include a statement "For use by healthcare professionals"	5	2	3	**30**
Mislabelling of expiration date	Misinformation of user. User may dispose of unexpired test kit causing wastage, or may use an expired test kit causing erroneous test results.	6	3	5	**90**	Carefully inspect label and cross-reference against production records to ensure printed expiry date is 24months from date of manufacture.	6	2	1	**12**
Mislabelling of lot numbers	Misinformation of user. Traceability not ensured.	2	3	5	**30**	Carefully inspect labels and cross-reference against production records.	2	2	1	**4**
Leakage of reagent bottles	Insufficient material to carry out test. Destruction of outer package.	5	3	5	**75**	Ensure all bottle lids are tight. Check each bottle for leaks by inverting and gently squeezing.	5	1	1	**5**
Foil pouch damaged	Humidity affects the test cassettes and causes degradation.	5	2	4	**40**	Inspect foil pouches and ensure vacuum seal integrity. Include a desiccant packet.	5	1	1	**5**
Antibody not properly coated on membrane	Caused by incorrect use of coating buffer, or insufficient coating time. Low or no reading. Difficult to	6	3	8	**144**	Ensure correct dilution of antibody is made according to manufacturing method. Ensure correct coating buffer is used. Assemble	6	1	2	**12**

Process step / component	Cause and effect of failure	S	O	D	RPN before	Preventative measure(s)	S	O	D	RPN after
	visually confirm strips are properly coated.					membrane into cassettes and seal the cassettes in a foil pouch with desiccant under vacuum. Carry out QC on membrane and ensure they meet batch release criteria.				
Use of incorrect antigen or antibody	Low or no reading and the test would not work.	5	3	4	**60**	Carefully check labels of antibody or antigen stock. Carry out QC to ensure batch release criteria are met.	5	2	1	**10**
Result read too early	Coloured lines on test strip may not be fully developed, could give misleading reading.	6	5	3	**90**	Ensure instructions clearly state to allow 10 minutes before reading results.	6	2	3	**36**
Result read too late	Coloured lines on test strip may begin to fade, giving misleading reading.	6	5	3	**90**	Ensure instructions clearly state not to read results after 20 minutes.	6	2	3	**36**

Declaration of Conformity

according to Directive 98/79/EC, on *in vitro* diagnostic medical devices

Manufacturer (Name, Address)	Biopanda Reagents Ltd Unit 14 Carrowreagh Business Park Carrowreagh Road Belfast, BT16 1QQ United Kingdom			
Product Details	Name	:	Dengue Combo Rapid Test Cassette (RAPG-DEC-001)	
	Classification of products according to directive	:	General IVDs (not under Annex II)	
Applicable coordination standards	EN ISO 14971:2012 ISO 13485:2016 EN ISO 18113:2011 EN 13612:2002 EN 23640:2015 EN ISO 15223-1:2016			

Signatory representative declares herein the above mentioned device meets the basic requirements of the European Parliament and the Council's *in vitro* diagnostic medical devices directive: 98/79/EC Annex III.

This declaration of conformity is based on European Parliament and the Council's 98/79/EC directive Annex III.

This CE Declaration of Conformity is issued under the full responsibility of the manufacturer.

14/10/2021, Belfast, UK

(date and place of issue)

Han Yan (Quality Manager)

(name, title and signature or equivalent marking of authorised person)