Dengue Virus NS1 Antigen (CLIA)
PACKAGE INSERT

IVD

INTENDED USE

Dengue Virus NS1 Antigen (CLIA) is a chemiluminescence immunoassay test for the qualitative measurement of circulating Dengue Virus NS1 antigen in human serum or plasma (heparin or EDTA), which is intended as an aid to assess the Dengue virus infection.
The test must be performed on the ACCRE series analyzers (including ACCRE 6 and, ACCRE 8, ACCRE90, ACCRE100, ACCRE120, and all other ACCRE series analyzers).

For *in vitro* diagnostic use only. For professional use only.

SUMMARY

Dengue Virus is a single chain RNA virus, belongs to the Flaviviridae family and flaviridae genera[1]. It is transmitted by Aedes aegypti and Aedes albopictus, Patients and latent infections are the main sources of infection. The incubation period after infection with dengue virus is 3-15 days, usually 5-8 days. New WHO guidelines in 2009 recommended that dengue be divided into non-severe and severe dengue. Clinical manifestations are mainly high fever, headache, muscle and joint pain, accompanied by rash, lymph node swelling, severe patients may appear plasma leakage, bleeding, shock and serious organ damage, high mortality[2].
The viral gene encodes three structural proteins (E protein, C protein, M protein) and seven non-structural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5). According to the antigenic specificity of virus E protein, it can be divided into four serotypes (DEN 1-4), with crossover antigenicity. NS1 is a highly conserved glycoprotein and a hallmark protein of dengue virus, which exists in infected dengue virus cells and can be detected in patients 1-9 days after onset.

ASSAY PRINCIPLES

The Dengue Virus NS1 Antigen (CLIA) hereinafter also referred to as the "Den NS1 Ag" is an antibody sandwich immunoassay. The principle is described as following:

Step1: The samples, alkaline phosphatase-labeled mouse anti-dengue NS1 antibody and mouse anti-dengue NS1 antibody coated magnetic beads were mixed and incubated, and the antigens in the samples combined with the antibodies on the magnetic beads and alkaline phosphatase-labeled antibodies to form a complex.

Step2: After the reaction, the magnetic field absorbs the magnetic beads, and the unbound material is removed by cleaning. After the substrate was added, the luminous intensity was measured by Photomultiplier tube (PMT) as relative light units (RLUs). The concentration of the substance to be detected in the sample is proportional to the luminescence intensity. At the end, the results were automatically calculated by the ACCRE analyzer in relation to the calibration curve stored.

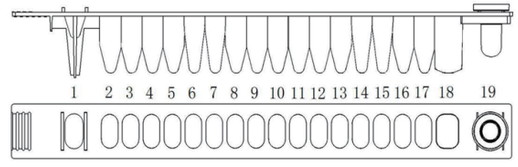
CONTENT OF THE KIT

Package: 60 tests/kit with quality controls, 36 tests/kit with quality controls, 60 tests/kit without quality controls, 36 tests/kit without quality controls

Contents	Quantities				Notice
	REF W115A	REF W115C	REF W133A	REF W133C	
Den NS1 Ag reagent cartridges	60 strips	36 strips	60 strips	36 strips	Ready to Use
Den NS1 Ag Calibrator 1 (CAL 1)	1.0 mL x 1 vial	1.0 mL x 1 vial	1.0 mL x 1 vial	1.0 mL x 1 vial	Tris buffer, 50mM; ProClin300, 0.5g/L; BND, 0.2g/L ; Recombinant Dengue NS1 antigen, 1RU/mL
Den NS1 Ag Calibrator 2 (CAL 2)	1.0 mL x 1 vial	1.0 mL x 1 vial	1.0 mL x 1 vial	1.0 mL x 1 vial	Tris buffer, 50mM; ProClin300, 0.5g/L; BND, 0.2g/L; Recombinant Dengue NS1 antigen , 25RU/mL
Den NS1 Ag Quality Control Level 1 (QC 1)	1.0 mL x 1 vial	1.0 mL x 1 vial	/	/	Tris buffer, 50mM; ProClin300, 0.5g/L; BND, 0.2g/L; Recombinant Dengue NS1 antigen , 0.5RU/mL Only for the configurations with Quality Controls
Den NS1 Ag Quality Control Level 2 (QC 2)	1.0 mL x 1 vial	1.0 mL x 1 vial	/	/	Tris buffer, 50mM; ProClin300, 0.5g/L; BND, 0.2g/L; Recombinant Dengue NS1 antigen , 20RU/mL Only for the configurations with Quality Controls
Instruction for Use	1	1	1	1	N/A
Reagent Registration Card	1	1	1	1	N/A
Calibrator Barcode Sheet	1	1	1	1	N/A
Quality Control Sheet	1	1	/	/	Only for the configurations with Quality Controls

Traceability: This method has been standardized against the internal reference. For calibrator concentration values, see the calibration card for each lot.

The Reagents Cartridge
The strip consists of 19 wells. The 1st well is the sample well and the last well 19th is the reading well for chemiluminescent signal. From the 2nd to the 18th well are all covered with a labeled, aluminum foil seal. The label comprises a barcode which mainly indicates the reagent assay information, reagent lot number and expiration date. All reagents components needed for the assay are ready-to-use and pre-dispensed in the cartridges. And please do not interchange integral component from different reagents or lots.



Description of The Reagent Cartridge

Well	Component	Content	Volume
3	Sample Diluent	Tris buffer, 50mmol/L;ProClin300,0.5g/L; BND,0.2g/L	50μL
5	Enzyme Label	ALP(alkaline phosphatase) labeled mouse anti- dengue NS1 antibody, 2.0mg/L, MES buffer, 50mmol/L; ProClin300,0.5g/L; BND, 0.2g/L	50μL
7	Magnetic Particles	Mouse anti-dengue NS1 antibody coated magnetic particles 0.2g/L; Tris buffer, 50mmol/L; ProClin300, 0.5g/L; BND,0.2g/L	50μL
10, 11, 12	Wash Solutions	Tris buffer, 50mmol/L; ProClin300,0.5g/L; BND,0.2g/L	350μL
16	Substrate	Disodium [(4-chlorophenyl)sulfonyl] (10-methyl-9(10H)-acridinylidene) methyl phosphate	100μL

Note: Well 1, 2, 4, 6, 8, 9, 13, 14, 15, 17, 18 are empty.

MATERIALS AND DISPOSABLES REQUIRED BUT NOT PROVIDED

- Sample diluent(manufactured by Tisenc).
- Pipette with disposable tip to dispense 200 μL.
- Powderless, disposable gloves.
- For other specific materials and disposables, please refer to the Instrument User Manual.
- Instruments of the ACCRE series analyzers.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- For professional use only by qualified laboratory personnel.
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (do not ingest; do not inhale).
- Do not use reagents after the expiration date indicated on the box label.
- Do not use if the packaging of reagent cartridge is damaged or leaked.
- Do not use the reagents if the quantity is obviously sufficient.
- If any component adheres to inside wall of wells or aluminum film of the reagent cartridge, please gently switch the reagent cartridge to let the component back the wells.
- Do not reverse the reagent cartridge. Otherwise, the result will be unreliable.
- Do not mix reagents (or disposables) from different lots.
- Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.
- Use powderless gloves, as powder has been reported to cause false results for certain enzyme immunoassay tests.

STORAGE

- Store the Dengue Virus NS1 Antigen (CLIA) kits **upright** at 2-8°C, kits can be stored for 18 months from manufactured date. After the reagent cartridge is on-board in the ACCRE analyzer, the testing should be finished within 2 hours.
- Store the calibrators and quality controls at 2-8°C till expiry date. When opened, the calibrators and/or controls will be stable for 30 days at 2-8°C and longer time at -20°C or below -20°C for 90 days. Calibrators and quality controls can only be frozen and thawed once.
- Keep away from sunlight.
- Do not freeze reagent cartridge.

SAMPLES

The sample volume for Dengue Virus NS1 Antigen assay is 50μL, please note that at least 75μL of sample has to be pipetted manually into the reagent cartridge.

Sample type and collection
Human serum or plasma (Heparin lithium, EDTA-K_2 or EDTA-K_3) are recommended.
Types of tubes:
- Plastic tube with clot activator,
- Plastic tube with clot activator and separation gel,
- Plastic tube with Heparin lithium,
- Plastic tube with EDTA-K_2,
- Plastic tube with EDTA-K_3,

It is recommended to validate collection tubes before use as some may contain substances which interfere with test results.
Note: Blood sampling tube results may vary from one manufacturer to another depending on the materials and additives used.
It is the responsibility of each laboratory to validate the type of sample tube used and to follow the manufacturer's recommendations for use.

Sample preparation
The current revision of the WHO/DIL/LAB/99.1 document provides recommendations for the preparation of samples.
For use of the sample tubes, follow the tube manufacturer's instructions for use.
The pre-analytical step, including the preparation of blood samples, is an essential part of medical analyses. In accordance with Good Laboratory Practice, this step is performed under the responsibility of the laboratory manager.
Insufficient clot time can result in the formation of fibrin with micro-clots that are invisible to the naked eye. The presence of fibrin, red blood cells, or suspended particles can lead to erroneous results.
Samples containing suspended fibrin particles or erythrocyte stroma should be centrifuged before testing.
If there is a lipid layer over the sample after centrifuging, please transfer the clear sample into a new sample tube without transferring the lipid layer.
Severe hemolysis samples (hemoglobin >1000mg/dL) or heat-inactivated samples are not recommended for applying on the reagents.
Preparation of frozen-stored samples: after thawing, these samples must be thoroughly mixed before testing.
Mix using a vortex-type mixer. If necessary, clarify samples by centrifuging before testing.

Sample stability
Samples (serum and plasma) can be stored at room temperature (15-30°C) for up to 4 hours or at 2~8°C for up to 2 days; if longer storage is required, freeze the serum or plasma at -20°C or below -20°C for up to 3 months, without exceeding 3 freeze/thaw cycles.

INSTRUCTIONS FOR USE

For complete instructions, see the Instrument User Manual.
When using the assay for the first time.
Register the assay using the registration code in the reagent kit before any assay is used in the ACCRE analyzer for the first time. Calibration, QC and sample test are only available after assay was registered successfully.
When opening a new lot of reagents.
Each new lot of reagent must be registered by using the registration code in

Reagent Registration Card within the reagent kit.

Calibration

Calibration must be performed with the reagents and calibrators from Manufacturer.

Calibration is necessary under the following conditions:

(1) New reagent lot number applied;

(2) Same lot of reagents used more than 4 weeks;

(3) Quality control out of control;

(4) When necessary, for example: system maintenance or repair that may affect the analytic performance.

Test Procedure

1. Remove the kit from storage at 2-8°C and take out the required number of reagent cartridges. Allow the reagent cartridges, specimens, calibrators, and/or controls to equilibrate to room temperature (15-30°C) prior to testing.
2. Use each assay strips for each sample, calibrator or control respectively.
3. Place the reagent cartridges into the reagent rack.
4. Manually pipette the Sample, Calibrator, or Control into the sample well of each reagent cartridge.

Please note that at least 75μL of sample has to be pipetted manually into the reagent cartridge and 50μL sample is needed for each test.

**Before pipetting: Mix the samples using a vortex-type mixer if necessary, and ensure that the samples and sample diluent (if applicable). DO NOT have any bubbles. The same for calibrators and controls.*

5. Put the sample rack into the reagent chamber of ACCRE analyzer.
6. Place the assay tips into the reagent chamber of the ACCRE analyzer.
7. Input Sample ID, sample type, dilution etc. into the software as directed by USER MANUAL.
8. Dengue Virus NS1 Antigen (CLIA) assays will be completed within approximately 15 minutes. Assays will be performed automatically by the ACCRE analyzer.
9. After assay is completed, remove the reagent rack from the ACCRE analyzer. Dispose the used reagent cartridge into an appropriate recipient.

Dilution

Samples with concentrations above 300RU/mL should be diluted with negative serum or plasma or sample diluent manually. The recommended dilution is 1:10. After manual dilution, multiply the result by the dilution factor. ACCRE analyzer cannot perform automatic dilution for samples.

Quality Control

Quality control should be performed in accordance with local regulations or requirements related to accreditation, as well as requirements defined in the laboratory's quality control procedure.

RESULTS AND INTERPRETATION

Calculation of Results

The results are automatically calculated by the ACCRE analyzer using stored calibration curve which generated by 2-point calibration. And calculated according to a predefined mathematical model, the results expressed in RU/mL. The upper normal range (cut-off value) for non-infected persons is defined as 1 RU/mL.

Interpretation of Results

COI>1.0 RU/mL indicates positive result.

COI<0.8 RU/mL indicates negative result.

When the COI value is between 0.8 RU/mL and 1.0 RU/mL, whether the Den NS1 Ag in the sample is positive or negative cannot be determined.

** In interpreting the results, patient's clinical condition including symptoms, diseases history, and other relevant data and information should be considered.*

LIMITATIONS

1. For diagnostic purposes, results should be used in conjunction with other data; e.g., symptoms, results of other tests, clinical impressions, etc.
2. Please note that heterophile antibody in human serum or plasma can interfere the test result.
3. High concentration of HAMA can also cause a false positive or false negative result.
4. If the test results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
5. The detection range is from 0 RU/mL to 300 RU/mL, Sample with Den NS1 antigen concentration below the upper detection limit can be quantified, if the concentration is above the upper detection limit , the reported results are reported as＞300 RU/mL. The product supports manual sample dilution of 1:10 and can be diluted with negative serum or plasma or sample diluent.

PERFORMANCE CHARACTERISTICS

Lower limit of measurement

LoD (Limit of detection) = 0.3 RU/mL.

The LoD was determined in accordance with the CLSI® (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

Measurement range

The measurement range of the Dengue Virus NS1 Antigen (CLIA) assay is 0.3RU/mL-300 RU/mL (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 0.3 RU/mL. Values above the measuring range are reported as >300 RU/mL.

Precision

The precision study was performed according to CLSI® EP05-A3 recommendations.

The within-lot precision CV% is less than 10%.

The between-lot precision CV% is less than 15%.

Interfering Substances

Potential interference substances were studied according to CLSI® EP07-A3 recommendations. No significant interference was detected up to the maximum concentrations tested.

Substance	Concentration
Hemoglobin	≤1000mg/dL
Bilirubin	≤20mg/dL
Triglyceride	≤1500mg/dL
Total Protein	≤9g/dL
Rheumatoid factor	≤1500IU/mL

Clinical coincidence

260 clinical samples were studied, of which 205 samples were negative and 55 samples were positive. The specificity was 100%, the sensitivity was 96.36% and the total coincidence rate was 99.23%. The study result was shown as below:

Methodology	positive (Reference)	negative (Reference)	Total
Den NS1 Ag positive	53	0	53
Den NS1 Ag negative	2	205	207
Total	55	205	260

WASTE DISPOSAL

Dispose of used or unused reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.

It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

INDEX OF SYMBOLS

Symbol	Meaning	Symbol	Meaning	Symbol	Meaning
	Consult instructions for use		Contains sufficient for <n> tests		Date of manufacture
	*In vitro* diagnostic medical device		Use-by date		Do not re-use
	Temperature limit		Batch code		Catalogue number
	Keep away from sunlight		Keep dry		Manufacturer
	Authorized representative in the European Community/ European Union		Do not use if package is damaged and consult instructions for use		Contains biological material of animal origin
	Biological risks		Caution		CE marking
	Upright		Fragile, handle with care		Do not roll
	Control				

REFERENCES

1. Henchal, EA and JR Putnak. 1990. The Dengue Viruses. Clin. Micro. Rev. #:376-396.
2. Halstead, SB. 1980. Immunological parameters of togavirus disease syndromes. p.107-173. In RW Schlesinger (ed.), The Toga Viruses. Academic Press,Inc., New York

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