Dengue Virus IgG Antibody (CLIA)
PACKAGE INSERT

IVD

INTENDED USE

Dengue Virus IgG Antibody (CLIA) is a chemiluminescence immunoassay test for the qualitative measurement of IgG antibody of Dengue Virus in human serum or plasma (Heparin lithium or EDTA), which is intended as an aid to assess the infection of dengue virus in the past or recently.

The test must be performed on the ACCRE series analyzers (including ACCRE 6, ACCRE 8, ACCRE90, ACCRE100，ACCRE120 and all other ACCRE series analyzers).

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

SUMMARY

Dengue fever is a serious viral infectious disease. The disease is mainly prevalent in tropical regions and sub-tropical regions. Dengue viruses belong to the flavivirus family, is a single stranded plus strand RNA virus. Dengue virus consists of a core and an outer envelope. The core is a 20-sided virus particle composed of RNA and protein, and the outer envelope is composed of two glycoproteins. The envelope contains type I and group specific antigens.

Dengue viruses are mainly transmitted by aedes aegypti and Aedes albopictus, it can cause dengue fever(DF)、dengue hemorrhagic fever（DHF）and Dengue shock syndrome (DSS)，which have a high incidence and mortality. Primary infection with dengue virus results in the level of dengue IgM antibody increased in 3 to 5 days and peaked at 2 weeks，this generally persists for 30 to 60 days. IgG levels also become elevated after 10 to 14 days and remain detectable for life. During secondary infection, IgM levels generally rise more slowly and reach lower levels than in primary infection, while IgG levels rise rapidly from 1 to 2 days after the onset of symptoms. Detection of dengue IgM antibody can preliminarily judge the primary infection and the secondary infection, which is contributes to the screening and diagnosis of dengue fever[1].

ASSAY PRINCIPLES

The Dengue Virus IgG Antibody (CLIA) hereinafter also referred to as the "Den IgG", is a two-step indirect immunoassay. The principle is described as following:

Step1: The sample was added into the dengue antigen coated magnetic particles. After incubating at 37°C for 5 minutes, the dengue IgG antibodies in the samples bound with the coated antigen to form an antibody-antigen complex.

Step2: The magnetic particles were separated through attaching to the side wall of assay tip. The unbound material in the sample was then washed away. Then anti-human IgG antibody labeled with alkaline phosphatase was added into the reaction system, mixed thoroughly and incubated at 37℃ for 5mins.

Step3: The magnetic particles were separated through attaching to the side wall of assay tip. The unbound material in the sample was then washed away. After the substrate was added, the luminous intensity was measured by Photomultiplier tube (PMT) as relative light units (RLUs). The content of IgG antibody to Dengue in the sample is proportional to the luminous intensity. At the end, the results were automatically calculated by the ACCRE analyzer in relation to the calibration curve stored.

CONTENT OF THE KIT

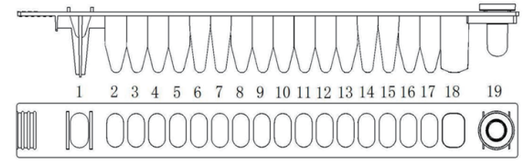
Package: 60 tests/kit with quality controls, 36 tests/kit with quality controls, 60 tests/kit without quality controls, 36 tests/kit without quality controls

Contents	Quantities				Notice
	REF W114A	REF W114C	REF W132A	REF W132C	
Den IgG reagent cartridge	60 strips	36 strips	60 strips	36 strips	Ready to Use
Den IgG 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; Antibody to dengue virus, About 1RU/mL.
Den IgG 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; Antibody to dengue virus，About 25RU/mL.
Den IgG 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; Antibody to dengue virus, About 0.5RU/mL. Only for the configuration with Quality Controls
Den IgG 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; Antibody to dengue virus，About 20 RU/mL. Only for the configuration 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 barcodes 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
2, 3	Wash Solutions	Tris buffer, 50mmol/L; ProClin300, 0.5g/L; BND,0.2g/L	350μL
5	Enzyme Label	ALP(alkaline phosphatase) labeled mouse anti-human IgG antibody ,2.0mg/L; MES buffer, 50mmol/L; ProClin300, 0.5g/L; BND,0.2g/L	50μL
7	Magnetic Particles	Dengue antigen 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
18	Sample diluent	Tris buffer, 50mmol/L; ProClin300,0.48g/L; BND,0.2g/L	350μL

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

MATERIALS AND DISPOSABLES REQUIRED BUT NOT PROVIDED

- 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.
- Sample diluent(manufactured by Tisenc).

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 infectiouscand 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 cartridgecplease 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 IgG Antibody (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 the reagent cartridge.

SAMPLES

The sample volume for Dengue Virus IgG Antibody (CLIA) 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 or EDTA-K_2) 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.

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.

Repeated freezing and thawing is not allowed.

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 8 hours or at 2~8°C for up to 7 days. If longer storage is required, freeze the serum or plasma at -20°C or below -20°C for up to 3 months.

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 reagent cartridges 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 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 into the software as directed by USER MANUAL.
8. Dengue Virus IgG Antibody (CLIA) assay 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 300 RU/mL can 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
Den IgG＞1.0 RU/mL indicates positive result.
Den IgG＜0.8 RU/mL indicates negative result.
When the Den IgG value is between 0.8RU/mL and 1.0RU/mL, whether the Dengue virus IgG antibody in the sample is positive or negative can not 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. In this case, direct nucleic acid assay for the Dengue virus should be considered.
5. The detection range is from 0 RU/mL to 300 RU/mL，Samples with Den IgG concentration below the upper detection limit can be quantified. If the concentration is above the upper detection limit, the results are reported ＞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 IgG Antibody (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	≤1000mg/dL
Total Protein	≤9g/dL
Rheumatoid factor	≤1000IU/ml

Clinical coincidence
86 positive samples and 557 negative samples were assayed by Dengue Virus IgG Antibody (CLIA), the specificity was 99.46%, and the sensitivity was 100%.

	positive（Reference）	negative（Reference）
Den IgG positive	86	3
Den IgG negative	0	554

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
IVD	*In vitro* diagnostic medical device		Use-by date		Do not re-use
	Temperature limit	LOT	Batch code	REF	Catalogue number
	Keep away from sunlight		Keep dry		Manufacturer
EU REP	Authorized representative in the European Community/ European Union		Do not use if package is damaged and consult instructions for use	BIO	Contains biological material of animal origin
	Biological risks		Caution	CE	CE marking
	Upright		Fragile, handle with care		Do not roll
CONTROL	Control				

REFERENCES

1. Rahman M, Rahman K, Siddique AK, Shoma S, Kamal AHM, AliKS, et al. First outbreak of dengue hemorrhagic fever, Bangladesh. Emerg Infect Dis 2002;8:738–40.
2. Boscato Lm, Stuart MC.Heterophilic antibodies: a problem for all immunoassays. Clin Chem 1988;34:27-33.
3. Kricka L. Interferences in immunoassays - still a threat. Clin Chem 2000; 46:1037-1038.
4. Bjerner J, et al. Immunometric assay interference: incidence and prevention. Clin Chem 2002; 48:613-621.

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