Folate (CLIA) PACKAGE INSERT

IVD

INTENDED USE

Folate (CLIA) is a chemiluminescence immunoassay test for the quantitative measurement of circulating Folate in human serum or plasma, which is intended as an aid to diagnosis of megaloblastic anemia.
The assay can be used for samples over the range of 1.0 ng/mL~20 ng/mL.
The test must be performed on the ACCRE series analyzers (including ACCRE 6, ACCRE 8, ACCRE 90, ACCRE 100, ACCRE 120and other ACCRE series analyzers).

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

SUMMARY

Folate and folic acid are forms of a water-soluble B vitamin. Folate occurs naturally in food, and folic acid is the synthetic form of this vitamin. Since 1998, folic acid has been added to cold cereals, flour, breads, pasta, bakery items, cookies, and crackers, as required by federal law. Foods that are naturally high in folate include leafy vegetables (such as spinach, broccoli, and lettuce), okra, asparagus, fruits (such as bananas, melons, and lemons) beans, yeast, mushrooms, meat (such as beef liver and kidney), orange juice, and tomato juice.
Folic acid is used for preventing and treating low blood levels of folate (folate deficiency), as well as its complications, including "tired blood" (anemia) and the inability of the bowel to absorb nutrients properly. Folic acid is also used for other conditions commonly associated with folate deficiency, including ulcerative colitis, liver disease, alcoholism, and kidney dialysis.
Folic acid supplements are standard for pregnant women and women who plan to become pregnant. Folic acid reduces the risk for birth defects of a baby's brain and spine-spina bifida and anencephaly - by 50% to 70%. Folic acid may also lower the risk of preeclampsia and early labor. Many doctors recommend that any women of childbearing age take either a multivitamin or a folic acid supplement. Folic acid can protect against birth defects that may form before a woman knows she is pregnant.

ASSAY PRINCIPLES

The principle of Folate (CLIA) hereinafter also referred to as the "Folate" combines a competitive immunoassay method with enzyme catalytic chemiluminescence detection (CLIA). The principle is described as following:
Reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent cartridges. All the assay steps are performed automatically by the ACCRE analyzer.
The sample is manually pipette into the sample well of reagent cartridge, then sample, pre-treatment solution 1 and pre-treatment solution 2 are mixed together, incubating at 37°C, the pre-treatment solutions served as a displacing reagent to release the Folate from its binding protein.
Then samples are mixed with biotin labeled folate binding protein and anti-biotin antibody coated magnetic particles, incubating at 37°C, the free folate in the sample bond to the biotin labeled folate binding protein, then the folate binding protein bond to the anti-biotin antibody coated in the magnetic particles, forming an antibody-antigen immuno-complex.
After the 2nd incubation, ALP labeled folate are added into the immuno-complex, incubating at 37°C for the 3rd time, the ALP labeled folate can bind to the immuno-complex. Then the mixture is washed in the magnetic field to eliminate the unbound components. The substrate (APS-5) is added to trigger the chemiluminescent reaction, the resulting chemiluminescent reaction is measured by photomultiplier tube (PMT) as relative light units (RLUs). The concentration of the substance to be detected in the sample is inversely proportional to the luminescence intensity. At the end, the results are automatically calculated by the ACCRE analyzer in relation to the calibration curve stored.

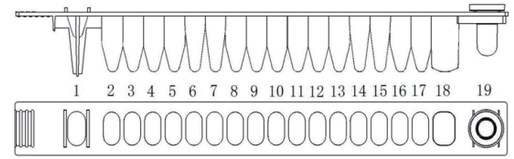
CONTENT OF THE KIT

Package: 60tests/kit with quality controls, 36 tests/kit with quality controls, 60 tests/kit without quality controls, 36 tests/kit without quality controls
The calibration of this kit has been traced to Roche Folate, which can be traced to WHO Standard 03-178.

Contents	Quantities				Notice
	REF W119A	REF W119C	REF W03A	REF W03C	
Folate reagent cartridges	60 strips	36 strips	60 strips	36 strips	Ready to Use
Folate Calibrator 1 (CAL1)	1.5 mL x 1 vial	1.5 mL x 1 vial	1.5 mL x 1 vial	1.5 mL x 1 vial	Tris Buffer contains (human serum albumin), 50mM; ProClin300, 0.5g/L; BND, 0.2g/ L
Folate Calibrator 2 (CAL2)	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 contains (human serum albumin), 50mM; ProClin300, 0.5g/L; BND, 0.2g/L; Folate, 2.5 ng/mL
Folate Calibrator3 (CAL3)	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 contains (human serum albumin), 50mM; ProClin300, 0.5g/L; BND, 0.2g/L; Folate, 10 ng/mL
Folate Quality Control Level 1 (QC1)	1.0 mL x 1 vial	1.0 mL x 1 vial	/	/	Phosphate buffer, 50mM; ProClin300, 0.48 g/L; BND, 0.2 g/L; Folate , 4 ng/mL Only for the configuration with Quality Controls
Folate Quality Control Level 2(QC2)	1.0 mL x 1 vial	1.0 mL x 1 vial	/	/	Phosphate buffer, 50mM; ProClin300, 0.48 g/L; BND, 0.2 g/L; Folate , 15 ng/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

Note: Refer to the target value list of the controls from Quality Control Sheet within the kit.

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
2	Pre-treatment Solution1 (PT1)	Sodium Citrate buffer,50mmol/L; ProClin300, 0.5g/L; BND, 0.2g/L; DTT, 1.024g/L	50μL
3	Pre-treatment Solution2 (PT2)	NaOH, 0.5mol/L	50μL
5	Enzyme Label (R2)	ALP (alkaline phosphatase) labeled folate, 5μg/mL; Tris Buffer, 50mmol/L; ProClin300, 0.5g/L; BND, 0.2g/L	50μL
7	Magnetic Particles (R1)	Anti-Biotin antibody coated magnetic particles, 0.2g/L; PBS Buffer, 200mmol/L; ProClin300, 0.5g/L; BND, 0.2g/L	50μL
9	Biotin Label (R3)	Biotin labeled folate binding protein, 2μg/mL; Tris Buffer, 50 mmol/L; ProClin300, 0.5g/L; BND, 0.2g/L	50μL
10, 11, 12, 13	Wash Solutions	Tris Buffer, 50mmol/L; ProClin300, 0.5g/L; BND, 0.2g/L	350μL
16	Substrate	APS-5	200μL

Note: Well 1, 4, 6, 8, 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 analyzer

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- For professional use only by qualified laboratory personnel.
- The kit contains products of human origin. No known analysis method can 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 (see Laboratory Biosafety Manual - WHO - Geneva - latest edition).
- 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 insufficient.
- 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 Folate (CLIA) kits including reagent cartridges, calibrators and quality controls **upright** at 2~8°C in dark place and the kit can be valid 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.
- When opened, the calibrators and quality controls will be stable for 30 days at 2~8°C.
- Keep away from sunlight.
- Do not freeze reagent cartridges.

SAMPLES

The sample volume for Folate (CLIA) assay is 60μL, please note that at least 85μL of sample has to be pipetted manually into the reagent cartridge.

Sample type and collection
Human serum or plasma (Heparin lithium, 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 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 (15-30°C for up to 4 hours or at 2~8°Cfor up to 3 days; if longer storage is required, freeze the serum or plasma at -20°C or below -20°C for up to 2 months, without exceeding 1 freeze/thaw cycle.

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 analytical 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 Folate strips for each sample, calibrator or control respectively.
3. Place the reagent cartridge into the reagent rack.
4. Manually pipette the Sample, Calibrator, or Controls into the sample well of each reagent cartridge.

Please note that at least 85μL of sample has to be pipetted manually into the reagent cartridge and 60μL 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. Folate (CLIA) assay will be completed within approximately 28 minutes. Assays will be performed automatically by the 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 20 ng/mL can be diluted with sample diluent manually. The recommended dilution is 1:2. And the concentration after dilution should be more than 5ng/mL. After manual dilution, multiply the result by the dilution factor. ACCRE analyzer cannot perform automatic dilution for samples.

Quality Control
Quality control can 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 generate by 3-point calibration, and calculated according to a predefined mathematical model, the results expressed in ng/mL. The results can be converted as the unit of nmol/L by manually through below conversion factors.
Conversion Factor: nmol/L×0.44=ng/mL, ng/mL×2.265=nmol/L.

Interpretation of Results
The detection range is from 1.0ng/mL to 20ng/mL. For samples with Floate below the upper limit of detection, quantitative determination can be performed; if the sample is lower than the lower limit of detection, the reported result is <1.0ng/mL; if the sample is higher than the upper limit of detection, the reported result is >20ng/mL.
Results of Folate (CLIA) assays are determined by testing serum specimens from a study in clinical centers with group of totally 235 healthy adult individuals in China, the reference rage determine by the range 2.5th percentile value and 97.5th percentile value is 3.67~21.73ng/mL.
** Please note that results may differ between laboratories due to variations in population and test method. If necessary, each laboratory should establish its own reference range.*
In interpreting the results, patient's clinical condition including symptoms, diseases history, and other relevant data and information should be considered.

LIMITATIONS

1. If the test results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
2. For diagnostic purposes, results should be used in conjunction with other data; e.g., symptoms, results of other tests, clinical impressions, etc.
3. Please note that heterophile antibody in human serum or plasma can interfere the test result.
4. High concentration of HAMA can also cause a false positive or false negative result.

PERFORMANCE CHARACTERISTICS

Detection limits
LOD (Limit of Detection) = 1.0 ng/mL
LOD value was determined according to CLSI® EP17-A2 recommendations.

Measurement range
The measurement range is the range of values corresponding to the acceptable performance limits (precision and linearity).
The measurement range of the Folate (CLIA) assay is: 1.0 ng/mL~20 ng/mL.

Linearity
Linearity was evaluated according to CLSI® EP06-A recommendations. The Folate (CLIA) assay is linear between 1.2 ng/mL to 20 ng/mL. The absolute linear correlation coefficient (r) should be bigger than 0.9900.

Precision
A 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 12%.

Accuracy
Add a high-value sample with known Folate concentration into a low-value serum sample for the detection, the recovery rate is between 85% and 115%.

Cross-reactivity
The cross-reactivity study of Folate (CLIA) assay was designed to evaluate potential cross-reactants. The results of this study are shown in below table:

Substance	Concentration	Cross Reaction rate
Aminopterin	750 ng/mL	<5%
Leukovorum	750 ng/mL	<5%
Methotrexate	750 ng/mL	<5%

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
Bilirubin	≤ 20 mg/dL
Triglyceride	≤1500 mg/dL
Total Protein	≤10 g/dL
Rheumatoid factors	≤1000 IU/mL

HAMA
Patient samples containing human anti-mouse antibodies (HAMA) may give falsely elevated or decreased values. Although HAMA-neutralizing agents are added, extremely high HAMA serum concentrations may occasionally influence resultss[11-13].

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				

LITERATURE REFERENCES

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Shenzhen Tisenc Medical Devices Co., Ltd.
Address: 11F, 11G, Kechuang Building, Quanzhi Science and Innovation Park, Maozhoushan Industrial Park, Shajing Community, BaoAn District, Shenzhen, 518104, P.R.China
Phone: +86-0755-23225620
E-mail: globalmarketing@tisenc.com

EC REP
Shanghai International Holding Corp. GmbH (Europe)
Address: Eiffestrasse 80, 20537 Hamburg, Germany
Phone: +49-40-2513175
Fax: +49-40-255726
DIMDI No.: DE/0000040627
E-mail: shholding@hotmail.com

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