Snibe Diagnostic

CE REF 130213001M: 100 tests
130613001M: 50 tests

MAGLUMI® FA (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the quantitative determination of Folic Acid (FA) in human serum with the MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer (including Maglumi 600, Maglumi 800, Maglumi 1000, Maglumi 1000 Plus, Maglumi 2000, Maglumi 2000 Plus, Maglumi 4000, Maglumi 4000 Plus, MAGLUMI X8, MAGLUMI X3 and MAGLUMI X6) and Biolumi series Integrated System (including Biolumi CX8).

SUMMARY AND EXPLANATION OF THE TEST

Folate, forms of which are known as folic acid and vitamin B9, is one of the B vitamins. It is on the World Health Organization's List of Essential Medicines, the most effective and safe medicines needed in a health system. Folic acid is essential for the body to make DNA, RNA, and metabolise amino acids which are required for cell division[1].

As humans cannot make folic acid, it is required from the diet, making it an essential vitamin. Folate deficiency can be caused by unhealthy diets that do not include enough fruits and vegetables, diseases in which folates are not well absorbed in the digestive system (such as Crohn's disease or celiac disease), some genetic disorders that affect levels of folate, and certain medicines (such as phenytoin, sulfasalazine, or trimethoprim-sulfamethoxazole)[2-3]. The risk of toxicity from folic acid is low, because folate is a water-soluble vitamin and is regularly removed from the body through urine. One potential issue associated with high dosages of folic acid is that it has a masking effect on the diagnosis of pernicious anaemia (vitamin B12 deficiency), and a variety of concerns [clarification needed] of potential negative impacts on health[4-5].

Not consuming enough folate can lead to folate deficiency. This may result in a type of anemia in which low numbers of large red blood cells occur. Symptoms may include feeling tired, heart palpitations, shortness of breath, open sores on the tongue and changes in the color of the skin or hair. Folate deficiency in children may develop within a month of poor dietary intake. Folate intake during pregnancy has been linked to a lessened risk of neural tube defects[6]. Likewise a meta-analysis of folate supplementation during pregnancy reported a 28% lower risk of newborn congenital heart defects[7].

PRINCIPLE OF THE TEST

The FA assay is a competitive chemiluminescence immunoassay.

Use ABEI to label purified FA antigen, and use FA-binding protein antibody to coat magnetic microbeads. FA-binding protein in the magnetic microbeads solution reacts with the FA-binding protein antibody on the magnetic microbeads. The sample, (or calibrator/control, if applicable), diluent, buffer are added and incubated. Then added ABEI label and magnetic microbeads and incubated, forming complexes; after precipitation in a magnetic field, decant the supernatant, then perform a wash cycle. Subsequently, the Starter 1+2 are added to initiate a flash chemiluminescent reaction. The light signal is measured by a photomultiplier as relative light units (RLUs), which is inversely proportional to the concentration of FA present in the test sample (or calibrator/control, if applicable).

KIT COMPONENTS

Material Provided

Components	Contents	100 tests (REF: 130213001M)	50 tests (REF: 130613001M)
Magnetic Microbeads	Magnetic microbeads coated with FA-binding protein antibody, containing FA-binding protein, BSA, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Calibrator Low	Containing BSA and FA antigen, NaN_3 (<0.1%).	3.0 mL	2.0 mL
Calibrator High	Containing BSA and FA antigen, NaN_3 (<0.1%).	3.0 mL	2.0 mL
Buffer	0.4 mol/L NaOH.	15.0 mL	15.0 mL
ABEI Label	Purified FA antigen labeled with ABEI, containing BSA, NaN_3 (<0.1%).	12.5 mL	7.5 mL
Diluent	Phosphate buffer, NaN_3 (<0.1%).	15.0 mL	15.0 mL
Internal Quality Control	Containing BSA and FA antigen, NaN_3 (<0.1%).	2.0 mL	2.0 mL
Sample Release Agent 1	DTT (30.0 mg, lyophilized).	1 bottle	1 bottle

Sample Release Agent 1 is lyophilized and must be reconstituted with Diluent.

Accessories Required But Not Provided

MAGLUMI and Biolumi Series:

Reaction Module	REF: 630003
Starter 1+2	REF: 130299004M, 130299027M
Wash Concentrate	REF: 130299005M
Light Check	REF: 130299006M
Reaction Cup	REF: 130105000101

Please order accessories from Shenzhen New Industries Biomedical Engineering Co., Ltd. (SNIBE) or our authorized representatives.

CALIBRATION

Traceability: This method has been standardized against the WHO 1st International Standard 95/528.

Test of assay specific calibrators allows the RLU values to adjust the assigned master curve. Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve (10 calibrations) provided via the reagent Radio Frequency Identification (RFID) CHIP.
Recalibration is recommended if any of the following conditions occurs:
- After each change of lots (Reagent or Starter 1+2).
- Every week and/or each time a new reagent kit is used (recommended)
- After instrument service is required.
- If control results lie outside the expected range.

QUALITY CONTROL

Follow government regulations or accreditation requirements for quality control frequency.
Internal quality control is only applicable with MAGLUMI and Biolumi systems. For instructions for use and target value refer to ***FA (CLIA) Quality Control Information***. User needs to judge results with their own standards and knowledge.
For detailed information about entering quality control values, refer to the corresponding Analyzer Operating Instructions.
To monitor system performance and chart trends, commercially available quality control materials are required. Treat all quality control samples the same as patient samples. A satisfactory level of performance is achieved when analyte values obtained are within the acceptable Control Range for the system or within your range, as determined by an appropriate internal laboratory quality control scheme. If the quality control results do not fall within the Expected Values or within the laboratory's established values, do not report results. Take the following actions:
- Verify that the materials are not expired.
- Verify that required maintenance was performed.
- Verify that the assay was performed according to the instructions for use.
- Rerun the assay with fresh quality control samples.
- If necessary, contact your local technical supporters or distributors for assistance.

SPECIMEN COLLECTION AND PREPARATION

- Use standard sampling tubes or tubes containing separating gel. Collect blood aseptically following the universal precautions for venipuncture.
- The sample which has been placed at the room temperature more than 8 hours cannot be used again. The Folic Acid is sensitive with light, please avoid exposure to sunlight when collect specimen.
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time.
- If the specimen is centrifuged before a complete clotting, the presence of fibrin may cause erroneous results. Samples must be free of fibrin and other particulate matter.
- Do not use hemolyzed or grossly lipemic specimens as well as specimens containing particulate substance or exhibiting obvious microbial contamination. Inspect all specimens for bubbles, and remove bubbles before analysis for optimal results.
- Avoid repeating freeze-thaw cycles. The serum sample can be only frozen and thawed one time. Specimens must be mixed thoroughly after thawing.
- Centrifuged specimens with a lipid layer on the top must be transferred to a sample cup or a secondary tube. Care should be taken to transfer only the clarified specimen without the lipemic material.
- All samples (patient specimens and controls) should be tested within 3 hours when placed on board the MAGLUMI and Biolumi Systems. Refer to the SNIBE service for more details of onboard sample storage constraints.
- Specimens removed from the separator, cells or clot may be stored up to 48 hours at 2-8°C. Protect from light.
- Specimens can be stored up to 30 days frozen at -20°C or colder. Stored samples should be thoroughly mixed prior to use (Vortex mixer).
- Before shipping specimens, it is recommended that specimens be removed from the serum separator, red blood cells or clot. When shipped, specimens should be packaged and labeled in compliance with applicable state, federal and international regulations covering the transport of clinical specimens and infectious substances. Specimens should be shipped frozen.
- The sample volume required for a single determination of FA is 80 µL.

WARNING AND PRECAUTIONS FOR USERS

- IVD
- For *In Vitro* Diagnostic Use.
- Follow the package insert carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Safety Precautions

- **CAUTION**: This product requires the handling of human specimens. It is recommended that all human sourced materials be considered potentially infectious and handled in accordance with the 29 CFR 1910.1030 Occupational exposure to bloodborne pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.
- All samples, biological reagents and materials used in the assay should be considered potentially able to transmit infectious agents. They should therefore be disposed in accordance with the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with prevailing regulatory requirements.
- This product contains Sodium Azide. Dispose of contents and containers must be in accordance with all local, regional and national regulations.
- Refer to safety data sheets which are available on request.

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- Do not interchange reagent components from different reagents or lots.
- Prior to loading the reagent kit on the system for the first time, the reagent kit requires mixing to re-suspend magnetic microbeads that have settled during shipment.
- For magnetic microbeads mixing instructions, refer to the Preparation of the Reagent section of this package insert.
- To avoid contamination, wear clean gloves when operating with a reagent kit and samples.
- Over time, residual liquids may dry on the septum surface. These are typically dried salts which have no effect on assay efficacy.
- For detailed discussion of handling precautions during system operation, refer to the SNIBE service information.

STORAGE AND STABILITY

- Sealed: Stored at 2-8°C until the expiration date.

- Opened at 2-8°C: Minimum stability is 4 weeks.
- On-board: Stability is 4 weeks.
- To ensure the best kit performance, it is recommended to place opened kits in the refrigerator after the end of the intraday test work. It is still possible to keep on using the kit beyond the opened or on-board period if the controls are found within the expected ranges.
- Keep upright for storage to facilitate later proper resuspension of magnetic microbeads.
- Keep away from sunlight.

TEST PROCEDURE

Preparation of Sample Release Agent 1

1. The Sample Release Agent 1 is provided in a lyophilized form. The vial containing the lyophilized Sample Release Agent 1 must be opened carefully and reconstituted with 1 mL Diluent (D box).
2. Cover the bottle stopper and gently shake to avoid producing bubbles.
3. Allow the dissolved Sample Release Agent 1 to stand for 3 minutes.
4. Transfer the dissolved Sample Release Agent 1 to the D box and slowly turn it up and down 10 times to make it well blended.
5. After using, the kits including the dissolved Sample Release Agent 1 should be stored at 2-8 °C in an upright position.

Preparation of the Reagent

- Resuspension of the magnetic microbeads takes place automatically when the kit is loaded successfully, ensuring the magnetic microbeads are totally resuspended homogenous prior to use.
- To ensure proper test performance, strictly adhere to the corresponding Analyzer Operating Instructions. Each test parameter is identified via a RFID CHIP on the Reagent kit. For further information please refer to the corresponding Analyzer Operating Instructions.

DILUTION

Sample dilution by analyzer is not available in this reagent kit.
Samples with concentrations above the measuring range can be diluted manually. After manual dilution, multiply the result by the dilution factor. Please choose applicable diluents or ask SNIBE for advice before manual dilution.

LIMITATIONS

- A skillful technique and strict adherence to the instructions are necessary to obtain reliable results.
- Bacterial contamination or heat inactivation of the specimens may affect the test results.
- A result within the expected range does not rule out the presence of disease and should be interpreted together with other diagnostic procedures.
- Test results are reported quantitatively. However, diagnosis of a disease should not be based on the result of a single test, but should be determined in conjunction with clinical findings in association with medical judgement.
- Any therapeutical decision should also be taken on a case-by-case basis.
- 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 results.

RESULTS

Calculation of Results

The analyzer automatically calculates the FA concentration of each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results are reported in the unit of ng/mL. For further information please refer to the corresponding Analyzer Operating Instructions.
Conversion factor: ng/mLx2.265=nmol/L.

Interpretation of Results

The expected range for the FA assay was obtained by testing 110 apparently healthy individuals in China, and gave the following expected value: 5.21-20 ng/mL (2.5^{th}-97.5^{th} percentiles).
If the sample's value is 3.21-5.21 ng/mL, it should be checked with the clinical diagnosis and observed continuously to identify whether the patient is FA-deficiency.
Results may differ between laboratories due to variations in population and test method. It is recommended that each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Precision

Precision for the FA assay was determined as described in the CLSI EP5-A2. 2 human serum pools and 3 controls containing different concentration of analyte were assayed in duplicate at two independent runs per day for 20 testing days. The results are summarized in the following table:

Sample	Mean(ng/mL) (N=80)	Within-Run		Between-Run		Total	
		SD(ng/mL)	%CV	SD(ng/mL)	%CV	SD(ng/mL)	%CV
Serum Pool 1	2.108	0.103	4.89	0.155	7.35	0.186	8.82
Serum Pool 2	20.766	0.253	1.22	1.021	4.92	1.052	5.07
Control 1	4.145	0.189	4.56	0.286	6.90	0.343	8.28
Control 2	10.216	0.402	3.94	0.349	3.42	0.532	5.21
Control 3	16.864	0.348	2.06	0.871	5.16	0.938	5.56

Limit of Blank (LoB)

The LoB for the FA assay is 0.375 ng/mL.

Limit of Detection (LoD)

The LoD for the FA assay is 0.5 ng/mL.

Measuring Range

0.375-24 ng/mL (defined by the limit of blank and the maximum of the master curve). Values below the limit of blank are reported as <0.375 ng/mL. Values above the measuring range are reported as >24 ng/mL.

Linearity
The assay is linear between 0.5 ng/mL and 24 ng/mL based on a study performed with guidance from CLSI EP6-A. Nine equally distributed levels of samples were prepared by blending a serum sample containing FA 25.4 ng/mL with a serum sample depleted of FA (0.0 ng/mL).The mean sample recovery ranged between 90% to 110%.

Method Comparison
A total of 100 samples in the range of 0.83 and 23.88 ng/mL were tested using the FA assay (y) and a commercially available immunoassay (x). The data from the resulting linear regressions are summarized as: y=1.010x-0.083, r^2=0.978.

Analytical Specificity
The specificity of the assay was obtained by adding Ferritin (100 ng/mL), Vitamin D (1000 pg/mL) and VB_{12} (1000 pg/mL) to two serum samples at the indicated concentrations. No interference was found.

Endogenous Interference
Substances up to the following concentrations did not interfere with the assay:
- Bilirubin 60 mg/dL
- Hemoglobin 900 mg/dL
- Triglyceride 2000 mg/dL

REFERENCES

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EC REP
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SYMBOLS EXPLANATIONS

	Consult instructions for use		Manufacturer
2°C 8°C	Temperature limit (Store at 2-8 °C)		Use-by date
Σ	Contains sufficient for		Keep away from sunlight
	This way up	EC REP	Authorized representative in the European Community
IVD	*In vitro* diagnostic medical device	CONTENTS	Kit components
REF	Catalogue number	LOT	Batch code