



MAGLUMI® HBsAg (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the quantitative determination of hepatitis B surface antigen (HBsAg) in human serum and plasma using the MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer and Biolumi series Integrated System, and the assay is used as an aid in the diagnosis of HBV infection and for screening of blood donations.

SUMMARY

Hepatitis B is an infectious disease caused by the hepatitis B virus (HBV) that affects the liver¹. It can cause both acute and chronic infections¹. Hepatitis B virus (HBV) is a member of the hepadnavirus family. The virus particle (virion) consists of an outer lipid envelope and an icosahedral nucleocapsid core composed of protein². The genome of HBV is made of circular DNA, but it is unusual because the DNA is not fully double-stranded³. There are four known genes encoded by the genome, called C, X, P, and S. The core protein is coded for by gene C (HBcAg), and its start codon is preceded by an upstream in-frame AUG start codon from which the pre-core protein is produced. HBsAg is produced by proteolytic processing of the pre-core protein. The DNA polymerase is encoded by gene P. Gene S is the gene that codes for the surface antigen (HBsAg)⁴. The function of the protein coded for by gene X is not fully understood but it is associated with the development of liver cancer⁵.

Hepatitis B diagnosis has been based on detection of serologic markers. Testing for these markers helps to determine the presence of past or ongoing HBV infection, the acute or chronic stage of the disease, response to therapy, and/or the immune status of the patient^{6,7}. HBsAg is the first serologic marker to appear and may be detected within 1 to 2 weeks after exposure. It precedes the development of symptoms by an average of 4 weeks. The presence of HBsAg indicates ongoing infection⁸. HBsAg assays are routinely used to aid in the diagnosis of suspected hepatitis B viral (HBV) infection and to monitor the status of infected individuals, i.e., whether the patient's infection has resolved or the patient has become a chronic carrier of the virus⁹.

TEST PRINCIPLE

Sandwich chemiluminescence immunoassay.

The sample, buffer, magnetic microbeads coated with anti-HBs monoclonal antibody are mixed thoroughly, incubating and performing a wash cycle after a precipitation in a magnetic field. ABEI labeled with another anti-HBs polyclonal antibody are then added, reacting to form sandwich complexes and incubating. After precipitation in a magnetic field, the supernatant is decanted and then a wash cycle is performed. Subsequently, the Starter 1+2 are added to initiate a chemiluminescent reaction. The light signal is measured by a photomultiplier as relative light units (RLUs), which is proportional to the concentration of HBsAg present in the sample. Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent RFID label.

REAGENTS

Kit Contents

Component	Description	100 tests/kit	50 tests/kit	30 tests/kit
Magnetic Microbeads	Magnetic microbeads coated with anti-HBs monoclonal antibody (~16.0 µg/mL) in PBS buffer, NaN ₃ (<0.1%).	2.5 mL	2.0 mL	1.5 mL
Calibrator Low	A low concentration of recombinant HBsAg in PBS buffer, NaN ₃ (<0.1%).	3.0 mL	2.0 mL	1.0 mL
Calibrator High	A high concentration of recombinant HBsAg in PBS buffer, NaN ₃ (<0.1%).	3.0 mL	2.0 mL	1.0 mL
Buffer	Tris-HCl buffer, NaN ₃ (<0.1%).	22.5 mL	12.5 mL	7.8 mL
ABEI Label	ABEI labeled with anti-HBs polyclonal antibody (~0.167 µg/mL) in Tris-HCl buffer, NaN ₃ (<0.1%).	22.5 mL	12.5 mL	7.8 mL
Diluent	Tris buffer, NaN ₃ (<0.1%).	25.0 mL	15.0 mL	10.0 mL
Negative Control	PBS buffer, NaN ₃ (<0.1%).	1.0 mL	1.0 mL	1.0 mL
Positive Control 1	A low concentration of recombinant HBsAg (1.00 IU/mL) in PBS buffer, NaN ₃ (<0.1%).	1.0 mL	1.0 mL	1.0 mL
Positive Control 2	A high concentration of recombinant HBsAg (100 IU/mL) in PBS buffer, NaN ₃ (<0.1%).	1.0 mL	1.0 mL	1.0 mL

All reagents are provided ready-to-use.

Warnings and Precautions

- For *in vitro* diagnostic use.
- For professional use only.
- Exercise the normal precautions required for handling all laboratory reagents.
- Personal protective measures should be taken to prevent any part of the human body from contacting samples, reagents, and controls, and should comply with local operating requirements for the assay.
- A skillful technique and strict adherence to the package insert are necessary to obtain reliable results.
- Do not use kit beyond the expiration date indicated on the label.
- Do not interchange reagent components from different reagents or lots.
- Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).
- All waste associated with biological samples, biological reagents and disposable materials used for the assay should be considered potentially infectious and should be disposed of in accordance with local guidelines.
- This product contains sodium azide. Sodium azide may react with lead or copper plumbing to form highly explosive metal azides. Immediately after disposal, flush with a large volume of water to prevent azide build-up. For additional information, see Safety Data Sheets available for professional user on request.

Note: If any serious incident has occurred in relation to the device, please report to Shenzhen New Industries Biomedical Engineering Co., Ltd. (Snibe) or our authorized representative and the competent authority of the Member State in which you are established.

Reagent Handling

- To avoid contamination, wear clean gloves when operating with a reagent kit and sample. When handling reagent kit, replace the gloves that have been in contact with samples, since introduction of samples will result in unreliable results.
- Do not use kit in malfunction conditions; e.g., the kit leaking at the sealing film or elsewhere, obviously turbid or precipitation is found in reagents (except for Magnetic Microbeads) or control value is out of the specified range repeatedly. When kit in malfunction conditions, please contact Snibe or our authorized distributor.
- To avoid evaporation of the liquid in the opened reagent kits in refrigerator, it is recommended that the opened reagent kits to be sealed with reagent seals contained within the packaging. The reagent seals are single use, and if more seals are needed, please contact Snibe or our authorized distributor.
- Over time, residual liquids may dry on the septum surface. These are typically dried salts and have no effect on assay efficacy.
- Use always the same analyzer for an opened reagent integral.
- For magnetic microbeads mixing instructions, refer to the Preparation of the Reagent section of this package insert.
- For further information about the reagent handling during system operation, please refer to Analyzer Operating Instructions.

Storage and Stability

- Do not freeze the integral reagents.
- Store the reagent kit upright to ensure complete availability of the magnetic microbeads.
- Protect from direct sunlight.

Stability of the Reagents	
Unopened at 2-8°C	until the stated expiration date
Opened at 2-8°C	6 weeks
On-board	4 weeks

Stability of Controls	
Unopened at 2-8°C	until the stated expiration date

C

€

0123

REF

130210009M:100 tests/kit

130610009M: 50 tests/kit

130710009M: 30 tests/kit

Opened at 10-30°C	6 hours
Opened at 2-8°C	6 weeks
Frozen at -20°C	3 months
Frozen and thawed cycles	no more than 3 times

SPECIMEN COLLECTION AND PREPARATION

Specimen Types

Only the specimens listed below were tested and found acceptable.

Specimen Types	Collection Tubes
Serum	Tubes without additive/accessory, or tubes containing clot activator or clot activator with gel
Plasma	sodium citrate (1:9), K2-EDTA, K3-EDTA, Na2-EDTA, Li-heparin, Na-heparin, ACD-B, CPD, CPDA and K-Oxalate/NaF

- The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. Follow tube manufacturers' instructions carefully when using collection tubes.

Specimen Conditions

- Do not use heat-inactivated samples or grossly hemolyzed/hyperlipidaemia specimens and specimens with obvious microbial contamination.
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some serum specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the serum 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.
- To prevent cross contamination, use of disposable pipettes or pipette tips are recommended.

Preparation for Analysis

- Inspect all specimens for foam. Remove foam with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.
- Frozen specimens must be completely thawed before mixing. Mix thawed specimens thoroughly by low speed vortexing or by gently inverting. Visually inspect the specimens. If layering or stratification is observed, mix until specimens are visibly homogeneous. If specimens are not mixed thoroughly, inconsistent results may be obtained.
- Specimens should be free of fibrin, red blood cells, or other particulate matter. Such specimens may give reliable results and must be centrifuged prior to testing. Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens with a lipid layer, transfer only the clarified specimen and not the lipemic material.
- The sample volume required for a single determination of this assay is 150 µL.

Specimen Storage

Specimens removed from the separator, red blood cells or clot may be stored up to 28 hours at 10-30°C or 14 days at 2-8°C, or 6 months frozen at -20°C or colder. Frozen specimens subjected to up to 6 freeze/thaw cycles have been evaluated.

Specimen Shipping

- Package and label specimens in compliance with applicable local regulations covering the transport of clinical specimens and infectious substances.
- Do not exceed the storage limitations listed above.

Specimen Dilution

- Samples, with HBsAg concentrations above the analytical measuring interval, can be diluted with Diluent either by following automated dilution protocol or manual dilution procedure. The recommended dilution ratio is 1:100. The concentration of the diluted sample must be >2.50 IU/mL.
- For manual dilution, multiply the result by the dilution factor. For dilution by the analyzers, the analyzer software automatically takes the dilution into account when calculating the sample concentration.

PROCEDURE

Materials Provided

HBsAg (CLIA) assay, control barcode labels.

Materials Required (But Not Provided)

- General laboratory equipment.
- Fully-auto chemiluminescence immunoassay analyzer Maglumi 600, Maglumi 800, Maglumi 1000, Maglumi 2000, Maglumi 2000 Plus, Maglumi 4000, Maglumi 4000 Plus, MAGLUMI X3, MAGLUMI X6, MAGLUMI X8, or Integrated System Biolumi 8000 and Biolumi CX8.
- Additional accessories of test required for the above analyzers include Reaction Module, Starter 1+2, Wash Concentrate, Light Check, Tip, and Reaction Cup. Specific accessories and accessories' specification for each model refer to corresponding Analyzer Operating Instructions.
- Please use accessories specified by Snibe to ensure the reliability of the test results.

Assay Procedure

Preparation of the Reagent

- Take the reagent kit out of the box and visually inspect the integral vials for leaking at the sealing film or elsewhere. If there is no leakage, please tear off the sealing film carefully.
- Open the reagent area door; hold the reagent handle to get the RFID label close to the RFID reader (for about 2s); the buzzer will beep; one beep sound indicates successful sensing.
- Keeping the reagent straight insert to the bottom along the blank reagent track.
- Observe whether the reagent information is displayed successfully in the software interface, otherwise repeat the above two steps.
- 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.

Assay Calibration

- Select the assay to be calibrated and execute calibration operation in reagent area interface. For specific information on ordering calibrations, refer to the calibration section of Analyzer Operating Instructions.
- Execute recalibration according to the calibration interval required in this package insert.

Quality Control

- When new lot used, check or edit the quality control information.
- Scan the control barcode, choose corresponding quality control information and execute testing. For specific information on ordering quality controls, refer to the quality control section of the Analyzer Operating Instructions.

Sample Testing

- After successfully loading the sample, select the sample in interface and edit the assay for the sample to be tested and execute testing. For specific information on ordering patient specimens, refer to the sample ordering section of the Analyzer Operating Instructions.

To ensure proper test performance, strictly adhere to Analyzer Operating Instructions.

Calibration

Traceability: This method has been standardized against the WHO NIBSC standard (Code number: 12/226; WHO Third International Standard for HBsAg, HBV genotype B4, HBsAg subtypes ayw1/adw2).

Test of assay specific calibrators allows the detected relative light unit (RLU) values to adjust the master curve.

Recalibration is recommended as follows:

- Whenever a new lot of Reagent or Starter 1+2 is used.
- Every 14 days.
- The analyzer has been serviced.
- Control values lie outside the specified range.

Quality Control

Controls are recommended for the determination of quality control requirements for this assay and should be run in singlicate to monitor the assay performance. Refer to published guidelines for general quality control recommendations, for example Clinical and Laboratory Standards Institute (CLSI) Guideline C24 or other published guidelines¹⁰.

Quality control is recommended once per day of use, or in accordance with local regulations or accreditation requirements and your laboratory's quality control procedures, quality control could be performed by running the HBsAg assay:

- Whenever the kit is calibrated.
- Whenever a new lot of Starter 1+2 or Wash Concentrate is used.

Controls are only applicable with MAGLUMI and Biolumi systems and only used matching with the same top seven LOT numbers of corresponding reagents. For each target value and range refer to the label.

The performance of other controls should be evaluated for compatibility with this assay before they are used. Appropriate value ranges should be established for all quality control materials used.

Control values must lie within the specified range, whenever one of the controls lies outside the specified range, calibration should be repeated and controls retested. If control values lie repeatedly outside the predefined ranges after successful calibration, patient results must not be reported and 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 package insert.
- If necessary, contact Snibe or our authorized distributors for assistance.

If the controls in kit are not enough for use, please order HBsAg (CLIA) Controls (REF: 160201123MT) from Snibe or our authorized distributors for more.

RESULTS

Calculation

The analyzer automatically calculates the HBsAg concentration in each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results are expressed in IU/mL. For further information please refer to the Analyzer Operating Instructions.

Interpretation of Results

The expected results for the HBsAg assay was obtained by testing 635 HBsAg positive patients and 546 HBsAg negative people in China, gave the following expected value by ROC curve:

- Non-reactive: A result less than 0.05 IU/mL (<0.05 IU/mL) is considered to be negative.
- Reactive: A result greater than or equal to 0.05 IU/mL is (≥0.05 IU/mL) is considered to be positive.

Results may differ between laboratories due to variations in population and test method. It is recommended that each laboratory establish its own reference interval.

LIMITATIONS

- Results should be used in conjunction with patient's medical history, clinical examination and other findings.
- If the HBsAg results are inconsistent with clinical evidence, additional testing is needed to confirm the result.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits which employ mouse monoclonal antibodies^{11,12}. Additional information may be required for diagnosis.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed¹³.
- Bacterial contamination or heat inactivation of the specimens may affect the test results.
- The intended testing population are individuals with suspected HBV infection and blood donors.
- Specimens from individuals recently vaccinated against HBV may score transiently positive for HBsAg because it is present in the vaccine. Reactivity to vaccine may vary with different manufacturers' tests.

SPECIFIC PERFORMANCE CHARACTERISTICS

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

Precision

2 serum samples and 2 controls containing different concentration of analyte were assayed in 5 replicates per run, 1 run per day for five operating days across three sites.

The result is summarized in the following table:

Sample	Mean (IU/mL) (n=75)	Repeatability		Between-Day		Between-Site		Reproducibility	
		SD(IU/mL)	%CV	SD(IU/mL)	%CV	SD(IU/mL)	%CV	SD(IU/mL)	%CV
ECV1	0.996	0.029	2.91	0.013	1.31	0.061	6.12	0.068	6.83
ECV2	100.054	2.974	2.97	0.512	0.51	5.509	5.51	6.281	6.28
PQC1	0.997	0.027	2.71	0.007	0.70	0.047	4.71	0.055	5.52
PQC2	100.276	2.496	2.49	0.012	0.01	5.413	5.40	5.961	5.94

Linear Range

0.050-250 IU/mL (based on a study performed with guidance from CLSI EP6-A).

Reportable Interval

0.020-25000 IU/mL (defined by the Limit of Blank and the maximum of the master curve×Recommended Dilution Ratio).

Analytical Sensitivity

Limit of Blank (LoB) =0.020 IU/mL.

Limit of Detection (LoD) =0.050 IU/mL.

Limit of Quantitation (LoQ) =0.050 IU/mL.

Detection limit (analytical sensitivity in CS)

The lowest concentration of the 3rd international standard for HBsAg (NIBSC code 12/266) still reactive with the MAGLUMI HBsAg (CLIA) is 0.039 IU/mL.

Detection efficiency on different genotypes

A similar detection efficiency was obtained for all genotypes of the 1st International Reference Panel for HBV genotypes for HBsAg assays (PEI 6100/09). All genotypes were detected at concentrations < 0.13 IU/mL.

Analytical Specificity

Interference

Interference was determined using the assay, three samples containing different concentrations of analyte were spiked with potential endogenous and exogenous interferents in a protocol (EP7-A2) of the CLSI. The measurement deviation of the interference substance is within ±10%. The following results were obtained:

Interference	No interference up to	Interference	No interference up to	Interference	No interference up to
Bilirubin	40 mg/dL	Cyclosporine	0.5 mg/dL	Acetaminophen	20 mg/dL
Hemoglobin	1000 mg/dL	Cefoxitin	250 mg/dL	Ibuprofen	50 mg/dL
Intralipid	2000 mg/dL	Levodopa	2 mg/dL	Theophylline	10 mg/dL
HAMA	40 ng/mL	Metronidazole	20 mg/dL	Lamivudine	30 mg/dL
Rheumatoid factor	1500 IU/mL	Tetracycline	5 mg/dL	Entecavir	0.5 mg/L
Acetylcysteine	15 mg/dL	Aspirin	100 mg/dL	Telbivudine	60 mg/dL
Ampicillin Sodium	100 mg/dL	Rifampicin	6 mg/dL	Adefovir	1 mg/dL
Ascorbic Acid	30 mg/dL	/	/	/	/

Cross-Reactivity

Clinical interference samples, which contain potential cross-reactants were used to evaluate the cross-reactivity of HBsAg assay. All samples were negative with the HBsAg assay. The results were summarized in the following table:

Condition	Number of samples tested	Number of HBsAg reactive
Anti-CMV	3	0
Anti-EBV	3	0
Anti-HAV	4	0
Anti-HCV	7	0
Anti-HEV	5	0
Anti-HIV	5	0
Anti-HSV	3	0

Anti-Syphilis	4	0
Anti-VZV	5	0
c-ANCA	5	0
Dialysis patients	7	0
HAMA	2	0
Hyper IgG	7	0
Hyper IgM	4	0
Influenza vaccine recipients	4	0
Pregnant women (multipara)	7	0
Pregnant women	20	0
Rheumatoid Factor	5	0
HBV vaccine recipients	10	0
Total	110	0

High-Dose Hook

No high-dose hook effect was seen for HBsAg concentrations up to 300000 IU/mL.

Clinical Sensitivity

411 samples from HBsAg infected patients with different stages of disease and infected with different HBsAg genotypes and subtypes, the diagnostic sensitivity of HBsAg assay was found to be 100%.

Group	Number of samples tested	Number of HBsAg reactive
HBsAg positive without genotype/subtype	89	89
HBsAg positive with known genotype	10	10
HBsAg positive with known subtype	7	7
HBsAg positive with known genotype and subtype	242	242
HBsAg mutant samples with known genotype	5	5
HBsAg mutant samples with known genotype and subtype	58	58
Total	411	411

Clinical Specificity

In a group of randomly selected blood donors, the diagnostic specificity was found to be 99.92%, and in a group of hospitalized patients, the diagnostic specificity was found to be 99.51%. The diagnostic specificity of the HBsAg assay was found to be 99.91%.

Group	Number of samples tested	Number of HBsAg reactive
Unselected donors	5101	4
Hospitalized patients	205	1
Total	5306	5

HBsAg Mutant Detection

A total of 67 mutants samples (63 native and 4 recombinant) were evaluated, all samples were positive with the HBsAg assay, resulting in a sensitivity of 100%.

Seroconversion Sensitivity

Seroconversion sensitivity of the HBsAg assay has been evaluated by testing 30 commercial seroconversion panels, which have been tested by commercially available CE-marked HBsAg assays. The HBsAg assay showed equivalent performance compared to the results from other commercially available assays.

REFERENCES

1. "Hepatitis B Fact sheet".World Health Organization. July 2014. Archived from the original on 9 November 2014. Retrieved 4 November 2014.
2. Zuckerman AJ (1996). "Hepatitis Viruses". In Baron S; et al. Baron's Medical Microbiology (4th ed.). University of Texas Medical Branch. ISBN 0-9631172-1-1. Archived from the original on 14 July 2009.
3. Kay A, Zoulim F (2007). "Hepatitis B virus genetic variability and evolution". Virus research. 127 (2): 164–176.
4. Buti M, Rodriguez-Frias F, Jardi R, Esteban R (December 2005). "Hepatitis B virus genome variability and disease progression: the impact of pre-core mutants and HBV genotypes". Journal of Clinical Virology. 34 Suppl 1: S79–82.
5. Li W, Miao X, Qi Z, Zeng W, Liang J, Liang Z (2010). "Hepatitis B virus X protein upregulates HSP90alpha expression via activation of c-Myc in human hepatocarcinoma cell line, HepG2". Virol. J. 7: 45.
6. G. DUSHEIKO, J.H. HOOFNAGLE Hepatitis B. In: Oxford Textbook of Clinical Hepatology, N. McIntyre et al. eds., Oxford University Press, p. 571-577 (1991).
7. M.R. ESCOBAR Chronic viral hepatitis. In: Clinical Virology Manual, S. Specter, G.J. Lancz eds., Elsevier, New York, p. 329-348 (1986).
8. Hoofnagle J, Seef L, Bales Z, et al. Serologic responses in hepatitis B. In: Vyas GN, Cohen SN, Schmidt R, editors. Viral Hepatitis: A Contemporary Assessment of Etiology, Epidemiology, Pathogenesis and Prevention. Philadelphia: Franklin Institute Press; 1978. p. 278.
9. Perrillo RP, Aach RD. The clinical course and chronic sequelae of hepatitis B virus infection. *Seminars in Liver Disease* 1981;1:15-25.
10. CLSI. Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions. 4th ed. CLSI guideline C24. Wayne, PA: Clinical and Laboratory Standards Institute; 2016.
11. Robert W. Schroff, Kenneth A. Foon, Shannon M. Beatty, et al. Human Anti-Murine Immunoglobulin Responses in Patients Receiving Monoclonal Antibody Therapy [J]. Cancer Research, 1985, 45(2):879-885.
12. Primus F J, Kelley E A, Hansen H J, et al. "Sandwich"-type immunoassay of carcinoembryonic antigen in patients receiving murine monoclonal antibodies for diagnosis and therapy [J]. Clinical Chemistry, 1988, 34(2):261-264.
13. Boscato L M, Stuart M C. Heterophilic antibodies: a problem for all immunoassays [J]. Clinical Chemistry, 1988,34(1):27-33.

SYMBOLS EXPLANATIONS

	Consult instructions for use		Catalogue number		Authorized representative in the European Community
	Temperature limit (Store at 2-8°C)		Manufacturer		Kit component
	Contains sufficient for<n> tests		Use-by date		Batch code
	This way up		Keep away from sunlight		CE marking with notified body ID number
	In vitro diagnostic medical device				

MAGLUMI® and Biolumi® are trademarks of Snibe. All other product names and trademarks are the property of their respective owners.

Summary of safety and performance is available at Eudamed.



Shenzhen New Industries Biomedical Engineering Co., Ltd.
No.23, Jinxiu East Road, Pingshan District, 518122 Shenzhen, P.R. China
Tel: +86-755-21536601 Fax: +86-755-28292740



Shanghai International Holding Corp. GmbH (Europe)
Eiffestrasse 80, 20537 Hamburg, Germany
Tel: +49-40-2513175 Fax: +49-40-255726