BIO TECHNOLOGY boditech

ichroma™ Total IgE

INTENDED USE

ichroma™ Total IgE is a fluorescence immunoassay (FIA) for the quantitative determination of total IgE in human whole blood/serum/ plasma. It is useful as an aid in diagnosis and management of allergic disease.

For *in vitro* diagnostic use only.

INTRODUCTION

Immunoglobulin E (IgE) was discovered for its involvement in allergic reactions (Type I hypersensitivity)[1]. Type I hypersensitivity is an allergic reaction provoked by re-exposure to a specific type of antigen referred to as an allergen.

The sequence of events in the allergic reaction consists of the production of IgE antibodies in response to an allergen, binding of IgE to of mast cells, cross-linking of the bound IgE by the allergen upon re-exposure, and release of mast cell mediators such as histamine, lipid mediators and cytokines. Some mast cell mediators cause rapid increase in vascular permeability and smooth muscle contraction, resulting in many of the symptoms.

The IgE concentration in serum is normally very low (<0.001% of the total serum immunoglobulin). The serum concentration of IgE is age- related, increasing during childhood until about 10 years of age, after which it reaches values that are maintained during adult life [2], [3].

Measurement of total IgE is often used as a tool in the diagnosis and management of atopic diseases, and elevated level of IgE can be found in patients with allergic disease such as asthma, hay fever, atopic dermatitis and urticarial [4], [5], [6].

It has been used to distinguish atopic from non-atopic individuals presenting allergy-like symptoms. In addition, studies have also shown that increased levels of IgE in cord blood and infants may be predictive of future atopic tendencies [7].

Serum IgE levels may vary as a result of diet, genetic background, geographical location and other factors. It is therefore recommended that total IgE measurements be used in conjunction with other clinical tests when establishing diagnoses [8].

PRINCIPLE

The test uses a sandwich immunodetection method; the detector antibodies in buffer bind to antigens in the sample, forming antigen-antibody complexes, and migrates onto nitrocellulose matrix to be captured by the other immobilized-antibodies on test strip.

More antigens in the sample will form more antigen-antibody complexes which lead to stronger fluorescence signal by detector antibodies, which is processed by instrument for ichroma tests to show total IgE concentration in the sample.

COMPONENTS

ichroma™ Total IgE consists of 'cartridges' and 'detection buffer'.

- The cartridge contains the membrane called a test strip which has anti IgE at the test line, with streptavidin at the control line. All cartridges are individually sealed in an aluminum foil pouch containing a desiccant in a box.
- The detection buffer contains anti-IgE fluorescence conjugate, BSA-Biotin fluorescence conjugate, bovine serum albumin (BSA) as a stabilizer and sodium azide in phosphate buffered saline (PBS) as a preservative.
- The detection buffer is dispensed in a tube. 25 detection buffer tubes are packaged in detection buffer box.

WARNING AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Follow the instructions and procedures described in this 'Instruction for use'.
- Use only fresh samples and avoid direct sunlight.
- Lot numbers of all the test components (cartridge, detection buffer and ID Chip) must match each other.
- Do not interchange the test components between different lots or use the test components after the expiration date, either of which might yield incorrect test result(s).
- Do not reuse cartridges or detection buffer. A cartridge should be used for testing one sample only. A detection buffer should be used for processing of one sample only.
- The cartridge should remain sealed in its original pouch until just before use. Do not use cartridge, if pouch is damaged or has already been opened.
- Frozen sample should be thawed only once. For shipping, samples must be packed in accordance with local regulations. Sample with severe hemolysis and/or hyperlipidemia must not be used.
- Allow cartridge, detection buffer and sample to be at room temperature for approximately 30 minutes before use.
- The instrument for ichroma™ tests may generate slight vibration during use.
- Used cartridges, detection buffer and pipette tips should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- An exposure to larger quantities of sodium azide may cause certain health issues like convulsions, low blood pressure and heart rate, loss of consciousness, lung injury and respiratory failure.
- **ichroma™ Total IgE** will provide accurate and reliable results subject to the below conditions.

- **ichroma™ Total IgE** should be used only in conjunction with instrument for ichroma™ tests.
- Have to use recommended anticoagulant sample.

Recommended anticoagulant
EDTA, heparin, sodium citrate

STORAGE AND STABILITY

Storage condition		
Component	Storage Temperature	Shelf life
Cartridge	4 - 30 °C	20 months
Detection buffer	2 - 8 °C	20 months

- After the cartridge pouch is opened, the test should be performed immediately.

LIMITATION OF THE TEST SYSTEM

- The test may yield false positive result(s) due to the cross-reactions and/or non-specific adhesion of certain sample components to the capture/detector antibodies.
- The test may yield false negative result(s) due to the non-responsiveness of the antigen to the antibodies which is the most common if the epitope is masked by some unknown components, so therefore not being able to be detected or captured by the antibodies. The instability or degradation of the antigen with time and/or temperature may also cause false negative result as it makes the antigen unrecognizable by the antibodies.
- An interference can be found for samples from patients treated with Xolair (omalizumab) or similar drugs containing anti IgE antibodies.
- Other factors may interfere with the test and cause erroneous results, such as technical/procedural errors, degradation of the test components/reagents or presence of interfering substances in the test samples.
- Any clinical diagnosis based on the test result must be supported by a comprehensive judgment of the concerned physician in conjunction with clinical symptoms and other relevant test results.

MATERIALS SUPPLIED

REF CFPC-91

Components of **ichroma™ Total IgE**

- Cartridge Box:
  - Cartridge 25
  - ID Chip 1
  - Instruction for Use 1
- Detection Buffer Box
  - Detection Buffer 25

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately from **ichroma™ Total IgE**. Please contact our sales division for more information.

- Instrument for ichroma™ tests
  - **ichroma™ II** REF FPRR021
- **Boditech Total IgE Control** REF CFPO-219

SAMPLE COLLECTION AND PROCESSING

The sample type for **ichroma™ Total IgE** is human whole blood/ serum /plasma.

- It is recommended to test the sample within 24 hours after collection.
- The serum or plasma should be separated from the clot by centrifugation within 3 hours after the collection of whole blood
- Samples may be stored for a month at 2-8 °C prior to being tested. If testing will be delayed more than a month, samples should be frozen at -20 °C
- Samples stored frozen at -20 °C for 3 months showed no performance difference.
- However, the whole blood sample should not be kept in a freezer in any case.
- As a repeated freeze-thaw cycle may affect the test result, do not refreeze previously frozen samples

TEST SETUP

- Check the contents of **ichroma™ Total IgE**: Sealed Cartridges, Detection buffers, ID Chip and Instruction for use.
- Ensure that the lot number of the cartridge matches that of the detection buffer as well as an ID Chip.
- If the sealed cartridge and the detection buffer have been stored in a refrigerator, place them on a clean and flat surface at room temperature for at least 30 minutes before testing.
- Turn on the instrument for ichroma™ tests.
  (Please refer to the 'Instrument for ichroma™ tests Operation Manual' for complete information and operating instructions.)

TEST PROCEDURE

1) Transfer of 50 µL (human serum/ plasma/ control) or 100 µL (human whole blood) of sample with a pipette dispense it into the detection buffer tube.
2) Shake 5 times or more the closed tube until the mixture mix well. The mixture has to be used within 30 seconds.
3) Pipette out 75 µL of a sample mixture and load it into the sample well of the cartridge.
4) Leave the cartridge at room temperature for 12 min before inserting the device into the holder.
   ⚠ *Scan the sample-loaded cartridge immediately when the incubation time is over. If not, it will cause inaccurate test result.*
5) To scan the sample-loaded cartridge, insert it into the cartridge holder of the instrument for ichroma™ tests. Ensure proper orientation of the test cartridge before pushing it all the way inside the test cartridge holder. An arrow is marked on the test cartridge especially for this purpose.
6) Tap the 'Start' button on the instrument ichroma™ for tests to start the scanning process.
7) The instrument for ichroma™ tests will start scanning the sample-loaded cartridge immediately.
8) Read the test result on the display screen of the instrument for ichroma™ tests.
   (Please refer to the ichroma™ II operation manual for complete information and operation instructions.)

INTERPRETATION OF TEST RESULT

- Instrument for ichroma™ tests calculates the test result automatically and displays total IgE concentration of the test sample in terms of IU/mL.
- To convert the IU/mL of the result to mass unit per volume, the conversion factor can be used as follow:
  1 IU/mL = 2.44 ng/mL
- The concentration of IgE in the serum is highly age dependent.
- Total IgE concentrations were measured in human serum samples from non-atopic healthy adult and child subjects using the **ichroma™ Total IgE**. The observed ranges of total IgE concentrations are shown below for each age group represented:

Age group	Geometric mean *	ichroma™ Total IgE
		Mean + 1SD
< 1 year	3.2 IU/mL	13.6 IU/mL
1 – 5 year	12.1 IU/mL	43.3 IU/mL
6 – 9 year	20.6 IU/mL	80.1 IU/mL
10 – 15 year	51.1 IU/mL	209.2 IU/mL
≥16 year	13.2 IU/mL	88.4 IU/mL

*Reference values for serum IgE in healthy non-atopic children and adults. *Clin Chem.* 1982;**28**(7):1556.

- Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.
- Working range : 1.00 IU/mL – 1,000 IU/mL

QUALITY CONTROL

- Quality control tests are a part of the good testing practice to confirm the expected results and validity of the assay and should be performed at regular intervals.
- The control tests should be performed immediately after opening a new test lot to ensure the test performance is not altered.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control materials are not provided on demand with **ichroma™ Total IgE**. For more information regarding obtaining the control materials, contact Boditech Med Inc.'s Sales Division for assistance.
  (Please refer to the instruction for use of control material.)

PERFORMANCE CHARACTERISTICS

- **Analytical sensitivity**
  - Limit of Blank (LoB) 0.50 IU/mL
  - Limit of Detection (LoD) 0.75 IU/mL
  - Limit of Quantification (LoQ) 1.75 IU/mL
  - Reportable range
    Reportable range of the undiluted sample is 1.00 IU/mL – 1,000 IU/mL. Samples with total IgE concentrations above 1,000 IU /mL can be diluted with saline (0.9% NaCl in distilled water, not provided). The recommended dilution is 1:10 or 1:100.
    After dilution, multiply the result by the dilution factor. Please follow the below equation to obtain final sample concentration.
    [Final sample conc. = Reported conc. x Dilution factor (10 or 100)]

- **Hook Effect**
  High concentration of the standard material was diluted, and there is no hook effect up to 15,000 IU/mL

- **Analytical specificity**
  - Cross-reactivity
    Biomolecules listed in the following table were added to the test sample(s) at concentrations much higher than their normal physiological levels in the blood. **ichroma™ Total IgE** test results did not show any significant cross-reactivity with these biomolecules.

Immunoglobulin	Concentration
Immunoglobulin G	20 mg/mL
Immunoglobulin A	20 mg/mL
Immunoglobulin M	20 mg/mL

  - Interference
    Interference materials listed in the following table were added to the test sample(s) the same as the below concentrations listed below. **ichroma™ Total IgE** test results did not show any significant interference with these materials.

Interference Materials	Concentration
Hemoglobin	200 mg/dL
Bilirubin	0.4 mg/mL
Triglyceride	2,000 mg/dL
Rheumatoid factor	78 IU/mL
Human serum albumin	12 g/dL
Biotin	10 ng/mL

- Precision
  - Between-lot
    One person tested three different lots of **ichroma™ Total IgE**, ten times at each sample.
  - Between person
    Three different persons tested **ichroma™ Total IgE**, ten times at each sample.
  - Between day
    One person tested **ichroma™ Total IgE** during five days, ten times at each sample.
  - Between site
    One person tested **ichroma™ Total IgE** at three different sites, ten times at each sample. The following results were obtained:

conc. (IU/mL)	Between-lot		Between-person	
	Mean	CV (%)	Mean	CV (%)
5.00	4.98	8.6	5.05	8.0
100.00	100.73	3.7	101.49	4.3
500.00	520.05	2.8	527.25	5.7
conc. (IU/mL)	Between-day		Between-site	
	Mean	CV (%)	Mean	CV (%)
1.00	4.90	8.1	5.07	9.5
10.00	101.18	3.6	104.76	7.2
100.00	514.54	2.6	534.28	5.6

- **Accuracy**

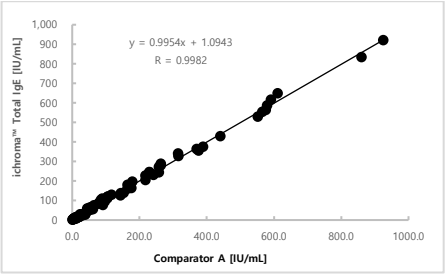
The accuracy was determined by 3 different lots testing six times each human serum.

Expected value [IU/mL]	Value [IU/mL]			Mean [IU/mL]	Bias (%)
	Lot 1	Lot 2	Lot 3		
15.00	14.97	15.15	15.29	15.14	1%
52.50	50.48	49.93	49.88	50.10	-5%
252.50	259.80	263.27	261.65	261.57	4%
400.00	420.10	423.13	420.40	421.21	5%

- Comparability

Total IgE concentration of 100 standard materials were quantified independently with **ichroma™ Total IgE (ichroma™ II)** and Comparator A as per prescribed test procedures. Test results were compared, and their comparability was investigated with linear regression and coefficient of correlation (R). Linear regression and coefficient of correlation between the two tests are below respectively.

	Comparator A	
	Linear regression	coefficient of correlation (R)
ichroma™ II	y = 0.9954x + 1.0943	0.9982



REFERENCES

1. "The biology of IGE and the basis of allergic disease". Annu Rev Immunol. 2003; 21: 579–628.
2. "Age-related serum immunoglobulin E levels in healthy subjects and in patients with allergic disease". J Allergy Clin Immunol. 1980; 66(4):305-313
3. "IgE concentrations measured by PRIST in serum of healthy adults and in patients with respiratory allergy. A diagnostic approach". Allergy. 1981; 36(8):537-547.
4. "Reference values of total serum IgE and their significance in the diagnosis of allergy in young European adults". Int Arch Allergy Immunol 2007; 142: 230-238.
5. "Allergen drives class switching to IgE in the nasal mucosa in allergic rhinitis". J Immunol. 2005; 174 (8): 5024–5032.
6. "Total serum IgE in a population-based study of Asian children in Taiwan: reference value and significance in the diagnosis of allergy". PLoS One. 2013; 8(11): e80996.
7. "Longitudinal observations of serum IgE and skin prick test response". Am J Respir Crit Care Med. 1995; 151:663-668.
8. "Genetic and developmental aspects of IgE". Pediatr Clin North Am. 1975; 22:17-32.
9. "Reference values for serum IgE in healthy non-atopic children and adults". Clin Chem. 1982;28(7):1556.

Note: Please refer to the table below to identify various symbols

Σ	Sufficient for <n> tests
	Read instruction for use
	Use by Date
LOT	Batch code
REF	Catalog number
	Caution
	Manufacturer
EC REP	Authorized representative of the European Community
IVD	In vitro diagnostic medical device
	Temperature limit
	Do not reuse
CE	This product fulfills the requirements of the Directive 98/79/EC on in vitro diagnostic medical devices