Rheumatoid Arthritis

ichroma™ ASO

INTENDED USE

ichroma™ ASO is a fluorescence immunoassay (FIA) for the quantitative determination of Anti Streptolysin O (ASO) in human serum/plasma. It is useful as an aid in management and monitoring of scarlet fever, rheumatic fever and post infectious glomerulonephritis along with several other conditions.

For *in vitro* diagnostic use only.

INTRODUCTION

ASO is an antibody produced in human blood against streptolysin O made from an infection of Streptococcus bacteria. An elevated or rising ASO titer may demonstrate recent streptococcal infections. Some autoimmune responses, glomerulonephritis, acute tonsillitis, scarlet fever and rheumatic fever may be associated with streptococcal infections. Even if ASO titers may vary due to a number of factors including population and age.

PRINCIPLE

The test uses a sandwich immunodetection method.

The detector recombinant proteins in buffer bind to ASO in the sample, forming recombinant protein-ASO complexes, and migrate onto nitrocellulose matrix to be captured by the other immobilized-proteins on a test strip.

More ASO in the sample will form more recombinant protein-ASO complexes which lead to stronger fluorescence signal by detector antibodies, which is processed by the instrument for ichroma™ tests to show ASO concentration in the sample.

COMPONENTS

ichroma™ ASO consists of 'cartridges', 'detector tube' and 'detector diluent'.

- The cartridge contains the membrane called a test strip which has SLO protein at the test line, and chicken IgY at the control line. All cartridges are individually sealed in an aluminum foil pouch containing a desiccant, and they are further packaged in a box.
- The detector tube has a granule containing SLO protein fluorescence conjugate, anti-chicken IgY fluorescence conjugate, and sodium azide in phosphate buffered saline (PBS) as a preservative. All detector tubes are packed in a pouch.
- The detector diluent contains sodium azide as a preservative in phosphate buffer saline (PBS) and it is pre-dispensed in a vial. The detector diluent packed in a box.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Follow the instructions and procedures described in this 'Instructions for use'.
- Use only fresh samples and avoid direct sunlight.
- Lot numbers of all the test components (cartridge, detector tube, detector diluent 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 detector tubes. A cartridge should be used for testing one sample only. A detector tube should be used for processing one sample only.
- The cartridge should remain sealed in its original pouch until just before use. Do not use the 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.
- If test components and/or sample are stored in refrigerator, then allow cartridge, detector tube, detector diluent 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, detector tube, detector diluent and pipette tips should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- The detector tube and the detector diluent contain sodium azide (NaN_3), and they may cause certain health issues like convulsions, low blood pressure, low heart rate, loss of consciousness, lung injury and respiratory failure. Avoid contact with skin, eyes, and clothing. In case of contact, rinse immediately with running water.
- **ichroma™ ASO** will provide accurate and reliable results subject to the below conditions.
  - **ichroma™ ASO** should be used only in conjunction with the Instrument for ichroma™ tests.
  - Have to use recommended anticoagulant.

Recommended anticoagulant
Na_2 EDTA, K_2 EDTA, Sodium citrate

STORAGE AND STABILITY

Storage condition			
Component	Storage Temperature	Shelf life	Note
Cartridge	2 – 30°C	20 months	Disposable
Detector tube	2 – 30°C	20 months	Disposable
Detector diluent	2 – 30°C	20 months	Unopened
		12 months	Opened

- 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 antigens 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 antigens with time and/or temperature may also cause false negative result as it makes antigens unrecognizable by the 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-46

Components of **ichroma™ ASO**

- Cartridge box:
  - Cartridge 25
  - Detector tube 25
  - Detector diluent 1
  - ID chip 1
  - Instruction for use 1

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately with **ichroma™ ASO**.

Please contact our sales division for more information.

- Instrument for ichroma™ tests
  - **ichroma™ Reader** REF FR203
  - **ichroma™ II** REF FPRR021
  - **ichroma™ III** REF FPRR037
  - **ichroma™ M3** REF FPRR035
- **Printer** REF FPRR007

SAMPLE COLLECTION AND PROCESSING

The sample type for **ichroma™ ASO** is human serum/ plasma.

- It is recommended to test the sample within 24 hours after collection when collected sample is stored at room temperature.
- The samples (serum, plasma) should be separated from the clot by centrifugation within 3 hours after the collection of whole blood.
- The samples (serum, plasma) may be stored for a week at 2 – 8 °C prior to being tested. If testing will be delayed more than a week, samples (serum, plasma) should be frozen at -20 °C.
- The samples (serum, plasma) stored frozen at -20°C for 2 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™ ASO**: Sealed cartridges, detector tubes, a detector diluent, an ID chip, an instruction for use.
- Ensure that the lot number of the cartridges matches that of the detector tube, detector diluent as well as an ID chip.
- If the sealed cartridge, the detector tube, and detector diluent 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

▶ ichroma™ Reader, ichroma™ II

Multi test mode

1) Select the sample type (whole blood or serum/plasma) on the screen.
2) Take 500 µL of detector diluent using a pipette and dispense it to the detector tube containing a granule. When the granule form is completely dissolved in the tube, it becomes detection buffer.
   (The detection buffer must be used immediately. Do not exceed 30 seconds.)
3) Take 5 µL of sample (serum/plasma) using a pipette and dispense it to the detector tube.
4) Close the lid of the detector tube and mix the sample thoroughly by shaking it about 10 times.
   (The sample mixture must be used immediately. Do not exceed 30 seconds.)
5) Take 75 µL of the sample mixture and dispense it into the sample well of the cartridge.
6) Leave the cartridge at room temperature for 12 minutes.
   ⚠ *Scan the sample-loaded cartridge immediately when the incubation time is over. If not, it will cause inaccurate test result.*
7) To scan the sample-loaded cartridge, insert it into the cartridge holder of the instrument for ichroma™ tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow is marked on the cartridge especially for this purpose.
8) Press the 'Select' or tap the 'Start' button on the instrument for ichroma™ tests to start the scanning process.
9) The instrument for ichroma™ tests will start scanning the sample-loaded cartridge immediately.
10) Read the test result on the display screen of the instrument for ichroma™ tests.

Single test mode

1) The test procedure is same with the 'Multi test mode 1) – 5)'.
2) Insert the sample-loaded cartridge into the holder of the instrument for ichroma™ tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow is marked on the cartridge especially for this purpose.
3) Press the 'Select' or tap the 'Start' button on the instrument for ichroma™ tests.
4) The cartridge goes inside the instrument for ichroma™ tests and will automatically start scanning the sample-loaded cartridge after 12 minutes.
5) Read the test result on the display screen of the instrument for ichroma™ tests.

▶ ichroma™ III

1) The test procedure is same with 'ichroma™ Reader, ichroma™ II 1) – 5)'.
2) Wait until the sample mixture flows into the windows, which may take about 10 seconds.
3) Insert the sample-loaded cartridge into the holder of the ichroma™ III. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow is marked on the cartridge especially for this purpose.
4) Tap the 'Start' button on the ichroma™ III to start the scanning process.
5) The cartridge goes inside and ichroma™ III will automatically start scanning the sample-loaded cartridge after 12 minutes.
6) Read the test result on the display screen of the ichroma™ III.

▶ ichroma™ M3

1) Select the sample type (whole blood or serum/etc.) on the screen. Press the key button to change the desired sample type.
2) The test procedure is same with the 'ichroma™ Reader, ichroma™ II test procedure 2) ~ 5)'.
3) Insert the cartridge approximately 1/3 into the slot of the ichroma™ M3.
   Before pushing the cartridge all the way in, ensure it is inserted in the correct orientation. An arrow is marked on the cartridge especially for this purpose.
4) Take 75 µL of the sample mixture and dispense it into the sample well of the cartridge.
5) Wait until the sample mixture flows into the window.
   ⚠ ***Insert the cartridge exactly 20 seceonds after applying the sample. Otherwise test results may be inaccurate.***
6) Insert the sample-loaded cartridge fully into the slot of the ichroma™ M3.
7) After inserting the cartridge, the result will be automatically displayed after 12 minutes.
8) Read the test result on the display screen of the instrument for ichroma™ tests.

INTERPRETATION OF TEST RESULT

- The instrument for ichroma™ tests calculates the test result automatically and displays ASO concentration of the test sample in terms of IU/mL.
- Reference range: 25-166 IU/mL

ASO Concentration	Result
< 166 IU/mL	Negative
≥ 166 IU/mL	Positive

- Working range: 25-800 IU/mL

PERFORMANCE CHARACTERISTICS

- **Analytical sensitivity**
  - Limit of Blank (LoB) 5.86 IU/mL
  - Limit of Detection (LoD) 9.18 IU/mL
  - Limit of Quantitation (LoQ) 25.0 IU/mL
- **Analytical specificity**
  - **Cross-reactivity**

    Biomolecules such as below the ones in the table were added to the test sample(s) at concentrations much higher than their normal physiological levels in the blood. **ichroma™ ASO** test results did not show any significant cross-reactivity with these biomolecules.

Cross-reactants	Concentration
CRP	40 mg/L
RF IgM	25 IU/mL

  - Interference

    Interferents listed in the following table were added to the test sample at the concentration mentioned below. **ichroma™ ASO** test results did not show any significant interference with these materials.

Interferents	Concentration
Hemoglobin	1,000 mg/dL
Bilirubin [unconjugated]	684 µmol/L
Phospholipid	20 g/L
Triglyceride	16.94 nmol/L
Human serum albumin	12 g/dL
Amoxicillin	54 µg/mL
Cephalexin	126 µg/mL

- **Precision**
  - **Single-site study**

    Repeatability (within-run precision)
    Within-laboratory precision (Total precision)
    Lot to lot precision

    3 Lots of **ichroma™ ASO** were tested for 20 days. Each standard material was tested 2 times per day. For each test, each material was duplicated.

Single-site study						
ASO [IU/mL]	Repeatability		Within-laboratory precision		Lot to lot precision	
	AVG [IU/mL]	CV (%)	AVG [IU/mL]	CV (%)	AVG [IU/mL]	CV (%)
125	127.24	8.18	126.10	8.18	126.38	7.85
250	248.06	5.98	248.91	5.55	251.86	5.96
500	498.19	5.81	498.21	6.00	500.46	6.11

  - **Multi-site study**

    Reproducibility

    1 Lot of **ichroma™ ASO** was tested for 5 days in 3 different sites (1 person per 1 site, 1 instrument per 1 site). Each standard material was tested 1 time per and 5 replicates per day.

Multi-site study		
ASO [IU/mL]	Reproducibility	
	AVG [IU/mL]	CV (%)
125	126.82	7.34
250	249.22	6.44
500	507.06	6.24

- **Accuracy**

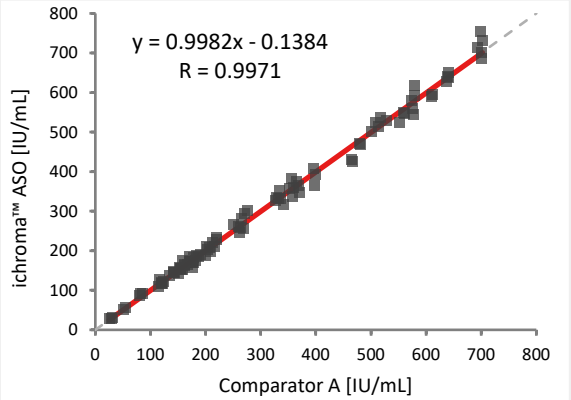
  The accuracy was confirmed by testing with 3 different lots of **ichroma™ ASO.** The tests are repeated 10 times in each different concentration.

ASO [IU/mL]	Lot 1	Lot 2	Lot 3	AVG	Recovery (%)
27.5	27.55	27.59	27.85	27.66	100.6%
125.0	127.13	126.44	124.67	126.08	100.9%
160.0	160.40	161.28	154.73	158.80	99.3%
250.0	254.14	245.80	255.45	251.80	100.7%
500.0	516.71	494.98	491.73	501.14	100.2%
720.0	736.18	717.68	722.67	725.51	100.8%

- **Comparability**

  ASO concentrations of 100 clinical samples were quantified independently with **ichroma™ ASO (ichroma™ II)** and **Comparator A** as per prescribed test procedures. Test results were compared, and their comparability was investigated with linear regression and correlation coefficient (R). The regression equation and correlation coefficient are as follows.

  * Solid line: regression line of ichroma™ ASO
  * Dash line: y = x



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

	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

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CE IVD