

QUANTITATIVE LATERAL FLOW ST2 TEST

Please read all of the information in these instructions for use for complete instructions before performing the test. If you have questions, call Critical Diagnostics Product Support for assistance. (See the Assistance section).

INDICATIONS FOR USE

The ASPECT-PLUS™ ST2 Test is a rapid lateral flow immunoassay to be used with the ASPECT Reader™ for the quantitative determination of ST2 in venous EDTA anticoagulated plasma and is intended for use by healthcare professionals only. The test is used as an aid for the risk stratification of heart failure (HF) or acute coronary syndrome (ACS) patients. The test can also be used to assess the risk of developing heart failure in individuals who are otherwise asymptomatic and apparently healthy.

INTRODUCTION

Heart disease is a leading cause of death worldwide affecting millions of patients annually. For example, heart disease is responsible for 25 percent of all the deaths in the United States, more than all forms of cancer combined. Many patients are treated for coronary artery disease (CAD) and/or acute coronary syndrome (ACS) but eventually develop heart failure. Heart failure is a chronic, progressive disease in which the ability of the heart to provide needed cardiac output weakens, thus impeding the heart's ability to pump enough blood to support the body's metabolic demands. The prevalence of heart failure is growing worldwide and is a major burden on hospital care costs. A major component to this burden is the fact that patients afflicted with advanced heart failure have high rates of hospitalization and resource utilization, and similarly have a high risk for death.

TEST PRINCIPLE

The ASPECT-PLUS ST2 Test is a quantitative sandwich monoclonal lateral flow immunoassay. Venous EDTA anticoagulated plasma is loaded into the sample well where it flows through the anti-ST2 antibody coated strip. Assay buffer is added to the second well. The cassette is then inserted into the ASPECT Reader for incubation, and ST2 is quantitatively determined and reported by the reader. Determination of a quantitative ST2 value using the ASPECT-PLUS ST2 Test is produced by standard linear regression analysis through a linear calibration curve unique to each lot of ASPECT-PLUS ST2 Test that is coded on the RFID tag included with each test cassette. The fluorescent signal measured on the test line is evaluated against the calibration curve to calculate an ST2 concentration.

MATERIALS PROVIDED:

The ASPECT-PLUS ST2 Test kit contains all the reagents needed for the generation of test results for measurement of ST2 in human EDTA plasma sample.

The ASPECT-PLUS ST2 Test cassette and ASPECT-PLUS ST2 Test buffer vial contain:

ASPECT-PLUS ST2 Test cassette [REF] PLUS0001-01 $\nabla \Sigma=20$

- Murine monoclonal antibodies against ST2
- Goat polyclonal antibodies against murine Ig-G
- Fluorescent dye

ASPECT-PLUS ST2 Test buffer [REF] PLUS0001-03 $\nabla \Sigma=1$

- Stabilizers
- Excipients



PLUS0001-02

MATERIALS REQUIRED BUT NOT PROVIDED

- ASPECT Reader (REF: READ0001)
- Electronic Quality Control (EQC) (REF: EQC0001)
- ST2 Controls, Level 1 and Level 2 (REF: LQC1111)
- ☣ Puncture-proof waste container for biohazard and sharps waste
- Timer
- Gloves
- Calibrated pipette

PRECAUTIONS AND WARNINGS

1. For in vitro diagnostic use.
2. For use by healthcare professionals.
3. Results should be interpreted along with clinical findings and other laboratory results.
4. Do not use the ASPECT-PLUS ST2 Test cassette in any other uses not identified in the Indications for Use.
5. Keep the ASPECT-PLUS ST2 Test cassette in the sealed pouch until ready to use. Discard after single use.
6. Do not use after the expiration date printed on the package.
7. ☣ Contains material of animal origin and should be handled as though capable of transmitting infectious disease. Follow proper infection control guidelines for handling all blood samples, EDTA plasma specimens, and related items. Parts of the kit that come in contact with blood or plasma are considered biohazardous and can potentially transmit infectious diseases, even after you have performed cleaning and disinfection.
8. Contains the active ingredient (0.05%) Proclin 300 as biocide preservative.
9. Avoid contact of assay reagents or specimen with skin, eyes, or mucous membranes. In the event of contact with skin or eyes, wash immediately with water.
10. Wash hands thoroughly after performing the test.
11. Dispose of waste as potentially infectious agents and in accordance with local requirements.

SAMPLE COLLECTION AND PREPARATION

1. Use only venous EDTA anticoagulated plasma. Other sample types, draw methods or anticoagulants have not been evaluated. Preparation of plasma should be performed as soon as possible after the specimen is collected.
2. The patient sample must be tested within 48 hours from collection when sample is stored room temperature (18-25C). If not able to test within 48 hours, store refrigerated (2-8C) up to seven (7) days or store frozen (< -20C) for a maximum of 18 months.⁶

Storage Condition (temperature)	Specimen Stability
20°C	48 hours
4°C	7 days
-20°C and -80°C	18 months

3. If the sample must be transported to a laboratory for testing, transport at room temp (18°-25°C) or refrigerated (2°-8°C) within 48 hrs of collection. Avoid exposure to temperatures >25°C.

NOTE: Current health status may affect test results or cause inaccurate results. It's important to take certain health factors into account when reading test results and setting a course of action.

STORAGE AND HANDLING

The ASPECT-PLUS ST2 Test is stable up to nine (9) months when stored in a controlled environment as follows:

Store cassettes as packaged in the sealed foil pouch until ready to use and until the expiration date printed on the package:

- Do not use the cassette if the foil pouch is torn, punctured, or not sealed completely. Discard the cassette and use another cassette.
- Store ASPECT-PLUS ST2 Test cassettes at 2°C to 8°C (35-46°F).
- Before using refrigerated ASPECT-PLUS ST2 Test cassettes, allow individual foil pouched cassettes to reach room temperature in 15 minutes prior to use.
- **Do not use after the expiration date printed on the package.**

NOTE: Use the cassette within ten (10) minutes of opening the foil pouch.

QUALITY CONTROL CONSIDERATIONS

Every ASPECT-PLUS ST2 Test cassette includes one internal control check (control line on test cassette) that is run automatically with every patient sample, external liquid control solution, or proficiency testing sample to check that there is proper flow in the ASPECT-PLUS ST2 Test cassette. If the automatic check of this built-in control shows that it is within a limit, the ASPECT Reader will report a result for the sample being tested. If the automatic check of this built-in control shows that is not within the limits, a test result will not be reported. Instead, the reader will display a warning or error message that is described in the ASPECT Reader User Guide.

Good Laboratory Practice suggests that external controls should be tested with each new lot or shipment of test materials, or every 30 days, and as otherwise required by your laboratory's standard quality control procedures. Controls should be tested in the same manner as if testing patient specimens. When running patient samples or external controls, if a test fails for any reason (built-in control failure or an external control out of range) no patient results will be reported.

Users should follow government guidelines (for example, federal, state or local) and/or accreditation requirements for quality control.

ELECTRONIC QUALITY CONTROL (EQC)

Use the EQC to ensure proper function of the ASPECT Reader. Perform the EQC testing for the following conditions:

- Initial setup of the ASPECT Reader.
- When the ASPECT Reader has been transported or moved.
- Whenever there is uncertainty about the performance of the ASPECT Reader.
- Whenever required by your laboratory's quality control requirements.
- Once at the beginning of each day.

NOTE: The EQC is light sensitive. Store the EQC in the provided foil pouch after each use.

NOTE: The EQC is ready to test. No sample or test buffer is required.

NOTE: Refer to the ASPECT Reader User Guide for complete instructions on use of the EQC.

PERFORM A TEST

Please read all of the information in this instructions for use before performing a test.

- Symbols used in these instructions are defined in Symbols Section.
- Gather the items needed (See the Materials Provided and Materials Required But Not Provided sections, above).
- Follow proper infection control guidelines for handling all blood samples and anything that comes in contact with blood.

1. Remove an ASPECT-PLUS ST2 Test cassette from refrigerated storage and warm to room temperature for 15 minutes. DO NOT open the foil pouch until the cassette is at room temp.
2. Prepare the test sample (venous EDTA plasma). See Sample Collection and Preparation section.
3. On the ASPECT Reader:
 - a. Enter appropriate information to LOG IN and perform a test.
 - b. From the main screen, select RUN PATIENT.
 - c. Enter Patient ID, then select RUN PATIENT.
4. Remove the test cassette from the foil pouch.

NOTE: You must use the cassette within 10 minutes of opening the foil pouch.

5. Using a calibrated micropipettor, pipette 35 uL patient sample into the sample, which is the middle well with the [🔴].
6. After 60 seconds, add 2 drops (~110 µL) test buffer into the test buffer well, which is the well beside the sample well on the edge of the test cassette. DO NOT add buffer to the sample well. Test buffer must be added ONLY to the buffer well of the test cassette.
7. Insert the test cassette loaded with the patient sample and test buffer.

NOTE: The test cassette must be inserted into the reader within 60 seconds from the time buffer is added. A delay longer than 60 seconds after test buffer addition may lead to inaccurate results.

8. Close reader drawer and reader will initiate analysis.
9. Results will be displayed on the screen in approximately 20 minutes.

NOTE: Refer to the ASPECT Reader User Guide for complete instructions on how to perform a test.

☣ Dispose of all items that have come in contact with plasma sample. Be sure to follow proper infection control guidelines.

READING RESULTS

The ASPECT Reader measures ST2 automatically. The result is displayed on the screen. The operator has the option to print the results. A value in ng/mL represents the concentration of ST2 in the sample.

NOTE: ST2 levels less than 12.5 ng/mL or greater than 250 ng/mL will be reported as <12.5 ng/mL and >250 ng/mL.

For additional information, refer to the ASPECT Reader User Guide.

INTERPRETATION OF RESULTS

- In self declared healthy individuals, normal patients, the interquartile values for ST2 are 15 to 25 ng/mL.
- A concentration of 35 ng/mL is between the 90th and 95th percentile of the normal population.¹
- In patients diagnosed with ACS or HF and who have ST2 concentrations ≥ 35 ng/mL risk of adverse events such as hospitalization or mortality within one (1) year is greater than for patients with ST2 concentrations below this level. Risk of mortality increases with increasing concentrations of ST2.^{2,3,4}
- In asymptomatic and apparently healthy individuals, an elevated ST2 concentration has been shown to be indicative of a greater than two-fold increased risk of developing heart failure.⁵
- Although a negative ST2 result, < 35 ng/mL, in patients with HF or ACS may be used to classify patients and individuals as low risk for adverse events such as rehospitalization or death, clinically appropriate therapy should be pursued.
- ST2 results should be used with other clinical and diagnostic information for forming a diagnosis and for patient management.

STANDARDIZATION

Concentration results of the ASPECT-PLUS ST2 Test cassette are traceable to reference standard solutions that contain defined concentration of ST2 protein. The ASPECT-PLUS ST2 Test cassette and ST2 Controls are traceable to the same reference standard solutions.

LIMITATIONS

- Testing in studies has been limited to people 18 years of age and older.
- Validated only with EDTA venous anticoagulated plasma samples.
- The presence of human anti-mouse antibody (HAMA) may produce falsely low ST2 concentration.
- There is a possibility that factors such as technical or procedural errors, as well as additional substances in blood specimens that are not listed below, may interfere with the test and cause erroneous results.

INTERFERING SUBSTANCES

The following analytes were tested separately and do not interfere with the expected results up to the concentrations shown:

Substance	Concentration
Bilirubin	0.1 mg/mL – 0.3 mg/mL
Cholesterol (Total)	1.5 mg/mL – 5 mg/mL
Hemoglobin	1 mg/mL – 2 mg/mL
Protein (BSA)	15 mg/mL – 60 mg/mL
Triglycerides/Lipids (Total)	1.5 mg/mL – 30 mg/mL

The following drugs were tested separately and do not interfere with the expected test results when spiked into EDTA plasma: acetaminophen, acetylsalicylic acid, allopurinol, ambroxol, amiodarone, amlodipine besylate, ampicillin, ascorbic acid, atenolol, bivalirudin, caffeine, captopril, chloramphenicol, cinnarizine, cyclosporine A, digitoxin, digoxin, diltiazem, dopamine, dipyridamole, enalapril maleate, eplerenone, erythromycin, fluvastatin, furosemide, glyburide, heparin sodium, hydralazine hydrochloride, hydrochlorothiazide, indomethacin, lisinopril, nicotine, nifedipine, nitrofurantoin, oxytetracycline, phenytoin, pravastatin sodium, probenecide, procainamide hydrochloride, (S)-(-)-propranolol hydrochloride,

quinidine, simvastatin, spironolactone, sulfamethoxazole, theophylline, L-thyroxine, trimethoprim, verapamil hydrochloride, and warfarin.

Specificity was tested against; human interleukin-1 soluble receptor type I (IL-1 sR-1), recombinant human IL-1 α and recombinant human IL-1 β . None of the three molecules tested, even at a concentration as high as 5x the measured ST2 concentration, exhibited measurable cross reactivity.

PERFORMANCE CHARACTERISTICS

PRECISION

Assay precision was determined per CLSI EP5-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition. Three test specimens, EDTA plasma sample along with the two (2) ST2 Controls were used for this evaluation. Intra-assay, inter-assay, and total precision were calculated for each specimen tested. The precision results are summarized in Table 1. The assay meets acceptable analytical performance criteria, exhibiting an average CV of 10.4% for intra-assay and 13.6% for inter-assay variation and does not exhibit any precision bias through the tested concentration range. The precision performance was validated through “Inter-Laboratory” testing following CLSI EP15-A3, User Verification of Precision and Estimation of Bias. Measurements were performed in four (4) laboratories once per day with three (3) replicates of two specimens (plasma) at each of two (2) concentrations and both levels of liquid controls daily for five (5) days. This testing showed no significant difference from the values summarized in Table 1.

Table 1: Precision Analysis Summary

ST2 Level	Intra-Assay CV	Inter-Assay CV	Total CV
Low (20 ng/mL)	15.4%	16.3%	22.4%
Clinical (32 ng/mL)	4.9%	13.3%	14.2%
Elevated (81 ng/mL)	10.8%	11.1%	15.5%

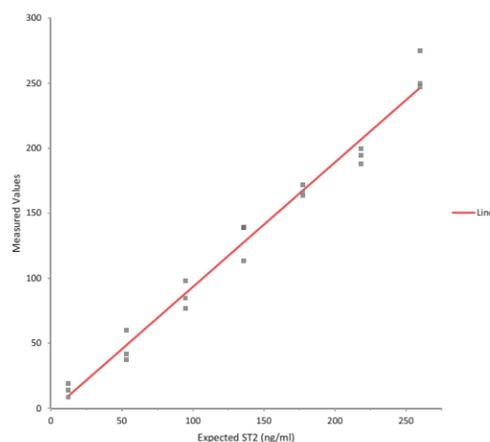
ANALYTICAL SENSITIVITY

Analytical sensitivity was determined per Clinical and Laboratory Standards Institute (CLSI) protocol EP17-A, Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline. To determine the Limit of Blank (LoB), fetal bovine serum (FBS) was used as the source of material without endogenous ST2. In accordance with the LoB determination protocol, a set of 60 replicates of FBS samples were measured over 4 days, with 15 replicates per day. Following determination of the LoB, the Limit of Detection (LoD) was ascertained. To determine the LoD, 60 replicates of 4 different low ST2 concentration human plasma samples were measured in sets of 15 replicates each over 4 consecutive days. Following determination of the LoD, the Limit of Quantitation (LoQ) was derived. To calculate LoQ, the same sample measurements used for the LoD determination were used to estimate bias and imprecision. Since the LoQ value is lower than the LoD value, then per the protocol, the LoQ will be defined as equal to the LoD at 12.5 ng/ml.

LINEARITY

Assay linear performance was determined per CLSI protocol EP6-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline. Frozen human EDTA plasma samples were used to produce plasma pools with which to perform this analysis. The ASPECT-PLUS ST2 Test has been designed with an upper linear limit of 250 ng/mL. For this analysis, a high concentration pool was prepared at a concentration of 425 ng/mL and a low concentration pool with a concentration of 13.6 ng/mL. A series of eleven (11) pools were created by combining varying amounts of each of the high and low concentration stock pools together. Each pool was measured in replicates of three (3). Linear regression analysis of the results from these pools shows that the correlation between the predicted and measured results are linear up to 257 ng/mL with an R² value of 0.9927 and a linear equation of $y = 1.0x + 3.6$, Figure 1.

Figure 1: Linearity of ASPECT-PLUS ST2 Test



HOOK EFFECT

No effect up to 2000 ng/mL.

CONCORDANCE ANALYSIS

ST2 is well established for use in conjunction with clinical evaluation as an aid in assessing the prognosis of patients diagnosed with chronic heart failure or acute coronary syndrome (ACS), as well as for determination of risk of developing heart failure in asymptomatic individuals. The Presage[®] ST2 Assay (ELISA format) is the reference assay for the ASPECT-PLUS ST2 Test. To verify concordance of the ASPECT-PLUS ST2 Test with the Presage ST2 Assay, an analysis of EDTA plasma from 60 donors was performed. This analysis showed a R² value of 0.92 and method comparison analysis by Passing-Bablok regression showed that the two assay techniques are comparable. The regression equation for this analysis is $y = 1.01x + 5.8$ and the Cusum test for linearity shows no significant deviation from linearity ($p=0.75$).

RETURN POLICY

If there is a problem with the ASPECT-PLUS ST2 Test, you may be asked to return unused cassettes in the foil pouch. Before returning cassettes please obtain a return authorization number from your local distributor. This return authorization number must be on the shipping carton for return.

ASSISTANCE OR ADDITIONAL INFORMATION

To order product, please contact your local distributor, or Critical Diagnostics Customer Service at +353 1 691 7061.

For Technical Support, please contact your local distributor, or Critical Diagnostics at +353 1 691 7061 or email, techsupport@criticaldiagnostics.com.

REFERENCES

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	Manufacturer
	In Vitro Diagnostic medical device
	Consult instructions for use
	Do not reuse
	Catalogue Number
	Contains sufficient for <n> tests
	Temperature limitation
	Batch Code /Lot Number
	Use by (date)
	Biological Risk: Disposable items pose biological risks.
	Patient Number
	European Authorized Representative
	CE Mark
	ASPECT-PLUS ST2 Test cassette
	ASPECT-PLUS ST2 Test buffer
	Instructions for use



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