

NoFACT XI_i

Immunodepleted Factor XI Deficient Substrate Plasma



The **NoFACT XI_i** Deficient Substrate Plasma is intended for the quantitative determination of Factor XI in patients suspected of having a congenital or acquired deficiency of this coagulation protein.

SUMMARY

At least eleven coagulation factors in human blood are required for normal blood clotting. A deficiency of Factors I, II, V, VII, VIII, IX, X, and XI can result in a clinically significant hemorrhagic condition, the severity of which is proportional to the degree of the deficiency, except for Factor XI. Factor deficiencies may be congenital or acquired. The congenital deficiencies are usually single deficiency states while the acquired deficiencies may be multiple in nature.

Factor XI deficiency is known as Rosenthal's disease or Hemophilia C. It is an autosomal recessive disease that occurs mainly in individuals of Ashkenazi Jewish ancestry. The disease occurs with equal frequency in males and females. Unlike most other Factor deficiency states, the risk of bleeding with Factor XI deficiency is not directly proportional to the degree of the deficiency measured by Factor XI assays. Acquired Factor XI deficiency occurs in liver disease.

The quantitative assay for Factor XI uses a modification of the activated partial thromboplastin time (APTT) test, and Factor XI deficient substrate plasma.

PRINCIPLE

Factor XI in its activated form converts Factor IX to IXa. Severe Factor XI deficient plasma (less than 1% activity) has a prolonged activated partial thromboplastin time (APTT). The degree of correction provided by the addition of various dilutions of an appropriate reference standard to the deficient plasma may be plotted on log-log graph paper. The degree of correction obtained using an unknown patient sample may then be read from the graph. The amount of correction is calculated and reported as a percentage of the reference plasma Factor XI activity.

REAGENTS

For In-Vitro Diagnostic Use Only.

Factor XI Deficient Substrate Plasma

Ingredients: The reagent is human plasma, which has been immunodepleted using immobilized goat anti human FXI antibody to contain less than 1% Factor XI activity. The plasma has been buffered and lyophilized to maximize stability.

WARNING: Potential Biohazard: The **NoFACT XI_i** Deficient Substrate Plasma has been found negative when tested for Hepatitis B Antigen (HBsAg) and antibodies to HCV and HIV by FDA licensed tests; however, the deficient plasma should be handled with the same precautions as those observed when handling patient plasmas.

Preparation for Use: Reconstitute each vial of **NoFACT XI_i** Deficient Substrate Plasma with 1.0 mL distilled water. Swirl gently; do not shake. Allow to stand for 20 minutes at room temperature to insure complete dissolution before use.

Storage and Stability: The lyophilized product is stable until the expiration date printed on the vial when stored at 2 to 8°C. The reconstituted product is stable for 8 hours when stored at 2 to 8°C. After reconstitution, the product should be kept on ice for the duration of testing.

TECHNIQUES

Factor XI assays using **NoFACT XI_i** Deficient Substrate Plasma can be performed by accepted manual methods or by using optical or electro-mechanical coagulation analyzers.

SPECIMEN COLLECTION AND PREPARATION

Specimen: Plasma obtained from whole blood anticoagulated with 0.1M sodium citrate.

Specimen Collection: Nine parts freshly collected whole blood should be immediately added to one part anticoagulant.

Specimen Preparation: Place the specimen on ice immediately after drawing and centrifuge as soon as possible. To insure platelet-poor plasma, centrifuge the specimen at 2500 x g for 15 minutes (NCCLS H21-A2, 1991). A refrigerated centrifuge is recommended. After centrifugation, the plasma should be removed immediately, using plastic pipettes only.

Storage and Stability: Before and during testing, keep the specimen on ice to insure stability of the factors being tested. If the specimen cannot be tested within 2 hours, quick-freeze the plasma at -70°C.

TEST PROCEDURE

Materials Provided:

10 vials **NoFACT XI_i** - 1 mL, 100 determinations

Other Supplies Available from R² Diagnostics

PlasmaRef ARN
PlasmaRef ARL-1
PlasmaRef ARL-2
PHOSPHOLIN ES
Calcium Chloride 0.025 M

Materials required but not provided:

Owren's Veronal buffer
12 x 75 mm plastic test tubes
Stopwatch
Log-log graph paper
Plastic serological pipettes

General Comments

1. Assay patient samples as soon after collection as possible.
2. Sample dilutions must be assayed within 30 minutes after preparation and maintained on ice until tested.
3. Sample dilutions exceeding 1:40 and serial dilutions are not recommended.
4. Run all four of the recommended dilutions on plasma samples to avoid potentially erroneous results due to dilution errors.
5. When performing factor assays, more than one vial of reagent may be needed. To eliminate vial-to-vial variation multiple vials should be reconstituted, allowed to dissolve and pooled, before use.
6. Prepare a new standard curve each time assays are performed. Even though the same lot of reagents may be used, vial-to-vial variation, technique differences and instrument variability require this procedure. **PlasmaRef ARN** is recommended for use in the preparation of the standard curve.

STEP-BY-STEP METHOD

A. Specimen and Reagent Preparation

1. Centrifuge the blood specimen for 15 minutes at 2500 x g using a refrigerated centrifuge. Remove plasma from the red blood cells immediately and place in a plastic test tube. Keep the plasma on ice for the duration of testing.
2. Reconstitute the appropriate number of vials of **NoFACT XI_i** Deficient Substrate with 1.0 mL distilled water. Swirl gently, do not shake and allow to stand approximately 20 minutes at room temperature to insure complete dissolution. Approximately 0.8 mL is required for each specimen assayed.
3. Reconstitute one vial of PlasmaRef ARN with 1.0 mL distilled water. Swirl gently, do not shake and allow to stand for 20 minutes to insure complete dissolution.
4. Prepare APTT reagent according to the package insert and transfer to a 37°C water bath or temperature controlled instrument reagent well.
5. Number a set of four 12 x 75 mm plastic test tubes for the standard curve and each test specimen.

B. Standard Curve Preparation

1. Prepare the following dilutions of **PlasmaRef ARN**, or fresh normal pool with Owren's Veronal buffer.

%	Dilution	mL	mL	Defined
Tube	Ratio	Standard	Buffer	Activity
1	1:5	0.1	0.4	100
2	1:10	0.1	0.9	50
3	1:20	0.1	1.9	25
4	1:40	0.1	3.9	12.5

2. Mix tubes gently but adequately. Avoid shaking and excess bubble formation.
3. Perform duplicate APTT tests on each of the standard dilutions as follows.

Pipette into the reaction cup in the order specified:

- 100 µL NoFACT XI/ Deficient Substrate
- 100 µL 1:5 dilution of PlasmaRef ARN
- 100 µL APTT Reagent

4. Start a stopwatch immediately and incubate the mixture according to the APTT reagent package insert.
5. At the end of the incubation period, remix the solution and add 100 µL pre-warmed calcium chloride reagent and simultaneously start a timer.
6. Record the times required for clot formation.
7. Repeat Steps 3-6 for each dilution in duplicate.

C. Testing of Unknown Specimen

1. Prepare dilutions of each unknown patient specimen as outlined in Step 1 above. Use the same volumes required for the standard.
2. Determine the clotting times of each specimen according to Steps 2-7.

Quality Control

Quality Control for factor assays involves multiple components. APTT reagents should be checked for their sensitivity to individual factor deficiencies.

A normal reference control plasma such as PlasmaRef ARL-1 and an abnormal reference control plasma such as PlasmaRef ARL-2 can be used to verify instrument and reagent performance.

Careful attention should be given to other test variables. These include; pipettes, calcium chloride, distilled water, timing devices, and buffers.

RESULTS

Standard Curve

1. Use standard log-log paper to plot the curve.
2. Use the X-axis (abscissa) to represent percent activity and the Y axis (ordinate) to represent the clotting time in seconds.
3. Determine the average clotting time for each dilution of the standard curve.
4. Plot the points where the line corresponding to the clotting times and the line corresponding to the known percent activity intersect.

EXAMPLE: Time/Sec	Dilution	% Activity	Clotting
	1:5	100	55
	1:10	50	65
	1:20	25	75
	1:40	12.5	85

5. Draw a line connecting all four points. This should be a straight line. The lines produced by plotting the clotting times of the standard dilutions and the patient dilutions should be parallel. If the two lines are not parallel, this may indicate the presence of an inhibitor in the patient plasma.

Calculation of Test Results

1. Calculate the mean clotting time for each of the test dilutions.
2. Plot the mean clotting time for each dilution and determine the percent activity.
3. Multiply the percent activity by the dilution factor.
4. Average the four percent activities calculated.
5. To calculate the actual percent factor activity of the mean of the unknown sample, multiply the percent activity of the mean of the sample by the activity of the reference standard (as provided by the manufacturer) used to prepare the curve.

EXAMPLE:

$$\frac{\text{Percent Activity of Unknown}}{\text{Defined Activity of Standard}} = \frac{\text{Actual Percent Factor Activity of Unknown}}{\text{Activity of Standard}}$$

If % activity of the unknown is 55.5 and the reference plasma assay value is 90% (or 0.90), the calculations would be:

$$55.5 \times 0.90 = 49.95\%$$

When using an un-assayed fresh normal plasma pool to prepare the standard curve, the percent activity is always assumed to be 100% (or 1.0). Fresh normal plasma pools should consist of at least ten healthy donors.

INTERPRETATION OF RESULTS

Factor XI activity is decreased in Rosenthal's disease or hemophilia C. The disorder tends to be mild, with bruising, epistaxis and menorrhagia as the most common clinical manifestations. Intramuscular hemorrhages and joint bleeds as seen with Hemophilia A and B are unusual. Affected individuals may have persistent bleeding following tooth extraction's, tonsillectomy or some other surgical procedure. Acquired Factor XI deficiency is seen in liver disease.

LIMITATIONS

1. The NoFACT XI/ Deficient Substrate Plasma is limited to Factor XI activity determinations based on a modified activated partial thromboplastin (APTT) test.
2. Specific variations in the test protocols may be required for use in automated coagulation instruments. The manufacturers recommendations should be followed carefully, especially when any recommendations may vary from those outlined above.

REFERENCE VALUES

Factor XI Expected Values: 50-150% of the normal reference plasma

For best results, each laboratory should determine a reference range for its particular population and instrument / reagent system.

