137 mm x 218 mm

FACTOR DEFICIENT PLASMAS

INTRINSIC PATHWAY

SUMMARY

Factor deficient plasmas are human plasmas deficient in a particular coagulation factor but has other coagulation factors within the normal range. A particular factor deficient plasma is useful in the quantitative determination of the activity of that factor using an appropriate clot based assay. The activities of the factors of intrinsic pathway (VIII, IX, XI & XII) is performed by using the APTT test.

REAGENTS

- **Factor VIII deficient Plasma (Cat. No.: 110500031)**: Factor VIII immune-depleted plasma with Factor VIII activity < 1% & other coagulation factors activity in the normal range.
- **Factor IX deficient Plasma (Cat. No.: 110510031)**: Factor IX immune-depleted plasma with Factor IX activity < 1% & other coagulation factors activity in the normal range.
- **Factor XI deficient Plasma (Cat. No.: 110520031)**: Factor XI immune-depleted plasma with Factor XI activity < 1% & other coagulation factors activity in the normal range.
- **Factor XII deficient Plasma (Cat. No.: 110530031):** Factor XII immune-depleted plasma with Factor XII activity < 1% & other coagulation factors activity in the normal range.

Available as individual factor deficient plasmas with respective cat nos. in 3 x 1 ml packs.

REAGENT STORAGE AND STABILITY

Store the reagents at 2-8C. DO NOT FREEZE.
The shelf life of the unopened factor deficient plasma vials is as per the expiry date mentioned on the respective vial label.
The reconstituted factor deficient plasma is stable for 8 hours at 2-8^{0}C or 1 month at -20^{0}C if rapidly frozen. Frozen plasma should be rapidly thawed at 37^{0}C. Thawed material should be discarded after use and should not be refrozen.

PRINCIPLE

The activity of the factor of interest is determined *in-vitro* through the use of APTT test. Patient plasma is diluted, mixed with the respective factor deficient plasma and the APTT test is performed. The result is interpolated from the calibration curve prepared with dilutions of reference plasma mixed with the respective factor deficient plasma. Correction of the clotting time of the deficient plasma is proportional to the activity of that factor in the patient plasma.

NOTE

In-vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
The reagents that are derived from human source have been tested for HBsAg and Anti-HIV antibodies & Anti-HCV antibodies and found to be non- reactive. However handle the material as if infectious.
The package insert is common for factor VIII, IX, XI & XII deficient plasmas.

SAMPLE COLLECTION AND PREPARATION

No special preparation of the patient is required prior to sample collection by approved techniques. Withdraw blood without undue venous stasis and without frothing into a plastic syringe fitted with short needle of 19 to 20 SWG. The vein puncture must be a clean one and, if there is any difficulty, take a new syringe and needle and try another vein. Transfer the blood to the tubes, after detaching needle from the syringe.
Mix exactly nine parts of freshly collected blood with one part of Tri-sodium citrate (0.11mol/l or 3.2%) or PROFACT (Cat. No.: 10660020) available from TULIP.
Centrifuge immediately for 15 minutes at 1500 g and transfer the plasma into a clean test tube. Plasma must be tested within three hours of blood collection.

ADDITIONAL MATERIAL REQUIRED

Owren's buffer, 12 x 75mm test tubes, pipettes and disposable pipette tips, stopwatch, water bath or heating block at 37^{0}C, reference plasma or Plasmatrol R from Tulip (Cat No.: 11043061), bi-distilled water, APTT reagent, $CaCl_2$.

REAGENT PREPARATION

Reconstitute the factor deficient plasma with the stated amount (refer vial label) of bi-distilled water. Avoid using water containing preservatives. Recap the vial and allow to stand until the hydration is complete (5-7 minutes). Mix by gentle swirling avoiding froth formation. Do not shake. Allow to stand and equilibrate for another 15 minutes before use.

TEST PROCEDURE

Prepare APTT reagent, $CaCl_2$, Owren's buffer and reference plasma according to manufacturer instructions.

Preparation of standard curve

Prepare serial dilutions of reference plasma with Owren's buffer as follows:

Tube No.	1	2	3	4	5
Vol. of reference plasma	0.2 ml	1 ml	1 ml	1 ml	1 ml
Vol. of owren's buffer	1.8 ml	1 ml	1 ml	1 ml	1 ml
Dilution	1:10	1:20	1:40	1:80	1:160
% factor Activity	100	50	25	12.5	6.25

Manual Method

(1) Take 0.1 ml of diluted reference plasma (Tube No.1 - 100%) in a test tube and add 0.1 ml of reconstituted factor deficient plasma of interest. Mix well and incubate for two minutes at 37°C. (2) Add 0.1 ml of prewarmed APTT reagent (LIQUICELIN – E, Cat. No.: 10630003/10630123). Incubate at 37°C for the recommended reagent activation time. (3) Add 0.1 ml of prewarmed $CaCl_2$ (0.025 mol/l, also available from Tulip as Calcium Chloride Cat No.: 10633010) and start the stop watch simultaneously. Shake the tube gently too mix contents. (4) Gently tilt the tube back and forth and stop the stopwatch as soon as the first fibrin strand is visible and the gel/clot formation begins. Record the time in seconds. (5) Repeat steps no 1-4 for all reference plasma dilutions (tube No. 2-6). (6) Test each reference plasma dilution in duplicates and use the mean value for plotting the standard curve. (7) Plot a standard curve of clotting time in seconds against % factor activity on a log-log graph paper (provided with the factor deficient plasma). (8) If a coagulation instrument is being used to perform the test the instrument manufacturer's instructions must be strictly followed.

Test procedure for Specimen (Patient plasma)

(1) Dilute the patient plasma 1:10 with Owren's buffer. (2) Follow steps 1-4 from the above mentioned test procedure replacing reference plasma dilution with diluted patient plasma. (3) Test the patient plasma in duplicate. Determine the mean clotting time.

CALCULATION OF RESULT

The activity of Factor of interest in the patient plasma can be interpolated form the respective standard curve using the mean clotting time of patient plasma.

Note:

100 % activity corresponds to the Activity of the respective factor present in reference plasma. For eg. If the FVIII activity mentioned on the reference plasma is X units, then X corresponds to 100 % activity in the standard curve).

REMARKS

(1) Incorrect Mixture of blood and tri-sodium citrate, insufficient prewarming of plasma and reagent, contaminated reagents and glasswares are potential source of errors. (2) Temperatures of all equipment must be calibrated regularly. (3) Glasswares and cuvettes used for testing must be scrupulously clean and free from even traces of acids/alkalies or detergents. (4) Hemolysed samples must not be used for testing. (5) Photoptical clot detection systems may have difficulty in determining clotting time of lipemic or icteric samples. Such samples must be tested with manual methods ar alternate clot detection methods. (6) Instrument, reagents method and user technique can affect the test results. Tulip factor deficient plasmas are subject to the limitations of the assay system used (instruments and reagents). (7) Deficiencies of one or more coagulation factors, whether congenital or acquired, can have differing clinical consequences. Therefore results must be interpreted in conjunction with clinical history of patients. (8) The validity of calibration curve must be checked periodically with known controls. Each laboratory must establish and maintain its own quality control ranges for each particular instrument – reagent system.

REFERENCE VALUES

The normal level for coagulation factors vary depending on the factor. The reference values are as mentioned below are for guidelines only: (a) Factor VIII - 50-150%, (b) Factor IX - 70-120%, (c) Factor XI - 60-120%, (d) Factor XII - 60-150%. Laboratories must determine their own normal values.

WARRANTY

The product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY

(1) Dacie and Lewis, Practical Hematology, Ninth edition. (2) Williamson MA,Synder LM,Wallach's interpretation of diagnostic tests. 9th ed. wolters Kluwer/Lippincott Williams & Wilkins Health: Philadelphia,2011. (3) Nicoll Diana MSJ, Pignone Michael, Lu Chuani Mark. Pocket Guide to Diagnostic Tests,5e ed:http://www.accessmedicine.com/pocket Diagnostic.aspx (4) NCCLS guideline H21-A3, Vol. 18, No. 20. (5) Data on file Tulip Diagnostics (P) Ltd.

Manufactured by:

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