**Factor VIII Deficient Plasma**

Immuno-depleted plasma for the determination of Factor VIII activity in citrated human plasma

<table>
<thead>
<tr>
<th>REF</th>
<th>R1 6 x 1 mL</th>
</tr>
</thead>
</table>

**TECHNICAL SUPPORT AND ORDERS**

Tel: (33) 03 23 25 15 50  
Fax: (33) 03 23 25 265

**CLINICAL SIGNIFICANCE (1) (5) (7) (8) (10) (11)**

This Reagent is used for the determination of the activity of Factor VIII (F.VIII) in human plasma by any method of measuring the clotting time on coagulometer or automated analyzer of haemostasis.

Factor VIII (antihemophilic factor A) is a glycoprotein present in the liver, spleen, kidneys and lymphocytes. It circulates in the plasma in the form of a non-covalent complex with Von Willebrand factor. The F.VIII is activated by Thrombin and F.Xa; the F.VII accelerates the activation of the F.X by the F.Ixa in the presence of phospholipids and Ca++. There are pathological changes in the F.VIII in the following cases:

- Haemophilia A: The seriousness of haemophilia is assessed on the basis of the concentration of F.VIII:
  - Severe haemophilia: < 0.1% (0.01 IU/mL)
  - Moderate haemophilia: 1-5% (0.01 - 0.05 IU/mL)
  - Hemophilia attenuated: 5-40% (0.05 - 0.40 IU/mL)

- Von Willebrand disease:
  - More or less pronounced decrease in the rate of F.VIII
  - The elevation of the F.VIII rate is a risk factor for thrombosis, including venous thrombosis. This elevation is observed in case of thrombocytopenic complications, coronary atherosclerosis, renal failure, diabetes, inflammatory syndrome...
  - There is a decrease in F.VIII in the presence of inhibitor of F.VIII

**PRINCIPLE (1)**

The assay consists in the measurement of the clotting time, in the presence of cephaline and activator, of a system in which all the factors are present in excess except of factor VIII which is derived from the sample being tested.

This determination may be realised with following BIOLABO reagent's:

- Human freeze dried citrated plasma from which Factor VIII has been removed by selective immune-adsorption.

**REAGENTS**

- Vial R1: Factor VIII Deficient Plasma

Human freeze dried citrated plasma from which Factor VIII has been removed by selective immune-adsorption.

**REAGENTS PREPARATION**

- Open the vial carefully and add exactly the volume of demineralised water stated on the label (usually 1 mL).
- Recap and let stand for 10 to 20 minutes at room temperature.
- Gently invert the vial several times to ensure homogeneity before use (avoid the formation of foam).

**SAFETY CAUTIONS**

Biolabo reagents are designated for professional, in vitro diagnostic use.

- Verify the integrity of the contents before use.
- Use adequate protections (overall, gloves, glasses).
- Do not pipette by mouth.
- In case of contact with skin and eyes, thoroughly wash affected areas with plenty of water and seek medical advice.
- Material Safety Data Sheet is available upon request.
- Waste disposal: Respect legislation in force in the country.

**STABILITY AND STORAGE**

Lyophilisate, unopened and stored at 2-8°C or –20°C: stable until expiry date stated in the label of the kit when stored and used as described in the insert.

- Once reconstituted: stable for 4 hours at room temperature.

Don't use reconstituted plasma after expiry date stated on the label of the Kit.

**SPECIMEN COLLECTION AND HANDLING (9) (12)**

Mix freshly drawn blood (9 Volumes) with buffered tri-sodium citrate solution 3.2% (1 Volume).

- Centrifuge for 10 min at 3000 g and extract supernatant.
- Storage in plastic tube:
  - 4h at 20-25°C
  - 15 days at -20°C, 1 month at -80°C (Thaw frozen plasmas at 37°C until complete thawing)

**INTERFERENCES (6)**

Heparins and Thrombin inhibitors (e.i. hirudin, argatroban ...) present in the specimen to be tested may lead to under-estimation of the factor VIII activity in the specimen.

The presence of Lupus anticoagulants may lead to an under-estimation of Factor VIII activity in the specimen.

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

**MATERIAL REQUIRED BUT NOT PROVIDED**

1. Basic medical analysis laboratory equipment.
2. Reagents as indicated in § PRINCIPLE
3. REF 13565 : CaCl Solution 0.025 M
4. REF 13970 : BIO-CAL Reference Plasma
5. REF 13971 and 13972 : COATROL 1 and COATROL 2
6. Paper graph

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Use by</th>
<th>In vitro diagnostic</th>
<th>Temperature limitation</th>
<th>Catalogue number</th>
<th>See insert</th>
<th>Batch number</th>
<th>Store away from light</th>
<th>sufficient for</th>
<th>dilute with</th>
</tr>
</thead>
</table>

Made in France  
Latest revision : www.biolabo.fr  
Revision : 26/03/2015
CALIBRATION
- **REF 13970**: BIO-CAL, reference plasma for calibration of coagulation tests.

The calibration frequency depends on proper instrument functions and on the preservation of reagent. It is recommended to calibrate in the following cases:
1. When using a new batch of reagent.
2. After maintenance operations on the instrument.
3. When control values are out of range, even after using a new vial of fresh plasma.

QUALITY CONTROL
- **REF 13971**: COATROL 1 Level 1
- **REF 13972**: COATROL 2 Level 2
- Assayed control sera referring to the same method.
- External quality control program.

It is recommended to control in the following cases:
- At least once a run.
- At least once within 24 hours.
- When changing vial of reagent.
- After maintenance operations on the instrument.

If control is out of range, apply following actions:
1. Repeat the test with the same control.
2. If control is still out of range, prepare fresh control plasma and repeat the test.
3. If control is still out of range, use a new vial of calibrator or a fresh calibrator and repeat the test.
4. If control is still out of range, calibrate with a new vial of reagent.
5. If control is still out of range, please contact BIOLABO technical support or your local Agent.

EXPECTED VALUES

**Plasma (adult)**

Usually 60-150%

Each laboratory should establish its own normal ranges for the population that it serves. Many factors may lead to increased F.VIII: C level:
- Use of birth control pills, pregnancy
- AVK and corticoid therapies
- Physical exercise, stress...

PERFORMANCES CHARACTERISTICS

Performances studies were realised on Thrombolyzer:

<table>
<thead>
<tr>
<th>Intra-Assay N = 20</th>
<th>level 1</th>
<th>level 2</th>
<th>Inter-Assay N = 20</th>
<th>level 1</th>
<th>level 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean %</td>
<td>127</td>
<td>52</td>
<td>Mean %</td>
<td>100</td>
<td>43</td>
</tr>
<tr>
<td>S.D. %</td>
<td>6.6</td>
<td>2.9</td>
<td>S.D. %</td>
<td>9.2</td>
<td>2.7</td>
</tr>
<tr>
<td>C.V. %</td>
<td>5.2</td>
<td>5.6</td>
<td>C.V. %</td>
<td>9.2</td>
<td>6.3</td>
</tr>
<tr>
<td>Criteria</td>
<td>&lt; 8%</td>
<td>&lt; 6%</td>
<td>Criteria</td>
<td>&lt; 10%</td>
<td>&lt; 10%</td>
</tr>
</tbody>
</table>

Detection limit:

When the plasma to be tested is diluted (1+9), detection limit of this method is 0.5 % of Factor VIII.

LINEARITY

The reaction is linear up to 150 % of factor VIII when the plasma to be tested is diluted 1/10

PROCEDURE

Manual Procedure
7. Prepare dilutions of **REF 13970**: BIO-CAL Reference Plasma in Owren Koller buffer as follows:

<table>
<thead>
<tr>
<th>Dilutions 1/d</th>
<th>1/10</th>
<th>1/20</th>
<th>1/40</th>
<th>1/80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference Plasma (mL)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Owren Koller Buffer (mL)</td>
<td>0.9</td>
<td>1.9</td>
<td>3.9</td>
<td>8.0</td>
</tr>
</tbody>
</table>

Pre-incubate Calcium chloride 0.025 M **REF 13965** in water bath at 37°C, and then dispense in test tubes as indicated in the table.

Determine clotting time for each dilution of reference plasma:

| Reference plasma diluted 1/10 to 1/80 | 0.1 mL |
| Deficient plasma | 0.1 mL |
| TCA Reagent : | 0.1 mL |
| Incubate 3 minutes at 37°C. |
| CaCl2 0.025 M : | 0.1 mL |

Simultaneously start a timer.

Gently tilt back and forth near horizontal position, until a solid gel clot appears. Operate under sufficient lighting.

Do the same for controls and specimens to be tested (pre-diluted 1/10 in Owren Koller buffer):

| Controls and specimens (diluted 1/10) | 0.1 mL |
| Deficient Plasma | 0.1 mL |
| TCA Reagent : | 0.1 mL |
| Incubate 3 minutes at 37°C. |
| CaCl2 0.025 M : | 0.1 mL |
| Thromboplastin (preincubated at 37°C): | 0.2 mL |

Automated Procedure

Use in preference reagent BIO SIL (TICA Silica Method). Refer to the instrument manufacturer’s instructions.

Note:

Application procedures on clinical chemistry analyzers are available upon request.

CALCULATION

Calculate the result as follows:
Plot a Standard Curve using results obtained with dilutions of reference plasma.

Concentration % = f (Clotting Time)

Read the concentration (%) of controls and samples reporting clotting time on the graph.

REFERENCES

(1) SOUILLER J.P., LARRIEU M-J.; Sang, 24, 3, 205-215, 1953
(2) CAEN J., LARRIEU M-J., SAMAMA M.; Paris, L’Exp. Scient., 181, 1975