Diagnostic Reagents Limited

UK CA

Bell and Alton Platelet Substitute

Catalogue Number: BAPS040

For *in vitro* diagnostic use only.

Intended Use
Diagen Bell and Alton Platelet Substitute is designed for use as the Phospholipid component in the Activated Partial Thromboplastin Time (APTT) see below.

Summary and Principle
Bell and Alton Platelet substitute is a freeze-dried buffered emulsion of chloroform extract of brain [1]. This material is manufactured under rigorously controlled conditions to ensure a homogeneous emulsion and standardised at optimum dilution. Below are listed some of the possible uses:

Two Stage Factor VIII Assay [2]
Dilute 1 in 2 in Saline.

Factor X Assay [2]
Russell's Viper Venom is diluted in the solution to a final concentration of 1 in 100,000 to 1 in 150,000.

Heparin Assay [2]
For therapeutic levels of heparin, the solution is used undiluted in the APTT test and in the anti Xa Heparin Assay.

Lupus Anticoagulant
For prolonged clotting times the following method is useful.
Reconstitute 1 vial of Bell and Alton Platelet Substitute in 2.5 mL of distilled H_2O.

Reconstitute a second vial of Bell and Alton Platelet Substitute (PS) at normal strength in 5.0 mL H_2O and from this make a one in two dilution in saline.

The following pattern of clotting times is obtained in the APTT.

	x 2 strength	x 1 strength	x 1/2 strength
Normal Plasma	51	44	47
Factor Deficiency	85	71	73
Ratio Abn/Norm	1.66	1.61	1.55
Lupus Inhibitor	70	85	100
Ratio Abn/Norm	1.37	1.93	2.12

Factor deficiency gives little or no change in ratio. Lupus Inhibitor gives an increase at half concentration and a decrease at twice concentration of PS.

Reagent
Bell and Alton Platelet Substitute 6 vials
A lyophilised, buffered, emulsion of chloroform extract of brain. For reconstitution, remove both the cap and bung and then add 5.0 mL of distilled water and leave for a minimum of 10 minutes to obtain complete solution.

Collection of Blood Samples
Blood (9 parts) is collected into 1 part of 3.2% trisodium citrate and the plasma obtained by centrifugation at 2500 g for 15 minutes. The plasma should be stored in stoppered tubes. The use of 3.2% citrate containing 5% HEPES buffer improves the stability of both fresh and deep-frozen plasma.

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Procedure
Materials Provided
Material needed for Activated Partial Thromboplastin Time (APTT) tests are shown below:
Cat. No.
BAPS040 – Bell and Alton Platelet Substitute (6 x 5 mL vials).

Materials and equipment required, but not provided:
1. General routine laboratory coagulation equipment.
2. Light Kaolin (BDH) 2.5 mg/mL in saline, or Imidazole buffer.
3. Reaction cups or test tubes (12 x 75 mm).
4. Pipettes delivering: 100 µL, 200 µL & 5.0 mL.
5. Distilled water.
6. 25mM CaCl2 solution (CTMM542).
7. Diagen Control plasmas: IQCN130 - Normal.
   IQCM140 - Abnormal 1 (Mild).
   IQCS150 - Abnormal 2 (Severe).

Manual Technique
1. To a clotting tube, add 100 µL of Kaolin suspension and 100 µL platelet substitute.
2. Incubate at 37°C for 1-2 minutes to reach temperature.
3. Add 100 µL of plasma and tilt the tube gently, at intervals, for exactly **2 minutes**.
4. 100 µL of $CaCl_2$ (pre warmed to 37°C) is added and the stopwatch started.
5. The tube is tilted at regular intervals (returning to the water bath between tilting) and the time for clot formation is recorded.

Notes:
1) Tubes should be new and scrupulously clean.
2) Water bath temperature should be 37°C.
3) Diagen Normal and Abnormal Control Plasma's have been standardised on an activation time of 2 minutes.

Activation Time with Kaolin
The two-minute activation time is optimum. Although longer times of 6 and 10 minutes have been used there is no evidence that the resulting minimum clotting times give greater sensitivity or precision. Fletcher factor deficiency may certainly be missed and there is some evidence that other deficiency states may be missed with prolonged activation with kaolin.

Order of Addition of Reagents
In the manual method, if the plasma is added first there is the risk of contamination by splash over into both Kaolin suspension and Platelet substitute. It is therefore recommended fresh pipettes or tips are used for each addition of the reagents.

Reporting Results
The normal range for APTT in our hands is 36 to 49 seconds but this may vary slightly in individual laboratories according to technique.

Quality Control
All laboratories should have in place a quality control system that uses normal and abnormal controls to evaluate reagent, instrument and user performance. Both normal and abnormal controls should be used prior to performing a test series to validate the patient results. We recommend Diagen control plasmas for this purpose, as these have been specifically manufactured for our reagents. If the controls do not perform within their reference ranges, a review of the instrument or test system is recommended.

Normal Range
The normal range should be determined locally in each laboratory, especially where photo optical or mechanical instruments are used. This may be obtained cumulatively by testing individual fresh normal plasma samples at the same time keeping the method "in control" by the use of a freeze-dried plasma control as a test of reagent, water bath temperature, calcium chloride etc. The normal range quoted is that obtained using the manual method.

Limitations
APTT values will differ between laboratories due to the many variables that can affect clotting times, particularly the use of coagulometers. All laboratories should therefore establish a quality control system that uses well-defined performance standards for control plasmas. The use of icteric, lipemic, or haemolyzed samples should be avoided as this may cause possible interference, especially when using photo-optical instruments.

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If the patient is on therapeutic drugs, it may influence interpretation of APTT test results. By obtaining accurate patient history and noting specific drug therapies we can better understand the potential impact on laboratory test results. The presence of heparin as a contaminant must always be considered in a sample where an abnormal result is obtained.

It should be remembered that a normal result obtained with this test might not exclude borderline or minor Factor deficiencies.

Storage and stability

The unopened freeze-dried vials are **best stored deep frozen** but may be stored for up to 3 years at 4° or below without deterioration. Once reconstituted the contents of the vial are then stable for up to 7 days when held at 2 - 8°C. The reconstituted product may also be deep frozen and then thawed once without loss of activity.

Packaging

6 x 5mL

References

1) Bell. W.N. and Alton. H.G. (1954) Nature (Lond.) 174.880.
2) Denson, K.W.E. In Human Blood Coagulation Thrombosis and Haemostasis. (Ed. R. Biggs). Blackwell Scientific Publications Oxford 1976.

Key guide to symbols

REF	Manufacturers catalogue number.		Consult instructions for use.
LOT	Manufacturers batch number.	Recon.	Requires reconstitution.
IVD	For *in vitro* diagnostic use only.		Product expiry date.
	Biological risks.	+4°C -20°C	Store at 4°C or below. Best stored deep frozen.

Manufacturer.

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