Intrinsin APTT Reagents
ILS & ILR

RESEARCH USE ONLY (RUO)

INTENDED USE
The INTRINSIN reagents are for carrying out Activated Partial Thromboplastin Time (APTT) clotting tests. They come in lupus inhibitor-sensitive (LS or "screen") and resistant (LR or "confirm") versions for the simple identification of lupus anticoagulants in test plasmas.

INTRODUCTION
The APTT is a widely accepted screening test for abnormalities in the intrinsic pathway of coagulation (1). It is also used for monitoring heparin, detecting inhibitors (including lupus anticoagulants) and for factor assays (2). A normal test result is dependent on the proper function of all the clotting factors and cofactors in the intrinsic and common pathways of the clotting mechanism. These include all known clotting factors except factor VII. Significantly decreased levels of any of these factors results in prolonged APTT results because the penultimate product thrombin, forms more slowly than usual.

Agents which interfere with any part of this mechanism may also prolong an APTT. Thus the APTT test can be used to monitor anticoagulants such as heparin, to assess resistance to activated protein C and detect antibodies against clotting factors in mixing tests. It can also be used to detect lupus inhibitors which interfere with the low, rate-limiting level of phospholipid in the Intrinsin LS reagent. Intrinsin LR contains excess phospholipid and is therefore more resistant to these agents. Thus the difference between ILS and ILR APTT results is a simple test for lupus inhibitors (eg, 3). These tests can be run on neat patient plasmas or their mixes with normal plasma to reduce the effect of factor deficiencies (4).

APTT tests are carried out by pre-incubating test plasma with APTT reagent 1:1 for 3-5 minutes at 37°C, during which time contact factors are activated. Then the mix is recalcified (2:1) and the time to a clotting endpoint is determined. Most APTT reagents are adjusted to give results on normal plasmas in the range 25-35 sec.

CONTENTS OF PRODUCT

Components in each test kit
INTRINSIN LS or LR, 5 x 10 ml vials. Ingredients include colloidal silicate contact activator, phospholipids, HEPES buffer, stabilisers and sodium azide (<0.1%).

Other materials required for test
Clot timing instrumentation or if for manual clotting tests, small test tubes, 37°C water bath, micropipette dispensers with tips and stopwatch. Calcium Chloride (0.025M) solution. QC plasmas.

Storage conditions

The liquid INTRINSIN reagents can be stored at 2-8°C for up to 2 years. Do not use the kits beyond the expiry dates shown on packaging. For longer term storage it is acceptable to freeze INTRINSIN reagents below -30°C for up to 2 years. Product deterioration may be indicated by test results on appropriate quality control plasmas outside the accepted laboratory range.

PRECAUTIONS
Handle all samples as if potentially infectious and use appropriate precautions. Use disposable gloves and contact a doctor immediately if a needle stick injury or unintentional blood person contact occurs.

Clean up any spillages with bleach or 70% ethanol and incinerate, sterilise or autoclave waste materials.
Plasmas containing heparin or DOACs may give10-20% longer APTT results with Intrinsin LR than with Intrinsin LS. Thus a weak LA might be missed if co-existing with such agents at higher levels. To avoid false negative LA results with heparin we suggest using heparin resistant recalcifying solution (HRRS) in the APTT. (Data on file at Haematex)

Borderline results should be considered in line with clinical circumstances and follow up tests requested if necessary. Results from samples which are partially haemolysed, icteric or lipaemic should be interpreted with caution. Partially filled blood collection tubes or samples containing visible clots may not be suitable.

Among the direct oral anticoagulant agents (DOACs) the direct thrombin inhibitor dabigatran has more prolonging effect than the FXa inhibitors rivaroxaban, apixaban or edoxaban at therapeutic levels.

SPECIMEN COLLECTION AND PREPARATION

Blood collection
Blood should be collected by clean venipuncture into one ninth its volume of 0.109M sodium citrate (3.2% tri-sodium citrate dihydrate). Guidelines from the CLSI should be followed (5).

Processing and storage
Citrated blood samples should be centrifuged initially at 1500g for 15min at 20°C. Supernatant plasma can then be tested fresh within 4 hours. If plasma is to be frozen for storage, it should be removed to a second tube and re-centrifuged for 10 minutes at a higher g force than initially (ie >2500g) for more complete removal of platelets and larger microparticles.

INSTRUCTIONS FOR USE

Procedural notes and precautions
Carry out tests in duplicate unless the instrument used routinely provides c.v below 5% with multiple tests on pooled abnormal plasma.

Quality control
Normal and abnormal QC plasmas must be included in each batch of 40-50 test samples. These results must be monitored on a regular basis and when values exceed mean +/- 2SD corrective action must be taken.

Test procedure
1. Prewarm appropriate volumes of APTT reagent and 0.025M calcium chloride at 37°C.
2. Dispense 0.1ml of test plasma and 0.1ml of pre-warmed APTT reagent into a test tube or reaction cuvette, mix and incubate for 3-5 min at 37°C.
3. Add 0.1ml of recalcifying solution and time to a clotting endpoint by tilt tube, instrument mechanical or photoelectric sensing.
4. Record the (mean) APTT clotting time.
5. Smaller volumes can be used but must be kept in the same proportions as indicated.
6. Tests can be carried out on mixes of patient: normal plasma in varying proportions to detect inhibitors, though 1:1 mixes are most widely used in screening.

PERFORMANCE CHARACTERISTICS

Setting reference ranges
A reference interval for normal individuals should be established using plasmas from 20 healthy individuals matched for age and sex with the patient population. This range should be calculated as mean +/-2SD.
Therapeutic range for unfractionated heparin should be established using plasmas from 20 patients being treated with heparin and monitored with an anti Xa method. APTTs corresponding to 0.2 and 0.4 u/ml anti Xa activity should be assigned as lower and upper treatment limits (2, 5).

Interpretation of results
APTT results longer than the upper limit of normal indicate a clotting abnormality. This may be due to an individual factor deficiency below 40% or a number of less severe factor deficiencies. Deficiencies in factors VIII, XI, and XI are most likely to be associated with a clinical bleeding problem and should then be assayed.

Plasmas which contain lupus anticoagulant (LA) usually give a more prolonged APTT with the LS reagent than with the LR reagent (3, 4). The abnormal result with LS reagent should also show less correction on mixing with pooled normal plasma (PNP) if due to LA than if due to other causes.

According to the latest guidelines of the ISTH (6), the Screen cutoff should be the 99th percentile which would require 120 healthy donors, though the CLSI recommends 3SD and only 40 donors (7).

The table below shows expectations of APTT outcomes with normal, factor deficient and lupus inhibitor test plasmas after using LS and LR APTT reagents on neat and 1:1 mixes with normal plasma.

Abnormality	APTT - LS		APTT - LR	
	neat	1:1 mix	neat	1:1 mix
Nil (normal)	N	N	N	N
Factor deficiency	Abn	N	Abn	N
LA	Abn	Abn	N	N
LA + defect	Abn	Abn	Abn	N

Expression of results for lupus inhibitors
Clotting time results with each APTT reagent can be expressed as a ratio relative to the mean normal result. Then this normalised ratio with APTT-LS can be divided by the similarly-derived normalised ratio with APTT-LR to yield an overall ratio (4). Most labs use a final cutoff near overall ratio 1.3 and 1.2 with mixes.

INDEMNITY NOTICE
Follow procedures and refer to precautions that may affect the stated or implied claims and performance of this product. Haematex Research Pty Ltd and its agents or distributors are not liable for damages.

REFERENCES

1. Proctor RR, Rapaport SI. The partial thromboplastin time with kaolin. A simple screening test for first stage plasma clotting factor deficiencies. Am J Clin Pathol.1961; 35; 212.
2. CLSI. One-stage prothrombin time (PT) test and activated partial thromboplastin time (APTT) test; Approved guideline - Second edition. CLSI document H47-A2. Wayne, PA, USA: Clinical and Laboratory Standards Institute; 2008.
3. Brancaccio V, Ames PR, Glynn J, et al. A rapid screen for lupus anticoagulant with good discrimination from oral anticoagulants, congenital factor deficiency and heparin is provided by a comparing a sensitive and insensitive APTT reagent. Blood Coag. Fibrinolys.1997; 8; 155-60.
4. Jacobsen EM, Barna-Cler L, Taylor JM, et al. The lupus ratio test - an interlaboratory study on the detection of Lupus Anticoagulants by an APTT-based, integrated and semi-quantitative test. Thromb. Haemost. 2000; 83; 704-8.
5. CLSI. Collection, transport and processing of blood specimens for testing plasma-based coagulation assays and molecular hemostasis assays: Approved guideline - Fifth edition. CLSI document H21-A5, Wayne, PA, USA: Clinical and Laboratory Standards Institute; 2008.
5. Smyth MA, Koerber JM, Westley SJ et al. Use of the activated partial thromboplastin time for heparin monitoring. Am J Clin Pathol. 2001;115; 148-155.
6. Pengo V, Tripodi A, Reber G, et al. Update of the guidelines for lupus anticoagulant detection. Thromb Haemost. 2009;7;1737-40.
7. CLSI. Laboratory testing for the lupus anticoagulant; Approved guidelines. CLSI document H60-A, Wayne, PA, USA: Clinical and Laboratory Standards Institute; 2014.

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