DIAGON
Solutions beyond imagination

INSTRUCTION FOR USE

Dia-DRVVT Confirm

Confirming test

CE

Cat. No.: 07405 Dia-DRVVT Confirm5 x 1 ml

Intended use

Lupus Anticoagulants (LA) are antibodies of the IgG and IgM type which are directed against a variety of anionic phospholipids. The presence of LA in plasma is increasingly associated with a variety of haemostatic problems such as recurrent foetal loss, thrombocytopenia, unexplained thrombosis and neurological disorders. LA prolongs phospholipid dependant *in vitro* clotting assays such as the activated partial thromboplastin time (aPTT). The Diagon Dia-DRVVT Confirm kit is intended for the qualitative confirmation of LA in human plasma. The reagent is designed to be used in conjunction with the Dia-DRVVT Screen Kit (Cat.No.: 07310) to discriminate between LA, factor deficiencies (II, V or X) or other inhibitors. If the clot time of the patient samples with the DRVVT Screen procedure are greater than 3 standard deviations above the mean of the normal range and are not corrected by mixing studies, a lupus anticoagulant may be present. Under these circumstances, samples should be re-tested using the Dia-DRVVT Confirm Reagent.

Warnings

The reagents contained in this kit are for *in vitro* diagnostic use only under professional's leadership.

Precaution

The reagents contained in this kit are for *in vitro* diagnostic use only - **DO NOT INGEST!** Wear gloves when handling all kit components.

Waste Material Treatement

Refer to the product safety data sheets for risk and safety phrases and disposal.

Materials provided

Component	Content	Description	Preparation
Dia-DRVVT Confirm	5 x 1 mL	Each vial contains a proprietary mixture of Russell's Viper Venom co-lyophilised with calcium chloride and concentrated phospholipid.	Reconstitute each vial with 1 mL of distilled / deionised water. Allow to stand for 10 minutes and mix well before use. **Do not shake.**

Each kit contains Instructions For Use.

Materials required but not provided

Cat.No.: 07310 Dia-DRVVT Screen

Cat.No.: 09305 Dia-CONT Spec N

Cat.No.: 09501 Dia-CONT LA P

Storage and stability

Unopened vials are stable until the given expiry date when stored under the related conditions.

Dia-DRVVT Confirm	Reconstituted vials are stable for 24 hours at $^{+}$15 –$^{+}$30°C, 5 days at $^{+}$2 –$^{+}$8°C or 2 weeks at -20°C. The reagent should be frozen in plastic test tubes and thawed at $^{+}$37°C before use.

Sample Collection and Preparation

Plastic or siliconised glass should be used throughout. Blood (9 parts) should be collected into 3.2% sodium citrate anticoagulant (1 part). Separate plasma after centrifugation at 1500 x g for 15 minutes. Plasma should be kept at $^{+}$2 – $^{+}$8°C or $^{+}$18 –$^{+}$24°C. Testing should be completed within 4 hours of sample collection, or plasma can be stored frozen at -20°C for 2 weeks or -70°C for 6 months. Thaw quickly at $^{+}$37°C prior to testing. Do not keep at $^{+}$37°C for more than 5 minutes[1]. If freezing, double centrifugation of the sample is recommended to ensure that the sample is platelet poor. Transfer the plasma following the initial centrifugation to a non-activating plastic tube using a plastic pipette, then re-centrifuge the plasma for an additional 10 minutes at a higher speed (>2500 x g). When aliquoting to a secondary tube, take care to not include the residual platelets that may have collected at the bottom of the centrifuge tube[2].

Procedure

A. Manual method

1. Pre-warm sufficient reconstituted reagent to $^{+}$37°C.
2. Pipette 0.2 mL of patient or control plasma into a reaction tube. Incubate at $^{+}$37°C for 2 minutes.
3. Add 0.2 mL of pre-warmed DRVVT Confirm reagent and start a timer.
4. Measure the clot formation time to the nearest 0.1 seconds.
5. Calculate the normalised ‘DRVVT Confirm’ ratio as:

Patient DRVVT Confirm Clot Time	/	Mean Normal DRVVT Confirm Clot Time

B. Automated method

Refer to the appropriate instrument operator manual for detailed instructions or contact Diagon Ltd. for instrument specific application guides.

Interpretation of results

Results are best expressed as a normalised ratio relative to the mean normal clot time obtained by each laboratory.

It is recommended that like for like sample types are used when calculating a normalised ratio. Both Dia-DRVVT Screen and Dia-DRVVT Confirm results can be ‘normalised’ in this way, reducing the effect of instrument variability and potentially improving discrimination between weak positive LA and normal samples. Results of the mixing tests can be treated in the same way.

- If patient samples with suspected LA (by DRVVT Screen testing) have a DRVVT Confirm clot time less than 3 SDs from the mean normal clot time for this kit, LA is strongly indicated. Mixing studies would also give normal clot times in this situation.
- Samples with an abnormal DRVVT Screen clot time and abnormal DRVVT Confirm clot time which are both normalised by mixing studies are most likely factor deficient (II, V or X). Samples with abnormal DRVVT Screen clot time and abnormal DRVVT Confirm clot time in which only the DRVVT Confirm clot time is

normalised in mixing studies suggests LA plus factor deficiency.

•Samples with abnormal DRVVT Screen clot time and abnormal DRVVT Confirm clot time which are not corrected by mixing studies indicate other inhibitors.
The results of DRVVT Screen and DRVVT Confirm testing when expressed as the 'normalised' ratio can also be used to indicate the level of LA present:

(DRVVT Screen Ratio)/(DRVVT Confirm Ratio)	> 2.0	Strong LA
(DRVVT Screen Ratio)/(DRVVT Confirm Ratio)	1.5 - 2.0	Moderate LA
(DRVVT Screen Ratio)/(DRVVT Confirm Ratio)	1.2 - 1.5	Weak LA

Limitations

Plasma deficiencies of Factors II, V or X may lead to abnormal results in neat plasma. Mixing studies should correct this. Plasma from patients with the following may give abnormal results when the plasma is tested neat, and these samples may not correct in mixing studies: heparin (>1 U/mL), oral anticoagulants, disseminated intravascular coagulation (DIC). Care must be taken to remove residual platelets from plasma by filtration or centrifugation, as platelet derived phospholipid can interfere with the test.

Quality control

Each laboratory should establish a quality control program. Normal and abnormal control plasmas should be tested prior to each batch of patient samples, to ensure satisfactory instrument and operator performance. If controls do not perform as expected, patient results should be considered invalid. Diagon Ltd. supplies the following controls available for use with this product:

Reference values

Reference values can vary between laboratories depending on the techniques and systems in use. For this reason each laboratory should establish its own reference ranges. The normal reference range (mean ± 3SDs) determined at Diagon Ltd. for the Dia-DRVVT Confirm test was 32.4 ± 6.0 seconds (range 26.4-38.4 seconds).

Performance characteristics

Each laboratory should establish its own performance data. Within run and between run CVs are expected to be <5%.

References

1. Clinical and Laboratory Standards Institute (2008) Collection, Transport and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Haemostasis Assays: Approved Guideline, 5th edn. CLSI: H21-A5
2. Pengo V *et al* (2009) Update of the guidelines for lupus anticoagulant detection, *J Thromb Haemost*, **7**: 1737-40
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Symbols			
IVD	In vitro diagnostics devices		Check in user manual
	Biohazard		Temperature range
	Manufacturer		Expiry date

LOT	LOT number	CE	CE conformity sign
REF	Catalogue number		