Lupus Anticoagulant diagnostic issues
APS is an autoimmune condition

Antiphospholipid antibodies (aPL) are:
- anticardiolipin antibodies (aCL antibodies) or,
- anti-β2GP1 antibodies (aβ2GPI antibodies) or,
- lupus anticoagulant

Vascular thrombosis
episodes of arterial/venous/small vessel thrombosis

Pregnancy morbidity
one or more unexplained fetal deaths, premature births, consecutive spontaneous abortions

No family history
Case Study 1

A 39 year old female complaining to the ER physician from persistently sore and swelling leg. She was subsequently admitted to the hospital and found to have a DVT with further documentation of PE.
## Case Study 1

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT</td>
<td>12.8 seconds</td>
<td>11 – 14 seconds</td>
</tr>
<tr>
<td>APTT</td>
<td>32.9 seconds</td>
<td>25 – 33 seconds</td>
</tr>
<tr>
<td>DRVV-S</td>
<td>40 seconds</td>
<td>30 – 41 seconds</td>
</tr>
<tr>
<td>DRVV-S ration</td>
<td>1.10</td>
<td>&lt; 1.2 No LA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥ 1.2 LA Suspected</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>236 mg/dL</td>
<td>170 – 410 mg/dL</td>
</tr>
<tr>
<td>Antithrombin</td>
<td>97%</td>
<td>80 – 127%</td>
</tr>
<tr>
<td>Protein S Activity</td>
<td>88%</td>
<td>57 – 105%</td>
</tr>
<tr>
<td>Protein C Activity</td>
<td>105%</td>
<td>69-133%</td>
</tr>
<tr>
<td>APCR</td>
<td>135 seconds</td>
<td>&gt; 120 sec No APRC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≤ 120 sec APRC</td>
</tr>
</tbody>
</table>
Choice of test - Screening step

- No single test sensitive to all LA
  - One screening test is not sufficient enough
- Two different tests based on different assay principles
- Increased risk of false-positive if more than two screening tests
  - More than 2 is not recommended
Choice of test - Screening step

The two recommended tests

- **dRVVT**
  - Widely used
  - Believed to be specific for LA for patients at high risk of thrombosis
  - Most robust in detecting LA (*EQA assessment*)

- **Sensitive aPTT**
  - Activator: silica
  - Low PL content
LA testing

Not recommended screening tests:

- **aPTT with**
  - Kaolin as activator
    - Insensitive to LA
    - Dedicated to Factor deficiencies
  - Ellagic acid as activator
    - Insensitive to LA

- **Dilute Prothrombin time**
  - High variability in thromboplastin reagents

- **Kaolin Clotting Time**
  - Poor reproducibility
  - Interfering with anticoagulant therapy

- **Ecarin/ Textarin based assays**
  - No standardized assays
  - Interfering with UFH therapy, FII and FV deficiencies
Confirmatory assays should be based on the method giving an abnormal screening assay.

There are available for each of the major screening test.

They may be achieved by addition of phospholipids, hexagonal phase phospholipids or platelet vesicles in the test system.
Confirmatory test

Same test principle than screening assay
- With increased Phospholipids concentration
- Bilayer or hexagonal phase PL should be used
- Freeze/thawed platelets not recommended “PNP”-(poor batch to batch consistency)
Stago LA screening and confirmatory tests

**Screening**
- APTT-based assays
  - PTT-LA
- Dilute Russel Viper Venom time (dRVVt)
  - STA® - Staclot® DRVV Screen

**Confirmation**
- Hexagonal phase phospholipid-based assay
  - Staclot® LA
- Dilute Russel Viper Venom confirm (dRVV Confirm)
  - STA® - Staclot® DRVV Confirm
PTT-LA contains a low phospholipid concentration with an optimal silica activator concentration

- LA-sensitized APTT reagent
The result is expressed as a clotting time:

- Comparison of the clotting time (CT) of the patient to the normal range (e.g. mean + 2 S.D.)

- If CT within range: LA not detected
- If CT prolonged > upper limit: presence of LA suspected, …
“PTT LA clearly showed the highest responsiveness among the APTT reagents. It is clear that the LA sensitivity of the APTT largely depends on the reagents used”

• J. Arnout and al, Lupus Anticoagulant testing in Europe (ECAT), Thromb Haemost 1999; 81, 929
Staclot® LA

Confirmatory test for PTT-LA

Cuvette 1
Low Phospholipid concentration

CT1

CaCl2 0.025M
PTT-LS
(Heparin Inhibitor)
Buffer
Patient plasma
25 µl

CT2

Compare the CT1 to the CT2: CT1 > CT2

LA

If CT1 - CT2 ≥ 8 seconds: LA presence confirmed

Cuvette 2
High Phospholipid concentration

CaCl2 0.025M
PTT-LS
(Heparin Inhibitor)
HPPE*
Patient plasma
25 µl

* Hexagonal Phase PhosphatidylEthanolamine
Staclot® LA

- Sensitive and specific to LA
  - Diagnose LA at low titre
  - Eliminates false positive

- Insensitive to heparin
  - Allows direct diagnosis of LA in heparinised patients

- Insensitive to factor deficiencies
  - Allows LA testing in warfarin patients, patients with specific factor deficiency or inhibitor

- Secure
  - Dedicated Controls
    - STA® Control LA 1+2 (negative and positive)
STA® Sta clot® DRVV Screen

Dilute Russell Viper Venom

\[ \text{STA® Sta clot®} \]

DRVV Screen

- RVV = Factor X activator
- PL: low phospholipid content in the reagent
- calcium, heparin inhibitor
The result is expressed as a Screen ratio:

- If Screen ratio < 1.2 : LA not detected
- Screen ratio ≥ 1.2 → presence of LA suspected, ...
STA® Staclot® DRVV Screen & Confirm

**STA® Staclot® DRVV Screen**

- Patient plasma + dilute PL + Venom + CaCl₂
  - **Clot** (time in seconds)
- RVV = Factor X activator
- PL: low phospholipid content in the reagent
- calcium, heparin inhibitor

**STA® Staclot® DRVV Confirm**

- Patient plasma + high level PL + Venom + CaCl₂
  - **Clot** (time in seconds)
- RVV = Factor X activator
- PL: high content of phospholipid in the reagent
- calcium, heparin inhibitor
**DRVV results Interpretation**

- **Screen ratio** = \( \frac{\text{CT Plasma to be tested}}{\text{Screen CT Reference Pool (Pool Norm)}} \) > 1.2

- **Confirm ratio** = \( \frac{\text{CT Plasma to be tested}}{\text{Confirm CT Reference Pool (Pool Norm)}} \)

- **Normalized Ratio** = \( \frac{\text{Screen ratio}}{\text{Confirm ratio}} \)

- Normalized ratio < 1.2 \( \Rightarrow \) Negative \( \Rightarrow \) inhibitor anti-factor?
- Normalized ratio \( \geq 1.2 \) \( \Rightarrow \) Positive \( \Rightarrow \) presence of LA

CT: clotting time
Case study 2

History: 5 year old female who presented a prolonged aPTT tested for a severe tonsillitis. Her meds included penicillin, acetaminophen and dilantin.
## Case Study 2

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT</td>
<td>12.0 Seconds</td>
<td>11 – 14 seconds</td>
</tr>
<tr>
<td>APTT</td>
<td>69.5 seconds</td>
<td>25 – 33 seconds</td>
</tr>
<tr>
<td>TT</td>
<td>16 seconds</td>
<td>&lt; 21 seconds</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>251 mg/dL</td>
<td>170 – 410 mg/dL</td>
</tr>
</tbody>
</table>
The result may be expressed by the calculation of an index of correction:

- **Rosner Index**

\[
\text{Index} = \frac{b - c}{a} \times 100
\]

- \( a \) = Clotting time (CT) of patient plasma
- \( b \) = CT of mixture
- \( c \) = CT of normal plasma (Pool Norm)

**Guidelines:**

- **Index < 12**: correction
  - Factor deficiency, (OAT)

- **Index > 15**: no correction
  - Presence of an inhibitor: LA suspected, …
Case study 2

Do we need to perform Staclot DRV screen and confirm

- Not required: One Positive test is enough to confirm LA.
### Case study 2

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRVVs</td>
<td>56 Seconds</td>
<td>31 – 42 seconds</td>
</tr>
<tr>
<td>DRVVs ratio</td>
<td>1.42</td>
<td>&lt; 1.2 No LA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt; 1.2 LA suspected</td>
</tr>
<tr>
<td>DRVVc</td>
<td>36</td>
<td>30 – 37 seconds</td>
</tr>
<tr>
<td>DRVVc Ratio</td>
<td>0.98</td>
<td>&lt; 1.2 No LA detected</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥ 1.2 LA detected</td>
</tr>
<tr>
<td>Normalized Ratio</td>
<td>1.45</td>
<td></td>
</tr>
</tbody>
</table>

- **DRVVs**: Ratio of DRVVs to DRVVc. DRVVs ≥ 1.2 indicates LA suspicion.
- **DRVVc**: DRVVc ratio. DRVVc < 1.2 indicates no LA. DRVVc < 1.2 indicates LA suspicion.
- **Normalized Ratio**: DRVVs divided by DRVVc.
Case Study 2

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTT-LA</td>
<td>37.4 seconds</td>
<td>35 – 45 seconds</td>
</tr>
<tr>
<td>DRVV-S</td>
<td>38 seconds</td>
<td>31- 42  seconds</td>
</tr>
<tr>
<td>DRVV-S ration</td>
<td>1.01</td>
<td>&lt; 1.2</td>
</tr>
</tbody>
</table>

- No further testing is required.
- Transient LA: infections, inflammations etc
- Any positive LA sample must be confirmed by a second sample 12 weeks later.

**Persistent positive test**

LA - aCL - aβ2GP1
Case Study 3

History: 72 year old male with adenocarcinoma of the prostate, and post-operative bleeding.
### Case Study 3

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT</td>
<td>12.0 Seconds</td>
<td>11 – 14 seconds</td>
</tr>
<tr>
<td>APTT</td>
<td>158 seconds</td>
<td>25 – 33 seconds</td>
</tr>
<tr>
<td>TT</td>
<td>14.5 seconds</td>
<td>&lt; 21 seconds</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>357 mg/dL</td>
<td>170 – 410 mg/dL</td>
</tr>
<tr>
<td>TT</td>
<td>13 seconds</td>
<td>&lt; 21 seconds</td>
</tr>
</tbody>
</table>
Case Study 3

Bestneda Assay: Anti FVIII = 9 BU
Case study 3

LA diagnosis should not be made on

- Finding of *prolonged* APTT
- Demonstration of inhibition on the mixing study
  - Uncorrected mixing study
- Heparin and Inhibitors against specific coagulation factors may interfere with these tests
- Confirmatory assay: Staclot LA should be used

Clinical history for patients tested for LA should be reviewed

- Need for factor and/ or Bethesda assays
Case Study 4

History: A 19 Month years old boy with Epstein-Barr virus infection.
## Case Study 4

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRVVs</td>
<td>48.5 Seconds</td>
<td>31 – 42 seconds</td>
</tr>
<tr>
<td>DRVVs ratio</td>
<td>1.24</td>
<td>&lt; 1.2 No LA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt; 1.2 LA suspected</td>
</tr>
<tr>
<td>DRVVc</td>
<td>32</td>
<td>30 – 37 seconds</td>
</tr>
<tr>
<td>DRVVc Ratio</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>Normalized Ratio</td>
<td>1.32</td>
<td>&lt; 1.2 No LA detected</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥ 1.2 LA detected</td>
</tr>
<tr>
<td>DRVVs mixing</td>
<td>11.9</td>
<td>&lt; 12 correction</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt; 15 no correction</td>
</tr>
<tr>
<td>Staclot LA</td>
<td>11.2</td>
<td>&lt; 8 seconds No LA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥ 8 seconds LA</td>
</tr>
</tbody>
</table>
Case Study 4

Mixing study may be corrected:
- The presence of mild LA.
- Time dependent LA

New guidelines allow the use of integrated tests
Integrated tests based on New ISTH recommendations

1- Include a screening and confirmatory step in single procedure.

2- Testing of the same plasma twice:
   - DRVV
   - APTT

3- Performed in Parallel at LOW and HIGH phospholipid concentration.
**Staclot® LA**

**Integrated screen/confirm test**

<table>
<thead>
<tr>
<th>Tube / cuvette</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient plasma</td>
<td>25 µl</td>
<td>25 µl</td>
</tr>
<tr>
<td>Buffer / HPPE*</td>
<td>25 µl</td>
<td>25 µl</td>
</tr>
<tr>
<td>Normal plasma</td>
<td>25 µl</td>
<td>25 µl</td>
</tr>
<tr>
<td>(heparin inhibitor)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>incubation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTT LS</td>
<td>50 µl</td>
<td>50 µl</td>
</tr>
<tr>
<td>CaCl$_2$ 0.025M</td>
<td>50 µl</td>
<td>50 µl</td>
</tr>
</tbody>
</table>

*Hexagonal Phase PhosphatidylEthanolamine*

New guidelines allow skipping of mixing study with integrated test*
Staclot LA as Integrated test

- Contains both screening and confirmation in a single procedure.
- Testing of plasma twice using an LA sensitive APTT.
- Performed in parallel at LOW and HIGH phospholipid concentration.
- Mixing study is integrated in the test.
New adapted solution

LA workup

Integrated test

Staclot LA

NEG

NEG

LA Not detected

DRVVS

POS

NEG

Staclot LA

POS

NEG

DrVVC

or

Staclot LA

DRVVS

POS

POS

DrVVC

LA Confirmed
**Case Study 5**

A 5-year-old girl presented with ecchymoses (bruise) and a hematoma after an upper respiratory illness.

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT</td>
<td>42.8 seconds</td>
<td>11 – 14 seconds</td>
</tr>
<tr>
<td>INR</td>
<td>4.52</td>
<td></td>
</tr>
<tr>
<td>APTT</td>
<td>126 seconds</td>
<td>25 – 33 seconds</td>
</tr>
<tr>
<td>APTTmix</td>
<td>38</td>
<td>&lt; 12 correction</td>
</tr>
<tr>
<td>Rosner Index</td>
<td></td>
<td>&gt; 15 no correction</td>
</tr>
</tbody>
</table>
### Case Study 5

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRVV-S</td>
<td>89 seconds</td>
<td>30 - 41 seconds</td>
</tr>
<tr>
<td>DRVV-S ratio</td>
<td>2.3</td>
<td>&lt; 1.2</td>
</tr>
<tr>
<td>DRVVc</td>
<td>38</td>
<td>31-38 seconds</td>
</tr>
<tr>
<td>DRVVc ratio</td>
<td>1.06</td>
<td>&gt; 1.2 detected</td>
</tr>
<tr>
<td>Normalized ratio</td>
<td>2.2</td>
<td>&lt; 1.2 No LA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥ 8 LA detected</td>
</tr>
<tr>
<td>Staclot LA</td>
<td>67</td>
<td>&lt; 8 seconds NO LA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥ 8 LA detected</td>
</tr>
</tbody>
</table>
Case study 5

- Bleeding may be associated with Lupus anticoagulant in cases associated an autoimmune prothrombin deficiency.
- PT and APTT will be prolonged.
LA testing with Stago reagents
(designation chart suggestion)

Screening test
- STA®-Staclot® DRVV Screen (dRVVI)
- PTT-LA (APTT)

Mixing studies
- Prolonged time
- CT correction
- Screen ratio > 1.2

Patient plasma + Pool Norm®
- LA Negative Factor assays

Confirmatory test
- STA®-Staclot® DRVV Confirm (dRVVI)
- STA®-Staclot® LA (APTT)
- NR > 1.2

OR
- LA Positive

NR: Normalized Ratio - CT: Clotting Time
* If OAT patient, mix 1:1 plasma patient + Pool Norm®
** According to Rosner index or ICA calculation (1)
LA testing flow chart adapted from the international guidelines (Stago reagents)

Screening tests  *For patient + Pool Norm*

Test 1  
PTT LA

- PTT-LA > 45 sec  
  Abnormal
  
- Normal  
  PTT-LA < 45 sec  
  Screen Ratio < 1.2

Stop evaluation for LA

Test 2  
STA Staclot DRVV Screen

- Normal plasma + Patient plasma
- Incubated Mixing Study
- Correction

Test 1  
Staclot LA

Confirmatory studies

Test 2  
STA Staclot DRVV Confirm

Factor assays

- Correction
  - LA confirmed
  - STA CLOT LA
    - Confirm for PTT-LA
      - LA1 – LA2
    - < 8 sec (NO LA)
    - > 8 sec (LA POS)

- No correction

- Correction
  - Factor deficiency
  - inhibitor anti-factor

*With screening test giving abnormal results

**Index** = \( \frac{b - c}{a} \times 100 \)

- a = Clotting time (CT) of patient plasma
- b = CT of mixture
- c = CT of normal plasma (Pool Norm)

< 12 = correction (?? Factor deficiency)

> 15 = NO correction (Inhibitor)

"Normalized Ratio = Screen ratio / Confirm Ratio"
Lupus Anticoagulant diagnostic issues

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