ANIARA DIAGNOSTICA LLC

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In the US, the product is intended **For Research Use Only. Not for Use in Diagnostic Procedures.**

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Manufactured By: HYPHEN BioMed

# ZYMUTEST ACA-APA, IgG or IgM isotype Technical File

## (# ARK029A / ARK029B)

**Assay of Anti-Cardiolipin/Anti-Phospholipid Antibodies, IgG or IgM isotype, by ELISA.**

**Assay range: 0 to >60 GPL/ml (IgG) or 0 to >35 MPL/ml (IgM)**

CE

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Form AH45
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# ZYMUTEST ACA-APA IgG/IgM technical file (Ref. ARK029A/B)

## Assay principle

- The ZYMUTEST ACA-APA, IgG or IgM ELISA kits, are standardised and optimised enzyme immuno-assays designed for measuring anti-cardiolipin/anti-phospholipid (ACA-APA) antibodies of the IgG or IgM isotype, in human plasma or serum, or in any biological fluid where these antibodies must be measured.

- The diluted assayed plasma sample or biological fluid is introduced into one of the microwells of the Cardiolipin coated plate.

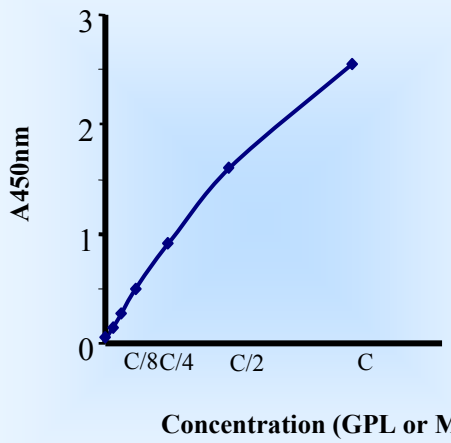
  When present, anti-Cardiolipin/anti-Phospholipid antibodies bind to immobilised Cardiolipin, overcoated with non denaturated, fully functional human B2GPI.

  Following a washing step, bound antibodies, of the IgG or IgM isotype, are revealed with a goat anti-human IgG (or IgM)(Fcγ or Fcμ specific)-peroxidase conjugate, which reacts specifically with IgG or IgM isotypes.

  Following a new washing step, the peroxidase substrate, Tetramethylbenzidine (TMB) in presence of hydrogen peroxide (H2O2), is introduced and a blue colour develops. The colour turns yellow when the reaction is stopped with sulfuric acid.

  The colour developed is directly proportional to the amount of anti-Cardiolipin/anti-Phospholipid antibodies, of the IgG or IgM isotype, present in the tested sample.

## Calibration curve




The assay has a dynamic range from 0 to > 60 GPL for IgG or 0 to > 35 MPL for IgM.

## Intended use: IVD

Assay of ACA-APA antibodies of the IgG or IgM isotype, in the following clinical situations:

- Anti-phospholipid syndrome (APS).
- Pregnancies with recurrent miscarriage.
- Unexplained thrombosis.
- Any clinical situation where the assay of ACA-APA antibodies is required.

## Kit presentation: 96 tests (microplate)

- 1 microELISA plate (12x8wells)
- 2 vials of specific sample diluent
- 3 vials of calibrator (lyophilised)
  (defined ACA concentration, in GPL or MPL units, according to the KAPS standards, duly indicated for each lot).
- 3vials of negative control (diluted normal human plasma) lyophilised
- 3 vials of immunoconjugate (lyophilised)
- 1 vial of conjugate diluent (ready to use)
- 1 vial of 20 fold concentrated wash solution
- 1 vial of substrate (ready to use)
- 1 vial of stop solution (ready to use)

## Procedure

- Specimen: citrate or Na2EDTA anticoagulated human plasma, or serum.
- Plasma Dilution: 1:100.
- Calibration: calibrator included, which concentration is defined (in GPL or MPL units) by reference to the KAPS standard preparation for anti-cardiolipin antibodies, IgG/IgM (N. Harris).
- Manual method or specific automates for ELISA.

## Assay Characteristics

- Total assay time : **about 1h 15min**
- Assay range : **0 to >60 GPL or >35 MPL** in plasma
- Reproducibility: **2 to 5 %** (specification **≤ 10 %**)
- Detection limit (blank+3SD, N≥10): **≤ 2 GPL** (IgG) **or ≤ 1 MPL** (IgM)
- Normal range (negative zone): **≤ 5 GPL** (IgG) **or ≤ 3 MPL** (IgM)
  Grey zone (high background): **5-10 GPL** (IgG) **or 3-7 MPL** (IgM)
  Positive zone : **≥10 GPL** (IgG) **or ≥7 MPL** (IgM)
- **Specificity:** Specific measurement of human ACA-APA antibodies of the IgG or IgM isotype, reactive with immobilised cardiolipin, then "overcoated" with non denatured, fully active human B2GPI.
  Other isotypes are not measured.
  Optimised assay designed with highly reactive cardiolipin, over-coated with semi-purified human B2GP1 (which binds onto cardiolipi/anionic phospholipids) stabilised, and saturated. This reliable method then provides high reproducibility, high sensitivity and high specificity, and offers an optimised discrimination between normal individuals and pathologies with presence of ACA-APA antibodies.



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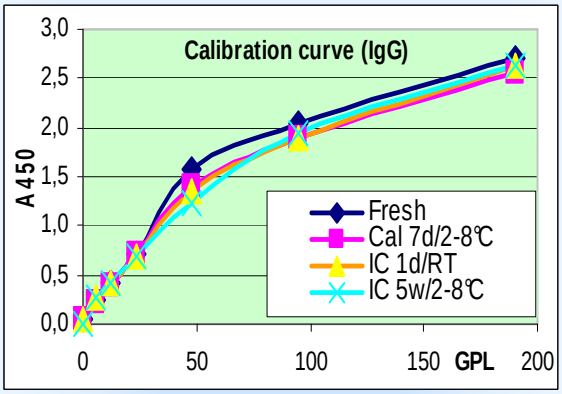
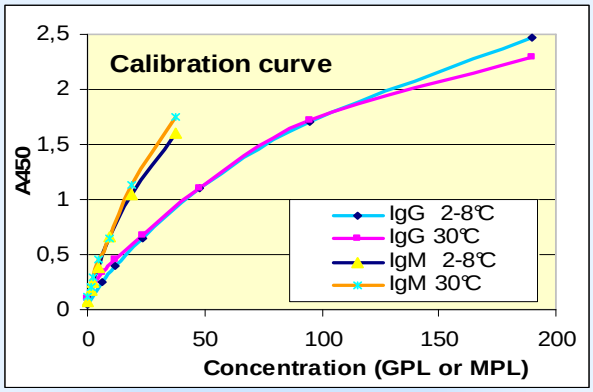
## Stability of reconstituted reagents




Excellent preservation of performances of reconstituted reagents stored at 2-8°C (calibrator for 7 days, immunoconjugate for 5 weeks) or at RT (immunoconjugate for 1 day), compared with freshly reconstituted vials.
(Performances are similar for IgM).

## Overheating study




Excellent performances preservation of lyophilised products stored for 3 weeks at 30°C, comparatively to those kept at 2-8°C.

Kits can be shipped at RT for a short period without damage.

## Normal range

The upper limit of the **normal range (negative zone)** is defined as the concentration corresponding to the "mean+2SD" obtained on a large panel of plasmas (or sera) from normal individuals (males or females), aged between 18 and 55 years, and out of any medication or disease.

### ACA-APA IgG:

| ZYMUTEST ACA-APA IgG | Lot 1: 041103J | Lot 2: 030919G | Lot 3: 051110F |
|---|---|---|---|
| N (plasmas) | 27 | 24 | 28 |
| Mean (GPL) | 1.6 | 2.4 | 2.34 |
| SD | 0.4 | 0.9 | 1.01 |
| Mean + 2SD (GPL) | 2.4 | 4.3 | 4.36 |

**Conclusion:** The normal range (Mean + 2SD **≤ 5 GPL**) is confirmed, and results are homogeneous between the various manufacturing lots.

### ACA-APA IgM:

| ZYMUTEST ACA-APA IgM | Lot 1: **041103K** | Lot 2: **031125E** | Lot 3: **051110G** |
|---|---|---|---|
| N | 25 | 24 | 29 |
| Mean | 0.75 | 0.28 | 0.86 |
| SD | 0.6 | 0.04 | 0.88 |
| Mean + 2SD (MPL) | 1.95 | 0.36 | 2.62 |

**Conclusion:** The normal range (Mean + 2SD **≤ 3 MPL**) is confirmed, and results are homogeneous between the various manufacturing lots.



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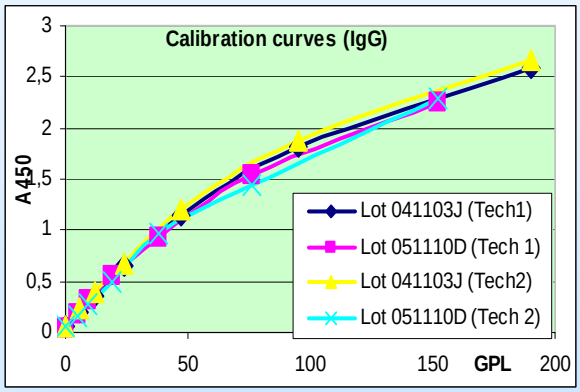
## Inter-lots performances comparison for ZYMUTEST ACA APA IgG device

59 normal plasma samples and 14 pathological samples (patients with elevated concentrations of ACA-APA IgG antibodies) were tested using 2 different lots of ZYMUTEST ACA-APA IgG device, and by 2 different technicians:

*** Calibration curves:**




*** Results obtained on normal samples:**

| ACA –APA IgG | Technician 1 | | | | Technician 1 | | | |
|---|---|---|---|---|---|---|---|---|
| | Lot 041103J | | Lot 051110D | | Lot 041103J | | Lot 051110D | |
| | A450 | GPL | A450 | GPL | A450 | GPL | A450 | GPL |
| Mean | 0.13 | 3.45 | 0.10 | 2.16 | 0.13 | 2.47 | 0.15 | 4.41 |
| SD | 0.03 | 1.29 | 0.03 | 1.06 | 0.03 | 1.18 | 0.04 | 1.53 |
| Mean+2 SD | / | 6.03 | / | 4.29 | / | 4.83 | / | 7.47 |
| Mean+5 SD | / | 9.9 | / | 7.5 | / | 8.4 | / | 12.1 |
| N (tested normals) | 59 | | 59 | | 59 | | 59 | |
| Negative | 50 | | 57 | | 58 | | 43 | |
| Grey zone | 9 | | 1 | | 1 | | 16 | |
| Positive | 0 | | 0 | | 0 | | 0 | |

Results are globally consistent with the definition of the negative zone (« normal » ≤5GPL) and « grey zone » (5-10 GPL). The repartition between the normal and grey zone can slightly vary for plasmas with OD values close to the « out off range » between the different zones, and according to the accuracy of the dilution (1:100), but is globally coherent between lots and technicians.

**•Results obtained on pathological samples:**

| ACA -APA IgG | Technician 1 | | | | Technician 2 | | | |
|---|---|---|---|---|---|---|---|---|
| | Lot 041103J | | Lot 051110D | | Lot 041103J | | Lot 051110D | |
| | A450 | GPL | A450 | GPL | A450 | GPL | A450 | GPL |
| Mean | 1.70 | 48.6 | 1.70 | 35.5 | 1.86 | 42.4 | 1,90 | 38,3 |
| SD | 1.42 | 58.5 | 1.34 | 34.89 | 1.41 | 44.9 | 1.45 | 46.5 |
| N (tested pathos) | 14 | | 14 | | 14 | | 14 | |
| Negative | 2 | | 1 | | 1 | | 1 | |
| Grey zone | 2 | | 1 | | 1 | | 1 | |
| Low Positive | 2 | | 4 | | 3 | | 4 | |
| Moderate Positive | 1 | | 2 | | 2 | | 1 | |
| High Positive | 7 | | 6 | | 7 | | 7 | |

Results obtained for pathological samples diagnosed as positive for ACA-APA IgG antibodies are globally coherent between series and kits.



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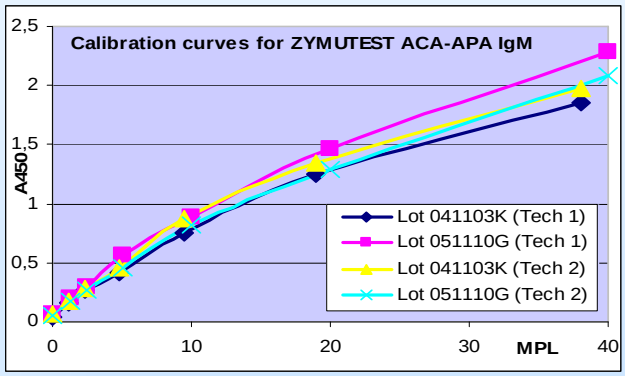
## Inter-lots performances comparison for ZYMUTEST ACA APA IgM device

62 normal plasma samples and 14 pathological samples (patients with elevated concentrations of ACA-APA IgM antibodies) were tested using 2 different lots of ZYMUTEST ACA-APA IgM device, and by 2 different technicians:

*** Calibration curves:**




*** Results obtained on normal samples:**

| ACA-APA IgM | Technician 1 | | | | Technician 2 | | | |
|---|---|---|---|---|---|---|---|---|
| | Lot 041103K | | Lot 051110G | | Lot 041103K | | Lot 051110G | |
| | A450 | MPL | A450 | MPL | A450 | MPL | A450 | MPL |
| Mean | 0,11 | 0,66 | 0,13 | 0,58 | 0,15 | 0,85 | 0,14 | 0,94 |
| SD | 0,04 | 0,39 | 0,05 | 0,48 | 0,05 | 0,54 | 0,05 | 0,52 |
| Mean+2 SD | / | 1,43 | / | 1,54 | / | 1,94 | / | 1,97 |
| Mean+5 SD | / | 2,6 | / | 3,0 | / | 3,6 | / | 3,5 |
| N (tested normals) | 62 | | 62 | | 62 | | 62 | |
| Negative (<3MPL) | 62 | | 62 | | 62 | | 62 | |
| Grey zone (3-7MPL) | 0 | | 0 | | 0 | | 0 | |
| Positive (>7MPL) | 0 | | 0 | | 0 | | 0 | |

Results are globally consistent with the definition of the negative zone (« normal » ≤3 MPL) and « grey zone » (3-7 MPL). The repartition between the normal and grey zone is coherent between lots and technicians.

**•Results obtained on pathological samples:**

| ACA-APA IgM | Technician 1 | | | | Technician 2 | | | |
|---|---|---|---|---|---|---|---|---|
| | Lot 041103K | | Lot 051110G | | Lot 041103K | | Lot 051110G | |
| | A450 | MPL | A450 | MPL | A450 | MPL | A450 | MPL |
| Mean | 0.71 | 6.1 | 0.93 | 7.8 | 0.83 | 6.9 | 0.92 | 9.7 |
| SD | 0.61 | 4.99 | 0.78 | 6.93 | 0.66 | 5.41 | 0.73 | 8.73 |
| N (tested pathos) | 14 | | 14 | | 14 | | 14 | |
| Negative (<3MPL) | 5 | | 5 | | 5 | | 3 | |
| Grey zone (3-7MPL) | 3 | | 2 | | 3 | | 4 | |
| Positive (low) | 4 | | 5 | | 4 | | 5 | |
| Positive (moderate/high) | 2 | | 2 | | 2 | | 2 | |

Results obtained for pathological samples diagnosed as positive for ACA-APA IgM antibodies are globally coherent between series and kits.



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## External comparison with 2 commercial devices

| ACA-APA kits | IgG isotype | | | IgM isotype | | |
|---|---|---|---|---|---|---|
| | Vita Diagnostika | Hyphen BioMed | Inova | Vita Diagnostika | Hyphen BioMed | Inova |
| N (samples) | 31 | 31 | 31 | 31 | 31 | 31 |
| Negative | 12 | 27 | 26 | 22 | 27 | 27 |
| Grey zone | 10 | 1 | 1 | 2 | 4 | 1 |
| Positive | 9 | 3 | 4 | 7 | 0 | 3 |

**Conclusion:** Results are globally coherent between the devices on normal plasmas, the "discrimination" obtained with HYPHEN BioMed kits being closer to Inova results than to Vita Diagnostika device ares.
*(For IgG: Some plasmas (9 for IgG) are found at the lower limit of the grey zone with Vita DiagnostiKa device, while diagnosed "normal" with the 2 other kits).*
"Discrepant results" between the kits are often within the grey zone or the low positive range. That could be explained by the referentials used.

## Clinical applications

Anti-cardiolipin/anti-phospholipid (ACA-APA) antibodies are usually absent in normal population.
Presence of ACA-APA antibodies at moderate or high concentrations is observed in the anti-phospholipid syndrome (APS), sometimes associated with thrombotic diseases, recurrent miscarriages, livedo reticularis, thrombocytopenia or neurological disorders.
The pathogenicity of ACA-APA antibodies is still under investigation. They are thought to contribute triggering various clinical manifestations (APS). Pathogenicity of the various isotypes is not completely documented, especially for IgM and IgA isotypes. Severity of the clinical manifestations associated with the presence of anti-cardiolipin/anti-phospholipid antibodies, increases with the IgG isotype, the antibody concentration and its affinity, and the time of exposure. IgG isotype is then, the most pathogenic.

***Note**: ACA-APA antibodies actually bind to a complex between anionic phospholipids and B2GP1. This complex is the true target antigen for those antibodies. The Zymutest ACA/APA kit is designed with this optimized complex in order to offer the right measurement of the true ACA/APA.*

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*12)* **« International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS);** Miyakis & al., *J Thromb Haemost*, 4 (2):295-306, Feb 2006.




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