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Coxsackie Virus IgA/IgG/IgM ELISA

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**For Research Use
Only – Not for Use
in Clinical
Procedures**

IBL-America Coxsackie virus IgA/IgG/IgM ELISA

Enzyme-immunoassay for determination of human antibodies

Coxsackie virus IgA	Order no.: IB05039
Coxsackie virus IgG	Order no.: IB05040
Coxsackie virus IgM	Order no.: IB05041

For Research Use Only – Not for Use in Clinical Procedures

1 INTENDED USE

The IBL-America ELISA Coxsackie virus IgA, IgG and IgM tests are qualitative immunoassays for the demonstration of human antibody classes IgA, IgG, and IgM directed against Coxsackie viruses. The assays are recommended for the sensitive detection of such antibodies in various kinds of samples.

2 BACKGROUND

Coxsackie viruses A and B, as well as the ECHO viruses, have a worldwide distribution and all belong to the *Picornaviridae* family, genus Enterovirus. These virus groups are similar in their biological characteristics and epidemiology with a primarily faecal-oral transmission strategy but also being capable of spread via aerosols. Person to person transmission is mainly by smear infection with the portal of entry being either the alimentary canal or the respiratory tract. The viruses reach the reticuloendothelial system and other target organs such as the myocardium, meninges or skin, via the blood circulation.

ELISA test-systems are especially suited for the differential analysis of immunoglobulin classes directed against the virus.

3 TEST PRINCIPLE

The ELISA (Enzyme Linked Immunosorbent Assay) is an immunoassay, which is particularly suited to the determination of antibodies in the field of infectious serology. The reaction is based on the specific interaction of antibodies with their corresponding antigen. The test strips of the microtiter plate are coated with specific antigens of the pathogen of interest. If antibodies in the sample are present, they bind to the fixed antigen. A secondary antibody, which has been conjugated with the enzyme alkaline phosphatase, detects and binds to the immune complex. The colourless substrate p-nitrophenylphosphate is then converted into the coloured product p-nitrophenol. The signal intensity of this reaction product is proportional to the concentration of the analyte in the sample and is measured photometrically.

4 KIT COMPONENTS

Test Components	Pieces / Volume
Break apart microtiter test strips each with eight antigen coated single wells, (altogether 96) [MTP] , 1 frame. The coating material is inactivated.	12 pieces
Standard serum (ready-to-use) [STD] , Human serum in protein containing phosphate buffer; negative for anti-HIV Ab, HBs-Ag (Hepatitis B-Virus surface antigen) and anti-HCV Ab; preservative: < 0.1 % sodium azide; colouring: Amaranth O.	2 x 2 ml
Negative control serum (ready-to-use) [NEG] , Human serum in protein containing phosphate buffer; negative for anti-HIV Ab, HBs-Ag (Hepatitis B-Virus surface antigen) and anti-HCV Ab; preservative: < 0.1 % sodium azide; colouring: Lissamin Green V.	2 ml
Anti-human IgA, IgG or IgM conjugate (ready-to-use) [APC] , Anti-human IgA, IgG or IgM polyclonal antibody, conjugated to alkaline phosphatase, stabilised with protein stabilisation solution; preservative: 0.01 % methylisothiazolone, 0.01 % bromnitrodioxane.	13 ml
Washing solution concentrate (sufficient for 1000 ml) [WASH] , Sodium chloride solution with Tween 20 and 30 mM Tris/HCl, pH 7,4; preservative: < 0.1 % sodium azide.	33.3 ml
Dilution buffer [DILB] , Protein containing phosphate buffer with Tween 20; preservative: < 0.1 % sodium azide; colouring: 0.01 g/l Bromphenol blue.	2 x 50 ml
Stopping solution [STOP] , < 0.1 N sodium hydroxide, 40 mM EDTA	15 ml
Substrate (ready-to-use) [pNPP] , Para-nitrophenylphosphate in solvent free buffer; preservative: < 0.1 % sodium azide	13 ml
Quality control certificate [INFO]	1 page

5 MATERIAL REQUIRED BUT NOT SUPPLIED

- common laboratory equipment
- for the IgM detection: Rf-Absorbent, order no. IB05998 (20 ml)
- photometer for microtitre plates with filter, wavelength 405 nm, recommended reference wavelength 620 nm - 690 nm (e.g. 650 nm)
- Microtiter plate washer
- incubator 37 °C
- moist chamber
- distilled water

Recommended but not required:

Control serum 5 x 3 ml order no.: IB05039CON for Coxsackie virus IgA
Control serum 5 x 3 ml order no.: IB05040CON for Coxsackie virus IgG
Control serum 5 x 3 ml order no.: IB05041CON for Coxsackie virus IgM

6 STORAGE AND STABILITY

Reagent	Storage	Stability
Microtiter strips (coated with antigen)	unopened after opening at 2 – 8 °C in closed aluminum bag with desiccant	see expiry date; 6 months
Control sera / Standard sera	Unopened after opening at 2 – 8 °C	see expiry date 6 months
Conjugate	Unopened after opening at 2 – 8 °C	see expiry date 6 months
Dilution buffer	Unopened after opening at 2 – 8 °C	see expiry date 6 months
Washing solution	Unopened / after opening at 2 – 8 °C working dilution at 2 – 8 °C working dilution at room temperature	see expiry date; 2 weeks; 1 week
Substrate	Unopened after opening at 2 – 8 °C	see expiry date 6 months
Stopping solution	Unopened after opening at 2 – 8 °C	see expiry date 6 months

7 TEST PROCEDURE

7.1 Evidence of Deterioration

Optimum results can only be achieved if the instructions are strictly followed. The components of the kit must not be exchanged for reagents of other manufacturers. Standard and control sera are defined exclusively for the test kit to be used and must not be used in other lots. Washing solution, substrate and stop solution can be used for all IBL-America immunoassays coded IB05xxx irrespective of the lot and the test.

Each test contains a ready-to-use sample dilution buffer. In some cases the use of special dilution buffers is necessary to guarantee consistent quality and reliable results. The dilution buffers can be used irrespective of the lots.

There are three different conjugate concentrations for each immunoglobulin class (IgA, IgG, IgM), indicated on the C of A as + (low), ++ (medium) and +++ (high). Conjugates with the same concentration and of the same immunoglobulin class are interchangeable and can be used for other IB05xxx immunoassays irrespective of the lot and the test. Dilution or alteration of the reagents may result in a loss of sensitivity. Use aseptic techniques when removing aliquots from the reagent tubes to avoid contamination.

Reproducibility of test results is dependent on thorough mixing of the reagents. Agitate the flasks containing control sera before use and also all samples after dilution (e.g. by using a vortex mixer).

Be sure to pipette carefully and comply with the given incubation times and temperatures. Significant time differences between pipetting the first and last well of the microtiter plate when dispensing samples and control sera, conjugate or substrate can result in different pre-incubation times, which may influence the precision and reproducibility of the results. Avoid exposure of reagents to strong light during storage and incubation.

Adequate washing avoids test unspecificities. Therefore, the washing procedure should be carried out carefully. All of the flat bottom wells should be filled with equal volumes of washing buffer. At the end of the procedure ensure that the wells are free of all washing buffer in order to avoid uncontrolled dilution effects. Avoid foaming!

Reagents must be tightly closed after use to avoid evaporation and contamination. Take care not to mix-up the caps of the bottles and/or vials.

The immunoassay is only valid if the lot-specific validation criteria on the quality control certificate are fulfilled.

7.2 Sample Preparation and Storage

Lipaemic, hemolytic or icteric samples (serum or plasma) should only be tested with caution. Obviously contaminated samples should not be tested. Serum or plasma (EDTA, citrate, heparin) collected according to standard laboratory methods are suitable samples. Samples must not be thermally inactivated.

7.2.1 Dilution of Samples

Before running the test, all samples (V_1) must be diluted in dilution buffer (V_2) as follows:

Coxsackie virus IgA ELISA

$V_1 + V_2 = 1+100$	add	10 μ l	sample
		each to 1000 μ l	dilution buffer

Coxsackie virus IgG ELISA

$V_1 + V_2 = 1+500$	add	10 μ l	sample
		each to 1000 μ l	dilution buffer (= 1+100)
		50 μ l	of the first dilution
		each to 200 μ l	dilution buffer (= 1+4)

After dilution and before pipetting into the microtiter plate the samples must be mixed thoroughly to prepare a homogenous solution.

Coxsackie virus IgM ELISA

Interference with rheumatoid factors

Rheumatoid factors are autoantibodies mainly of the IgM class, which preferably bind to IgG immune complexes. The presence of non-specific IgM antibodies (rheumatoid factors) can lead to false-positive results in the IgM assay. Furthermore, the possibility exists, that weak-binding pathogen-specific IgM antibodies may be displaced by stronger-binding IgG antibodies leading to a false negative IgM result. Therefore it is necessary to pretreat samples with rheumatoid factor-absorbents prior to IgM detection (Rf-Absorbent). Rf-absorption is performed by incubation of the sample in Rf-dilution buffer for 15 minutes at room temperature or over night at 4 °C. The test procedure is described in a separate instruction manual.

Before running the test, rheumatoid factor-absorbent (V_1) must be diluted 1+4 in dilution buffer (V_2).

$V_1 + V_2 = V_3 (1 + 4)$	add	200 μ l	Rf-absorbent
	each to	800 μ l	dilution buffer

The samples (V_4) must be diluted in this Rf-dilution buffer (V_3):

$V_4 + V_3 = 1+100$	add	10 μ l	Sample
	each to	1000 μ l	Rf-dilution buffer

After dilution and before pipetting into the microtiter plate the samples must be mixed thoroughly to prepare a homogenous solution.

7.2.2 Sample Storage

The samples should not be stored for more than 7 days at 2 – 8 °C. Extended storage is possible at ≤ -20 °C. Avoid repeated freezing and thawing of samples. Diluted samples can be stored at 2 – 8 °C for one week.

7.3 Preparation of Kit Reagents

Bring all reagents to room temperature before testing.

7.3.1 Microtiter Test Strips

The microtiter test strips labeled with abbreviations for pathogen and immunoglobulin class are packed with a desiccant in an aluminum bag. To open the aluminum bag of the microtiter plate please cut off the top of the marked side only, in order to guarantee proper resealing. Take unrequired cavities out of the frame and put them back into the aluminum bag. Close bag carefully to ensure airtight conditions. Do not use the strips if the aluminum bag is damaged or if the bag with remaining strips and desiccant was not properly resealed.

7.3.2 Control Sera / Standard Sera

Negative control and standard sera are ready-to-use and must not be diluted any further. For each test run (independent of the number of microtiter test strips to be used) negative control and standard sera must be included. Standard sera should be set up in duplicate. Do not treat negative control and standard sera with Rf-absorbent.

7.3.3 Anti-human IgA, IgG or IgM AP-Conjugate (ready-to-use)

The required conjugate concentration (+, ++, +++) is indicated on the quality control certificate. Avoid contamination.

7.3.4 Washing Solution

Dilute washing buffer concentrate (V_1) 1:30 with aqua dest. to a final volume of V_2 . Bottles used for the working dilution should be cleaned regularly. Discard cloudy solutions.

Example:

Buffer concentrate (V_1)	Final volume (V_2)
33.3 ml	1000 ml
1.0 ml	30 ml

7.3.5 Dilution Buffer for Samples (ready-to-use)

Discard cloudy solutions.

7.3.6 Substrate (ready-to-use)

Substrate in unopened bottle may have a slightly yellow coloring, which does not reduce the quality of the product! Avoid contamination of the ready-to-use substrate solution e. g. by using sterile tips.

7.3.7 Stopping Solution (ready-to-use)

7.4 Overview - Test Procedure

IBL-America Coxsackievirus IgA/IgG/IgM

In case of IgM detection absorption of rheumatoid factor, see No. 7.2.1;
Incubation 15 minutes at room temperature or over night at 4°C

sample dilution¹

IgG: 1 + 500

IgM/IgA: 1 + 100

Pipette diluted samples and ready-to-use control /
standard sera into the microtest wells (100 µl)



INCUBATION 60 Min./ 37 °C
moist chamber



WASH (4 x 300 µl [DIL] [WASH])²



Pipette conjugate solution [APC] (100 µl)



INCUBATION 30 Min./ 37 °C
moist chamber



WASH (4 x 300 µl [DIL] [WASH])²



Pipette substrate solution [pNPP] (100 µl)



INCUBATION 30 Min./ 37 °C
moist chamber



Pipette stopping solution [STOP] (100 µl)



READ EXTINCTION at 405 nm

¹Special dilution buffers for the following IBL-America tests:
Borrelia burgdorferi IgG, IgM, EBV EA IgG

²For manual use:
tap plate at the end of the wash procedure on paper towel.

7.5 Manual Test Procedure

1. Place the required number of **cavities in the frame** and prepare a protocol sheet.
2. Add each **100 µl of diluted sample or ready-to-use controls** into the appropriate wells of microtiter test strips. Spare one well for substrate blank, e.g.:

IgA/IgG/IgM well no.	
well A1	Substrate blank
well B1	Negative Control
well C1	Standard serum
well D1	Standard serum
well E1	Sample 1....

3. **Sample incubation** for 60 minutes (+/- 5 min) at 37 °C (+/- 1°C) in moist chamber
4. After incubation **wash** all wells with washing solution (by automated washer or manually):
 - aspirate or shake out the incubation solution
 - fill each well with 300 µl washing solution
 - aspirate or shake out the washing buffer
 - repeat the washing procedure 3 times (altogether 4 times!)
 - dry by tapping the microtiter plate on a paper towel
5. **Addition of conjugate**
Add 100 µl of the ready-to-use IgA/IgG/IgM conjugate to the appropriate wells (except substrate blank)
6. **Conjugate incubation** for 30 minutes (+/- 1 min) at 37 °C (+/- 1 °C) in moist chamber.
7. After incubation **wash** all wells with washing solution (see above)
8. **Addition of substrate**
Add 100 µl of ready-to-use substrate solution to each well (including well for substrate blank!)
9. **Substrate incubation** for 30 minutes (+/- 1 min) at 37 °C (+/- 1 °C) in moist chamber.
10. **Stopping of the reaction**
Add 100 µl stopping solution to each well, shake microtiter plate gently to mix.
11. **Read extinction**
Read optical density (OD) within 60 minutes at 405 nm against substrate blank, reference wave length between 620 nm and 690 nm (e.g. 650 nm).

7.6 Automated Test Procedure

This ELISA also is suited for processing on automats and evaluated for use with Immunomat™ (using the following consumables: VT124, VT111, VT112) and suited for processing on similar analyzers. For processing on the Immunomat the current software version including reagent check has to be used. The automated processing is performed analogous to manual use. Please note, that under special working-conditions internal laboratory adaptations of the substrate incubation times may be necessary.

7.7 Positive Control / Accuracy Control

For the periodic verification of the test method, in order to fulfil the requirements of laboratory internal quality management systems, we recommend using IBL-America ELISA controls (cat.-no. IB05xxxCON, see also chapter 5) to determine precision and accuracy of the test runs. The use of IBL-America ELISA controls is described in specific instruction manuals.

8 TEST EVALUATION

For qualitative interpretation of serum samples a lot specific correction factor as well as a lot specific grey zone is calculated by manufacturer for each kit lot. These values can be found on the lot specific quality certificate included in each test kit.

For test run control a standard serum is used in each individual test run. For this control serum a reference value with a validity range is determined by the quality control of the manufacturer. Within this range a correct cut-off interpretation is ensured.

8.1 Criteria of Validity

The substrate blank must be < 0.25 OD

The negative control must produce a negative test result.

The mean OD-value (after subtraction of the substrate blank!) of the standard serum must be within the validity range, which is given on the lot specific qualitycontrol certificate.

The variation of OD-values of the standard serum may not be higher than 20%.

If these criteria are not met, the test is not valid and must be repeated.

8.2 Cut-off Calculation

A lot specific quality control certificate is included in the test kit so that the obtained OD values can be interpreted qualitatively. The substrate blank must be subtracted from all OD values prior to evaluation.

To fix the cut-off ranges multiply the mean value of the measured standard OD with the lot specific correction factor from the quality certificate. Then add and subtract the lot specific grey zone percentage mentioned on the quality certificate to obtain the upper and lower cut-off. The following numbers are an example only, the valid data you will find in the lot-specific QC certificate which comes with each kit.

Lot specific correction factor: 0.805

Lot specific grey zone: 15%

If the measured mean absorbance value of the standard serum is 0.84 OD, the range of the cut-off is:

Lower cut-off: $(0.84 * 0,805) - 15\% = \text{OD } 0,575$

Upper cut-off: $(0.84 * 0,805) + 15\% = \text{OD } 0,778$

8.3 Interpretation of Results

A positive test result confirms the presence of specific antibodies. A negative result indicates that no relevant antibodies against the pathogen are present in the sample, but does not exclude the possibility of an acute reaction. In case of a borderline result a reliable evaluation is not possible. A definitive determination can only be achieved by testing paired serum samples, taken at one to two weeks intervals, in parallel.

The Coxsackievirus IgA, IgG and IgM tests are based on a mixture of recombinant antigens derived from conserved and subtype specific epitopes of the VP1 proteins of Coxsackieviruses B1, B3 and B5. As a result, the Coxsackievirus tests allow for the demonstration of heterotypical antibodies directed against the conserved epitopes of VP1 proteins and the detection of other Coxsackievirus serotypes. Furthermore, Coxsackievirus tests are suitable for the determination of homotypical antibodies directed against subtype specific epitopes of the VP1 proteins of Coxsackieviruses B1, B3 and B5.

9 PERFORMANCE CHARACTERISTICS

9.1 Sensitivity and Specificity

IBL-America Coxsacki IgA, IgG and IgM

The IBL-America Coxsacki IgA, IgG and IgM ELISA was verified in an internal study. The sensitivity and specificity in all cases exceeded 90%.

9.2 Reproducibility

Intraassay reproducibility was determined by testing samples of different reactivities 20 times in one test run. Interassay reproducibility was determined by testing samples of different reactivities 10 times in 10 independent assays performed on 5 different days.

$$\text{Coefficient of Variation (CV \%)} = \frac{\text{Standard deviation}}{\text{Mean value}} \times 100$$

Coxsackievirus IgA ELISA:

Sample	Mean Value (OD)	Intraassay (CV %)	Mean Value (OD)	Interassay (CV %)
Sample 1	0.179	4.7	0.213	7.4
Sample 2	0.445	2.9	0.528	7.3
Sample 3	0.774	2.8	0.859	6.1

Coxsackievirus IgG ELISA:

Sample	Mean Value (OD)	Intraassay (CV %)	Mean Value (OD)	Interassay (CV %)
Sample 1	0.411	1.7	0.418	9.7

Sample 2	0.718	1.7	0.724	9.4
Sample 3	1.324	5.0	1.339	8.4

Coxsackievirus IgM ELISA:

Sample	Mean Value (OD)	Intraassay (CV %)	Mean Value (OD)	Interassay (CV %)
Sample 1	0.065	7.5	0.077	12.1
Sample 2	0.638	3.4	0.747	5.3
Sample 3	1.281	2.4	1.500	3.0

9.3 Cross-reactivities

Coxsackievirus IgA ELISA:

To determine detection of cross-reactive antibodies directed against different parameters sera were analyzed with Coxsackievirus IgA and a commercially available anti-Coxsackie IgA ELISA. Positive sera (10 sera each) for Adenovirus IgA, Cytomegalovirus IgA, Epstein-Barr Virus IgA, Herpes Simplex Virus ½ IgA, Influenza A Virus IgA, Influenza B Virus IgA, Parainfluenza IgA and Varicella Zoster Virus IgA have been tested as well as sera positive for rheumatoid factor (RF) and anti-nuclear antibodies (ANA). Within this internal evaluation potential cross-reactivities with one Cytomegalovirus IgA, one Influenza A Virus and three ANA positive serum have been observed. All reactivities have been confirmed by positive or borderline results in the reference assay. Other cross-reactivities cannot be ruled out in general.

Coxsackievirus IgG ELISA:

To determine detection of cross-reactive antibodies directed against different parameters sera were analyzed with the Coxsackievirus IgG and a commercially available anti-Coxsackievirus IgG ELISA. Positive sera (10 sera each) for Adenovirus IgG, Cytomegalovirus IgG, Epstein-Barr Virus IgG, Herpes Simplex Virus ½ IgG, Influenza A Virus IgG, Influenza B Virus IgG, Parainfluenza IgG and Varicella Zoster Virus IgG have been tested as well as sera positive for rheumatoid factor (RF) and anti-nuclear antibodies (ANA). Within this internal evaluation potential cross-reactivities with one Adenovirus IgG, one Herpes Simplex Virus ½ IgG, five Influenza A Virus IgG, one Influenza B Virus, one Varicella Zoster Virus IgG, one RF and three ANA positive sera have been observed. The majority of reactivities have been confirmed by positive or borderline results in the reference assay. Other cross-reactivities cannot be ruled out in general.

Coxsackievirus IgM ELISA:

To determine detection of cross-reactive antibodies directed against different parameters sera were analyzed with the Coxsackievirus IgM and a commercially available anti-Coxsackie IgM ELISA. Positive sera (10 sera each) for Epstein-Barr Virus IgM, Cytomegalovirus IgM and Mycoplasma pneumoniae IgM have been tested as well as sera positive for rheumatoid factor (RF) and anti-nuclear antibodies (ANA). Within this internal

evaluation potential cross-reactivities with six Epstein-Barr Virus IgM, two Cytomegalovirus IgM and one Mycoplasma pneumoniae IgM positive samples have been observed. Other cross-reactivities cannot be ruled out in general.

9.4 Interfering Substances

Coxsackievirus IgA/IgG/IgM


To determine the influence of interfering substances, sera with different reactivities were analyzed with the Coxsackievirus IgA/IgG/IgM. No interferences have been detected for sera with concentrations up to 2.00 g/L hemoglobin, 11.50 g/L lipemia/triglyceride or 0.201 g/L bilirubin (conjugated and unconjugated).

10 SAFETY MEASURES

10.1 Statements of Warning

The IBL-America ELISA test kits are designed for use by qualified personnel who are familiar with good laboratory practice.

All kit reagents and specimens should be handled carefully, using established good laboratory practice.

- This kit contains human blood components. Although all control- and cut-off sera have been tested and found negative for anti-HIV-ab, HBs-Ag (*Hepatitis B-Virus-surface Antigen*) and anti-HCV-ab, they should be considered potentially infectious.
- Do not pipette by mouth.
- Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.
- Wear disposable gloves, laboratory coat and safety glasses while handling kit reagents or specimens. Wash hands thoroughly afterwards.
- Sample material and other potentially infectious material should be decontaminated after the test run.
- Reagents should be stored safely and be inaccessible to unauthorized access e.g. children.
- Stopping solution:  corrosive (C); causes acid burn (R34)
Use safety glasses, gloves and laboratory coat while handling!

10.2 Disposal

Please observe the relevant statutory requirements!

11 REFERENCES

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