



# Enterovirus IgA/IgG/IgM ELISA

## CONTENTS

- 1 INTENDED USE**
- 2 BACKGROUND**
- 3 TEST PRINCIPLE**
- 4 KIT COMPONENTS**
- 5 MATERIAL REQUIRED BUT NOT SUPPLIED**
- 6 STORAGE AND STABILITY**
- 7 TEST PROCEDURE**
  - 7.1 Evidence of Deterioration
  - 7.2 Sample Preparation and Storage
  - 7.3 Preparation of Kit Reagents
  - 7.4 Overview - Test Procedure
  - 7.5 Manual Test Procedure
  - 7.6 Automated Test Procedure
  - 7.7 Positive Control / Accuracy Control
- 8 TEST EVALUATION**
  - 8.1 Criteria of Validity
  - 8.2 Cut-off Calculation
  - 8.3 Borderline Ranges
- 9 PERFORMANCE CHARACTERISTICS**
  - 9.1 Sensitivity and Specificity
  - 9.2 Reproducibility
- 10 SAFETY MEASURES**
  - 10.1 Statements of Warning
  - 10.2 Disposal
- 11 REFERENCES**

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

# **IBL-America Enterovirus IgA/IgG/IgM ELISA**

## **Enzyme-immunoassay for determination of human antibodies**

|                              |                    |
|------------------------------|--------------------|
| <b>Enterovirus IgA ELISA</b> | Order no.: IB05051 |
| <b>Enterovirus IgG ELISA</b> | Order no.: IB05052 |
| <b>Enterovirus IgM ELISA</b> | Order no.: IB05053 |

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

#### **1 INTENDED USE**

The IBL-America ELISA Enterovirus IgA, IgG and IgM tests are qualitative immunoassays for the demonstration of human antibodies in serum or plasma directed against Enteroviruses. The Enterovirus IgA, IgG and IgM ELISA are recommended for the sensitive detection of such antibodies in various kinds of samples.

#### **2 BACKGROUND**

Coxsackieviruses 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 ELISA classic 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 |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------|
| <b>Break apart microtiter test strips each with eight antigen coated single wells,</b> (altogether 96) <b>[MTP]</b> , 1 frame. The coating material is inactivated.                                                                                                                | 12 pieces       |
| <b>Standard serum (ready-to-use) [STD]</b> ,<br>Human serum in protein containing phosphate buffer; negative for anti-HIV Ab, HBs-Ag (Hepatitis B-Virus surface antigen) and anti-HCV Ab;<br>preservative: < 0.1 % sodium azide; colouring: Amaranth O.                            | 2 x 2 ml        |
| <b>Negative control serum (ready-to-use) [NEG]</b> ,<br>Human serum in protein containing phosphate buffer; negative for anti-HIV Ab, HBs-Ag (Hepatitis B-Virus surface antigen) and anti-HCV Ab;<br>preservative: < 0.1 % sodium azide; colouring: Lissamin Green V.              | 2 ml            |
| <b>Anti-human IgA, IgG or IgM conjugate (ready-to-use) [APC]</b> ,<br>Anti-human IgA, IgG or IgM polyclonal antibody,<br>conjugated to alkaline phosphatase, stabilised with protein stabilisation solution;<br>preservative: 0.01 % methylisothiazolone, 0.01 % bromnitrodioxane. | 13 ml           |
| <b>Washing solution concentrate (sufficient for 1000 ml) [WASH]</b> ,<br>Sodium chloride solution with Tween 20 and 30 mM Tris/HCl, pH 7,4;<br>preservative: < 0.1 % sodium azide.                                                                                                 | 33.3 ml         |
| <b>Dilution buffer [DILB]</b> ,<br>Protein containing phosphate buffer with Tween 20;<br>preservative: < 0.1 % sodium azide; colouring: 0.01 g/l Bromphenol blue.                                                                                                                  | 2 x 50 ml       |
| <b>Stopping solution [STOP]</b> ,<br>< 0.1 N sodium hydroxide, 40 mM EDTA\                                                                                                                                                                                                         | 15 ml           |
| <b>Substrate (ready-to-use) [pNPP]</b> ,<br>Para-nitrophenylphosphate in solvent free buffer;<br>preservative: < 0.1 % sodium azide                                                                                                                                                | 13 ml           |
| <b>Quality control certificate [INFO]</b> .                                                                                                                                                                                                                                        | 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 1 x 3 ml order no.: IB05051CON for Enterovirus IgA

Control serum 1 x 3 ml order no.: IB05052CON for Enterovirus IgG

Control serum 1 x 3 ml order no.: IB05053CON for Enterovirus IgM

## 6 STORAGE AND STABILITY

| Reagent                                    | Storage                                                                                                                                                         | Stability                            |
|--------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------|
| Microtiter strips<br>(coated with antigen) | Unopened/<br>after opening at 2 – 8 °C in closed aluminum bag with desiccant<br><i>Strips which are not used must be stored dry in the closed aluminum bag.</i> | see expiry date<br><br>6 months      |
| Control sera /<br>Standard sera            | unopened / after opening at 2 – 8 °C                                                                                                                            | see expiry date<br>6 months          |
| Conjugate                                  | unopened / after opening at 2 – 8 °C<br><i>Avoid contamination e.g. by using sterile tips.</i>                                                                  | see expiry date<br>6 months          |
| Dilution buffer                            | unopened / after opening at 2 – 8 °C                                                                                                                            | see expiry date<br>6 months          |
| Washing solution                           | unopened / after opening at 2 – 8 °C<br>working dilution at 2 – 8 °C<br>working dilution at room temperature                                                    | see expiry date<br>2 weeks<br>1 week |
| Substrate                                  | unopened / after opening at 2 – 8 °C                                                                                                                            | see expiry date<br>6 months          |
| Stopping solution                          | unopened / after opening at 2 – 8 °C                                                                                                                            | see expiry date<br>6 months          |

## **7 TEST PROCEDURE**

### **7.1 Evidence of Deterioration**

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. Dilution buffer, washing solution, substrate and stop solution can be used for all IBL-America immunoassays coded IB05xxx irrespective of the lot and the test.

Unopened, all components of this ELISA may, if stored accordingly, be used up to the expiry dates given on the labels. Reagents may not be used after date of expiry.

Dilution or alteration of the reagents may result in a loss of sensitivity.

Avoid exposure of reagents to strong light during storage and incubation. Reagents must be tightly closed after use to avoid evaporation and contamination.

To open the aluminum bag of the microtiter plate please cut off the top of the marked side only, in order to guarantee proper reclosing. Do not use the strips if the aluminum bag is damaged or if the bag with remaining strips and desiccant was not properly reclosed.

Use aseptic techniques when removing aliquots from the reagent tubes to avoid contamination. To avoid false positive results ensure not to contact or splash the top-walls of wells while pipetting conjugate. Take care not to mix the caps of the bottles and/or vials.

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.

Optimum results can only be achieved if the instructions are strictly followed.

The results of this ELISA are only valid if the lot-specific validation criteria on the quality control certificate are fulfilled.

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!

Take care not to damage the inscription (pathogen / antibody class) on the microtiter test strips during washing and aspiration to avoid confusion.

## 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:

#### Enterovirus IgA

|                     |         |                              |
|---------------------|---------|------------------------------|
| $V_1 + V_2 = 1+100$ | add     | 10 $\mu$ l sample            |
|                     | to each | 1000 $\mu$ l dilution buffer |

#### Enterovirus IgG

|                     |         |                                        |
|---------------------|---------|----------------------------------------|
| $V_1 + V_2 = 1+500$ | add     | 10 $\mu$ l sample                      |
|                     | to each | 1000 $\mu$ l dilution buffer (= 1+100) |
|                     |         | 50 $\mu$ l of the first dilution       |
|                     | to each | 200 $\mu$ 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.

## Enterovirus IgM

### 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 IB05998). Rf-absorption is performed by incubation of the samples 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 $\mu$ l | Rf-absorbent    |
|                           | each to | 800 $\mu$ l | dilution buffer |

Samples ( $V_4$ ) must be diluted in this Rf-dilution buffer ( $V_3$ ):

|                     |         |              |                    |
|---------------------|---------|--------------|--------------------|
| $V_4 + V_3 = 1+100$ | add     | 10 $\mu$ l   | sample             |
|                     | each to | 1000 $\mu$ 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  $\leq -20$  °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

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 - control and standard sera must be included. The standard sera should be set up in duplicate.

Do not treat control sera with Rf-absorbent.

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

Conjugates with the same concentration and of the same immunoglobulin class are interchangeable. Avoid contamination of ready-to-use conjugates e. g. by using sterile tips.

#### 7.3.4 Washing Solution

Dilute washing buffer concentrate ( $V_1$ ) 1:30 with aqua dest. to a final volume of  $V_2$ .

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 Enterovirus 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 dilution<sup>1</sup>

IgA/IgM: 1 + 100

IgG: 1 + 500

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** )<sup>2</sup>



Pipette conjugate solution **APC** (100 µl)



INCUBATION 30 Min./ 37 °C  
moist chamber



WASH (4 x 300 µl **DIL** **WASH** )<sup>2</sup>



Pipette substrate solution **pNPP** (100 µl)



INCUBATION 30 Min./ 37 °C  
moist chamber / dark incubation



Pipette stopping solution **STOP** (100 µl)



READ EXTINCTION at 405 nm

<sup>1</sup>Special dilution buffers for the following IBL-America tests:  
Borrelia burgdorferi IgG, IgM, EBV EA IgG, Parvovirus B19 IgM and Hantavirus Puumala IgG, IgM

<sup>2</sup>For 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<br>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).

\* Please note, that under special working-conditions internal laboratory adaptations of the incubation times may be necessary.

## **7.6 Automated Test Procedure**

The automated processing is performed analogous to manual use. Please note, that under special working-conditions internal laboratory adaptations of the 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. IB0xxxCON, 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 Borderline Ranges

The borderline range indicates the range for borderline test results. Values obtained, when testing a sample, which fall below this range indicate a negative test result; values above the borderline range are interpreted positive. In cases where the results are within the borderline range a definitive interpretation of the result is not possible. In such cases, the test should be repeated in parallel with a follow-up sample taken one to two weeks later (serum pair).

# 9 PERFORMANCE CHARACTERISTICS

## 9.1 Sensitivity and Specificity

### IBL-America Enterovirus IgA, IgG and IgM

The IBL-America Enterovirus IgA, IgG and IgM ELISA tests were verified in an internal study. The sensitivity and specificity in both cases exceeded 92%.

## 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 sera of different reactivities 10 times in 10 independent assays performed on 5 different days.

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

**Enterovirus IgA:**

| Sample  | Mean Value<br>(OD) | Intraassay<br>(CV %) | Mean Value<br>(OD) | Interassay<br>(CV %) |
|---------|--------------------|----------------------|--------------------|----------------------|
| Serum 1 | 0.103              | 4.0                  | 0.118              | 10.4                 |
| Serum 2 | 0.358              | 3.9                  | 0.414              | 6.2                  |
| Serum 3 | 1.140              | 2.4                  | 1.347              | 5.1                  |

**Enterovirus IgG:**

| Sample  | Mean Value<br>(OD) | Intraassay<br>(CV %) | Mean Value<br>(OD) | Interassay<br>(CV %) |
|---------|--------------------|----------------------|--------------------|----------------------|
| Serum 1 | 0.326              | 2.0                  | 0.321              | 10.9                 |
| Serum 2 | 0.837              | 2.1                  | 0.876              | 9.6                  |
| Serum 3 | 1.550              | 2.2                  | 1.629              | 8.2                  |

**Enterovirus IgM:**

| Sample  | Mean Value<br>(OD) | Intraassay<br>(CV %) | Mean Value<br>(OD) | Interassay<br>(CV %) |
|---------|--------------------|----------------------|--------------------|----------------------|
| Serum 1 | 0.140              | 4.2                  | 0.153              | 6.1                  |
| Serum 2 | 0.413              | 3.6                  | 0.445              | 3.6                  |
| Serum 3 | 1.233              | 2.0                  | 1.333              | 3.5                  |

## 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 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

- [1] Cello, J., Svennerholm, B. (1994) Detection of enterovirus-specific total and polymeric IgA antibodies in serum using a synthetic peptide or heated virion antigen in ELISA. *J. Med. Virol.* 44, 422-7.
- [2] King, M.L., Shaikh, A., Bidwell, D., Voller, A., Banatvala, J.E. (1983) Coxsackie-B-virus-specific IgM responses in children with insulin-dependent (juvenile-onset; type I) diabetes mellitus. *Lancet* 1, 1397-9.
- [3] Modlin, J. F. (1990) Coxsackieviruses, echoviruses, and newer enteroviruses. In: *Principles and practice of infectious diseases*. Mandel, Douglas, Bernaett (Eds.), 3. Auflage, 1367-83.
- [4] Muir, P., Singh, N. B., Banatvala, J. E. (1990) Enterovirus-specific serum IgA antibody responses in patients with acute infections, chronic cardiac disease, and recently diagnosed insulin-dependent diabetes mellitus. *J. Med. Virol.* 32, 236-42.
- [5] Muir, P. (1992) The Association of Enteroviruses with chronic heart disease. *Med. Virol.* 2, 9-18.
- [6] Pozzetto, B., Gaudin, O. G., Lucht, F. R., Hafid, J., Ros, A. (1990) Detection of immunoglobulin G, M, and A antibodies to enterovirus structural proteins by immunoblot technique in echovirus type 4-infected patients. *J. Virol. Methods* 29, 143-56.
- [7] Reigel, F., Burkhardt, F., Schilt, U. (1985) Cross-reactions of immunoglobulin M and G antibodies with enterovirus-specific viral structural proteins. *J. Hyg. Camb.* 95, 469-81.
- [8] Roth, B., Enders, M., Arents, A., Pfitzner, A., Terletskaia-Ladwig, E. (2007) Epidemiologic Aspects and Laboratory Features of Enterovirus Infections in Western Germany, 2000–2005. *J. Med. Virol.* 79, 956-62.
- [9] Samuelson, A., Skoog, E., Forsgren, M. (1990) Aspects on the serodiagnosis of enterovirus infection by ELISA. *Serodiagn. Immunother. Infect. Dis.* 4, 395-406.
- [10] Samuelson, A., Glimaker, M., Skoog, E., Cello, J., Forsgren, M. (1993) Diagnosis of enteroviral meningitis with IgG-EIA using heat-treated virions and synthetic peptides as antigens. *J. Med. Virol.* 40, 271-7.
- [11] Samuelson, A., Forsgren, M., Johansson, B., Wahren, B., Sällberg, M. (1994) Molecular Basis for Serological Cross-Reactivity between Enteroviruses. *Clin. Diagn. Lab. Immunol.* 1, 336-41.
- [12] Schwarz, T. F. (1994) Coxsackie- und Echoviren. In-vitro Diagnostica Nachrichten: Diagnostische Bibliothek 24, 1-8.
- [13] Searle, K., Dirmeier, D., Metzger, C., Enders, G. (1997) A semi-automated PCR-ELISA as an alternative to virus isolation in cell culture for the routine diagnosis of enterovirus infection. *Clin. Lab.* 43, 659-64.

Manufactured for Immuno-Biological Laboratories Inc.  
(IBL-America)  
8201 Central Avenue, NE, Suite P  
Minneapolis, MN 55432  
Tel: 763-780-2955  
Toll Free: 1-888-523-1246

