DRG® Yersinia IgM (EIA-2568)

Revised 20 Apr. 2010 rm (Vers. 4.0) **USA: RUO**

Please use only the valid version of the package insert provided with the kit.

INTENDED USE

Enzyme immunoassay for the qualitative and quantitative determination of IgM antibodies against Yersinia enterocolitica in human serum and plasma.

SUMMARY AND EXPLANATION

The pathogenic germs Yersinia pestis, Y. pseudotuberculosis, Y. enterocolitica and Y. ruckeri belong to the genus Yersinia as a member of the enterobacteriacea family. All the species of medical importance occur facultatively inside the cells, which leads to the characteristical inflammation of the lymphatic tissue in the course of an illness. Yersinia enterocolitica is taken up orally, and the symptoms in a patient are terminal ileitis as well as diarrhoea. It is difficult to make a separation from appendicitis by differental diagnosis. In the course of a retarded immunological reaction, extraintestinal manifestations like erythema nodosum, uveitis, and arthritis can appear. It has been claimed that the background for a reactive arthritis caused by Yersinia consists in the local synthesis of antibodies in the joints (synovial fluid).
Yersinia can be characterized without problems by standardized bacterial tests, after isolation in a pure culture. Following a human disease, above all the serotypes O3, O8 and O9 are found. Cross-reactivities to Brucella are described and have to be taken into account by the differential diagnosis. The classical serological detection method has always been the Widal test. Further increasingly popular methods are the HAT, the KBR test as well as the ELISA. The enzyme immunoassay is characterized on the one hand side by a high sensitivity, but also by the possibility to differentiate between IgG and IgA/IgM antibodies. The immunoglobulin classes IgA and IgM should be referred to as a criterium of interpretation for an active process, when an arthritic disease is suspected. Because there exists also an immunological similarity with the thyrotropin receptor, the test can also be employed for the confirmation of the autoimmune Graves' disease. As a confirmatory test with the possibility of an identification of separate bacterial proteins as well as for the exclusion of cross-reactivities, the Western Blot method has proved successful.

TEST PRINCIPLE

Solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The wells are coated with antigen. Specific antibodies of the sample binding to the antigen coated wells are detected by a secondary enzyme conjugated antibody (E-Ab) specific for human IgM. After the substrate reaction the intensity of the color developed is proportional to the amount of IgM-specific antibodies detected. Results of samples can be determined directly using the standard curve.

WARNINGS AND PRECAUTIONS

1. For in-vitro use only. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. In case of severe damage of the kit package please contact your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Observe lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.

5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available upon request.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. Avoid contact with Stop solution. It may cause skin irritations and burns.
9. Some reagents contain sodium azide (NaN_3) as preservatives. In case of contact with eyes or skin, flush immediately with water. NaN_3 may react with lead and copper plumbing to form explosive metal azides. When disposing reagents, flush with a large volume of water to avoid azide build-up.
10. All reagents of this kit containing human serum or plasma have been tested and were found negative for anti-HIV I/II, HBsAg and anti-HCV. However, a presence of these or other infectious agents cannot be excluded absolutely and therefore reagents should be treated as potential biohazards in use and for disposal.

STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2-8°C. Keep away from heat or direct sun light. The storage and stability of specimen and prepared reagents is stated in the corresponding chapters.
The microtiter strips are stable up to the expiry date of the kit in the broken, but tightly closed bag when stored at 2–8°C.

SPECIMEN COLLECTION AND STORAGE

Serum, Plasma (EDTA, Heparin)
The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material.

Storage:	2-8°C	-20°C	Keep away from heat or direct sun light.
Stability:	7 d	> 7 d	Avoid repeated freeze-thaw cycles.

MATERIALS SUPPLIED

1 x 12 x 8	MTP	**Microtiter Plate** Break apart strips. Coated with specific antigen.
1 x 14 mL	ENZCONJ IgM	**Enzyme Conjugate IgM** Red colored. Ready to use. Contains: anti-human IgM, conjugated to peroxidase, protein-containing buffer, stabilizers.
4 x 2 mL	CAL A-D	**Standard A-D;** 1; 10; 30; 110 U/mL. Ready to use. Standard A = Negative Control Standard B = Cut-Off Control Standard C = Weakly Positive Control Standard D = Positive Control Contains: IgM antibodies against Yersinia, PBS, stabilizers.
1 x 60 mL	DILBUF	**Diluent Buffer** Ready to use. Contains: PBS Buffer, BSA, < 0.1 % NaN_3.
1 x 60 mL	WASHBUF CONC	**Wash Buffer**, Concentrate (10x) Contains: PBS Buffer, Tween 20.
1 x 14 mL	TMB SUBS	**TMB Substrate Solution** Ready to use. Contains: TMB.
1 x 14 mL	TMB STOP	**TMB Stop Solution** Ready to use. 0.5 M H_2SO_4.
2 x	FOIL	**Adhesive Foil** For covering of Microtiter Plate during incubation.
1 x	BAG	**Plastic Bag** Resealable. For dry storage of non-used strips.

Revised 20 Apr. 2010 rm (Vers. 4.0) **USA: RUO**

MATERIALS REQUIRED BUT NOT SUPPLIED

1. RF Adsorbent (can be ordered separately from DRG under REF KIRF561)
2. Micropipettes (Multipette Eppendorf or similar devices, < 3% CV). Volumes: 5; 50;100; 500 µL
3. Calibrated measures
4. Tubes (1 mL) for sample dilution
5. 8-Channel Micropipettor with reagent reservoirs
6. Wash bottle, automated or semi-automated microtiter plate washing system
7. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 600-650 nm)
8. Bidistilled or deionised water
9. Paper towels, pipette tips and timer

PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. Use a pipetting scheme to verify an appropriate plate layout.
5. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
6. Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
7. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.

PRE-TEST SETUP INSTRUCTIONS

⚠ In order to avoid interferences of specific IgG and rheumatoid factors, patient sera should be treated with RF absorbent (REF KIRF561).

Preparation of Components

⚠ The contents of the kit for 96 determinations can be divided into 3 separate runs.
The volumes stated below are for one run with 4 strips (32 determinations).

Dilute/ dissolve	Component		Diluent	Relation	Remarks	Storage	Stability
20 mL	Wash Buffer	200 mL	bidist. water	1:11	Warm up at 37°C to dissolve crystals, if necessary. Mix vigorously.	2-8°C	8 w
1 mL	RF-Absorbent	20 mL	Diluent Buffer	1:21	Incubate ≥ 1 min.	2-8°C	8 w

Dilution of Samples

Sample	to be diluted	with	Relation	Remarks

Serum / Plasma	generally	Diluent Buffer (+ RF-Absorbent)	1:101	e.g. 5 µL + 500 µL

Samples containing concentrations higher than the highest standard have to be diluted further.

Samples with RF-Absorbent: Do not incubate >20 min to avoid adsorption of specific antibodies.
Pretreated samples may be turbid.

TEST PROCEDURE

1. Pipette **100 µL** of each **Standard and diluted sample** into the respective wells of the Microtiter Plate. In the qualitative test only Standard B is used.
2. Cover plate with adhesive foil. Incubate **60 min** at **18-25°C.**
3. Remove adhesive foil. Discard incubation solution. Wash plate **3 x** with **300 µL** of **diluted Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
4. Pipette **100 µL** of **Enzyme Conjugate** into each well.
5. Cover plate with new adhesive foil. **Incubate 30 min** at **18-25°C**.
6. Remove adhesive foil. Discard incubation solution. Wash plate **3 x** with **300 µL** of **diluted Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
7. For adding of Substrate and Stop Solution use, if available, an 8-channel Micropipettor. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution. Use positive displacement and avoid formation of air bubbles.
8. Pipette **100 µL** of **TMB Substrate Solution** into each well.
9. Incubate **20 min** at **18-25°C** in the dark (without adhesive foil).
10. Stop the substrate reaction by adding **100 µL** of **TMB Stop Solution** into each well. Briefly mix contents by gently shaking the plate. Color changes from blue to yellow.
11. **Measure** optical density with a photometer at **450 nm** (Reference-wavelength: 600-650 nm) within **60 min** after pipetting of the Stop Solution.

QUALITY CONTROL

The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. All standards/controls must be found within the acceptable ranges as stated on the QC Certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls.

In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

It is recommended to participate at appropriate quality assessment trials

CALCULATION OF RESULTS

The evaluation of the test can be performed either quantitatively or qualitatively.

Qualitative evaluation

The Cut-off value is given by the optical density (OD) of the Standard B (Cut-off standard). The Cut-off index (COI) is calculated from the mean optical densities of the sample and Cut-off value. If the optical density of the sample is within a range of 20 % around the Cut-off value (grey zone), the sample has to be considered as borderline. Samples with higher ODs are positive, samples with lower ODs are negative.

For a quantification, the Cut-off index (COI) of the samples can be formed as follows:

$$COI = \frac{\text{OD Sample}}{\text{OD Standard B}}$$

Quantitative Evaluation

The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 Parameter Logisitcs or Logit-Log.

For the calculation of the standard curve, apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used).

The concentration of the samples can be read directly from the standard curve.

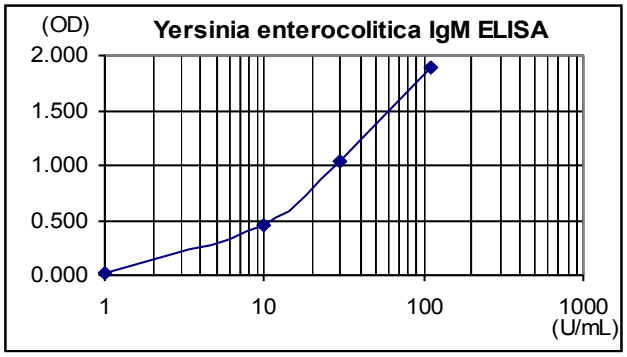
The initial dilution has been taken into consideration when reading the results from the graph. Results of samples of higher predilution have to be multiplied with the dilution factor.

Samples showing concentrations above the highest standard have to be diluted as described in PRE-TEST SETUP INSTRUCTIONS and reassayed.

Typical Calibration Curve

(Example. Do not use for calculation!)

Standard	U/mL	Mean OD
A	1	0.023
B	10	0.463
C	30	1.030
D	110	1.899



INTERPRETATION OF RESULTS

Method	Range	Interpretation
Quantitative (Standard curve)	< 8 U/mL	negative
	8 - 12 U/mL	equivocal
	> 12 U/mL	positive
Qualitative (Cut-off Index, COI)	< 0.8	negative
	0.8 – 1.2	equivocal
	> 1.2	positive

The results themselves should not be the only reason for any therapeutical consequences. They have to be correlated to other clinical observations and diagnostic tests.

EXPECTED VALUES

In an in-house study, apparently healthy subjects showed the following results:

Ig Isotype	n	Interpretation		
		positive	equivocal	negative
IgM	56	0 %	0 %	100 %

LIMITATIONS OF THE PROCEDURE

Specimen collection has a significant effect on the test results. See SPECIMEN COLLECTION AND STORAGE for details.

For cross-reactivities, see PERFORMANCE.

Azide and thimerosal at concentrations > 0.1 % interfere in this assay and may lead to false results.

The following blood components do not have a significant effect (+/- 20 % of expected) on the test results up to the concentrations stated below:

Hemoglobin	8.0 mg/mL
Bilirubin	0.3 mg/mL
Triglyceride	5.0 mg/mL

PERFORMANCE

Analytical Specificity (Cross Reactivity)	No cross-reactivities were found to:		Helicobacter	
Precision	Mean (U/mL)	CV (%)		
Intra-Assay	36	10.3		
Inter-Assay	27	10.3		
Linearity	Range (U/mL)	Serial dilution up to	Range (%)	
	3.2 – 109	1/8	66 - 114	
Recovery	74 – 98 %	% Recovery after spiking (n = 3)		
Method Comparison versus ELISA	Rel. Sensitivity	> 95 %		
	Rel. Specificity	> 95 %		

DRG® Yersinia IgM (EIA-2568)

Revised 20 Apr. 2010 rm (Vers. 4.0) **USA: RUO**

Symbols used with DRG Assays

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità