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Yersinia enterocolitica IgA ELISA

RUO

For Research Use Only – Not for Use in Diagnostic Procedures

REF **DEYERA0990**

Σ **96**

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1. INTENDED USE

The Yersinia enterocolitica IgA ELISA is intended for the determination of IgA class antibodies against antigens of the 70 kb virulence plasmid of Yersinia enterocolitica in human serum or plasma (citrate, heparin).

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2. PRINCIPLE OF THE ASSAY

The immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader.

3. MATERIALS

3.1. Reagents supplied

1. **SORB MT Microtiterplate**: 12 break-apart 8-well snap-off strips coated with specific antigen; in resealable aluminium foil.
2. **SAM DIL Sample Dilution Buffer**: 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap; ≤ 0.0015 % (v/v) CMIT/MIT (3:1).
3. **STOP SOLN Stop Solution**: 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
4. **WASH SOLN 20x Washing Buffer (20x conc.)**: 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap; 0.2 % (w/v) 5-Bromo-5-nitro-1,3-dioxane.
5. **ENZ CONJ Conjugate**: 1 bottle containing 20 mL of peroxidase labelled antibody to human IgA in phosphate buffer (10 mM); coloured violet; ready to use; black cap.
6. **SUB TMB TMB Substrate Solution**: 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap.
7. **CAL C Positive Control**: 1 vial containing 2 mL control; coloured yellow; ready to use; red cap; ≤ 0.02 % (v/v) MIT.
8. **CAL B Cut-off Control**: 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; ≤ 0.02 % (v/v) MIT.
9. **CAL A Negative Control**: 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; ≤ 0.0015 % (v/v) CMIT/MIT (3:1).

*For hazard and precautionary statements see 11.1
For potential hazardous substances please check the safety data sheet.

3.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

3.3. Materials and Equipment needed

- ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing Microtiterplates
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

4. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

5. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

5.1. Microtiterplate

The break-apart snap-off strips are coated with specific antigen. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

5.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 mL Washing Buffer + 190 mL distilled water. The diluted buffer (1x Washing Buffer) is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37 °C e.g., in a water bath. Mix well before dilution.

5.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the TMB Substrate Solution turns into blue, it may have become contaminated and should be thrown away.

6. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise, they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.

Heat inactivation of samples is not recommended.

6.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with Sample Dilution Buffer. Dispense 10 µL sample and 1 mL Sample Dilution Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

7. ASSAY PROCEDURE

Please read the instruction for use carefully before performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems, we recommend increasing the washing steps from three up to five and the volume of 1x Washing Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 11. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of 1x Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark**. A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

7.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.
If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!
Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.
Bichromatic measurement using a reference wavelength of 620 nm is recommended.
Where applicable calculate the mean absorbance values of all duplicates.

8. RESULTS

8.1. Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

- **Substrate Blank:** Absorbance value **< 0.100**
- **Negative Control:** Absorbance value **< 0.200 and < Cut-off**
- **Cut-off Control:** Absorbance value **0.150 – 1.300**
- **Positive Control:** Absorbance value **> Cut-off**

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

8.2. Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43

Cut-off = 0.43

8.3. Results in Units [U]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[Units = U]}$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ U (Units)

8.4. Evaluation of Results

10 U	Cut-off
> 11 U	Antibodies against the antigen are present.
9 – 11 U	Antibodies against the antigen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks.
< 9 U	The sample contains no antibodies against the antigen.

9. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.
For further information about the specific performance characteristics please contact Demeditec.

9.1. Precision

Evaluation of precision of the assay was performed according to "CLSI. Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition. CLSI document EP05-A3. Wayne, PA: CLSI; 2014".

9.1.1. Single-Site Study

The precision study was performed at a single site. Samples were run in 4 replicates, two times per day for 12 days for a total of 96 results.

Sample	n	Mean (U)	Repeatability		Between Run		Within Day		Between Day		Within lab	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	96	25.03	1.3174	5.3	1.3537	5.4	1.8889	7.5	0.8140	3.3	2.0568	8.2
2	96	10.01	0.7302	7.3	0.7926	7.9	1.0777	10.8	0.4165	4.2	1.1554	11.5
3	96	6.81	0.6429	9.4	0.0912	1.3	0.6494	9.5	0.3696	5.4	0.7472	11.0
4	96	2.82	0.4468	15.8	0.1387	4.9	0.4678	16.6	0.2013	7.1	0.5093	18.0

9.1.2. Multisite-Study

The precision study was performed at three different sites. Samples were run in 5 replicates, once a day for 5 days for a total of 75 results.

Sample	n	Mean (U)	Repeatability		Within Site		Reproducibility	
			SD	CV	SD	CV	SD	CV
1	75	23.10	1.0818	4.7	1.8064	7.8	2.0543	8.9
2	75	9.98	0.6410	6.4	0.9572	9.6	1.3167	13.2
3	75	6.13	0.4330	7.1	0.6231	10.2	0.6688	10.9
4	75	2.14	0.2894	13.5	0.4488	20.9	0.6611	30.8

9.2. Specificity

The specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 97.32 % (95 % confidence interval: 92.37 % - 99.44 %).

9.3. Sensitivity

The sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 93.94 % (95 % confidence interval: 79.77 % - 99.26 %).

9.4. Interferences

The assay was evaluated for interferences according to guideline EP07-A3 ("Interference Testing in Clinical Chemistry" from the CLSI). Three samples, covering the relevant measuring range, were spiked with high levels of interferents and were tested along with the unspiked sample. The following table shows the tested substances added to samples at the indicated concentrations.

Interferent	Concentration tested
Albumin	60 mg/mL
Bilirubin, unconjugated	0.4 mg/mL
Bilirubin, conjugated	0.4 mg/mL
Cholesterol	4 mg/mL
Hemoglobin	10 mg/mL
Triglycerides	15 mg/mL

No significant interference effect was found for all tested substances.

9.5. Cross Reactivity

A minimum of 5 samples with antibody activities to potentially cross-reacting parameters (Adenovirus, Borrelia burgdorferi, Brucella, Campylobacter jejuni, Chlamydia pneumoniae, Enterovirus, Epstein-Barr virus, Helicobacter pylori, Parvovirus B19, Salmonella typhi) or samples positive for ANA or rheumatoid factors, and samples from pregnant women were tested to evaluate the cross reactivity of the assay. Positive findings were additionally analyzed with a reference assay. The results are shown in the following table.

Antigen	Samples tested	Number of positive samples	
		Yersinia enterocolitica IgA	reference assay
Adenovirus	15	3	0
Antinuclear antibodies (ANA)	15	5	1
Borrelia burgdorferi	10	1	1
Brucella	10	6	5
Campylobacter jejuni	9	1	0
Chlamydia pneumoniae	12	4	2
Enterovirus	7	2	2
Epstein-Barr virus (EBV)	13	4	0
Helicobacter pylori	13	2	1
Pregnancy samples	13	2	0
Rheumatoid factor (RF)	11	3	0
Salmonella typhi	9	3	1

Cross-reactions with antibodies against Borrelia burgdorferi, Chlamydia pneumoniae, Epstein-Barr virus, Helicobacter pylori, Enterovirus, Adenovirus, Campylobacter jejuni, Salmonella typhi, Brucella as well as with samples from pregnant women or samples positive for rheumatoid factor (RF) or antinuclear antibodies (ANA) cannot be excluded

10. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

11. PRECAUTIONS AND WARNINGS

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results. The manufacturer is not liable for any results by visual analysis of the samples.
- For research use only.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or Microtiterplates of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
- For further internal quality control each laboratory should additionally use known samples.

11.1. Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to **Fehler! Verweisquelle konnte nicht gefunden werden.**).
Therefore, the following hazard and precautionary statements apply.

Warning	H317	May cause an allergic skin reaction.
	P261	Avoid breathing spray.
	P280	Wear protective gloves/ protective clothing.
	P302+P352	IF ON SKIN: Wash with plenty of soap and water.
	P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
	P362+P364	Take off contaminated and Wash it before reuse.

Reagents may contain 5-Bromo-5-nitro-1,3-dioxane (refer to **Fehler! Verweisquelle konnte nicht gefunden werden.**)
Therefore, the following hazard and precautionary statements apply.

Warning	H315	Causes skin irritation.
	H317	May cause an allergic skin reaction.
	P280	Wear protective gloves/ protective clothing.
	P302+P352	IF ON SKIN: Wash with plenty of soap and water.
	P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
	P337+P313	If eye irritation persists: Get medical advice/attention.

Further information can be found in the safety data sheet

11.2. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

12. REFERENCES

1. Granfors K, Isomäki H, Essen R von, Maatela J, Kalliomäki JL, Toivanen A. 1983. Yersinia antibodies in inflammatory joint diseases. Clin Exp Rheumatol 1:215–218.

2. Strieder TGA, Wenzel BE, Prummel MF, Tijssen JGP, Wiersinga WM. 2003. Increased prevalence of antibodies to enteropathogenic Yersinia enterocolitica virulence proteins in relatives of patients with autoimmune thyroid disease. Clin Exp Immunol 132:278–282. doi:10.1046/j.1365-2249.2003.02139.x

SYMBOLS USED WITH DEMEDITEC ASSAYS

Symbol	English	Deutsch	Française	Espanol	Italiano
CE	European Conformity	CE-Konformitäts-kennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	utilisation Diagnostic in vitro	Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungs-zwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Note warnings and precautions	Warnhinweise und Vorsichtsmaßnahmen beachten	Avertissements et mesures de précaution font attention	Tiene en cuenta advertencias y precauciones	Annoti avvisi e le precauzioni
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservacion	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	Distributed by	Vertrieb durch	Distribution par	Distribución por	Distribuzione da parte di
V<x>	Version	Version	Version	Versión	Versione
	Single-use	Einmalverwendung	À usage unique	Uso único	Uso una volta