Equine Neutrophil Myeloperoxidase ELISA

ENGLISH (en)

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ISO15223	MEDICAL DEVICES SYMBOL
	STORAGE TEMPERATURE LIMITATION
LOT	BATCH CODE
	USE BY
	CONSULT OPERATING INSTRUCTIONS
	MANUFACTURED BY
REF	CATALOGUE NUMBER

	SYMBOLS (EDMA recommendations)
	number of determinations (96)
CAL	Calibrator
CONTROL	Control serum
SORB MTP	Microtiterplate
CONJ AP	PA conjugate
DIL	Sample Diluent
SUBS pNPP	Substrate pNPP
BUF WASH 10X	Washing solution to be diluted ten-fold
STOP SOLN	Blocking reagent

ENGLISH

IMMUNOENZYMOMETRIC ASSAY FOR THE QUANTATIVE DETERMINATION OF NEUTROPHIL MYELOPEROXIDASE IN HORSE EDTA PLASMA

E-IV-96 - 96 Assays

CLINICAL APPLICATIONS

Myeloperoxidase (MPO; 1.11.1.7) is a heme-containing peroxidase enzyme specific of primary (azurophilic) granules of neutrophils.
MPO participates to the pathogen destruction in the phagocytosis vacuole of the neutrophils. MPO is specifically responsible for the production of a powerful oxidant agent, hypochlorous acid (HOCl) synthetized from chloride anion (Cl^-) and hydrogen peroxide.
In the horse, the field of research in inflammatory diseases (e.g. colic, septic choc, articular pathologies) is growing. In a number of inflammatory pathologies, the activation of the neutrophils is excessive and becomes uncontrolled. Activated neutrophils released MPO and reactive oxygen species in the extracellular medium. Hence the measurement of MPO can be used as an index of neutrophil activation.

PRINCIPLE OF THE ASSAY

96 microwells are coated with purified rabbit polyclonal anti-Neutrophil MPO. MPO, when present in the sample, will bind to the solid phase.
After removing the unbound fraction, i.e. non specific protein by a washing process, the immune complexes are detected by alkaline phosphatase conjugated to guinea pig polyclonal anti-Neutrophil MPO.
After removing the unbound conjugate by another washing step, the chromogen/substrate is added, which turns from clear to yellow if the antibody being tested for is present. The intensity of the yellow color, directly proportional to the amount of MPO present in the sample, is measured using a spectrophotometer with a 405 nm filter.
Sample concentrations are read from a calibration curve.

REAGENTS PROVIDED WITH THE KIT

- Reagents are sufficient for 96 wells.
- Store the reagents at 2-8°C.
- The expiry date of each reagent is shown on the vial label.

1. **Coated microtiterplate :** a microtiter plate of 96 breakable wells coated with rabbit polyclonal anti-MPO. Keep unused wells at 2-8°C, protected from moisture in the provided aluminium bag and carefully sealed.
   Store at 2 – 8°C

2. **Calibrator:** 1 vial of diluted Equine Neutrophil MPO lyophilized in buffer. Reconstitute with 2ml of distilled water. The exact concentration value is stated on the quality control sheet.
   For storage, immediately prepare aliquots and store at –20°C.
   Contains NaN_3 < 0,1% (w/w).

3. **Control plasma :** 2 vials with validated amount of Equine MPO lyophilized in buffer. Reconstitute with 1ml of distilled water.
   For storage, immediately prepare aliquots and store at –20°C.
   Contains NaN_3 < 0,1% (w/w).

4. **Conjugate-AP :** 1 vial of 12 ml of polyclonal Anti-MPO antibodies coupled to phosphatase alkaline (AP).
   Ready for use.

5. **Diluent Buffer:** 1 vial (50ml) of serum sample dilution buffer. Ready for use.
   Contains NaN_3 < 0,1% (w/w).

6. **Washing solution :** 1 vial of 50 ml of phosphate buffer. Bring the vial content to 500 ml (final volume) with distilled water. The diluted washing solution is stable for 1 month at 2 - 8°C.
   If undissolved crystals are detected, put them back into solution by placing the vial at 37°C for few minutes.

7. **Chromogenic substrate :** 1 vial (15ml) of p-Nitrophenylphosphate (PNPP) diluted in buffer containing preservatives. Ready for use.

8. **Blocking reagent :** 1 vial (15ml) of diluted buffer containing preservative (NaN_3 < 0,1% (w/w). Ready for use.

KIT REAGENTS

Reagents	Quantity	Physical state
Microtiterplate	1 x 96	Ready for use
Calibrator	1 x 2 ml	Lyophilized
Control plasma	2 x 1 ml	Lyophilized
AP conjugate	1 x 12 ml	Ready for use
Diluent Buffer	1 x 50 ml	Ready for use
Washing Solution	1 x 50 ml	Concentrated 10 x
Chromogenic substrate	1 x 15 ml	Ready for use
Blocking Reagent	1 x 15 ml	Ready for use

MATERIAL REQUIRED BUT NOT SUPPLIED

- Adjustable, automatic micropipettes with disposable tips.
- Graduated cylinder.
- Aspiration pump or automated well washing device.
- Microtiterplate spectrophotometer able to read absorbances within a 0-3.0 OD interval at 405 and 620nm.
- A refrigerator (2-8°C).
- Microtiterplate incubator (37°C).
- Distilled Water.

WARNINGS AND PRECAUTIONS

In order to obtain reproducible results, the following rules must be observed:

- Do not mix reagents from different lot number or from other manufacturers.
- Do not freeze kits.
- Strict adherence to the specific time and temperature of incubations is recommended for accurate results.
- Allowing the micro well strip and reagents to equilibrate to room temperature before used.
- Do not use reagents beyond their expiry date.
- Incomplete or inefficient washing will cause poor precision and high background.
- Use thoroughly clean glassware.
- Use distilled water, stored in clean containers.
- Avoid any contamination among samples; for this purpose, disposable tips should be used for each sample and reagent.
- Microbial contaminated sample or specimen containing heavy, visible particulate should not be used.
- Cross contaminations of reagents or samples could cause false results. Use a clean, fresh, disposable pipette tips for each reagent or specimen manipulation.
- Do not expose the substrate to light storage or incubation.
- Follow exact incubation times. Dispense Chromogen and blocking reagent in no more than 3-4 minutes ; Dispense the two reagents in the same sequence.

In order to avoid personal and environmental contamination, the following precautions must be observed:

- Use disposable gloves while handling potentially infectious material and performing the assay.
- Do not pipette reagents by mouth.
- Do not smoke, eat, drink or apply cosmetics during the assay.
- Animal origin materials are used in this kit. These are provided with sanitary certificate. However, no known test can guarantee that such material does not contain any of these infectious agents or other infectious agents. This product must be considered as potentially infectious and handled with care.
- Disposable ignitable material must be incinerated; disposable non-ignitable material must be sterilized in autoclave for at least 1 hour at 121°C. Liquid wastes must be added with sodium hypo chlorite at a final concentration of 3%. Let the hypochlorite act for at least 30 minutes. Liquid wastes containing acid must be neutralized with appropriate amounts of base, before treating with sodium hypochlorite.

- Chromogen and Blocking Reagent should be handled with care. Avoid contact with skin, eyes and mucous membranes. In case of accident rinse thoroughly with running water.
- Avoid splashing and aerosol formation; in case of spilling, wash carefully with a 3% sodium hypochlorite solution and dispose of this cleaning liquid as potentially infectious waste.
- Some reagents contain sodium azide as preservative; to prevent build-up of explosive metal azides in lead and copper plumbing, reagents should be discarded by flushing the drain with large amounts of water.

SPECIMEN COLLECTION AND PREPARATION

The Equine Neutrophil Myloperoxidase ELISA is intended to use with EDTA plasma samples.
Avoid using hemolized, lipemic or contaminated samples.
Thoroughly mix thawed specimens before assay and avoid repeated freeze/thawing cycles, which may cause loss of antibody activity and give erroneous results.
Plasma may be assayed immediately, within 12 hours if stored at 2°-8°C. For long-term storage, samples should be store d frozen at -20°C or lower.
Other biological fluids can be used as sample. But we recommend that each laboratory establish its own normal range.

ASSAY PROCEDURE

- Allow reagents and samples to warm up to room temperature.
- Mix samples by inversion before use.

SAMPLE PRE-DILUTION

It is recommended to dilute 1/41 the plasma samples with the diluent buffer : 25µl sample + 1000µl diluent buffer.

PROCEDURE

1. Allocate the wells of microtiterplate for calibrators, control plasma and samples. Leave the well A1 for the substrate blank.
2. Performed serial dilutions of the MPO calibrator solution with the Diluent Buffer.
3. Dispense 100µl of each calibrator, controls and diluted samples into the wells.
4. Incubate overnight at 4°C after covering the plate with the plate sealer.
5. Wash the wells 4 times with 300µl of diluted washing solution. Aspirate all liquid from the wells.
6. Dispense 100µl of AP conjugate into the wells except well A1.
7. Incubate for 120 minutes at 37°C after covering the plate with a plate sealer.
8. Wash the wells 4 times with 300µl of diluted washing solution. Aspirate all liquid from the wells.
9. Pipette 100µl of substrate-chromogen solution to all wells.
10. Incubate 30 minutes at 37°C. Avoid direct light exp osure (NB. The incubation time begins after the first substrate addition).
11. Pipette 100µl of blocking reagent to all wells.
12. Read the absorbance of the wells 405nm (620nm as reference). Reading must be completed within 20 minutes from the end of the assay.

ASSAY SCHEME

Wells / Reagents	Blank	Calibrator	Control plasma	Samples
Diluted Calibrator	----	100 µl	----	----
Control Serum	----	----	100 µl	----
Samples	----	----	----	100 µl
- Incubate: overnight in a refrigerator (2-8°C) - Aspirate and wash: 4 X 300 µl.				
AP-Conjugate	------	100 µl	100 µl	100 µl
- Incubate: 120' at 37°C - Aspirate and wash: 4 x 300 µl				
Chrom-Substrate	100 µl	100 µl	100 µl	100 µl
- Incubate: 30' at 37°C				
Blocking Reagent	100 µl	100 µl	100 µl	100 µl
- Read : 405 nm and 620 nm as reference.				

CALCULATION OF RESULTS

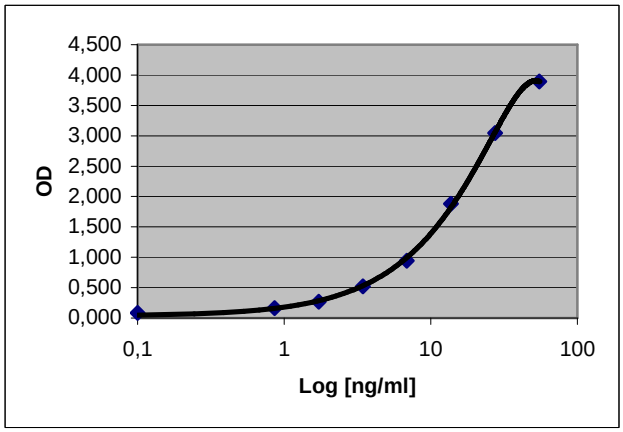
Draw a calibration curve on millimetric graph paper, by plotting the calibrators concentration (x-axis, log) against the absorbance obtained for each standard (y-axis).
If a computer is used to calculated the results, the data can be fitted to the appropriate equation : sigmoid, polynomial (Cubic Spline).
Since the dilution factor has not been considered in the standard curve calibration, the MPO concentrations needs to be corrected by the appropriate dilution factor. Any sample values above the standard range should be more diluted and retested.

EXAMPLE OF CALCULATION

The values shown below must be considered as an example and must not be used in place of experimental data.

Description	Concentration (ng/ml)	O.D
Calibrator 7	55	3.892
Calibrator 6	27.5	3.048
Calibrator 5	13.75	1.881
Calibrator 4	6.88	0.945
Calibrator 3	3.44	0.521
Calibrator 2	1.72	0.269
Calibrator 1	0.86	0.163
Calibrator 0	0.0	0.082
Control plasma 1	4.4	0.741
Control plasma 2	15.2	1.928

TYPICAL CALIBRATION CURVE



NORMAL VALUES

The normal values determined are only indicative since they may be affected by various agents. We recommend that each laboratory establishes its own normal range.
According to the data available, mean plasma concentration for healthy horses is 181,8 ± 64,74 ng/ml.

LIMITATION OF THE PROCEDURE

- The results obtained from this or any other diagnostic kits should be used and interpreted only in the context of an overall clinic picture.
- Special care is needed to prevent contamination of the substrate by the conjugate. The substrate should be uncolored or pale yellow, a more intense yellow indicate that the reagent has been contaminated and must be discarded. Substrate degradation is increased at temperatures above 25°C.

PERFORMANCES OF THE ASSAY

SENSITIVITY

Analytical sensitivity

The sensitivity was calculated based upon the calibration curve and expressed as the minimal dose showing a significant difference from the Zero Calibrator (mean value + 2 S.D.). This dose is 0,1 ng/ml.

PRECISION

Intra-assay

Sample	Mean	±	S.D.	C.V. (%)	Replicates no.
	(ng/ml)				
1	3,7	±	0,1	4%	18
2	6,3	±	0,3	5%	18
3	19,4	±	1,3	7%	18

Inter-assay

Sample	Mean	±	S.D.	C.V. (%)	Replicates No.
	(ng/ml)				
1	4,6	±	0,5	11%	5
2	8,3	±	0,8	9%	5
3	23,9	±	2,7	11%	5

ACCURACY

Recovery Test

Samples (p/mol/l)	Measured (ng/ml)	Expected (ng/ml)	Recovery (%)
S1	15,3		
S1 + Cal 1	8,1	7,6	107%
S1 + Cal 2	8,4	8,3	101%
S1 + Cal 3	8,8	9,0	98%
S1 + Cal 4	9,5	10,4	91%
S1 + Cal 5	10,8	13,1	82%

Parallelism Test

Dilution	Measured	Expected	Recovery
	(ng/ml)	(ng/ml)	(%)
1/1	38,0		
1/2	19,2	19,0	101%
1/4	9,4	9,5	99%
1/8	5,1	4,7	108%
1/16	2,6	2,4	108%

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