




User's Manual

Feline COOMBS

ELISA

The feline COOMBS test is designed to detect immune mediated erythrocyte destruction in EDTA or Heparin blood

REF F-COOMBS

 96

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Gebruik alleen de juiste versie van het protocol dat meegestuurd wordt met de kit.

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

Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Arbeitsanleitung.

Si prega di usare la versione valida dell'inserto del pacco a disposizione con il kit.

Por favor, se usa solo la version valida de la metodico técnico incluido aqui en el kit.

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1. Introduction

The Feline COOMBS test, also called direct anti-globulin test, is designed to detect immune mediated erythrocyte destruction which occurs in auto-immune hemolytic anemia, Feline systemic erythematosis and in some cases with infections and neoplastic disorders. Hemolysis in these diseases is caused by the erythrocytes being coated with antibody (IgG, IgM) and/or complement components (C3). This process makes the erythrocytes weak and will cause leakage of the cell-membrane and finally complete destruction of the red blood cells.

Erythrocytes for testing can be obtained in two ways and are listed in order of preference:

1. Blood collected in Ethylene-Diamine-Tetra-Acetic-acid (EDTA).
2. Blood collected in heparin.

Note: Blood from a healthy non-anemic cat should always be evaluated along with blood from the anemic dog. Blood from the normal dog will serve as a negative control

2. Intended use of the test kit

All cats with anemia (including that caused by intra-vascular and extra-vascular hemolysis) of unknown origin are reasonable candidates for evaluation by COOMBS-testing. This testkit is suitable for 12 tests.

3. Contents

- 12 x 8 round bottomed plate
- 1 x 2,5ml Feline COOMBS-reagent (2,0 IU)
- 1 x 18 ml Feline COOMBS buffer (green cap)
- 1 x plastic cover seal
- 1 x User's manual

Supplies needed (not included)

- Validated precision pipettes
- Pipette tips and clean containers/tubes (EVL)

4. Handling and storage of specimens

The kit should be stored at 4°C.

An open packet should be used within 10 days.

Samples may be used fresh or may be kept frozen below -20°C before use.

Positive and negative controls may be stored after reconstitution in aliquots at -20°C and used until the expiry date.

Avoid repeated freezing and thawing as this increases non-specific reactivity.

5. Sample preparation

Washing of erythrocytes:

1. Remove 20 µl of blood (III) and add 1 ml Phosphate Buffered Saline (PBS, **not provided**) or normal saline solution. (**Note: Other solutions may influence results**).
2. Mix the erythrocytes and PBS. Centrifuge the mixture (Standard tabletop centrifuge for 5 minutes at room temperature, 1000-1200 rpm) and remove the supernatant. Re-suspend the blood in 1 ml of PBS.
3. Repeat the washing procedure in the previous step three more times. This provides for four washings of the erythrocytes.
4. At the end of the last wash, remove the supernatant and re-suspend the pellet in 1 ml of PBS. This provides a ±2% suspension of erythrocytes

6. Test protocol see figure 2

Before starting this test read “preparations”

1. Take the 96 well round bottom plate and number consecutively 4 wells (for example; A1, B1, C1 and D1).
2. Add 100µl Feline COOMBS-buffer to wells 2, 3 and 4.
3. Add 200µl of Feline COOMBS-reagent (2,0 IU) to well number 1, transfer 100µl of this mixture to well number 2. Mix well 2 and then transfer 100µl to well number 3. Mix well 3 and then remove and discard 100µl.
4. At the end of this process, well 1 should contain 100µl of 2,0 IU of the Feline COOMBS-reagent, well 2 100µl of 1.0 IU, well 3 100µl of 0,5 IU and well 4 should contain PBS.
5. Steps 1 to 4 should be repeated for each sample to be tested, including the **negative** control (III, **Note**).
6. Add 100µl of washed erythrocytes (VI) from the dog to be tested to wells 1 through 4. Mix gently.
7. Incubate for 30 minutes at 37°C

7. Precautions

- Handle all biological material as though capable of transmitting infectious diseases.
- Do not pipette by mouth.
- Do not eat, drink, smoke or prepare foods, or apply cosmetics within the designated working area.
- TMB substrate (buffer B) is toxic by inhalation, through contact with skin or when swallowed; observe care when handling substrate.
- Do not use components past the expiry date and do not mix components from different serial lots.
- Optimal, results will be obtained by strict adherence to this protocol. Careful pipetting and washing throughout this procedure are necessary to maintain precision and accuracy.
- Each well is ultimately used as an optical cuvette. Therefore, do not touch the under-surface of the microtiter plate and protect it from damage and dirt.

8. Interpretation of the test results

To evaluate the contents of each well there are three possibilities:











1. Put the 96 well round bottom plate under an angle of 45 degrees and let it rest for 5-10 minutes. When after 10 minutes the dog erythrocytes are packed in a point the test may be considered positive. The test is negative when there is no point showing.
2. Evaluate the contents of each well by placing the 96 well round bottom plate under a phase-contrast microscope (40-100X magnification is suitable).
3. Evaluate the contents of each well by placing a small amount of the solution on a slide and viewing with a phase-contrast microscope (40-100X magnification is suitable).

Negative: Erythrocytes are not clumped or agglutinated.

Positive: There are clumps and large aggregates of erythrocytes. The clumps should not be present in the control cells.

Remark: Occasional clumps (3 or 4 per slide) may occur in test and control erythrocytes and should be disregarded. Hemolysis should be considered a positive reaction.

9. Symbols used with EVL 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
	European Conformity	CE-Konformitätskennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
	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	Mindesthaltbarkeitsdatum	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

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