Uricult® Vet CLED EMB

Instructions for use

Intended use

A urine culture-paddle method for diagnosing urinary tract infections (UTI). Uricult Vet CLED EMB is intended for veterinary use only.

Principles of the procedure

The Uricult Vet CLED EMB culture-paddle system is based on two culture media for the detection of microbes causing urinary tract infections in animals. One side of the plastic paddle is covered with green CLED medium and the other with reddish EMB medium. The CLED medium is intended for determining the total microbial count. The EMB media is intended for detecting gram-negative* microbes.

Reagents

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Typical formulation

CLED medium		EMB medium	
Peptone	10.0 g/l	Peptone	10.0 g/l
Meat extract	3.0 g/l	Lactose	5.0 g/l
Lactose	10.0 g/l	Sucrose	5.0 g/l
L-Cystine	0.13 g/l	Dipotassium Phosphate	2.0 g/l
Bromthymol blue		Eosin Y	
		Methylene Blue	

Storage

Uricult Vet CLED EMB is stored at 45...77°F (7...25°C) in the package provided. Protection from light, air and temperature fluctuations will ensure product stability to the expiration date. A small amount of condensed water (<0.5 mL) may accumulate on the bottom of the tube during storage. This water does not affect the performance of the test nor does it shorten the shelf-life.
Avoid drafts and storage near heat-generating appliances. **Do not allow to freeze**. The expiration date is marked on the box.

Warnings and precautions

- Uricult Vet is for ***in vitro* diagnostic** use only.
- Do not use the product beyond the expiration date marked on the box.
- Wear protective clothing and disposable gloves while handling samples or tests, and wash hands thoroughly afterwards.
- Do not use the product if you detect discoloration or dehydration of the culture media, separation of the culture media from the plastic paddle or evidence of microbial growth on the culture media.
- Because any colonies growing on the Uricult Vet CLED EMB culture media are actual or potential pathogens, do not touch the growth.
- To avoid contamination**, do not touch the surfaces of uninoculated culture media.

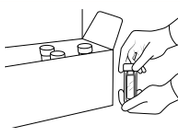
Sample collection and preparation

Ideally, urine for bacterial culture should remain in the bladder for four hours prior to sampling. The veterinarian can take a sample via catheterization or cystocentesis as required. Cystocentesis is preferred method when taking sample from animals suspected of having a UTI[1,2].
The urine should be inoculated onto the Uricult Vet CLED EMB culture-paddle immediately after collection. The paddle should then at once be returned into its protective tube and the cap closed.
If the urine sample needs to be stored prior to inoculation onto Uricult Vet CLED EMB, it should be kept refrigerated at 36…46 °F (2...8°C) no longer than 24 hours.
Antibiotics may affect the result of the Uricult Vet CLED EMB test. Therefore, the test should not be performed until 48 hours after the final dose of antibiotics.

Inoculated paddle may be
a) incubated immediately or
b) stored at 36...46°F (2...8°C) for up to 48 hours or
c) transported to a laboratory for incubation and/or interpretation

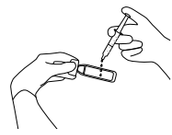
Procedure

1



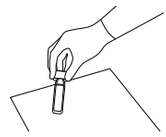
Unscrew the paddle from the tube without touching the surfaces of the culture media.

2



Holding Uricult Vet CLED EMB by the cap, inoculate the culture media by placing drops of urine from the syringe onto both sides while tilting the paddle from side to side to ensure complete wetting.

3



Allow excess urine to drain from the paddle and blot the last drops on absorbent paper.

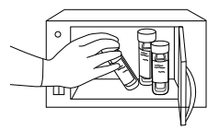
4

Return the paddle into the tube and close the tube.

5

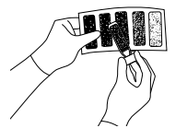
Fill in the patient label and attach it to the tube.

6



To incubate*** Uricult Vet CLED EMB, place the tube upright in an incubator (97°F ± 4°F / 36°C ± 2°C) for 16–24 hours. The tube may also be sent to a laboratory for incubation and interpretation.

7



To obtain a colony forming units per milliliter (CFU/ml), remove the slide from the tube and compare the colony density with the model chart provided in the kit.

Note:

1. Negative cultures may be incubated for additional 24 hours at 97°F ± 4°F (36°C ± 2°C) to ensure that slow growing microbes are detected.
2. The inoculated slide may be incubated immediately or stored or transported to a laboratory for incubation and interpretation. Storage or transportation should not exceed 48 hours at 45...77°F (7…25°C) for 16 – 24 hours. If the slide has been stored or transported for up to 48 hours, only the presence of growth and the colony count should be recorded from it; the color reaction may be atypical.
3. The inoculated slide may be incubated at room temperature for 24 – 72 hours, after which positive cultures may be interpreted.

Quality control

Quality control tests are performed on each lot of Uricult Vet CLED EMB culture-paddle at the time of manufacture.

Results' interpretation

Following the incubation of an inoculated culture-paddle, the presence of bacteria may be evidenced by visible signs of colony growth on the culture medium. Separate, distinct areas of the bacterial growth on the agar surface are called "colonies". Since the formation of a colony results from the natural multiplication of a single bacterial cell, and since the agar surfaces on Uricult Vet CLED EMB culture-paddle are uniform in dimension, the number of colonies can indicate the "colony count" which is the approximate number CFU/ml of urine.
At the end of the incubation period, check the agar surfaces on both sides of the Uricult Vet CLED EMB culture-paddle for colony growth. If all visible bacterial colonies are similar in characteristics, compare the number of colonies on each side of the

Contact information

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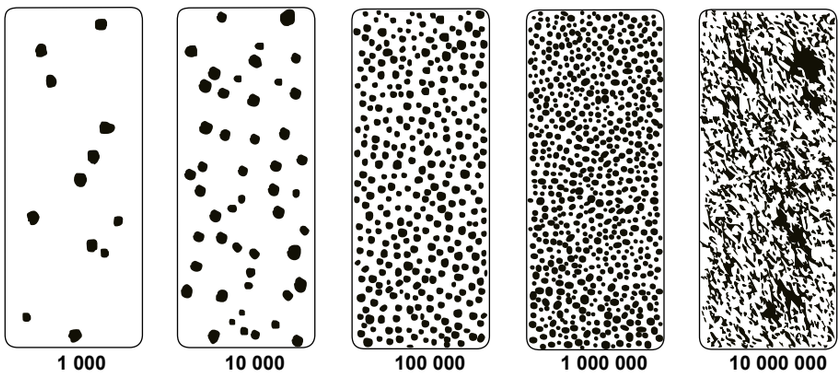
Colony Density Chart



CFU/ml	1 000	10 000	100 000	1 000 000	10 000 000
	10^3	10^4	10^5	10^6	10^7

The colony count is determined from the originally green CLED media by matching the colony density with the model chart it most closely resembles. Low volume of urine may cause it to spread unevenly on the different media. In these cases, it is advisable to evaluate the growth on both media. If there is a significant difference in the number of colonies on each side, the side with the greater number should be used for determining the colony count.
It is important to compare the number of colonies, not their size.
A growth consisting of several species of bacteria is termed mixed flora and is most likely due to contamination of the urine sample.

culture-paddle. If there is a significant difference in the number of colonies on each side, the side with the greater number should be used for determining the "colony count". In making the determination, the number of colonies and not the dimensions of the individual colonies should be considered. Match the "colony density" on the agar surface with the printed example its most closely resembles on the Colony Density Chart. If the character¬istics of visible colonies on either side of the culture-paddle differ enough to indicate more than one type of bacteria, the colony count match-up procedure should be performed and reported for each organism.

The colonies on the agar may also be observed at this time for the morphology and the media color reactions which may also be used for presumptive identification of the bacterial growth.

Presumptive identification
Staphylococcus aureus:
Gram-positive
Growth of yellow colonies with a color change towards yellow on the CLED media.

Enterococcus faecalis:
Gram-positive
Growth of yellow colonies with a color change towards yellow on the CLED media and with a growth of pin point colonies on the EMB media.

Proteus vulgaris:
Gram-negative
Growth of translucent colonies with a color change towards blue on the green CLED media. Growth of colorless colonies on the EMB media.

Escherichia coli:
Gram-negative
Growth of yellow colonies with a color change towards yellow on the CLED media and growth of purple or metallic green colonies on the EMB media.

	Gram (+) or (–)		
Organism	**CLED**	**EMB**	**Gram**
S. aureus	G	TNG	+
E. faecalis	G	G	+
P. vulgaris	G	G	–
E. coli	G	G	–

G = Growth TNG = Typically No Growth

Confluent growth
"Confluent growth" (complete coverage of the agar surfaces) can be interpreted as a negative result. Therefore, any culture media surfaces that appear negative should be examined under a reflecting light; absence of reflection suggests confluent growth. A bright light also facilitates the detection of pinpoint colonies.
A change in color of the CLED media is also an indication of growth.
A growth consisting of several species of bacteria is termed mixed flora and is most likely due to contamination of the urine sample.

Further confirmation of a negative culture may be obtained by gently swabbing part of the agar surface. Bacterial growth will be evident on the swab itself, and by a difference in appearance be-tween the swabbed and unswabbed portions of the agar surface.

Limitations of procedure
Uricult Vet is capable of detecting urinary bacterial concentrations between 10^3 and 10^7 CFU/ml. The colony density chart allows the determination of colony counts to the nearest power of 10. When the method is used according to instructions, the colony counts show a 99% correlation with the conventional pour plate method[3].

Expected values
High bacterial number in a properly collected and cultured sample indicates bacterial UTI[1].

Method of sampling	Significant colony count CFU/ml[4]	
	Dog	Cat
Bladder aspiration	≥ 1 000	≥ 1 000
Catheterization	≥ 10 000	≥ 1000

Performance characteristics
Uricult Vet ● CLED medium[5]

Number of samples	140
Sensitivity	100%
Specificity	99%
PPV	98%
NPV	100%
Reference method	Pour plate (Nutrient agar)

Disposal
- Dispose of contents according to national and local law.
- All patient samples and used components should be handled and disposed of as potentially infectious material.
- Materials of the components:
  Paper: Instructions for use, patient labels
  Cardboard: Kit box
  Plastic: Tubes, caps and dipslides
- When used in accordance with Good Laboratory Practice, good occupational hygiene and the instructions for use, the reagents supplied should not present a hazard to health.

Glossary
*** gram-positive and gram-negative:**
Gram staining is the most important method of grouping bacteria, allowing bacteria to be classified either as gram-positive (staining blue) or gram-negative (staining red). The differential staining is due to differences in the structure of the bacterial cell wall.
**** Contamination** denotes the presence of microbes in the urine that were introduced by the sampling procedure.
***** Incubation** denotes growing of culture-paddles in an incubator.

References

1. Bartges JW. Diagnosis of urinary tract infections. Vet Clin Small Anim. 2004; 34: 823-933.
2. Weese JS, Blondeau JM, Boothe D *et al.* Antimicrobial use guidelines for treatment of urinary tract disease in dogs and cats: antimicrobial guidelines working group of the international society for companion animal infectious diseases. Veterinary Medicine International 2011; article ID: 263768. doi:10.4061/2011/263768
3. McAllister TA, Arnell GC, Barr W, & Kay P. Assessment of plain dipslide quantitation of bacteriuria. Nephron 1973; 11: 111–122.
4. Lulich JP, Osborne CA. Bacterial urinary tract infection. In: Ettinger SJ, Feldman EC, editors. Textbook of veterinary internal medicine. 4th edition. Philadephia: WB Saunders, 1999: 1775-1788.
5. Arneil GC, McAllister TA, & Kay P. Detection of bacteriuria at room temperature. Lancet 1970; January 17: 119–121.

Explanation of symbols