FLEXICULT® VET URINARY TEST

SSI DIAGNOSTICA

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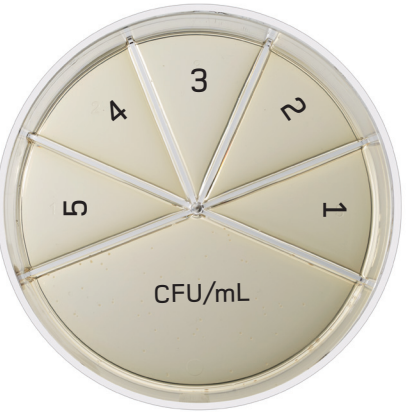
Flexicult® Vet is a 24 hour culture test designed for diagnosing urinary tract infections (UTI) in dogs and cats by:

- identification and quantitative enumeration of bacteria in the urine
- predicts the susceptibility of the bacteria against 5 antibiotics

The golden standard for diagnosis of UTI is urine culturing. Beside this it is recommended that antibiotic treatment is based on the susceptibility of the bacteria.

Flexicult® Vet is designed as an agar plate with a large compartment for the quantitative determination (CFU/mL) and 5 small compartments each containing a specific antibiotic.



Flexicult® Vet contains the following antibiotics:

1 = Ampicillin (predictor for amoxicillin)
2 = Amoxicillin/clavulanate
3 = Oxacillin (indicator for methicillin resistant Staph. pseudintermedius (MRSP))
4 = Enrofloxacin
5 = Trimethoprim/sulfamethoxazole (predictor of potentiated sulfonamides with trimethoprim)

The ease of use makes Flexicult® Vet particularly well suited for use in the veterinary clinic as well as in laboratory settings. For *in vitro* veterinary diagnostic use only.

Background

Criteria for urinary tract infection

The incidence of urinary tract infections is higher among dogs than cats and is reported very differently in the literature. The diagnosis of UTI is made by demonstrating bacteria in the urine (bacteriuria).

If a dog or a cat presents with one or more of the below mentioned symptoms and uro-pathogens are detected in a concentration of > 10^3 CFU/mL in a urine sample collected by cystocentesis then there is indication of a urinary tract infection.

Uncomplicated bacterial UTI are those where no underlying structural, neurologic or functional abnormality is identified.

Symptoms of UTI at dogs/cats:

- Frequent urination
- Painful urination
- Malodorous urine
- Cloudy urine
- Hematuria

Principle

Flexicult® Vet is designed as an agar plate with elevated sides and divided into compart-ments with partitions and a lid. There is one large compartment for quantitative analysis and 5 small compartments each containing a specific antibiotic. The compartments are marked with numbers 1 to 5 in the bottom of the plate. Since the agar contains a fixed quantity of the different antibiotics the plate must be read with growth/no growth.

The agar plate is briefly flooded with the urine sample from the dog or cat. This brings the urine sample in contact with the agar and any bacteria in the urine sample will stick to the agar surface. The urine is poured off and the Flexicult™ Vet plate is incubated with the bottom facing upwards. After incubation overnight at 35 °C the individual bacteria will have grown into visible colonies in the large compartment. Bacteria will not appear in the antibiotic compartments in which they are susceptible to the relevant antibiotic.

Sample collection

The urine sample should preferably be collected as sterile as possible by cystocentesis. By this technique normal microflora from the animals urinary tract are avoided. If cystocentesis is not possible catherization or collection of urine from a midstream void can be used.

The fresh urine sample should be applied on Flexicult® Vet within 30 min. after collection.

Urine samples with added boric acid **cannot** be used in Flexicult® Vet since the acid will affect the growth of certain bacteria and thereby the antibiotic result.

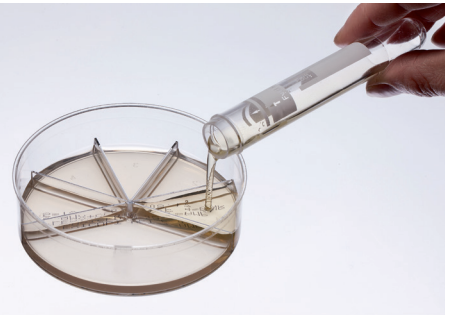
Procedure for large volume samples (> 1 mL)

1. The urinary sample is poured briefly (3 - 5 sec.) over the agar. If the urine sample does not cover the total agar surface in all compartments the plate is tilted so that urine from the large counting compartment floods the small compartments containing antibiotics.

2. The remaining urine is poured off so that urine having been in contact with the small antibiotic compartments don't come in contact with the counting compartment. Let any excess of urine drip from the plate – ex. by tapping it against the waste container.

3. Apply the lid and incubate Flexicult® Vet with the bottom facing upwards at 35°C in 18-24 hours.

4. The plate is read the next day.



Procedure for small volume samples (< 1 mL)

1. There has to be at least 0.8 mL of urine.

2. Apply 0.1 mL (2-3 drops)* urine in each of the 5 small compartments.

3. Apply 0.3 mL (6-8 drops)* of urine in the large compartment.

4. Tilt the plate gently for 3-5 sec. to ensure that the surface of agar is covered with urine in each compartment.

5. Let any excess of urine drip from the plate – ex. by tapping it against the waste con tainer.

6. Apply the lid and incubate Flexicult® Vet with the bottom facing upwards at 35°C in 18-24 hours.

7. The plate is read the next day.

* A drop is defined as 0.05 mL. Use ex. a steri sterile pipette to apply the drops.

Diagnostics

The agar plate

The agar plate contains a chromogenic substrate, which causes bacterial colonies or the agar to appear with different colours depending on the bacterium species (see table 2). More than 98 % of all *E. coli* become red/reddish brown while *Klebsiella* sp. become dark blue. Most uropathogenic bacteria become stained in different colors making it possible to determine the species (ex. *E. coli*, *Ps. aeruginosa*) or the family (ex. *Klebsiella* sp., *Proteus* sp., *Enterococcus* sp.).

Examples of Flexicult® Vet inoculated with E. coli (left) and E. faecalis (right).

Bacteria

Infections caused by *E. coli* are the most common, accounting for about half of all organisms isolated from the urine of both dogs and cats. The remaining infections are diagnosed with Gram-positive cocci (staphylococci, enterococci and streptococci) or Gram-negative rods (*Proteus* sp., *Klebsiella* sp., *Pseudomonas* sp. etc.) with a variation in frequency. Infections with more pathogens at the same time are seen in app. 20% of the urine samples.

Resident flora

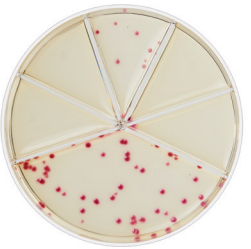
Although urine in the bladder is normally sterile, urine that passes through the distal urogenital tract often becomes contaminated with resident flora. Therefore, interpretation of bacteria in urine collected by catheterization or voiding is often difficult to interpret. If there is doubt as to whether there is growth of an uropathogen or normal flora a new urine sample should be examined. Alternatively a new urine sample or the cultivated Flexicult® Vet plate could be sent for analysis at a microbiological laboratory.

Reading of Flexicult® Vet

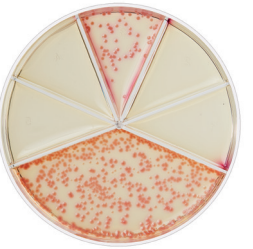
After incubation over night (18-24 hours) the agar plate is examined for growth of bacteria.

Counting compartment

The more bacteria in the urine sample, the more bacteria will grow in the counting compartment. A comparison of the colony density in the counting compartment with the examples below with *E. coli* gives an indication of the number of bacteria in the urine sample (CFU/mL urine).



E. coli 10^3 CFU/mL



E. coli 10^4 CFU/mL

E. coli 10^5 CFU/mL

The shown *E. coli* is sensitive to ampicillin, amoxicillin/clavulanate, enrofloxacin and trimethoprim/sulfamethoxazole since there is no growth in these antibiotic compartments. The *E. coli* is resistant towards the last antibiotic, oxacillin.

Table 1. Limits for significant bacteriuria (CFU/mL) (modified from Bartges et al., 2004) [1]

Collection method	Dog	Cat
Cystocentesis[a]	≥ 10^3 CFU/mL	≥ 10^3 CFU/mL
Catheterization	≥ 10^4 CFU/mL	≥ 10^3 CFU/mL
Midstream voiding and catheterization[b]	≥ 10^5 CFU/mL	≥ 10^3 CFU/mL

[a] A lower number of bacteria in the urine can be significant and represent an infection.
[b] Even at these high limits there is a risk of false positive results. Therefor culturing of voiding is not recommended.

From the pictures on page 11-20 and the limits in Table 1 it is concluded if there is growth of an uropathogen and if the number of CFU/mL is causing significant bacteriuria. If growth is seen in the compartments with antibiotics the density (CFU/mL) is compared with the density in the counting compartment.

Animals with repeating urinary tract infections should always have the urine sample collected by cystocentesis.

If there is growth of several species of bacteria the quantity for each bacterium is assessed.

- If there is one pathogenic bacteria in a mixture with normal flora the normal flora must be disregarded.
- If there is one dominating pathogen the reading of both the counting and the antibiotic compartments are done only for this pathogen.
- If there are more than two uropathogens the sample cannot be evaluated and a new urine sample should be taken.

Size and colours

E. coli and *Proteus* sp. will grow well with large colonies, i.e. a diameter of 2-4 mm. *Klebsiella* sp. and *Enterobacter* sp. will grow with even larger, fat colonies. Enterococci and streptococci will grow with small colonies (0.5-1 mm) and staphylococci a little larger (1-2 mm).

Identification of the bacteria is assisted by the colour of the colonies and the colour of the agar according to the table 2 below.

Table 2. Usual colony size, colony colour and agar colour for common urinary tract pathogens.

Bacteria	Colony size	Colony colour	Agar colour
E. coli	Large	Red/redbrown	-
Klebsiella sp.	Large, fat	Dark blue/purple*	-
Enterobacter sp.	Large	Dark blue/purple*	-
Proteus sp.	Large	Light brown	Brown
Proteus vulgaris	Large (swarm)	Green/brown	Brown
Ps. aeruginosa	Large	Greyish white/greenish	Greenish
E. faecalis	Small	Green/green blue	-
E. faecium	Small	Green/grey	-
Staph. pseudintermedius	Small	White/rosa	-
Strep. canis	Small	Offwhite	-

* In some cases with heavy growth the colour of the bacteria can be reddish.

Resistance

If there is no growth at all in one of the antibiotic compartments the bacteria is considered to be susceptible (S) towards the specific antibiotic.

Table 3 show the natural and acquired resistance for the uropathogens towards the antibiotics in Flexicult® Vet.

Natural resistance towards ampicillin is found at *Klebsiella* sp., *Enterobacter* sp., *P. vulgaris* and *Ps. aeruginosa*. *Enterobacter* sp. and *Ps. aeruginosa* are naturally resistant towards amoxicillin/clavulanacid. Besides this *Ps. aeruginosa* is naturally resistant towards trimethoprim/sulfamethoxazole. *Enterococci* sp. must always be regarded as resistant towards trimethoprim/sulfamethoxazole, even if the bacteria are read as susceptible on Flexicult® Vet.

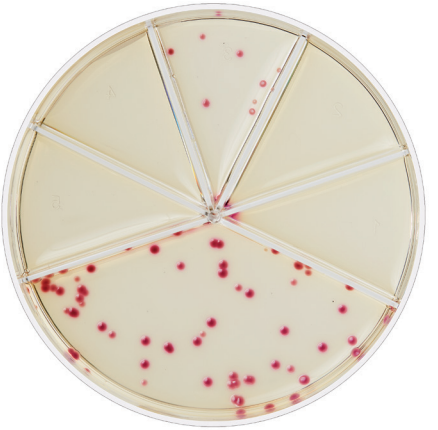
Table 3. List of expected susceptibiity pattern.

***R** indicates natual resistance, R indicates often resistant, S indicates usually susceptible.*

Bacteria	Ampicillin	Amoxicillin/ clavunate	Oxacillin	Enrofloxacin	Trimethoprim/ sulfamethoxazole
E. coli	S	S		S	S
Klebsiella sp./*Enterobacter* sp.	**R/R**	S/**R**		S/S	S/R
Proteus sp.	S	S		S	S
Proteus vulgaris	**R**	S		S	S
Ps. aeruginos	**R**	**R**		S	**R**
E. faecalis	S	S		S	**R**
E. faecium	R	S		R	**R**
Staph. pseudintermedius	R	S	S (R if MRSP)	S	S
Strep. canis	S	S		S	S

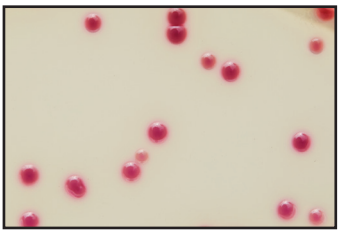
E. coli

E. coli are large Gram-negative, often motile rods.



The bacteria grows with large red/reddish brown colonies.

10^3 CFU/mL



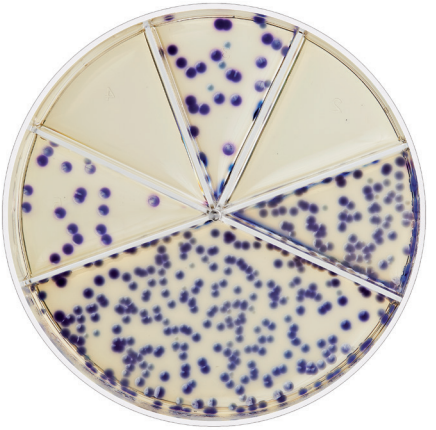
Close up picture of E. coli

10^5 CFU/mL

The pictures show examples of an *E. coli* in different concentrations susceptible towards ampicillin, amoxicillin/clavulanate, enrofloxacin and trimethoprim/sulfamethoxazole.

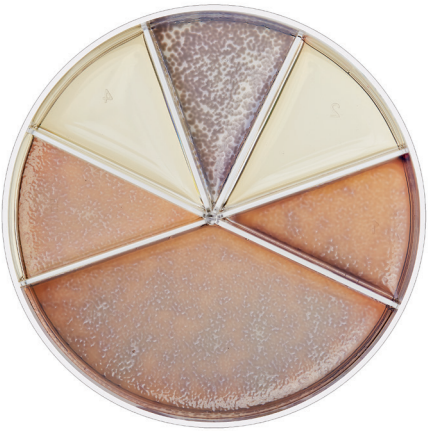
Klebsiella sp. and Enterobacter sp.

Klebsiella sp. are large Gram-negative, non-motile rods. *Enterobacter* sp. are large Gram-negative, motile rods.

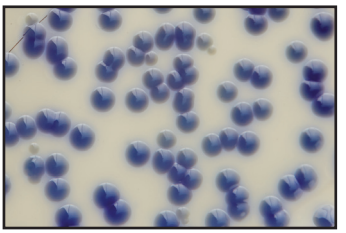


10^3 CFU/mL

Klebsiella sp. grows with large dark blue/dark purple colonies.



10^5 CFU/mL



Close up picture of Klebsiella sp.

The pictures show examples of a *Klebsiella* sp. in different concentrations susceptible towards amoxicillin/clavulanate and enrofloxacin.
REMEMBER: *Klebsiella* sp. and *Enterobacter* sp. should always be read as resistant towards ampicillin/clavulanate.

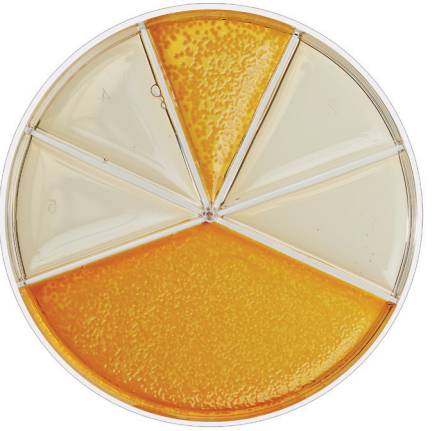
Proteus sp.

Proteus sp. are large Gram-negative motile rods.

10^3 CFU/mL

The bacteria grow with large light brown/ brown colonies. The agar will be brown around the bacterium.

Proteus sp. cleaves urea into ammonia making the urine alkaline. This makes ex. calciumphosphate precipitate resulting in formation of stones in the urinary tract.



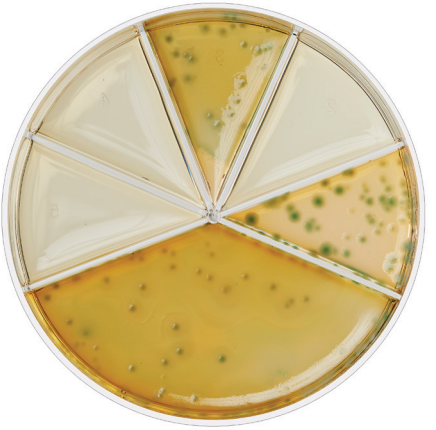
10^5 CFU/mL

Close up picture of P. mirabilis

The pictures show examples of a *Proteus mirabilis* in different concentrations susceptible towards ampicillin, amoxicillin/clavulanate, enrofloxacin and trimethoprim/sulfamethoxazole. *P. mirabilis* is often susceptible towards ampicillin whereas *P. vulgaris* is resistant.

Proteus vulgaris

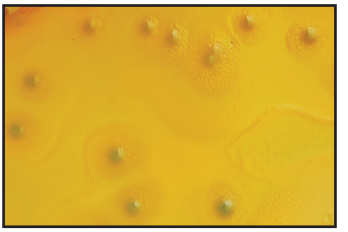
Proteus vulgaris are large Gram-negative motile rods.



10^3 CFU/mL

The bacteria grow with large green brownish colonies. The agar will be brown around the bacterium.

Proteus sp. cleaves urea into ammonia making the urine akaline and precipitation of ex. calciumphosphate. The result can be stones in the urinary tract.



Close up picture of P. vulgaris

10^5 CFU/mL

The pictures show examples of a *P. mirabilis* in different concentrations susceptible towards amoxicillin/clavulanate, enrofloxacin and trimethoprim/sulfamethoxazole.

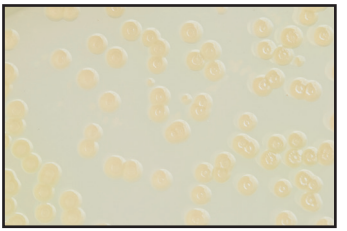
Pseudomonas aeruginosa

Ps. aeruginosa are thin Gram-negative motile rods.

10^3 CFU/mL

The bacteria grows with large greyish white/ greenish colonies. Often the agar will have a greenish color and the plate will have a sweet odour.

Beside this the colonies often will have a metallic sheer.



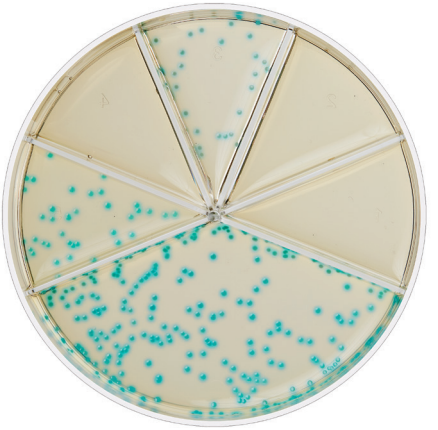
Close up picture of Ps. aeruginosa

10^5 CFU/mL

The pictures show examples of a *Ps. aeruginosa* in different concentrations susceptible towards enrofloxacin and naturally resistant towards all other antibiotics.

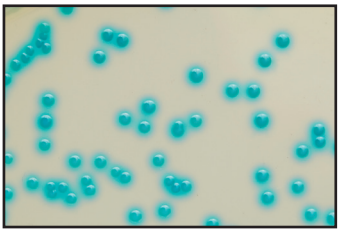
Enterococcus faecalis

E. faecalis are Gram-positive cocci in short and long chains.

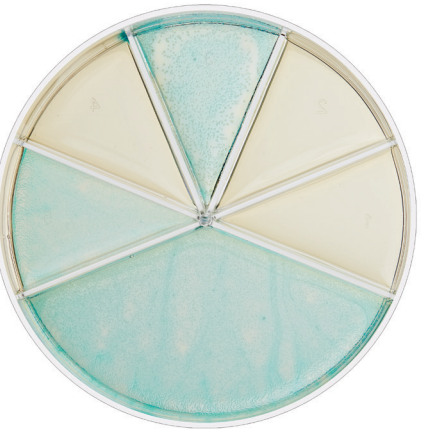


The bacteria grows with small green/ greenish blue colonies.

10^3 CFU/mL



Close up picture of E. faecalis



10^5 CFU/mL

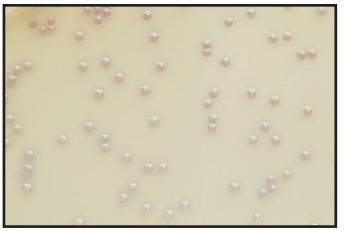
The pictures show examples of an *E. faecalis* in different concentrations susceptible towards ampicillin, amoxicillin/clavulanate and enrofloxacin.
REMEMBER: *E. faecalis* should always be interpreted as resistant towards trimethoprim/ sulfamethoxazole even if there is no growth in compartment no. 5.

Enterococcus faecium

E. faecium are Gram-positive cocci in short and long chains.

10^3 CFU/mL

The bacteria grows with small green/ grey colonies.



Close up picture of E. faecium

10^5 CFU/mL

The pictures show examples of an *E. faecium* in different concentrations susceptible towards ampicillin and amoxicillin/clavulanate.
REMEMBER: *E. faecium* should always be interpreted as resistant towards trimethoprim/ sulfamethoxazole even if there is no growth in compartment no. 5.

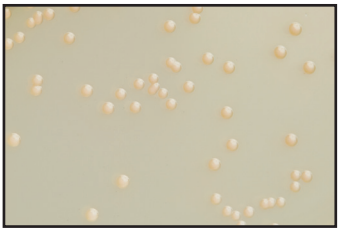
Staphylococcus pseudintermedius

S. pseudintermedius are Gram-positive cocci in cluster.

10^3 CFU/mL

The bacteria grows with small white/rose colonies.

10^5 CFU/mL



Close up picture of S. pseudintermedius

The pictures show examples of an *S. pseudintermedius* in different concentrations susceptible towards all antibiotics.

Streptococcus canis

S. canis are group G beta-haemolytical Gram-positive cocci.

The bacteria grows with small off-white colonies.

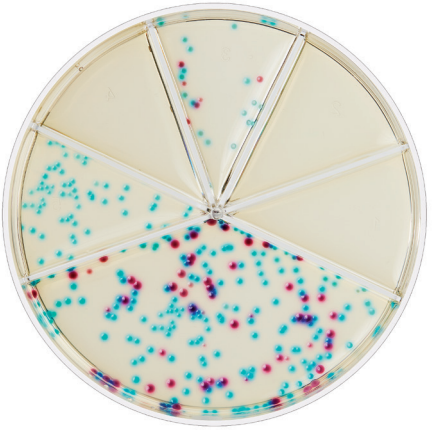
10^3 CFU/mL

Close up picture of S. canis

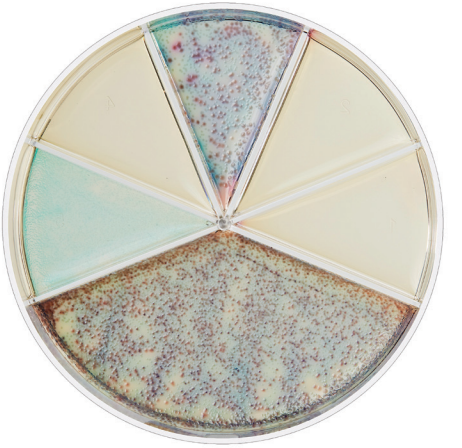
10^5 CFU/mL

The pictures show examples of an *S. canis* in different concentrations susceptible towards all antibiotics.

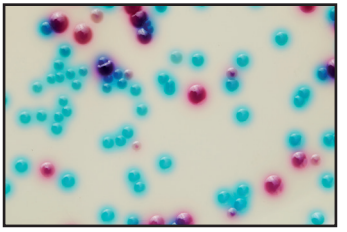
Mixed bacteria (ex.)



Mix of 10^3 CFU/mL *E. coli* and 10^3 CFU/mL *E. faecalis.*



Mix of 10^5 CFU/mL *E. coli* and 10^5 CFU/mL *E. faecalis*



Close up picture of mixed E. faecalis og E. coli

The reading of the plate should be done for each pathogen.
E. coli is susceptible towards ampicillin, amoxicillin/clavulanate, enrofloxacin and trimethoprim/sulfamethoxazole. *E. faecalis* is susceptible towards ampicillin, amoxicillin/clavulanate and enrofloxacin.

Notes

Notes

General information

References

1. Bartges, J. W. 2004. Diagnosis of urinary tract infections. Vet Clin Small Anim. 34: 923-933
1. Guardabassi et al. Optimization and evaluation of Flexicult® Vet for detection, identification and antimicrobial susceptibility testing of bacterial uropathogens in small animal veterinary practice. Acta Vet Scand (2015) 57:72

Storage and shelf life

Flexicult® Vet must be stored in the refrigerator (2-8°C). The agar plates should be stored in the cardboard packaging protected against light until use.

Expiration date is printed on the label of the cardboard packaging and on the bottom of each plate.

Disposal

After use Flexicult® Vet may contain infectious material. Handle and discard all waste as infectious waste.

Ordering

Item no. 97543 Flexicult® Vet 3 pcs.

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