VetoRapid

FOR THE EASY IDENTIFICATION OF BACTERIA

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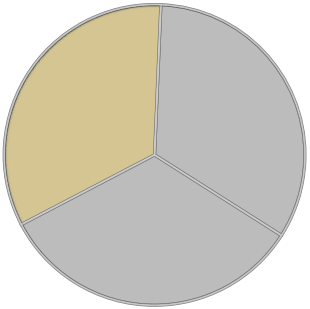
Vetorapid is a diagnostic tool that has been designed to aid in the easy identification of the most common bacteria found in mastitic milk samples. It is not intended to replace a high quality diagnostic laboratory but is a useful first line tool in basic identification of the main pathogens that cause clinical mastitis; *E.coli*, Staphylococci and Streptococci[1].

A university of Glasgow trial found that Vetorapid provided a rapid preliminary identification of five common causes of bovine mastitis (*Escherichia coli*, *Staphylococcus aureus*, coagulase-negative staphylococci, *Streptococcus uberis* and *Enterococcus* species) under field conditions[2].

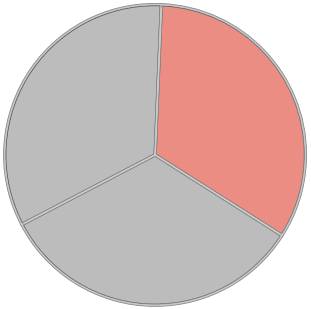
Introducing Vetorapid

Vetorapid dish compartments for bacterial identification:



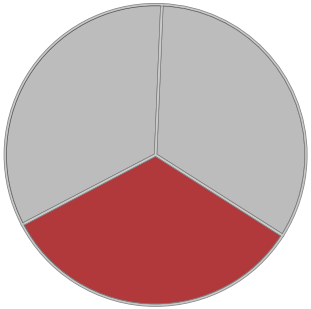
Section 1

Selective for Gram negative bacteria



Section 2

Selective for staphylococci



Section 3

Selective for streptococci and enterococci

How to correctly take a sample for Vetorapid testing

1. Take a clean milk sample from the affected quarter - See milk sampling procedure below for details
2. Place the sample in the fridge for storage
3. Prepare your plate in a clean area and take a sterile swab or a loop to dip into the milk sample

NB. Plates should be dry. If there is condensation on the agar, let it dry in the incubator before use.

4. Spread the milk sample on the agar plate, using the zig-zag technique
5. Label the plate and incubate at 35-37°C with the lid on (lid must be facing downwards)
6. Do the next sample the same way, with a new plate. Only one sample should be analysed per plate
7. Results within 18-36 hours*
8. Follow the diagnostic table, sector by sector.

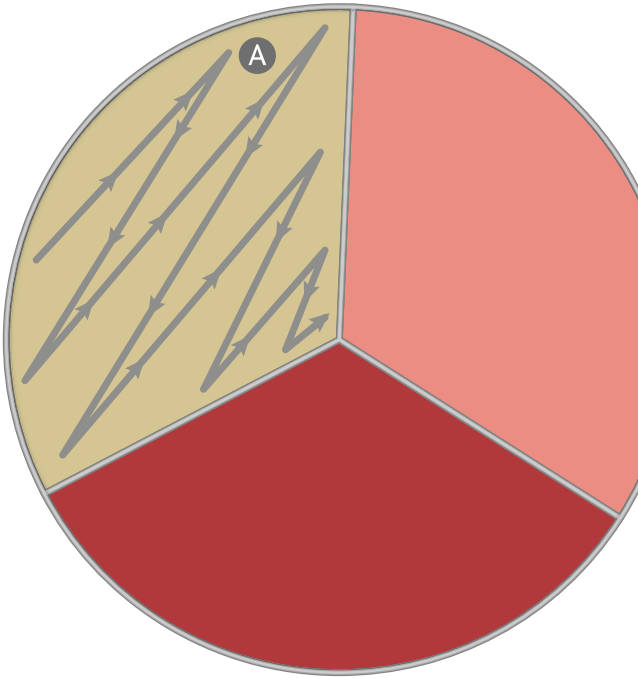
Taking a milk sample on farm:

How to apply the milk sample to the Vetorapid plate

We recommend the use of a loop (metal loop or disposable loop) to apply the milk sample to the Vetorapid plate as results are easier to analyse with this method.

- single colonies are easier to detect and there is better differentiation between possible growth and cloudiness caused by the smeared milk
- plate the milk as shown in the picture A. Do this for all three sections.

(N.B. Sterile cotton swabs can also be used but can cause cloudiness making it harder to differentiate between this and possible growth; however this method can improve sensitivity).



Incubation of Vetorapid

Once the sample has been applied to the Vetorapid plate, label and incubate it at 35-37oC with the lid on facing downwards.

Results should show within 18-36 hours*.
Most colonies are detectable within 24 hours.

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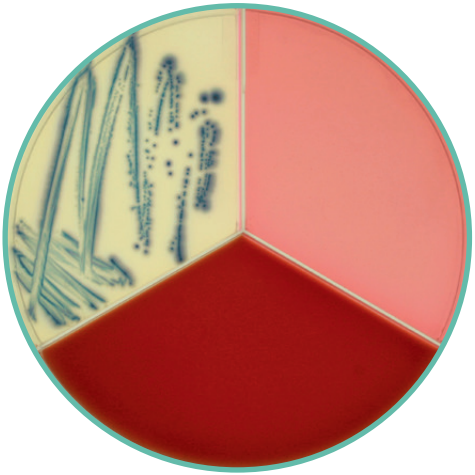
*Some cultures can take up to 48 hours.

How to read the results by plate sector

Gram negative coliform agar (Sector 1)

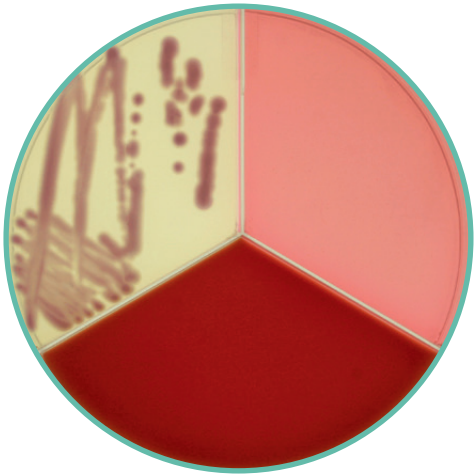
Generally *E. coli* and other coliforms are clearly detectable in less than 18 hours.



E. Coli;

Colony colour: Dark blue-violet

Agar colour: No change in agar colour



Other coliforms,

example: Klebsiella;

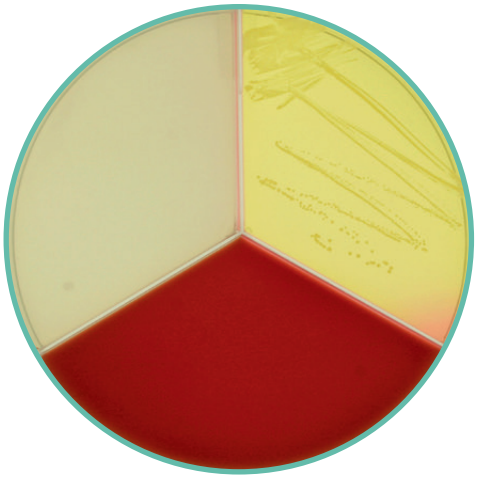
Colony colour: Red-purple

Agar colour: No change in agar colour

How to read the results by plate sector

Staphylococcus agar (Sector 2)

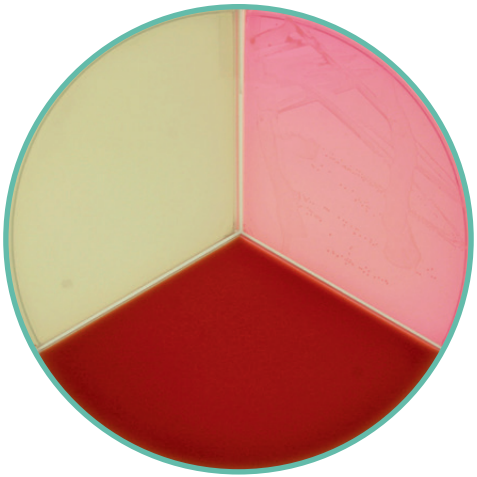
Generally staphylococci (e.g. *Staph. aureus* and coagulase negative staphyloccoci) are clearly detectable after 24 hours.



Staph. aureus;

Colony colour: Yellow-golden

Agar colour: Gold



Coagulase-Negative Staph. (CNS),
example: Staph. epidermidis;

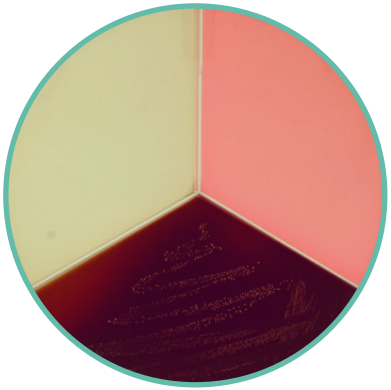
Colony colour: Clear

Agar colour: No change in agar colour

How to read the results by plate sector

Streptococcus and enterococcus agar (sector 3) - agar aesculin positive

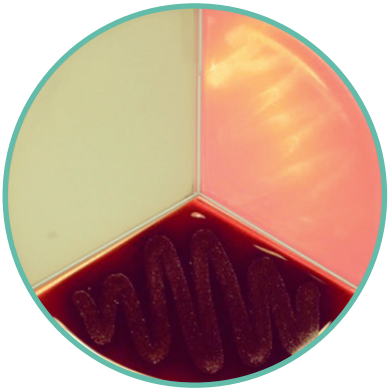
Aesculin positive bacteria are clearly detectable by the black discolouration of the agar after 24 h; of the aesculin positive bacteria, Strep. uberis accounts for ≥ 95% of cases and enterococci ≤ 5 % of cases.



Aesculin positive streptococci, *example: Strep. uberis;*

Colony colour: Black

Agar colour: Black



Aesculin positive enterococci;

Colony colour: Black (Yellow in sector 2)

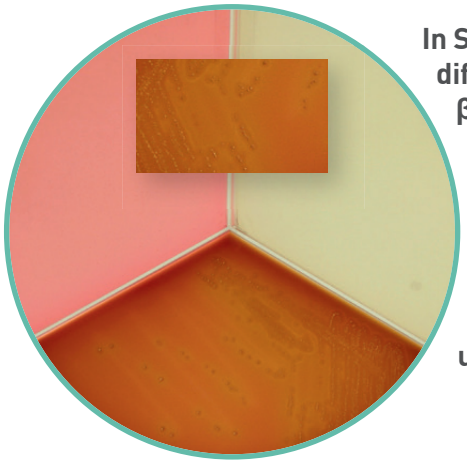
Agar colour: Black

How to differentiate between *Strep. uberis* and enterococci:

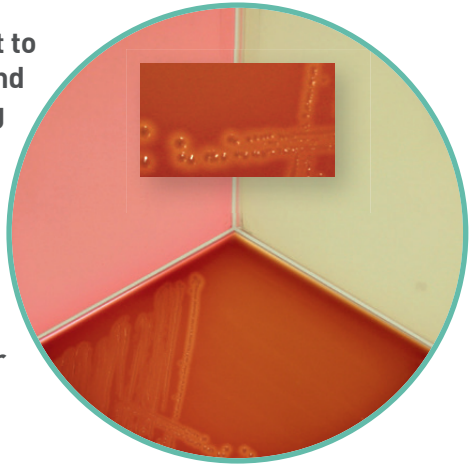
- Generally enterococci and *Strep. uberis* cannot be differentiated by this mastitis test (a salt tolerance test is necessary).
- Some enterococci strains will also grow slightly on the Sector 2 agar. Look for black discolouration of the Sector 3 agar after 24 hours, combined with a yellow discolouration of the Sector 2 agar after 48 hours (less obvious than the yellow discolouration caused by *Staph. aureus*).

How to read the results by plate sector

Streptococcus and enterococcus agar (sector 3) - agar aesculin negative



In Sector 3 agar it is difficult to differentiate between α- and β- haemolysis after using a swab, we recommend using loops. Smaller colonies of aesculin negative streptococci are difficult to detect before 24 hours but are usually more visible after 48 hours.



Aesculin negative *Strep. dysgalactiae;*

Colony colour: Clear

Agar colour: Red-brown

α-haemolysis: Green coloured corona around the colonies.

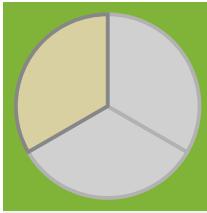
Aesculin negative *Strep. agalactiae;*

Colony colour: Clear

Agar colour: Red-brown

β-haemolysis: Clear bright corona around the colonies

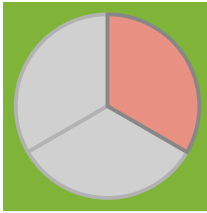
Summary of all streptococcus and enterococcus agar readings:



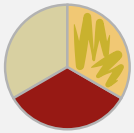
Growth on Section 1
Gram negatives

Dark blue colony
E.coli

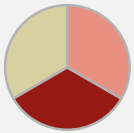
Red-purple colony
Coliform



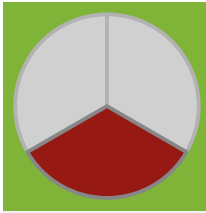
Growth on Section 2
Staphylococci



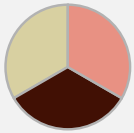
Golden agar and colony
Staph. aureus



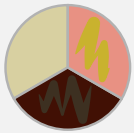
No change in agar and clear colony
Coag. Neg.Staph.



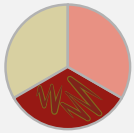
Growth on Section 3
Streptococci



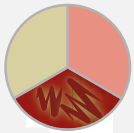
Black agar and colony = Aesculin +ve
Strep. uberis
Strep. bovis



Black agar and colony = Aesculin +ve
Enterococci



Red-brown agar = Aesculin -ve Alpha haemolitic
Strep. dysgalactiae



Red-brown agar = Aesculin -ve Alpha haemolitic
Strep. agalactiae
Strep. canis

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*Some cultures can take up to 48 hours.

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References: 1. Bradley A.J, Leach K.A, Breen J.E, Green L.E, Green M.J. Survey of the incidence and aetiology of mastitis on dairy farms in England and Wales. 2007 Veterinary Record 160, 253-258. 2. Viora L, Graham E.M, Mellor D.J, Reynolds K, Simoes P.B.A, Geraghty, T.E. Evaluation of a culture-based pathogen identification kit for bacterial causes of bovine mastitis. 2014 Veterinary record 175, 89.

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