Test Validation

Test is valid if the standard tube contents become blue within 2 to 3 minutes after addition of reagent B (substrate).

Interpretation of Results

Compare the colour of the standard and of the sample tubes and refer to the table hereafter:

<table>
<thead>
<tr>
<th>Use</th>
<th>Day of Sampling</th>
<th>Test Result</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recycling after calving</td>
<td>Two samples at 10 days interval</td>
<td>Sample darker than standard</td>
<td>Lack of active corpus luteum. Cow is still in anestrus or close to oestrus. Observe for visual signs of heat.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Same colour for standard and sample</td>
<td>Result inconclusive.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sample paler than standard</td>
<td>Presence of an active corpus luteum. Cow has started cycling.</td>
</tr>
<tr>
<td>Heat check before insemination</td>
<td>Day of intended insemination</td>
<td>Sample darker than standard</td>
<td>Lack of active corpus luteum. Cow is close to oestrus.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Same colour for standard and sample</td>
<td>Result inconclusive. Sample one to two days later and re-test.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sample paler than standard</td>
<td>Presence of an active corpus luteum. Cow not in heat.</td>
</tr>
<tr>
<td>Non-pregnancy indication</td>
<td>21 to 24 days after insemination</td>
<td>Sample darker than standard</td>
<td>Lack of active corpus luteum. Cow unlikely to be pregnant. Observe for return to heat.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Same colour for standard and sample</td>
<td>Result inconclusive. Sample one to two days later and re-test.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sample paler than standard</td>
<td>Presence of an active corpus luteum. Cow may be pregnant. Pregnancy has to be confirmed later by veterinary examination.</td>
</tr>
</tbody>
</table>

OVUCHECK® RAPID TUBE is an immunoenzymatic test intended to evaluate the level of progesterone in the milk of dairy cows. This test is simple and rapid. It provides a reliable aid to improve cow fertility management.

Progesterone is a hormone produced by the corpus luteum which develops in the ovary following ovulation. Progesterone is present in the blood as well as in the milk. During oestrus and the following 2 to 3 days progesterone levels are very low. Subsequently the concentration progressively increases and tops after 5 to 6 days. If the cow is not pregnant 17 to 18 days after breeding, the corpus luteum quickly regresses and the progesterone concentration falls. By contrast, if the cow is pregnant, the progesterone concentration remains high until calving. Low progesterone levels may also occur during anoestrus (non cycling cows) and high levels may result from luteal cysts.

PRINCIPLE OF THE TEST

The OVUCHECK® RAPID TUBE test is based on the competition between unlabelled progesterone present in the milk sample (or the standard) and the progesterone labelled with an enzyme (conjugate), to binding sites of specific progesterone antibodies coated on the wells of the tubes.

After incubation, the labelled progesterone and the unlabelled progesterone which have not been bound by the antibodies are washed away.

The amount of labelled progesterone fixed to the antibodies is inversely proportional to the quantity of unlabelled progesterone present in the samples (or the standard).

The bound labelled progesterone is measured by making the enzyme react with its substrate during a second incubation.

The colour produced is interpreted visually or measured using a spectrophotometer. The colour intensity is inversely proportional to the concentration of the unlabelled progesterone present in the samples (or the standard).
### MATERIAL

<table>
<thead>
<tr>
<th>Components</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test tubes</td>
<td>24</td>
</tr>
<tr>
<td>Ready-to-use standard (S)</td>
<td>1 X 3 mL</td>
</tr>
<tr>
<td>Ready-to-use conjugate (A)</td>
<td>1 X 20 mL</td>
</tr>
<tr>
<td>Ready-to-use substrate (B)</td>
<td>1 X 15 mL</td>
</tr>
<tr>
<td>Ready-to-use stop solution (C)</td>
<td>1 X 10 mL</td>
</tr>
<tr>
<td>Plastic pipettes</td>
<td>26</td>
</tr>
<tr>
<td>Test tube rack (for 6 tubes)</td>
<td>1</td>
</tr>
</tbody>
</table>

Materials Required but not Provided:
- Tap water
- Spectrophotometer equipped with a 650 nm filter (not necessary if the colour is interpreted visually)

### PRECAUTIONS
- Store the kit at 2-7°C. NEVER FREEZE.
- For *in vitro* veterinary diagnostic use only.
- Do not use the kit after the expiry date indicated on the package.
- Do not mix the reagents from different serial numbers.
- Keep out of the reach of children.
- Do not take by mouth.
- If eyes or skin are splashed, wash thoroughly with tap water.
- When emptying the tubes contents into a sink, thoroughly flush away with excess volume of tap water.
- At the end of testing, return components to the refrigerator. Unused tubes should be resealed in the plastic pouch. Do not use a tube twice.
- Do not expose substrate (B) to direct light

### EXECUTION

#### A. Sample Collection

**From Collecting Jar:**
Whole milk samples can be taken from the collecting jar (any milking) into a clean container marked with the cow’s number.

**Sampling by Hand:**
If sampling by hand from the udder, discard the first five squirts from each quarter. Then collect an equal amount from each quarter into the container.

Samples not to be tested immediately can be stored for 24 hours in the refrigerator.

#### B. Test Procedures

- The kit’s components should be allowed to reach room temperature before testing begins (30 minutes or more may be necessary depending on room temperature).
- Milk samples should be allowed to reach room temperature before testing begins.
- Make sure that samples and all reagents (S, A, B, C) are properly mixed before use.
- No more than 5 milk samples should be tested with the standard on a single occasion to assure similar incubation time for samples and standard.
- The standard (S) needs to be used every time an assay is performed.

1. Take out the required number of tubes from the plastic bag. Select the necessary number of tubes (1 + n, n being the number of milk samples to test). Unused tubes should be kept in the plastic bag.
2. Place a tube in the rack in position marked S for the standard and in positions marked 1 to 5 for the samples. Good record keeping of the position of each milk sample is essential.
3. Squeeze the bulb of a clean dropper so that it is fully compressed. Keeping the dropper vertical, dip the tip in the standard and release the pressure on the bulb. Standard will be sucked up into the barrel of the dropper. Position the dropper near the base of tube “S” and fully squeeze the bulb so that the standard is completely ejected into the tube. Using a fresh dropper for each milk sample, repeat this method to add milk samples. Each milk sample to be tested shall be added to a different tube. Always use a new pipette for each milk sample or standard. Tubes must either contain a standard or a milk sample, not both.
4. Keeping bottle A vertical, add 10 drops of reagent A to each tube. If standard or milk has stuck on the inner walls of the tube, wash it down with solution A during this step.
5. Mix contents by moving rack quickly from side to side for 3 to 4 seconds. Leave tubes for 3 minutes to allow the reaction to develop.
6. Empty the contents of the tubes into the sink and gently rinse the tubes 5 times, by filling the tubes with tap water. After the 5th washing dry by tapping the tube onto absorbent paper. Do not scrub the inside of the tube.
7. Keeping bottle B vertical, add 10 drops of reagent B to each tube.
8. Repeat step 5.
9. Check that a blue colour develops in the standard tube. Mix contents by moving rack quickly from side to side.
10. Keeping bottle C vertical, add 5 drops of reagent C to each tube. This step is only necessary if the reaction is read using a spectrophotometer (step 11).
11. Mix contents by moving rack quickly from side to side then compare the colour in each milk sample tube with that of the standard tube or read the optical density using a spectrophotometer with a 650 nm filter.