The quality of samples taken for any diagnostic procedure is extremely important. Correct identification and a truly representative sample are critical for obtaining optimal diagnostic test results. Below is a protocol for the manual collection of milk samples for chemical-based pregnancy testing. Use bovine milk samples from ≥ 28 days postbreeding and ≥ 60 days postcalving.

For optimal timing of sample collection in other species, refer to the test’s user instructions.

**Materials for sampling**
1. Clean vials or tubes 30 mL–50 mL capacity (Standard herd testing tubes will suffice.)
2. Preservative (e.g., tablet containing bronopol) prefilled in the vial or tube
3. Wet wipes or towel
4. Permanent marker or labels for identifying sample vials/tubes

**Sampling technique**
1. Label tube prior to sampling. Include date and individual cow ID. It is essential to ensure that the correct tube is used for the correct animal. Failure to do so may result in an incorrect status being assigned to the cow.
2. Using the wet wipes or towel, remove dirt and detritus from the teats. Ensure udder is clean and dry prior to starting sample collection.
3. Samples may be taken from individual quarters or as a composite sample of all four quarters.
4. Discard a few streams of milk and remove the cap from the tube or vial. Angle tube at 45º to the teat end.
5. Fill the tube or vial three-quarters full from one or multiple quarters and replace the cap.
6. Place samples in a refrigerator prior to shipping to the laboratory.
7. Samples should be well-packaged and kept cool during shipping (especially if preservative has not been used).

**Sample quality and handling at the laboratory**
- Bronopol or a similar preservative may be used to maintain sample quality—this will not affect test results.
- The assay can be run on whole or skim milk samples and samples that have been exposed to heat treatment (during milk component analysis, usually up to 20 minutes at 40°C/105°F).
- Poor-quality samples may compromise the accuracy of test results. Prior to testing, milk samples will be checked to ensure that they have not soured or separated and are free from contamination.
- Do not heat milk samples in microwave as this procedure will destroy the pregnancy-associated glycoproteins (PAGs).

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Sample storage conditions and time frames</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room temperature</td>
<td>Refrigerator (2°C–8°C)</td>
</tr>
<tr>
<td>Native milk samples</td>
<td>Less than a few hours</td>
</tr>
<tr>
<td>Preserved milk samples (bronopol or other preservative)</td>
<td>3–5 days</td>
</tr>
</tbody>
</table>

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