Test Kit for Determination of Drug Residues in Milk and Milk Products

Self Food Quality Care

Drug administration for prevention and treatment in dairy cattle causes the problem of drug residues in their tissues and milk which will have and effect on consumers and industry of yoghurt and cheese. Easy and rapid test for screening drug residues in milk and dairy products in necessary to be developed in order to control milk quality for the safety of consumers.

Health impact

Prolonged consumption of milk with drug residues can cause drug resistance and allergenicity to sensitive consumers.

Target Sample

- Raw milk
- Pasteurized / Sterilized / UHT milk
- Powdered milk
- Other dairy products

Number of Tests / Kit

50 Tests
Test Kit Tool

- 50 prepared tubes
- 10 droppers
- Accessory tool: water bath (need accurate temperature control at 64+2 °C and 82 + 2°C)

Collection and Preparation of Milk Sample

- Raw Milk will be sampled from truck or milk container and immediately tested. If it is not possible to do so, the samples must be frozen and the test should be conducted within 7 days. Before performing the analysis, the samples must be heated in water bath at 82 + 2°C for 2 minutes to destroy heat-labile natural inhibitors and microorganisms contaminated in raw milk.
- Pasteurized Milk or liquid dairy products can be analyzed without heat treatment at 82 + 2°C. If analysis cannot be conducted immediately, the samples should be frozen for not exceeding 7 days.
- Powdered Milk and Dairy Products will be dissolved in sterile distilled water or phosphate buffer pH 6.0 with the proportion of 1:3 (weight per volume) before the test.

Procedure

There are 3 levels of the test according to user’s purpose:
A. Test for presence or absence of drug residues
   1. Add 3 drops (~0.1mL) of milk sample into the prepared tube.
2. Add 3 drops (~0.1 ml) of UHT fresh milk into another prepared tube for negative control.
3. Incubate all the tubes for 2 hours 45 minutes in water bath when the temperature reaches 64 ± 2°C, keeping medium in the tube under water level, or incubate until the color of medium in negative control tube changes completely from purple to yellow. Observe the color change of medium in sample tubes.

B. Confirmatory test for penicillin group
Add 0.05 ml of penicillinase enzyme into 2-3 ml of positive milk sample and mix together. Then, add 3 drops of mixture into another prepared tube and follow step A.2 and 3.

C. Quantitative test for drug residues of penicillin group
The quantity of Penicillin residue is indicated by the level of purple color in medium which can be read in the range of 1-2, 2-4, 4-8, 8-16,…, 128-256 µg/L (ppb) by comparing it with standard chart.

Evaluation

Observe the color change of medium in each tested tube and interpret as follows:
A. Test for presence or absence of drug residues

The level of purple color in medium indicates the quantity of drug residues.

B. Confirmatory test for penicillin group

- The results of milk sample before adding penicillinase:

- The results of milk sample after adding penicillinase:

1. 

The color of medium changes completely from purple to yellow
2. positive for other groups

The level of purple color in medium is the same as before adding penicillinase.

3. positive for penicillin and other groups

The level of purple color in medium is lower than before adding penicillinase.

C. Quantitative test for penicillin group

Standard chart of penicillin residues in milk

<table>
<thead>
<tr>
<th>Control</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>16</th>
<th>32</th>
<th>64</th>
<th>128</th>
<th>256</th>
</tr>
</thead>
<tbody>
<tr>
<td>µg/L (ppb)</td>
<td>0</td>
<td>1.95</td>
<td>3.58</td>
<td>5.64</td>
<td>7.35</td>
<td>8.51</td>
<td>9.71</td>
<td>10.84</td>
<td>11.77</td>
</tr>
</tbody>
</table>

Level of purple color (mm)
For a penicillin positive sample, if the level of purple color in medium is measures 3.2 mm, it can be concluded that there is 1-2 µ/L of penicillin residues.

Effectiveness of Test Kit

This test kit has 91.7% accuracy, 100% sensitivity and 90.5% specificity. It can be tested for the presence of at least 12 kinds of drug residues: ampicillin, amoxicillin, bacitracin, chlortetracycline, erythromycin, oxytetracycline, penicillin, rifampicin, sulfadimethoxine, tetracycline and tylosin. However, after this screening test, the kinds of drug residues in most cases may be indentified, if needed, by using chemical or immunological techniques. Only penicillin and sulfonamide group can be confirmed by using microbiological technique with the aid of some enzymes of chemicals.

Keeping / Age

Always keep prepared tubes at 4-8°C during 3-month shelf-life. Storage prepared tubes outside refrigerator for hours can retard the microbial growth which results in prolong incubation time needed and so, false positive results are possible to obtain.

Procedure after test

Soak the tested medium in disinfectant by adding it into the tube and leave it there for 30 minutes or immerse the tube in boiling water for 15 minutes, and later discard it.