Dynamic Test Kits for R&D
and Quality Control

CV Test II

# IMMUNOLOGICAL TEST FOR THE DETECTION BY IMMUNO-DIFFUSION OF COWS MILK POSSIBLY TO SUPPLIES OF GOATS OR EWES MILK

**INRA procedure**
Official method (Official Journal of the French Government, June 1978)

# Instructions For Use: CV Test II

Version: May 2014

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**References (components):**
- 250101-1 (1 kit = 5 petri-dishes of 10 tests + 1 range 0, 2%, 10%, 25%)
- 250101-2 (2 kits = 10 petri-dishes of 10 tests + 1 range 0, 2%, 10%, 25%)
- 250101-3 (3 kits = 15 petri-dishes of 10 tests + 1 range 0, 2%, 10%, 25%)
- 250101-4 (4 kits = 20 petri-dishes of 10 tests + 1 range 0, 2%, 10%, 25%)
- etc…

**READ CAREFULLY BEFORE USE**
**REFER TO THE INSTRUCTIONS FOR USE INCLUDED IN THE KIT, THE VERSION AND VERIFY IF CHANGES OCCURRED**

## I - PRINCIPLE

Sample (milk or cheese) for analysis is deposited in pits made in a layer of agar containing a specific antiserum to cows milk. As diffusion occurs, the constituents of cow milk recognized by the antiserum are precipitated. The diameter of this precipitate is proportional to the amount of cow milk present in the sample.

## II – AREA OF APPLICATION

The method applies to untreated or heated (up to +60°C for 30 seconds at most) ewes and goats milk, fresh or preserved by freezing or addition of dichromate of potassium or chloride mercuric, as fresh and matured cheeses.

## III – SAMPLES PREPARATION

A. MILK

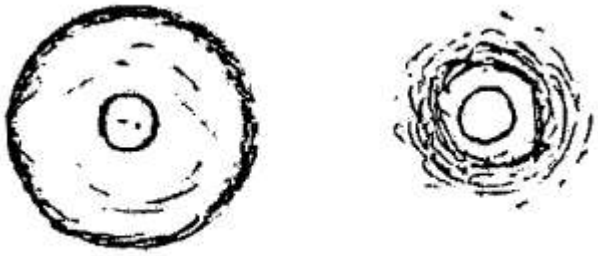
- Add two drops of concentrated rennet to approximately 5 ml of milk, incubate for 15 minutes at +37°C, then centrifuge for 10 minutes à 3,000 rpm. (It's better to use rennet of animal origin other one than bovine).
It is possible to replace the addition of concentrated rennet by the addition of 50 µl of icy acetic acid for 1 ml of milk.
Three phases are obtained: an upper layer of fats, an opalescent layer containing lacto serum and the deposit of caseins.

- Carefully draw off 0.5 ml of lacto serum, avoiding taking any fat and store it at +4°C or in the freezer for a storage of more long-lasting.

Remark:
This method can be also applied to whole milk provided that it is very fresh (absence of clumps which block the needle of the micro-syringe), Otherwise make it spin-dry

- However, the use of whole milk is inadvisable when dishes are to be stained since washing is then imperfect and a colored halo persists.

- Careful examination nevertheless enables differentiation of this halo from a specific precipitate (very clear-cut borders):




B - CHEESE

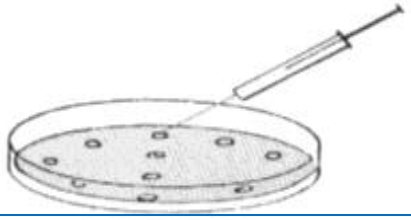
- Weigh 2 grams of cheese, add 2 ml of water at pH approximately 4 (acidified with acetic acid) and 4 ml of chloroform.

- Homogenize using a homogenizer, then centrifuge (3,000 rpm for. 10 min).
(This step can be replaced by an incubation of homogenized cheese with the acidified water and the chloroform at ambient temperature for 24 hours: centrifuge for 5 min)

- Store the clear upper phase.

## IV – RANGE PREPARATION

- Using a micro-syringe deposit 10µL of the sample for analysis in one of the pits of a titration dish.



- In each titration plate the three samples of the range should be included in three pits.
The 0% of the range could be used for obtaining another concentration in the range (for example: 5 % can be obtained by dilution 1/2 of the 10 % sample with the 0 %).

- TO ENSURE PRECISE incubate for 24 hours in a damp room at +37°C

- TO ENSURE READING incubate 3 hours à +45°C
(**CAUTION**: the ratio diameter of precipitates/amount of cow milk is not linear at amounts higher than 10 %, Therefore, these samples have to be re-analyzed at +37°C for 24 hours, in order to obtain an acute result.)

- Then cover the agar with 2 % acetic acid for 5 minutes.

- Empty acetic acid and rinse with 1 change of distilled water.

- Examine the dish in oblique light.

## V – READING OF RESULTS

- The diameter of precipitates can be measured with or without staining, using a two-sided ruler with half-millimeter graduations or, better, with a magnifying glass fitted with a micrometer.

- In the graphic, diameters measured with the standard range provide a reference curve on which the diameters obtained for each sample can be read off. So for each sample, the percentage of cow milk in goats or ewes milk is determined.

See the example in the graphic: last page

Measured diameter: 80mm → Percentage of cow milk: 10%

## VI - INTERPRETATION OF RESULTS

1. For sample containing more than 50 % cow milk, it is advisable to repeat the analysis with 1/2dilution of the sample.

2. Correction factor for analysis of whole milk:
Whole milk diffuses slightly less rapidly than lacto serum. The standard range having been prepared using lacto serum, it will be necessary to add 10%to the value found for whole milk:
(Actual % cow's milk) = 1.10 x (measured % cow's milk).

3. Correction factor for analysis of cheeses:
The dry extract of cheeses analyzed must be taken into consideration.
In comparison with the lacto serum range, the result obtained must be multiplied by a correction factor which varies according to the dry extract of the cheese:

| **DRY EXTRACT AS %** | **30** | **40** | **50** | **60** | **70** |
|---|---|---|---|---|---|
| correction factor x result by | 3 | 2.5 | 2 | 1.5 | 1 |

## VII - SENSITIVITY

The technique described can reveal 1 % of cow milk in goats or ewes milk.

It is possible to increase this sensitivity by reloading the pits after 30 minutes to 1 hour of diffusion at laboratory temperature (without cover) with 10µL of sample .The standard range is reloaded in the same way.

It is also possible to extract cheeses with a lower percentage dry extract using a smaller amount of acidified water. We increase then all the more the sensibility.

## VIII – SHELF-LIFE

- The deadline of use of the CV tests and ranges is mentioned on their label.

- Keep PETRI dishes upside down (with agar upwards) in a damp plastic container (between +15°C and +18°C).

- Keep the range, which is reconstituted, at +4°C (expire date: 6 months).

## IX – TO STORE RESULTS

- Incubate for 24 hours à 37°C in a damp room (do not cover with acetic acid).

- Then wash the dishes with 6 à 7 baths in a 9 ‰ sodium chloride solution, for 10 minutes with mixing from time to time

- Empty sodium chloride solution.

- Fill the with 5% glycerinated water. Allow to stand for 10 minutes.

- Deposit on the surface of the water a filter paper disc with the same diameter as that of the dish, allow the disc to sink, then hold it against the agar while tipping away the glycerinated water. This should avoid the presence of bubbles (in particular in the pits) between the agar and filter paper.

- Allow to dry overnight on a hot surface (radiator, etc.) or in keeping a drier (or hair-drier) from the agar, in order to maintain the temperature below +45 °C

- Cover the agar for a few minutes with distilled water to allow the filter paper to detach gradually.

- Remove the filter paper with forceps and tip away water.

- Stain dishes for 15 minutes with 1 ‰ Amid Black in 2 %.

- Rinse with 5 or 6 changes of 2 % acetic acid each lasting 2 minutes with removing from time to time in order to discolor the zones without precipitate .Then rinse the dishes for 2 minutes with 2% acetic containing 5% glycerin.

- Empty and dry.

- For the reading, see the part V.

## XI - EXAMPLE