Stick cGMP Rapid Test

Cat. No.:DTS714
Pkg.Size:5 tests

Intended use

Stick cGMP has been designed for the detection of casein glycomacropeptide(cGMP) in milk. This molecule concentration may increase when milk has been adulterated with whey.

General Description

Immunochromatographic test for the qualitative detection of casein glycomacropeptide (cGMP) in milk. The cGMP is produced by enzymatic or bacterial induced degradation of the casein present in milk. This degradation takes place during the cheese manufacturing process, where specific proteases are added to act on the milk casein. Therefore its presence in milk is an indication of milk adulteration with whey. Other degradation processes (mostly related to the bacterial activity present in lactic products) are also capable of increasing the cGMP concentration but not as much as adulteration.
This immunochromatographic test is very sensitive, detecting even GMP levels that could indicate adulterations of the 4% of whey in milk. If the conditions of milk, conservation, transport and process are optimal, levels as low as 2% of whey in milk could be detected.

Principle Of The Test

The cGMP present in the milk samples will react with coloured latex particles coated with monoclonal antibodies against the glycomacropeptide. This complex of latex particles/monoclonal antibodies/GMP reaches, through a chromatographic process, the reaction zone, where there are another anti-GMP antibodies that react with the complex. This reaction causes the formation of a red line.
In parallel a similar reaction occurs with the colloidal particles that originate a blue precipitation line.

Reagents And Materials Provided

1. Test strips 5
2. Sample dilution buffer bottle (25 mL) 1

Materials Required But Not Supplied

1. Trichloroacetic acid (TCA) for casein precipitation.
2. Bench top centrifuge and 5.0 um filters (or 0.2 um filters).
3. Eppendorf tubes for each supernatant sample dilution: one for TCA casein precipitation/centrifugation and three for serial dilutions- one for each sample.
4. Flat bottom 96-well Microtitter plates for the test (one well per sample).
5. 100 ul, 900 ul or 1000 ul calibrated micropipettes to carry out the dilutions.
6. Calibrated micropipettes for sample addition to the Eppendorf tube (250 – 500 ul) or to the microtitter well (150 ml) where the stick test will be placed.
7. Timer/Stopwatch.

Storage

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The reactive strips can be stored at temperatures between 2°C and 30°C. Their expiry date are printed in the envelope.
The sample dilution buffer must be removed from the box and stored at 4°C.
VERY IMPORTANT. Do not leave the test sticks exposed to the atmospheric humidity. Close the tube after taking out the strip.

Specimen Collection And Preparation

Samples will be collected in clean containers and will be kept refrigerated to diminish bacterial activity which could increase cGMP concentration. Long time sample storage will be done at –18°C or –70°C (if possible). Samples can be frozen and defrosted just one time. Defrosted samples must reach room temperature before use.
Milk powder: Reconstitute milk powder by solubilizing 10 g of sample in 100 ml water (distilled water). Stir for 10 minutes at 40°C for complete dissolution.

Assay Procedure

1. Procedure of treatment of sample (milk)

1. The milk sample will be pretreated to separate the glycomacropeptide (cGMP) from the casein. Add TCA to the milk sample to reach a final concentration of 8% (e.g. to 6 mL milk sample add 4 mL of 20% TCA). Mix and wait for 10 minutes.
2. Centrifuge the mixture from approximately 100 x g during 20 minutes to 2000 x g during 10 minutes to separate the casein precipitate (non soluble at this pH) from the supernatant containing cGMP.
3. Filter supernatant through a 5 um low protein adsorption filter (other pore size could also be used)
4. Add 900 ul of sample diluent to 3 vials (assay tubes or Eppendorf)
5. Prepare the 1/10 dilution by adding 100 ul of supernatant from step 3 to an Eppendorf tube containing 900 ul of sample diluent buffer (pump carefully several times the 100 ul) Mix well using the micropipette.
6. Prepare the 1/100 dilution by adding 100 ul from the 1/10 dilution to an Eppendorf tube containing 900 ul of sample diluent buffer (pump carefully several times the 100 ul) Mix well using the micropipette.
7. Prepare the 1/1000 dilution by adding 100 ul from the 1/100 dilution to an Eppendorf tube containing 900 ul of sample diluent buffer (pump carefully several times the 100 ul). Mix well using the micropipette. We call this dilution as 1/1.000 dilution.
8. It is recommended to start with a 1/4000 ditution (4% whey in milk) diluting 1/4 the 1/1000 dilution (add 100 ul of supernatant from step 7 to an Eppendorf tube containing 300 ul of sample diluent buffer) If the most of the results are negative or the milk quality is very good, try with the 1/2000 dilution (2% limit) and even 1/1000 (1% limit) The optimal dilution will depend on the microbial quality of the sample, that depend itself on the conditions of handling and conservation of the milk and also on the farming and country of origin.

2. Test developing:

1. Using a micropipette transfer 500 ul supernatant of the milk dilution into an Eppendorf tube or we can also transfer 150 – 250 ul supernantant of the milk if we use a flat bottom 96-well microtitter plates.
2. Dip a test stick into the Eppendorf tube or microtitter plate well with the arrows pointing to the bottom.

IMPORTANT: the liquid should never overpass the arrows; if necessary use a bigger tube or reduce the sample amount.

3. Read the test result in the central white area of the strip after 5 minutes.

Alternatively, the stick can be placed in the Eppendorf tube or microtitter plate well for 10-20 seconds after which is removed and placed on a horizontal surface.

Quality Control

If no blue band appears the test is invalid, since improper test procedure was carried out or deterioration of the reagents has occurred.
It is recommended to repeat the test.

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Interpretation of Results

NEGATIVE: (depending on the dilution milks will be between 4-1% of cGMP) Only one BLUE band appears across the result window. This line must always appear.
POSITIVE: In addition to the BLUE control band, a distinguishable RED band also appears across the result window. The intensity of the line depends on the concentration of sample.
If no blue line appears, the test is INVALID, it has not been correctly run, reagents has been damaged or the sample added was incorrect. Run another test.
Any line or colour that should appear after 5 minutes has no diagnostic value.
The final diagnosis should take into account all factors that could increase glycomacropeptide(cGMP) concentration in the milk and not related to its adulteration.

Sensitivity

The sensitivity limit is 15 – 30 ng/mL with GMP Sigma (Ref. C-7278). This means that the test can detect 15 –30 ug/mL in the precipitated sample (1- 2 % of whey added to milk), taking into consideration the dilution used (usually 1/1000) and the milk content in the precipitate (usually 60% and depending of the sample preparation protocol of TCA+milk).
Sensitivity determination has been carried out using serial dilutions of 1 ug/mL GMP Sigma (Ref. C-7278). Two determinations have been done: qualitative or visual determination and quantitative using an immunochromatographic test-strip reader.

Specificity

Stick cGMP contains monoclonal antibodies that assure the high specificity for GMP or k-casein detection and no other species; however, since casein and GMP are detected in a similar fashion, it is necessary the previous k-casein separation by TCA precipitation.

Compound	Starting concentration (before dilution)	Result
κ-casein	3 g/L	+
Total casein	3 g/L	+

Hook Effect

In order to verify whether a high concentration of cGMP may affect efficiency of test (prozone or high dose effect) a large number of GMP concentrations from 1.106 to 1 µg/ml were analysed. Close to 4 orders can be visually detected with the Stick cGMP and the higher detectable concentrations (higher than 50 µg/ml) are much higher than those from pure cheese whey after one 1000 fold dilution.

Comparative Trial

A survey for the detection of added rennet whey in raw milk was carried out in 60 samples from Brazil. The results obtained with the strip test were compared with those obtained with the official HPLC method and the colorimetric sialic acid method.
The strip could always detect the presence of rennet whey in samples in which an amount of rennet whey higher than 4% was found by the HPLC. The only difference takes place in samples between 2-4%, close to the limit of detection of the test. In these cases, the amount of added rennet whey was very low and unlikely to have any technological impact.
The sialic acid method showed the whey rennet was not always correctly detected, the two samples detected as negative (in the case >4%) had in fact an amount higher than 10%.

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HPLC		Stick cGMP		Sialic acid method	
cGMP percentage	Nº of samples				
<2%	6	All negatives		2 slightly higher than blank	4 same than blank
2 – 4%	18	8 negatives (values between 2 and 2.5%)	10 positives	3 same than blank 5 lower than standard 2%	10 same than standard 2%
>4%	36	All positives		2 lower than standard 2% 11 same than standard 2% 10 between standards 2 – 5 %	8 same than standard 5% 5 higher than standard 5%

Repeatability

Ten replicates of three concentrations (Negative control; Low positive control and Positive control) from the sensitivity curve are tested and the same results are obtained.

Reproducibility

INTER-DAY PRECISION

Using 1 lot of the product, ten duplicates of the sensitivity curve are performed throughout ten consecutive days. There are no differences in the evaluation.

INTER-LAB PRECISION

Three different laboratories-operators test those same samples, presenting high precision and concordance. Only a difference of one ½ dilution is observed, acceptable and tolerable for the assay.

INTER-LOT PRECISION

Based on the historical data of all manufactured lots and studying the sensitivity curve, there is only a difference of one ½ dilution, acceptable and tolerable for the assay.

Interferences

None of the possible interfering substances listed below had any effect on the test even though they were assayed at higher concentrations that those found in the milk.

Compound	Starting concentration (before dilution)	Result
BSA	10 %	-
Bovine antibodies (PAb IgG's)	1%	-
Lactose	10%	-
Lactoalbumins (α y β)	4 g/L (total LA)	Not available

Precautions

1. Do not interchange kit components from different kit lot numbers.
2. Allow kit components and specimens to reach the room temperature before use, as cold reagents and/or specimens may decrease assay performances. 20-30 minutes are recommended.
3. Do not use kit components beyond labelled expiration date.
4. It is very important to prepare the dilutions very accurate. Otherwise, sensitivity or specificity are not guaranteed
5. It is very important to add the correct quantity of sample. If it is lower than the suggest one, the sample may not arrive to the reaction area and the test would not run. If it is higher, the reactive could be diluted and the line very slight.
6. All product used should be rejected according to the legislation in force.
7. Do not use the test if any coloured line can be seen in the test zone before performing the test.
8. Very important: In Stick in tube final format is very important to keep the tube closed after taking a reactive strip. In case of a high environmental humidity the strips that remain in the tube can turn out to be damaged by this environmental factor.

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Limitations

It is recommended not to test milk samples from:

1. Inadequate cold storage. Storage temperature not maintained until consumption.
2. Very acid milk.

Degradation processes will take place, in the above mentioned conditions, which could result in the non-specific casein degradation and in an increase in casein glycomacropeptide (cGMP) concentration.

REFERENCES

1. Isolation of Monoclonal Antibodies Monoespecific for Bovine k-Casein. Konrad M. Kuzmanoff, John W. Andresen, and Craig W. Beattie. 1990 J.Dairy Sci 73:2741-2748
2. Determination of caseinmacropeptide with capillary zone electrophoresis end its application to the detection and estimation of rennet whey solids in milk and buttermilk powder. J.van Riel and C. Olieman. Electrophoresis 1995, 529-533.
3. Detection of rennet whey solids in UHT milk by capillary electrophoresis. Isidra Recio, Mónica R.García-Risco, Rosina López-Fandiño, Agustín Olano, Mercedes Ramos. International Dairy Journal 10 (2000) 333-338.
4. Detection of rennet whey solids in skim milk powder and buttermilk powder with reversed-phase HPLC. C. Olieman and J.A.: van Riel. Neth.Milk Dairy J. (1989) 171-184
5. Isolation and Analysis of k-Casein Glycomacropeptide from Goat Sweet whey. Eryck R. Silva-Hernandez, Takuo Nakano, and Lech Ozimek . J.Agric.Food Chem. 2002, 50, 2034-2038.
6. Purification of k-casein glycomacropeptide from Sweet Whey with Undetectable Level of Phenylalanine. Takuo Nakano, Eryck R. Silva-Hernandez, Noriaki Ikawa, and Lech Ozimek Biotechnol. Prog. 2002, 18, 409-412.
7. Ciencia de la leche. Principios de técnica lechera. Capítulo 5. Editorial Reverté, S.A.

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