

STABILITY TEST FOR MASTITIS REAGENT ad us.vet.

V.Vuković¹, M.Vičentijević¹, N. Plavša²

¹Institute of Veterinary Medicine of Serbia, 11070 Belgrade, Auto put 3, Republic of Serbia

²University of Novi Sad, Faculty of Agriculture, Department of Animal Science, Trg Dositeja

Obradovića 8, 21000 Novi Sad, Republic of Serbia

Corresponding author: vujadin.vukovic@gmail.com

Communication

Abstract: In the stability test of the mastitis reagent ad us.vet., as the finished product, three production series were tested in quantities of 500 ml of the sample, under appropriate storage conditions. For the testing, the appropriate uniformity of temperature and relative humidity was provided. Also, the procedure of the stability test was determined, which included the initial state, then every three months until the end of the first trial and a final testing at the end of shelf life (0, 3, 6, 9, 12 and 18 months). Of the tested parameters the following were included: appearance, pH value of the solution, dry residue (in %) and microbiological purity.

Key words: mastitis reagent, stability, appearance, dry residue, microbiological purity

Introduction

Mastitis is one of the most widespread diseases in domestic dairy animals (cows, sheep and goats) and requires permanent activity in its suppression, including prevention, diagnosis, treatment (*Adams, 1995; Jezdimirović, 2000; Vukovic et al., 1988*).

Mastitis reagent is a diagnostic tool for the detection of udder secretion disorders in order to achieve accurate diagnosis of the mastitis in dairy cows, sheep and goats (*Batis, 1976; Miljkovic, 1977; Ruegg, 2002; Wilson and Gonzales, 1997*). The procedure is based on the indirect method of determining the number of somatic cells in milk (*Dohoo et al., 1984; Vukovic, 1998*). The reaction is performed in the milk testator composed of four separate parts and based on the effect of surface-active substance (surfactant) alkyl aryl sulfonate in reagent on leukocytes in the milk, releasing the DNA that polymerizes and produces visible gel. The complete reaction is accompanied by changes in the pH visible in indicator colour change (brome cresol red).

The result can be displayed as: negative (-), suspicious (\pm), weakly positive (+), positive (+ +) or strongly positive (+ + +) and is in proportion to the respective range of somatic cell count (*Miljkovic, 1977*). In this way, the assessment of udder health status ranges from healthy to clinically manifested mastitis. Further procedure is taking samples of milk for bacteriological examination to determine the cause and to determine the appropriate therapy, therapy.

Mastitis reagent is not used in advanced pregnancy, and two weeks after birth, due to obtaining of inadequate results.

Stability testing of active and auxiliary substances and finished products includes the chemical, physical and microbiological stability. This stability is under the influence of external factors (temperature, humidity, storage method and conditions, etc.) and internal factors (characteristics of active and auxiliary substances: pH, particle size, pharmaceutical/dosage form, the characteristics of the technological process, etc.). Also, the shelf life of the product determines the procedure of the stability test in order to obtain sufficient data for parameter testing at specified intervals, with a final examination at the end of shelf life.

Materials and Methods

Mastitis reagent stability test, as the finished product, includes examining the relevant parameters, appropriate procedures and methods and their compliance with the requirements of the test. Mastitis reagent in 1 ml of solution contains 50.0 mg of alkyl aryl sulfonate (*Valčić, 1995*) and each of the series that will be included in the stability test must meet quality specifications. Assessment of results for individual parameters is expressed with: adequate/compliant (+) or not adequate/non-compliant (-) with the requirement or numerically compared with the limits required.

The quality specification of the mastitis reagent, the appearance/exterior was tested sensory (*Ph.Jug.IV, 1984; Ph.Jug.V, 2000; Ph.Eur.VI, 2006*). At the same time, the shape and colour were observed, and the requirement is that the colour is red-purple clear liquid and foam of yellowish purple colour. Of general tests (*Ph.Eur.VI, 2006*) the verification of charging is provided for, and the requirement is $500\text{ml} \pm 2,0\%$. The identification was made according to internal regulation (*QCC-P-162-1, 2004*) by adding in the surplus 10% NaOH and after the flakes deposition, addition in excess of 10% HCl leading to intense blurring of the solution with a milky-yellow discoloration.

Of the constants, the pH value was investigated by potentiometric titration (*Ph.Eur.VI, 2006*), where the claim for pH value (6.5 to 7.0 by), and the limits required for the dry residue ranged from 4.75 to 5, and 25% were investigated by

the internal regulation (*QCC-P-162-1, 2004*) by a 3-4 ml solution steamed and dried in an oven at 105⁰C for 3 hours.

Microbiological purity test procedure was carried out according to regulation Ph.Eur.VI, 2006. Microbial limit was that in 1 ml sample up to 10³ aerobic bacteria is allowed and up to 10² of fungi yeast and mould spores. On the other hand, in 1 ml of the sample there must not be any: *E. coli* and other enterobacteria, *Salmonella* spp., *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

After confirming that all testing parameters match or are within quality specifications, it was decided to the stability test will encompass three series of mastitis reagent. In addition, for each series the same storage conditions, the same procedure with the relevant test parameters investigated will be applied.

For adequate storage during the test, along with keeping out of reach of children and protection from light and moisture, the constant temperature (25⁰C ± 2⁰) and a relative humidity of 60 ± 5% were provided. Further, based on the shelf life, stability test procedure was determined. In addition, it included the beginning, then testing every three months until the end of the first year of shelf life, and a final examination at the end of the stability test aligned with the expiry of its shelf life (0, 3, 6, 9, 12 and 18 months).

Of the tested parameters the stability test included appearance/exterior, pH value, dry residue and microbiological purity.

Results and Discussion

Total of three series of mastitis reagent were tested in the study of stability within the shelf life of the finished product (18 months). Also provided were the storage conditions: adequate temperature and relative humidity, shielding from light and humidity and placed out of reach of children.

From Table 1, it is apparent that all three series examined, in all observed periods, and in terms of the parameter of appearance/exterior meet the requirements of the test. This means that it was clear liquid, red-purple in colour, the foam of yellowish-purple colour appeared when shaking and that these characteristics did not change during the experiment.

Also, the pH value from the test requirements (6.5 to 7.0) corresponded (+) in all three tested series and in all observed time intervals. In all three series, the pH value fluctuated slightly in the observed times, and after 18 months in the first and second series the lowest values were registered, which amounted to 6.75 and 6.54.

Table 1. Stability test for mastitis agents within the shelf life periodTemperature: $25^{\circ}\text{C} \pm 2^{\circ}$ Relative humidity: $60 \pm 5\%$

protected from the light, moisture and out of reach of children

| Parameter | Series | Stability test time (months) | | | | | |
|-------------------------------|--------|------------------------------|------|------|------|------|------|
| | | 0 | 3 | 6 | 9 | 12 | 18 |
| Appearance/ exterior | 1 | + | + | + | + | + | + |
| | 2 | + | + | + | + | + | + |
| | 3 | + | + | + | + | + | + |
| pH (6,50-7,00) | 1 | 6.85 | 6.88 | 6.85 | 6.82 | 6.80 | 6.75 |
| | 2 | 6.70 | 6.68 | 6.71 | 6.70 | 6.65 | 6.54 |
| | 3 | 6.62 | 6.60 | 6.60 | 6.61 | 6.62 | 6.64 |
| Dry residue 4,75- 5,25 (%) | 1 | 5.00 | 4.96 | 4.95 | 4.98 | 4.94 | 4.88 |
| | 2 | 5.02 | 5.00 | 4.98 | 4.97 | 4.92 | 4.85 |
| | 3 | 5.10 | 5.06 | 5.10 | 4.98 | 4.99 | 4.90 |
| Microbiological purity | 1 | + | + | + | + | + | + |
| | 2 | + | + | + | + | + | + |
| | 3 | + | + | + | + | + | + |

*The + = compliant/corresponds

Also, the values obtained from the dry residue corresponded/compliant with the requirement (4.75 to 5.25%) in all three series examined in all periods of observation. In addition, these values generally showed slight continuous decrease from the start of the testing to the end of the shelf life period (18 months), from 5.00 to 4.88% for Series 1, also from 5.02 to 4.85% for the Series 2 and from 5.10 to 4.90% for the third series (Table 1).

Microbiological purity was compliant/corresponding (+) in all three series examined and all tested periods of observation.

According to reports on sensory evaluation of the active and auxiliary substances and finished products ad.us.vet. (NIVS, 2012), of the total of 35 samples examined, the parameter appearance/exterior for all samples was compliant/corresponded with the requirements and the Law on Medicines and Medical Devices (*Official Gazette of RS 30/2010 and 107/2012*). The same results were obtained in our stability test for mastitis reagent, for the tested parameter appearance/exterior.

Also, according to the manufacturer attesting analysis (*QCC-P-162-1, 2004*): pH value of the mastitis reagent was 6.60, i.e. it was within the requirements of 6.50 to 7.00. Also, values obtained in the pH stability test for mastitis reagents ranged within the limits of the requirement in the observed periods of time within the shelf life. Identical data were obtained for microbiological purity.

According to some authors (*Gašić and Orešković, 2008*), by determining the moisture content % of dry residue was calculated, and the data were used for correlation with the content of active substance for several finished products. Dry

residues of atrazine were 48.30 to 52.53%, for 475 to 525 g/l of active substance. In our experiment, the dry residue of mastitis reagent ranged within the limits of the requirement - from 4.75 to 5.25%.

Conclusion

Based on the results obtained in the test of stability of the finished product mastitis reagent, the following can be concluded:

- that the storage conditions, temperature and relative humidity were adequate;
- appearance/exterior of the reagent didn't change within the shelf life;
- pH value and the dry residue were within the limits of the requirement;
- microbiological purity was adequate also at the end of expiration time;
- the quality of mastitis reagent, in above-mentioned storage conditions, was ensured/provided within a period of 18 months.

Acknowledgment

Projects MNTR Government of Serbia no, 46009 and no. 31034 for the period (2011-2014).

Test stabilnosti za mastitis reagens ad us.vet.

V.Vuković, M.Vičentijević, N. Plavša

Rezime

U okviru testa stabilnosti za mastitis reagens ad us.vet., kao gotovog proizvod, ispitane su tri proizvedene serije, u količinama od po 500 ml uzorka, pri odgovarajućim uslovima čuvanja. Za to je obezbeđena ujednačenost odgovarajuće temperature ($25^{\circ}\text{C} \pm 2^{\circ}$) i relativne vlažnosti ($60 \pm 5\%$). Isto tako, određena je i procedura testa stabilnosti koja obuhvata početno stanje, zatim tromesečno ispitivanje do isteka prve godine i jedno završno ispitivanje na kraju roka trajanja (0, 3, 6, 9, 12 i 18 meseci). Od testiranih parametara obuhvaćeni su: izgled, pH vrednost rastvora, suvi ostatak (u %) i mikrobiološka čistoća. Za vreme ispitivanja uzorci su bili zaštićeni od uticaja svetlosti, vlage i bili su van domašaja dece.

References

- ADAMS R.H. (1995): Veterinary pharmacology and therapeutics. 7th Edition, Iowa State Univ. Press/Ames, Iowa.
- BATIS J. (1976): Problemi dijagnostike mastitisa krava. Veterinarski glasnik, 7,30
- DOHOO I.R., MEEK A.H., MARTIN S.W. (1984): Somatic cell counts in bovine milk: Relationships to production and clinical episodes of mastitis. Can J Comp Med Rev Can Med Comp. 48, 130-135.
- GAŠIĆ S., OREŠKOVIĆ Z. (2008): Determination of active ingredient content based on correlation with dry content in suspension concentrates. Određivanje sadržaja aktivne materije u koncentrovanim suspenzijama na osnovu korelacije rezultata sa sadržajem suvog ostatka. Pestic.Phytomed, Belgrade, 23, 59-62.
- JEZDIMIROVIĆ M. (2000): Veterinarska farmakologija. II prerađeno izdanje, Fakultet veterinarske medicine Beograd.
- MILJKOVIĆ V. (1977): Higijena i tehnologija mleka. Fakultet veterinarske medicine Beograd.
- NAUČNI INSTITUT ZA VETERINARSTVO SRBIJE (NIVS) (2012): Knjiga laboratorijskih pregleda Odeljenja za ispitivanje lekova.
- PH.EUR.(2006): European Pharmacopoeia - 6th edition, EDQM, Strasbourg.
- PH.JUG. IV (1984): Farmakopeja SFRJ. Savezni zavod za zdravstvenu zaštitu, Beograd.
- PH.JUG. V (2000) Farmakopeja. Savezni zavod za zdravstvenu zaštitu, Beograd.
- POUTREL B., RAINARD P (1981): California mastitis test guide of selective dry cow therapy. J Dairy Sci 64, 241-248.
- PROPIS QCC-P-162-1 (2004): Mastitis reagens ad.us.vet. Farmaceutsko hemijsko biološka dokumentacija.
- RUEGG L. PAMELA, REINEMANN J. DOUGLAS (2002): Milk Quality and Mastitis Tests. University of Wisconsin, Madison
- VALČIĆ J. (1995): Lekovi ad us.vet. i zakonska regulativa. Savezno ministarstvo za privredu.
- VUKOVIĆ V. (1998): Određivanje broja somatskih ćelija u zbirnim uzorcima mleka sa većine otkupnih mesta u Srbiji. VII Kongres veterinarara Jugoslavije, sa međunarodnim učešćem, Beograd, Zbornik radova, 203.
- VUKOVIĆ V., ERSKI-BILJIĆ MILANKA, JAKIĆ DOBRILA (1988): Raširenost mastitisa na jednoj društvenoj farmi krava u SR Srbiji. XXX Naučni sastanak mikrobiologije, epidemiologije i infektologa Jugoslavije, Pula, 963.
- Sl. GLASNIK RS 30/2010: Zakon o lekovima i medicinskim sredstvima.
- WILSON D.J., GONZALEZ R.N. (1997): Evaluation of milk culture, SCC and CMT for screening herd additions. Proceedings of the 36th Annual Meeting of National Mastitis Council, Albuquerque, NM. Natl Mast Coun. Madison WI, 127-131