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Strip-dried whole milk sampling technique for progesterone detection in cows by ELISA

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ABSTRACT

New sampling format of whole cows' milk in strip-dried form was proposed. Few methodological issues of whole milk progesterone ELISA using samples dried on a membrane carrier in a form of strip were investigated and optimized: width of a strip, shape of punched/cut-off part of membrane, sample application method. It was shown that distribution of the hormone along narrow strip was even except the initial part of a strip (the first 0.5 × 0.5 cm piece) where recovered concentration of progesterone was higher. Storage stability of progesterone in strip-dried whole cows' milk samples at 4 °C, ambient temperature, 37 °C and 60 °C was investigated. Rising of the detected progesterone concentration over storage period at elevated temperatures was observed predominantly in milk samples with low hormone concentration (from non-pregnant cows). Strip-dried whole milk samples can be used for collection, transportation, storage and ELISA analysis of progesterone level which is correlated with reproductive status of cows.

1. Introduction

Technology based on the application of dried samples for storage, transportation and analysis of biological fluids for the presence of high and low molecular weight antigens and DNA/RNA is used in laboratory diagnostics, pharmacokinetic and toxicokinetic studies [1–3]. Originally the technology was realized in a form of Dried Blood Spots (DBS) in neonatal screening [4]. Nowadays a variety of samples such as saliva, urine, human milk, blood serum and plasma are prepared in a form of dried spots and analysed for diverse diagnostic purposes [5,6]. However DBS technology is well established for medical screening it is still not wide spread for veterinary diagnostics. Among examples of DBS veterinary application are: analysis of small animals for toxic heavy metals exposure [7] and wild birds for exposure to environment toxicants [8], detection of some infections in canine and water buffaloes blood samples [9,10], avian sexing [11] and some others. Cows' and saws' pregnancy diagnosis as well as monitoring of progesterone (P4) levels during estrous cycle were also performed by the analysis of dried blood spots [12,13]. DBS technology is usually realized in a form of round spots of biological fluids placed onto a special sample collection card made of cellulose fibers. After sample application and drying a part of membrane is sub-punched and analysed for the presence of target antigen. Application of DBS technology in quantitative assay is associated with some limitations, for instance, hematocrit effect [1,3]. Hematocrit value (the volume percentage of red blood cells in whole blood) varies from sample to sample and influences the spreading of whole blood on filter paper. Therefore volume of sample taken for analysis (sub-punched disc of fixed diameter with dried blood sample) and analyte quantity recovered under analysis will be dependent on hematocrit value that leads to an assay bias. In recent years some new approaches were proposed to overcome these limitations: new format of sample pretreatment [14], new sampler [15] and new material for sample collection [16].

Early detection of cows' pregnancy is an important task for modern dairy husbandry. Measurement of reproductive hormones or pregnancy associated substances in serum and milk by immunochemical methods such as RIA and ELISA helps to detect the reproductive status in cows around 20–30 day post insemination [12,17,18]. Moreover the analysis of P4 in serum or milk allows to evaluate the status of cow's reproductive system and to detect existing reproductive disorders. An inline P4 monitoring system that works automatically and provides real-time physiological information about lactating dairy cows was developed recently [19]. The milk P4 assay is based on the fact that concentration of P4 for pregnant and non-pregnant cow at the end of the oestrus cycle (19–21 day post insemination) is different [20]. The accuracy of pregnancy diagnosis using a P4 milk (or serum) test is usually around 80% however the method has 100% sensitivity for detecting non-pregnant cows [21]. Dried milk sampling on filter paper

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was pioneered by Brown et al. in 1982 on the bovine and human milk samples [22]. In later works human milk was analysed in conventional spot-dried format to detect a number of infections [6] but investigations of paper dried cows' milk were not described. Dried form of cows' milk is a convenient way of samples shipment to a laboratory. After dried samples prepared on-site, for instance, on farm, their transportation to laboratory is less consuming compared to liquid whole milk samples when special temperature conditions should be provided. So, frequent noninvasive monitoring of P4 in cows based on modern analytical techniques can become more available for any farm with dried form of whole milk samples easily prepared on-site and transported to analysis spot.

In our previous works the successful application of a new format of sample pretreatment in strip-dried form for milk P4 ELISA and some infectious cattle diseases analysis was demonstrated [23–25]. The aim of the present study was to investigate some methodological aspects associated with sample preparation and P4 analysis of strip-dried whole cows' milk: format of a membrane strip, the shape of the punched/cut-off piece of membrane, method of sample application on a strip, distribution of sample (antigen) along a strip. Suitable conditions of strip-dried whole cows' milk samples transportation and short- and long-term P4 storage stability were also investigated. Optimisation of sampling, storage and analysis conditions of whole milk strip-dried samples can provide reliable and efficient tool for P4 level monitoring in dairy cattle, first of all with the aim of early detection of non-pregnant cows (on 21st day after artificial insemination).

2. Material and methods

0.5 cm and 1 cm-width milk sampling strips were fabricated from glass fiber membrane (MAPDS-0300, Arista Biologicals, USA) and supplied by Immunoved, Russia. 0.5 cm width membrane was supplied with black marks at every 0.5 cm.

Inorganic salts, acids and organic solvents were obtained from Chimmed (Russia). Ready-to-use substrate solution containing TMB was supplied by Immunoved (Russia).

The following buffers were used: 0.01 M K-phosphate (K_2HPO_4-KH_2PO_4), 0.15 M NaCl, pH 7.4 (PBS) and 0.01 M K-phosphate (K_2HPO_4-KH_2PO_4), 0.15 M NaCl, 0.05% Tween 20, pH 7.4 (PBST).

2.1. Analysis of native and strip-dried whole cows' milk samples by ELISA

Whole milk samples were collected in clean tubes at the end of afternoon milking of Holstein Friesian breed cows. Samples were frozen at −20 °C and also prepared in strip-dried form. For this an aliquot of fresh milk (100–150 µl) was applied onto the end of membrane strip (0.5 cm or 1 cm width) or the end of membrane strip was dipped into sample to saturate with milk (Fig. 1). After drying (1–2 h at ambient temperature) samples were stored in zip-lock plastic bags with desiccant. For the analysis of strip-dried whole milk samples P4 standard solutions in strip-dried form were prepared similarly. For the analysis, we used a 10 µl aliquot of a defrosted (liquid) sample and a piece of membrane with strip-dried sample: manually punched 0.5 cm (Ø) or 0.5 × 0.5 cm square piece cut-off by scissors. A piece of membrane was put into microtiter plate well and wetted with 15 µl distilled water. All other steps were performed according to the kit instruction ("ELISA-progesterone-milk", Immunoved, Russia). All samples were analysed in duplicates.

2.2. Investigation of strip-dried milk P4 storage stability

Strip-dried whole cows' milk samples prepared as described above were stored at 37 °C, 4 °C and ambient temperature (20–25 °C) and checked by ELISA after 7 days, 1 and 6 months storage. Samples were also incubated for 24 h at 60 °C and analysed.

3. Results and discussion

In our previous work dried milk sampling technique and its application for P4 analysis in whole cows' milk was described [23]. Milk was applied onto 1 cm-width sampling strip fabricated of membrane material and dried. On the next step round pieces of membrane (discs) were sub-punched and analysed by ELISA. It was shown that results of P4 analysis of liquid and dried samples were correlated (r = 0.911). Recently we proposed a new format of sample pretreatment to obtain strip-dried sample [24–26]. The approach is based on the application of biological fluid onto narrow strip (0.5 cm width) (Fig. 1). For the analysis a piece of membrane in a form of square (0.5 × 0.5 cm) was cut-off. New sampling format utilizing strip-dried samples has a few advantages over conventional DBS format where biofluid is applied dropwise onto special cellulose card. The applied sample distributes evenly along a strip and after drying a piece of membrane in a form of square (0.5 × 0.5 cm) can be cut-off by ordinary scissors and analysed by ELISA or PCR [25,26]. One strip-dried sample (sampling strip) can provide up to ten square pieces (0.5 × 0.5 cm) of carrier with equal amount of trapped biofluid (target antigen). This type of sample pretreatment represents an example of volumetric sampling and can be used for quantitative analysis [26]. One of the advantages of strip-dried approach is the convenience of non-coloured biofluids sampling, for instance milk. Conventional DBS sampling cards intending to collection of non-coloured biofluids are usually saturated with visualizing agents. In the case of sampling onto narrow strip there is no need to use special agents; one just should be sure that the migrated biofluid wets the defined amount of marked pieces (squares) of the strip. Fluid migration along a strip can be easily controlled by eye.

Few methodological issues of P4 ELISA using whole cows' milk samples dried on a membrane carrier in a form of narrow strip were investigated and optimized: width of a strip, shape of punched/cut-off part of membrane, sample application method.

3.1. Investigation the influence of application strip/analysed piece of membrane format on ELISA performance

Format of membrane strip used in our previous work (1 cm-width) [23] was compared with new narrow strip format (0.5 cm-width) to detect P4 in dried whole milk samples (Fig. 2). Strip-dried standards were prepared on 1 cm and 0.5 cm-width strips by saturation from standard solutions of ELISA kit. Shape of the membrane piece separated for analysis was also different for both cases. In the first case a disc (Ø0.5 cm) was sub-punched from a strip, in the second – 0.5 × 0.5 cm square piece of a strip was cut-off. Alternatively 10 µl of standard solutions were applied onto empty sub-punched discs or square pieces (0.5 × 0.5 cm) of membrane and dried. Then separated membrane pieces with dried standard solutions were used for the generation of P4 standard curve in ELISA. It should be stressed that while membrane pieces in a form of square (0.5 × 0.5 cm) were used the immmunoassay sensitivity was slightly higher than for ELISA with membrane discs (Fig. 2). The same pattern was observed for discs and square pieces prepared by separation from a strip saturated with standard solution or by the application an aliquot of standard solution onto free sub-punched/cutted-off membrane piece. The difference can be seen at the beginning of calibration curves for the points where P4 concentration is low. It could be explained as follows. As far as the location of membrane piece in a microtiter plate well is concerned, a round piece of membrane (disc) lays on well bottom whereas a square piece inclines in a well (Fig. 2). It is likely that in the second case a dried sample with target analyte is solubilised quicker and evenly from membrane support. In this case both sides of membrane are in contact with working buffer whereas a disc freely faces the buffer in well only by its upper part. At the same time the disc laying on well bottom can shield immobilized antibodies from free interaction with target antigen and conjugate in reaction mixture. Probably all these factors resulted in lower value of optical density which occurred at low P4 concentrations.

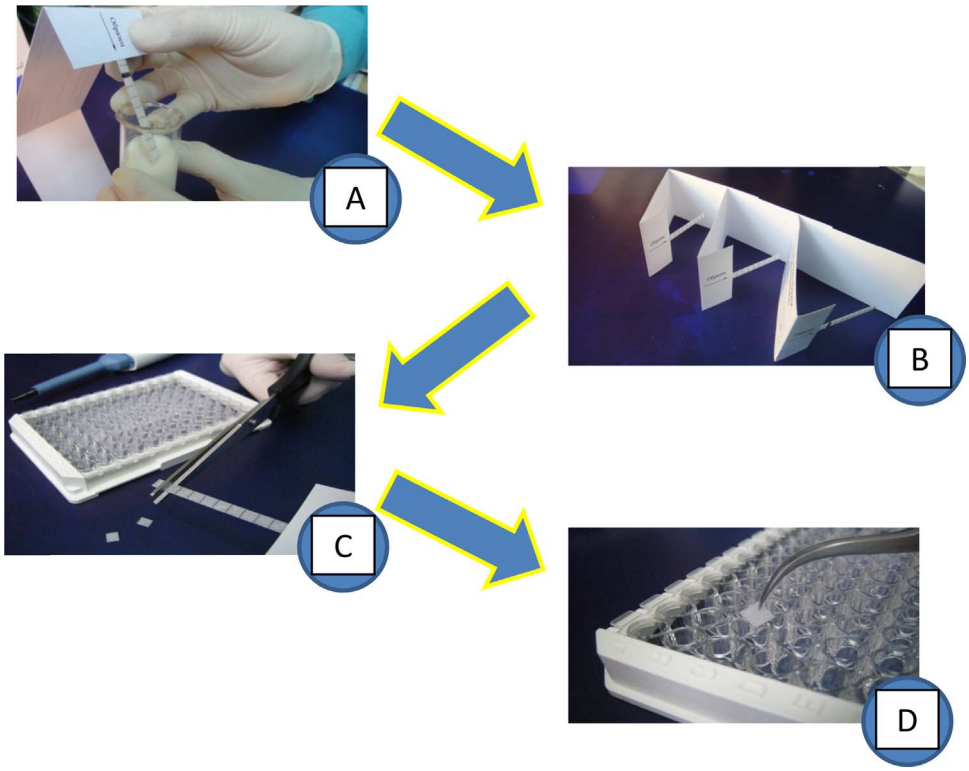

Fig. 1. General scheme of preparation and analysis of strip-dried whole milk samples using membrane sampling strip. (A) – saturation of a 0.5 × 5 cm sampling strip with whole milk sample; (B) – strip drying at room temperature; (C) - cutting of square pieces of a strip; (D) – a piece of a strip with dried sample is put into microtiter plate well and analysed (ELISA).

Strip-dried milk samples were prepared by similar way on 0.5 cm and 1 cm-width membrane strips. Then separated membrane pieces were analysed in ELISA. In both cases (disc or square piece of membrane) similar correlation was observed for liquid and dried whole milk samples (n = 18) measured in P4 ELISA (Fig. 3). Coefficient of correlation was more than 0.97 for both strip/analysed piece of membrane format (Fig. 3). Thus, a narrow strip provides convenient way of whole milk sampling and sensitive assay allowing P4 detection in physiologically important concentration range (0–30 ng/mL in milk). 0.5-width strip showed better performance in terms of assay sensitivity and convenience of strip-dried sample utilizing.

3.2. Investigation of P4 distribution along a membrane strip with dried whole cows' milk

The assay reproducibility was investigated under measurement of P4 concentration for all sequential pieces of membrane (from no. 1 to no. 6) with strip-dried sample for analysed disc (Fig. 4A) or square

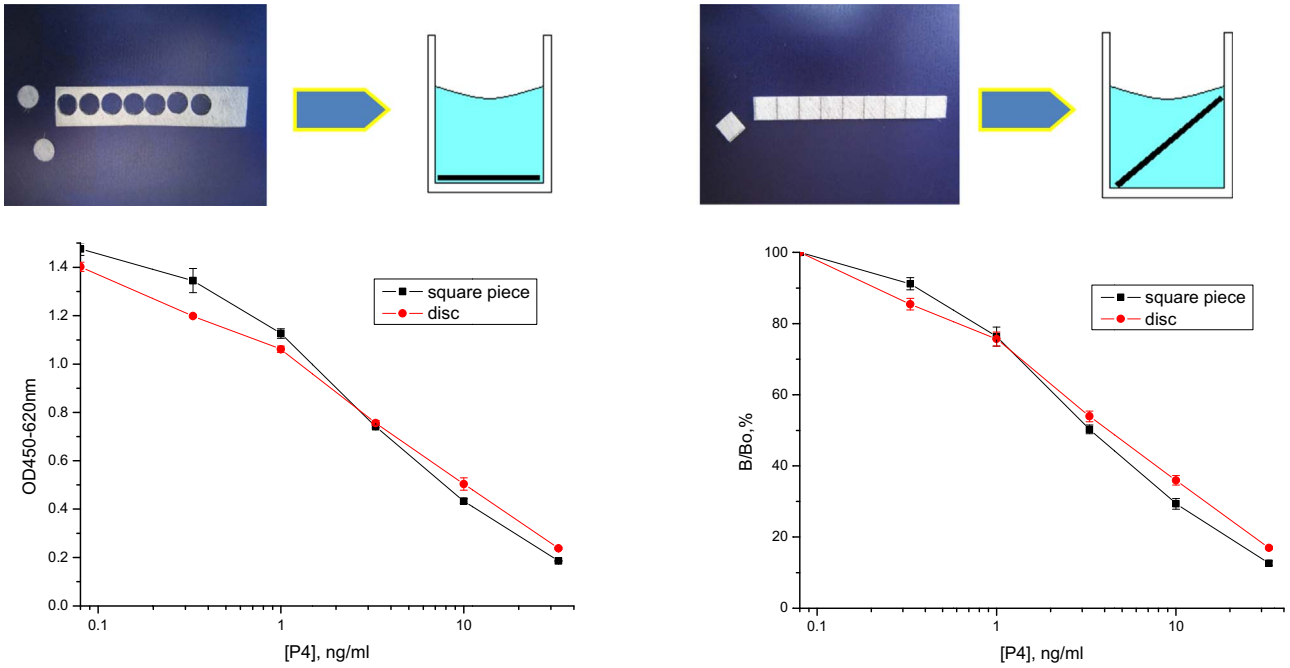

Fig. 2. Calibration curves of P4 ELISA for 0.5 cm (square pieces) and 1 cm-width strips (discs).

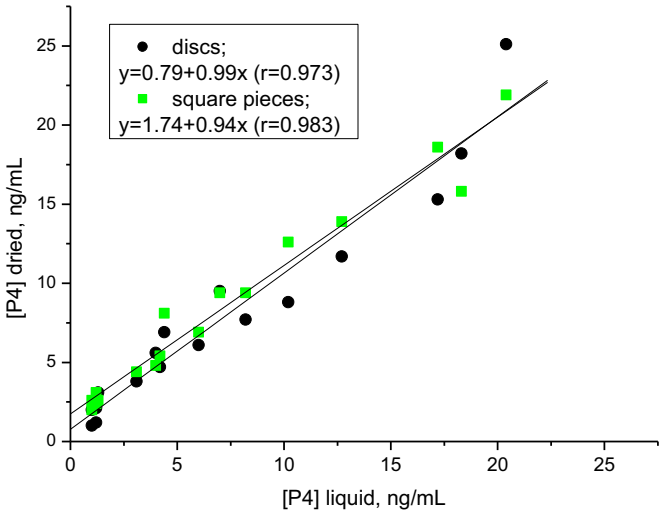

Fig. 3. Correlation of P4 ELISA results for native (liquid) and strip-dried samples of whole cows' milk samples prepared on 0.5 cm (square pieces) and 1 cm-width stripes (discs).

piece (Fig. 4B). For the experiment six cows' milk samples contained different P4 concentration (preliminary checked by ELISA) were applied onto 0.5 cm and 1 cm-width membrane strips and dried. Reproducibility of P4 detection for sequential pieces of membrane from no. 1 to no. 6 was similar and did not exceed 7%. In the first square piece (0.5 × 0.5 cm) of membrane the co-called edge effect was observed i.e. the recovered P4 concentration was higher than in sequential parts. According to our results P4 concentration recovered in the initial (the first) square piece of 0.5-width sampling strip ranged from 6 up to 60% of the mean P4 concentration detected in square pieces from no. 2 to no. 6 (Fig. 4B). The similar findings were observed earlier for a range of low and high molecular weight antigens in strip-dried human serum samples and were attributed to effect resulted from strip drying process [26]. On average in our previous work the recovered analyte concentration in square no. 1 was 10–15% higher than in other membrane pieces with dried serum [26]. Higher recovery values for the initial part of sampling strip with dried whole milk are probably attributed to variability of fat content of whole milk that changes from sample to sample (animal to animal). As the narrow strip starts drying from its edges, the redistribution of a sample has resulted in higher antigen concentration on the very end of the strip. For 1 cm-width strip the edge effect wasn't observed because edges of the strip were almost not trapped in sequentially subpunched discs (Fig. 4A). So any strip format represents applicable way to biofluid sampling and analysis but narrow (0.5-width) strip with square pieces (0.5 × 0.5 cm) has some advantages such as convenience of work with it. Moreover narrow strip (0.5 cm) can be wetted quicker and two times lower application volume of biofluid is needed. So, P4 distributes predominantly even along a strip and for quantitative P4 analysis in strip-dried whole milk samples any piece of membrane can be used except the first one that should be omitted.

3.3. Investigation of the method of sample application onto a strip

Sample application method onto a strip was also investigated. 0.5 cm and 1 cm-width strips were saturated with whole milk sample by two ways. Milk aliquot was applied onto the end of a strip and distributed along it (Fig. 5A) or the end of a strip was dipped into a

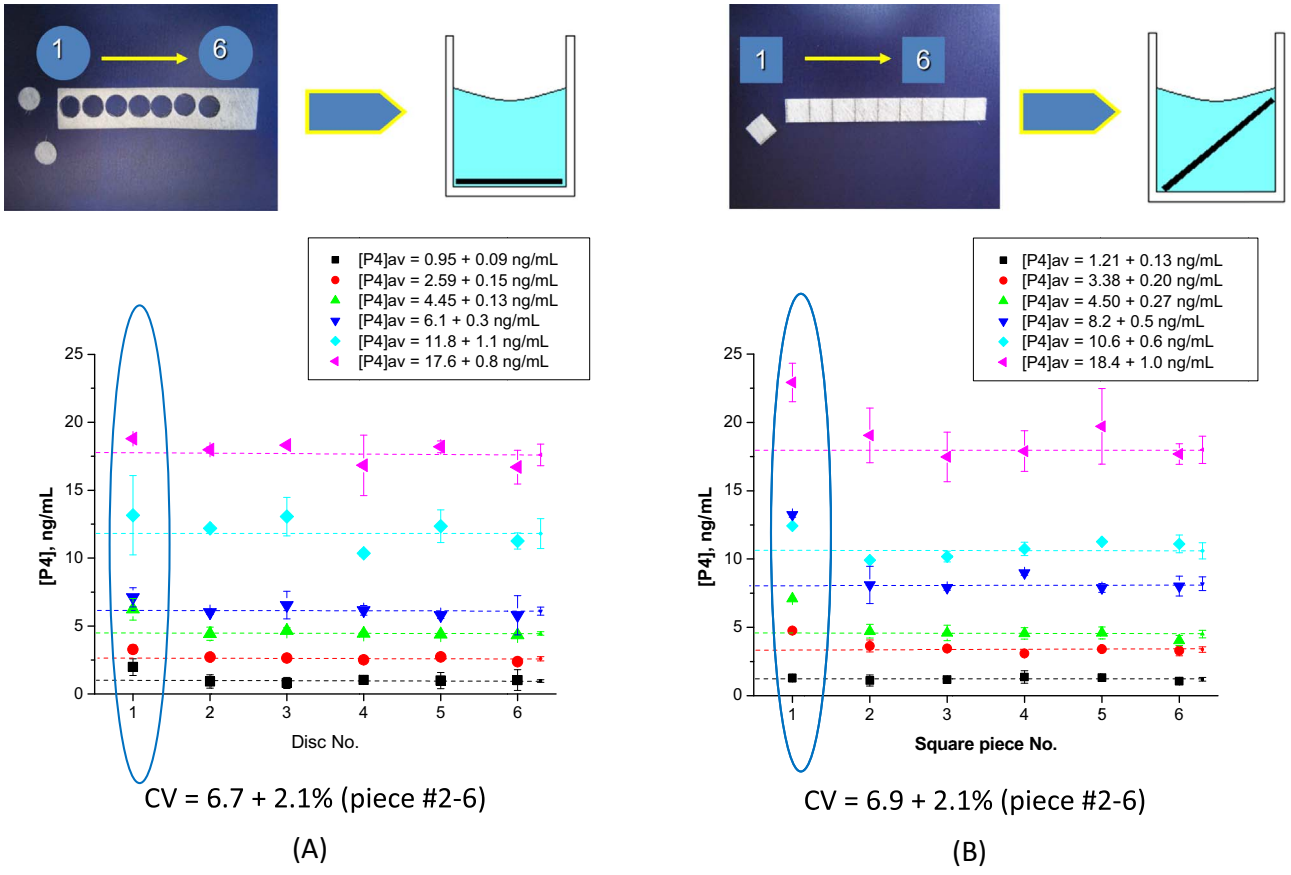

Fig. 4. P4 concentration detected in sequential parts of sampling strip with dried whole cows' milk (from membrane piece no.1 to no. 6). (A) – strip with subpunched discs, (B) – strip with cut-off square pieces. X axis– no. of piece of a sampling strip analysed in ELISA, y axis– P4 concentration detected in membrane piece. An antigen concentration (mean + SD) detected for piece no.2 to piece no.6 is presented as a line (i.e. mean of measurements of pieces no. 2–6).

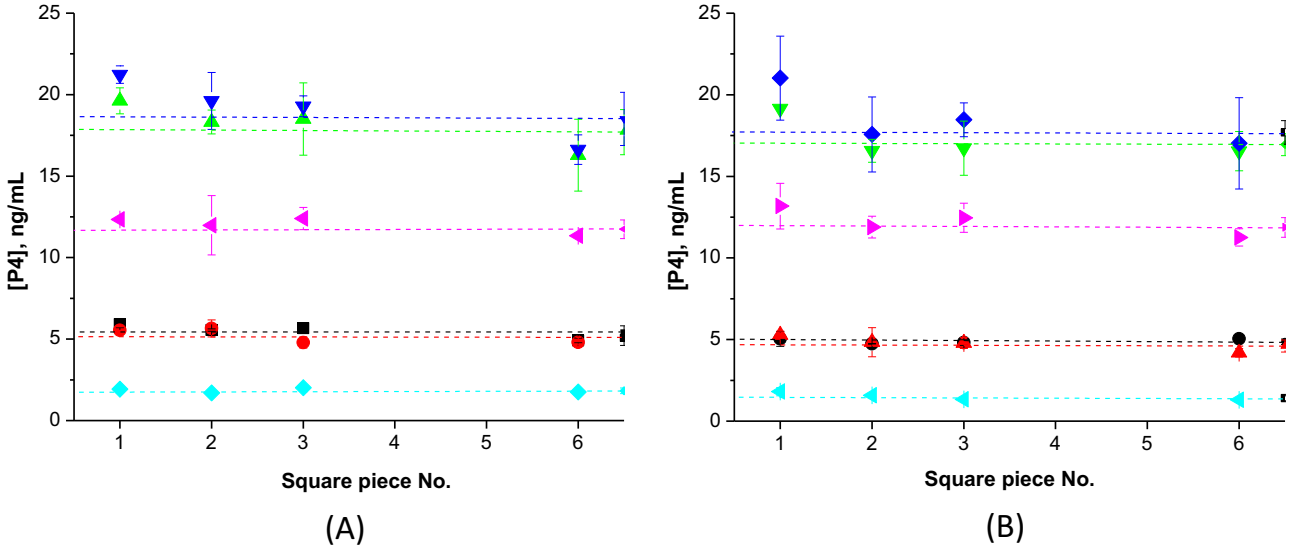

Fig. 5. P4 concentration detected in sequential parts of sampling strip with dried whole cows' milk (from square piece no.1 to no. 6). (A) – aliquot of a sample (100 µl) was applied onto a strip, (B) – strip was saturated with sample by dipping the end of a strip into milk. X axis– no. of piece of a sampling strip analysed in ELISA, y axis– P4 concentration detected in square piece. An antigen concentration (mean ± SD) detected for square piece no.2 to square piece no.6 is presented as a line (i.e. mean of measurements of pieces no. 2–6).

tube with sample and kept there until being fully saturated (Fig. 5B). As it could be seen any of the sampling method can be used and the antigen concentration recovered from sequential strip pieces are identical (Fig. 5). Assay variation did not exceed 7% for sequential membrane pieces in a form of disc or square (0.5 × 0.5 cm) starting from no. 2 to no. 6 along a strip.

The influence of a strip position during drying onto assay results was also investigated. For this milk samples were applied onto narrow strips (0.5 cm-width) and then positioned vertically, horizontally or laterally to dry. In all cases the results of P4 determination in sequential membrane pieces of a strip were identical and similar to the results of P4 distribution along a strip in previous experiment (data not shown). So, whole milk sample can be aliquoted onto a strip or strip can be saturated with sample (by dipping) and dried in any position that simplifies sampling process providing convenient dried form of whole cows' milk for the following quantitative analysis.

3.4. P4 storage stability in strip-dried whole cows' milk samples

It was found earlier that P4 in dried blood (on filer paper) was stable at 4 °C and ambient temperature for at least 9 and 15 weeks, respectively [27]. P4 was also stable for at least 9 weeks at 37 °C in the presence of desiccant, although in the presence of high humidity significant losses occurred within one week storage [27]. In this work P4 storage stability in strip-dried whole milk samples was investigated. In total 42 milk samples were stored at 37 °C, 4 °C and ambient temperature and checked by ELISA after 7 days and then after 1 and 6 months storage (Fig. 6). It should be stressed that majority of investigated whole milk samples showed storage stability for 1 month at 4 °C and ambient temperature (Fig. 6A, B). However for strip-dried samples stored at ambient temperature for 1 month larger dispersion of recovery results was observed (Fig. 6B). After 6 months storage average recovery for samples stored at 4 °C raised up to 138 ± 33% (Fig. 6C). At the same time after 6 months storage at ambient temperature milk samples with P4 concentration less than 10 ng/mL showed obvious trend towards higher percentage of P4 recovery (Fig. 6D). Long-term storage (6 months) was performed during spring and summer period when ambient temperature in some days raised up to 30–33 °C. Elevated temperature could probably cause P4 release from milk fat fraction. Fat content of milk can be different from animal to animal. It is known that P4 predominantly accumulates in the lipid fraction of milk. It was found earlier that P4 concentration in fat fraction of milk is about 150 times higher than in aqueous phase [28]. So the effect observed probably is not resulted from P4 stability issue but from some changes which occurred during the particular milk sample storage.

Majority of samples except a few one with low P4 concentration showed good stability during 7 days storage at 37 °C (Fig. 6E). One week storage at 37 °C is usually considered to be equivalent one year storage at 4 °C but the assumption could not be accepted in the case because of the results of milk samples storage at 4 °C for 6 months (see above). One week storage at elevated temperatures it is also a modeling of strip-dried samples transportation to laboratory without could chain. Strip-dried whole milk samples were also incubated at 60 °C for 24 h and checked. Majority of samples showed good stability however for six samples recovered P4 concentration was more than 150% (Fig. 6F).

In all the cases a few milk samples with low hormone concentration from non-pregnant cows taken at the end of estrous cycle showed the rising of the detected P4 concentration even after short term storage (Fig. 6). The reason of this finding is not quite clear but it might be assumed that partial P4 release from milk fat could occur. It should be noted that the detected P4 concentration in these samples was still within limits of hormone concentration usually observed for non-pregnant cows, i.e. < 4 ng/mL. So whole milk samples from non-pregnant cows can be transported, stored and after being analysed can be correctly identified as belonged to non-pregnant animals (as contained low P4 concentration).

On the whole, the following recommendations over samples storage should be proposed: strip-dried whole cows' milk samples can be transported to the laboratory during a week at temperature not higher than 37 °C and stored for up to a month for 4 °C without significant influence on the detected cows' status.

4. Conclusions

Simple and effective system of sampling and transportation of strip-dried whole milk samples to laboratory for the following quantitative P4 detection was developed. The preparation of whole milk strip-dried samples represents an alternative to conventional drop sampling. Drop sampling needs quite precise drop application close to the middle of marked circle on a card. Moreover the edge of white-coloured biofluid like milk dried as a spot on white application paper could be difficult to identify while punching. In the case of membrane strip a sample can be

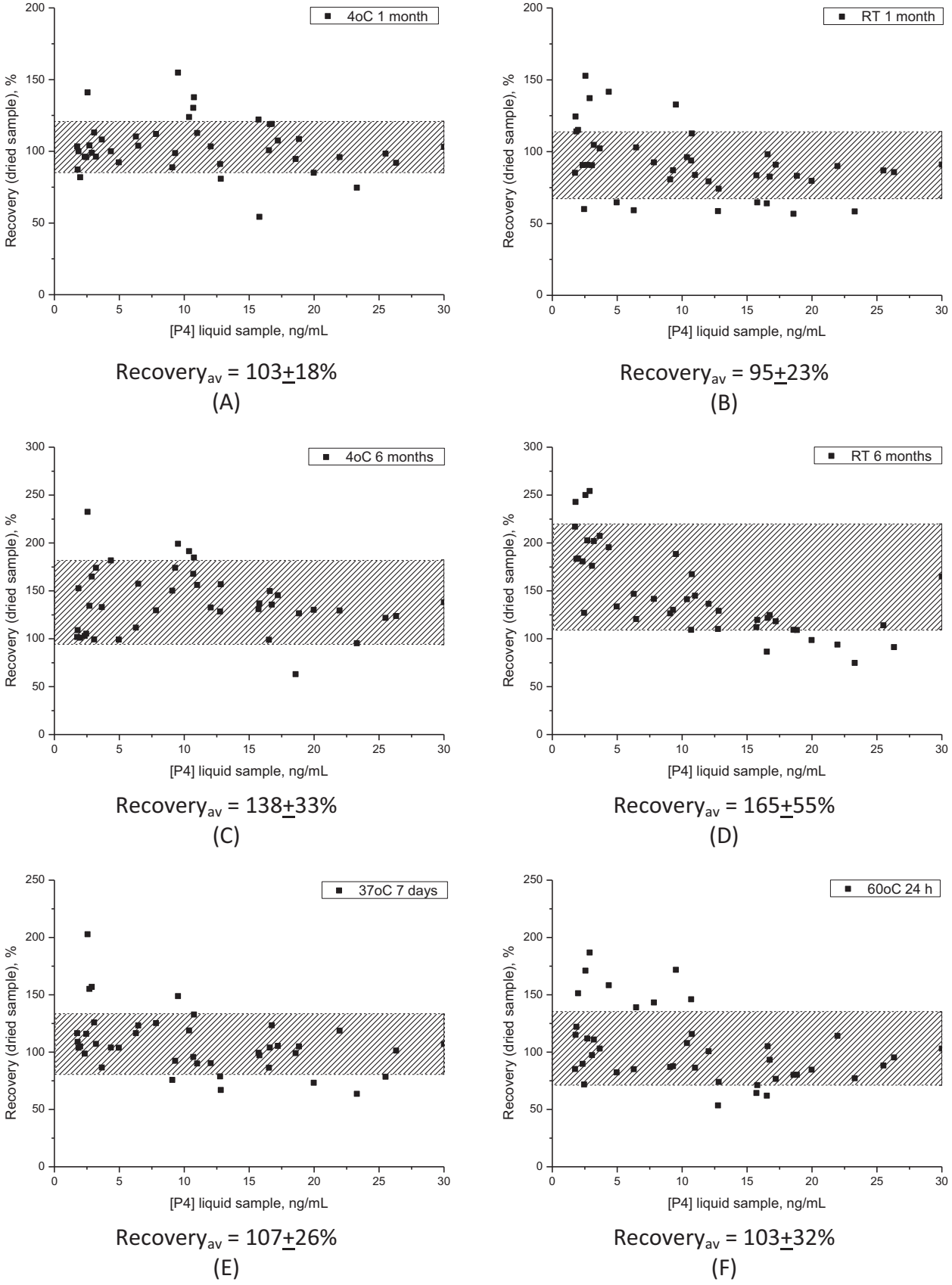

Fig. 6. P4 stability in strip-dried whole cows' milk stored at 4 °C (A, C) and ambient temperature (20–25 °C) (B, D), 37 °C (E) and 60 °C (F). X axis – P4 concentration in liquid samples, ng/mL; Y axis – recovery of corresponding strip-dried samples after storage period, %.

applied onto a strip by different ways providing even distribution of the sample with target analyte along a strip. The wetted part of membrane strip can be easily identified by eye and whole strip could be used for analysis as soon as applied biofluide migrated up to bold line on a strip (ten square pieces). New format of sample pretreatment when liquid is applied onto a narrow membrane strip, dried and a square piece (0.5 × 0.5 cm) is used for the following analysis has some advantages in preparation, storage and analysis of whole milk samples. Stripes with dried cows' milk are rigid and a piece of membrane can be easily separated. Strip-dried whole milk samples can be transported to laboratory without cold chain for short period and stored for a month at 4 °C without significant influence on detected cows' status.

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Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

References

[1] A.J. Wilhelm, J.C. den Burger, E.L. Swart, Therapeutic drug monitoring by dried blood spot: progress to date and future directions, Clin. Pharmacokinet. 53 (2014) 961–973. http://dx.doi.org/10.1007/s40262-014-0177-7.

[2] R.J. Meesters, G.P. Hooff, State-of-the-art dried blood spot analysis: an overview of recent advances and future trends, Bioanalysis 5 (2013) 2187–2208. http://dx.doi.org/10.4155/bio.13.175.

[3] A. Sharma, S. Jaiswal, M. Shukla, J. Lal, Dried blood spots: concepts, present status, and future perspectives in bioanalysis, Drug Test. Anal. 6 (2014) 399–414. http://dx.doi.org/10.1002/dta.1646.

[4] P.A. Demirev, Dried blood spots: analysis and applications, Anal. Chem. 85 (2013) 779–789. http://dx.doi.org/10.1021/ac303205m.

[5] J. Henion, R.V. Oliveira, D.H. Chace, Microsample analyses via DBS: challenges and opportunities, Bioanalysis 5 (2013) 2547–2565. http://dx.doi.org/10.4155/bio.13.197.

[6] E.M. Miller, M.O. Aiello, M. Fujita, K. Hinde, L. Milligan, E.A. Quinn, Field and laboratory methods in human milk research, Am. J. Hum. Biol. 25 (2013) 1–11. http://dx.doi.org/10.1002/ajhb.22334.

[7] A.F. Lehner, W. Rumbeiha, A. Shlosberg, K. Stuart, M. Johnson, R. Domenech, H. Langner, Diagnostic analysis of veterinary dried blood spot for toxic heavy metal exposure, J. Anal. Toxicol. 37 (2013) 406–422. http://dx.doi.org/10.1093/jat/bkt048.

[8] A. Shlosberg, W.K. Rumbeiha, A. Lublin, K. Kannan, A database of avian blood spot examinations for exposure of wild birds to environmental toxicants: the DABSE biomonitoring project, J. Environ. Monit. 13 (2011) 1547–1558. http://dx.doi.org/10.1039/c0em00754d.

[9] W.G. Holland, N.G. Thanh, L.N. My, E. Magnus, D. Verloo, P. Büscher, B. Goddeeris, J. Vercruysse, Evaluation of whole fresh blood and dried blood on filter paper discs in serological tests for *Trypanosoma evansi* in experimentally infected water buffaloes, J. Acta Trop. 81 (2002) 159–165. http://dx.doi.org/10.1016/S0001-706X(01)00211-X.

[10] H. Tani, Y. Tada, K. Sasai, E. Baba, Improvement of DNA extraction method for dried blood spots and comparison of four PCR methods for detection of *Babesia gibsoni* (Asian genotype) infection in canine blood samples, J. Vet. Med. Sci. 70 (2008) 461–467. http://dx.doi.org/10.1292/jvms.70.461.

[11] G. Suriyaphol, N. Kunnasut, S. Sirisawadi, W. Wanasawaeng, S. Dhitavat, Evaluation of dried blood spot collection paper blotters for avian sexing by direct PCR, Br. Poult. Sci. 55 (2014) 321–328. http://dx.doi.org/10.1080/00071668.2014.925087.

[12] D. Sun, Y.I. Cho, P. Comyn, K.J. Yoon, Use of blood collected onto and dried on filter paper for diagnosing pregnancy in cattle, Vet. J. 198 (2013) 494–497. http://dx.doi.org/10.1016/j.tvjl.2013.09.016.

[13] S. Chadio, E. Xylouri, D. Kalogiannis, E. Michalopoulou, S. Evagelatos, I. Menegatos, Early pregnancy diagnosis in swine by direct radioimmunoassay for progesterone in blood spotted on filter paper, Anim. Reprod. Sci. 69 (2002) 65–72. http://dx.doi.org/10.1016/S0378-4320(01)00145-2.

[14] A.C. Rosypal, L.D. Pick, J.O. Esquivel Hernandez, D.S. Lindsay, Evaluation of a novel dried blood spot collection device (HemaSpotTM) to test blood samples collected from dogs for antibodies to Leishmania infantum, Vet. Parasitol. 205 (2014) 338–342. http://dx.doi.org/10.1016/j.vetpar.2014.07.031.

[15] P. Denniff, N. Spooner, Volumetric absorptive microsampling: a dried sample collection technique for quantitative bioanalysis, Anal. Chem. 86 (2014) 8489–8495. http://dx.doi.org/10.1021/ac5022562.

[16] L.E. Eibak, A.B. Hegge, K.E. Rasmussen, S. Pedersen-Bjergaar, A. Gjelstad, Alginate and chitosan foam combined with electromembrane extraction for dried blood spot analysis, Anal. Chem. 84 (2012) 8783–8789. http://dx.doi.org/10.1021/10.1021/ac301996n.

[17] A. Waldmann, A. Raud, Comparison of a lateral flow milk progesterone test with enzyme immunoassay as an aid for reproductive status determination in cows, Vet. Rec. 178 (2016) 260. http://dx.doi.org/10.1136/vr.103605.

[18] O. Barbato, M. Merlo, P. Celi, N.M. Sousa, L. Guarneri, J.F. Beckers, G. Gabai, Relationship between plasma progesterone and pregnancy-associated glycoprotein concentrations during early pregnancy in dairy cows, Vet. J. 195 (2013) 385–387. http://dx.doi.org/10.1016/j.tvjl.2012.06.028.

[19] Guang-Min Yu, T. Maeda, Inline progesterone monitoring in the dairy industry, Trends Biotechnol **37** (2017) 579–582.

[20] P. Blavy, M. Derks, O. Martin, J.K. Höglund, N.C. Friggens, Overview of progesterone profiles in dairy cows, Theriogenology 86 (2016) 1061–1071. http://dx.doi.org/10.1016/j.theriogenology.2016.03.037.

[21] G. Purohit, Methods of pregnancy diagnosis in domestic animals: the current status, WebmedCentral REPRODUCTION 1: WMC001305 (2010). ⟨http://dx.doi.org/10.9754/journal.wmc.2010.001305⟩.

[22] S.E.I.I.I. Brown, K.T. Sauer, M. Nations-Shields, D.S. Shields, J.G. Araujo, R.L. Guerrant, Comparison of paired whole milk and dried filter paper samples for anti-enterotoxin and anti-rotavirus activities, J. Clin. Microbiol. 16 (1982) 103–106.

[23] J.V. Samsonova, A.P. Osipov, S.E. Kondakov, A new dried milk sampling technique and its application for progesterone detection in cows, Vet. J. 199 (2014) 471–472. http://dx.doi.org/10.1016/j.tvjl.2013.10.031.

[24] N.Yu Saushkin, J.V. Samsonova, A.P. Osipov, S.E. Kondakov, M.A. Efimova, A.N. Chernov, A new sampling format for the diagnostics of bovine infectious diseases in dried blood spots by ELISA, Mosc. Univ. Chem. Bull. 71 (2016) 253–257 (http://dx.doi.org/10.3103%2FS0027131416040088).

[25] N.Yu Saushkin, J.V. Samsonova, A.P. Osipov, S.E. Kondakov, N.I. Khammadov, K.V. Usoltsev, Kh.Z. Makaev, A.N. Chernov, Comparison of PCR and ELISA methods for the detection of bovine leucosis in dried blood spots, Mosc. Univ. Chem. Bull. 71 (2016) 319–323 (http://dx.doi.org/10.3103%2FS0027131416050084).

[26] J.V. Samsonova, A.D. Chadina, A.P. Osipov, S.E. Kondakov, Porous membrane strip microsampling: a dried biofluid collection format and application for quantitative enzyme immunoassay, Anal. Methods 24 (2016) 4835–4843. http://dx.doi.org/10.1039/C6AY00724D.

[27] P. Petsos, W.A. Ratcliffe, D.C. Anderson, Assessment of corpus luteum function by direct radioimmunoassay for progesterone in blood spotted on filter paper, Clin. Chem. 31 (1985) 1289–1293.

[28] A. Waldmann, E. Ropstad, K. Landsverk, K. Sørensen, L. Sølverød, E. Dahl, Level and distribution of progesterone in bovine milk in relation to storage in the mammary gland, Anim. Reprod. Sci. 56 (1999) 79–91. http://dx.doi.org/10.1016/S0378-4320(99)00036-6.