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Research article

Clinical trials of a test kit for the visual diagnosis of uterine diseases in cows

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Abstract

Background and Aim. The prevalence of uterine diseases in cows is reported to range from 14.3% to 50% of obstetric and gynecologic disorders, compromising reproductive function and increasing economic losses. Clinical diagnosis is most informative in the early postpartum period, whereas laboratory methods, despite high specificity, are often impractical for routine on-farm use. This study aimed to develop a test kit for visual diagnosis of uterine diseases in cows and to determine its diagnostic performance.

Materials and Methods. The study was performed on a commercial dairy farm («En-Dala» LLP) in Holstein-Friesian cows (n=100) in their first or second lactation. The design features of the test kit for visual diagnosis of uterine diseases in cows (catheter length, catheter diameter) were determined using a comparative approach. The performance of clinical methods (rectal and vaginal examinations), laboratory methods (Whiteside test and cytology), and two prototype reagents included in the visual test kit was evaluated by calculating sensitivity, specificity, diagnostic accuracy, PPV, and NPV. The diagnostic parameters of the test (foam formation, discoloration time) were determined using descriptive statistics and a comparative method.

Results. An optimal catheter length of 40 cm and diameter of 0.6 cm were established, and a 50-mL syringe with appropriate design features was selected for aspiration of cervicovaginal mucus (CVM). Prototype 1 (10% H_2O_2 + 0.01% methylene blue) showed the most favorable diagnostic profile (Sens. 90.3%, Spec. 100.0%, Acc. 94.2%, PPV 100.0%, NPV 87.5%) versus the other methods, maintaining maximal specificity regardless of days in milk. Diagnostic criteria for test use were defined as foam formation >1 cm and discoloration within ≤4 min, with stratification by clinical course.

Conclusion. The developed test kit for visual diagnosis of uterine diseases in cows is a rapid and highly specific diagnostic method, enabling its use for screening the herd for uterine disease regardless of the days in milk.

Keywords: clinical diagnosis; clinical effectiveness; dairy cattle; uterine disease.

Introduction

Uterine diseases in cows remain among the most significant causes of impaired reproductive performance and economic losses in dairy cattle production (add a reference). The prevalence of these conditions may range from 14.3% to 50% of all obstetric and gynecologic disorders recorded in dairy cows, which contributes to cow infertility and represents a limiting factor for agriculture sector development [1, 2]. According to data reported by international researchers within the European Union, costs associated with endometritis are approximately €30 per cow [3]. A key feature of the clinical course of uterine diseases in cows is their confusing dynamic: variation in the inflammatory process, the severity of clinical signs, and the differential effectiveness of diagnostic methods depending on days in milk necessitate a stratified diagnostic approach to these conditions [4].

Under on-farm conditions, the most accessible approaches for diagnosing uterine disease remain clinical methods (rectal and vaginal examination) add reference. However, the effectiveness of these methods depends on the veterinarian's expertise and the days in milk, whereas in subclinical cases they are largely uninformative [5, 6]. In recent years, ultrasonography has also been widely implemented on farms for the diagnosis of uterine disease, based on detection of intrauterine exudate, measurement of endometrial wall thickness, and identification of abnormal contents within the uterine lumen; however, these findings are not universal diagnostic indicators in subclinical disease [7, 8]. Therefore, a combined «clinical examination + ultrasonography + cytology» approach is currently recommended when monitoring reproductive tract status in cows at different days in milk points to improve diagnostic accuracy [9].

Research efforts are ongoing to identify rapid diagnostic solutions for uterine diseases in cows that enable accurate diagnosis based on characteristics of uterine contents and the micro and macroscopic structure of the organ itself: changes in the structure of the organ, the cellular composition of the uterine walls, changes in the chemical composition of uterine secretions. For example, histologic examinations have revealed endometrial changes in cows with subclinical endometritis, characterized by a vascular response, strands of collagen fibers, and focal inflammatory lesions [10]. Morphometric measurements of the reproductive tract in cows make it possible to develop a standardized sampling protocol for histologic assessment during biopsy, which in turn facilitates procedural standardization and, consequently, improves diagnostic accuracy [11]. The use of endoscopic systems of various designs may also represent an effective approach for diagnosing uterine disease in cows, as supported by the use of visual insemination system – the AlphaVision device (IMV Technologies, France) which not only increased the proportion of successful inseminations but also identified reproductive system abnormalities in 14% of the study animals [12].

An additional approach involves using the physicochemical properties of uterine contents as a source of diagnostic markers for uterine disease in cows. One example is the Metrisor device (Firat University, Turkey), developed based on gas sensors that detect intrauterine gases produced by pathogenic microflora in the presence of inflammation and enable on-farm diagnosis of metritis in cows with up to 80% accuracy [13]. Evaluation of the enzymatic activity of cervicovaginal mucus has shown that, in combination with dyes, this marker may also serve as a valuable diagnostic tool; accordingly, a new laboratory method for diagnosing uterine diseases in cows was developed [14]. A logical continuation of this work was to adapt the laboratory method for field use by developing an appropriate device and test reagent formulation, followed by validation under farm conditions.

The aim of this study was to develop a test kit for visual diagnosis of uterine diseases in cows and to determine the diagnostic performance parameters when the test is applied. It was expected that, thanks to the optimal design of the test and the composition of the reagents, a method would be developed for diagnosing uterine diseases in cows, allowing for diagnoctics to be carried out in a short time.

Materials and Methods

Ethical approval

The Local Ethics Committee of S. Seifullin Kazakh Agrotechnical Research University (KATRU) approved the study (Protocol No. 3, November 3, 2022). Study planning and implementation were conducted in accordance with the Law of the Republic of Kazakhstan «On Responsible Treatment of Animals» (No. 97-VII LRK, December 30, 2021) [15].

The study was conducted from May to August 2025 at the commercial dairy farm «En-Dala LLP» (Akmola Region) and at the Veterinary Medicine Research Laboratory of the Institute of Animal Science and Veterinary Medicine, KATRU.

The study population comprised Holstein-Friesian cows in their first (n=50) or second (n=50) lactation at 10-91 and more days in milk (d.m.), maintained under a free-stall housing system with ad libitum access to feed and water and milked twice daily.

To determine the sampling device parameters for cervico-vaginal mucus (CVM) collection, catheters with a length of 40 cm and diameters of 0.4 cm, 0.6 cm, and 0.8 cm were compared by assessing insertion depth and the volume of mucus collected. The mucus volume within the catheters was calculated using the cylinder volume formula:

$$V = \pi \times r2 \times h \qquad (1)$$

where:

V – the volume of the contents;

π – a constant approximately equal to 3.14;

r – the radius of the catheter lumen;

h – the height of the column of collected mucus.

For these measurements, cows (n=100) were randomly selected into 5 groups depending on the days in milk: Group I, cows (n = 20) at 10–20 days in milk; Group II, cows (n = 20) at 21–30 days in milk; Group III, cows (n = 20) at 31–60 days in milk; Group IV, cows (n = 20) at 61–90 days in milk; and Group V, cows (n = 20) at ≥91 days in milk.

To evaluate the effectiveness of the prototype diagnostic reagents, a comparative analysis of diagnostic methods for uterine diseases was performed. These included clinical methods (rectal and vaginal examination) and laboratory methods (Whiteside test, cytologic examination, and the prototype diagnostic reagents for the visual diagnostic test kit).

Rectal examination was used to assess uterine position, size, consistency, and rigidity, as well as the condition of the uterine horns and body; particular attention was paid to uterine wall thickening, fluctuation, asymmetry of the horns, and pain on palpation. Indicators of uterine disease were considered to include uterine enlargement, uneven wall consistency, and the presence of fluctuation [16].

Vaginal examination included inspection of the mucosa and cervix and collection of CVM. The collected discharge samples were placed in a sterile container with a screw-cap lid, and CVM was evaluated according to a test card for diagnosing the physiologic status of the reproductive tract in cows [17].

The Whiteside test of CVM was performed by placing 1 mL of CVM into a glass test tube, followed by addition of 1 mL of 5% sodium hydroxide solution. The resulting mixture was heated over an alcohol burner to boiling and then cooled under running tap water. An animal was considered diseased if the tube contents turned lemon yellow; in clinically healthy animals, the tube contents did not change [18].

For cytologic evaluation of CVM, mucus samples were collected using Minitube cytology brushes (Minitube GmbH, Germany), and smears were stained using the Romanowsky-Giemsa method. In each smear, 200–300 cells were counted at 1000 × magnification using an Olympus CX41 microscope (Olympus Corporation, Japan). Smears were evaluated by quantifying epithelial cells and leukocytes, specifically polymorphonuclear neutrophils (PMNs) [19].

The first prototype diagnostic reagent for the visual diagnostic test kit for uterine diseases in cows was based on a method for the visual diagnosis of acute and chronic uterine disorders in cows [20]. Using this prototype, a CVM sample was collected, and the free end of the catheter was sealed with a cap. Next, 1 mL of a 10% hydrogen peroxide solution and 1 mL of a 0.01% methylene blue solution were sequentially introduced into the mucus through the catheter lumen. An animal was classified as diseased if foam formation and discoloration of the device contents occurred; in clinically healthy animals, no foam formation was observed and the contents retained their original color [21].

As the second prototype diagnostic reagent for the visual diagnostic test kit for uterine diseases in cows, a 10% hydrogen peroxide solution and a 0.03% 4-chloro-1-naphthol solution were used. The procedure was performed analogously to that of the first prototype. An animal was classified as diseased if foam formation occurred and the device contents turned dark gray; in clinically healthy animals, foam formation did not occur or was minimal, and the color of the contents remained unchanged.

To determine the clinical effectiveness of diagnostic methods for uterine diseases in cows, sensitivity (Sens.), specificity (Spec.), diagnostic accuracy (Acc.), positive predictive value (PPV), and negative predictive value (NPV) were calculated using the following formulas [22]:

$$Sens. = \frac{TP}{TP + FN} \quad (2)$$

where:
Sens. – sensitivity;
TP – number of true positive samples;
FN – number of false negative samples.

$$Spec. = \frac{TN}{FP + TN} \quad (3)$$

where:
Spec. – specificity;
TN – number of true negative samples;
FP – number of false positive samples.

$$\boldsymbol{Acc.=Sens.\times Prev.\times Spec.\times(1\text{-}Prev.)} \quad (4)$$

where:
Acc. – diagnostic accuracy;
Sens. – sensitivity;
Prev. – prevalence;
Spec. – specificity.

$$PPV \ = \frac{Sens.\times\ Prev.}{Sens.\times\ Prev. + (1 - Spec.) \times (1 - Prev.)} \quad (5)$$

where:
PPV – positive predictive value;
Sens. – sensitivity;
Prev. – prevalence;
Spec. – specificity.

$$NPV \ = \frac{Spec.\times\ (1 - Prev.)}{(1 - Sens.) \times Prev. + Spec.\times\ (1 - Prev.)} \quad (6)$$

where:
NPV – negative predictive value;
Spec. – specificity;
Prev. – prevalence;
Sens. – sensitivity.

This study used a combined reference in the form of vaginal examination and cytology. To avoid incorporation bias, the performance of the vaginal examination was evaluated against a composite reference excluding vaginal examination, and the performance of the cytology was evaluated against a composite reference excluding cytology.

Diagnostic parameters obtained when using the visual diagnostic test were determined using standard metric measurements and descriptive statistical methods. The evaluated parameters included foam height and the time to color change of the catheter contents.

Statistical analyses were performed using Jamovi (The jamovi project, version 2.7.6) and MedCalc (MedCalc Software Ltd, version 23.4.8) [23, 24]. The statistical analysis was predominantly descriptive. Continuous (quantitative) variables were summarized as the mean and standard deviation (M ± SD); the standard deviation was calculated using the standard sample formula and used as the primary measure of variability. Diagnostic performance was assessed using 2×2 contingency tables (true positives, false positives, false negatives, and true negatives) against the reference diagnosis (clinical examination combined with cytology), with calculation of sensitivity, specificity, diagnostic accuracy, and the positive (PPV) and negative (NPV) predictive values. All hypothesis tests were two-sided, and results were considered statistically significant at $P < 0.05$.

Results

To determine the catheter specifications for CVM collection for the visual diagnostic test kit for uterine diseases in cows, 40 cm catheters with diameters of 0.4 cm, 0.6 cm, and 0.8 cm were used. Catheter insertion depth was measured using a ruler placed inside the catheter, and the volume of collected mucus was calculated using the cylinder volume formula. The measurement results are presented in Table 1.

Table 1. Determination of catheter length and diameter for a visual diagnostic test kit for uterine diseases in cows at different days in milk

Group no.	Clinical status	D=0.4 см		D=0.6 см		D=0.8 см	
		l, cm	V, mL	l, cm	V, mL	l, cm	V, mL
I (10-20 d.m.)	diseased	30.1 ± 0.53	0.97 ± 0.04	31.8 ± 0.42	1.12 ± 0.06	29.5 ± 0.61	0.95 ± 0.02
	clinically healthy	24.73 ± 0.32	0.51 ± 0.05	25.68 ± 0.39	0.71 ± 0.04	23.4 ± 0.44	0.63 ± 0.04
II (21-30 d.m.)	diseased	27.5 ± 0.55	0.95 ± 0.04	28.3 ± 0.63	1.16 ± 0.06	27.3 ± 0.65	0.97 ± 0.05
	clinically healthy	21.8 ± 0.4	0.52 ± 0.03	22.4±0.5	0.69 ± 0.05	21.5 ± 0.64	0.62 ± 0.04
III (31-60 d.m.)	diseased	23.5 ± 0.56	0.88 ± 0.05	24.4 ± 0.35	1.01 ±0.05	22.5 ± 0.34	0.91 ± 0.05
	clinically healthy	20.1 ± 0.61	0.49 ± 0.03	20.2 ± 0.76	0.65 ± 0.04	19.3 ± 0.82	0.57 ± 0.05
IV (61-90 d.m.)	diseased	23.4 ± 0.65	0.76 ± 0.05	24.47 ± 0.75	1.01 ± 0.03	22.5 ± 0.43	0.85 ± 0.03
	clinically healthy	18.21 ± 0.21	0.45 ± 0.06	18.53 ± 0.26	0.61 ± 0.06	17.81 ± 1.29	0.51 ± 0.05
V (≥91 d.m.)	diseased	21.75 ± 0.82	0.71 ± 0.03	22.85 ± 1.31	0.98 ± 0.02	21.47 ± 0.92	0.76 ± 0.03
	clinically healthy	17.75 ± 0.43	0.45 ± 0.05	18.06 ± 0.33	0.5 9 ± 0.04	17.4 ± 0.56	0.47 ± 0.04

The data in Table 1 show that, across all groups and for all catheter diameters, the mean insertion depth was statistically significantly greater in diseased animals ($P < 0.05$) than in clinically healthy animals. In Group I, when using a catheter with a 0.6-cm diameter, the insertion depth into the vagina up to the cervix was 31.8 ± 0.42 cm in diseased cows, whereas it was 25.68 ± 0.39 cm in clinically healthy cows. A similar trend was observed for the volume of collected CVM samples, with values in diseased animals being 1.5–2.0 times higher than those in clinically healthy animals.

When analyzing data from cows with uterine disease, a catheter with a diameter of 0.6 cm – particularly at 10–60 days in milk – enabled collection of the largest volume of CVM compared with catheters of 0.4 cm and 0.8 cm. This may be attributable to mucus viscosity and catheter lumen patency: a 0.4 cm catheter may become occluded, whereas a 0.8 cm catheter may generate insufficient negative pressure for aspiration.

These results indicate that the initially selected catheter length of 40 cm for the CVM sampling device is sufficient for aspiration of the contents, and that the optimal catheter diameter is 0.6 cm, which allows collection of an adequate amount of CVM for analysis.

The next stage of the study was to compare the clinical effectiveness of diagnostic methods for uterine diseases in cows at different days in milk. For this purpose, clinical and laboratory diagnostic methods were used, including the prototype diagnostic reagents for the visual diagnostic test for uterine diseases in cows. The results are presented in Table 2.

Table 2. Clinical effectiveness of diagnostic methods for uterine diseases in cows at different days in milk (n=65)

Days in milk	Diagnostic method	Diagnostic performance metrics (%)				
		Sens.	Spec.	Acc.	PPV	NPV
10–20	Rectal examination	94.7	33.3	86.4	90.0	50.0
	Vaginal examination	94.7	75.0	91.3	94.7	75.0
	Whiteside test	42.9	50.0	44.4	75.0	20.0
	Cytology	44.4	100.0	54.6	100.0	28.6
	Prototype 1	94.1	100.0	95.0	100.0	75.0
	Prototype 2	84.2	100.0	86.4	100.0	50.0
21–30	Rectal examination	66.7	66.7	66.7	66.7	66.7
	Vaginal examination	83.3	83.3	83.3	83.3	83.3
	Whiteside test	33.3	25.0	28.6	25.0	33.3
	Cytology	33.3	100.0	66.7	100.0	60.0
	Prototype 1	83.3	100.0	91.7	100.0	85.7
	Prototype 2	60.0	100.0	75.0	100.0	60.0
31–60	Rectal examination	16.7	57.1	38.5	25.0	44.4
	Vaginal examination	66.7	85.7	76.9	80.0	75.0
	Whiteside test	75.0	25.0	50.0	50.0	50.0
	Cytology	33.3	100.0	62.5	100.0	53.6
	Prototype 1	83.3	100.0	91.7	100.0	85.7
	Prototype 2	80.0	100.0	90.9	100.0	85.7
61–90	Rectal examination	50.0	85.7	77.8	50.0	85.7
	Vaginal examination	66.7	71.4	70.0	50.0	83.3
	Whiteside test	50.0	50.0	50.0	50.0	50.0
	Cytology	50.0	100.0	88.9	100.0	87.5
	Prototype 1	85.7	100.0	91.7	100.0	83.3
	Prototype 2	50.0	75.0	66.7	50.0	75.0
≥91	Rectal examination	25.0	87.5	66.7	50.0	70.0
	Vaginal examination	33.3	87.5	72.7	50.0	77.8
	Whiteside test	50.0	50.0	50.0	50.0	50.0
	Cytology	33.3	100.0	75.0	100.0	71.4
	Prototype 1	80.0	100.0	87.5	100.0	75.0
	Prototype 2	50.0	50.0	50.0	50.0	50.0
Total	Rectal examination	65.7	73.3	69.2	74.2	64.7
	Vaginal examination	79.4	86.7	82.8	87.1	78.8
	Whiteside test	53.9	40.0	47.8	53.9	40.0
	Cytology	40.5	100.0	66.7	100.0	56.7
	Prototype 1	90.3	100.0	94.2	100.0	87.5
		79.3	83.3	80.9	88.5	71.4

According to the data in Table 2, the highest diagnostic performance parameters were observed for Prototype 1 of the visual diagnostic test: sensitivity (Sens.) 90.3%, specificity (Spec.) 100.0%, diagnostic accuracy (Acc.) 94.2%, positive predictive value (PPV) 100.0%, and negative predictive value (NPV) 87.5%. This method exceeded vaginal examination in diagnostic accuracy by 11.4% and exceeded Prototype 2 of the visual diagnostic test kit by 13.3%. Cytology demonstrated absolute specificity (100.0%) and positive predictive value (100.0%); however, it showed low sensitivity (40.5%), which resulted in moderate diagnostic accuracy (66.7%) and a limited negative predictive value (56.7%). Among the clinical diagnostic methods in cows, vaginal examination showed the most balanced performance metrics (sensitivity 79.4%, specificity 86.7%, diagnostic accuracy 82.8%); rectal examination was inferior to vaginal examination across all effectiveness parameters, by 13.7% in sensitivity, 13.4% in specificity, and 13.6% in diagnostic accuracy. The Whiteside test was the least clinically effective diagnostic method, underperforming Prototype 1 of the visual diagnostic test kit by 36.4% in sensitivity, 60.0% in specificity, and 46.4% in diagnostic accuracy.

Based on the stratified analysis, a pronounced dependence of several diagnostic methods on days in milk was identified. At 10–20 days in milk, clinical diagnostic methods demonstrated high sensitivity (Sens. 94.7%); however, for rectal examination, specificity was 41.7% lower than that of vaginal examination, with a diagnostic accuracy of 86.4%. Vaginal examination showed a more balanced sensitivity/specificity profile (94.7%/75.0%) and high diagnostic accuracy (91.3%). During the same period, sensitivity of Prototype 1 and Prototype 2 was 94.1% and 84.2%, respectively, and diagnostic accuracy was 95.0% and 86.4%, respectively, with absolute specificity (100.0%). In contrast, cytological examination, despite also showing absolute specificity (100.0%), had low sensitivity (44.4%) and diagnostic accuracy (54.6%). The Whiteside test exhibited the lowest diagnostic informativeness among the methods evaluated.

During the 21–30 days in milk, vaginal examination showed stable sensitivity, specificity, and diagnostic accuracy values of 83.3%, exceeding rectal examination by 16.6% for these parameters. Sensitivity of Prototype 1 was higher than that of Prototype 2 by 23.3% (83.3% vs 60.0%, respectively), and diagnostic accuracy was higher by 16.7% (91.7% vs 75.0%), while maintaining absolute specificity (100.0%). During this period, cytological examination demonstrated absolute specificity (100.0%); however, as in the previous time interval, it had low sensitivity (33.3%) and diagnostic accuracy (66.7%). The Whiteside test was non-informative during this postpartum interval (Sens. 33.3%, Spec. 25.0%, Acc. 28.6%).

Beginning in the 31-60 days in milk, the informativeness of clinical diagnostic methods declined substantially: sensitivity, specificity, and diagnostic accuracy for rectal examination were 16.7%, 57.1%, and 38.5%, respectively. In contrast, vaginal examination yielded higher sensitivity (+50.0%), specificity (+28.6%), and diagnostic accuracy (+38.4%) than rectal examination. During this interval, Prototype 1 and Prototype 2 showed the highest sensitivity and diagnostic accuracy (Sens. 83.3% and 80.0%; Acc. 91.7% and 90.9%, respectively) while maintaining absolute specificity (100.0%). Cytology results in this period were similar to those observed at 21–30 days in milk: despite absolute specificity (100.0%), sensitivity was 33.3% and diagnostic accuracy was 62.5%. The Whiteside test demonstrated relatively high sensitivity (75.0%) but low specificity and diagnostic accuracy (25.0% and 50.0%, respectively).

During the 61–90 days in milk, clinical diagnostic methods showed moderate performance. Sensitivity of vaginal examination (66.7%) exceeded that of rectal examination by 16.7%, whereas specificity of rectal examination (85.7%) was higher than that of vaginal examination by 14.3%, and diagnostic accuracy (77.8%) was higher by 7.8%. Prototype 1 exhibited the highest diagnostic performance in this period and markedly outperformed Prototype 2: sensitivity of Prototype 1 (85.7%) was higher than that of Prototype 2 by 35.7%, and specificity (100.0%) and diagnostic accuracy (91.7%) were each higher by 25.0%. Cytology during this period also demonstrated absolute specificity (100.0%); although sensitivity remained low (50.0%), diagnostic accuracy was the highest across all time intervals (88.9%). The Whiteside test was of limited informativeness, as in the previous intervals (Sens., Spec., and Acc. each 50.0%).

In the latest period (≥91 days in milk), clinical rectal and vaginal diagnostic methods were characterized by high specificity (87.5%) and moderate diagnostic accuracy (66.7% and 72.7%, respectively), but low sensitivity (25.0% and 33.3%). In this period, Prototype 1 was the most effective diagnostic method, with sensitivity of 80.0%, specificity of 100.0%, and diagnostic accuracy of 87.5%, which were 30.0%, 50.0%, and 37.5% higher, respectively, than those of Prototype 2. Cytology showed diagnostic performance similar to that in the previous period; however, sensitivity decreased by 16.7% and diagnostic accuracy by 13.9%. The clinical effectiveness of the Whiteside test did not change compared with the previous interval, and the method remained of limited informativeness.

Thus, considering overall diagnostic performance and the stability of results across days in milk intervals, Prototype 1 of the visual diagnostic test kit was the most effective. Among the clinical methods, vaginal examination was the most informative. Cytological examination provided maximal specificity but had low sensitivity and moderate diagnostic accuracy. The Whiteside test exhibited the lowest diagnostic informativeness across postpartum periods.

The next stage of the study was to determine the diagnostic parameters of the developed visual diagnostic test kit for uterine diseases in cows. For this purpose, during CVM testing, foam height (cm) and the time to color change of the catheter contents (min) were measured in both diseased and clinically healthy animals. The measurement results are presented in Table 3.

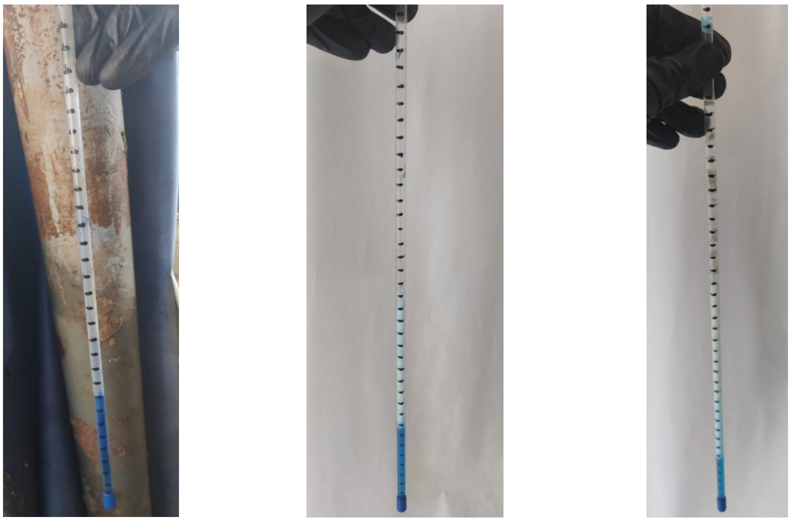
Table 3. Determination of diagnostic parameters of the visual diagnostic test kit for uterine diseases in cows (n = 45)

Clinical course of uterine disease	n	Reaction readout	
		foam height (cm)	time to color change of catheter contents (min)
Clinically healthy animals	15	0.22 ± 0.42	25.5 ± 3.5
Acute	15	17.5 ± 10.1	3.5 ± 0.35
Chronic	15	5.62 ± 3.93	4.5 ± 0.45

According to Table 3, clinically healthy animals exhibited a minimal reaction when the visual diagnostic test for uterine disease was applied, characterized by negligible foam formation (0.22 ± 0.42 cm) and the longest time to color change of the catheter contents (25.5 ± 3.5 min), reflecting low reaction intensity in the absence of pathology. In contrast, animals with uterine disease showed an accelerated color change: 3.5 ± 0.35 min in acute cases and 4.5 ± 0.45 min in chronic cases. These findings indicate a clear separation between diseased and clinically healthy animals based on the time parameter, which was markedly reduced in the presence of pathology.

With respect to foam formation, the highest value was observed in acute cases (17.5 ± 10.1 cm), whereas in chronic cases the value was substantially lower (5.62 ± 3.93 cm). In clinically healthy animals, foam formation was virtually absent, indicating that foam height reflected not only the presence or absence of pathology but also gradation by clinical course: the most pronounced reaction corresponded to acute disease, whereas a moderate reaction was characteristic of chronic disease.

Collectively, these data indicate that the developed visual diagnostic test kit produces a characteristic reaction pattern depending on the animals' clinical status: clinically healthy animals show no foam or only minimal foam formation with a prolonged time to color change (Figure 1a); chronic disease is characterized by moderate foam formation with rapid color change (Figure 1b); and acute disease is characterized by abundant foam formation and the fastest color change of the catheter contents (Figure 1c).



a b c

a – clinically healthy animal; b – chronic course; c – acute course

Figure 1. Results of using the test for visual diagnosis of uterine diseases in cows

Discussion

Uterine diseases in cows, particularly metritis and endometritis of various forms, remain among the leading causes of deteriorated herd reproductive performance and reduced cow productivity, while the severity of clinical signs becomes «attenuated» as time postpartum increases [25, 26]. This factor drives demand for rapid diagnostic methods that meet the following requirements: ease of use under on-farm conditions and the ability to screen the herd for uterine disease, thereby reducing the time from diagnosis to initiation of potential treatment when pathology is detected [27, 28].

When using the visual diagnostic test for uterine diseases in cows, two diagnostic criteria were selected: an animal was considered diseased if foam formation exceeded 1 cm and discoloration of the catheter contents occurred within 4 min. This approach yields high specificity because it requires the simultaneous presence of two visual reaction indicators, thereby reducing the likelihood of false-positive results when pathology is absent and helping to minimize unjustified costs and production losses [29].

The obtained reaction parameter data in diseased and clinically healthy animals confirm the validity of the selected approach: in clinically healthy cows, the reaction was virtually absent (foam 0.22 ± 0.42 cm; discoloration within 25.5 ± 3.5 min), whereas acute disease showed a distinct reaction pattern (foam 17.5 ± 10.1 cm; discoloration within 3.5 ± 0.35 min) and chronic disease was associated with a moderate reaction (foam 5.62 ± 3.93 cm; discoloration within 4.5 ± 0.45 min). The more moderate reaction observed in chronic cases is consistent with the literature, which indicates that chronic or subclinical uterine disease is characterized by less pronounced or absent clinical signs, thereby necessitating more sensitive markers or adjustment of diagnostic parameters [25, 30].

Comparative analysis of the diagnostic performance of methods for detecting uterine disease in cows showed that Prototype 1 (10% H_2O_2 + 0.01% methylene blue) of the visual diagnostic test provided the most stable effectiveness metrics: overall sensitivity reached 90.3%, specificity 100.0%, diagnostic accuracy 94.2%, PPV 100.0%, and NPV 87.5%. Notably, the specificity of Prototype 1 remained at 100.0% regardless of days in milk, which is critically important for on-farm screening because it minimizes unnecessary therapeutic interventions. In contrast, Prototype 2 (10% H_2O_2 + 0.03% 4-chloro-1-naphthol) ultimately showed more variable performance, with sensitivity of 79.3%, specificity of 83.3%, and diagnostic accuracy of 80.9%; a trend toward reduced performance was observed in later postpartum intervals, declining to 50.0% for all parameters at ≥91 days in milk. This may indicate lower reaction robustness or greater dependence on mucus composition as the inflammatory profile changes over the course of disease. Such postpartum-period dependence is conceptually consistent with the notion that the pathogenesis and manifestations of inflammatory uterine disease differ by clinical course (acute, chronic, and subclinical) and, consequently, that a universal marker or diagnostic feature may behave differently across postpartum time points [31, 32].

With respect to clinical diagnostic methods, the following pattern was observed: vaginal examination showed a higher overall performance profile (Sens. 79.4%, Spec. 86.7%, Acc. 82.8%) compared with rectal examination (Sens. 65.7%, Spec. 73.3%, Acc. 69.2%). The effectiveness of clinical methods for diagnosing uterine disease in cows was maximal in the early postpartum period (10–20 days in milk), which is consistent with literature reporting more overt clinical signs during the acute course of uterine disease [25, 33].

In our study, cytological examination demonstrated maximal specificity (100.0%) with relatively low sensitivity (40.5%), which is consistent with available literature describing cytology as a highly specific tool for diagnosing uterine disease [34]. In our study, cytology was used specifically as a high-specificity reference method, whereas the developed test was intended as a screening approach designed to identify a larger number of disease cases while maintaining a low false-positive rate. Population-based studies emphasize the need for a multimodal diagnostic strategy, as some animals may be negative based on clinical findings yet positive on cytology; a combined approach provides a more objective assessment of herd-level disease prevalence [32, 35].

It should be noted that for methods based on assessment of vaginal discharge or cervicovaginal mucus, a standardized sampling procedure is essential. Comparative studies have shown that the diagnostic performance of different discharge assessment approaches (Metricheck and vaginal speculum) differs in sensitivity and specificity despite similar diagnostic workflows [36]. Therefore, the objective of determining the device parameters for the visual diagnostic test helped reduce sample variability, which in turn improved the accuracy of the study.

From a practical perspective, the results suggest that the visual diagnostic test aligns with a cow-side diagnostic approach: it is rapid, reproducible, highly specific, and provides an easily interpretable readout. For example, reagent strip-based tests and leukocyte esterase assays are also positioned as rapid diagnostic methods that enable assessment of inflammatory status under on-farm conditions without laboratory infrastructure [28]. From the standpoint of commercial dairy production, a test with high sensitivity and specificity could potentially reduce the proportion of undiagnosed uterine disease cases, which is important for preserving herd reproductive performance. This is critical because reproductive system disorders are associated with both a lower probability of achieving pregnancy and a higher risk of gestational pathologies in breeding animals [37].

Based on the study results, the proposed visual diagnostic test for uterine diseases in cows can be integrated into on-farm diagnostic protocols for the breeding herd as a rapid, reproducible, and accurate diagnostic method, enabling timely diagnosis and implementation of necessary therapeutic and preventive measures. Subsequently, it is planned to validate the developed test on a larger number of animals on other dairy farms in Kazakhstan.

Conclusion

A test kit was developed for the visual diagnosis of uterine diseases in cows at different days in milk, based on collection of cervicovaginal mucus and detection of an inflammation biomarker via a chemical reaction. The visual diagnostic test kit for uterine diseases in cows includes a graduated catheter (40 cm in length and 0.6 cm in diameter), a 50- µL syringe, a coupling for attaching the syringe to the catheter, a protective cap for the free end of the catheter, and two 5-µL syringes containing the diagnostic reagents. When two reagent formulations were compared, Prototype 1 (10% H_2O_2 + 0.01% methylene blue) was preferred over Prototype 2 (10% H_2O_2 + 0.03% 4-chloro-1-naphthol) and demonstrated high diagnostic performance (sensitivity 90.3%, specificity 100.0%, diagnostic accuracy 94.2%). The established criterion for a positive test result (foam formation >1 cm and discoloration of the catheter contents within 4 min) ensured unambiguous result interpretation and high test specificity. Based on clinical trial results, the visual diagnostic test demonstrated high diagnostic effectiveness relative to a composite reference standard (clinical signs plus cytology findings), supporting its use for screening the breeding herd at different postpartum time points.

Authors' Contributions

IJ: developed the study concept, justified the study design, supervised all stages of the research, edited the initial version of the manuscript; AM: developed the experimental protocol, conducted the animal study, contributed to the writing of the methodological section; ZhZh: prepared the literature review, introduction, reference list, edited the manuscript text; AA: performed the statistical analysis, interpreted the results, contributed to the preparation of the Results and Discussion sections; YeK: conducted the animal study, was responsible for the technical implementation of the device, preparation of reagents, contributed to the preparation of the Introduction section; AZ: provided project administration, conducted the animal study, was responsible for preparing the device and reagents for research, served as the corresponding author.

Statement on Competing Interests (Conflict of Interest)

The authors declare that they have no conflicts of interest.

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