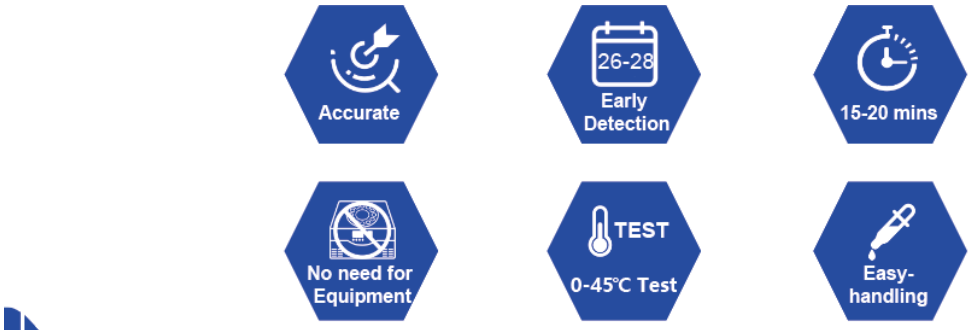
GM-DIAGNOSTICS
Innovating for Health

PregAccu™

Bovine Pregnancy Rapid Test Kit

Validation Report

Proven Accuracy for Bovine Pregnancy Testing



GeneMedi biotechnology Co. ltd
Phone: +86-021-50478399
Web: www.pagtest.net

The GeneMedi PregAccu™ Bovine Pregnancy Rapid Test Kit is a lateral flow immunoassay (LFIA) designed to detect pregnancy-associated glycoproteins (PAGs) in bovine serum, EDTA plasma, and whole blood as an early marker of pregnancy. The test employs a strip coated with a specific anti-PAG antibody at the test line. A sample applied to the sample pad flows along the strip, interacting with the antibody and a labeled detection reagent. Unbound materials are carried beyond the test and control lines. A visible line at the test region indicates the presence of PAGs, with intensity varying based on PAG levels from 24 to 40 days post-Artificial Insemination (AI). A line at the control region confirms the test's proper function. The kit achieves 100% accuracy and sensitivity with blood samples at 30–35 days post-AI, showing equivalence to plasma samples. The test is not suitable for diagnosing pregnancy in cows within 60 days postpartum, as PAG levels from a previous pregnancy may interfere with reliable detection.

I. Glossary of Terms

The following definitions are adapted from the Glossary of Terms section of the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (2012) to describe the performance characteristics of the GeneMedi PregAccu™ Bovine Pregnancy Rapid Test Kit in this validation report.

- **Repeatability**: The extent of agreement in results from repeated testing of a sample, evaluated both within a single run and across multiple runs using the same method in one laboratory.
- **Reproducibility**: The capability of the test method to produce consistent results when applied to the same sample using the same procedure across different laboratories.
- **Sensitivity (diagnostic)**: The percentage of confirmed pregnant animals correctly identified as pregnant by the assay; pregnant animals misclassified as non-pregnant (open) are considered false negatives.
- **Specificity (diagnostic)**: The percentage of confirmed non-pregnant (open) animals correctly identified as open by the assay; non-pregnant animals misclassified as pregnant are considered false positives.
- **Accuracy**: The overall proportion of correct identifications (true positives and true negatives) among all tested animals, indicating the test's ability to reduce both false positives and false negatives, thus offering a comprehensive measure of reliability.

1. Proven Accuracy of GeneMedi PregAccu™ in Post-AI and Open Bovine Samples Compared to I-Company On-Farm Pregnancy Test, with Ultrasound as Reference

Purpose:

Verify the accuracy of the GeneMedi PregAccu™ Bovine Pregnancy Rapid Test Kit in detecting pregnancy-associated glycoproteins (PAG) for bovine pregnancy diagnosis.

Method:

A total of 4,000 bovine serum and plasma samples were tested and compared against ultrasound as the established reference method.

Key performance metrics, including sensitivity, specificity, and overall accuracy, were thoroughly evaluated.

The predefined success criteria for this evaluation were a sensitivity of ≥ 99%, a specificity of ≥ 99 % ≥99%, and an accuracy of ≥ 99%

Table 1: GeneMedi PregAccu™ Performance in Post-AI and Open Bovine Samples, Demonstrating Superior Accuracy Over I-Company's Bovine Pregnancy Test Kit with Ultrasound (35th day) as a Reference Results.

Sample categories	True positive			False positive	True negative	False negative	Sensitivity (%)	Specificity (%)	Accuracy (%)	Strong positive/true positive (%)
	Strong positive	Week positive	Total positive							
GeneMedi PregAccu™ Bovine Pregnancy Rapid Test Kit										
Post AI (28 days) (n=2957)	1771	0	1771	4	1173	6	99.66	99.66	99.66	100
Post AI (26 days) (n=300)	182	2	184	1	113	1	99.45	99.12	99.33	94.73
Post AI (24 days) (n=300)	151	26	177	1	113	9	95.16	99.2	96.66	57.89
open cows (n=200)	0	0	0	0	200	0	NA	100	100	NA
I - company's Bovine Pregnancy Test Kit										
Post AI (28 days) (n=183)	108	2	110	1	71	0	99.0990991	98.61	98.9	98.18
open cows (n=15)	0	0	0	0	15	0	NA	100	100	NA

Post-AI: Days after artificial insemination (e.g., 24–35 days). **Open Cows:** Non-inseminated, non-pregnant cows (ultrasound-confirmed). **True Positive:** Correctly detects pregnancy (ultrasound-confirmed). **False Positive:** Incorrectly detects pregnancy (non-pregnant by ultrasound). **True Negative:** Correctly detects non-pregnancy (ultrasound-confirmed). **False Negative:** Incorrectly identifies as non-pregnant (pregnant by ultrasound). **Weak Positive:** Thin test line, low PAG levels post-AI. **Strong Positive:** Clear test line, sufficient PAG levels post-AI. **Total positive:** Includes both week positive and strong positive

Inference:

The 26–28-day post-AI intervals achieve high accuracy (99.33–99.66%), consistent with ultrasound results.

The 24-day accuracy (96.66%) is lower due to reduced PAG levels, increasing false negatives.

Use the kit after 26 days post-AI for optimal results.

The GeneMedi PregAccu™ kit outperforms the I-company On Farm Pregnancy Test due to greater consistency.

2. Proven Accuracy of GeneMedi PregAccu™ Compared to Ultrasound in Blood and Plasma Samples at 30–35 Days Post-AI

Purpose:

To evaluate the accuracy of the GeneMedi PregAccu™ Bovine Pregnancy Rapid Test Kit in detecting pregnancy-associated glycoproteins (PAG) for diagnosing bovine pregnancy using blood samples, with a comparison to plasma samples.

Method:

A total of 311 bovine blood and plasma samples were collected from animals at 30 and 35 days post-Artificial Insemination (AI).

Samples were tested using the GeneMedi PregAccu™ Bovine Pregnancy Rapid Test Kit, with results compared to ultrasound as the reference method.

Blood and plasma samples were analyzed to assess equivalence in test performance.

Predefined success criteria included a sensitivity of ≥99%, a specificity of ≥99%, and an accuracy of ≥99%.

Table 2: Diagnostic Performance Summary for Blood and Plasma Samples at 30–35 Days Post-AI, Demonstrating Equivalent Accuracy to Ultrasound (35th day) Results.

Sample categories	True positive	False positive	True negative	False negative	Sensitivity (%)	Specificity (%)	Accuracy (%)
Validation with blood samples							
Post AI (30 days) (n=311)	187	0	124	0	100	100	100
Post AI (35 days) (n=311)	187	0	124	0	100	100	100
Validation with plasma samples							
Post AI (30 days) (n=311)	187	0	124	0	100	100	100
Post AI (35 days) (n=311)	187	0	124	0	100	100	100

Post-AI: Days after artificial insemination (e.g., 24–35 days). **True Positive:** Correctly detects pregnancy (ultrasound-confirmed). **False Positive:** Incorrectly detects pregnancy (non-pregnant by ultrasound). **True Negative:** Correctly detects non-pregnancy (ultrasound-confirmed). **False Negative:** Incorrectly identifies as non-pregnant (pregnant by ultrasound).

Inference:

The GeneMedi PregAccu™ Bovine Pregnancy Rapid Test Kit achieved 100% accuracy at 30–35 days post-AI, matching ultrasound results, with no differences observed between blood and plasma samples, confirming their equivalence.

Blood samples demonstrated 100% reliability and sensitivity for detecting PAG when tested at 30 days post-AI and beyond, making them as effective as plasma samples for bovine pregnancy diagnosis.

For optimal results, use the kit with blood samples collected at least 30 days post-AI to ensure 100% sensitivity and accuracy in diagnosing bovine pregnancy.

3. Proven Stability of GeneMedi PregAccu™ Under Accelerated Temperature Conditions

Purpose:

Confirm the performance consistency of the GeneMedi PregAccu™ Bovine Pregnancy Rapid Test Kit over time under high-temperature storage conditions.

Method:

Test kits were subjected to controlled storage conditions of 55°C and 60% relative humidity (RH) for a duration of three weeks.

Twenty samples, with pregnancy status confirmed by ultrasound, were tested using strips from the stored kits at weekly intervals (0, 1, 2, and 3 weeks).

The acceptance criteria mandated that sensitivity and specificity deviations must not exceed 0.1% and no degradation of test kit components should be observed.

Table 3: Performance Summary of GeneMedi PregAccu™ Accelerated Temperature Stability Testing, Demonstrating Consistent Performance.

Time Point	*Sensitivity Deviation*	*Specificity Deviation*	*Accuracy*	*Component Integrity*	*Status*
0 weeks	0% (Baseline)	0% (Baseline)	100% (Baseline)	Intact	Pass
1 weeks	0%	0%	100%	Intact	Pass
2 weeks	0%	0%	100%	Intact	Pass
3 weeks	0%	0%	100%	Intact	Pass*

***Note:** No deviations observed; stability confirmed within criteria.

Inference:

The kit remains stable for 3 weeks at 55°C with no deviations, meeting acceptable limits.

4. Proven Reliability of GeneMedi PregAccu™ Across Various Ambient Temperatures.

Purpose:

To evaluate the consistency and reliability of the GeneMedi PregAccu™ Bovine Pregnancy Rapid Test Kit when operated across a range of temperatures representative of typical field conditions.

Method:

The test was performed at 4°C, 16°C, 25°C, 37°C, and 45°C sing a panel of 20 samples (10 pregnant and 10 non-pregnant, all confirmed by ultrasound).

The criteria for success required sensitivity and specificity to remain within ±0.1% of the 25°C baseline, with no instances of test failures.

Table 4: Performance Summary of GeneMedi PregAccu™ Under Different Ambient Temperature Conditions, Demonstrating Reliability.

Temperature	Sensitivity	Specificity	Accuracy	Deviation from baseline	Test failures	Status
4°C	100%	100%	100%	0%	None	Pass
16°C	100%	100%	100%	0%	None	Pass
25°C	100%	100%	100%	0% (baseline)	None	Pass
40°C	100%	100%	100%	0%	None	Pass

Inference:

The kit performs reliably from 4°C to 45°C, suitable for field diagnostics. Data sourced from calibrated chambers (±0.2°C accuracy).

5. Proven repeatability of GeneMedi PregAccu™ by Different Farm staffs

Purpose:

To evaluate the consistency of results from the GeneMedi PregAccu™ Bovine Pregnancy Rapid Test Kit when tested by different farm staffs, ensuring reliable visual assessment.

Method:

Three distinct farm staffs independently tested four bovine serum samples (two pregnant, two non-pregnant), with status confirmed by ultrasound or palpation.

All farm staffs adhered strictly to the standard PregAccu™ Bovine Pregnancy Rapid Test Kit protocol, conducting tests at 25°C under consistent standard lighting.

The success criterion mandated 100% agreement among all farm staffs, with their results precisely matching the reference statuses.

Table 5: Performance Summary of GeneMedi PregAccu™ by different farm staffs, Demonstrating Repeatability.

Sample ID	Reference Status	Dairy Farm staff - 1	Dairy Farm staff - 2	Dairy Farm staff - 3	Agreement	Status
1	Pregnant	Positive	Positive	Positive	100%	Pass
2	Pregnant	Positive	Positive	Positive	100%	Pass
3	Non-Pregnant	Negative	Negative	Negative	100%	Pass
4	Non-Pregnant	Negative	Negative	Negative	100%	Pass

Inference: The PregAccu™ Bovine Pregnancy Rapid Test Kit demonstrates perfect inter-operator consistency, with all farm staffs correctly interpreting results, confirming robust repeatability. Data sourced from different farm staff, compliant with veterinary standards**.**

6. Proven repeatability of GeneMedi PregAccu™ Among Different Farm House

Purpose: To ascertain the consistency of the GeneMedi PregAccu™ Bovine Pregnancy Rapid Test Kit's performance when utilized across various testing locations.

Method:

A standardized panel of four bovine serum samples (two pregnant, two non-pregnant, confirmed by ultrasound) was tested at three distinct farm house.

The same lot of the PregAccu™ Bovine Pregnancy Rapid Test Kit was used for all tests across the different farm house.

Operators at each farm house meticulously followed the established PregAccu™ Bovine Pregnancy Rapid Test Kit protocol.

The validation criterion required 100% agreement across all sites, with test results consistently matching the established reference statuses.

Table 6: Performance Summary of GeneMedi PregAccu™ among different farm house, Demonstrating Repeatability.

Sample ID	Reference Status	Farm House A	Farm House B	Farm House C	Agreement	Status
1	Pregnant	Positive	Positive	Positive	100%	Pass
2	Pregnant	Positive	Positive	Positive	100%	Pass
3	Non-Pregnant	Negative	Negative	Negative	100%	Pass
4	Non-Pregnant	Negative	Negative	Negative	100%	Pass

Inference:

The PregAccu™ Bovine Pregnancy Rapid Test Kit shows perfect consistency across different farm house, confirming robust reproducibility.

7. Proven Uniformity of GeneMedi PregAccu™ Among Different Batches

Purpose:

To assess the level of variability between different manufacturing batches of the GeneMedi PregAccu™ Bovine Pregnancy Rapid Test Kit.

Method:

A consistent set of four bovine serum samples (two pregnant, two non-pregnant, confirmed by ultrasound) was tested by a single operator using multiple distinct batches of the GeneMedi PregAccu™ Bovine Pregnancy Rapid Test Kit.

The success criterion for this evaluation was 100% agreement across all tested lots.

Table 7: Performance Summary of GeneMedi PregAccu™ batch to batch variations, Demonstrating Repeatability Uniform Results

Sample ID	Reference Status	Batch A	Batch B	Batch C	Agreement	Status
1	Pregnant	Positive	Positive	Positive	100%	Pass
2	Pregnant	Positive	Positive	Positive	100%	Pass
3	Non-Pregnant	Negative	Negative	Negative	100%	Pass
4	Non-Pregnant	Negative	Negative	Negative	100%	Pass

Inference:

Test results across multiple batches of the PregAccu™ Bovine Pregnancy Rapid Test Kit show 100% agreement, correctly identifying open and pregnant samples based on ultrasound, demonstrating good repeatability across different batches.

8. Summary of Robust Validation Outcomes of GeneMedi PregAccu™

- The GeneMedi PregAccu™ Bovine Pregnancy Rapid Test Kit achieves a high accuracy of 99.33–99.66% at 26–28 days post-AI, consistent with ultrasound results.
- Accuracy drops to 96.66% at 24 days due to lower PAG levels, thus recommending use after 26 days post-AI for optimal performance.
- The GeneMedi PregAccu™ Bovine Pregnancy Rapid Test Kit delivers 100% accuracy in both blood and plasma samples after 30 days post-AI.
- Serum/plasma samples after 26 days post-AI and blood samples after 30 days post-AI are highly suitable for early pregnancy testing.
- The kit maintains stability for 3 weeks at 55°C with no deviations, meeting established stability criteria.
- It performs reliably across temperatures from 4°C to 45°C, making it ideal for field use.
- Consistency and reproducibility across different farm staff, farmhouses, and batches are perfect, confirming robust repeatability and uniform performance.
- The GeneMedi PregAccu™ kit outperforms the I-Company On-Farm Pregnancy Test in consistency, although some metrics fall slightly below the ≥99% target.