

INSTRUCTIONS

REPROTEST for Bulls

CHUTE SIDE TEST FOR FAA IN BULLS

The **REPROTEST FOR BULLS** is a qualitative test to measure presence of a **PROTEIN** on **BOVINE SPERM** which corresponds to higher fertility.

The **REPROTEC CASSETTE** is designed to screen a semen sample for **FERTILITY-ASSOCIATED ANTIGEN (FAA)**, a protein which is produced in the Seminal Vesicles, Prostate, and Cowper's Glands and binds to the sperm during ejaculation. The **TEST CASSETTE** is a rapid test format and eliminates the need for laboratory equipment, fragile instruments, or a specific temperature to measure **FAA**. The **REPROTEST FOR BULLS** provides a yes/no answer within 20 minutes regarding **FAA** status of a bull.

THE IMPORTANCE OF FAA

Since 1992, field trials have been conducted utilizing various detection methods to screen for **FAA** on sperm. **FAA** was chemically identified in 1998. To date, over 15,000 cows have been bred to bulls categorized as **FAA**-positive or **FAA**-negative after testing their semen. Cows have been pasture-bred in single sire or multiple-site matings or were artificially inseminated. Collectively, data from over 600 bulls revealed that **FAA-positive bulls were 17-19% more fertile than their own contemporary FAA-negative herdmates.**

FAA is a unique protein, which is produced by the male accessory sex glands (seminal vesicles, prostate, and Cowper's gland) after puberty is attained. Production of **FAA** is dependent upon presence of testosterone which is secreted by the testes at puberty. Therefore, screening for **FAA** with the **REPROTEST FOR BULLS** cassette cannot be conducted in pre-pubertal animals. During ejaculation, as sperm traverse the male reproductive tract, they acquire secretions from the accessory organs, including **FAA**. A composite **SEMEN SAMPLE** is diluted with a **BUFFER** in the **TEST KIT**, and a simple color change in a narrow band serves as the detection system.

Approximately 1 out of 4 (26.5%) bulls were categorized as **FAA**-negative utilizing the test cassette on 914 animals in 18 herds in spring, 2003. In those herds, **FAA**-negative bulls were obviously occurring within sire families. Therefore, culling of animals deemed to be **FAA**-negative should be encouraged.

Testing for **FAA** will not replace a breeding soundness evaluation (BSE) ordinarily performed by a veterinarian. Ideally, once a bull qualifies as a potential satisfactory breeder based upon his BSE results, that same **SEMEN SAMPLE** should be tested for **FAA**.

PREPARATION - PRIOR TO TESTING

Usually, if large numbers of bulls are tested (10 or more) it is easier to pre-number the **REPROTEC DATA SHEET**. Number the samples 1, 2, 3, 4 etc. As bulls are BSE tested, record the bull's **PERMANENT ID NUMBER** with the corresponding **SAMPLE NUMBER**.

Remove the tray from its outer box. The tray contains **BUFFER DILUTION VIAL** and **CRYO STORAGE VIALS**. Number each **CRYO STORAGE VIAL** in the same manner as the **REPROTEC DATA SHEET** 1, 2, 3 etc. Number the corresponding location of each **CRYO STORAGE VIAL** 1, 2, 3, etc. on the cardboard **TEST TRAY**. At this point you should have the **DATA SHEET**, **CRYO STORAGE VIAL** and its location on the **TEST TRAY** all numbered sequentially 1, 2, 3, etc. Remove the **DISPOSABLE SYRINGE** from its plastic cover. Mark the **DISPOSABLE SYRINGE** the same as the **CRYO STORAGE VIAL** and place in the small hole behind the **BUFFER DILUTION VIAL**. You are now ready for **STEP 1**.

SEE TIPS & SUGGESTIONS #1 WHERE CAN I PERFORM THE CHUTESIDE TEST?

Step 1: SEMEN SAMPLE COLLECTION / HANDLING

In order to ensure optimum chances for detecting **FAA**, a freshly collected SEMEN SAMPLE should be used. The following recommendations should be heeded:

- (1) Test a SEMEN SAMPLE as soon as possible after BSE results are apparent.
- (2) Transfer 1.5-2 ml of SEMEN from BSE COLLECTION TUBE into 2 ml CRYO STORAGE VIAL and cap vial.
- (3) Be sure to write the BULL'S PERMANENT ID on the DATA SHEET.
- (4) If samples are to be tested concurrent with BSE Exams, Proceed to STEP 2: **TESTING PROCEDURE**. If SAMPLES are to be tested at a later time proceed as follows.
- (5) Place CRYO STORAGE VIAL in ICE CHEST with CRUSHED ICE or preferably in an ICE BATH. If tests are to be performed within 1 hour the ice bath is sufficient. For longer periods freeze SAMPLE.
SEE TIPS & SUGGESTIONS #3 PROPER SEMEN STORAGE & HANDLING.
- (6) Do NOT use EXTENDED SEMEN SAMPLES which were processed by an A.I. center. The egg yolk or milk which are found in extenders can interfere with the results.

Step 2: TESTING PROCEDURE

1. Obtain sample (see **SEMEN SAMPLE COLLECTION / HANDLING**) Thaw SAMPLE fully if necessary at room temperature. Do not use heat or microwave to thaw the semen.
2. Remove the CASSETTE from the foil pouch by tearing at the notched end.
3. Discard the foil pouch and desiccant.
4. Label the CASSETTE with it's appropriate SAMPLE NUMBER 1, 2, 3, etc; the same as the CRYO STORAGE VIAL AND DATA SHEET.
5. Take the corresponding DISPOSABLE SYRINGE from the plastic needle cover and draw .5ml (1/2ml) of SEMEN from the CRYO STORAGE VIAL into the DISPOSABLE SYRINGE.
6. Remove the aluminum cap from the BUFFER DILUTION VIAL, exposing the rubber top. Insert the SYRINGE NEEDLE (with 1/2ml semen) into the BUFFER DILUTION VIAL and draw out 1/2ml of BUFFER. This will result in a 1:1 mixture of BUFFER to SEMEN. The syringe should contain 1ml of solution.
7. Draw the SYRINGE Plunger to the 2 1/2-3ml mark and invert syringe several times to mix the BUFFER-SEMEN solution. Avoid shaking. Shaking could harm the SAMPLE and possibly effect the accuracy of the test.
8. Once sample is thoroughly mixed, purge 3 or 4 drops of diluted SAMPLE into appropriate WASTE CONTAINER (paper towel).
9. Express 4 to 5 drops (Fill Well) of the diluted SAMPLE into the SAMPLE WELL of the CASSETTE, making sure that it is on a level surface.
10. Replace the DISPOSABLE SYRINGE with the remaining diluted SAMPLE. into it's plastic needle cover behind it's corresponding BUFFER DILUTION VIAL.
11. Allow the test to proceed for 5-10 minutes then read the results for accuracy. It may take up to 20 minutes for a TEST LINE to appear - this is not unusual. The TEST LINE may be more visible after the Test membrane dries in 20 to 30 minutes. Remember, the CONTROL LINE MUST APPEAR.

IMPORTANT

If the diluted SAMPLE does NOT start to migrate across the membrane (looks wet) within 10 minutes, the SAMPLE may need to be diluted more.

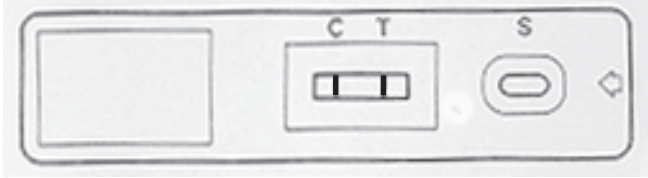
SEE TIPS & SUGGESTIONS #2 IF THE SAMPLE DOES NOT RUN.

Step 3: INTERPRETATION OF TEST RESULTS

Examples of **FAA** test CASSETTE results
(control = "C," test = "T" and sample = "S")

- A. A **POSITIVE TEST** for **FAA** is indicated by **TWO** lines; One at the "T" position and One at the "C" position.
Even a faint line at the "T" position indicates a **FAA POSITIVE TEST**.

FAA POSITIVE TEST



- B. A **NEGATIVE TEST** for **FAA** is indicated by **ONE** line at the "C" position only.

FAA NEGATIVE TEST – NO LINE AT "T" POSITION



Footnote: Independent from **FAA** content in a SEMEN SAMPLE, a line must appear in the "C" (control) window to assure that all reagents on the CASSETTE are working together.

READING THE TEST

Once the SEMEN SAMPLE is placed into the SAMPLE WELL on the CASSETTE you should observe the "running" of the test within 2-5 minutes. The TEST strip membrane will appear wet and will slowly move toward the left side of the CASSETTE. Next the CONTROL LINE should appear. If the bull is **POSITIVE** for **FAA** a 2nd line will start to appear below the "T" on the CASSETTE. Color should appear within 5-10 minutes after start of the TEST. In some instances, the TEST LINE will start to develop within 2-3 minutes. In other SAMPLES it takes up to 10 minutes for the TEST LINE to appear.

IMPORTANT

Color intensity of TEST LINES will vary from intense to vague. Both indicate a **POSITIVE TEST**.

****IMPORTANT****

**IF FOR ANY REASON YOU BELIEVE THE CASSETTE
HAS NOT FUNCTIONED PROPERLY, FREEZE SAMPLE
IN CRYO STORAGE VIAL AND CALL REPROTEC INC.
1-800-533-8115 AND ASK FOR TECHNICAL
ASSISTANCE.**

TIPS & SUGGESTIONS

#1 WHERE CAN I PERFORM THE CHUTESIDE TEST?

The **REPROTEST FOR BULLS** can be accurately and quickly performed Chuteside, providing the following conditions are followed:

(A) Provide a secure flat level surface with room for necessary materials.

(B) Avoid direct sun on hot days. Avoid dusty or windy or freezing conditions if at all possible.

*** A small folding table or cardtable placed inside a stock trailer with a covered top, provides an adequate working area. Place the trailer as close to the chute area as possible.

#2 IF THE SAMPLE DOES NOT RUN?

If the diluted SAMPLE does NOT start to migrate across the membrane (does not look wet) within 10 MINUTES the SAMPLE may need to be diluted more.

(A) Take CASSETTE and expell the existing SAMPLE (liquid) from the TEST WELL. Expell into WASTE CONTAINER or on a PAPER TOWELL. Tap the CASSETTE gently to remove all of the liquid SAMPLE. Blot any existing liquid out of the well with a paper towell. Blot gently and carefully.

(B) Take corresponding SYRINGE from TEST TRAY.

Draw 1/2ml of BUFFER from the BUFFER DILUTION VIAL into the DISPOSABLE SYRINGE and mix gently.

(C) Expell 4-5 drops of the newly diluted SAMPLE into the SAMPLE WELL. If TEST does not run in 5-8 minutes discard CASSETTE and redo using a new CASSETTE and the highly DILUTED SAMPLE solution.

*** In order to minimize the need to additionally dilute highly concentrated, viscous samples, usually from older Bulls (over 30 months of age) the following procedure should be followed.

1. During the BSE exam score the CONCENTRATION of the SEMEN SAMPLE from 1 to 4. 1 is low CONCENTRATION of SPERM, 4 is high CONCENTRATION of SPERM. This will be a judgement call determined by the Veterinarian.

2. Note the CONCENTRATION Score (1-4) on the DATA SHEET.

3. On SAMPLES that score 4 (HIGHLY CONCENTRATED) dilute the sample 2:1 BUFFER to SEMEN. Draw 1/2 ml of SEMEN and 1.0 ml of BUFFER into the SYRINGE.

4. The SAMPLE should run within 5 - 8 minutes.

5. SAMPLES with a CONCENTRATION SCORE of 1 - 3 should run at normal dilution rates (1:1) BUFFER to SEMEN.

#3 PROPER SEMEN STORAGE & HANDLING.

Regardless of whether you plan to run the **ReproTest for Bulls** concurrent with the BSE, the SEMEN SAMPLES must be properly handled.

(A) CRUSHED ICE is one method of storage. Ice must be crushed like a Sno Cone - not in ice cubes. Place the CRUSHED ICE in a six pack size or SMALL ICE CHEST.

(B) ICE BATH is the preferred method of storage. To do this place ICE CUBES and water into a SMALL ICE CHEST. This will chill the samples down to 32 F and should prevent the **FAA** protein from degradation. The CRYO STORAGE VIAL may be placed into a small zip lock bag for ease of retrieval. Be sure the zip lock bag is left open or holes are punched in the bag so that cold water can surround CRYO STORAGE VIAL.

(C) STORAGE/FREEZING IN DRY ICE is best, especially if you are planning to freeze the semen samples and test them at a later date. Place 4 - 5lbs of dry ice into the SMALL ICE CHEST. As samples are collected place CRYO STORAGE VIALS into ICE CHEST. They may be placed in a zip lock bag for easier retrieval. The samples will freeze within a matter of minutes. THIS IS THE RECOMMENDED PROCEDURE IF SAMPLES MUST BE STORED MORE THAN 1 ½ - 2 HOURS. BEFORE TESTING. FREEZING in your home freezer is adequate to freeze the samples for longer term storage.

IMPORTANT

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** Even if you plan to run the CHUTE SIDE TEST FOR FAA concurrent with the BSE, be prepared to place the CRYO STORAGE VIALS into an ice bath in an ice chest.

** If for any reason, the TEST does not function properly or if you want to recheck a negative test, you must have properly handled the SEMEN SAMPLE. Once the SEMEN SAMPLE is frozen you have many options to retest those SAMPLES. The worst case scenario is that you send the frozen SAMPLES to **ReproTec** for retesting

#4 LABELING.

If a SHARPIE PERMANENT MARKER is not available, a standard ball point ink pen will work on the CASSETTE and CRYO STORAGE VIAL. However, you will need to place a SCOTCH TAPE strip around the top of SYRINGE barrel. Then a ball point pen will mark on this surface.

#5 RECORD KEEPING.

It is imperative that you keep accurate records (Sample Number to Permanent ID Number) so that there will be no confusion as to which Bulls tested positive or negative for **FAA**.

#6 DISPOSAL.

Be sure to dispose of all kit components in an appropriate manner.

#7 MATERIALS NEEDED FOR COLLECTION/TESTING.

- (A) CLIPBOARD to hold your DATA SHEET(s)
- (B) A SMALL ICE CHEST
- (C) Ice and water for an ICE BATH or DRY ICE
- (D) PAPER TOWELS
- (E) SHARPIE PERMANENT MARKERS-Fine or Ultra Fine points
- (F) Small ZIP LOCK baggies
- (G) Clear SCOTCH TAPE
- (H) Small FOLDING TABLE
- (I) TRASH CAN/bags

STORAGE

- Avoid prolonged temperature and humidity extremes, store at room temperature.
- **DO NOT REMOVE THE CASSETTE FROM THE FOIL POUCH UNTIL READY FOR USE.** Even though the foil pouch includes a desiccant packet, exposure to high humidity conditions should be minimized.
- If the BUFFER in the DILUTION VIAL freezes, thaw before using.

PRECAUTIONS / WARNINGS

- Do not use components past expiration date and do not mix components from kits with different lot numbers.
- The BUFFER SOLUTION in this kit contains Sodium Azide. Sodium Azide may react with lead or copper plumbing to form highly explosive metal Azides. On disposal, flush with a large volume of water to prevent Azide build-up. For further information, refer to the manual issued by the Centers for Disease Control.
- Do not ingest desiccant.
- Dispose of all kit components in an appropriate manner.

Each test contains:

(1) CASSETTE, (1) 3ml DISPOSABLE SYRINGE, (1) BUFFER DILUTION VIAL, (1) CRYO STORAGE VIAL, DATA SHEET and INSTRUCTIONS.

Kits are packaged and sold in the following sizes:

Package of 1	RTBK1
Package of 5	RTBK5
Package of 10	RTBK10

For more information please contact us:

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ReproTest for Bulls

CHUTE SIDE TEST FOR FAA IN BULLS



ReproTEC inc

FERTILITY TECHNOLOGY FOR THE 21ST CENTURY