SQA-Vb

User Guide

Version 2.00

Catalog #7401

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Section 1: Overview

The SQA-Vb is a high performance analytical veterinary device that combines state-of-the-art technology in electro-optics, computer algorithms and video microscopy. The system has a built-in heating system to maintain samples at body temperature during the entire testing cycle and can be used to conduct automated or manual testing. The SQA-Vb performs a rapid and reliable automated analysis of both FRESH and FROZEN Bull semen and, together with the B-Sperm PC software, dosing can be conducted by a variety of methods. A video visualization system allows the user the flexibility to view specimens at X300 and X500.

The SQA-Vb is perfect for any AI STUD facility for testing **FRESH** samples, dosing by a variety of methods and performing quality control on the resulting frozen samples. Dosing is automatically performed by **B-sperm** software which supplies all the dosing tables, calculations, tracking and analysis.

The **QC-Frozen** mode of the SQA-Vb is used to analyze the quality of frozen bull semen prior to insemination and/or for batch approval at the AI stud production site.

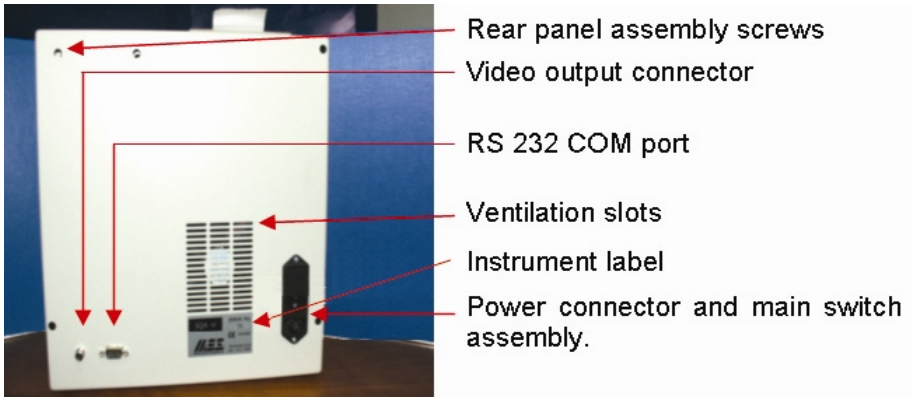
In addition to running samples for AI preparations, the SQA-Vb has a **BSE (Breeding Soundness Evaluation)** mode that analyzes fresh semen samples. Data from the SQA-Vb can be downloaded to a PC where **B-Sperm** software reports all the results and provides data management including herd histograms.

Reported Semen Parameters	
FRESH SAMPLES and FROZEN	
Total Sperm Concentration	Millions/milliliter
Motility	%
Morphology	% Normal
Progressive Motility	%
Motile Sperm Concentration	Millions/milliliter
Progressively Motile Sperm Concentration	Millions/milliliter
Velocity	Microns/second
# Sperm	Billions per sample
# Progressively Motile Sperm	Billions per sample
# Motile Sperm	Billions per sample
FROZEN (Milk Based Freezing Media only)	
Motile Sperm Concentration	Millions/milliliter
Progressively Motile Sperm Concentration	Millions/milliliter
Velocity	Microns/second
Motile Sperm	Per Staw/Semen Volume
Progressively Motile Sperm	

Section 2: System Overview

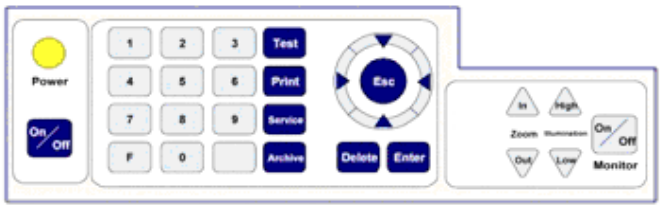
The Front Panel

The Rear/Side Panel



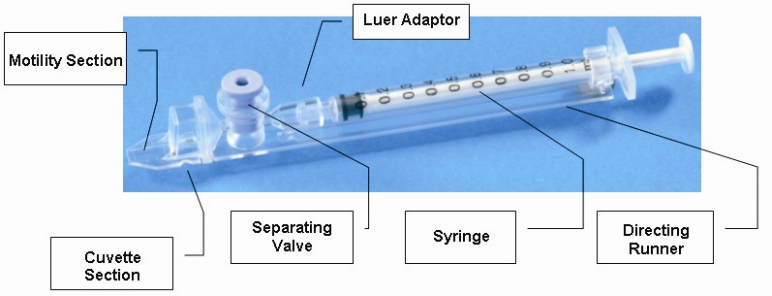
I-button

Keypad and Navigation



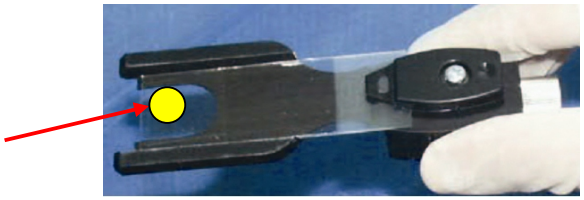
- Use the ARROW keys to move within the screens.
- Press ENTER to confirm menu options and to move to the next screen.
- Using ESC will return the user to a previous screen/row.

SQA-Vb Testing Capillary



- Plastic, multi-use (animal use only), disposable.
- Can be used in both measurement and visualization chambers of the SQA-Vb.
- Refer to the appendix section of this guide for instructions on how to use the capillary and how to clean it.

Slide Adaptor



NOTE: Place the sample on the top end of the slide for proper use (see arrow).

- For use with a standard laboratory slide 76 x 25.6 mm and 22 x 22 mm cover-slip.
- Sample should be placed where indicated by the yellow dot.

Section 3: Operating the SQA-Vb

- Turn on the main switch on the rear panel of the SQA-Vb.
- The power indicator on the front panel will illuminate.
- Press the On/Off key on the SQA-Vb keypad.
- The system will now automatically perform auto-calibration and self-testing.
- The **# tests remaining** will be displayed.
- Press ENTER to view the **MAIN MENU**.

Four options are available from the **MAIN MENU**:

- **TEST NEW SAMPLE**
- **ARCHIVE**
- **CONTROL**
- **SERVICE**

When using the system for the first time please note the following set-up requirements which are also explained in the **SERVICE** section of this user guide:

- I-button tests must be loaded. Go to **MAIN MENU>SERVICE>ADD TESTS TO COUNTER**
- System and sample defaults must be set-up: Go to **MAIN MENU>SERVICE>SETTINGS**
- B-Sperm setting defaults (Go to B-Sperm: **SET UP>SYSTEM SETTINGS>ADMINISTRATOR**)
- Control setting defaults (Go to B-Sperm: **CONTROLS>SET-UP**)

Section 4: Sample Testing

FRESH Sample Testing: BSE or AI-Dosing

To begin testing FRESH samples in the SQA-Vb, first select the type of sample to test: BSE or DOSING by going to: **MAIN MENU>SERVICE>SETTINGS**. Select either BSE or DOSING as the testing default for FRESH (see SET-UP section for complete details)

- **TEST NEW SAMPLE** from the MAIN MENU
- The **ENTER SAMPLE DATA** screen will be displayed
- Enter data using the SQA-Vb keypad:
  - Herd/Breed #: Up to 10 digits (mandatory entry).
  - Bull ID: Up to 10 digits
  - Semen Volume: Volume of the entire specimen **(must be ≤ 20 ml)**
  - Sample #: Up to 10 digits

NOTE: Load I-Button tests before starting. Select: SERVICE > ADD I-BUTTON TESTS from the MAIN MENU for instructions.

NOTE: Data entry fields have limited space for Semen Volume and ID #'s, please be aware when entering values/numbers.

NOTE: See the Appendix section for sample preparation and capillary filling guidelines.

ENTER SAMPLE DATA: FRESH

HERD/BREED #:	12
BULL ID:	145
SEMEN VOLUME:	5.0 ml
SAMPLE #:	2

The following screen will explain how to prepare the sample for testing while the system performs auto-calibration. **DO NOT TOUCH or MOVE THE SYSTEM UNTIL THE BEEP!**

TO TEST A FRESH SAMPLE:

1. PRE-HEAT EMPTY CAPILLARY > 4 MIN
2. PRE-HEAT 2.0 ML DILUENT: 4 MIN
3. ADD SEMEN TO DILUENT 100 ul

MIX SAMPLE, FILL AND CAPILLARY
WAIT FOR BEEP!
AUTOCALIBRATION – DO NOT TOUCH UNIT

- When a "beep" is heard, insert the testing capillary into the measurement compartment:

TO TEST A FRESH SAMPLE:
4. PRE-HEAT EMPTY CAPILLARY > 4 MIN
5. PRE-HEAT 2.0 ML DILUENT: 4 MIN
6. ADD SEMEN TO DILUENT 100 ul

MIX SAMPLE, FILL AND CAPILLARY
WAIT FOR BEEP!
INSERT CAPILLARY INTO CHAMBER

- Testing will begin automatically and takes about 40 seconds.
- A "beep" will indicate that testing is complete.
- Test results will be automatically saved and the Semen Analysis Report will be displayed on the screen:

SEMEN ANALYSIS REPORT: FRESH SAMPLE

DATE/TIME:	08/05/05 11:35
HERD/BREED #:	12
BULL ID:	145
SEMEN VOLUME:	5.0 ml
SAMPLE #:	2353344
TEST:	AI DOSING (or BSE)

- Press **Enter** to view the test results:

TEST RESULTS: FRESH SAMPLE

CONC.	332.6 M/ml	MSC	259.1M/ml
MOTILITY	77.9 %	PMSC	183.9 M/ml
PR. MOT.	55.3 %	VELOC.	69 mic/sec
MORPHOLOGY	81.0 %		

TOTALS PER EJACULATE

SPERM #	1.66 Bil	MOT. SPERM	1.30 Bil
PR. SPERM	0.92 Bil		

NOTE: The option to activate the QwikClick counter is ONLY for the AI DOSING testing mode.

- Next, if the AUTOMATICALLY SEND TO PC (for BSE and AI DOSING) or QWIKCLICK COUNTER (Only for AI DOSING): YES option was set in the SQA-Vb Sample Default Settings screen (See Set-up Section) one of the IMPORT TEST screens below will be displayed:

FOR DOSING CALCULATION
PRESS: "IMPORT TEST" BUTTON
IN B-SPERM

PRESS: "IMPORT TEST" BUTTON
IN B-SPERM
TO ACTIVATE QWIKCLICK COUNTER

PREPARE A SLIDE FOR QWIKCLICK TESTING

- To IMPORT TEST: When one of the above screens is displayed, go to the **B-Sperm** software main navigation menu and press the **Import Test** button in the B-Sperm.
- Test results will import automatically and the **BSE** spreadsheet (or **AI DOSING** screen) will be displayed. If QwikClick is activated, the QwikClick Counter will be displayed.
- If test results are not set to automatically print, press the **Print** button on the SQA-Vb key pad to print.

After testing is completed the MAIN MENU will be displayed with additional options:

- **RECALL LAST TEST RESULTS** – View last test results
- **RETEST SAME SAMPLE** – Test the same sample again without re-entering data

Frozen Sample Testing

Freezing Media Settings

NOTE: Select one of five freezing media options in the default setting screen (See SERVICE Section). The selected option should correspond to the media used to freeze the sample.

- To begin, select **FROZEN sample type** and a default **Freezing Media** in **SERVICE> SETTINGS > SYSTEM DEFAULT** settings (See SERVICE Section of this guide).
- Select **TEST NEW SAMPLE** in the **MAIN MENU**
- The screen below will then be displayed and the requested data can be entered:

ENTER SAMPLE DATA: FROZEN	
HERD/BREED #:	XXXXXXXXXX
BULL ID:	XXXXXXXXXX
STRAW/TABLET DATE:	01/04/05
SEMEN VOLUME:	XX.XXX ml
FRESH SAMPLE #:	XXXXXXXXXX

- Press ENTER and follow the screen instructions for preparing the **STRAW** sample for testing (See the appendix section of this guide for **FROZEN SEMEN SAMPLE Preparation).**
- Fill the testing capillary according to the Capillary Filling Instructions in the appendix section of this guide.
- **DO NOT INSERT TESTING CAPILLARY UNTIL THE SYSTEM BEEPS!**
- **DO NOT TOUCH or MOVE THE SYSTEM – WAIT FOR THE BEEP!**

TO TEST A FROZEN STRAW:

1. PRE-HEAT EMPTY CAPILLARY > 4 MIN
2. PRE-HEAT 500 ul DILUENT: 4 MIN
3. ADD SEMEN TO DILUENT: 200 ul

MIX SAMPLE, FILL AND CLEAN CAPILLARY
WAIT FOR BEEP
INSERT CAPILLARY INTO CHAMBER

- Insert the testing capillary, testing will begin automatically and take about 40 seconds.
- A “beep” will indicate that testing is complete.
- Test results will be saved automatically and the Semen Analysis Report will be displayed:

NOTE:

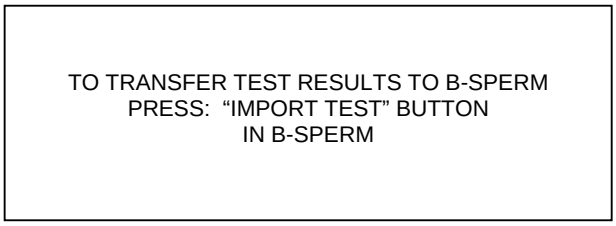
Test results for FROZEN samples are as shown EXCEPT when testing MILK BASED extenders. Only motility parameters will be reported when testing MILK BASED extenders.

SEMEN ANALYSIS REPORT: FROZEN SAMPLE	
DATE/TIME:	08/05/05 11:35
HERD/BREED #:	1234
BULL ID:	12334545
STRAW DATE:	01/04/08
SEMEN VOLUME	0.25 ml
FRESH SAMPLE #:	232456
FREEZING MEDIA:	CLEAR

- Press **Enter** to view the test results:

TEST RESULTS: FROZEN SAMPLE			
CONC.	82.6 M/ml	MSC	65.1M/ml
MOTILITY	78.8 %	PMSC	58.9 M/ml
PR. MOT.	71.3 %	VELOC.	32 mic/sec
TOTAL # / AI DOSE			
SPERM #	20.65 M		
MOT. SPERM	16.28 M		
PR. SPERM	14.73 M		

- Next, if the AUTOMATICALLY SEND TO PC YES option was set in the SQA-Vb Sample Default Settings screen the IMPORT TEST screen below will be displayed and the test record can be transferred to B-Sperm.



- **To IMPORT TEST**: When the above screen is displayed, go to the PC and start **B-Sperm**. Press the **Import Test** button in the B-Sperm.
- The test results will be displayed in the **QC-Frozen** spreadsheet.
- Leave the B-Sperm software on in order to import the next test results into B-Sperm.
- If test results are not set to automatically print, press the **Print** button on the SQA-Vb key pad to print.

After testing is completed the **MAIN MENU** will be displayed with additional options:

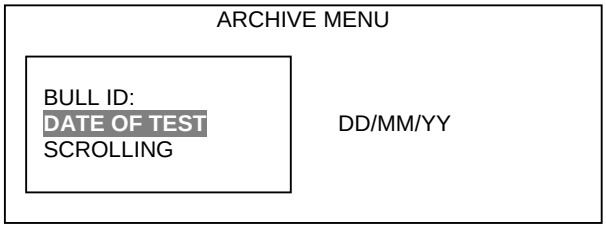
- **RECALL LAST TEST RESULTS** – View last test results
- **RETEST SAME SAMPLE** – Test the same sample again without re-entering data

Section 5: Archive

NOTE:

To import the SQA-Vb archive to the PC please follow the instructions in the B-Sperm™ User Guide.

Select **ARCHIVE** in the MAIN MENU to view the screen below with three record search options:



- Highlight the search option by using the arrow key on the SQA-Vb and press **ENTER**
- Input the required information and press ENTER to display the appropriate records
- Select SCROLLING to view/search the entire archive for a record:
  - Press **ENTER** after highlighting the **SCROLLING** option
  - Select the desired test record using the directional arrows
  - Press **Print** for a copy of the test results

Section 6: Running Controls

NOTE:

Controls can be run only after the defaults have been set-up in B-sperm.

The SQA-Vb runs QwikCheck™ beads for concentration. These beads are assayed for the SQA-Vb and produced by Medical Electronic Systems. Other commercially supplied latex beads will need to be assayed in order to run on the SQA-Vb.

The beads are aspirated into the testing capillary and run in the same manner as a normal volume specimen in the testing compartment of the SQA-Vb. Each new lot of beads must be set-up (based on the target value and +/- range in the product labeling) in the B-Sperm prior to running on the SQA-Vb. If running a non-assayed control, the target value and +/- range must be established by the user.

SET UP

Set-up the control defaults in B-Sperm as follows:

- From the SQA-Vb **MAIN MENU** go to: **SERVICE>SERVICE DATA**
- Connect the SQA-Vb to the B-Sperm PC and activate the B-Sperm program.
- **From B-Sperm:**
  - Go to: **Controls>Set Up**.
  - Press: **Continue** to display the Control Set-up screen in B-Sperm.
  - Enter all the data required in the table (view B-Sperm CONTROLS section for details).
  - Press: **Apply** to transfer the set-up data to the SQA-Vb.

The SQA-Vb is ready to run Controls. For each new lot of beads, repeat this process.

Control Testing

To run Controls on the SQA-Vb go to: **MAIN MENU>CONTROL,** select the Control **LEVEL** and press **ENTER**:

```
CONTROL: LATEX BEADS
SELECT:

CONTROL TEST: AUTOMATED
CONTROL LEVEL:
LEVEL #1/LEVEL #2/NEGATIVE CONTROL
PRESS ENTER TO CONTINUE
```

- Follow the instructions:

```
CONTROL: LATEX BEADS, LEVEL #1 (#2 or #3)
PLEASE WAIT
DO NOT INSERT CAPILLARY
SYSTEM PERFORMING AUTOCALIBRATION
PLEASE NOTE:
BE SURE TO VERIFY THAT LOT #, EXP DATE
AND TARGET VALUES ARE CURRENT
```

- A “beep” will indicate that a testing capillary can be inserted:

```
CONTROL: LATEX BEADS, LEVEL #1 (#2 or #3)

FILL, CLEAN AND WIPE CAPILLARY
INSERT IN CHAMBER
TESTING WILL BEGIN
AUTOMATICALLY
```

- Another “beep” will indicate that testing is complete:

```
CONTROL TEST RESULTS: AUTOMATED
DATE  01/12/03                    TIME 15:09:08
LEVEL #1 (#2)      LOT#   080505001
EXP. DATE  04/06 MM/YY
TYPE: LATEX BEADS
TARGET VALUE: 45 +/- 6.3 M/ml
CONC. RESULTS: 45.1 M/ml NORM (LOW, HIGH)
ACEPTABLE RANGE: 38.7 – 51.3 M/ml
```

- Press **Enter** to both print and save the controls test results.
- Controls can be imported on-line the same way as other SQA-Vb tests.

Section 7: SERVICE MENU

From the **MAIN MENU** select: **SERVICE** to display five SERVICE options:

- **SERVICE DATA**
- **SERVICE PERSONNEL**
- **PRINT DEFAULT SETTINGS**
- **SETTINGS**
- **ADD TESTS TO COUNTER**

SERVICE DATA:

Click SERVICE DATA to display three additional screens: Service Data, Self-Test Data and Algorithm. All three of these screens are used for technical troubleshooting and might be required if a problem occurs with the system.

In addition, the Service Data screen needs to be displayed when transferring data between the SQA-Vb and the PC with B-Sperm™ software such as test results and CONTROL default settings.

SERVICE PERSONNEL: For technical service personnel only (requires a password).

PRINT SQA-Vb DEFAULT SETTINGS:

To print Self-Test Data, Default Settings and Control Settings simply select which test is desired and press **Enter.**

SETTINGS:

Open SETTINGS and find three options: System Default Settings, Freezing Media Default Settings and Operational Default Settings.

System Default Settings:

- **DATE FORMAT**: Select the format **MM/DD/YY** or **DD/MM/YY** using the right/left arrows on the keypad. Press **Enter** to confirm.
- **DATE/TIME SETTING**: Enter current date and time.
- **SAMPLE TYPE: FRESH / FROZEN** – Select sample type to run.
- **FRESH: BSE / DOSING** – Select BSE or DOSING default when testing FRESH semen.
- **CONC. STANDARD:** – S Select "1" for Neubauer standard; "2" for Nucleocounter standard.

Press **ENTER** to select the FREEZING MEDIA DEFAULT SETTINGS. Move the cursor to the desired default and press **ENTER**:

FREEZING MEDIA DEFAULT SETTINGS
1. CLEAR
2. SEMI-CLEAR
3. SEMI-DENSE
4. DENSE
5. MILK

Freezing Media Default Settings: Five media settings are available

- **CLEAR:** Completely clear, transparent extenders that do not contain ANY turbid components such as soybean proteins or egg yolk.
- **SEMI-CLEAR:** Slightly turbid extenders containing soybean protein or a synthetic based media.
- **SEMI-DENSE:** Egg Yolk media prepared with fresh FILTERED egg yolk (homemade) and CSS or TRIS buffer. These extenders appear semi-translucent and darker than the SEMI CLEAR extenders. Commercially available Egg Yolk extenders which are denser than the previous category (more opaque).
- **DENSE:** Dark yellow media that are dense in nature. Egg Yolk based media prepared with fresh NON FILTERED egg yolk (homemade). Egg yolk particles can be seen under the microscope.
- **MILK:** All milk-based extenders.

CLASSIFICATION OF EXTENDERS (OPTICAL DENSITY AND NAME)				
#	FREEZING MEDIA	OD RANGE*		EXTENDER
		FROM	TO	
1	CLEAR	0.00	0.00	Optidyl®, Triladyl®, Biladyl®
2	SEMI-CLEAR	0.10	0.20	Andromed®, Bioxcell
3	SEMI-DENSE	0.25	0.35	Homemade fresh, FILTERED egg yolk based
4	DENSE	0.40	0.60	Homemade fresh NON-FILTERED Egg Yolk based extenders
5	MILK	0.70	1.8	All milk based extenders

- ***OD = Optical Density. If this is not known, a sample of non-diluted freezing media can be run on the system to obtain this value. Contact your local distributor or the manufacturer @ www.a-tech-global.com for instructions on how to run this test.

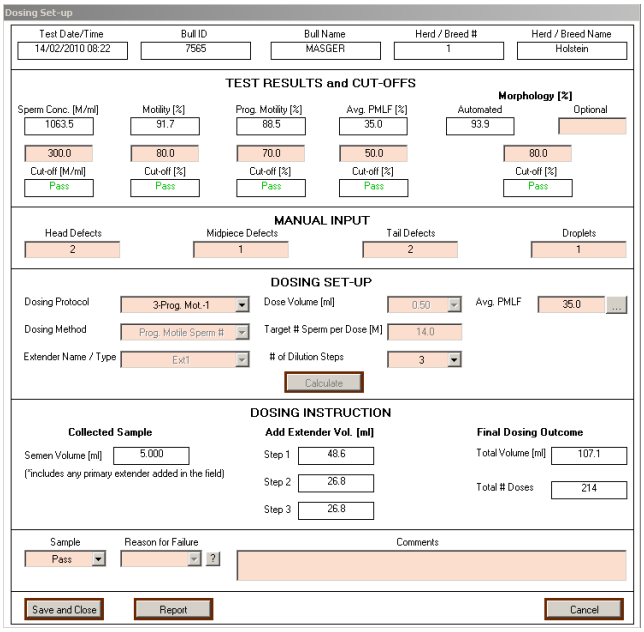
Operational Default Settings:

OPERATIONAL DEFAULT SETTINGS		
SAMPLE TYPE:	FRESH	FROZEN
AUTOMATICALLY PRINT	YES/NO	YES/NO
DISPLAY TEST RESULTS	YES/NO	YES/NO
AUTOMATICALLY SEND TO PC	YES/NO	YES/NO
BARCODE DATA TRANSFER:	YES/NO	
QWIKCLICK COUNTER:	YES/NO	

- **AUTOMATICALLY PRINT**: Select YES to print a test report from the SQA-Vb immediately after each test is run.
- **DISPLAY TEST RESULTS**: Select YES to display test results on the SQA-Vb, or NO to save time and go right to dosing on B-Sperm.
- **AUTOMATICALLY SEND TO PC**: Select YES to automatically transfer test results to B-Sperm with a click on the B-Sperm "IMPORT TEST" button. This feature should be set to YES when dosing fresh samples (see screen below).

FOR DOSING CALCULATION
PRESS: "IMPORT TEST" BUTTON
IN B-SPERM

- The screen above will be displayed after a FRESH AI DOSING test has been run. Click the IMPORT TEST button in B-Sperm and the Dosing Set-Up screen (below) will be displayed.



- Select NO and import all SQA-Vb tests at once via B-Sperm.
- **BARCODE DATA TRANSFER: YES/NO** – NO is the manufacturer default – this option requires technical support.

NOTE: If the I-Button is not properly inserted a message: **I-BUTTON NOT PROPERLY ACTIVATED OR NOT RECOGNIZED BY SYSTEM** will be displayed. Remove the button, press ESC and try again.

ADD TESTS TO COUNTER:

The SQA-Vb requires that tests be "loaded" using a new I-Button:

- Select **ADD TESTS TO COUNTER** and press **ENTER**.
- Follow instructions: **HOLD NEW I-BUTTON AGAINST PORT AND PRESS ENTER**.
- Make sure the I-Button touches both the internal surface and the edges of the port.
- Press and HOLD the I-button firmly in the port during the entire loading process
- The **# TESTS ADDED** and the **# OF TESTS NOW REMAINING** will be displayed
- The screen will warn if an **EMPTY I-BUTTON** (was) **INSERTED**.

Section 8: Troubleshooting

Stabilization Failed:

STABILIZATION FAILED TURN OFF MAIN SWITCH ON REAR PANEL REACTIVATE UNIT IF PROBLEM PERSIST, CALL FOR TECHNICAL SUPPORT

- Make sure there is no testing capillary in the measurement compartment.
- Remove the SQA-Vb from sources of electronic noise (cell phones, etc.).
- Clean the measurement compartment (refer to Appendix III).
- Reboot the SQA-Vb without a testing capillary in the chamber:
  - Turn system **OFF** then back **ON** at the main switch on the rear panel.
  - Press the front panel **ON/OFF** key to begin Auto-Calibration /Stabilization.
- Call technical support if failure recurs.

Self-Test Failed:

FAILED SELF TEST TURN OFF MAIN SWITCH ON REAR PANEL CLEAN OPTICAL CHAMBER REACTIVATE UNIT IF PROBLEM PERSIST, CALL FOR TECHNICAL SUPPORT

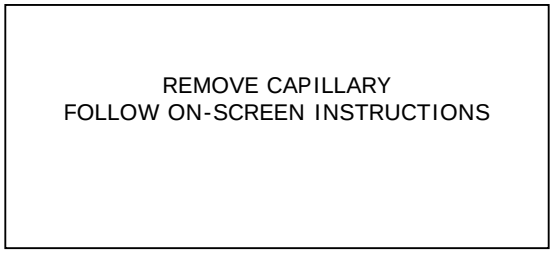
- Make sure there is no testing capillary in the measurement compartment.
- Remove the SQA-Vb from sources of electronic noise (cell phones, etc.).
- Clean measurement compartment (refer to Appendix III).
- Reboot the SQA-Vb without a testing capillary in the chamber:
  - Turn the system **OFF** then back **ON** at the main switch on the rear panel.
  - Press the front panel **ON/OFF** key to begin Auto-Calibration and Stabilization.
- Call technical support if this message is displayed again. Prepare for technical support by printing a copy of the SQA-Vb service data:
  - Press **SERVICE** key. The SERVICE menu will be displayed.
  - Select **PRINT SQA-Vb DEFAULT SETTINGS>SELF-TEST DATA**
  - Press **Enter** to print the SELF-TEST results.

Electronic Noise:

ELECTRONIC NOISE TURN OFF MAIN SWITCH ON REAR PANEL REACTIVATE UNIT IF PROBLEM PERSIST, CALL FOR TECHNICAL SUPPORT

- Make sure there is no testing capillary in the measurement compartment.
- Remove SQA-Vb from sources of electronic noise (cell phones, etc.).
- Clean measurement compartment (refer to Appendix III).
- After cleaning:
  - Turn **ON** the SQA-Vb.
  - Select **TEST NEW SAMPLE** and re-run test.
- If this message is displayed again, reboot the SQA-Vb:
  - Turn the system **OFF** then back **ON** at the main switch on the rear panel.

- Press the front panel **ON/OFF** key to begin Auto-Calibration and Stabilization.
- From MAIN menu: Select **TEST NEW SAMPLE** and re-run.
- Call technical support if this message is displayed again. Prepare for technical support by printing a copy of the system parameters:
- Press **SERVICE** key. The **SERVICE** menu will be displayed.
- Select **PRINT SQA-Vb DEFAULT SETTINGS** option.
- Select **SELF-TEST DATA** and press **ENTER** and the service parameters will be printed.



- This message is displayed prior to running a new test if the capillary from the previous test was left in the measurement slot.

Appendix I: Semen Sample Preparation

EQUIPMENT REQUIRED:

- Testing Media:
  - o QwikCheck™ Diluent for FRESH BULL semen
  - o QwikCheck™ Diluent for FROZEN BULL semen
- Diluent Dispenser
- 10 ml Plastic Containers
- Positive Displacement Pipette with tips
- SQA-Vb capillary
- Heating stage

Figure 1: Diluent Dispenser

FRESH SEMEN SAMPLES:

DILUENT/TESTING CAPILLARY PREPARATION (prior to testing the sample)

- Pre-heat testing capillaries to 37^{0}C / 98.6^{0}Ffor at least for 4 minutes.
- Bring **QwikCheck™ Diluent for FRESH BULL semen** to room temperature (RT: 22^{0}C-26^{0}C or 72^{0}F-79^{0}F).
- Extract 2.0 ml of **QwikCheck™ Diluent for FRESH BULL semen** into a 10 ml plastic container using the dispenser (Fig. 1).
- Pre-heat the **QwikCheck™ Diluent for FRESH BULL semen** at 37^{0}C / 98.6^{0}Ffor at least for 4 minutes. Place 10 ml plastic container with the diluent into the heating device (see appendix section for how to operate)

Figure 2: Sample Preparation

FRESH SAMPLE PREPARATION

1. Extract exactly 100 µl of semen using a positive displacement pipette (Fig. 2).
2. Wipe the tip of the pipette to remove any excess semen.
3. Add the pipetted semen to the 2.0 ml of **QwikCheck™ Diluent for FRESH BULL semen** in the plastic container (Fig 3).
4. Gently but thoroughly mix the sample for 10-20 seconds (Fig 4) to insure that the diluent and semen are homogeneously mixed.
5. The sample is now ready for testing. Fill the testing capillary per the instructions in the Appendix II section of this SQA-Vb User Guide.

Figure 3: Add semen to the diluent

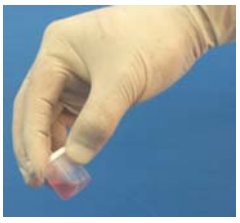


Figure 4: Mixing the sample

FROZEN SEMEN SAMPLES:

STRAWS

NOTE: Use **QwikCheck™ Diluent for FRESH BULL** semen when testing samples frozen in **MILK** based freezing media

DILUENT/ TESTING CAPILLARY PREPARATION – STRAWS

- Pre-heat testing capillaries to 37^{0}C / 98.6^{0}Ffor at least for 4 minutes.
- Bring **QwikCheck™ Diluent for FROZEN BULL semen** to room temperature (RT: 22^{0}C-26^{0}C or 72^{0}F-79^{0}F).
- Extract 500 microliters (0.5 ml) of **QwikCheck™ Diluent for FROZEN BULL semen** into a 10 ml plastic container using the dispenser. Use **QwikCheck™ Diluent for FRESH BULL** semen when testing samples frozen in **MILK** based freezing media.
- Pre-heat the **QwikCheck™ Diluent for FROZEN BULL semen** in a 10 ml plastic container to 37^{0}C for 4 minutes by placing it into a heating device (Please see Appendix section for how to use the sample heating system).

FROZEN SAMPLE PREPARATION – STRAWS

1. Thaw one or two straws in a water bath set at 37^{0}C (98.6^{0}F) in order to obtain a 200-microliter sample.
2. Express the semen from the thawed straw(s) into a separate plastic container.
3. Mix the semen thoroughly.
4. Extract exactly 200 µl of semen using a positive displacement pipette.
5. Wipe the tip of the pipette to clean of excess semen.
6. Add the 200 microliters of semen to 500 microliters of pre-heated **QwikCheck™ Diluent for FROZEN BULL semen** in the 10 ml plastic container. Use **QwikCheck™ Diluent for FRESH BULL semen** for samples frozen in **MILK** based freezing media.
7. If the volume of the thawed specimen is <200 µl and only ONE straw can be used, disregard the on-screen instructions for straw sample preparation and follow the instructions in the dilution table below:

Semen Volume, µl	QwikCheck™ Diluent for FROZEN BULL semen, µl
180	450

8. Gently but thoroughly mix the sample for 10-20 seconds (Fig 4) to make sure that the diluent and sperm are homogeneously mixed (Pre-heat the mixture to 37^{0}C / 98.6^{0}F for 4 minutes).
9. The sample is now ready for testing. Fill the testing capillary per the instructions in the Appendix section of this SQA-Vb User Guide.

Appendix II: Frozen Media Types and Settings

Freezing Media Default Settings: Five media settings are available:

FREEZING MEDIA DEFAULT SETTINGS
6. CLEAR
7. SEMI-CLEAR
8. SEMI-DENSE
9. DENSE
10. MILK

- **CLEAR:** Completely clear, transparent extenders that do not contain ANY turbid components such as soybean proteins or egg yolk.
- **SEMI-CLEAR:** Slightly turbid extenders containing soybean protein or a synthetic based media.
- **SEMI-DENSE:** Egg Yolk media prepared with fresh FILTERED egg yolk (homemade) and CSS or TRIS buffer. These extenders appear semi-translucent and darker than the SEMI CLEAR extenders. Commercially available Egg Yolk extenders which are denser than the previous category (more opaque).
- **DENSE:** Dark yellow media that are dense in nature. Egg Yolk based media prepared with fresh NON FILTERED egg yolk (homemade). Egg yolk particles can be seen under the microscope.
- **MILK:** All milk-based extenders.

CLASSIFICATION OF EXTENDERS (OPTICAL DENSITY AND NAME)				
#	FREEZING MEDIA	OD RANGE*		EXTENDER
		FROM	TO	
1	CLEAR	0.00	0.00	Optidyl®, Triladyl®, Biladyl®
2	SEMI-CLEAR	0.10	0.20	Andromed®, Bioxcell
3	SEMI-DENSE	0.25	0.35	Homemade fresh, FILTERED egg yolk based
4	DENSE	0.40	0.60	Homemade fresh NON-FILTERED Egg Yolk based extenders
5	MILK	0.70	1.8	All milk based extenders

- ***OD = Optical Density. If this is not known, a sample of non-diluted freezing media can be run on the system to obtain this value. Contact your local distributor or the manufacturer @ www.a-tech-global.com for instructions on how to run this test.

Instructions for measuring the OD of the extender in the SQA-Vb:

- Thoroughly mix EXTENDER/FREEZING MEDIA to be tested. Do not dilute with any other media or diluent. Aspirate into an SQA-V testing capillary for a normal volume sample.
- From the MAIN MENU select: SERVICE/SETTINGS
- Select: SYSTEM DEFAULT SETTINGS and set:
  - SAMPLE TYPE: FROZEN
  - FROZEN TYPE: STRAW
- Select: FREEZING MEDIA DEFAULT SETTINGS > MILK
- From the MAIN MENU select: TEST NEW SAMPLE and enter:
  - 1 for Bull ID
  - 1 for Semen Volume
  - 1 for Herd ID
- Disregard the STRAW SAMPLE PREPARATION onscreen instructions
- Insert the SQA-V testing capillary into the measurement chamber when prompted
- After testing go to: MAIN MENU > SERVICE > PRINT SQA-Vb DEFAULT SETTINGS > SELF-TEST DATA
- The SERVICE DATA report will be printed. Item #7 is the OD value.
- View the table above and select the correct extender default (based on the OD value).

Appendix III: Capillary Filling Instructions: FRESH and FROZEN SAMPLES

SQA-Vb Testing Capillary

NOTE: If air bubbles are still present in the capillary after tapping on the syringe, dip the capillary into the semen sample again and aspirate a small quantity of semen to draw air bubbles into the syringe.

NOTE: It is important to remove all semen from the exterior of the capillary in order to prevent the SQA-Vb optical chamber from becoming clogged.

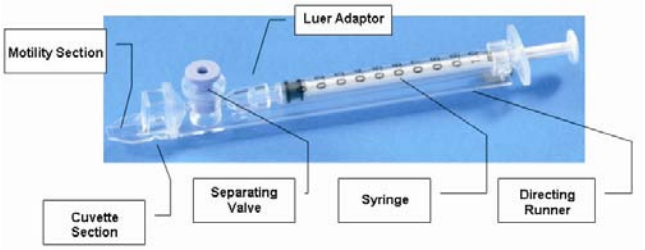


Figure 1

1. Push the syringe piston in fully. Place only the thin part of the capillary into the bottom of the diluted sample - Figure 1.
2. Placing two fingers below the piston head pull the piston back slowly while keeping the tip of the capillary well below the sample level and below any surface bubbles (Figure 1). Continue to aspirate the sample until it appears in the Luer adaptor.
3. Hold the capillary in a vertical position and visually confirm that the sample has completely filled the thin section and the cuvette section and appears in the Luer adaptor (Figure 2).
4. Tap on the syringe to make sure there are no air bubbles in the sample.
5. Quickly and thoroughly wipe both the top and bottom of the outer surface of the capillary with a tissue such as Kimwipes, etc. (Figure 3). Visually confirm that the capillary chambers are still full after wiping. If some of the sample has been depleted, a meniscus will be visible in the thin section of the capillary. If this is evident, push very slightly on the piston to re-fill the thin capillary section.
6. Slowly and carefully push-in the separating valve until it is level with the plastic. The capillary is now ready for testing (Figure 4)
7. Insert the capillary into the SQA-Vb (Figure 5)

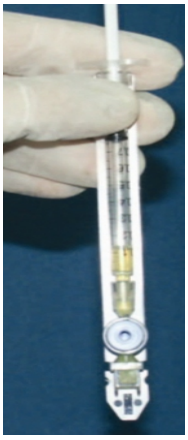


Figure 2

Figure 3

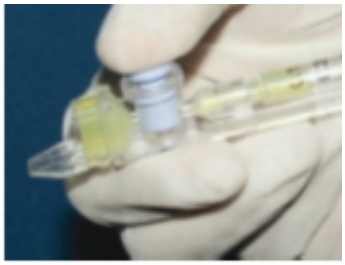


Figure 4

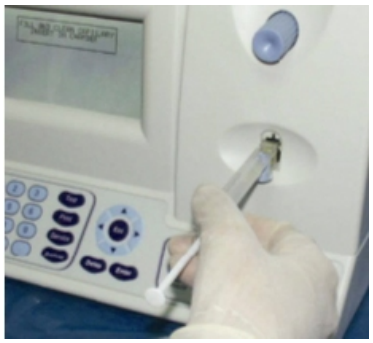


Figure 5

Appendix IV: SQA-Vb Cleaning Instructions

When to clean:

Daily or after every 25 tests
If the system fails **SELF-TEST**

Cleaning kit components:

Blue Dot capillaries (fig 1)
Sponge-tipped drying capillaries (fig 2)
Cleaning brush -wooden-handled (fig 4)
Cleaning fluid

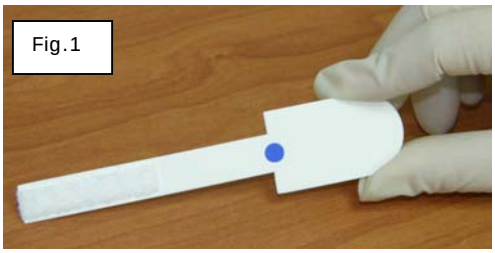


Fig.1

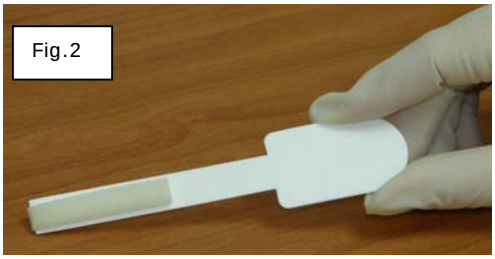


Fig.2

CLEANING: STEP 1

1. **TURN OFF** SQA-Vb and unplug it at main electrical outlet.
2. Select a **BLUE DOT cleaning** capillary (fig 1).
   - Moisten with **ONE** drop of cleaning fluid, shaking off excess fluid.
   - Insert into the measurement compartment - fibrous material facing up, and move back and forth a few times in the directional runner.
   - Repeat with fibrous material facing down
   - Select a sponge material capillary (Fig 2) and insert it in the same compartment in order to dry the chamber (fig 3)


Fig.3


Fig.4

CLEANING: STEP II

Clean the channel that measures concentration using the cleaning brush (fig 4):

1. Insert the brush (bristle-side down) fully into the upper portion of the lower chamber of the SQA-Vb in same manner as a testing capillary (fig 5).
2. Pull the brush out of the chamber while sweeping or "dusting off" the LED (you will feel a step or shelf at the back and top of the chamber – this is the top of the LED). (Fig 6)
3. Switch SQA-Vb unit **ON** and observe self-test results. The SQA-Vb should now PASS the self-test. If not, repeat cleaning procedure with the brush.


Fig.5


Fig.6

CLEANING THE VISUALIZATION COMPARTMENT:

Open the visualization compartment door (upper slot) and swing the cover above the lens to the left. Wipe the lens with 98% alcohol (not provided).

Appendix V: Capillary Washing/Drying Instructions

Testing Capillary

Repositioning the blue valve with the jig

Removing the plunger

Reassembled capillary

Washing 10 ml sample collection cups

(For animal applications ONLY!)

Both testing capillaries and 10ml sample collection cups can be washed and re-used up to 10 times by following this EASY procedure:

Step 1 After running a test:

- Use the white capillary jig to re-position the blue capillary valve
- Expel semen by pumping the plunger a couple of times
- Soak the testing capillary in tap water until ready to wash

Step 2 Set-up: Fill with 1 liter/2 quarts of solution as follows:

- Bowl #1: Tap water (marked “TAP WATER”)
- Bowl #2: Distilled water (marked “DISTILLED WATER”)
- Bowl #3: Isopropyl Alcohol 70% - 100%

Step 3: Remove all liquid from the testing capillary:

- Pump the syringe plunger a couple of times to remove liquids.

Step 4: Capillary Washing – Follow this order:

- Bowl #1 Tap Water: Completely fill each capillary with tap water. Expel the solution into a hazardous waste container. **Repeat 2 times** then go to Bowl 2.
- Bowl #2 Distilled Water: Completely fill each capillary with distilled water. Expel the solution into a hazardous waste container. **Repeat 2 times** then go to Bowl 3.
- Bowl #3 Isopropyl Alcohol: Completely fill each capillary with isopropyl alcohol and expel the solution into a hazardous waste container. **Repeat 2 times**.
- Remove the plunger from the syringe.

Step 5: Capillary Drying Options:

- Place the capillaries:
  - On a flat surface and dry overnight.
  - In a commercial desiccator - follow manufacturer instructions.
  - In an oven on low heat for a few hours.

Step 6: Final Preparation/Inspection:

- Replace the plunger into the syringe and inspect the capillary.
- Discard capillaries with debris, cracks or broken parts.
- Make a dot on the capillary with a water proof marker after each washing cycle.

Capillary re-assembly

- Place the syringe plunger back into the SQA-Vb capillary.
- Confirm (using the jig) that the blue stopper to the correct position.
- Inspect the capillary for cracks, broken parts or remaining semen. Discard capillaries that are not acceptable. Check syringe by aspirating air in and out twice.
- Mark a dot on the capillary after washing to indicate the # washings.

Washing 10 ml sample collection cups

Refer to Step 4 and Step 5 of the Capillary Washing Procedure above - follow the same process for washing in solution bowls #1; #2 and #3. Turn upside down on absorbent paper to dry overnight or place in a commercial warming oven for a few hours.

Appendix VI: The Visualization System

The SQA-Vb Visualization System permits the user to analyze/view semen samples using either a standard slide or the SQA-Vb testing capillary. Additionally, the visualization system is a critical "link" to B-Sperm which allows the user to view samples on a PC monitor and capture and save video images. The visualization system:

- Accommodates both an SQA-Vb testing capillary and a standard slide.
- Control knobs located below the visualization screen can be used to set the focus, brightness and contrast.
- Operate the zoom, illumination and on/off setting from the SQA-Vb keypad.
- Magnification range: x300 through x500.

Operating Instructions

Slide Preparation:

- Use 10 µl of semen (20 micron sample depth).
- Use only a standard slide with a 22mm x 22mm cover-slip.
- Load the prepared slide into the SQA-Vb slide adaptor and insert into the visualization compartment of the SQA-Vb.

Testing Capillary Preparation:

- Fill the SQA-Vb testing capillary following the instructions in the appendix section of this guide.
- Insert the capillary into the visualization compartment of the SQA-Vb.

Testing Process:

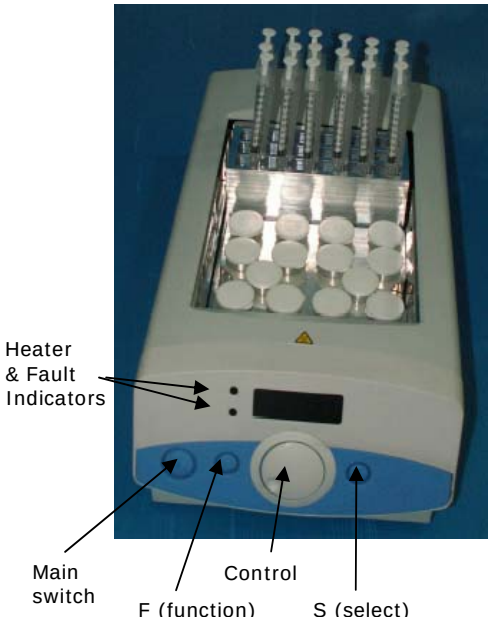
1. The video display will automatically illuminate when the SQA-VB is turned on.
2. Use the monitor **ON/OFF** key on the keypad to independently operate the video display.
3. To ensure that the visualization system is working properly prior to use:
    a. Press the **HIGH ILLUMINATION** key multiple times to ensure a maximum level setting.
    b. Turn **BRIGHTNESS**, **CONTRAST** and **COLOR** buttons all the way counterclockwise.
    c. Turn FOCUS knob fully clockwise.
4. Use **ZOOM IN** for maximum magnification (x500) and **ZOOM OUT** for minimum magnification (x300).
5. Insert the semen sample into the visualization chamber.
6. Turn the **BRIGHTNESS** knob clockwise until the video screen just begins to lighten-up.
7. Turn the **FOCUS** knob counter-clockwise until the image is in focus.
8. Adjust **CONTRAST, COLOR, BRIGHTNESS, FOCUS** and object **ILLUMINATION** controls for optimal image quality.

NOTE: The focus knob should not be forced to turn beyond the initial stopping point. Turn the knob gently and when resistance is felt it is at the maximum (or minimum) position. Forcing this knob beyond the stopping point will cause extensive damage to the SQA-Vb.

Appendix VII: Block Heater Operating Instructions
For Use with A-Tech Sperm Quality Analyzers

Safety:

- Do not pre-fill the SQA testing capillaries with semen prior to heating.
- Do not touch the heating elements to check the temperature.
- If transported or stored in humid conditions, dry the unit before connecting it to power.
- Plug into a grounded electrical outlet that delivers the appropriate voltage indicated on the rear panel of the heater.
- If liquid is spilled inside the unit, disconnect the power supply, take out the metal heating blocks and wipe the spilled material with a damp cloth. Do not use chemical cleaning agents.
- Place the system on a level surface that is free from flammable materials, insuring that all ventilation slots on the base of the system are clear of obstructions.



Heater Operation:

- Place the metal heating racks for capillaries and sample containers into the heating system.
- Plug the electrical cord into the socket at the rear of the unit.
- Turn the unit on by pressing the Main Switch on the front panel - The display will illuminate showing the current temperature of the block.
- Press the 'S' button and adjust the temperature to 40°C using the Control knob. This will ensure a 37°C temperature for the testing capillary and semen samples.
- Press 'S' to confirm the temperature setting or press 'F' to exit without changing the value.
- The block heater will now begin to heat the racks.
- A light will indicate that the system is heating and will begin to flash when the set temperature is approached.
- The temperature setting will be stored in the memory.

Timer Operation:

- Press 'F' button and turn the Control knob until 'CLOC' is seen.
- Press 'S' button and turn the Control knob until 'On' is seen.
- Press 'S' button and turn the Control knob to select the required time (Example: 4 minutes (004) for Equine).
- Press 'S' button: The time and the Temperature will be displayed intermittently.
- A beep will sound and 'End' will be displayed when the time has expired; press 'S' to stop beeping.

Heating the SQA Testing Capillaries and Samples:

- Place **empty** testing capillaries in the heating rack (as shown in the picture above).
- Place **empty** 10-ml plastic containers in the appropriate heating rack.
- Wait 5-7 minutes for the heating unit to pre-heat the containers and testing capillaries.
- Distribute the semen sample into 10-ml container following User Guide instructions.
- Close the containers during pre-heating.
- Remove testing capillaries as needed for testing. Fill with semen per user guide instructions.

Note: If the processor detects an error in heating the fault indicator will illuminate, the buzzer will beep and the display will flash. To reset this fault, switch the unit off and on. If the fault re-occurs contact the service personnel at your local distributor.

Appendix VIII: Glossary of Terms

	SQA-Vb Terms	Definition
	SN	Serial Number of the SQA-Vb
	DATE/TIME	The date and time the test was performed
Sample/Test Data	SAMPLE #	The number assigned to the semen sample
	BULL ID	The identifying number of the bull being tested
	HERD/BREED #	The number that identifies the herd of the bull being tested
Test Results	CONC.	Total Sperm Concentration expressed in millions/ml
	MSC	Motile Sperm Concentration expressed in millions/ml
	PMSC	Progressively Motile Sperm Concentration expressed in millions/ml
	MOTILITY %	% of Motile Sperm
	PROGRESSIVE MOTILITY %	% of Progressively Motile Sperm
	MORPHOLOGY	% of Morphologically Normal Sperm
	VELOCITY	The average velocity of the progressively motile sperm cells (microns/sec) in the sample
	TOTAL SPERM NUMBER	The total number of sperm cells per ejaculate (FRESH Semen) or per AI dose (FROZEN Semen)
	TOTAL MOT. SPERM	The total number of motile sperm cells per ejaculate (FRESH) or per AI dose (FROZEN)
	TOTAL PR.SPERM	The total number of progressively motile sperm cells per ejaculate (FRESH) or per AI dose (FROZEN)
Dosing Calculations	EXTENDER VOLUME	The amount (ml) of extender to add to the semen in order to produce the desired number of doses
B-Sperm	TOTAL VOLUME	Semen volume + extender volume (ml)
	NUMBER OF DOSES	The total number of doses that can be produced from the semen based upon the users set-up parameters
Dosing Set-up	DOSING METHOD	Option to dose by: Total Sperm #; Motile Sperm #; or Progressively Motile Sperm # in an A.I. dose
B-Sperm	DOSE VOLUME	The desired A.I. dose volume (ml): 0.25/0.5/other
	TARGET # SPERM	The total number of spermatozoa desired in an A.I. dose (millions/ml)

Appendix IX: SQA-Vb System Specifications

Dimensions: 40 x 30 x 15 cm

Weight: 4 kg

AC power supply: 100 to 250 VAC, 50/60 Hz, 10 VA

Measurement Compartment

- **Sources of radiant energy** - two 880 nm LEDs for motility and spectrophotometry channels
- **Detector system** – 2 photo detectors - Motility and Optical Density

Visualization Compartment

- Green LED illumination system
- CCD, 350 TV lines
- Objective lens: Standard, x20
- Signal Output: PAL standard
- Zoom system for smooth magnification transition from x300 to x500
- Focus regulator

Display(s)

- Operational backlight LCD (16 lines x 40 characters)
- Video backlight LCD (8 x 10 cm)

Printer

- Built-in, Dot Matrix
- Non-thermostatic narrow paper with 20 characters per line (Citizen)
- Ribbon cassette (Citizen)

Keypad

- **Operational keys:** ON/OFF, TEST, PRINT, SERVICE, ARCHIVE, DELETE, ENTER, four cursor buttons, ESC, numeric buttons (0-9)
- **Video control keys**: ZOOM IN/OUT, ILLUMINATION HIGH/LOW, and MONITOR ON/OFF

Front Panel

- Built-in printer
- Visualization compartment
- LCD video display and controls
- Focus knob
- LCD operational display
- Measurement compartment
- Multi-button keypad

Rear/Side Panel

- Power connector with fuse-holder (fuse 250V, 1A)
- Video connector
- RS232 cable outlet
- I-Button port (side panel)

Specimen Testing Supplies

- **Measurement capillary:** Disposable, multi use plastic, positive displacement testing capillary (purchase from manufacturer).
- **Standard lab slide:** 76 X 25.6 mm with 22 x 22 mm cover-slip.
- **I-Button:** Required to run tests (purchase from manufacturer)

Archive Capacity

- 500 test records in each archive (Test and Controls)

Operating System

- **Control:** Keypad
- **Analysis Time:** Normal Test – 50 seconds
- **Software:** Resides on flash memory and drives all man-machine interface functions, runs algorithms for test measurements and operational screens. System can be upgraded from a PC CD-ROM.
- **Sample Testing Temperature:** Calibrated for room temperature only. Motility results will be impacted by heating the specimen.
- **Motility channel input signal:** Analog, up to 5V.
- **Spectrophotometer channel input signal:** Modulated (1 kHz) analog, up to 5V.

Quality Control

- **Internal:** Electronic Self-Test and Auto-Calibration.
- **External:** Latex beads control material (QwikCheck™ beads)

PC Compatibility

Minimum requirements for B-Sperm™ software

- **PC:** 1 GHz processor, Pentium 3
- **RAM:** 256 MB
- **AGP-video display card** with at least 16 MB of RAM memory
- **Video color**: At least 16 bit (65,535)
- **CD ROM drive**
- **40 GB free hard disk space** for image capturing/storage
- **Video resolution:** Minimum 640 x 480

Operating system compatibility

- **Windows** XP or VISTA or WIN7
- **Ports:** One serial; two USB ports
- **Monitor:** 15" color

Additional Software (supplied with system)

- **B-Sperm software:** Real time visualization interface between PC and SQA-Vb visualization system, data transfer, video/picture capture and archive.

Operational Temperature and Humidity

- System is operational at 15-38°C.
- *NOTE*: SQA-Vb operates in a wide range of ambient temperatures however the system is calibrated to measure semen samples at room temperature: 22-26°C (68-79°F). Semen samples can be pre-heated to 37^{0}C / 98.6^{0}F prior to testing if required (refer to the SQA-Vb User Guide Appendix I: Semen Sample Preparation).
- *NOTE*: Variations in ambient temperature may impact the accuracy of test results because of the effect of temperature on semen.
- System is fully operational at up to 80% humidity and 31°C.

Maintenance Schedule

- Cleaning daily or after every 25 tests (refer to the SQA-Vb User Guide Appendix III: SQA-Vb Cleaning Instructions).

Manufacturer Recommendations

- Operate the SQA-Vb away from devices that may cause electronic noise (cell phones) or other devices causing vibrations such as centrifuges.
- Turn system **OFF** at the rear-panel when not in use for extended period of time.
- Variations in ambient temperature can affect semen samples. The SQA-Vb is calibrated to conduct tests at room temperature: 22-26ºC (68-79ºF). Nevertheless semen samples can be run by the system after pre-heating to 37^0C / 98.6^0F.
- Semen is considered a biologically hazardous material and is subject to individual laboratory protocols for handling such materials.

Factory Default Settings

Date format: **DD/MM/YY**

Date/Time: Manufacturer's local date/time

Sample Type: **FRESH**

Fresh: **DOSING**

Frozen Type: **STRAW**

Freezing Media Default Settings: **SEMI CLEAR**

CONC STANDARD**: 1 (Neubauer)**

Operational Default Settings:

- Automatically Print: **YES**
- Display Test Results: **YES**
- Automatically Send to PC: **YES**
- Barcode Data Transfer: **NO**
- QwikClick Counter**: NO**

Appendix X: Product Performance Data

Abbreviations:

CONC:	Sperm Concentration
MSC:	Motile Sperm Concentration
PMSC:	Progressively Motile Sperm Concentration
Mic.	Micron
Sec.	Second
M/ml	Million per milliliter

Performance Data Summary:
The performance of the SQA-Vb system for bull semen analysis is summarized in the text, tables and graphs below. Sperm concentration measurements are expressed as $x10^6$ sperm cells per milliliter (M/ml). Motility and Morphology values are expressed as a percent (%). Unless otherwise noted all testing was performed using fresh and frozen bull semen samples.

Calibration:
Each SQA-Vb device is biologically calibrated against two reference systems at Medical Electronic System's laboratory using bull semen.

Dynamic Range:

Sample Type	Test Mode	Conc. M/ml	Motility %	Morphology %	MSC M/ml	PMSC M/ml	Velocity, mic./sec.
Fresh	Fresh	0-2000	0-95	0-100	0-1900	0-1800	0-130
Frozen	Frozen	0-100	0-95	-	0-950	0-900	0-80

Precision and accuracy established against a known target (Latex beads)

Background: The precision and accuracy of the SQA-V was compared to a known target value using commercially available latex beads of two concentrations. Latex beads are run on the SQA-V in the same manner as semen samples.

Limitations of method:
Latex beads cannot:

- Measure sperm motility or morphology
- Correct for inaccurate chamber depths or technician errors

Method comparison:
A total of 320 latex bead samples were tested on ten SQA-V systems (32 samples per SQA-V). Precision of the SQA-V was estimated (Table 1). SQA-V concentration readings were compared to the established target values +/- the acceptable range for the latex beads (Fig. 1 & 2).

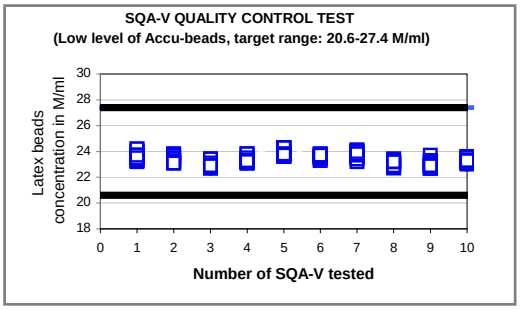
Accu-beads® published acceptable ranges (*Hemacytometer*):

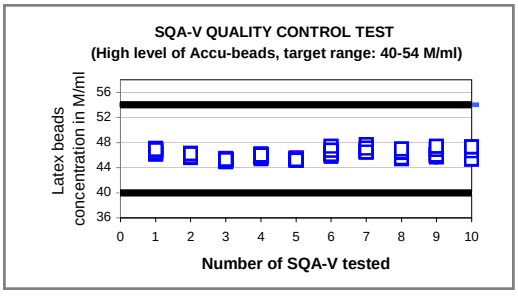
- Vial #1: 47 +/- 7.0 M/ml
- Vial #2: 24 +/- 3.4 M/ml

Table 1: Precision

SQA-V	Accu-beads®	CV %
Intra-device Variability	**High 47± 7.0 M/ml**	**≤ 0.01**
	Low 24 ± 3.4 M/ml	**≤ 0.01**
Inter-device Variability	**High 47± 7.0 M/ml**	**≤ 2.00**
	Low 24 ± 3.4 M/ml	**≤ 2.50**

Fig. 1- 2 Accuracy
Low/High Level Controls:





Conclusions:

The CONTROL mode software of the SQA-Vb (bull) device is exactly the same as the SQA-V (human) system. Both systems also have the same hardware platform. Therefore, the accuracy and precision results obtained on the CONTROL mode of the SQA-V will be the same as that of the CONTROL mode of the SQA-Vb.

Sensitivity, specificity, precision, accuracy and correlation to manual method established in the MES laboratory and field clinical trials using bull semen samples

FRESH SEMEN Performance Claims (Table 2-5)

Sensitivity

- Concentration: 90%
- Motility: 85%
- Morphology 80%

Specificity

- Concentration: 90%
- Motility: 80%
- Morphology 90%

Precision (CVs)

- Conc.: 3%
- Motility: 5%
- MSC: 7%
- Velocity: 10%

Accuracy (regression coefficients of "live/dead" trend line) Claims:

- Motility: 0.85
- MSC: 0.9
- PMSC: 0.9

Correlation to Manual Method Claims:

- Concentration: 0.9
- Motility: 0.8
- Morphology 0.7
- MSC: 0.9
- PMSC: 0.8
- Velocity: 0.75

FROZEN SEMEN Performance Claims (Table 3, 5)

Precision (CVs)

- MSC: 7%
- Velocity: 4%

Correlation to Manual Method

- MSC: 0.8
- PMSC: 0.7
- Velocity: 0.85

Table 2: Sensitivity/Specificity

SQA-Vb vs. Microscope	Sensitivity	Specificity
FRESH SEMEN		
Sperm Concentration M/ml	**100.0%**	**98.4%**
Motility, %	**96.3%**	**85.0%**
Morphology	**85.7%**	**91.7%**

Table 3: Precision
SQA-Vb intra-device Variability (CV, %)

Parameter	Sample Type	
	Fresh	Frozen
Sperm Concentration M/ml	**2.4**	**-**
Motility, %	**4.1**	**-**
Morphology %	**5.0**	**-**
MSC, M/ml	**6.1**	**6.4**
Velocity, microns/sec.	**9.9**	**3.4**

Table 4: Accuracy:
Regression coefficients from "live/dead" experiments

Parameter	SQA-Vb	Manual
Motility, %	**0.9135**	**0.8325**
MSC, M/ml	**0.9348**	**0.7547**
PMSC, M/ml	**0.9340**	**-**

Notes:

- Sensitivity and specificity claims are lower than actual values noted (Table 2).
- Precision CV claims are higher (lower precision) than actual values noted (Table 3).
- Accuracy regression coefficient claims are less than actual values noted (Table 4).
- Correlation to Manual Method claims are less than actual correlations noted (Table 5).

Method comparison:

SQA-Vb was compared to the microscope based on WHO '99 guidelines. The SQA-Vb automated readings of the sperm concentration, motility, MSC, PMSC and velocity were compared to microscopic results. A Makler chamber was used according to manufacturer's instructions for the manual sperm concentration measurements. A standard slide and B-Sperm software were used to assess manual motility, progressive motility and velocity measurements. Manual MSC and PMSC parameters were calculated from experimental results. The protocols were based on WHO '99 and MES guidelines. The alpha-site clinical trials were conducted at the Sion farm. A total of 104 fresh and 138 frozen semen samples were analyzed.

Analytical Specificity:

- To achieve analytical specificity a specific wave length of light which is maximally absorbed by sperm cells and minimally absorbed by other cells and seminal plasma is used.
- Low noise and high electronic resolution hardware components and compensation circuits ensure that analytical specificity is optimized.

Limitations of method:

Samples were assessed in duplicate on the automated SQA-Vb system and manually using a microscope. Statistical counting errors and intra-operator variability (subjectivity) may have affected the results of the study.

Accuracy assessment: "live/dead" sperm plots.

The SQA-Vb accuracy was assessed by "live/dead" bull sperm experiments. Fresh bull semen was distributed into two aliquots. The first aliquot was intact ("live") and the second one was treated with the liquid nitrogen ("dead"). Then different "live-to-dead" proportions were created providing a constant Sperm Concentration but varying MSC, PMSC and Motility. The samples were tested using the SQA-Vb device and under the microscope and the results plotted. The linear trend lines were established for motility, MSC and PMSC variables vs. "Live/Dead" sperm ratio.

Table 5: Correlation to manual method

Parameters	Correlation coefficients	
	Fresh semen	Frozen semen
Sperm Concentration, M/ml	0.93	-
Motility, %	0.81	-
Morphology %	0.71	-
MSC, M/ml	0.94	0.84
PMSC, M/ml	0.86	0.74
Velocity, mic./sec.	0.81	0.91

Fig. 3: Method comparison: Regression plot of SQA-Vb Sperm Concentration in fresh bull semen vs. manual results

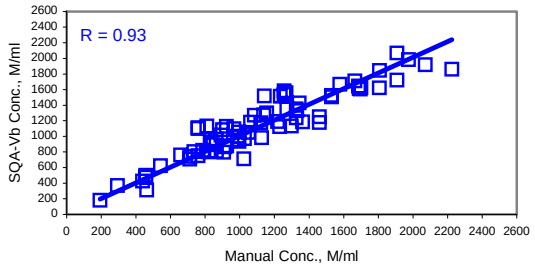


Fig. 4: Method comparison: Regression plot of SQA-Vb Motility in fresh bull semen vs. manual results

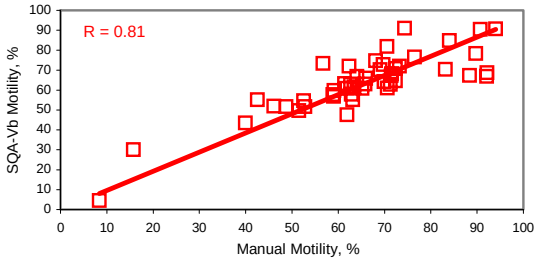
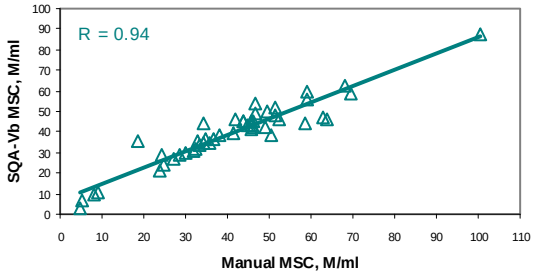


Fig. 5: Method comparison: Regression plot of SQA-Vb MSC in fresh bull semen vs. manual results



Performance parameters:

- Sensitivity and specificity were calculated using ROC analysis formulas. The cutoffs normally used for the sperm concentration and motility of the fresh semen samples were used for calculation of sensitivity and specificity. As for the frozen samples there are no cutoffs, sensitivity and specificity were not calculated for this type of samples (Table 2).
- Precision of the SQA-Vb device was estimated by calculation of the intra-device coefficients of variation (CV) of the duplicate measurements (Table 3). CV is calculated according to the formula:

  CV = SD / MEAN x 100
- The accuracy of the SQA-Vb device was characterized by regression coefficients of the trendline obtained in the “live/dead” experiment (Table 4).
- Correlation to manual method was established by calculation of correlation coefficients (Table 5, Fig. 3-5).

Conclusions:

- High levels of sensitivity, specificity and correlation of the SQA-Vb device results to the manual method were found. Therefore the instrument can be used in the field for the semen quality assessment, dose preparation and in the frozen semen QC.
- SQA-Vb provides the precise and accurate results with low coefficients of variation (<6%) and high regression coefficients of the “live/dead” trend lines (>0.95).

B-Sperm™

User Guide

Version 3.00

Catalog #7410

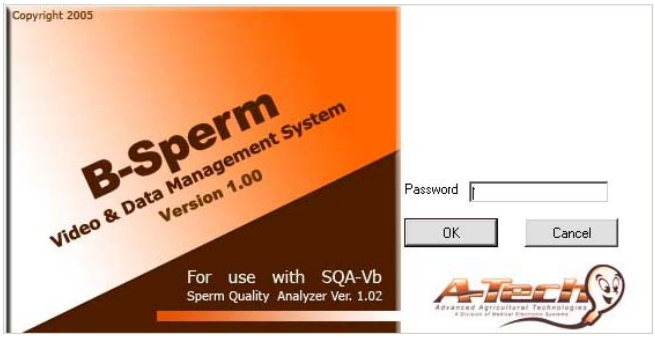
June 2010

TABLE OF CONTENTS

Section 1: Overview

B-Sperm software is included with each SQA-Vb. Some of the B-Sperm features are listed below:

- Tests results run on the SQA-Vb are saved and can be used to assess bull fetility, AI dose productivitiy and ejaculate quality for specified timeframes.
- The B-Sperm DOSING function interacts with the SQA-Vb to automatically provide information for dividing the ejaculate into AI doses by # CELLS, # MOTILE CELLS or # PROGRESSIVELY MOTILE CELLS per dose.
- Bull morphology can be assessed using the QwikClick Counter in B-Sperm and the SQA-Vb visualization system.
- Cut-offs can be established for automatic sample qualifying or disqualifying.
- Dosing protocols unique to the AI Stud can be pre-set for easy dosing.
- Graphs, video clips, pictures, reports and, herd data can be captured.
- The Average Progressive Motility Loss Factor (AVG % PMLF) will be calculated and updated automatically (see User Guide Section for information about this value).
- Information is secure – access to B-Sperm requires a password



The B-Sperm package includes:

- B-Sperm User Guide
- Installation CD
- Security Key
- Video Frame Grabber (or internal PC card)
- Video Cables

System Requirements:

- SQA-Vb with RS232 communication cable and power cable
- PC requirements:

Hardware requirements:

- 1Ghz or higher CPU
- 256 MB RAM
- AGP Video Display Card with at least 16 MB of RAM memory
- 15 inch or more color monitor
- CD-ROM compatible drive
- RS232 communication port (serial)
- Two available USB ports

Software requirements:

- Compatible operation system:
- Windows XP, Windows VISTA, Windows 2007
- EXCEL for data export

Section 2: Software and Hardware Installation

Frame Grabber USB Device Installation

Warning: The frame grabber should be installed prior to using B-Sperm!

The frame grabber provided by the manufacturer needs to be installed prior to using B-Sperm. Please refer to the package insert in the B-Sperm box for complete directions.

B-Sperm Installation

Warning: Do not connect the video capture device or security key until the software installation is complete and the PC has been re-started.

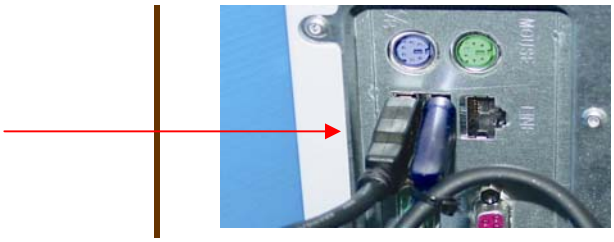
NOTE: It is highly recommended to use the manufacturer's default settings.

1. Close any open programs before beginning the upgrade process.
2. Insert the B-Sperm CD into the PC's CD-ROM. The installation process will begin automatically.
3. The screen will display: Initializing Wise Installation Wizard.
4. Click NEXT when the "Welcome" screen is displayed.
5. Click NEXT when the "Choose Destination Location" screen is displayed to accept the B-Sperm default folder or click "Browse" to select another folder.
6. Click NEXT when "Select Program Manager Group" is displayed. If a message "Digital Signature Not Found" is displayed, click YES.
7. Installation complete: Click OK to restart the PC.

Security Key Installation

Warning: B-Sperm will not work without the "security key" connected to the PC.

- Plug the B-Sperm security key into a second free USB port on the computer
- The B-Sperm security key will automatically install.



SQA-Vb Communication Cable Connection

- Connect one end of the RS232 communication cable to the PC.
- Connect the other end of the RS232 communication cable to the SQA-Vb.

Video Capture Device Settings

- Go to the B-Sperm: Real Time Video > Video Settings.
- Using the drop-down menus, make the appropriate setting selections for the video capture device that has been installed.
- Refer to the Video Capture Installation guide provided with the video capture hardware.

Section 3: System Navigation Overview

Eight navigation buttons allow easy access to the B-Sperm features:

- B-Sperm is set-up for easy use by simply clicking on main navigation buttons that are always available in the left margin of the screen.
- When a main navigation button is selected, sub-menu buttons appear across the top of the B-Sperm screen displaying more options and features.
- At the top of every screen are directions indicator buttons – use these to move back to the previous menu.

Test Data
Controls
Import Test
Dosing: Pooled
Import/Export
Qwik Click/Video
Set-Up
Exit

The icons below are used to indicate a variety of messages and warnings:

<> Test results out of normal range: This icon indicates that one or more of the test results are **out of normal range** that has been set-up by the user (please refer to Section 10: Set-Up). Click on this icon to display the semen analysis results compared to the normal ranges for the selected record.

! Dosing mismatch: This icon is displayed when the semen cannot be diluted to produce AI doses that meet the requirements the user has "set-up". This happens when:

- Set-up values have been entered incorrectly
- The semen sample is of low quality

Picture indicator: A camera icon indicates that a picture is attached to the test results. Click the camera icon to view the attached picture. Only one picture can be attached to each test.

Video Indicator: A video camera icon shows that a video clip is attached to the test results. Click the video icon to view the attached video clip. Only one clip can be attached to each test.

Graphs: This icon indicates that a graph of the test results is attached to a bull file. Click on the graph icon to display the chart. The icon will only be displayed when a bull has more than one test record.

Section 4: Start-up

Click the B-Sperm icon located on the PC desktop to enter the system. Enter the **temporary (factory default) password: fertility** and then click OK.

NOTE: A new password is required when entering the system for the first time.

WARNING: Please remember the new password!

A screen will automatically appear. Enter and confirm a new password.

Test Data

Section 5: Test Data (See Section 10: Set-Up before testing)

WARNING: Set-Up system defaults before testing (See Set-up section of this user guide).

Click on **TEST DATA** to locate, select and analyze test results that have been imported from the SQA-Vb. After clicking **TEST DATA,** two sub-buttons will appear: **AI–Fresh & Frozen** (FRESH samples tested and dosed for artificial insemination and the FROZEN samples that have been tested/QC'ed) and **BSE** (FRESH samples run for Breeding Soundness Evaluation).

AI - Fresh & Frozen

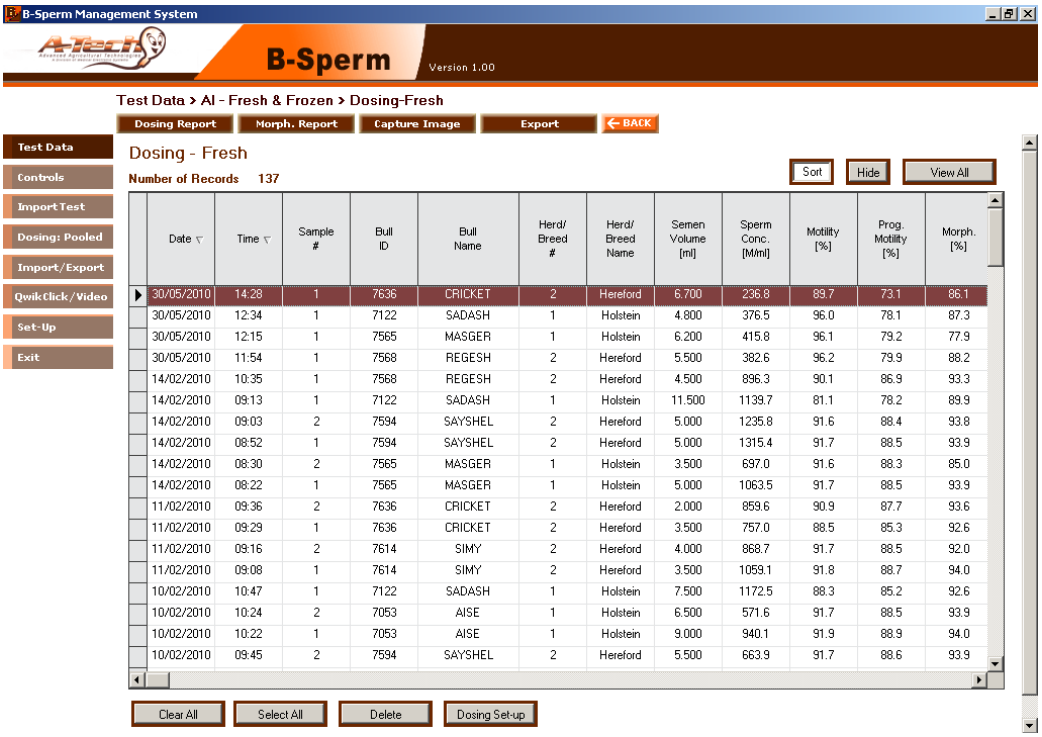
AI – Fresh & Frozen

Click on the **AI – Fresh & Frozen** navigation button and click on one of three options:

- **Dosing-Fresh:** A table containing all of the fresh semen samples in B-Sperm will be displayed. From this table, the dosing set-up screen can be accessed to calculate insemination doses (see **Dosing Set-up** instructions to follow).
- **QC-Frozen:** Displays a table of all the frozen sample tests.
- **Daily Report:** A table displaying all the FRESH tests and their associated FROZEN tests for a specific date can be viewed.
- **Bull Report:** View the table that contains all the FRESH tests and their associated FROZEN tests for a specific bull.

Dosing-Fresh GRID

Dosing-Fresh:



Click on the **Dosing-Fresh** button to view complete information about the Fresh samples imported from the SQA-Vb. The **Dosing–Fresh** table displays:

Sample/Bull/Herd/Breed Information

- Date – The date the sample was tested.
- Time – The time the sample was tested.
- Sample # - The Sample # that was designated by the user.
- Bull ID - The Bull ID that was designated by the user.
- Bull Name – The Bull Name entered into the database.
- Herd/Breed # - The Herd/Breed # designated by the user.
- Herd/Breed Name – The Herd/Breed Name entered into the database.
- Semen Volume – The sample (ejaculate) volume in ml.

Dosing-Fresh GRID Continued

Test Parameters

- Sperm Concentration [M/ml]
- Motility [%]
- Progressive Motility [%]
- MSC [M/ml] – motile sperm concentration.
- PMSC [M/ml] – progressively motile sperm concentration.
- Normal Morphology [%]
- Velocity [microns/second]
- # Sperm [Bil] – # of sperm cells in the entire semen ejaculate.
- # Motile Sperm [Bil] – # of motile sperm cells in the entire semen ejaculate.
- # Progressively Motile Sperm [Bil] – # of progressively motile sperm cells in the entire semen ejaculate.
- Avg. PMLF – Averaged Progressive Motility Loss Factor. This value is expressed as a percentage reflecting the LOSS of progressively motile cells in a bull's semen as a result of the freezing/thawing process. Please see below more extensive information about this parameter:

PMLF Progressive Motility Loss Factor

PMLF (Progressive Motility Loss Factor):

It is evident that preparing AI doses by the # progressively motile cells per AI straw makes biological sense due to the fact that moving cells impregnate cows. It is also true that progressive motility is lost during the freezing/thawing process AND this loss is unique to each bull. Therefore, to accurately prepare AI straws by the post thaw # progressively motile cells, the AI Stud must consider the PMLF (Progressive Motility Loss Factor) for each bull.

What is PMLF and Avg % PMLF?

- A new parameter that permits dosing fresh bull semen by a post-thaw progressively motile sperm target that is unique to each bull.
- PMLF is expressed as a percentage of Progressive Motility LOSS due to freezing-thawing process. Avg. % PMLF is calculated over time (set by the user) and includes an average of all PMLF values during the set timeframe.
- PMLF can be calculated only when both a FRESH sample and its associated FROZEN sample are tested. The # progressively motile cells contained in the FROZEN sample are compared with the associated FRESH sample that was used to create the AI batch. A percentage is established reflecting the loss of cells.
- The AVG PMLF TIMEFRAME is set in the Bull Set-up table for each individual bull and this timeframe will remain until it is changed by the operator.
- When AVG PMLF cannot be calculated because there is no linked FRESH/FROZEN test record in the database within the selected timeframe, the following message will be displayed in the Dosing Set-up screen:

  "This sample cannot be dosed based on progressively motile sperm #. Please manually enter AVG PMLF or change the AVG PMLF timeframe."
- The timeframe is from date of test backwards in time (week(s), moth(s), year(s)). FROZEN tests may not have been run on every FRESH sample. Today's test will not be included in today's AVG PMLF (it will be shown tomorrow).
- The manufacturer's default for AVG PMLF TIMEFRAME is 1 year (this includes ANY data from the last year).

Dosing-Fresh
GRID
Continued

- If there is no data available for calculating AVG PMLF and no manual input, the AVG PMLF will be reported as N.A.
- Manual PMLF input is not considered when establishing the Avg % PMLF.
- For reporting purposes in the INDIVIDUAL BULL REPORT and the DAILY REPORT the values for PMLF and AVG PMLF are calculated only once and are not dynamically updated each time the daily report is opened.

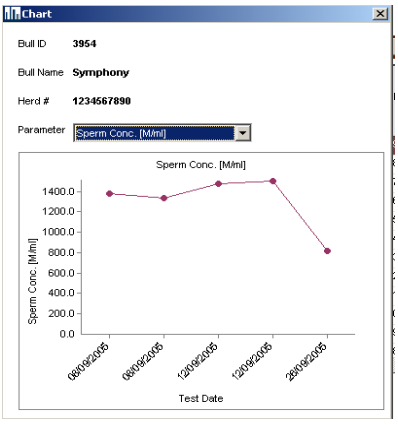
Dosing Information

The following dosing information is displayed on the DOSING FRESH GRID (some information is displayed only if previously set–up in the **Dosing Set-up**):

- **Dosing Protocol**: The predefined (in the set-up) protocol name that was used to define how to dose the sample.
- **Dosing Method**: The parameter selected for dosing: # Total Sperm, # Motile Sperm, or # Progressively Motile Sperm per AI dose.
- **Dose Volume (ml)**: The dose volume selected by the user.
- **Target # Sperm per Dose (M)**: The amount of total, motile or progressively motile sperm cells/dose desired by the user.
- **Sperm Conc. Cut-off**: The minimum acceptable Concentration (M/ml) threshold.
- **Motility Cut-off**: The minimum acceptable % Motility threshold.
- **Progressive Motility Cut-off**: The minimum acceptable % Progressive Motility.
- **Morphology Cut-off**: The minimum acceptable % Morphology threshold.
- **Avg. PMLF Cut-off**: The maximum acceptable average % PMLF threshold.
- **Extender Name/Type**: The name or type of extender used for dosing.
- **Total # Doses**: The total # of doses that will be made from the extended sample.
- **Extender Volume (ml) Step 1/Step 2/Step 3**: The volume of freezing extender to add to the sample for each dilution step.
- **Total Volume (ml)**: The sum of the semen plus the extender volume.
- **Sperm Conc., Motility, Prog. Motility, Morphology, Avg. PMLF, Sample**: Pass/Fail vs. the Cut-offs.
- **Reason for Failure**: The reason for failing the sample - selected from a pre-defined list.

Informational Icons

Graphs: Click on this icon, then select the parameter from the drop down menu to graph (graphs can be generated only if a bull has more than one test in the B-Sperm database).



Other icons on the table indicate:

- Test results out of normal range
- Dosing errors (mismatch)
- Clips/videos attached to the record

Unit #: The serial number of the SQA-Vb used for testing.

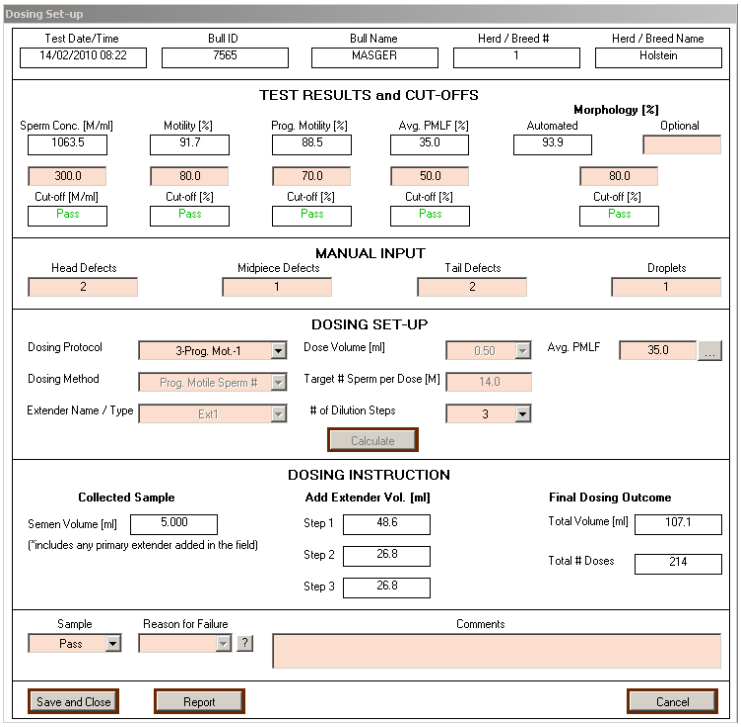
The following buttons appear below the Dosing-Fresh Grid:

- **Clear All:** Clears ALL of the selection criteria.
- **Select All:** Marks ALL of the records.
- **Delete:** Deletes only the marked records (drag the arrow to select more than one record or use Select All to delete everything).
- **Dosing Set-up:** See below…

NOTE: The DOSING SET-UP screen automatically appears when test results are imported on-line from the SQA-Vb immediately after testing an AI FRESH sample.

Dosing Set-Up: To dose samples:

- Click in the first column to select the desired record from the Dosing-Fresh grid.
- Click the **Dosing Set-up** button to display the calculation screen below.



- Enter the dosing settings as follows:
  - **Dosing Protocol** – Select a customized protocol from the drop-down menu. These are pre-defined in the SET-UP of P-Sperm. If a protocol is used other fields may be disabled.
  - **Dosing method** – Select options from the drop-down menu: # Total Sperm, # Motile Sperm or # Progressively Motile Sperm.
  - **Extender Name/Type** – Select from a drop-down menu or type the name/type of extender used for dosing.
  - **Dose Volume (ml)** – Select: 0.25, 0.5 or "other" from the drop-down menu.
  - **Target # Sperm per Dose (M)** – Enter the target # Sperm (Total/Motile/Prog. Motile) per dose.
  - **# of Dilution Steps –** Select 1, 2 or 3 step dilution process.
  - **Avg. PMLF** – Enter this parameter manually if it is not computed automatically or change AVG PMLF TIMEFRAME.

- Click the **CALCULATE** button to display the dosing instructions and parameters. B-Sperm will keep these settings for the next dosing calculation.
- Click **SAVE AND CLOSE** to save the information.
- Click **CANCEL** to delete the calculations for this sample (and start over).
- Click **REPORT** to display and print a report of the dosing instructions.

Dosing Instruction Report

Sample Data			
Bull ID	7565	**Herd / Breed**	
Bull Name	MASGER	#	1
Test Date/Time	14/02/2010 08:22	Name	Holstein
Test Results		**Cut-off**	**Sample Status**
Sperm Conc. [M/ml]	1063.5	300.0	Pass
Motility [%]	91.7	80.0	Pass
Prog. Motility [%]	88.5	70.0	Pass
Morph. [%]	93.9	80.0	Pass
Avg. PMLF [%]	35.0	50.0	Pass
		Sample	Pass

Comments	Dosing Set-up		
	1	Dosing Protocol	3-Prog. Mot.-1
	2	Dosing Method	# Prog. Motile
	3	Target # Sperm per Dose [M]	14.0
	4	Extender Name/Type	Ext1
	5	# of Dilution Steps	3
		Dosing Instructions:	
	6	Extender Volume, Step 1 [ml]	48.6
	7	Extender Volume, Step 2 [ml]	26.8
	8	Extender Volume, Step 3 [ml]	26.8
	9	Semen Volume [ml]	5.000
	10	Total Volume [ml]	107.1
	11	Dose Volume [ml]	0.50
	12	Total # Doses	214

Customize the display of information in the Dosing-Fresh table using the buttons in the upper right hand corner of the table:

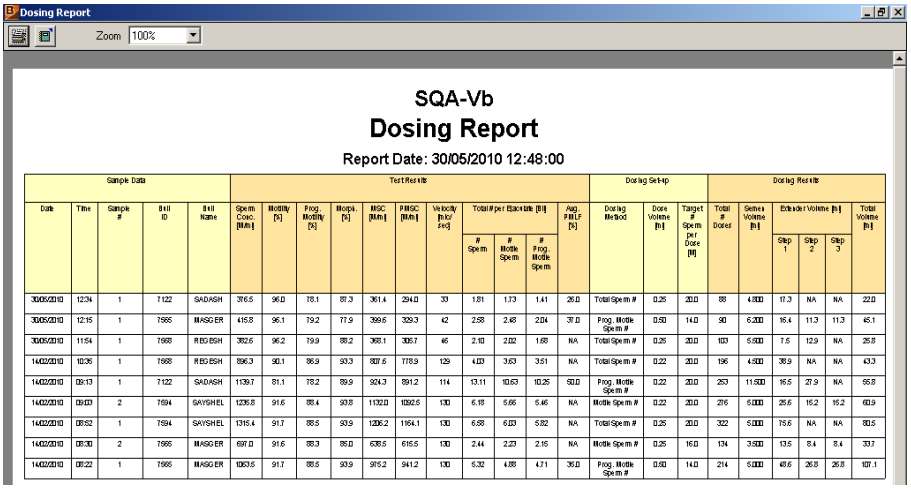
- **SORT:** Click this button and then click on the header column to sort the data.
- **HIDE**: Select the columns to hide and click the **Hide** button.
- **VIEW ALL:** Click this button to display all of the columns again.

Four additional sub-menu buttons are displayed at the top of the **Dosing-Fresh** table: **Dosing Report, Morph. Report, Capture Image** and **Export**.

Dosing Report: Select the record(s) in the Dosing-Fresh grid and press the Dosing Report button to view, print and export the Dosing Report:

Click on this icon to print a copy of the report.

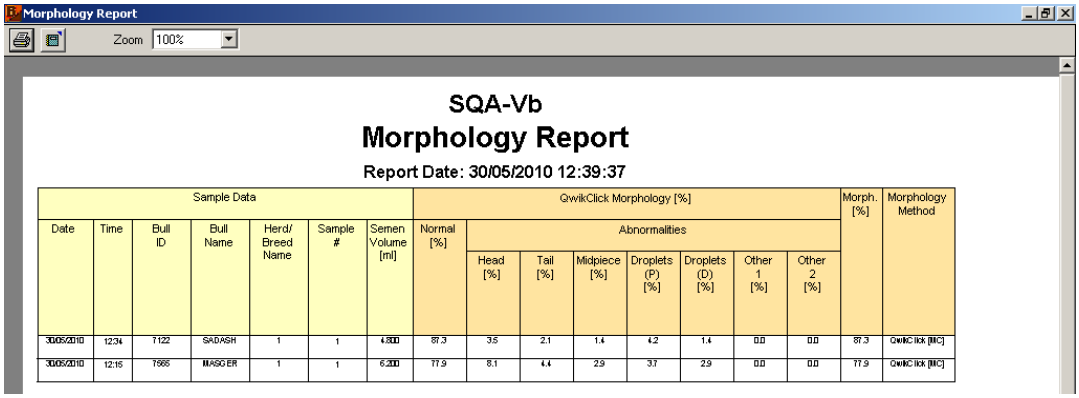
Click on this icon to export the semen analysis report to Excel file.



- Use the page bar at the bottom of the report to move between pages.
- Click the printer icon to print the report.
- Click on the X in the upper right hand corner of the screen to exit.
- Click the EXPORT icon to send the report to an external file.

Morph. Report

Morphology Report: Select the record(s) in the Dosing-Fresh grid and press the Morph. Report button to display the report below:

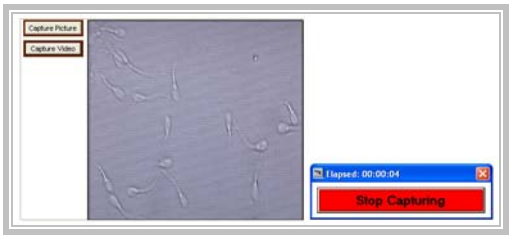


Morphology differential abnormalities (head, tail defects, etc.) will be reported only for samples run using the QwikClick Morphology Counter.

- Use the page bar at the bottom of the report to move between pages.
- Click the printer icon to print the report.
- Use the ZOOM to minimize/maximize the report view.
- Click on the X in the upper right hand corner of the screen to exit.
- Click the EXPORT icon to send the report to an external file.

Capture Image

Capture Image: Click this button to activate the "real time" video:



NOTE: If no record is selected the video or picture will be attached to the latest record imported into B-Sperm.

To save a video clip or picture and attach it to a record:

- Import the test records from the SQA-Vb to B-Sperm.
- Go to: **TEST DATA> AI-FRESH & FROZEN > DOSING-FRESH**
- Insert a prepared slide into the visualization system of the SQA-Vb.
- Highlight the desired record in B-Sperm to attach a video/picture.
- Click: **CAPTURE IMAGE** to activate the video screen in B-Sperm
- Click: **CAPTURE PICTURE** to attach a picture to the selected record
- Click: **CAPTURE VIDEO** to attach a video clip to the selected record; Click Stop Capturing to end the video capture process.
- A camera/video icon will now appear with this record in the table
- To **delete** a picture or video, click on the icon and click "DELETE".

Export

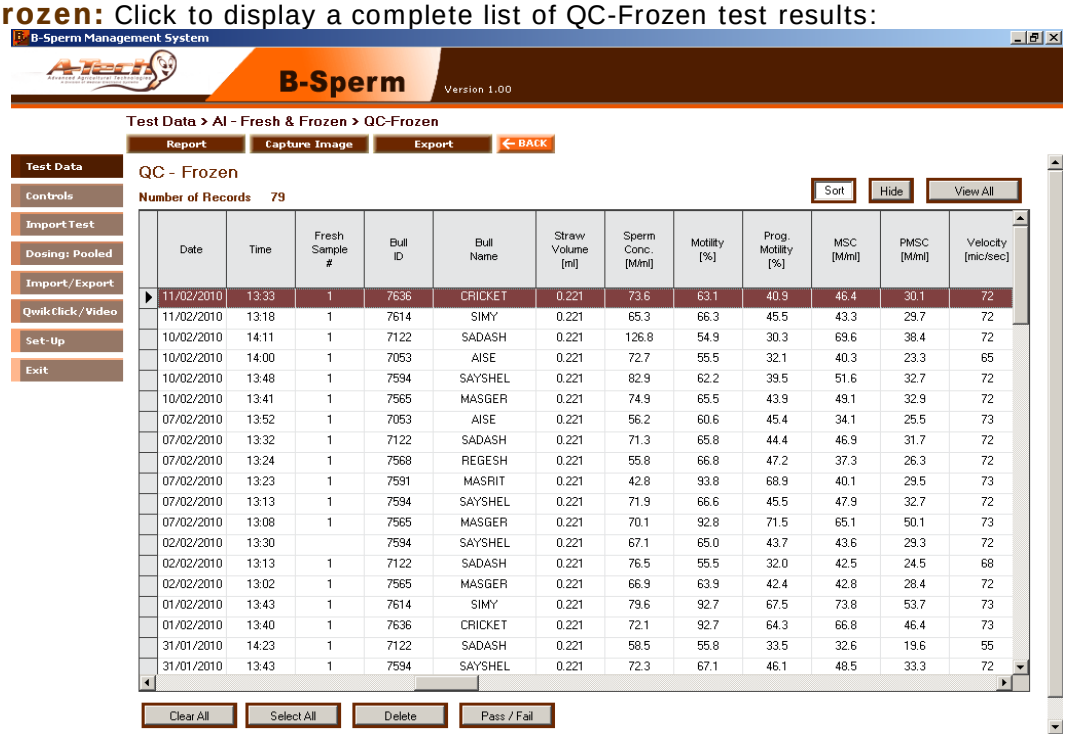
Export: Click to export selected records to an external file in Excel format. To export the entire archive to an external Excel file, use the IMPORT/EXPORT main navigation button.

AI - Fresh & Frozen

QC-Frozen

AI – FRESH AND FROZEN

QC-Frozen: Click to display a complete list of QC-Frozen test results:



Date	Time	Fresh Sample #	Bull ID	Bull Name	Straw Volume [ml]	Sperm Conc. [M/ml]	Motility [%]	Prog. Motility [%]	MSC [M/ml]	PMSC [M/ml]	Velocity [mic/sec]
11/02/2010	13:33	1	7636	CRICKET	0.221	73.6	63.1	40.9	46.4	30.1	72
11/02/2010	13:18	1	7614	SIMY	0.221	65.3	66.3	45.5	43.3	29.7	72
10/02/2010	14:11	1	7122	SADASH	0.221	126.8	54.9	30.3	69.6	38.4	72
10/02/2010	14:00	1	7053	AISE	0.221	72.7	55.5	32.1	40.3	23.3	65
10/02/2010	13:48	1	7594	SAYSHEL	0.221	82.9	62.2	39.5	51.6	32.7	72
10/02/2010	13:41	1	7565	MASGER	0.221	74.9	65.5	43.9	49.1	32.9	72
07/02/2010	13:52	1	7053	AISE	0.221	56.2	60.6	45.4	34.1	25.5	73
07/02/2010	13:32	1	7122	SADASH	0.221	71.3	65.8	44.4	46.9	31.7	72
07/02/2010	13:24	1	7568	REGESH	0.221	55.8	66.8	47.2	37.3	26.3	72
07/02/2010	13:23	1	7591	MASRIT	0.221	42.8	93.8	68.9	40.1	29.5	73
07/02/2010	13:13	1	7594	SAYSHEL	0.221	71.9	66.6	45.5	47.9	32.7	72
07/02/2010	13:08	1	7565	MASGER	0.221	70.1	92.8	71.5	65.1	50.1	73
02/02/2010	13:30		7594	SAYSHEL	0.221	67.1	65.0	43.7	43.6	29.3	72
02/02/2010	13:13	1	7122	SADASH	0.221	76.5	55.5	32.0	42.5	24.5	68
02/02/2010	13:02	1	7565	MASGER	0.221	66.9	63.9	42.4	42.8	28.4	72
01/02/2010	13:43	1	7614	SIMY	0.221	79.6	92.7	67.5	73.8	53.7	73
01/02/2010	13:40	1	7636	CRICKET	0.221	72.1	92.7	64.3	66.8	46.4	73
31/01/2010	14:23	1	7122	SADASH	0.221	58.5	55.8	33.5	32.6	19.6	55
31/01/2010	13:43	1	7594	SAYSHEL	0.221	72.3	67.1	46.1	48.5	33.3	72

The QC-Frozen table displays the following information:

Sample/Bull/Herd/Breed Information

- Date/Time - The date/time the sample was tested.
- Fresh Sample # - The sample # from which the AI Dose was prepared.
- Bull ID - The Bull ID that was designated by the user.
- Bull Name - The Bull Name (see Set-Up > BULL/HERD).
- Herd/Breed # - The Herd/Breed # (see Set-up > BULL/HERD).
- Herd/Breed Name - The Herd/Breed Name (see Set-up > BULL/HERD).
- Straw Date - The date the AI Dose was prepared.
- Freezing Media - The freezing media used for the AI dose.
- Straw Volume - The AI Dose volume in ml.

Test Parameters

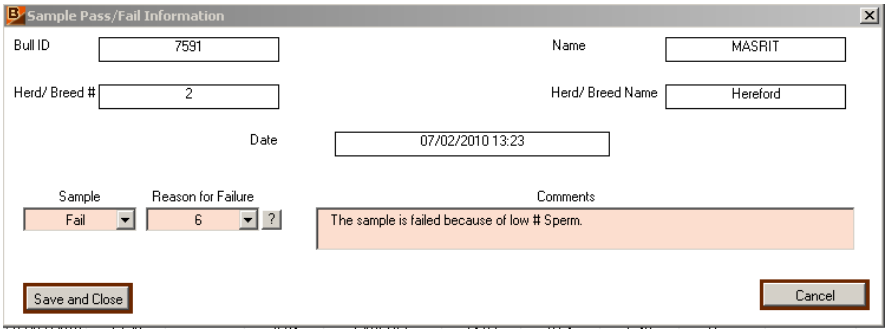
- Sperm Concentration [M/ml], Motility [%], Progressive Motility [%]
- MSC [M/ml] – motile sperm concentration, PMSC [M/ml] – progressively motile sperm concentration, Velocity [microns/second]
- # Sperm [M] – total # sperm cells/AI dose
- # Motile Sperm [M] – # motile sperm cells/AI dose
- # Progressively Motile Sperm [M] – # progressively motile cells/AI dose

Pass/Fail: Manually Pass/Fail the QC-Frozen results.

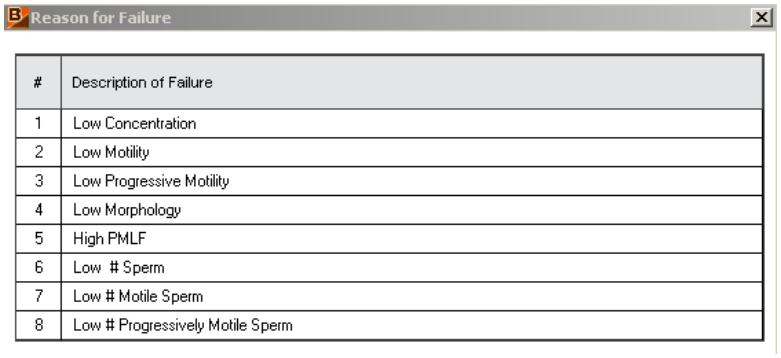
Reason for Failure: The reason for failing the sample can be selected from a pre-defined drop-down menu.

The following buttons appear below the QC-Frozen table:

- **Clear All:** Clears ALL of the selection criteria.
- **Select All:** Marks ALL of the records.
- **Delete:** Deletes only the marked records (drag the arrow to select more than one record or use Select All to delete everything).
- **Pass/Fail:** Clicking this button to open up the Sample Pass/Fail Information window in order to pass or fail the sample based on the QC results:



Click the ? button to view the Reason for Failure table containing failure codes set-up by the administrator:



#	Description of Failure
1	Low Concentration
2	Low Motility
3	Low Progressive Motility
4	Low Morphology
5	High PMLF
6	Low # Sperm
7	Low # Motile Sperm
8	Low # Progressively Motile Sperm

Customize the display of information in the QC-Frozen table using the buttons in the upper right hand corner of the table:

- **SORT:** Click this button and then click on the header column to sort the data.
- **HIDE**: Select the columns to hide and click the **Hide** button.
- **VIEW ALL:** Click this button to display all of the columns again.

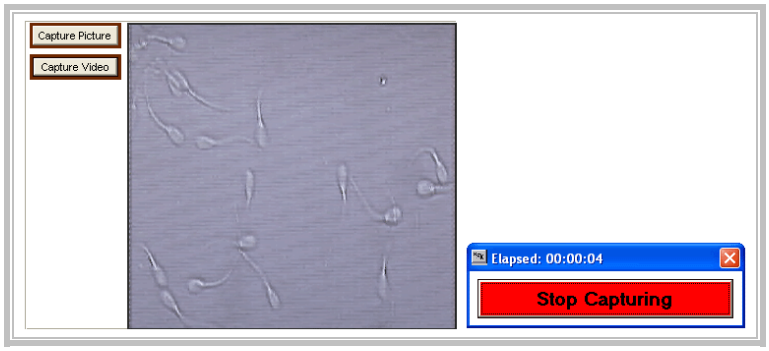
Three additional sub-menu buttons are displayed at the top of the **QC-Frozen** table: **Report, Capture Image** and **Export**.

Report: Click on this button to view, print and export the QC-Frozen semen analysis report.

Capture Image

NOTE: If no record is selected the video or picture will be attached to the latest record imported into B-Sperm.

Capture Image: Click this button to activate the "real time" video:



To save a video clip or picture and attach it to a record:

- Import the test records from the SQA-Vb to B-Sperm.
- Go to: **TEST DATA > AI-FRESH & FROZEN > QC-FROZEN**
- Insert a prepared slide into the visualization system of the SQA-Vb.
- Highlight the desired record in B-Sperm to attach a video/picture.
- Click: **CAPTURE IMAGE** to activate the video screen in B-Sperm.
- Click: **CAPTURE PICTURE** to attach a picture to the selected record.
- Click: **CAPTURE VIDEO** to attach a video clip to the selected record; Click **STOP CAPTURING** to end the video capture process.
- A camera/video icon will now appear with this record in the table.
- Click **DELETE** (lower left corner of the viewing screen) to remove a picture/video.

Export

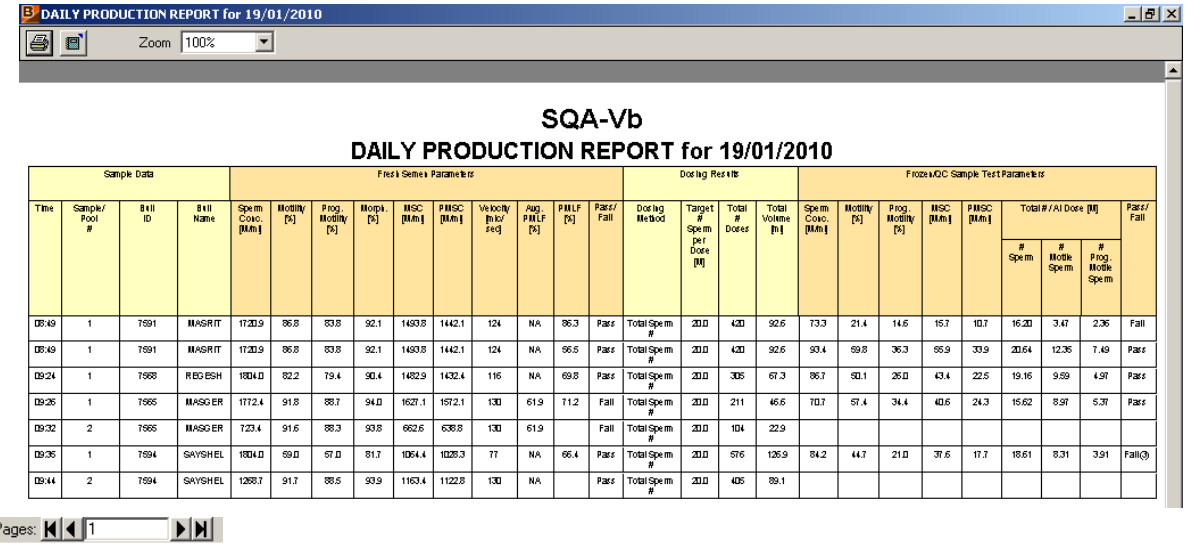
Export: Click to export selected records to an external file in Excel format. Exporting the entire database archive to an external Excel file is available from the IMPORT/EXPORT main navigation menu.

AI – FRESH AND FROZEN

Daily Report

Daily Report: Click on **DAILY REPORT** to run a report of all the FRESH samples and FROZEN tests associated with them for a given day.

- Select the desired date(s) and click **ENTER** to display the report below:



- Use the page bar at the bottom of the report to move between pages.

Click on this icon to print a copy of the report

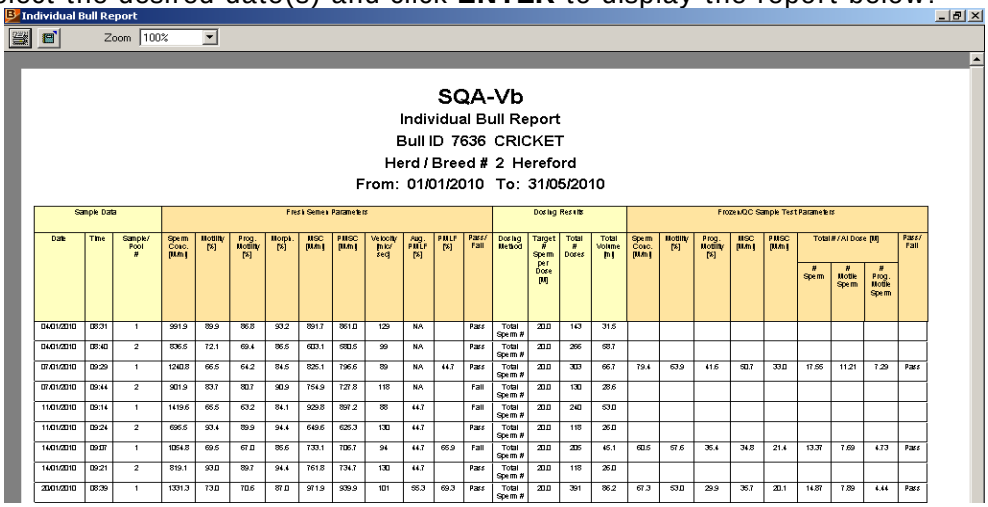
Click on this icon to export the semen analysis report

- Click the printer icon to print the report.
- Use the ZOOM option to minimize/maximize the report view.
- Click the **X** in the upper right hand corner to exit the screen.
- Click the EXPORT button to send the report to an external Excel file.

Bull Report

Individual Bull Report: Click to run a report of all the FRESH samples and the related FROZEN tests for a given bull.

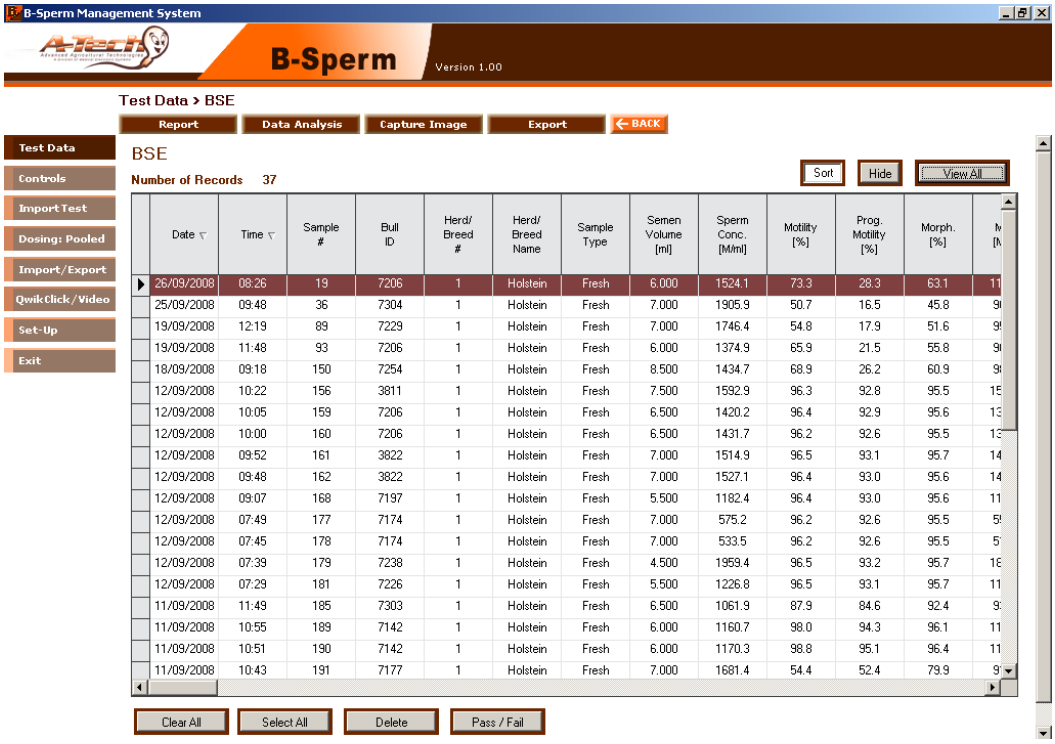
- Select the desired date(s) and click **ENTER** to display the report below:



BSE

BSE: Breeding Soundness Evaluation

Go to: **TEST DATA > BSE** to view the table below. The BSE table displays sample, bull, herd/breed information and test results. From this table, histograms can be run as well.



Date	Time	Sample #	Bull ID	Herd/ Breed #	Herd/ Breed Name	Sample Type	Semen Volume [ml]	Sperm Conc. [M/ml]	Motility [%]	Prog. Motility [%]	Morph. [%]
26/09/2008	08:26	19	7206	1	Holstein	Fresh	6.000	1524.1	73.3	28.3	63.1
25/09/2008	09:48	36	7304	1	Holstein	Fresh	7.000	1905.9	50.7	16.5	45.8
19/09/2008	12:19	89	7229	1	Holstein	Fresh	7.000	1746.4	54.8	17.9	51.6
19/09/2008	11:48	93	7206	1	Holstein	Fresh	6.000	1374.9	65.9	21.5	55.8
18/09/2008	09:18	150	7254	1	Holstein	Fresh	8.500	1434.7	68.9	26.2	60.9
12/09/2008	10:22	156	3811	1	Holstein	Fresh	7.500	1592.9	96.3	92.8	95.5
12/09/2008	10:05	159	7206	1	Holstein	Fresh	6.500	1420.2	96.4	92.9	95.6
12/09/2008	10:00	160	7206	1	Holstein	Fresh	6.500	1431.7	96.2	92.6	95.5
12/09/2008	09:52	161	3822	1	Holstein	Fresh	7.000	1514.9	96.5	93.1	95.7
12/09/2008	09:48	162	3822	1	Holstein	Fresh	7.000	1527.1	96.4	93.0	95.6
12/09/2008	09:07	168	7197	1	Holstein	Fresh	5.500	1182.4	96.4	93.0	95.6
12/09/2008	07:49	177	7174	1	Holstein	Fresh	7.000	575.2	96.2	92.6	95.5
12/09/2008	07:45	178	7174	1	Holstein	Fresh	7.000	533.5	96.2	92.6	95.5
12/09/2008	07:39	179	7238	1	Holstein	Fresh	4.500	1959.4	96.5	93.2	95.7
12/09/2008	07:29	181	7226	1	Holstein	Fresh	5.500	1226.8	96.5	93.1	95.7
11/09/2008	11:49	185	7303	1	Holstein	Fresh	6.500	1061.9	87.9	84.6	92.4
11/09/2008	10:55	189	7142	1	Holstein	Fresh	6.000	1160.7	98.0	94.3	96.1
11/09/2008	10:51	190	7142	1	Holstein	Fresh	6.000	1170.3	98.8	95.1	96.4
11/09/2008	10:43	191	7177	1	Holstein	Fresh	7.000	1681.4	54.4	52.4	79.9

The following information can be viewed from the BSE table:

Sample/Bull/Herd/Breed Information

- Date/Time – The date/time the sample was tested.
- Sample # - The Sample # that was designated by the user.
- Bull ID - The Bull ID that was designated by the user.
- Herd/Breed # - The Herd/Breed # designated by the user.
- Herd/Breed Name – The Herd/Breed Name.
- Sample Type – Will always be FRESH in the BSE mode.
- Semen Volume – The ejaculate volume in ml.

Test Parameters

- Sperm Concentration [M/ml], Motility [%], Progressive motility [%], Normal Morphology [%], MSC [M/ml], Velocity [microns/second], PMSC [M/ml] – progressively motile sperm concentration.
- # Sperm [Bil] – total # sperm cells in the entire semen volume (ejaculate).
- # Motile Sperm [Bil] – # motile sperm cells in the entire semen volume (ejaculate).
- # Progressively Motile Sperm [Bil] – # progressively motile sperm cells/volume (ejaculate).

Pass/Fail: Manual Pass/Fail of the test results.

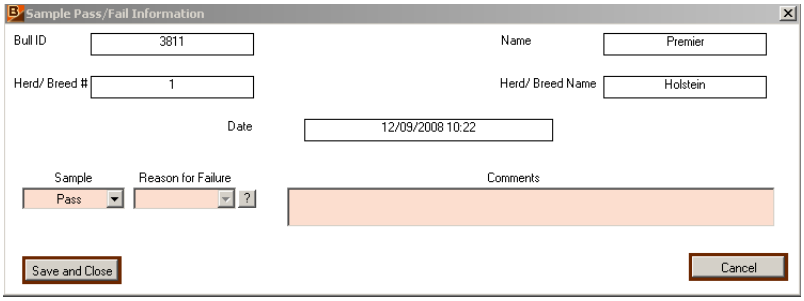
Reason for Failure: The reason of the sample failure (select from a drop-down menu).

Informational Icons: (See Section 3: System Navigation Overview).

Unit #: The serial number of the SQA-Vb used for testing.

The following buttons appear below the BSE table:

- **Clear All:** Clears ALL of the selection criteria.
- **Select All:** Marks ALL of the records.
- **Delete:** Deletes only the marked records (drag the arrow to select more than one record or use Select All to delete everything).
- **Pass/Fail:** Click this button to open the Sample Pass/Fail Information window to pass or fail a sample based on the test results:



Select the reason for failure from a drop-down menu. Click the ? button to view the Reason for Failure table with the failure codes pre-set by the administrator:

Reason for Failure

#	Description of Failure
1	Low Concentration
2	Low Motility
3	Low Progressive Motility
4	Low Morphology
5	High PMLF
6	Low # Sperm
7	Low # Motile Sperm
8	Low # Progressively Motile Sperm

Customize the display of information in the BSE table using the buttons in the upper right hand corner of the table:

- **SORT:** Click this button and then click on the header column to sort – all the data will be sorted based on this selection.
- **HIDE**: Select the columns to hide and click the **Hide** button.
- **VIEW ALL:** Click this button to display all of the columns again.

Four additional sub-buttons are displayed at the top of the **BSE** table:

Report

Report: Click to view, print and export a BSE Report for FRESH sample records selected in the BSE grid:

BSE Report

Zoom 100%

SQA-Vb

BSE Report

Report Date: 02/06/2010 12:16:05

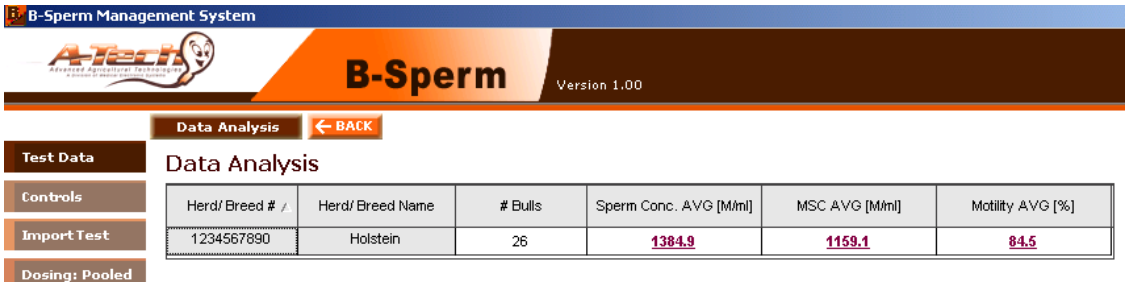
Sample Data								Test Results									Out of Range <>	Pass/ Fail
Date	Time	Sample #	Bull ID	Herd/ Breed #	Herd/ Breed Name	Sample Type	Semen Volume [ml]	Sperm Conc. [M/ml]	Motility [%]	Prog. Motility [%]	Morph. [%]	MSC [M/ml]	PMSC [M/ml]	Velocity [mic/ sec]	Total # per Ejaculate [Bil]				
															# Sperm	# Motile Sperm	# Prog. Motile Sperm		
11/09/2008	11:49	185	7303	1	Holstein	Fresh	6.500	1061.9	87.9	84.6	92.4	933.6	898.5	42	6.90	6.07	5.84		Pass
11/09/2008	10:55	189	7142	1	Holstein	Fresh	6.000	1160.7	98.0	94.3	96.1	1136.9	1094.2	41	6.96	6.82	6.57		Pass
11/09/2008	10:51	190	7142	1	Holstein	Fresh	6.000	1170.3	98.8	95.1	96.4	1156.2	1112.4	40	7.02	6.94	6.67		Pass
11/09/2008	10:43	191	7177	1	Holstein	Fresh	7.000	1681.4	54.4	52.4	79.9	915.5	881.2	43	11.77	6.41	6.17	< >	Fail(2)
11/09/2008	10:22	195	7262	1	Holstein	Fresh	5.000	1333.7	96.3	92.8	95.5	1284.7	1237.5	46	6.67	6.42	6.19		Pass
11/09/2008	10:18	196	7262	1	Holstein	Fresh	5.000	1323.0	96.4	92.9	95.6	1274.8	1228.4	48	6.62	6.37	6.14		Pass
11/09/2008	10:06	198	7223	1	Holstein	Fresh	7.000	1236.9	99.1	95.4	96.6	1225.6	1180.2	44	8.66	8.58	8.26		Pass

Data Analysis

Herd Data Analysis:

To run histograms on Herds:

- Mark all desired records or click **Select All** button.
- Click the **Data Analysis** button to display the table below:

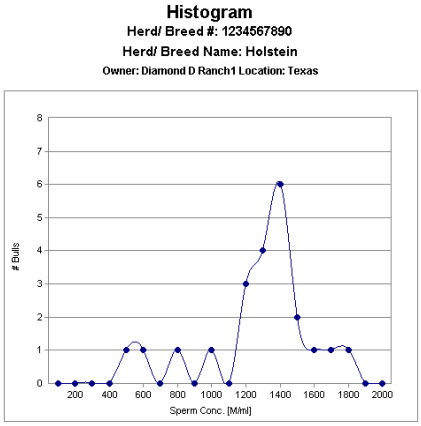


This table averages the parameters below for each herd based on the selected tests.

- Sperm Concentration average [M/ml]
- MSC average [M/ml]
- Motility average [%]

To run the Histogram:

- Click on a RED value from the Data Analysis table to select the parameter to run.
- The report below will be run.



Sperm Conc. [M/ml]	# Bulls
0-100	0
100-200	0
200-300	0
300-400	0
400-500	1
500-600	1
600-700	0
700-800	1
800-900	0
900-1000	1
1000-1100	0
1100-1200	3
1200-1300	4
1300-1400	6
1400-1500	2
1500-1600	1
1600-1700	1
1700-1800	1
1800-1900	0
1900-2000	0
>2000	4
Total	**26**

- To **EXIT**, click the **X** in the upper right hand corner of the screen.

Capture Image and Export: See Section 4: Test Data>AI-Fresh & Frozen>Dosing-Fresh for details.

Controls

Section 6: Controls

The SQA-Vb runs QwikCheck™beads controls to verify the concentration channel and to indicate a potential problem (obstruction of the concentration channel with dirt or debris) BEFORE test results are impacted.

Control defaults need to be set-up in B-Sperm and transferred to the SQA-Vb before running control tests on the SQA-Vb the first time with each new box of beads. Once tests are run on the SQA-Vb they need to be imported into B-Sperm where reports can be run.

To run QwikCheck™beads controls:

Click on the **Controls** tab and two buttons will appear: **Set-Up** and **Test Results**.

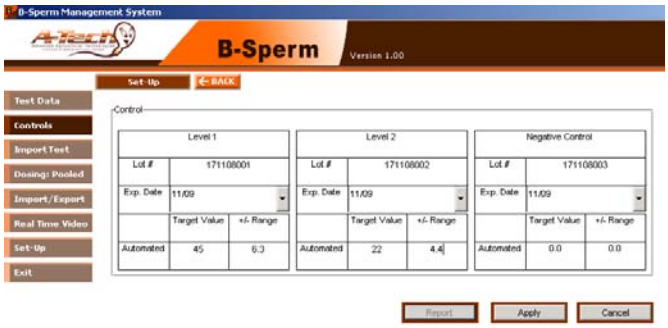
Set-Up

NOTE: Remember to set-up Controls before running QwikCheck™ Beads.

NOTE: The **Set-up** data transfer may take several minutes! Please wait…..

Set-Up Controls:

- Turn on both the SQA-Vb and B-Sperm.
- From the SQA-Vb go to: **MAIN MENU > SERVICE > SERVICE DATA**
- To display the screen below go to: **Controls > Set-Up**

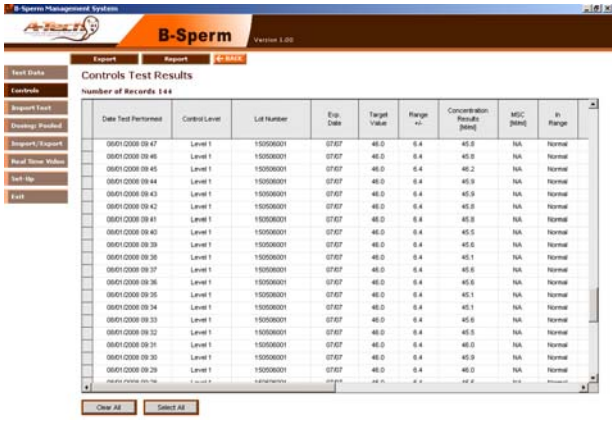


- From the set-up screen enter default settings for all **THREE** levels of beads:
  Lot Number, Expiration date and Target Value and +/- Range
- Press **Apply** to accept and the settings will be transferred to the SQA-Vb.

Test Results

Test Results:

Click the navigation button **Controls > Test Results** to display the test screen below:

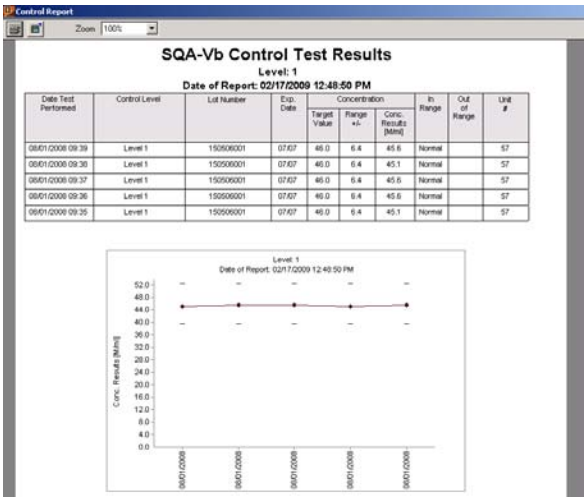


Report

Report:

Select individual records or click and drag the mouse over a series of tests and click the **REPORT** button.

NOTE: The Controls report needs to be run for each level seperately.



Import Test

Section 7: Import Test

To automatically import test results into B-Sperm after performing a test in the SQA-Vb, go to the SQA-Vb:

- **MAIN MENU > SERVICE > SETTINGS > SAMPLE DEFAULT SETTINGS**
- Select: **Automatically send to PC?** **YES** (this is the factory default setting).
- Select: **QwikClick Counter?** **YES/NO** (the factory default is NO).

If "QwikClick Counter? NO" is selected, after running a FRESH sample for DOSING in the SQA-Vb the following message will appear:

FOR DOSING CALCULATION
PRESS: "IMPORT TEST" BUTTON
IN B-SPERM

If "QwikClick Counter? NO" is selected, after running a FRESH BSE or FROZEN test in the SQA-Vb the following message will appear:

TO TRANSFER TEST RESULTS TO
B-SPERM
PRESS: "IMPORT TEST" BUTTON
IN B-SPERM

If "QwikClick Counter? YES" is selected, after running a test in the SQA-Vb the following message will appear:

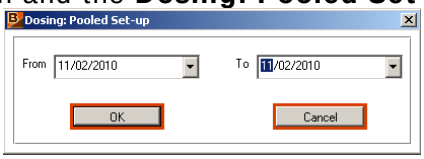
PRESS: "IMPORT TEST" BUTTON
IN B-SPERM
TO ACTIVATE QWIKCLICK
COUNTER
PREPARE A SLIDE FOR
QWIKCLICK TESTING

- Click on the **IMPORT TEST** navigation button in B-Sperm and the test results will be transferred immediately.
- When a Fresh sample is tested for Dosing, results will be transferred and the Dosing Set-up screen will automatically appear in B-Sperm if "QwikClick Counter? NO" is selected. Alternatively ("QwikClick Counter? YES" is set in the SQA-Vb) the QwikClick Morphology Counter or Droplet Assessment screen will be opened after importing the test results (For more detailed information about QwikClick feature refer to the QwikClick Counter section).

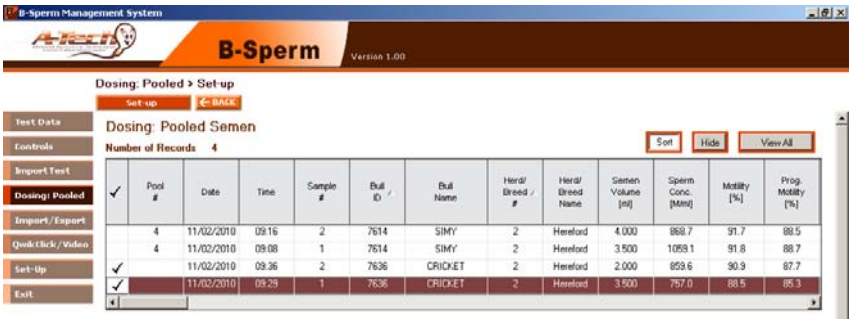
Dosing: Pooled

Section 8: Dosing: Pooled

- To dose two FRESH pooled bull semen samples, click on: **Dosing: Pooled**
- Two buttons will appear: **Set-Up** and **Report**.
- Click the **Set-up** button and the **Dosing: Pooled Set-up** window will appear:

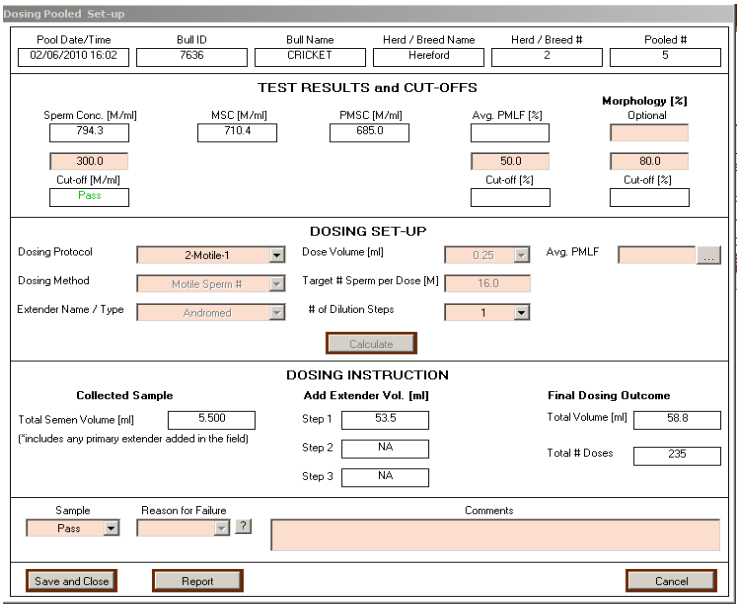


- Select the date(s) the duplicate ejaculates were collected and tested and press **OK**.
- The **Dosing: Pooled Semen** grid below will display all the duplicate FRESH test records for the bull ejaculates that were tested during the designated date.



Dosing Set-up

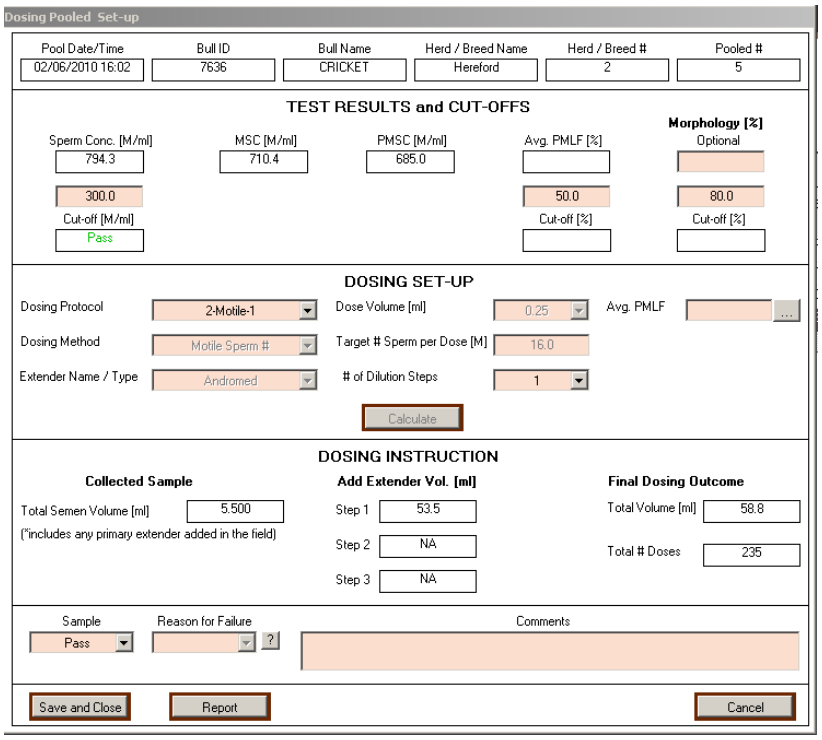
- Select the two records (samples) of the same bull to be pooled/dosed by clicking in the column marked with a √.
- Click on: **Dosing Set-up** (lower left hand corner) and the **Dosing Pooled Set-up** window displayed below will appear.



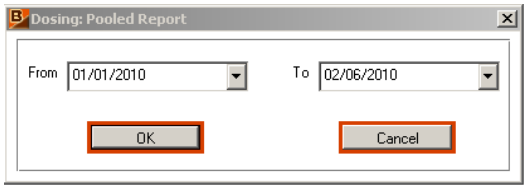
- The B-Sperm will assign a unique system generated **Pool #.**
- Automated Test Results will be calculated for the pooled sample.
- Enter the required information in the **Dosing Set-up** section:

- **Dosing Protocol**: Select from a drop-down menu. The Dosing Protocols can be pre-set in: Set-up > System Settings > Administrator > Dosing Protocol. If a pre-set protocol is used, some fields below will be frozen.
- **Dosing Method** – # Total, # Motile or # Progressively Motile Sperm.
- **Extender Name/Type** – Select from a drop-down menu or type the name/type of extender used for dosing.
- **Dose Volume (ml)** – Select from drop-down menu: 0.25, 0.5 or other.
- **Target # Sperm per Dose (M)** – Target # Sperm desired for each dose.
- **# of Dilution Steps** – Select a 1, 2 or 3 dilution step process.
- **Avg. PMLF** – Enter this parameter manually if it is not computed automatically or change AVG PMLF TIMEFRAME if the dosing is to be processed by # of Progressively Motile Sperm.

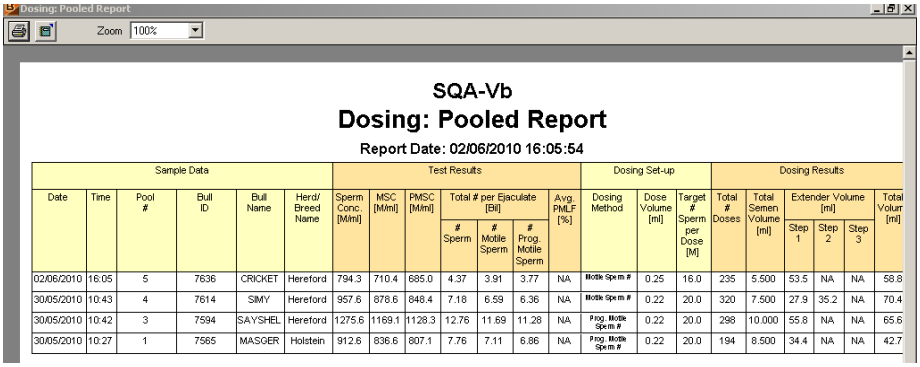
- Click: **CALCULATE** to display the dosing results. B-Sperm saves these settings until new dosing information is entered.



- Click **Save and Close** button to save the Pooled Dosing results or **Cancel** to quit. Click **Report** button for displaying the printable **Pooled Dosing Instruction Report**.
- Click Dosing Pooled>Report button and the **Dosing: Pooled Report** window will appear:



- Select the desired date(s) and press **OK** to view the report below:


Dosing: Pooled Report

Zoom 100%

SQA-Vb

Dosing: Pooled Report

Report Date: 02/06/2010 16:05:54

Sample Data						Test Results							Dosing Set-up			Dosing Results					
Date	Time	Pool #	Bull ID	Bull Name	Herd/ Breed Name	Sperm Conc. [M/ml]	MSC [M/ml]	PMSC [M/ml]	Total # per Ejaculate [Bil]			Avg. PMLF [%]	Dosing Method	Dose Volume [ml]	Target # Sperm per Dose [M]	Total # Doses	Total Semen Volume [ml]	Extender Volume [ml]			Total Volur [ml]
									# Sperm	# Motile Sperm	# Prog. Motile Sperm							Step 1	Step 2	Step 3	
02/06/2010	16:05	5	7636	CRICKET	Hereford	794.3	710.4	685.0	4.37	3.91	3.77	NA	Motile Sperm #	0.25	16.0	235	5.500	53.5	NA	NA	58.8
30/05/2010	10:43	4	7614	SIMY	Hereford	957.6	878.6	848.4	7.18	6.59	6.36	NA	Motile Sperm #	0.22	20.0	320	7.500	27.9	35.2	NA	70.4
30/05/2010	10:42	3	7594	SAYSHEL	Hereford	1275.6	1169.1	1128.3	12.76	11.69	11.28	NA	Prog. Motile Sperm #	0.22	20.0	298	10.000	55.8	NA	NA	65.6
30/05/2010	10:27	1	7565	MASGER	Holstein	912.6	836.6	807.1	7.76	7.11	6.86	NA	Prog. Motile Sperm #	0.22	20.0	194	8.500	34.4	NA	NA	42.7

Import/Export

Section 9: Import / Export

Click: **Import/Export** to import SQA-Vb test results into B-Sperm or export test results to another file from B-Sperm.

Import

Import: Click on this button to bring data into B-Sperm from the SQA-Vb. To successfully **Import** data:

- The SQA-Vb and B-Sperm must running and the SQA-Vb SERVICE DATA screen must be displayed.
- From the SQA-Vb go to: **MAIN MENU > SERVICE > SERVICE DATA**
- From B-Sperm click: **Import/Export>Import > Import test results > Continue**

NOTE: The SQA-Vb checks for duplicate records and will not overwrite records already imported.

NOTE: Records can be deleted from the SQA-Vb archive after successfully importing to B-Sperm!

NOTE: The SQA-Vb will display a warning message when it is full of tests. Records MUST be imported to B-Sperm and deleted from the SQA-Vb archive when this warning message is displayed in the SQA-Vb!

- The series of screens above will be displayed to confirm that the test records were successfully imported into B-Sperm.
- Select YES to delete the test records from the SQA-Vb archive that have been transferred to B-Sperm. Select **NO** and the test data will remain in both the SQA-Vb and B-Sperm.
- All test data (Dosing-Fresh, QC-Frozen and BSE) is imported at the same time, however, CONTROL test results are imported separately.

Export

NOTE: Set the page print default to "landscape" when priting exported tables in Excel.

Export: Click on this button to send data from B-Sperm to an external file (Excel required):

- From B-Sperm click: **Import/Export > Export > Export Test Results (Export Controls)**
- If **Export Test Results** was selected, chose: **Export Dosing, QC-Frozen or BSE**
- A message box will appear to confirm the export
- Click **YES** and enter a path and file name; then click **SAVE**.

Qwik Click / Video

Section 10: QwikClick/Video

Qwik Click

QwikClick Droplet and Morphology Counter

The **QwikClick Droplet Counter** works with the AUTOMATED Morphology results reported by the SQA-Vb to account for DROPLETS. This is especially useful for young bull morphology assessment and will provide a high level of morphology accuracy.

The **QwikClick Morphology Counter** is used to obtain a full morphology differential of defects. This counter can be connected to a test result or used off-line.

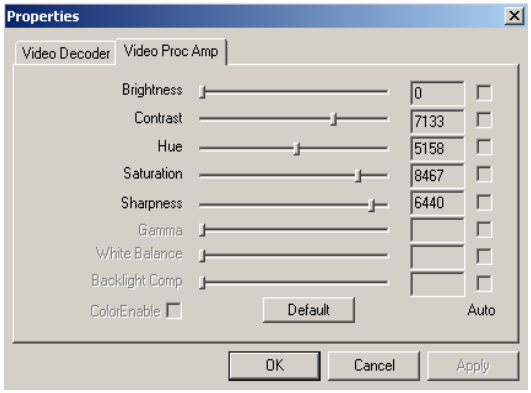
In order to use QwikClick:

Note: There are three options for QwikClick counters: Droplet Counter; Morphology Counter On-line and Off-line.

- From the **SQA-Vb MAIN MENU** select: **SERVICE > SETTINGS**
- **Select YES** to the question "**QWIK CLICK COUNTER?**"
- From the **B-Sperm** go to: **SET-UP > SYSTEM SETTINGS > ADMINISTRATOR** > **QwikClick Setting** and select either **Morphology Counter** or **Droplet Counter** from the drop-down menu.

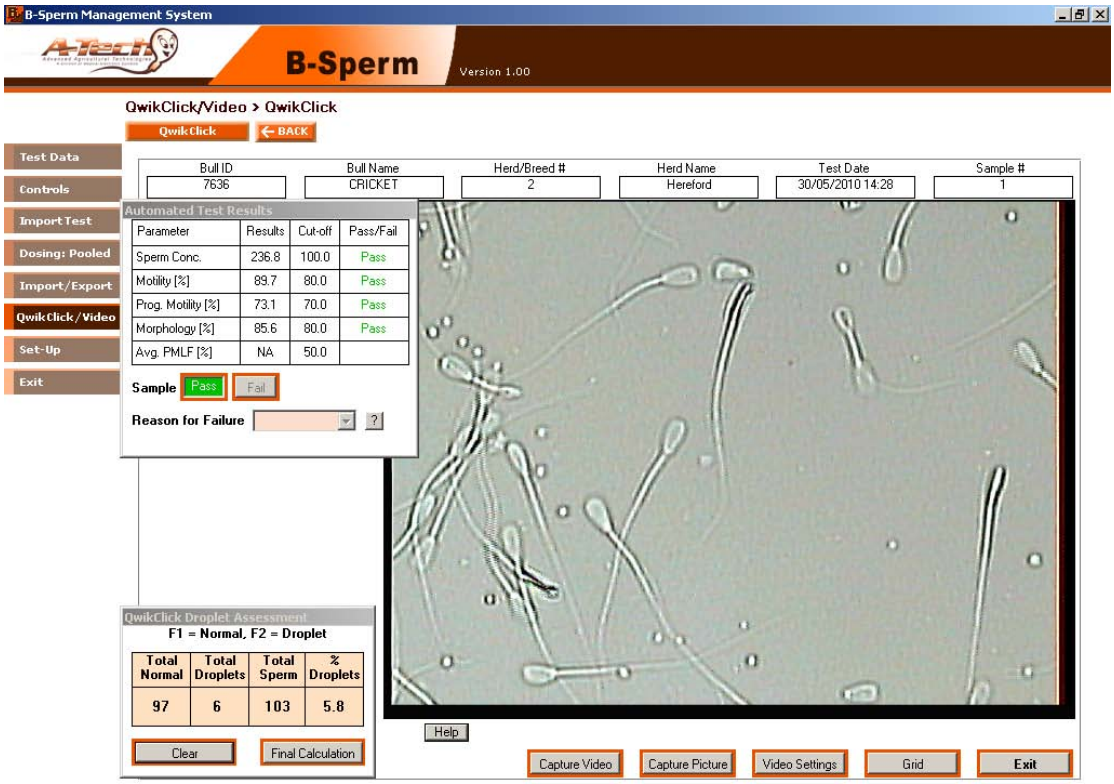
The QwikClick counter selected as a default will automatically appear when importing a new test from the SQA-Vb.

- When using the feature for the first time, Click the Video Settings button of the QwikClick feature to set the resolution (minimize the Brightness and maximize Contrast, Saturation and Sharpness).



- Click OK to accept the settings.

- The **QwikClick Droplet Assessment** screen below will be displayed:



How to use the QwikClick Droplet Counter:

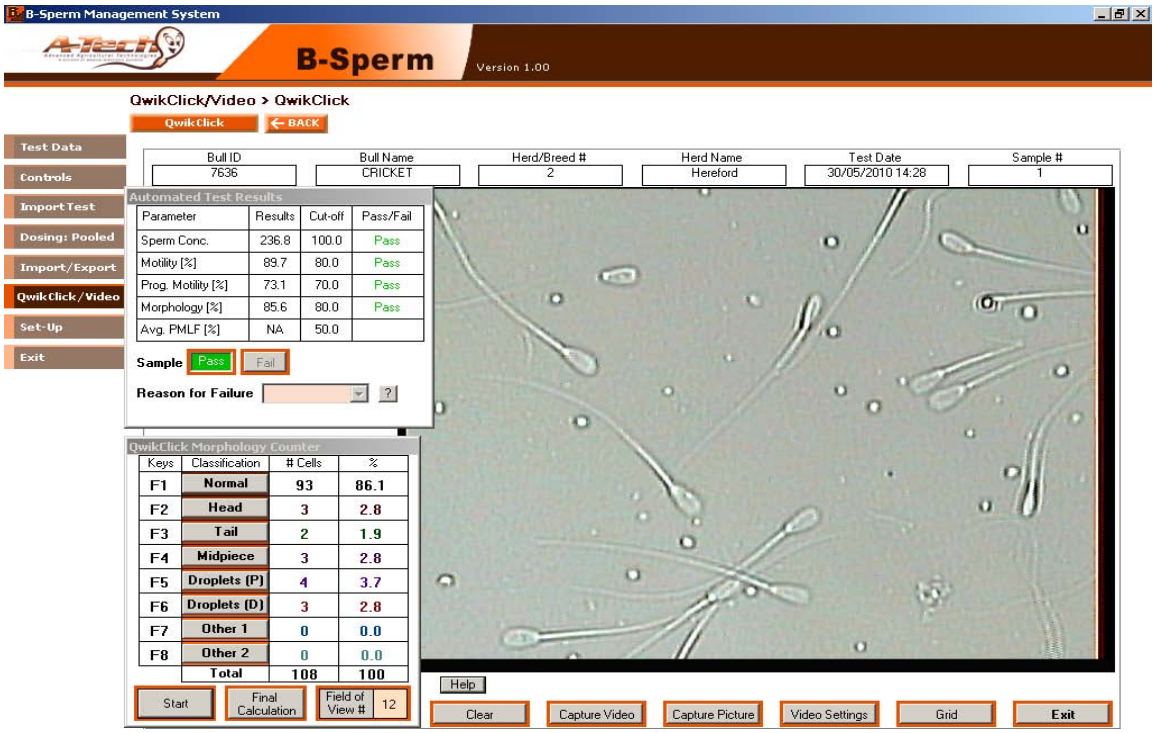
Click **HELP** to display the instructions for how to use the QwikClick Droplet Assessment:

- Prepare a slide of non-motile cells and place it in the SQA-Vb visualization system.
- Focus the SQA-Vb and begin "counting" all cells:
  - Press the F1 key on the computer keyboard for all sperm cells without droplets (even if they have other morphological defects – these will be assessed by the SQA-Vb automatically).
  - Press the F2 key on the computer keyboard for all sperm cells with droplets.
- Move to additional fields of view until the desired amount of cells are counted
- Click the FINAL CALCULATION button to complete the droplet assessment.
- The % droplet result will be used to calculate normal morphology.
- The results will be automatically sent to the dosing table and dosing report.

Features of the QwikClick Droplet Assessment:

- An Automated Test Results table is automatically displayed with the test results, cut-off criteria and Pass/Fail results of the sample just tested.
- Cut-off criteria can be set-up by the administrator (see SET-UP).
- If cut-offs have NOT been set-up, the manufacturer default cut-offs will be used.
- The Sample will PASS if all of the semen parameters have passed the cut-offs. The sample will FAIL if one semen parameter fails.
- If the system default is to automatically PASS or FAIL the sample, the user cannot change it. If this is not a default, the operator can still PASS the sample.

The screen below displays the QwikClick Morphology Counter:



How to use the QwikClick Morphology Counter:

Click **HELP** to display the instructions for how to use the QwikClick Morphology Counter:

- Prepare a slide of non-motile cells and place in the SQA-Vb visualization system.
- Be sure that the computer FUNCTION keys and morphology defects/cells have been defined in the B-Sperm SET-UP.
- Press: START and from the FN keys of the QwikClick Morphology Counter, assess the cells views on the QwikClick screen.
- Move to another field of view by slightly turning the silver know of the SQA-Vb slide holder.
- Click on NEXT FIELD and repeat the process until the desired amount of cells have been assessed.
- Click FINAL CALCULATION to save the results. The dosing screen will be automatically updated and the morphology results will be seen on reports. If an off-line morphology assessment was performed, the results will be printed.

Features of the QwikClick Morphology Counter:

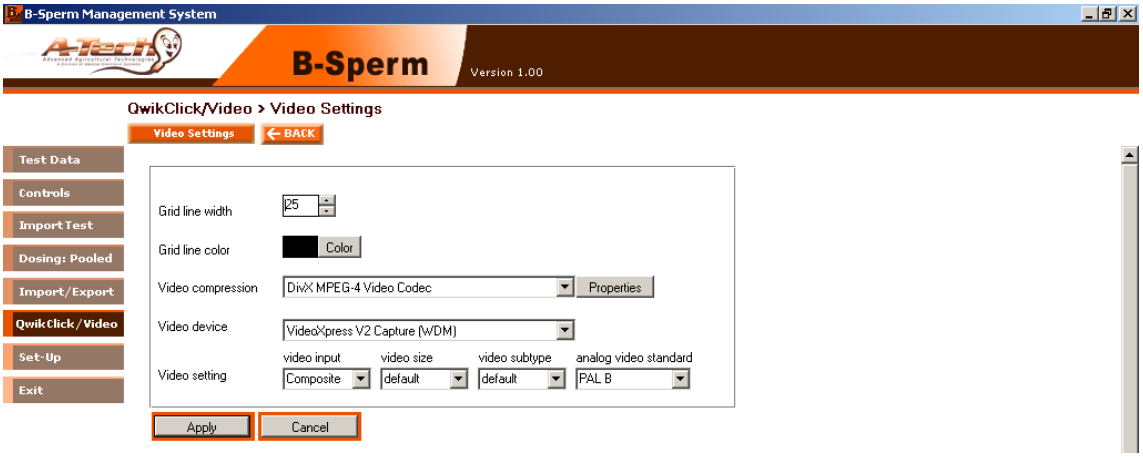
- An Automated Test Results table is automatically displayed with the test results, cut-off criteria and Pass/Fail results of the sample just tested.
- Cut-off criteria can be set-up by the administrator (see SET-UP).
- If cut-offs have NOT been set-up, the manufacturer default cut-offs will be used.
- The Sample will PASS if all of the semen parameters have passed the cut-offs. The sample will FAIL if one semen parameter fails.
- If the system default is to automatically PASS or FAIL the sample, the user cannot change it. If this is not a default, the operator can still PASS the sample.

Video Settings

Video Settings

Click: **Video Settings** to select grid line width, color and other video default settings.

- Optimal video device settings are described in the installation procedure supplied with the video device hardware.

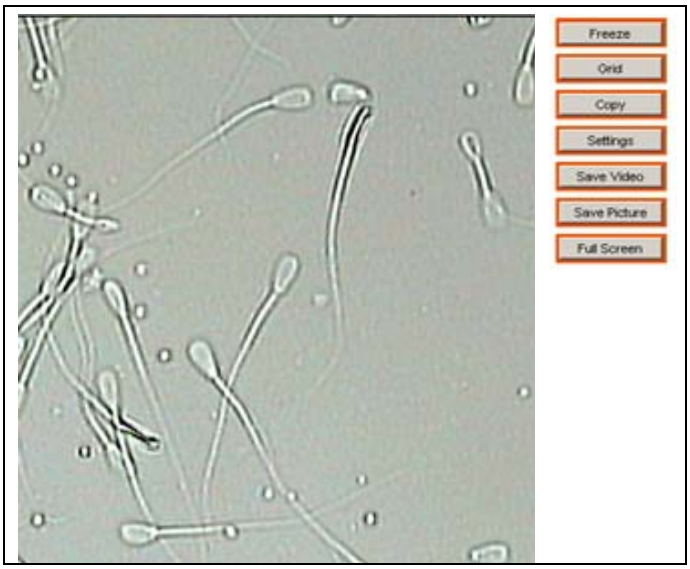


Real Time Video

View "live" samples using the **REAL TIME VIDEO** feature of B-Sperm.

Click the **Real Time Video** to display the following features:

- **Freeze** – To freeze the screen. Click the **Real Time** button to un-freeze.
- **Grid** – Add a grid to the screen to make cell counting easier.
- **Copy** – Click this button to copy pictures and then paste in an external file.
- **Settings** – Video settings can be selected by the user.
- **Save Video** – Video clips can be saved in an .avi format.
- **Save Picture** – Pictures can be saved in a .bmp format.
- **Full Screen** – The screen can be maximized and then minimized (Click the **Close** button).



Set-Up

Data Settings

Bull/Herd Setting

Note: First set-up the Herd\Breed information. The Herd\Breed will then be automatically availalble in the Bull Set-up as a drop-down menu for Herd/Breed#. See next page for instructions.

Section 11: Set-Up

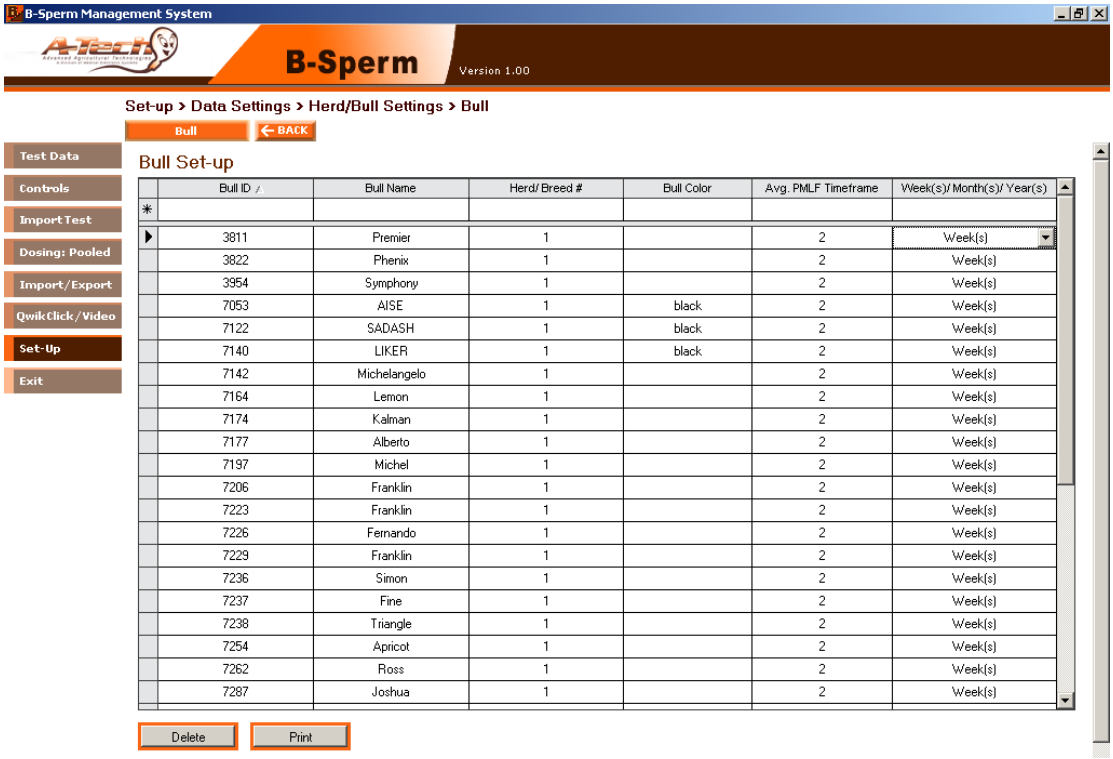
Before beginning the testing process, set-up the B-Sperm defaults by clicking: **Set-Up.** Two options will appear: **Data Settings** and **System Settings**.

Data Settings:

Click: **SET-UP > DATA SETTINGS** to view two options: **Bull/Herd Settings** & **Normal Ranges**.

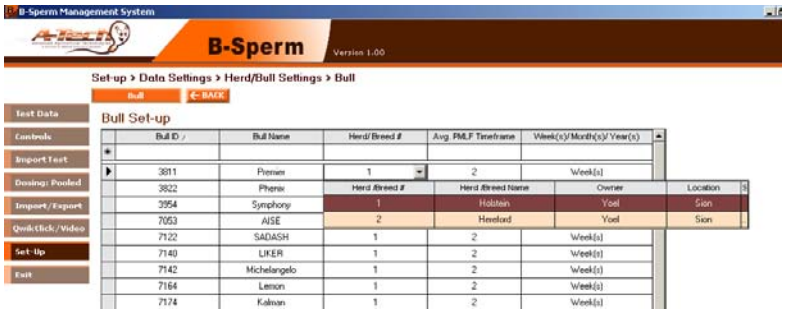
Bull/Herd Settings: Click **Bull/Herd Settings** to set-up **Bull** and **Herd\Breed** information.

Bull: Click **Bull** and the **Bull Set-up** table below will be displayed**:**



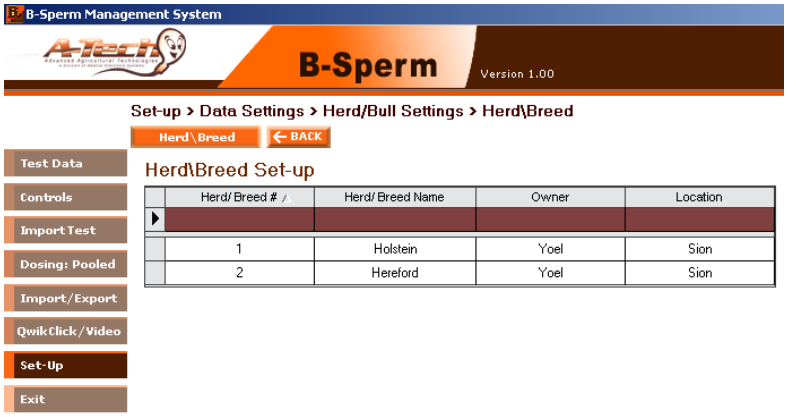
Bull ID	Bull Name	Herd/ Breed #	Bull Color	Avg. PMLF Timeframe	Week(s)/ Month(s)/ Year(s)
3811	Premier	1		2	Week(s)
3822	Phenix	1		2	Week(s)
3954	Symphony	1		2	Week(s)
7053	AISE	1	black	2	Week(s)
7122	SADASH	1	black	2	Week(s)
7140	LIKER	1	black	2	Week(s)
7142	Michelangelo	1		2	Week(s)
7164	Lemon	1		2	Week(s)
7174	Kalman	1		2	Week(s)
7177	Alberto	1		2	Week(s)
7197	Michel	1		2	Week(s)
7206	Franklin	1		2	Week(s)
7223	Franklin	1		2	Week(s)
7226	Fernando	1		2	Week(s)
7229	Franklin	1		2	Week(s)
7236	Simon	1		2	Week(s)
7237	Fine	1		2	Week(s)
7238	Triangle	1		2	Week(s)
7254	Apricot	1		2	Week(s)
7262	Ross	1		2	Week(s)
7287	Joshua	1		2	Week(s)

- Enter Bull ID, NAME, HERD/BREED #, Bull Color, Avg. PMLF Timeframe numeric value and Week(s), Month(s) or Year(s) of the timeframe. Use the drop-down menu where available. Highlight and click to select parameter for each bull in the entire herd.
- To Delete: Click the **Delete** button after highlighting a row to remove a bull record (cannot be deleted if a test has been run).
- Click on the Herd\Breed# to view the Herd\Breed Name, Owner and Location:



Herd\Breed: Click Herd\Breed to display the table below.

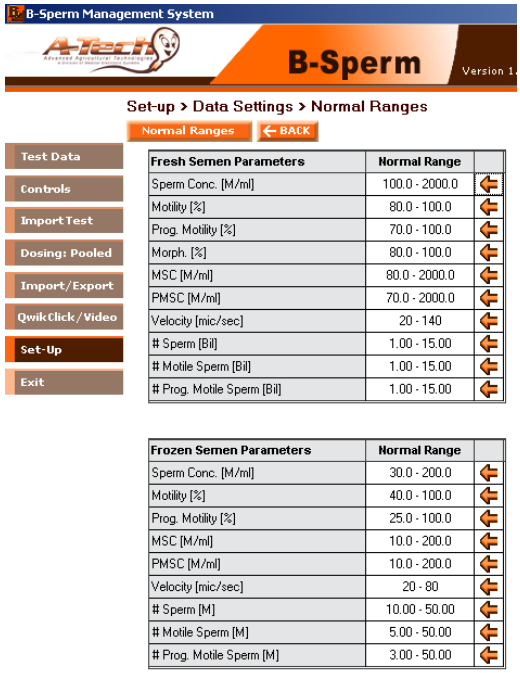
- Assign a Herd\Breed#.
- Enter the Herd\Breed Name, Owner and Location associated with this number.
- This information will automatically link to each bull tested.



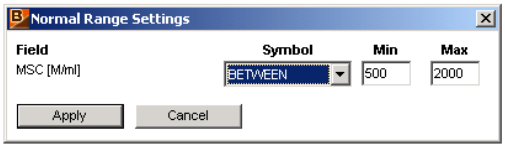
Normal Ranges

Normal Ranges: Click this button to set-up the normal ranges for the FRESH and FROZEN semen test results:

NOTE: The manufacturer has set default normal ranges which can be re-set based on the laboratory preference.



- Click on the ARROW which points to the parameter to be set-up and the **Normal Range Settings** screen below will appear:



- Enter the desired symbol (≤, ≥, =, BETWEEN, etc.)
- Enter the desired values and click **APPLY** to enter.

System Setting

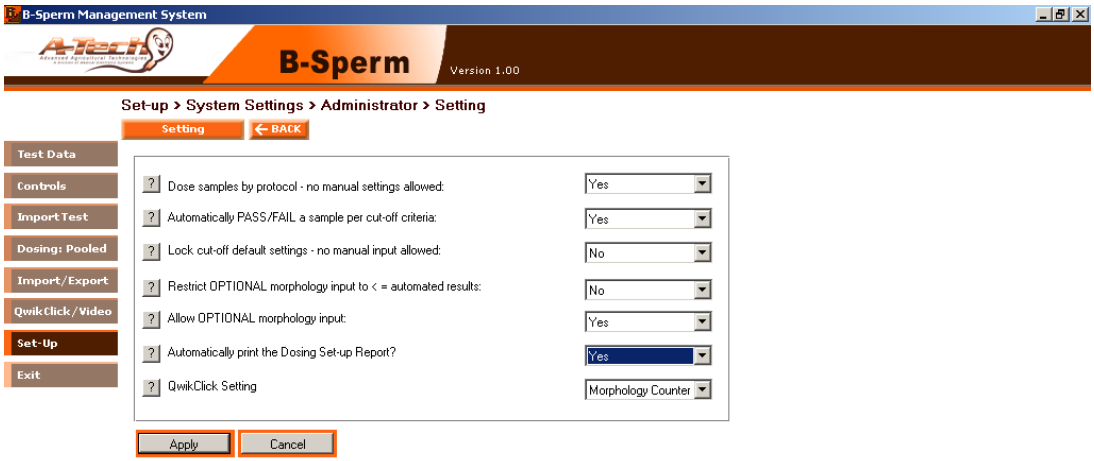
Administrator

Setting

System Settings: Opens **Administrator, Language**, **Password**, **Port, User Fields** and **Auto Export** options.

- Click: **SET-UP>SYSTEM SETTING>Administrator** to display the buttons:

- Click the **Setting** button to display the table below. An explanation of each setting will be displayed by pressing the **?** icon:



Dose samples by protocol – no manual settings allowed: The Administrator can set-up pre-programmed dosing protocols that can be used instead of manually selecting each dosing criteria. After set-up, these protocols are selected from a drop-down menu on the dosing screen. The following options are available for this default setting:

- Select: YES to dose (single or multiple) only by the pre-set Administrator dosing protocols. All manual entry fields will be locked.
- Select: NO to have the option to dose by EITHER a pre-set protocol or by manual selection/entry. All manual entry fields will be un-locked.

Automatically PASS/FAIL a sample per cut-off criteria: After setting up cut-offs for various semen parameters, there is an option to automatically PASS or FAIL a sample based on these cut-offs. If a sample FAILS automatically, the dosing screen will not be visualized. The test results will still be reported. To use this feature:

- Select: YES and a sample will automatically PASS or FAIL based on pre-set cut-offs. If the sample FAILS, the QwikClick will be locked and only the EXIT button will be activated. Press EXIT and the test results grid will be displayed instead of the dosing screen.
- Select: NO to manually PASS/FAIL a sample and have the option to use the QwikClick Droplet Assessment and view the dosing screen.

Lock cut-off default settings – no manual input allowed: Default settings for cut-offs can be set by the Administrator. Once these are set, they are reported in the QwikClick and Dosing Screens and PASS/FAIL results are displayed based on these cut-off values. Access to change these fields can be restricted:

- Select: YES to LOCK the Administrator defined cut-offs. The fields displayed in the QwikClick and Dosing screens cannot be changed.
- Select: NO and the Administrator defined cut-offs can be updated manually in the QwikClick and Dosing screens.

Restrict optional morphology input to < = automated results: Manual morphology results can be run off-line and entered into a field on the dosing screen. The B-Sperm will then report manual morphology instead of automated. The manual morphology field can be restricted in the following way:

- **Select: YES** and manual morphology results that are greater than the automated results cannot be entered into the manual morphology field. Manual morphology will be reported only if less than or equal to the automated results.
- **Select: NO**, enter manual morphology results and the manual results will be reported instead of the automated results.

Allow optional morphology input: In the dosing screen, manual morphology can be entered into a field. If it is entered, the manual morphology results will be reported instead of the automated or QwikClick morphology results:

- **Select: YES** to report manual morphology based on the explanation above.
- **Select: NO** to block the entry of manual morphology and only AUTOMATED/QwikClick morphology will be reported.

Automatically print the Dosing Set-up Report:

- **Select YES** to automatically print the Dosing Instruction Report for after the dosing information has been saved.
- **Select NO** to print manually (click the PRINT button after dosing).

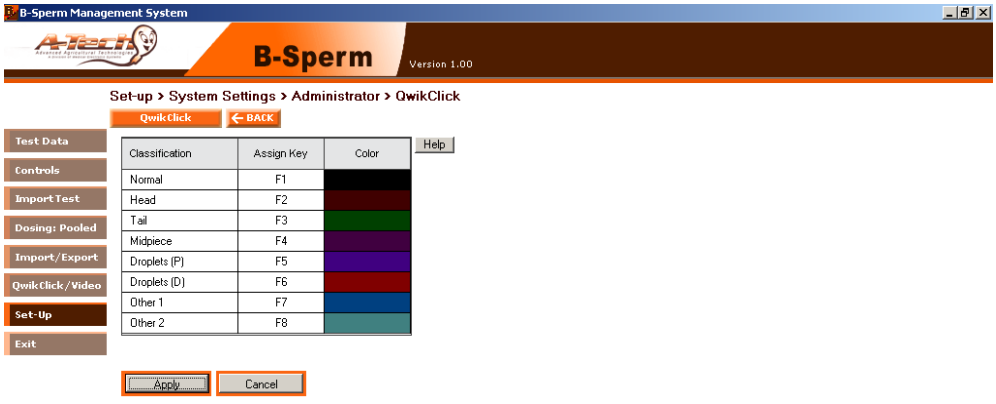
QwikClick Setting: Press HELP for an explanation of the types of QwikClick options:

- **Option 1**: **Droplet Assessment** – The QwikClick Droplet Assessment screen will automatically be seen when importing a test from the SQA-Vb. Use this feature to assess the % droplets seen in the bull sample. This information is then used to re-calculate the automated morphology for VERY HIGHLY accurate morphology.
- **Option 2**: **Morphology Counter** – The QwikClick Morphology Counter will automatically be seen when importing a test from the SQA-Vb. Use this feature to perform an on-line fully manual morphology assessment that includes an assessment of all defects. The results can be seen on a separate morphology report.

QwikClick

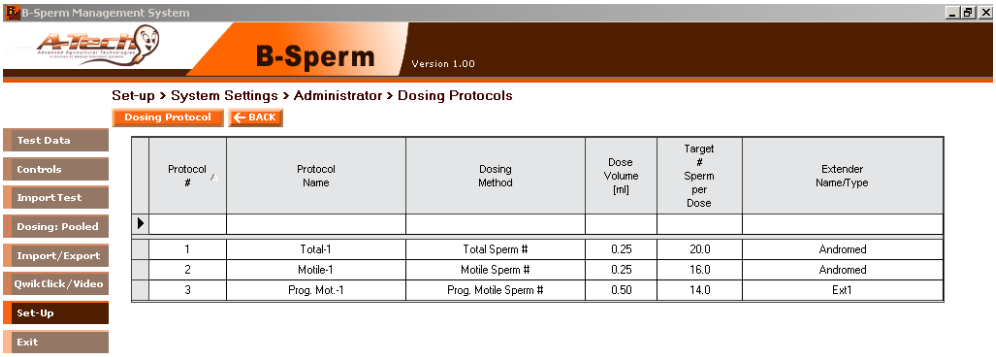
Go to **SET-UP > SYSTEM SETTINGS > ADMINISTRATOR > QwikClick** to define the QwikClick Morphology FUNCTION keys used from the P-Sperm computer keyboard:

- All abnormalities/defects can defined AND assigned to FN keys (see table below). The NORMAL is fixed and cannot be assigned another FN key.



Dosing Protocol

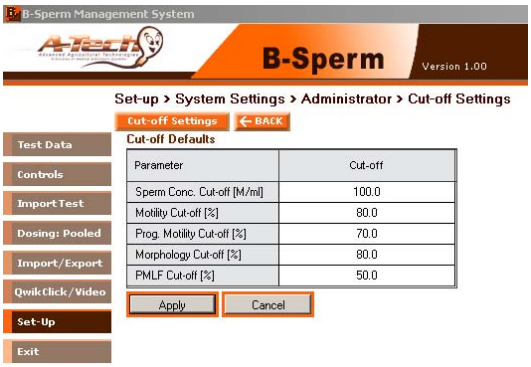
To set-up standardized dosing protocols to use in dosing production, go to: **SET-UP > SYSTEM SETTINGS >ADMINISTRATOR > Dosing Protocols** to display the table:



- Click in the left hand column to enter a new protocol. Enter information into each field. All fields must contain information.
- These protocols can now be viewed in a drop-down menu on the dosing screen or one can be used as a dosing protocol default.

Cut-off Settings

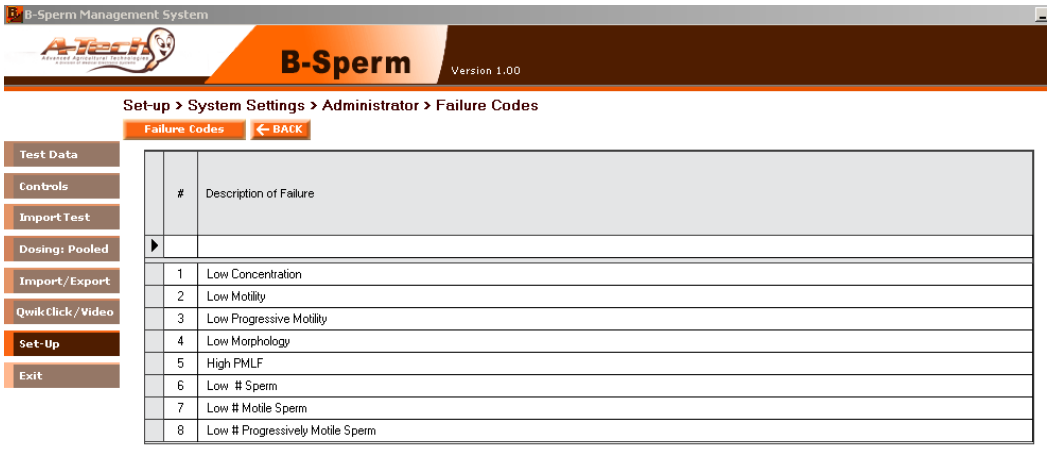
To set-up **Cut-off Settings** go to: **SET-UP** > **SYSTEM SETTINGS > ADMINISTRATOR > Cut-off Settings** and the screen below will be displayed**:**



- Enter the desired cut-off values and click: APPLY.

Failure Codes

To set-up **Failure Codes go to: SET-UP > SYSTEM SETTINGS > ADMINISTRATOR > Failure Codes**:



- Click in the left hand column to assign a number and description of the type of sample failure. This list will be available in the TEST GRID.

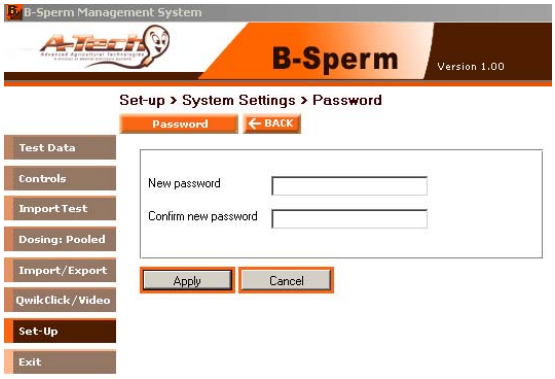
Language

Click **SET-UP > SYSTEM SETTINGS > Language** to display the table below:

- Choose "Other" from the "Language" drop-down screen.
- Edit the table as desired and click **Apply** to save.

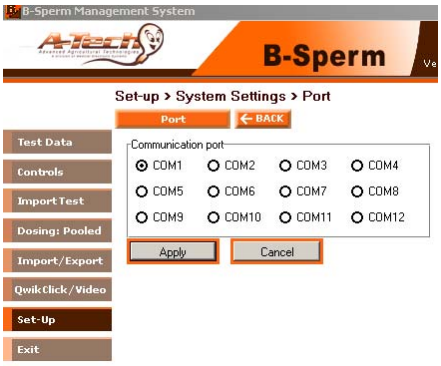
Password

Click: **SET-UP > SYSTEM SETTING > Password** to change the password.
Click **APPLY.** The password is immediately re-set. **REMEMBER YOUR PASSWORD!**



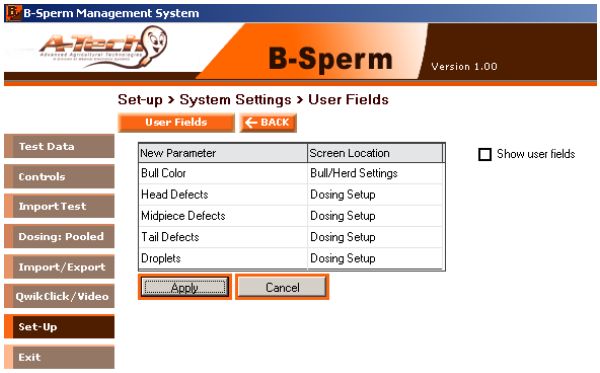
Port

Go to: SET-UP > SYSTEM SETTINGS > Port set the communication port for the PC.



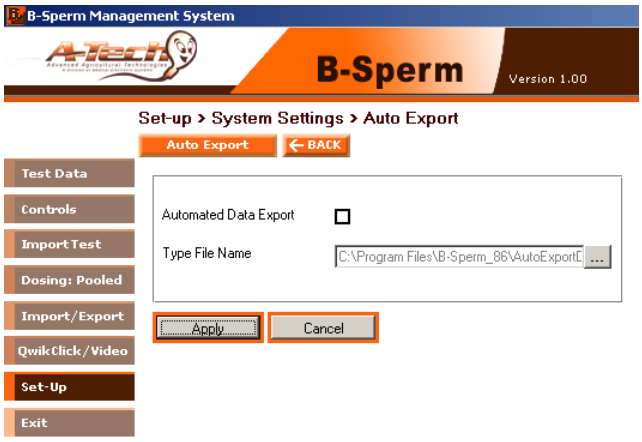
User Fields

Click: SET-UP > SYSTEM > User Fields to label optional user fields which appear on the **Dosing Set-up** or **Bull/Herd Settings** screens if the "**show user fields**" box is checked.



Auto Export

Go to: SET-UP > SYSTEM SETTINGS > Auto Export to set the automated export of the sample data from the PC to the SQA-Vb Enter Sample Data screen. Please contact you distributor for details how to set-up this feature.



Exit

Section 12: Exit

Exit the B-Sperm system by clicking the **Exit** button. Confirm with a click: