SQA-Ve
Equine
User Guide

Catalog # 7650

Version 2.00

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Section 1: Overview

The SQA-Ve is a high performance analytical veterinary system that combines state-of-the-art technology in electro-optics, computer algorithms and video microscopy. The system can be used to conduct automated or manual testing. The SQA-Ve performs a rapid and reliable automated analysis of Raw, Extended, Cooled and Frozen equine semen at 37^0C (98.6^0F).

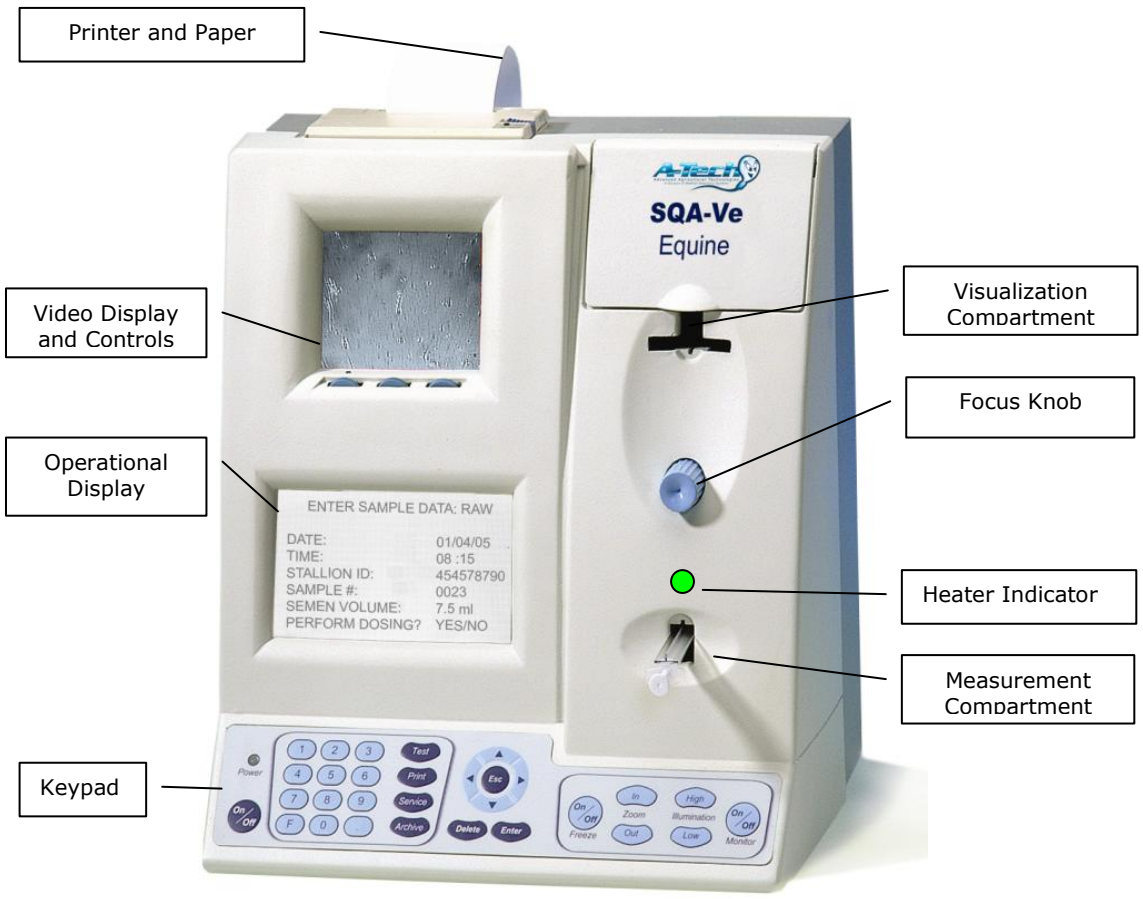
The SQA-Ve video visualization system allows the user the flexibility to view specimens at X300 through X500. Both the testing capillary and the standard slide placed onto the slide adaptor can be used in the SQA-Ve visualization system.

RAW samples: The SQA-Ve automatically analyzes, reports and prints test results. These test results can be automatically transferred to E-Sperm (PC software included with each SQA-Ve) which has an automated AI dosing feature and many reports available for analysis.

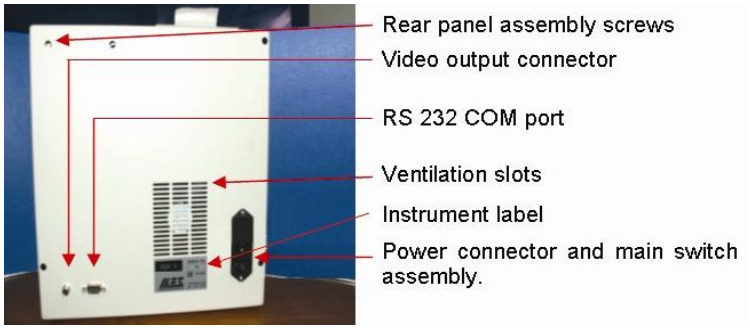
EXTENDED/COOLED/FROZEN samples: This feature is used to automatically assess the quality of extended/cooled/frozen semen.

Section 2: System Overview

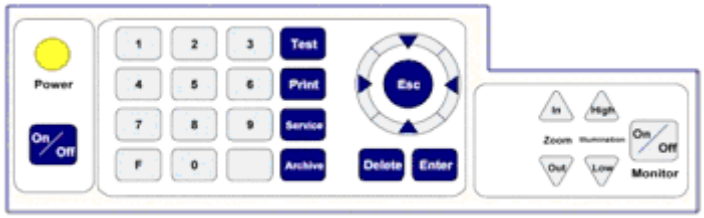
The Front Panel



Rear/Side Panel

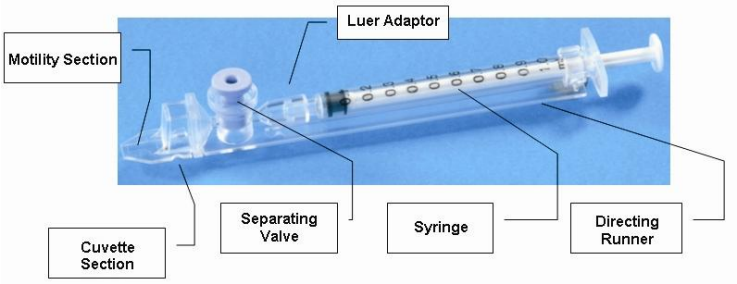


Keypad and Navigation



- Use numeric keys for entering sample data.
- The TEST key is for service personnel only
- Use the PRINT key for printing the test results.
- The SERVICE key is for entering the service menu (please see Section 5).
- Archive key is disabled (Please see Section 6).
- Use the ARROW keys to move within the screens.
- Press ENTER to select menu options and to move to the next screen or row.
- Press ESC to return to a previous screen or row.
- Press DELETE to correct a sample data entry error prior to testing.

SQA-Ve Testing Capillary



The re-usable, washable testing capillary consists of the following parts:

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

Slide Adaptor

- Use 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-Ve

- Turn on the main switch on the rear panel of the SQA-Ve.
- The power indicator and 37^0C heating indicator will illuminate.
- Press the On/Off key on the SQA-Ve keypad.
- The system will automatically auto-calibrate and then display the **# Tests Remaining** on the I-button.
- Press the **ENTER** key to view the **MAIN MENU.**

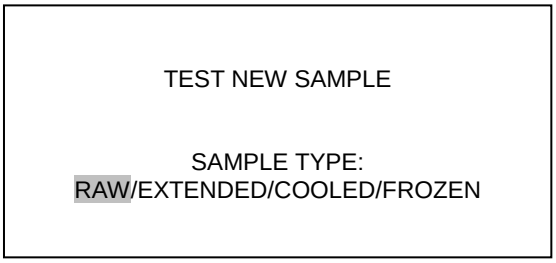
Two options are available from the **MAIN MENU** after the system is turned on:

- **TEST NEW SAMPLE**
- **SERVICE**

Section 4: Sample Testing

NOTE: Load I-button tests and set system defaults **PRIOR** to testing (see Section 5 for full instructions)

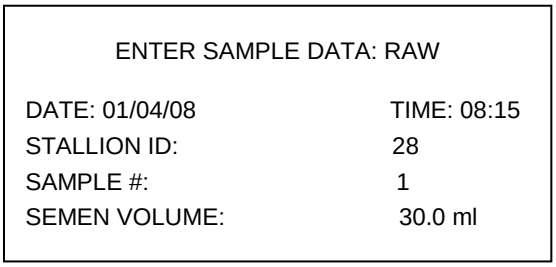
- Select **TEST NEW SAMPLE** from the MAIN MENU to open the screen below which displays four sample type testing options:



Raw Samples

Select **RAW** sample type and the screen below will be displayed:

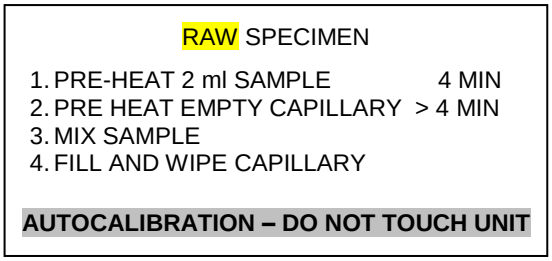
NOTE: Raw sample (2 ml) need to be preheated to 37^0C for 4 minutes prior to testing.



Enter the following:

- **Stallion ID**: up to 8 digits
- **Sample #**: up to 10 digits
- **Semen Volume** (Gel free): up to 3 integers and 1 decimal point.

Press ENTER and the system will autocalibrate – do not touch!



- Prepare a **RAW** sample for testing when the screen above is displayed.
- Instructions for preparing a **RAW** sample:

- Take 2 ml aliquots of the **RAW** semen.
- Pre-heat the semen to 37^{0}C (98.6^{0}F) for 4 minutes (Please refer to Appendix I: Semen Sample Preparation and Appendix VIII: Block Heater Operating Instructions).
- Pre-heat the testing capillaries to 37^{0}C (98.6^{0}F) in the Block Heater.
- Fill a pre-heated testing capillary but do not insert it into the system until a beep is sounded (Refer to the SQA-Ve User Guide Appendix section for instructions how to fill a capillary with a Normal Volume specimen.)

- A BEEP will sound AND the following screen will be displayed when the system is prepared to accept a testing capillary (do not touch the system or insert a testing capillary until this screen is displayed).

RAW SPECIMEN

1. PRE-HEAT 2 ml SAMPLE 4 MIN
2. PRE HEAT EMPTY CAPILLARY > 4 MIN
3. MIX SAMPLE
4. FILL AND WIPE CAPILLARY

INSERT CAPILLARY INTO CHAMBER

- Insert the testing capillary into the measurement chamber of the SQA-Ve.
- The SQA-Ve will now pre-heat the capillary for about 60 seconds.

PLEASE WAIT
PRE-HEATING CAPILLARY

- Testing will begin automatically and take approximately 45 seconds.
- A "beep" will sound and the screen below will automatically display test results.

SEMEN ANALYSIS REPORT: RAW SAMPLE

DATE: 01/04/08	TIME: 08:15
STALLION ID:	28
SAMPLE #:	1
SEMEN VOLUME:	30.0 ml

- Press ENTER to view the test results which now need to be transferred to E-Sperm

TEST RESULTS: RAW SAMPLE

CONC.	332.6 M/ml	MSC	259.1 M/ml
MOTILITY	77.9 %	PMSC	183.9 M/ml
PROG. MOT.	55.3 %	VELOC.	32 mic/sec
MORPHOLOGY	73.3 %		
TOTALS		# SPERM	9.97 Bil
PER		MOT. SPERM	7.77 Bil
EJACULATE		PROG. SPERM	5.52 Bil

NOTE: Morphology results will be generated by the system **only** for Raw samples.

- Transfer results to E-Sperm following the instructions on the screen:

NOTE:
There is no archive in the SQA-Ve – test result must be transferred to E-Sperm to save them for future printing/viewing

```
PRESS: "IMPORT TEST" BUTTON IN E-SPERM
TO TRANSFER AND SAVE TEST RESULTS

WARNING: TEST RESULTS MUST BE
IMPORTED TO
E-SPERM TO SAVE

PRESS ENTER TO RETURN TO MAIN MENU
```

Extended/Cooled Samples

EXTENDED samples are defined as RAW equine semen diluted with a commercial extender.

COOLED samples are defined as EXTENDED samples kept in cooling conditions.

- Select: TEST NEW SAMPLE from the Main Menu
- Select: EXTENDED or COOLED and the screen below will be displayed:

```
ENTER SAMPLE DATA: EXTENDED/COOLED

DATE: 01/04/08          TIME: 08:15
STALLION ID:                    28
SAMPLE #:                        1
SEMEN VOLUME:            20.0 ml
```

Enter the following:

- **Date** and **Time** will be displayed by the unit
- **Stallion ID**: up to 8 digits
- **Sample #**: up to 10 digits
- **Semen Volume** (the actual dose volume): up to 3 integers and 1 decimal point.

When a COOLED sample is run, it is necessary to know if the sample has been cooled for more than 24 hours. Select YES/NO in the screen below:

```
PLEASE SELECT:

SAMPLE COOLED > 24 HOURS

YES/NO
```

NOTE:
Preheat the sample (2 ml) to 37^0C;
EXTENDED: 4 minutes
COOLED: 7 minutes

Press **ENTER** and the system will autocalibrate – do not touch! Do not insert the testing capillary!

```
EXTENDED SAMPLE

5. PRE-HEAT 2 ml SAMPLE          4 MIN
6. PRE-HEAT EMPTY CAPILLARY  > 4 MIN
7. MIX SAMPLE
8. FILL AND WIPE CAPILLARY

AUTOCALIBRATION – DO NOT TOUCH UNIT
```

COOLED SPECIMEN

1. PRE-HEAT 2 ml SAMPLE 7 MIN
2. PRE-HEAT EMPTY CAPILLARY > 4 MIN
3. MIX SAMPLE
4. FILL AND WIPE CAPILLARY

AUTOCALIBRATION – DO NOT TOUCH UNIT

Fill a pre-heated testing capillary with pre-heated (4 MINUTES) EXTENDED or pre-heated (7 MINUTES) COOLED sample following the on-screen instructions.

- A BEEP will sound AND the following screen will be displayed when the system is prepared to accept a testing capillary (do not touch the system or insert a testing capillary until this screen is displayed).

EXTENDED SAMPLE

1. PRE-HEAT 2 ml SAMPLE 4 MIN
2. PRE HEAT EMPTY CAPILLARY > 4 MIN
3. MIX SAMPLE
4. FILL AND WIPE CAPILLARY

INSERT CAPILLARY INTO CHAMBER

COOLED SAMPLE

1. PRE-HEAT 2 ml SAMPLE 7 MIN
2. PRE HEAT EMPTY CAPILLARY > 4 MIN
3. MIX SAMPLE
4. FILL AND WIPE CAPILLARY

INSERT CAPILLARY INTO CHAMBER

- **Insert the testing capillary into the measurement chamber.** The testing capillary will now be pre-heated for about 60 seconds.

PLEASE WAIT
PRE-HEATING CAPILLARY

- Testing will begin automatically and take approximately 40 seconds.
- A "beep" will sound and the screen below will display the automatically saved semen analysis report:

SEMEN ANALYSIS REPORT:
EXTENDED/COOLED SAMPLE

DATE:	01/04/08	TIME:	8:15
STALLION ID:		2825841	
SAMPLE #:		114	
SEMEN VOLUME:		20.0 ml	
COOLING TIME > 24H		YES (COOLED ONLY)	

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

TEST RESULTS: EXTENDED/COOLED SAMPLE			
CONC.	67.5 M/ml	MSC	45.0 M/ml
MOTILITY	66.6 %	PMSC	27.2 M/ml
PROG. MOT.	40.3 %	VELOC.	66 mic/sec
TOTALS PER SEMEN VOLUME			
# SPERM	1.35 Bil		
MOT. SPERM	0.90 Bil		
PROG. SPERM	0.54 Bil		

- If Motility is <= 30% and >= 10% in the sample, the report will display only motile and progressively motile spermatozoa per semen volume (AI dose).

TEST RESULTS:

MOTILITY <= 30%

TOTALS PER SEMEN VOLUME

MOT. SPERM	1.62 Bil
PROG. SPERM	0.83 Bil

- If Motility is < 10% in the sample, semen parameters cannot be accurately measured and the screen below will be displayed.

TEST RESULTS:

MOTILITY < 10%

SEMEN PARAMETERS CANNOT BE MEASURED

Frozen Samples

- Select: **FROZEN** from the TEST NEW SAMPLE screen in the Main Menu.

ENTER SAMPLE DATA: FROZEN	
DATE: 01/04/08	TIME: 08:15
STALLION ID:	28
STRAW DATE:	10/09/07
SAMPLE #:	1
SEMEN VOLUME:	0.500 ml

Enter the following:

- **Stallion ID**: up to 8 digits
- **Straw Date**: according to the straw labeling
- **Sample #**: up to 10 digits
- **Semen Volume** (actual straw volume): up to 2 integers and 3 decimal points.

Press **ENTER** and the system will autocalibrate – do not touch! Do not insert the testing capillary!

FROZEN SPECIMEN

1. PRE-HEAT THAWED SAMPLE 4 MIN
2. PRE-HEAT EMPTY CAPILLARY > 4 MIN
3. MIX SAMPLE
4. FILL CAPILLARY 20 microliters
5. WIPE TESTING CAPILLARY

AUTOCALIBRATION – DO NOT TOUCH UNIT

- Fill a pre-heated testing capillary with pre-heated FROZEN sample (Refer to Appendix I: Semen Sample Preparation and Appendix VIII: Block Heater Operating Instructions).
- Follow the instructions in the SQA-Ve User Guide Appendix III for Filling a Capillary with a Low Volume sample (20µl).
- A BEEP will sound AND the following screen will be displayed when the system is prepared to accept a testing capillary (do not touch the system or insert a testing capillary until this screen is displayed).

FROZEN SPECIMEN

1. PRE-HEAT THAWED SAMPLE 4 MIN
2. PRE-HEAT EMPTY CAPILLARY > 4 MIN
3. MIX SAMPLE
4. FILL CAPILLARY 20 microliters
5. WIPE TESTING CAPILLARY

INSERT CAPILLARY INTO CHAMBER

- **Insert the testing capillary into the measurement chamber.**
- The SQA-Ve will now pre-heat the capillary in the measurement chamber for approximately 60 seconds.

NOTE:
Preheat the frozen sample (0.5 ml) to 37^0C for 4 minutes prior to testing.

PLEASE WAIT
PRE-HEATING CAPILLARY

- Testing will begin automatically and will take about 45 seconds.
- A "beep" will indicate when the testing is done, the results will be automatically saved and the following screen will be displayed:

SEMEN ANALYSIS REPORT: FROZEN SAMPLE

DATE: 01/04/08	TIME: 8:15
STALLION ID:	28
STRAW DATE:	10/09/07
SAMPLE #:	1
SEMEN VOLUME:	0.500 ml

- Press ENTER to view the test results:

TEST RESULTS: FROZEN SAMPLE			
CONC.	NA M/ml	MSC	259.1 M/ml
MOTILITY	77.9 %	PMSC	183.9 M/ml
PROG. MOT.	55.3 %	VELOC.	32 mic/sec
TOTALS PER SEMEN VOLUME			
# SPERM	NA M		
MOT. SPERM	129.6 M		
PROG. SPERM	92.0 M		

  - Sperm Concentration and # Sperm per semen volume are not reported for the Frozen semen.
  - If Motility is <= 30% and >= 10% in the Frozen sample only motile and progressively motile spermatozoa per semen volume is reported.

TEST RESULTS:

MOTILITY <= 30%

TOTALS PER SEMEN VOLUME

MOT. SPERM	50.0 M
PROG. SPERM	30.0 M

  - If Motility is < 10% in the Frozen sample semen parameters cannot be accurately measured and no test results will be reported.

MAIN MENU:

The MAIN MENU is displayed after each test is run:

MAIN MENU

TEST NEW SAMPLE
RECALL LAST TEST RESULTS
RETEST SAME SAMPLE
SERVICE

- RECALL LAST TEST RESULTS: Test results can be recalled for viewing.
- RETEST SAME SAMPLE: Re-run the sample without re-entering the sample data. To run duplicate tests for REPEATABILITY, **keep the testing capillary in the measurement slot and select RETEST SAME SAMPLE without removing the testing capillary.** Both replicates will be saved automatically and can be transferred to E-Sperm for analysis.

Section 5: SERVICE MENU

Select the **SERVICE MENU** and the following options are available:

- **SERVICE DATA**
- **SERVICE PERSONNEL**
- **PRINT SELF-TEST DATA & SETTINGS**
- **SETTINGS**
- **ADD I-BUTTON TESTS**

SERVICE DATA:

Select this option to view three service screens:

- **Service Data screen (Communication screen)**:
  - Activate this screen to establish a communication between the SQA-Ve and the PC in order to transfer test results to E-Sperm.
  - Service information for technical support is viewed from this screen.
- **Self-Test Data screen:** This screen provides information for troubleshooting testing errors by displaying Self-Test and Internal Data.
- **Algorithm data screen:** This screen displays algorithm calculations.

SERVICE PERSONNEL: For Technical service support (requires a password).

PRINT SELF-TEST DATA & SETTINGS:

- Highlight this option in the **SERVICE MENU** and press **ENTER**.
- Select: **Self-Test Data** or **Test Settings** and press **ENTER** to print**.**

SETTINGS:

Select this option to set the system and sample defaults.

System Default Settings:

- **LOCAL TIME:** enter local time
- **DATE FORMAT:** Select the format **DD/MM/YY or MM/DD/YY** using the right/left arrows on the keypad.
- **DATE SETTING:** enter current date
- **AUTOMATICALLY PRINT?** Select **YES/NO** to automatically print test results after running a test (recommended setting: YES)
- **RICH FRACTION COLLECTED:** The default is **NO**. If a rich ejaculate fraction is collected select **YES**.
- **CONC STD:** Select "1" for Neubauer (default) or "2" for Nucleocounter

ADD I-BUTTON TESTS:

The SQA-Ve requires tests be "loaded" using an I-Button.

- Select **SERVICE > SERVICE MENU >ADD I-BUTTON TESTS** and press **ENTER.**
- The SQA-Ve screen will instruct the user:

  TO ADD I-BUTTON TESTS:

  - CONNECT THE SQA-Ve TO THE PC
  - ACTIVATE E-SPERM ON THE PC
  - FROM E-SPERM SELECT: SET-UP/I-BUTTON
  - FOLLOW THE INSTRUCTIONS

- Hold the I-Button firmly in the port making sure it touches both the internal surface and the edges of the port.
- Continue to hold the I-Button in place until the **#TESTS ADDED** and the cumulative **#TESTS REMAINING** are displayed on the E-Sperm screen.
- The user is informed when:
  - Less than 10 tests are available.
  - No more tests are available.

Section 6: Troubleshooting

Stabilization Failed:

STABILIZATION FAILED
TURN OFF MAIN SWITCH ON REAR PANEL
REACTIVATE UNIT

IF PROBLEM PERSISTS,
CALL FOR TECHNICAL SUPPORT

- Ensure there is no testing capillary in the measurement compartment.
- Remove the SQA-Ve from sources of electronic noise and/or vibration (centrifuge, cell phones, etc.).
- Clean measurement compartment (refer to Appendix IV).
- Reboot the SQA-Ve 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/Self-Test
  - Call for technical support if failure recurs.

Failed Self-Test:

FAILED SELF-TEST
TURN OFF MAIN SWITCH ON REAR PANEL
CLEAN OPTICAL CHAMBER
REACTIVATE UNIT

IF PROBLEM PERSISTS,
CALL FOR TECHNICAL SUPPORT

- Ensure there is no testing capillary in the measurement compartment.
- Remove the SQA-Ve from sources of electronic noise and/or vibration (centrifuge, etc.).
- Clean measurement compartment (refer to Appendix IV).
- Reboot the SQA-Ve without a testing capillary in the chamber:
  - From the rear panel switch, turn the system **OFF** then back **ON.**
  - Press the front panel **ON/OFF** key to begin Auto-Calibration, Stabilization and Self-Test.
- Call for technical support if this message is displayed again. Prepare for technical support by printing a copy of the SQA-Ve Self-Test/Service Data:
  - Press **SERVICE** key. The **SERVICE MENU** will be displayed.
  - Select **PRINT SELF-TEST DATA & SETTINGS** option.
  - Select **SELF-TEST DATA** and press **ENTER** to print a copy of the self-test/service data.

Electronic Noise:

ELECTRONIC NOISE
TURN OFF MAIN SWITCH ON REAR PANEL
REACTIVATE UNIT

IF PROBLEM PERSISTS,
CALL FOR TECHNICAL SUPPORT

- Ensure there is no testing capillary in the measurement compartment.
- Remove SQA-Ve from sources of electronic noise and/or vibration (centrifuge, etc.).
- Clean measurement compartment (refer to Appendix IV) and then:
  - Activate **MAIN MENU > TEST NEW SAMPLE** and re-run the test.
- If this message is displayed again, reboot the SQA-Ve:
  - Turn the system **OFF** then **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 Self-Test/Service parameters:
  - Press **SERVICE** key. The **SERVICE MENU** will be displayed.
  - Select **PRINT SELF-TEST DATA & SETTINGS**.
  - Select **SELF-TEST DATA > ENTER** to print the service information.

Remove Capillary:

If the testing capillary has been left in the measurement chamber after testing a sample and prior to testing the next sample this screen will be displayed (Except when the RETEST SAME SAMPLE function has been selected):

REMOVE CAPILLARY
FOLLOW ON-SCREEN INSTRUCTIONS

Appendix I: Semen Sample Preparation

EQUIPMENT REQUIRED:

- 10-ml plastic container
- Regular volumetric and/or Pasteur pipette
- SQA-Ve capillary
- Heater (Please refer to Appendix VIII: Block Heater Operating Instructions)
- 20-micron Nylon Filter to filter samples with debris

RAW/EXTENDED/COOLED SEMEN SAMPLES:

SAMPLE PREPARATION

1. Place SQA-V capillaries and 10-ml plastic containers in the heating unit.
2. Distribute 2-ml aliquots of semen into 10-ml containers.
3. Close the plastic containers and pre-heat the semen to 37^0C (98.6^0F) for 4 minutes (RAW/EXTENDED room temperature samples) or for 7 minutes (COOLED samples).
4. Gently but thoroughly mix the sample for 10 seconds (Figure 1).
5. The sample is now ready for testing.
6. Fill a pre-heated to 37°C (98.6°F) SQA-Ve testing capillary following the instructions in the Appendix II: Capillary Filling Instructions: Normal Volume (Raw Specimens).

NOTE: When using the external heating device, set the temperature to 40^0C in order to heat the samples to 37^0C.

FROZEN SEMEN SAMPLES:

SAMPLE PREPARATION

1. Thaw a frozen straw in a 37°C (98.6°F) water bath.
2. Expel the thawed sample (20 microliters required for testing) into a pre-heated 10-ml plastic container.
3. Heat the sample to 37°C / 98.6°F for 4 minutes.
4. Gently but thoroughly mix the sample.
5. The sample is now ready for testing.
6. Fill a pre-heated to 37°C / 98.6°F SQA-Ve testing capillary with the semen sample following the instructions in the Appendix III: Capillary Filling Instructions: Low Volume Sample (Frozen Specimens).

How to quickly observe samples for debris

NOTE: Semen containing a lot of debris should be filtered with a 20-micron mesh filter prior to testing.

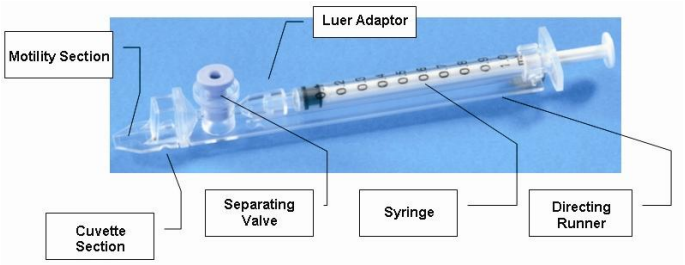
<u>Option 1:</u>

- Insert the testing capillary into the visualization compartment of the SQA-Ve (Refer to Appendix VII: The Visualization System).
- Optimize the image and scan the capillary depth using the focus knob.

<u>Option 2:</u>

- Place 10 µl of sample onto a standard slide, cover it with 22 mm x 22 mm coverslip and insert into the visualization compartment of the SQA-Ve using the slide adapter.
- If debris (big aggregates of epithelial cells) is observed, use a 20-micron nylon mesh to filter ~ 2 ml of sample (place the filter on a 10-ml plastic container and place the semen on the filter using a Pasteur pipette).

Appendix II: Capillary Filling Instructions: Normal Volume (Raw/Extended/Cooled Specimens)



1. Push the syringe piston in fully. Place only the thin part of the capillary into the bottom of the 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 (Figure 3).
6. 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.
7. Slowly and carefully push-in the separating valve until it is level with the plastic. The capillary is now ready for testing (Figure 4).
8. Insert the capillary into the SQA-Ve (Figure 5)

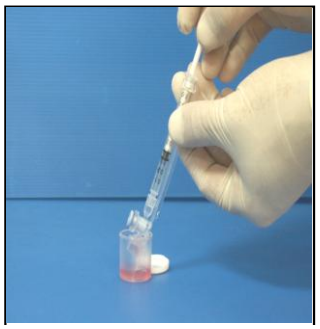


Figure 1

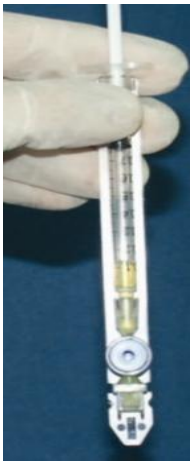


Figure 2

Figure 3

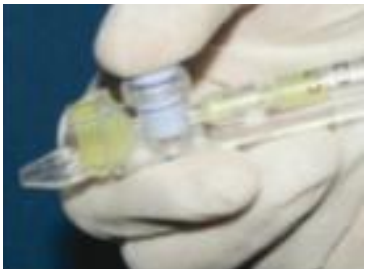


Figure 4

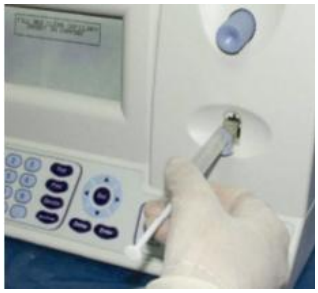


Figure 5

APPENDIX III: Capillary Filling Instructions: Low Volume Sample (Frozen Specimens)

Sample size, collection container and preparation:

1. For Low Volume Sample Testing, a minimum of 20 microliters is required to fill just the thin section of the testing capillary (Figure 1).
2. The semen sample must be **well mixed prior to aspiration**. Gently rotate the container to fully mix the specimen. ***WARNING:*** *Do not shake nor use a pipette to aspirate and dispense specimen in order to mix, otherwise air bubbles will form.*
3. **Carefully check that the specimen is free of air bubbles** before immersing the capillary into the specimen.

Filling the capillary:

1. **Push the syringe piston in fully**. Place only the thin part of the capillary into the bottom of the sample.
2. **Pull the piston back slowly** without withdrawing the capillary from the sample. **Fill only the (thin) capillary chamber** with 20 micro liters of semen. Aspirate the sample until it just appears in the cuvette part while keeping the tip of the capillary well below the sample level.
3. Visually inspect the capillary to ensure that the sample has completely filled the thin section (no meniscus).
4. Quickly and **thoroughly wipe the outer surface of the capillary** - It is important to remove all semen from the exterior of the capillary in order to prevent the SQA-Ve from becoming clogged.
5. Visually confirm that the thin chamber of the capillary is still full of semen after completing the cleaning process. If some of the sample is missing push-in the piston slightly until a drop appears on the capillary tip and then fill the capillary again from the sample container.

Figure 1

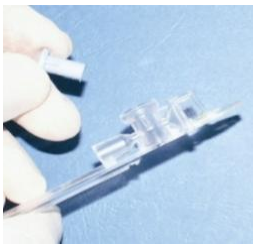


Figure 3

6. The separating valve must now be removed.
   - Use the black jig (white jig shown) to firmly **push-out the separating valve** from the underside of the capillary (Figure 1).
   - Completely detach the separating valve (Figure 2). The capillary is now ready to be inserted into the SQA-Ve.
7. PLEASE NOTE: Test Low Volume samples as soon as the sample is aspirated into the capillary!

APPENDIX IV: Cleaning the Capillary/Slide Compartment

When to clean:

Daily or after every 10-15 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

PLEASE NOTE: ALL CLEANING/DRYING CAPILLARIES ARE FOR **SINGLE** USE ONLY

CLEANING: STEP 1

1. **TURN OFF** the SQA-Ve
2. Use a **BLUE DOT** fibrous material capillary (fig 1)
3. Moisten with ONE drop of cleaning fluid, shaking off excess fluid.
4. Insert into the measurement compartment - fibrous material facing up. Move back and forth a few times. Repeat with the material facing down.
5. Use a sponge-tipped drying capillary to dry the same compartment. (fig 3)

CLEANING: STEP II

1. Insert the brush (bristle-side down) into the lower chamber of the SQA-V (fig 5)
2. Pull the brush out of the chamber while sweeping or "dusting off" the lens (you will feel a step or shelf at the back and top of the chamber – this is the top of the lens).
3. Switch SQA-V ON and observe self-test results. The SQA-V should now PASS the self-test. If not, repeat cleaning procedure with the brush.

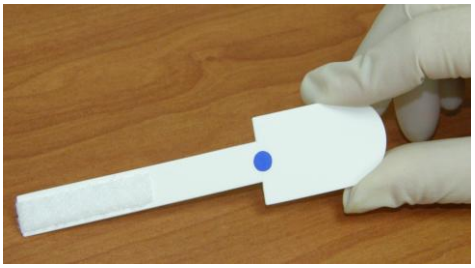


Figure 1

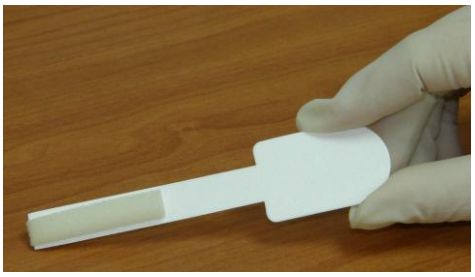


Figure 2

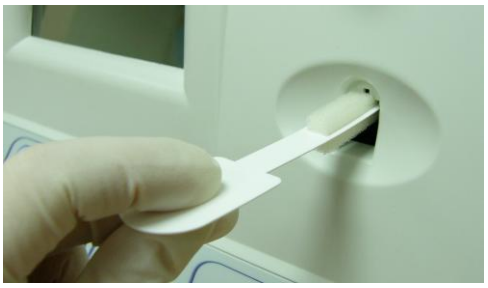


Figure 3

Figure 4

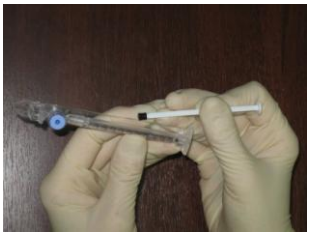
Figure 5

Appendix V: Capillary Washing Instructions

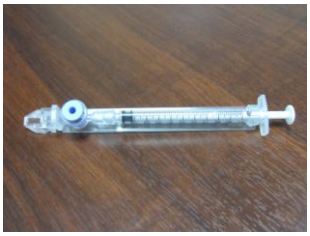
FOR ANIMAL SYSTEMS ONLY!!
Both testing capillaries and 10ml sample collection cups can be washed and re-used up to 5 times by following this EASY procedure:

Testing Capillary

Reposition the blue valve with the jig



Remove the plunger



Reassemble the capillary

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 expel all remaining 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.**
- After final washing, remove the plunger from the syringe.

Step 5: Drying the Capillaries:

- Place the capillaries on a flat surface to dry overnight or use the silica bead desiccator described in the Appendix section or place in a low heat oven for a few hours to dry.

Step 6: Final Preparation/Inspection:

- Replace the plunger into the syringe.
- Inspect the capillary and throw away if cracked, broken or semen remains.
- Note the number of washings by making a dot on the capillary with a water proof marker after each washing cycle.

Sample collection cups

Washing – Please refer to Step 4 Capillary Washing and follow the same process for washing in the solutions in bowls #1; #2 and #3. Turn upside down on absorbent paper to dry overnight.

Appendix VI: Capillary Drying Instructions

For Animal Applications ONLY!

A simple desiccator can be made to dry the washed QwikCheck™ QC testing capillaries using the silica gel beads provided in the QwikCheck™ QC start-up kit. The testing capillaries will dry in approximately 12-24 hours.

Materials/Equipment Required

- 1 kg of blue Silica gel beads
- 1 large airtight plastic box/jar container.
- Plastic netting to hold capillaries above the silica beads
- 50 washed capillaries

Drying Instructions

Silica Gel Beads

Capillaries Drying

Step 1: Assemble the desiccator:

- Pour all of the Silica gel beads provided into the desiccation container.
- Put plastic net over the silica beads.
- Place 50 washed capillaries on the net.

Step 2: Close the desiccator:

- Tightly close the cover of the desiccator

Step 3: Capillary Drying :

- After 12-24 hours see if the capillaries are dry (without opening the cover).
- If the capillaries appear dry, open the desiccator and check closely to see if all of the water has evaporated from the capillaries.
- If they are still wet, check again in 2 hours.
- When dry, remove capillaries from the desiccator and tightly close the desiccator to preserve the silica beads.

Step 4: Capillary re-assembly

- Slide the plunger back into the syringe.
- Check the capillary for cracks, broken parts or remaining semen.
- Throw away capillaries that are damaged or contaminated.
- Mark the capillary with a dot after each washing-drying cycle.
- The capillaries are ready for use.

Step 5: Silica gel re-activation

- When the BLUE silica gel beads turn PURPLE/PINK they need to be dried.
    - Heat for 3 – 4 hours at 130 degrees C.
    - Mix the beads 3-4 times during the drying.
    - When the color of the beads turns BLUE, place the beads into the desiccator and close tightly.

Appendix VII: The Visualization System

Samples can be viewed on the SQA-Ve Visualization System. Both the SQA-Ve testing capillary and a standard slide can be used in the visualization system. The system:

- Operates via control knobs to set focus, brightness, contrast and color, and via the keypad zoom, illumination, and monitor on/off functions.
- Has a magnification range: x300 to x500.

Operating Instructions:

Slide Preparation:

- Place 10 µl of semen approximately 20 mm from the edge of the slide.
- Use only a standard slide with a 22 mm x 22 mm cover-slip.
- Load the prepared slide into the SQA-Ve slide adaptor and insert into the visualization compartment of the SQA-Ve.

Preparation of the SQA-Ve Testing Capillaries:

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

Operating Process:

NOTE: Use caution when turning the focus knob. If resistance is felt it is at the maximum (or minimum) position. Forcing this knob beyond the stopping point may damage the focus system.

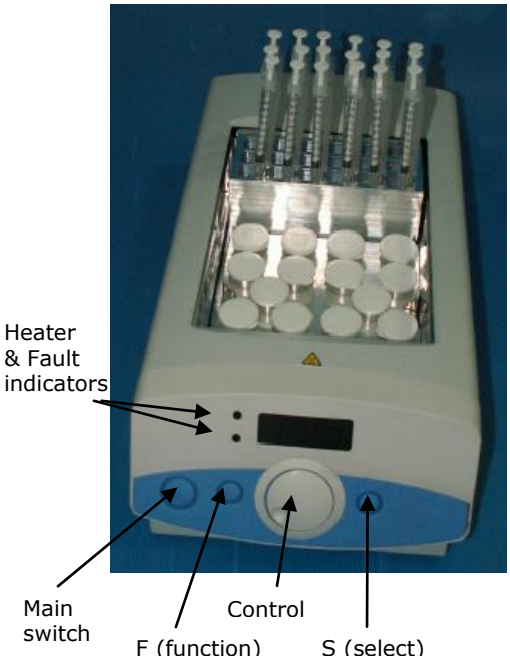
1. The video display illuminates when the SQA-Ve is turned on.
2. To ensure that the visualization system is working properly prior to use:
   a. Press the **HIGH ILLUMINATION** key multiple times to achieve the maximum level setting.
   b. Turn **BRIGHTNESS**, **CONTRAST** and **COLOR** buttons all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

Appendix VIII: Block Heater Operating Instructions

For Use with A-TECH Sperm Quality Analyzers with Heating Requirements

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 IX: Glossary of Terms

	Parameter	SQA-Ve Terms	Definition
Sample Data	Serial number	SN	Serial Number of the SQA-Ve
	Date & time	DATE/TIME	The date and time the test was performed
	Stallion ID	STALLION ID	The identifying number of the stallion being tested
	Straw date	STRAW DATE	The date and time the frozen semen straw was produced
	Sample number	SAMPLE #	The number assigned to the semen sample
	Semen volume	SEMEN VOLUME	Raw, extended, cooled or frozen semen volume expressed in ml
Test Results	Sperm Concentration	CONC.	Total sperm concentration expressed in millions/ml
	Motile Sperm Concentration	MSC	Motile sperm concentration expressed in millions/ml
	Motility	MOTILITY	The ratio between the motile and total sperm cells in the sample, expressed in %.
	Progressive Motility	PROG. MOT.	The ratio between the progressively motile and total sperm cells in the sample, expressed in %.
	Normal Morphology	MORPHOLOGY	The ratio between the morphologically normal and total sperm cells in the sample, expressed in %.
	Average velocity	VELOC.	The average velocity of the motile spermatozoa in the sample, in microns per second.
	Total # sperm cells	# SPERM	The total number of sperm cells per ejaculate (Raw semen) or per semen volume (Frozen/Extended/Cooled)
	Total Motile Sperm	MOT. SPERM	The total number of motile sperm cells per ejaculate (Raw samples) or per semen volume (Frozen/Extended/Cooled)
	Total Progressively Motile Sperm	PROG. SPERM	The total number of progressively motile sperm cells per ejaculate (Raw samples) or per semen volume (Frozen/Extended)

Appendix X: SQA-Ve 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 (disabled), 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, 22 x 22 mm cover-slip.
- **I-Button:** Required to run tests (purchase from manufacturer)

Operating System

- **Control:** Keypad
- **Analysis Time:** 45 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:** 37^{0}C (98.6^{0}F).
- **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.

PC Compatibility

Minimum requirements for E-Sperm™ software

- **PC:** 1 GHz processor, Pentium 3
- **RAM:** 256 MB
- **CD ROM drive**
- **Ports:** One serial

Operating system compatibility: Windows XP and VISTA

Operational Temperature and Humidity

- System is operational at 15-40^{o}C.
- *NOTE*: SQA-Ve operates in a wide range of ambient temperatures however the system is calibrated to measure semen samples at 37^{0}C (98.6^{0}F).
- System is fully operational at up to 80% humidity and 31^{o}C.

Maintenance Schedule

- Cleaning daily and after every 50 tests (refer to User Guide – "Cleaning Instructions").

Manufacturer Recommendations

- Operate the SQA-Ve 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.
- 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

Time/Date: Manufacturer's local time/date

Automatically print: YES

Appendix XI: SQA-Ve EQUINE Product Performance Data

Performance Data Summary:

The performance of the SQA-Ve is summarized in the text, tables and graphs below. All values concerning sperm concentration measurements are expressed as 10^6 sperm cells per milliliter (M/ml). Motility values are expressed as a percent (%). Unless otherwise noted, all testing was performed using raw, extended, cooled and frozen equine semen samples. Manufacturers claims are generally lower than actual performance data. Please also note that Sensitivity & Specificity are clinical screening parameters that demonstrate the accuracy of device. Sensitivity demonstrates the ability of the SQA-Ve to correctly detect ABNORMAL cases. Specificity demonstrates the ability of the SQA-Ve to correctly detect NORMAL cases. Sensitivity & Specificity results are based on the cutoffs established by Society of Theriogenology. Each SQA-Ve device is biologically calibrated against two reference systems at Medical Electronic System's laboratory.

Abbreviations:

CONC: Sperm Concentration
CV: Coefficient of Variation
M/ml: Million per milliliter

Table 1. Dynamic Range

Sample Type	Concentration M/ml	% Motility	% Progressive Motility	% Normal Morphology
Raw	0-800	0-100	0-100	0-100
Extended	0-400	0-100	0-100	-
Frozen	0-1000	0-100	0-100	-

Sensitivity, specificity, precision and correlation to manual method established in the in-house and field clinical trials using equine semen samples.

Clinical claims:

Sensitivity
- Concentration: 90%
- Motility: 90%
- Prog. Motility: 90%

Specificity
- Concentration: 90%
- Motility: 90%
- Prog. Motility: 80%
- Morphology: 85%

Precision (Intra-device CVs)
- Conc.: 3%
- Motility: 3%
- Prog. Motility: 7%
- Morphology: 3%

Precision (Inter-device CVs)
- Conc.: 10%
- Motility: 10%
- Prog. Motility: 10%
- Morphology: 10%

Accuracy (regression coefficients of the dilution trend line)
- Conc.: 0.9
- MSC: 0.9

Table 2. Sensitivity/Specificity

SQA-Ve vs. Microscope	Sensitivity %	Specificity %	% False Positive	% False Negative
Sperm Concentration	**96.4**	**100.0**	**0**	**2.2**
Motility	**95.0**	**96.3**	**2.1**	**2.1**
Progressive Motility	**100.0**	**90.0**	**4.3**	**0**
Morphology	**-**	**93.3**	**6.3**	**6.3**

Table 3. Precision: SQA-Ve intra- and inter-device variability

Semen Parameters	Intra-device CV, %	Inter-device CV, %
Sperm Concentration	**2.0**	**7.0**
Motility	**0.3**	**7.2**
Prog. Motility	**5.6**	**8.6**
Morphology	**0.3**	**2.6**

Correlation to Manual Method

- Concentration: 0.9
- Motility: 0.9
- Prog. Motility: 0.8
- Morphology: 0.7

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).*
- *Correlation to Manual Method claims are less than actual correlations noted (Table 4).*

Method comparison:
The SQA-Ve was compared to the microscope based on WHO'99 manual guidelines. The SQA-Ve automated readings for sperm concentration, motility, progressive motility and morphology were compared to microscopic results. A Makler chamber was used according to the manufacturer's instructions for manual sperm concentration measurements. A microscope and standard slide were used to manually assess motility. Stained slides were used for the manual morphology examination. The protocols were based on WHO'99 manual and MES guidelines. The clinical trials were conducted at the Medisoos vet clinic. A total of 201 raw, extended, cooled and frozen semen samples were analyzed.

Accuracy: Dilution plots.
The accuracy of the SQA-Ve was assessed by diluting equine semen and analyzing the resulting sperm concentrations. Raw stallion semen was gradually diluted with commercial extender. Dilutions provided varying motile and total sperm concentrations. Semen samples were tested using the SQA-Ve and the results were plotted. Linear trend lines were established for Concentration and MSC vs. expected values.

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 analytical specificity optimization.

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

Table 4: Correlation to Manual Method

Semen Parameters	Correlation coefficients
Sperm Concentration, M/ml	**0.996**
Motility, %	**0.956**
Progressive Motility, %	**0.892**
Morphology, %	**0.744**

Fig. 1. Method comparison: Regression plot of SQA-Ve Sperm Concentration in Raw equine semen vs. manual results

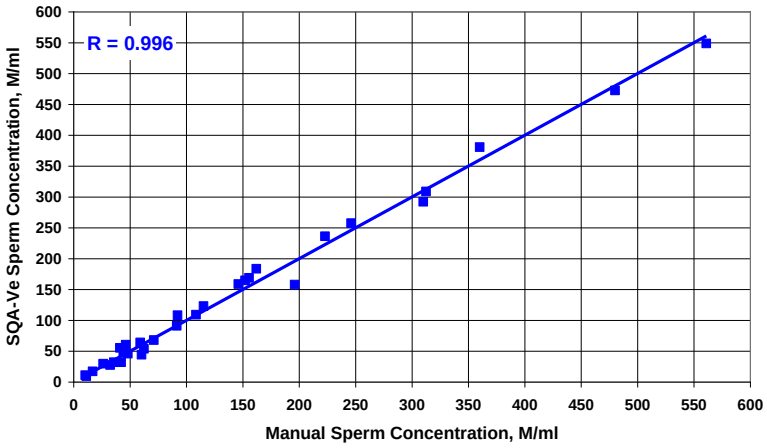
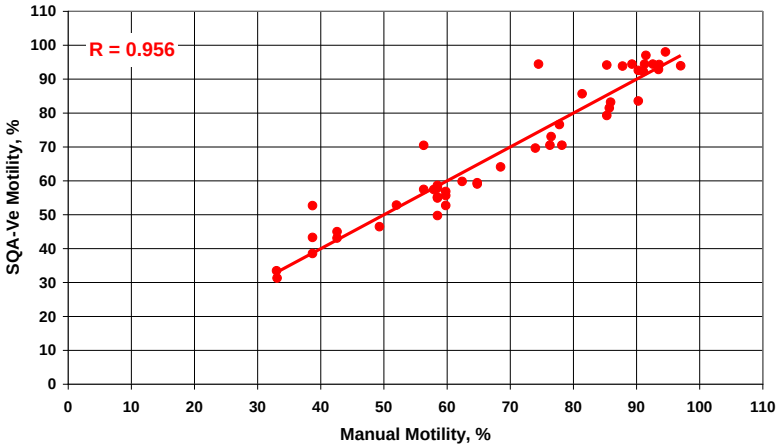


Fig. 2. Method comparison: Regression plot of SQA-Ve Motility in Raw equine semen vs. manual results



Performance parameters:

- Sensitivity and specificity were calculated using ROC analysis. Cutoffs normally used for sperm concentration, motility and morphology were used for the calculation of sensitivity, specificity, false positive and false negative parameters (Table 2).
- Precision of the SQA-Ve was estimated by calculation of the intra-device and inter-device coefficients of variation (CV) of duplicate measurements (Table 3). CV is calculated according to the formula:

CV = SD / MEAN x 100

The lower CV, the higher precision of the method.

- Correlation to manual method was established by calculating correlation coefficients (Table 4, Fig. 1-3).
- The accuracy of the SQA-Ve was determined by the regression coefficients of the dilution trendline (Fig. 4).

Fig. 3. Method comparison: Regression plot of SQA-Ve Morphology in Raw equine semen vs. manual results

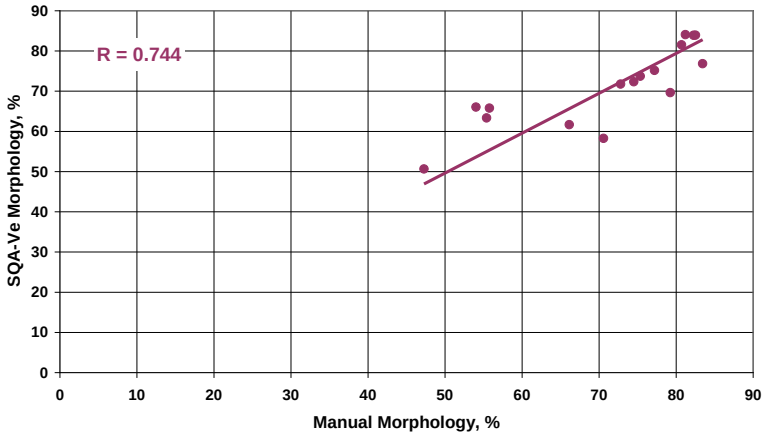
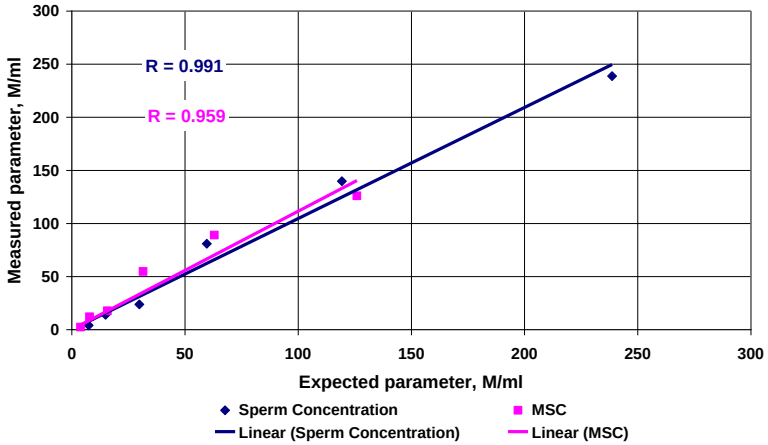


Fig. 4. Regression plot of SQA-Ve Conc. & MSC in Extended equine semen vs. expected values



Conclusions:

- *The SQA-Ve demonstrated high levels of sensitivity, specificity and correlation to the manual method.*
- *The SQA-Ve is precise and accurate with low coefficients of variation for all semen parameters assessed (<10%).*
- *The SQA-Ve can be used for semen quality assessment, dose preparation and to QC frozen equine semen.*