

1. Introduction

This working procedure refers to the verification of AT1000/KF1000 syringes by gravimetric analysis, based on ISO 8655 standard.

2. Principle

The AT1000/KF1000 syringe delivers chosen volumes with a chosen number of replicates.

The weight of each addition is recorded and converted to a volume to evaluate the syringe accuracy.

3. Setup

The syringe verification kit includes all of the necessary items: dispensing tips, tubes, syringe holder, pipette holder, support stand, software key and label with the license number. Make sure to keep the label with the license number. A license key is necessary to enable the syringe verification option on the instrument. The first time the option is selected, the instrument shows the MAC address and the Firmware version. Send the MAC address, firmware version and the license number to the manufacturer or a sales representative to get the license key.

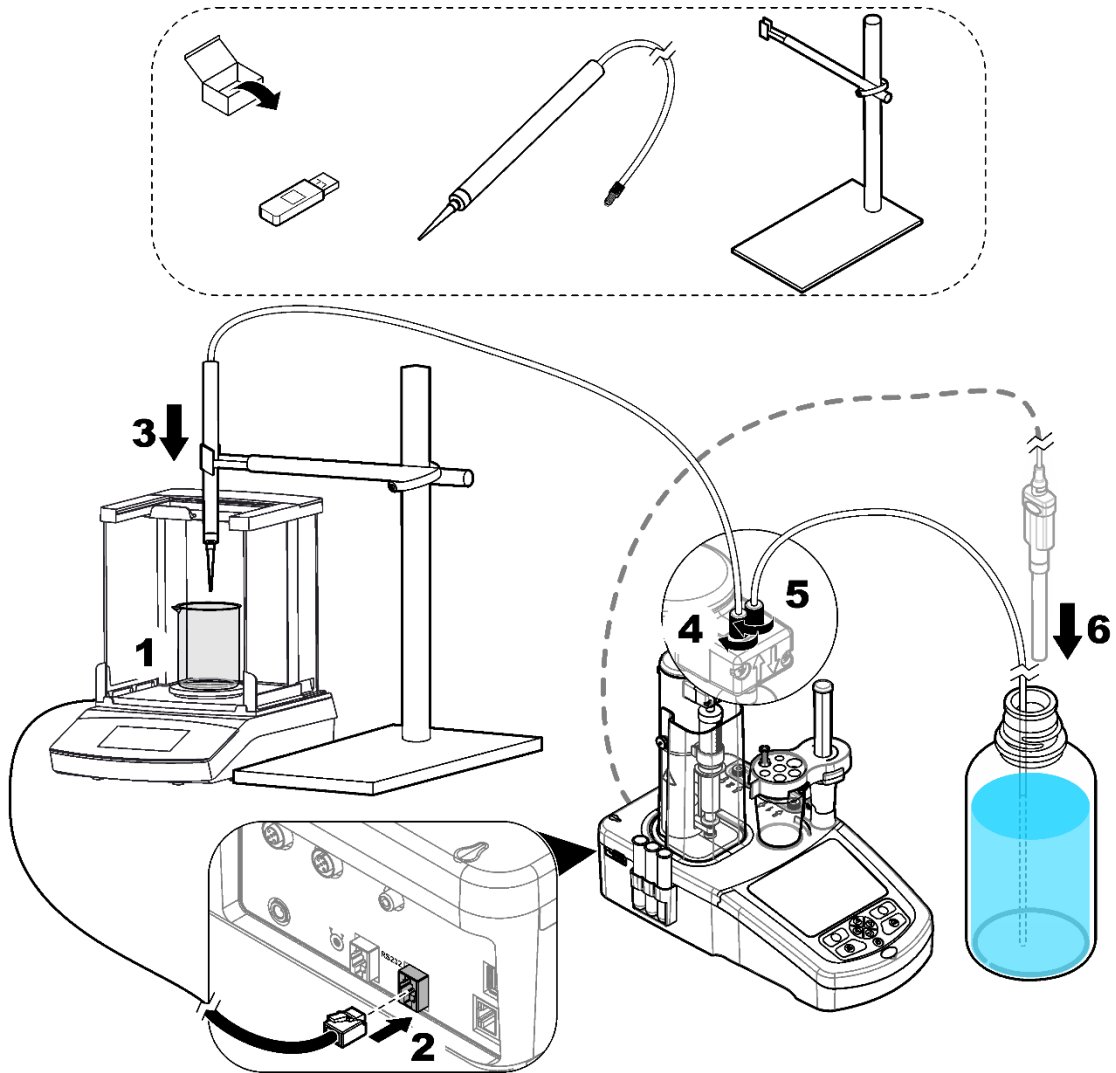
Note: Make sure that the AT1000/KF1000 Firmware version is 1.25 or higher.

Items to collect:

- Balance with resolution 0.1 mg, repeatability 0.2 mg and uncertainty 0.2 mg.
Note: For FullyAutomated mode, the recommended model is Sartorius Quintix224 plus applicable cables
- Support stand, bosshead and clamp
- Delivery tip plus tubing
- Temperature sensor, clean, put in the deionized water container
- Deionized water "quality 3" (ISO 3696)

Prepare the syringe verification kit as follows:

1. Put an empty beaker on the balance. Refer to the illustrated steps that follow.
2. For the fully automated mode, connect the balance to the instrument.
3. Install the support stand near the balance. Put the pipette tip adapter above the beaker on the balance.
4. Connect the tube from the pipette holder to the syringe outlet port.
5. Connect the tube from the water bottle to the syringe inlet port.
6. On the instrument, manually enter the temperature. As an alternative, connect and put a temperature sensor on the water bottle to get the temperature automatically.



4. Test conditions

Make sure to follow the test conditions given in the ISO 8655 standard. Some test conditions follow:

	Value	Uncertainty of measurement
Humidity	> 50 %	10%
Temperature	15-30°C (59-86°F) must be stable +/-0.5°C	0.2°C
Pressure	-	1 kPa
Duration of weighing for 1 volume	< 60 s	1 s

Put the system (AT1000/KF1000, syringe, balance, water) for a minimum of 2 hours in the lab before the test to get the lab temperature.

5. Settings

5.1. Default parameters

The default values are based on the ISO 8655 standard:

- 3 volumes: 10%; 50% and 100% of the syringe volume
- 10 replicates for each volume
- Error limits:

Syringe volume	Systematic error		Random error	
	µL	%	µL	%
2.5 mL	15.0	0.30	5.0	0.10
5 mL	15.0	0.30	5.0	0.10
10 mL	20.0	0.20	7.0	0.07
25 mL	50.0	0.20	17.5	0.07

5.2. Modes

DispenseOnly

The system only makes the additions. The user must do the validation between each addition. The balance weight must be taken by hand and no calculations are made.

Note: It is recommended to select the Dispense Only mode for 25-mL syringes, because with the standard test conditions the maximum weight of the balance will be reached.

PartiallyAutomated

The system makes the additions. The balance is not connected to the AT1000/KF1000: the user must enter the balance weight on the AT1000/KF1000 between each addition. The system makes the calculations.

FullyAutomated

The system makes the additions. The balance is connected to the AT1000/KF1000: the AT1000/KF1000 automatically retrieves balance weight between each addition. The system makes the calculations.

5.3. Temperature input

Water temperature is needed for Fully Automated and Partially Automated modes, as it is used in calculations. By default, temperature input is set on Manual and the water temperature must be entered before the test. To use Automatic temperature input, a temperature sensor must be plugged on the AT1000/KF1000. The sensor must be clean and must not release any electrolyte that might pollute the deionized water container. IntelliCAL CDC401 can be used, or a Legacy adapter with a suitable temperature sensor.

5.4. Balance settings for FullyAutomated mode

On the AT1000/KF1000

In Settings > Options: check Balance

On the balance Sartorius Quintix224

1) In Menu > Settings > USB port > RS232 Configuration:

Baudrate	Databits	Parity	Stopbits	Handshake
9600	8	None	1	Off

2) In Menu > Settings > Printout > Print function: chose **Autom. at stability**

cf the SOP HACH for AT/KF1000 Series "Use AT-TM-KF with Sartorius Balance"

6. Verification procedure

Important note: On KF1000 instruments, make sure to fully rinse the syringe with Methanol dry before the test to remove traces of titrant and fully rinse the syringe with Methanol dry after the test to remove traces of water.

6.1. Start screen

Syringe

If the AT1000/KF1000 has two syringes, select syringe 1 or syringe 2

Operator

Select user

Configure Syringe Test

mode: FullyAutomated, PartiallyAutomated, DispenseOnly

number of tested volumes: 1 to 10

volume unit: mL or %

tested volumes: 0 to maximum volume of the syringe*

number of replicates of each tested volume: 1 to 10

temperature mode: Manual or Automatic

error limits for results acceptance

test conditions: pressure, temperature, humidity

*errors due to evaporation are not considered in calculations so do not test volumes under 50 μ L

6.2. Syringe verification

Make sure there is an empty beaker on the balance, under the delivery tip. Make sure to record the weight after a whole drop is added from the tip to the beaker.

Syringe verification always starts with a priming step. The priming is a short purge cycle to avoid air bubbles in the tubing.

DispenseOnly

Press **Start**.

Wait for priming step to complete.

Press **Continue**.

After each addition, note the weight of the added volume and press **Continue**.

No error calculations are made by the AT1000/KF1000.

PartiallyAutomated

Press **Start**.

Wait for priming step to be done.

Enter the balance weight after priming and press **OK**.

After each addition, enter the balance weight and press **OK**. Do not make the balance zero between additions.

Error calculations are made by the AT1000/KF1000 and result of the test (passed or failed) is displayed according to the acceptance limits defined in the **Configure** menu.

FullyAutomated

Make sure the balance is connected to the AT1000/KF1000.

Press **Start**.

When the priming step is complete, the syringe test automatically starts.

The balance weight is automatically retrieved after each addition.

Error calculations are made by the AT1000/KF1000 and result of the test (passed or failed) is displayed according to the acceptance limits defined in the **Configure** menu.

NB: Push **Stop** to go back to the start screen and discard data (nothing is saved in the datalog). Push **Skip** to show and save results only for volumes that have been fully tested.

7. Results

7.1. Results calculations

The ISO 8655 standard gives the following equations:

Average measurement in mL:

$$\bar{V} = \frac{1}{n} \times \sum_{i=1}^n V_i$$

n being the number of replicates and V_i the added volume.

Systematic error in μL :

$$e_s = \bar{V} - V_s$$

and in %:

$$e_s = 100 \times (\bar{V} - V_s) / V_0$$

V_s being the tested volume and V_0 the nominal volume of the syringe.

Random error in μL :

$$s_r = \sqrt{\frac{\sum_{i=1}^n (V_i - \bar{V})^2}{n - 1}}$$

and in %:

$$CV = 100 \times \frac{s_r}{\bar{V}} \times \frac{V_s}{V_0}$$

7.2. Experimental results

These results are indicative and have been obtained for a 10 mL syringe, 3 volumes, 10 replicates for each volume.

Tested volume	[mL]	1	5	10
Average measurement	[mL]	0.9944	4.9984	9.9983
Systematic error	[μL]	5.6	1.6	1.7
Systematic error	[%]	0.06	0.02	0.02
Random error	[μL]	1.0	0.9	3.1
Random error	[%]	0.01	0.01	0.03

8. Recommendations

This application note is a memo aiming to gather the main information, make sure to read and follow the instructions on the ISO 8655 standard.