New Technological Guidelines Required for Pipette Management

(Proposal of a Pipette Management Method)

R&D Division 5
A&D Company, Limited
2010/9/2

First of All

Differences in dispensing volume of pipettes

- Directly influence the test results

Compliance with standards such as GLP or GCP

- For creating a SOP (Standard Operating Procedure), checking pipette performance

Problems in the workplace

- Avoiding troubles due to pipette inaccuracy
- Maintaining and controlling pipette performance

Pipette Management Tools

Pipette Leak Tester

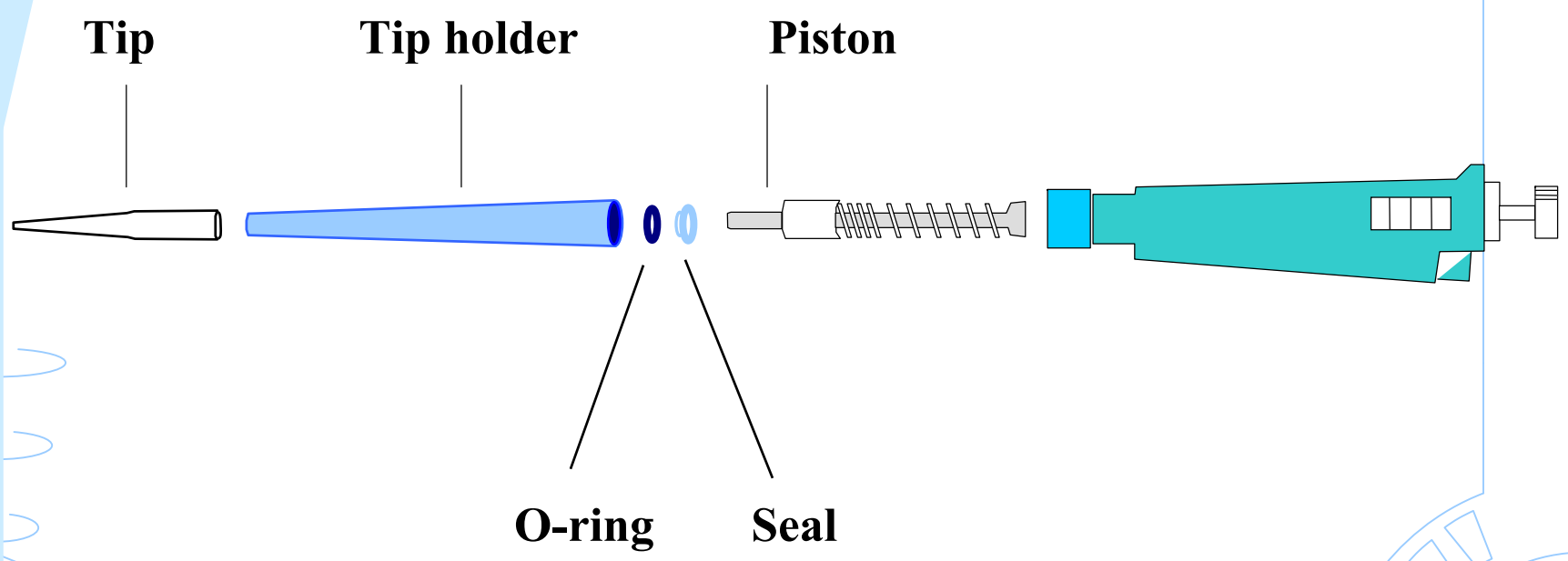
AD-1690

AD-4212B-PT
AD-4212A-PT

Pipette Accuracy Testers

FX-300i-PT

Pipette Structure (Air-displacement Type)



What Is a "Leak?"

- **Fissures/scratches on the tip holder**
- **Deterioration of the O-ring**
- **Abrasion/scratches of the seal**
- **Rust on the piston, etc.**

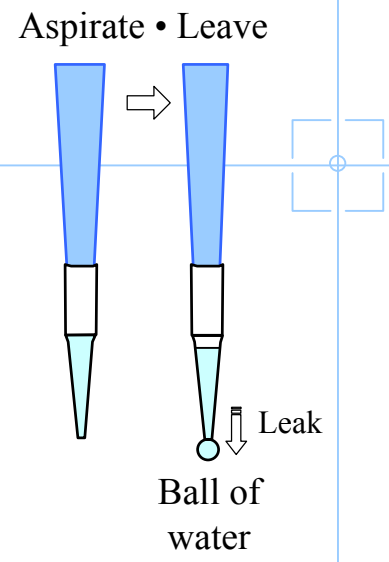
Unable to maintain the airtightness when moving the piston

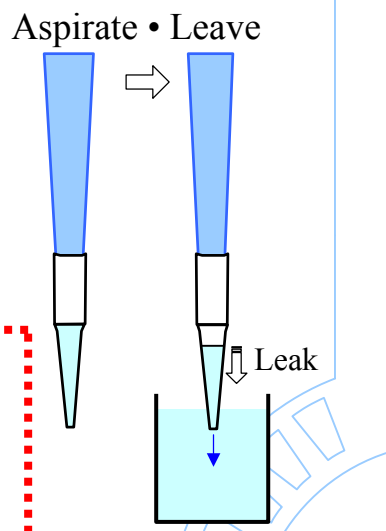
=> Unable to aspirate the set volume into the tip

Decreased dispensing volume from the pipette

General Leak Detection Method

- **Aspirate water and leave it. Check whether a ball of water appears at the tip end.**
- **Aspirate water and dip the tip end into the water container. Check whether the water level inside the tip lowers.**



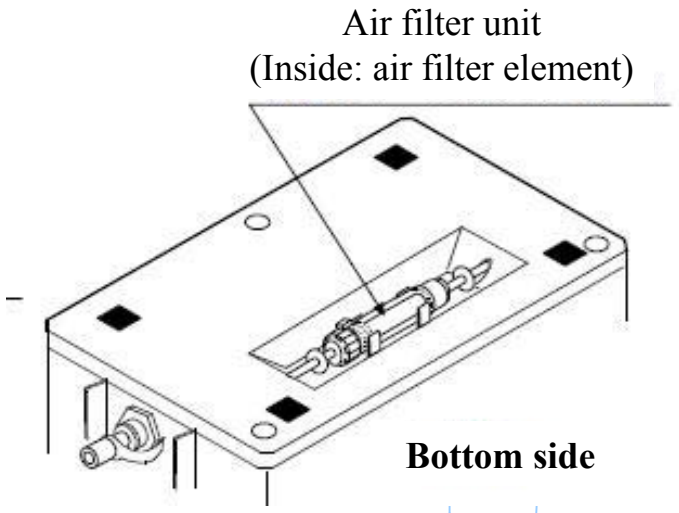


Visual check

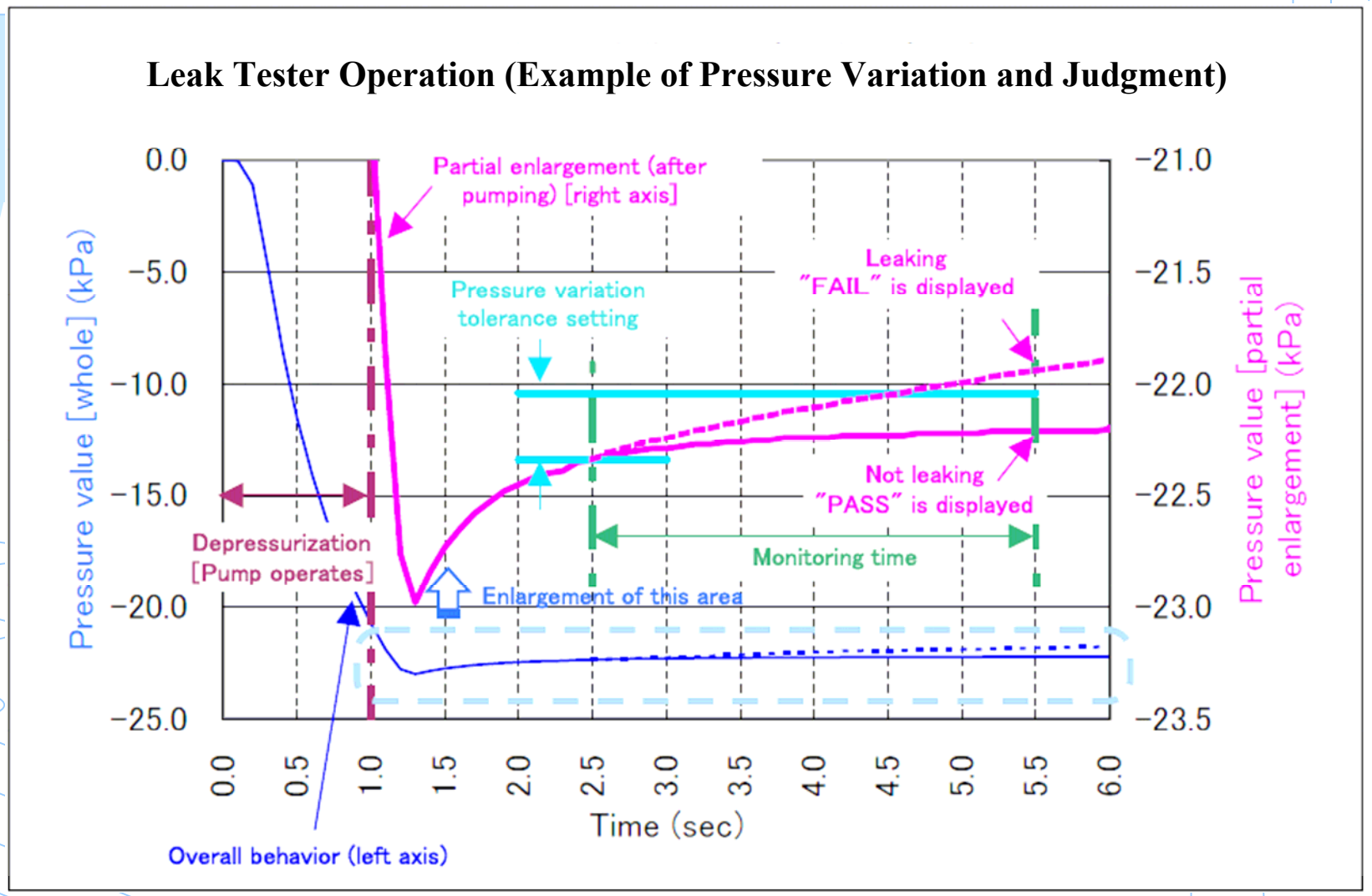
- **Difficult to make judgments with consistent criteria**
- **Some leaks may be missed depending on the degree of the leak.**

Checking Pipettes Using the Leak Tester

- **Actively reproduces the negative pressure that arises inside the pipette**
- **Detects leakage by monitoring pressure variation after the negative pressure is generated**
- **Prevents contamination (ingress of dust inside the pipette) by using the depressurization method**

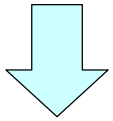


How the Leak Tester Operates



Benefits of Management With the Leak Tester

Easy (no water required), quick (approx. 6 sec.), and judgment using uniform criteria



Possible to detect pipette leaks efficiently as a daily task

Leak-test Results and Necessary Actions

- Pipette with leakage

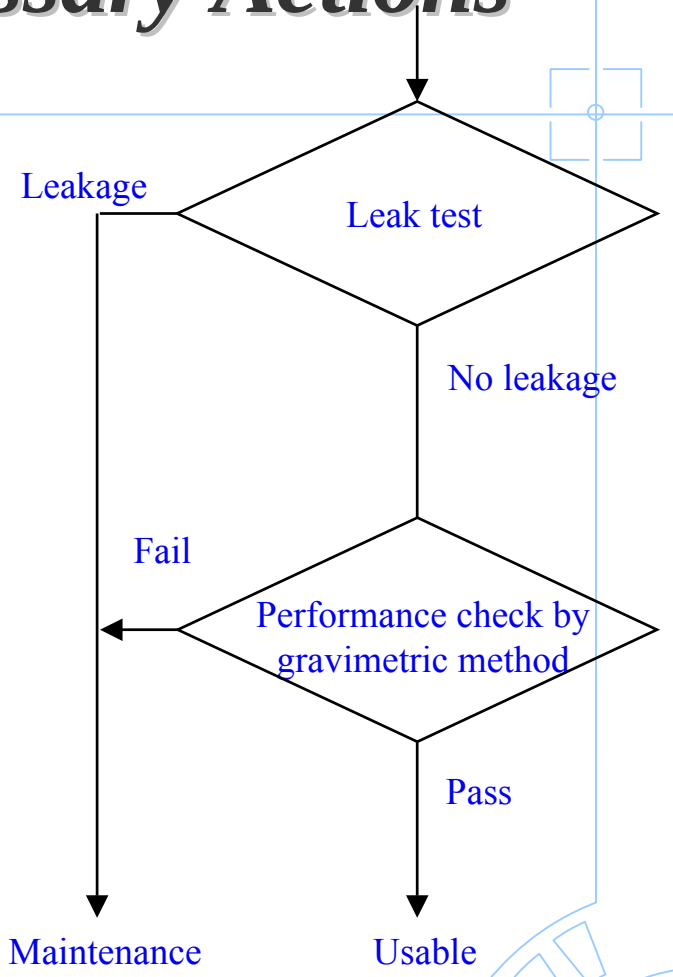
✈Unable to dispense the set volume

Maintenance required

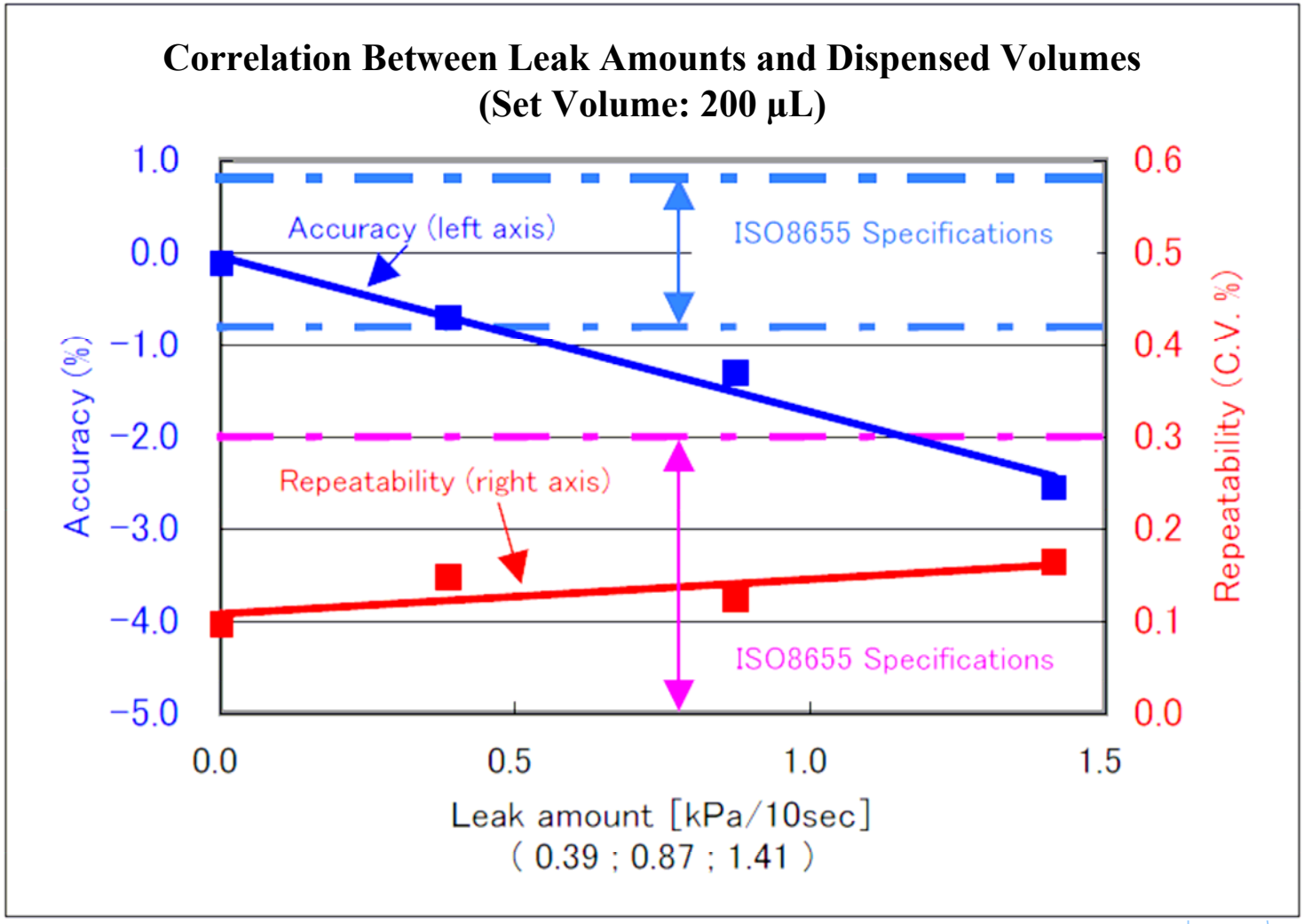
- Pipette without leakage

✈Not always able to dispense the set volume

Performance check by gravimetric method



Correlation Between Leak Amounts and Dispensed Volumes



Performance Check by Gravimetric Method

Measure the mass of the distilled water dispensed from the pipette and then convert it to a volume.

- ISO 8655(2002)

  ISO 8655-2 : Piston pipettes (specifications requirements)

  ISO 8655-6 : Gravimetric methods for the determination of measurement error

- JIS K0970(1989)

*** Distilled water is a standard substance with known properties**

*** ISO 8655 is commonly practiced.**

Mass-to-volume Conversion (ISO 8655 Gravimetric Method)

The mass of the distilled water dispensed from the pipette is multiplied with a conversion factor called Z factor to obtain the volume.

$$V_i = m_i \times Z$$

V_i : Volume(µL)
m_i : Mass of the distilled water (mg)
Z : Z factor (conversion factor, µL/mg)

"Z factor"
Factor to calculate a volume from a mass of distilled water, taking the temperature of the distilled water and the atmospheric pressure as parameters
The following are taken into account:
- **Density based on the water temperature**
- **Buoyancy of the distilled water (from atmospheric pressure and temperature)**

Influences of Water Temperature and Atmospheric Pressure

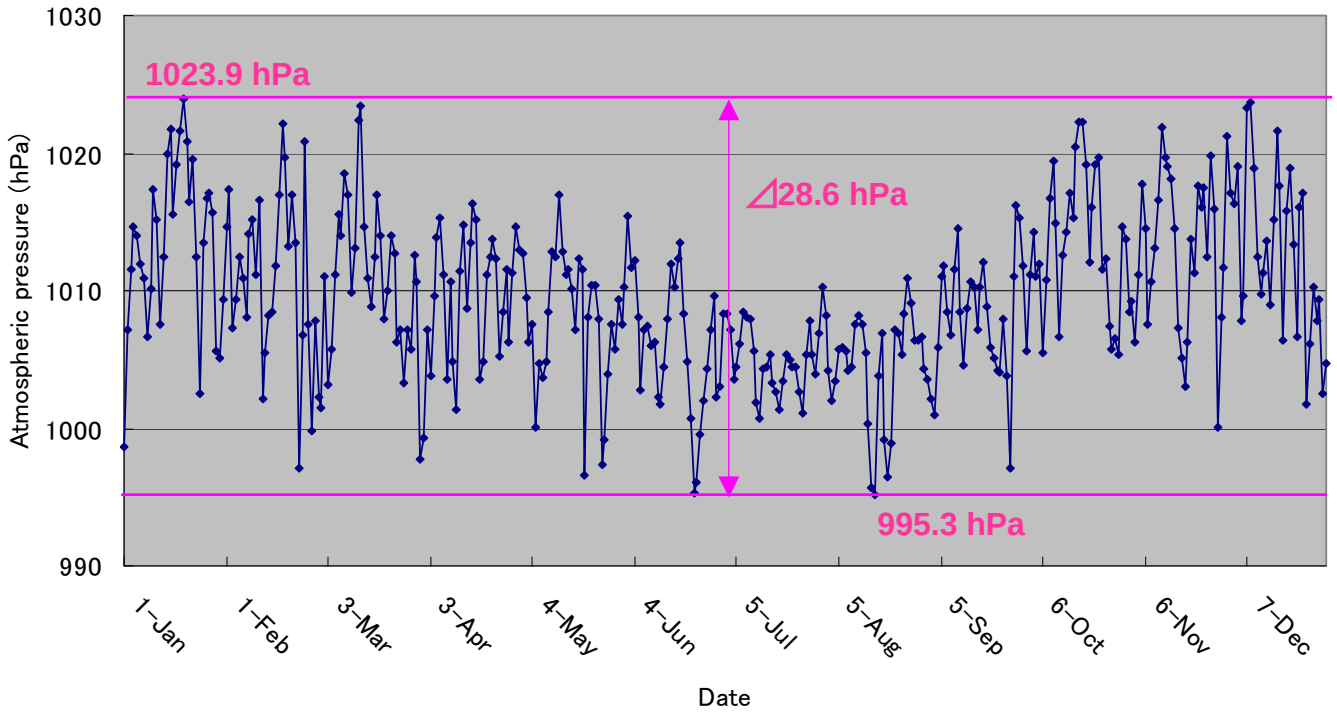
- **Influence of water temperature**
  Approx. 0.1% per change of 5 ˚C between 15 ˚C and 30 ˚C
- **Influence of atmospheric pressure**
  Approx. 0.005% per change of 50 hPa between 850 hPa and 1050 hPa

Z factor matrix shown by ISO8655 (extract)

Temp ˚C	Atmospheric pressure hPa						
	800	850	900	950	1000	1013	1050
15.0	1.0017	1.0018	1.0019	1.0019	1.0020	1.0020	1.0020
15.5	1.0018	1.0019	1.0019	1.0020	1.0020	1.0020	1.0021
16.0	1.0019	1.0020	1.0020	1.0021	1.0021	1.0021	1.0022
16.5	1.0020	1.0020	1.0021	1.0021	1.0022	1.0022	1.0022
17.0	1.0021	1.0021	1.0022	1.0022	1.0023	1.0023	1.0023
17.5	1.0022	1.0022	1.0023	1.0023	1.0024	1.0024	1.0024
18.0	1.0022	1.0023	1.0023	1.0024	1.0025	1.0025	1.0025
18.5	1.0023	1.0024	1.0024	1.0025	1.0025	1.0026	1.0026
19.0	1.0024	1.0025	1.0025	1.0026	1.0026	1.0027	1.0027
19.5	1.0025	1.0026	1.0026	1.0027	1.0027	1.0028	1.0028
20.0	1.0026	1.0027	1.0027	1.0028	1.0028	1.0029	1.0029
20.5	1.0027	1.0028	1.0028	1.0029	1.0029	1.0030	1.0030
21.0	1.0028	1.0029	1.0029	1.0030	1.0031	1.0031	1.0031
21.5	1.0030	1.0030	1.0031	1.0031	1.0032	1.0032	1.0032

Influence of Atmospheric Pressure

Changes in atmospheric pressure in Tokyo (in 2008)



- **Pressure changes at one location is normally with ±15 hPa.**
- **Even though the average (fixed value) is used, the pressure fluctuation can be locked in easily between ±30 hPa.**
- **Influence on the mass-to-volume conversion is within ±0.003%.**

✈Influence of atmospheric pressure is negligible. => A representative value of the measurement location is acceptable.

Pipette Accuracy Tester

Includes the following:

- A balance that measures the mass of the distilled water dispensed from the pipette
- An evaporation trap that effectively prevents the evaporation of the dispensed water
- A thermometer that measures the temperature of the distilled water
- Software (WinCT-Pipette) that performs mass-to-volume conversion based on the mass output from the balance and the preliminarily input temperature of the distilled water and atmospheric pressure and then makes pass/fail judgments

Models	AD-4212B-PT	AD-4212A-PT	FX-300i-PT
Weighing Capacity	110 g / 31 g /5.1 g	110 g	320 g
Minimum Weighing Value	0.1 mg / 0.01 mg / 0.001mg	0.1 mg	1 mg

AD-4212B-PT

Pipette specifications in accordance with ISO 8655

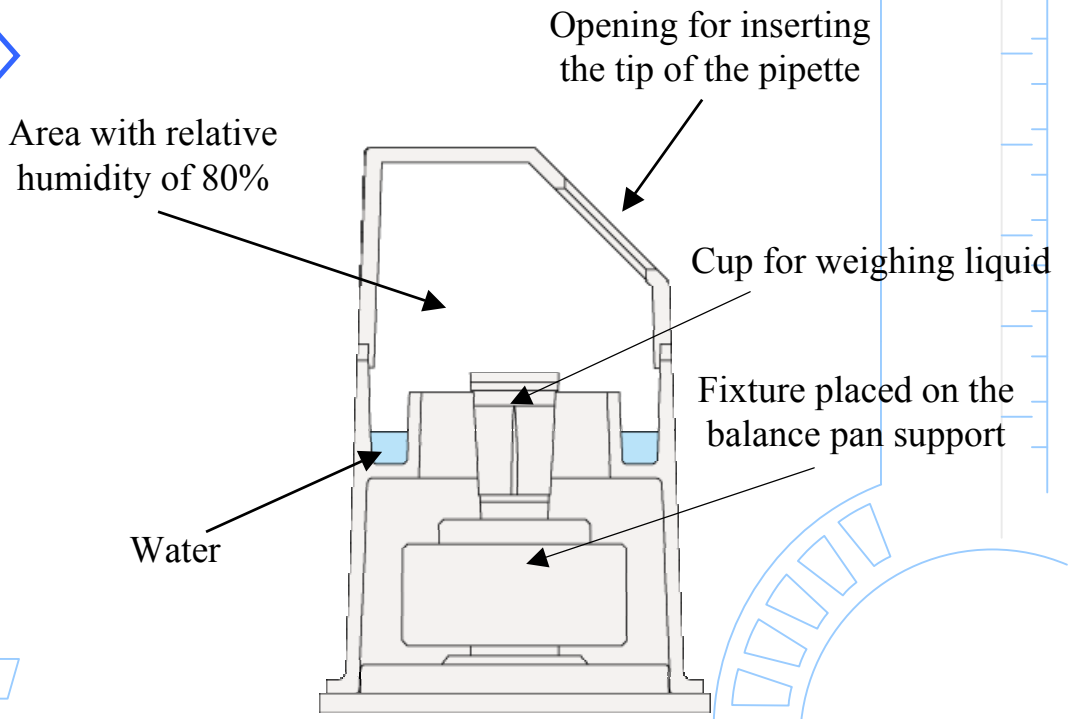
Pipette Nominal Volume	ISO8655 Requirements: Maximum Permissible Error – Accuracy		Repeatability		Balance Minimum Weighing Value
µL	± %	± µL	%	µL	mg
1	5.0	0.05	5	0.05	0.001
2	4.0	0.08	2	0.04	
5	2.5	0.125	1.5	0.075	
10	1.2	0.12	0.8	0.08	
20	1.0	0.2	0.5	0.1	0.01
50	1.0	0.5	0.4	0.2	
100	0.8	0.8	0.3	0.3	
200	0.8	1.6	0.3	0.6	0.1
500	0.8	4	0.3	1.5	
1000	0.8	8	0.3	3.0	
2000	0.8	16	0.3	6.0	
5000	0.8	40	0.3	15	
10000	0.6	60	0.3	30	
Daily inspection, simplified verification					

Pipette Accuracy Tester corresponding to the requirements

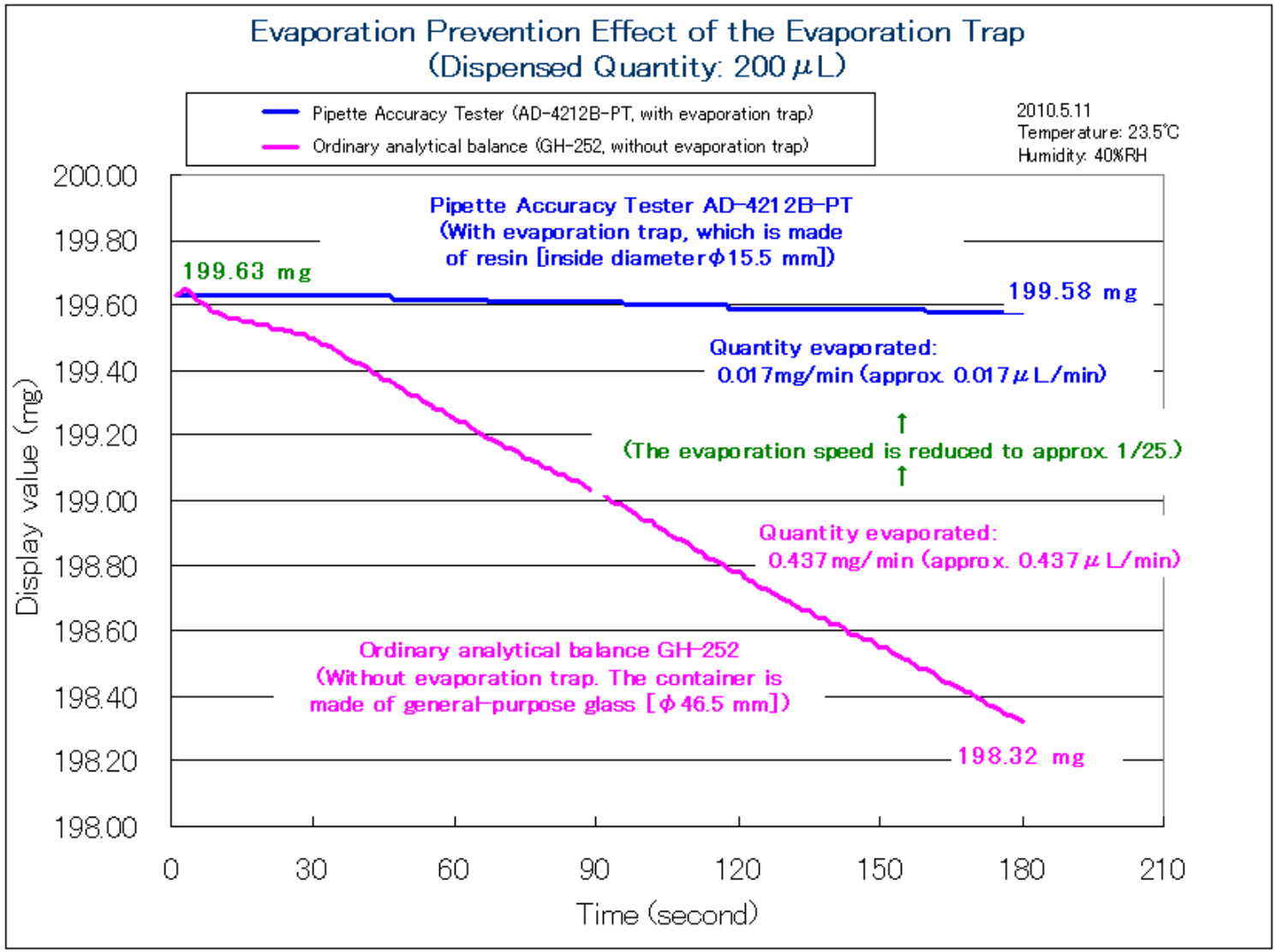
- AD-4212B-PT
- AD-4212A-PT
- FX-300i-PT

Evaporation of the Distilled Water Dispensed From the Pipette

Structure of the evaporation trap

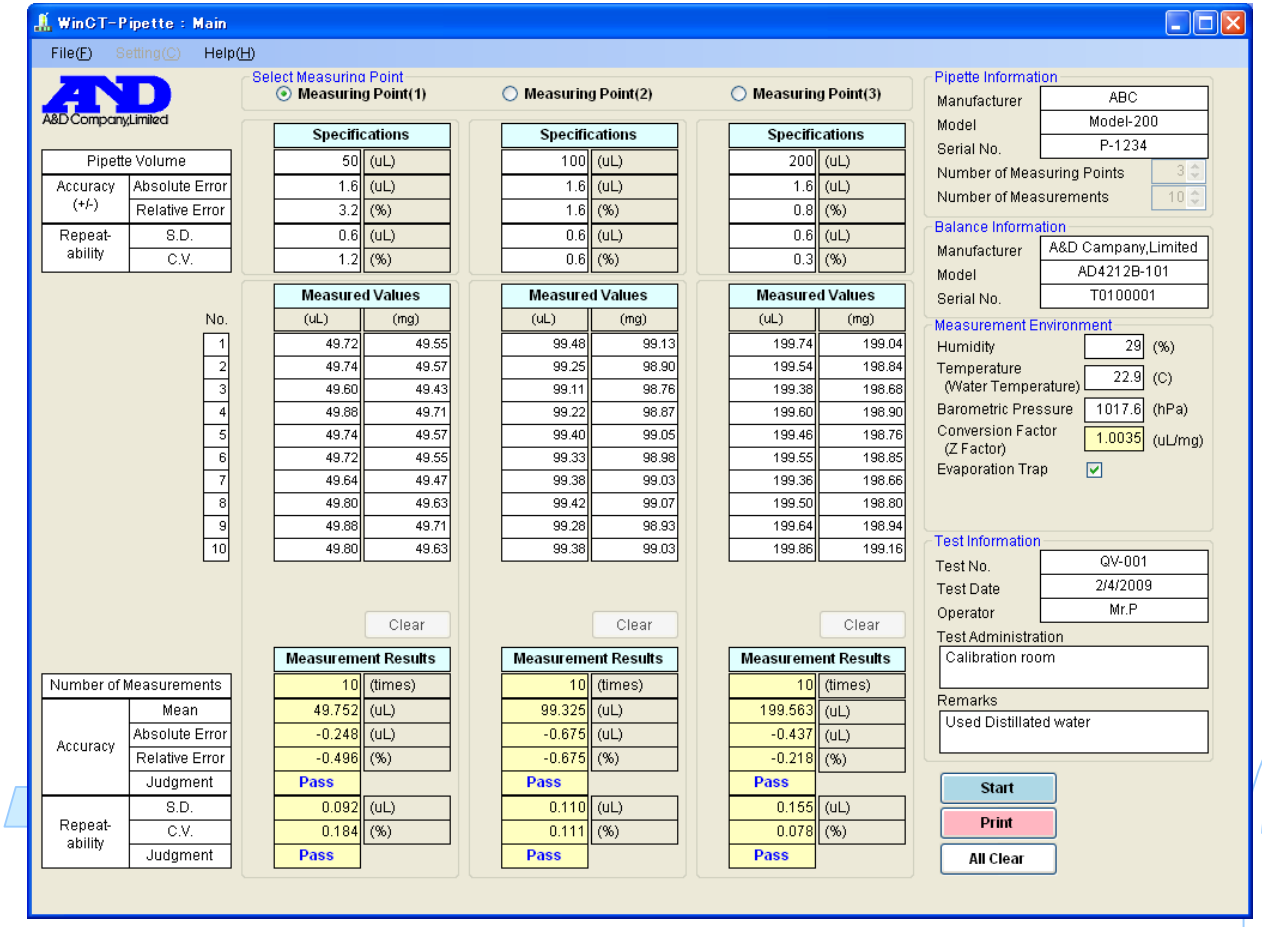


Effectiveness of the Evaporation Trap



WinCT-Pipette (Software to Calculate Volumes)

- **Possible to make pass/fail judgments according to the required level of accuracy**
- **Possible to save information on pipettes, balance used, and environmental conditions, and output the results**



Summary

- **Use of the leak tester for pipettes**
  Possible to detect leaks using uniform criteria easily and quickly

- **Use of the pipette accuracy testers**
  Possible to measure actual volumes dispensed from pipettes

→ Adoption of these testers in the workplace

Detect pipette failures efficiently as a daily task
→ Improve productivity

Make a quality management report at the actual workplace
→ Exercise effective QMS

A&D Won the Kardux Cup for the AD-4212B-PT Pipette Accuracy Tester!

The Kardux Cup is awarded by the International Society of Weighing and Measurement (ISWM) "to recognize a company, group or individual for the most outstanding new technical achievement of the past two years that positively impacts the weighing and measurement industry."

May 2010


Thank You for Your Attention