

Transferpettor

Testing Instructions (SOP)

February 2016

1. Introduction

The standards DIN EN ISO 8655 describe both the design and the testing of the piston operated pipette Transferpettor. The following Testing Instructions describe how to apply the ISO standard in practice.

We recommend a testing of the Transferpettor every 3 - 12 months. This interval may be adjusted to individual requirements. For example, when working very frequently or when using aggressive media, the instrument should be tested more frequently.

These Instructions may also be used as a basis for the supervision of testing devices to DIN EN ISO 9001, DIN EN ISO 10012 and DIN EN ISO/IEC 17025.

For the regular examinations required by DIN EN ISO 9001, DIN EN ISO 10012, DIN EN ISO/IEC 17025 and the GLP Guidelines, BRAND (see chapter 7) additionally provides a calibration service. Your instrument will be returned within a few days together with a test report. For more detailed information, please contact your labware supplier.



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2. Preparation for testing and visual examination

2.1 Instrument identification

- Read instrument type and nominal capacity ⇒ Enter in Test Record (1).
- Read Serial Number ⇒ Enter number in Test Record (1).
- Read customer's identification, if present ⇒ Enter identification in Test Record (1).

2.2 Minimal configuration of the Transferpettor

- Transferpettor ⇒ Use only manufacturer's original parts.
- Cap / capillary
- Seal

2.3 Cleaning

- Cap / capillary clean?
 - ⇒ No media residues!
 - ⇒ For cleaning, remove the cap / capillary.
 - ⇒ Wipe exterior with soft cloth.
- Is the exterior sufficiently clean?
 - ⇒ Sight soiling is permissible.

2.4 Visual examination for damage

- Housing ⇒ Mechanical damages?
- Cap / capillary / Seal
 - ⇒ Deformations? Mechanical damages?
 - ⇒ Enter result in the Test Record (2).

Possible faults and resulting measures:

Fault	Possible causes	Measures
Cap / capillary damaged	<ul style="list-style-type: none">■ Liquid containing solids;■ wear	⇒ Replace cap / capillary; see Operating Manual
Seal damaged	<ul style="list-style-type: none">■ Liquid containing solids;■ wear	⇒ Replace seal; see Operating Manual

2.5 Functional test

- Mount the seal and the cap / capillary properly.
 - ⇒ Carefully observe the Operating Manual.
 - ⇒ Make sure that the cap / capillary is placed properly

- Set to nominal volume, and move lever to "fix".
 - ⇒ Volume adjusting knob easy to turn?
Locking mechanism firm?
 - ⇒ Instruments up to 200 µl: The lower end of the seal must be flush with the ring mark of the capillary; this is important for an accurate volume check.

- Press pipetting button to the stop.
 - ⇒ Instruments from 0.5 ml upward: Button and seal must reach their stop simultaneously; important for volume check.

- Immerse the cap / capillary into the water.
 - ⇒ Submerge a few millimeters into the deionized water. The liquid must be sucked in slowly and evenly.

- Wipe off the outside of the cap / capillary.
 - ⇒ Use a lint-free cloth.
Avoid touching the opening; this would extract liquid.

- Hold the filled cap / capillary vertically, and observe if a drop forms at the tip.
 - ⇒ Hold for approx. 10 seconds.

- Release the testing liquid.
 - ⇒ Lean the cap / capillary against the wall of the vessel.
Wipe off the last drop against the wall.
 - ⇒ The pipetting button must move smoothly and without jerks.
 - ⇒ Enter the result of the functional test in the Test Record (3).

Possible faults and resulting measures:

Fault	Possible causes	Measures
Aspiration not possible or very slow	■ Cap / capillary or seal is soiled	⇒ Clean the instrument; see Operating Manual
Drop forming at the pipette tip	■ Cap / capillary damaged	⇒ Replace cap / capillary; see Operating Manual
	■ Seal damaged	⇒ Replace the seal; see Operating Manual

3. Equipment required for testing

3.1 For Transferpettor with nominal volume $\geq 50 \mu\text{l}$

- **Recipient vessel** filled with deionised or distilled water (e.g., Erlenmeyer flask, glass beaker) (according to ISO 3696, at least quality 3). \Rightarrow Match temperature of water and room to 1 °C accuracy. Prevent cooling of the water in the container due to evaporation.
- **Weighing vessel** filled with some water. (e.g., Erlenmeyer flask, glass beaker). \Rightarrow Bottom must be covered at least. In case of testing volumes < 100 μl , protect against evaporation (see 3.2)
- **Balance**, recommended specifications:

Selected volume ^a of apparatus under test V	Resolution mg	Repeatability and linearity mg	Standard uncertainty of measurement μl
20 μl < V \leq 100 μl	0,01	0,02	0,02
100 μl < V \leq 1000 μl	0,1	0,2	0,2
1 ml < V \leq 10 ml	0,1	0,2	0,2

^a For practical purposes, the nominal volume may be used to choose the balance.

- **Thermometer** with a measuring error of maximum $\Rightarrow \pm 0,2 \text{ }^\circ\text{C}$
- **Hygrometer**: Considering the measuring tolerance of the hygrometer a relative atmospheric humidity of at least 40% should be reached.
- Place the Transferpettor including accessories into the testing room for at least 1 hour (unpacked). \Rightarrow Allow instrument to adjust to room temperature.

3.2 For Transferpettor with nominal volume $\leq 50 \mu\text{l}$

- **Recipient vessel** filled with deionised or distilled water (e.g., Erlenmeyer flask, glass beaker) (according to ISO 3696, at least quality 3). \Rightarrow Match temperature of water and room to 1 °C accuracy. Prevent cooling of the water in the container due to evaporation.
- **Disposable micro pipettes** intraEND 100 μl ; Pipette holder. \Rightarrow Supplier: BRAND GMBH + CO KG
Postfach 11 55
97861 Wertheim
 \Rightarrow Ordering information: IntraEND 100 μl Cat. No. 7091 44
Pipette holder Cat. No. 7086 05
- **Balance**, recommended specifications:

Selected volume ^a of apparatus under test V	Resolution mg	Repeatability and linearity mg	Standard uncertainty of measurement mg
1 μl < V \leq 10 μl	0,001	0,002	0,002
10 μl < V \leq 50 μl	0,01	0,02	0,02

^a For practical purposes, the nominal volume may be used to choose the balance.

- **Thermometer** with a measuring error of maximum $\Rightarrow \pm 0,2 \text{ }^\circ\text{C}$
- **Hygrometer**: Considering the measuring tolerance of the hygrometer a relative atmospheric humidity of at least 40% should be reached.
- Place the Transferpettor including accessories into the testing room for at least 1 hour (unpacked). \Rightarrow Allow instrument to adjust to room temperature.

Traceability of test results to national standards

Through the use of calibrated testing devices (balance and thermometer), the requirement of DIN EN ISO 9001, DIN EN ISO 10012 and DIN EN ISO/IEC 17025 to refer the test to the national standard is fulfilled. The calibration of the balance e.g. can be carried out either by DAkkS calibration or official certification of the balance, or by calibrating the balance with appropriate weights that are traced to the national standard. The calibration of the thermometer, hygrometer and barometer can also be carried out by DAkkS calibration or official certification, or by a comparison with thermometers that are traced to the national standard (under defined conditions).

4. Gravimetric test (Calibration)

4.1 For Transferpettor with nominal volume $\geq 50 \mu\text{l}$

1. Set the nominal volume.
2. Determine temperature of the deionized water for testing. \Rightarrow Enter temperature into Test Record (4).
3. Pre-rinse the cap / capillary once. \Rightarrow Take in testing liquid once, and release again, the cap/capillary must be bubble-free.
4. Place the weighing vessel (filled with some deionized water) on the balance, and tare the balance.
5. Press the pipetting button to the stop.
6. Immerse the cap / capillary into the water. \Rightarrow Immerse vertically approx. 2 - 3 mm.
7. Take up testing liquid from the recipient. \Rightarrow Release pipetting button steadily.
 \Rightarrow Leave tip immersed for approx. 1 second.
 \Rightarrow Lightly wipe off pipette tip in an angle about $30^\circ - 45^\circ$ against wall of vessel.
Capillary: The meniscus must be level with the lower edge of the capillary.
8. Wipe the outside of the cap / capillary. \Rightarrow Use a lint-free cloth. Avoid touching the opening; this would extract liquid.
9. Remove weighing vessel from the balance.
10. Release the sample into the weighing vessel. \Rightarrow Lean cap / capillary against wall of vessel in an angle about $30^\circ - 45^\circ$.
 \Rightarrow Press pipetting button at steady speed to its stop and keep it there.
 \Rightarrow Wipe off cap / capillary against the wall of the vessel in an angle about $30^\circ - 45^\circ$; withdraw the pipette upwards while doing so.
 \Rightarrow Lightly wipe off pipette tip against wall of vessel (approx. 10 mm).
11. Place the weighing vessel on the balance. \Rightarrow Enter weighing values into Test Record (5).
12. Re-tare the balance.
13. Follow steps 5 - 10 times. \Rightarrow Enter weighing values into Test Record (5).
14. Along the same lines, pipette 50 % and then 20 % of the nominal volume. \Rightarrow Only applicable for digital instruments.
 \Rightarrow Enter weighing values into Test Record (5), resulting in a total of 30 values (Digital type) or 10 values (Fix type).

4.2 For Transferpettor with nominal volume $\leq 50 \mu\text{l}$

Note:

With pipettes of a nominal volume $\leq 50 \mu\text{l}$, the tolerance limits are usually smaller than $0.5 \mu\text{l}$. Due to this small tolerance limit, the evaporation of water during the test procedure has a relatively large influence on the result. Therefore, the testing of pipettes of this size requires a test procedure which largely prevents evaporation. If a special pipette calibration balance with a so-called evaporation trap is used, the process described in 4.1 can be used. For this purpose, BRAND has specially developed the following test procedure. The weighing vessel used is a disposable micropipette which virtually eliminates evaporation.

1. Set the nominal volume.
2. Determine temperature of the deionized water for testing. \Rightarrow Enter temperature into Test Record (4).
3. Pre-rinse the capillary once. \Rightarrow Take in testing liquid once, and release again, the cap/capillary must be bubble-free.
4. Mount a disposable micropipette on the pipette holder. Place upon the balance and tare the balance.
5. Press the pipetting lever to first stop.
6. Dip the capillary into the liquid. \Rightarrow Immerse vertically approx. 2 - 3 mm.
7. Take up testing liquid from the recipient. \Rightarrow Release pipetting lever steadily.
 \Rightarrow Leave tip immersed for approx. 1 second.
 \Rightarrow Lightly wipe off pipette tip against wall of vessel in an angle about $30 - 45^\circ$. The meniscus must be level with the lower edge of the capillary.
8. Wipe the outside of the capillary. \Rightarrow Take care not to touch the capillary orifice because this would cause liquid to sucked out.
9. Remove disposable micropipette on the balance. \Rightarrow The pipette holder facilitates handling.
10. Release testing liquid into the disposable micropipette. \Rightarrow Push the disposable micropipette upon the pipette tip as far as it will go.
 \Rightarrow Press pipetting lever at steady speed to its stop and keep it there.
 \Rightarrow **Keep pipetting lever at second stop** while pulling the disposable micropipette off the pipette tip. No wiping off is needed.
 \Rightarrow Release pipetting lever.
11. Place the disposable micropipette on the balance. \Rightarrow Enter weighing values into Test Record (5).
12. Re-tare the balance with a new disposable micropipette.
13. Repeat steps 4 - 10 ten times. \Rightarrow Enter weighing values into Test Record (5).
14. Along the same lines, pipette 50 % and then 20 % of the nominal volume. \Rightarrow Only for digital type instruments!
 \Rightarrow Enter weighing values into Test Record (5), resulting in a total of 30 values (Digital type) or 10 values (Fix type).

5. Evaluation of gravimetric test results

The values obtained by weighing during the gravimetric test are only the mass values of the dispensed volume. In order to obtain the actual volume, an adjustment calculation must be carried out.

To facilitate your calculations and evaluations, we recommend the use of the Windows-compatible calibration software EASYCAL™ from BRAND.

The following calculations must be carried out:

1. Mean weight:

$$\bar{x} = \frac{x_1 + x_2 + x_3 + x_4 + x_5 + x_6 + x_7 + x_8 + x_9 + x_{10}}{10}$$

2. Mean volume:

$$\bar{V} = \bar{x} \cdot Z$$

⇒ For factor Z, see Table 1.

⇒ Enter value into Test Record (6a).

3. Standard deviation:

$$s = Z \cdot \sqrt{\frac{(x_1 - \bar{x})^2 + (x_2 - \bar{x})^2 + (x_3 - \bar{x})^2 + (x_4 - \bar{x})^2 + \dots + (x_{10} - \bar{x})^2}{9}}$$

⇒ For factor Z, see Table 1

⇒ Enter value into Test Record (6b)

4. Accuracy:

$$A [\%] = \frac{\bar{V} - V_{\text{nominal value}}}{V_{\text{nominal value}}} \cdot 100$$

⇒ Enter value into Test Record (6c)

5. Coefficient of variation:

$$CV [\%] = \frac{s \cdot 100}{\bar{V}}$$

⇒ Enter value into Test Record (6d)

Comparison actual/nominal values:

- See tables of error limits and accuracy for each instrument on the following pages, or define your own error limits.

⇒ Enter value into Test Record (6e, f)

Result:

⇒ Enter value into Test Record (6g)

If calculated values A [%] and CV [%] are smaller than or equal to the error limits, the instrument is in good working order.

If the calculated values are **larger** than the error limits:

- Verify if the above instructions have been carefully followed step by step.
- Observe the suggestions under "Troubleshooting" in the Operating Manual.
- Calibrate the Transferpettor as described in the Operating Manual (recalibration is only possible with recent models).

If these measures are not successful, we recommend to use the BRAND calibration service (see page 11).

Possible volumetric faults and resulting measures:

Fault	Possible causes	Measures
Volume too small	<ul style="list-style-type: none"> ■ Pipetting button not pressed all the way ■ Cap / capillary not positioned properly ■ Liquid residue in cap / capillary ■ Air bubbles in the liquid taken up inside cap / capillary 	<ul style="list-style-type: none"> ⇒ Press the pipetting button to its stop. ⇒ Mount the cap/capillary properly; see Operating Manual ⇒ Cap / capillary or seal damaged; replace as described in Operating Manual ⇒ Liquid taken up too fast.
Volume too large	<ul style="list-style-type: none"> ■ Cap / capillary not positioned properly 	<ul style="list-style-type: none"> ⇒ Mount the cap / capillary properly; see Operating Manual

Table 1:

Excerpt from DIN EN ISO 8655, part 6.
Table refers to 1013 hPa
Validity range 980 hPa to 1040 hPa.

Temperature °C	Factor Z ml/g	Temperature °C	Factor Z ml/g
15	1.0020	23	1.0035
15.5	1.0020	23.5	1.0036
16	1.0021	24	1.0038
16.5	1.0022	24.5	1.0039
17	1.0023	25	1.0040
17.5	1.0024	25.5	1.0041
18	1.0025	26	1.0043
18.5	1.0026	26.5	1.0044
19	1.0027	27	1.0045
19.5	1.0028	27.5	1.0047
20	1.0029	28	1.0048
20.5	1.0030	28.5	1.0050
21	1.0031	29	1.0051
21.5	1.0032	29.5	1.0052
22	1.0033	30	1.0054
22.5	1.0034		

Table 2:

Excerpt from DIN EN ISO 8655, part 2.

Nominal volume µl	1	2	5	10	20	50	100	200	500
A ≤ ±%	5	4	2.5	1.2	1.0	1.0	0.8	0.8	0.8
CV ≤ %	5	2	1.5	0.8	0.5	0.4	0.4	0.4	0.3
Nominal volume ml	1.0	2.0	5.0	10					
A ≤ ±%	0.8	0.8	0.8	0.6					
CV ≤ %	0.3	0.3	0.3	0.3					

Table 3:

Volume error limits for piston operated pipettes:

The stated volume error limits are final test values relative to the nominal capacity. These error limits refer to new instruments under optimized testing conditions (qualified operators and standardized ambience conditions).

Nominal volume µl	Accuracy Value 6e ≤ ± %	Coefficient of variation Value 6f ≤ %
Fix type		
1	4	4
2	2.5	2
3	1.7	1.5
4	1.3	1
5	1	0.8
10	1	0.8
20	0.8	0.5
25	0.8	0.4
50	0.6	0.4
100	0.6	0.4
200	0.5	0.2
Digital type		
10/5/2.5	1/2/5	0.8/1.6/4
25/12.5/5	0.8/1.6/4	0.5/1/2.5
50/25/10	0.6/1.2/3	0.4/0.8/2
100/50/20	0.6/1.2/3	0.4/0.8/2
500/250/100	0.5/1/2.5	0.2/0.4/1
1000/500/200	0.5/1/2.5	0.2/0.4/1
Digital type (ml)		
5/2.5/1	0.5/1/2.5	0.2/0.4/1
10/5/2	0.5/1/2.5	0.2/0.4/1

For calibration, the error limits to be observed by the operator must be individually defined by the user. For this purpose, the following methods can be applied:

- If required by the application and if the optimized conditions for measuring are present, the stated error limits can also be expected in the case of used volumetric instruments in good working order.
- In analogy to the German regulations for official testing, it is also admissible to apply the limits which are typical for practice. These practice limits correspond to double the limits for official testing. In this case, the values found in Table 3 should be **doubled**.
- The user may also define his own individual error limits corresponding to his particular application, and apply these tolerances for the calibration of his instrument.

The above procedures fulfil the requirements of DIN EN ISO 9001, DIN EN ISO 10012 and DIN EN ISO/IEC 17025.

Test Record for Volumetric Instruments (EX)

1. Instrument: <input type="checkbox"/> Titrette® <input type="checkbox"/> Digital Burette <input type="checkbox"/> Dispensette® <input type="checkbox"/> Transferpette® <input type="checkbox"/> Transferpette® S <input type="checkbox"/> Transferpette® electronic <input type="checkbox"/> Transferpettor <input type="checkbox"/> _____	Type: <input type="checkbox"/> fix <input type="checkbox"/> analog <input type="checkbox"/> digital/adjustable volume Nominal capacity: _____ Serial number: _____ Customers identification: _____
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2. Damage:

None
 Type of damage: _____
 Damage repaired

3. Functional defects:

None
 Type of functional defect: _____
 Functional defect repaired

4. Water temperature: _____ °C

Balance: _____

Thermometer: _____

Corrective factor Z: _____

Relative atmospheric humidity (at least 35 %): _____

5. Weighing values from gravimetric test

Weighing No.	Nominal volume	50 %	20 %
x ₁			
x ₂			
x ₃			
x ₄			
x ₅			
x ₆			
x ₇			
x ₈			
x ₉			
x ₁₀			

6. Evaluation of gravimetric test

Procedure	Nominal volume	50 %	20 %
a	\bar{V}		
b	s		
c	A [%] found		
d	CV [%] found		
e	A [%] nominal		
f	CV [%] nominal		
g	result		

The testing was carried out according to DIN EN ISO 8655.

Date

Signature



6. Declaration on the Absence of Health Hazards

To be sent together with the instruments or via Mail (if urgent by Fax in advance).

To
BRAND GMBH + CO KG
Otto-Schott-Straße 25
97877 Wertheim
Fax: 09342 808-91290

We intend to give our staff a maximum of protection from health hazards caused by contaminated instruments. We therefore ask for your understanding that we cannot carry out any calibration / repair unless this declaration is submitted completed and signed.

Re: Instrument Consignment dated _____ / Re: Delivery Note No. _____

The Undersigned hereby declares:

- That the instruments have been carefully cleaned and decontaminated before shipment.
- That the instruments pose no danger through bacteriological, chemical, radiological or viral contamination.
- To be authorised to make declarations on behalf of the Institution represented.
- For calibrating service only: minor repairs of a value up to € 25,- + VAT will be carried out and invoiced without further queries (cross out if not applicable).

Company / Laboratory (Stamp)

Tel. / Fax / E-Mail

Name

Position

Date, Signature

- In case of Return for Repair, please provide us with the following supplementary information:

Detected defect _____

Media which the instrument has been used with: _____

7. Calibration Service from BRAND

BRAND offers a full service including calibration and adjustment of Brand- and foreign instruments as well as maintenance and repair if necessary - only for BRAND- instruments. This saves money and adds the benefit of an independent review organisation for the calibration of the instruments. Further information and the order form for repair- and calibration service are found on www.brand.de.

7.1 Range of instruments covered

1. Piston-operated pipettes (single- and multichannel)
2. Bottletop dispensers
3. Piston burettes (bottle-top burettes)
4. Repetitive pipettes

7.2 Testing according to DIN EN ISO 8655

At BRAND, a team of qualified staff, working in temperature and humidity controlled rooms and using the state-of-the-art balances and calibration software, calibrates Liquid Handling instruments, regardless of their make, according to ISO 8655.

Instruments with adjustable volumes such as HandyStep® electronic, Transferpette®, Transferpette® S, Transferpette® electronic, Transferpette® S -8/-12, Transferpette® or Dispensette®, Digital Burette or Titrette® are tested at nominal volume, and at 50%, 10% or 20% of nominal volume. To document the results, a detailed Test Report is compiled which fully complies with all relevant regulations.

The BRAND Calibration Service provides:

1. Calibration of Liquid Handling instruments, regardless of their make
2. Detailed calibration certificate
3. Return of instrument within a few working days
4. Cost-efficient handling

9. DAKKS-Calibration Service for Volumetric Instruments at BRAND

9.1 DAKKS – Deutsche Akkreditierungsstelle GmbH and DKD



The German Calibration Service (DKD) was founded in 1977 as a joint task of state and economy and constitutes the link between the measuring equipment in industrial and research laboratories, testing institutions and authorities and the national standards of the PTB (the German Institute of Physics and Metrology). It effectively supplements the existing verification system which serves above all the purposes of consumer protection. Based on the legal requirements the DKD Accreditation was successively transformed to the DAKKS Accreditation (Deutsche Akkreditierungsstelle GmbH), starting from 2010. BRAND has been accredited by the DAKKS since Apr. 23, 2013, with the certificate number D-K-18572-01-00.



9.2 DAKKS-Calibration Certificate and Calibration Symbol

The DAKKS-Calibration Certificate documents officially on a high level the traceability of measuring results to national and international standards and to international SI-units, as required by standards as DIN EN ISO 9001 and DIN EN ISO/IEC 17025 for monitoring of measuring devices.

DAKKS-Calibration Certificates are issued when calibrations of an accredited laboratory are requested, when high level calibrations are necessary, when national and international standards are demanded and when reference instruments have to be calibrated.

9.3 DAKKS – A member in the International Accreditation Network

DAKKS is a member of the **International Laboratory Accreditation Cooperation (ILAC)**, the highest level international institution for laboratory calibration, and is a signatory to the MRA – Mutual Recognition Agreements.

The accreditation bodies that are signatories to the ILAC mutual recognition agreements (MRAs) recognize their mutual equivalence, and the equivalence of the calibration certificates issued by those same signatories. Likewise, signatories are obliged generally to promote and recommend recognition of the calibration certificates of other signatories (excluding factory calibration certificates).

The DAKKS is a member of the EA (European Co-operation for Accreditation), which again is a member of the ILAC (International Laboratory Accreditation Cooperation). A multilateral agreement assures obligatory recognition of the DAKKS calibration certificate in a variety of countries.

9.4 DAKKS-Calibration Laboratory at BRAND

In 1998 a calibration laboratory for volumetric instruments at BRAND has been accredited by the German Calibration Service according to DIN EN ISO/IEC 17025. Our calibration laboratory is therefore authorized to issue DAKKS-Calibration Certificates (in several languages) for the volumetric instruments listed below. Furthermore we offer adjustment and – for BRAND Liquid Handling instruments – repair and maintenance.

For ordering information on DAKKS-Calibration Certificates for volumetric instruments please consult our General Catalog.

9.5 Volumetric instruments for which BRAND issues DAKKS Calibration Certificates

BRAND calibrates the following volumetric instruments (new or already in use and regardless of their make):

- **Piston-operated pipettes**, from 0.1 µl to 10 ml
- **Multichannel piston-operated pipettes**, from 0.1 µl to 300 µl
- **Piston-operated burettes**, from 5 µl to 200 ml
- **Dispensers, Dilutors**, from 5 µl to 200 ml
- **Volumetric instruments of glass**, calibrated to contain (TC, In) from 1 µl to 10000 ml
- **Volumetric instruments of glass**, calibrated to deliver (TD, Ex) from 100 µl to 100 ml
- **Volumetric instruments of plastic**, calibrated to contain (TC, In) from 1 ml to 2000 ml
- **Volumetric instruments of plastic**, calibrated to deliver (TD, Ex) from 1 ml to 100 ml
- **Density bottles of glass**, from 1 cm³ to 100 cm³

DOMINIQUE DUTSCHER SAS