PERFORMANCE TEST

INSTRUCTIONS

FOR

BIOHIT PROLINE

PIPETTORS

VER3150999

Content:

1.0 Introduction:

The purpose of this manual is to give the main frame of the Standard Operation Procedure (SOP) how to execute the performance and calibration service to all Biohit Proline pipettors. This manual follows the latest features of the future standards and their demands to field technical service.

2.0 Existing Standards:

Following is short description of all main standards and their ”special” features.

1) DIN 12650,”Piston-operated volumetric apparatus”

Is a German standard series which was launched 1997. Today major part of the pipettor manufacturers are following this standard. DIN 12650 describes in detail how volumetric instruments are defined and should be tested.
The specifications in this standard are introduced as a calculation result, which is named as the F-value, German “Fehlergrenze”.
This F-value formula is: | accuracy | + 2 x CV.
DIN 12650 is specifying the maximum error in µl and relative maximum error.
When this manual is produced (September -99) the Biohit specifications are calculated in relation to DIN 12650. However the Biohit specifications are better and more strict than what the mentioned standard demands.

2) NCCLS and BS 7532:1991

NCCLS = National Committee for Clinical Laboratory Standards has published the proposed guideline 18-P “ Determining Performance of Volumetric Equipment “.
BS 7532 is a British standard “ Accuracy and precision of mechanical hand pipettes of capacity less than 50µl down to and including 10µl”.
The NCCLS and BS 7532:1991 are describing accuracy and precision terms while DIN 12650 describes the F-value.
Also some difference can be found how to make the gravimetric test.
The basics and principles of NCCLS and BS7532 are very close to e.g. DIN 12650 and therefore do not have big significance against what standard a pipettor is tested

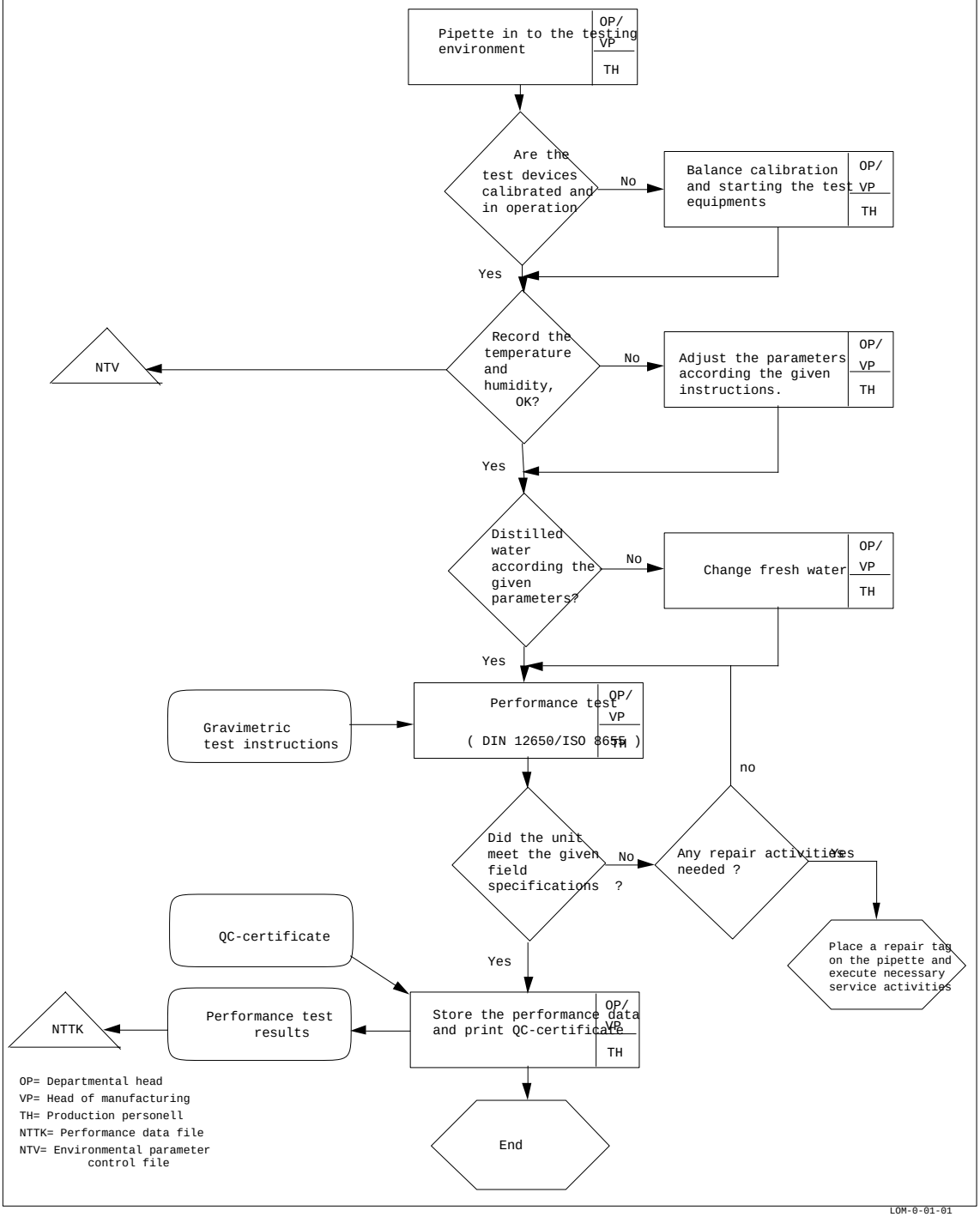
3) ISO 8655

Is a new series of standard, which is at the moment (September -99) in Committee Draft (CD) stage. This new international standard will most obviously be approved to have the draft International standard (DIS) level before end 1999.
The basis for ISO 8655 is the corresponding DIN 12650 and its parts. However quite many changes have been made to original working drafts (WD) and the ISO 8655 will differ from the DIN 12650.
The ISO 8655 will describe “trueness” and “precision” and also the field technical service is taken in notice. Also the new ISO will refer also to field service performance testing and specifications.

3.0 The Biohit Gravimetric test process, flow chart

BIOHIT OYJ	Name of the quality managerment protocol: Logistics/ Production	
Acitivity defidition: Performance test		Page: 1
Designed by: S. Riikonen	Approved: O.Suovaniemi	Date: 170899	Code: TKK - 3C- 08 - 01

= File | = Document | = Task, Responsility, Participate | = Decision | = Transfer or End



OP= Departmental head
VP= Head of manufacturing
TH= Production personell
NTTK= Performance data file
NTV= Environmental parameter control file

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4.0 The Calibration service

Major part of Biohit technical service departments can already offer to their customers a pipettor performance service or calibration service.
However there are several kind of methods in field and misunderstandings how to execute the performance test and what specifications should be used.
With the help of following instructions we like to give tools to you for proper calibration/performance service.
The original purpose of the calibration service is that a pipettors performance; the present accuracy and precision levels are tested using either gravimetric (or photometric methods). Very rarely it means that pipettor should perform like a new one after several years use.
The nature of a calibration or performance service is only to check the pipettor. Therefore our recommendation is:

- Pipettor cleaning and regreasing according given instructions
- Either gravimetric (or photometric) test.
- Calibration / performance certificate.

However many times the market situation can demand to include additional features to above content. All these additional features should be decided locally. Also the service price varies depending of the market area.

5.0 Performance test method, according ISO 8655.

Pipette manufacturer can decide in which and (in how many) volume(s) the pipettor is performance tested before shipping. This factory test is a QA-test (Quality Assurance), which confirms the performance of the pipettor. This factory QA-test is also called a suppliers declaration.
However manufacturers must publish type test or conformity test specifications to following volumes:

- nominal volume =upper limit of useful volume range.
- 50 % off nominal volume.
- the smallest settable volume and/or 10% of the nominal volume.

The conformity test is always related to entire system, pipettor and tip used. The original ISO 8655 standard method describes how the type test (or conformity test) should be done. Unfortunately it is not clearly stating how pipettors should be tested in field and normal laboratory environment.

The ISO 8655 separates two specification levels:

1) **Manufacturers specifications, (type test specifications , conformity test specifications or type evaluation)**

The specks defined by the manufacturer with their internal conformity testing. These specks are achieved in strictly controlled conditions with optimal pipetting features and technique. The type test is done to one or more representative specimens of an instrument type.

2) **User specifications (field specifications)**

ISO 8655 allows users to exceed given specifications by the standard not more than 100%, multichannel pipettors. The main responsibility is user to establish the accuracy and precision levels for their own needs and performance demands.
Please note that specifications mentioned in ISO 8655 are the maximum error levels defined to pipettor/tip manufacturers to fulfil the demands of this standard.
However main part of the pipettor manufacturers introduces specifications which are tighter and better that mentioned in ISO 8655. This is due the result of their own production process quality and /or market demands.
When pipettors are tested in field conditions it must be confirmed against what specifications the pipettors are referred to.

NOTE!
The ISO 8655 allows the technical services to use specification levels given in this standard.

5.1.Equipment needed:

Analytical balance: - analytical balance min. 0.01mg readability
- weighing vessel with relatively small inner diameter

The balance should be calibrated in intervals recommended by the balance manufacturer or when ever relevant.
Thermometer: 20- 25 °C ± 0.5 °C

The main purpose is to control that pipettor, tips used, balance and water used are at the same testing temperature.

Water: grade 3, according ISO 3696

The water should be changed in regular basis and not reused at all.

5.2 Simplified field (user) test instructions:

1) Temperature :
   Take care that pipettor, tip, balance and water used are within the same temperature.
2) Pipetting technique:
   Tip should be immersed into the liquid only 2-3 mm into a vessel containing water.
   The pipettor should not be tilted.
   There must be a 1-2 second pause before the tip is removed from water.
   It is important that using same pipetting technique and smooth plunger operation carry out the complete performance test when testing mechanical pipettors.
3) Disposable tip:
   The performance of a pipettor is a combination of the pipettor and tip used. Tips of different origin can cause problems to achieve proper performance level. Therefore the original manufacturers specks are valid only when test is executed with the original manufacturers tips.
4) Prerinsing:
   Prerinsing it 3-5 times prior to test should wet the tip used.
5) Number of repetitions:
   Since the accuracy (trueness) and precision are statistical values it is recommended to execute 10 subsequent measurements.
6) Microliters vs. milligrams:
   The milligram reading (mg) is not directly a test result in microliters (µl) and the conversion factor (Z-factor) is dependent on temperature.

The detailed test procedure can be found from publications ISO 8655-6.

6.0 The Biohit Specifications in field service:

The specks defined by the manufacturer are for their internal conformity testing. These specks are achieved in strictly controlled conditions with optimal pipetting features and technique.
Unfortunately in field service the control or adjustment of the environment parameters is difficult or many times even impossible.
The main purpose of the performance test in field is to find out how good or bad the pipettor is performing in normal environment. Therefore it is not sensible to performance test pipettors against manufacturers original type test specifications or trying to achieve the same performance level as in the strict controlled conditions.
The DIN 12650 allows user to exceed the specks mentioned in the standard not more than 100%. However the user should establish their own specification levels related to their needs.

NOTE!
The specification mentioned in ISO 8655 are not same as the Biohit type specifications.

Biohit Oyj recommends that all Biohit technical service centers should take in use field specifications, which are:

The basic rule: The original Biohit type specks + 50 %

For example;

Original accuracy: 1.00%	Field: 1.50%
Original precision: 0.40%	Field: 0.60%

Following pages describes **The Biohit Field Test Specifications**.

6.1.The Biohit Field specifications

Code	Volume range	Volume	Type specifications			Service specifications		
			Inacc. %	Impr. %	DIN 12650,Fehlerg./µl	Inacc.%	Impr. %	DIN12650,Fehlerg./µl
710520	0,2-10	10	0,9	0,5	0,2	1,4	0,8	0,3
		5	1	0,7	0,2	1,5	1,1	0,3
		1	2,5	1,5	0,2	3,8	2,3	0,3
		0,2	6	6	0,2	9,0	9,0	0,3
710010	5-100	100	0,4	0,15	0,7	0,6	0,2	1
		50	0,7	0,3	0,7	1,1	0,5	1
		10	2	1	0,7	3	1,5	1
		5	2,5	1,8	0,7	3,8	2,7	1
710030	10-250	250	0,4	0,15	1,2	0,6	0,2	2,5
		125	0,6	0,2	1,2	0,9	0,3	2,5
		25	1,5	0,8	1,2	2,3	1,2	2,5
		10	2	1	1,2	3,0	1,5	2,5
710100	10-500	500	0,4	0,15	3,5	0,6	0,2	5
		250	0,7	0,2	3,5	1,1	0,3	5
		50	1,5	0,8	3,5	2,3	1,2	5
		10	4	1,5	3,5	6	2,3	5
710020	50-1000	1000	0,4	0,15	7	0,6	0,2	10
		500	0,6	0,2	7	0,9	0,3	10
		100	1,5	0,5	7	2,3	0,8	10
		50	2	1	7	3	1,5	10
710040	50-1200	1200	0,4	0,15	8,4	0,6	0,2	12
		600	0,7	0,2	8,4	1,1	0,3	12
		100	1,5	0,5	8,4	2,3	0,8	12
		50	2	1	8,4	3	1,5	12
710500	100-5000	5000	0,5	0,15	40	0,8	0,2	58
		2500	0,8	0,2	40	1,2	0,3	58
		500	0,8	0,3	40	1,2	0,5	58
710410	5-100/4	100	0,5	0,2	1,5	0,8	0,3	2,3
		50	0,8	0,3	1,5	1,2	0,5	2,3
		10	1,5	1,5	1,5	2,3	2,3	2,3
		5	4	2,5	1,5	6,0	3,8	2,3

Code	Volume	Volume	Type specifications			Service specifications		
	range		Inacc. %	Impr. %	DIN 12650,Fehlerg./µl	Inacc.%	Impr. %	DIN12650,Fehlerg./µl
710420	25-250/4	250	0,4	0,15	1,75	0,6	0,2	2,5
		125	0,7	0,2	1,75	1,1	0,3	2,5
		25	1,5	1	1,75	2,3	1,5	2,5
710200	0,2-10/8	10	0,9	0,5	0,2	1,4	0,8	0,3
		5	1,5	0,8	0,2	2,3	1,2	0,3
		1	4	4	0,2	6	6	0,3
710210	5-100/8	100	0,5	0,2	0,9	0,8	0,3	1,4
		50	0,7	0,3	0,9	1,1	0,5	1,4
		10	1,5	1,5	0,9	2,3	2,3	1,4
		5	4	2,5	0,9	6,0	3,8	1,4
710220	25-250/8	250	0,4	0,15	1,75	0,6	0,2	2,5
		125	0,6	0,2	1,75	0,9	0,3	2,5
		25	1,5	1	1,75	2,3	1,5	2,5
710800	50-1200/8	1200	0,5	0,15	9,6	0,8	0,2	13,8
		600	1	0,2	9,6	1,5	0,3	13,8
		100	4	0,8	9,6	6	1,2	13,8
		50	8	1,5	9,6	12	2,3	13,8
710300	0,2-10/12	10	0,9	0,5	0,2	1,4	0,8	0,3
		5	1,5	0,8	0,2	2,3	1,2	0,3
		1	4	4	0,2	6	6	0,3
710310	5-100/12	100	0,5	0,2	0,9	0,8	0,3	1,4
		50	0,8	0,4	0,9	1,2	0,6	1,4
		10	1,5	1,5	0,9	2,3	2,3	1,4
		5	4	2,5	0,9	6	3,8	1,4
710320	25-250/12	250	0,4	0,15	1,75	0,6	0,2	2,5
		125	0,6	0,2	1,75	0,9	0,3	2,5
		25	1,5	1	1,75	2,3	1,5	2,5
710810	50-1200/12	1200	0,8	0,15	9,6	1,2	0,2	13,8
		600	1	0,2	9,6	1,5	0,3	13,8
		100	4	0,8	9,6	6	1,2	13,8
		50	8	1,5	9,6	12	2,3	13,8

Code	Volume range	Volume	Type specifications			Service specifications		
			Inacc. %	Impr. %	DIN 12650,Fehlerg./µl	Inacc.%	Impr. %	DIN 12650,Fehlerg./µl
720000/	0,5-10	10	1	0,8	0,3	1,5	1,2	0,4
720001		5	1,5	1,5	0,3	2,3	2,3	0,4
		1	2,5	1,5	0,3	3,8	2,3	0,4
720080	2-20	20	0,9	0,4	0,3	1,4	0,6	0,5
		10	1,2	1	0,3	1,8	1,5	0,5
		2	3	2	0,3	4,5	3	0,5
720020/	5-50	50	0,6	0,3	0,6	0,9	0,5	1
720021		25	0,9	0,6	0,6	1,4	0,9	1
Calib.vol.		20	0,9	0,6	0,6	1,4	0,9	1
		5	2	2	0,6	3	3	1
720050	10-100	100	0,8	0,15	1,1	1,2	0,2	1,6
		50	1	0,4	1,1	1,5	0,6	1,6
Calib.vol.		25	1,3	0,5	1,1	2,0	0,8	1,6
		10	3	1,5	1,1	4,5	2,3	1,6
720070	20-200	200	0,6	0,15	1,8	0,9	0,2	2,6
		100	0,8	0,3	1,8	1,2	0,5	2,6
Calib.vol.		50	0,7	0,3	1,8	1,1	0,5	2,6
		20	3	1	1,8	4,5	1,5	2,6
720030/	50-200	200	0,6	0,15	1,8	0,9	0,2	2,6
720031		100	0,8	0,3	1,8	1,2	0,5	2,6
		50	1	0,4	1,8	1,5	0,6	2,6
Calib.vol.		50	0,7	0,3	1,8	1	0,5	2,6
720060	100-1000	1000	0,6	0,2	10	0,9	0,3	15
		500	0,7	0,25	10	1,1	0,4	15
Calib.vol.		200	0,6	0,3	10	0,9	0,5	15
		100	2	0,7	10	3	1,1	15
720040/	200-1000	1000	0,6	0,2	10	0,9	0,3	15
720041		500	0,7	0,25	10	1,1	0,4	15
		200	0,9	0,3	10	1,4	0,5	15
Calib.vol.		200	0,6	0,3	10	0,9	0,5	15

Code	Volume range	Volume	Type spesifications			Service specifications		
			Inacc. %	Impr. %	DIN 12650,Fehlerg./µl	Inacc.%	Impr. %	DIN 12650,Fehlerg./µl
720110/	1-5 ml	5	0,5	0,15	40	0,8	0,2	60
720111		2,5	0,6	0,3	40	0,9	0,5	60
		1	0,7	0,3	40	1,1	0,5	60
720120	5-50/4	50	1	0,5	1	1,5	0,8	1,5
		25	1,5	1	1	2,3	1,5	1,5
Calib.vol.		20	1,5	1	1	2,3	1,5	1,5
		5	3	2	1	4,5	3	1,5
720130	50-250/4	250	0,7	0,25	3	1,1	0,4	4,8
		125	1	0,5	3	1,5	0,8	4,8
Calib.vol.		100	1	0,5	3	1,5	0,8	4,8
		50	1,5	0,8	3	2,3	1,2	4,8
720210/	0,5-10/8	10	1,5	1,5	0,45	2,3	2,3	0,7
720211		5	2,5	2,5	0,45	3,8	3,8	0,7
		1	4	4	0,45	6	6	0,7
720220/	5-50/8	50	1	0,5	1	1,5	0,8	1,5
720221		25	1,5	1	1	2,3	1,5	1,5
Calib.vol.		20	1,5	1	1	2,3	1,5	1,5
		5	3	2	1	4,5	3	1,5
720230/	50-250/8	250	0,7	0,25	3	1,1	0,4	4,7
720231		125	1	0,5	3	1,5	0,8	4,7
Calib.vol.		100	1	0,5	3	1,5	0,8	4,7
		50	1,5	0,8	3	2,3	1,2	4,7
720240/	50-300/8	300	0,7	0,25	3,6	1,1	0,4	5,6
720241		150	1	0,5	3,6	1,5	0,8	5,6
Calib.vol.		100	1	0,5	3,6	1,5	0,8	5,6
		50	1,5	0,8	3,6	2,3	1,2	5,6
720310	0,5-10/12	10	1,5	1,5	0,45	2,3	2,3	0,7
		5	2,5	2,5	0,45	3,8	3,8	0,7
		1	4	4	0,45	6	6	0,7

Code	Volume range	Volume	Type specifications			Service specifications		
			Inacc. %	Impr. %	DIN 12650,Fehlerg./µl	Inacc.%	Impr. %	DIN 12650,Fehlerg./µl
720320/	5-50/12	50	1	0,5	1	1,5	0,8	1,5
720321		25	1,5	1	1	2,3	1,5	1,5
Calib.vol.		20	1,5	1	1	2,3	1,5	1,5
		5	3	2	1	4,5	3	1,5
720330/	50-250/12	250	0,7	0,25	3	1,1	0,4	4,7
720331		125	1	0,5	3	1,5	0,8	4,7
Calib.vol.		100	1	0,5	3	1,5	0,8	4,7
		50	1,5	0,8	3	2,3	1,2	4,7
720340/	50-300/12	300	0,7	0,25	3,6	1,1	0,4	5,6
720341		150	1	0,5	3,6	1,5	0,8	5,6
Calib.vol.		100	1	0,5	3,6	1,5	0,8	5,6
		50	1,5	0,8	3,6	2,3	1,2	5,6

Code	Volume range	Volume	Type specifications			Service specifications		
			Inacc. %	Impr. %	DIN 12650,Fehlerg./µl	Inacc.%	Impr. %	DIN 12650,Fehlerg./µl
722001	5	5	1,3	1,2	0,2	2,0	1,8	0,3
722004	10	10	0,8	0,8	0,2	1,2	1,2	0,4
722010	20	20	0,6	0,5	0,3	0,9	0,8	0,5
722015	25	25	0,5	0,3	0,3	0,8	0,5	0,4
722020	50	50	0,5	0,3	0,55	0,8	0,5	0,8
722025	100	100	0,5	0,3	1,1	0,8	0,5	1,7
722030	200	200	0,4	0,2	1,6	0,6	0,3	2,4
722035	250	250	0,4	0,2	2	0,6	0,3	3
722040	500	500	0,3	0,2	3,5	0,5	0,3	5,3
722045	1000	1000	0,3	0,2	7	0,5	0,3	11
722050	2000	2000	0,3	0,15	12	0,5	0,2	17
722055	5000	5000	0,3	0,15	30	0,5	0,2	43

7.0 Quality Control

More and more demands are directed to technical service and traceability of the gravimetric performance testing.
The traceability of the gravimetric test is understood to include:

- balance calibration
- certified calibration weights
- test method and standard used
- environmental control

Therefore Biohit Oyj has launched a quality control protocol for field service departments which are executing performance or calibration tests to end user.

Protocol:

1) Biohit Oyj will send a set of pipettors to be performance tested by the local technical service center.
2) After the pipettors are tested the units and their test results are returned back to Biohit Quality Control who will check the results. The service partner is not allowed to do any cleaning service or calibration adjustment to pipettors in concern.
3) Biohit Quality Control will send The Competence Certificate of the test results.

This Quality control test is done once in a year, after 9 months intervals.

8.0 The Main Biohit Service Center Approval:

Those service partners which attends the Quality Control program, will get the Biohit Performance Test Certificate where Biohit Oyj approves the partner to execute the performance tests. This certificate also conforms testing is regularly controlled by Biohit Oyj; Quality Control Department.
Please note that the traceability of the balance is not included with this protocol.
See enclosed certificate copy.

9.0 Uncertainty of the measurement

The performance test or calibration test of a pipettor suffers from two different sources of uncertaintes:

- the delivering process itself i.e. pipettor itself
- uncertaintes related to gravimetric process

Both of these sources have to be taken care if special quality control management activities has to be fulfilled.The complete description and calculating formulas are mentioned in ISO-document ” Guide to Expression of Uncertainty in Measurement (GUM) , 1993.

When the performance test or calibration test is executed in field environment, all uncertaintes cannot take in notice and experience shows that the uncertainty budget resulting from gravimetric measuring apparatus is small related to delivering process itself. The gravimetric apparatus in this case are; analytical balance,barometer, humidity meter and thermometer. It is , of course obvious that these apparatus must be handled according the given instructions eg. ISO 8655 or DIN 12650.

The uncertainties caused by the incomplete knowledge of air temperature, pressure and humidity are so small ,that is most of the cases it is justified to work standard values rather than measured values.

The evaporation of the liquid has to be taken care when volumes below 100µl are measured.

9.1 Temperature

In field performance test a reliable (preferably calibrated) thermometer is needed and it should be placed as close the balance weighing pan as possible.

The temperature alters the water density and is therefore needed to control. The water density or z-factor ca be easily to achieved form a separate water density chart.

9.2 Evaporation

The water evaporation speed is necessary to know when volumes below 100µl are measured.

Both the DIN 12650 and ISO 8655 are describing that a stop watch must be used when the gravimetric test is done and after the evaporation must be measued within same time limit.

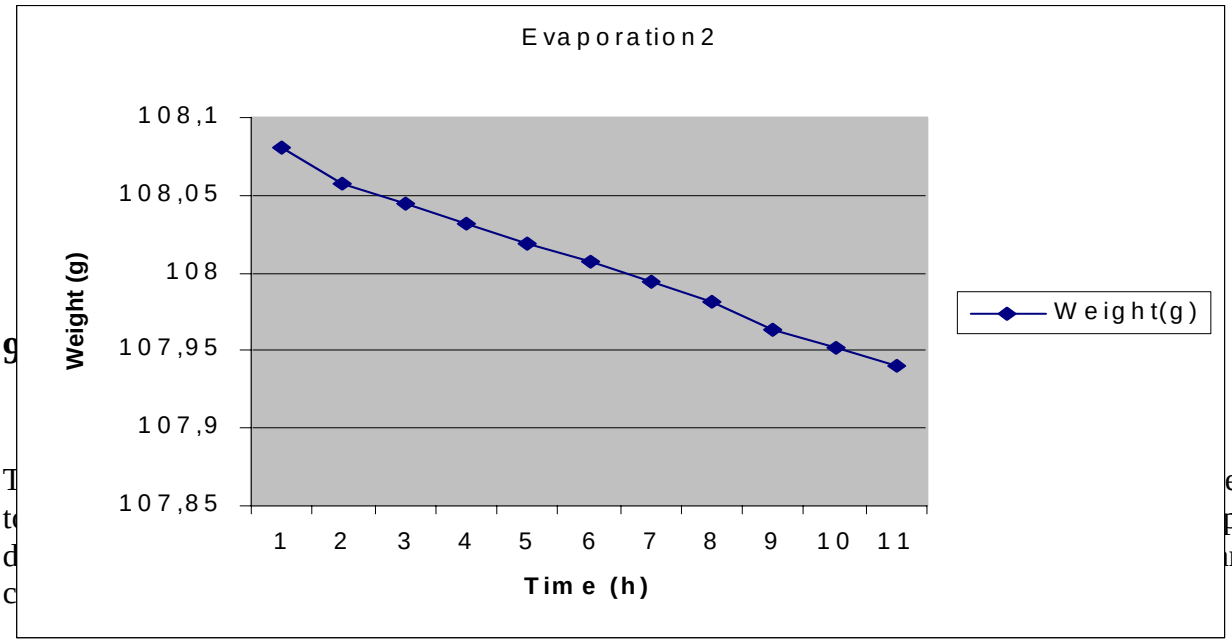
There are in the market a special evaporation traps combined with the analytical balances. However it is recommended to measure what is the exact evaporation speed in individual test environments.

If a dedicated evaporation trap is not used, a set of additional water containers should be used to reduce the evaporation speed during the gravimetric test. Please note that DIN 12650 is stating that the RH% should be 60-90% around (close) the weighing area.

The evaporation speed can be experimentally measured by controlling the balance reading up to a certain period of time.

Next page descibes a one experiment and corresponds the evaporation speed of 0.07µl in 10 seconds. The time is coming from an average stabilizing time of the balance.

	Time (h)	Weight(g)						
T0	0,0	108,0805		Balance, Ohaus, HN01				
	0,5	108,0572		Balance calibrated before the test				
T1	1,0	108,0446		4 pcs additional moisture traps used. Size: 14 x 54 mm				
	1,5	108,0319						
T2	2,0	108,0192		Glass container: 22 x 90 mm				
	2,5	108,0073						
T3	3,0	107,9946						
	3,5	107,9817						
T4	4,0	107,9631						
	4,5	107,9519						
T5	5,0	107,9402						
Total:	5 x 60min.	0.1403	(=T0-T5)		**Evaporation, average:**			
	=300 min.				**0.07 µl = 10 sec.**			
	0.1403g=140.3 mg							
	140.3 mg/300min= 0.467mg/min							
	0.467mg / 6 = 0.078mg/ 10 sec.							
	1 mg = 1 µl							



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T e in this case is
t pecially
d nce weighing
c

9.4. Air Pressure

The air pressure is depending of the height distance to the sea level. Following chart is describing the differences volumes referred to sea level.

200 ul Manual Pipette; calibrated and adjusted at NTP to provide 200 ul at 200 ul setting							
Dead Volume		540	ul				
Height at 200 ul		36	mm				
Air Pressure (NTP)		10282	mmH2O				
Altitude (km)	Diff	Error (ul)	Error (%)				
0	1,90	0,00	0,00				
1	2,09	0,19	0,10				
2	2,32	0,42	0,21				
3	2,61	0,71	0,36				
4	2,99	1,09	0,55				
5	3,49	1,59	0,80				