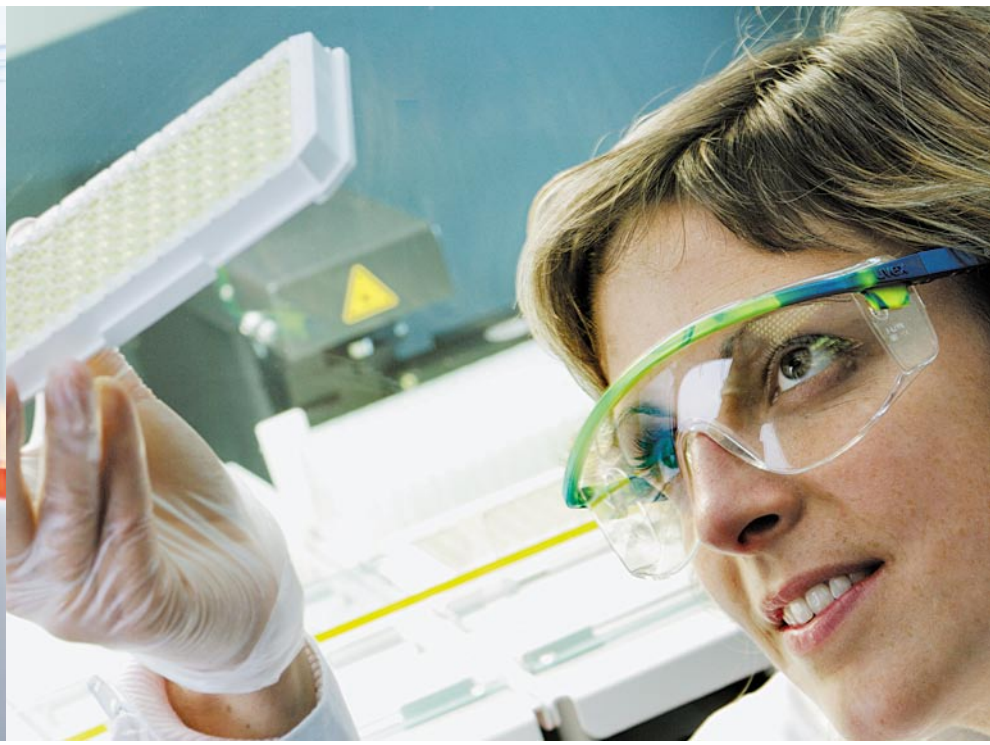
TECAN.



Correct use of Tecan liquid handling platforms

Instructions and recommendations for routine operation in the field of clinical diagnostics according to the requirements of In Vitro Diagnostic Directive

Scope of this instruction guide

Tecan liquid handling platforms are state-of-the-art systems that enable a user to raise productivity and improve throughput, accuracy and reproducibility of test performance in diagnostic laboratories. This good usage guide concerns the platforms that are no longer commercially available but are still in use, with the guaranteed support of the manufacturer. This guide is intended to provide an overview of:

- relevant pre-analytical steps for sample treatment
- understanding general liquid handling mechanisms
- key maintenance information for instruments
- training information.

This guide summarizes good usage of Tecan liquid handling platforms in diagnostic laboratories for pipetting body fluids, such as whole blood, serum or plasma, and concerns the following Tecan liquid handling platforms and software packages:

- Freedom EVO® Clinical with Logic™ Software
- Genesis RSP™ with Logic Software
- Genesis RMP™ with TOPS™ Software.

This guide also contains input from field use and, therefore, is a valuable information source to supplement the corresponding operating manuals. It is intended for use by technicians, laboratory supervisors, Tecan application specialists and field service engineers.

This guide can also serve for establishing standard operating procedures (SOP). It assumes adherence to the applicable operating manual. Any change to the set-up in your application software must be carried out only by persons trained by Tecan or by Tecan application specialists.
As for all other Tecan liquid handling platforms please refer to the corresponding operating manuals.

Collection and handling of clinical samples

There are two prerequisites for collecting clinical samples:

- samples should remain as unchanged as possible to provide a valid clinical laboratory result
- for automation of analytical tests the samples must be prepared in such a way as to prevent generation of artifacts such as clots, foam and bubbles that impair proper pipetting.

Sample definitions (ref. [1])
Whole blood
A venous, arterial or capillary blood sample stabilized with anti-coagulants.

Plasma
Almost cell-free supernatant of blood sample containing anti-coagulants obtained after centrifugation.

Serum
Undiluted, extra-cellular portion of a whole blood sample after adequate coagulation (does not contain anti-coagulants) and subsequent centrifugation.

Sample collection (ref. [1])

Detailed information on specifications of sample tubes and use of anti-coagulants is available from manufacturers of sample collection devices. Otherwise refer to information available in scientific articles, reviews and recommendations of the National Societies for Clinical Chemistry on how to collect, store, transport and prepare blood, serum and plasma.

Recommendations for processing patient samples prior to sample handling (ref. [1])

Whole blood
Immediately after filling, tilt the sample tube thoroughly to mix with the anti-coagulant (do not shake, to avoid foaming). For storage at room temperature see reference [1].

Plasma
Centrifuge the anticoagulated blood (e.g. citrated or EDTA blood) for at least 15 minutes at 2000 to 3000 xg to obtain cell-free plasma.

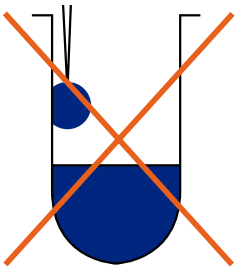
Serum
When plasma coagulation is complete, the sample should be centrifuged for at least 10 minutes at a minimum speed of 1500 xg. When separating serum or plasma, the temperature should not drop below 15 °C or exceed 24 °C.

Handling of stored samples
Serum or plasma samples that have been stored at 4–8 °C or frozen should be thawed and must reach ambient temperature before pipetting. Please note that the subsequent coagulation during the storage of serum may occur. Therefore, centrifugation of samples for a minimum of 15 minutes at 2000–3000 xg prior to use is strongly recommended or, at least, the visual control of samples should be done to prevent possible clogging of liquid handling needles or disposable tips.

Handling and inspection of sample tubes prior to pipetting

Samples and reagents must reach ambient temperature before pipetting. The samples and the sampling tubes must be free of:

- blood clots
- foam
- droplets or liquid films on the wall.



Droplets on the wall

The sample tubes should not be more than 80% full.

The sample tubes must not contain any additional inserts or have any caps or other covers.

When using plunger monovettes, the plunger must be completely retracted and broken off before placing the tube on a sample rack to ensure proper liquid detection. It is essential that there is sufficient plasma for pipetting.

Use only the labware that was approved or set up in the software by the application specialist. Please contact the Tecan application specialist if you intend to use any labware that is not specified in the software. This will avoid unexpected malfunctions of the liquid handling platform.

References:
[1] Guder WG, Ehret W, Fonseca-Wollheim F, Heil W, Schmitt Y, Wisser H, Zawata B, et al. Use of anticoagulants in diagnostic laboratory investigations. WHO/DIL/LAB/99.1 Rev.2
http://www.diagnosticsample.com

Preparation of the instrument

System liquid
Use distilled or deionized water as system liquid, or an aqueous buffer solution based on distilled or deionized water. The conductivity of the system liquid must be between 0.5 and 500 µS/cm (micro Siemens per centimeter).

The system liquid must be free of any particles. Use clean containers only.

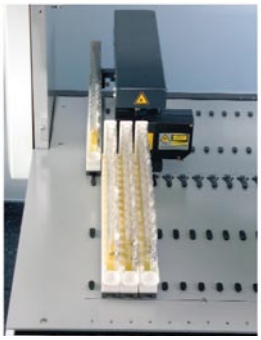
The system liquid must be free of any air bubbles to ensure accurate pipetting. Replenishing the system with liquid that is colder than room temperature may lead to air bubble formation. Therefore the system liquid containers should be filled the day before and placed next to the pipetting robot to adapt the system liquid to ambient temperature. In addition, degassing the system liquid with vacuum or helium helps to prevent formation of air bubbles.

When setting up an application it should be verified that the pipetting accuracy under the prevailing conditions is adequate for the application.

For further information, please contact the responsible application specialist.

Worktable
All carriers must be placed at the predefined grid positions. For proper operation ensure that all carriers are fully pushed back. Carriers which, for example, are placed to the left of grid 1, can lead to mechanical problems (collisions) or to errors in the identification of barcode-labeled samples.

Wrong: Carrier next to grid 1.



Correct: The carriers are only in the programmed positions.

Ensure that all tubes are correctly placed in the proper carrier and that they touch the bottom of the carrier. Microplates must also be placed correctly on the carrier, ie. they must be well seated in the intended site. Make sure that the microplates do not rest on the edge of the carrier site (slanting).

The capacitive detection of liquids requires surface contact of the carriers with the worktable. Regular cleaning of the carriers and of the worktable ensures good surface contact. Carriers and labware must not have any mechanical damage.

Only tubes with the outer diameter specified below can be used. The appropriate outer diameter must match the tube carrier and insert combination. The following sample tube carrier parameters are predefined for the TOPS and Logic application software.

Tube carrier with	Sample tube (outer diameter)
Insert black	Ø 10 mm
Insert blue	Ø 13 mm
No insert (white)	Ø 16 mm

Only one size of sampling tube must be used per carrier; height, inner and outer diameters of the sampling tubes within one particular carrier must be identical. However, it is possible to have different types of tube carriers in one run, but this must be defined in the application software configuration.

Dimensions of all labware (eg. deep-well plates, microplates) including the definitions (X, Y, Z dimensions) of carriers must be specified and verified with the settings in the corresponding database.

Ensure that all co-ordinates (X, Y, Z) of the used carriers and labware have been correctly measured. Ensure that outer and inner diameters of sample tubes are defined correctly in the corresponding database (accessible labware editor). In case of deviating parameters (height and inner diameter), these must be corrected in the application software. Properly defined carriers and labware prevent collisions and malfunctions.

Use carriers recommended by Tecan. If different kinds of carriers are intended for use, they must be validated.

Disposable tips must be checked for transportation and storage damage. The packaging must be opened and the tips visually inspected before usage:

- disposable tips must not be damaged
- disposable tips must not be bent
- disposable tips must be free of dust or moisture.

Disposable tips must be properly seated in the rack and carrier intended for this purpose. Only original Tecan disposable tips should be used, because they are tested for use with Tecan liquid handling platforms.

Configuration of racks/Z-height settings

Z-travel

Corresponds to the Z-height, which is higher than all obstacles of the corresponding carrier.

Z-dispense

Corresponds to the Z-height at which liquid is dispensed in air (free dispense). It must be set so that that no drops get into adjacent wells, to avoid cross-contamination. Check settings with the recommendations in the applicable operating manual.

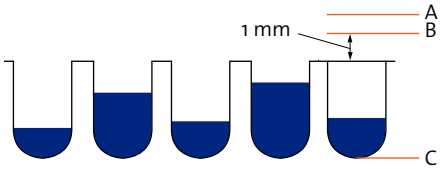
Z-start

Corresponds to the Z-height at which the liquid detection is switched on. Z-start must be at least 1 mm below the edge of the tube and above the surface of the liquid. An exception is the microplate (including deep-well plates), where Z-start is defined as 1 mm above the top of the well. Verify settings using the corresponding software database.

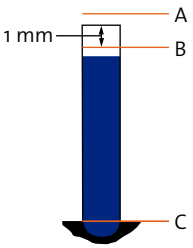
Z-max/Z-bottom

Corresponds to the Z-height, which is minimally above the lowest point of the well or tube bottom without touching it. Verify settings using the corresponding software database.

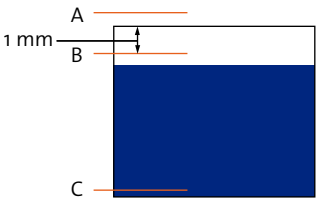
The use of other settings must be clarified with the responsible application specialist.



Microplate Z-heights
A Z-travel
B Z-start, Z-dipense
C Z-max/Z-bottom



Sample tubes Z-heights
A Z-travel
B Z-start, Z-dipense
C Z-max/Z-bottom



Reagent trough Z-heights
A Z-travel
B Z-start, Z-dipense
C Z-max/Z-bottom

Liquid handling

This information concerns 2-, 4- and 8-tip pipetting robots with 1,000 µl syringes (plunger stroke volume).

Warning: imprecise definition of the inner diameters of used sample tubes may lead to pipetting errors. An inner diameter that is too large may lead to air aspiration due to wrongly calculated volume of the sample. An inner diameter that is too small causes deeper submersion of tips and may provoke a "not enough liquid" message.

Deeper submersion of tips can lead to insufficient cleaning of the outside of the tips and, therefore, increases the risk of cross-contamination.

Minimum volumes
For free dispense in air, the following minimum volumes are applicable for the tip types listed:
- standard fixed tips: 3–10 µl*
- 200 µl disposable tips: 5–10 µl*
- 1,000 µl disposable tips: 25 µl.

*Minimum volumes depend on physicochemical properties (eg. viscosity) of samples and reagents and have to be validated by the user.

Aspiration speed and delay
In order to achieve optimum pipetting results for serum-like liquids, the following settings for the aspiration speed should be taken into account:

Aspiration volume	Aspiration speed
10 µl	20 µl/s
≥ 100 µl	100 µl/s

A sufficiently long delay after the aspiration (between 200 and 500 ms) must be applied.

In the case of viscous samples and solutions, such as serum or highly concentrated reagents, a delay of at least 500 ms should be implemented.

Air gaps
Recommended air gap volumes are given in the table below; deviations from the standard set-up in essential cases are allowed.

		STAG	LAG	TAG
Standard Tip Disposable	S	Σ ≤ 30 µl		5–20 µl 10 µ ideal
	M	Σ ≤ 30 µl		0–20 µl
Tip 200 µl	S	Σ ≤ 40 µl		5–20 µl 10 µ ideal
	M	Σ ≤ 30 µl		0–20 µl
Disposable Tip 1000 µl	S	Σ ≤ 40 µl		5–20 µl 10 µ ideal
	M	Σ ≤ 30 µl		10–20 µl

S: Single Pipetting Mode
M: Multi Pipetting Mode
STAG: System trailing air gap
TAG: Trailing air gap
LAG: Leading air gap

Dispense speed, break-off speed and delay
In order to achieve optimum results with the standard pipetting method (non contact dispense out of air), we recommend the following settings:

- rapid dispense speed of between 250 and 600 µl/s; ideal dispense speed: 400 µl/s
- break-off speed is ideally 70% of the dispense speed, but the minimum break-off speed should be 150 µl/s
- sufficiently long delays (≥ 200 ms) might be necessary in case of viscous samples and solutions such as serum or highly concentrated reagents.

Multi-pipetting
Multi-pipetting is a pipetting technique with one aspiration step of a larger amount of liquid followed by subsequent dispensing of multiple aliquots. For this pipetting technique, all the rules listed in this guide apply and in addition the following parameters are required.

A conditioning volume is required in order to achieve similar conditions for the first and all subsequent aliquots. The conditioning volume should be ≥ 30 µl or, ideally, correspond to the volume of one aliquot. The conditioning volume can be dispensed back into the original container or into the wash station.

An excess volume is required in order to achieve similar conditions for the last and all preceding aliquots. The excess volume should be ≥ 30 µl or, ideally, correspond to 15 % of the overall volume. The excess volume is dispensed into the wash station.

For multi-pipetting, the best precision is achieved with 4–12 aliquots.

Liquid detection
Liquid detection must always be switched on during the aspiration step. Switching off the liquid detection option is allowed in exceptional cases only, and after consultation with the Tecan application specialist.

Recommendations for pipetting blood, serum, plasma and isotonic buffers out of sample tubes:
- detection mode: 'detect twice with separate tips with retract' or 'detect twice with odd/even tips'
- conductivity: good (corresponds to sensitivity setting 'high')
- clot limit: set to 4 mm.

At the aspiration position, the offset (depth of submersion) must be set as follows:
- microplates: ≥ 1 mm
- sampling tubes: ≥ 2 mm
- troughs: ≥ 3 mm.

Note: deviations from these settings must be consulted in advance with the Tecan application specialist and validated by the end user.

Clot detection
We recommend particular care with the following sample preparation steps:
- during the pre-analytic step, in particular with the centrifugation step
- during sample collection and distribution of the samples
- after thawing stored serum samples.

To generally prevent problems with clots (clogging of the tips or sticking of particles to the tips) the samples must be properly centrifuged.

Clot detection must always be switched on during aspiration of the samples to detect possible clogging of the tips through blood clots. The application range of the clot detection is dependent on the geometry of the sample tubes as well as on the volume and adhesion of the aspirated sample.

Clot detection functions most reliably when certain volumes of samples to be aspirated are observed (please refer to the applicable operating manuals).

Clot detection is also recommended for aspiration for all liquid classes and detection modes as an additional safety function to detect possible air aspiration. Cylindrical and cubic-shaped tubes enable optimum tracking, clot detection and volume metering.

Clot detection does not apply on troughs and microplates.

Carryover
For any application where carryover might lead to erroneous results, the actual carryover properties must be measured using reference samples (positive and negative). The measurements must be conducted with test conditions identical to those of the application.

To prevent carryover, disposable tips (DiTis) or DiTis with filters must be used.

Wash procedure for fixed tips
The appropriate wash procedure is strongly dependent on the sample matrix and physico-chemical properties of a particular analyte. The wash procedure must be adapted according to test requirements and should be validated by the end user.

Disposable tips
For disposable tips, all the rules listed in this guide apply. Disposable tips with or without filters should be used whenever contamination and carryover are to be prevented.

Re-use of disposable tips is not recommended.

Tecan-branded disposable tips guarantee that the specified performance of the Tecan pipetting instruments is achieved.

Correct positioning of the barcode labels on the sampling tubes

Reading barcodes
The barcodes must be accurately aligned both horizontally as well as vertically on all sampling tubes and containers used. The recommendations in the operating manual must be adhered to.

The barcodes must have at least the following quality:
- class A, B or C according to ANSI
- quality grade 4, 3, or 2 to according to ISO 15416
- yellowed, shiny, dirty, folded, wet or damaged barcode labels must not be used
- the adhesive labels must be flat and not peeling off at the edges.

Note: manufacturer's performance specifications for PosID™ are guaranteed with code class 4 (A). The scanning performance can be diminished with code classes 3 (B) and 2 (C).

We recommend ensuring the quality of the barcodes by means of a local SOP according to ISO 15416 and the applicable specification of the symbologies:

- code 128 – ISO/IEC 15417
- code 39 – ISO/IEC 16388
- interleaved 2/5 – ISO/IEC 16390
- codabar – EN 798.

Use a barcode verifier according to ISO 15426-1.

The carrier ID of the tube carrier must match the size of the sampling tubes used. In the software, each carrier ID includes corresponding liquid handling parameters, geometrical and kinematical data for reading barcodes.

Only those barcode types that are actually present on the worktable must be activated.

Barcodes protected by check digits should be used (code 128 always has a check digit by definition). In case of barcodes without a check digit, at least the number of characters has to be defined.

The codabar barcode must never be used without a defined code length and check digit (modulo 16). Start and stop characters should be used and activated.

The interleaved 2 of 5 barcode must never be used without a defined code length and check digit (modulo 10). At least 6 characters are recommended for safe operation.

Log files handling
The log file function in the Logic Software must always be switched on, including serial data. The storage duration of log files (days) should be selected as appropriate to ensure the files cannot be overwritten too soon.

User management must be enabled if supported in the applied software version. The rights are assigned by the administrator and must correspond to the necessary knowledge and tasks that the user has to perform. This prevents any undesirable changes to the application being carried out by any unauthorized or untrained users.

The run of routine jobs must be performed in the operator mode (standard user) only, not as an administrator or power user!

Maintenance

Daily, weekly and periodical maintenance
Regular service and daily maintenance are prerequisites for optimal functioning of the platform to maintain high standards of accuracy and precision of pipetting, as well as minimizing instrument downtime. A precise description of the maintenance procedure can be found in the corresponding operating manuals.

Your local Tecan office offers a range of preventive maintenance contracts that will keep your equipment in the optimal condition.

For instruments with standard washable fixed tips, daily maintenance and extensive flushing with the system liquid should be carried out. Efficient cleaning of liquid system can be only achieved with the fast wash pump with high flow rate (FaWa).

We recommend:

- after switching on the instrument, flush system with ≥ 50 ml system liquid using FaWa and subsequently with ≥ 5 ml using dilutors (syringes)
- prior to starting a new application, flush system with ≥ 20 ml system liquid using FaWa and subsequently with ≥ 2 ml using syringes.

Verify that the pipetting tubing is free of any air bubbles. If any air bubbles are visible, the flushing operation should be repeated until air bubbles disappear.

Prior to switching off the instrument, standard fixed tips should be cleaned. For this purpose a syringe volume of cleaning agent is aspirated and left to soak for 10 minutes. Subsequently, flush the instrument extensively with system liquid. Thereafter the same procedure is repeated with one syringe volume of detergent and flush.

The selection of an appropriate cleaning agent strongly depends on the application. Recommendations for cleaning agents are provided in the appropriate operating manual.

Training courses

Training courses are an exceedingly effective measure for acquiring the necessary specialist knowledge for the correct use of Tecan instruments. Tecan offers a comprehensive range of courses in which in-depth theory is combined with hands-on coaching sessions. These training courses give the trainees a thorough basic knowledge of the liquid handling and pipetting platform.

We recommend that the end users of instruments participate in the appropriate training courses.

For further information visit the website www.tecan.com, contact your local Tecan representative or call the Tecan Helpdesk.

Note: not all instruments and software packages mentioned in this instrument guide are CE-marked according to the In Vitro Diagnostic Directive 98/79/EC.

Validity
This guide addresses Tecan customers in Europe and the rest of the world, but is not valid in the USA and Canada.

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