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# A Liquid Handling Robot for Robust and Reproducible Preparation of Standard and Quality Control Samples in Bioanalysis

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**Abstract**

We describe a unique liquid handling platform, based on a Tecan EVO, specifically designed for the preparation of analytic calibrators and quality control samples according to the requirements of the Good Laboratory Practice (GLP). The platform utilizes a combination of off-the-shelf software (Tecan Gemini 4.2) and custom-programmed SPIKE 1.0. The system convinces with robust and reproducibly quality and a very easy, intuitive user interface. All security requirements as per FDA 21CFRpart 11 were considered, when programming the software bundle.

**Keywords:** Robust; Bioanalysis; Liquid handling robot; SPIKE 1.0

## Introduction

The preparation of analytical standards/calibrators and quality control samples (QCs) in a GLP laboratory is one of the key challenges during the conduct of preclinical and clinical studies. The regulations of GLP demand the independent preparation of calibrators and QCs, which, being measured simultaneously, shall match each other within strictly defined criteria [1,2]. These preparations may need to be repeated, if the study runs over longer time, or an unexpected number of repetitions lead to further demand of standard and QC material.

A manual approach is time and labor intensive. Moreover, mismatches between calibrators and QCs tend to happen, with consequently repeated preparations. This leads to a higher consumption of valuable reference substance used, such as stock material and blank matrix (e.g. analyte-free serum), and a longer lead-in until calibrators and QCs are available for sample measurement.

A robotic approach in contrast could guarantee constant and robust day-by-day or bulk preparations of calibrators and QCs and may help to overcome waste of material and time. This paper describes a unique liquid handling platform allowing for an easy, robust and reproducible fully automated production of calibrators and QCs, utilizing a custom-made software application being in full compliance with 21 CFR part 11 [3].

## System Description

The robotic platform consists of a 150cm Tecan Freedom EVO equipped with an 8-channel liquid handling arm (LiHa), a barcode scanner (PosID), two on deck shakers and diverse carriers for tubes and troughs (Tecan). Gemini 4.2 was chosen as the robotic software administering the deck, labware and liquid classes, and the custom-made SPIKE 1.0 (Fornax), administering the pipetting scheme in a database and driving the Gemini to execute the steps defined in a sqlite database. The software package runs on Windows 7 and can be installed on multiple PCs to allow for separation in a development and production environment. A full computerized system validation was applied [4,5] before setting the system into productive usage.

## Software Description

Apart from the deck layout with all aspiration and dispense vectors, the Gemini administers liquid class settings, such as aspiration and dispense height and speed for any solvent used in the different bioanalytical methods (e.g. serum, urine, methanol, acetonitrile, buffer etc.).

The Gemini is driven by the SPIKE 1.0, which provides a surface with all functionalities to create/edit methods, create and execute worklists, allocate pipetting locations, administer user rights and export and import methods from other PCs (Figure 2). This information is stored in a sqlite database, where it is retrieved by the SPIKE.exe.

The Gemini 4.2 software (Tecan) controls the robotic workstation and the peripheral equipment, based on a standard deck layout (Figure 1) that differentiates between source and destination locations for high and low volumes, including two shakers for 30 mL and 50 mL tubes. Eight carriers are reserved for barcode-driven aliquoting of the prepared liquids into small cryotubes for later usage in the laboratory. The system checks before dispensing the aliquots, whether the destination tubes have the correct barcodes for the respective solution. This avoids accidental mixing of incorrectly labeled tubes into one series and later unpleasant surprises when e.g. the calibrator level 3 shows the analytic response on calibrator level 4.

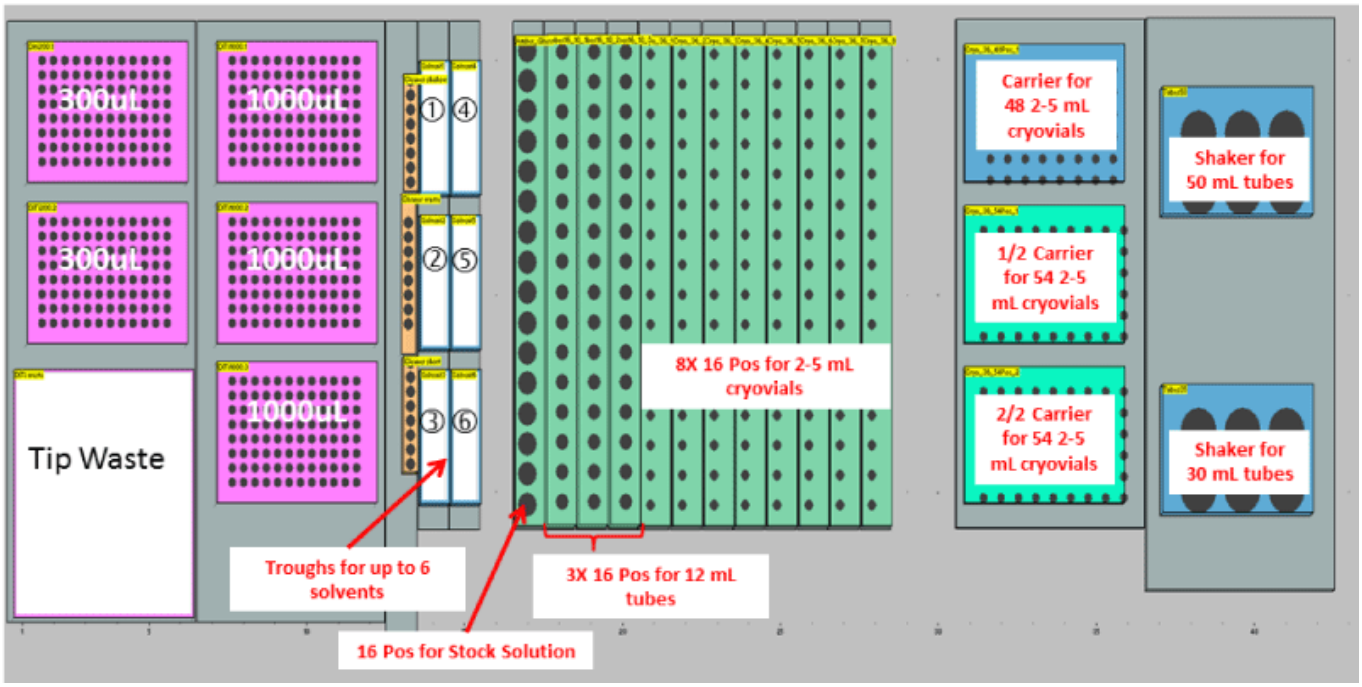


**Figure 1:** Gemini 4.2. deck layout for robotic preparation of calibrators and WCs. The deck constantly contains several tip carriers, dedicated racks for source and destination tubes (e.g. stock solutions to be located on one rack, high and low volume tubes for calibrator and QC 5 preparation on subsequent racks, carriers and shakers. Up to six different solutions can be added to the reagent troughs.

The main frame of the SPIKE appears as a table with predefined columns. In a "Create Method" mode, the user enters any information on IDs and concentrations of the source and destination solvents and the final volume to be prepared.

Since calibrators and quality control samples in most cases cannot be prepared directly from the high concentrated stock solution, interim solutions (= Working Solutions) are required. For multi-analyte methods, the SPIKE can handle the preparation of working solutions containing multiple stock solutions, with the diluent volume being correctly calculated (Figure 3).

Subsequently, a "set" is created, allocating pipetting steps to carrier positions on the deck layout and to liquid classes optimized for the solvents of interest. By saving the method, the table and set will get accessible to the end user to execute the method and prepare the desired solvents in the defined volumes.

Since calculations are embedded into the SPIKE, the end user can scale the final volume up and down on demand, while the software will automatically adjust the required volumes of stock solution and diluent. Moreover, the SPIKE recognizes, whether the volume to be transferred requires high or low volume tips and will adjust the aspirated volume per pipetting channel accordingly.

If solutions are prepared on the embedded mixers, mixing mode (speed, direction etc) can be defined within the set. If no shaker is chosen as destination location (e.g. a tube carrier for 10 mL tubes), the SPIKE will generally use the stroke of the pipetting channels (utilizing fresh tips) to do the mixing, with the number of mix cycles automatically adapted to the volume in the tube.

By creating a worklist, all steps or only parts of the table can be chosen for execution. When the worklist is prepared and visible in the run window, the method can be executed. After execution, a pdf report is generated listing the accomplished steps and volumes together with the login information (user, date and time).

The spike has several control functionalities for testing a method working as expected. First, a "Validate Entry" tool checks, whether the volumes, concentrations and locations are reasonable. SPIKE would not allow running an application with e.g. the destination concentration being higher than the source concentration.

To check liquid volumes for correctness, the "Create Worklist PQ" tool is extremely helpful. It requests the user to weigh the empty tube and will interrupt the preparation of the solution after each step to allow the user to weigh the transferred liquid volumes. By comprehension of the specific weight of the solvents, the SPIKE will calculate whether the transferred volumes are correct, or whether adjustments are necessary to the affected liquid class.

Regarding data security, access levels and user rights define which user can create or modify a method within SPIKE and Gemini, and who is limited to executing methods. Electronic signatures and audit trail round the software security requirements as per FDA 21 CFR part 11 [3].

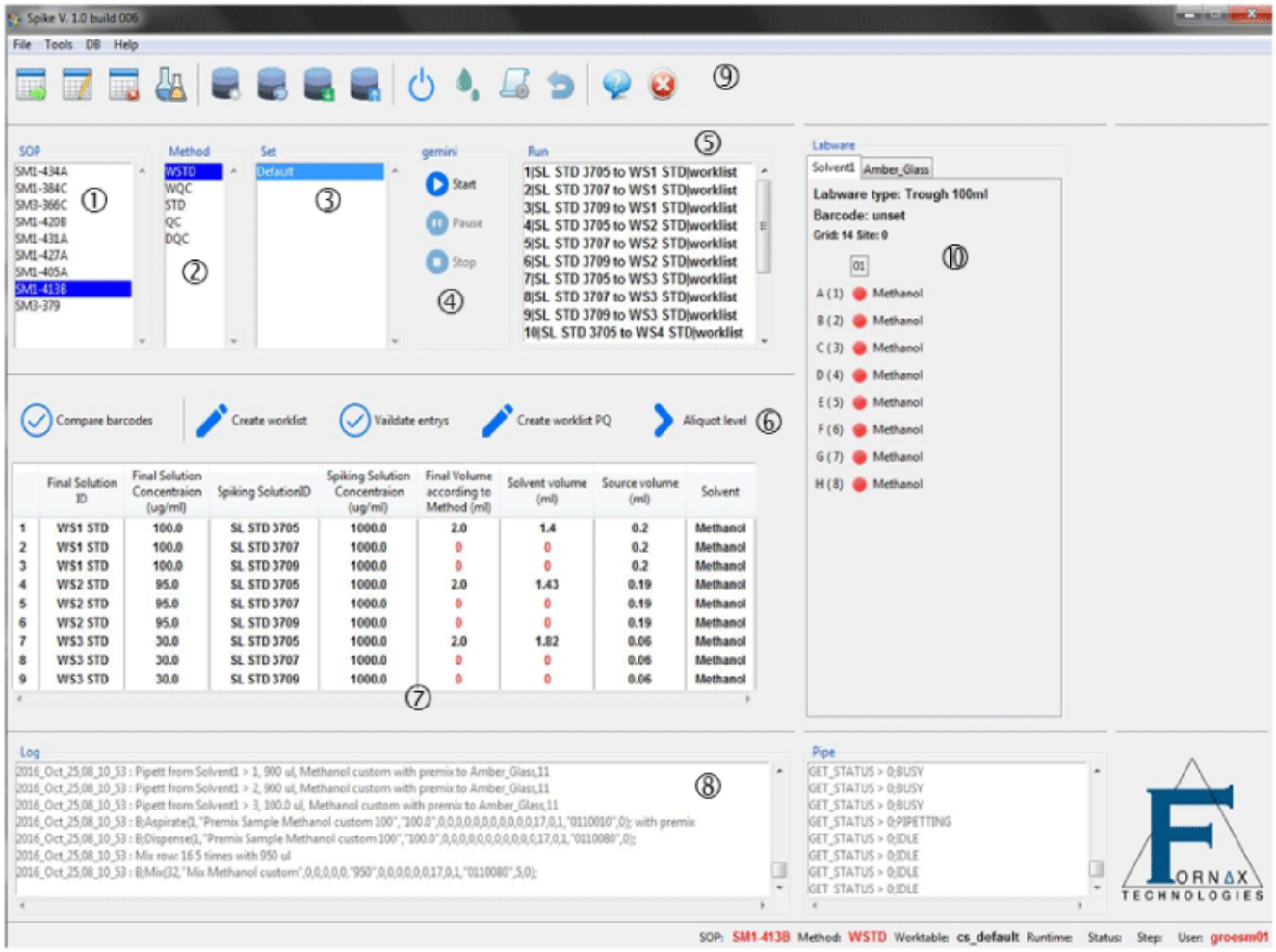


**Figure 2:** Main frame in SPIKE 1.0: 1. Display of all bioanalytical methods 2. Display of associated preparations (WSTD = Working solution standard, WQC = Working solution QC, WIS = Working solution internal standard, STD = Standard, QC = Quality control) 3. Display of sets (link between pipetting scheme and deck layout) 4. Control panel for executing a method 5. Run window with status of processed steps of worklist 6. Control panel for barcode check, worklist generation, and aliquoting 7. Table presenting the pipetting scheme, including source and destination IDs, concentrations, volumes and liquid class. Required volumes automatically get calculated by concentrations and final volume 8. Log-File display 9. Utility icons for creating/editing methods, liquid classes, Back-up and restore of methods, flushing the system, reset Tip counter) 10. Labware display for rapid check of tube or trough locations

## Results

For biosimilars as an emerging market, it is recommended to assess independently prepared standard curves, derived from stock material from the originator drug and the biosimilar drug. The idea is to avoid variabilities from the preparation as much as possible, in order to keep the reference substances the only relevant variable. For a biosimilar ELISA method, we used our robotic approach and prepared these standard curves, via several dilution steps, and sorted them in alternating order on the assay plate as recommended by Marini et al [6].

The standard curves yielded from these independent robotic preparations were found to be well in accordance to each other, not only within the preparations from one reference substance but also when curves were compared between originator and biosimilar (Figure 4). The back-calculated difference between the individual levels to the target concentration never exceeded 3%. Calibrators and QCs, which shall not exceed a difference by 15% [1], never exceeded 5%.

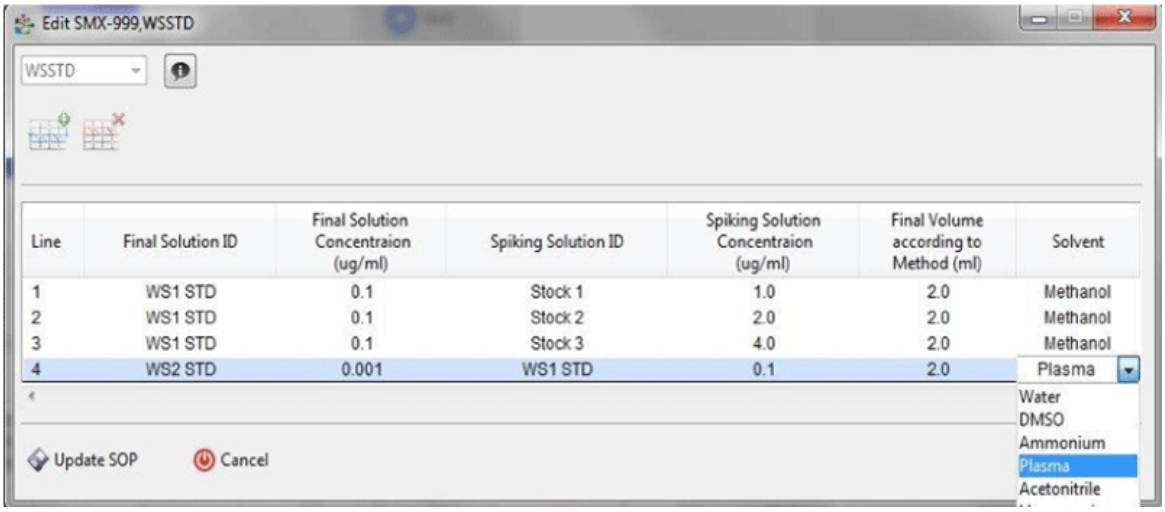


**Figure 3:** Creating a method for two working solutions (WS1 STD and WS2 STD). WS1 STD is prepared from three different stock solutions (Stock 1, 2, 3) with different original concentrations, but requiring the same final concentration in the mixture. Methanol serves as diluent for this WS1 STD preparation, while WS2 STD derives from WS1 STD with plasma as diluent.

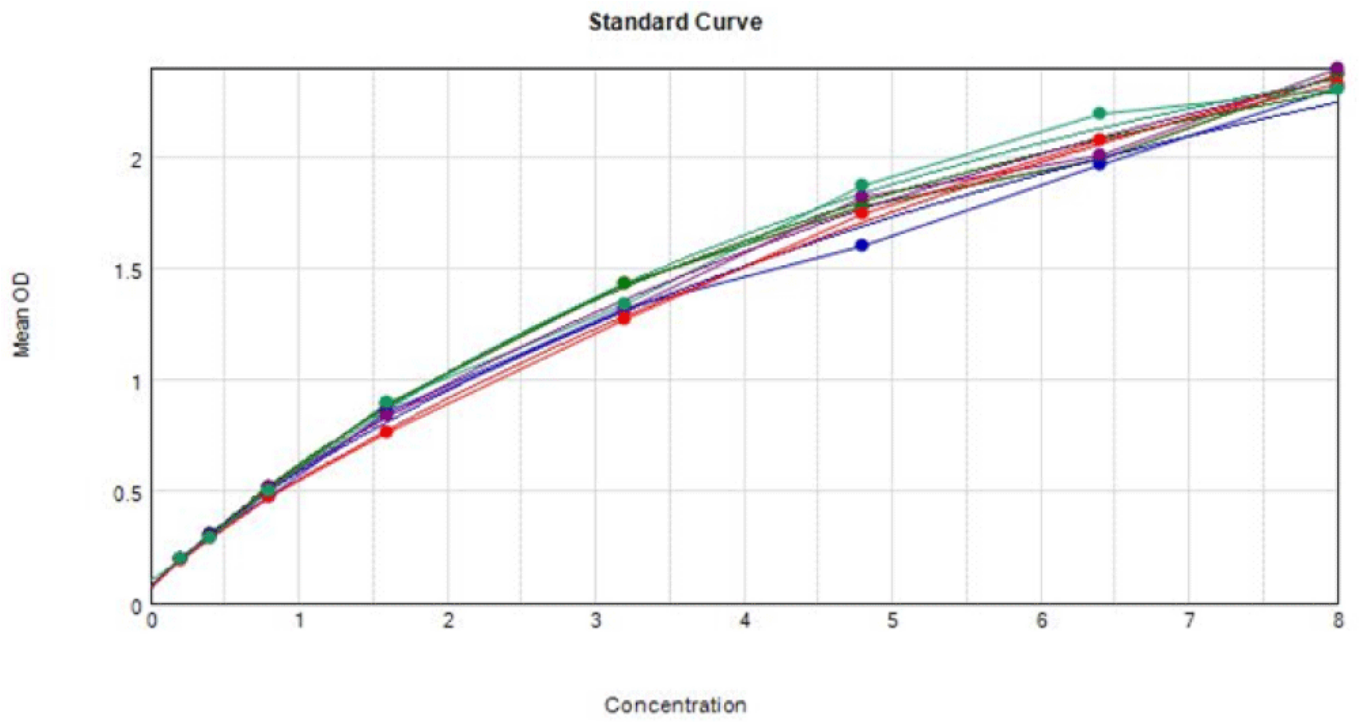


**Figure 4:** Overlay of six standard curves separately prepared by the described robotic system. The back-calculated difference between the individual levels to the defined concentration never exceeds 3%.

## Conclusion

The automated system and software described here has been proven to be a valuable tool for improving robustness and reproducibility of calibrator and quality control sample preparation. The methods can easily be entered to the SPIKE 1.0 and security tools avoid unauthorized creation or modification of methods. By introducing this unique platform in cooperation with Fornax Technologies GmbH und Co KG, Celerion Switzerland is the first Contract Research Organization to utilize robotic liquid handling systems not only for sample processing, but also for the time and material sensitive steps during the lead-in of preclinical or clinical studies.

## References

1. EMA (2011) Guideline on bioanalytical method validation pp: 1-23.
2. FDA (2001) Guidance for Industry. Bioanalytical Method Validation, CDER, CVM, pp: 1-25.
3. FDA, Electronic Records, Electronic Signatures; Final Rule. Federal Register March 21.
4. CFR Part 11 (1997) GAMP 5: A Risk based Approach to Compliant GxP Computerized Systems, ISPE.
5. Bansal SK, Thomas L, Bush ED, Hamilton M, Edward AH (2004) Qualification of Analytical Instruments for Use in the Pharmaceutical Industry: A Scientific Approach. AAPS Pharm SciTech 5(1): 151.
6. Marini JC, Michael A, Xiao-Yan Cai, Chappell J, Coffey T (2014) Systematic verification of bioanalytical similarity between a biosimilar and a reference biotherapeutic: committee recommendations for the development and validation of a single ligand-binding assay to support pharmacokinetic assessments. AAPS J 16(6): 1149-58.