



Guideline for validation of analytical methods using Cedex Bio, Cedex Bio HT and Cedex HiRes Analyzers

Application Note

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Objective

The following summarizes a brief guideline for validation of analytical methods (e.g., on Cedex Bio, Cedex Bio HT, Cedex HiRes Analyzers) based on interpretation of the current ICH-Q2 guideline.* This guideline provides suggestions for developing methods for validation and appropriate analytic data generation during manufacturing of active pharmaceutical ingredients (APIs) in compliance with good manufacturing practice (GMP) specifications. This guideline is meant to support a company's quality authorities; accountability remains within the respective quality departments. Implementation of validation processes may differ between companies and sites.

Scope

Parameters used for in-process control (IPC-parameter) are of special significance and should be validated. This guideline focuses on validation design of experiments and excludes any additional recommendations, such as documentation, storage etc.

* International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use: ICH Harmonized Tripartite Guideline Validation of Analytical Procedures: Text and Methodology Q2

Validation – Preparation

Materials

Quality of validation data is highly dependent on the quality of materials used. Sampling, sample handling, and appropriate storage must be monitored by the operators. Expiration dates of all consumables must be checked.

Documentation

GMP-compliant documentation should be maintained. Usually a validation plan describing methodologies and acceptance criteria must be set up and approved before starting validation. Ensure data integrity as well as appropriate deposition of raw data planned also to be implemented within the validation report.

Qualification

Guarantee that only qualified devices are used.

Validation – Design and criteria

Design

We recommend validating the analytical methodology by following the listed criteria within dedicated conditions, such as (production) process, (production) clone, matrix, and measurement range. Since every analytical methodology has its own benefit (or drawback), perform an independent validation instead of a comparison against an established methodology.

However, comparability to previous methods should be one part of evaluation to assess the impact of change in your particular analytical methods on your particular process. Depending on the parameter, characteristics of the following criteria should be checked during validation design.

Usually, an evaluation is performed before the validation to define the ranges to be used as acceptance criterion for each parameter.

Specificity

Influence of your test result has to be shown to be independent from impurities, degradation products, or matrix effects which could differ from process to process. Even though Roche CustomBiotech test kits have been shown to be highly specific, we recommend checking for substances within your sample matrix which might interfere with the test or analyte. Interference could be shown by, for example, spiking experiments or using additional analytical methodologies to distinguish between analyte and potential interference. By including your sample matrix for spiking/dilution series experiments, validation of specificity can be easily combined with determination of accuracy, linearity and precision by repeatability.

Measurement range and sample preparation

The measurement range defines the upper and lower limit of values for validation of precision, linearity, and accuracy. Define your desired final measurement range and consider if it is possible to check an additional range above the upper limit and below the lower limit. Proceed using your sample exactly the same way you handle your sample during routine measurements. Use a sufficient number of measurement points (as per your definition) around concentrations of special interest, such as range or borders of in process control (IPC) values.

Cedex Bio/Bio HT Analyzer

Every metabolite test has its defined test range described within the Instructions for Use. Your targeted validation test range might be different and dependent on your process. Note that by defining your own validation test range inside (or outside) the defined range of the respective test, you might adapt automated flagging and reference range by the instrument. Use the Roche CustomBiotech Control Set Level 3 to spike your sample matrix (e.g., medium). If your analyte baseline within your sample matrix is above your desired minimum measurement range, use water or buffer as diluent. Turn off the rerun function if your validation range is too close to the upper limit of the specific range of the test kit.

Cedex HiRes Analyzer

Desired measurement range of your clone/process might be above the density of the Cedex HiRes Control Beads. Nevertheless, use the Cedex HiRes Control Beads in parallel with cell culture samples of different origin or density to check linearity. For example, use a high cell culture sample to generate a serial dilution within your sample matrix (fermentation media).



Cedex HiRes Analyzer



Cedex Bio Analyzer



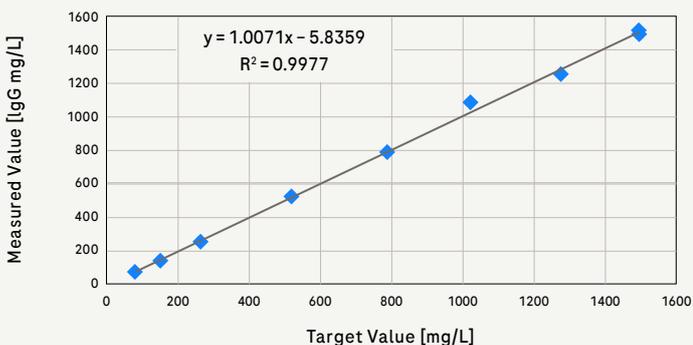
Cedex Bio HT Analyzer

Linearity

A linear correlation between target value and measured value of your analyte must be shown. Perform at least 3 sample requests (better 5 – 7) of at least 5 different analyte/sample concentrations within your measurement range to be validated.

Cedex Bio/Bio HT Analyzer

Use the Roche CustomBiotech Control Set Level 3 to spike your sample matrix.



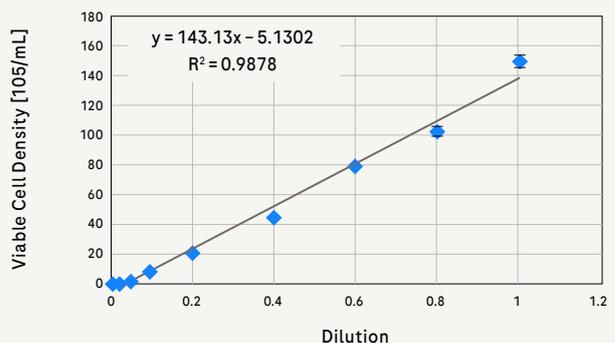
01a

Cedex Bio HT linearity of IgG determination: An IgG molecule was spiked into sample matrix (Roche evaluation data).

Cedex HiRes Analyzer

Start checking the linearity of live cell density using the Roche CustomBiotech Cedex HiRes Control Beads within an appropriate density range (up to 106), according to the directions in the Instructions for Use. Additionally, use a serial dilution of your cell clone covering your entire desired target range. You should also check the linearity of viability using different kinds of Cedex Viability Beads.

Plot the measured values to the (calculated) target values and determine the coefficient of correlation (r), intercept to y-axis, slope of regression line, and square sum of residues. For some analytical methods, a mathematical transformation will be necessary to show linearity.



01b

Cedex HiRes linearity determination: A high cell culture sample was used for a serial dilution (Roche evaluation data).

Accuracy (Recovery)

Accuracy defines the correctness of determined values over the complete measurement range. Combine a specificity check by also testing the influence of possible impurities, degradation products, or matrix effects. Accuracy can be determined by comparing measured values to given values (of known purity), or to values generated by an independent established methodology. Perform at least 3 sample requests (better 5 - 7) of at least 3 different analyte/sample concentrations within your desired measurement range.

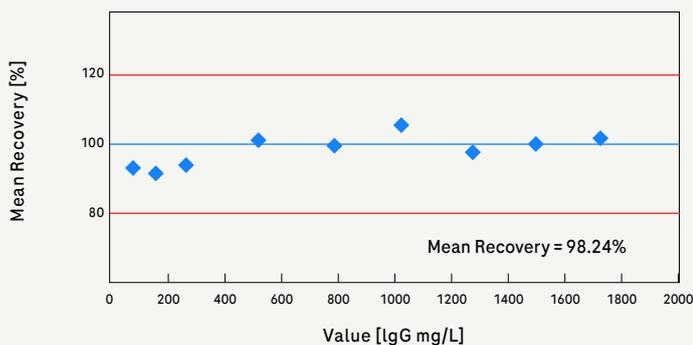
Cedex Bio/Bio HT Analyzer

Use the Roche CustomBiotech Control Set Level 3 to spike your sample matrix. Check for any additional influence within your sample/matrix which might have a negative effect on the test (function) to be validated.

Cedex HiRes Analyzer

Use the Roche CustomBiotech Density Reference Standard Beads to check the accuracy of the system, and then monitor trends in system performance over time with the appropriate Cedex HiRes Control Beads. Follow the directions in the respective Instructions for Use.

Use samples of your cell clone and check the Cedex HiRes Analyzer raw data picture analysis. Verify correct detection and discrimination of living cells. Sometimes standard operator settings may need to be optimized. If possible, compare Cedex HiRes Analyzer data with a reliable alternative cell counting methodology. When changing the methodology, by definition, the previous device (if validated) is considered reliable. For each sample/dilution values, calculate the recovery obtained to given value [%], as well as a confidence interval of recovery.

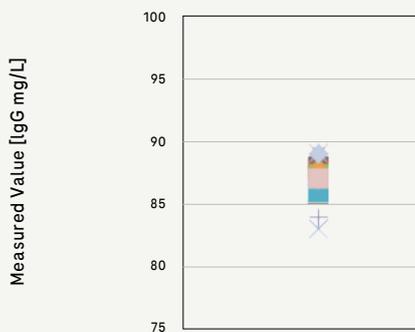


Precision by repeatability

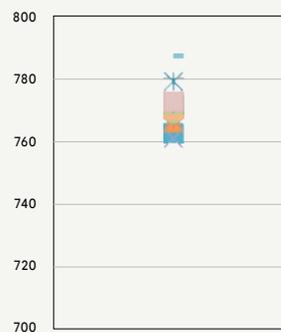
Check repeatability by repeating measurements of the same sample individually requested on one device. Perform at least 3 sample requests (better 5 - 7) of at least 3 different analyte/ sample concentrations within your measurement range to be validated.

Cedex Bio/Bio HT, Cedex HiRes Analyzer

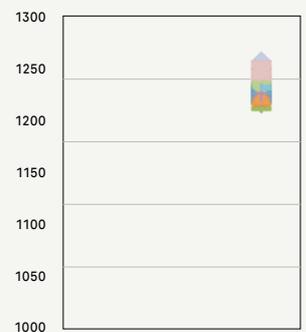
Use the different spiking/dilution samples from linearity determination within your upper, middle, and lower measurement range. Calculate the standard deviation, relative standard deviation, and confidence interval for each concentration within the tested measurement range.



RSD = 1.74 %



RSD = 0.77 %



RSD = 0.88 %

Precision by intermediate precision

Intermediate precision considers stability of values over different instruments, operators, or days. The Cedex Bio/Bio HT Analyzer as well as the HiRes Analyzer generally show high device-to-device and day-to-day comparability. If necessary, repetition of linearity, accuracy, and repeatability by variation of those conditions may be required. When comparing different instruments, use the same, i.e. replicate, samples. When validating day-to-day performance or different operators, consider using fresh (spiked) samples. According to precision of repetition, calculate the standard deviation, relative standard deviation, and the confidence interval for each concentration within the tested measurement range.

Detection limit and quantitation limit

Detection and quantitation limits are described within the documentation of the respective tests. Your sample matrix might influence limits. Evaluate the minimum level at which a signal can be reliably detected, determined, and quantified with acceptable accuracy and precision. Typically your quantitation limit is equal to your lower measurement range.

Robustness

In addition to reagent stability, assay robustness also correlates to sample stability, which is highly dependent on the sampling process, sample handling, storage time, and storage conditions. Analyze your desired process and consider evaluating whether one of your parameters of interest might be sensitive to stability and continuity. Also be aware of defined borders and flags, calibration cycles, and stability of reagents described within the instrument manuals, as well as the Instructions for Use of the reagents.

Cedex Bio/Bio HT Analyzer

In order to generate appropriate data for metabolite analysis, spin down samples as early as possible since analyte concentrations of cell-free supernatants are much more stable compared to cell-containing samples. For some analytes, it might also be possible to use whole broth samples. However, note that such a procedure biologically would have a major effect on robustness and would need to be validated over time.

Terms of revalidation

We recommend revalidation of your analytical method when changing processes, compounds (e.g., matrix), specifications (e.g., test range), and device, or when changing regulatory authority requirements.

Regulatory Disclaimer

For use in quality control/manufacturing process only.

Trademark

CEDEX is a trademark of Roche.

Note: Roche Technical Service provides documentation and assistance for Installation Qualification (IQ) and Operation Qualification (OQ) but cannot perform, or provide support for Performance Qualification (PQ). PQ design, performance, and evaluation is done entirely under the responsibility of the customer.



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