Immunoassay, Clinical Chemistry and Specific Protein Calibration
Definitions:

Calibration curve: Is a plot of the “Analytical Signals” (ex. Optical Density) against the concentration of the analyte (substance to be measured). This curve allows to give the results of samples in terms of concentration starting from absorbance.

Master curve: The master curve is calculated from a number of calibration curves derived from different instruments by Adaltis QC. The parameters for the curve are stored on the Smart-Card supplied with each kit pack of Specific Reagent.

C1&C2 Controls (Immunoassay): 2 controls at different concentrations (low and high) and target absorbance, specific for each method and inserted into the specific reagent IA kit. They are used to validate the calibration curve.

Calibration kit (calibration set): it is a kit (external to specific kit for all line of ECL product). It is used to perform a multi-point calibration.

Common Blank for IA: it is the background for all IA methods; it is done by reading 50 µl of substrate + 450 µl of stop.

Reagent blank for CC and SP: it is the indicator of the validity of the specific reagent. It is done by reading CC diluent + specific reagent.

Control C1 Clinical Chemistry: Diluent for CC used to do a reagent blank.

Sample blank for SP: measures the eventual sample interferences in the reaction measurement. is a reading of sample + Reagent1.
Immunoassay Calibration

• 2-point calibration
  Eclectica checks the calibration curve of the smart card (master curve) by testing the two controls (C1 & C2),

• 6-point calibration
  If after twice run the C1 & C2 are not accepted the 6-point calibration is required.

• The algorithm used by the software to elaborate the calibration curve is a 5 parameters.

• The validity checks of the calibration curve include:
  ✓ OD Range of Calibrator A
  ✓ For all points (standards) of the curve, the difference between backfit* and theoretical concentration.

*Backfit is the concentration of the calibrator, read on the curve done.
Immunoassay Controls

- The C1/C2 controls must be included in the first run of a new lot of Specific Reagents. This to verify or to adjust the master curve (if necessary) depending on the local conditions of the instrument.

- This check of the master curve is stable within a period that depends on the analyte (2-4 weeks); after this period the user should repeat the C1&C2. In case they are not run again the results are given as “temporary result”.

- When the C1&C2 controls are run, some checks are performed to accept the C1&C2 OD.
Immunoassay Controls

Master curve
Supplied by Adaltis on a smart card

Calibration curve

Active curve

C1 and C2 Controls
Immunoassay - Controls acceptance criteria and re-calibration rules

1. Precision criteria: the %CV between the first and second replicate of C1 and the first and second replicate of C2. The limit depends on the analyte. Beyond this range, the accuracy is reduced and the software will invalidate the master-curve.

2. C1 & C2 Acceptability limit: OD target ± certain percentage. This limit of acceptability on the controls is up to 35% for competitive assays and up to 45% for direct assays (Adaltis R&D has established these limits where the performances of the methods are respected). Beyond this range, the accuracy is reduced and the software will invalidate the test.

3. C1 & C2 Recalibration limit: for each lot of analyte, the smart card supplies a target OD for both C1 and C2. The recalibration limit for competitive assays is up to 10% and up to 20% for direct assays.
   - If C1 & C2 fall both into these limits the accuracy is excellent, the software will accept the master-curve without modifying it.
   - If the OD of C1 or C2 are over these limits the software will shift the master-curve (using only the OD of the control that is over the limit) before accepting it. In this case C1 and C2 act as calibrators and not as controls.
Sample Result conditions

- C1/C2 are accepted: VALID TEST
- The curve is still valid (C1/C2 have not expired): VALID TEST
- A new kit lot is loaded → No valid curve. C1/C2 fail: INVALID TEST (no results: only ODs). You have 24 hours to repeat C1 and C2.
- C1/C2 expired and they fail: TEMPORARY RESULTS. You have 24 hours to repeat C1 and C2.
- C1/C2 repeated and accepted: TEST CONFIRMED. This situation applies to both the previous cases.
- C1/C2 are not repeated within 24 hours: TEST NOT CONFIRMED

NOTE: If you run a 6-points standardization there’s no need to check C1 and C2 the first time (only at curve’s validity expiry)
Clinical Chemistry Calibration

- The master curve for the clinical chemistry methods doesn’t exist
- The user must perform the calibration curve when a new lot of specific reagent changes
- The calibration curve is stable within a period that depends on the analyte and after this period the user should do a new calibration curve
  - The Sw allows also to report the results with the invalid curve.
- Normally the “calibration kit” is a single point calibrator but there are few methods that have a dedicated multi-point calibration kit
- The algorithm used by the software for the calibration curve is a linear regression for the single or two point calibration otherwise is a 4 o 5 parameters.
- There are certain validity checks that the software must do to verify the calibration curve:
  - Range for OD of Calibrator A (reagent blank)
Clinical Chemistry Control

• The true meaning of the control (C1) in clinical chemistry is the reagent blank.
• For clinical chemistry methods there isn’t control C2.
• The user should do a control every day.
• When the control (C1) is run, the result must fall within acceptable limits:
  ✓ Range for OD of Control C1 (reagent blank)
Clinical Chemistry Calibration

- Some enzymes (AST-GOT, ALT-GPT, Gamma GT, ALP, CK Nak, CK MB) do not have a calibration curve because they use a factor.
- The factor, related to the temperature and time, is fixed and cannot be modified.
Specific Protein Calibration

• The “calibrator set” is used to perform the calibration curve at any new lot of specific reagents
• The calibration curve is stable within a period of time that depends on the analyte. Beyond this period the user should do a new calibration curve (The Sw allows to report the results with also the invalid curve)
• Normally the “calibrator set” is a single point calibrator that the instrument serially dilutes to have the whole range of calibrators
• ASO, Lipoprotein A, Beta2 Microglobulin have a calibration set (calibrators pre diluted by Adaltis)
• The algorithm used by the software for the calibration curve is 5 parameters
• Checks on the calibration curve:
  ✓ OD Range for Calibrator A
  ✓ Difference between backfit and theoretical concentration for all single calibrators
• For the specific proteins methods there are no control C1 & C2
SP Calibration

• The calibration process requires the Samples Carousel.

• All assays can be divided in 2 groups depending of their composition:

  – **1st Group:** IgA, IgG, IgM, C1r, Ceruloplasmin, AntiTrombin 3, Alfa-Glycoprotein, Albumin, C3, C4, AAT, Apatoglobin, Transferrin, Kappa and Lambda chains, Prealbumin, Microalbumin, Fibrinogen, Fibronectin, CRP, Apo A/B

    1 vial for the Standard Calibration (only 1 tube)
    2 tubes for the dilution of the standard (the other dilutions are made with the needle)

  – **2nd Group:** ASO, b2-microglobulin, Lp(a)

    4 vials (4 tubes)