P0320
SeraQ Alinity V2

REF P0320

The kit insert contains a detailed protocol and should be read carefully before testing the run control to ensure optimal performance.

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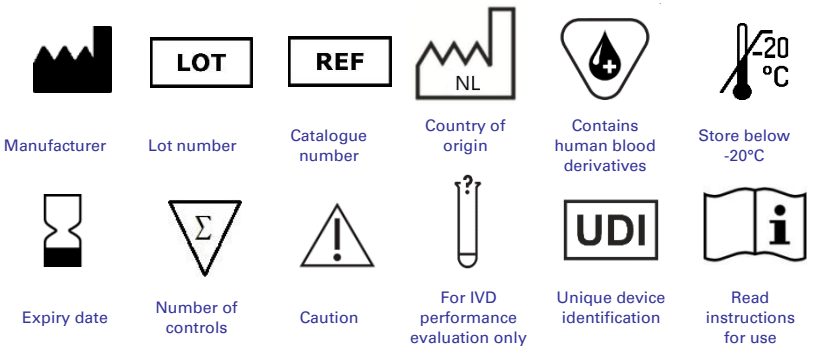
Intended Use

P0320 SeraQ Alinity V2 is intended to be used on the Abbott Alinity ci series® platform in diagnostic and blood screening laboratories as an external run control in combination with the assays for the detection of hepatitis B surface antigen (HBsAg), antibodies to hepatitis B core antigen (anti-HBc), antibodies to hepatitis C virus (anti-HCV), antibodies to human immunodeficiency virus types 1 and 2 (anti-HIV-1/2) and antibodies to human T-cell leukemia virus type I and II (anti-HTLV I/II) (see Table 1). P0320 SeraQ Alinity V2 is a multi-marker mixture of inactivated HBsAg, anti-HBc, anti-HCV, anti-HIV-1 and anti-HTLV I standards in defibrinated plasma giving a low reactive result in the Abbott Alinity ci series® assays. The run control is intended for repeated testing in consecutive runs of the immunoassays over time by trained laboratory workers. By comparison of the sample to cut off (S/CO) values for the five markers found on P0320 SeraQ Alinity V2 one can monitor whether the analytical sensitivity of test runs is consistent. The run control should not be used to replace internal controls or calibrators in the test kits. The test result on the run control should not be used to reject the run or delay the release of test results on donor or patient samples. This product is used for performance evaluation only.

Table 1 Test kits and platform covered by this run control

Equipment	Agent	Assays
Abbott Alinity ci series®	Hepatitis B surface antigen (HBsAg)	Alinity ci HBsAg
	Anti-hepatitis B core antigen (anti-HBc)	Alinity ci Anti-HBc
	Anti-hepatitis C virus (anti-HCV)	Alinity ci Anti-HCV
	Anti-human immunodeficiency virus type 1 (anti-HIV-1)	Alinity ci HIV Ag/Ab Combo
	Anti- Human T-cell leukemia virus type I (anti-HTLV I)	Alinity ci HTLV I/II

Key to Symbols Used



Principle of method

A series of SeraQ multi-marker run controls has been designed for monitoring HBsAg, anti-HBc, anti-HCV, anti-HIV-1 and anti-HTLV I test performance. The run control tubes are barcoded and can be placed at random positions in sample racks of the blood screening device. The tubes are comparable in size to donor blood collection tubes. The run controls are designed to mimic naturally occurring serum specimens with low reactivity for HBsAg, anti-HBc, anti-HCV, anti-HIV-1 and anti-HTLV I. The analytical sensitivity of test kits from

different manufacturers varies and therefore for each combination of test kits a separate multi-marker run control has been designed. This SeraQ run control family includes the product P0320 SeraQ Alinity V2 for which the composition is optimised for use with the Abbott Alinity ci® test system. The P0320 SeraQ Alinity V2 run control is designed to generate assay response values (i.e. S/CO ratios) positioned in the low positive range of the assays. Routine use of external run controls enables laboratories to monitor day-to-day test performance and *in-vitro* medical diagnostic device (IVD) reagent lot variation. After approval of the Notified Body a summary of the safety and performance of the P0320 SeraQ Alinity V2 run control will be published at the EUDAMED website of the European Union[1].

Traceability of antigen and antibody concentrations

For each HBsAg, anti-HBc, anti-HCV, anti-HIV-1 and anti-HTLV I an internal serum standard has been established[2] from which reference panels and run controls are prepared by gravimetrically recorded dilution steps. The undiluted S0001 standard for HBsAg is derived from the same purified heat-inactivated source material as is used for preparation of the 2nd WHO HBsAg adw2 (00/588) International Standard (IS)[3,4]. Studies with the later established WHO international hepatitis B virus genotype reference panel showed that the heat-inactivation of HBsAg in the International Standard had little impact on the detectability in immuno-assays[5]. The HBsAg concentration in the run control has been set at 0.088 IU/mL based on the dilution factor of the HBsAg standard[2,3]. During manufacturing of SeraQ run controls the measurable HBsAg concentration reduces to a certain extent depending on the test method. One IU of heat inactivated HBsAg was found to be equivalent to 0.67 nanogram (ng) of HBsAg when historically calibrated against the first HBsAg standard established by the Paul Ehrlich Institute (1st PEI HBsAg standard), comparable to conversion factors of 0.58 and 0.71 reported in WHO collaborative studies[3,4,6]. The S0001 HBsAg standard used for preparation of the SeraQ run controls has been instrumental in studies to establish the length of the pre-HBsAg infectious window period and the infectivity of HBsAg positive blood without detectable hepatitis B virus (HBV)-DNA[7,8]. No unitage could be assigned to the internal standards for anti-HBc, anti-HCV, anti-HIV-1 and anti-HTLV-I since international reference preparations are not available. The consistent concentration of the analytes in consecutive SeraQ run control batches is guaranteed by release testing against a reference batch of the run control kept frozen at -30°C. These reference batches are derived from the same undiluted internal standards that are used for manufacturing of the SeraQ run controls.

Materials Provided

The run control contains human serum and 0.01% (w/v) Thimerosal as preservative and is provided in two formats as detailed in Table 2.

Table 2. Description of P0320 SeraQ Alinity V2 kit formats and contents

Cat. Code	GTIN/UDI-DI code^	Quantity run control	Tube size	Claimed sample volume	Secondary packaging
P0320/01	8718719831588	60 x 3.0 mL	10 mL	2.9 mL (+overfill)	60 tube rack in box
P0320/02	8718719832226	10 x 3.0 mL	10 mL	2.9 mL (+overfill)	10 tubes in bag

^ Global Trade Item Number = Unique Device Identification - Device Identifier (UDI-DI) code

The basic UDI code (or Global Model Number (GMN)) of the P0320 SeraQ Alinity V2 run control is 871871983P0320EG.

To facilitate automation the run control is presented in a polypropylene tube with screw cap comparable in size to vacutainer tubes used for donor sample collection. In addition, the label includes a barcode identifying the product, sequential batch number and multi-marker: MM. The barcode of each run control tube can be read by the Abbott Alinity ci series instruments.

Materials not provided

Pipetting devices in IVD test systems, a vortex instrument for thorough mixing of samples prior to use and a water bath of 37°C for quickly thawing of run control are not provided.

Storage Instructions

Store unopened tubes at or below -20°C. For each Alinity instrument thaw one run control tube in a water bath of 37°C until ice clot has disappeared. After thawing, the run control tubes should be stored at 2°C to 8°C for no longer than one week.

Warning and precautions

P0320 SeraQ Alinity V2 run controls are prepared from serum standards, in which virus has been inactivated by validated methods applied in the plasma industry[2]. Infectivity and inactivation data have been analysed to demonstrate absence of residual infectivity of HBV, HCV, HIV-1 and HTLV I in the run controls[2]. The serum matrix in the run controls has been tested for infectious disease markers by serologic and molecular screening methods. However, no screening strategy can offer complete assurance that products derived from human blood cannot transmit undetected infectious agents. The run control should only be used by trained laboratory workers who are aware of the potential risk of infectious agents in human serum samples and take the necessary precautions.

- SeraQ run controls should be handled with the normal preventive measures in a serology laboratory[9,10].
- This product contains human plasma and traces of biological source material of non-human origin (bovine thrombin).
- The use of the run control in other assay configurations should be avoided and is not supported by the manufacturer.
- Wear disposable gloves when handling samples.
- Do not eat drink, smoke or apply cosmetics in areas where specimens are handled.
- Do not pipette by mouth.
- If skin or mucous membrane exposure occurs, immediately wash the area with copious amounts of water.
- Disinfect spills using a 0.5% hypochlorite solution (1:10 v/v household bleach) or equivalent disinfectant.
- Dispose unused or spilled materials according to the normal practices for biological waste disposal in your institution.
- If precipitates are visible, mix the run controls for 2 minutes thoroughly using a vortex instrument.
- Do not use run controls beyond one-week storage at 2-8°C.
- Store run controls in an upright position.
- Validation of the diagnostic test results must be based on the specifications set by the manufacturer of the test kit and not be influenced by the test result on the run control.

Reagent preparation

- For first use of the run control thaw the tube quickly in a water bath at 37°C.
- Mix gently during thawing until contents are just thawed.
- Immediately after ice clot has disappeared remove the run control tube from the water bath.
- Before testing allow the run control tube to adapt to room temperature.
- Mix the run control tube thoroughly prior to use with a vortex instrument.
- Place the run control tube at the specified positions in the sample racks of the Alinity system for regular donor or patient samples.
- Test on the Abbott Alinity platform with the assays mentioned in Table 1 according to the manufacturer's instructions.
- Store the opened tube immediately after use at 2-8 °C (see storage instructions).

Analytical Performance Characteristics

SeraQ run controls have been designed by examination of the response curves on dilutions of the internal standards and as such relate to the analytical sensitivity of immunoassays. In the following paragraphs the essential analytical performance characteristics of SeraQ run controls are presented.

Dose response and analytical sensitivity

By analysing standard dilution series, the relationship between S/CO values and concentration of the analyte can be established[11,12]. Plotting Log transformed Alinity S/CO values against Log concentration of analyte using linear regression analysis enables calculation of correlation coefficients. Figures 1a-e show linear dose response relations in the Abbott Alinity HBsAg, anti-HBc, anti-HCVII, HIV-Ag/Ab Combo and anti-HTLV I/II assays obtained after Log transformation of dilution factor and S/CO values.

Figure 1a. Dose response in Abbott Alinity HBsAg assay. Log HBsAg S/CO values are plotted against log dilution of HBsAg standard (r^2=0.99).

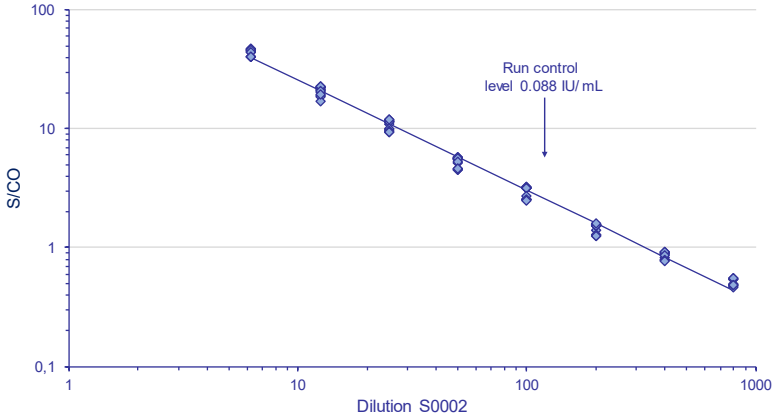


Figure 1b. Dose response in Abbott Alinity anti-HBc assay. Log anti-HBc S/CO values are plotted aginst log dilution of anti-HBc standard (r^2=0.99).

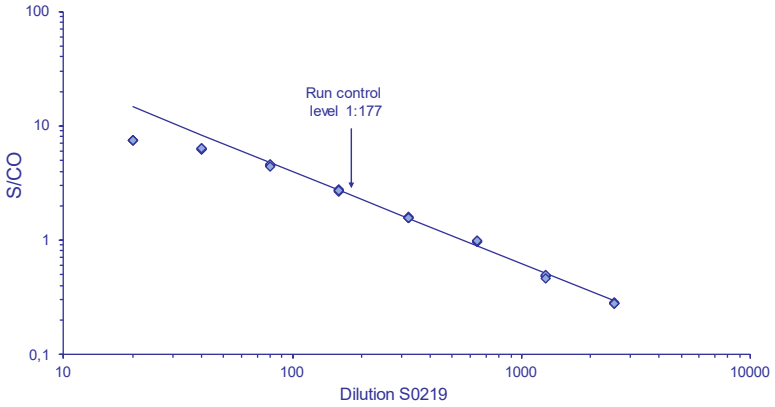


Figure 1c. Dose response in Abbott Alinity anti-HCV assay. Log anti-HCV S/CO values are plotted aginst log dilution of anti-HCV standard (r^2=0.99).

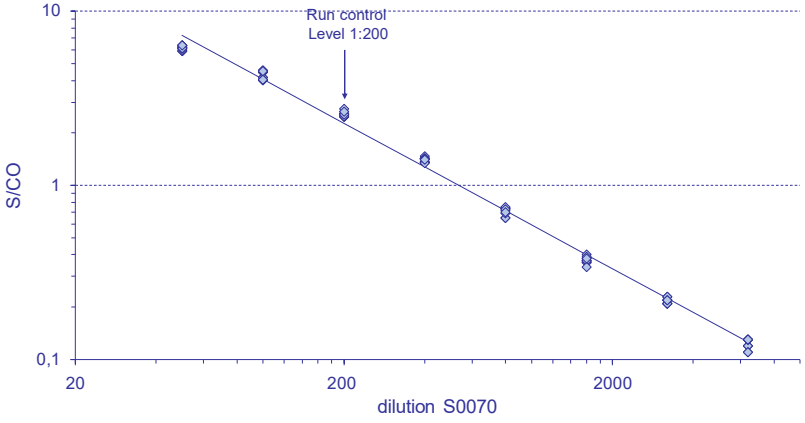


Figure 1d. Dose response in Abbott Alinity HIV Ag/Ab Combo assay. Log anti-HIV-1 S/CO values are plotted aginst log dilution of anti-HIV-1 standard (r^2=0.99).

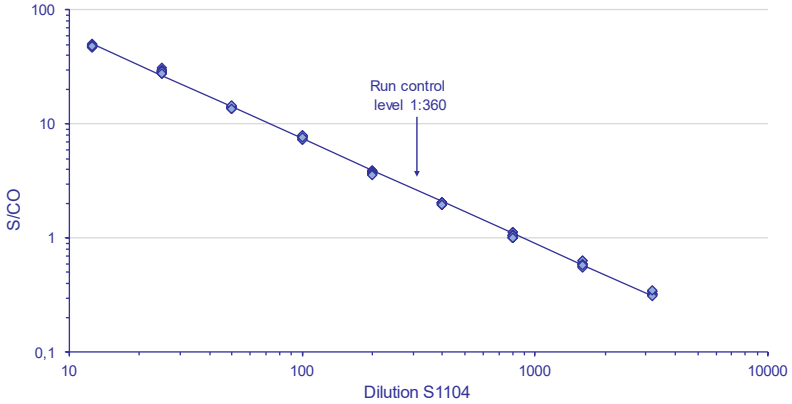
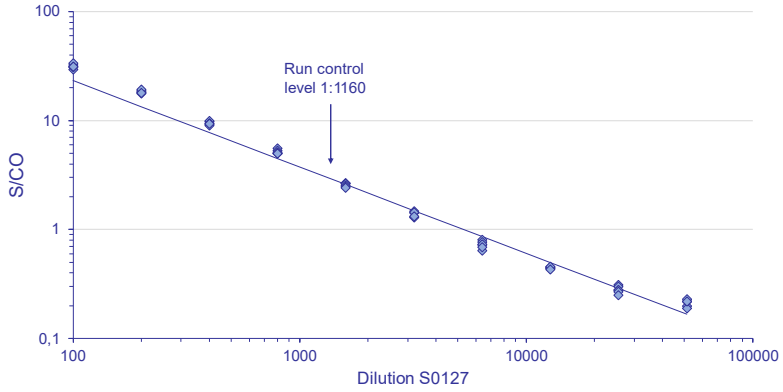


Figure 1e. Dose response in Abbott Alinity HLTV I/II assay. Log anti-HTLV-I S/CO values are plotted aginst log dilution of anti-HTLV-I standard (r^2=0.99).



Expected assay response values on run control

The expected results for the P0320 Abbott Alinity V2 run control are as follows:

1. HBsAg range S/CO ratio: 2.0 – 3.4
2. Anti-HBc range S/CO ratio: 1.9 – 2.7
3. Anti-HCV range S/CO ratio: 2.0 – 3.2
4. Anti-HIV-1 range S/CO ratio: 1.6 – 4.5
5. Anti-HTLV I range S/CO ratio: 2.0 – 2.7

Each Alinity reagent lot appears to have its own dose response curve and distribution of S/CO values on SeraQ run controls. This depends on the analytical sensitivity of the Abbott Alinity reagent lots that are in use. Thus, it cannot be guaranteed that the assay response values will always fall within these ranges. P0320 SeraQ Alinity V2 run control serves as an independent standard for monitoring consistent analytical sensitivity of Abbott Alinity reagent lots over time.

Interpretation of Results

Calculations

Subsequent test runs can be analysed by appropriate statistical approaches on the S/CO ratios obtained on the external control samples. A software system (DataQ Analytics) is available via the website www.bioqcontrol.com for entering S/CO values and generating a statistical report with the following calculations for preparing a Levey-Jennings Chart:

Transforming assay response values

To obtain the test kit batch specific reference values for each marker, an initial collection of at least 30 consecutive test results is required. Upon collecting additional data, the chart characteristics may be updated.

- The S/CO values for HBsAg, anti-HBc, anti-HCV, anti-HIV and anti-HTLV are 'log normally' distributed. For the Abbott Alinity assays one should use the logarithm of S/CO ratios for calculation of the geometric mean and predictive interval (pred. int.) as follows:
  - Calculate from each measurement the log S/CO value.
  - Calculate average and standard deviation on this log transformed values; Average (log) and Standard Deviation (log).
  - Calculate the (geometric) mean in S/CO ratio by taking the anti-log value of the Average (log).
  - Calculate Student-t-values belonging to the 95% and 99% pred. int. for different number of observations (n) (Table 3).
  - Calculate the 95% and 99% pred. int. (log) as follows:

    99% Lower limit (log): Average (log) – (99%) Student-t-Value x Standard Deviation (log)
    95% Lower limit (log): Average (log) – (95%) Student-t-Value x Standard Deviation (log)
    95% Upper limit (log): Average (log) + (95%) Student-t-Value x Standard Deviation (log)
    99% Upper limit (log): Average (log) + (99%) Student-t-Value x Standard Deviation (log)
  - Take the anti-log values for calculating the predictive limits in S/CO ratio. To visualize the individual S/CO values make a Levey-Jennings control chart on a linear scale. S/CO ratios plotted on a linear scale depict the upper 95% and 99% predictive limits at greater distance from the geometric mean S/CO value than the lower predictive limits (see Figure 2).

Levey-Jennings Chart

Figure 2a-e shows examples of Levey-Jennings charts for different Alinity assays on the P0320 SeraQ Alinity V2 run control as can be obtained from the statistical reports by the DataQ Analytics software system. The Levey-Jennings chart is a graph in which quality control results are plotted over subsequent test runs in time to give a visual indication when a laboratory test is (not) working well. The data points for each test run in the scatter plots in Figure 2 show the distance from the geometric mean S/CO ratio (green line in graph) which is the expected response level for the run control. The orange and red lines represent the 95% and 99% predictive intervals respectively. The data represents individual measurements of different laboratories and instruments.

Table 3. Relation of Student t value and numbers of runs (n) to calculate predictive intervals.

Runs (n)	t-value at 95% pred. int.	t-value at 99% pred. int.
10	2.262	3.250
20	2.093	2.861
30	2.045	2.756
Infinite	1.960	2.576

Infinite equals the normal distribution

Figure 2. Levey-Jennings charts of P0320 SeraQ Alinity V2 run control results in Abbott Alinity ci series assays from different laboratories represented by the orange dots and blue dots for one laboratory and its peer group respectively. The average (green line) and 95% and 99% predictive intervals (orange and red lines) are log transformed as explained in the text.

Figure 2a. Abbott Alinity ci series HBsAg assay.

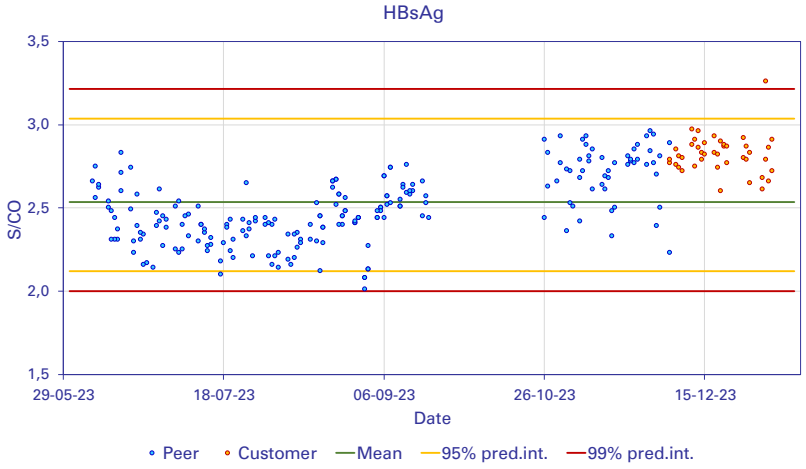


Figure 2b. Abbott Alinity ci series anti-HBc assay.

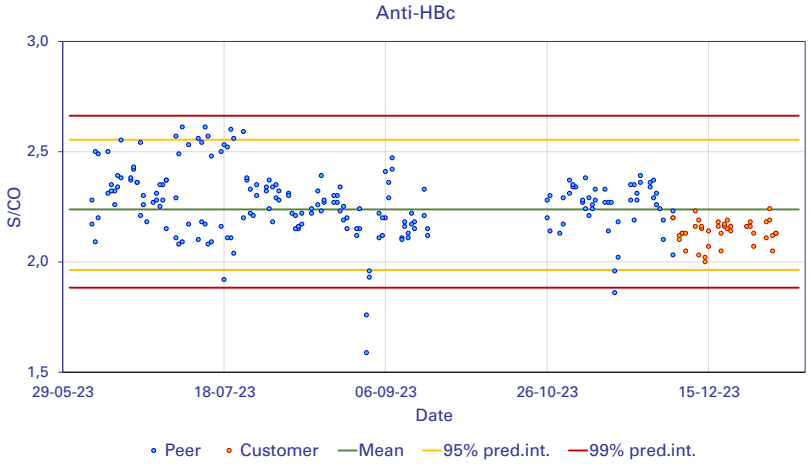


Figure 2c. Abbott Alinity ci series anti-HCV assay.

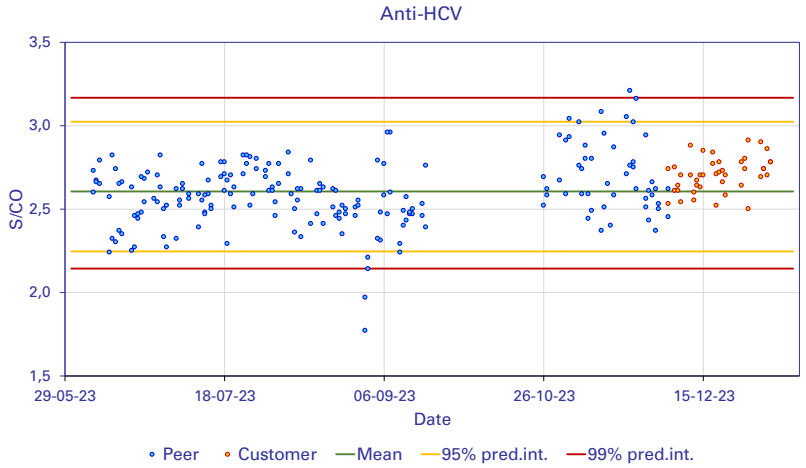


Figure 2d. Abbott Alinity ci series HIV-Ag/Ab Combo assay

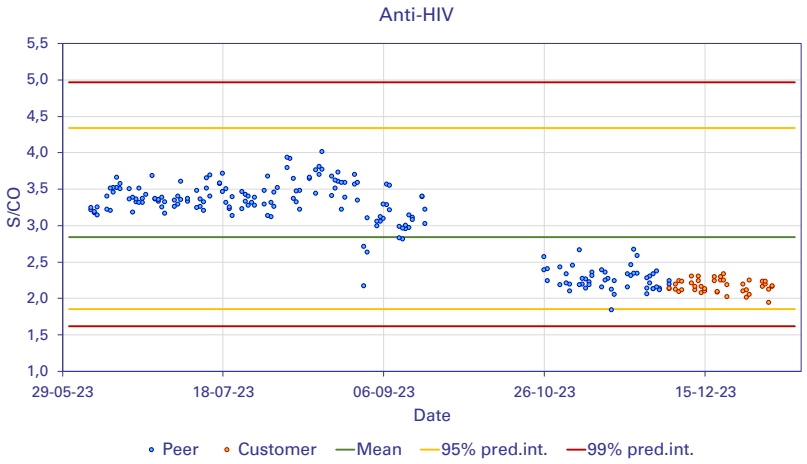
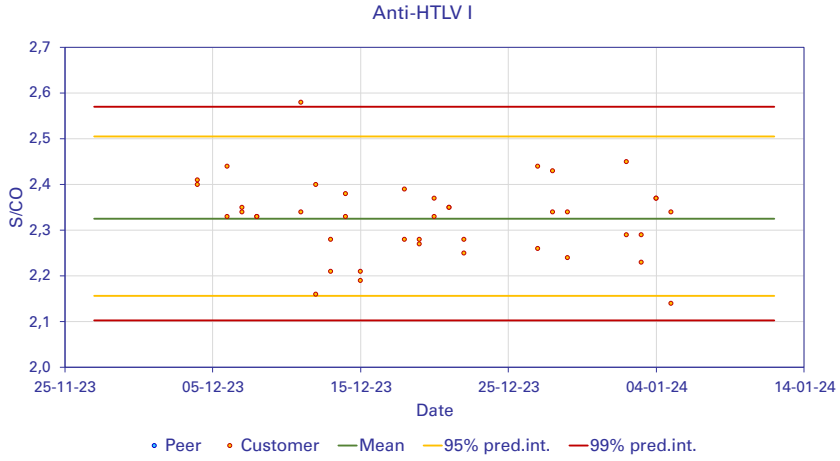


Figure 2e. Abbott Alinity ci series Anti-HTLV I/II assay



Interpretation

Knowing the 95% and 99% predictive intervals for generating a Levey-Jennings chart one can use Nelson rules[13] to interpret values outside the predictive limits and for identifying trends and aberrant results. The statistical report generated by the DataQ Analytics system (on the website www.bioqcontrol.com) identifies these trends and outliers for the laboratory requesting the report.

- Negative or positive trends resulting from gradual changes in test performance and not reported by the internal kit controls and/or alert systems in the test robot, are indicative for a lack of maintenance, the need for recalibration of equipment, or degradation of reagents. These are systematic errors. In case a trend is recognised, the laboratory is encouraged to identify the root cause of the deviation.
- Aberrant results like a negative response on the run control or a result outside the 99% predictive interval are indicative for incidental errors that need further investigation to identify the root cause. The identification of the root cause of aberrant results is beyond the scope of the intended use of the run controls.
- Differences between S/CO values of laboratories could be attributed to different reagent lots or run control batches that are in use. The statistical report that can be obtained from the DataQ Analytics system (available on www.bioqcontrol.com) compares the assay response values on different lab instruments, test reagent lots and run control batches.

Abbott Alinity Assay response values on P0320 SeraQ Alinity V2 run control

Table 4 gives an example of the test results reported by two laboratories that tested the P0320 SeraQ Alinity V2 run control in different Alinity ci series reagent lots and instruments.

Table 4. Abbott Alinity ci Assay response values on P0320 SeraQ Alinity V2 run control reported by a few laboratories over a period of two years.

Alinity s Assay	n	geomean S/CO	95% pred. int. S/CO	99% pred. int. S/CO
HBsAg	255	2.54	2.12 – 3.04	2.00 – 3.09
Anti-HBc	236	2.24	1.96 – 2.55	1.88 – 2.66
Anti-HCV II	236	2.61	2.25 – 3.02	2.14 – 3.17
HIV Ab/Ag	235	2.84	1.86 – 4.34	1.62 – 4.97
Anti-HTLV-I	42	2.32	2.16 – 2.51	2.10 – 2.57

Variation in immunoassay reagent lots and run control batches

Variation in S/CO ratio on run controls reflects the difference in analytical sensitivity of assay runs and reagent lots. Different batches of SeraQ run controls are prepared from the same standards. Therefore, the composition of the multi-marker run controls is consistent from batch-to-batch. Multi-variance analysis on another Alinity platform (Alinity s) shows that test reagent lots are a larger source of variation in S/CO values than run control batches. Insufficient data are obtained with the Alinity ci assays to compare the variation in S/CO response values on Alinity reagent lots and SeraQ run control batches.

Limitations

- SeraQ run controls were designed for monitoring the analytical performance of serologic test systems. They cannot be used to evaluate the diagnostic sensitivity of the assays.
- The run control must not be substituted for the mandatory controls or calibrators provided with IVD test kits for calculating the cut off and/or criteria for releasing test results.
- The response values on the run controls should not be used to release or reject the test run but can be used as an aid in the assessment of analytical performance.
- The expected S/CO values and 99% predictive intervals have been established with a limited number of Alinity reagent lots. It cannot be guaranteed that S/CO values obtained with new reagent lots will always fall within these limits.
- Although the batch-to batch composition of SeraQ run controls is consistent some variation in the measurable potency of the serum standards in the run control batches cannot be avoided due to matrix effects and other manufacturing variables.

References

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Biologicals Quality Control B.V.
Droogmakerij 31h
1851 LX Heiloo
The Netherlands

Tel: +31 (0)72 2020 730
Internet: www.BioQControl.com

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