Bio QControl

P0180 SeraQ LIAISON

CE 0088

IVD

REF P0180

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

Table of contents

Intended Use

P0180 SeraQ LIAISON is intended to be used on the DiaSorin LIAISON® platform as an external run control in combination with the assays for the detection of hepatitis B surface antigen (HBsAg), antibodies to hepatitis C virus (anti-HCV) and antibodies to human immunodeficiency virus types 1 and 2 (anti-HIV-1/2) (see Table 1) performed in diagnostic and blood screening laboratories. P0180 SeraQ LIAISON is a multi-marker mixture of inactivated HBsAg, anti-HCV and anti-HIV-1 standards in defibrinated plasma giving a low reactive result in the DiaSorin LIAISON® Assays. The run control is intended to be tested in consecutive runs of the immunoassays over time. By comparison of the sample to cut off (S/CO) values for the three markers found on P0180 SeraQ LIAISON one can monitor the consistent analytical sensitivity of test runs. The run control should not be used to replace internal controls or calibrators in the test kits.

Table 1. Test kits covered by this run control

Equipment	Agent	Tests
LIAISON®	Hepatitis B virus	DiaSorin LIAISON® HBsAg QUANT
	Hepatitis C virus	DiaSorin LIAISON® HCV-Ab
	Human immunodeficiency virus	DiaSorin LIAISON® HIV Ab/Ag

Key to Symbols Used

Manufacturer

Lot number

Catalogue number

In vitro diagnostic medical device

Store below -20°C

Expiry date

Contents

Caution

CE mark with Notified Body number

Read instructions for use

Principle of method

A series of SeraQ run controls for monitoring HBsAg, anti-HCV and anti-HIV-1 test performance have been designed. 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-HCV and anti-HIV-1. 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 series includes the product P0180 SeraQ LIAISON for which the composition is optimised for use with the DiaSorin LIAISON® test system. The P0180 SeraQ LIAISON run control is designed to generate assay response values (i.e. S/CO ratios and International Units per mL (IU/mL)) 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 IVD batch variation.

Traceability of antigen and antibody concentrations

For each HBsAg, anti-HIV-1 and anti-HCV, an internal (secondary) serum standard has been established[1] from which reference panels and run controls are prepared by gravimetrically recorded dilution steps. The undiluted secondary standard for HBsAg is derived from the same purified heat-inactivated source material as is used for preparation of the WHO HBsAg adw2 (00/588) International Standard (IS).[1-4]. 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[4]. The HBsAg concentration in the run control has been set at 0.084 IU/mL[1]. One IU heat-inactivated HBsAg was found to be equivalent to 0.67 ng HBsAg when historically calibrated against the first WHO standard established by the Paul Ehrlich Institute[1], comparable to conversion factors of 0.58 and 0.71 reported in WHO collaborative studies[3,4]. No unitage could be assigned to the internal standards for anti-HIV-1 and anti-HCV since international reference preparations are not available. The consistent concentration of the analytes in consecutive seraQ run control batches is guaranteed by batch release control testing against suitable reference samples kept frozen at -40°C. These reference samples are derived from the same undiluted internal (secondary) standards that are used for manufacturing of the seraQ run controls.

Materials Provided

Ten (10) polypropylene tubes (10 mL) with screw caps, each contains 2.0 mL of P0180 SeraQ LIAISON run control and 0.01% (w/v) Thimerosal as preservative.

Materials not provided

Pipettes or pipetting devices for use in IVD test systems.

Storage Instructions

Store unopened tubes below -20°C. After use, the run control tubes should be stored to 2°C to 8°C (< 1 month)

Warning and precautions

P0180 SeraQ LIAISON run controls are prepared from secondary serum standards, in which virus has been inactivated by *in vivo* validated methods applied in the plasma industry[1]. Infectivity and inactivation data have been analysed to demonstrate absence of residual infectivity of HBV, HCV and HIV-1 in the run controls[1]. The serum matrix in in the run controls has been tested for infectious disease markers by serologic and molecular screening methods. However, no screening procedure can offer complete assurance that products derived from human blood cannot transmit undetected infectious agents.

- SeraQ run controls should be handled with the normal preventive measures in a serology laboratory[5,6].
- 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.
- Do not use run controls beyond one month 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.

Test Procedure

- Allow a run control tube to adapt to room temperature.
- Mix the tubes thoroughly prior to use (any visible precipitate will then easily disappear).
- For automated test systems, place the run control tube at the specified positions in the sample racks for regular donor or patient samples. Otherwise, pipet run controls manually as with regular test specimens at the target position in test plates.
- Test on the DiaSorin LIAISON platform with the assays mentioned in Table 1 according to the manufacturers instructions
- Store the opened tube immediately after use at 2-8 °C (see storage instructions).

Expected assay response values

The expected results for the P0180 SeraQ LIASON run control are follows:

1. HBsAg range: 0.05 – 0.15 IU/mL
2. anti-HIV range S/CO ratio: 1.5 – 3.0
3. anti-HCV range S/CO ratio: 1.5 – 3.0

Each test kit batch 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 DiaSorin reagent batches that are in use. Thus it can not be guaranteed that the assay response values will always fall within these ranges. P0180 SeraQ LIAISON run control serves as

an independent secondary standard or external quality control sample for monitoring consistent analytical sensitivity of DiaSorin reagent batches over time.

Interpretation of Results

Calculations

Subsequent test runs can be analysed by appropriate statistical approaches on the S/CO ratios and HBsAg concentrations obtained on the external control samples.

Assay response values

To obtain the test kit batch specific reference values for each marker, an initial collection of 10-30 consecutive test results is required. Upon collecting additional data the chart characteristics may be updated.

- HBsAg concentrations are 'normally' distributed. For the HBsAg assay the LIAISON software calculates a quantitative HBsAg value in IU/mL. This value can be directly used for calculation of the average and confidence intervals:
  - Calculate average expressed in IU/mL and its standard deviation
  - Use Table 2 to obtain Student-t-values belonging to the 95% and 99% confidence interval (CI) for different number of observations (n)
  - Calculate the 95% and 99% CI as follows:
    99% Lower limit: Average – (99%) Student-t-Value x Standard Deviation
    95% Lower limit: Average – (95%) Student-t-Value x Standard Deviation
    95% Upper limit: Average + (95%) Student-t-Value x Standard Deviation
    99% Upper limit: Average + (99%) Student-t-Value x Standard Deviation
  - To visualize the individual IU/mL values make a Levey-Jennings plot on a linear scale.
- The S/CO values for anti-HIV and anti-HCV are 'log normally' distributed. For the LIAISON anti-HCV and HIV-combo assays one should use the logarithm of S/CO ratios for calculation of the geometric mean and confidence interval.
  - Calculate from each measurement the log S/CO value.
  - Calculate average and standard deviation on these log transformed values; log (Average) and log (Standard Deviation).
  - Calculate the (geometric) mean in S/CO ratio by taking the anti-log value of the log (Average)
  - Use Table 2 to obtain Student-t-values belonging to the 95% and 99% CI for different number of observations (n)
  - Calculate the log(95% and 99% CI) as follows:
    Log (99% Lower limit):log (Average) – (99%) Student-t-Value x log (Standard Deviation)
    Log (95% Lower limit):log (Average) – (95%) Student-t-Value x log (Standard Deviation)
    Log (95% Upper limit): log (Average) + (95%) Student-t-Value x log (Standard Deviation)
    Log (99% Upper limit): log (Average) + (99%) Student-t-Value x log (Standard Deviation)
  - Take the anti-log values for calculating the confidence 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% confidence limits at greater distance from the geometric mean S/CO value than the lower confidence limits (see example Figure 1).

Levey-Jennings chart

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 plot below (Figure 1) show the distance from the geometric mean S/CO ratio (green line in graph) which is the

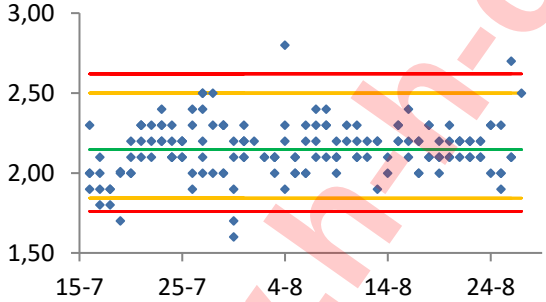
expected response level for the run control. The orange and red lines represent the 95% and 99% CI, respectively. The data represents individual measurements of three instruments.

Table 2. Relation of Student t value and numbers of runs (n) to calculate confidence intervals.

Runs (n)	t-value at 95% CI	t-value at 99% CI
10	2.306	3.355
20	2.101	2.878
30	2.048	2.763
Infinite	1.960	2.576

Infinite equals the normal distribution

Figure 1. Example of a Levey-Jennings control chart for DiaSorin anti-HCV.
The confidence limits for the S/CO ratio are log transformed as explained in the text.



Interpretation

Knowing the 95% and 99% CI for generating a Levey-Jennings chart one can use Westgard rules[7] to interpret values outside the confidence limits for identifying trends and aberrant results. One can find guidance on how to identify trends and outliers on the website www.westgard.com.

- 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 range of 99% CI are indicative for (incidental) random 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.

Analytical Performance Characteristics

SeraQ run controls have been designed by examination of the response curves on dilutions of the internal (secondary) 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[8,9]. Plotting (transformed) S/CO values against (log) concentration analyte using linear regression analysis enables calculation of correlation coefficients. It is recommended to use a transformation resulting in an optimal correlation. Figure 2a shows a linear dose response relation between the input HBsAg concentration and the reported IU/mL values by the DiaSorin HBsAg quant assay for which no transformation is required. The arrow indicates the expected response at the run control concentration set at 0.084 IU/mL. Figures 2b and 2c show linear dose response relations in the DiaSorin LIAISON® anti-HCV and HIVAb/Ag assays obtained after log transformation of dilution and S/CO values.

Figure 2a. Dose response in HBsAg quant assay. IU/mL input plotted against IU/mL measured (r^2=0.99).

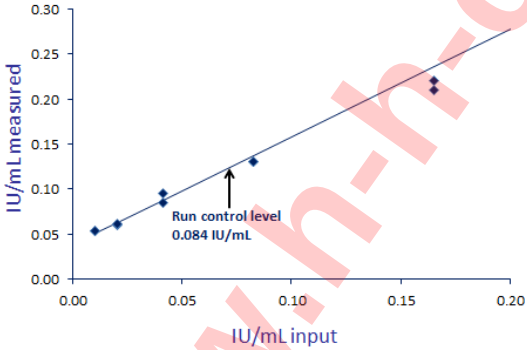


Figure 2b. Dose response in anti-HCV assay. Log anti-HCV S/CO values are plotted against log dilution (r^2 = 0.99).

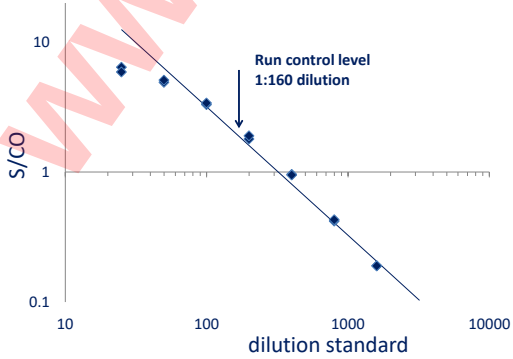
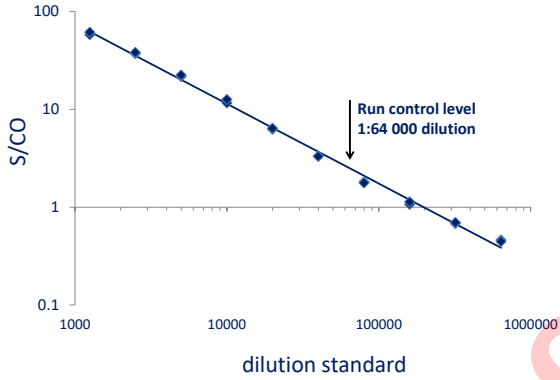


Figure 2c. Dose response in a-HIV/HIV-Ag assay. Log HIVAb/Ag S/CO values are plotted against log dilution (r^2 = 0.998).



DiaSorin assay response values on P0180 SeraQ LIASON run control

Table 3 gives an example of results obtained within the same DiaSorin reagent batches. When a new DiaSorin reagent batch is introduced the values in Table 3 need to be reassessed (see Interpretation of Results).

Table 3. DiaSorin Assay response values on P0180 SeraQ LIAISON run control

DiaSorin Assay	n	average IU/mL	95% CI*	99% CI*
HBsAg QUANT	165	0.079	0.050-0.107	0.038-0.119
		geomean S/CO		
HCV-Ab	195	2.15	1.79-2.56	1.71-2.69
HIV Ab/Ag	166	1.98	1.70-2.33	1.58-2.50

*for calculation see instructions above.

Variation in immune-assay reagent batches

Variation in S/CO ratio on run controls reflects the difference in analytical sensitivity of assay runs and reagent batches. Different batches of SeraQ run controls are prepared from the same secondary standards. As a consequence the composition of the multi-marker run controls is consistent from batch to batch. This is confirmed by multi-variance analysis on large data sets showing that immuno-assay reagent batches are the major source of variation in analytical sensitivity. Figure 3a and 3b show examples of the S/CO distribution for four different Abbott PRISM HBsAg reagent batches and two SeraQ run control batches. Similar results were observed for other serologic assays.

Figure 3a. Frequency distribution of HBsAg S/CO ratios on one batch of SeraQ run control and four PRISM batches (n=1992)

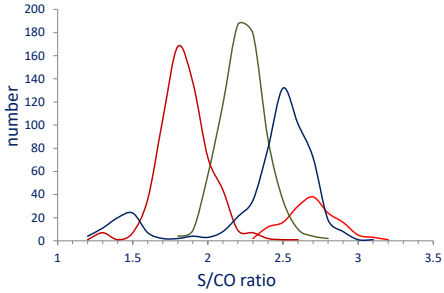
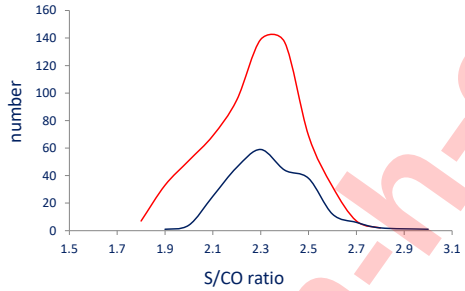


Figure 3b. Frequency distribution of HBsAg S/CO ratios on two batches of SeraQ run control and one PRISM batch (n=879)



Limitations

SeraQ run controls were designed for monitoring the analytical performance of IVD kits. They cannot be used to evaluate the diagnostic sensitivity of IVD kits. 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.

References

1. Van Drimmelen A.A.J., Lelie PN. Preparation of inactivated secondary viral standards: Safety assessment of quality control samples for viral serology and NAT assays in blood screening laboratories. BQC document number CE4006 (manuscript in preparation).

2. Schüttler GG, Wend UC, Faupel FM, Lelie PN, Gerlich HW. J Antigenic and physiochemical characterization of the 2nd International Standard for hepatitis B virus surface antigen (HBsAg). J Clin Virol 2010;47:238-42

3. Ferguson M, Heath A, Lelie N, Nübling M, Nick S, Gerlich W, et al.WHO Working Group on Hepatitis and HIV Diagnostic Kits. Report of a collaborative study to (1) assess the suitability of a candidate replacement International Standard for HBsAg and a reference panel for HBsAg and (2) to calibrate the candidate standard in IU. 2003. http://www.who.int/bloodproducts/cs/en/031987.pdf.

4. Chudy M, Scheiblauer H, Hanschmann H-M, Kress J, Nick S, Wend U, Schüttler C, Nübling CM, Gerlich WH. Performance of hepatitis B surface antigen tests with the first WHO international hepatitis B virus genotype reference panel. J Clin Virol. 2013;58:47-53

5. Centers for Disease Control (CDC). Update: Universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus, and other bloodborne pathogens in health-care settings. MMWR 1988; 37:377-388.

6. Centers for Disease Control (CDC). Guidelines for prevention of transmission of human immunodeficiency virus and hepatitis B virus to health-care and public-safety workers. MMWR 1989; 38(S-6): 1-36.

7. Westgard rules, www.westgard.com

8. Plikaytis BD, Turner SH, Gheesling LL, Carlone GM.Comparisons of standard curve-fitting methods to quantitate Neisseria meningitidis group A polysaccharide antibody levels by enzyme-linked immunosorbent assay. J Clin Microbiol. 1991 Jul;29(7):1439-46

9. Bank HL. A quantitative enzyme-linked immunosorbent assay for rat insulin J Immunoassay. 1988; 9(2):135-58.

BioQControl B.V.
Droogmakerij 31h
1851 LX Heiloo
The Netherlands

Tel: +31 (0)72 2020 730
Fax: +31 (0)72 2020 731
Internet: www.bioQControl.com

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