P0313 SeraQ
BIO-RAD Syphilis

REF **P0313**

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

P0313 SeraQ BIO-RAD Syphilis is intended to be used on a open platform as an external run control in combination with the assay for the detection of antibodies to *Treponema pallidum* (see Table 1) performed in diagnostic and blood screening laboratories. P0313 SeraQ BIO-RAD Syphilis is a single-marker dilution of an internal anti-*Treponema pallidum* standard in defibrinated plasma giving a low reactive result in the BIO-RAD Syphilis Total Ab assay. 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 found on P0313 SeraQ BIO-RAD Syphilis 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	Test
Open platform	*Treponema pallidum*	BIO-RAD Syphilis Total Ab

Key to Symbols Used

Manufacturer

Lot number

Catalogue number

Performance evaluation only

Store below -20°C

Expiry date

Contents

Caution

Read instructions for use

Principle of method

A number of SeraQ run controls have been designed for monitoring the performance of different anti-*Treponema pallidum* assays. 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 anti-*Treponema pallidum*. The analytical sensitivity of test kits from different manufacturers varies and therefore for each test kit a separate single marker run control has been designed. The SeraQ run control series includes the product P0313 SeraQ BIO-RAD Syphilis for which the composition is optimised for use with the BIO-RAD Syphilis Total Ab test system. The P0313 SeraQ BIO-RAD Syphilis run control is designed to generate assay response values (i.e. S/CO ratios) positioned in the low positive range of the assay. Routine use of external run controls enables laboratories to monitor day-to-day test performance and IVD batch variation.

Traceability of antibody concentrations

For anti-*Treponema pallidum*, an internal serum standard has been established[1] from which reference panels and run controls are prepared by gravimetrically recorded dilution steps. No unitage could be assigned to the internal standard for anti-*Treponema pallidum* 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 -30°C. These reference samples are derived from the same undiluted internal standards that are used for manufacturing of the SeraQ run controls.

Materials Provided

Ten (10) polypropylene tubes with screw caps, each contains 2.0 mL of P0313 SeraQ BIO-RAD Syphilis 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 at or below -20°C. After use, the run control tubes should be stored to 2°C to 8°C (< 1 month).

Warning and precautions

P0313 SeraQ BIO-RAD Syphilis run controls are prepared from an internal serum standard, in which the bacterium has been inactivated by refrigerated storage[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[2,3].
- 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 cobas platform with the assay 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 S/CO value on the P0313 SeraQ BIO-RAD Syphilis run control is between1.4 and 2.9. Each batch of BIO-RAD Syphlis has its own dose response curve and distribution of S/CO values on SeraQ run controls. This depends on the analytical sensitivity of the r eagent batches that are in use. P0313 SeraQ BIO-RAD Syphilis serves as an independent external run control for monitoring consistent analytical sensitivity of reagent batches 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. Calculations should be made within each BIO-RAD Syphilis Total Ab batch.

Assay response values

To obtain the BIO-RAD Syphilis Total Ab 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.

The S/CO values for anti-*Treponema pallidum* are 'log normally' distributed. For the BIO-RAD Syphilis Total Ab assay 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 within one test batch.

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

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[4].

- 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.

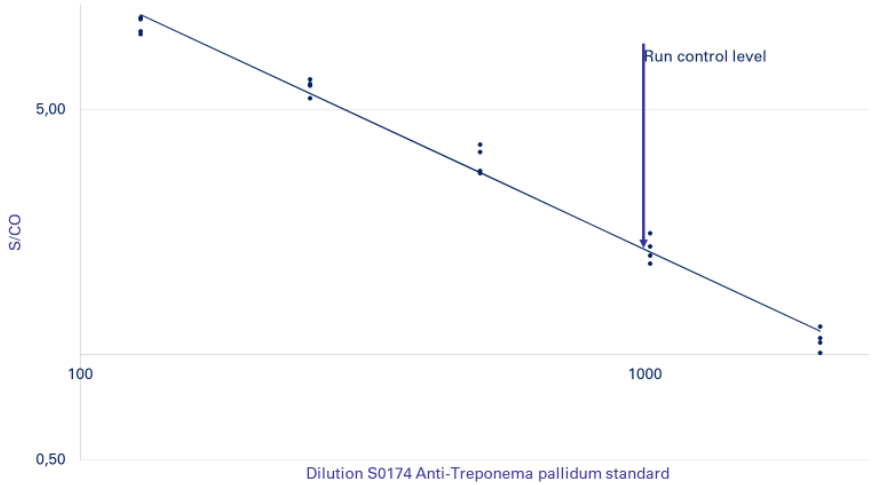
Analytical Performance Characteristics

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[5,6]. 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 1 show linear dose response relations in the BIO-RAD Syphilis assay obtained after log transformation of dilution and S/CO values.

Figure 1. Dose response in BIO-RAD Syphilis Total Ab assay. Log S/CO values are plotted against log dilution (r^2 = 0.993).



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 2a and 2b 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 (see table 3).

Figure 2a. Frequency distribution of HBsAg S/CO ratios on one batch of SeraQ run control and four PRISM batches (n=1992)

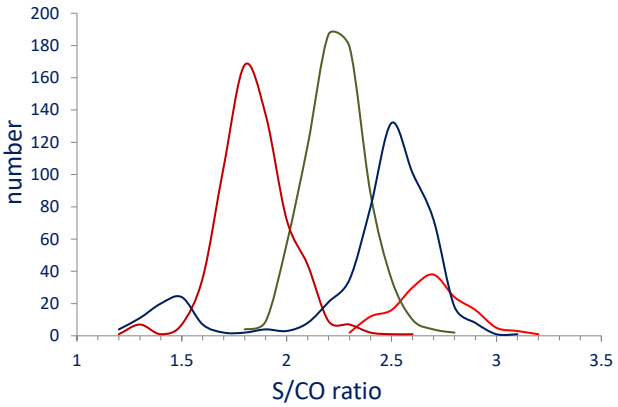
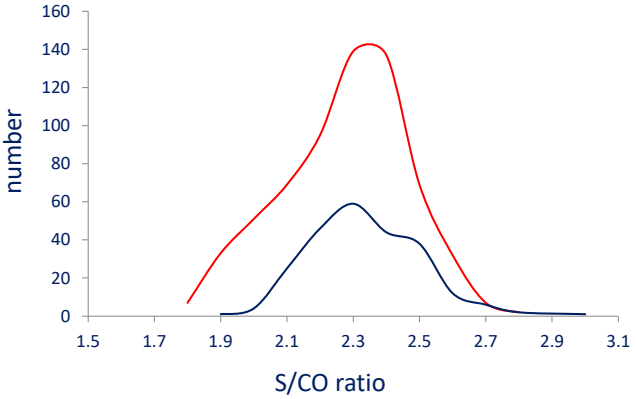


Figure 2b. Frequency distribution of HBsAg S/CO ratios on two batches of SeraQ run control and one PRISM batch (n=879)



Thus, the variability in results is mainly caused by differences in test batches.

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

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4. Westgard rules, www.westgard.com
5. Plikaytis B.D., Turner S.H., Gheesling L.L., Carlone G.M. 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
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