Bio QControl

P0260 SeraQ Murex Syphilis

REF P0260

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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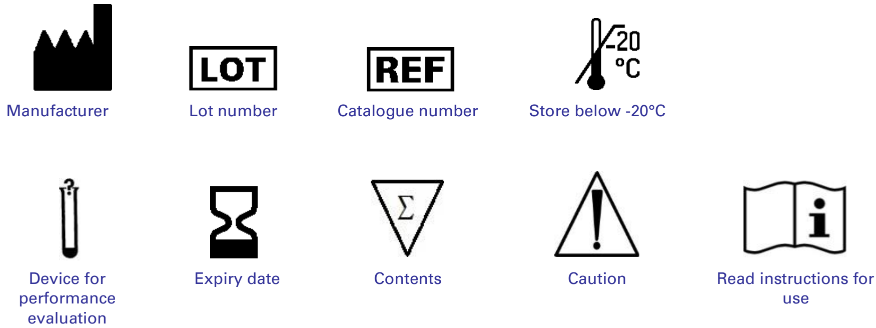
Intended Use

P0260 SeraQ Murex Syphilis is intended to be used on an open platform (96 wells EIA) as an external run control in combination with the Murex assay for the detection of anti-*Treponema pallidum* (see Table 1) performed in diagnostic and blood screening laboratories. P0260 SeraQ Murex Syphilis is a single-marker mixture of inactivated anti-*Treponema pallidudum* standard in defibrinated plasma giving a low reactive result in the Murex Assay. The run control is intended to be tested in consecutive runs of the immunoassay over time. By comparison of the sample to cut off (S/CO) values found on P0260 SeraQ Murex 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. The product is intended for In Vitro Diagnostic (IVD) performance evaluation only.

Table 1 Test kits covered by this run control

Agent	Tests
Anti-*Treponema pallidum*	Murex ICE Syphilis

Key to Symbols Used



Principle of method

A series of SeraQ run controls for monitoring anti-*Treponema pallidum* 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 closely naturally occurring serum specimens with low reactivity for anti-*treponema pallidum*. The analytical sensitivity of test kit from different manufacturers varies and therefore for each test kit a separate single-marker run control has been designed. This series of SeraQ run controls includes the product P0260 SeraQ Murex Syphilis for which the composition is optimised for use with the Murex ICE Syphilis. The P0260 SeraQ Murex Syphilis run control is designed to generate assay response values (i.e. S/CO ratios) positioned in the low positive range of the assays taking into account the batch-to-batch variation of the test kits for which the run control is designed. 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 anti-*Treponema pallidum*, an internal (secondary) serum standard has been established from which reference panels and run controls are prepared by gravimetrically (accurate) recorded dilution steps. No unitage could be assigned to the internal standard since an international reference preparation is 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 P0260 SeraQ Murex 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

P0260 SeraQ Murex Syphilis run controls is prepared from an secondary serum standard, in which the bacterium has been inactivated by *in vivo* validated methods applied in the plasma industry[1]. The serum matrix 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 is manufactured from human plasma and contains traces of biological source material of non-human origin (bovine thrombin).
- 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.

Test Procedure

- Allow a run control tube to adapt to room temperature.
- Mix the tubes thoroughly prior to use (visible precipitates are unlikely to occur, but if present 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 in the Murex ICE Syphilis assay 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 S/CO, 99.5 % confidence interval in P0260 SeraQ Murex Syphilis run control are follows:

1. anti-*Treponema pallidum* range S/CO ratio: 1.4 – 2.9

Each test kit batch appears to have its distribution of S/CO values on SeraQ run controls. This depends on the analytical sensitivity of the Murex ICE Syphilis reagent batches that are in use. Thus it cannot be guaranteed that the assay response values will always fall within these ranges.

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.

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.

- The S/CO values for anti-*Treponema pallidum* are 'log normally' distributed.
  - 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).

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

Runs (n)	t-value at 95% CI	t-value at 99% CI

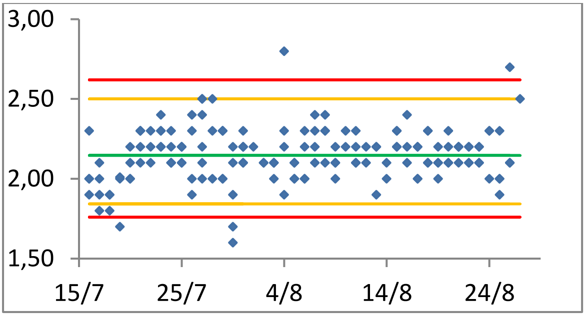
10	2.306	3.355
20	2.101	2.878
30	2.048	2.763
Infinite	1.960	2.576

Infinite equals the normal distribution

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.

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



The orange and red lines represent the 95% and 99% CI, respectively. The data represents individual measurements of three instruments.

Interpretation

Knowing the 95% and 99% CI for generating a Levey-Jennings chart one can use Westgard rules[4] 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

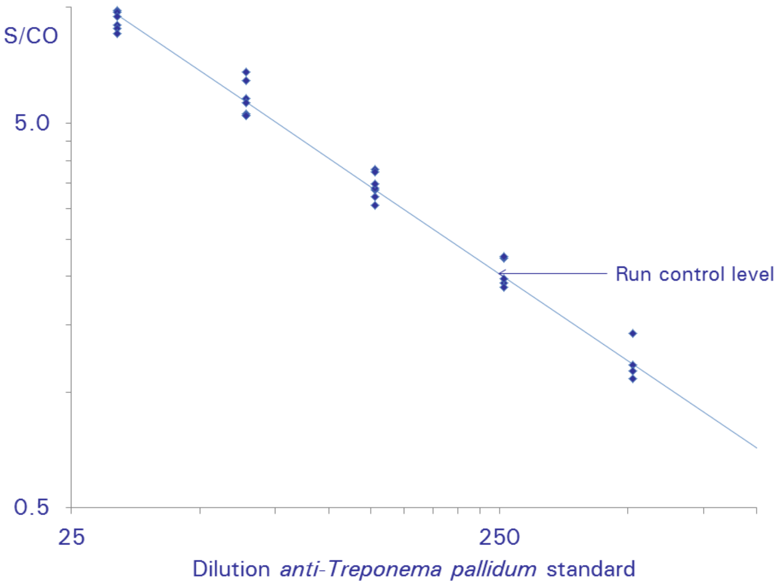
SeraQ run controls have been designed by examination of the response curves on dilutions of the internal (secondary) standards and as such relate to the analytical sensitivity of immunoassays. In the following paragraphs the essential analytical performance characteristics of SeraQ run controls are presented.

Analytical sensitivity and positioning of the run control

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. Figure 2 show S/CO ratios of the Murex ICE Syphilis vs the standard dilution. The run control is positioned such that a positive result is expected in

99.5% to 99.9% of the cases. This is based on descriptive statistics using the variation in S/CO ratios resulting in the respective Confidence Intervals.

Figure 2. anti-*Treponema pallidum* S/CO values are plotted against standard dilution (r^2 = 0.99).



General information on 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 single-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)

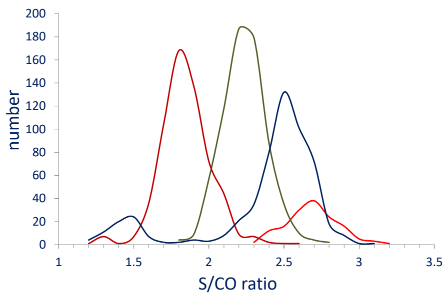
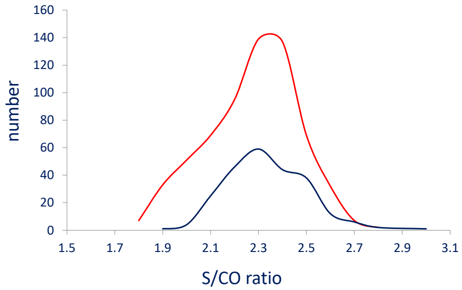


Figure 3b. Frequency distribution of HBsAg S/CO ratios on two batches of SeraQ run control and one PRISM batch (n=879)



Note: The information presented in this section is intended to show the general principle of batch-to-batch variation in test kits versus batch-to-batch variation in run controls. Data for estimating the "Murex" variation are not available.
Note: the information presented in this section is intended to show the general principle of batch to batch variation in test kits in relation to batch to batch variation in run controls. No such data are available for the Murex system.

Limitations

- The use of the run control in other assay configurations should be avoided and is not supported by manufacturer.
- 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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2. 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.
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4. Westgard rules, www.westgard.com
5. 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
6. Bank HL. A quantitative enzyme-linked immunosorbent assay for rat insulin J Immunoassay. 1988; 9(2):135-58.

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