

Assurance™ Interference Test Kit Instructions for Use

Interferents are provided in a concentrated form to minimize change to typical sample matrix.

Notes on the Preparation of Base Pool, Test Pools, Control Pools, and Experiments

The Base Pool is the sample matrix of interest, most often serum or plasma. Sample may be collected from a donor or leftover specimens can be pooled. For most experiments 4-6 mL should be sufficient, half for the Test Pool and half for the Control Pool. Each Interferent requires a matched Test Pool and a Control Pool.

The Test Pool is the Base Pool with added Interferent. The Control Pool is Base Pool with same volume of solvent as used in preparation of the corresponding Interferent Pool. This solvent can be normal saline (0.9%NaCl) or water, 0.1M NaOH for use with unconjugated bilirubin, ethanol, etc. Aside from saline or water, the solvent needed to prepare the Control Pool is included with the Kit.

The number of replicates (sample size) and also the volume of Pools depends on both assay variability and the size of the Interferent effect to be detected (maximum difference or d_{max}). Prepare enough volume of each pool for the required number of replicate measurements. The Assurance™ Excel spreadsheet, available at www.sundiagnosics.us, provides a Table of the replicates required, as calculated from d_{max} and within-run precision, as recommended by CLSI EP7. The spreadsheet can also be used to directly calculate the required sample size.

Recommendations

- If feasible, analyze replicates in alternating pattern (control, test, control, test etc.). This may increase the volume requirement because each sample cup will include a “dead volume”.
- Establish a maximum clinically significant difference (D_{max}). We suggest using $\frac{1}{2}$ or $\frac{1}{4}$ of the total allowable error, TEa, as defined by CLIA other published guidance documents, or as defined by your Laboratory Director. A table of recommended allowable errors is available on our website: sundiagnosics.us/wp-content/uploads/2018/04/SUNDX-TAE-LIMITS-TABLE-20180405.pdf.
- Perform screening experiments and dose-response experiments with both low and high analyte pools, as interference may be dependent on analyte concentration.

Preparation of Base Pool

1. After reading the Instructions for Use, calculate the volume of Base Pool required for conducting all planned Interference experiments. An approximate minimum requirement is 5 mL per experiment.
2. Prepare a Base Pool for all Interference experiments by collecting or pooling specimens without any visible lipemia, hemolysis, or icterus and with little or no measurable concentration of the interferent of interest.

Hemolysis Screening Study

1. Thaw Hemolysate Interference Material and remove an aliquot for measurement of hemoglobin concentration.
2. Prepare a Test Pool by adding enough hemolysate to the Base Pool to bring the hemoglobin concentration to ~1000 mg/dL. Use the Assurance™ Excel spreadsheet Test Pool Calculator spreadsheet, available at www.sundiagnosics.us, to calculate the required amount of Hemolysate to add or assume that the hemoglobin concentration is 0.0 mg/dL or 7.0 mg/dL (assuming a normal plasma hemoglobin is 0.0 to 15 mg/dL). Mix gently by inversion.

3. Prepare a Control Pool by adding the same volume of water or saline, instead of Hemolysate, to an equal volume of the Base Pool. Mix gently by inversion.

For example, if 4 mL Base Pool is enough for the planned experiment, transfer 2 mL of the Base Pool for the Test Pool and 2 mL for the Control Pool. If 100 μ L Hemolysate is required for the Test Pool, then add 100 μ L saline or water to the Control Pool so that analyte or measurand of interest is the same in both the Test and Control Pools.

4. Analyze each pool for each analyte of interest using the required number of replicates. The Assurance™ Excel worksheet will calculate the required number of replicates. You may also refer to the Table in the Excel workbook. Enter the data into the Assurance™ Excel spreadsheet. Enter information and data in cells highlighted in yellow. The spreadsheet will automatically calculate the differences between Test and Control Pools and interpret results.

Lipemia Screening Study

1. Thaw the Triglyceride-Rich Lipoprotein (TRL) material and remove an aliquot for measurement of triglyceride concentration.
2. Prepare a “Base Pool” using serum without any visible lipemia, hemolysis, or icterus. For Lipemia testing it is best to choose sera with very low triglycerides as this will be the concentration in the Control Pool.
3. Prepare a Test Pool by adding enough TRL to the Base Pool to bring the triglyceride concentration to 1500 mg/dL. Use the Assurance™ Excel Test Pool Calculator, available at www.sundiagnosics.us, to calculate the required amount of TRL. Mix gently by inversion.
4. The TRL Interference material includes an aliquot of saline/sucrose solution for use in the Control Pool. Prepare the Control Pool by adding the same volume of saline/sucrose solution, in the same volume as TRL, to an equal volume of the Base Pool. Mix gently by inversion.

For example, if 4 mL Base Pool is sufficient for the planned experiment, transfer 2 mL of the Base Pool for the Test Pool and 2 mL for the Control Pool. If 200 μ L TRL is added to the Test Pool, then add 200 μ L saline/sucrose solution to the Control Pool.

5. Analyze each pool for each analyte using the required number of replicates. The Assurance™ Excel spreadsheet will calculate the required number of replicates or you can refer to the Replicate Table in the same document. Enter the data into the Assurance™ Excel spreadsheet.

Unconjugated Bilirubin Screening Study

1. Thaw Unconjugated Bilirubin (Bu) and 0.1N NaOH and remove an aliquot for measurement of bilirubin concentration.
2. Prepare a “Base Pool” using serum without any visible lipemia, hemolysis, or icterus.
3. Prepare a Test Pool by adding enough Bu to the Base Pool to bring the concentration of bilirubin to ~40 mg/dL. Use the Assurance™ Excel Test Pool Calculator, available at www.sundiagnosics.us, to calculate the required amount of Unconjugated Bilirubin to add, or assume that base pool concentration is 0.0 mg/dL

or 0.4 mg/dL (assuming a normal serum unconjugated bilirubin concentration of 0.1 to 0.7 mg/dL). Mix gently by inversion.

4. Prepare a Control Pool by adding the same volume of 0.1N NaOH, instead of Unconjugated Bilirubin Concentrate, to an equal volume of the Base Pool. Mix gently by inversion.

For example, if 4 mL Base Pool is sufficient for the planned experiment, transfer 2 mL of the Base Pool for the Test Pool and 2 mL for the Control Pool. If 100 μ L Bu Concentrate is added to the Test Pool, then add 100 μ L 0.1N NaOH to the Control Pool.

5. Analyze each pool for each analyte using the required number of replicates. The Assurance™ Excel spreadsheet will calculate the required number of replicates. You may also refer to the Replicate Tables in the workbook. Enter the data into the Assurance™ Excel spreadsheet.

Conjugated Bilirubin Screening Study

1. Thaw Conjugated Bilirubin (Bc) and remove an aliquot for measurement of bilirubin concentration.
2. Prepare a “Base Pool” using serum without any visible lipemia, hemolysis, or icterus.
3. Prepare a Test Pool by adding enough Bc to the Base Pool to bring the concentration of bilirubin to ~40 mg/dL. Use the Assurance™ Excel Test Pool Calculator, available at www.sundiagnosics.us, to calculate the required amount of conjugated bilirubin. Mix gently by inversion.
4. Prepare a Control Pool by adding the same volume of saline or water, instead of Conjugated Bilirubin Concentrate, to an equal volume of the Base Pool.

For example, if 4 mL Base Pool is enough for the planned experiments, transfer 2 mL of the Base Pool for the Test Pool and 2 mL for the Control Pool. If 100 μ L Bc Concentrate is added to the Test Pool, then add 100 μ L saline or water to the Control Pool.

5. Analyze each pool for each analyte using the required number of replicates. The Assurance™ Excel spreadsheet will calculate the required number of replicates. You may also refer to the Replicate Tables in the workbook. Enter the data into the Assurance™ Excel spreadsheet.

Protein Interference Study

1. Thaw Protein Concentrate and remove an aliquot for measurement of total protein concentration.
2. Prepare a “Base Pool” using serum without any visible lipemia, hemolysis, or icterus, and ideally, relatively low concentration of total protein.
3. Prepare a Test Pool by adding enough Protein Concentrate to the Base Pool to bring the concentration of total protein to ~12 g/dL. Use the Assurance™ Excel Test Pool Calculator, available at www.sundiagnosics.us, to calculate the required amount of Protein Concentrate. Mix gently by inversion.
4. Prepare a Control Pool by adding the same volume of saline or water, instead of Protein Concentrate, to an equal volume of the Base Pool.

For example, if 2 mL Base Pool is enough for the planned experiments, transfer 1 mL of the Base Pool for the Test Pool and 1 mL for the Control Pool. If 1 mL concentrate is added to the Test Pool, then add 1 mL saline or water to the Control Pool. Note that the Protein Interference material is as concentrated as possible, 25 g/dL total protein containing equal amounts of human albumin and human gamma-globulins. Therefore, we recommend mixing equal parts of Base Pool and Protein Interferent to create a Test Pool with 12.5 g/dL protein.

5. Analyze each pool for each analyte using the required number of replicates. The Assurance™ Excel spreadsheet will calculate the required number of replicates. You may also refer to the Replicate Table in the workbook. Enter the data into the Assurance™ Excel spreadsheet.

Biotin Interference Study

1. Thaw Biotin Interference material and mix well by inversion. Biotin Interference material is prepared gravimetrically at 20-fold the CLSI recommended test concentration and is therefore easily prepared with a 1 part:19-part dilution in Test Pool, if the test pool contains relatively low biotin concentrations.
2. Prepare a “Base Pool” using serum without any visible lipemia, hemolysis, or icterus.
3. Prepare a Test Pool by adding 1-part Biotin Interference material to 19 parts Base Pool. Mix gently by inversion.
4. Prepare a Control Pool by adding the same volume of 0.01M HCl, included with the Biotin Kit, to an equal volume of the Base Pool.

For example, if 4 mL Base Pool is enough for the planned experiments, transfer 1.9 mL of the Base Pool for the Test Pool and 1.9 mL for the Control Pool. Add 0.1 mL Biotinrug Interferent to the Test Pool, then add 0.1 mL 0.01M HCl to the Control Pool.

5. Analyze each pool for each analyte using the required number of replicates. The Assurance™ Excel spreadsheet will calculate the required number of replicates. You may also refer to the Replicate Table in the workbook. Enter the data into the Assurance™ Excel spreadsheet.

Drug Interference Study

1. Thaw Drug Interference materials and mix well by inversion. These materials are all prepared gravimetrically at 20-fold the recommended test concentration and are therefore easily prepared with a 1:19 part dilution in Test Pool, assuming that the test pool does not contain measurable amounts of the drug of interest.
2. Prepare a “Base Pool” using serum without any visible lipemia, hemolysis, or icterus.
3. Prepare a Test Pool by adding 1 part Drug Interference material to 19 parts Base Pool. Mix gently by inversion.
4. Prepare a Control Pool by adding the same volume of saline or water, instead of Drug Interferent, to an equal volume of the Base Pool.

For example, if 4 mL Base Pool is enough for the planned experiments, transfer 1.9 mL of the Base Pool for the Test Pool and 1.9 mL for the Control Pool. Add 0.1 mL Drug Interferent to the Test Pool, then add 0.1 mL saline or water to the Control Pool.

5. Analyze each pool for each analyte using the required number of replicates. The Assurance™ Excel spreadsheet will calculate the required number of replicates. You may also refer to the Replicate Table in the workbook. Enter the data into the Assurance™ Excel spreadsheet.

Endogenous Interferent Study

1. Thaw Drug Interference materials and mix well by inversion.
2. Prepare a “Base Pool” using serum without any visible lipemia, hemolysis, or icterus.
3. Prepare a Test Pool by adding enough Endogenous Interferent material to the Base Pool to bring the target concentration. Use the Assurance™ Excel Test Pool Calculator, available at www.sundiagnosics.us, to calculate the required amount of Endogenous Interferent. Mix gently by inversion.
4. Prepare a Control Pool by adding the same volume of saline or water, instead of Interferent, to an equal volume of the Base Pool.

For example, if 4 mL Base Pool is enough for the planned experiments, transfer 1.9 mL of the Base Pool for the Test Pool and 1.9 mL for the Control Pool. Add 0.1 mL Endogenous Interferent to the Test Pool, then add 0.1 mL saline or water to the Control Pool.

5. Analyze each pool for each analyte using the required number of replicates. The Assurance™ Excel spreadsheet will calculate the required number of replicates. You may also refer to the Replicate Table in the workbook. Enter the data into the Assurance™ Excel spreadsheet.

HAMA and RF Interference

We recommend using a dose-response experimental design to assess interference from human anti-mouse antibodies (HAMA) and rheumatoid factor (RF).

Dose-Response Experiments

1. Determine the range of interferent concentrations to be tested.
2. Determine the concentration of interferent in the Low Pool, which contains low or “normal” levels of interferent and High Pool, which contains very high concentrations of analyte. Refer to CLSI EP7¹ for recommended concentrations.
3. The High Pool (Level 5) is intermixed into the Low Pool (Level 1) to create the Intermediate Pool (Level 3). Intermix Levels 1 and 3 to create Level 2; Intermix Levels 3 and 5 to create Level 4.
4. Divide into three (3) sample cups for each level, for triplicate analysis, first in ascending order, then in descending order, and again in ascending order. This will help “average out” systemic drift effects. Alternately, assay each aliquot in random order.

Data Entry and Analysis

We encourage you to use the Assurance™ Excel spreadsheets, available at www.sundiagnosics.us, for data entry and to perform the required statistics or refer to the CLSI guidelines.¹

The spreadsheets are designed to CLSI recommendations. The cells highlighted in yellow are cells that require input of information and data.

1. The first worksheet in the workbook is a Test Pool Calculator. Use this sheet to verify concentrations of interferent in the Assurance Interference Kits and also to calculate the required amount of Interference Material to add to the Base Pool to create the Test Pools. The section on the right side of the page is useful if the Base Pool contains endogenous interferents such as protein and triglycerides. The worksheet will calculate the amount of additional Interferent to add to reach the target concentration for testing.
2. The second page includes Replicate Tables to help determining the required number of replicates of Test and Control Pool for use with the Screening experiments. However, the Interference Screening Study worksheet will also calculate the number of replicates required, if Within-Run Standard Deviation and maximum allowable difference (Dmax) between Test and Control Pools are defined.

If in doubt, we suggest using a two-tailed test with an alpha of 5% (0.05) and a beta of 0.1 (10%).

3. For the Screening Experiment worksheets, enter the test results for Control and Test Pools, and the spreadsheet will calculate the difference, percent difference, upper and lower 95% confidence intervals for the difference, and a p-value for a two-sample t-test with equal variances. The t-test and 95% confidence intervals are provided for information purposes only; CLSI recommends interpreting the difference in terms of clinical significance—defined as a difference less than the Dmax.

Also, the spreadsheet will interpret the results for statistical and clinical significance.

The Worksheet includes a signature line and can be printed out to provide a summary of the experiments and the conclusions.

4. The Workbook also includes worksheets for dose-response experiments. Enter the Interferent concentrations of levels 1 and 5 and the intermediate concentrations will be calculated. Enter the test results and the means, difference, and percent difference will be calculated, in comparison to Interferent level 1.

The worksheet will also plot analyte concentration (Y) versus interference concentration (X) and calculate slope, intercept, and whether the slope is significantly different from zero. A slope of zero indicates that there is no association between analyte concentration and interferent concentration. This statistical test is provided for informational purposes only as CLSI recommends an interpretation based on clinical difference (Dmax) only.

Also, the worksheet will interpret concentration differences and/or percent differences depending on whether Dmax is defined as an absolute concentration or a percent difference. If Dmax is defined as both an absolute difference and percent difference the interpretation of clinical difference will require both to be greater than Dmax.

Finally, the worksheet will interpolate between each level of interferent to calculate the exact interferent concentration that is $> D_{max}$. If multiple concentrations are provided, the correct concentration is the one that corresponds to the lowest interferent level that exceeds D_{max} .

The Dose-Response worksheet also includes a signature line and can be printed out as a summary report.

For Technical Assistance or Support, email support@sundiagnosics.us or call 1-877-SUN-DIAG (1-877-786-3424).

¹CLSI, Interference Testing in Clinical Chemistry; Approved Guidelines, 2ND ed, CLSI Document EP07-A2, Wayne, PA, 2005.

²Sonntag O, Scholer A. Drug interference in clinical chemistry: recommendations of drugs and their concentration to be used in drug interference studies. Ann Clin Biochem 2001; 38:376-385.