ASSURANCE™
INTERFERENCE TEST KIT

Sun Diagnostics

This document provides recommendations for performing Interferent screening and dose-response experiments using ASSURANCE™ Interference Test Kit. The experiments outlined are based on CLSI EP7-A: Interference Testing in Clinical Chemistry; Approved Guidelines.[1]

ASSURANCE™ Interference Test Kits include interferents in concentrated form to minimize change to typical sample matrix.

Interferent	Test Concentration	Typical Assurance™ Concentrate values
Triglycerides	1000 mg/dL *(CLSI recommendation 3000 mg/dL)*	>15,000 mg/dL (15X)
Hemolysate	500 mg/dL	>10,000 mg/dL (20X)
Total Protein *(from Albumin and gamma-globulins)*	12 g/dL	~25 g/dL (2X)
Bilirubin, conjugated	20 mg/dL	>400 mg/dL (20X)
Bilirubin unconjugated	20 mg/dL	>400 mg/dL (20X)

Preparation of Base Pool, Test Pools, Control Pools, and Experiments

- **Base Pool**: 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 of Base Pool should be sufficient, using half for the Test Pool and half for the Control Pool. Each Interferent Screening Experiment requires a matched Test Pool and a Control Pool. Analysis of the Base Pool for starting concentrations of the interferents prior to spiking with concentrated components is recommended.
- **Test Pool:** Base Pool with added Interferent. *Before preparing Test Pool, a 20X dilution (4X for total protein) in saline is recommended for measurement of actual interferent concentration. Actual concentrations are used to determine volume of interferent to add to the test pool.* Refer to the ASSURANCE™ Dilution Calculator, available at www.sundiagnostics.us for assistance with this calculation.
- **Control Pool:** Base Pool with same volume of solvent as used in preparation of corresponding Test Pool. This solvent can be normal saline (0.9%NaCl) or water. Unconjugated bilirubin requires 0.1N NaOH, included with the ASSURANCE™ Kit.
- **Sample Sizes and Pool Volumes**: The number of replicates (sample size) and volume of Pools depend on assay variability and the size of the Interferent effect to be detected. Prepare enough volume of each pool for the required number of replicate measurements. To estimate the replicates and Base Pool volume required, refer to the ASSURANCE™ Excel spreadsheet, available at www.sundiagnostics.us, or to the CLSI EP-7A guidelines[1].

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 ½ of the total allowable error, TEa, as defined by CLIA, other published guidance documents, or as defined by your Laboratory Director. Email: support@sundiagnostics.us for a list of CLIA TEa recommendations.
- Analyze the Base Pool and ASSURANCE™ concentrate values for actual concentrations of interferents before performing experiments.
- Perform screening experiments with both low and high analyte pools, as interference may be dependent upon analyte concentration.

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Preparation of Base Pool

1. After reading the Instructions for Use, calculate the volume of Base Pool required for conducting all planned Interference Experiments.
2. Prepare a Base Pool for all Interference Experiments (screening and/or dose response) by collecting or pooling specimens without any visible lipemia, hemolysis, or icterus.
3. Analysis of the base pool for starting concentrations of the interferents is recommended.

SCREENING STUDIES

1. Thaw interferent vial at room temperature.
2. Perform a 20X dilution (4X for Protein Component) of the Interferent to determine actual concentration.
3. Choose the appropriate Test Pool Calculator. **Steps 4-7** correspond to the calculator for ***negligible base pool interferent concentration*** and **Steps 8-10** correspond to the calculator for ***significant base pool interferent concentration*** (highly recommended for Protein Interference Testing).
4. Input actual concentration of interferent and desired Test Pool Size into the ASSURANCE™ Dilution Calculator to determine the amount of concentrated interferent to add to the Test Pool.
5. Remove the determined amount of base pool from the test pool sample. For example, if you are required to add 100µL of Interferent to the Base Pool to create your test pool, to avoid volume changes, you should remove 100µL of base before adding the interferent.
6. Add calculated amount of interferent to Test Pool. Mix by inversion.
7. Remove the same volume of base from the Control Pool and add the same volume of water or saline (Bu requires addition of 0.1N NaOH), instead of interferent to the Control Pool. Mix by inversion. For example, if 100 µL of interferent was added to the 1 mL test pool, add 100µL to the 1 mL Control Pool. Proceed to Step 11.

   OR

8. Input actual concentration of ASSURANCE™ interferent and base pool interferent concentration into the calculator. Enter the desired test pool volume and target interferent concentration.
9. Select the addition that best approximates your desired concentration.
10. Measure the calculated amount of base pool and calculated amount of concentrated interferent and mix. This is now your test pool. Proceed to Step 11.
11. If performing High and Low interferent evaluations, repeat Steps 4-7 or 8-10 for the additional level.
    NOTE: Do not allow samples to sit before analysis. Bilirubin materials, and other components, are temperature and light sensitive.
12. Analyze each pool for desired analyte(s) using the appropriate number of replicates.
13. Analyze results using the ASSURANCE™ results spreadsheet(s) or refer to CLSI EP-7A[1] for guidance.

DOSE RESPONSE STUDIES

1. Determine the range of interferent concentrations to be tested. Prepare High and Low Pools as described above in the screening experiments. The low pool should contain low or "normal" levels of interferents
2. Prepare intermixtures: Test Pools Level 1 to Level 5. Six (6) mL of High and Six (6) mL of Low Pools will provide 1 mL of Test Pools at each level. NOTE: Do not allow samples to sit before analysis. Bilirubin materials, and other components, are temperature and light sensitive.

    1) 1 part low
    2) 3 parts low + 1 part high
    3) 1 part low + 1 part high
    4) 1 part low + 3 parts high
    5) 1 part high

3. Divide each pool into three (3) sample cups for triplicate analysis. To help "average out" systemic drift effects, analyze first in ascending order, descending order, then ascending order again. Alternately, assay each aliquot in random order.
4. Analyze results using the ASSURANCE™ results spreadsheet(s) or refer to CLSI EP-7A[1] for guidance

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

[1]CLSI, INTERFERENCE TESTING IN CLINICAL CHEMISTRY; APPROVED GUIDELINE, 2ND ED, CLSI DOCUMENT EP07-A2, WAYNE, PA, 2005.