

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

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

Drug Interferent	Test Concentration ^{1,2}	Typical Assurance™ Concentrate values
Acetaminophen	1,324 µmol/L	26,480 µmol/L
Acetylcysteine	10.2 mmol/L	204 mmol/L
Acetylsalicylic acid	3.62 mmol/L	72.4 mmol/L
Ampicillin	152 µmol/L	3,040 µmol/L
Cefoxitin	1,546 µmol/L	30,920 µmol/L
Cyclosporine	5 mg/L	100 mg/L
Doxycycline	67.5 µmol/L	1,350 µmol/L
Heparin	3,000 U/L	60,000 U/L
Ibuprofen	2,425 µmol/L	48,500 µmol/L
Levodopa	20 mg/L	400 mg/L
Methyldopa	20 mg/L	400 mg/L
Metronidazole	701 µmol/L	14,020 µmol/L
Phenylbutazone	400 mg/L	8,000 mg/L
Rifampicin	78.1 µmol/L	1,562 µmol/L
Theophylline	222 µmol/L	4,440 µmol/L

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.
- **Test Pool:** Base Pool with added Interferent. Because the drug solutions are a 20-fold concentrate of recommended test concentrations a 1:20 dilution is recommended, e.g. 50 µL of drug solution + 950 µL Base Pool.
- **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 for the aqueous drug solutions and ethanol for the ethanolic drug solutions. Absolute Ethanol (1 mL) is 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.sundiagnosics.us, or to the CLSI EP-7A guidelines¹.

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, TE_a, as defined by CLIA, other published guidance documents, or as defined by your Laboratory Director. Email: support@sundiagnosics.us for a list of CLIA TE_a recommendations.

SCREENING STUDIES

1. Thaw interferent vial at room temperature.
2. Determine the amount of concentrated interferent to add to the Test Pool.
3. Remove the determined amount of base pool from the test pool sample. For example, if you are required to add 100 µL of Interferent to the Base Pool to create your test pool, to avoid volume changes, you should remove 100 µL of base before adding the interferent.
4. Add calculated amount of interferent to Test Pool. Mix by inversion.
5. Remove the same volume of base from the Control Pool and add the same volume of water (or saline) for aqueous drug solutions or ethanol for ethanolic drugs. 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.
6. Analyze each pool for desired analyte(s) using the appropriate number of replicates.
7. Analyze results using the ASSURANCE™ results spreadsheet(s) or refer to CLSI EP-7A¹ for guidance.

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.