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1.0 Purpose and Scope

1.1 This manual describes the method used for screening for drugs in whole blood samples using the Randox Evidence Investigator.

1.2 Samples to be analyzed may include calibration materials, control standards, and casework samples.

1.3 The scope of this manual includes reagent preparation, retrieval and opening of evidentiary samples, sample preparation and analysis, instrument set-up, data review and release of reports, documentation, and quality control criteria.

2.0 Responsibility

2.1 All analysts having the responsibility for screening blood samples for drugs as part of their prescribed job duties are responsible for following these procedures as written.

2.2 These procedures are reviewed periodically by the Toxicology Section staff. Revisions are made at that time or when there is an identified need to change this written manual to be compatible with changing needs in the analytical process. In the event that there are changes to be made to this manual, the analyst must report those changes in detail to the Toxicology Section Supervisor in a timely manner.

2.3 All analysts performing and reporting analytical results for forensic purposes must be fully trained and authorized in the use of these procedures in accordance with the Toxicology Training Manual (TOX_P301). All analysts must demonstrate initial competency in the method and must show ongoing proficiency by successfully analyzing at least one internal or external proficiency test annually.

2.3.1 Proficiency samples will be treated as casework. Results will be unknown to the analyst prior to testing. Samples that screen positive will be confirmed using currently validated confirmatory methods.

2.3.2 Analysts trained and authorized to perform both toxicological screening and confirmation procedures must perform analysis using both procedures on at least one proficiency sample per year regardless of screening results.

2.3.3 Proficiency test results for the screening method must be consistent with the reported results of the provider.

2.4 Analysts will ensure that an adequate amount of sample processing supplies are on hand at all times. Orders should be placed when supplies are low to ensure that new stock arrives before supplies are completely empty.

3.0 Emergency or High Priority Situations

3.1 The Commissioner of Public Safety, Laboratory Director, or Toxicology Section Supervisor can designate samples as high priority.

3.2 High priority samples are analyzed as soon as possible.

3.3 Priority sample results are reviewed and released as soon as they are available and once they pass the quality assurance criteria.

4.0 Quality Assurance

4.1 It is expected that the analyst will report any unacceptable or anomalous behavior of any analytical system immediately to the Toxicology Section Supervisor. It is further expected that appropriate actions will follow as soon as possible and be properly documented.

4.2 Equipment

4.2.1 Pipettes

4.2.1.1 Measurements made by the Toxicology Section using pipettes are critical.

4.2.1.2 Pipettes used by the Toxicology Section, including fixed and variable volume pipettes have their calibration evaluated and certified by an approved vendor. The resulting documentation will be maintained.

4.2.1.3 If a pipette is sent out for service, an inspection of the package/pipette will be performed to check for any shipping and handling concerns prior to being returned for use. The calibration certificate will be reviewed in accordance with QA_P100_6.4 Equipment QC.

4.2.1.4 If a question arises regarding the proper functioning of a pipette, a performance check or calibration service by an approved vendor may be initiated.

4.2.1.4.1 Performance checks will be performed in accordance with QA_P100_6.4_Equipment QC.

4.2.1.4.2 Performance checks will be reviewed and filed in the VFL Pipettes Equipment QA/QC binder.

4.2.2 Thermoshaker

4.2.2.1 Temperature will be checked annually using a NIST traceable thermometer. The temperature must read 37°C ± 2°C. This check will be documented using the thermoshaker temperature check worksheet. The performance check will be reviewed by the Toxicology Section Supervisor, or their designee, and stored with the instrument maintenance records.

4.2.3 Centrifuge

4.2.3.1 Centrifugation times and velocities will be performed in accordance with the manufacturer recommendations but are not considered critical to this analysis. Samples are centrifuged to avoid pipetting any solid particulate onto the biochips that could interfere with analysis.

4.2.3.2 The centrifuge is checked annually.

4.3 Instrumentation

4.3.1 All maintenance performed, including routine and preventative maintenance as well as troubleshooting activities, should be recorded in the Instrument Maintenance Log.

4.3.2 The Instrument Maintenance Log and the listed resources are kept near the instrument.

4.3.3 Randox Evidence Investigator

4.3.3.1 Day of use

4.3.3.1.1 Check kit to ensure there is sufficient volume of reagents. If previously prepared, ensure materials have not expired.

4.3.3.1.2 Check biochip cassettes.

4.3.3.2 Monthly

4.3.3.2.1 Clean carrier loading bay with disinfectant.

4.3.3.3 Analytical data is exported with each run and saved in the lab document storage system. The Randox database should be archived onto long term storage media at least annually.

4.3.3.4 The Randox Evidence Investigator is designed to require minimal maintenance. If there are any issues with instrument performance contact the manufacturer to schedule a service check.

4.3.3.4.1 The instrument will periodically initiate an internal diagnostic check. While shutting down the software, the instrument will prompt user to allow a diagnostic to run. Select OK and allow instrument to perform check, which takes approximately 45 minutes. Do not power down instrument or close out of software during this process.

4.3.3.5 Refer to Randox Evidence Investigator Operator Manual for additional information on maintenance and troubleshooting.

4.4 Reference Materials

4.4.1 Purchased Standards

4.4.1.1 All standards will be stored as specified by the manufacturer until their expiration date and then discarded.

4.4.1.2 Calibration materials

4.4.1.2.1 Lyophilized multi-analyte calibration materials are included in the Randox DOA Ultra WB biochip kits. Upon receipt, kits will be stored in the refrigerator until use. Prior to use, calibrators must be reconstituted. The date and initials of the analyst reconstituting the materials will be written on the kit.

4.4.1.2.2 Once reconstituted, calibrators are stable for up to 14 days when refrigerated.

4.4.1.3 Quality control mixtures

4.4.1.3.1 Lyophilized multi-analyte controls are available from Randox and will be used as quality control checks. Upon receipt, control kits will be stored in the refrigerator until use. Prior to use, controls must be reconstituted. The date and initials of the analyst reconstituting the materials will be written on each vial.

4.4.1.3.2 Once reconstituted, controls are stable for up to 14 days when refrigerated.

4.4.2 In-House Preparations

4.4.2.1 Solutions prepared in-house will be performance checked prior to use in casework.

4.4.2.2 Negative Control Stock

4.4.2.2.1 Negative Control Stock (NEG) will be prepared by pooling whole blood samples.

4.4.2.2.2 Each source of whole blood must be screened and shown to be free from target analytes prior to pooling. Results from the analysis will be reviewed and documentation of passing QC recorded in the Reagent

Preparation Log. Analytical results will be kept on file with the Toxicology Section.

4.4.2.2.3 Each NEG will be assigned a unique lot number NEG-MMDDYYYY, where MMDDYYYY is the date of pooling. Document the preparation in the Reagent Preparation Log.

4.4.2.2.4 NEG may be used for screening analysis after pooling.

4.4.2.2.5 NEG is stored in the refrigerator and is approved for use until consumed or contamination is suspected.

5.0 Evidence Handling

5.1 Evidence Storage and Retention

5.1.1 Samples submitted for toxicology analysis are stored in an evidence intake refrigerator (Room 155A) until brought to the toxicology lab for analysis. Samples in personal custody, but not currently being analyzed, are stored in a refrigerator.

5.1.2 Subsequent to analysis, ensure all blood tubes are sealed using evidence tape and returned to the evidence refrigerator for storage.

5.1.3 Evidentiary blood tubes are kept for at least 90 days subsequent to analysis. They may be disposed of after that time in accordance with the Evidence Handling Manual (EH_P100).

5.2 Opening Evidentiary Blood Kits and Blood Tube Labeling

5.2.1 Kits must be opened and the corresponding blood tubes labeled one at a time at the lab bench.

5.2.2 Compare the evidence with the information in FA.

5.2.3 Compare any identifying information written on seals, blood tubes, the kit, and submission form(s). If there are any noteworthy discrepancies, make a comment on the worksheet. If there is a question regarding the identification of a sample, contact the Toxicology Section Supervisor or designee.

5.2.4 It is permissible to use an expired kit; the expiration date refers to the vacuum of the blood tubes. If the tubes filled, regardless of the expiration date, the sample is deemed acceptable.

5.2.5 Note in FA the number of tubes submitted in the kit (typically three) and whether or not each tube was sealed. Label each blood tube with the corresponding identification number (e.g. A1-1, A1-2, A1-3).

5.2.5.1 Any unsealed tubes will be sealed with evidence tape, dated and initialed.

5.2.5.2 One tube from each kit should be kept unopened for independent testing at the subject's discretion.

5.2.5.3 Evidentiary samples submitted for analysis should be collected in grey-topped tubes. Each tube should contain at least 2 mL of blood and be fluid enough to aliquot.

5.2.6 Any anomalies in the evidence submitted will be documented in the case record.

6.0 Drug Screening by Biochip Immunoassay

6.1 Principle of Measurement

6.1.1 The Randox Evidence Investigator instrument is an immunoassay analyzer which functions on the principles of competitive antibody binding, using proprietary Biochip Array Technology. A number of discrete testing areas containing immobilized antibodies are arranged across the substrate of each biochip. Each area is specific to a particular analyte of interest and exhibits cross reactivity with related drug compounds. When an unknown blood sample containing a drug analyte is introduced to the biochip, the drug compound binds to the immobilized antibody, creating an antibody-antigen complex attached to the biochip substrate. Concurrent with the unknown sample, a conjugate reagent containing multiple drug compounds labeled with horseradish peroxidase (HRP) enzyme is introduced to the biochip. These enzyme-labeled compounds will compete for antibody binding sites with free unlabeled drug compounds present in the unknown sample. After incubation, the biochip is washed to remove any residual unbound sample and conjugate material. A signal reagent, consisting of luminol/enhancer solution and hydrogen peroxide, is added to the biochip. The HRP enzyme attached to the antigen-antibody conjugate reacts with hydrogen peroxide and luminol to generate chemiluminescence. The amount of light emitted from each discrete testing region on the surface of the biochip corresponds inversely to the amount of drug compound bound to those regions. A charge-coupled device (CCD) camera in the instrument captures the light emitted from the biochip, which is translated into an electronic signal. Further software processing of this signal will validate and quantify the amount of analyte present in the sample. These results are semi-quantitative and function as a screening tool.

6.2 Equipment and Materials

6.2.1 Randox Evidence Investigator immunoassay analyzer

6.2.2 Thermoshaker unit

6.2.3 Biochip carrier handling tray

6.2.4 Barcode scanner

6.2.5 Pipettes and pipette tips

6.2.6 Lint free tissues

6.2.7 Vortex mixer

6.2.8 Rocker mixer

6.2.9 Centrifuge

6.3 Standards and Controls

6.3.1 Randox DOA Ultra WB biochip kit or equivalent. See manufacturer insert for specific information. Reagents from different kits may be used as long as they are of the same lot. Kit includes:

6.3.1.1 Six vacuum-sealed foil bags containing biochip carriers

6.3.1.2 Sample prep diluent (DIL SPE)

6.3.1.3 Assay diluent (DIL ASY)

6.3.1.4 Multi-analyte conjugate solution (CONJ)

6.3.1.5 Wash buffer concentrate (BUF WASH)

6.3.1.6 Luminol/enhancer solution (LUM-EV481)

6.3.1.7 Hydrogen peroxide solution (PX)

6.3.1.8 Nine multi-analyte calibrators

6.3.1.9 CD containing kit information, instructions, and calibration concentration data

6.3.2 Calibration material

6.3.2.1 Lyophilized multi-analyte calibration materials are included in the Randox DOA Ultra WB biochip kits.

6.3.3 Quality control material

6.3.3.1 A negative control sample prepared from Negative Control Stock.

6.3.3.2 Lyophilized multi-analyte controls are available from Randox.

6.3.3.2.1 Two levels of positive quality control material are provided by the manufacturer. Actual concentrations of analyte present in quality control material may differ by lot number; target values and standard deviation ranges will be updated in the analysis software when placing a new lot number of control material into service.

6.4 Procedure

6.4.1 Additional instructions are provided in each kit by the manufacturer. Unless otherwise noted, follow manufacturer's recommendations included in the kit.

6.4.2 Instrument, kit, and sample preparation

6.4.2.1 If calibration materials have not been previously prepared, add 1 mL of diH2O to each vial of calibration material and gently mix using the rocker at room temperature for at least 30 minutes.

6.4.2.2 If controls have not been previously prepared, add 1 mL of diH2O to each vial and gently mix using the rocker at room temperature for at least 30 minutes.

6.4.2.3 If needed, prepare fresh wash buffer using the wash buffer concentrate provided in kit.

6.4.2.3.1 Wash buffer is prepared by adding approximately 32 mL of wash buffer concentrate to 1 L of diH2O.

6.4.2.3.2 Alternate volumes of wash buffer may be prepared using the same ratio of wash buffer concentrate to diH2O.

6.4.2.3.3 Diluted wash buffer is suitable for use up to 30 days when refrigerated.

6.4.2.4 If using a new lot, install the CDs provided with the DOA Ultra WB kit and the DOA Ultra WB Control kit. This must be done before the Evidence Investigator software is opened.

6.4.2.5 Turn on the thermoshaker unit and the Evidence Investigator and initialize the software.

6.4.2.6 Remove evidential sample kits from refrigerated storage and allow them to reach room temperature. Follow procedure listed in Section 5.2 regarding documentation of evidential kits and blood tubes.

6.4.2.7 Prepare a whole blood negative control sample using previously verified NEG.

6.4.2.8 Whole blood samples require a dilution and centrifugation step prior to analysis. Due to varying sample volumes, samples will be vortexed and prepared as a 1:3 dilution in SPE diluent by adding 100 µL of blood to 300 µL of SPE diluent prior to centrifugation. See manufacturer's instructions included in package insert regarding centrifuge parameters.

6.4.2.9 In the analyzer software, prepare a worklist of calibrators, controls, and samples to be run in the analytical batch. Accept worklist.

6.4.3 Assay Procedure

6.4.3.1 Remove biochip carriers from their packaging and label carriers using the Randox Sample Prep Sheet as a guide. Insert the biochip carriers into the handling tray.

6.4.3.2 Each biochip, including the calibrators and controls, requires the addition of assay diluent, sample, and conjugate. See manufacturer insert for instructions.

6.4.3.3 After the addition of these reagents, incubate the handling tray with the biochip carriers in the thermoshaker per the manufacturer recommendations.

6.4.3.4 Prepare signal reagent by mixing luminol/enhancer and hydrogen peroxide solutions 1:1 and rock for at least 15 minutes. Add peroxide to amber vial first and then add luminol. A total volume of ~3 mL of signal reagent is needed per each biochip carrier.

6.4.3.5 Upon completion of the incubation period, a series of wash steps are performed. Remove the carrier from the thermoshaker and quickly invert the handling tray over the sink to discard the liquid.

6.4.3.6 Add wash buffer to each well being careful not to over fill the wells. Repeatedly tap each side of the handling tray for a few seconds then quickly invert the tray over the sink to discard the wash buffer solution. Repeat for a minimum of 6 total quick washes.

6.4.3.7 Repeat wash step, but allow wash buffer to soak for 2 minutes after tapping each side of the handling tray. Repeat for a

minimum of 6 total 2-minute washes, discarding the liquid between each cycle.

6.4.3.8 After the last wash, fill all wells with wash buffer and place the handling tray in dark storage until analysis. Biochip carriers can remain stored with wash buffer for up to 30 minutes in a dark environment.

6.4.3.9 Remove the first biochip carrier from the tray and discard the liquid into the sink. Wrap the carrier in a lint-free tissue, and tap the back of the carrier to remove any excess liquid from the wells.

6.4.3.10 Add prepared signal reagent to the biochips and incubate for 2 minutes in a dark environment.

6.4.3.11 Once prompted, insert the carrier into the instrument for imaging.

6.4.3.12 Repeat the signal addition and imaging step for all remaining biochip carriers.

7.0 Quality Control and Corrective Action

7.1 Analytical batches should contain:

7.1.1 Nine multi-analyte calibration standards

7.1.1.1 Each assay calibration line curve fit (R^2) must be greater than the manufacturer target curve fit.

7.1.1.2 Calibration results must meet all manufacturer requirements and report a status of “PASS”.

7.1.1.2.1 The instrument software will automatically exclude any outlying data points; if more than 3 data points are excluded from the calibration, it cannot be used for casework batches.

7.1.2 A negative control sample, yielding no results above analytical threshold for any assay. At a minimum, a negative control sample is run subsequent to the calibration and at the end of the run.

7.1.3 Positive controls are analyzed at two different concentrations for each assay. At a minimum, positive controls are run subsequent to the calibration and at the end of the run.

7.1.3.1 All control results at each concentration should fall within 3 standard deviations of the target value in order to report screening results for an assay.

7.1.3.1.1 In instances where one control result is outside 3 SD of the target value, all other control results at that concentration must be within 2 SD of the target value.

7.1.3.1.2 If control results fail high for an assay, e.g. > 3SD above the target value for both replicates, negative results may still be reported.

7.1.3.2 If control results do not meet acceptance criteria for 3 or more assays, all samples in the batch must be re-analyzed.

7.1.3.3 If control results do not meet acceptable criteria for an assay, samples may be re-analyzed, passed on to in-house confirmatory testing or reported as not meeting quality control criteria for that drug class at the analyst's discretion and with approval from the Toxicology Section Supervisor, or their designee.

7.2 Results above the cutoff concentration, including borderline results, will be forwarded on to confirmatory testing for all analytes for which a validated confirmatory test is available.

7.2.1 Cut off concentrations

Assay	Cutoff (ng/mL)	Assay	Cutoff (ng/mL)
Meth /Amphetamines	20	Meprobamate	100
Barbiturates	50	Methadone	10
Benzodiazepines	10	Opiates	10
Buprenorphine	1	Opioids	10
Cannabinoids	10	Phencyclidine	5
Benzoylecgonine	50	TCA	25
Dextromethorphan	5	Tramadol	5
Fentanyl	1	Zolpidem	10

7.2.2 Samples within 20% of the cutoff concentration are considered "borderline" results.

7.2.3 Results greater than the highest calibrator will be passed on to confirmatory testing. No dilution is required.

7.3 Results above the cutoff concentration, including borderline results, for which a validated confirmatory test is not available, will be reported as preliminarily positive.

7.4 Samples with results below the borderline concentration will be reported as below the laboratory's reporting threshold.

8.0 Data Review and Reporting

8.1 Procedure

8.1.1 Samples that screen positive for a compound for which a validated confirmatory test is available will be passed on to confirmatory testing and a report will be issued once the confirmatory test is complete and the results are reviewed.

8.1.2 Generate a raw data output file using the Evidence Investigator Software.

8.1.3 Enter the raw data and control assignment data into the Randox Result Sheet (TOX_F600_1).

8.1.4 Upon successful completion of analysis, the analyst must perform a primary data review of the screening data packages.

8.1.5 The completed Case File includes:

8.1.5.1 Toxicology Case Specific Review Checklist (QA_F100_7.7_17).

8.1.5.2 VFL lab report, if applicable, and FA worksheet.

8.1.5.3 Sample result summary sheet from TOX_F600_1.

8.1.5.4 A request for analysis form (EH_F100_2).

8.1.6 The completed Batch File includes

8.1.6.1 Blood Drug Screening Batch File Review Checklist (QA_F100_7.7_19).

8.1.6.2 QC summary and control assignment sheets from QA_F600_1.

8.1.6.3 Sample preparation worksheet.

8.1.6.4 Run worklist.

8.1.6.5 Calibration graphs.

8.1.6.6 Analytical results summary.

8.1.7 Analyst Review

8.1.7.1 Ensure the criteria defined in Section 7.0 are met.

8.1.7.2 Verify the Randox Sample Prep Sheet is completely filled out and all reagents were used within their expiration dates/times.

8.1.7.3 Ensure that all transcription is correct and results are reported appropriately.

8.1.8 Technical Review:

8.1.8.1 A qualified analyst must perform a technical review of the complete Batch and Case Specific data packages.

8.1.8.2 Ensure that the data packages are complete and all forms are complete and accurate.

8.1.8.3 Confirm all calculations that are not part of a validated worksheet.

8.1.8.4 If data quality issues have been identified during review, the reviewer must attempt resolution through discussion with the analyst and/or Toxicology Section Supervisor. If issues cannot be resolved, it may be necessary to prepare and analyze new aliquots of the submitted sample.

8.1.9 Administrative and Director Review:

8.1.9.1 Upon completion of the technical review, an administrative and director review of the Batch and Case Specific data packages will be completed.

8.1.10 All review criteria must be met before the final report can be released.

9.0 Backup Procedures

9.1 Any secure storage refrigerator in rooms 155A, 266, or 265B may be used to store samples.

10.0 References

10.1 Toxicology Training Manual (TOX_P301)

10.2 Randox Evidence Investigator Operator Manual

10.3 Reagent Preparation Log

10.4 Evidence Handling Manual (EH_P100)

10.5 Request for Analysis for Alcohol/Drugs in Blood (EH_F100_2)

10.6 Instrument Maintenance Log

10.7 Toxicology Confirmation Manual (TOX_P700)

10.8 Blood Drug Screening Batch File Review Checklist (QA_F100_7.7_19)

10.9 Toxicology Case Specific Review Checklist (QA_F100_7.7_17)

10.10 Randox Result Sheet (TOX_F600_1)

10.11 Randox Sample Prep Sheet

DOCUMENT HISTORY			
DATE	**VERSION**	**APPROVED BY**	**ACTIVITY OR REVISION**
2/11/2019	1	Lab Director	First edition
11/2/2020	2	Lab Director	Rephrased pipette qc section to match Equipment QC manual. Clarified NEG preparation instructions. Updated the evidence handling section. Updated reporting language section. Removed abbreviations section, see lab-wide abbreviations list.
1/13/2021	3	Lab Director	Removed approximate control concentration table (section 6.3.3.3) and replaced with section 6.3.3.2.1; added cutoff concentration table to section 7.2; TOX_F600_1 updated (added the assay Benzodiazepine3 and lowered the cutoff for Buprenorphine to 1ng/ml; updated the formula to source and index the data by assay instead of by column location)
6/14/2021	4	Lab Director	Minor formatting changes throughout for consistency between manuals
8/10/2022	5	Lab Director	Updated section 8 for batch and case file parameters; changed pipette interval; removed section 9.2; minor formatting throughout; TOX_F600_1 was updated to allow 24 samples in the spreadsheet rather than 12