SCREENING OF BIOLOGICAL SPECIMENS FOR THE PRESENCE OF CYANIDE

19.1 POLICY

This test method may be used to qualitatively identify cyanide in biological specimens. Reporting of results following the application of this method will be contingent upon a thorough review and acceptance of quality control data and the qualification of individual results under the criteria for acceptance.

Any adjustments or deviations from the procedures below must be approved by a member of TLD Management, and appropriately documented in the testing paperwork.

19.2 PURPOSE

The purpose of this standard operating procedure (SOP) is to provide technical direction for the presumptive screening of cyanide in biological specimens. This procedure will serve as the laboratory document describing sample preparation and criteria for acceptance and reporting of the specified compound.

19.3 PRINCIPLE

The cyanide anion is liberated from the sample by reaction with sulfuric acid to form HCN gas. The HCN gas reacts with the dye in the CYANTESMO® test strip. A color change, from pale green to blue, indicates the possible presence of cyanide in the sample.

19.4 SPECIMENS

19.4.1 The specimen volume is 1 mL.

19.4.2 Specimens include whole blood, serum, plasma, urine, vitreous, and tissue homogenate.

19.4.3 Dilutions of specimens may be analyzed at the Forensic Scientist's discretion; however, this should be done in addition to testing the standard specimen volume, unless sample quantity dictates otherwise.

19.4.4 Analysis of larger specimen volumes must be approved and documented.

19.5 REAGENTS, MATERIALS AND EQUIPMENT

19.5.1 REAGENTS

19.5.1.1 Certified blank blood

19.5.1.2 Deionized water (DI H_2O)

19.5.1.3 Potassium cyanide (KCN)

19.5.1.4 1N Sodium hydroxide

Add 400 mL DI H2O to a glass flask. Add 50mL concentrated NaOH. Dilute to 500 mL with DI H_2O and mix. Store the solution in a glass or plastic bottle at room temperature for up to one year. Adjustments to final volume are permitted as long as proportions are maintained.

19.5.1.5 Sodium hydroxide (NaOH), concentrated 10N

19.5.1.6 3.6N Sulfuric acid

Add 300 mL DI H_2O to a glass flask. Add 180 mL concentrated H_2SO_4. Dilute to 500 mL with DI H_2O and mix. Store the solution in a glass bottle at room temperature for up to one year. Adjustments to final volume are permitted as long as proportions are maintained.

19.5.1.7 Sulfuric acid (H_2SO_4), concentrated 10N

19.5.2 MATERIALS

19.5.2.1 Amber glass vials and bottles

19.5.2.2 CYANTESMO® test strip (stored in a sealed container with desiccant)

19.5.2.3 Disposable 16 x 100mm tubes

19.5.2.4 Disposable 16 x 150mm tubes

19.5.2.5 Disposable pipette tips

19.5.2.6 Disposable safety closures for 16 mm tubes

19.5.2.7 Laboratory glassware (graduated cylinders, flasks)

19.5.2.8 Volumetric glassware (flasks, pipettes)

19.5.3 EQUIPMENT

19.5.3.1 Calibrated, adjustable piston pipettes

19.5.3.2 Heat source (sand bath, heating block, etc.)

19.5.3.3 Vortex mixer

19.6 STANDARDS, CALIBRATORS AND CONTROLS

19.6.1 STANDARDS

19.6.1.1 Stock standard (100 mg/L)

a. Using a calibrated balance, weigh 25 mg KCN (10 mg CN^-) and transfer to a 100 mL class-A volumetric flask.
b. Add 10 mL 1N NaOH.
c. Add DI H_2O to the flask to the designated volume.
d. The final concentration of the stock standard is 100 mg/L. The stock standard is stored in an amber glass bottle at approximately 4°C and expires 6 months from the date of preparation. Adjustments to final volume are permitted as long as proportions are maintained.

19.6.1.2 Working standard (10 mg/L)

a. Using a calibrated pipette, measure 1 mL of the stock standard into a 10 mL class-A volumetric flask.
b. Add DI H_2O to the flask to the designated volume.

c. The final concentration of the working standard is 10 mg/L. The working standard is stored in an amber glass bottle at approximately 4°C and expires 6 weeks from the date of preparation (if the stock standard expires prior to 6 weeks from the date of preparation of the working standard, the expiration date of the working standard is the expiration date of the stock standard). Adjustments to final volume are permitted as long as proportions are maintained.

19.6.2 CALIBRATORS

19.6.2.1 Calibrators are prepared in DI H_2O at the time of analysis using the working standard. The preparation of the calibrators is detailed in 19.7 SAMPLE PREPARATION.

19.6.3 CONTROLS

19.6.3.1 Negative Control

a. At least one negative control is tested with every batch. The negative control is prepared using certified blank blood.
b. When testing different sample types, wherever possible, include a negative control prepared from that matrix. (For example, when analyzing whole blood and urine samples the batch shall include at least one negative whole blood control and at least one negative urine control.)

19.6.3.2 Positive Controls

a. The calibrators act as positive controls for this assay. (There are no commercially available cyanide controls.)
b. When testing different sample types, wherever possible, include at least one positive control prepared from that matrix.

19.7 SAMPLE PREPARATION

19.7.1 Label a clean 16 x 100mm tube for each of the five calibrators.

19.7.2 Using the working standard, spike the calibrators according to the following table, adding the appropriate amount of working standard and DI H_2O as necessary, cap, and vortex-mix.

Calibrator Description	Volume (µL) Added Working Std	Volume (µL) Added DI H_2O
Calibrator 1 (0.25 µg/mL)	50	1950
Calibrator 2 (0.50 µg/mL)	100	1900
Calibrator 3 (1.0 µg/mL)	200	1800
Calibrator 4 (3.0 µg/mL)	600	1400
Calibrator 5 (6.0 µg/mL)	1200	800

19.7.3 Label a clean 16 x 150mm tube for each member of the test batch. (i.e. calibrator, negative control, or case samples).

19.7.4 Pipette 1 mL of each of the five prepared calibrators prepared in 19.7.2 into the appropriately labeled 16 x 150mm tube.

19.7.5 Pipette 1 mL certified blank blood into the appropriately labeled 16 x 150mm tube to act as the negative control.

19.7.6 Sample 1 mL of each case sample into its respective tube.

19.7.7 Add 1 mL of 3.6N H_2SO_4 to each tube, cap immediately, and vortex mix.

19.7.8 Tear off 2-3 cm pieces of the CYANTESMO® test strips and suspend them above the liquid in each tube. Use the cap to hold the strip in place. The length of strip inside each tube should be consistent.

19.7.9 Place the capped tubes in a heating block or sand bath at 50-60°C for 30 minutes. **(HEATING BLOCK OR SAND BATH MUST BE LOCATED INSIDE A FUME HOOD, AS THE GASSES PRODUCED IN THIS ASSAY ARE HAZARDOUS).**

19.7.10 Arrange the strips on a piece of paper, tape the strips down using clear tape, and label each strip with its indentifying information. Prepare a color photo copy of the page to be included in the file for each case in the batch. Submit the original and color photo copies for peer review. **Following peer review, discard the paper holding the CYANTESMO® test strips as this could pose a hazard if kept in the case file.**

19.8 CRITERIA FOR BATCH ACCEPTANCE

If the analysis of the batch meets the criteria listed below, the results for the specimens are accepted.

19.8.1 Calibrators

19.8.1.1 The blue color change of the calibrators should increase in intensity based on the increasing concentration of standard added.

19.8.1.2 **All photo copies should be a true and accurate reflection of the original CYANTESMO® test strips, as the original will be discarded following review.**

19.8.1.3 Any original notes or paperwork relevant to the test batch (e.g. worklist, deviation approvals, sample volume adjustments) will be retained in the case file of first case in the batch.

19.8.2 Controls

19.8.2.1 The negative control should show relatively no color change.

19.9 CRITERIA FOR CASE SAMPLE ACCEPTANCE

19.9.1 If criteria described in 19.8 above has been met, the case samples may be reported as negative or presumptive positive for cyanide, as indicated by the relative color change.

19.10 REPORTING

19.10.1 Compare the strips from the unknowns to those from the calibrators and negative controls. A blue color change is considered a positive result.

19.10.2 The case may then be reported as either presumptive positive or negative for cyanide, accordingly.

19.10.3 Positive results must be confirmed by a separate method.

19.11 METHOD PERFORMANCE

19.11.1 Limit of detection: 0.25 µg/mL

19.12 TRACEABILITY

19.12.1 Traceability of the reference materials is provided through the certificates of analysis provided by the approved reference material supplier.

LIST OF CHANGES

Revision Date	Description	Page Number
06/26/13	Method approved by the State Toxicologist. See DRA dated 6/25/13. Method released for evidentiary use as of 06/26/13.	All
9/30/16	Modified deviation approval in 19.1 to “TLD Management” and changed pipette type to “piston” in 19.5.3.1. Wording was added to 19.6.1.1.d and 19.6.1.2.c to allow for volume adjustments and a note regarding stock standard expiration date was added to 19.6.1.2.c. Added use of negative and positive controls for matrices other than blood in 19.6.3. Removed “to SI units” from 19.12.1 and added “Printed Copies are Uncontrolled” to footer.	All