SpOtOn Clinical Diagnostics

The SpOtOn SCD001 kit for newborn dried blood spot haemoglobinopathy screening using electrospray mass spectrometry (MSMS) has been modified to enable simultaneous screening for clinically significant haemoglobinopathies
biotinidase deficiency
type 1 tyrosinaemia (surrogate porphobilinogen synthase)

WARNING:
SpOtOn Clinical Diagnostics strongly recommends that the user contacts SpOtOn before first use of SCD001 for advice on equipment to be used and tips on using the test

STORAGE

Kits should be stored at or below -70ºC until required for use. Individual reagent vials should only be thawed on the day of use, do not re-freeze

ASSAY PRINCIPLE

The reagents are designed for the simultaneous measurement of clinically significant haemoglobinopathies, biotinidase activity, for the detection of biotinidase deficiency, and porphobilinogen synthase activity, for the detection of type 1 tyrosinaemia (fumarylacetoacetase deficiency), in newborn dried blood spot screening programmes.

Note succinylacetone, the pathognomic metabolite in type 1 tyrosinaemia, inhibits porphobilinogen synthase activity

The enzyme reagent, a mix of the relevant substrates (biocytin and 5-aminolevulinic acid), is added to dried blood spots and incubated for 60 min. The standard SpOtOn haemoglobin kit reagents are then added, which the stops the reaction. Following a further 30minute incubation, haemoglobin variants (sickle cell disease and thalassaemias) and substrate and product ions for biotinidase and PBG synthase are monitored by MSMS analysis in multiple reaction monitoring (MRM) mode. Hb data are analysed using variant peptide/wild-type peptide area ratios and substrate/product ion area ratios are used to determine biotinidase and PBG synthase activities.

ASSAY PROCEDURE

Dried blood spots (3mm) should be punched into the specified 96 deep well plates

Each reagent vial contains sufficient reagent for two polypropylene 96 deep well plate containing DBS

Reagents should only be thawed on the day of use, do not re-freeze

Working reagents should be used within 2h following preparation

1. Thaw **Enzyme Reagent for MSMS biotinidase & PBG synthase activity** (dual substrate).

2. Prepare working **Enzyme Reagent**. Dilute the complete contents of **Enzyme Reagent for MSMS biotinidase & PBG synthase activity** in 12mL of deionised water, taking care to ensure complete mixing.

3. Using an 8 channel manual pipettor, (automated pipetting stations can be used but are not recommended) add 50µL of working reagent, using a reverse pipetting technique, to each well containing a DBS.

5. Mix the plate gently using lateral movement. Ensure that the DBS are immersed in the liquid. It is good practice to centrifuge the plate at 1500 rpm for 1 minute to ensure that the substrate covers each DBS. Seal the plate with a specified sealing mat.

8. Incubate the plate at 37^{o}C for 60 min with gentle shaking.

9. During incubation thaw reagent 1 (vial **Reagent 1**), modified stable isotope labeled sickle T1 peptide, and reagent 2 (vial **Reagent 2**), TPCK treated trypsin.

10. Prepare working **Reagent 1** reagent. Dilute the complete contents of **Reagent 1** in 12mL of deionised water, taking care to ensure complete mixing.

11. Prepare working **Reagent 2** reagent. Dilute the complete contents of **Reagent 2** in 12mL of deionised water, again taking care to ensure complete mixing.

12. After incubation with working **Enzyme Reagent**, remove the plate from the incubator, remove the sealing cap, and stop the enzyme reaction by adding the haemoglobin reagents as specified in steps 13-16.

13. Using an 8 channel manual pipettor, (automated pipetting stations can be used but are not recommended) add 50µL of working reagent **Reagent 1**, using a reverse pipetting technique, to each well.

14. Mix the plate gently using lateral movement. Ensure that the DBS are immersed in the liquid.

15. Using an 8 channel manual pipettor, (automated pipetting stations can be used but are not recommended) add 50µL of working reagent **Reagent 2**, using a reverse pipetting technique, to each well.

16. Mix the plate gently using lateral movement. Ensure that the DBS are immersed in the liquid. Seal with a specified sealing cap.

17. Incubate the plate at 37^{o}C for 30-45 min with gentle shaking.

18. After incubation, remove the plate from the incubator, remove the sealing cap, and carefully, to avoid splashing, add 1 mL stopping solvent (acetonitrile:water, 90:10 with 0.025% formic acid) to each well using an Eppendorf repeating dispenser, or equivalent.

13. Reseal, making sure the lid position is the same as during incubation, and mix the plate gently using lateral movement.

14. Place the sealed plate into the autosampler for MSMS analysis.

Data acquisition and processing

We recommend that data be acquired using appropriate newborn screening software, e.g. Chemoview, NeoLynx, that defines the signal acquisition window by the total ion signal.

Area ratios should be calculated:
variant peptide/wild-type peptide
biotin/biocytin
5-aminolevulinic acid /porphobilinogen (Q&C)

We recommend that a blank filter paper spot be included in each assay, the SpOtOn DBS QC and, where available, third party positive and negative controls

Safety Information

Enzyme Reagent includes biocytin and 5-aminolevulinic acid. These substances are not hazardous substances or mixtures and not classified as dangerous according to Regulation (EC) No. 1272/2008.

Reagent 1 includes <1% w/v modified stable isotope labeled sickle T1 peptide. This substance is not a hazardous substance or mixture neither is it classified as dangerous according to Regulation (EC) No. 1272/2008.

Reagent 2 includes <1% w/v TPCK treated trypsin.
Trypsin may cause eye irritation or skin irritation. Use appropriate personal protective equipment (gloves, eye protection).
In case of skin contact Wash off with soap and plenty of water and consult a physician. **In case of eye contact** Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

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