

## SpO<sup>t</sup>On Clinical Diagnostics

Newborn dried blood spot haemoglobinopathy screening using electrospray mass spectrometry-mass spectrometry (MSMS)

**These reagents should not be used for clinical diagnosis.**

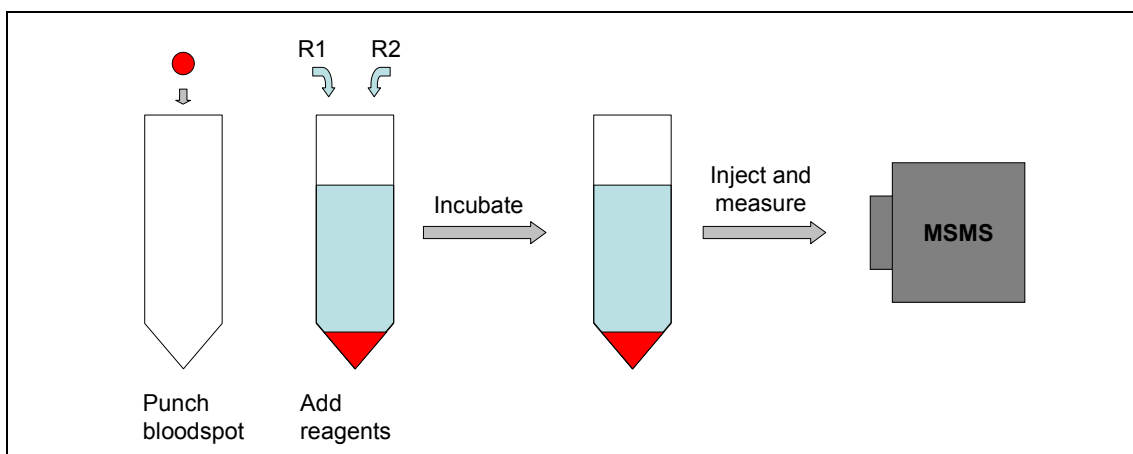
### ASSAY PROCEDURE

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

**Each reagent vial contains sufficient reagent for a single 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 reagent 1 (vial **R1**), modified stable isotope labeled sickle T1 peptide, and reagent 2 (vial **R2**), TPCK treated trypsin.
2. Working **R1** reagent. Dilute the complete contents of **R1** in 6mL of deionised water, taking care to ensure complete mixing.
3. Working **R2** reagent. Dilute the complete contents of **R2** in 6mL of deionised water, again taking care to ensure complete mixing.
4. Using an 8 channel manual pipettor, (automated pipetting stations can be used but are not recommended) add 50µL of working reagent **R1**, using 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.
6. Using an 8 channel manual pipettor, (automated pipetting stations can be used but are not recommended) add 50µL of working reagent **R2**, using reverse pipetting technique, to each well containing a DBS and an aliquot working reagent **R1**.
7. Mix the plate gently using lateral movement. Ensure that the DBS are immersed in the liquid. Seal with a specified sealing cap.
8. Incubate the plate at 37°C for 30-45 min with gentle shaking.
9. After incubation, remove the plate from the incubator, remove the sealing cap, and carefully, to avoid splashing, add 1 mL of MSMS mobile phase to each well using an Eppendorf repeating dispenser, or equivalent.
10. Reseal, making sure the position is the same as during incubation, and mix the plate gently using lateral movement.
11. Place the sealed plate into the autosampler.