Lamp Human HemoglobinS&C mutation KIT (LC-HbS/C-LP-24)

PROTOCOL

Prepare the whole blood samples as follows:
Pipette up and down to homogenize the lysis buffer (LC-HbS/C-LB).
Dispense 1000 µl of lysis buffer (LC-HbS/C-LB) in the microtubes 1.5 mL. Add 5µl of whole blood sample to the lysis buffer. Vortex in order to homogenize and wait 10 minute at room temperature. Use this solution as "specimen lysed".

Prepare the dried blood cards as follows:
Pipette up and down to homogenize the lysis buffer (LC-HbS/C-LB).
Dispense 1000 µl of lysis buffer (LC-HbS/C-LB) in the microtubes 1.5 mL. Add 2 punches of 3mm of the dried blood card to the lysis buffer. Vortex in order to homogenize and wait 10 minute at room temperature. Vortex two-three times during these ten minutes. Use this solution as "specimen lysed".

Prepare the positive and negative controls as follows:
Dispense 1000µl of lysis buffer (LC-HbS/C-LB) in the microtubes 1.5 mL. Add 5µl of positive (LC-HbS/C-Ctrl+) or negative (LC-HbS/C-Ctrl-) control to the lysis buffer. Pipette up and down or vortex in order to homogenize. Use these two solutions as "controls for reaction".

Prepare the Lamp reaction as follows:
Add 5 µl of "specimen lysed, or controls for reaction" to the 20 µl of Reaction Buffer (LC-HbS/C-RB) previously dispensed in the LC-Genie strips 0.2 mL set over the LC-Genie strip holder. Avoid air bubble formation while pipetting. Close the tubes correctly with the caps. Load the tube onto the adapted system in the LC-Genie III machine and close the cover.

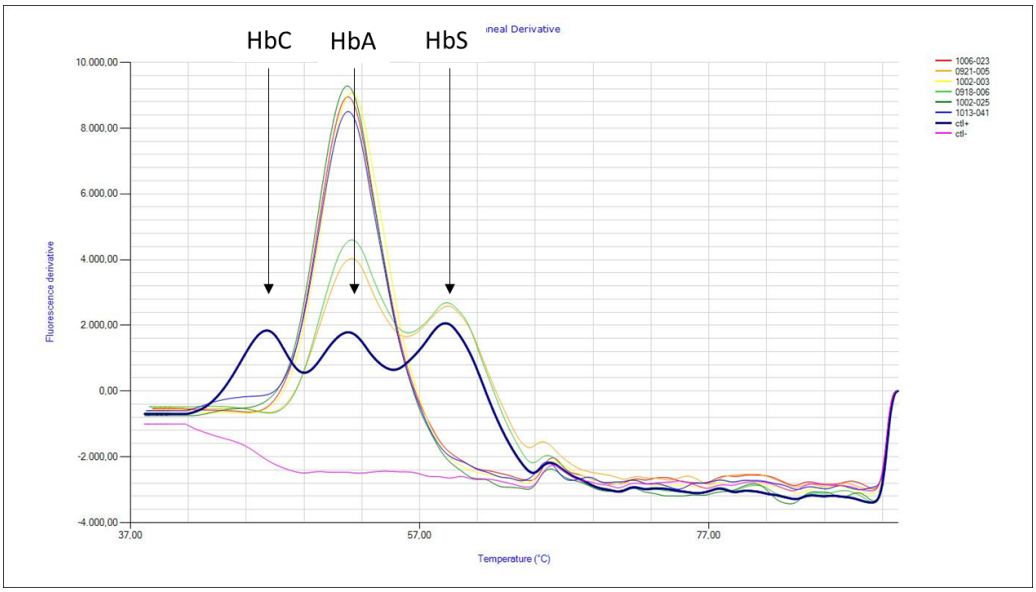
In order to validate a run, the positive and the negative controls must be run in each LAMP run.

LC-Genie III

Select in the main menu the predefined profile ‘HbS/C melting” saved on the instrument. Add all required information and press the ‘Start’ button to launch the run.

All LC-Genie III runs are automatically saved in the ‘LOG’ folder and classified corresponding to the year/month/day of the run.

Peak value for the positive and negative control must be within the specified limits. If controls correspond to the results above the run is valid and test sample results will be interpreted.



Controls:

Sample	Peaks – Tm (°C)	Level of fluorescence
Positive control	46.4 +/- 1°C 52.0 +/- 1°C 58.7 +/- 1°C	Up 500 Up 500 Up 500
Negative control	No peak up the threshold	No peak up the threshold

Fresh and dried blood samples:

Genotype:	Peaks – Tm (°C)	Level of fluorescence
HbA/HbC	46.6 +/- 1°C 52.1 +/- 1°C	Up 500 Up 500
HbA/HbS	52.1 +/- 1°C 59.1 +/- 1°C	Up 500 Up 500
HbS/HbC	46.6 +/- 1°C 59.1 +/- 1°C	Up 500 Up 500
HbS/HbS	59.1 +/- 1°C	Up 500
HbA/HbA	52.1 +/- 1°C	Up 500

Samples with melting temperatures outside the criteria are considered as invalid.

A pdf report containing all run information and results can be created by clicking the pdf button in the “interpret” window. The report is saved in the folder “REPORT”.