Troubleshooting Guide Malaria-LAMP

1. BLOOD SAMPLE PROCESSING

Problem	Solution
Pipette has touched blood tube walls during the transfer.	Discard the tip and wipe the pipette with 0.5 % bleach to avoid cross contamination with other samples. Remove the bleaching solution with distilled water.
One dried blood spot fell on the table/working area during punching the filter paper.	Discard the blood spot and use a new one. If the blood spot has fallen on the tissue on the table, discard only the tissue and use an new one. If the dried blood spot has fallen on the table surface, clean the affected area with 0.5% bleach solution (5 min incubation) to avoid cross contaminations. Remove the bleaching solution with distilled water.
Any blood spatter on the tissue.	To avoid cross contaminations, discard the tissue and use a new one.
Any blood spatter on the surface of the table (working area).	Clean the affected area with 0.5% bleach solution (5 min incubation) for avoiding of cross contaminations. Remove the bleaching solution with distilled water.
Problems with collecting blood directly from the fingertip after pricking.	Collect the sample in a heparin tube or transfer the blood to a filter paper and let it dry (then use the dried blood spot as sample).
You accidentally put a pipette tip contaminated with blood into the NaCl solution.	Discard the NaCl solution and prepare/mix a new solution.

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1. BLOOD SAMPLE PROCESSING

Problem	Solution
There are air bubbles in the filter tip after pipetting the blood sample.	Release the blood out of the tip, put the pipette tip deep enough in the blood and pipette again slowly.
You accidently pipetted NaCl solution twice in the same Heating Tube.	Discard the Heating Tube and use a new one. To avoid this issue, always pipette the samples one by one.
The lysis buffer spilled from the Heating Tube.	Discard the Heating Tube & prepare a new one. Wipe the working area with 0.5% bleaching solution and remove it with distilled water.
The skin or eyes came in contact with the lysis buffer.	Rinse your skin/eyes immediately and seek medical help.

2. DNA EXTRACTION WITH THE LOOPAMP™ PURE DNA EXTRACTION KIT

Problem	Solution
There is no colour change of the powder and lysed sample (from white and blue to creamy white).	Shake the Adsorbent Tube again until all powder is dissolved. If the powder cannot be dissolved discard the Adsorbent Tube and repeat the extraction of the patient sample.
There is red a colour onto the bottom of the Adsorbent Tube.	Shake the Adsorbent Tube again until all powder is dissolved. If the powder is dissolved completely the red colour should vanish.
The Adsorbent Tube drops.	Discard and prepare a new Adsorbent Tube. Upon contamination with fluids clean the surface with 0.5% bleaching solution (5 minutes incubation).
The Injection Cap drops.	Discard the Injection Cap and use a new one. Upon contamination with fluids clean the surface with 0.5% bleaching solution (5 minutes incubation).
No drops came out when the Adsorbent Tube is squeezed.	Keep The Adsorbent Tube squeezed and wait until the extracted DNA solution drops out of the tube. If necessary, use the Squeezing Tool to ease the process. Shaking the Adsorbent Tube again might also be helpful.
The DNA solution is turbid/coloured.	Discard the DNA solution and repeat the extraction.

2. DNA EXTRACTION WITH BOIL & SPIN METHOD

Problem	Solution
No pellet after centrifugation.	Repeat the centrifugation step.
You destroyed the pellet with the pipette tip during pipetting of the supernatant and pipetted accidentally parts of the red coloured pellet.	Release the solution and discard the pipette tip. If the pellet is destroyed and the supernatant red coloured, repeat the centrifugation step and pipette the supernatant again.
The DNA solution is turbid/coloured.	Discard the DNA solution and repeat the extraction.

3. LOOPAMP™ REACTION

Problem	Solution
The Reaction Tube is broken.	Discard the tube and use a new one.
There is no colour in the lid of the Reaction Tube.	No colour in the lid could mean that there are no reagents in the lid. Discard the tube and use a tube with colour in the lid.
The volume of the DNA solution exceeds the upper line.	If the mixing was not performed, remove the volume with a pipette and a filter tip (a separate pipette is required) until the volume of the DNA solution reaches the middle of the two lines or discard the tube and use a new Reaction Tube. If the mixing was already performed, use a new Reaction Tube and extract again. The pipette and filter tips box must be decontaminated with 0,5% bleach, otherwise you are not allowed to bring them into the amplification area.
The volume of the DNA solution is below the lower line.	Squeeze the Adsorbent Tube again until the volume of the DNA solution reaches the middle of the two lines.
Sample, negative and positive control solutions were spilled outside the Reaction Tube.	Discard the tubes and wipe the working area immediately with 0.5 % bleaching solution due to the high risk of contamination! Change the gloves. Repeat the procedure with controls only to confirm that there is no contamination. Retest the blood sample (use an aliquot of the stored material if necessary).

4. FLUORESCENCE DETECTION

Problem	Solution
The negative control shows green fluorescence.	Check the actual temperature and time setting on the display. Check the room temperature! If the room temperature is above 35°C the risk of false positive results increases. Use a fan or air conditioner to decrease the room temperature. Repeat the procedure with negative controls. If they are correct, retest the blood samples. If the result is still positive, the reason could be a contamination. In this case, discard all open reagents, wipe the working space and the instrument with 0.5 % bleaching solution. Perform the amplification reaction again with a negative control and retest the blood sample. If the negative controls become positive again, replace all reagents, repeat the procedure with new reagents and verify all steps with a supervisor and instruction manuals.
The positive control shows no green fluorescence.	Check the actual temperature and time setting on the display. Repeat the procedure with positive and negative controls only. If they are correct, retest the blood samples. If the same results are obtained, replace all reagents, repeat the procedure with new reagents and verify all steps with a supervisor and instruction manuals.

Problem	Solution
Samples with doubtful fluorescence	If samples show at least brighter fluorescence than the negative controls, report them as positive.
Reaction Tubes got opened during or after amplification.	Close the tubes immediately! Discard if scattered. If scattered in the HumaLoop instrument, turn off the instrument, clean the cavities with a cotton-swab soaked in 0.5 % bleaching solution. Clean surfaces and working areas with 0.5% bleaching solution and water. Repeat the testing procedures with negative and positive controls.