PanAfrican Medical Journal

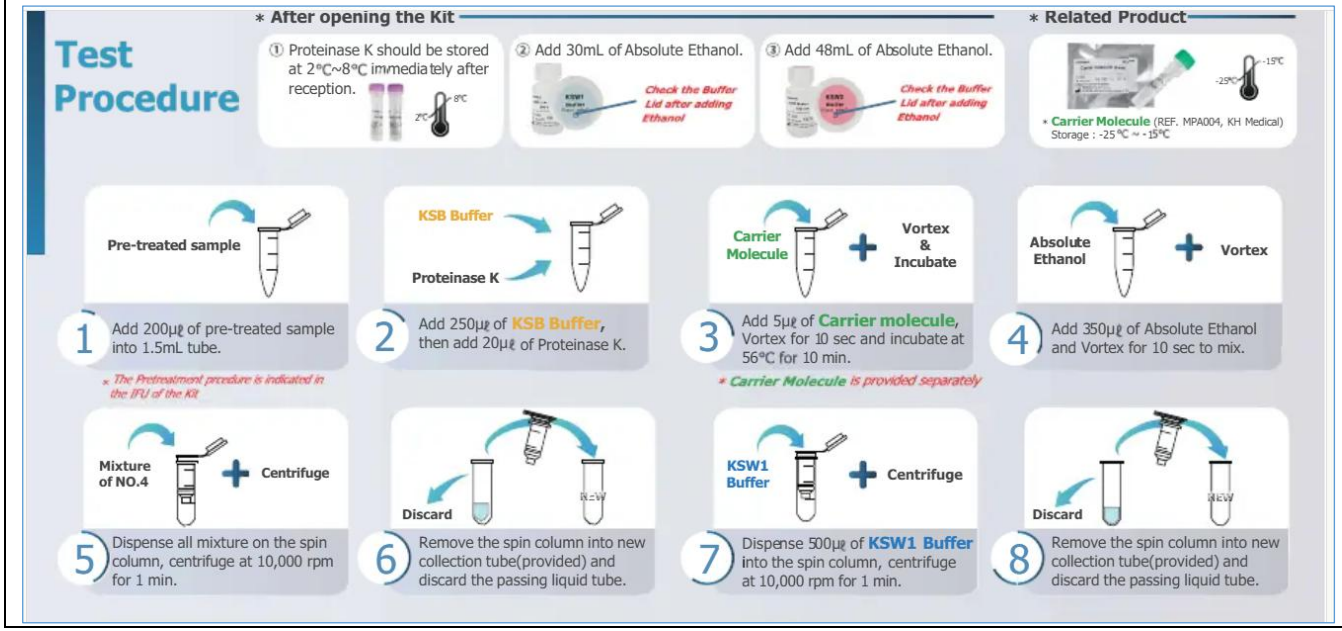
**Annex 3:** analysis of breast milk samples in the laboratory

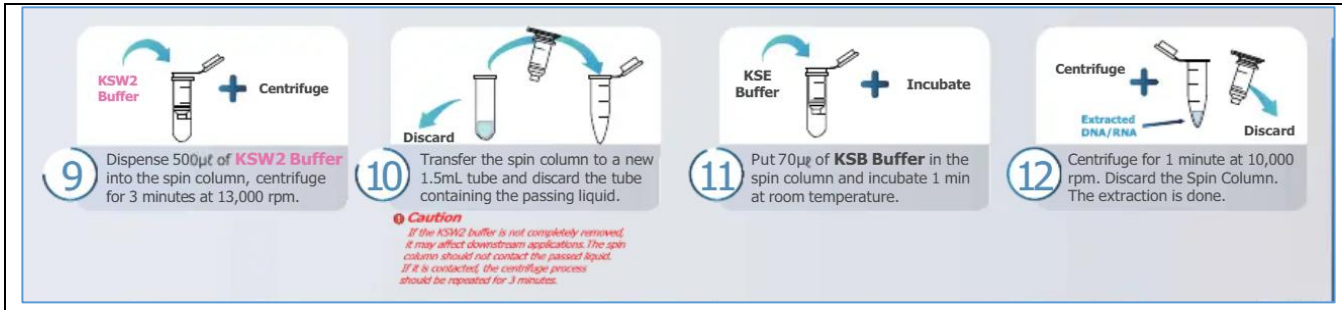
This appendix describes the procedures for the preparation and PCR testing of breast milk samples. All analyses were carried out at the laboratory of the National Institute of Public Health (INSP).

**Sample preparation**

Milk should be pre-processed to reduce its lipid and cellular component content.
Centrifuge the milk at 2500g (3500rpm) for 15 min
Recovering the supernatant
Dilute the sample (supernatant) to 1/10

**Extraction**






**Amplification**

**Preparation of the reaction medium (Master Mix)**

Briefly vortexing the reagents

Prepare the Master mix in the following proportions

Calculate the amount of PCR mixture as follows: Volume/ reaction*N (samples + PC+NC+NTC+1)

| | | |
|---|---|---|
| Number of reactions | 1 | 10 |
| 2X RADI FAST MasterMix | 10µl | 100µl |
| Mpox Primer and Probe Mixture | 5µl | 50µl |
| Volume du Master Mix | 15µl | 150µl |
| Samle, PC or NC | 5 | - |

**Preparing the Reaction**

Pipete 15µl of Master Mix into each reaction well of the PCR tubes

Add 5µl of sample (DNA extracts) or 5µl of control (CP, CN, or NTC)

Make sure all 3 controls are used for each handling

**Thermal Cycler Programming (CFX96)**

This programming may vary depending on the type of instrument to be used

**Settings**

Define the following

| **Settings** | |
|---|---|
| Reaction Volume | 20µl |
| Ramp rate | Default |
| Passive reference | None |

**Selecting fluorophores**

| Target | Detector Name | Reporter |
|---|---|---|
| Monkeypox virus specific DNA<br>Clade 2 (W. African) | MPXV | FAM |
| Clade1 (Congo Basin) | MPXV | Hex |
| IC | Internal Control | Cy5 |

**Temperature profile and acquisition**

| | Stage | Cycle repeats | Acquisition | Temperature [°C] | Time |
|---|---|---|---|---|---|
| Denaturation | Hold | 1 | - | 95 | 20sec |
| Amplification | Cycling | 45 | - | 95 | 2sec |
| | | | Yes | 60 | 5sec |

**Data analytics**

For basic information regarding data analysis on a specific RT-PCR instrument, refer to the instrument's user manual.

**Interpretation of qualitative results**

| **Sensing Channels** | | | **Interpretation of the results** |
|---|---|---|---|
| FAM (Clade2) | HEX(Clade1) | Cy5 (IPC) | |
| **≤40** | **>40 or -** | **≤40** | Clade 2 Positive |
| **≤40** | **>40 or -** | **-*** | Clade 2 Positive |
| **>40 or -** | **≤40** | **≤40** | Clade 1 Positive |
| **>40 or -** | **≤40** | **-*** | Clade 1 Positive |
| **>40 or -** | **>40 or -** | **≤40** | Negative |
| **>40 or -** | **>40 or -** | **-** | **invalid. PCR inhibition or reagent problem. Resume the analysis from the primary sample or collect and analyse a new sample. |
| *: When the CPI Ct value is 40, the test is valid. In positive cases, IPC may not be detected due to competitive reaction with target genes. **: Results are invalid. A new test is necessary. | | | |

**Quality control procedures**

The quality of the sample is assessed during amplification. The amplification reagent contains a Sample control (SC), detected by the Cy5 fluorophore. In addition, the IC, always added at the beginning of the process (during extraction), allows you to control all the steps from extraction to amplification.