Human Milk Analyzer

Photo Martina Sjaunja

USER MANUAL

PREFACE

Thank you for selecting the MIRIS™ HMA (Human Milk Analyzer). Please read this manual carefully before starting to use the instrument.

Miris promotes the use of the MIRIS™ Human Milk Analyzer by health professionals to assist them, together with the parents, to offer new-borns and infants the nutrition they need.

Miris is not liable for technical failures or inaccuracies of the results resulting from misuse of MIRIS™ devices. The use of MIRIS™ devices and the decisions on the measures to be taken based upon the results obtained with these, must be determined by a healthcare professional.

With the MIRIS™ HMA it is possible to quantitatively measure the concentration of fat, carbohydrate, protein, total solids, and energy of human milk. It is a compact, robust instrument without moving parts and it is easy to handle with a broad application area. The analytical technique used in MIRIS™ HMA is a combination of established mid-infrared (mid-IR) transmission spectroscopy principles and a patented innovation. The analytical accuracy of the instrument is compatible with other mid-IR technologies and several milk components are analysed simultaneously in one single run, requiring a small sample volume.

READ THE MANUAL BEFORE USING THE INSTRUMENT

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IMPORTANT USER INFORMATION

WARRANTIES AND DUTIES

By operating this Human Milk Analyzer, the USER and MIRIS agree to the following responsibilities, which constitute contractual warranties and conditions between MIRIS and USER for the maximum benefit and usefulness of the Human Milk Analyzer.

MIRIS AB WARRANTS THAT IT:

- Knows of no defects in the construction of the HMA or its materials used
- Will replace or repair the Human Milk Analyzer according to the guarantee in the product warranty

USER WARRANTS THAT:

- The Human Milk Analyzer will be used according to the instructions given in the user manual
- The Human Milk Analyzer will not be altered without written approval of MIRIS AB. Miris will not be held responsible for the analytical results from an altered machine, nor will the user be allowed to mention Miris in combination with any results obtained from an altered machine
- MIRIS should be notified within 48 hours if any injury occurs in any association with the Human Milk Analyzer, and will be allowed prompt and thorough examination of the Human Milk Analyzer in question
- MIRIS will not be held responsible in cases of injury arising from use of the Human Milk Analyzer:
    a. When the Human Milk Analyzer is not used according to the instructions in this manual
    b. When MIRIS is not notified within 5 days of said injury

INTENDED USE OF THE HUMAN MILK ANALYZER

The MIRIS™ Human Milk Analyzer (HMA) quantitatively measures the concentration of fat, protein, carbohydrate, total solids, and energy in human milk. These measurements may be used to aid in the nutritional management of new-borns, including preterm, and infants. This device is intended for use in health care by trained healthcare personnel at clinical laboratories.

SAFETY INFORMATION

To avoid damaging the HMA and the optical unit (cuvette), please read this information before installing or using the instrument.

Never inject any liquids outside the temperature range 35 - 40° C (95 - 104° F)

Never force liquid into the system

Never inject other liquids than milk, Miris CHECK™, Miris CLEANER™, or deionised or distilled water into the system

Never leave milk idle in the system for more than five minutes

Storage or transport of the HMA at temperatures below 0° C (32° F) must always be done with the cuvette completely empty of any liquid

Never open the instrument, breaking of the void seal will invalidate the product warranty

Always leave the instrument filled with de-ionized or distilled water when not in use and make sure the system is closed. At long-time storage, inject fresh distilled/deionised water minimum every second week

Ensure that the in- and outlet ports are in place in order to avoid dirt entering the cuvette

Always leave the protective cap covering the RS232 connection when not in use

Never use any other power supply than ADAPTER (ELPAC POWER SYSTEMS) MODEL MWA10018A-12, input voltage 100-240 V ~ 50/60 Hz, 2.3A, output voltage 18 V DC, 100VA

Consider using UPS (uninterruptible power supply) protection to shield the instrument from power failures and power spikes

Let the HMA completely adjust to room temperature (20-30° C) before switching the power on

After switching the power on, allow the system to warm up by waiting 30 minutes before proceeding

Please ensure to read the full User Manual in order to secure proper handling

Please refer to the Installation Qualification/Operational Qualification documentation (enclosed in the instrument carrying case) to secure proper installation and functional control

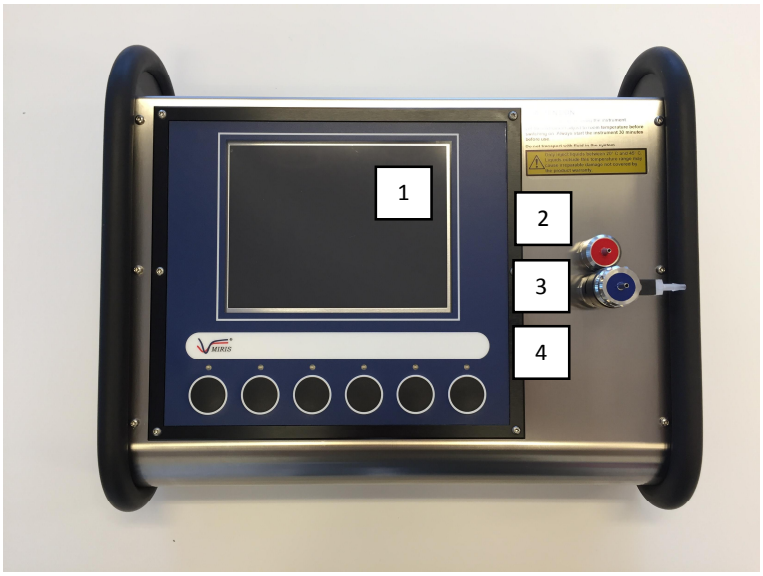
The performance of the instrument is only guaranteed if the instructions provided are followed carefully

MIRIS HUMAN MILK ANALYZER USER GUIDE

Chapter 1 INSTALLATION AND OPERATION

In order to secure proper installation of the instrument and to ensure correct function, please refer to the Installation Qualification/Operational Qualification documentation (enclosed in the instrument carrying case). There are different types of inlets, please identify your type and follow the handling instructions accordingly.

THE INSTRUMENT



1. Display
2. Outlet
3. Inlet
4. Buttons

Figure 1. HMA front.

Figure 2. HMA back.

5. On/off button
6. Power connector 
7. Reset
8. Computer connection (OPTIONAL, RS232)
9. Computer connection (USB A)
10. Computer connection (USB B) 
11. Ethernet 

IN- AND OUTLETS

There are three types of inlets, please identify your type (Figure 3-5) and follow the handling instructions accordingly.

Figure 3. Inlet Syringe.

Figure 4. Inlet Pressure Guard (IPG).

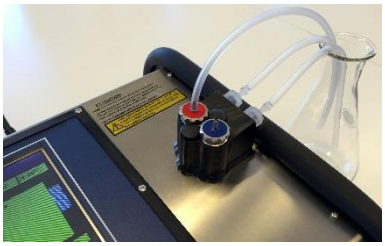


Figure 5. Cuvette Pressure Guard (CPG).

Inlet Syringe

The Inlet Syringe consists of Inlet Syringe (22-02-001) and Outlet Syringe (22-03-001) (Figure 6-7 and 8-9 respectively).

Inlet

Figure 6. The inlet separated in two parts.

Figure 7. The inlet separated in all parts.

The inlet parts and article numbers from the left:
Filter House 23-03-004
Inlet Filter 33-01-002
O-ring 25-01-007
Filter Washer 23-03-006
Nipple Seal 26-03-003
Valve Case 23-03-003
Rubber Gasket 26-03-001 (not pictured)

Outlet

Figure 8. The outlet separated in two parts.

Figure 9. The outlet separated in all parts.

The outlet parts and article numbers from the left:
Stop Cover 23-03-005
Stop Gasket 26-03-004
Stop Washer 23-03-007
Nipple Seal 26-03-003
Valve Case 23-03-003
Rubber Gasket 26-03-001 (not pictured)

Inlet Pressure Guard (IPG)

Inlet Pressure Guard, IPG, (22-02-004) protects the cuvette from being exposed to harmful pressures at injection via the blue inlet part. Liquid injected through the IPG at pressures exceeding 3 bar, will be diverted via the vent at the side of the IPG and will not pass through the cuvette (Figure 10-13).

Miris recommends new users of the IPG to perform a few test injections with distilled water to become acquainted with the amount of pressure needed to inject liquid through the cuvette, while avoiding any diversion via the IPG vent.

Inlet

Figure 10.
Filter House 23-03-004
Inlet Filter 33-01-002
O-ring 25-01-007

Figure 11.
Cylindric House 23-11-002
Relief Valve 24-08-005
House Fitting 24-10-001

Figure 12.
Adapter 23-11-001
O-ring 25-01-011

Figure 13.
Nipple Seal 26-03-003
Valve Case 23-03-003
Rubber Gasket 26-03-001

Outlet

The outlet is the same as in the Inlet Syringe, see Outlet Syringe 22-03-001 on page 7.

Cuvette Pressure Guard (CPG)

The Cuvette Pressure Guard, CPG, (22-02-101) protects the cuvette from being exposed to harmful pressures at injection both via the blue inlet part and the red outlet part. Fluid injected through the CPG at pressures exceeding 3 bar, will be diverted via the vent at the side of the CPG and will not pass through the cuvette (Figure 14-16).

Miris recommends new users of the CPG to perform a few test injections with distilled water to become acquainted with the amount of pressure needed to inject liquid through the cuvette, while avoiding any diversion via the CPG vent.

Inlet and outlet

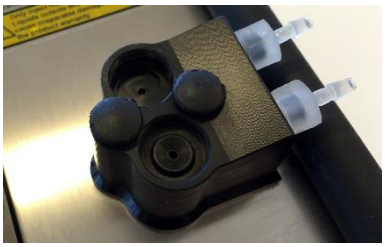


Figure 14.
Restrictor House 23-11-003

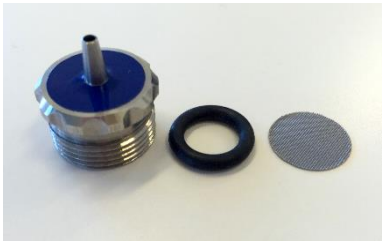


Figure 15.
Filter House Restrictor 23-03-025
O-ring 25-01-011
Inlet Filter 33-01-001

Figure 16.
Stop Cover House Restrictor
23-03-026

ELECTRICAL REQUIREMENTS

The Human Milk Analyzer requires 12-20 V ⎓, 100VA. Do not use any other adapter than MWA10018A-12A (Figure 17).

Figure 17. Adapter and power cable.

INSTRUMENT PLACEMENT

The HMA should be placed in an area free from dust, dirt, explosives, corrosive fumes, and extremes of temperature and humidity (see Technical specifications on page 47). Place the instrument on a stable work bench or similar. Avoid draft and vibrations that can influence the accuracy of results and prolong the analysis time. Never place the instrument in direct sunlight, which may disturb the function of the instrument.

If the HMA is permanently connected to a computer or if you use accessories, please read the relevant manual for information about placement guidance and electrical security.

Figure 18-21. Description of how to correctly insert the power plug.

1. Do not use any other adapter than the one received with the instrument. It has a plug designed for HMA. Pull back the plastic cover.

2. Keep the cover pulled back while you insert the plug. Attach the instrument to a power source and turn on the instrument by pressing the on/off button. The licensed application software will automatically start when the instrument is turned on.

OPERATING THE INSTRUMENT

The start-up procedure

Before start-up, make sure the HMA is completely adjusted to room temperature (20-30° C) before switching the power on. After switching the power on, allow the system to warm up by waiting 30 minutes before proceeding.

Place a tube on the red outlet port and on the vents if your HMA is equipped with an Inlet Pressure Guard or Cuvette Pressure Guard. For the first start-up, and after transportation or long-time storage, start by rinsing the system by injecting at least 3 x 5 ml Miris CLEANER. For correct temperature of injected liquids, see Table 4.

When turning on the instrument, it will start up with the licensed application software and the following main menu (Figure 22). The instrument is ready to operate after an initial warming period which takes a few minutes; the display will read “Ready – Press a button”.

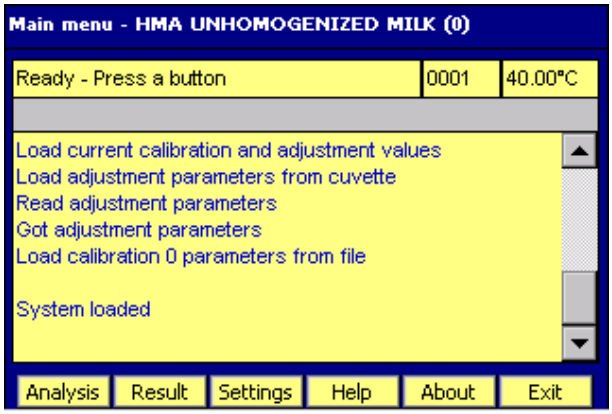


Figure 22. Main menu.

The stand-by procedure

To leave the HMA in stand-by mode, clean according to the instructions and finish by filling the instrument with deionised or distilled water (see Chapter 3, Cleaning at the end of the day). At continuous daily use, leave the HMA constantly turned on to keep the system stable. If the HMA will not be used for two days or more, turn the instrument off. Always make sure the system is closed, by leaving the syringe on the inlet or by attaching the end of the outlet tube to the inlet, see storage on page 41.

BASIC PRINCIPLES

This instrument can be used as a standalone unit or with USB accessories, such as mouse, external keyboard, barcode reader and printer. There is only one USB A connection, but it is possible to connect a USB hub allowing use of several accessories at the same time. Miris recommends using a mouse for most convenient operation of the instrument (especially settings such as date/time and slope/bias adjustments). The instrument can, however, be operated by only using the buttons to navigate the cursor. By clicking with the mouse or pressing the instrument’s buttons, operation is straightforward following the instructions on the screen. The active menu will always be presented at the top of the screen.

See Figure 23 for a schematic drawing of the instrument’s menu system.

Note! If you press ‘Exit’ the licensed application software will close. Miris is not responsible for actions done outside the application software. To restart the software, turn the instrument off and on using the on/off button.

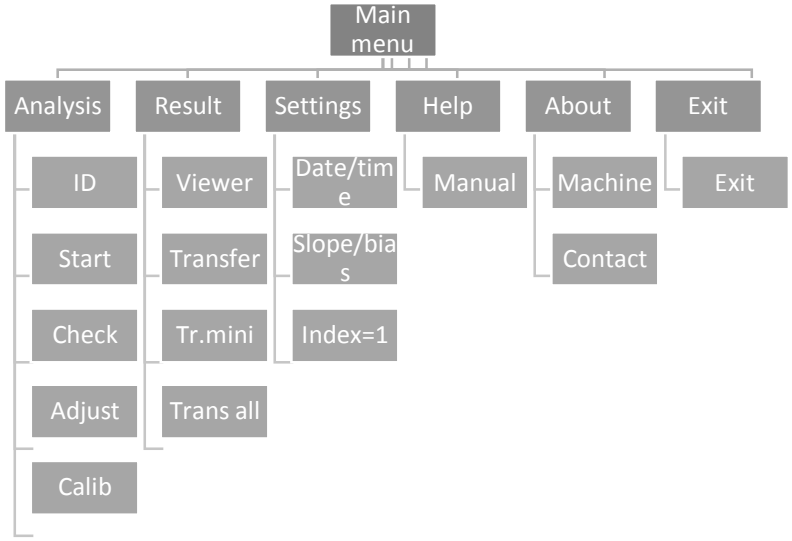


Figure 23. Menu system of the instrument. **Note!** In older versions of the software some of the options are not available.

Chapter 2 HUMAN MILK ANALYSIS WITH THE HMA

For the first start-up of a new instrument, refer to the start-up procedure on page 10.

When performing a measurement or zero-setting check it is very important to leave the syringe on the inlet for the entire procedure, see Figure 24. Leave some of the fluid in the syringe to avoid introduction of air into the cuvette, as this may lead to inaccurate results.

Figure 24. Leave the syringe on the inlet during the measurement.

STANDARD OPERATING PROCEDURE (SOP)

Equipment	**Required solutions**
Miris HMA [Miris SONICATOR, for analysis on Calibration 1] Water bath at 40° C Syringes, 2 ml and 5 ml Waste container	Miris CHECK Miris CLEANER Distilled/deionised water **Note!** All solutions must be 35-40° C when injected into the instrument.

According to milk sample type, analyse on Calibration 1 (homogenized milk), see following **SOP table A**, or Calibration 0 (unhomogenized milk), see following **SOP table B**

For detailed instructions, go to the specific chapters referred to.

For further details on use and handling of Miris SONICATOR, refer to instructions in the Sonicator manual.

Notes on sample handling (further information in Chapter 5)

Control the integrity of each sample before use by checking that specified storage temperature has been maintained and that there are no signs of leakage or breakage of the sample container.

Defrost frozen samples at room temperature or in a refrigerator.

Control the sample for quality issues: e.g. the milk is churned, if a distinct smell from free fatty acids is noticeable, if during, or after, preparation of the sample, white particles are visible on the walls of the sample container, fat droplets float on the surface of the sample. If any of these issues are noted the HMA analysis might not be accurate and the sample should be discarded.

Always keep sample containers securely sealed in order to avoid evaporation, only remove the lid briefly to take out samples.

Mix the milk thoroughly by gentle inversion or swirling of the sample container. No shaking in order to avoid foaming.

If foam is formed in the sample, this needs to disintegrate completely before sample withdrawal for analysis.

A sample withdrawn by syringe must be immediately injected into the HMA and immediately analysed.

A new sample withdrawal and injection needs to be made for each analysis.

Avoid multiple sample container transfers.

Table 1. SOP table A - Calibration 1.

	A. SOP: Analysis on Calibration 1 (Homogenized milk)
A1. Set-up	Defrost frozen samples at room temperature or in a refrigerator Turn on the water bath to warm up to 40° C Place bottles of distilled water, and working solutions of Miris CHECK and Miris CLEANER in the water bath Turn on the Sonicator and the HMA, wait at least 30 minutes before proceeding Place a waste container by the HMA outlet
A2. Instrument preparation	Select 'Analysis' in the main menu Press 'Calib' and select Calibration 1, confirm by pressing 'OK' Set the Sonicator on the appropriate sample volume to get correct processing time (1.5 s/ml) Control that the Sonicator probe is clean and that the tip is evenly polished
A3. Zero-setting check *Further information in Chapter 4*	Select 'Analysis' in the main menu **STEP I**: Inject 3 ml Miris CHECK Press 'Check' and wait for the result, approximately one minute Display message **"No adjustment necessary":** continue to A4 Display message **"Adjustment necessary":** go to STEP II **STEP II**: Inject 2 ml Miris CHECK Press 'Check' and wait for the result, approximately one minute Display message **"No adjustment necessary":** continue to A4 Display message **"Adjustment necessary":** go to STEP III **STEP III**: Press 'Adjust', wait for the display message "New adjustment done" Inject 1 ml Miris CHECK Press 'Check' and wait for the result, approximately one minute Display message **"No adjustment necessary":** continue to A4 Display message **"Adjustment necessary":** repeat STEP III
A4. Sample preparation *See Chapter 5*	Place the sample in the water bath. Keep the sample in the water bath for another 5-10 minutes after reaching 40° C. Homogenize the sample with the Sonicator (1.5 s/ml) Analyse immediately or keep the sample in the water bath until analysis, max another 15-20 minutes

A5. Analysis	Select 'Analysis' in the main menu  If required, press 'ID' and enter a sample name, confirm by pressing 'OK' (see Chapter 7) Mix the sample thoroughly Withdraw a 3 ml sample by syringe Immediately inject into the HMA, leaving 0.5 ml in the syringe and the syringe on the inlet Initiate the analysis by pressing 'Start' Results for fat, crude protein, true protein, carbohydrate, total solids and energy are presented on the display after approximately one minute [Repeat the A5 steps to do a replicate analysis, and for subsequent samples] After 10 analyses, clean the HMA (A7) followed by a zero-setting check (A3) before continuing analysing
A6. Results *Further information in Chapter 6*	The most recent result will stay on the display until the next result is presented for the following analysis To view or export results, select 'Result' in the main menu Press 'Viewer' to view the results on the instrument display Press 'Transfer' to export the results file to a USB memory attached to the instrument
A7. Cleaning *Full instructions in Chapter 3*	Clean the HMA every 10th analysis, or if the instrument is idle for more than 5 minutes with milk in the cuvette **If continuing analysing samples, HMA cleaning must always be followed by a zero-setting check (A3)** Disassemble the inlet and outlet to clean the parts every 100th analysis, or once a week After each use of the Sonicator, wipe the probe clean with a dampened cloth and polish the tip with emery cloth
A8. Stand-by mode *See Chapter 3, Chapter 1, and Chapter 10*	After cleaning at the end of the day the HMA must be injected with distilled water Close the system by leaving the syringe on the inlet, or by attaching the end of the outlet waste tube to the inlet At continuous daily use, leave the HMA constantly turned on At non-use exceeding two days, turn the HMA off At long-time storage, inject fresh distilled or deionised water minimum every second week, make sure the system is closed

Table 2. SOP table B - Calibration 0.

	B. SOP: Analysis on Calibration 0 (Unhomogenized milk)
B1. Set-up	Turn on the water bath to warm up to 40° C Place bottles of distilled water, and working solutions of Miris CHECK and Miris CLEANER in the water bath Turn on the HMA, wait at least 30 minutes before proceeding Place a waste container by the HMA outlet
B2. Instrument preparation	Select 'Analysis' in the main menu Press 'Calib' and select Calibration 0, confirm by pressing 'OK'
B3. Zero-setting check *Further information in Chapter 4*	Select 'Analysis' in the main menu **STEP I**: Inject 3 ml Miris CHECK Press 'Check' and wait for the result, approximately one minute Display message **"No adjustment necessary":** continue to B4 Display message **"Adjustment necessary":** go to STEP II **STEP II**: Inject 2 ml Miris CHECK Press 'Check' and wait for the result, approximately one minute Display message **"No adjustment necessary":** continue to B4 Display message **"Adjustment necessary":** go to STEP III **STEP III**: Press 'Adjust', wait for the display message "New adjustment done" Inject 1 ml Miris CHECK Press 'Check' and wait for the result, approximately one minute Display message **"No adjustment necessary":** continue to B4 Display message **"Adjustment necessary":** repeat STEP III
B4. Sample preparation *See Chapter 5*	Place the sample in the water bath. Keep the sample in the water bath for another 5-10 minutes after reaching 40° C. Analyse immediately or keep the sample in the water bath until analysis, max another 15-20 minutes

B5. Analysis	Select 'Analysis' in the main menu If required, press 'ID' and enter a sample name, confirm by pressing 'OK' Mix the sample thoroughly Withdraw a 3 ml sample by syringe Immediately inject into the HMA, leaving 0.5 ml in the syringe and the syringe on the inlet  Initiate the analysis by pressing 'Start' Results for fat, crude protein, true protein, carbohydrate, total solids and energy are presented on the display after approximately one minute [Repeat the B5 steps to do a replicate analysis, and for subsequent samples] After 10 analyses, clean the HMA (B7) followed by a zero-setting check (B3) before continuing analysing
B6. Results *Further information in Chapter 6*	The most recent result will stay on the display until the next result is presented for the following analysis To view or export results, select 'Result' in the main menu Press 'Viewer' to view the results on the instrument display Press 'Transfer' to export the results file to a USB memory attached to the instrument
B7. Cleaning *Full instructions in Chapter 3*	Clean the HMA every 10th analysis, or if the instrument is idle for more than 5 minutes with milk in the cuvette **If continuing analysing samples, HMA cleaning must always be followed by a zero-setting check (B3)** Disassemble the inlet and outlet to clean the parts every 100th analysis, or once a week
B8. Stand-by mode *See Chapter 3 and Chapter 1 Table 1*	After cleaning at the end of the day the HMA must be injected with distilled water Close the system by leaving the syringe on the inlet, or by attaching the end of the outlet waste tube to the inlet At continuous daily use, leave the HMA constantly turned on At non-use exceeding two days, turn the HMA off At long-time storage, inject fresh distilled or deionised water minimum every second week, make sure the system is closed

Chapter 3 HMA CLEANING ROUTINES

Note! Miris CLEANER, Miris CHECK and distilled/deionised water needs to be 35-40° C when injected into the instrument. Sudden temperature changes may cause irreparable damage to the cuvette not covered by the product warranty.

The cuvette in the Miris HMA must be cleaned minimum **every 10th analysis, or when the instrument is idle more than five minutes with** milk in the cuvette.

The inlet should be disassembled and cleaned **once a week or every 100th analysis**, whichever comes first. Use Miris CLEANER and a small brush, e.g. a tooth brush, and a toothpick or similar.

After the final analysis of the day is performed, leave the instrument filled with distilled/deionised water.
Please note that **Miris CLEANER, Miris CHECK and distilled/deionised water** must be heated to **40° C** before use. The cuvette is sensitive to sudden temperature changes, which may cause irreparable damage, and the cleaning efficiency of Miris CLEANER is improved by the temperature.

All volumes specified in this chapter are minimum volumes, if necessary the volumes can be increased. Figure 25 shows a cross section of a cuvette which explains the importance of a correct cleaning routine.

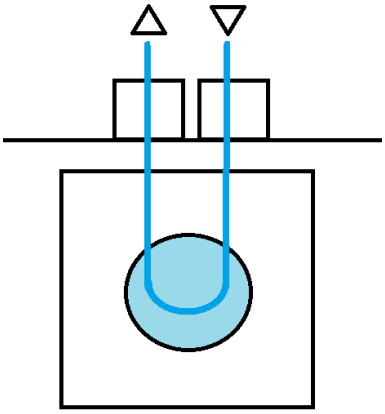


Figure 25. Schematic drawing of a cuvette and in- and outlets in cross section. The cuvette comprises of two CaF2 (calcium fluoride) windows separated by a spacer (50 µm) which needs several injections of Miris CLEANER to completely rinse any milk residues.

A video to instruct how to perform cleaning of the in- and outlet can be obtained, please contact Miris at support@miris.se.

Please note that the cleaning routines differ with the type of inlet the HMA is equipped with. Identify your inlet type and follow the instructions accordingly (Figure 26-28).

Figure 26. Inlet Syringe.

Figure 27. Inlet Pressure Guard (IPG)

Figure 28. Cuvette Pressure Guard (CPG).

CLEANING OF THE INSTRUMENT SURFACE

Clean the surface of the instrument using a cloth dampened with Miris CLEANER. Use a mild disinfectant if necessary.

CLEANING ROUTINES OF THE INLET SYRINGE

Cleaning during analysing – Minimum every 10th analysis

1. Place the waste tube on the blue inlet.
2. Inject 2 x 5 ml of Miris CLEANER in the red outlet, reversed fluid direction.
3. Put the waste tube back on the outlet and inject 5 ml Miris CLEANER in normal fluid direction.
4. If further analyses are planned, perform a Check. If it is the final analysis for the day, follow the instructions below.

Cleaning at the end of the day

1. Clean the cuvette as instructed above.
2. Inject 2 x 5 ml distilled/deionised water, leave about 0.5 ml in the syringe.
3. Leave the instrument with the syringe on the inlet. If needed, the instrument can now be turned off. For longer storage periods, follow the storage instructions on page 41.

Cleaning of the in- and outlet – Every 100th analysis or once a week

It is important to make sure that the instrument in- and outlets are clean. Clean the entire in- and outlets minimum once a week, or every 100 milk samples, whichever comes first. Use Miris CLEANER and a small brush, e.g. a tooth brush, and a toothpick or similar.

Note! Remove only one port at a time. Remove the inlet, with the outlet still attached to the instrument, and vice versa.

1. Use the Nipple Key provided to unscrew the inlet from the instrument. Open the inlet, carefully remove the gasket and the filter by using a toothpick or similar. Clean the parts using Miris CLEANER. Rinse with distilled/deionised water. The filter is the most important part to clean.

2. Assemble the inlet and mount it onto the instrument.

3. Use the Nipple Key provided to unscrew the outlet from the instrument. Open the outlet, carefully remove the gaskets by using a toothpick or similar. Clean the parts using Miris CLEANER. Rinse with distilled/deionised water.

4. Assemble the outlet and mount it onto the instrument. Put back the waste tube and inject distilled/deionised water as described in Cleaning at the end of the day.

CLEANING ROUTINES OF THE INLET PRESSURE GUARD (IPG)

Cleaning during analysing – Minimum every 10th analysis

1. Inject 5 ml Miris CLEANER in the blue inlet with pressure so the fluid is diverted via the vent on the IPG.
2. Inject 2 x 5 ml Miris CLEANER in the blue inlet, through the cuvette and outlet.
3. If further analyses are planned, perform a Check. If it is the final analysis for the day, follow the instructions below.

Cleaning at the end of the day

1. Clean the cuvette as instructed above.
2. Inject 5 ml distilled/deionised water in the blue inlet with pressure so the fluid is diverted via the vent on the IPG.
3. Inject 2 x 5 ml distilled/deionised water in the blue inlet, through the cuvette and outlet, leave about 0.5 ml in the syringe.
4. Leave the instrument with the syringe on the inlet. If needed, the instrument can now be turned off. For longer storage periods, follow the storage instructions on page 41.

Cleaning of the IPG and outlet – Every 100th analysis or once a week

It is important to make sure that the instrument in- and outlets are clean. Clean the entire in- and outlets minimum once a week, or every 100 milk samples, whichever comes first. Use Miris CLEANER and a small brush, e.g. a tooth brush, and a toothpick or similar.

Note! Remove only one port at a time. Remove the inlet, with the outlet still attached to the instrument, and vice versa.

1. Remove the Filter House and clean the parts with Miris CLEANER and rinse with distilled/deionised water. Carefully remove the gasket and the filter by using a toothpick or similar. The filter is the most important part to clean.


2. Remove the Adapter and the Cylindric House using the Nipple Key and clean the parts with Miris CLEANER. Rinse with distilled/deionised water. If the Adapter and Cylindric House are hard to separate, let them soak in Miris CLEANER for a while and try again.


3. Remove the Valve Case using the Nipple Key and clean the parts using Miris CLEANER. Rinse with distilled/deionised water.

4. Attach the Valve Case to the instrument.

Note! It is important to remove only one port at a time. Use the Nipple Key carefully, not to scratch the instrument surface.

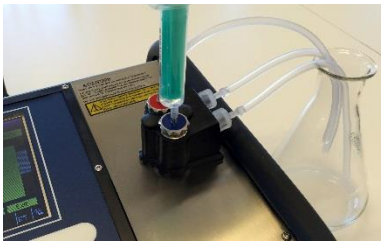
5. Remove the outlet using the Nipple Key, separate the gaskets and clean the parts using Miris CLEANER. Rinse with distilled/deionised water, reassemble the outlet and attach to the instrument. See Figure 9 for a detailed picture of the outlet.


6. Place the Adapter on the Valve Case and put the Nipple Key in the cuts on the side of the Adapter, while rotating the Cylindric House in the opposite direction to tighten. Position the Cylindric House with the vent to the side of the instrument.


7. Attach the Filter House on the Adapter and connect tubes to the vent on the side of the IPG, and the outlet. Test the mounted IPG by injecting a few syringes with distilled water. At pressures below 3 bar (normal injection) fluid should not pass via the vent on the side of the IPG with a squeaky sound. Also make sure it is not leaking. If water is leaking, tighten the Adapter and test again.

CLEANING ROUTINES OF THE CUVETTE PRESSURE GUARD (CPG)

Cleaning during analysing – Minimum every 10th analysis



1. Inject 5 ml Miris CLEANER in the blue inlet with pressure so the fluid is diverted via the front vent on the CPG.
2. Inject 5 ml Miris CLEANER in the blue inlet, through the cuvette and outlet.
3. Move the waste tube from the outlet to the inlet.
4. Inject 5 ml Miris CLEANER in the red outlet, reverse fluid direction, with pressure so the fluid is diverted via the rear vent on the CPG.

5. Put the waste tube back on the outlet.
6. If further analyses are planned, perform a Check. If it is the final analysis for the day, follow the instructions below.

Cleaning at the end of the day

1. Clean the cuvette as instructed above.
2. Move the waste tube from the outlet to the inlet.
3. Inject 5 ml distilled/deionised water in the red outlet, reverse fluid direction, with pressure so the fluid is diverted via the rear vent on the CPG.
4. Put the waste tube back on the outlet.
5. Inject 5 ml distilled/deionised water in the blue inlet with pressure so the fluid is diverted via the front vent on the CPG.
6. Inject 5 ml distilled/deionised water, leave about 0.5 ml in the syringe.
7. Leave the instrument with the syringe on the inlet.

If needed, the instrument can now be turned off. For longer storage periods, follow the storage instructions on page 41.

Cleaning of the CPG and outlet – Every 100th analysis or once a week

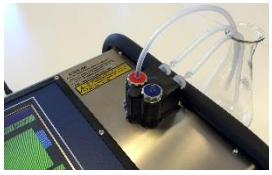
It is important to make sure that the instrument in- and outlets are clean. Clean the entire in- and outlets minimum once a week, or every 100 milk samples, whichever comes first. Use Miris CLEANER and a small brush, e.g. a tooth brush, and a toothpick or similar.

1. Remove the Filter House Restrictor and Stop Cover House Restrictor, use a poly grip plier if necessary. Clean the parts using Miris CLEANER and rinse with distilled/deionised water. Carefully remove the gasket and filter by using a toothpick or similar. The filter is the most important part to clean.

2. Clean the CPG from any milk residues using a cloth and Miris CLEANER.

3. Reassemble the Filter House Restrictor and Stop Cover House Restrictor and attach to the instrument.

4. Put back the waste tubes and inject distilled/deionised water as described in Cleaning at the end of the day.



Chapter 4 HMA QUALITY CONTROL

The check procedure with zero level adjustment (Figure 53) is a required quality control of the HMA. This procedure must be performed at start-up and after cleaning the instrument if continuing analysing samples. By doing a check, and an adjust when this is indicated by the instrument software, the validity of the internal calibration is ensured by control of, and if necessary adjustment to, the correct zero-level. This chapter provides further explanation of this function, described step-by-step in the Standard Operating Procedure in Chapter 2.

Further control procedures as described in this chapter are recommended to be routinely implemented by the user.

CHECK PROCEDURE WITH ZERO LEVEL ADJUSTMENT

At start-up and after cleaning the instrument if continuing analysing, i.e. every 10th analysis, always perform a zero-setting check. This function initiates a validation of the linearity (and of the instrument internal calibration and zero setting). Inject at least 3 ml Miris CHECK into a cleaned instrument. The Check procedure is described step-by-step in Figure 54. HMA check procedure with zero level adjustment. To be performed at start-up, and after cleaning the HMA if continuing analysing (i.e. every 10th analysis). Select 'Analysis' in the main menu, begin with Step 1 and continue until message 'No adjustment necessary" appears, see Figure 53.

The check procedure takes approximately one minute and when the process is completed, a pass or fail message will appear. If the check passes ("Result%" is within ±0.05), the text "No adjustment necessary" is shown and the instrument is ready for analysis.

If the test fails, the text **"Adjustment needed!**" is shown. This may be an indication of a contaminated sample cuvette. It is then important to carefully clean the system again. Ensure also that the check solution is not contaminated.

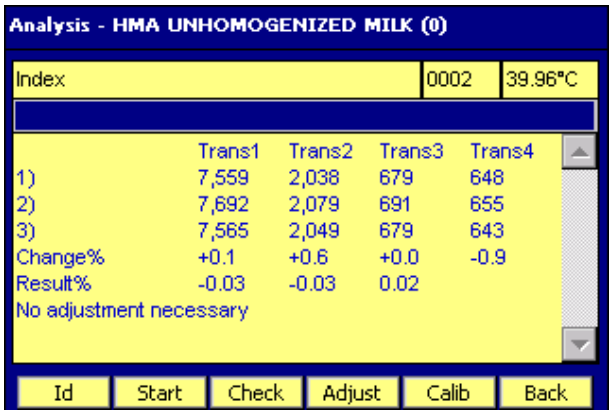


Figure 53. Check procedure results table. Note that in 2.84 and earlier versions of the software, the "Change%" is presented as a quotient.

Note! In software version 2.87 the fourth row "Change%" describes the transmission difference in percent from the original values at the time of factory calibration. In earlier software versions this is presented as a quotient.

Zero level adjustment

At "**Adjustment needed!**" perform at least one more check after injecting new Miris CHECK solution. If the check still fails it may be necessary to perform adjust of the instrument, this is done by pressing the key marked 'Adjust'.

When the 'Adjust' key is pressed, the instrument adjusts the zero level of the internal calibration. This function must always be preceded by the Check procedure. Press 'Check' again after the message "New adjustment done" appears. The calibration setting is completed when the message "No adjustment necessary" appears on the screen, as in Figure 53.

Note! Be observant of the “Change%”-line. This percentage shows, for each instrument filter 1-4, how much the instrument transmission has altered since the factory calibration at Miris. In software 2.84 and later versions, the instrument will alert the user by displaying a warning message at the event of a transmission change of 10% or more for any one filter. If this happens, click ‘OK’ on the message, clean the instrument and try the check procedure again. If the problem persists, stop using the instrument and contact Miris (support@miris.se) or your local distributor for an instrument check-up. In software version 2.84 or earlier the warning will appear if “Change%” is below 90% or above 110%, in software version 2.87 or later if “Change%” is more than ±10%.

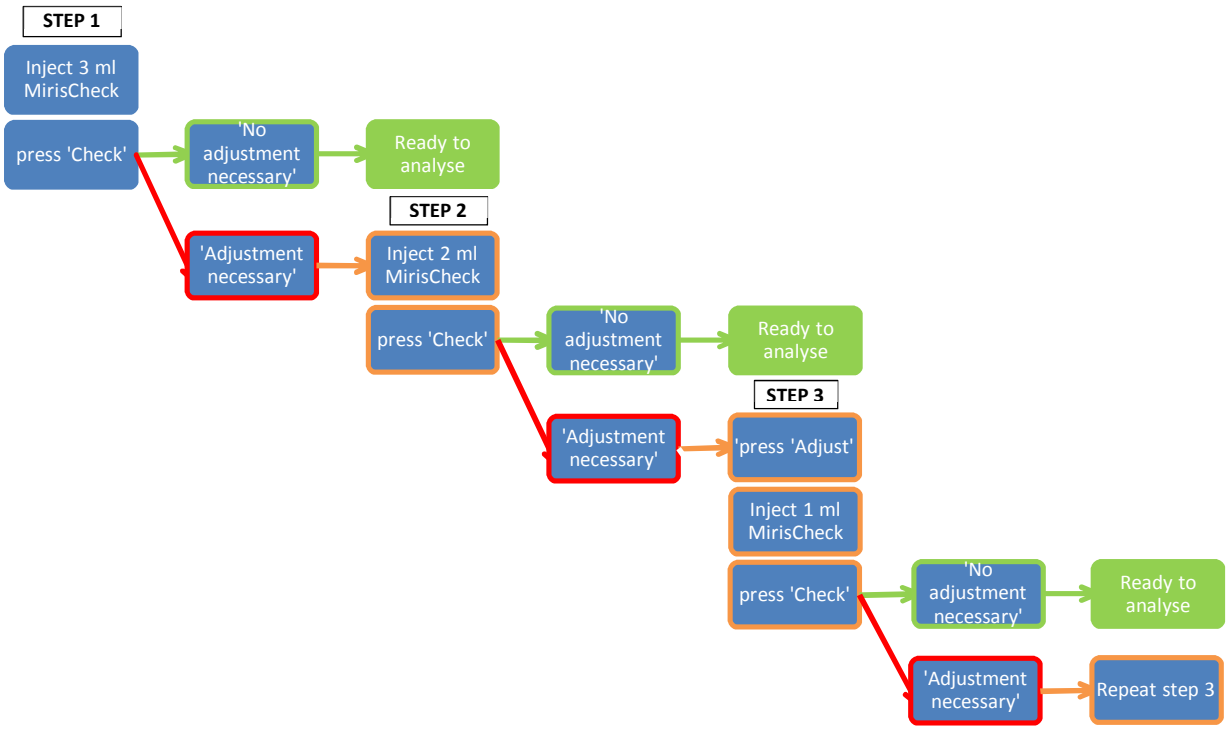


Figure 54. HMA check procedure with zero level adjustment. To be performed at start-up, and after cleaning the HMA if continuing analysing (i.e. every 10th analysis). Select ‘Analysis’ in the main menu, begin with Step 1 and continue until message ‘No adjustment

REPEATABILITY TEST

Recommended as a weekly or monthly control of instrument stability.

At least 10 replicates of a representative uniform human milk sample are analysed.

Procedure

Set the HMA on the chosen calibration and go through the check procedure to obtain the "No adjustment necessary" message.

If required, enter sample ID in the menu 'Analysis' – 'ID'

Warm about 35 ml human milk in a 40° C water bath (20 min)

Mix carefully and if Calibration 1 is set, homogenize the milk with Miris SONICATOR (1.5 s/ml)

Analyse 10 replicates of the sample on the HMA, injecting 3 ml for each analysis

Clean the HMA

Evaluation

Calculate the repeatability standard deviation (SD) on the results for fat, protein, and carbohydrates (CHO), respectively:

$$SD = \sqrt{\frac{\sum(x - \bar{x})^2}{n - 1}}$$

Repeatability SD should meet the HMA specification ≤ 0.05 g/100 ml for fat and protein, and ≤ 0.08 g/100 ml for CHO.

At deviation from the specification, it must first be determined whether this was caused by sample factors or other external factors. Repeat the test with a new sample, and control the environment, the instrument, and sample and instrument handling routines. If problems persist, contact your local distributor (or Miris directly at support@miris.se).

REPLICATE SAMPLES CONTROL

Recommended as a control of the level of the internal calibration, as a daily control, or in each run.

From a uniform batch of representative human milk, a number of replicate samples are prepared. These samples are analysed on the HMA e.g. in each analytical run or daily, to control that the instrument is stable and maintains at the same level.

Replicate sample preparation

Add Bronopol (20% m/m in distilled water, 1 ml/l milk) to a batch of human milk. Warming the milk to 40° C is recommended in order to obtain a uniform batch. Under constant gentle agitation, divide the milk into as many replicate samples of a suitable volume as required. Use air tight containers. Freeze the samples at max -20° C immediately after preparation and keep frozen until use.

Uniformity control

Control the uniformity of the replicate samples by selecting <10 random replicates and analyse them as a series of single determinations on the HMA. The standard deviation (SD) for the fat results should be ≤ 0.05 g/100 ml in order for the uniformity to be acceptable and the sample batch ok to use.

$$SD = \sqrt{\frac{\sum(x - \bar{x})^2}{n - 1}}$$

Note! As these samples are frozen, they should only be used for controlling HMA Calibration 1 (homogenized milk). For controlling Calibration 0 (unhomogenized milk), prepare both unhomogenised and homogenised samples from suitable fresh milk. Analyse the fresh unhomogenised sample on Calibration 0, and the fresh homogenised sample and the replicate sample on Calibration 1. Use the replicate sample as a reference.

The stability of the instrument can e.g. be monitored by setting up a statistical quality control chart of the replicate sample results in accordance with ISO 8196-2 [1].

Target values

Determine the target values for fat, protein and carbohydrate, respectively. Use the results from the uniformity control and calculate the average values for each parameter. The average values will be the target values.

Reference analysis can also be performed on the replicate samples in order to obtain target values for each parameter. Send >3 replicate sample aliquots to an external reference laboratory for analysis of contents of fat, protein and carbohydrate. Calculate the average values for each parameter. The average values will be the target values.

Procedure

Thaw a replicate sample at room temperature (20-30° C) or in a refrigerator.

Set the HMA on Calibration 1 and go through the check procedure to obtain the “No adjustment necessary” message.

If required, enter sample ID in the menu ‘Analysis’ – ‘ID’

Warm the thawed sample in a 40° C water bath (20 min)

Mix the sample carefully and homogenise with Miris SONICATOR (1.5 s/ml)

Analyse the sample in duplicate on the HMA, injecting 3 ml for each analysis

Evaluation

Calculate the average values for fat, protein and carbohydrate, respectively, of the duplicate analysis and compare with the target values.

The internal calibration is operating at a correct level if the results are within the acceptable range:

for fat and protein	target value ±0.2 g/100 ml
for carbohydrate	target value ±0.5 g/100 ml

If results should fall outside the specified acceptable range, it must first be determined whether this was caused by sample factors or other external factors. Repeat the test with a new sample, and control the environment, the instrument, and sample and instrument handling routines. If problems persist, contact your local distributor (or Miris directly at support@miris.se).

REFERENCES

[1] ISO, “*8196-2:2009 Milk -- Definition and evaluation of the overall accuracy of alternative methods of milk analysis -- Part 2: Calibration and quality control in the dairy laboratory*” International Organization for Standardization, Geneva, Switzerland, 2009.

Chapter 5 MILK COLLECTION AND HANDLING

Before starting to analyse human milk samples it is important to ensure that proper procedures are followed regarding milk collection, handling, sampling for analysis, and sample pre-treatment. This chapter contains Miris' recommendations for milk collection intended for HMA analysis, as well as references to more information on the topic.

COLLECTION AND SAMPLING

Milk collection for analysis should be performed under standardized conditions and the method should be chosen according to the purpose of the analysis or study, e.g. 24h-collection, full breast expression, fore and hind sampling, mid-feed sampling [1]. Efforts should be made to obtain a representative sample that will reflect this purpose, taking into consideration possible diurnal variation in milk composition, longitudinal changes associated with postpartum duration, possible seasonal effects, time since last feed, volume of milk consumed at the prior feed, and maternal physiological let down [1]. If local or national guidelines exist, they should be followed (e.g. [2] [3]). For a representative sample for nutritional analysis, 24h-collection sampling is recommended [3] [4]. If the intention is to use the HMA in a clinical research setting, a clinical study protocol defining the milk collection procedure is required.

Milk collected for analytical purposes is preferably immediately preserved by adding Bronopol solution (20% m/m in distilled water, 1 ml solution per litre of milk).

If the collected milk is to be separated into smaller sample aliquots, which is recommended as refreezing or other repeated temperature changes of the milk should be avoided, the milk should be warmed and mixed thoroughly prior to aliquoting in order to ensure homogeneity. A good aliquot size purposed for HMA analysis is 10 ml, enough for a triplicate measurement. The container with the human milk should be gently swirled or inverted, never shaken, and inspected that no fat is stuck to the walls before transfer to the smaller test tube or container. Any extra manipulation should be avoided, e.g. multiple sample container transfers.

Choice of sample container is also to be considered as fat and protein may adhere more to some materials.

STORAGE

If the milk will not be analysed within 2 hours of collection or thawing, the sample should be stored in a refrigerator for a maximum of 48 h (Bronopol preserved milk 72 h). Fresh milk should be immediately frozen at max -20°C to preserve the overall quality of the sample, if it is to be kept beyond 3 days for preserved milk or beyond 2 days for unpreserved milk. The freezing process should be rapid (keep milk volumes <200 ml) and the thawing process slow. Milk that has been frozen and then thawed must not be frozen again, as it will cause deterioration of the physicochemical quality. Storage recommendations are summarized in Table 3.

DEFROSTING/THAWING

Frozen milk should be defrosted slowly overnight in a refrigerator (4° C). Smaller sample volumes (<200 ml) may also be defrosted at room temperature (20-30° C), or in a water bath with cold water fresh from the tap. This should take about 2-3 hours for 100-200 ml. Thawing in a microwave oven is strongly advised against because of the uneven temperature distribution, which may cause hot-spots and protein denaturation.

Thawed milk can be kept at room temperature (20-30° C) for a maximum of 2 hours, or refrigerated for max 48 h (unpreserved)/max 72 h (preserved), respectively.

Table 3. Milk storage recommendations [2] [3].

Sample	Location	Temperature	Max recommended storage duration	Comment
Preserved/unpreserved fresh/thawed milk	Room temperature	max 30° C	2 hours	
Unpreserved fresh/thawed milk	Refrigerator	max 4° C	48 hours	Warm milk is not to be mixed with refrigerator-cold or frozen milk, but must first be chilled in a separate container
Preserved fresh/thawed milk	Refrigerator	max 4° C	72 hours	Warm milk is not to be mixed with refrigerator-cold or frozen milk, but must first be chilled in a separate container
Unpreserved fresh milk	Freezer	max -20° C	6 months	If the milk is to be frozen, this should be done within 24 hours of collection. Freeze rapidly, thaw slowly. Once thawed do not refreeze.
Preserved fresh milk	Freezer	max -20° C	6-12 months	If the milk is to be frozen, this should be done within 24 hours of collection. Freeze rapidly, thaw slowly. Once thawed do not refreeze.
Any milk sample	Water bath	40° C	20 minutes after reaching 40° C	

WARMING

Samples should be warmed in a 40° C water bath prior to analysis. The entire sample should be 40° C and the HMA will also operate at 40° C. Never warm the milk using a microwave oven because of the uneven temperature distribution, which may cause hot-spots and protein denaturation.

ULTRASONIC HOMOGENIZATION (SONICATION)

Milk is an oil-in-water emulsion and fat separation (creaming, oiling-off) and protein aggregation are processes common in stored, particularly frozen, milk. This can be due to a slow freezing process or long storage time (age gelation) of the milk. For the reduction of such effects, rapid freezing is recommended and repeated freeze/thaw cycles of milk samples should be avoided. Aggregates can cause blockage or bring air into the measuring unit of the HMA and fat separation will make representative sampling difficult.

Note! Homogenization is a required step in milk sample preparation in order to achieve the expected performance of Calibration 1 (Homogenized milk) of the HMA.

The HMA Calibration 1 is adapted to homogenisation by Miris SONICATOR, a high-intensity ultrasonic liquid processor, where ultrasonic waves will generate the homogenising effect by cavitation. This method is suitable for homogenising small sample volumes as required for the HMA. The Miris SONICATOR has a 3 mm probe and settings optimised for human milk (amplitude 75% full scale, no pulsation). The energy output is approximately 20 J/sec per ml of human milk. Prior to analysis on calibration 1 on Miris HMA, frozen human milk should be thawed at room temperature (20-30° C) or overnight at 4° C. In order to obtain high homogenisation efficiency, the milk should be pre-warmed to 40° C and homogenised 1.5 s/ml using Miris SONICATOR.

CONTROL OF SAMPLE QUALITY

Good physical quality of the milk is essential for an accurate HMA analysis and the integrity of the milk sample should always be carefully controlled.

If part of the milk fat appears as oil droplets on the sample surface (oiling off), the test sample injected into the instrument will not be representative of the fat content of the sample. Oiled-off samples should therefore be avoided. Care should be taken to re-incorporate cream layers sticking to the walls of containers and caps. Check the sample for other quality issues such as e.g. the milk is churned, a distinct smell from free fatty acids is perceptible, if during or after preparation of the test sample white particles are visible on the walls of the sample bottle. If any of these items are noted the HMA analysis might not be accurate and the sample should be discarded.

SAMPLE PRETREATMENT FOR HMA ANALYSIS

Calibration 0: Unhomogenized milk

Heat the milk in a 40° C water bath. Gently swirl the container or tube to ensure homogeneity and make sure no fat is stuck to the container walls. No shaking in order to avoid foaming. If foam is formed in the sample, this needs to disintegrate completely before sample withdrawal for analysis. Withdraw a milk sample by syringe, inject immediately into the HMA and analyse. Always keep sample containers securely sealed in order to avoid evaporation, only remove the lid briefly to take out samples.

Calibration 1: Homogenized milk

Defrost the milk sample if necessary. Heat the milk in a 40° C water bath. Homogenize the sample 1.5 s/ml using Miris SONICATOR. Gently swirl the container or tube to ensure homogeneity and check no fat is stuck to the container walls. No shaking in order to avoid foaming. If foam is formed in the sample, this needs to disintegrate completely before sample withdrawal for analysis. Withdraw a milk sample by syringe, inject immediately into the HMA and analyse. Always keep sample containers securely sealed in order to avoid evaporation, only remove the lid briefly to take out samples.

SAFETY ASPECTS ON HUMAN MILK

Care should be taken when handling material of human origin. All material should be handled as potentially infectious. No test method can offer complete assurance that Hepatitis B, HCV, HIV 1 and 2 or other infectious agents are absent. Handling of samples, their use, storage and disposal should be in accordance with local regulation and institutional biohazard safety guidelines.

REFERENCES

[1] E. Miller, M. Aiello, M. Fujita, K. Hinde, L. Milligan and E. Quinn. ”Field and laboratory methods in human milk research,” *American Journal of Human Biology,* vol. 25, pp. 1–11, 2013.

[2] The Academy of Breastfeeding Medicine Protocol Committee. “Human milk storage information for home use for full-term infants”. *Breastfeeding Medicine*, vol. 5, pp.127-130, 2010.

[3] Milknet. “*Guidelines for use of human milk and milk handling in Sweden*“, available at http://neoforeningen.se/dokument/vardprogram/Milknet_english_2011.pdf.

[4] M. Picciano. “What constitutes a representative human milk sample?” *Journal of Pediatric Gastroenterology and Nutrition*, vol. 3, pp. 280-283, 1984.

Chapter 6 RESULT REVIEW

This chapter describes how results can be shown on the instrument display, saved on a USB memory stick and exported from the instrument to a PC. The memory of the instrument can save approximately 4000 measurements. When more than 4000 analyses have been done, the system will automatically discard the oldest data.

To take a screen shot of the result, press button 1 and 6 simultaneously. The buttons 2-5 will turn of momentarily to acknowledge this. The screen shot is saved as a png-file to the USB flash drive. Note that if no USB flash drive is not connected, an error message appears. This feature is only available in software version 2.87 and later.

VIEW LAST RESULTS

In the menu 'View' it is possible to see the results from previous measurements. Open the menu 'Result' and then 'View'. To exit the menu, press 'Finished'.

The first screen shows a summary of the last actions performed by the instrument. Number of measurements, checks, adjustments, slope/bias changes and resets appears on the screen.

To view the result, choose what to be shown by marking ID, Index, Date and Time and the Number of Samples.

To see results from measurements, press 'M' (marked blue in Figure 55). The results will be presented in a table, scroll to see all information.

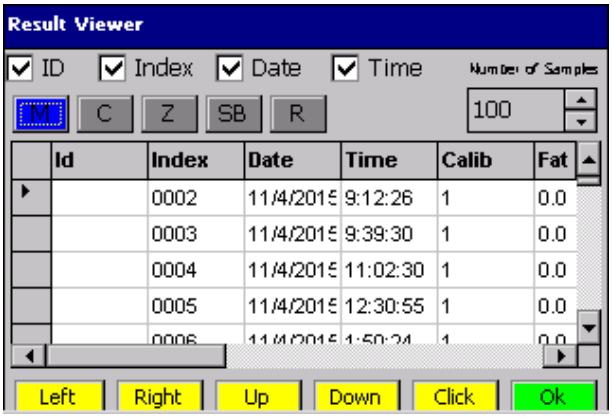


Figure 55. Results viewer screen.

Press 'C' to see performed checks
Press 'Z' to see performed adjustments
Press 'S/B' to view slope and bias settings changes
Press 'R' to see when the instrument has been reset

TRANSFER THE RESULTS

The transfer of results can be done in different ways depending on the software version installed on the HMA. To identify software version, press 'About' and then 'Machine', the software version is printed in line 6 "Application software".

Transfer the results to a USB memory stick

Connect a USB memory stick.

Software 2.53 and earlier

Open the menu 'Results' and press 'Transfer'. The results are transferred as a text file (.txt): *result_datetime.txt*.

If 'Transfer' is pressed without a USB memory stick connected, the message "No USB memory detected" will be shown. Connect a USB memory stick and press 'Transfer' again.

For information on how to convert the text file into an Excel file, see below.

Software 2.84 and later

Open the menu 'Results' and press 'Transfer'. Two results files will automatically be saved on the memory stick as text files (.txt): *result_datetime.txt* and *result_meas_datetime.txt*. The latter file only contains measurements (M) and has column headings.

Other transfer options are available: 'Tr.mini' and 'TransAll'. They transfer a minilogg.txt file and the content under bin respectively, and are used in support matters.

For information on how to convert the text file into an Excel file, see below.

If 'Transfer' is pressed without a USB memory stick connected, the message "No USB memory detected" will be shown. Connect a USB memory stick and press 'Transfer' again.

Converting a text file to Excel

1. Open Excel
2. Click on the Data tab
3. In the Get External Data group, click From Text.
4. Double-click the text file that you want to import in the Import Text File dialogue box.
5. Click Import
6. Select Delimited and click Next
7. Uncheck Tab and select Comma
8. Click Finished. The data is presented as in Figure 56.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
4	MIndex0001	11/18/2015	3:51:06 PM	1	3.5	1.3	7.5	12.5	68	1	0	0	HMA HOMOGENIZED MILK	VC	
5	MIndex0002	11/18/2015	3:52:19 PM	1	3.5	1.3	7.5	12.5	69	1	0	0	HMA HOMOGENIZED MILK	VC	
6	MIndex0003	11/18/2015	3:53:47 PM	1	3.5	1.3	7.5	12.5	68	1	0	0	HMA HOMOGENIZED MILK	VC	
7	MIndex0004	11/18/2015	3:54:59 PM	1	3.5	1.3	7.5	12.5	68	1	0	0	HMA HOMOGENIZED MILK	VC	
8	MIndex0005	11/18/2015	3:56:25 PM	1	3.5	1.3	7.5	12.5	68	1	0	0	HMA HOMOGENIZED MILK	VC	
9	MIndex0006	11/18/2015	3:57:45 PM	1	3.5	1.3	7.5	12.5	68	1	0	0	HMA HOMOGENIZED MILK	VC	
10	MIndex0007	11/18/2015	3:59:51 PM	1	3.5	1.3	7.4	12.4	68	1	0	0	HMA HOMOGENIZED MILK	VC	
11	MIndex0008	11/18/2015	4:01:13 PM	1	3.5	1.3	7.5	12.5	68	1	0	0	HMA HOMOGENIZED MILK	VC	
12	MIndex0009	11/18/2015	4:02:40 PM	1	3.5	1.3	7.5	12.5	68	1	0	0	HMA HOMOGENIZED MILK	VC	
13	MIndex0010	11/18/2015	4:03:51 PM	1	3.5	1.3	7.4	12.5	68	1	0	0	HMA HOMOGENIZED MILK	VC	
14	MIndex0011	11/18/2015	4:05:00 PM	1	3.5	1.3	7.5	12.5	68	1	0	0	HMA HOMOGENIZED MILK	VC	
15	MIndex0012	11/18/2015	4:06:14 PM	1	3.5	1.3	7.5	12.5	68	1	0	0	HMA HOMOGENIZED MILK	VC	
16	Reset	Index0012	1/4/2016	8:12:10 AM											
17	CIndex0013	1/4/2016	8:25:26 AM	1	0	0	0	-0.6	-2	0.1	0	0	6175.76	2059.6	629.86
18	QIndex0013	1/4/2016	9:52:52 AM	Calibration	HMA UNHOMOGENIZED MILK (0)										
19	CIndex0014	1/4/2016	9:53:56 AM	0	0	0	0	-0.7	-3	0.1	0	0	6175.76	2059.6	629.86
20	MIndex0001	1/4/2016	10:13:12 AM	0	4.3	1.2	7.4	13.1	74	1	0	0	HMA UNHOMOGENIZED MILK	VC	
21	MIndex0002	1/4/2016	10:14:21 AM	0	4.1	1	7.3	12.6	72	0.8	0	0	HMA UNHOMOGENIZED MILK	VC	
22	MIndex0003	1/4/2016	10:16:34 AM	0	4.3	1.1	7.5	13.1	75	0.9	0	0	HMA UNHOMOGENIZED MILK	VC	
23	MIndex0004	1/4/2016	10:17:43 AM	0	4.2	1.2	7.3	12.9	73	1	0	0	HMA UNHOMOGENIZED MILK	VC	

Figure 56. The logg file opened in Excel. The columns are from the left: ID, date, time, calibration number, fat, crude protein, carbohydrates, TS, energy, and true protein. One row represents one measurement.

TRANSFER RESULTS DIRECT TO COMPUTER

Depending on your computers' operative system there are different procedures for transferring results. Make sure the correct sync program is installed before starting.

Windows 7 and later

Windows Mobile Center is downloaded automatically when the HMA is connceted to a computer, via the USB B conncetion.

In the computer, choose 'Connect without setting up your device'. Under 'File management', press 'Browse the concent of your device'. Open 'Bin' and copy the logg.txt file it to your computer and convert it to an excel file following the steps described on page 33.

Note! Software version 2.84 can not start without the logg file in the bin folder, make sure not to remove it.

Windows Vista

Windows Vista requires Mobile Center 6.1, available at https://www.microsoft.com/en-us/download/details.aspx?id=14.

Connect the instrument and the computer with the USB cable, using the USB B connection. In the computer, Windows Mobila Device Center starts automaticly. Choose 'Connect without setting up your device'. Under 'File management', press 'Browse the concent of your device'. Open 'Bin' and copy the logg.txt file it to your computer and convert it to an excel file following the steps described on page 32.

Windows XP and older

Windows XP (or older) requires ActiveSync, available at https://www.microsoft.com/en-us/download/details.aspx?id=15.

Connect the instrument and the computer with the USB cable, using the USB B connection. In the computer, open ActiveSync, select 'File', 'Explore' and choose 'Pocket PC' (depending on Active Sync version Windows might need to be opened) and copy the logg.txt file it to your computer and convert it to an excel file following the steps described on page 32.

ALTERNATIVE WAYS TO TRANSFER RESULT FILES

To manually copy files from the instrument, use a USB hub (unit allowing several USB accessories to be connected to the instrument) and direct transfer to PC.

1. Connect a mouse and USB memory stick to a USB hub and connect it to the HMA
2. Press 'Exit' and 'Exit' to close the software
3. Open 'My Device' found on the Desktop
4. Open 'Bin'
5. Copy the logg file (right click on the file and choose 'copy')
6. Go back to 'My Device'
7. Open 'Hard Disk' (the USB memory stick) and paste the file

Chapter 7 SETTINGS

This chapter describes how to change date and time, how to reset the index number, how to assign samples with an ID of your choice, and how to change the slope and bias. Changing instruments settings is easier using a USB-connected mouse.

CHANGE DATE AND TIME

Press 'Settings' and 'DateTime'. Set the correct date and time by clicking on the month, day and clock. Use a mouse or direct the pointer. Press 'Finished' when done.

RESET THE INDEX NUMBER

The index number is reset to 0 in the menu 'Settings' by pressing 'Index=1'.

IDENTITY OF SAMPLE

Each sample or sample batch can be given a unique ID (max 20 characters), which will stay the same until changed. Every sample will also get a four-digit index number after the unique ID. If an ID is not given, samples will get an index number only.

It is recommended not to use commas (,) in ID's, since this complicates the data interpretation in Microsoft Excel.

Under 'Analysis', press 'ID'.

There are three different ways to type the ID:

- Using a mouse or direct via the instrument keys, turn on the keyboard and enter the ID. Turn off the keyboard and press 'OK'.
- Using an external keyboard, connect it to the instrument's USB port and type in the ID. Press 'OK'.
- Using a barcode reader, connect the device to the instrument via USB and read the barcode. Press 'OK'.

CHANGE SLOPE, BIAS, OR SLOPE AND BIAS

Note! Before changing slope and bias, please read all information to make sure what method to use and how to calculate. Read more in Chapter 8 Slope and Bias. This section will only describe how to make the settings in the instrument. When doing these settings, a mouse is recommended.

Press 'Settings' and 'Slope/Bias'.

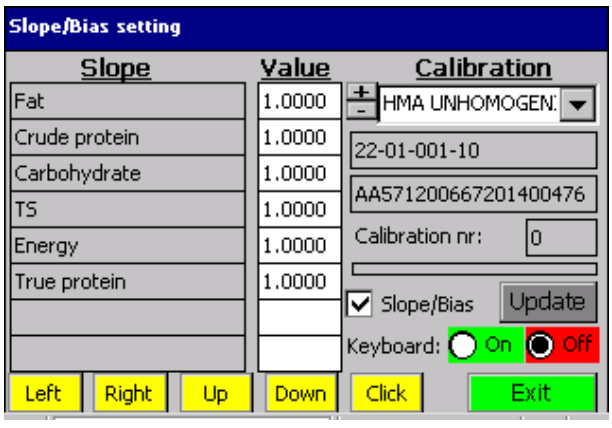


Figure 57. The Slope/Bias menu.

CHANGE SLOPE SETTINGS

1. When the box Slope/Bias is marked the instrument is set to change the slope. Slope will also read in the left upper corner of the screen (Figure 57).

2. Change the value for each parameter by using the keyboard on the screen or by using an external keyboard. It is possible to move the keyboard on the screen by using the mouse (Figure 58).

3. Figure 58. The Slope/Bias menu with keyboard.When done, turn off the keyboard.

4. Press 'Update'. The Update button will turn red while processing the changes, and grey when the changes are set.

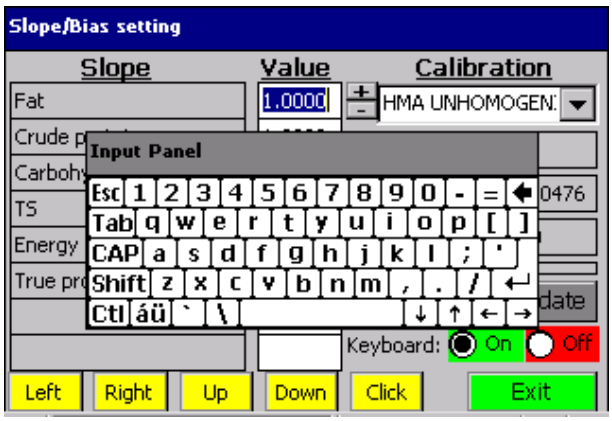


Figure 58. The Slope/Bias menu with keyboard.

CHANGE BIAS SETTINGS

1. When slope/bias is unmarked, the instrument is set to change the bias settings. Bias will also read in the left upper corner of the screen.

2. Change the value for each parameter by using the keyboard on the screen or by using an external keyboard. It is possible to move the keyboard on the screen by using the mouse (Figure 58).

3. When done, turn off the keyboard.

4. Press 'Update'. The Update button will turn red while processing the changes, and grey when the changes are set.

Chapter 8 SLOPE AND BIAS

Under 'Slope/Bias' in the 'Settings' menu an external calibration program can be accessed, giving the opportunity to adjust the level of the internal calibration by entering correction factors determined by the user.

With proper monitoring controls and instrument maintenance the internal calibration of the HMA is valid for the instrument life-time. There may however be situations when correction of the internal calibration is wanted, e.g. to align the instrument towards a specific reference method, or towards a specific reference laboratory.

The internal calibration of the HMA is based on chemical reference methods as specified in Table 10. In case of adjusting the level of the internal calibration using the slope/bias function it is important to carefully consider which reference method to use as the comparative method. Miris recommends Röse-Gottlieb [1] or Mojonnier [2] for analysis of fat, Kjeldahl [3] or Dumas [4] for analysis of crude protein, and to calculate the total carbohydrate content by difference from the total solids value (Table 11).

This procedure requires reliable reference samples with known contents of the parameter of interest, and a zero-set instrument. Miris recommends to consult an experienced person for test design and sample set-up.

CORRECTION FACTORS

Slope is a factor adjusting the internal calibration to align the HMA results with the results of the reference method. Default value is 1.000.

Bias is an additional term adjusting the internal calibration to align the HMA results with the results of the reference method. Default value is 0.000.

METHOD SELECTION

There are three methods to adjust the internal calibration of the instrument. Applying only bias, applying only slope, or applying bias and slope.

Miris recommends using the slope method.

SAMPLE PREPARATION AND ANALYSIS

To determine the magnitude of the slope and bias error, reference samples with at least two distinct concentration levels of the parameter of interest are needed (low and high). Different fat level samples can be obtained by splitting milk in two parts and allowing the fat to separate. Skim one part using a spoon or similar, transferring the fat to the second part.

Analyse each reference sample both on the HMA, as well as with the comparative reference method. Duplicate analysis is recommended.

Calculate the average value for the parameter of interest, for the HMA analysis (Fat_{HMA}, $Protein_{HMA}$, CHO_{HMA}) and for the reference analysis (Fat_{ref}, $Protein_{ref}$, CHO_{ref}).

DETERMINING THE SLOPE AND BIAS ERROR

Calculate the slope factor or bias term for each milk parameter separately. Remember that the current slope or bias error must be considered in the calculation.

SLOPE method

The new slope is calculated for each parameter by dividing the mean values of two reference samples by the mean values from the HMA measurements, times the current bias and slope settings:

$$Slope = (mean_{ref}/mean_{HMA})*(bias_{current} + slope_{current})$$

Example 1 – Slope on the fat parameter

Reference measurements	HMA measurents	Current slope and bias settings
Ref_{low}:2.5	HMA_{low}:2.7	Bias: 0.0000 (default)
Ref_{high}: 4.0	HMA_{high}:4.2	Slope: 1.0000 (default)

$Slope = (mean_{ref}/mean_{HMA})*(bias_{current} + slope_{current})$ = ((2.5 + 4.0) / 2) / ((2.7 + 4.2) / 2) * (0.0000 + 1.0000) = **0.9420**

Example 2 – New slope on the fat parameter

Reference measurements	HMA measurements	Current slope and bias settings
Ref_{low}:2.5	HMA_{low}:2.7	Bias: 0.0000 (default)
Ref_{high}: 4.0	HMA_{high}:4.2	Slope: 0.9420

$Slope = (mean_{ref}/mean_{HMA})*(bias_{current} + slope_{current})$ = ((2.5 + 4.0) / 2) / ((2.7 + 4.2) / 2) * (0.0000 + 0.9420) = **0.8874**

BIAS method

The new bias is calculated according to:

$New\ bias = Bias_{current} – (parameter_{HMA} – parameter_{ref})$

Example 1 Bias

Bias = 0.000 – (4.2 – 4.0) = **– 0.2**

Example 2 New bias

New bias = 0.100 – (4.2 – 4.0) = **– 0.1**

SLOPE AND BIAS method

With skills in statistics and regression analysis it is possible to make a slope and bias adjustment, this is not described in this manual.

SETTING THE SLOPE AND BIAS IN THE INSTRUMENT

For instructions on how to enter the new correction factors into the instrument, see Chapter 7.

VALIDATION OF THE CORRECTED INTERNAL CALIBRATION

After the new correction factors have been entered into the instrument, analyse a number of milk samples to validate that the results from the HMA are at the expected level.

REFERENCES

[1] ISO, "*1211:2010 Milk - Determination of fat content - Gravimetric method (reference method)*," International Organization for Standardization, Geneva, Switzerland, 2010.

[2] AOAC, *"989.05-1992, Fat in Milk - Modified Mojonnier Ether Extraction method"*.

[3] ISO, "*8968-1:2014 Milk and milk products - Determination of nitrogen content - Part 1: Kjeldahl principle and crude protein calculation*," International Organization for Standardization, Geneva, Switzerland, 2014.

[4] ISO, *"ISO 14891:2002 Milk and milk products - Determination of nitrogen content - Routine method using combustion according to the Dumas principle"*, International Organization for Standardization, Geneva, Switzerland, 2002.

Chapter 9 CONSUMABLES - MIRIS CLEANER AND MIRIS CHECK

REQUIRED CONSUMABLES

Miris HMA requires Miris CLEANER, Miris CHECK and distilled or deionised water to function properly. Contact Miris to obtain safety data sheets for these products.

All liquids need to be 35-40° C when injected into the instrument. Sudden temperature changes may cause irreparable damage to the cuvette not covered by the product warranty.

Miris CLEANER™

Cleaning solution to be used after every 10th sample or when the instrument is idle for more than five minutes.

Instructions: Dilute one tube (50 ml) with 950 ml distilled/deionised water. Warm the solution to 40° C before use and follow the instructions in Chapter 3.

Storage: Store at room temperature (20-30° C), out of direct sunlight. Keep away from foodstuff and animal feed.

Use before: Expiry date for unopened tubes is 2 years from production date. Use solution within 3 months of preparation.

Miris CHECK™

Solution for zero-setting the internal calibration, to be used when preparing the instrument for analysis and also after cleaning if analysis is continued.

Instructions: Dilute 1 tube (10 ml) with 90 ml distilled/deionised water. Warm the solution to 40° C before use and follow the standard operation in Chapter 2 or the instructions in Chapter 4.

Storage: Store dark at room temperature (20-30° C). Keep away from foodstuff and animal feed.

Use before: Expiry date for unopened tubes is 1 year from production date. Use solution within 3 months of preparation.

Distilled or deionised water (not provided by the manufacturer)

Distilled or deionised water is required to dilute concentrates of Miris CHECK and Miris CLEANER to working solutions, and for stand-by and storage of the HMA.

Chapter 10 STORAGE AND TRANSPORTATION

Storage or transport of the HMA at temperatures below 0° C (32° F) must always be done with the cuvette completely empty of any liquid. Empty the cuvette by injecting air using a new syringe. If the HMA is equipped with an IPG, empty the vent on the side of the IPG by applying gentle pressure when the air is injected. If the HMA is equipped with a CPG, empty both vents at the side of the CPG by applying gentle pressure at injection through the inlet and outlet respectively.

Note! Correct transportation and storage routines are essential as sudden temperature changes may cause irreparable damage to the cuvette not covered by the product warranty.

Transportation

The cuvette must be completely empty of any liquid before transportation. Transportation must be careful, avoiding impacts, preferably using the carrying case the instrument is delivered in. After transport, let the HMA stand 4 hours in room temperature (20-30° C, 68-86° F) before switching the power on. Follow the start-up procedure instructions on page 10.

Storage

Always leave the instrument filled with distilled/deionised water when not in use and make sure the system is closed (Figure 59-61) when stored for longer periods.

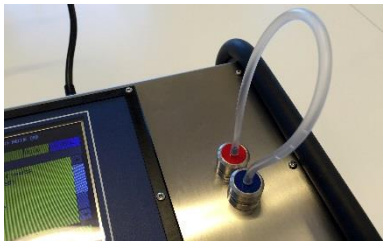


Figure 59. Inlet Syringe.

Figure 60. Inlet Pressure Guard (IPG).

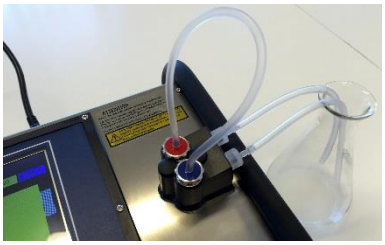


Figure 61. Cuvette Pressure Guard (CPG)

At long-time storage, inject fresh distilled/deionised water minimum every second week. Pay attention the temperature of the distilled/deionised water injected depending on if the HMA is switched on or off, see Table 4.

Table 4. Temperature of liquids to be injected into an instrument switched on or off.

	Ambient temperature	Temperature of distilled/deionised water
Instrument switched on	20-30° C, 68-86° F	35-40° C, 95-104° F
Instrument switched off	20-30° C, 68-86° F	20-30° C, 68-86° F
Instrument switched off	10-20° C, 50-68° F	10-20° C, 50-68° F

Chapter 11 TROUBLESHOOTING AND PROBLEM SOLVING

If any problem should occur that you are unable to solve by referring to this manual, please contact your distributor or Miris. When doing so, please include the serial number and software version of your instrument. The serial number is printed on the label placed at the back of the HMA. Software version can be found in the menu 'About', 'Machine'.

TROUBLESHOOTING

Table 5. Troubleshooting guide.

	Error	Cause	Action	If the error persists
Error messages	"Air in the system"	Sample is not properly injected; cause may be a foamy sample, a worn-out syringe or worn-out rubber gaskets in the in- and outlets	Repeat the measurement with a new sample, and/or new syringe Replace rubber gaskets	Contact Miris (support@miris.se) or your local distributor
	"No energy in the system"	The measurement cell is blocked, the cause may be an improper sample, insufficient cleaning, or a hardware error	Clean the system and repeat the measurement	Contact Miris (support@miris.se) or your local distributor
	"Bad sample, check the inlet and try again"	Sample is not properly injected; cause may be a foamy sample, a worn-out syringe or worn-out rubber details in the in- and outlets	Repeat the measurement with a new sample, and/or new syringe Replace rubber gaskets	Contact Miris (support@miris.se) or your local distributor
	"Data limited has been reached. Dumping data, please wait"	Minilogg file too big	Wait Oldest data is erased automatically	Contact Miris (support@miris.se) or your local distributor
	"Error"		Restart the instrument with the on/off button	Contact Miris (support@miris.se) or your local distributor
	"Transmission change"	The transmission has decreased more than 10% on one or several instrument filters (Tr1-Tr4)	Click 'ok' on the message Clean the system and repeat the check procedure Ensure the Miris CHECK solution is not contaminated	Stop using the instrument and contact Miris (support@miris.se) or your local distributor
Problem solving	Blocked fluids system	If the sample injection needs higher mechanical force than	Do not force liquid through the system! Clean the filter, then	Remove filter housing and outlet valve using

		normal, this may be an indication of a dirty fluids system, typically a dirty filter	reverse the fluid direction and rinse with Miris CLEANER, see instructions in Chapter 3.	the spanner provided and clean the parts. Contact Miris (support@miris.se) or your local distributor
	No response when pressing the buttons or using the mouse		Turn off the instrument and restart it again.	Contact Miris (support@miris.se) or your local distributor
	The application software is closed		Restart the instrument by pressing the on/off-button	Contact Miris (support@miris.se) or your local distributor

FAQ – FREQUENTLY ASKED QUESTIONS

Q. What happens if I analyse the milk with the wrong calibration (i.e. Calibration 1 Homogenized instead of Calibration 0 Unhomogenized)?
A. The results will be affected as the two different calibrations depend on the milk properties. Miris can help you with recalculation of the results. Contact support@miris.se.

Q. What routines should my milk bank use?
A. Miris can only be of help setting up routines for proper use of the HMA. For other issues regarding milk handling etc. please consult the authorities in your country. See Chapter 5.

Q. What is the difference between true/crude/total protein?
A. Crude protein, also referred to as total protein, is the protein content based on the total amount of nitrogen (N) in a sample. This means that non-protein nitrogen (NPN) compounds will also be included in this value. True protein on the other hand is corrected for this and represents only the content of actual protein, hence the denotation true. Human milk contains a large proportion of NPN, about 20-25 % of the total N. As it is the protein that will contribute to infant growth it is important to know whether your analysis yields crude or true protein. It is also necessary to keep in mind when comparing results and reference samples analysed by different methods. The Miris HMA gives both crude protein and true protein to avoid misunderstandings. Miris uses the factor 6.38 to convert N content to protein content.

Q. How do I get a representative sample if I can't warm the whole bottle of milk?
A. Try mixing gently by turning the bottle back and forth. Keep in mind that this might be a non-representative sample when performing the analysis – the instrument measures only the part you inject.

Q. How do I know when the milk is of "bad quality"?
A. If the milk has started to curdle or has oil droplets on the surface (oiling-off) then it should not be analysed. See Chapter 5.

Q. How do I know if my results are reasonable?
A. Apply Miris quality control procedures to ensure that the instrument is functioning in a correct way, see Chapter 4. Perform double analyses in order to detect and exclude outlying results. Make notes of sample, its origin, sampling procedure etc. Compare results to literature values, see ranges of macronutrients in Chapter 12.

Q. What kind of service has to be done and how often?
A. Make sure to keep the instrument clean, and pay special attention to the in- and outlets. Use the check function to see that the instrument is stable and working properly. Be observant of the “Change%”-line. This percentage shows, for each instrument filter 1-4, how much the instrument transmission has altered since the factory calibration at Miris. The instrument software will alert the user by displaying a warning message at the event of a transmission change of 10% or more for any one filter. This feature is only available in software 2.84 and later. If this happens, click ‘OK’ on the message, clean the instrument and try the check procedure again. If the problem persists, stop using the instrument and contact Miris (support@miris.se) or your local distributor for an instrument check-up. In software version 2.84 or earlier the warning will appear if “Change%” is below 90% or above 110%, in software version 2.87 or later if “Change%” is more than ±10%.

Q. Who can perform service?
A. Service can only be performed by an authorized service technician. Ask your local distributor or contact Miris.

Chapter 12 HUMAN MILK COMPOSITION - EXPECTED RANGE

The composition of human milk is highly variable, with factors such as diurnal variation, longitudinal changes associated with postpartum duration, time since last feed, volume of milk consumed at the prior feed, time during feed, and maternal physiological let down to consider.

Data from a meta-analysis of 41 studies on the nutrient content of human milk [1], term (37-42 weeks of gestation) and preterm (<37 weeks of gestation), are presented in Table 6 and Table 7. For estimates of fat and energy only studies using 24-h collection as the sampling method were included, due to the high variability in milk fat content depending on foremilk/hindmilk, time of day, and time since last feed. See the original article for full inclusion criteria.

Data from two studies specifically on the composition of donor human milk are given in Table 8.

Note that the HMA measures the total carbohydrate content, i.e. including the content of lactose and oligosaccharides.

Table 6. Meta-analysis results of the macronutrient composition of term (37-42 weeks of gestation) human milk [1].

Time after delivery	**Fat** (g/100mL)		**Crude protein** (g/100mL)		**True protein** (g/100mL)		**Lactose** (g/100mL)		**Oligosaccharides** (g/100mL)		**Energy** (kcal/100mL)	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Day 1-3	1.8	0.7	2.0	0.6	2.0	0.9	5.6	0.6	1.6	0.2	54	8
Day 4-7	2.6	0.8	2.0	0.5	1.6	0.3	6.0	1.0	1.9	0.4	66	9
Week 2	3.0	0.9	1.8	0.4	1.3	0.2	6.2	0.6	1.9	0.4	66	9
Week 3-4	3.4	0.8	1.5	0.3	1.1	0.2	6.7	0.7	1.6	0.3	66	8
Week 5-6	3.6	1.1	1.1	0.2	1.0	0.1	6.1	1.0	1.4	0.3	63	7
Week 7-9	3.4	0.8	1.3	0.2	0.9	0.1	6.5	0.5	1.3	0.3	63	7
Week 10-12	3.4	0.9	1.2	0.2	1.0	0.1	6.7	0.7	-		63	8
Colostrum (day 1-3)	1.8				2.0		5.6				54	
Mature milk (week 5-12)	3.4				1.0		6.5				63	

Table 7. Meta-analysis results of the macronutrient composition of preterm (<37 weeks of gestation) human milk [1]

Time after delivery	**Fat** (g/100mL)		**Crude protein** (g/100mL)		**True protein** (g/100mL)		**Lactose** (g/100mL)		**Oligosaccharides** (g/100mL)		**Energy** (kcal/100mL)	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Day 1-3	2.2	0.9	2.8	1.1	2.7	1.5	5.1	0.7	-		49	7
Day 4-7	3.0	1.2	2.1	0.5	1.7	0.5	6.3	1.1	2.1	0.4	71	9
Week 2	3.5	1.1	1.9	0.4	1.5	0.4	5.7	0.8	2.1	0.5	71	12
Week 3-4	3.5	1.0	1.6	0.4	1.4	0.4	6.0	0.5	1.7	0.3	77	8
Week 5-6	3.2	0.8	1.4	0.3	1.1	0.2	5.8	0.6	-		70	5
Week 7-9	3.3	0.9	1.1	0.2	1.1	0.2	6.3	0.4	-		76	8
Week 10-12	3.7	1.5	1.3	0.3	1.0	0.2	6.8	0.3	-		-	
Colostrum (day 1-3)	2.2				2.7		5.1				49	
Mature milk (week 5-12)	3.3				1.1		6.2				73	

Table 8. Macronutrient composition of donor human milk.

Reference	Fat (g/100mL)		Crude protein (g/100mL)		True protein (g/100mL)		Carbohydrate (g/100mL)		Energy (kcal/100mL)	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
[2]	3.2	1.0	1.2	0.5	-		7.8	0.9	65	9
[3]	3.6		0.9		-		7.2		67	

REFERENCES

[1] D. Gidrewicz and T. Fenton. “A systematic review and meta-analysis of the nutrient content of preterm and term breast milk”, *BMC Pediatrics* 14:216, 2014.

[2] K. Wojcik, D. Rechtman, M. Lee, A. Montoya, and E. Medo. “Macronutrient analysis of a nationwide sample of donor breast milk”. *J Am Diet Assoc*, vol. 109, pp. 137-140, 2009.

[3] K. Michaelsen, L. Skafte, J. Badsberg, and M. Jorgensen. ”Variation in macronutrients in human bank milk: Influencing factors and implications for milk banking”. *Journal of Pediatric Gastroenterology and Nutrition*, vol. 11, pp. 229-239, 1990.

ABOUT MIRIS HUMAN MILK ANALYZER

TECHNICAL SPECIFICATIONS

Table 9. Technical specifications.

Dimensions (HxWxL)	9 x 26 x 31 cm	
Weight	3 kg	
Power supply Adapter	Input voltage 100-240 V ~ 50/60 Hz, 2.3A	
Power supply Instrument	Output voltage 18 V DC, 100VA	
Battery	Li-ion battery to keep date and time (lifetime > 10 years)	
PC connections	USB B for transfer of results and software update via ActiveSync or Windows Mobile Device Center. USB A for memory stick and devices e.g. keyboard, mouse, scanner, etc. RS232 and Ethernet	
Display	TTFT QVGA 320*240	
Sample temperature	+35° C (95° F) to +40° C (104° F)[1]	
Internal Storage capacity	4000 measurements	
Backup of measurement data	Internal persistent flash memory	
Operative system	Windows Compact 7 or later	
Measurement performance	Repeatability (SD): fat, crude protein, true protein ≤0.05 g/100ml; carbohydrate ≤0.08 g/100ml Accuracy (SD): fat, crude protein, true protein ≤0.2 g/100 ml; carbohydrate ≤0.4 g/100 ml	
Components tested	Fat [g/100 ml], Crude protein [g/100 ml], True protein [g/100 ml], Carbohydrate [g/100 ml]	
Components calculated	Total solids (TS) [g/100 ml], Energy [kcal/100 ml]	
Measuring range	Fat 0.5 - 8 g/100 ml, Crude protein 0.5 - 3 g/100 ml, True protein 0.4 - 2.4 g/100 ml, Carbohydrate 4 - 8 g/100 ml	
Shown value	1 decimal	
Time for analysis	60 seconds / measurement	
Analytical method	Mid-infrared transmission spectroscopy	
Standards	CE, IVD class General, FCC	
ENVIRONMENTAL	OPERATIONAL	NOT OPERATIONAL (freight empty cuvette)
Temperature	+20° C (68° F) to +30° C (86° F)	-10° C (14° F) to +50° C (122° F)
Humidity	10-80% not condensed	20-80 % not condensed

[1]For optimum results preheating the samples to 40° C (104° F) is recommended

PRECAUTIONS AND LIMITATIONS

For in vitro diagnostic use

The HMA is intended for analysis of non-diluted human milk without any additives (except for the preservative 2-bromo-2-nitropropane-1,3-diol)

The HMA is not intended to measure the nutritional content of any other liquid, e.g. fortified human milk or infant formula

PERFORMANCE CHARACTERISTICS

Table 10. Performance charachteristics of the HMA

Components measured*	Fat, crude protein, true protein, carbohydrate		
Components calculated	Total solids, energy		
Analysis time	Approximately one minute		
Measuring range	Fat 0.5 - 8 g/100 ml Crude protein 0.5 - 3 g/100 ml True protein 0.4 - 2.4 g/100 ml Carbohydrate 4 - 8 g/100 ml		
Repeatability (SD)	Fat, crude protein, true protein ≤ 0.05 g/100ml Carbohydrate ≤ 0.08 g/100ml		
Accuracy (SD)†	Fat, crude protein, true protein ≤ 0.2 g/100 ml Carbohydrate ≤ 0.4 g/100 ml		
	Inlet Syringe	**Inlet Pressure Guard**	**Cuvette Pressure Guard**
Purging efficiency# at a sample injection volume of ≥ 3 ml	99 %	99 %	99 %
Purging efficiency# at a sample injection volume of 2 ml	98 %	98 %	99 %
Purging efficiency# at a sample injection volume of 1 ml	-	-	98 %

* All components are analysed simultaneously

†Under stipulated experimental conditions when reference methods used are Röse-Gottlieb for fat [1], Kjeldahl for protein [2], carbohydrate value calculated by difference [8] from oven drying method for total solids [3]. Contact Miris for more information.

Purging efficiency is a measure of carry over-effect from previous sample [7].

WORKING PRINCIPLES OF THE INSTRUMENT

TECHNOLOGICAL CHARACTERISTICS

The HMA device is comprised of a sample cuvette and assisting hardware components. The cuvette is a mid-infrared measurement cell with an inlet and an outlet. Liquids are injected via the inlet and pass between two CaF_2 (calcium fluoride) windows separated by a spacer (50 µm). On one side of the windows is an infrared radiation source (emitter) and on the other side is a four channel detector receiving the radiation transmitted through the liquid. The detector specification is based on the US patent No US 7,132,660 B2. The filters in the detector are selected to absorb only mid-infrared radiation correlated to fat, protein and carbohydrates, respectively. The fourth filter acts as a reference filter. Figure 62 provides a schematic drawing representing the HMA hardware components.

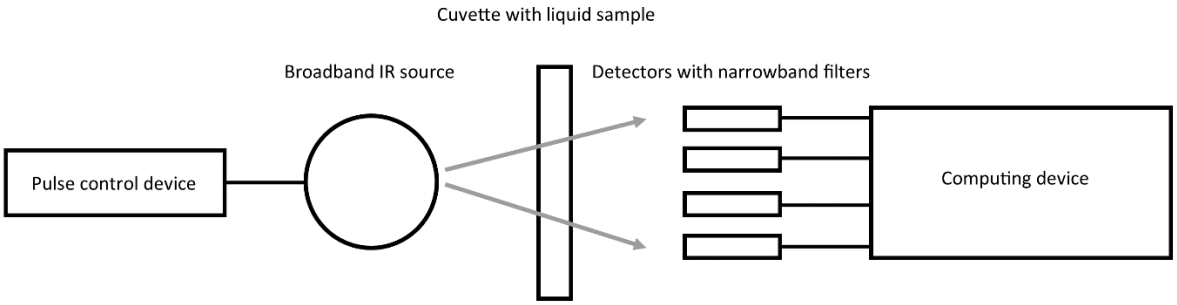


Figure 62. Schematic drawing of HMA hardware components.

PRINCIPLES OF OPERATION

The actual analysis time depends on the ambient and sample temperatures, approximately one minute.

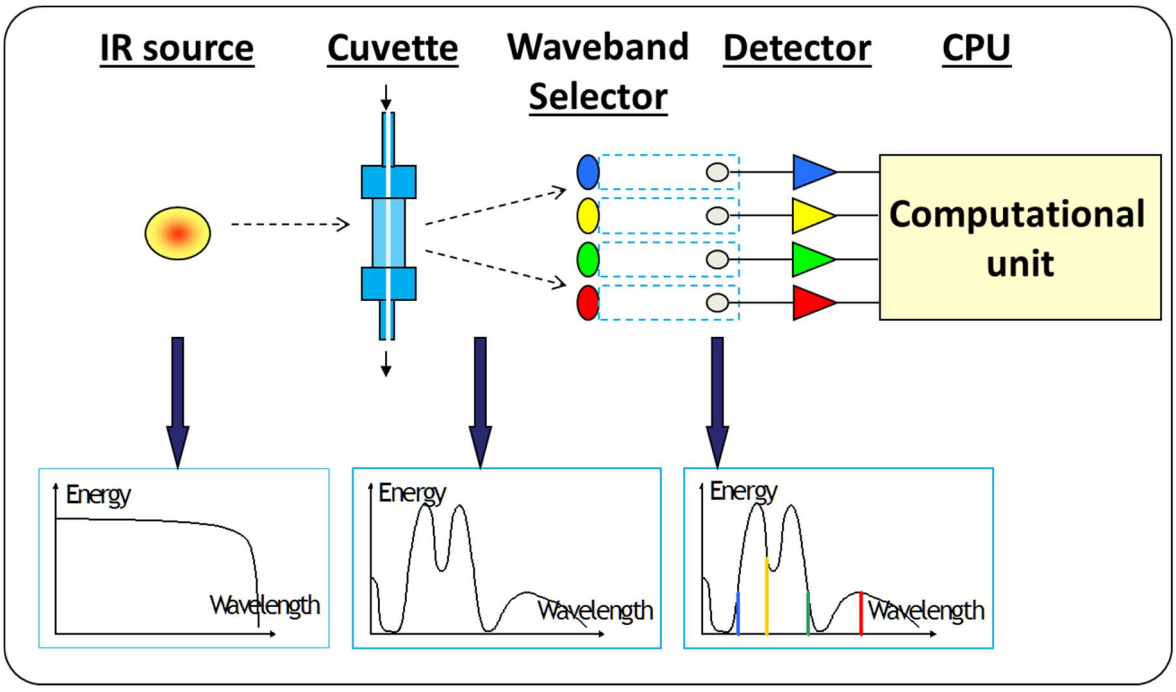


Figure 63. Working principle of the HMA.

Figure 63 illustrates the working principle of the instrument. The radiation from an IR-source penetrates the transparent cuvette containing the liquid sample. After passing through the cuvette chamber the quantities of radiation absorbed by specific functional groups of fat, protein and carbohydrate (Table 11), respectively, are evaluated. The quantitative determination of fat, protein and carbohydrate is performed according to Beer's law - the absorbance is proportional to concentration. The HMA software processes the measurement data by the internal calibration and the results are presented to the user.

The internal calibration is made at Miris' production laboratory, using a matrix of human milk samples covering the HMA measuring range for each component. The calibration samples are analysed both by the HMA and by the reference method. The HMA results and the reference method results are combined to fit calibration curves for fat, protein and carbohydrate, respectively, which will constitute the internal calibration.

The HMA internal calibration is based on chemical reference methods (Table 11) commonly used for analysis of human milk. These reference methods are ISO/AOAC certified and IDF recommended; Röse-Gottlieb for fat [1], Kjeldahl for crude protein [2]. True protein measurement equals crude protein minus non-protein-nitrogen (NPN). The reference analysis of total carbohydrate (lactose and oligosaccharides) content is a calculation of total solids minus fat, protein and a mineral constant [8]. Total solids are measured by drying-oven [3].

Table 11. Description of components in human milk analysed by Miris HMA.

Component	Reportable unit	Definition	Mid-IR absorbance		Reference method
			Chemical bond	λ	
Fat (F)	g/100 ml	Triacylglycerides (≈98-99%), di- and monoglycerides, FFA, phospholipids, sterols	Carbonyl group of ester bonds of glycerides	5.7 µm	Röse-Gottlieb ISO 1211 [1]
Crude protein (CP)	g/100 ml	Total nitrogen content*6.38. Includes both protein N and non-protein N (oligosaccharides, urea etc.).	Secondary amide (II) groups of peptide bonds	6.5 µm	Kjeldahl ISO 8968-1 [2]
True protein (TP)	g/100 ml	Crude protein*0.8. Non-protein N (≈20% of total N [4]) excluded.	Secondary amide (II) groups of peptide bonds	6.5 µm	Kjeldahl ISO 8968-1 [2]
Carbohydrate (CHO)	g/100 ml	Total carbohydrate content. Lactose (≈70-85%), mono-/oligosaccharides (≈15-30%) [5, 6]	Hydroxyl groups of lactose and mono-/oligosaccharides	9.6 µm	Drying oven ISO 6731 for TS [3], then by [8]: CHO_{ref} = TS_{ref} - F_{ref} - CP_{ref} - 0.2 (minerals)
Total solids (TS)	g/100 ml	Dry matter	Calculated from the HMA measurement results	-	Formula: $TS_{HMA}=F_{HMA}+CP_{HMA}+CHO_{HMA}+0.2$ (minerals)
Energy (E)	kcal/100 ml	Energy content	Calculated from the HMA measurement results	-	Formula [4]: $E_{HMA} = 9.25*F_{HMA} + 4.40*CP_{HMA} + 4.00*CHO_{HMA}$

REFERENCES

[1] ISO, "*1211:2010 Milk - Determination of fat content - Gravimetric method (reference method)*," International Organization for Standardization, Geneva, Switzerland, 2010.

[2] ISO, "8968-1:2014 Milk and milk products - Determination of nitrogen content - Part 1: Kjeldahl principle and crude protein calculation," International Organization for Standardization, Geneva, Switzerland, 2014.

[3] ISO, "6731:2010 Milk, cream and evaporated milk - Determination of total solids content (reference method)," International Organization for Standardization, Geneva, Switzerland, 2010.

[4] S. Polberger and B. Lönnerdal, "Simple and rapid macronutrient analysis of human milk for individualized nutrition: Basis for improved nutritional management of very-low-birth-weight-infants?," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 17, pp. 283-290, 1993.

[5] D. Viverge, L. Grimmonprez, G. Cassanas, L. Bardet and M. Solere, "Variations in oligosaccharides and lactose in human milk during the first week of lactation," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 11, pp. 361-364, 1990.

[6] O. Gabrielli, L. Zampini, T. Galeazzi, L. Padella, L. Santoro, C. Peila, F. Giuliani, E. Bertino, C. Fabris and G. V. Coppa, "Preterm milk oligosaccharides during the first month of lactation," *Pediatrics*, vol. 128, pp. 1520-1531, 2011.

[7] ISO "9622:2013 Milk and liquid milk products - Guidelines for the application of mid-infrared spectrometry", International Organization for Standardization, Geneva, Switzerland, 2013.

[8] A. Merrill and B. Watt. "Energy value of foods, basis and derivation", *Agriculture Handbook No 74 Slightly revised 1973.* Human Nutrition Research Branch, Agricultural Research Service, United States Department of Agriculture, 1973