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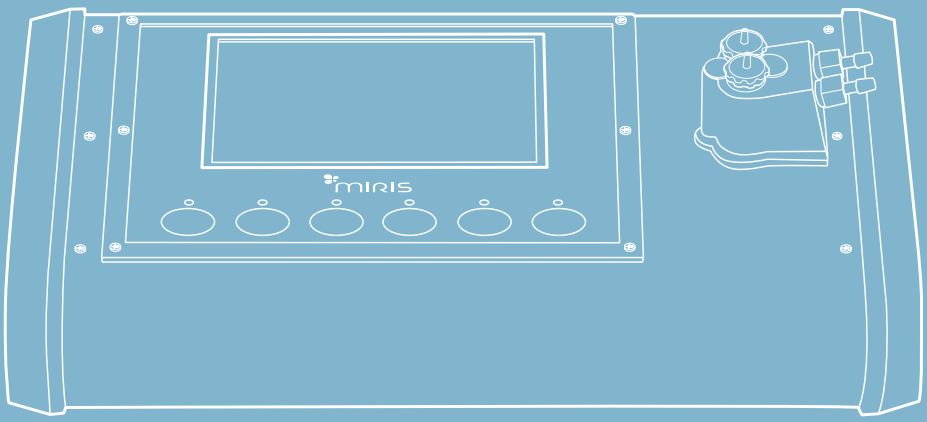
Miris HMA™
Human Milk Analyzer



USER MANUAL

READ THE MANUAL BEFORE USING THE INSTRUMENT

PREFACE

Thank you for choosing the Miris HMA™ - Human Milk Analyzer. Please read this manual carefully before starting to use the instrument.

Miris is not liable for technical failures or inaccuracies 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.

The Miris HMA™ quantitatively measures the concentration of fat, carbohydrate and protein in human milk. The Miris HMA™ also provides calculated values for total solids and energy. It is a compact, robust instrument without moving parts. The analytical technique used in Miris HMA™ is a combination of established mid-infrared (mid-IR) transmission spectroscopy principles and a patented innovation.

Manufacturer contact information

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Document information

name	Miris HMA™ User Manual
ID	DOC-510
revision	3
issue date	2023-07-28
article number	36-03-101

Product information

name	Miris HMA™
article number	08-02-102
software version	3
standard	European Commission Directive 98/79/EC on in vitro diagnostic medical devices

CE

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IMPORTANT USER INFORMATION

To operate the instrument, the user must be trained by MIRIS or a MIRIS authorised distributor.

S1

INTENDED USE OF THE MIRIS HUMAN MILK ANALYZER

The Miris Human Milk Analyzer (HMA) quantitatively measures the concentration of fat, protein and carbohydrate in human milk. The Miris HMA™ also provides calculated values for total solids and energy. These measurements in conjunction with other clinical assessments, may be used to aid in the nutritional management of newborns, including preterm, and infants. This device is intended for use in healthcare by trained healthcare personnel at clinical laboratories.

The Miris HMA is also intended for use by personnel trained in the use of the device at donor human milk banks, for the purpose of labeling milk donations and in the processing of milk donations.

WARRANTIES AND DUTIES

By operating this Miris HMA™ 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 Miris HMA™.

MIRIS AB WARRANTS THAT IT:

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

USER WARRANTS THAT:

- The Miris HMA™ will be used according to the instructions given in the user manual
- The Miris HMA™ 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 immediately if any injury occurs in any association with the Miris HMA™, and will be allowed prompt and thorough examination of the Miris HMA™ in question
- MIRIS will not be held responsible in cases of injury arising from use of the Miris HMA™:

  a. When the Miris HMA™ is not used according to the instructions in this manual
  b. When Miris is not notified within 5 days of said injury

REGULATORY REPORTING

Any serious incident that has occurred involving the Miris HMA™ shall be reported to MIRIS and to the Competent Authority of the Member State in which the USER is established.

PRECAUTIONS AND LIMITATIONS

For in vitro diagnostic use only.

Do not dilute milk samples. The only acceptable preservative that can be used is Bronopol (additive).

Do not use the Miris HMA™ with fortified human milk or infant formula.

The Miris HMA™ is not the sole basis for nutritional management of the newborn, use of the Miris HMA™ device is intended as part of an overall treatment plan and nutritional measures for newborns. The Miris HMA™ is an aid to the healthcare providers' standard of care assessment of nutritional management of newborns through monitoring of weight gain and growth.

Clinicians should follow clinical practice guidelines and standard of care when supplementing or fortifying human milk.

INTERFERENCE STATEMENT

Do not analyze milk that may contain any of the drugs listed below, i.e. milk from mothers taking the drug:

Citalopram	Results in a negative bias on fat measurements
Sertraline	Results in a negative bias on fat measurements
Ampicillin	Results in a negative bias on fat measurements
Vancomycin	Results in a positive bias on fat measurements
Clindamycin	Results in a negative bias on fat measurements
Cephalexin	Results in a negative bias on fat measurements
Pseudoephedrine	Results in a negative bias on fat measurements

Human milk may be contaminated with hemoglobin, from whole blood. This will result in a positive bias on protein measurements. If the milk is visibly pink, presumed from blood contamination, macronutrient analysis by Miris HMA™ is not recommended.

For further information on the interference testing, see section Summary of Bench Testing.

SAFETY INFORMATION

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

Let the Miris HMA™ completely adjust to room temperature (20-30°C, 68-86°F) before switching the power on

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

When the instrument is switched on, 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 consumables 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 Miris 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 deionised 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 are protected by protection caps or tubing when the instrument is not in use to avoid dirt entering the cuvette

User must ensure that accessories used with the Miris HMA™ will not contaminate the sample or influence the measurement results

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

Do not use any other power supply than the one supplied by Miris, included in the instrument starting kit

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

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

Please refer to the Installation Qualification/Operational Qualification documentation (www.MirisSolutions.com) to secure proper installation and functional control

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

SYMBOLS GLOSSARY

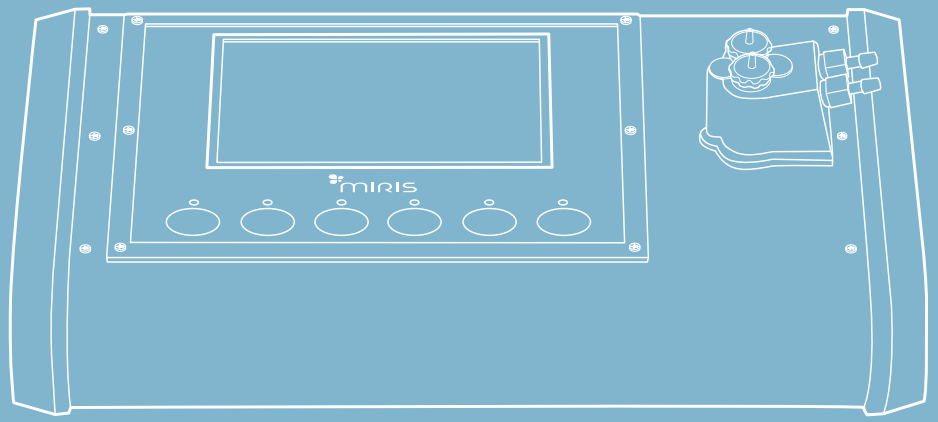
ISO 15223-1:2021 Medical devices — Symbols to be used with medical device labels, labelling and information to be supplied — Part 1: General requirements

Symbol	Description
	Read the user manual
CE	CE marking declaring conformity with 98/79/EC IVDD
IVD	In Vitro Diagnostic medical device
	Warning, biological hazard
	Caution
	Date of manufacture YYYY-MM-DD
LOT	Batch code
	Use by date YYYY-MM-DD
	Temperature limits for storage and transport
	Keep away from rain
	The product should not be discarded as unsorted waste but must be sent to separate collection facilities for recovery and recycling, in accordance with Directive 2012/19/EU on waste electrical and electronic equipment (WEEE)
	Direct current
	Warning, causes serious eye irritation
	This way up
FRAGILE	Fragile content

MIRIS HMA™ USER GUIDE

Chapter 1 INSTALLATION AND OPERATION

- **THE INSTRUMENT**
- **IN- AND OUTLETS**
- **ELECTRICAL REQUIREMENTS**
- **OPERATING THE INSTRUMENT**
- **BASIC PRINCIPLES**



MIRIS HMA™ 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 (www.MirisSolutions.com).

S1 THE INSTRUMENT

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

Figure 1. Miris HMA™ front.

Figure 2. Miris HMA™ back.

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

IN- AND OUTLETS

Cuvette Pressure Guard (CPG)
The Cuvette Pressure Guard, CPG, protects the cuvette from being exposed to harmful pressures at injection both via the blue inlet and the outlet. Fluid injected through the CPG at pressures exceeding 3 bar, will be diverted via the valve at the side of the CPG and will not pass through the cuvette. The CPG parts are given in Figures 3 to 5.

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 valve.

Inlet and outlet CPG

Figure 3.
Restrictor House

Figure 4.
Filter House Restrictor, O-ring, Inlet Filter

Figure 5.
Stop Cover House Restrictor, O-ring

The Miris HMA™ requires 12-20 V ⎓ , 100VA.

Do not use any other power supply than the one supplied by Miris, included in the instrument starting kit (Figure 6). Consider using UPS (uninterruptible power supply) protection to protect the instrument from power failures and power spikes.

Figure 6. Power supply and cable.

Description of how to correctly insert the power supply connector

1. Pull back the plastic cover.

Figure 7.

Figure 8.

2. Keep the cover pulled back while you insert the connector. 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.

Figure 9.

Figure 10.

INSTRUMENT PLACEMENT

The Miris HMA™ should be placed in an area free from dust, dirt, explosives, corrosive fumes, and extremes of temperature and humidity (see Technical specifications). 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. Keep the area around and beneath the instrument clean and free from dust. Always position the power supply so that it can easily be disconnected from the power grid.

The Miris HMA™ must only be used by personnel who has received training in using the instrument.

The start-up procedure
Follow this instruction for the first start-up after transportation or long time storage. The start-up procedure will take approximately 5 hours.

1. Before start-up, make sure the Miris HMA™ is completely adjusted to room temperature (20-30°C, 68-86°F) before switching the power on.
2. Switch the power on. The Miris HMA™ licenced application software will start automatically and after an initial warming period the display will read “Ready - Press a button”, see Figure 11.
3. Allow the system to warm up completely by waiting 30 minutes before proceeding.
4. Place the plastic tubes supplied with the instrument on the outlet port and on the valves.
5. Inject 3 x 5 ml Miris Cleaner™ followed by 2 x 5 ml distilled or deionized water. Make sure the temperature of the liquids is within 35-40°C (95-104°F).
6. The instrument is now ready to use. See instructions in Chapter 2 for instructions on milk analysis with Miris HMA™.
7. The instrument will start up in Operator user mode, see log-in information in Chapter 7

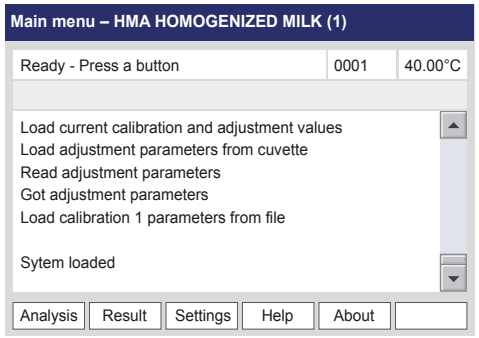


Figure 11.
Main menu.

The stand-by procedure
To leave the Miris 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 Miris HMA™ constantly turned on to keep the system stable. 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 in Chapter 9.

The Miris HMA™ 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. The instrument can also, however, be operated by 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 12 for a schematic drawing of the instrument's menu system.

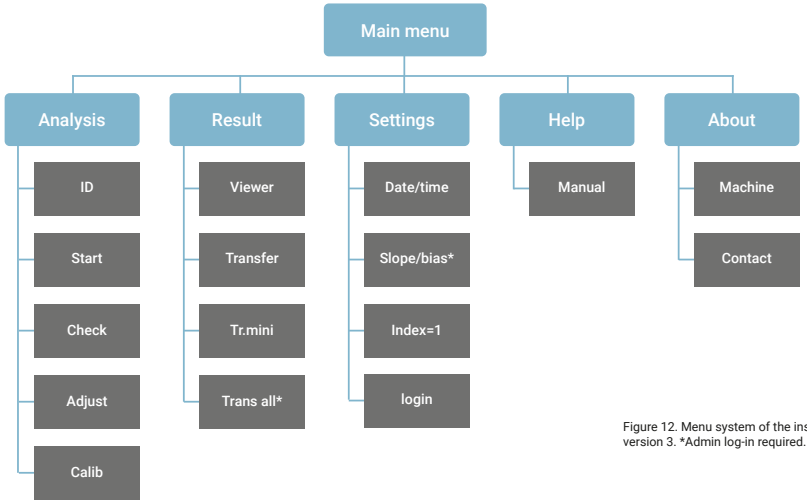


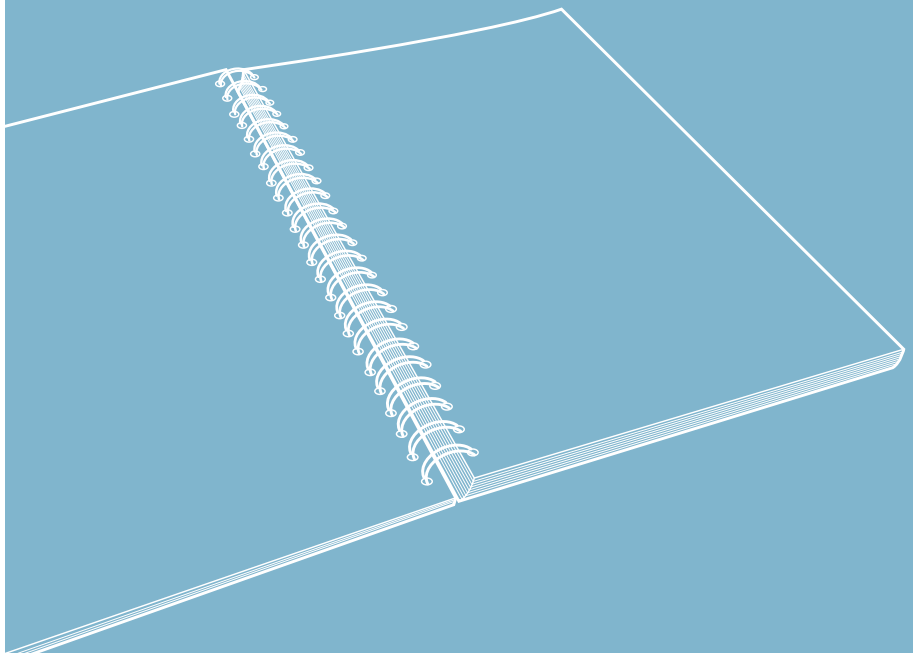
Figure 12. Menu system of the instrument, software version 3. *Admin log-in required.

Instrument identification
The serial numbers and product numbers of the Miris HMA™ can be found on the instrument label found on the instrument underside. This information is also available in the menu 'About' in the application software, by pressing 'Machine'. The installed version of the application software is also specified here, printed on line 6 'Application software'.

MIRIS HMA™ USER GUIDE

Chapter 2 DAILY START-UP PROCEDURES AND HUMAN MILK ANALYSIS

→ **STANDARD OPERATING PROCEDURE (SOP)**



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Chapter 2 DAILY START-UP PROCEDURES AND HUMAN MILK ANALYSIS

This chapter describes routines for human milk sample analysis with the Miris HMA™.

For the first start-up of a new instrument, refer to the start-up procedure in Chapter 1. Follow the cleaning instructions in Chapter 3, zero-setting check procedure in Chapter 4 and the instructions for milk sample preparation in Chapter 5 accordingly.

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 13. Leave some of the fluid in the syringe to avoid introduction of air into the cuvette, as this may lead to inaccurate results.

Figure 13.
Leave the syringe on the inlet during the measurement.

STANDARD OPERATING PROCEDURE (SOP)

Equipment	Consumables
Miris HMA™ Ultrasonic processing device, Miris Ultrasonic Processor or equivalent device Heating bath at 40°C (104°F) Thermometer (not provided by the manufacturer) **Note!** All solutions must be 35-40°C (95-104°F) when injected into the instrument.	Miris Check™ Miris Cleaner™ Distilled/deionised water (not provided by the manufacturer) Syringes, 2 ml and 5 ml Waste container (not provided by the manufacturer) Emery cloth for the ultrasonic processing device Miris Calibration Control Kit™

For detailed instructions, go to the specific chapters referenced in this SOP.

For further details on use and handling of Miris Ultrasonic Processor, refer to instructions in the Miris Ultrasonic Processor 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, or in a cold water bath.

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 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 instrument and immediately analysed.

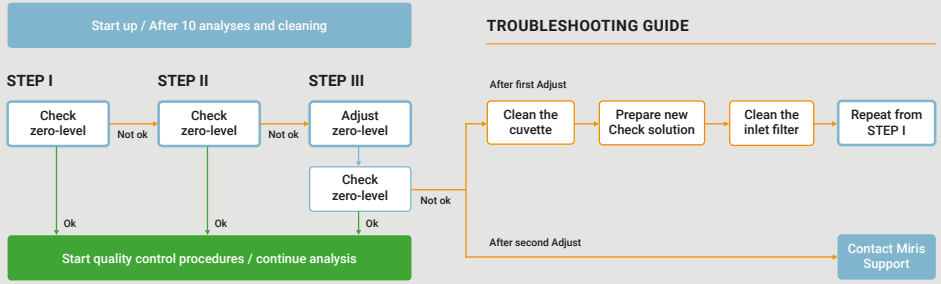
A new sample withdrawal and injection needs to be made for each analysis.

Avoid multiple sample container transfers.

Injection technique:
Make sure liquids are injected with correct pressure. Too high pressure (>3 bar) will divert the liquid via the valve at the side of the CPG (see Chapter 1, in- and outlets).

Table 1. Standard Operating Procedure A

A1. Set-up	Defrost frozen samples at room temperature or in a refrigerator or a cold water bath Turn on the heating bath to warm up to 40°C (104°F) Prepare working solutions of Miris Check™ and Miris Cleaner™ Pour the daily requirement of working solutions of Miris Check™ and Miris Cleaner™, and of distilled water, into separate bottles to use for the day's work Place bottles of distilled water, and working solutions of Miris Check™ and Miris Cleaner™ in the heating bath Turn on the Miris Ultrasonic Processor and the Miris HMA™ Place a waste container by the Miris HMA™ outlet Wait at least 30 minutes before proceeding to A2
A2. Instrument preparation	Select 'Analysis' in the main menu Control that the Miris Ultrasonic Processor probe is clean and that the tip is evenly polished
A3. Zero-setting check *Further information in Chapter 4* *Watch the 'Zero-setting check procedure'-video at MirisSolutions.com for details*	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 needed! (Repeat check twice before adjust)"**: 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 needed! (Repeat check twice before adjust)"**: 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 needed! (Repeat check twice before adjust)"**: repeat STEP III



Overview of the Miris HMA™ zero-setting check procedure with troubleshooting guide. 'Check zero-level' includes injection of Miris Check™ solution and starting the Check analysis. After completing the zero-setting check procedure, run control samples before any sample analysis.

Table 1. Standard Operating Procedure A, continued.

A4. Run control samples, calibration control *Miris recommend following national and local guidelines for testing of quality control materials*	**Sample Preparation, Miris Calibration Control Kit™** 1. Place one vial each of Miris Calibration Control™ 1 and Miris Calibration Control™ 2 in a heating bath 40° C (104° F) for 10-15 minutes 2. Mix the Miris Calibration Control™ 1 vial by 10 times gentle inversion 3. Open the vial and use a thermometer to check the temperature, 40°C (104° F) 4. Homogenize the Miris Calibration Control 1 sample for 12 seconds (1.5 s/ml) with the Miris Ultrasonic Processor **Analyse** 5. Inject 3 ml Miris Calibration Control 1 sample and analyse by pressing 'Start' - ensure there are no air bubbles or foam in the sample - ensure correct injection (sample not diverted via the safety valve) 6. Compare the results for fat, crude protein, true protein and carbohydrate with the reference values, see label on the Miris HMA front If the results are within the accepted range for Miris Calibration Control™ 1, repeat step 2-6 with Miris Calibration Control™ 2 If the results are within the accepted range for Miris Calibration Control™ 2, continue to step 7 If quality control results do not meet the defined acceptance criteria, patient values may be suspect. Follow the established quality control procedures at your laboratory. A satisfactory level of performance is achieved when the analyte values obtained are within the expected control intervals on label on the Miris HMA™ front. Follow your laboratory's quality control procedures if the results obtained do not fall within the acceptable limits. If an out of range warning message appears while running Miris Calibration Control™ 2 this can be ignored If unacceptable deviation remains, contact Miris at support@mirissolutions.com 7. The internal calibration is validated, the Miris HMA™ can be used for analysing milk samples. Continue to step A5 Sample preparation.
A5. Sample preparation *See Chapter 5 for correct collection and handling of milk samples*	Place the milk sample in the heating bath and use a thermometer to check sample temperature. Keep the sample in the heating bath for another 5-10 minutes after reaching 40°C (104°F) Homogenise the sample with the Miris Ultrasonic Processor (1.5 s/ml) Analyse immediately or keep the sample in the water bath until analysis, maximum another 20 minutes
A6. Analysis	Select 'Analysis' in the main menu If required, press 'ID' and enter a sample name using the internal keyboard or barcode reader, confirm by pressing 'OK' (see Chapter 7) Mix the sample thoroughly, by 10 times gentle inversion Withdraw a 3 ml sample by syringe - ensure there are no air bubbles or foam in the sample Immediately inject into the Miris HMA™, leaving about 0.5 ml in the syringe and the syringe on the inlet - ensure correct injection (sample not diverted via the CPG safety valve) 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 A6 steps above to do a replicate analysis (recommended) Repeat the A6 steps above for subsequent samples After 10 analyses, clean the Miris HMA™ (step A8) followed by a zero-setting check (step A3) before continuing analysing 

A7. 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 flash drive (FAT-32 formatted) connected to the instrument
A8. Cleaning *Full instructions in Chapter 3*	Clean the Miris HMA™ every 10th analysis, or if the instrument has been idle for 5 minutes with milk in the cuvette. **If continuing analysing samples, Miris HMA™ cleaning must always be followed by a zero-setting check (step A3)** Disassemble the inlet and outlet to clean the parts every 100th analysis, or once a week After each use of the Miris Ultrasonic Processor, wipe the probe clean with a dampened cloth and polish the probe tip with the emery cloth
A9. Stand-by mode *See Chapter 3, Chapter 1, and Chapter 9*	After cleaning at the end of the day the Miris 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 Discard any left-overs of the prepared daily requirements of Miris Check™, Miris Cleaner™, Miris Calibration Control Kit™ and distilled water At continuous daily use, leave the Miris HMA™ constantly turned on At long-time storage, inject fresh distilled or deionised water minimum every second week, make sure the system is closed

NOTE!

A warning message will appear if the measurement result is out-of-range, higher or lower than the instrument measuring range, for a particular parameter (see section Performance Characteristics).

If out-of-range, the measurement result cannot be regarded as with normal accuracy

Press OK

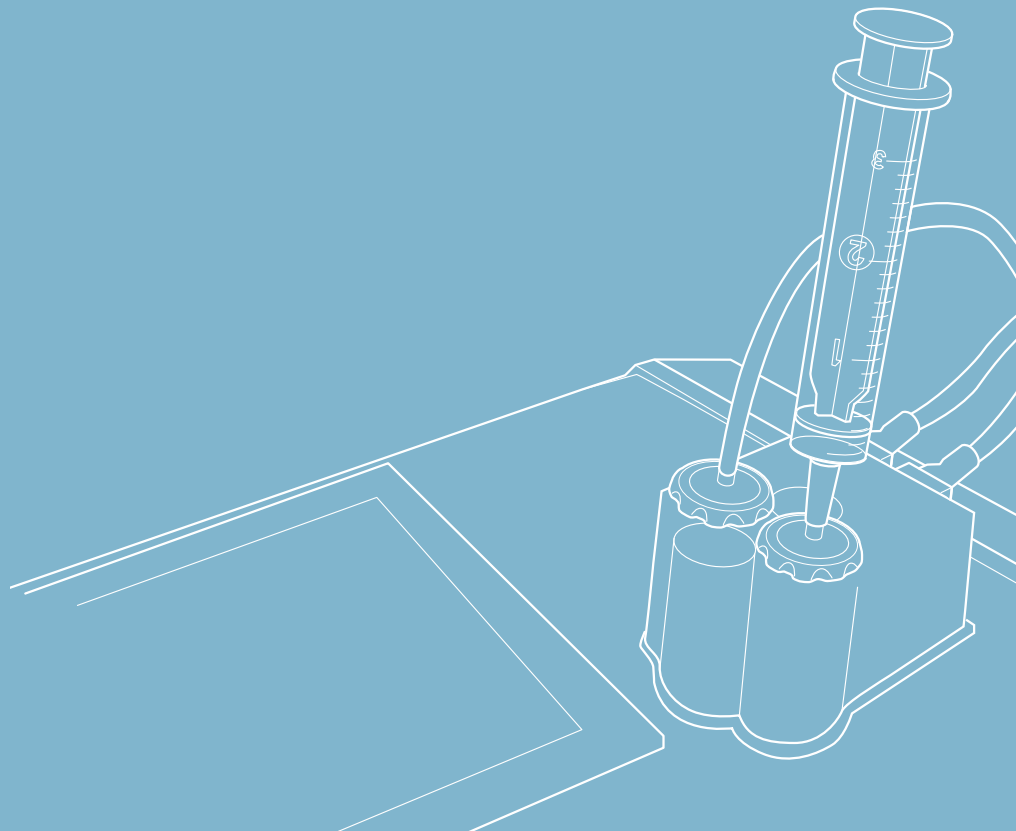
Reject the result

Out-of-range measurement results are available in the results file, marked with an asterisk (*)
(see further information in Chapter 6)

MIRIS HMA™ USER GUIDE

Chapter 3 CLEANING ROUTINES

- **CLEANING OF THE INSTRUMENT SURFACE**
- **CLEANING OF THE INSTRUMENT IN- AND OUTLET**



MIRIS HMA™ USER GUIDE

Chapter 3 CLEANING ROUTINES

NOTE!

All liquids must be 35-40°C (95-104°F) 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 has been idle for 5 minutes with** milk or Miris Calibration Control Kit solutions 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.

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 warmed to **35-40°C (95-104°F)** 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 warm temperature.

All volumes specified in this chapter are minimum volumes, if necessary the volumes can be increased. Figure 14 shows a cross section of a cuvette which explains the importance of a correct cleaning routine. For hygienic reasons, it is recommended to keep the daily required volume of consumables in separate bottles, and to discard any left-overs at the end of the day.

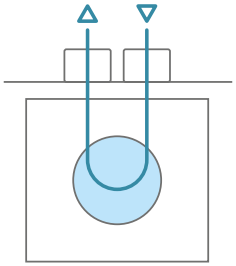


Figure 14. Schematic drawing of a cuvette and in- and outlets in cross section. The cuvette comprises of two windows separated by a spacer (50 µm) which needs several injections of Miris Cleaner™ to completely rinse any milk residues.

An instruction video of how to perform cleaning of the in- and outlet is available at www.MirisSolutions.com

CLEANING OF THE INSTRUMENT SURFACE

Clean the surface of the instrument using a cloth dampened with Miris Cleaner™. Use a mild disinfectant if necessary. Keep the area around and beneath the instrument free from dust.

CLEANING OF THE INSTRUMENT IN- AND OUTLET

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 valve 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 outlet, reverse fluid direction, with pressure so the fluid is diverted via the rear valve 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.

Figure 15.

Figure 16.

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 outlet, reverse fluid direction, with pressure so the fluid is diverted via the rear valve 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 valve 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. At continuous daily use leave the instrument constantly turned on. For longer storage periods, follow the storage instructions in Chapter 9.

Cleaning of the CPG – 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.

1. Remove the Filter House Restrictor and Stop Cover House Restrictor. Clean the parts using Miris Cleaner™ and rinse with distilled/deionised water. Carefully remove the gasket and filter. The filter is the most important part to clean. Use Miris Cleaner™ and a small brush.

Figure 17.

Figure 18.


Figure 19.


Figure 20.

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

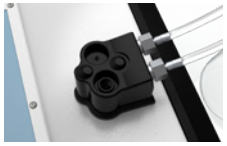


Figure 21.

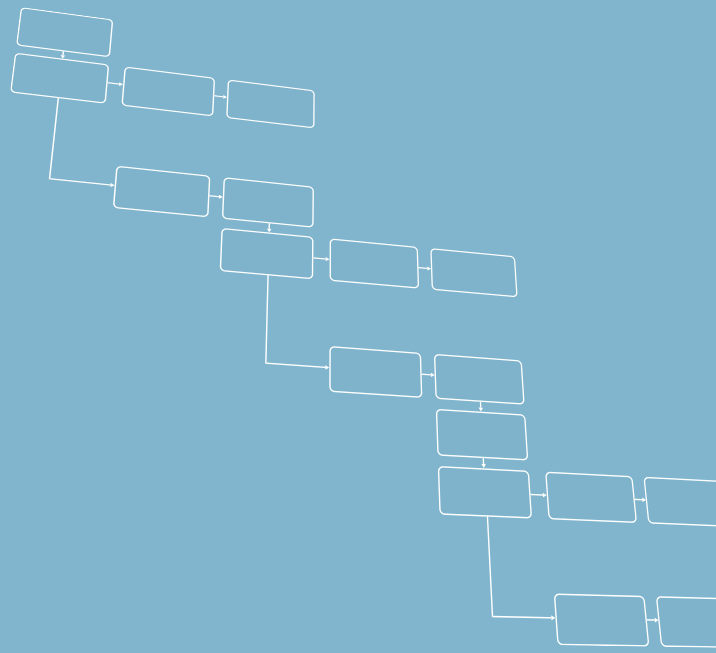
3. Reassemble the Filter House Restrictor and Stop Cover House Restrictor and attach to the instrument.
4. Put back the waste tubes and inject distilled/deionised water as described in Cleaning at the end of the day.


Figure 22.

Watch the 'Cleaning procedure CPG and filters'- video on MirisSolutions.com for further details.

MIRIS HMA™ USER GUIDE

Chapter 4 ZERO-SETTING AND CONTROL PROCEDURES



MIRIS HMA™ USER GUIDE

Chapter 4 ZERO-SETTING AND CONTROL PROCEDURES

GENERAL INFORMATION

The zero-setting check procedure with zero-level adjustment and the calibration control procedure are required controls for use of the Miris HMA™. Both procedures are described step-by-step in the Standard Operating Procedure (SOP) in Chapter 2 and are implemented in the Miris HMA™ workflow as described in figure 23.

Figure 23. Miris HMA™ workflow

The zero-setting check with zero-level adjustment (Figure 24b) is a validation of the zero-level of the internal calibration and must be performed at start-up and after cleaning the instrument if continuing analysing samples. This chapter provides further explanation of this function.

The calibration control, with analysis of control samples, is a validation of the instrument's internal calibration and must be performed at daily start-up before performing any sample analysis.

Miris recommend following national and local guidelines for testing of quality control materials.

Repeatability testing as described in this chapter is recommended to be routinely implemented by the user to ensure instrument stability.

ZERO-SETTING CHECK PROCEDURE WITH ZERO-LEVEL ADJUSTMENT

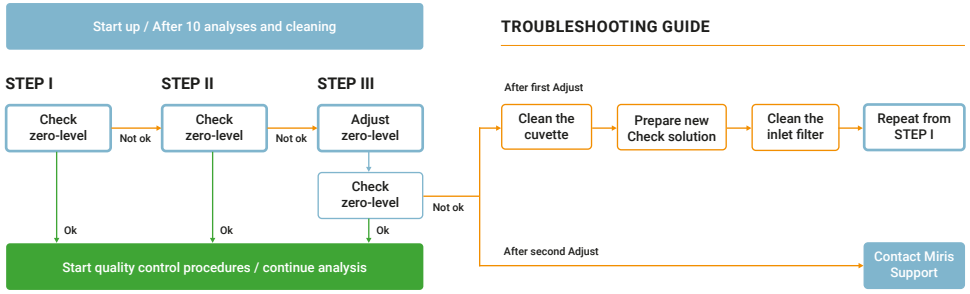


Figure 24a. Overview of the Miris HMA™ zero-setting check procedure with troubleshooting guide. 'Check zero-level' includes injection of Miris Check™ solution and starting the check analysis. After completing the zero-setting check procedure, run control samples before any sample analysis.

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 internal calibration and zero setting. The check procedure is described step-by-step in Figure 24b, an overview is given in Figure 24a.

If the test fails, the text **"Adjustment needed! (Repeat check twice before adjust)"** 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 Miris Check™ solution is not contaminated and that the inlet filter is clean.

The zero-setting check procedure takes approximately one minute and when the process is completed, a pass or fail message will appear. If the check passes ("Result g/100ml" is within ±0.05), the text "No adjustment necessary" is shown and the instrument is ready for analysis (see Figure 24c).

Zero-level adjustment
When the 'Adjust' key is pressed, the instrument adjusts the zero level of the internal calibration. This function must always be preceded by the zero-setting check procedure. Inject new Miris Check solution and press 'Check' again after the message "New adjustment done" appears. The zero-setting check procedure is completed when the message "No adjustment necessary" appears on the screen, as in Figure 24c.

Figure 24b. Miris HMA™ check procedure with zero level adjustment. To be performed at start-up, and after cleaning the Miris 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' is shown on the instrument display. Watch the 'Zero-setting check procedure'-video at MirisSolutions.com for a visual overview.

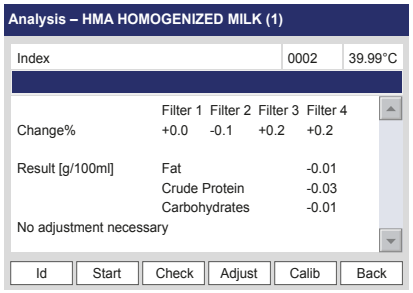
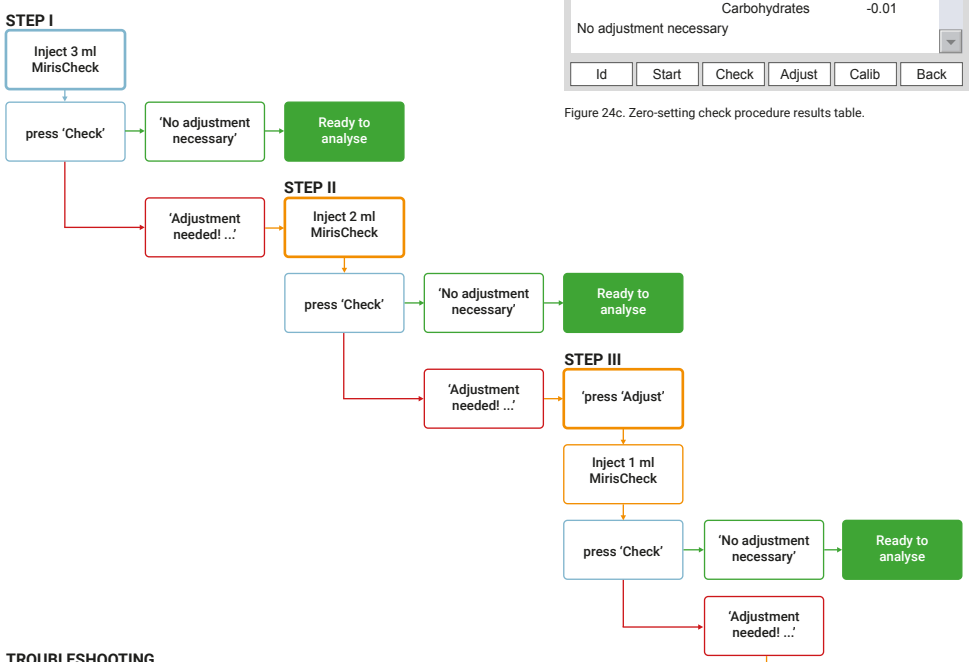
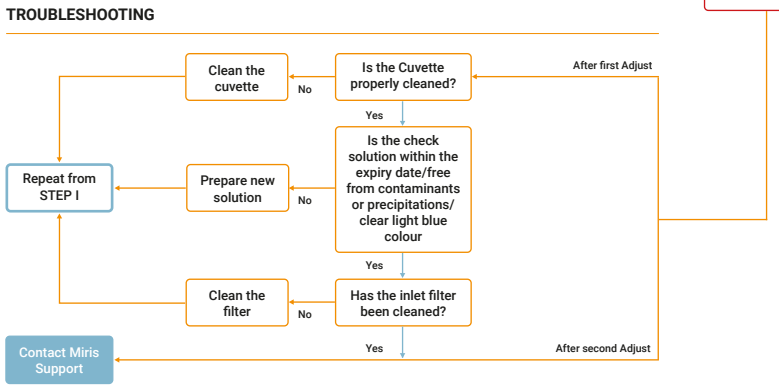


Figure 24c. Zero-setting check procedure results table.





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. 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@MirisSolutions.com) or your local distributor for an instrument check-up.

REPEATABILITY TEST

Recommended as a monthly control of instrument stability.
At least 10 replicates of standard solution or a representative uniform human milk sample are analysed.

Procedure
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 of the sample in a 40°C (104°F) water bath (20 min)
- Mix carefully and homogenize the milk with Miris Ultrasonic Processor (1.5 s/ml)
- Analyse 10 replicates of the sample on the Miris HMA™, injecting 3 ml for each analysis
- Clean the Miris HMA™

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

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

Instructions on how to calculate standard deviation using Microsoft Excel are given in Chapter 6 Result review.

Repeatability SD should meet ≤ 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@MirisSolutions.com).

MIRIS HMA™ USER GUIDE

Chapter 5 MILK COLLECTION AND HANDLING

MIRIS HMA™ USER GUIDE

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 Miris HMA™ analysis, as well as references to more information.

COLLECTION AND SAMPLING

Milk collection for analysis should be performed under standardised 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 Miris 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% mass/mass 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 Miris 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 is not 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 (-4°F) 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 summarised in Table 3.

DEFROSTING/THAWING

Frozen milk should be defrosted slowly overnight in a refrigerator (4°C, 39°F). Smaller sample volumes (<200 ml) may also be defrosted at room temperature (20-30°C, 68-86°F), 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, 68-86°F) 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 type	Location	Temperature	Max recommended storage duration	Comment
Preserved/unpreserved fresh/thawed milk	Room temperature	max 30°C (86°F)	2 hours	
Unpreserved fresh/thawed milk	Refrigerator	max 4°C (39°F)	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 (39°F)	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 (-4°F)	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 (-4°F)	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 (104°F)	20 minutes after reaching 40°C (104°F)	

WARMING

Samples should be warmed in a 40°C (104°F) water bath prior to analysis. The entire sample should be 40°C (104°F) and the Miris HMA™ will also operate at 40°C (104°F). Never warm the milk using a microwave oven because of the uneven temperature distribution, which may cause hot-spots and protein denaturation.

40°C (104°F)

ULTRASONIC HOMOGENISATION (SONICATION)

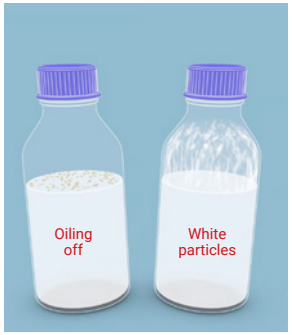
Milk is an oil-in-water emulsion and fat separation (creaming, oiling-off) and protein aggregation are processes common in stored, particularly frozen, milk. This can be due to a slow freezing process or long storage time (age gelation) of the milk. For the reduction of such effects, rapid freezing is 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 Miris HMA™ and fat separation will make representative sampling difficult.

The Miris HMA™ Calibration 1 is adapted to homogenisation by Miris Ultrasonic Processor (or equivalent device), 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 Miris HMA™. The Miris Ultrasonic Processor 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, 68-86°F) or overnight at 4°C (39°F). In order to obtain high homogenisation efficiency, the milk should be pre-warmed to 40°C (104°F) and homogenised 1.5 s/ml using Miris Ultrasonic Processor.

SAMPLE QUALITY

Good physical quality of the milk is essential for an accurate Miris 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 Miris HMA™ analysis might not be accurate and the sample should be discarded.



SAMPLE PRETREATMENT FOR MIRIS HMA™ ANALYSIS

Calibration 1: Homogenized milk
Defrost the milk sample if necessary. Heat the milk in a 40°C (104°F) water bath. Homogenize the sample 1.5 s/ml using Miris Ultrasonic Processor (or equivalent device). 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 a sample is drawn for analysis. Draw a milk sample by syringe, inject immediately into the Miris 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. This also includes handling and disposal of consumables such as syringes and tubes and re-usable parts such as waste containers.

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: https://neo.barnlakarforeningen.se/wp-content/uploads/sites/14/2014/03/Guidelines-2017-English.pdf

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

MIRIS HMA™ USER GUIDE

Chapter 6 RESULT REVIEW

- **VIEW LAST RESULTS**
- **TRANSFER THE RESULTS**
- **DATA INTERPRETATION**

MIRIS HMA™ USER GUIDE

Chapter 6 RESULT REVIEW

This chapter describes how results can be shown on the instrument display, saved on a FAT-32 formatted USB flash drive 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 screenshot of the result, press button 1 and 6 simultaneously. The buttons 2-5 will turn off momentarily to acknowledge this. The screenshot is saved as a png-file to the USB flash drive. Note that if no USB flash drive is connected, an error message appears.

NOTE!

The result presented on the instrument display is the origin measurement value.

Out-of-range measurement results, higher or lower than the instrument measuring range for a particular analyte (see section Performance Characteristics), are not presented in the Result Viewer.

Out-of-range measurement results are presented on the instrument display at analysis and can also be obtained after transfer of the results, where such results are marked with an asterisk (*).

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 and resets appear 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 25). The results will be presented in a table, scroll to see all information.

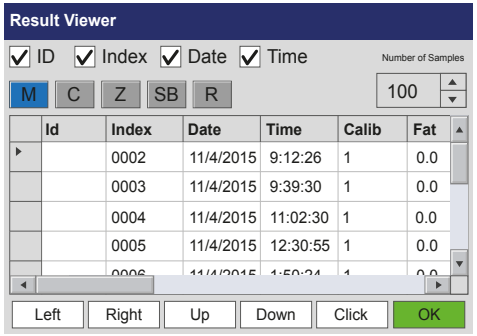


Figure 25. Results viewer screen.

Press M to see performed measurements

Press C to see performed zero-setting checks

Press Z to see performed zero-level adjustments

SB Not available

Press R to see when the instrument has been reset

TRANSFER THE RESULTS

Transfer the results to a USB flash drive
Connect a USB flash drive (FAT-32 formatted).

Open the menu 'Results' and press 'Transfer'. Two results files will automatically be saved on the flash drive 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' (Admin users only). They transfer a minilogg.txt file and the content in the 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 flash drive connected, the message "No USB memory detected" will be shown. Connect a USB flash drive and press 'Transfer' again.

Results in the results files marked with an asterisk (*) indicate that at least one analyte was measured higher or lower than the instrument measuring range (see section Performance Characteristics). Out-of-range measurement results cannot be regarded as with normal accuracy and should be rejected.

DATA INTERPRETATION

Miris recommends the use of Microsoft Excel to process data for i.e. repeatability test or replicate samples control. Since the result file is saved in txt-format a conversion is needed, see below.

	A	B	C	D	E	F	G	H	I	J
1	index	date	time	Calibratio	fat	crude protein	carbohydrates	ts	energy	true protein
2	MIndex0001	11/9/2016	11:46:56 AM	1	4,3	2,1	7,2	13,8	78	1,7
3	MIndex0002	11/9/2016	11:48:23 AM	1	4,2	2,0	7,3	13,7	77	1,7
4	MIndex0003	11/9/2016	11:50:08 AM	1	4,3	2,0	7,2	13,7	77	1,7
5	MIndex0004	11/9/2016	11:51:58 AM	1	4,3	2,0	7,2	13,7	77	1,6
6	MIndex0005	11/9/2016	11:53:10 AM	1	4,3	2,0	7,2	13,8	78	1,6
7	MIndex0006	11/9/2016	11:54:15 AM	1	4,3	2,1	7,3	13,8	78	1,7
8	MIndex0007	11/9/2016	11:55:17 AM	1	4,3	2,1	7,3	13,9	78	1,7
9	MIndex0008	11/9/2016	11:56:24 AM	1	4,3	2,0	7,2	13,7	77	1,7
10	MIndex0009	11/9/2016	11:57:35 AM	1	4,3	2,1	7,2	13,8	78	1,7
11	MIndex0010	11/9/2016	11:58:38 AM	1	4,3	2,1	7,3	14,0	79	1,7
12	MIndex0011	11/9/2016	11:59:57 AM	1	4,3	2,1	7,2	13,8	77	1,7
13	MIndex0012	11/9/2016	12:01:02 PM	1	4,3	2,1	7,2	13,7	77	1,7

Figure 26. The result_meas file opened in Excel.

Converting a text file to Excel format

1. Open Microsoft Excel
2. Click on the Data tab
3. In the Get External Data group, click From Text.
4. Select 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.

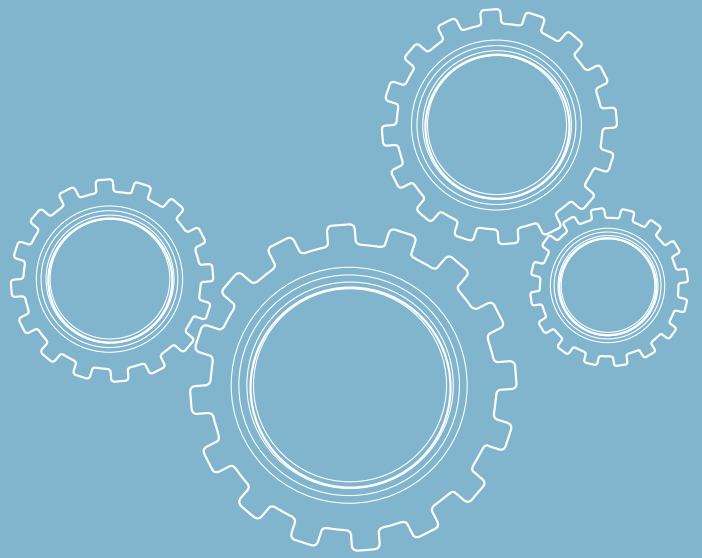
Interpret results from a repeatability test
Open the result data in Excel. Enter the formula =STDEV in an empty cell and mark the column with the parameter you wish to interpret.

Interpret the results from a replicate samples control
Open the result data in Excel. Enter the formula =AVERAGE in an empty cell and mark the column with the parameter you wish to interpret.

MIRIS HMA™ USER GUIDE

Chapter 7 SETTINGS

- CHANGE DATE AND TIME
- RESET THE INDEX NUMBER
- IDENTITY OF SAMPLE
- LOG-IN
- CHANGE USER LEVEL



MIRIS HMA™ USER GUIDE

Chapter 7 SETTINGS

This chapter describes how to change date and time, how to reset the index number and how to assign samples with an ID of your choice, and how to change user log-in-levels. 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 using the buttons. 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'.

LOG-IN

The software has a log-in function with user ID and password. There are three user levels giving different access to the instrument's functions. Default user is Operator. It is not possible to change passwords. The Admin password can only be acquired from the manufacturer.

User ID	Main menu access	Available functions	Password (case sensitive)
GUEST	Analysis About	'ID' – change sample ID 'Start' – perform an analysis 'Machine' – instrument information 'Contact' – MIRIS or distributor contact information	Guest
OPERATOR	All	Access to all functions except 'Slope/Bias', 'TransAll'; these functions are NOT available	Operator
ADMIN	All	Access to all functions	Contact Miris support@MirisSolutions.com

CHANGE USER LEVEL

At initial startup, the instrument will be in Operator user mode.

1. Under 'Settings' press 'Login' to open the login screen, see figure 27.
2. Click the desired rights-box
3. Turn the on-screen keyboard on
4. Type in the password and turn the keyboard off. The password is case sensitive.
5. Press "OK"
6. The logged-in user level is confirmed by a message at the top of the screen, see figure 28.

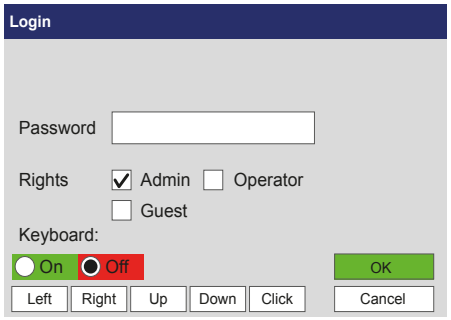


Figure 27. The Log-in screen.

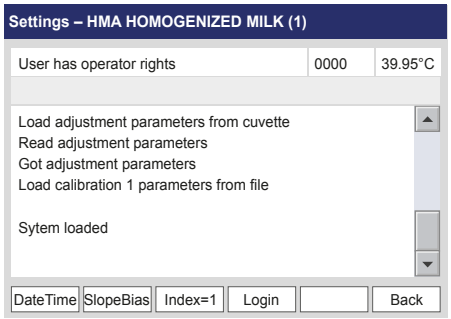


Figure 28. The Log-in as operator successful.

To check the current user level at any time, press 'Settings' and 'Login' and see which rights box is ticked.
If an action is selected that is not allowed at the current user level, a message will appear at the top of the screen (Figure 29-30)

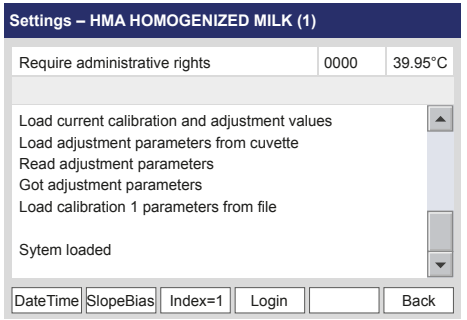


Figure 29. Unauthorized action selected.

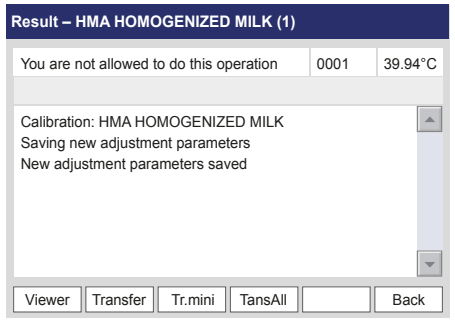


Figure 30. Unauthorized action selected.

MIRIS HMA™ USER GUIDE

Chapter 8 CONSUMABLES AND SPARE PARTS

- MIRIS CLEANER™
- MIRIS CHECK™
- MIRIS CALIBRATION CONTROL KIT™
- DISTILLED OR DEIONISED WATER
- SYRINGES
- SAMPLE TUBES
- SPARE PARTS

MIRIS HMA™ USER GUIDE

Chapter 8 CONSUMABLES AND SPARE PARTS

REQUIRED CONSUMABLES

S10 Miris HMA™ requires Miris Cleaner™, Miris Check™, Miris Calibration Control Kit™, and distilled or deionised water to function properly. Contact Miris to obtain safety data sheets for these products.

All liquids must be 35-40°C (95-104°F) when injected into the instrument. Sudden temperature changes may cause irreparable damage to the cuvette not covered by the product warranty.

Miris Cleaner™

Description: Cleaning agent (liquid concentrate) for Miris HMA™.

Application: Only for cleaning of Miris instruments. For in vitro diagnostic use.

Storage: Store the concentrate dark, at room temperature (20-30°C, 68-86°F), sealed in the container in which it is supplied. Store the diluted solution in glass or plastic containers, out of direct sunlight. Keep away from foodstuff and animal feed.

Instructions: Dilute 50 ml concentrate (1 tube) with 950 ml distilled or deionized water and mix. Warm to 40° C before use and follow the instructions in Chapter 3.

The estimated time for preparation is 20 minutes for mixing the ingredients and to warm the solution to 40°C (104°F). The ready to use dilution should have no colour and faint odour (soapy, chemical). Cleaning solution to be used after every 10th sample, when the instrument is idle for 5 minutes or end of day and weekly cleaning.

Materials provided:
Liquid concentrate in 50 ml containers

Materials required but not provided:
1000 ml glass or plastic container
950x5 ml distilled or deionized water
Heating bath at 40°C (104°F)

Do not use if the liquid is cloudy or precipitations are visible.

Use before: Expiry date for unopened tubes is stated on the product label. Use solution within 3 months of preparation.

Environment and health: Small spillages may be flushed away with plenty of water to a drain or sewer. Miris Cleaner™ is biodegradable. Ready biodegradability by OECD 301E (ISO method 7287 - 1986(E)).

Non-hazardous. May be mildly irritating on the skin, in the eyes and in the respiratory system. In case of contact with eyes, rinse opened eye for several minutes under running water. Keep eyelids apart. Remove contact lenses if present. Consult a doctor if symptoms persist. In case of contact with skin, rinse with soap and water. If ingested, rinse the mouth with running water. Drink a few glasses of water. Consult a doctor if symptoms persist.

Miris Check™

Description: Zero-setting solution (liquid concentrate) to use with Miris HMA™.

Application: Only for quality control of the preset internal calibration of the Miris HMA™. For in vitro diagnostic use.

Storage: Store the concentrate dark, at room temperature (20-30°C, 68-86°F), sealed in the container in which it is supplied. Store the diluted solution in glass or plastic containers, out of direct sunlight. Keep away from foodstuff and animal feed.

Instructions: Dilute 10 ml concentrate (1 tube) with 90 ml distilled or deionized water and mix. Warm to 40°C (104°F) before use. The estimated time for preparation is 15 minutes for mixing the ingredients and to warm the solution to 40°C (104°F). The solution is to be used when preparing the instrument for analysis and also after cleaning if analysis is continued.

For use, follow the standard operating procedure in Chapter 2 or the instructions in Chapter 4.
The ready to use dilution should have light blue colour and no odour.

Materials provided:
Liquid concentrate in 10 ml containers

Materials required but not provided:
100 ml glass or plastic container
90x5 ml distilled or deionised water
Heating bath at 40°C (104°F)

Do not use if the blue colour has faded, the liquid is cloudy or precipitations are visible.

Use before: Expiry date for unopened tubes is stated on the product label. Use solution within 3 months of preparation.

Environment and health: Safety Data Sheet available on request. Miris Check™ concentrate is classified as not harmful to the environment, however do not empty into drains. Small spillages of diluted concentrate may be flushed away with plenty of water to a drain or sewer. The product is irritant for eyes and causes pain. May mildly irritate skin and cause itching. In case of contact with eyes, rinse opened eye for at least five minutes under lukewarm running water. Keep eyelids apart. Remove contact lenses if present. Consult a doctor if symptoms persist. In case of contact with skin, rinse with soap and water. If ingested, rinse the mouth with running water. Drink a few glasses of water. Consult a doctor if symptoms persist.

Miris Calibration Control Kit™

Description: Control material for Miris HMA™. Includes standardised solutions in two concentrations, Miris Calibration Control™ 1 and Miris Calibration Control™ 2.

Application: Only for quality control of the preset internal calibration of Miris HMA™.
For professional use only. For in vitro diagnostic use only.

Storage: Store unopened vials dark, at room temperature (20-30° C, 68-86° F). Do not freeze.

Instructions: Prone to sedimentation and separation, mix thoroughly but gently. Vials are opened by carefully flipping the cap upwards to remove the seal. Estimated time for preparation is 15 minutes, to warm the solution to 40° C (104° F) and to homogenise using an ultrasonic processing device before use. The control material is to be used daily before any milk sample analysis, follow the standard operating procedure in Chapter 2. The solutions should have an even creamy-white colour and a scent of boiled milk.

Materials provided:
Miris Calibration Control™ 1
Miris Calibration Control™ 2
Instructions for Use

Materials required but not provided:
Miris Ultrasonic Processor (or equivalent device)
with a 3 mm probe
Heating bath at 40° C (104°C)

Do not dilute or reconstitute. Do not use if precipitations are visible or the colour has changed.

Use before: Expiry date for unopened vials is stated on the product label. Use opened vials directly. Do not store opened vials to re-use the content.

Environment and health: Non-hazardous. Contains no substances which results in any labelling according to the current legislation. The product is not harmful at skin contact, ingestion or inhalation. Spillages may be flushed away with plenty of water to a drain or sewer.

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 Miris HMA™.

SYRINGES

For injection of liquids into the Miris HMA™, Miris recommends syringes Injekt Luer Solo (B Braun, Melsungen, Germany). These syringes are tested and approved by Miris to securely fit the Miris HMA™ inlet.

SAMPLE TUBES

Suitable milk sample tubes for the milk analysis process i.e. storage, warming and homogenization.

SPARE PARTS

Spare rubber gaskets, inlet filter and outlet tubes are supplied in the starting kit, and can also be obtained as spare parts. Contact order@MirisSolutions.com or your distributor.

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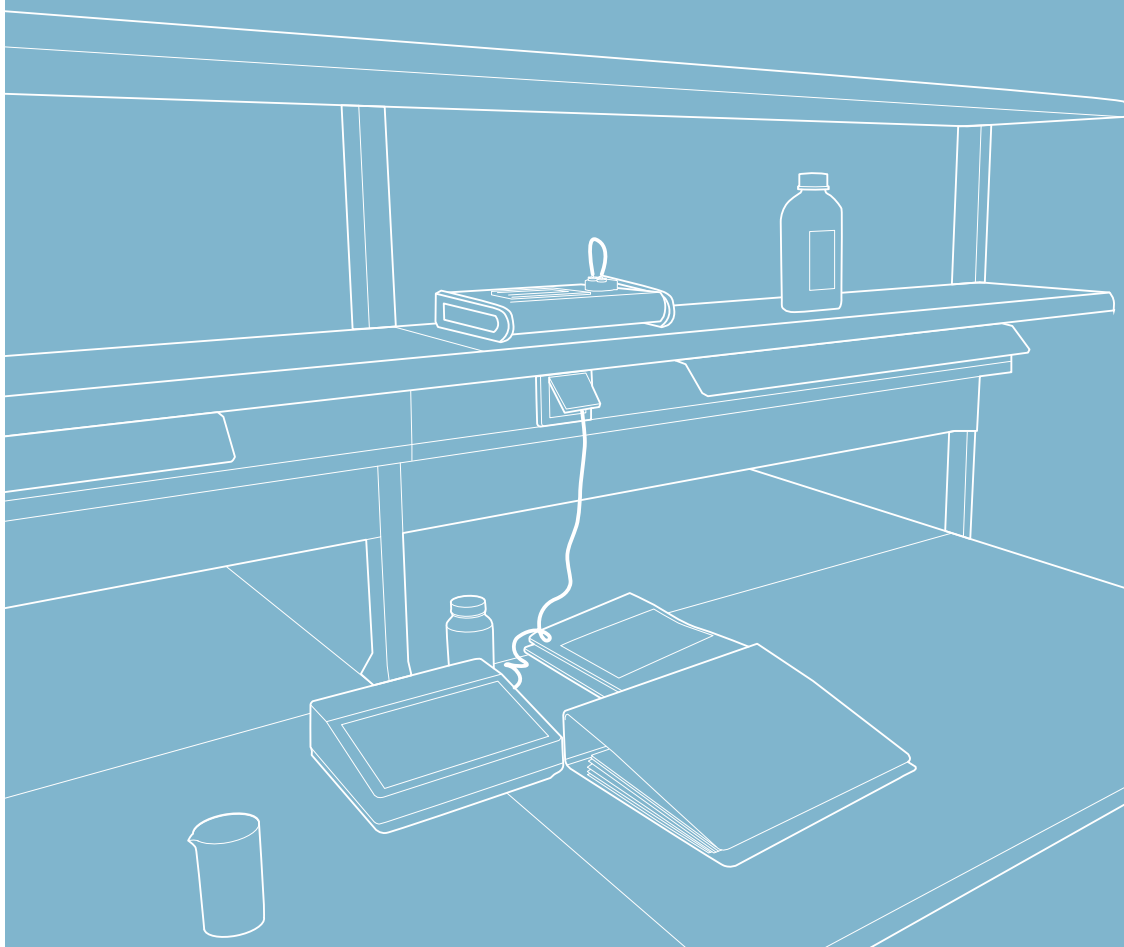
Chapter 9 STORAGE AND TRANSPORTATION



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. Empty the cuvette by injecting air using a new syringe. Empty both valves at the side of the CPG by applying gentle pressure at injection through the inlet and outlet respectively. Transportation must be careful, avoiding impacts, preferably using the box the instrument is delivered in. After transport, let the Miris HMA™ stand 4 hours in room temperature (20-30°C, 68-86°F) before switching the power on. Follow the start-up procedure instructions in Chapter 1.

Storage
Storage instructions only apply to instruments that have been in use, i.e. excluding new and unused instruments.
Store at normal temperatures (18-25°C). Always leave the instrument filled with distilled/deionised water when it is not in use and make sure the system is closed (Figure 31) when it is stored for longer periods.

Figure 31 Closed system CPG

At long-time storage, inject 2 x 5 ml of fresh distilled/deionised water minimum every second week. Pay attention to the temperature of the distilled/deionised water injected depending on if the Miris HMA™ is switched on or off:

- If the instrument is turned on the temperature of injected liquids must be 35-40°C (95-104°F)
- If the instrument is turned off the temperature of injected liquids must correspond to the ambient temperature

Watch instruction videos on how to prepare Miris HMA™ for transportation and storage on MirisSolutions.com

MIRIS HMA™ USER GUIDE

Chapter 10 TROUBLESHOOTING AND PROBLEM SOLVING

MIRIS HMA™ USER GUIDE

Chapter 10 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 Miris HMA™. Software version can be found in the menu 'About', 'Machine'.

TROUBLESHOOTING

Table 3. Troubleshooting guide.

	Error	Cause	Action	If the error persists
Error message	"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@MirisSolutions.com) or your local distributor
Error message	"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@MirisSolutions.com) or your local distributor
Error message	"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@MirisSolutions.com) or your local distributor
Error message	"Data limited has been reached. Dumping data, please wait"	Minilogg file too big	Wait Oldest data is erased automatically	Contact Miris (support@MirisSolutions.com) or your local distributor
Error message	"Warning, out-of-range measurement on parameter..."	Measurement result is out-of-range, higher or lower than the instrument measuring range for a particular parameter (see section Performance Characteristics) Milk sample composition	The measurement result cannot be regarded as with normal accuracy Disregard the result Let the sample stand in the water bath 40°C a while longer. Homogenise the sample. Ensure that any foam disintegrates before taking a sample for injection	Control sample handling routines For concentrations beyond the range of the HMA, consider using a different method
Error message	"Error"		Restart the instrument with the on/off button	Contact Miris (support@MirisSolutions.com) or your local distributor

Table 4. Troubleshooting guide, continued.

	Error	Cause	Action	If the error persists
Error message	"Transmission change"	The transmission has changed 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@MirisSolutions.com) or your local distributor
Problem solving	Blocked fluids system	If the sample injection needs higher mechanical force than normal, this may be an indication of a dirty inlet filter	Do not force liquid through the system! Clean the filter, then reverse the fluid direction and rinse with Miris Cleaner™, see instructions in Chapter 3.	Remove filter housing and outlet valve using the spanner provided and clean the parts. Contact Miris (support@MirisSolutions.com) or your local distributor
Problem solving	No response when pressing the buttons or using the mouse		Turn off the instrument and restart it again.	Contact Miris (support@MirisSolutions.com) or your local distributor
Problem solving	Zero values for protein	May indicate protein aggregation or air in the sample	Let the sample stand in the water bath 40°C (104°F) a while longer. Homogenise the sample. Ensure that any foam disintegrates before taking a sample for injection	Control sample handling routines Contact Miris (support@MirisSolutions.com) or your local distributor
Problem solving	Low protein values (around 0.5-0.6 g/100 ml or lower)	May indicate protein aggregation or air in the sample	Let the sample stand in the water bath 40°C (104°F) a while longer. Homogenise the sample. Ensure that any foam disintegrates before taking a sample for injection Discard the sample, disregard the result	Control sample handling routines Contact Miris (support@MirisSolutions.com) or your local distributor
Problem solving	High variation in a parameter, high standard deviation, at repeat analysis of the same sample	May indicate poor sample homogeneity, fat separation, protein aggregation, air in the sample May indicate insufficient cleaning May indicate incorrect injection, see separate problem solving item below	Inspect the sample visually, see 'control of sample quality' in Chapter 5. Mix the sample thoroughly. Homogenise the sample. Ensure that any foam disintegrates before taking a sample for injection. Discard the sample. Clean the instrument.	Control sample handling routines and the overall quality of your samples. Control storage conditions and storage times of samples. See Chapter 5. Contact Miris (support@MirisSolutions.com) or your local distributor
Problem solving	Cuvette is not clean	Issues with the cuvette can occur if either there is milk left or if air bubbles has been introduced into the system.	Clean the cuvette in accordance with the procedure described in Chapter 3	Contact Miris (support@MirisSolutions.com) or your local distributor

Table 4. Troubleshooting guide, continued.

	Error	Cause	Action	If the error persists
Problem solving	Suspected deterioration of Miris Check™ solution	Contaminated, degraded or expired Miris Check™ solution	Inspect your Miris Check™ solution visually to see i) that the color of the solution is blue and ii) that there is no precipitation or particles in the solution. If any of these points occur, or if the solution has expired, discard the solution. Prepare new Miris Check™ solution.	Control sample handling routines and the overall quality of your samples. Control storage conditions and storage times of samples. See Chapter 5. Contact Miris (support@MirisSolutions.com) or your local distributor
Problem solving	Cuvette is blocked/ it is difficult to inject	May indicate insufficient cleaning of the filters in the in- and outlets or that the in- and outlets have not been put together correctly which can result in air being introduced into the cuvette or samples not coming trough the cuvette correctly.	Clean the filters in accordance with the procedure 'Cleaning of the CPG' described in Chapter 3. Ensure the probe is placed low in the sample vial while sonicating the milk sample.	Watch the video 'Cleaning Procedure CPG & filters' in Miris 'How-to' video guides om Miris web page www. MirisSolutions.com. Contact Miris (support@MirisSolutions.com) or your local distributor
Problem solving	Foamy sample	Probe is not placed right while sonicating a milk sample	Foaming has occured, place your sample in the heating bath until the foam has disapeard beore injecting the sample into the Miris HMA™.	Watch the video and 'Sample placement in Miris Ultrasonic Processor' in Miris Preparation 'How-to' video guides on Miris web page www.MirisSolutions.com. Contact Miris (support@MirisSolutions.com) or your local distributor
Problem solving	Incorrect injection	Sample injected is diverted through the side valve of the Cuvette Pressure Guard (CPG) instead of through cuvette and outlet	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 side valve.	Contact Miris (support@MirisSolutions.com) or your local distributor
Problem solving	Leakage of liquids on or underneath the instrument.	May be caused by insufficiently tightened inlet or outlet, worn-out rubber details in the in-/outlets, or may indicate a hardware error.	At leakage on instrument front - check in-/outlets and ensure they are clean and tightened properly. Replace rubber gaskets. At leakage underneath the instrument - stop using the instrument and contact Miris (support@mirissolutions.com) or your local distributor.	Contact Miris (support@mirissolutions.com) or your local distributor.

FAQ – FREQUENTLY ASKED QUESTIONS

Q. What routines should my site use?
A. Miris can only be of help setting up routines for proper use of the Miris 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. If during or after preparation of the test sample white particles are visible on the walls of the sample bottle, analysis might not be accurate and the sample should be discarded. See Chapter 5. You can also watch the 'Sample preparation for milk samples'-video on MirisSolutions.com

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 11.

Q. How do I clean the filters?
A. Clean the filters in accordance with the procedure 'Cleaning of the CPG' described in Chapter 3. You can also watch the video 'Cleaning Procedure CPG & filters' in Miris 'How-to' video guides om Miris web page www.MirisSolutions.com

Q. What kind of service has to be done and how often?
A. Miris recommends yearly maintenance service of the instrument, contact Miris at support@MirisSolutions.com.

Miris may need to provide user updates, fixes or patches to control cybersecurity risks. If required, Miris will contact the customer for further instructions.

Q. How can the instrument be maintained in a proper way?
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. 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@MirisSolutions.com) or your local distributor for an instrument check-up.

Q. Who can perform service?
A. Service can only be performed by an authorized service technician. Ask your local distributor or contact Miris (support@MirisSolutions.com).

MIRIS HMA™ USER GUIDE

Chapter 11 HUMAN MILK COMPOSITION

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.

The following tables illustrate the variation in macronutrients present in human milk. The information is based on meta-analysis results from the literature. The tables are not intended to be used as expected values.

Data from a meta-analysis of 41 studies on the nutrient content of human milk [5], term (37-42 weeks of gestation) and preterm (<37 weeks of gestation), are presented in Table 5 and Table 6. 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 7.
Note that the Miris HMA™ measures the total carbohydrate content, i.e. including the content of lactose and oligosaccharides.

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 and the factor 0.8 to estimate true protein from crude protein.

Table 5. Meta-analysis results of the macronutrient composition of term (37-42 weeks of gestation) human milk [5].

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 6. Meta-analysis results of the macronutrient composition of preterm (<37 weeks of gestation) human milk [5]

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 7. 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
[6]	3.2	1.0	1.2	0.5	-		7.8	0.9	65	9
[7]	3.6		0.9		-		7.2		67	

REFERENCES

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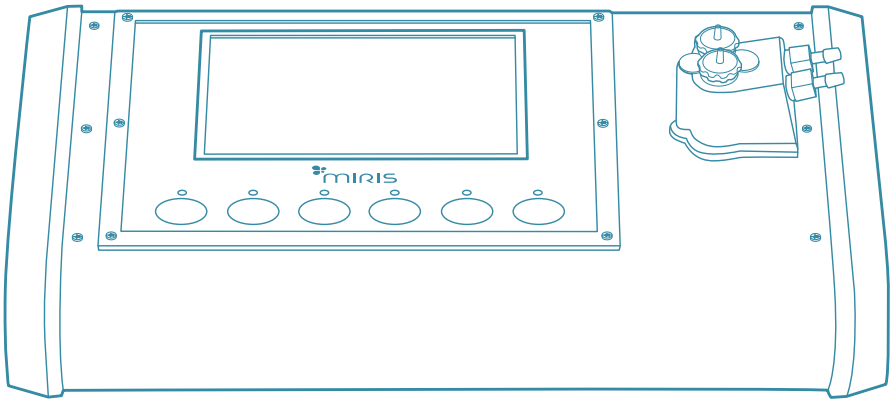
[6] K. Wojcik, D. Rechtman, M. Lee, A. Montoya, and E. Medo. "Macronutrient analysis of a nationwide sample of donor breast milk". J Am Diet Assoc, vol. 109, pp. 137-140, 2009.

[7] 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 HMA™



TECHNICAL SPECIFICATIONS

Table 10. Technical specifications.

	Dimensions (HxWxL)	11 x 26 x 31 cm	
	Weight	3.8 kg	
S9	**Power supply unit**	Input voltage 100-240 V ~ 50/60 Hz, 2.3A	
	Power supply Instrument	Normal input 18V DC 60VA	
	Mains supply voltage fluctuations	Max variations in mains voltage +/- 10 %	
	Overvoltage category	Overvoltage category II	
	Battery	Li-ion battery to keep date and time (lifetime > 5 years)	
S5	**PC connections**	USB A for flash drive and devices e.g. keyboard, mouse, scanner, etc. RS232	
	Display	TTFT QVGA 320*240	
	Sample temperature	+35°C (95°F) to +40°C (104°F)	
S5	**Internal Storage capacity**	4000 measurements	
	Backup of measurement data	Internal persistent flash memory	
	Operative system	Windows Compact 7 or later	
	Measurement performance	Repeatability (CV): Fat, crude protein, true protein ≤ 5% at concentration < 1 g/100 ml ≤ 3% at concentration > 1 g/100 ml Carbohydrate, total solids, energy ≤ 3%	
S7		Accuracy: Fat, crude protein, true protein, total solids, energy ±10%, Carbohydrate ±13%	
S3	**Components tested**	Fat [g/100 ml], Crude protein [g/100 ml], True protein [g/100 ml], Carbohydrate [g/100 ml]	
S4	**Components calculated**	Total solids (TS) [g/100 ml], Energy [kcal/100 ml]	
S6	**Measuring range**	Fat 0.6 - 6 g/100 ml, Crude protein 0.8 - 3 g/100 ml, True protein 0.6 - 2.4 g/100 ml, Carbohydrate 6.6 - 8.7 g/100 ml	
	Shown value	1 decimal	
S8	**Time for analysis**	Approximately one minute / measurement	
S2	**Analytical method**	Mid-infrared transmission spectroscopy	
	Standards	CE marked in accordance with 98/79/EC IVDD	
	ENVIRONMENTAL	OPERATIONAL	NOT OPERATIONAL (freight empty cuvette)
	Temperature	+20°C (68°F) to +30°C (86°F)	0°C (32°F) to +50°C (122°F)
	Humidity	20-80% not condensed	
	Altitude	Max operational altitude 2000 m	
	Ambient air pressure	600 - 1100 mbar	
	Pollution degree of intended environment	Pollution degree 2	

PERFORMANCE CHARACTERISTICS

Table 11. Performance characteristics of the Miris HMA™. Contact Miris for more information.

Components measured*	Fat, crude protein, true protein, carbohydrate
Components calculated	Total solids, energy
Analysis time	Approximately one minute
Measuring range	Fat 0.6 - 6 g/100 ml Crude protein 0.8 - 3 g/100 ml True protein 0.6 - 2.4 g/100 ml Carbohydrate 6.6 - 8.7 g/100 ml
Repeatability CV	Fat, crude protein, true protein ≤ 5% at concentration < 1 g/100 ml ≤ 3% at concentration > 1 g/100 ml Carbohydrate, total solids, energy ≤ 3%
Reproducibility CV	Fat, crude protein, true protein ≤ 9% at concentration < 1 g/100 ml ≤ 6% at concentration > 1 g/100 ml Carbohydrates, total solids, energy ≤ 6%
Accuracy †	Fat, crude protein, true protein, total solids, energy ±10% Carbohydrate ±13%
Carry-over# at a sample injection volume of ≥ 3 ml	< 2 %

* All components are analysed simultaneously
† Under stipulated experimental conditions and single analysis when standard methods used are Röse-Gottlieb for fat [8], Kjeldahl for crude protein [9], carbohydrate value calculated by difference [15] from oven drying method for total solids [10]. Contact Miris for more information.
# Carry over-effect of previous sample on current sample [14].

SUMMARY OF BENCH TESTING

This section contains summaries of performance studies on Miris HMA™, by standard methods of the CLSI (Clinical and Laboratory Standards Institute) and ISO (International Organization for Standardization).

The evaluated characteristics include precision, trueness in terms of bias, linearity, detection capability, interference, and carry-over.

MIRIS HMA™ PRECISION STUDY

Objective
Determine repeatability and reproducibility of the Miris HMA™ for measuring fat, crude protein, true protein, and carbohydrate content in a human milk sample, following the CLSI EP05-A3 standard [17]. The calculated variables total solids and energy were also evaluated.

Study design
Precision was tested in two separate studies, one multicenter study at three sites, and one subsequent single-site study. Study design as described below, with one device per site.

Site type	Number of sites	Devices/site	Number of samples	Number of days	Runs/day	Replicates/sample/run
Multicenter main (A)	1	1	5	20	2	2
Multicenter secondary (B, C)	2	1	5	5	2	3
Single site (D)	1	1	5	20	2	2

At the multicenter main site (A), 5 human milk samples were tested on 1 device by 1 operator over 20 days, with 2 runs per day, and 2 replicates of each sample per run.

At each of the two multicenter secondary sites (B, C), the same 5 samples were tested on 1 device by 1 operator over 5 days, with 2 runs per day, and 3 replicates of each sample per run.

At the single site (D), a separate set of 5 human milk samples was tested on 1 device by 1 operator over 20 days, with 2 runs per day, and 2 replicates of each sample per run.

Study samples
Study samples in the two study sample sets were prepared using human milk, pooled from different mothers, collected according to milk bank/hospital laboratory standard procedures, stored max 12 months at -20°C or lower. Milk was screened for a specific content, or modified by cream separation. Sample composition is given below.

Sample ID	Fat (g/100mL)	CP (g/100mL)	TP (g/100mL)	CHO (g/100mL)	TS (g/100mL)	Energy (kcal/100mL)
1_{A-C}	0.7	2.8	2.3	8.5	12.2	53
2_{A-C}	5.6	1.3	1.0	7.7	14.8	88
3_{A-C}	1.9	0.8	0.6	4.8	7.6	40
4_{A-C}	3.4	1.3	1.0	6.6	11.4	64
5_{A-C}	3.9	1.1	0.8	7.5	12.7	71
1_{D}	0.4	3.1	2.5	8.6	12.3	52
2_{D}	5.3	1.5	1.2	8.1	15.2	88
3_{D}	1.8	1.0	0.8	5.1	8.1	42
4_{D}	2.3	1.2	1.0	7.5	11.2	56
5_{D}	3.5	1.6	1.3	8.1	13.4	72

In the multicenter study, frozen aliquots of each sample were prepared by the main site and distributed to each of the secondary sites.

In the single-site study, the sponsor was responsible for sample preparation and distribution to the laboratory site.

Results
Summary of results in the Miris HMA™ precision studies. Each of the following tables presents precision estimates for one analyte.

			FAT	Repeatability (within-run variation)		Intermediate Precision (within-lab variation)		Reproducibility (total variation)	
Description	**Sample**	**N**	**Mean (g/100 mL)**	**SD (g/100 mL)**	**CV%**	**SD (g/100 mL)**	**CV%**	**SD (g/100 mL)**	**CV%**
Multicenter study, overall (sites A, B, C)	$1_{A\text{-}C}$	139	0.8	0.04	4.8	-	-	0.07	8.9
	$2_{A\text{-}C}$	140	5.5	0.22	4.1	-	-	0.23	4.3
	$3_{A\text{-}C}$	140	1.9	0.13	6.9	-	-	0.13	7.1
	$4_{A\text{-}C}$	140	3.2	0.04	1.3	-	-	0.10	3.1
	$5_{A\text{-}C}$	138	3.7	0.05	1.2	-	-	0.15	4.0
Multicenter study, per site (sites A, B, C)	$1_{A\text{-}C}$	80	0.8	0.04	5.6	0.06	7.7	Site A	
	$2_{A\text{-}C}$	80	5.5	0.10	1.8	0.10	1.8		
	$3_{A\text{-}C}$	80	1.9	0.14	7.7	0.15	7.7		
	$4_{A\text{-}C}$	80	3.2	0.04	1.3	0.05	1.7		
	$5_{A\text{-}C}$	78	3.7	0.05	1.3	0.07	1.8		
	$1_{A\text{-}C}$	29	0.7	0.03	3.7	0.05	6.9	Site B	
	$2_{A\text{-}C}$	30	5.5	0.45	8.1	0.48	8.7		
	$3_{A\text{-}C}$	30	1.8	0.12	6.6	0.13	7.3		
	$4_{A\text{-}C}$	30	3.3	0.04	1.1	0.20	6.1		
	$5_{A\text{-}C}$	30	3.8	0.05	1.3	0.32	8.3		
	$1_{A\text{-}C}$	30	0.8	0.03	4.0	0.06	7.5	Site C	
	$2_{A\text{-}C}$	30	5.6	0.04	0.7	0.08	1.4		
	$3_{A\text{-}C}$	30	1.8	0.03	1.8	0.04	2.3		
	$4_{A\text{-}C}$	30	3.2	0.04	1.4	0.06	1.8		
	$5_{A\text{-}C}$	30	3.7	0.03	0.9	0.05	1.4		
Single-site study (site D)	1_{D}	80	0.5	n/a	n/a	n/a	n/a	Site D	
	2_{D}	80	5.3	0.06	1.1	0.07	1.4		
	3_{D}	80	1.9	0.05	2.7	0.06	3.2		
	4_{D}	80	2.3	0.05	2.3	0.06	2.6		
	5_{D}	80	3.4	0.05	1.4	0.07	2.2		

			CRUDE PROTEIN	Repeatability (within-run variation)		Intermediate Precision (within-lab variation)		Reproducibility (total variation)	
Description	**Sample**	**N**	**Mean (g/100 mL)**	**SD (g/100 mL)**	**CV%**	**SD (g/100 mL)**	**CV%**	**SD (g/100 mL)**	**CV%**
Multicenter study, overall (sites A, B, C)	$1_{A\text{-}C}$	139	2.8	0.03	1.2	-	-	0.09	3.2
	$2_{A\text{-}C}$	140	1.3	0.12	9.2	-	-	0.13	9.9
	$3_{A\text{-}C}$	140	0.8	0.04	4.7	-	-	0.05	6.8
	$4_{A\text{-}C}$	140	1.3	0.03	2.3	-	-	0.07	5.0
	$5_{A\text{-}C}$	138	1.0	0.03	3.0	-	-	0.06	5.6
Multicenter study, per site (sites A, B, C)	$1_{A\text{-}C}$	80	2.8	0.04	1.4	0.09	3.2	Site A	
	$2_{A\text{-}C}$	80	1.3	0.04	3.2	0.06	4.5		
	$3_{A\text{-}C}$	80	0.8	0.04	5.0	0.05	6.4		
	$4_{A\text{-}C}$	80	1.3	0.03	2.1	0.04	3.0		
	$5_{A\text{-}C}$	78	1.0	0.02	1.5	0.05	4.8		
	$1_{A\text{-}C}$	29	2.8	0.02	0.7	0.08	2.7	Site B	
	$2_{A\text{-}C}$	30	1.3	0.23	17.4	0.25	18.9		
	$3_{A\text{-}C}$	30	0.8	0.03	4.1	0.05	6.1		
	$4_{A\text{-}C}$	30	1.4	0.03	2.3	0.08	5.7		
	$5_{A\text{-}C}$	30	1.1	0.04	4.3	0.09	8.2		
	$1_{A\text{-}C}$	30	2.9	0.03	1.1	0.05	1.9	Site C	
	$2_{A\text{-}C}$	30	1.3	0.04	3.3	0.07	5.6		
	$3_{A\text{-}C}$	30	0.8	0.04	4.8	0.05	6.6		
	$4_{A\text{-}C}$	30	1.3	0.04	2.7	0.07	5.3		
	$5_{A\text{-}C}$	30	1.0	0.04	3.6	0.05	4.8		
Single-site study (site D)	1_{D}	80	3.1	0.03	1.1	0.03	1.1	Site D	
	2_{D}	80	1.5	0.05	3.5	0.05	3.6		
	3_{D}	80	1.0	0.04	4.3	0.05	5.6		
	4_{D}	80	1.2	0.03	2.3	0.03	2.9		
	5_{D}	80	1.6	0.05	3.1	0.05	3.1		

			TRUE PROTEIN	Repeatability (within-run variation)		Intermediate Precision (within-lab variation)		Reproducibility (total variation)	
Description	**Sample**	**N**	**Mean (g/100 mL)**	**SD (g/100 mL)**	**CV%**	**SD (g/100 mL)**	**CV%**	**SD (g/100 mL)**	**CV%**
Multicenter study, overall (sites A, B, C)	1_{A-C}	139	2.3	0.04	1.6	-	-	0.07	3.1
	2_{A-C}	140	1.0	0.09	9.4	-	-	0.10	10.0
	3_{A-C}	140	0.6	0.03	5.4	-	-	0.05	8.0
	4_{A-C}	140	1.1	0.03	2.8	-	-	0.06	5.2
	5_{A-C}	138	0.8	0.04	4.7	-	-	0.06	6.8
Multicenter study, per site (sites A, B, C)	1_{A-C}	80	2.2	0.04	1.7	0.07	2.9	Site A	
	2_{A-C}	80	1.0	0.04	4.2	0.04	4.5		
	3_{A-C}	80	0.6	0.03	5.2	0.05	7.8		
	4_{A-C}	80	1.1	0.03	3.0	0.05	4.8		
	5_{A-C}	78	0.8	0.03	3.0	0.05	5.8		
	1_{A-C}	29	2.3	0.03	1.2	0.07	3.1	Site B	
	2_{A-C}	30	1.1	0.19	17.7	0.20	18.9		
	3_{A-C}	30	0.6	0.04	6.6	0.05	7.9		
	4_{A-C}	30	1.1	0.04	3.4	0.06	5.9		
	5_{A-C}	30	0.8	0.06	7.3	0.09	10.4		
	1_{A-C}	30	2.3	0.04	1.6	0.05	2.3	Site C	
	2_{A-C}	30	1.0	0.03	2.6	0.04	4.5		
	3_{A-C}	30	0.6	0.03	4.2	0.04	6.7		
	4_{A-C}	30	1.1	0.02	1.7	0.04	3.3		
	5_{A-C}	30	0.8	0.03	3.9	0.04	4.6		
Single-site study (site D)	1_{D}	80	2.5	0.03	1.3	0.04	1.5	Site D	
	2_{D}	80	1.2	0.04	3.6	0.05	4.1		
	3_{D}	80	0.8	0.04	5.5	0.05	5.9		
	4_{D}	80	1.0	0.04	4.0	0.05	5.0		
	5_{D}	80	1.3	0.04	2.8	0.04	2.9		

			CHO	Repeatability (within-run variation)		Intermediate Precision (within-lab variation)		Reproducibility (total variation)	
Description	**Sample**	**N**	**Mean (g/100 mL)**	**SD (g/100 mL)**	**CV%**	**SD (g/100 mL)**	**CV%**	**SD (g/100 mL)**	**CV%**
Multicenter study, overall (sites A, B, C)	1_{A-C}	139	8.5	0.05	0.6	-	-	0.31	3.6
	2_{A-C}	140	7.7	0.57	7.4	-	-	0.63	8.1
	3_{A-C}	140	4.8	n/a	n/a	-	-	n/a	n/a
	4_{A-C}	140	7.2	0.08	1.1	-	-	0.27	3.7
	5_{A-C}	138	8.0	0.07	0.9	-	-	0.29	3.7
Multicenter study, per site (sites A, B, C)	1_{A-C}	80	8.5	0.05	0.6	0.09	1.1	Site A	
	2_{A-C}	80	7.6	0.77	10.2	0.77	10.2		
	3_{A-C}	80	4.8	n/a	n/a	n/a	n/a		
	4_{A-C}	80	7.1	0.09	1.3	0.12	1.6		
	5_{A-C}	78	7.9	0.09	1.1	0.10	1.3		
	1_{A-C}	29	8.2	0.05	0.6	0.22	2.7	Site B	
	2_{A-C}	30	7.7	0.05	0.7	0.18	2.4		
	3_{A-C}	30	4.7	n/a	n/a	n/a	n/a		
	4_{A-C}	30	7.2	0.05	0.7	0.31	4.3		
	5_{A-C}	30	7.8	0.04	0.6	0.50	6.3		
	1_{A-C}	30	8.8	0.05	0.6	0.15	1.7	Site C	
	2_{A-C}	30	8.0	0.05	0.7	0.15	1.8		
	3_{A-C}	30	4.9	n/a	n/a	n/a	n/a		
	4_{A-C}	30	7.5	0.06	0.8	0.09	1.2		
	5_{A-C}	30	8.2	0.06	0.7	0.11	1.4		
Single-site study (site D)	1_{D}	80	8.5	0.06	0.7	0.08	0.9	Site D	
	2_{D}	80	8.1	0.04	0.5	0.05	0.6		
	3_{D}	80	5.2	0.05	0.9	0.06	1.2		
	4_{D}	80	7.5	0.07	0.9	0.08	1.0		
	5_{D}	80	8.0	0.07	0.8	0.07	0.9		

			TOTAL SOLIDS	Repeatability (within-run variation)		Intermediate Precision (within-lab variation)		Reproducibility (total variation)	
Description	**Sample**	**N**	**Mean (g/100 mL)**	**SD (g/100 mL)**	**CV%**	**SD (g/100 mL)**	**CV%**	**SD (g/100 mL)**	**CV%**
Multicenter study, overall (sites A, B, C)	1_{A-C}	139	12.3	0.08	0.6	-	-	0.40	3.2
	2_{A-C}	140	14.7	0.64	4.4	-	-	0.72	4.9
	3_{A-C}	140	7.7	0.26	3.4	-	-	0.68	8.8
	4_{A-C}	140	12.0	0.10	0.8	-	-	0.34	2.8
	5_{A-C}	138	12.9	0.11	0.8	-	-	0.37	2.9
Multicenter study, per site (sites A, B, C)	1_{A-C}	80	12.2	0.09	0.7	0.14	1.2	Site A	
	2_{A-C}	80	14.5	0.86	5.9	0.86	5.9		
	3_{A-C}	80	7.8	0.34	4.4	0.82	10.5		
	4_{A-C}	80	11.8	0.12	1.0	0.17	1.4		
	5_{A-C}	78	12.8	0.13	1.0	0.17	1.4		
	1_{A-C}	29	12.0	0.04	0.4	0.33	2.7	Site B	
	2_{A-C}	30	14.8	0.20	1.3	0.40	2.7		
	3_{A-C}	30	7.5	0.19	2.6	0.27	3.7		
	4_{A-C}	30	12.1	0.08	0.6	0.53	4.4		
	5_{A-C}	30	12.9	0.07	0.5	0.82	6.4		
	1_{A-C}	30	12.7	0.08	0.6	0.23	1.8	Site C	
	2_{A-C}	30	15.0	0.07	0.5	0.24	1.6		
	3_{A-C}	30	7.7	0.04	0.5	0.15	2.0		
	4_{A-C}	30	12.3	0.08	0.6	0.15	1.2		
	5_{A-C}	30	13.1	0.08	0.6	0.16	1.2		
Single-site study (site D)	1_{D}	80	12.2	0.07	0.6	0.11	0.9	Site D	
	2_{D}	80	15.1	0.09	0.6	0.12	0.8		
	3_{D}	80	8.2	0.10	1.2	0.12	1.5		
	4_{D}	80	11.1	0.13	1.2	0.14	1.3		
	5_{D}	80	13.3	0.10	0.7	0.12	0.9		

			ENERGY	Repeatability (within-run variation)		Intermediate Precision (within-lab variation)		Reproducibility (total variation)	
Description	**Sample**	**N**	**Mean (kcal/100 mL)**	**SD (kcal/100 mL)**	**CV%**	**SD (kcal/100 mL)**	**CV%**	**SD (kcal/100 mL)**	**CV%**
Multicenter study, overall (sites A, B, C)	1_{A-C}	139	53	0.4	0.8	-	-	1.8	3.4
	2_{A-C}	140	87	3.3	3.7	-	-	3.6	4.1
	3_{A-C}	140	40	1.6	4.1	-	-	2.5	6.3
	4_{A-C}	140	64	0.6	0.9	-	-	1.7	2.7
	5_{A-C}	138	71	0.7	1.0	-	-	2.1	2.9
Multicenter study, per site (sites A, B, C)	1_{A-C}	80	53	0.4	0.7	0.8	1.4	Site A	
	2_{A-C}	80	87	3.9	4.5	3.9	4.5		
	3_{A-C}	80	40	2.1	5.1	3.0	7.4		
	4_{A-C}	80	64	0.7	1.1	1.0	1.5		
	5_{A-C}	78	70	0.9	1.2	1.0	1.5		
	1_{A-C}	29	52	0.4	0.8	1.4	2.7	Site B	
	2_{A-C}	30	88	3.0	3.5	3.7	4.2		
	3_{A-C}	30	39	1.5	3.7	1.8	4.5		
	4_{A-C}	30	65	0.4	0.7	3.0	4.6		
	5_{A-C}	30	71	0.4	0.6	4.8	6.7		
	1_{A-C}	30	55	0.5	0.9	1.3	2.4	Site C	
	2_{A-C}	30	89	0.5	0.5	1.4	1.6		
	3_{A-C}	30	40	0.2	0.5	0.8	2.0		
	4_{A-C}	30	66	0.5	0.7	0.9	1.4		
	5_{A-C}	30	71	0.5	0.7	0.8	1.2		
Single-site study (site D)	1_{D}	80	52	0.4	0.9	0.7	1.3	Site D	
	2_{D}	80	88	0.7	0.8	0.9	1.0		
	3_{D}	80	42	0.6	1.5	0.8	1.9		
	4_{D}	80	56	0.7	1.2	0.9	1.5		
	5_{D}	80	71	0.6	0.9	0.8	1.2		

MIRIS HMA™ PRECISION STUDY HUMAN DONOR MILK BANKS

Objective
Determine repeatability and reproducibility of the Miris HMA™ for measuring fat, crude protein, true protein, and carbohydrate content in a human milk sample, following the CLSI EP05-A3 guideline [17]. The calculated variables total solids and energy were also evaluated. This study was done to obtain further precision data collected at human donor milk banks.

Study design
Precision was tested in a multicenter study at three human donor milk bank sites. Study design as described below, with one device per site.

Site type	Number of sites	Devices/site	Number of samples	Number of days	Runs/day	Replicates/sample/run
Multicenter main (E)	1	1	5	20	2	2
Multicenter secondary (F, G)	2	1	5	5	2	3

At the main site (E), 5 human milk samples were tested on 1 device by 1 operator over 20 days, with 2 runs per day, and 2 replicates of each sample per run.

At each of the two secondary sites (F, G), the same 5 samples were tested on 1 device by 2-3 operators over 5 days, with 2 runs per day, and 3 replicates of each sample per run.

Study samples
Study samples were prepared using human milk, pooled from different mothers, collected according to milk bank/hospital laboratory standard procedures, stored max 12 months at -20°C or lower. Milk was screened for a specific content, or modified by cream separation. Sample composition is given below.

Sample ID	Fat (g/100mL)	CP (g/100mL)	TP (g/100mL)	CHO (g/100mL)	TS (g/100mL)	Energy (kcal/100mL)
1_{E-G}	0.7	2.9	2.3	8.6	12.4	53
2_{E-G}	5.4	1.3	1.1	8.0	14.9	88
3_{E-G}	2.7	0.9	0.7	6.8	10.6	56
4_{E-G}	3.5	1.1	0.9	7.7	12.4	68
5_{E-G}	4.4	1.7	1.3	7.7	14.0	79

Frozen aliquots of each sample were prepared at the main site and distributed to each of the secondary sites.

Results
Summary of results in the Miris HMA™ human donor milk bank precision study. Each of the following tables presents precision estimates for one analyte.

			FAT	Repeatability (within-run variation)		Intermediate Precision (within-lab variation)		Reproducibility (total variation)	
Description	Sample	N	Mean (g/100 mL)	SD (g/100 mL)	CV%	SD (g/100 mL)	CV%	SD (g/100 mL)	CV%
Overall (sites E, F, G)	1_{E-G}	139	0.7	0.03	3.9	0.05	7.4	0.05	7.7
	2_{E-G}	140	5.5	0.07	1.3	0.08	1.4	0.30	5.4
	3_{E-G}	140	2.8	0.03	1.2	0.05	1.8	0.19	6.8
	4_{E-G}	140	3.5	0.05	1.4	0.06	1.7	0.24	6.7
	5_{E-G}	140	4.2	0.10	2.3	0.11	2.5	0.30	7.1
Per site (sites E, F, G)	1_{E-G}	80	0.7	0.02	2.4	0.04	6.7	Site E	
	2_{E-G}	80	5.4	0.07	1.3	0.07	1.3		
	3_{E-G}	80	2.7	0.03	1.2	0.05	1.7		
	4_{E-G}	80	3.4	0.06	1.6	0.07	1.9		
	5_{E-G}	80	4.1	0.10	2.6	0.11	2.8		
	1_{E-G}	29	0.6	0.03	5.2	0.05	8.5	Site F	
	2_{E-G}	30	5.9	0.08	1.4	0.09	1.5		
	3_{E-G}	30	3.0	0.00	0.0	0.06	2.0		
	4_{E-G}	30	3.8	0.04	1.0	0.05	1.2		
	5_{E-G}	30	4.5	0.06	1.3	0.07	1.6		
	1_{E-G}	30	0.6	0.03	5.3	0.06	8.6	Site G	
	2_{E-G}	30	5.4	0.07	1.3	0.08	1.5		
	3_{E-G}	30	2.7	0.05	1.8	0.06	2.2		
	4_{E-G}	30	3.5	0.04	1.3	0.07	1.9		
	5_{E-G}	30	4.3	0.10	2.4	0.11	2.5		

			CRUDE PROTEIN	Repeatability (within-run variation)		Intermediate Precision (within-lab variation)		Reproducibility (total variation)	
Description	Sample	N	Mean (g/100 mL)	SD (g/100 mL)	CV%	SD (g/100 mL)	CV%	SD (g/100 mL)	CV%
Overall (sites E, F, G)	1_{E-G}	139	2.9	0.04	1.2	0.04	1.5	0.09	3.1
	2_{E-G}	140	1.4	0.03	2.3	0.04	3.2	0.06	4.5
	3_{E-G}	140	0.9	0.03	3.5	0.04	4.7	0.06	6.3
	4_{E-G}	140	1.1	0.03	2.7	0.04	3.9	0.07	5.7
	5_{E-G}	140	1.7	0.03	1.8	0.05	2.8	0.07	3.7
Per site (sites E, F, G)	1_{E-G}	80	2.9	0.04	1.2	0.04	1.4	Site E	
	2_{E-G}	80	1.3	0.03	2.5	0.05	3.6		
	3_{E-G}	80	0.9	0.02	2.5	0.04	4.5		
	4_{E-G}	80	1.1	0.04	3.5	0.05	4.6		
	5_{E-G}	80	1.7	0.03	1.5	0.05	2.8		
	1_{E-G}	29	2.9	0.04	1.4	0.05	1.8	Site F	
	2_{E-G}	30	1.4	0.02	1.3	0.02	1.3		
	3_{E-G}	30	1.0	0.04	4.5	0.05	4.9		
	4_{E-G}	30	1.2	0.02	2.1	0.03	2.8		
	5_{E-G}	30	1.8	0.02	1.0	0.02	1.0		
	1_{E-G}	30	3.0	0.04	1.2	0.04	1.2	Site G	
	2_{E-G}	30	1.3	0.03	2.5	0.05	3.5		
	3_{E-G}	30	0.9	0.04	4.1	0.04	4.1		
	4_{E-G}	30	1.1	0.00	0.0	0.00	0.0		
	5_{E-G}	30	1.8	0.05	2.9	0.06	3.4		

			TRUE PROTEIN	Repeatability (within-run variation)		Intermediate Precision (within-lab variation)		Reproducibility (total variation)	
Description	**Sample**	**N**	**Mean (g/100 mL)**	**SD (g/100 mL)**	**CV%**	**SD (g/100 mL)**	**CV%**	**SD (g/100 mL)**	**CV%**
Overall (sites E, F, G)	1_{E-G}	139	2.3	0.03	1.2	0.03	1.3	0.07	3.0
	2_{E-G}	140	1.1	0.03	3.0	0.04	3.2	0.04	3.3
	3_{E-G}	140	0.7	0.03	4.4	0.05	6.1	0.06	8.0
	4_{E-G}	140	0.9	0.03	3.5	0.04	4.1	0.04	4.1
	5_{E-G}	140	1.4	0.03	1.8	0.03	2.4	0.04	2.6
Per site (sites E, F, G)	1_{E-G}	80	2.3	0.02	0.8	0.02	1.0	Site E	
	2_{E-G}	80	1.1	0.04	3.3	0.04	3.4		
	3_{E-G}	80	0.7	0.03	3.6	0.05	6.2		
	4_{E-G}	80	0.9	0.04	3.9	0.04	4.7		
	5_{E-G}	80	1.4	0.03	2.0	0.04	2.8		
	1_{E-G}	29	2.4	0.05	1.9	0.05	2.1	Site F	
	2_{E-G}	30	1.1	0.02	1.7	0.02	1.7		
	3_{E-G}	30	0.8	0.04	5.0	0.04	4.8		
	4_{E-G}	30	0.9	0.04	4.1	0.04	4.2		
	5_{E-G}	30	1.4	0.02	1.3	0.02	1.3		
	1_{E-G}	30	2.4	0.03	1.1	0.03	1.1	Site G	
	2_{E-G}	30	1.1	0.04	3.5	0.04	3.9		
	3_{E-G}	30	0.7	0.04	5.5	0.04	5.7		
	4_{E-G}	30	0.9	0.00	0.0	0.00	0.0		
	5_{E-G}	30	1.4	0.03	1.8	0.03	1.9		

			CHO	Repeatability (within-run variation)		Intermediate Precision (within-lab variation)		Reproducibility (total variation)	
Description	**Sample**	**N**	**Mean (g/100 mL)**	**SD (g/100 mL)**	**CV%**	**SD (g/100 mL)**	**CV%**	**SD (g/100 mL)**	**CV%**
Overall (sites E, F, G)	1_{E-G}	139	8.9	0.05	0.5	0.06	0.6	0.55	6.2
	2_{E-G}	140	8.2	0.05	0.6	0.06	0.7	0.30	3.7
	3_{E-G}	140	7.0	0.05	0.7	0.06	0.8	0.28	4.0
	4_{E-G}	140	7.8	0.05	0.7	0.06	0.8	0.31	3.9
	5_{E-G}	140	8.0	0.06	0.8	0.07	0.9	0.35	4.3
Per site (sites E, F, G)	1_{E-G}	80	8.6	0.04	0.5	0.05	0.6	Site E	
	2_{E-G}	80	8.0	0.05	0.6	0.05	0.7		
	3_{E-G}	80	6.8	0.04	0.6	0.05	0.8		
	4_{E-G}	80	7.6	0.05	0.6	0.06	0.8		
	5_{E-G}	80	7.8	0.05	0.6	0.05	0.7		
	1_{E-G}	29	9.3	0.04	0.5	0.05	0.6	Site F	
	2_{E-G}	30	8.4	0.05	0.6	0.06	0.7		
	3_{E-G}	30	7.2	0.05	0.7	0.05	0.7		
	4_{E-G}	30	8.0	0.05	0.7	0.06	0.7		
	5_{E-G}	30	8.3	0.05	0.6	0.05	0.6		
	1_{E-G}	30	9.3	0.06	0.6	0.07	0.8	Site G	
	2_{E-G}	30	8.3	0.04	0.5	0.07	0.8		
	3_{E-G}	30	7.1	0.06	0.8	0.08	1.1		
	4_{E-G}	30	8.0	0.06	0.8	0.07	0.8		
	5_{E-G}	30	8.2	0.09	1.1	0.12	1.4		

			TOTAL SOLIDS	Repeatability (within-run variation)		Intermediate Precision (within-lab variation)		Reproducibility (total variation)	
Description	**Sample**	**N**	**Mean (g/100 mL)**	**SD (g/100 mL)**	**CV%**	**SD (g/100 mL)**	**CV%**	**SD (g/100 mL)**	**CV%**
Overall (sites E, F, G)	1_{E-G}	139	12.7	0.06	0.5	0.09	0.7	0.61	4.8
	2_{E-G}	140	15.2	0.09	0.6	0.12	0.8	0.56	3.7
	3_{E-G}	140	10.8	0.07	0.6	0.10	0.9	0.46	4.2
	4_{E-G}	140	12.7	0.09	0.7	0.12	0.9	0.54	4.2
	5_{E-G}	140	14.1	0.12	0.8	0.15	1.1	0.67	4.7
Per site (sites E, F, G)	1_{E-G}	80	12.4	0.05	0.4	0.09	0.7	Site E	
	2_{E-G}	80	15.0	0.08	0.6	0.11	0.8		
	3_{E-G}	80	10.6	0.05	0.5	0.10	0.9		
	4_{E-G}	80	12.4	0.10	0.8	0.13	1.1		
	5_{E-G}	80	13.8	0.12	0.9	0.16	1.2		
	1_{E-G}	29	13.1	0.06	0.5	0.09	0.7	Site F	
	2_{E-G}	30	15.8	0.08	0.5	0.14	0.9		
	3_{E-G}	30	11.3	0.07	0.6	0.08	0.7		
	4_{E-G}	30	13.2	0.08	0.6	0.09	0.7		
	5_{E-G}	30	14.7	0.08	0.5	0.10	0.7		
	1_{E-G}	30	13.1	0.08	0.6	0.08	0.6	Site G	
	2_{E-G}	30	15.3	0.09	0.6	0.11	0.7		
	3_{E-G}	30	11.0	0.09	0.9	0.10	0.9		
	4_{E-G}	30	12.8	0.06	0.5	0.09	0.7		
	5_{E-G}	30	14.4	0.15	1.0	0.14	1.0		

			ENERGY	Repeatability (within-run variation)		Intermediate Precision (within-lab variation)		Reproducibility (total variation)	
Description	**Sample**	**N**	**Mean (kcal/100 mL)**	**SD (kcal/100 mL)**	**CV%**	**SD (kcal/100 mL)**	**CV%**	**SD (kcal/100 mL)**	**CV%**
Overall (sites E, F, G)	1_{E-G}	139	55	0.37	0.7	0.52	1.0	2.4	4.4
	2_{E-G}	140	90	0.68	0.8	0.84	0.9	3.7	4.2
	3_{E-G}	140	57	0.46	0.8	0.64	1.1	2.8	4.9
	4_{E-G}	140	69	0.59	0.9	0.77	1.1	3.4	4.9
	5_{E-G}	140	79	0.90	1.1	1.10	1.4	4.2	5.4
Per site (sites E, F, G)	1_{E-G}	80	53	0.35	0.7	0.52	1.0	Site E	
	2_{E-G}	80	88	0.70	0.8	0.82	0.9		
	3_{E-G}	80	56	0.37	0.7	0.60	1.1		
	4_{E-G}	80	67	0.63	0.9	0.84	1.2		
	5_{E-G}	80	77	0.89	1.2	1.20	1.5		
	1_{E-G}	29	56	0.30	0.5	0.54	1.0	Site F	
	2_{E-G}	30	94	0.66	0.7	1.00	1.1		
	3_{E-G}	30	60	0.49	0.8	0.77	1.3		
	4_{E-G}	30	72	0.56	0.8	0.57	0.8		
	5_{E-G}	30	83	0.70	0.9	0.74	0.9		
	1_{E-G}	30	56	0.48	0.9	0.49	0.9	Site G	
	2_{E-G}	30	89	0.64	0.7	0.78	0.9		
	3_{E-G}	30	58	0.61	1.0	0.61	1.1		
	4_{E-G}	30	69	0.53	0.8	0.64	0.9		
	5_{E-G}	30	80	1.10	1.4	1.10	1.3		

MIRIS HMA™ TRUENESS: METHOD COMPARISON AND BIAS ESTIMATION

Objective
Compare the Miris HMA™ analysis to analysis by chemical standard methods, by estimating the bias for measuring fat, crude protein, true protein, and carbohydrate in unmodified human milk samples according to the CLSI EP09c standard [18]. The calculated variables total solids and energy were also evaluated.

Study design
Individual, unmodified human milk samples were tested on one device by one operator. Each sample was also analyzed by biochemical standard methods to provide comparative data.
The comparative methods were biochemical standard methods, which had been validated for analysis on human milk:

Röse Gottlieb (ISO 1211) for fat [8];
Kjeldahl (ISO 8968-1) for crude protein [9];
Kjeldahl crude protein*0.8 for true protein [11];
Total carbohydrate content calculated by difference [15], from values obtained by the comparative methods;
Drying oven for total solids (ISO 6731) [10];
Bomb calorimetry for energy (ISO 1928) [22].

Bias to the comparative methods was based on single HMA analysis results.

Study samples
Unmodified, human milk from different mothers was used, collected according to milk bank/hospital laboratory standard procedures, stored max 12 months at -20°C or lower. Each sample was an individual sample.

The study samples covered the following concentration ranges (HMA results):

Fat	0.6 - 6.4 g/100 mL
Crude protein	0.9 - 3.5 g/100 mL
True protein	0.7 - 2.8 g/100 mL
Carbohydrate	6.1 - 8.8 g/100 mL
Total solids	9.8 - 15.6 g/100 mL
Energy	44 - 111 kcal/100 mL

Results
Results based on first HMA replicate and mean of comparative replicates, with 95% confidence intervals (CI), are given below.

Variable	Correlation coefficient	Bias (95% CI)	Bias % (95% CI)	Slope (95% CI)	Intercept (95% CI)
Fat (g/100 mL) (N=112)	0.98	0.22 (0.01 - 0.44)	6.34 (5.72 - 6.82)	1.12 (1.065 - 1.182)	-0.17 (-0.377 - 0.036)
Crude protein (g/100 mL) (N=112)	0.96	-0.07 (-0.15 - 0.00)	-6.02 (-6.76 - -4.64)	1.19 (1.02 - 1.369)	-0.33 (-0.582 - -0.088)
True protein (g/100 mL) (N=112)	0.96	-0.06 (-0.11 - 0.00)	-5.13 (-6.26 - -3.65)	1.19 (1.019 - 1.371)	-0.27 (-0.463 - -0.068)
Carbohydrate (g/100 mL) (N=106)	0.85	-0.16 (-0.24 - -0.08)	-3.07 (-3.50 - -2.44)	0.90 (0.759 - 1.041)	0.62 (-0.527 - 1.757)
Total solids (g/100 mL) (N=112)	0.96	-0.10 (-0.30 - 0.20)	-1.00 (-1.00 - -0.75)	0.97 (0.929 - 1.013)	0.32 (-0.224 - 0.861)
Energy (kcal/100 mL) (N=105)	0.96	1.70 (-1.10 - 4.57)	0.31 (-0.23 - 0.87)	0.95 (0.902 - 0.995)	5.01 (1.734 - 8.291)

Difference in N due to missing values

Point bias estimates and limits for the 95% confidence interval (CI) at low, medium, and high levels of fat, protein, carbohydrate, and energy, respectively, were calculated:

Fat	-0.1 (-0.3 - 0.2) g/100 mL at 1 g/100 mL; 0.2 (-0.2 - 0.6) g/100 mL at 3 g/100 mL; 0.6 (0.0 - 1.1) g/100 mL at 6 g/100 mL
Crude protein:	-0.1 (-0.4 - 0.1) g/100 mL at 1 g/100 mL; 0.0 (-0.3 - 0.3) g/100 mL at 1.5 g/100 mL; 0.1 (-0.3 - 0.6) g/100 mL at 2.5 g/100 mL
True protein:	-0.1 (-0.3 - 0.1) g/100 mL at 0.8 g/100 mL; 0.0 (-0.35 - 0.3) g/100 mL at 1.1 g/100 mL; 0.1 (-0.2 - 0.45) g/100 mL at 2.0 g/100 mL
Carbohydrate:	0.0 (-2.1 - 2.0) g/100 mL at 6.6 g/100 mL; 0.1 (-2.3 - 2.1) g/100 mL at 7.5 g/100 mL; -0.2 (-2.6 - 2.1) g/100 mL at 8.5 g/100 mL
Energy	3 (-3 - 8) kcal/100 mL at 45 kcal/100 mL; 2 (-5 - 8) kcal/100 mL at 70 kcal/100 mL; 1 (-9 - 8) kcal/100 mL at 110 kcal/100 mL

Conclusion
Overall, bias estimates were ±7% (95% CI) for all Miris HMA™ variables. For energy ±1% (95% CI).

MIRIS HMA™ LINEARITY STUDY

Objective
Establish the linear range of the Miris HMA™ method for measuring fat, crude protein, true protein, and carbohydrate content in a human milk sample, according to the CLSI EP06-A standard [19]. The calculated variables total solids and energy were also evaluated.

Study design
- A total of 38 samples were tested on 1 device over 1 day, with 2 replicates of each sample.
- A dilution series in 9 samples was used for evaluation of the HMA fat variable (0.4, 0.6, 0.9, 1.4, 2.5, 3.7, 4.8, 5.9, 7.0 g/100 mL);
- a dilution series in 12 samples was used for evaluation of the HMA protein variables (CP, TP) (0.2, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.7, 3.1 g/100 mL);
- a dilution series in 7 samples was used for evaluation of the HMA carbohydrate variable (6.1, 6.6, 7.0, 7.5, 8.0, 8.4, 8.9 g/100 mL);
- linearity of variables total solids (0.7 - 20.8 g/100 mL) and energy (4 - 143 kcal/100 mL) was evaluated from all 38 samples.

Study samples
Samples were prepared from pooled human milk from different mothers, collected according to milk bank/hospital laboratory standard procedures, stored max 12 months at -20°C or lower. To obtain samples of required composition, the milk was modified.

The expected concentrations of fat, crude protein, true protein, carbohydrate, total solids, and energy in a sample was calculated by the measured concentration in the stock solution (max sample) and in the diluent solution (min sample), multiplied by the weighed-in amounts of the stock and the diluent, respectively, when preparing the sample.

Results
Regression analysis was used to check for non-linear response patterns. Measured concentrations (y) were plotted vs expected (x). The difference between the linear and the non-linear models was assessed, and the difference compared with the preset criterion ±10% (allowable non-linearity) for all variables. The outcome is summarised below.

Variable	Linear interval	Unit	Intercept	Slope	R^2	HMA measuring range
Fat	0.4 - 7.0	g/100 mL	-0.1122	1.0284	0.9981	0.6 - 6
Crude protein	0.4 - 3.8	g/100 mL	0.0364	0.9925	0.9964	0.8 - 3
True protein	0.3 - 3.1	g/100 mL	0.0647	0.9811	0.9967	0.6 - 2.4
Carbohydrate	6.1 - 8.9	g/100 mL	0.5095	0.9457	0.9854	6.6 - 8.7
Total solids	0.7 - 20.8	g/100 mL	0.2079	0.9890	0.9974	n/a
Energy	8 - 143	kcal/100 mL	0.0135	1.0067	0.9982	n/a

Conclusion
Linearity was demonstrated in intervals corresponding to the Miris HMA™ measuring range of fat, crude protein, true protein, and carbohydrate, and in ranges relevant for total solids and energy.

MIRIS HMA™ DETECTION CAPABILITY STUDY

Objective
Determine the detection capability of the Miris HMA™, i.e. limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ), for analysing fat, crude protein, true protein, and carbohydrate in a human milk sample, according to the CLSI EP17-A2 standard [20]. The calculated variables total solids and energy were also evaluated.

Study design
For the LoB estimate the study design consisted of 3 replicate measurements on 4 blank samples across 5 days, with a single instrument and one operator. Total of 60 measurement values for each variable.
For the LoD and LoQ estimates the study design consisted of 3 replicate measurements on 14 low level samples across 5 days, with a single instrument and one operator. Total of 105 replicates per variable.
To determine LoQ the 14 low level test samples were also analysed by comparative standard methods, acceptance criteria for Total Error was <30%.
The comparative methods were biochemical standard methods, which had been validated for analysis on human milk:

Röse Gottlieb (ISO 1211) for fat [8];
Kjeldahl (ISO 8968-1) for crude protein [9];
Kjeldahl crude protein*0.8 for true protein [11];
Total carbohydrate content calculated by difference [15], on values obtained by the comparative methods;
drying oven for total solids (ISO 6731) [10];
Energy calculated by formula (see Table 12) [11], on values obtained by the comparative methods

Study samples
Human milk was used to prepare the 14 low level milk samples, modified to contain required levels of fat, protein and carbohydrate. The milk was pooled samples from different mothers, collected according to milk bank/hospital laboratory standard procedures, stored max 12 months at -20°C or lower. The blank samples were deionised water. To obtain samples of required composition, the milk was modified.

Seven of the low-level samples were designed for evaluation of the HMA fat variable. The composition showed a fat content range 0.08 - 0.41 g/100 mL, and stable crude protein (1.2-1.3 g/100 mL) and carbohydrate contents (8 g/100 mL).

Seven of the low-level samples were designed for evaluation of the HMA protein and CHO variables. The composition showed a crude protein content range 0.42 - 0.75 g/100 mL; true protein content range 0.34 - 0.60 g/100 mL; carbohydrate content range 3.0 - 5.8 g/100 mL; and stable fat content (1.1-2.0 g/100 mL).

Detection capability of variables total solids and energy were evaluated from all 14 samples. Total solids range 4.6 - 10.0 g/100 mL; energy range 24 - 44 kcal/100 mL.

Results
Detection capability characteristics of the Miris HMA™ were determined as follows:

Characteristic	Fat (g/100 mL)	Crude protein (g/100 mL)	True protein (g/100 mL)	Carbohydrate (g/100 mL)
LoB (N=60)	0.06	0.06	0.05	0.04
LoD (N=105)	0.11	0.25	0.20	0.35
LoQ (N=105)	0.41	0.42	0.34	3.00

Conclusion
LoB was below 0.1 g/100 mL for fat, crude protein, true protein and carbohydrate.
LoB ≤ LoD < LoQ.
LoQ was below the low end of the Miris HMA™ measuring range for all variables.

MIRIS HMA™ INTERFERENCE TESTING

Objective
Determine whether bronopol, hemoglobin, or drug residuals causes interference when measuring fat, crude protein, true protein, and carbohydrate content in a normal human milk sample using the Miris HMA™, following the CLSI EP07-A2 standard [21]. The calculated variables total solids and energy were also evaluated.

Study design
The study included evaluation of bronopol, hemoglobin, and 28 drugs. Each of the 30 substances were tested in two human milk samples of different composition and at two concentrations (expected and high) – four test samples per substance. Each substance was tested on two devices during one analytical run, with four replicates of each test sample (two per device). Bias was evaluated relative to a control portion, blank, of the same sample (paired-difference test).

Study samples
Samples were prepared from human milk, pooled from different mothers, collected according to milk bank/hospital laboratory standard procedures, stored max 12 months at -20°C or lower. Samples were modified to contain high or medium levels of fat, protein and carbohydrate, see below.

Sample	Fat (g/100 mL)	Crude protein (g/100 mL)	True protein (g/100 mL)	Carbohydrate (g/100 mL)	Total solids (g/100 mL)	Energy (kcal/100 mL)
High	4.2 - 4.6	2.8 - 3.1	2.3 - 2.4	7.6 - 8.0	15.1 - 15.5	83 - 86
Medium	1.0 - 1.3	1.0 - 1.4	0.8 - 1.1	6.1 - 7.6	8.5 - 10.1	39 - 45

Substances were dissolved in DMSO to make a stock solution and then added to the milk samples, except bronopol which was dissolved in water, and hemoglobin which was dissolved directly in the milk sample. Blanks were the same milk samples, with only the solvent added (DMSO, water).

Criteria for interference
The point estimate of the observed interference effect was calculated as the difference between the means of the test samples and the blank samples. If this was less than or equal to a cut-off value ($d_{cut\text{-}off}$) it was concluded that there was no interference. Otherwise, the hypothesis that the substance interferes was accepted.

The cut-off values ($d_{cut\text{-}off}$) were:
0.10 g/100 mL for fat, crude protein, and true protein
0.15 g/100 mL for carbohydrate
0.36 g/100 mL for total solids
1.8 kcal/100 mL for energy

Results
Substances tested for interference at Miris HMA™ analysis, the substance levels tested, and if interference was detected is given below. A substance was classed as interfering if the criteria were fulfilled on one or more analytes, in one or more of the four test samples. Interferents are marked by * and substance name in bold.

Class	Substance	Test level expected	Test level high	Unit	Interference detected
Analgesics	Acetaminophen	15	45	mg/L	-
	Ibruprofen	400	1200	µg/L	-
	Oxycodone	200	600	µg/L	-
	Aspirin	5	15	mg/L	-
Antidepressants	**Citalopram***	250*	750	µg/L	Negative bias on fat measurements
	Paroxetine	50	150	µg/L	-
	Sertraline*	50	150*	µg/L	Negative bias on fat measurements
Antibiotics	**Ampicillin***	1*	3*	mg/L	Negative bias on fat measurements
	Gentamicin	200	600	µg/L	-
	Cefazolin	1	3	mg/L	-
	Vancomycin*	10	30*	mg/L	Positive bias on fat measurements
	Clindamycin*	2*	6	mg/L	Negative bias on fat measurements
	Cephalexin*	0.5*	1.5	mg/L	Negative bias on fat measurements
Antihistamines/ decongestants	Diphenhydramine	100	300	µg/L	-
	Pseudoephedrine*	300*	900	µg/L	Negative bias on fat measurements
	Loratidine	30	90	µg/L	-
Antiepileptics	Phenytoin	1	3	mg/L	-
	Carbamazepine	2.5	7.5	mg/L	-
Diuretics, beta blockers	Hydrochlorothiazide	200	600	µg/L	-
	Propranolol	30	90	µg/L	-
	Metoprolol	150	450	µg/L	-
Steroids	Progestin only contraceptives	0.25	0.75	µg/L	-
	Prednisolone	250	750	µg/L	-
Galactogogues	Domperidone	5	15	µg/L	-
Miscellaneous	Morphine	50	150	µg/L	-
	Fluoxetine	100	300	µg/L	-
	Methyldopa	1	3	mg/L	-
	Caffeine	5	15	mg/L	-
	Hemoglobin*	150*	700*	mg/dL	Positive bias on protein measurements
Preservatives	Bronopol	0.02	0.10	%	-

Conclusion
Interference with the Miris HMA™ measurement results was found from the following substances: Citalopram, Sertraline, Ampicillin, Vancomycin, Clindamycin, Cephalexin, Pseudoephedrine, and Hemoglobin.

Macronutrient analysis by Miris HMA™ is not recommended on milk that may contain any of these drugs, i.e. milk from mothers taking the drug.

Human milk may be contaminated with hemoglobin, from whole blood. If the milk is visibly pink, presumed from blood contamination, macronutrient analysis by Miris HMA™ is not recommended.

MIRIS HMA™ CARRY-OVER TESTING

Objective
Evaluate carry-over in the Miris HMA™ when measuring fat, crude protein, true protein, and carbohydrate in human milk samples following the ISO 9622:2013 guideline [14]. The calculated variables total solids and energy were also evaluated.

Study design
By running samples of milk and water, or milk and Miris Cleaner™, through the Miris HMA™, the carry-over effect was assessed.

When running two milk samples after each other, in a water-filled system (or Miris Cleaner™-filled), the potential effect of any residual water was noted as a measurement difference between the first and second milk sample (E_W).

Similarly, when injecting water (or Miris Cleaner™) twice in a milk-filled system, the effect of any residual milk was noted as a measurement difference between the first and second water (or Miris Cleaner™) sample (E_M).

Study samples
The human milk used was pooled samples from different mothers, collected according to milk bank/hospital laboratory standard procedures, stored max 12 months at -20°C or lower.

The test milk used for the study was modified to have a fat content, protein content and carbohydrate content corresponding to the upper end of the Miris HMA™ measuring range, to illustrate worst case.

Descriptive	Fat (g/100 mL)	Crude protein (g/100 mL)	True protein (g/100 mL)	Carbohydrate (g/100 mL)	Total solids (g/100 mL)	Energy (kcal/100 mL)
Mean (N=40)	5.9	3.1	2.4	8.6	17.8	102.9
SD	0.04	0.07	0.05	0.07	0.11	0.57

Results
Carry-over (%) of water or Miris Cleaner™ to a following milk sample, E_W, is given below:

Characteristic	Fat	Crude protein	Carbohydrate	Total solids	Energy	True protein
E_W (%) Water 3 mL injection	0.84	0.72	0.74	0.58	0.70	0.75
E_W (%) Miris Cleaner™ 3 mL injection	0.74	-0.06	-0.04	0.50	0.47	0.55

Carry-over (%) of milk to a following water or Miris Cleaner™ sample, E_M, is given below:

Characteristic	Fat	Crude protein	Carbohydrate	Total solids	Energy	True protein
E_M (%) Water 3 mL injection	1.19	1.28	1.23	0.63	0.94	1.01
E_M (%) Miris Cleaner™ 3 mL injection	0.10	-0.28	-0.35	-0.08	-0.05	-0.00

Conclusion
The results showed carry-over < 2% in all tests, at 3 mL sample injections.

WORKING PRINCIPLES OF THE INSTRUMENT

TECHNOLOGICAL CHARACTERISTICS

The Miris 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 32 provides a schematic drawing representing the Miris HMA™ hardware components.

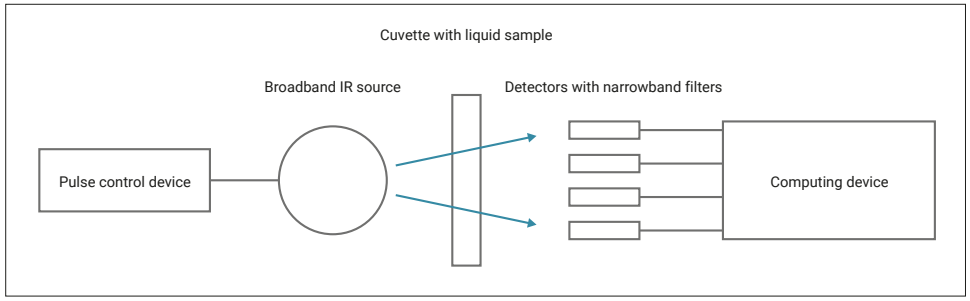


Figure 32. Schematic drawing of Miris HMA™ hardware components.

PRINCIPLES OF OPERATION

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

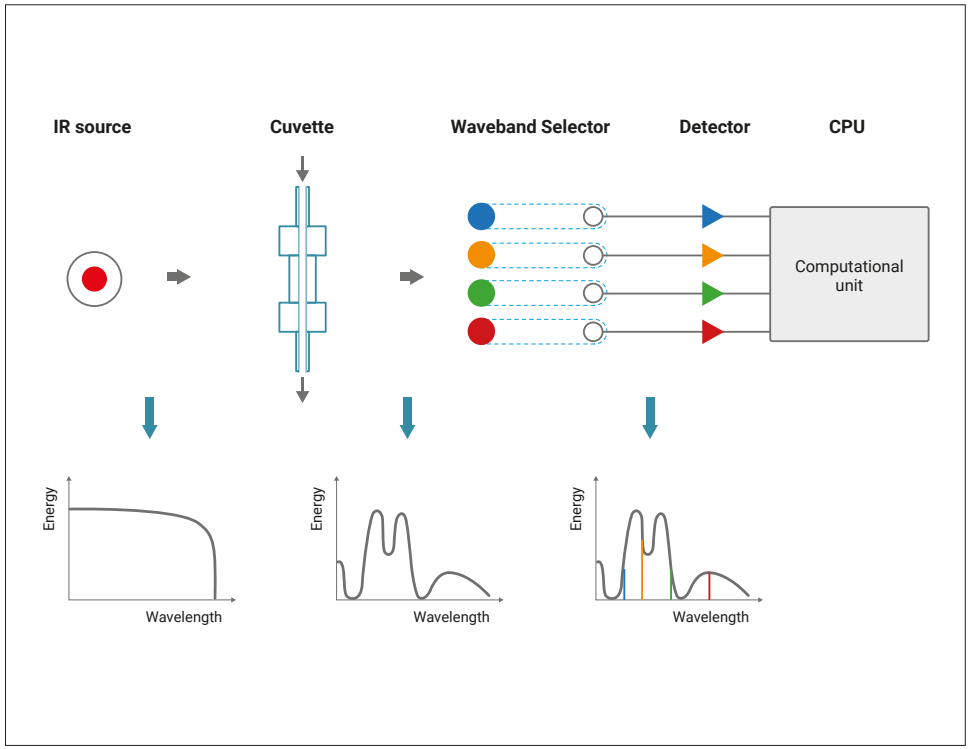


Figure 33. Working principle of the Miris HMA™.

Figure 33 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 12), respectively, are evaluated. The quantitative determination of fat, protein and carbohydrate is performed according to Lambert Beer's law - the absorbance is proportional to concentration. The Miris 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 Miris HMA™ measuring range for each component. The calibration samples are analysed both by the Miris HMA™ and by a standard method. The Miris HMA™ results and the standard method results are combined to fit calibration curves for fat, protein and carbohydrate, respectively, which will constitute the internal calibration.

The Miris HMA™ internal calibration is based on chemical standard methods validated for analysis of human milk. These standard methods are ISO/AOAC certified; Röse-Gottlieb for fat [8], Kjeldahl for crude protein [9]. True protein is crude protein multiplied by 0.8, to correct for non-protein-nitrogen (NPN).

The value of total carbohydrate (lactose and oligosaccharides) content is a calculation of total solids minus fat, protein and ash [15]. Total solids are measured by drying-oven [10].

Table 12. Description of components in human milk analysed by Miris HMA™.

Component	Reportable unit	Definition	Mid-IR absorbance Chemical bond	 λ	Standard method
Fat (F_{HMA})	g/100 ml	Triacylglycerides (≈98-99%), di- and monoglycerides, FFA, phospholipids, sterols	Carbonyl group of ester bonds of glycerides	5.7 µm	F_{ref} Röse-Gottlieb ISO 1211 [8]
Crude protein (CP_{HMA})	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	CP_{ref} Kjeldahl ISO 8968-1 [9]
True protein (TP_{HMA})	g/100 ml	Crude protein*0.8 Non-protein N excluded (≈20% of total N [11])	Secondary amide (II) groups of peptide bonds	6.5 µm	$TP_{ref} = CP_{ref}$*0.8
Carbohydrate (CHO_{HMA})	g/100 ml	Total carbohydrate content Lactose (≈70-85%), mono-/oligo-saccharides (≈15-30%) [12, 13]	Hydroxyl groups of lactose and mono-/oligosaccharides	9.6 µm	CHO_{ref} = TS_{ref} - F_{ref} - CP_{ref} - ash_{ref} [15] (TS_{ref} ISO 6731 [10], ash_{ref} NMKL 173 [16])
Total solids (TS_{HMA})	g/100 ml	TS_{HMA} = F_{HMA} + CP_{HMA} + CHO_{HMA} +0.2(ash)	Calculated from the Miris HMA™ measurement results	-	Not a calibrated component (TS_{ref} ISO 6731 [10])
Energy (E_{HMA})	kcal/100 ml	Gross energy content E_{HMA} = 9.25* F_{HMA} + 4.40* CP_{HMA} + 4.00* CHO_{HMA} [11]	Calculated from the Miris HMA™ measurement results	-	Not a calibrated component (E_{ref} ISO 1928 [22])

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MANUFACTURER'S DECLARATION - ELECTROMAGNETIC EMISSION AND IMMUNITY

Guidance and manufacturer's declaration – electromagnetic emissions		
The Miris HMA™ is intended for use in the electromagnetic environment specified below. The customer or the user of the Miris HMA™ should assure that it is used in such an environment.		
Emissions test	**Compliance**	**Electromagnetic environment – guidance**
RF emissions CISPR 11	Group 1	The Miris HMA™ uses RF energy only for its internal function. Therefore, its RF emissions are very low and are not likely to cause any interference in nearby electronic equipment.
RF emissions CISPR 11	Class B	The Miris HMA™ is suitable for use in all establishments, including domestic establishments and those directly connected to the public low-voltage power supply network that supplies buildings used for domestic purposes.
Harmonic emissions IEC 61000-3-2	Class A	
Voltage fluctuations/ flicker emissions IEC 61000-3-3	Complies	

Guidance and manufacturer's declaration – electromagnetic immunity			
The Miris HMA™ is intended for use in the electromagnetic environment specified below. The customer or the user of the Miris HMA™ should assure that it is used in such an environment.			
Immunity test	**IEC 60601 test level**	**Compliance level**	**Electromagnetic environment – guidance**
Electrostatic discharge (ESD) IEC 61000-4-2	±6 kV contact ±8 kV air	±6 kV contact ±8 kV air	Floors should be wood, concrete or ceramic tile. If floors are covered with synthetic material, the relative humidity should be at least 30 %.
Electrical fast transient/burst IEC 61000-4-4	±2 kV for power supply lines ±1 kV for input/output lines	±2 kV for power supply lines ±1 kV for input/output lines	Mains power quality should be that of a typical commercial or hospital environment.
Surge IEC 61000-4-5	±1 kV line(s) to line(s) ±2 kV line(s) to earth	±1 kV line(s) to line(s) ±2 kV line(s) to earth	Mains power quality should be that of a typical commercial or hospital environment.
Voltage dips, short interruptions and voltage variations on power supply input lines IEC 61000-4-11	<5 % U_T (>95 % dip in U_T) for 0,5 cycle 40 % U_T (60 % dip in U_T) for 5 cycles 70 % U_T (30 % dip in U_T) for 25 cycles <5 % U_T (>95 % dip in U_T) for 5 sec	<5 % U_T (>95 % dip in U_T) for 0,5 cycle 40 % U_T (60 % dip in U_T) for 5 cycles 70 % U_T (30 % dip in U_T) for 25 cycles <5 % U_T (>95 % dip in U_T) for 5 sec	Mains power quality should be that of a typical commercial or hospital environment. If the user of the Miris HMA™ requires continued operation during power mains interruptions, it is recommended that the Miris HMA™ be powered from an uninterruptible power supply or a battery.
Power frequency (50/60 Hz) magnetic field IEC 61000-4-8	3 A/m	300 A/m	Power frequency magnetic fields should be at levels characteristic of a typical location in a typical commercial or hospital environment.
NOTE U_T is the a.c. mains voltage prior to application of the test level.			

Guidance and manufacturer's declaration – electromagnetic immunity			
The Miris HMA™ is intended for use in the electromagnetic environment specified below. The customer or the user of the Miris HMA™ should assure that it is used in such an environment.			
Immunity test	**IEC 60601 test level**	**Compliance level**	**Electromagnetic environment – guidance**
Conducted RF IEC 61000-4-6	3 Vrms 150 kHz to 80 MHz	10 Vrms	Portable and mobile RF communications equipment should be used no closer to any part of the Miris HMA™, including cables, than the recommended separation distance calculated from the equation applicable to the frequency of the transmitter. **Recommended separation distance** $d = 0{,}4\sqrt{P}$ $d = 0{,}4\sqrt{P}$ 80 MHz to 800 MHz $d = 0{,}7\sqrt{P}$ 800 MHz to 2,5 GHz
Radiated RF IEC 61000-4-3	3 V/m 80 MHz to 2,5 GHz	10 V/m (80-1000MHz) 10 V/m (1-2,7 GHz)	where P is the maximum output power rating of the transmitter in watts (W) according to the transmitter manufacturer and d is the recommended separation distance in metres (m). Field strengths from fixed RF transmitters, as deter-mined by an electromagnetic site survey,[a] should be less than the compliance level in each frequency range.[b] Interference may occur in the vicinity of equipment marked with the following symbol:
NOTE 1 At 80 MHz and 800 MHz, the higher frequency range applies.			
NOTE 2 These guidelines may not apply in all situations. Electromagnetic propagation is affected by absorption and reflection from structures, objects and people.			

[a]Field strengths from fixed transmitters, such as base stations for radio (cellular/cordless) telephones and land mobile radios, amateur radio, AM and FM radio broadcast and TV broadcast cannot be predicted theoretically with accuracy. To assess the electromagnetic environment due to fixed RF transmitters, an electromagnetic site survey should be considered. If the measured field strength in the location in which the [EQUIPMENT or MOBILE] is used exceeds the applicable RF compliance level above, the [EQUIPMENT or MOBILE] should be observed to verify normal operation. If abnormal performance is observed, additional measures may be necessary, such as re-orienting or relocating the Miris HMA™.

[b]Over the frequency range 150 kHz to 80 MHz, field strengths should be less than 10 V/m.

Recommended separation distances between portable and mobile RF communications equipment and the Miris HMA™

The Miris HMA™ is intended for use in an electromagnetic environment in which radiated RF disturbances are controlled. The customer or the user of the Miris HMA™ can help prevent electromagnetic interference by maintaining a minimum distance between portable and mobile RF communications equipment (transmitters) and the Miris HMA™ as recommended below, according to the maximum output power of the communications equipment.

Rated maximum output power of transmitter W	**Separation distance according to frequency of transmitter m**		
	150 kHz to 80 MHz $d = 0{,}4\sqrt{P}$	**80 MHz to 800 MHz** $d = 0{,}4\sqrt{P}$	**800 MHz to 2,5 GHz** $d = 0{,}7\sqrt{P}$
0,01	0,04	0,04	0,07
0,1	0,12	0,12	0,22
1	0,4	0,4	0,7
10	1,2	1,2	2,2
100	4.0	4.0	7,0

For transmitters rated at a maximum output power not listed above, the recommended separation distance d in metres (m) can be estimated using the equation applicable to the frequency of the transmitter, where P is the maximum output power rating of the transmitter in watts (W) according to the transmitter manufacturer.

NOTE 1 At 80 MHz and 800 MHz, the separation distance for the higher frequency range applies.

NOTE 2 These guidelines may not apply in all situations. Electromagnetic propagation is affected by absorption and reflection from structures, objects and people.

Miris HMA™ User Manual

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