



RespiSensor - KIT120 - User Manual

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Unisensor

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1. Foreword

Thank you for purchasing our product. This user manual describes how to use the kit. Please read it carefully before using the kit, and keep it for future reference.

Opening Check

Please check the kit when you first open it. If you find any problem or missing item, please contact your distributor.



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2. Introduction

RespiSensor™ is a veterinary diagnostic kit that identifies the pathogens behind bovine respiratory disease complex (BRDC), directly from a respiratory sample taken on the farm. A single run screens seven pathogens at once, alongside a positive control, and the analysis takes 40 minutes.

Intended use

The RespiSensor test is intended **exclusively for veterinary use**. It detects the DNA or RNA of the targets listed below, from either of two sample types: a nasopharyngeal swab, or a bronchoalveolar lavage (BAL).

TARGET	TYPE
<i>Histophilus somni</i>	Bacterium
<i>Mycoplasma bovis</i>	Bacterium
<i>Pasteurella multocida</i>	Bacterium
<i>Mannheimia haemolytica</i>	Bacterium
Bovine coronavirus (BCoV)	Virus
Bovine parainfluenza virus 3 (BPIV3)	Virus
Bovine respiratory syncytial virus (BRSV)	Virus
Positive control	Run control



The PCR strip carries **8 reaction tubes** – one per target above, the eighth being the positive control. The analysis itself takes **40 minutes**.

How the test works

Detection relies on **LAMP**, for Loop-mediated Isothermal Amplification: an amplification technique for DNA and RNA carried out at a single, constant temperature. Where other methods alternate between heating and cooling cycles, LAMP holds one temperature throughout, which removes the need for a thermal cycler. A polymerase with strand-displacement activity, guided by a set of target-specific primers, copies the target sequence quickly and in large quantity. Fast, simple to run and highly sensitive, the method is now widely used in both diagnostics and research.

Four stages, from lysis to detection:

1. **Lysis** – the buffer and a lysis bead release the genetic material of the pathogens present in the sample.
2. **Filtration** – the lysate passes through a column that retains debris and the compounds liable to inhibit the reaction.
3. **Amplification** – the LAMP reaction copies whichever target sequences are present, in large numbers.

4. **Detection** – a fluorescent probe binds to the amplification products and emits a signal, which the instrument measures target by target.

The chapters that follow describe each of these stages in practice.

Why it matters

Bovine respiratory disease is rarely traced to a single, obvious cause, and treating it blind is expensive, and rarely effective. Knowing **which** pathogen is involved, on the day the animal is examined, supports a targeted treatment decision: it avoids therapies that were never going to work, spares the cost that goes with them, and limits selection pressure on antimicrobials. At herd level, it turns respiratory disease management from reactive to informed.

3. Preparation and Precautions for Use of the Kit

Kit Storage

The kit is supplied in **two boxes**.

- The **sampling box** contains the nasopharyngeal swabs and the lysis buffer tubes. It is designed to be carried by the veterinarian in the field and can be stored at room temperature. It may also be kept between 2 and 8 °C, so that both boxes can be stored together.
- The **analysis box** contains the reagents and consumables required for the test. It must be stored between 2 and 8 °C upon receipt, and returned to the refrigerator after use.

Usage Precautions

- To minimise contamination risks, it is recommended to wear a new pair of gloves for each analysis and to use only filtered pipette tips.
- Always check the kit's expiration date before use. Never use reagents past their expiration date.
- Never touch the heating block or the hot metallic surfaces around it. Burn hazard.
- Follow the safety guidelines established by the laboratory or the relevant authority.

 To avoid any risk of contamination, never open PCR tubes after the amplification reaction.

 The equipment needed to run the test is listed in [Check List](#).

4. Kit composition

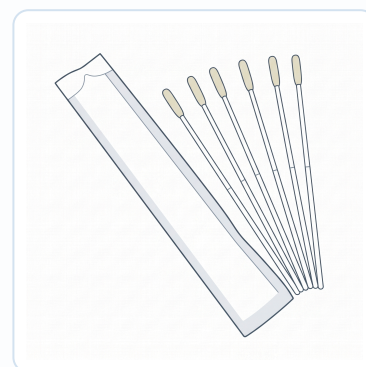
The kit is supplied in two boxes: a **sampling box**, carried in the field, and an **analysis box**, kept refrigerated at the workstation.

Sampling box

Lysis buffer tube – 8 mL screw-cap tube, prefilled with lysis buffer



Nasopharyngeal swab – sterile nylon flocked swab, 15 cm

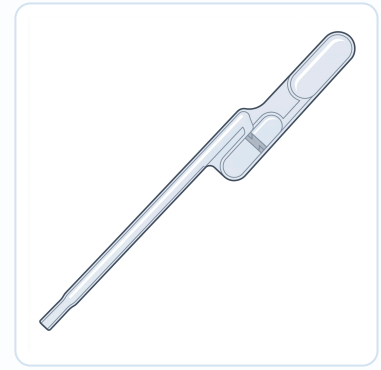


Analysis box

Lysis bead – one bead per test, in a 1.5 mL tube supplied in the aluminium pouch together with the PCR strip and the desiccant



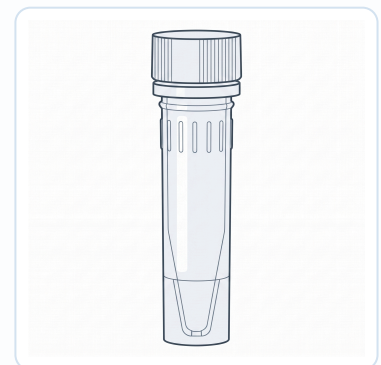
Pasteur pipette – 300 µL transfer pipette, supplied in the aluminium pouch



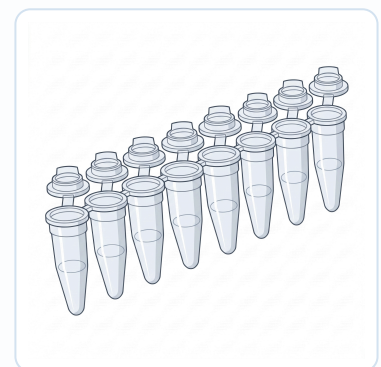
Spin filter column – filter insert and collection tube



Resuspension buffer tube – graduated tube, white screw cap



PCR strip – 8 tubes with attached lids, one per target



5. Check List

Required Equipment (not provided with the kit)

The following equipment is necessary for proper test execution but is **not included** in the detection kit:

- 20–200 µL adjustable micropipette
- Nuclease-free 200 µL filtered pipette tips
- Disposable gloves
- Combined rack for tubes (1.5 mL) and PCR tubes (0.2 mL)
- Biological waste container
- Vortex mixer
- Mini centrifuge accepting both 1.5 mL tubes and PCR strips, with a tube available as a counterweight
- Permanent marker
- [Heating block \(100 °C\) with power cable](#)
- ReadSensor LAMP reader with connection cable

Unisensor starter pack

Most of this equipment is available from Unisensor as a **starter pack**, ordered separately from the kit:

- ReadSensor LAMP reader
- Heating blocks (×3)
- Mini centrifuge
- Vortex mixer
- Adjustable micropipettes, 20–200 µL and 100–1000 µL
- Nuclease-free filtered pipette tips, 2 boxes
- Tube rack

The starter pack is common to several Unisensor tests, which is why it contains three heating blocks; the RespiSensor procedure uses one of them. Every item of the starter pack can also be ordered individually.



Ensure that all equipment is available, clean, and in perfect working order before starting the test.

6. Sample Collection Protocol

The RespiSensor test can be run from two sample types: a **nasopharyngeal swab** or a **bronchoalveolar lavage (BAL)**. Both are collected into the same lysis buffer tube.

 **Viral RNA is highly prone to degradation. It is therefore essential to ensure rapid transport of samples.**

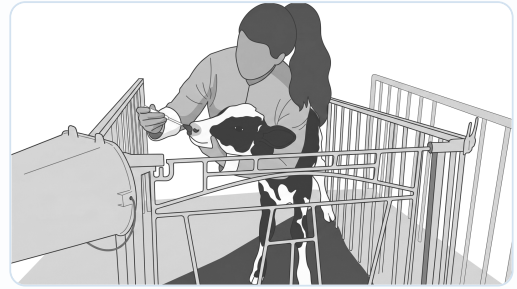
Required Materials

- Sterile nylon nasopharyngeal swab provided in the kit, or 300 µL Pasteur pipette provided in the aluminium pouch of the kit
- Lysis buffer tube, prefilled with lysis buffer
- Disposable gloves
- Sample identification materials (date, animal, site)

 **Sampling must be performed by trained personnel (e.g., a veterinarian) in compliance with biosafety standards and animal welfare. Always use a new pair of gloves for each animal.**

6.1. Nasopharyngeal swab

1. Gently insert a sterile swab into the bovine nostril.
2. Rotate the swab at least 5 times, gently, and keep it in place for a few seconds to absorb respiratory secretions.
3. Remove the swab while avoiding contact with external surfaces.
4. Cut the swab shaft so that the absorbent head stays in contact with the lysis buffer in the provided tube; close the tube tightly.
5. Immediately label the tube.



✿ Up to 3 swabs may be pooled in the same lysis buffer tube.

✿ Visually check that the buffer inside the tube is in contact with the swab tip.

6.2. Bronchoalveolar lavage (BAL)

1. Perform the bronchoalveolar lavage in accordance with good veterinary practice.
2. Back at the clinic, draw **300 µL** of the BAL collected in saline solution, using the 300 µL Pasteur pipette provided in the aluminium pouch of the kit.
3. Transfer the 300 µL directly into the lysis buffer tube; close the tube tightly.
4. Immediately label the tube.



! Do not discard the Pasteur pipette at this stage. The same pipette is used again at step 5 of the test procedure, to transfer the lysate into the spin filter column.

6.3. Contact time and sample holding



Whichever sample type is used, the sample must remain in contact with the lysis buffer for **at least 15 minutes** before the test procedure is started.

Once the sample is in the lysis buffer tube, two limits apply, both counted from the moment the sample was collected:

- the tube must be placed at **4 °C within 6 hours** of collection;
- the analysis must be carried out **within 12 hours** of collection.

Additional Recommendations

To ensure optimal result quality, it is recommended to discard any sample presenting one of the following issues:

- Poorly identified or unlabeled sample
- Tube not tightly sealed
- Missing lysis buffer or evidence of leakage
- Sample transported outside the recommended temperature range
- Sample held beyond the maximum holding time stated above

7. Before You Start



Read all of these instructions carefully before starting the test procedure.

Preparation Before Handling



Perform the steps below before starting the procedure, in a clean and safe environment.

1. Thoroughly clean the work surface.
2. Put on a pair of clean gloves.
3. Take the analysis box out of the refrigerator.
4. If samples are frozen, allow them to thaw completely before use.
5. Switch on the heating block now, so that it reaches temperature while the rest of the preparation is carried out. Check its settings: 100 °C for 10 minutes. Refer to the [heating block user manual](#).
6. Briefly centrifuge the resuspension buffer tube (5 seconds) to bring the liquid down to the bottom and prevent reagents from remaining in the cap.

Two Conditions to Check Before Starting



The heating block must have reached 100 °C before step 2 of the test procedure is started.



The sample must have been in contact with the lysis buffer for at least 15 minutes.

During Step 2 — Do Not Exceed 5 Minutes



Once the lysis bead has been added, the sample must not be left at room temperature for more than 5 minutes. As soon as the 5 minutes have elapsed, proceed immediately to the 100 °C incubation.

8. Test Procedure

The step numbers used below are those of the validated RespiSensor test protocol. Step 1 – the collection of the sample – has its own chapter, and is recalled here so that the sequence can be followed from beginning to end.

Step 1 — Sample collection



Collect the sample into the lysis buffer tube, either as a nasopharyngeal swab (step 1A) or as a bronchoalveolar lavage (step 1B), following the procedure described in [Sample Collection Protocol](#).



Before starting step 2, check the two conditions listed in [Before You Start](#) – heating block at 100 °C and at least 15 minutes of contact with the lysis buffer – and note the 5-minute limit that applies once the lysis bead has been added.

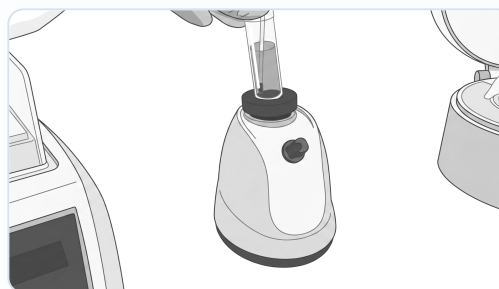
8.1. Lysis

Step 2 — Lysis bead

Add **one lysis bead** to the lysis buffer tube.



Vortex thoroughly.

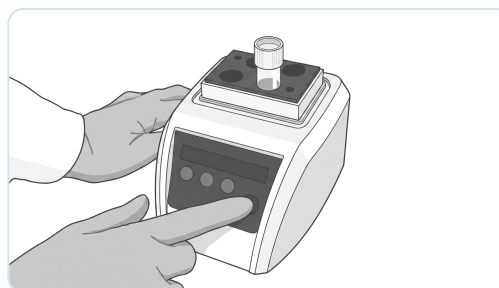


Incubate for **5 minutes** at room temperature. Do not exceed this time.



Step 3 — Thermal lysis

After the 5 minutes, vortex again and immediately place the lysis buffer tube in the heating block for **10 minutes at 100 °C**. The block must already read 100 °C before the tube goes in.



Beware of burn risks when placing the tube into the heating block, when removing it, and when touching hot metallic surfaces.

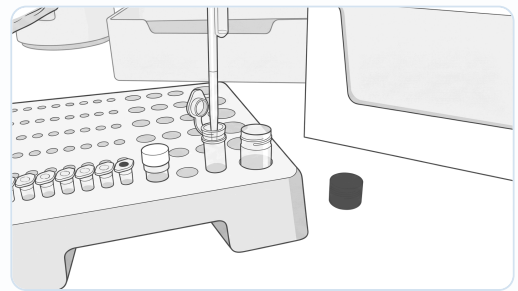
8.2. Cooling and filtration

Step 4 — Cooling

When the end-of-incubation alarm sounds, remove the tube from the heating block and allow it to cool for **3 minutes** at room temperature.

Step 5 — Transfer of the lysate

Once the tube has cooled, vortex again. Using the 300 µL Pasteur pipette provided in the kit, transfer **300 µL of the lysate** into the spin filter column.



! Discard the Pasteur pipette after this step.

Step 6 — Filtration

Centrifuge the spin filter column for **30 seconds**.

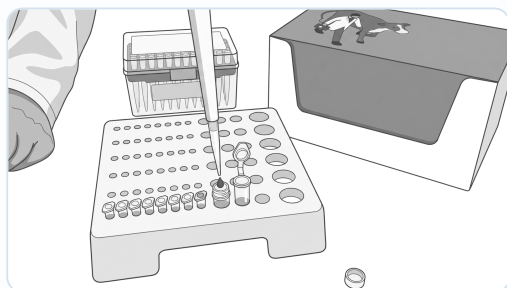


✿ Always balance the centrifuge by placing a tube opposite to counterbalance the weight.

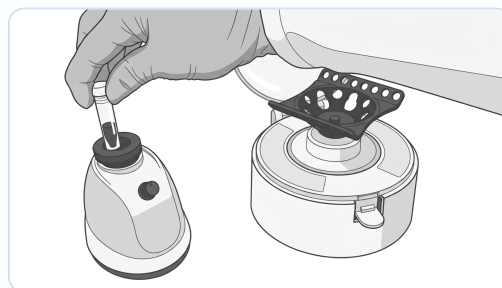
8.3. Reaction mix and PCR strip

Step 7 — Reaction mix

Collect 25 μ L of the filtered lysate and transfer it into the resuspension buffer tube (white cap). Vortex thoroughly, then centrifuge briefly.



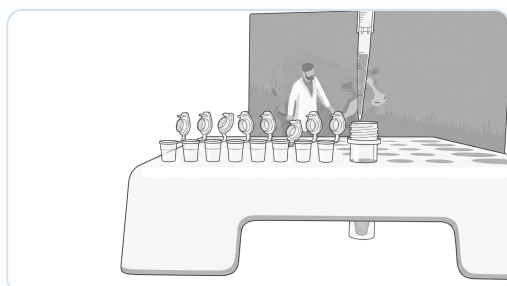
25 μ L into the resuspension buffer tube



Vortex, then a brief spin

Step 8 — Filling the PCR strip

Dispense 25 μ L of the mixture into **each** of the 8 tubes of the PCR strip, then close the lids carefully, one by one.



25 μ L per tube, all 8 tubes



Close the lids one by one

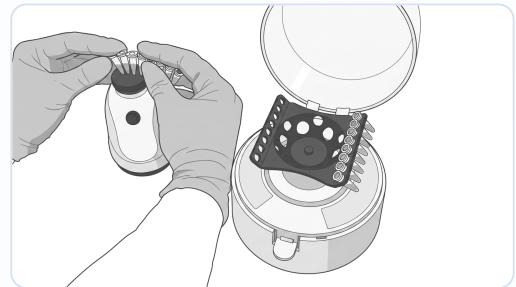


Do not touch the dried reagent bead at the bottom of the tubes with the pipette tip. If this happens, change the tip before filling the next tube.

8.4. Reading

Step 9 — Vortex and spin

Vortex the strip along its entire length, then centrifuge briefly.



* Check that the beads are fully rehydrated and that no air bubble remains at the bottom of the reaction tubes.

* Always balance the centrifuge by placing a tube or strip opposite to counterbalance the weight.

Step 10 — Reading

Switch on the ReadSensor LAMP and insert the strip into the instrument. Select the KIT120 protocol, then start the analysis.



! The strip must be placed in a specific orientation: the tube marked with a **green dot** must always be positioned on the left-hand side of the instrument.

* The analysis takes **40 minutes** in total.

Run profile

The KIT120 protocol runs at two successive temperatures, which the instrument applies automatically once the analysis is started.

Step	Temperature	Duration
------	-------------	----------

1	57 °C	5 min
2	62 °C	35 min

The two steps together make up the 40 minutes of the run.

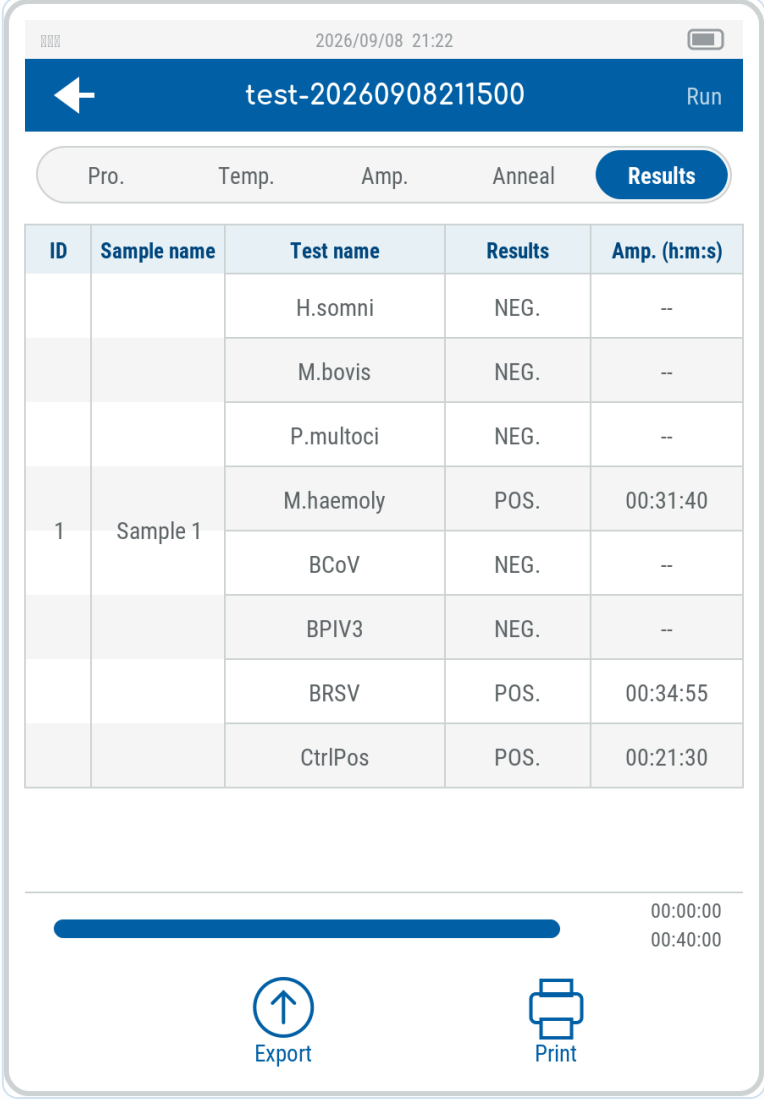
9. Interpretation of results

! Before interpreting the targets, check that the run is valid – see [Run validity and troubleshooting](#).

The analysis takes **40 minutes** in total; at the end of the run, the ReadSensor LAMP reports the presence or the absence of each target in the tested sample.

Reading the result table

The **Results** tab lists one line per target. A target that amplified is reported **POS.**, together with the time at which amplification was detected. A target that did not amplify is reported **NEG.**, with no time.



The screenshot shows the 'Results' tab of the ReadSensor LAMP app. The interface includes a header with the test ID 'test-20260908211500' and a 'Run' button. Below the header are tabs for 'Pro.', 'Temp.', 'Amp.', 'Anneal', and 'Results'. The 'Results' tab is active, displaying a table with the following data:

ID	Sample name	Test name	Results	Amp. (h:m:s)
		H.somni	NEG.	--
		M.bovis	NEG.	--
		P.multoci	NEG.	--
1	Sample 1	M.haemoly	POS.	00:31:40
		BCoV	NEG.	--
		BPIV3	NEG.	--
		BRSV	POS.	00:34:55
		CtrlPos	POS.	00:21:30

At the bottom of the screen, there is a progress bar and two buttons: 'Export' (with an upward arrow icon) and 'Print' (with a printer icon). The progress bar shows a duration of 00:00:00 to 00:40:00.

Example of a co-infection: *Mannheimia haemolytica* and BRSV both positive, the five other targets negative

Reading the amplification curves

The **Amp.** tab plots fluorescence against time for the eight tubes of the strip. A positive target produces a curve that leaves the baseline and rises; negative targets stay flat for the whole run. Each tube keeps the same colour in the chart and in the channel selector below it.



The same run: three curves rise, the earliest being the positive control

9.1. Run validity and troubleshooting

Run validity

The eighth tube of the PCR strip carries the positive control. It is what determines whether the run is valid.

Validation decision	Criteria
Test is valid	The positive control is detected (POS.)
Test is invalid	The positive control is not detected (NEG.)



If the result is invalid, repeat the test on the same sample, following the instructions of this user manual.

If the second run is also invalid

Work through the checks below before contacting Unisensor.

Check	What is expected
Was the sample collected following good veterinary practice, using a 0.9% physiological saline solution?	The sample should be collected according to good veterinary practice and using a 0.9% physiological saline solution, as specified in this manual. Improper sampling may affect assay performance and lead to invalid or inaccurate results.
Were nylon swabs used?	Unisensor recommends the swabs supplied with the RespiSensor kit. If they are not available, only nylon swabs should be used. Other types of swab may affect sample collection efficiency and assay performance.
Was the sample stored and held under the conditions described in this manual?	Inappropriate storage conditions may degrade the sample and give unreliable results.
Were the instruments correctly set up – pipettes calibrated and set to the right volume, heating block at the right temperature?	Verify that all equipment was working properly and configured according to the assay requirements before the test was run.
Was the protocol followed exactly as described in this manual?	Any deviation from the recommended procedure may affect test performance and result accuracy.
Was the KIT120 method selected on the ReadSensor LAMP?	RespiSensor has been validated only with the KIT120 method. Methods developed for other kits are not compatible and may lead to incorrect results.
Was the sample taken from a bovine animal?	RespiSensor has been validated for bovine samples only. It has not been validated for samples from other species such as sheep, goats or buffalo, and performance can only be guaranteed for bovine samples.
Did the ReadSensor LAMP complete the full 40-minute run?	The assay requires the complete 40-minute run. If the run was interrupted or stopped early, the result may be invalid and the test should be repeated.

If every point above is in order and the run is still invalid, contact support@unisensor.be.

10. Kit Performance and Compliance

Limits of detection

The limits below are validated for both sample types: nasopharyngeal swab and bronchoalveolar lavage.

Target	Limit of detection (copies of RNA or DNA per µL of matrix)
<i>Histophilus somni</i>	$>5 \times 10^2$
<i>Mycoplasma bovis</i>	$>10^3$
<i>Pasteurella multocida</i>	$>10^3$
<i>Mannheimia haemolytica</i>	$>10^3$
Bovine coronavirus (BCoV)	$>10^4$
Bovine parainfluenza virus 3 (BPIV3)	$>5 \times 10^4$
Bovine respiratory syncytial virus (BRSV)	$>10^4$
Positive control	$>10^4$

Quality Control

In accordance with Unisensor’s ISO-certified quality management system, each batch of RespiSensor test kits is tested according to predefined controls and specifications to ensure consistent product quality.

11. Symbols

SYMBOL	DESCRIPTION OF SYMBOL
	Indicates the manufacturer's catalogue number.
	Indicates the temperature limits.
	Indicates the expiry date.
	Caution.
	Indicates the manufacturer.

12. Disclaimer

The information contained in this document is provided in good faith, and every reasonable effort is made to ensure that it is correct and up to date.

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