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Urine sensor development project for MAFpol

Final report

31 July 2008

2020 SCIENCE

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Urine sensor development project for MAFpol

AgResearch

July 2008

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Table of Contents

1. Objectives

1. Prove the concept that a urine sensor can be developed for sheep that is capable of predicting nitrogen (N) content of urine as it is excreted
2. Evaluate the urine sensor on six sheep indoors over a period of up to six days

2. Concept

Refractive Index (RI) is one of the few methods we know of which offers the opportunity to indirectly estimate N% of urine *in situ*. The RI sensor is to be calibrated against standard solutions covering the expected range of urinary N concentration, and is validated against actual urine samples collected as they are excreted by sheep, constrained within a pen indoors. These samples will be assayed for total N in a laboratory test.

Plate 1: RI sensor apparatus fitted to a sheep.

3. The urine sensor

To provide a measure of RI for urine, a lab-grade RI sensor was attached to the sheep. The method of attachment consisted of a platform located over the sheep's rump, fastened with a harness (Plate 1). Urine enters a funnel which captures all of the urine, but excludes faeces and other contaminants. The RI sensor is located in a small chamber which retains a sub-sample of voided urine (~30 ml) that is refreshed during each urination event. The urine in this chamber prevents the RI sensing surface from drying out between urination events thereby allowing an accumulation of urinary solids on the sensor surface (urine solids contain 80+% urea). The sensing head and urine chamber is enclosed in foam plastic for protection and to exclude ambient light.

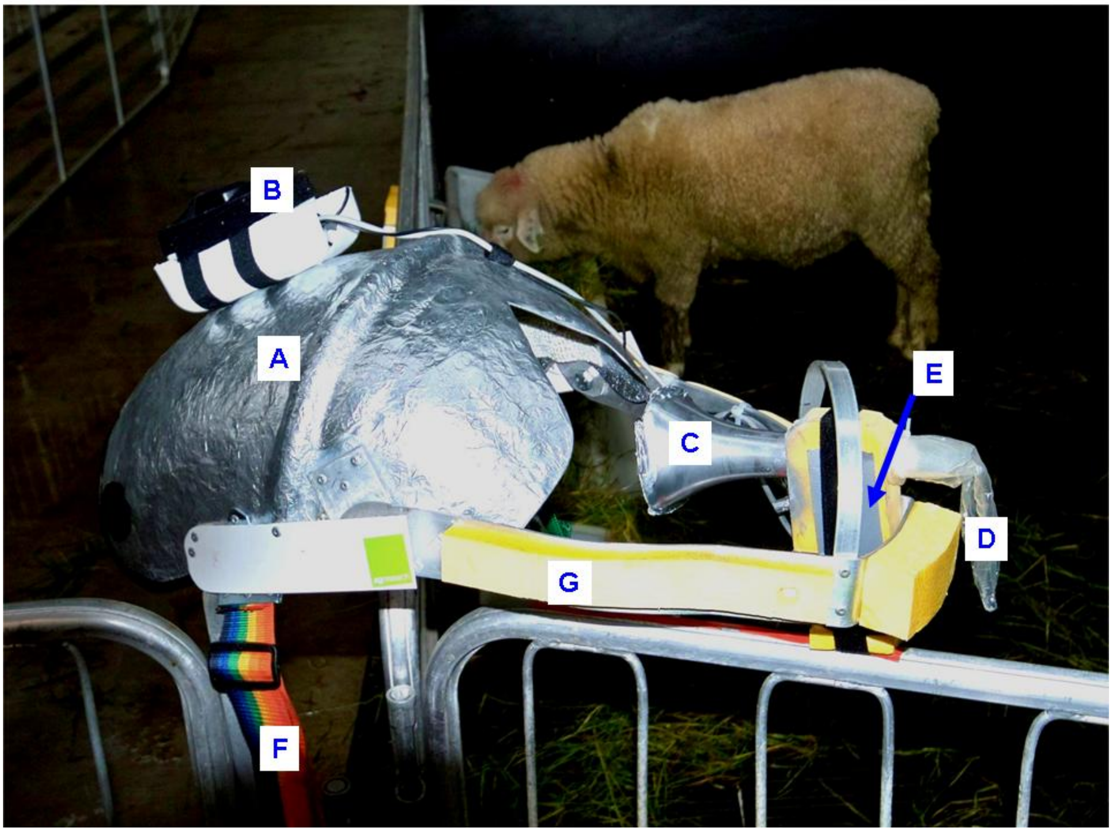


Plate 2: Close-up of urine sensor apparatus (resting on steel gates). A – fibreglass mould to locate urine sensor on rear of sheep; B - electronics box containing batteries, RI sensor control and GPS circuitry - a GPS antenna is located on top of this box; C - funnel through which urine passes; D - condom for collecting urine sample (for validation work only); E - The RI sensor is contained within a foam block to provide protection and to exclude sunlight; F - one of 3 straps that holds apparatus on the sheep; G – adjustable aluminium hoop with rubber protection to hold urine sensor in position. As the sheep squats to urinate the apparatus pivots about the hind leg joints such that urine passes into funnel.

When a urination event occurs, the electronic circuit senses a rise in temperature near the RI sensor. The RI sensor is activated to obtain a position fix with its built-in GPS receiver, and RI and temperature readings, logged for 20 seconds, at a rate of one per second. The 20 second group of RI readings will in many cases be repeat readings of the residual urine sample following the new ‘event’, since many urination events last only 5-10 seconds.

The circuit turns on the RI sensor for 30 seconds when the temperature within the sensing device rises above a threshold, presently set at 25ºC. This conserves battery life. Ten minutes later, the RI sensor is again turned on to log data for 30 seconds. Data from this period is the most accurate, assuming no further urine events occur in the 10 minute delay period. The delay ensures that the RI sensor and urine are at the same temperature, since urine RI is greatly affected by errors temperature compensation.

If the measured RI value falls below the pre-set 'normal range' for urine, a default value is entered. This is likely to happen where there is insufficient urine in the sensor chamber to cover the RI sensing surface, or where there is ambient light entering the device, such that the internal light sensor is compromised. The date, time, GPS position, RI and temperature log is transferred to a PC by removing the data card from the control box and placing it into a standard SD-card reader on a PC.

The sampler and RI sensor head weighs 300 g and occupies the dimensions 200 mm L x 60 mm W x 40 mm deep.

4. Activities undertaken during this contract

A laboratory 'model' of the rear end of a sheep was created to enable us to create urination events by the flick of a switch and to test our modified urine sensor at full-scale indoors. This has allowed us to advance our thinking and do preliminary tests on how best to capture all excreted urine: a future requirement is to measure the volume of urine at every urination event.

Two types of sensor – conductivity and RI sensors – were considered. The conductivity sensor was ranked second since it was more sensitive to sodium and potassium ions in urine than to urinary N, compared to the RI sensor. Furthermore, we have experienced problems with conduction sensors used for other purposes in the early development of the urine sensor.

The urine flow path within the RI sensor mock-up, mounting platform and control box,were all tested on a live sheep. Locally developed and constructed hardware and software to integrate with the RI sensors was manufactured for the six modified urine sensors.

The RI sensor was made to our specification by MISCO, USA. MISCO is a company specialising in making RI sensors for industry. Although delivery of the prototype was promised for mid-March, it arrived in late May 2008. Following 3 days of testing in the lab, a fault was found and the sensor had to be returned to MISCO. The repaired prototype was returned to us on 24 June. A further 6 complete systems were promised and scheduled to be working on our sheep by 16 July 2008. These sensors did not arrive by 21 July and so the apparatus evaluation was undertaken on just one sheep between 21-25 July. Samples were analysed at Massey University on 29 July.

A large number of urine samples from other trials were made available to us for N assay and use in validation of the RI calibration curve. These were tested using a Bausch & Lomb 33-45-58 bench top RI instrument. Laboratory N analyses were based on Kjeldahl digestion of urine samples, followed by colorimetric readings in an auto-analyser (Massey University Feed Analyses Laboratory).

The harness (Plate 1) was made to enable the fixing of the sampling device to the sheep. This was not envisaged in the original design concept which we based on using a CIDR to anchor the urine sensor to the sheep. However, due to the heavier than expected weight and greater size of the RI sensor apparatus, the harness shown in Plate 1 needed to be made. The harness also provides the opportunity to fix other devices to the sheep, such as for measuring urine volume.

Each urine concentration has a different temperature compensation equation. We now have an algorithm to cope with this. Given this, and the lab work showing the relationship of N to RI (temperature compensated to 20ºC), MISCO have promised to deliver an in-field device capable of measuring N from RI . This work is being done at no cost to the programme in recognition of their failure to deliver sensors within the promised timeframe.

5. Evaluation

The sensor apparatus was worn by one sheep held in a 4m x 6m pen during five days. Two other sheep were kept in this pen as companions, but were not monitored. The sheep were fed cut grass twice a day and had water available at all times.

A condom was fitted to the outflow pipe of the sensor to catch a sample of voided urine. During work hours, the condom was checked hourly and emptied if it contained urine. This sample was analysed for RI in the lab and for N content at Massey University.

RI values were determined on fresh urine only. Samples analysed for total N were acidified to ensure ammonia was not lost during Kjeldahl digestion.

6. Results

6.1 Laboratory calibration test of urine sensor

Urine samples from 16 cows were analysed for N content and had their RI value determined at 20ºC using the benchtop Bausch & Lomb refractive index meter (Fig. 1). This regression equation had a goodness of fit (R^2) of 96%, indicating that RI values taken by our urine sensor could be used to confidently predict N content of each urination.

6.2 Indoor validation of urine sensor on one sheep

The on-board RI sensor detected 8 urination events during Day 1 that exceeded the 25ºC threshold. A further 3 events are indicated by a small change in urine temperature (Fig. 2). Of the 8 urinations that exceeded the 25ºC during Day 1, the RI sensor logged 7 estimates of RI. The remaining event was below the normal range, indicating insufficient urine, or excessive ambient light on the sensor. Of the 5 urination events that occurred during work hours of Day 1, only two sub-samples (labelled "4" and "5") were successfully collected for validation analysis.

Only 3 urination events were detected by the sensor on Day 3, and 2 were detected Day 4 (24-25 July).

On Day 2, the wire from the battery to the circuit board was broken as the lid to the electronics box was being fitted but this could not be detected until the following day when the box was opened for the data to be downloaded. On Days 3 and 4 we assume that the urine sensing apparatus was not properly aligned with the sheep's vulva, or that the sheep changed its posture while urinating. At the end of Day 4 urine was collected from 3 sheep by nasal

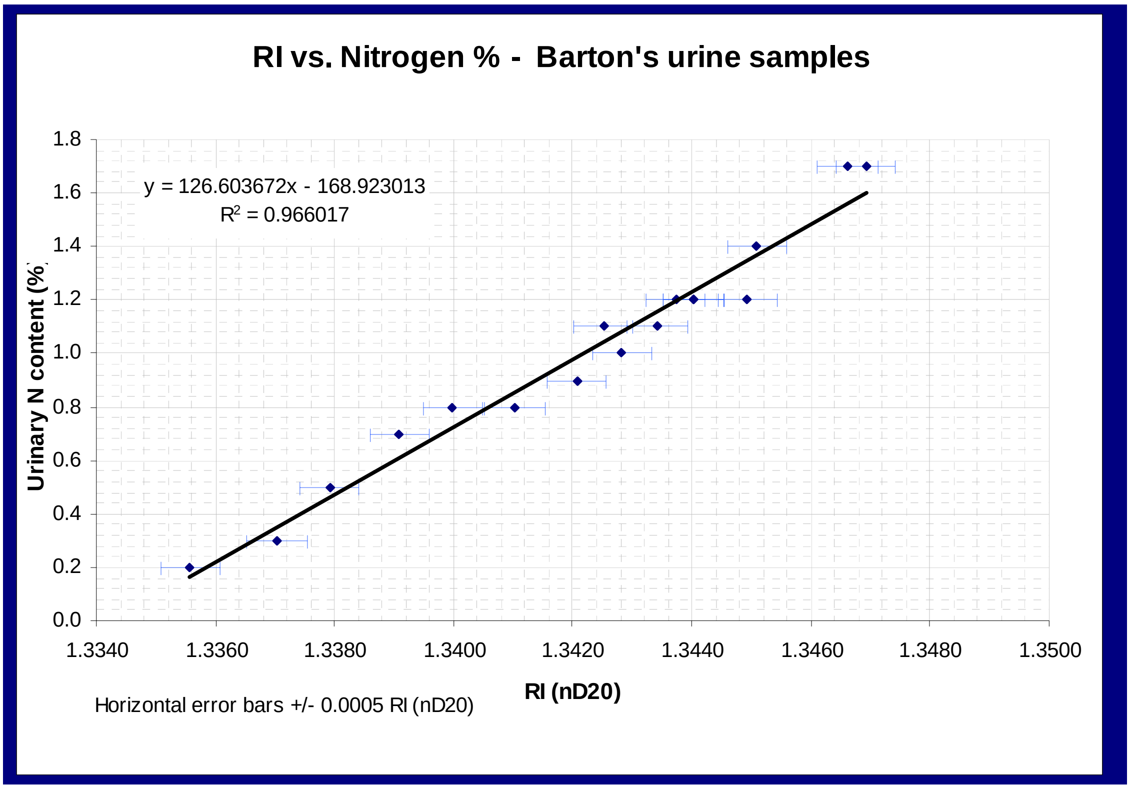


Figure 1 Refractive index measured by laboratory RI meter against measured urinary N content.

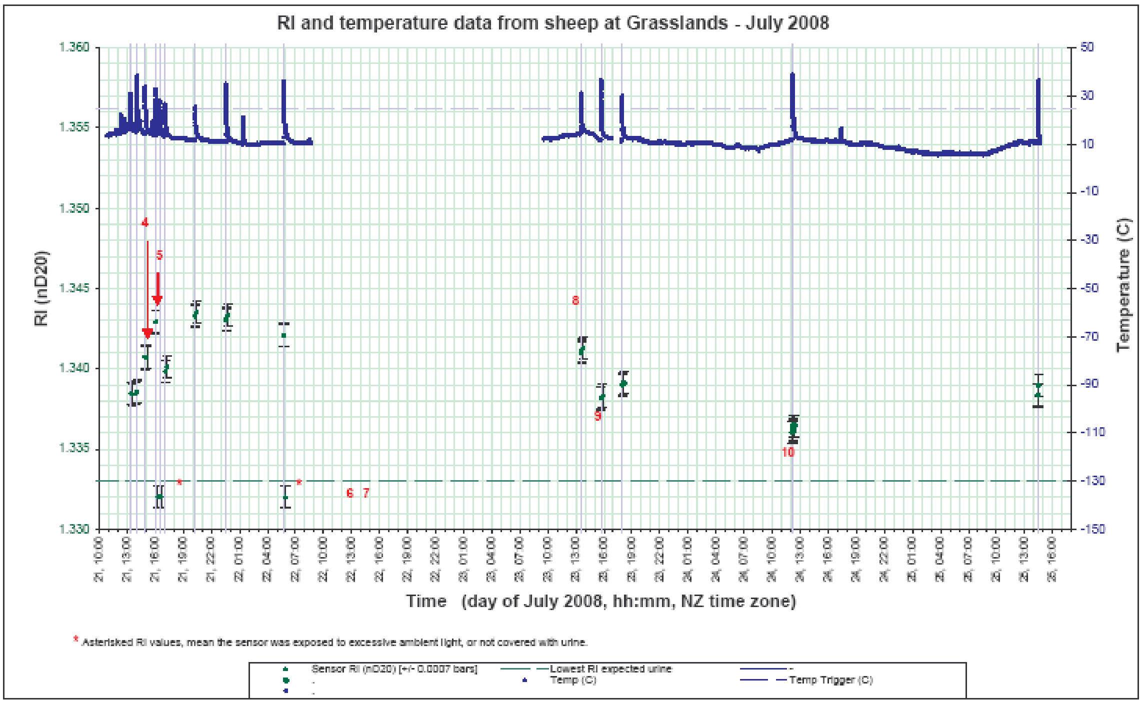


Figure 2 Urine sensor printout from a sheep fitted with the refractive index sensor. The blue line plot is of temperature which rose rapidly when the thermocouple was covered with urine, at body temperature. Green dots with error bars are RI values measure *in situ*. RI was measured only when the 25ºC threshold temperature was exceeded. On the second day (22-23 July) a wire from the battery had been broken and no data were recorded. On Day 3 only 4 urination events were logged because the apparatus was apparently poorly aligned.

occlusion which illicits a stress urination event. This urine was manually passed through the apparatus to obtain RI values to match samples analysed for N content.

Ten urine samples that had passed through the sensor were analysed for N content at Massey University. Two good urine samples were lost during removal of the condom from the sheep, because the sheep moved, causing the sample to spill. Another sample was missed when the sheep urinated while the condom was being changed. These problems relate only to validation work: physical samples are not required in grazing trials using this equipment.

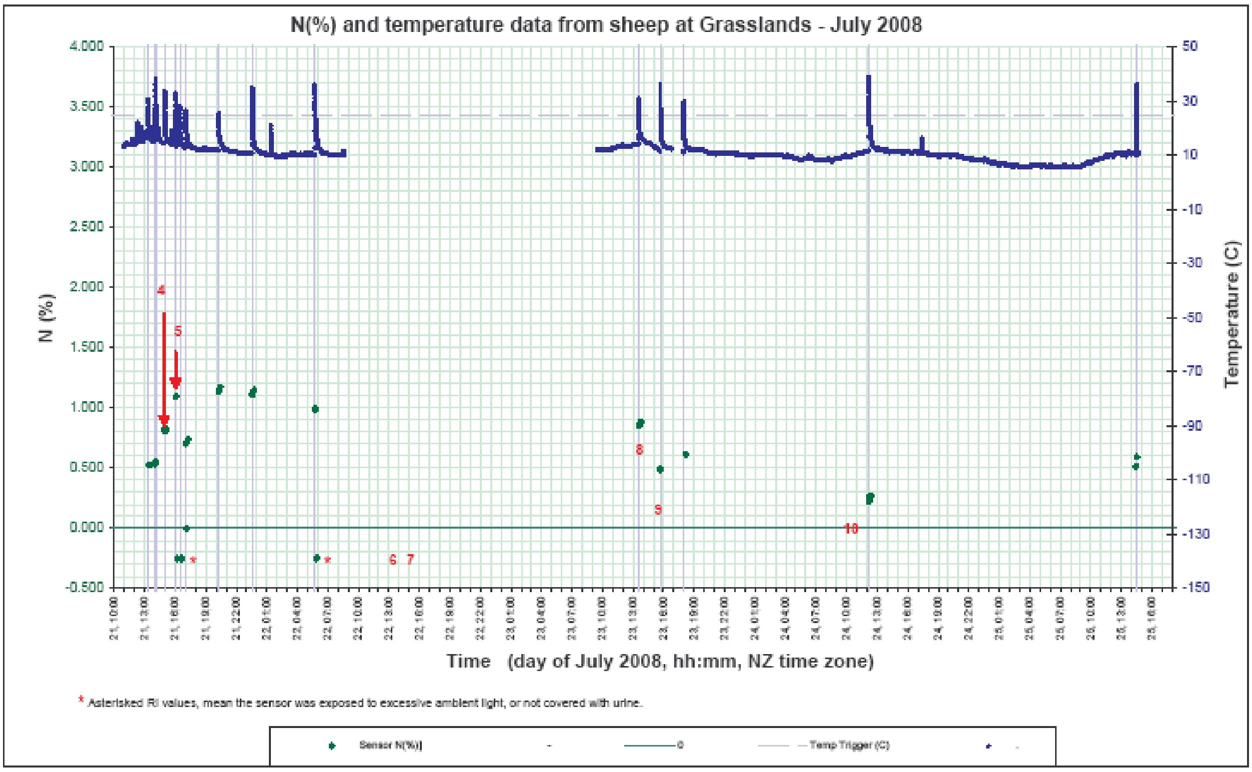


Figure 3 Predicted nitrogen content of urine based on RI measured *in situ* by the urine sensor.

No good relationship between assayed N and RI measured both on the sheep or in the laboratory could be established (Fig. 4). Similarly, there was no good relationship between predicted and assayed N content (Fig. 5).

The difference between RI measured on the animal and that measured on the respective validation urine samples in the laboratory, with the handheld sensor (Atago PAL-RI, Table 1), was 0.18%. Thus, our inability to predict urinary N using either RI values taken from the urine sensor or from the laboratory instrument, appears to relate to the handling of the urine samples and/or it's subsequent N assay.

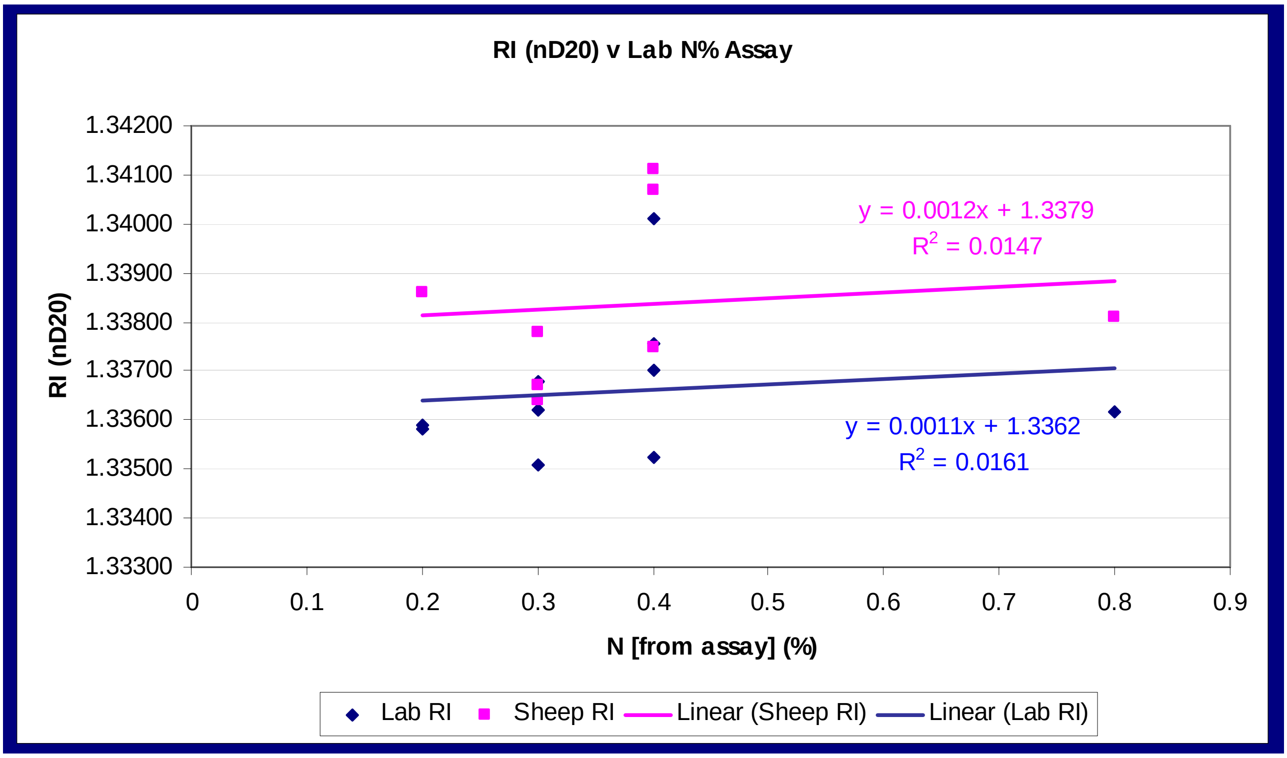


Figure 4 Urinary N content determined from the RI standard curve at 20ºC compared with urinary N content measured by wet chemistry. Pink data are based on *in situ* RI determinations and blue data are based on laboratory RI determinations.

Table 1: Comparison of fresh sheep urine RI values from sheep sensor, and handheld sensor (Atago PAL-RI).

Sample No.	Lab RI Sensor (nD20)	Handheld RI Sensor (nD20) [Difference WRT lab RI]	On-sheep RI Sensor (nD20) [Difference WRT lab RI]
1	1.3368	1.3369 [0.0001]	1.3378 [0.0010]
2	1.3370	1.3371 [0.0001]	1.3375 [0.0005]
3	1.3362	1.3362 [0.0000]	1.3367 [0.0005]

6.3 Discussion

The manufacturer of the RI sensor, MISCO, designed a unit that we successfully used to measure the refractive index of urine excreted by one sheep constrained within an open, indoor pen over 4 days. Where RI data were obtained, with only one exception, they corresponded with the presence of urine in the sensor, as determined by the elevated temperature measured by an independent thermocouple. The data from the exception could be automatically eliminated, as the RI sensor indicated there was insufficient urine available and thus the urination event would have been very small or perhaps non-existent. There was also excellent agreement between the sheep RI sensor and the handheld RI sensor when using fresh urine.

We conclude: *The concept of measuring the RI of urine in situ is proven.*

We were unable to predict urinary N content from RI in the 10 validation samples when using the calibration curve developed from urinary N and RI of independent urine samples from cattle (Figure 1). The range in urinary N concentration amongst our small number of validation samples was small and would thus partially explain the weak correlation coefficient between N estimated by RI and wet chemistry (r = 0.49) (J. Koolaard, pers comm).

We think that the poor prediction of N content most probably relates to the N assay. Since assays were done using standard laboratory Quality Assurance methods, including duplication of assays for 2 of the 9 urine samples and the use of standard reference samples, these lab results are not questioned. This then leaves only the sample handling as the most probable source of error related to N assay – contamination, inadequate sub-sampling, the acidification procedure, and N loss during storage prior to analysis. Whereas stored samples are known to result in precipitation of some nitrogenous components (e.g. uric acid, creatinine), precipitates were not seen in our samples. This probably occurs when samples sit around for several days before being analysed without having been frozen.

Our samples were frozen within two hours of collection and analysed within 1 week, which is considered to be adequate (Leiza Turnbull, Massey University, pers. comm.). We are unaware of how contamination may have occurred following measurement of RI in the laboratory. In future validation work we will pay greater attention to handling of samples.

Because we have still not received the remaining six, prepaid RI sensors from MISCO, we could not fulfil the contractual agreement to test the device on six sheep over six days. We still plan to undertake this work as soon as we are able, and without further cost to MAFpol.

In doing so, we will attend to the following points:

- Better alignment of the collection funnel with the vulva, by ensuring that the apparatus can not move on that sheep's back.
- Studying camera footage of urinating sheep, fitted with the apparatus, to determine optimal placement of the funnel, and hence the apparatus, when the sheep squats to urinate. We will also try to see if this posture changes with time.

- Replace wires within the electronics box so they cannot be broken while fitting the lid to the box. We will also fit a waterproof viewing 'window' that allows us to confirm an intact electronic circuit, as indicated by 3 flashing diodes on the printed circuit board.
- Urine collected for validation of the RI sensor will be sub-sampled and frozen immediately, and thawed immediately before being assayed. This will minimise the risk of nitrogenous precipitates before analysis.
- Sheep used for validation studies will be managed to induce a wide range in urinary N content by, for example, restricting access to water for some of the day, or by feeding diets of different N content during the study period.
- Reduce the size of the apparatus – if possible

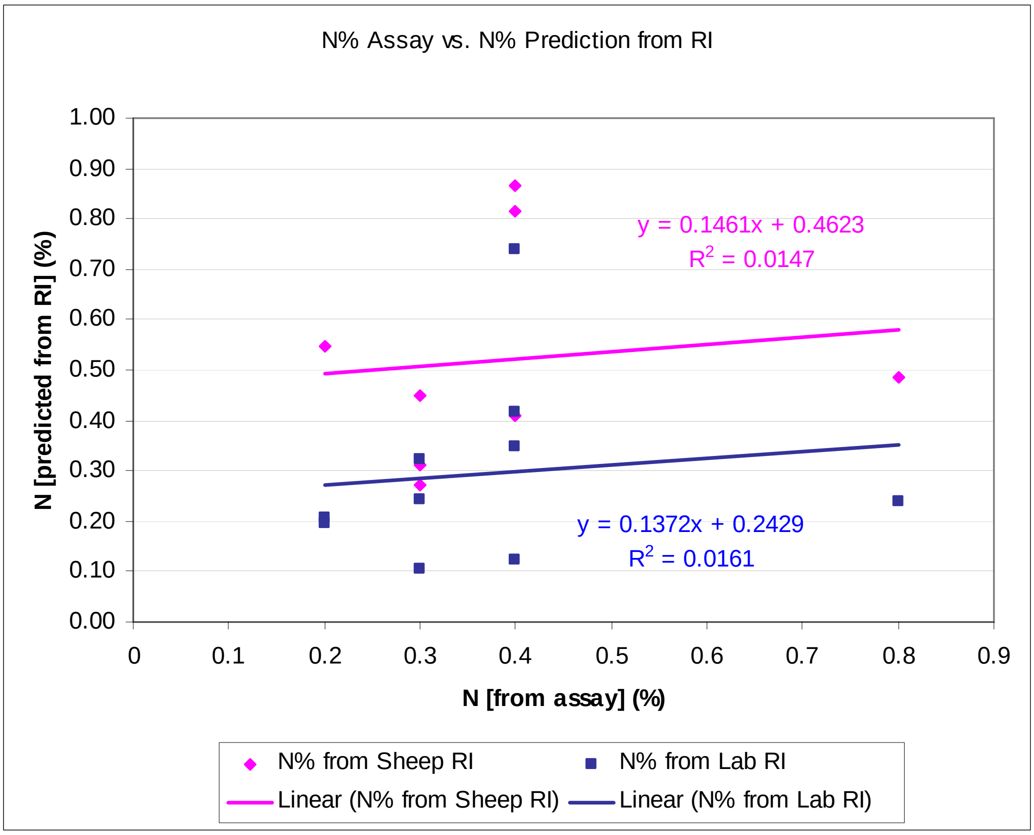


Figure 5 Plot of urinary N content determined by laboratory assay versus N content predicted from RI estimate made by the urine sensor on one sheep (pink) and RI measured in the laboratory (blue).

7. General Discussion

MISCO will use our RI calibration data to develop an on-board algorithm to calculate urinary N content for logging into memory. This will be given to us at no further cost to this programme.

As cattle are known to leach more N than sheep (Hoogendoorn et al., Int. Grassland Congress, 2008), the urine sensor needs to be adapted for use on cows to enable us to better understand urinary N dynamics within the nitrogen cycle of cattle. This will be more challenging than for sheep as attachment of the apparatus on these larger animals will require a different approach.

Further development of the urine sensor for both animal species is required to enable determination of the volume of each urination event. With knowledge of both volume and N content of each urination event, we will know the *load of N* deposited within a urine patch. The GPS will give the location of these patches within the field. Such data are crucial for better understanding N dynamics in grazed pastures and how these impact nitrous oxide emissions and nitrogen leaching.

A literature search has indicated a wide variation exists in daily urinary N excretion and daily urine volume amongst animals fed a common diet. Compared to animals with a low urine volume output, high-volume-output animals tend to excrete urine with a lower N content and urinate more frequently. This should result in less emissions of N from urine patches created by these high-urine-volume animals. Our ‘completed’ urine sensor will enable us to screen for animals with this high volume urination trait.

A research programme would then be required to determine the heritability of the trait, with the view that it might be possible to breed animals that mitigate nitrogen losses to the environment.

The improved urine sensor would also be used to validate other mitigation techniques where measurement of deposited urinary N is important.

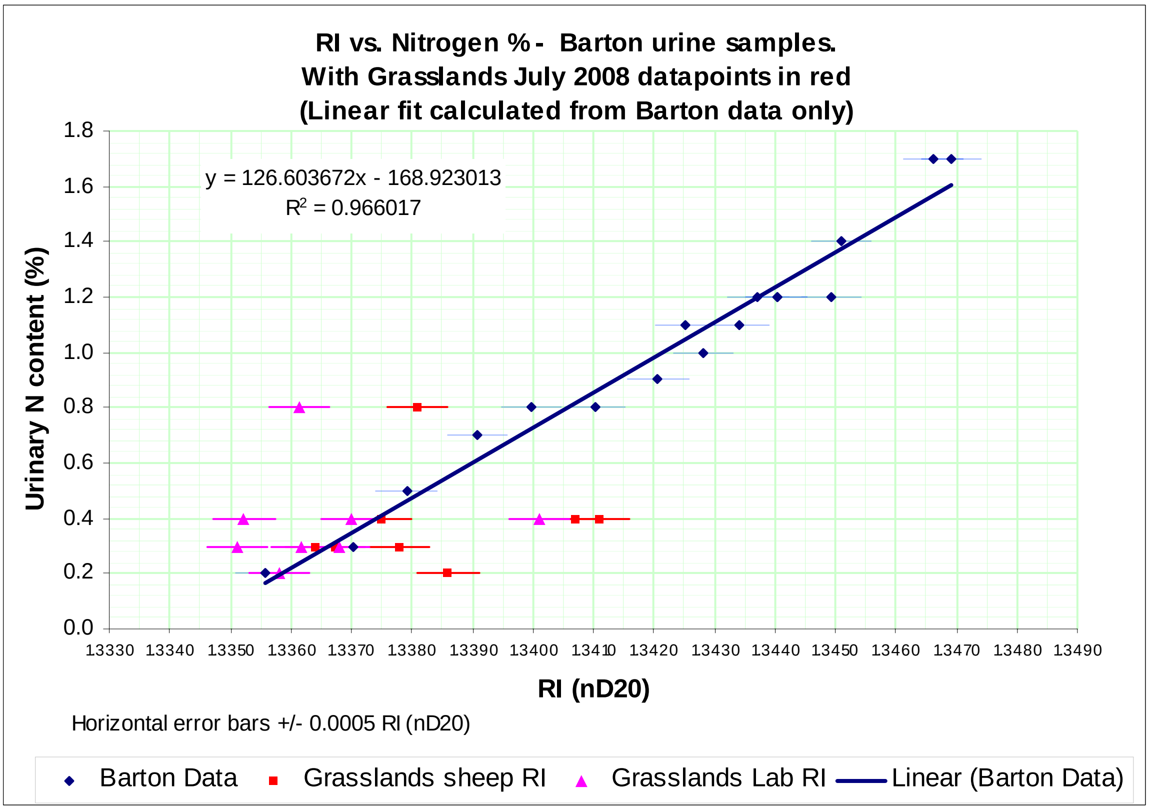


Figure 6 Urine from the validation dataset overlaid upon calibration curve of urinary nitrogen v RI developed with urine from Barton's cattle trial. The shorter the vertical distance from the plotted point to the regression line the better the predicted N content. The regression equation relates only to the data from cow urine samples.

8. Acknowledgements

Mark Carter assisted with circuit design and adapted the existing urine sensor to accommodate the RI sensor. Nitrogen assays were conducted by Ms Leiza Turnbull and her team in the Animal Nutrition Feed Laboratory, Massey University. AgResearch staff Fiona Burke made the animal housing facilities available for this trial, Hailey Hillson sourced and fed the sheep, and Kate Lowe assisted in preparation of urine samples prior to N assays. Gerald Cosgrove, Chairman of the AgResearch Grasslands, Animal Ethics Committee, assisted in many ways to ensure the trial was not delayed as trial protocols were altered to meet changing plans.

Addendums and Appendices

MISCO IRIS VIP RI Sensor - ver 0.12.doc

Important notes

1. This document is controlled by Peter Cross peter.cross@agresearch.co.nz +64 7 838 5361.
2. The window must be shielded from light to obtain correct results.
3. When the sensor is first turned on it goes through an initialization that is typically about five to eight readings. Ignore these readings until the RI results stabilizes.
4. It's not unusual for the temperature reported by the sensor to be slightly different than the ambient temperature. Typically, at 20 deg. C, serial number 0 reads 3.8 C higher than ambient. Use the temperature values as reported by the RI sensor for temperature compensation calculations.

Contents

Overview

The MISCO IRIS VIP 201 Refractive Index Sensor provides a lab-grade Refractive Index measurement capability in a compact, portable, low power, robust package. See "Appendix I: Specifications37" on page 37.
A Micro-Flow adapter is available (see **Micro-Flow adapter** drawing on page 24).

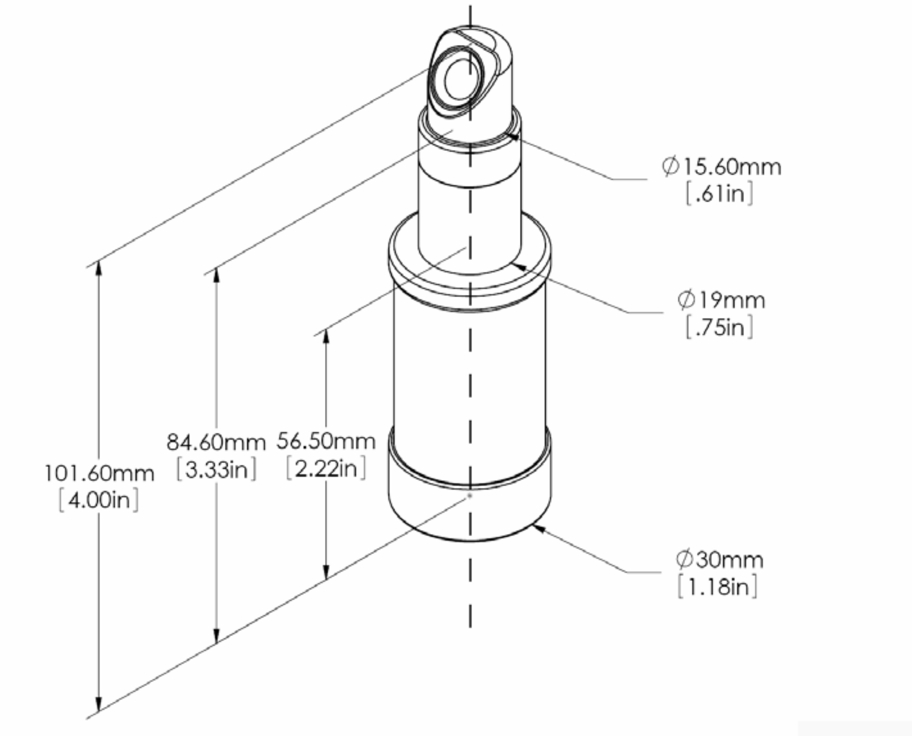


Figure 2: Misco IRIS VIP 201 Refractive Index Sensor

Communications protocol

Applicability

This document applies to the first sensor, model VIP203, (quote #Q080325-1, 25 March 2008) and the remaining 6 sensors, model VIP201, (quote #Q080424-1kw, 24 April 2008). I.e. all sensors from both quotes should be supplied as per this document.
The first sensor is to be returned to the manufacture in exchange for another VIP201 (7 total).

Power on and result transmission sequence

1. Power supply voltage =0V for at least 1 second.
2. Power supply voltage >5.0V
3. Wait for system initialisation: maximum 3 seconds.
4. Begin one transmission of the sensor identification string followed immediately by the sensor result string.
5. Every second thereafter: begin transmission of the sensor result string.

String definitions

All values output from the sensor are printable ASCII characters except for
`<CR>` (carriage return, decimal 13), and,
`<LF>` (line feed, decimal 10).
See "Appendix D: Allowable ASCII characters".

Sensor identification string

`$,S,`*`aaaa,bbbb,cccc,...`*`<CR><LF>`

Where *`aaaa,bbbb,cccc,...`* represents a string of up to 70 characters representing the fields describing model, hardware version, firmware version and serial number of the sensor. Only printable ASCII characters are allowed in each field and they must not be special characters defined in "Appendix D: Allowable ASCII characters". Each field is separated by a comma and a comma is not allowed within a field.
E.g.

`$,S,VIP203,HW1.2,FW3.11,SN0001<CR><LF>`

`VIP201` is hardware model VIP201
`HW1.2` is hardware version 1.2
`FW3.11` is firmware version 3.11
`SN0001` is serial number 0001

More comma separated fields are allowed to be present after the firmware version, e.g. calibration constants.

Sensor result string

`$,R,`*`r.rrrrr`*`,T,`*`tt.tt`*`<CR><LF>`

Where *`r.rrrr`* is the refractive index reading (nD) and *`tt.tt`* is the temperature reading in degrees Celsius.
E.g.

`$,R,1.33340,T,19.85<CR><LF>`

Negative values are not allowed.
Leading and trailing zeros are allowed to be present or not present. E.g. the following are all valid and treated as equivalent:

`$,R,1.340,T,9.2<CR><LF>`

`$,R,1.34000,T,09.20<CR><LF>`

RI result errors

If a correct RI result cannot be produced, the RI result is replaced by the word ERROR*x*, where *x* is the error number defined in Table 1. E.g.

`$,R,ERROR1,T,19.85<CR><LF>`

Table 1: RI result error numbers

Error Text	Error Description
Error0	**No error**
Error1	**No sample detected**
Error2	**nD is out of range (RI reading out of range)**
Error3	**Reserved, not used**
Error4	**Reserved, not used**
Error5	**Measurement is terminated by external command**
Error6	**Calibration data is lost or insufficient**
Error7	**Bad calibration standard (on calibration only)**

Temperature result errors

If a correct temperature result cannot be produced, the temperature result is replaced by the word ERROR E.g.

`$,R,1.3510,T,ERROR<CR><LF>`

Power supply

Power cycling

See Table 2 for power supply parameters.

If the sensor power supply drops below the minimum value defined in Table 2, invalid characters are allowed to be output.

Sensor power supply must drop to zero and remain at zero for at least 1 second before sensor is powered up again. Power supply ramp-up voltage must be of a positive slope throughout power up of at least ?V/s.

Calibration

The unit must be returned to the manufacturer for calibration.

Physical interface – communications and power

See Table 2 for physical interface characteristics.

Table 2: MISCO VIP Physical interface – communications and power characteristics.

Item	Value				Comments
	Min	Typical	Max	Units	
Communications					
Physical interface					**Serial RS232**
Baud rate		**19200**		**Baud**	
Parity		**None**			**No parity bit**
Start bits		**1**			
Stop bits		**2**			
Flow control		**None**			**No hardware or software flow control**
Character pacing	**0**	**0**	**10**	**ms**	**Character pacing is the time between the end of the last stop bit and the start of the next start bit during a string.**
Power					
Wires					
Power (+V)	**5.0**		**5.1**	**V**	**Regulated**
Ground (GND)					
Current (Peak)	**?**	**50**	**150**	**mA**	
Current (average)	**?**	**45**	**50**	**mA**	**75mA if using the in-line CAN to RS232 converter PCB.**

Figure 3: Connector pin-out. View of solder termination side of male contacts. Centre pin is pin 8.

Note: Figure 3 is a view of the solder termination side of **male** contacts (not female contacts).

Table 3: Connector pinout.

Pin on RI Sensor	Pin on In-line PCB	Label on In-line PCB	Function	Colour Scheme 1	Colour Scheme 2
1			**Do not connect**		
2			**Do not connect**		
3	**4**	**XT4**	**+5V**	**Red**	**Red**
4	**2**	**XT2**	**CAN_L**	**White**	**Blue**
5	**1**	**XT1**	**CAN_H**	**Yellow**	**Yellow**
6			**Do not connect**		
7			**Do not connect**		
8	**5**	**XT5**	**GND**	**Black**	**Green**

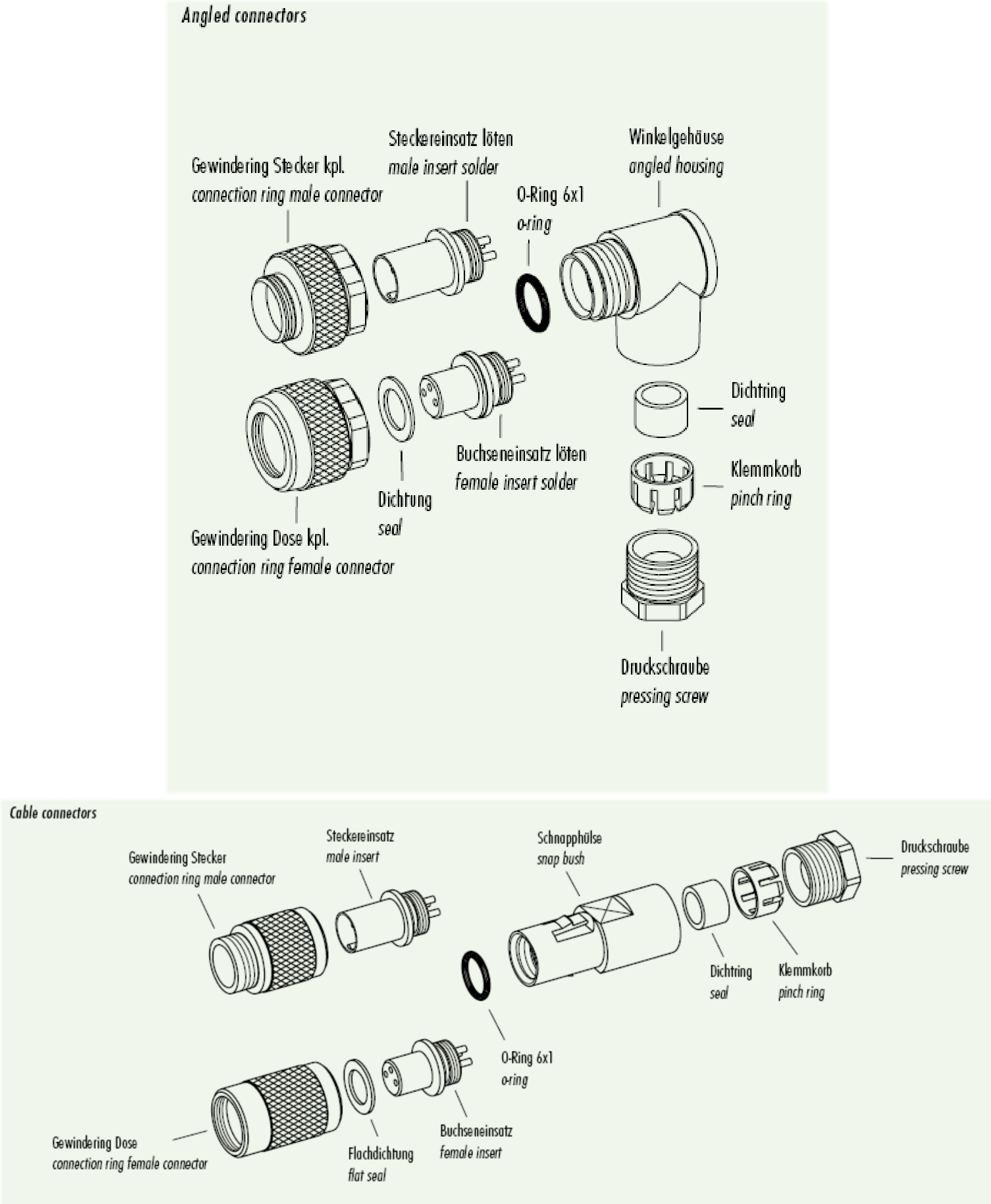


Figure 4: Connector assembly diagrams. See "M9 connectors IP67" catalogue from http://www.binder-usa.com/subminiature-cylindrical-connectors/

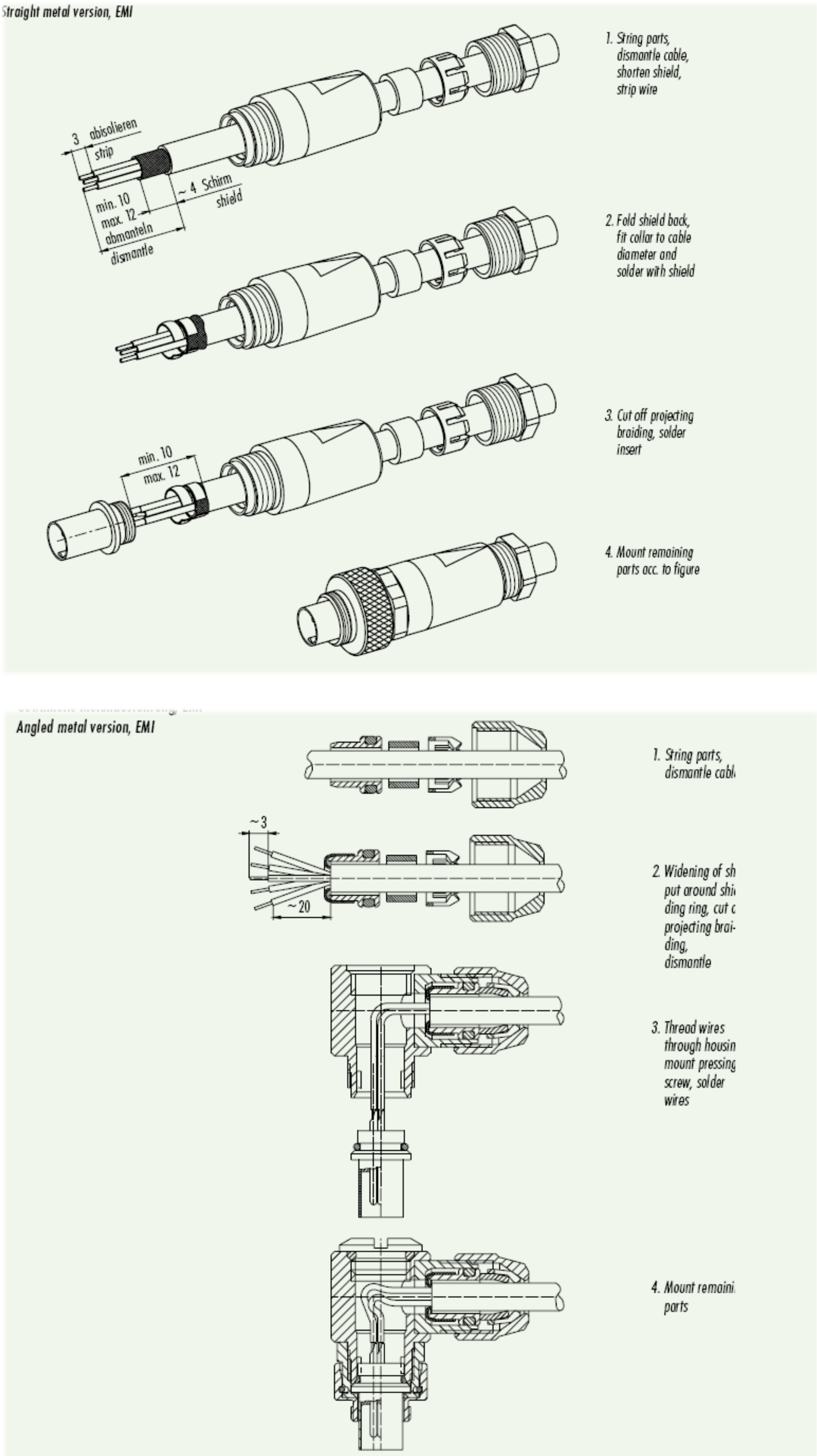


Figure 5: These wiring instructions are for the full metal version of the connector but can be used as a guide to the other types. See http://www.binder-usa.com/subminiature-cylindrical-connectors/

Table 4: Connector description and ordering information.

Connector	
RI sensor	**Description: Connector, circular, sub-miniature, 8-way, male contacts, male thread, M9x0.5 thread, chassis, 712 series.** **Probably front fastened part number 09-0427-90-08.**
Matching cable	**Description: Connector, circular, sub-miniature, 8-way, female contacts, female thread, M9x0.5 thread, free, 712 series.** Cable OD 3.5 to 5.0mm, max wire gauge 0.14mm^2 (AWG 26)**.**

Manufacturer:

http://www.binder-usa.com/subminiature-cylindrical-connectors/

http://www.binder-connector.de/e/index.html

Binder series 711 is the economical (IP40) version, series 712 series is the high performance (IP67) version, series 702 is the over-moulded cord sets (IP67) for 711 and 712 series.

Parts:

99-0426-00-08, connector, circular, sub-miniature, 8-way, female contacts, female thread, M9 thread, free, 712 series, straight**, solder contacts.**

99 0426 70 08 Female right-angled **connector, 8-way female, IP67**

79 1426 72 08 Series 702 Single-ended over-moulded **cordset with** 90° **female plug, IP67, 8-way**

Suppliers:

Binder

http://www.binder-usa.com

Lowest cost. No MOQ.

They don't accept pre-pay on shipping -- must use account

number of your own courier company.

Farnell

http://nz.farnell.com/jsp/home/homepage.jsp

No MOQ.

Binder part number 99-0426-00-08 line, straight, socket, series 712 version IP67 (Farnell 1122561 $47)

A.C. & E. Sales Pty. Ltd.

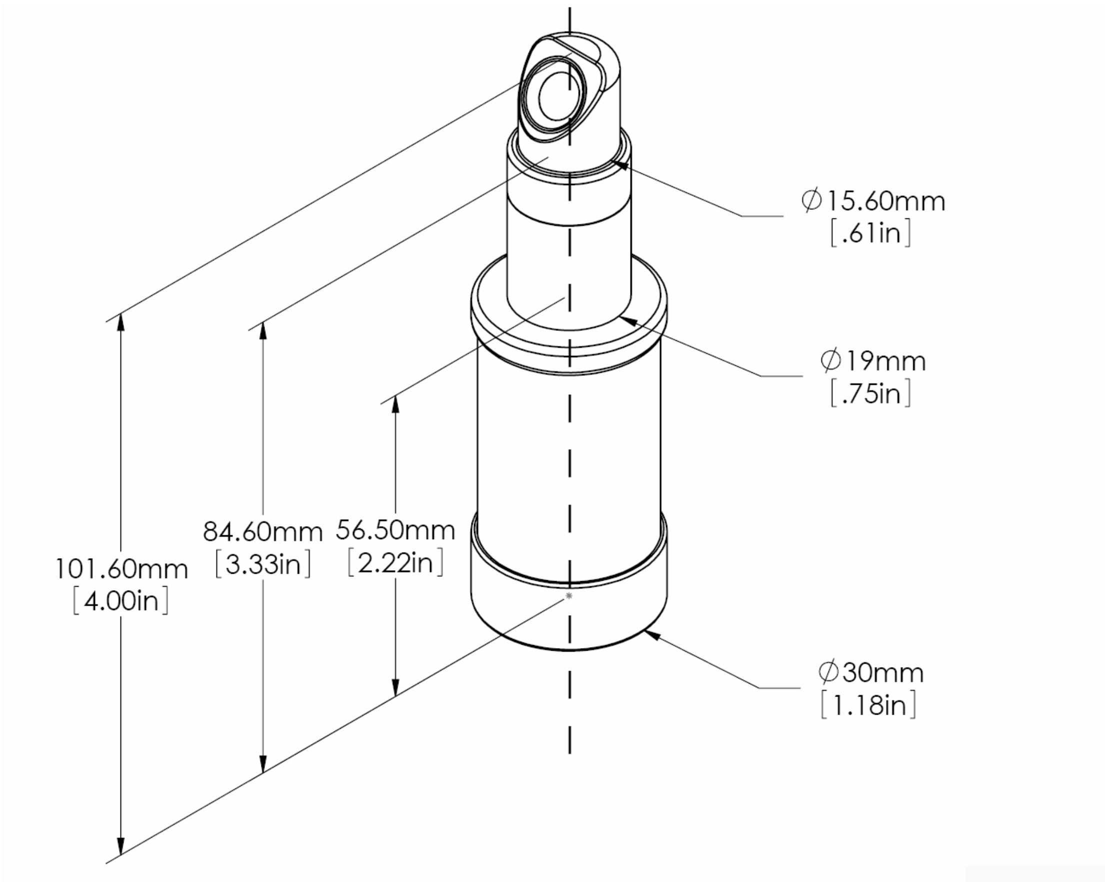
MOQW = 20, $70 each

http://www.acande.com.au/

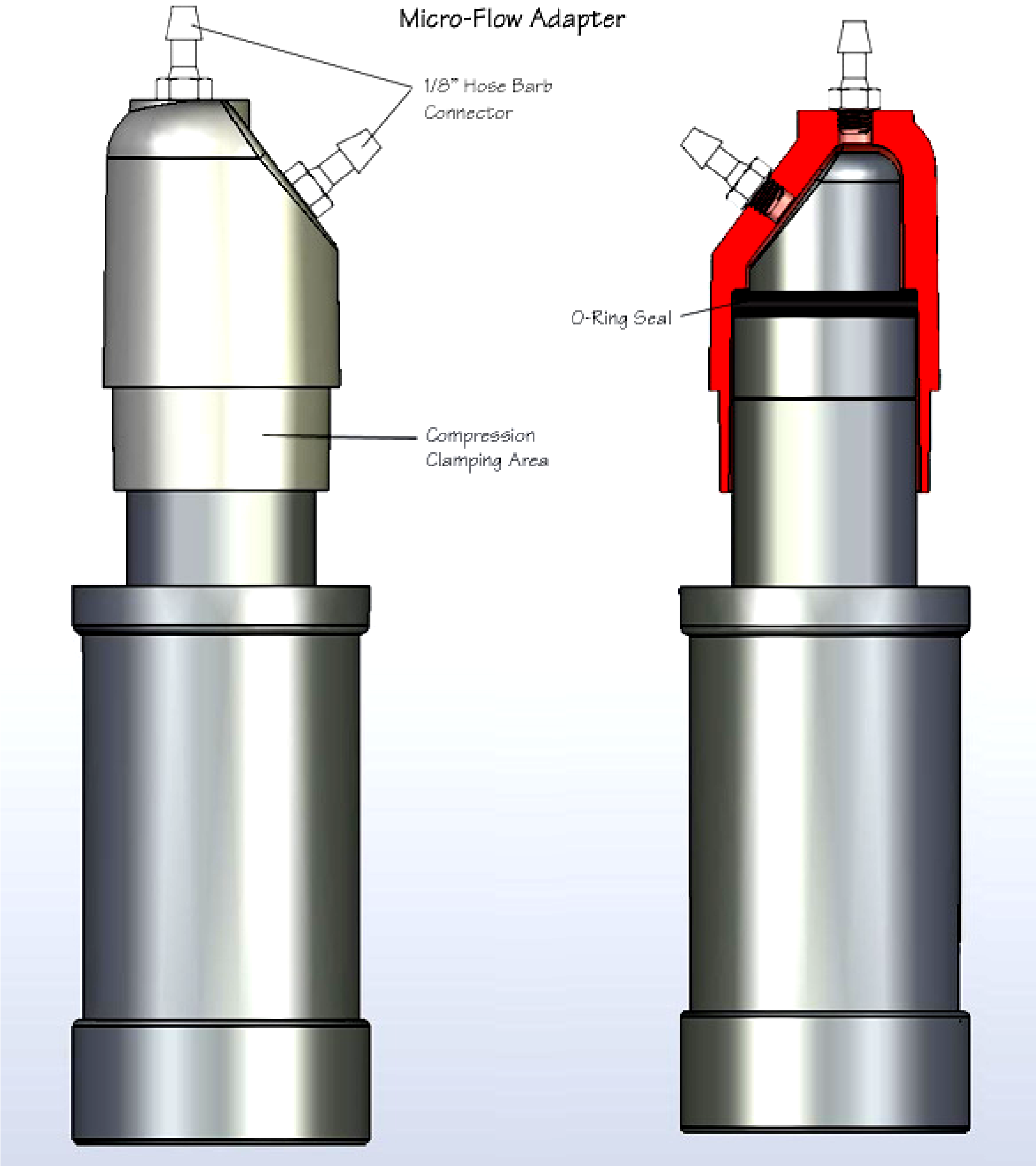
prichardson@acande.com

Appendix A: Drawings

Production version



Micro-Flow adapter



Sensor serial number 0

Sensor serial number 0. The first sensor received by AgR in May 2008. Subsequent sensors will be of slightly different dimensions.

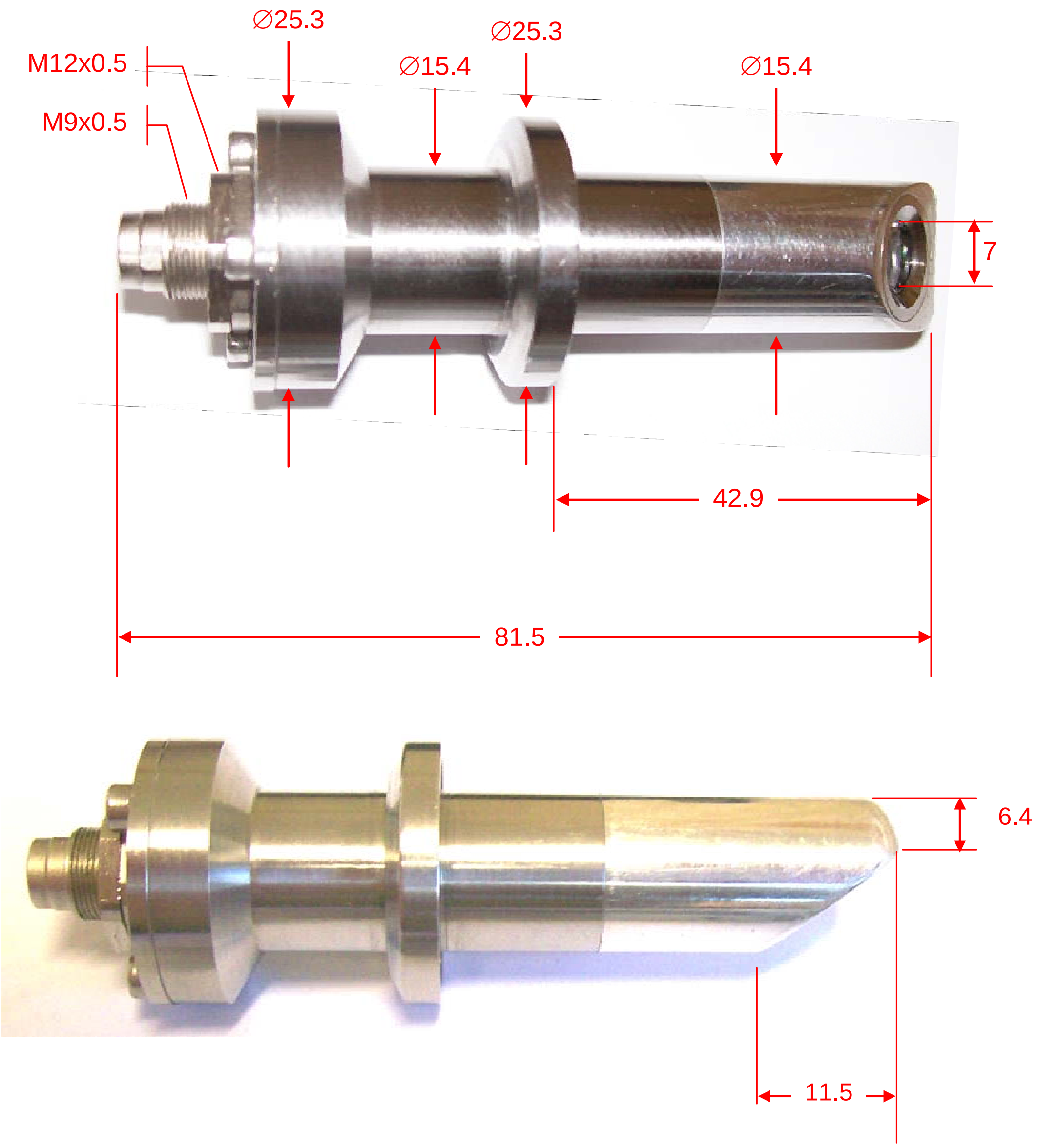


Figure 6: Dimensioned photo of sensor serial number 0. Dimensions in mm.

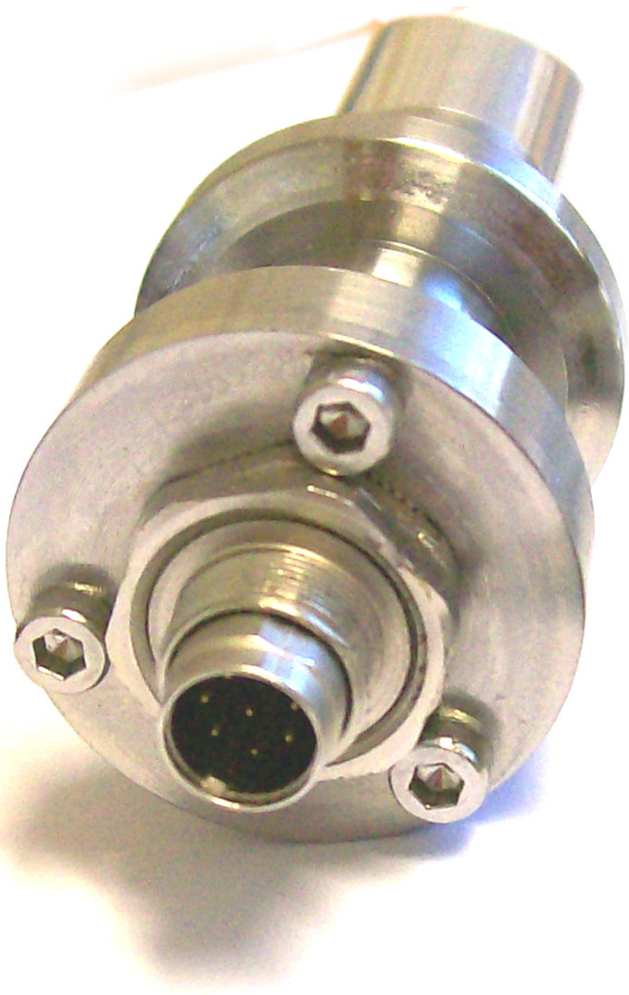


Figure 7: View from electrical connector end. Sensor serial number 0.

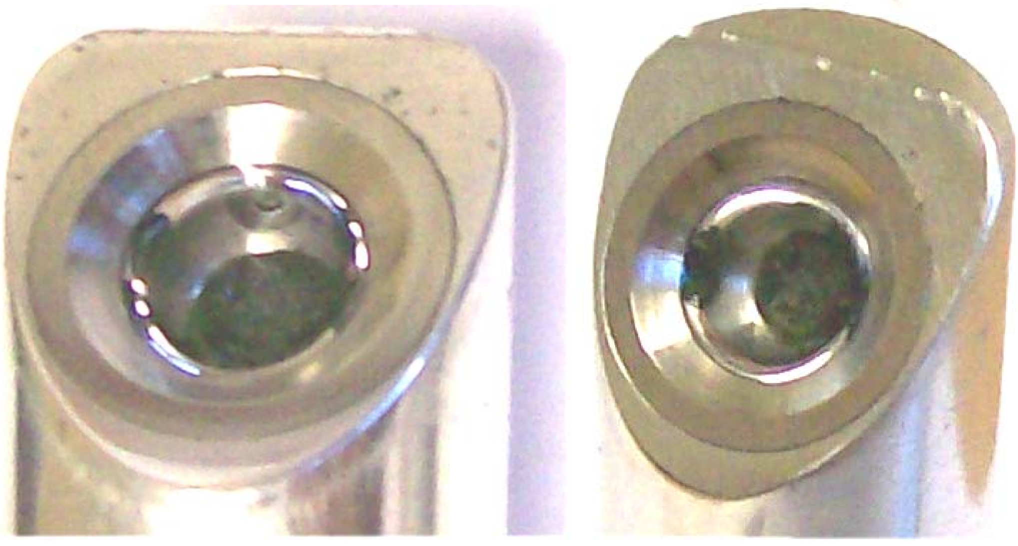


Figure 8: Close-up of sensor window. Sensor serial number 0.

Appendix B: In-line CAN-RS232 PCB

Dimensions of the PCB are 45mm x 65mm. This is supplied without an enclosure. Connector X3, pins 2 and 3 are at RS232 (+/-12V) levels. Mass is 22g including 9-pin D-sub connector (not shown in figure).

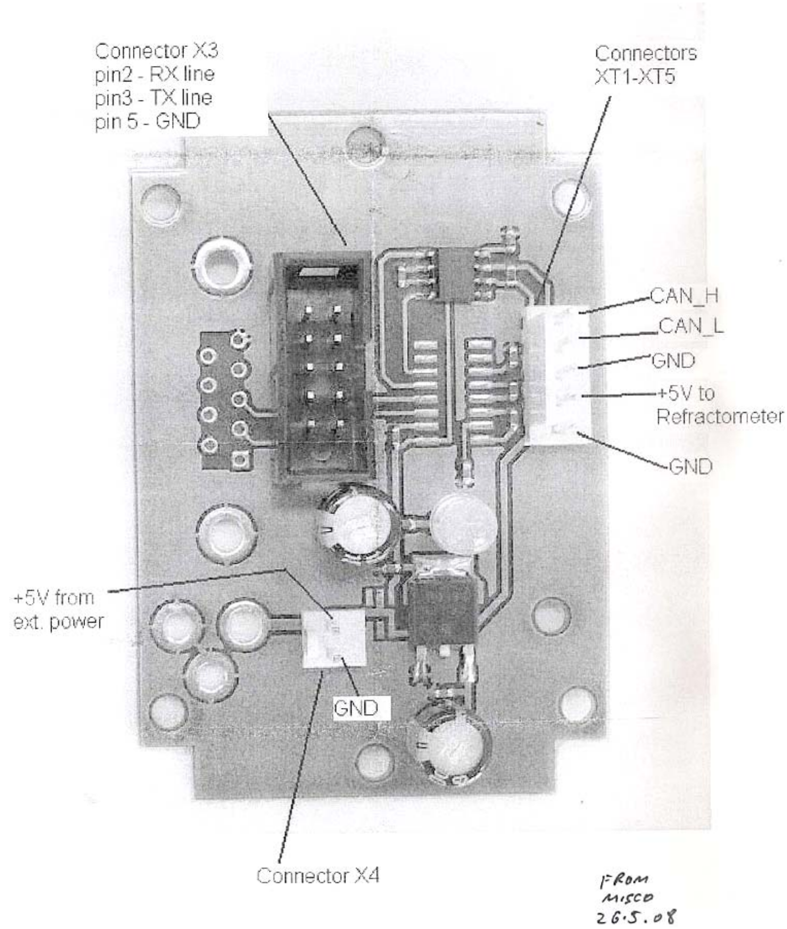


Figure 9: Photo of In-line CAN-RS232 PCB supplied by MISCO

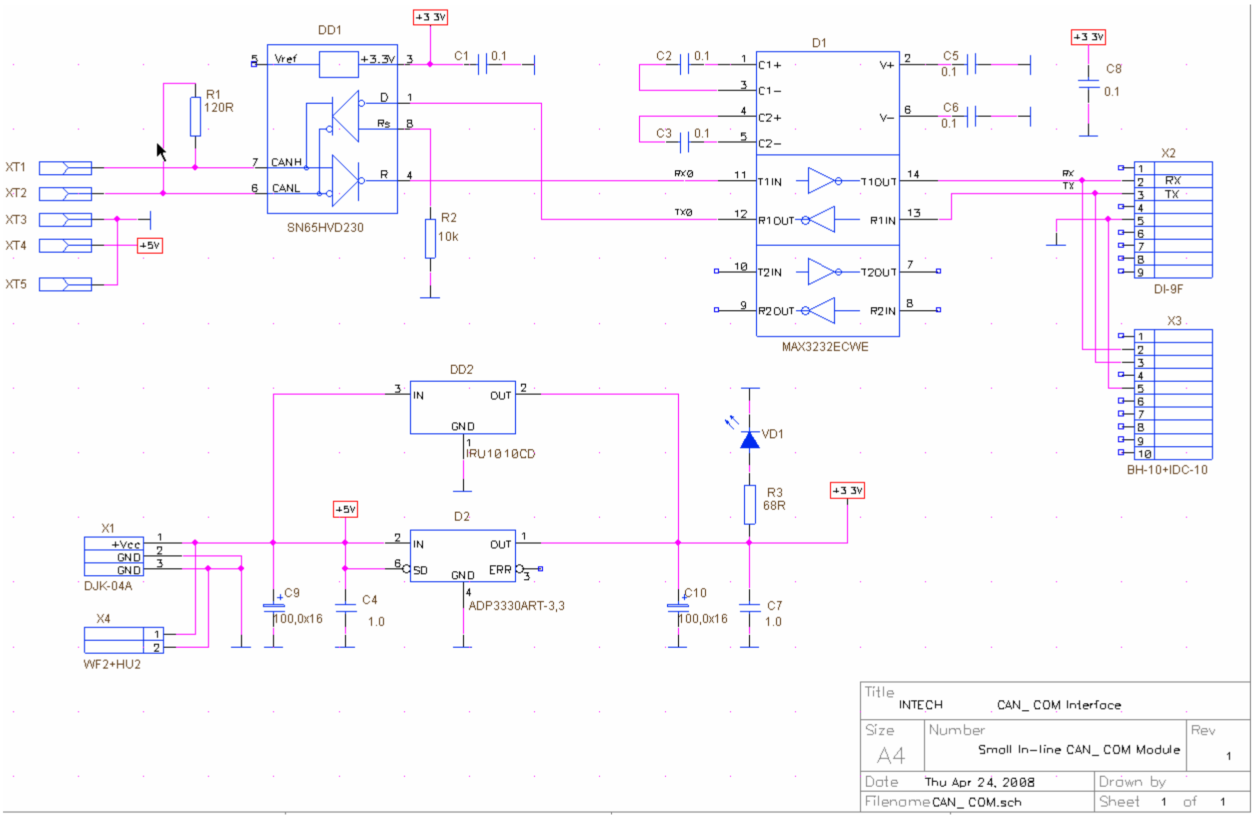


Figure 10: Schematic of In-line CAN-RS232 PCB supplied by MISCO

Appendix C: Wiring block diagrams

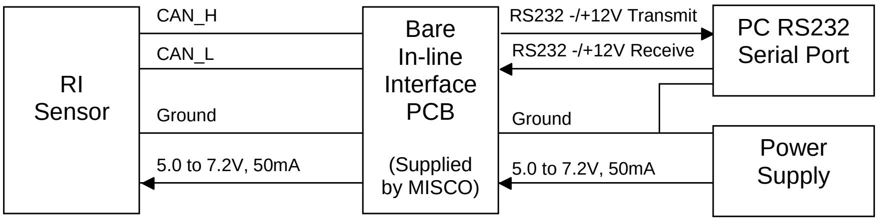


Figure 11: Configuration for bench testing.

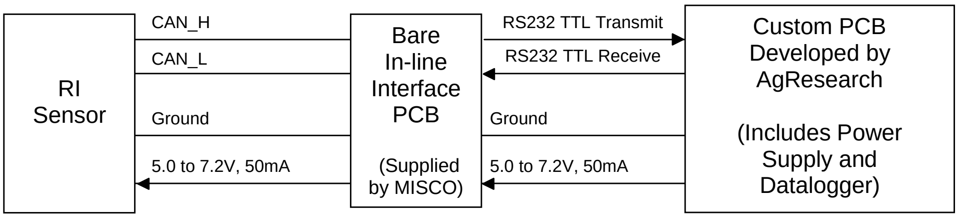


Figure 12: Configuration for use in applications without CANbus support.

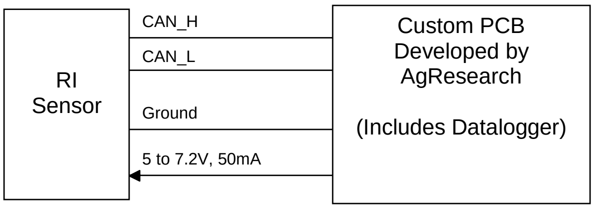


Figure 13: Configuration for use future application.

Appendix D: Allowable ASCII characters

Greyed out characters are not allowed to be output from the sensor (except when the power supply voltage is below the minimum stated in Table 2). Characters with specified functions have been underlined.

Table 5: Allowable ASCII characters

Decimal	Hex	Value	
000	000	NUL	(Null char.)
001	001	SOH	(Start of Header)
002	002	STX	(Start of Text)
003	003	ETX	(End of Text)
004	004	EOT	(End of Transmission)
005	005	ENQ	(Enquiry)
006	006	ACK	(Acknowledgment)
007	007	BEL	(Bell)
008	008	BS	(Backspace)
009	009	HT	(Horizontal Tab)
010	00A	LF	(Line Feed or Newline) <LF>
011	00B	VT	(Vertical Tab)
012	00C	FF	(Form Feed)
013	00D	CR	(Carriage Return) <CR>
014	00E	SO	(Shift Out)
015	00F	SI	(Shift In)
016	010	DLE	(Data Link Escape)
017	011	DC1	(XON) (Device Control 1)
018	012	DC2	(Device Control 2)
019	013	DC3	(XOFF)(Device Control 3)
020	014	DC4	(Device Control 4)
021	015	NAK	(Negative Acknowledgement)
022	016	SYN	(Synchronous Idle)
023	017	ETB	(End of Trans. Block)
024	018	CAN	(Cancel)
025	019	EM	(End of Medium)
026	01A	SUB	(Substitute)
027	01B	ESC	(Escape)
028	01C	FS	(File Separator)
029	01D	GS	(Group Separator)
030	01E	RS	(Req to Send)(Record Separator)
031	01F	US	(Unit Separator)
032	020	SP	(Space)
033	021	!	(exclamation mark)
034	022	"	(double quote)
035	023	#	(number sign)
036	024	$	(dollar sign)
037	025	%	(percent)
038	026	&	(ampersand)
039	027	'	(single quote)
040	028	(	(left/opening parenthesis)
041	029	)	(right/closing parenthesis)
042	02A	*	(asterisk)
043	02B	+	(plus)
044	02C	,	(comma)

045	02D	-	(minus or dash)
046	02E	.	(dot)
047	02F	/	(forward slash)
048	030	0	
049	031	1	
050	032	2	
051	033	3	
052	034	4	
053	035	5	
054	036	6	
055	037	7	
056	038	8	
057	039	9	
058	03A	:	(colon)
059	03B	;	(semi-colon)
060	03C	<	(less than)
061	03D	=	(equal sign)
062	03E	>	(greater than)
063	03F	?	(question mark)
064	040	@	(AT symbol)
065	041	A	
066	042	B	
067	043	C	
068	044	D	
069	045	E	
070	046	F	
071	047	G	
072	048	H	
073	049	I	
074	04A	J	
075	04B	K	
076	04C	L	
077	04D	M	
078	04E	N	
079	04F	O	
080	050	P	
081	051	Q	
082	052	R	
083	053	S	
084	054	T	
085	055	U	
086	056	V	
087	057	W	
088	058	X	
089	059	Y	
090	05A	Z	
091	05B	[	(left/opening bracket)
092	05C	\	(back slash)
093	05D	]	(right/closing bracket)
094	05E	^	(caret/circumflex)
095	05F	_	(underscore)
096	060	`	
097	061	a	
098	062	b	
099	063	c	
100	064	d	
101	065	e	
102	066	f	
103	067	g	

104	068	h	
105	069	i	
106	06A	j	
107	06B	k	
108	06C	l	
109	06D	m	
110	06E	n	
111	06F	o	
112	070	p	
113	071	q	
114	072	r	
115	073	s	
116	074	t	
117	075	u	
118	076	v	
119	077	w	
120	078	x	
121	079	y	
122	07A	z	
123	07B	{	(left/opening brace)
124	07C	|	(vertical bar)
125	07D	}	(right/closing brace)
126	07E	~	(tilde)
127	07F	DEL	(delete)

Appendix E: Undocumented communications

The sensor was originally designed to operate at 115200 Baud and on a CANBus.
When the device is powered-up, communication parameters are 115200 etc.
If there are no commands from the PDA for long time (5-10 sec. for example), the device will reconfigure UART to 19200 baud etc. and send the strings according to the protocol in this documents.
If there are commands from the PDA, then the device will continue with 115200, 2 stop and will not send RS232 strings.

Appendix F: Manufacturer and Supplier

MISCO Refractometer
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USA
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Fax: +1 (216) 831-1195
http://www.misco.com

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Manager / sales
Kathy Widing
Extension 210
kwiding@misco.com

Appendix G: Instructions for use

a. Configure the power supply and display/datalogger as per “Appendix C: Wiring block diagrams” on page 29. Set current limit on power supply to 100mA and Voltage to 5.0V before connecting it. **NB:**

   i. **Do not set above 5.0V.**

   ii. **Ensure correctly polarity of power supply.**

b. Setup the communications protocol as per “Table 2: MISCO VIP Physical interface – communications and power characteristics.” on page 17. If using a PC as a display/datalogger, a serial terminal program and serial port are required. The standard serial terminal program on AgR PC’s is HyperTerminal. To use HyperTerminal:

   i. Click `start | run` and type `hypertrm.exe`.

   ii. You must disconnect to change port parameters. (Click `disconnect`)

   iii. Select `Properties | Connect to | Connect using | Comm`*x*. Where *x* is the serial port number you are using.

   iv. Select `Properties | Settings | backscroll lines` and change the value to 500.

   v. Configure serial port communications as per “Table 2: MISCO VIP Physical interface – communications and power characteristics.” on page 17. The parameters that must be set are Baud rate, parity, start bits, stop bits and flow control.

   vi. Click `connect`.

   vii. Click `File | Save as` and give a filename to save the configuration of the terminal program.

   viii. Close HyperTerminal and open it again by double-clicking the icon of the configuration file you just saved.

   ix. Click `Transfer | capture text` to start a log.

   x. **NB:** When finished later, you must click `Transfer | capture text | stop` to correctly save the data file.

   xi. You can also copy and paste values from the screen.

c. Switch power supply on.

d. Apply fluid to the window (optionally through Micro-Flow adapter) so that the well is full.

e. The sensor window must be shielded from most light to obtain correct results.

f. Allow temperature and RI readings to stabilise — probably around 30 seconds.

g. Wash out well with deionised water and wipe with alcohol and a tissue between samples. Allow alcohol to evaporate fully.

h. Errors are described in “Table 1: RI result error numbers” on page 16.

i. When finished click `Transfer | capture text | stop` to correctly save the data file.

Appendix H: Tips for accurate lab testing of refractive index

1. Stir samples immediately before use.
2. Keep covers on samples when not being accessed to reduce evaporation. This is very important, especially at the higher temperatures.
3. Ensure sample is at the correct temperature before reading. (Temperature reading is not changing by more than 0.05ºC over 10 seconds)
4. Evaporation can affect the reading of small samples on the order of 1ml within a minute or two.
5. Check with traceable standards
6. Use disposable pipettes to transfer samples to prevent any cross-contamination.
7. Take at least five readings of every sample and average them for a final reading. Re-do measurements with large standard deviations between the five readings.

See "

8. **Important notes**" on page 13.

Appendix I: Specifications

Table 6: Specifications.

Model	**IRIS VIP-201**
List Price	**US$1,500**
Mass	**Serial number 0 is partly Stainless steel and weighs 68g without cable. The rest should weigh less due to being slightly shorter and changing to aluminium.**
Dimensions	**See "Appendix A: Drawings" on page 23"**
Ambient light rejection	**None**
Fluid Resolution	**0.50%**
Fluid Precision	**+/- 0.5**
Internal	**Refractive Index**
Wavelength	**589.29 nm**
Range	**1.3330 to 1.3900 nD** **(The first sensor supplied had a range of 1.3330 t0 1.5000, but was returned to MISCO)**
Resolution	
Accuracy	**0.0002 nD**
Output Isolation	**Non-Isolated**
Display	**None**
Operating Range	**10 to 40 °C**
Automatic Temperature Compensation to standard temperature	**No: The RI value is reported for the temperature output by the RI sensor.[1]**
Pressure	**50 psi**
Sensor Head Material	**Anodized aluminum**

[1] See "

Important notes" on page 13.

	(Serial number 0 is partly Stainless steel)
Measuring Surface	**Sapphire**
Waterproof	
Fitting	**Swagelok. Optional Micro-Flow adapter.**