



WOOL CONTAMINATION

Detection of dark fibre contamination of Merino Wool

Abstract

Two laboratories testing replicate samples have investigated the Detection of Dark Fibre Contamination in core samples from commercial sale lots of merino ewe fleece wool after the ewes have been in contact with Damara Rams and Damara crossbred lambs.

Summary

- This fact sheet reports on a trial designed by AWTA Ltd, using a technique developed by SARDI (South Australian Research & Development Institute) based on the CSIRO dark fibre detector, to assess levels of Dark Fibre contamination in **core samples** from **sale lots** of wool from merino ewes known to have been in contact with **Damara rams**. This assessment was replicated by SARDI.
- The sale lots were separated into two groups. The first consisted of dry ewes that had been in contact with the Damara rams for 6 weeks, then drafted off and shorn separately from the main shearing. These were run into the shearing shed using a new system that ensures that there is minimal risk of contamination from the yards and races. The second consisted of wet ewes, in contact with the rams for the same period, with the lambs at foot for 18 weeks. The lambs were separated from their mothers 6 weeks prior to shearing. A control group of sale lots, from sheep known not to have been in contact with Damara Rams or Damara crossbred lambs was also included.
- The examinations showed that despite the subjective nature of the measurement technique, AWTA Ltd and SARDI were clearly able to separate wools expected to have no contamination from wools likely to have heavy contamination. Although the wool from the dry ewes tended to be less contaminated than that from the wet ewes, contamination of both groups was unacceptably high.
- It is **technically feasible** for AWTA Ltd to provide guidance about the level of Dark Fibre contamination (i.e. whether or not any contamination is present) in Merino Fleece wool after contact with Damara, and possibly other exotic, sheep using an adaptation of the technique developed by SARDI.
- However, it is not **commercially feasible** to routinely provide this service for all presale lots, or even a significant subset of all presale lots, given the labour input required.
- The guidance test technique is based, to a significant extent, on the subjective judgment of operators. There was considerable difference between the AWTA Ltd and SARDI Dark Fibre counts for individual samples. However, both laboratories have clearly separated wools expected to have no contamination from wools expected to have heavy contamination.
- Given that in such instances the contamination is distributed throughout the fleece it should be technically feasible, given sufficient commitment of funds, to develop a commercial screening test.

OBJECTIVES

1. To examine the detection rates by SARDI and AWTA Ltd of Dark and Medullated Fibres from Sale Lot core samples of Merino wools with a known history of contact with Damara sheep.
2. To record the times taken to perform the test as a basis for determining the cost of any future Dark Fibre testing by AWTA Ltd.

METHOD AND MATERIALS

Samples

Three (3) sets of samples with different histories of contact with Damara sheep were chosen for this trial:

- 5 lots as a control set (no contamination);
- 6 sale lots prepared from dry ewes (expected low levels of contamination); and
- 14 sale lots prepared from wet ewes (expected heavy contamination).

For the potentially contaminated lots, core samples were obtained from 20 Sale Lots from three properties in one region in Western Australia with a history of contact with Damara sheep. The wool from 6 of these lots was from dry ewes from flocks in which Damara lambs were being raised. It was expected that these lots would have a low to negligible level of Dark Fibre contamination. The ewes were drafted off from the main mobs and shorn separately.

The remaining lots were from the ewe mothers of these mobs. Because of the continuing contact of these ewes with the crossbred lambs it was expected, based on research conducted by SARDI, that the wool from these ewes would have significant levels of contamination.

Core samples from a further set of 5 Sale Lots with no contact with exotic sheep were also included in the trial as a control set.

Trial Design

Six (6) 10g sub-samples were drawn from the presale core samples from each of the 25 lots. Two of these were measured as blind replicates by AWTA Ltd. The other 4 were provided to SARDI as follows:

- 2 x 25 x 10g webs Shirley Analysed at AWTA Ltd; and,
- 2 x 25 x 10g scoured core material (fibres to be separated and blended by SARDI).

The reason for this was to check whether or not the preparation systems used by both laboratories affected the results.

Thus three sets of results are reported as follows:

- AWTA results;
- SARDI (AWTA preparation); and,
- SARDI (SARDI preparation).

Procedures:

Testing was performed using a CSIRO Dark Fibre Detector (DFD). SARDI supplied a prototype DFD to AWTA Ltd for the period of the trial. The procedure is essentially in two parts:

- Detection and classification of the level of darkness, of non-white, fibrous contamination in 0.5g webs of wool using the DFD, as illustrated in Plate 1, and the CSIRO Dark Fibre Scale* (rating levels 5, 6, 7 or 8); and,

- Verification of the source of the detected fibres, e.g. fibre type and origin; dyed or naturally discoloured; medullated or not, using a microscope (up to 200x magnification) as shown in Plate 2.

*Note that research has shown that fibres of levels 5 to 8 (inclusive) on the CSIRO Dark Fibre scale are visible in undyed wool fabric (e.g. underwear) and in fabric dyed to light or pastel shades. Fibres of level 5 appear brown and are barely visible using the DFD; level 6 fibres are a darker brown; level 7 fibres are a dark chocolate colour; and, level 8 fibres are black.



Plate 1: Detection and classification. Prototype CSIRO Dark Fibre Detector used by AWTA Ltd.



Plate 2: Verification. Microscopic examination of Dark Fibres extracted from the samples. Note the metal bar added to the microscope stage to accommodate an A4 transparency capable of holding up to 25 extracted fibres.

Two (2) x operators were used at each site. At AWTA Ltd 2 operators worked in tandem to detect classify and verify the dark fibre counts of each sample. At SARDI the operators detect and classify but the scientist, Malcolm Fleet, performs the verification. The times recorded in the trial for SARDI relate only to the detection and classification phase.

The normal SARDI procedures were used in this trial, except for the following:

- A maximum of 10g or a maximum of 50 Dark Fibres (df), whichever comes first, was measured for each sample. Mass of sample tested was recorded if fewer than 51 fibres were counted.

Results and Discussion:

Summaries of the results are presented in the following Tables and Figures.

1. Overall level of contamination

Results in Table 1 show that AWTA Ltd testing detected greater numbers of fibres of levels 5 and 6, and fewer fibres of levels 7 and 8 than detected by either of the SARDI tests. The average differences per 10g of sample were that, compared to SARDI, AWTA Ltd detected an average of:

- 9 (approx.) more fibres equal to or greater than level 5;
- 7 (approx.) more fibres equal to or greater than level 6; and,
- 4 (approx.) fewer fibres equal to or greater than level 7.

Table 1. Average numbers of Dark Fibres/10g for all samples tested

Dark Fibre Level	AWT A	SARDI-AWTA Prep	SARDI – SARDI Prep
5,6,7or 8	21	11.8	12.8
6,7 or 8	18	11	12.3
7 or 8	3.7	7.4	8

The inclusion of the AWTA Ltd Shirley Analyser into the SARDI procedure does not introduce a major change to the SARDI results, indicating that any extra contamination introduced by the AWTA Shirley Analysing procedure was minimal.

Inspection of Figure 1 shows that the differences between AWTA Ltd and SARDI are structured rather than random. Some of the differences between the labs may be due to classification of the level of darkness of the extracted fibres, rather than the ability and reliability of detecting fibres, i.e. AWTA Ltd may assess fibres as level 6 when SARDI would assess these same fibres as level 7.

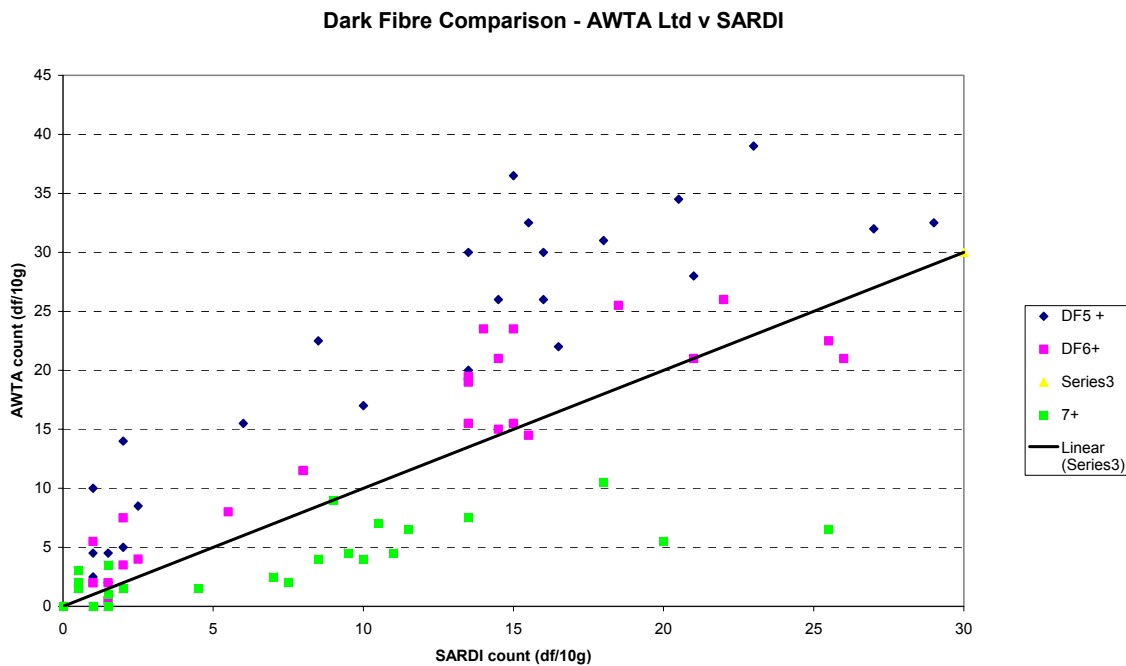


Figure 1. Relationship between Dark Fibre detection results for AWTA Ltd and SARDI (AWTA-prep).

2. Test variation

The numbers in Table 2 show that there is considerable variation in Dark Fibre counts between the replicate 10g samples. As expected, the variability between the labs is generally higher than the within lab variability. For Darkness levels 5 and 6 AWTA Ltd has greater variability than does SARDI, though the opposite is the case for levels 7 and 8.

Importantly for determination of very low levels of contamination, the between replicate variation is level dependent, being much lower at low contamination levels, as shown in Figure 2.

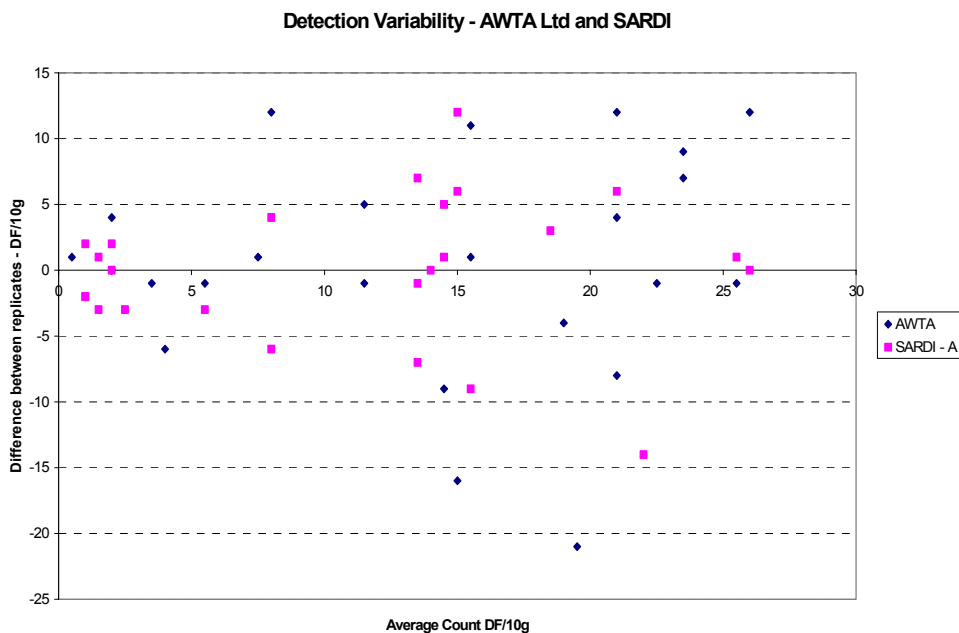
The AWTA Ltd Shirley Analysing step adds slightly to the variability between replicates of the SARDI testing procedure.

It is worth noting that the between replicate variability for the level 7 and 8 fibres of approximately 6df/10g to 8df/10g is high relative to the average level of contamination (4df/10g to 8df/10g).

Table 2. Average differences in Dark Fibre counts between replicate 10g samples (df/10g)

Dark Fibre Level	AWT A	SARDI (A prep)	SARDI (S prep)	AWTA/SARDI - A
All Samples:				
5,6,7or 8	5.9	4.5	5.6	9.2
6,7 or 8	6.8	4	5.2	6.7
7 or 8	2.0	4.9	6.7	4.3
<10df/10g:				
5,6,7or 8	2.9	2.6	1.9	4.8
6,7 or 8	3	2.7	1.9	3.5
>10df/10g:				
5,6,7or 8	7.1	5.1	8.9	11
6,7 or 8	8.2	5.9	8.2	8.1

Figure 2. The increase in variation between replicate subsamples as Dark Fibre count increases for both AWTA Ltd and SARDI (AWTA preparation).



The medullation counts of the two laboratories are very similar as shown in Table 3.

Table 3. Average Levels of Medullation

	AWT A	SARDI(AWTA- Prep)	SARDI(SARDI- Prep)
Average values	2.5	2.4	2.3
Differences between replicates	3.9	2.4	2.7

3. Inspection time

Table 4 shows the average inspection times and the ranges of times from the trial. AWTA Ltd operators used an extra 40 minutes of time/10g sample on average. The average time to test 2x10g subsamples at AWTA Ltd is 4.5 hours.

Table 4. Inspection Times (hours: minutes) for 1x 10g Samples. Note that 2 x 10g is the recommended rate of testing.

	AWT A	*SARDI(AWTA- Prep)
Average	2:15	1:35
Minimum	1:10	0:45
Maximum	3:15	3:15

***Note:** SARDI inspection time was for detection and classification only; the AWTA Ltd time also includes the verification phase.

4. Comparison with expected levels of contamination

Tables 5 and 6 show the levels of contamination for the three sets of samples with different levels of expected contamination, viz. 'control' (no contamination), 'mild' and 'heavy'.

Table 5 shows the very clear distinctions between the three sets of samples based on their average values. Table 6, on the other hand, shows the level of overlap between the ranges of values for each set. This overlap is shown clearly in Figures 3, 4 and 5 which show the Dark Fibre counts for the AWTA Ltd tests of the 1 x 10g sample, and the AWTA Ltd and SARDI (AWTA-prepared) lots as the average of 2 x 10g subsamples, respectively. In each case there was no overlap in Dark Fibre counts between the control and the heavy contamination sets, indicating that the procedures were providing the expected level of separation.

Table 5. Average Dark Fibre Counts for the Three Sets of Samples with Different Levels of Expected Contamination

	Contro l	Mild	Heavy
AWTA Ltd:			
5,6,7or 8	3.9	16.0	29.4
6,7 or 8	2.6	13.1	24.7
7 or 8	0.6	3.0	5.1
Medullation	0.6	2.8	5.5
SARDI(AWTA-prep):			
5,6,7or 8	1.4	7.7	17.4
6,7 or 8	1.4	7.6	16.0
7 or 8	0.9	4.8	10.9
Medullation	0.7	1.3	3.5

Table 6. Ranges of Dark Fibre Counts from the Three Sets of Samples with Different Expected Level of Contamination. Average of 2 x 10g Samples (Dark Fibres/10g rounded to nearest whole number).

Classification	Control	Mild	Heavy
AWTA Ltd:			
5,6,7or 8	2 – 5	8 – 28	17 – 39
6,7 or 8	0 – 4	5 – 26	17 – 32
7 or 8	0 – 2	0 – 8	1 – 11
Medullation	0 - 2	1 - 5	2 - 13
SARDI (AWTA-prep):			
5,6,7or 8	1 – 2	1 – 21	9 – 29
6,7 or 8	1 – 2	1 - 21	8 – 26
7 or 8	0 – 2	0 – 14	2 – 26
Medullation	0 - 4	0 – 3	0 - 9

Spread of Dark Fibre results amongst the 3 groups
AWTA Ltd Testing 1 x 10g

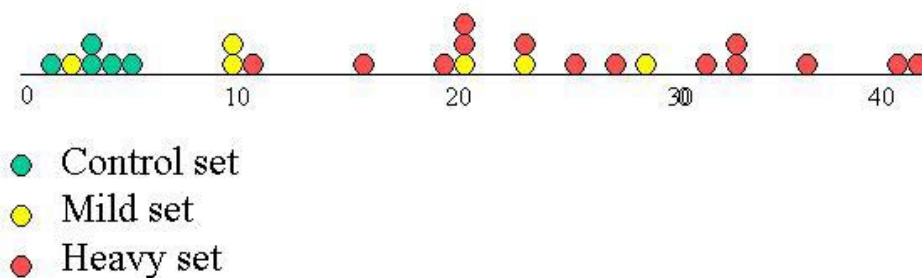


Figure 3. The overlap of Dark Fibre counts amongst ‘Control’, ‘Mild contamination’ and ‘Heavy contamination’ sets of samples, for AWTA testing of 1x10g sample/test.

Spread of Dark Fibre results amongst the 3 groups

AWTA Ltd Testing 2 x 10g

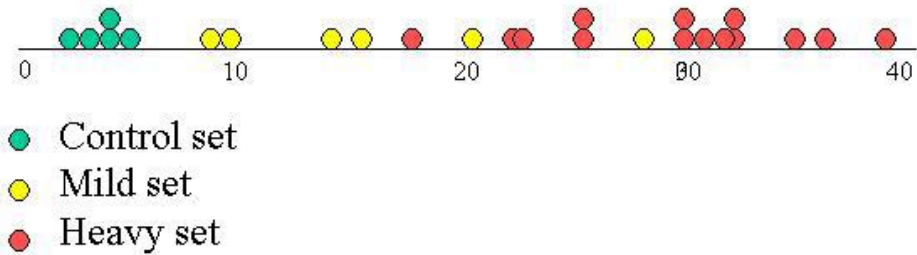


Figure 4. The overlap of Dark Fibre counts amongst ‘Control’, ‘Mild contamination’ and ‘Heavy contamination’ sets of samples, for AWTA Ltd testing of 2x10g samples/test.

Spread of Dark Fibre results amongst the 3 groups

SARDI Testing 2 x 10g

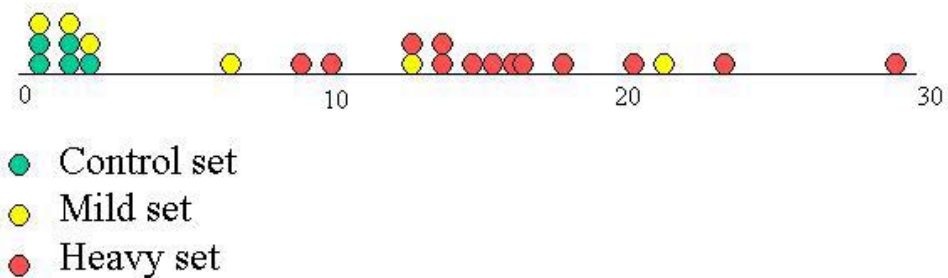


Figure 5. The overlap of Dark Fibre counts amongst ‘Control’, ‘Mild contamination’ and ‘Heavy contamination’ sets of samples, for SARDI(AWTA-prep) testing of 2x10g samples/test.

5. Operational issues for Dark Fibre testing

The Dark Fibre test is unlike any other test currently performed by AWTA Ltd. The technique incorporates a large degree of subjectivity, requires concentration and attention to detail by the operator, is prone to cross contamination at a level far more demanding than other tests conducted, and, in its current form, takes an average of 4.5 hours per test. The long time per test means that turnaround time for a Dark Fibre guidance test would be very slow if more than a few tests were received simultaneously.

These problems with the test appear insurmountable in the short term. Consequently the test will be expensive to perform routinely.

In this trial, 2 x operators worked an average of 2.25 hours per 10g sample, i.e. 4.5 hours per test and 9 x operator hours/test.

The cost of a Dark Fibre Detector is approximately \$8,000 to \$10,000 from CSIRO. The cost of a microscope is \$3,000 to \$6000.

The 2 x 10g Shirley Analysed subsamples could be obtained from the workflow associated with the existing Yield & Micron test with minimal extra effort.

Quality control of any Dark Fibre test would need to be strict and between lab monitoring would be required if the service were to be provided from more than 1 lab.

Summary:

The AWTA Ltd adaptation of the SARDI technique for Dark Fibre detection has shown that:

- The 3 sets of samples with different expected levels of Dark Fibre contamination from Damara sheep can be clearly separated on **average** into low, mild and heavy Dark Fibre contamination with mean detected counts (AWTA Ltd testing) of 4, 16 and 29 dark fibres/10g, respectively.
- There are overlaps amongst the 3 sets of results in the ranges of their Dark Fibre counts (refer Figure 4).
- 2 x 10g samples can be assessed with 2 x operators at an average testing time of 2.25 hours/10g sample, i.e. 4.5hours/test and 9 operator hours/test.
- The 95% Confidence Interval of the 2 x 10g assessment **within a lab** is approximately 6 df/sample on average and approximately 3 df/sample for samples with fewer than 10df/10g. The equivalent values for the **between lab** variability are 9df/10g and 5df/10g, respectively.
- There is a bias in test results of approximately 9 counts between the 2 labs. AWTA Ltd found more low-level contamination than did SARDI.

FURTHER INFORMATION

Peter Sommerville
Corporate Development Manager
AWTA Ltd
Ph: 03 9371 4105
Fax: 03 9371 4191
Email: peter.sommerville@awta.com.au