Scanning Electron Microscopy Scheme

BACKGROUND

This report covers the fourth round of the SEMS asbestos fibre counting PT scheme. The scheme is operated by HSL, in collaboration with APC, Germany and TNO, Netherlands.

SAMPLES

Four samples were circulated representing a range of different fibre densities and fibre types. All samples were produced at HSL using the modified sputnik multi-port sampling instrument.

INTRODUCTION

A total of 61laboratories enrolled for Round 4 (including the validating laboratories) and results were received from 58 laboratories. Laboratories were able to submit up to three results per sample and many laboratories took advantage of this with a total of 379 data sets submitted.

The samples were as follows:

4SEM1XX – Very low fibre density (<10 fibres/mm^2) - amphibole asbestos fibres

4SEM2XX – Low density (10-20 fibres/mm^2) - amphibole asbestos fibres

4SEM3XX – Low density (~20 fibres/mm^2) – amphibole asbestos fibres

4SEM4XX – Very low density (<10 fibres/mm^2) – amphibole asbestos fibres

INFORMATION SUBMITTED BY LABORATORIES

Laboratories were asked to supply:

- The number of fibres >5µm long counted (amphibole, chrysotile and other inorganic)
- The number of fields of view searched
- The area of the field of view
- The magnification and the method used

Laboratories were asked to calculate the fibre density (in fibres/mm^2) for each fibre type identified. There was also an option to include the number of fibres ≤5µm in length.

LABORATORY ASSESSMENT

RESULTS

Screen area – The fibre densities submitted by laboratories have not been recalculated and the density calculation and therefore screen area has not been verified.

Magnification – As was the case in earlier rounds, some laboratories used an operating magnification outside the range defined in ISO 14966 (or VDI 3492).

Magnifications of 5000x, 4000x, 3000x, 1000x, 1000x, 700x and 500x were recorded.

Results for total asbestos fibre densities for each laboratory are summarised in Appendix 1.

Data Analysis

Data analysis is based upon the total asbestos fibre densities (amphibole & chrysotile) derived from fibre numbers counted and the area of the filter searched. The distribution of fibres on a filter derived from airborne sampling is normally described as being Poisson-distributed. For Poisson-distributed counts, the variance (standard deviation squared) is equal to the mean. However, in practice the variation may be larger due to differences in sample production, laboratories and individual microscopists. A comparison of the observed standard deviations with the expected standard deviations (expected under Poisson distribution) show that the observed variation is larger than that expected, and it is difficult to quantify how much of this may be due to differences in sample production, and how much is due to differences between labs/microscopists.

Two approaches have been used to analyse the data for this round. The data have been compared against the criteria used in the UK phase contrast fibre counting proficiency testing scheme RICE and a modification of the analysis used in Rounds 1 and 2 (GLMM). Details of the analysis used can be found in Appendix 2.

Some laboratories have now analysed four rounds of the SEMS scheme and a brief summary on this is given at the end of Appendix 1 on page 15.

Sample 1 (4 SEM1) - Total asbestos fibre density (fmm^{-2})

Lab No	Total Asbestos	GLMM	RICE
7	1.5	A	A
7	2.9	A	A
139	2.0	A	A
139	7.5	A	A
300	8.0	A	A
709	3.9	A	A
807	2.3	A	A
807	1.8	A	A
1187	3.8	A	A
1267	4.8	A	A
1267	6.0	A	A
1277	6.2	A	A
1458	6.7	A	A
1477	0.0	B	C
1477	0.0	B	C
1507	6.6	A	A
1562	3.8	A	A
1575	5.5	A	A
1579	4.0	A	A
1579	1.5	A	A
1579	5.0	A	A
1582	2.0	A	A
1582	2.0	A	A
1592	3.0	A	A
1620	6.5	A	A
1620	9.0	A	A
1628	3.1	A	A
1628	2.6	A	A
1628	4.7	A	A
1638	8.0	A	A
1639	3.0	A	A
1640	3.0	A	A
1669	9.0	A	A
1669	6.0	A	A
1669	1.0	B	A
1680	7.8	A	A
1680	6.3	A	A
1680	7.0	A	A
1684	4.0	A	A
1687	8.9	A	A
1715	6.9	A	A
1716	5.0	A	A
1717	2.0	A	A

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1719	4.0	A	A
1719	4.5	A	A
1720	6.0	A	A
1722	6.9	A	A
1722	4.8	A	A
1722	5.4	A	A
1745	7.4	A	A
1759	8.9	A	A
1759	8.9	A	A
1759	12.5	B	A
1761	0.0	B	B
1764	1.0	B	A
1765	9.0	A	A
1767	2.0	A	A
1768	5.0	A	A
1774	2.5	A	A
1776	3.0	A	A
1776	5.0	A	A
1812	0.0	B	B
1812	0.0	B	B
1812	0.0	B	B
1814	4.4	A	A
1817	6.5	A	A
1826	4.0	A	A
1829	5.3	A	A
1829	4.2	A	A
1831	4.8	A	A
1831	7.2	A	A
1831	3.2	A	A
1852	0.0	B	B
1852	0.0	B	B
1852	0.0	B	B
1871	6.5	A	A
1875	5.0	A	A
1876	1.8	A	A
1879	6.0	A	A
1879	4.0	A	A
1879	7.0	A	A
1880	13.0	B	A
1880	12.0	B	A
1880	17.0	B	A
1881	4.8	A	A
1882	9.0	A	A
1882	14.0	B	A
1882	5.0	A	A
1884	14.0	B	A
1884	17.0	B	A
1884	13.0	B	A

1885	3.0	A	A
1885	3.0	A	A
1885	2.0	A	A
1892	3.0	A	A
1892	3.0	A	A
1894	4.0	A	A
1894	4.0	A	A
1894	8.0	A	A

4 SEM1

Mean	5.15
Median	4.80
STDev	3.65
Min	0.00
Max	17.00

glmm mean (mixed effects model)	*4.73*
Poisson lower limit of CI for mean	*1.35*
Poisson upper limit of CI for mean	*10.96*
Random effects sd	0.19

RICE A (Lower)	RICE A (Upper)	RICE B (Lower)	RICE B (Upper)	RICE C (Lower)	RICE C (Upper)
0.39	17.23	0.02	30.15	<0.02	>30.15

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Sample 2 (4 SEM2) - Total asbestos fibre density (fmm^{-2})

Lab No	Total Asbestos	GLMM	RICE
7	10.30	A	A
7	10.30	A	A
139	14.50	A	A
139	13.00	A	A
300	18.00	A	A
709	20.80	A	A
807	13.46	A	A
807	9.37	A	A
1187	8.64	B	A
1267	12.00	A	A
1267	22.00	A	A
1277	20.50	A	A
1458	12.50	A	A
1477	0.00	B	C
1477	0.00	B	C
1507	17.92	A	A
1562	14.80	A	A
1575	11.88	A	A
1579	15.50	A	A
1579	18.00	A	A
1579	20.00	A	A
1582	7.00	B	A
1582	8.00	B	A
1592	15.00	A	A
1620	30.00	B	A
1620	25.00	A	A
1628	12.27	A	A
1628	23.57	A	A
1628	19.25	A	A
1638	16.00	A	A
1639	12.00	A	A
1640	14.80	A	A
1669	9.00	A	A
1669	21.50	A	A
1669	11.50	A	A
1680	18.00	A	A
1680	19.70	A	A
1680	20.30	A	A
1684	14.00	A	A
1687	26.10	B	A
1715	31.68	B	A
1716	26.00	B	A
1717	6.11	B	A

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1719	18.00	A	A
1719	18.50	A	A
1720	16.00	A	A
1722	7.90	B	A
1722	9.70	A	A
1722	10.00	A	A
1745	34.10	B	A
1759	20.54	A	A
1759	24.11	A	A
1759	27.23	B	A
1761	2.50	B	C
1764	20.00	A	A
1765	20.97	A	A
1767	2.00	B	C
1768	19.84	A	A
1774	15.80	A	A
1776	21.00	A	A
1776	17.00	A	A
1812	0.00	B	C
1812	0.00	B	C
1812	0.00	B	C
1814	13.00	A	A
1817	21.00	A	A
1826	18.00	A	A
1829	12.70	A	A
1829	18.30	A	A
1831	15.10	A	A
1831	15.90	A	A
1831	19.10	A	A
1852	12.06	A	A
1852	6.43	B	A
1852	8.04	B	A
1871	22.00	A	A
1875	10.00	A	A
1876	8.24	B	A
1879	13.00	A	A
1879	16.00	A	A
1879	18.00	A	A
1880	44.00	B	B
1880	36.00	B	B
1880	43.00	B	B
1881	13.10	A	A
1882	90.00	B	B
1882	99.00	B	B
1882	97.00	B	B
1884	3.00	B	B
1884	5.00	B	B
1884	6.00	B	A

1885	14.00	A	A
1885	14.00	A	A
1885	9.00	A	A
1892	19.50	A	A
1892	21.00	A	A
1894	21.00	A	A
1894	18.00	A	A
1894	21.00	A	A

4 SEM2

Mean	18.12
Median	15.90
STDev	16.23
Min	0.00
Max	99.00
glmm mean (mixed effects model)	15.64
Poisson lower limit of CI for mean	8.77
Poisson upper limit of CI for mean	25.36

RICE A (Lower)	RICE A (Upper)	RICE B (Lower)	RICE B (Upper)	RICE C (Lower)	RICE C (Upper)
5.84	35.37	2.71	53.11	<2.71	>53.11

APPENDIX 1

Sample 3 (4 SEM 3) - Total asbestos fibre density (fmm^{-2})

Lab No	Total Asbestos	GLMM	RICE
7	15.70	A	A
7	16.20	A	A
139	18.50	A	A
139	29.50	A	A
300	33.00	A	A
709	23.70	A	A
807	12.88	B	A
807	17.56	A	A
1187	11.27	B	A
1267	19.00	A	A
1267	23.00	A	A
1277	23.10	A	A
1458	26.00	A	A
1477	0.01	B	C
1477	0.00	B	C
1507	20.76	A	A
1562	21.80	A	A
1562	21.10	A	A
1575	24.68	A	A
1579	27.50	A	A
1579	23.50	A	A
1579	29.50	A	A
1582	14.00	A	A
1582	14.00	A	A
1592	19.00	A	A
1620	20.00	A	A
1620	27.00	A	A
1628	10.18	B	A
1628	23.61	A	A
1628	18.19	A	A
1638	27.00	A	A
1639	18.00	A	A
1640	15.80	A	A
1669	29.00	A	A
1669	26.50	A	A
1669	16.00	A	A
1680	33.90	B	A
1680	34.80	B	A
1680	26.40	A	A
1684	26.00	A	A
1687	21.70	A	A
1715	37.62	B	A
1716	26.20	A	A
1717	18.34	A	A

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1719	26.00	A	A
1719	27.50	A	A
1720	27.00	A	A
1722	20.20	A	A
1722	16.30	A	A
1722	16.00	A	A
1745	36.00	B	A
1759	28.13	A	A
1759	31.25	A	A
1759	16.52	A	A
1761	7.50	B	A
1764	24.00	A	A
1765	16.97	A	A
1767	2.00	B	C
1768	28.77	A	A
1774	13.00	B	A
1776	26.00	A	A
1776	25.00	A	A
1812	0.00	B	C
1812	0.00	B	C
1812	0.00	B	C
1814	22.00	A	A
1817	37.00	B	A
1826	18.00	A	A
1829	24.10	A	A
1831	18.30	A	A
1831	21.40	A	A
1831	17.00	A	A
1852	46.62	B	B
1852	32.96	A	A
1852	39.39	B	A
1871	18.50	A	A
1875	14.00	A	A
1876	8.25	B	B
1879	21.00	A	A
1879	26.00	A	A
1879	29.00	A	A
1880	28.00	A	A
1880	31.00	A	A
1880	25.00	A	A
1881	15.00	A	A
1882	3.00	B	C
1882	35.00	B	A
1882	71.00	B	C
1884	13.00	B	A
1884	9.00	B	A
1884	21.00	A	A
1885	17.00	A	A

1885	17.00	A	A
1885	25.00	A	A
1892	23.00	A	A
1892	31.50	A	A
1894	27.00	A	A
1894	21.00	A	A
1894	23.00	A	A

4 SEM3

Mean	21.73
Median	21.80
STDev	10.50
Min	0.00
Max	71.00
glmm mean (mixed effects model)	22.17
Poisson lower limit of CI for mean	13.79
Poisson upper limit of CI for mean	33.31

RICE A (Lower)	RICE A (Upper)	RICE B (Lower)	RICE B (Upper)	RICE C (Lower)	RICE C (Upper)
9.60	43.94	5.42	63.51	<5.42	>63.51

APPENDIX 1

Sample 4 (4 SEM4) - Total asbestos fibre density (fmm^{-2})

Lab No	Total Asbestos	GLMM	RICE
7	0.00	A	A
7	0.00	A	A
139	0.00	A	A
139	0.00	A	A
300	0.00	A	A
709	0.00	A	A
807	0.00	A	A
807	0.59	A	A
1187	0.75	A	A
1267	0.40	A	A
1267	1.00	A	A
1277	0.00	A	A
1458	1.00	A	A
1477	0.00	A	A
1477	0.00	A	A
1507	0.94	A	A
1562	1.00	A	A
1575	0.00	A	A
1579	0.00	A	A
1579	0.00	A	A
1579	0.00	A	A
1582	0.00	A	A
1582	0.00	A	A
1592	1.00	A	A
1620	0.00	A	A
1620	0.00	A	A
1628	0.52	A	A
1628	0.00	A	A
1628	0.00	A	A
1638	2.00	A	A
1639	0.00	A	A
1640	1.00	A	A
1669	0.00	A	A
1669	0.00	A	A
1680	0.40	A	A
1680	0.00	A	A
1680	0.40	A	A
1684	2.00	A	A
1687	0.00	A	A
1715	0.00	A	A
1716	0.00	A	A
1717	0.00	A	A
1719	0.00	A	A
1719	0.00	A	A

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1720	1.00	A	A
1722	0.60	A	A
1722	0.00	A	A
1722	0.00	A	A
1745	1.90	A	A
1759	1.34	A	A
1759	0.89	A	A
1759	0.45	A	A
1761	0.00	A	A
1764	2.00	A	A
1765	0.00	A	A
1767	4.00	B	B
1768	0.00	A	A
1774	0.00	A	A
1776	1.00	A	A
1776	0.00	A	A
1812	0.00	A	A
1812	0.00	A	A
1812	0.00	A	A
1814	0.00	A	A
1817	0.50	A	A
1826	0.00	A	A
1829	0.00	A	A
1831	0.00	A	A
1831	0.80	A	A
1831	0.00	A	A
1852	0.00	A	A
1852	0.00	A	A
1852	0.00	A	A
1871	1.50	A	A
1875	0.00	A	A
1876	0.92	A	A
1879	1.00	A	A
1879	0.00	A	A
1879	0.00	A	A
1880	0.00	A	A
1880	0.00	A	A
1880	0.00	A	A
1881	0.60	A	A
1882	9.00	B	B
1882	6.00	B	B
1882	3.00	A	A
1884	0.00	A	A
1884	0.00	A	A
1884	0.00	A	A
1885	0.00	A	A
1885	2.00	A	A
1885	0.00	A	A

1892	0.00	A	A
1892	0.00	A	A
1894	0.00	A	A
1894	0.00	A	A
1894	0.00	A	A

4 SEM 4

Mean	0.53
Median	0.00
STDev	1.27
Min	0.00
Max	9.00
glmm mean (mixed effects model)	0.28
Poisson lower limit of CI for mean	0.00
Poisson upper limit of CI for mean	3.69
random effects sd	1.35

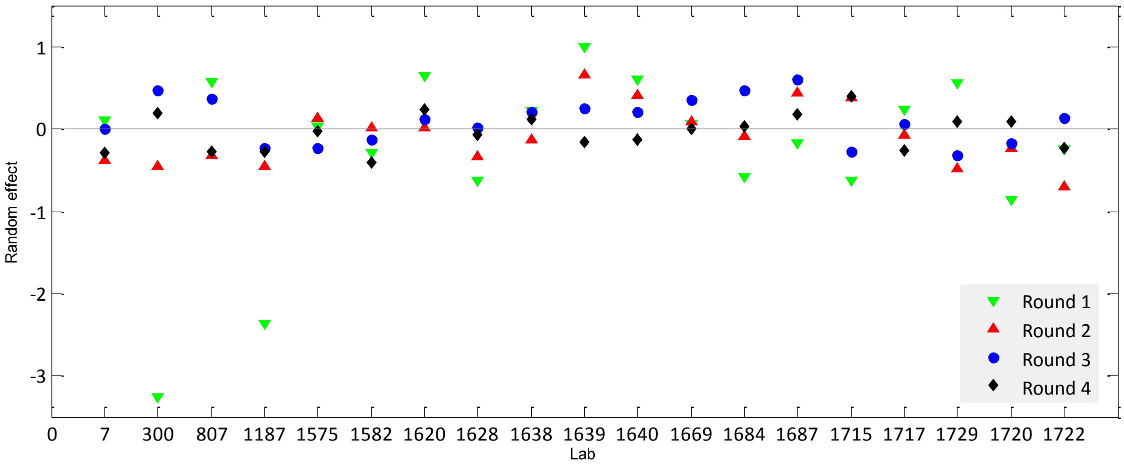
RICE A (Lower)	RICE A (Upper)	RICE B (Lower)	RICE B (Upper)	RICE C (Lower)	RICE C (Upper)
0.00	3.84	-	10.89	-	>10.89

APPENDIX 1

Overview of Last Four Rounds

Of the 19 labs who have participated in the first four rounds of SEMS, most have performed 'better' (i.e. closer to the average) in Round 4 than in Round 1 (the random effects being closer to zero in Round 4 than Round 1). Ten labs performed better in Round 4 than any of the previous three rounds. These are labs 300, 807, 1575, 1638, 1639, 1640, 1669, 1684, 1719 and 1720.

All 19 labs are generally performing better with each round (the standard deviation of the random effects has decreased with each round). In Round 1, the standard deviation was 1.04, declining to 0.37 (Round 2), 0.28 (Round 3) and 0.22 (Round 4). In comparison, the standard deviation for Round 4 across all 58 labs is 0.27, meaning that there is more variation between labs when all 58 are considered. The results of the validating laboratories are not included.



Standard deviation of random effects

	Round 1	Round 2	Round 3	Round 4
Lab	**1.04**	**0.37**	**0.28**	**0.22**
7	0.10	-0.38	0.00	-0.29
300	-3.25	-0.46	0.47	0.20
807	0.58	-0.32	0.36	-0.28
1187	-2.36	-0.46	-0.23	-0.28
1575	0.03	0.13	-0.23	-0.02
1582	-0.29	0.01	-0.12	-0.40
1620	0.65	0.01	0.12	0.23
1628	-0.63	-0.34	0.01	-0.08
1638	0.23	-0.13	0.21	0.12
1639	0.99	0.66	0.25	-0.15
1640	0.60	0.41	0.20	-0.12
1669	0.06	0.09	0.35	0.00
1684	-0.59	-0.08	0.47	0.03
1687	-0.17	0.44	0.60	0.17
1715	-0.62	0.38	-0.28	0.40
1717	0.23	-0.07	0.06	-0.26
1719	0.55	-0.49	-0.32	0.09
1720	-0.85	-0.23	-0.17	0.08
1722	-0.25	-0.70	0.13	-0.23

APPENDIX 2

DATA ANALYSIS – METHOD 1

Regular Inter-laboratory Counting Exchange (RICE) Criteria

Where ***R*** is the reference value – in this case the Median value.

High density slides ($R > 63.7$ fibres. mm^{-2})

Target band A: $> 0.65R$ to $<1.55R$

Target band B: $> 0.50R$ to $0.65R$ [band -B] and $> 1.55R$ to $2.00R$ [band +B]

Target band C: $< 0.50R$ [band -C] and $> 2.00R$ [band +C]

Low density slides ($R \leq 63.7$ fibres. mm^{-2})*

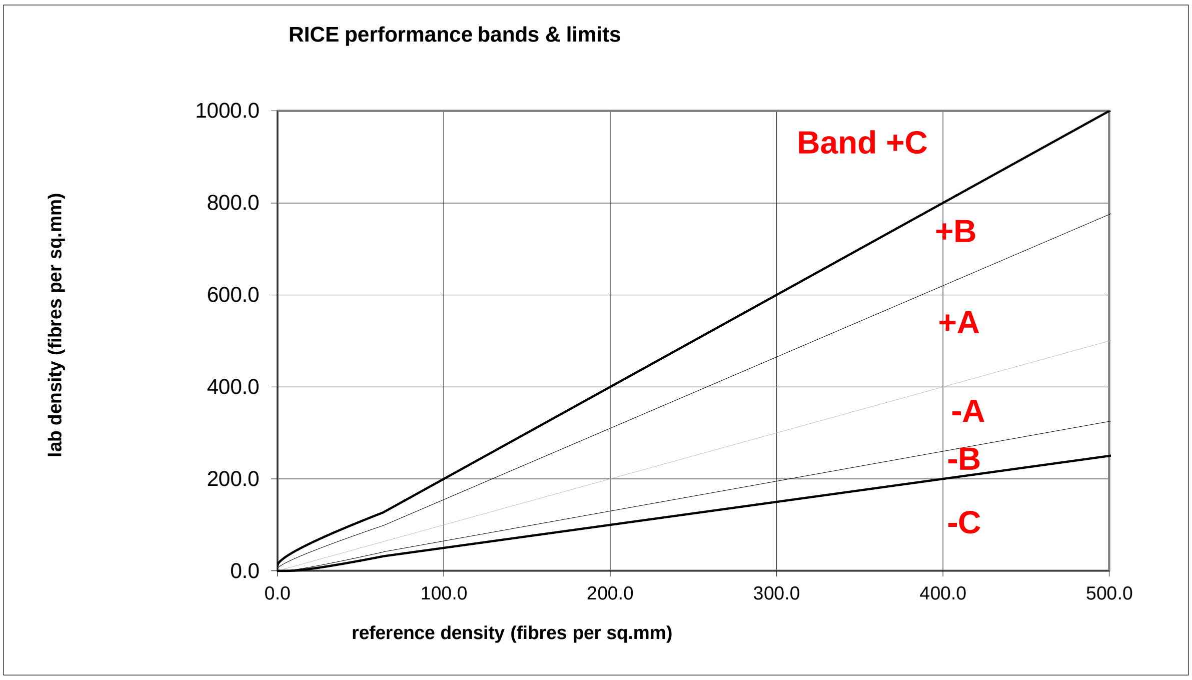
Target band A: $(\sqrt{R}-1.57)^2$ to $(\sqrt{R}+1.96)^2$ [band A]

Target band B: $<(\sqrt{R}-2.34)^2$ to $(\sqrt{R}-1.57)^2$ [band -B]
$>(\sqrt{R}+1.96)^2$ to $(\sqrt{R}+3.30)^2$ [band +B]

Target band C: $<(\sqrt{R}-2.34)^2$ [band -C]
$>(\sqrt{R}+3.30)^2$ [band +C]

* For samples less than 5.5 fibres.mm^{-2} the lower limit is set to zero when the component within the brackets ($\sqrt{R}$-n) is less than zero.

The plot below shows the positions of the performance limits in relation to the reference counts up to reference density 500 fibres per mm^2.



APPENDIX 2

DATA ANALYSIS – METHOD 2

Mixed effects model for fibre counting

Data analysis is based upon the calculated total asbestos (amphibole & chrysotile) fibre densities derived from fibre numbers counted and the area of the filter searched. The distribution of fibres on a filter derived from airborne sampling is normally described as being Poisson-distributed. For Poisson-distributed counts, the variance (standard deviation squared) is equal to the mean. However, in practice the variation may be larger due to differences in sample production, laboratories and individual microscopists. A comparison of the observed standard deviations with the expected standard deviations (expected under Poisson distribution) show that the observed variation is larger than that expected, and it is difficult to quantify how much of this may be due to differences in sample production, and how much is due to differences between labs/microscopists.

For each sample, it has been assumed that there are no production differences between samples, and that the fibre densities are Poisson distributed with mean "λ" (λ is unknown but is estimated from the fibre counts). For samples where each lab submits just one reading, an estimate of "λ" is the observed mean density count across all participating labs. However, when laboratories submit more than one reading per sample, taking simply the mean of all the submitted results to estimate lambda may lead to a biased estimate. Therefore, although the mean may be a close approximation to "λ"; a more appropriate method would be to use a mixed effects regression model to estimate "λ". Therefore, 95% confidence limits for "λ" can also be calculated from this, whichever method is used to estimate "λ". For a Poisson random variable with mean "λ", the variance is equal to the mean, i.e. if fibre counts truly follow a Poisson distribution with mean "λ", the variance should also equal "λ".

Calculating Confidence Limits for a Poisson Mean

The fibre densities are assumed to follow a Poisson distribution with unknown mean "λ". When each lab submits just one result, the maximum likelihood estimate of "λ", (which we denote as *s*) is the mean of the observed fibre densities across all laboratories, i.e.

$$s = \frac{\sum_{i=1}^{N} x_i}{N}$$

Where x_i is the observed fibre densities and N is the number of observations.

APPENDIX 2

When some labs submit more than one result, to account for variability between labs and reduce bias, we assume the following generalised linear mixed model (glmm):

$$E\ X_{ij}\ = \lambda_i$$

$$\log\ \lambda_i\ = a + b_i$$

$$b_i \sim N(0, \sigma_b{}^2)$$

Where a is the logarithm of the general mean density (i.e. exp (a) represents the general mean density), and b_i are random effects representing the systematic differences between the general mean density and the lab's measured densities (the b_i are normally distributed with mean 0 and variance $\sigma_b{}^2$). The model presented above can be fitted using statistics software such as R, providing us with estimates of the model parameter *a,* as well as the random effects b_i. The penalised quasi-likelihood estimate of λ is simply s=exp(a), and is presented in the table below, for each round and fibre type, e.g. the estimate of λ for total fibres in Sample 1 is s=9.39, so the total fibre densities in Sample 1 are can be assumed to be Poisson distributed with an estimated mean of 9.39.

Sample	Linear mixed effects estimate of fibre density s = exp(a)	
	Total fibres	Total asbestos
1	9.39	6.60
2	3.71	2.37
3	12.42	8.32
4	1.82	1.18

Once *s* has been calculated using the maximum likelihood method or the glmm method, the 95% confidence interval for the Poisson mean can be determined:

$$\left[\frac{\chi^2_{2s,0.025}}{2}, \frac{\chi^2_{2s+2,0.975}}{2}\right]$$

Where $\chi^2_{2s,0.025}$ (lower limit of the confidence interval for the Poisson mean) and $\chi^2_{2s+2,0.975}$ (upper limit of the confidence interval for the Poisson mean) are calculated as the chi-square quantiles with lower tail probabilities 0.025 and 0.975 on 2s and 2s+2 degrees of freedom respectively.

Laboratory results have been compared against the 95% confidence intervals as follows:

1. Where the total asbestos fibre density falls within the 95% confidence intervals, the result is classified as "A"

APPENDIX 2

2. Where the total asbestos fibre density falls outside the 95% confidence intervals, the result is classified as “B”

Results for total asbestos fibre densities for each laboratory are summarised in Appendix 1.

Appendix 2 summarises the total fibre, total asbestos, amphibole, chrysotile and other inorganic fibre densities for all samples.