Group Report
Round 5
December 2015

HEALTH & SAFETY LABORATORY

Final

Scanning Electron Microscopy Scheme

BACKGROUND
This Interim Report covers the fifth 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 69 laboratories participated in this round (including the validating laboratories). Laboratories were able to submit up to three results per sample and many laboratories took advantage of this with a total of 470 results submitted.

The samples were as follows:

5SEM1 – Medium density (<50 fibres/mm2) - chrysotile asbestos fibres

5SEM2 – Medium – high density (100 fibres/mm2) - chrysotile asbestos fibres

5SEM3 – Very low density (<10 fibres/mm2) – wollastonite – no asbestos fibres

5SEM4 – Very low density (<10 fibres/mm2) – 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

Calculations - At least two laboratories are known to have submitted incorrectly calculated results. In both cases the laboratory was informed.

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 6000x, 4000x, 3000x and 1000x were recorded.

Samples – 5SEM1 and 5SEM2 were chrysotile samples and both included a number of very fine chrysotile bundles and fibres

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). The GLMM method was not used for samples 5SEM3 and 5SEM4 Details of the analysis used can be found in Appendix 2.

For the next round the performance of laboratories that have completed 4, 5 or 6 rounds will be included.

APPENDIX 1

Sample 1 (5 SEM1) - Total asbestos fibre density (fmm^{-2})

Lab Number	Total Asbestos	RICE	GLMM
7	12.29	B	B
7	15.24	B	A
139	30.50	A	A
139	32.00	A	A
300	24.00	A	A
709	30.80	A	A
807	32.81	A	A
807	30.80	A	A
818	46.95	A	A
1181	22.44	A	A
1187	20.03	A	A
1276	39.00	A	A
1276	30.00	A	A
1277	27.60	A	A
1282	20.83	A	A
1477	69.99	B	B
1477	72.16	B	B
1477	77.81	B	C
1507	24.56	A	A
1562	68.80	B	B
1562	64.50	B	B
1575	22.85	A	A
1579	33.50	A	A
1579	35.00	A	A
1579	36.00	A	A
1582	22.00	A	A
1592	48.00	A	A
1592	46.00	A	A
1620	26.50	A	A
1620	22.00	A	A
1620	42.00	A	A
1628	34.11	A	A
1628	25.31	A	A
1628	51.75	A	A
1638	14.00	B	A
1639	24.00	A	A
1640	37.20	A	A
1658	14.00	B	A
1658	15.50	B	A
1669	34.50	A	A
1669	23.50	A	A
1669	39.00	A	A
1675	31.60	A	A
1680	56.70	B	A
1680	61.50	B	A

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1680	66.80	B	B
1684	47.50	A	A
1687	26.70	A	A
1715	17.82	A	A
1717	9.81	C	C
1719	7.00	C	C
1719	7.00	C	C
1720	14.00	B	A
1722	22.60	A	A
1722	21.10	A	A
1722	28.10	A	A
1734	20.51	A	A
1734	14.22	B	A
1759	28.60	A	A
1759	46.90	A	A
1759	38.40	A	A
1761	19.16	A	A
1764	46.50	A	A
1767	10.50	B	C
1767	11.70	B	B
1768	26.79	A	A
1774	25.50	A	A
1776	63.00	B	A
1776	57.00	B	A
1812	19.00	A	A
1812	16.50	A	A
1812	27.50	A	A
1814	48.50	A	A
1817	45.50	A	A
1826	14.43	B	A
1827	21.00	A	A
1827	22.00	A	A
1829	26.89	A	A
1829			
1830	44.64	A	A
1830	47.62	A	A
1830	32.74	A	A
1831	14.30	B	A
1831	19.00	A	A
1831	22.20	A	A
1832	33.50	A	A
1832	48.50	A	A
1836	35.70	A	A
1836	37.56	A	A
1836	37.70	A	A
1848	14.30	B	A
1852	14.00	B	A
1871	46.50	A	A

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1871	43.50	A	A
1874	10.00	C	C
1879	37.00	A	A
1879	38.00	A	A
1879	48.00	A	A
1885	28.00	A	A
1885	30.00	A	A
1885	26.00	A	A
1889	39.40	A	A
1889	53.10	A	A
1903	6.40	C	C
1903			
1910	214.00	C	C
1923	41.50	A	A
1923	44.00	A	A
1928	18.80	A	A
1928	20.10	A	A
1928	23.50	A	A
1937	48.60	A	A
1937	51.50	A	A
1938	24.00	A	A
1938	16.00	A	A
1939	42.47	A	A
1939	48.46	A	A
1939			
1940	36.00	A	A
1941	31.00	A	A
1941	63.00	B	A
1948	29.00	A	A

Mean	34.2
Median	**30.5**
STDev	22.9
Min	6.4
Max	214.0

RICE A (Lower)	RICE A (Upper)	RICE B (Lower)	RICE B (Upper)	RICE C (Lower)	RICE C (Upper)
15.6	56.0	10.1	77.8	<10.1	>77.8

glmm mean (mixed effects model)	28.9
Poisson lower limit of CI for mean	19.0
Poisson upper limit of CI for mean	41.1

Sample 2 (5 SEM2) - Total asbestos fibre density (fmm^{-2})

Lab Number	Total Asbestos	RICE	GLMM
7	34.41	A	A
7	42.28	A	A
139	54.50	A	A
139	33.50	A	A
300	35.00	A	A
709	28.50	A	A
807	37.61	A	A
807	46.16	A	A
818	68.93	A	A
1181	20.94	B	B
1187	30.67	A	A
1276	75.00	A	A
1276	65.00	A	A
1277	59.40	A	A
1282	38.69	A	A
1477	59.56	A	A
1477	65.21	A	A
1477			
1507	35.09	A	A
1562	105.20	C	B
1562	75.90	A	A
1575	44.79	A	A
1579	120.00	C	C
1579	95.00	B	A
1579	103.50	C	B
1582	20.00	B	B
1592	75.00	A	A
1592	66.00	A	A
1620	51.50	A	A
1620	32.00	A	A
1620	108.50	C	B
1628	42.04	A	A
1628	30.67	A	A
1628	50.72	A	A
1638	30.50	A	A
1639	19.00	B	C
1640	47.10	A	A
1658	19.89	B	B
1658	19.00	B	C
1669	40.00	A	A
1669	56.00	A	A
1669	66.00	A	A
1675	41.60	A	A

1680	118.60	C	C
1680	98.30	B	B
1680	95.40	B	A
1684	75.00	A	A
1687	111.10	C	B
1715	26.73	B	A
1717	29.44	A	A
1719	22.00	B	B
1719	24.00	B	A
1720	30.00	A	A
1722	46.20	A	A
1722	51.00	A	A
1722	41.70	A	A
1734	19.72	B	B
1734	32.39	A	A
1759	46.00	A	A
1759	40.20	A	A
1759	38.40	A	A
1761	5.00	C	C
1764	87.00	B	A
1767	29.50	A	A
1767	6.40	C	C
1768	39.68	A	A
1774	32.00	A	A
1776	72.00	A	A
1776	61.00	A	A
1812	40.00	A	A
1812	28.50	A	A
1812	31.50	A	A
1814	98.10	B	B
1817	106.00	C	B
1826	52.93	A	A
1827	36.50	A	A
1827	41.00	A	A
1829	59.80	A	A
1829	60.40	A	A
1830	89.29	B	A
1830	103.17	C	B
1830	91.27	B	A
1831	34.10	A	A
1831	31.00	A	A
1831	45.30	A	A
1832	67.50	A	A
1832	69.00	A	A
1836	45.25	A	A
1836	56.40	A	A
1836	38.45	A	A
1848	19.80	B	B

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1852	53.67	A	A
1871	37.50	A	A
1871	48.00	A	A
1874	20.00	B	B
1879	46.00	A	A
1879	56.00	A	A
1879	58.00	A	A
1885	41.00	A	A
1885	30.00	A	A
1885	41.00	A	A
1889	92.50	B	A
1889	94.50	B	A
1903	18.70	C	C
1903	11.30	C	C
1910	127.00	C	C
1923	74.50	A	A
1923	71.30	A	A
1928	46.30	A	A
1928	49.60	A	A
1928	46.90	A	A
1937	39.10	A	A
1937	37.10	A	A
1938	36.00	A	A
1938			
1939	78.94	B	A
1939	78.44	B	A
1939			
1940	99.00	B	B
1941	83.50	B	A
1941	81.00	B	A
1948	31.00	A	A

Mean	53.3
Median	**46.0**
STDev	27.4
Min	5.0
Max	127.0

RICE A (Lower)	RICE A (Upper)	RICE B (Lower)	RICE B (Upper)	RICE C (Lower)	RICE C (Upper)
27.2	76.4	19.7	101.7	<19.7	>101.7

glmm mean (mixed effects model)	46.8
Poisson lower limit of CI for mean	34.1
Poisson upper limit of CI for mean	61.9

Sample 3 (5 SEM 3) - Total asbestos fibre density (fmm^{-2})

Lab Number	Total Asbestos	RICE
7	0.00	A
7	0.98	A
139	0.00	A
139	0.00	A
300	0.00	A
709	0.00	A
807	0.00	A
807	0.00	A
818	0.00	A
1181	0.00	A
1187	0.00	A
1276	1.50	A
1276	0.40	A
1277	0.00	A
1282	0.00	A
1477	0.00	A
1477	0.00	A
1477	0.00	A
1507	0.00	A
1562	0.00	A
1562	0.00	A
1575	0.00	A
1579	0.00	A
1579	0.00	A
1579	0.00	A
1582	0.00	A
1592	0.00	A
1592	0.00	A
1620	0.00	A
1620		
1620		
1628	0.00	A
1628	0.00	A
1628	0.00	A
1638	0.00	A
1639	0.00	A
1640	0.00	A
1658	0.00	A
1658	0.00	A
1669	0.00	A
1669	0.00	A
1669	0.00	A
1675	0.00	A
1680	0.00	A
1680	0.00	A
1680	0.00	A
1684	0.00	A
1687	0.00	A
1715	0.00	A
1717	0.00	A
1719	0.00	A
1719	0.00	A

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1720	0.00	A
1722	0.00	A
1722	0.00	A
1722	0.00	A
1734	0.00	A
1734	0.00	A
1759	0.00	A
1759	0.00	A
1759	0.00	A
1761	0.00	A
1764	0.00	A
1767	0.00	A
1767	0.00	A
1768	0.00	A
1774	0.00	A
1776	0.00	A
1776	0.00	A
1812	0.00	A
1812	0.00	A
1812	0.00	A
1814	0.00	A
1817	0.00	A
1826	0.00	A
1827	0.00	A
1827	0.00	A
1829	0.00	A
1829		
1830	0.00	A
1830	0.00	A
1830	0.00	A
1831	0.00	A
1831	0.00	A
1831	0.00	A
1832	0.00	A
1832	0.00	A
1836	0.00	A
1836	0.00	A
1836		
1848	0.00	A
1852	0.00	A
1871	0.00	A
1871	0.00	A
1874	0.00	A
1879	0.00	A
1879	0.00	A
1879	0.00	A
1885	0.00	A
1885	0.00	A
1885	0.00	A
1889	0.00	A
1889	0.00	A
1903	0.00	A
1903		
1910	0.00	A
1923	0.00	A
1923	0.00	A

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1928	0.00	A
1928	0.00	A
1928	0.00	A
1937	2.90	A
1937	1.90	A
1938	0.00	A
1938	0.00	A
1939	0.00	A
1939	1.00	A
1939		
1940	0.00	A
1941	0.00	A
1941	0.00	A
1948	0.00	A

Mean	0.1
Median (Ref)	**0.0**
STDev	0.4
Min	0.0
Max	2.9

RICE A (Lower)	RICE A (Upper)	RICE B (Lower)	RICE B (Upper)	RICE C (Lower)	RICE C (Upper)
-	3.8	-	10.9	-	>10.9

Sample 4 (5 SEM4) - Total asbestos fibre density (fmm^{-2})

Lab Number	Total Asbestos	RICE
7	0.98	A
7	1.47	A
139	2.00	A
139	0.00	A
300	4.00	A
709	3.70	A
807	1.60	A
807	1.20	A
818	2.00	A
1181	1.99	A
1187	2.50	A
1276	2.00	A
1276	2.40	A
1277	0.80	A
1282	0.00	A
1477	7.83	A
1477	2.17	A
1477		
1507	1.75	A
1562	3.90	A
1562	1.80	A
1575	1.83	A
1579	5.00	A
1579	4.00	A
1579	4.00	A
1582	3.00	A
1592	1.00	A
1592	3.00	A
1620	2.00	A
1620		
1620		
1628	1.56	A
1628	1.60	A
1628	1.04	A
1638	2.00	A
1639	0.00	A
1640	2.90	A
1658	1.00	A
1658	0.00	A
1669	0.00	A
1669	2.00	A
1669	2.00	A
1675	2.50	A
1680	1.80	A
1680	2.70	A
1680	2.70	A
1684	1.00	A
1687	0.00	A
1715	0.99	A
1717	2.94	A
1719	2.00	A

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1719	2.50	A
1720	2.00	A
1722	1.20	A
1722	2.40	A
1722	1.20	A
1734	0.94	A
1734	0.00	A
1759	3.10	A
1759	4.00	A
1759	3.10	A
1761	1.66	A
1764	1.00	A
1767	1.00	A
1767	0.60	A
1768	0.99	A
1774	2.00	A
1776	1.00	A
1776	3.00	A
1812	1.00	A
1812	1.00	A
1812	0.00	A
1814	4.60	A
1817	1.00	A
1826	2.41	A
1827	0.00	A
1827	0.00	A
1829	0.50	A
1829		
1830	1.98	A
1830	1.98	A
1830	0.00	A
1831	1.60	A
1831	0.00	A
1831	0.00	A
1832	2.50	A
1832	1.00	A
1836	6.00	A
1836	2.56	A
1836		
1848	3.00	A
1852	0.78	A
1871	3.50	A
1871	2.00	A
1874	0.80	A
1879	3.00	A
1879	2.00	A
1879	4.00	A
1885	2.00	A
1885	2.00	A
1885	2.00	A
1889	1.97	A
1889	0.00	A
1903	2.00	A
1903		
1910	0.00	A
1923	1.10	A

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1923	3.20	A
1928	2.00	A
1928	2.70	A
1928	2.00	A
1937	2.90	A
1937	1.90	A
1938	2.00	A
1938	0.00	A
1939	1.00	A
1939	2.00	A
1939	3.00	A
1940	2.00	A
1941	1.50	A
1941	2.00	A
1948	1.00	A

Mean	1.9
Median (Ref)	**2.0**
STDev	1.3
Min	0.0
Max	7.8

RICE A (Lower)	RICE A (Upper)	RICE B (Lower)	RICE B (Upper)	RICE C (Lower)	RICE C (Upper)
0.0	11.4	-	22.2	-	>22.2

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 samples (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 samples (R ≤ 63.7 fibres. mm^{-2})*

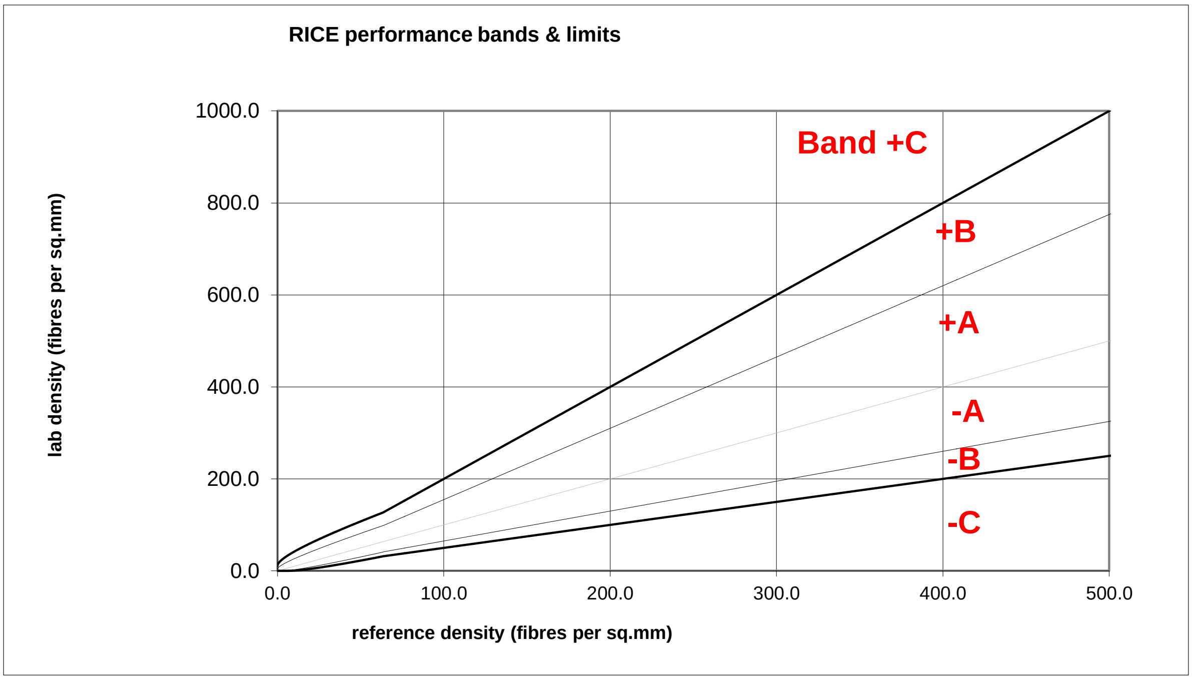
Target band A: $(\sqrt{R}-1.57)^2$ to $(\sqrt{R}+1.96)^2$ [band A]

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"

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