

|                    |   |
|--------------------|---|
| Customer Name      | Maeda-Kougyou Japan<br>218-2 Norimatsu Yahatanishi ward<br>Kitakyushu City<br>Japan   |
| Contact            | Dr. Khaled Hussein  |
| Customer PO no.    | N/A   |
| Test Requested     | BS ISO 27447: 2009 Fine ceramics<br>(advanced ceramics, advanced<br>technical ceramics) — Test method<br>for antibacterial activity of<br>semiconducting photocatalytic<br>materials (Test method modified due<br>to client's requests)                   |
| Sample Description | Stainless steel: non-coated and<br>coated with Miracle Titanium (Primary<br>and MVX),<br>Clear plastic: non-coated and coated<br>with Miracle Titanium (Primary and<br>MVX),<br>Textile: non-coated and coated with<br>Miracle Titanium (Primary and MVX) |
| Date of Receipt    | 27 <sup>th</sup> April 2012   |
| Project Number     | ASCR0092008   |
| Report Date        | 5 <sup>th</sup> July 2012   |



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### Description of Test Items

The following items were tested. The samples to be tested all measured 50 x 50 mm.

| Test Item                 | Product Code |
|---------------------------|--------------|
| Treated Stainless Steel   | ASC002162    |
| Untreated Stainless Steel | ASC002163    |
| Treated Clear Plastic     | ASC002164    |
| Untreated Clear Plastic   | ASC002165    |
| Treated Textile           | ASC002166    |
| Untreated Textile         | ASC002167    |

### Introduction

The purpose of the project was to ascertain the effect of the MVX coating on bacterial viability when applied to stainless steel, plastic and textile following exposure to UV and incandescent light as per the client's request. The test was agreed to be performed in accordance with BS ISO 27447: 2009 Fine ceramics (advanced ceramics, advanced technical ceramics) — Test method for antibacterial activity of semiconducting photocatalytic materials. As the client's requests included alterations in the type of materials to be tested however these have been subsequently noted.

### Procedure

The experimental procedure was performed in accordance with BS ISO 27447: 2009 Fine ceramics (advanced ceramics, advanced technical ceramics) — Test method for antibacterial activity of semiconducting photocatalytic materials, with alterations made to accommodate the client's sample and microbiological challenge specifications. The method for analysis was as follows:

#### Modules 1 and 2: Stainless steel and Plastic (Film adhesion method)

1. Cultures of *Staphylococcus aureus* ATCC6538P were grown under aerobic conditions at 37°C for 18 ± 1 hours. The concentration of bacterial cells was adjusted to a target concentration of  $2.6 \times 10^6$  cells ml<sup>-1</sup> in 1/500 nutrient broth. The adjustment in cellular concentration was calculated from previously performing serial dilutions of an overnight culture and correlating the bacterial concentration against the level of absorbance at an optical density of 600 nm using a spectrophotometer.



2. Prior to bacterial inoculation, all of the samples were surface sterilised with 70% (v/v) ethanol and were selected at random.
3. Specimens were individually placed in sterile petri-dishes. The specimens were placed on top of glass slides that separated the sample from the sterile wet filter paper, which was used as a moisture control measure.
4. A 150 µl aliquot of bacterial suspension was placed on top of the samples and immediately covered with sterilised film.
5. Treated and untreated samples were kept in a dark place or exposed to the light source as specified in the standard with the additional incandescent light source as per the client's request.
6. Three untreated samples were immediately withdrawn at  $t = 0$  to ascertain the recovery of bacteria immediately after inoculation. Bacteria were extracted by washing in 10 mls of Tryptic Soy Broth 0.05% (v/v) Tween-80.
7. A 1 ml aliquot of the washout was withdrawn and was serially diluted in phosphate buffered saline (PBS).
8. A 200 µl aliquot of the neat washout and serial dilutions was placed in a sterile petri dish. Approximately 15 – 20 ml Tryptic Soy Agar was added in order to enumerate viable cells by the pour plate method.
9. The solidified plates were allowed to set at room temperature and were incubated overnight at  $37 \pm 1^\circ\text{C}$ .
10. Following incubation the agar plates were counted for the presence of colony forming units (cfu) and the results were recorded. Where the number of cfu exceeded 300 the plates were recorded as TNTC (Too numerous to count).

#### Module 3: Textile Samples (Glass adhesion method)

1. Prior to inoculation all samples and coverslips were sterilised by autoclaving at  $121^\circ\text{C}$  for 15 minutes.
2. *S. aureus* was grown under aerobic conditions in Tryptic Soy Broth at  $37^\circ\text{C}$  for 18 hours.
3. The concentration of bacteria was adjusted to a target concentration of  $1 \times 10^5$  cells  $\text{ml}^{-1}$  using 1/500 nutrient broth.
4. Specimens were individually placed in sterile petri dishes. In order to preserve moisture a sterile filter paper was moistened with sterile water with the specimens to be tested separated by a glass slide.

5. A 150 µl aliquot of the adjusted bacterial suspension was placed on the surface of the textile samples and a glass slide was placed on top to press the bacterial suspension uniformly under the glass.
6. Treated and untreated samples were kept in a dark place or exposed to the light source as specified in the standard with the additional incandescent light source as per the client's request.
7. Three untreated samples were immediately washed in 20 ml PBS. A 2 ml aliquot of this washout was serially diluted in sterile PBS.
8. A 500 µl aliquot of the neat washout and the serially diluted samples was plated in duplicate on sterile petri-dishes.
9. Approximately 15 – 20 ml of Tryptic Soy Agar was placed into each petri dish in order to enumerate viable colony forming units by the pour plate method following incubation at 37°C for 24 – 48 hours. Where the number of cfu exceeded 300 the plates were recorded as TNTC (Too numerous to count).

#### **Satisfaction of criteria for a valid test and calculations**

The test requirement fulfilment validation follows the raw data in the results section (see below). In addition the results expressing photocatalyst antibacterial activity value for hard surfaces ( $R_L$ ) and on textiles ( $S_L$ ) and the photocatalyst antibacterial activity value with UV and incandescent light irradiation for hard surfaces ( $\Delta R$ ) and on textiles ( $\Delta S$ ).

#### **Film adhesion method**

$$N = P \times V$$

N is the number of viable bacteria

P is the bacteria concentration (cells/ml)

V is the volume of extraction buffer used in the test

1. The logarithmic value of the number of viable bacteria of non-treated specimens after inoculation is:

$$(L_{\max} - L_{\min}) / (L_{\text{mean}}) < 0.2$$

$L_{\max}$  is the maximum logarithmic value of viable bacteria

$L_{\min}$  is the minimum logarithmic value of viable bacteria

$L_{\text{mean}}$  is the average logarithmic value of viable bacteria for 3 specimens



2. The logarithmic value of viable bacteria of non-treated specimens after inoculation shall be within the  $1.0 \times 10^5$  -  $4.0 \times 10^5$  range.
3. The viable bacteria of non-treated specimens after light exposure shall be more than  $1 \times 10^3$  cells for all three specimens.
4. After being kept in a dark place the viable bacteria of non treated specimens shall be more than  $1 \times 10^3$  cells for all three specimens.

*Photocatalyst antibacterial activity value calculation*

$$R_L = [\log(B_L/A) - \log(C_L/A)] = \log[B_L/C_L]$$

- $R_L$  is the photocatalyst antibacterial activity value after light exposure  
 $A$  is the average number of viable bacteria of non-treated samples just after inoculation  
 $B_L$  is the average number of viable bacteria of non treated specimens after light exposure  
 $C_L$  is the average number of viable bacteria of photocatalytic treated specimens after light exposure

$$\Delta R = \log[B_L/C_L] - \log[B_D/C_D]$$

- $\Delta R$  is the photocatalyst antibacterial activity value with UV irradiation  
 $B_D$  is the average number of viable bacteria of non – treated specimens after being kept in a dark place  
 $C_D$  is the average number of viable bacteria of photocatalytic treated specimens after being kept in a dark place

Glass adhesion method

$$M = P \times 20$$

- $M$  is the number of cells of viable bacteria  
 $P$  is the bacteria concentration (cells/ml)  
 $20$  is the quantity of PBS used for washout (ml)

*Propagation values for validation of conditions for a valid test*

$$F_{BL} = M_{BL} - M_{BA}$$

- $F_{BL}$  is the growth value after light exposure

$M_{BL}$  is the average logarithmic value of the number of bacteria for 3 non treated specimens after light exposure

$M_{BA}$  is the average logarithmic value of the number of viable bacteria for three non treated specimens just after inoculation

$$F_{BD} = M_{BD} - M_{BA}$$

$F_{BD}$  is the growth value after being kept in a dark place

$M_{BD}$  is the average logarithmic value of the number of viable bacteria for three non treated specimens after being kept in a dark place

*Photocatalyst antibacterial activity value calculation*

$$S_L = M_{BL} - M_L$$

$S_L$  is the photocatalyst antibacterial activity value after light exposure

$M_L$  is the average logarithmic value of the number of viable bacteria for 3 photocatalytic treated specimens after light exposure

$$\Delta S = (M_{BL} - M_L) - (M_{BD} - M_D)$$

$\Delta S$  is the photocatalyst antibacterial value with light exposure

$M_D$  is the average logarithmic value of the number of viable bacteria for three photocatalytic treated specimens after being kept in a dark place

## Results

### 1. Stainless Steel

| Sample description   | Dilution             | Colony count | Number of viable bacteria recovered per specimen | Log values |
|----------------------|----------------------|--------------|--|------------|
| Untreated 1<br>t = 0 | 1 x 10 <sup>-1</sup> | TNTC         | 275000   | 5.43933269 |
|                      | 1 x 10 <sup>-2</sup> | 50, 60       |  |            |
|                      | 1 x 10 <sup>-3</sup> | 3, 7         |  |            |
| Untreated 2<br>t=0   | 1 x 10 <sup>-1</sup> | TNTC         | 327500   | 5.5152113  |
|                      | 1 x 10 <sup>-2</sup> | 60, 71       |  |            |
|                      | 1 x 10 <sup>-3</sup> | 5, 8         |  |            |
| Untreated 3<br>t=0   | 1 x 10 <sup>-1</sup> | TNTC         | 365000   | 5.56229286 |
|                      | 1 x 10 <sup>-2</sup> | 70, 76       |  |            |
|                      | 1 x 10 <sup>-3</sup> | 5, 8         |  |            |
| Light untreated 1    | 1 x 10 <sup>0</sup>  | TNTC         | 20500  | 4.31175386 |
|                      | 1 x 10 <sup>-1</sup> | 50, 32       |  |            |
|                      | 1 x 10 <sup>-2</sup> | 6, 2         |  |            |
| Light untreated 2    | 1 x 10 <sup>0</sup>  | TNTC         | 11250  | 4.05115252 |
|                      | 1 x 10 <sup>-1</sup> | 24, 21       |  |            |
|                      | 1 x 10 <sup>-2</sup> | 4, 1         |  |            |
| Light untreated 3    | 1 x 10 <sup>0</sup>  | TNTC         | 31250  | 4.49485002 |
|                      | 1 x 10 <sup>-1</sup> | 61, 64       |  |            |
|                      | 1 x 10 <sup>-2</sup> | 10, 4        |  |            |
| Light treated 1      | 1 x 10 <sup>0</sup>  | 0, 0         | 0  | 0          |
|                      | 1 x 10 <sup>-1</sup> | 0, 0         |  |            |
|                      | 1 x 10 <sup>-2</sup> | 0, 0         |  |            |
| Light treated 2      | 1 x 10 <sup>0</sup>  | 0, 0         | 0  | 0          |
|                      | 1 x 10 <sup>-1</sup> | 0, 0         |  |            |
|                      | 1 x 10 <sup>-2</sup> | 0, 0         |  |            |



| Sample description | Dilution           | Colony count | Number of viable bacteria recovered per specimen | Log values |
|--------------------|--------------------|--------------|--|------------|
|                    |                    |              |  |            |
|                    | $1 \times 10^{-1}$ | 0, 0         |  |            |
|                    | $1 \times 10^{-2}$ | 0, 0         |  |            |
| Dark untreated 1   | $1 \times 10^0$    | TNTC         | 30500  | 4.48429984 |
|                    | $1 \times 10^{-1}$ | 54, 68       |  |            |
|                    | $1 \times 10^{-2}$ | 8, 4         |  |            |
| Dark untreated 2   | $1 \times 10^0$    | TNTC         | 29000  | 4.462398   |
|                    | $1 \times 10^{-1}$ | 50, 66       |  |            |
|                    | $1 \times 10^{-2}$ | 2, 0         |  |            |
| Dark untreated 3   | $1 \times 10^0$    | TNTC         | 30750  | 4.48784512 |
|                    | $1 \times 10^{-1}$ | 51, 72       |  |            |
|                    | $1 \times 10^{-2}$ | 6, 3         |  |            |
| Dark treated 1     | $1 \times 10^0$    | TNTC         | 16500  | 4.21748394 |
|                    | $1 \times 10^{-1}$ | 36, 30       |  |            |
|                    | $1 \times 10^{-2}$ | 3, 1         |  |            |
| Dark treated 2     | $1 \times 10^0$    | TNTC         | 9250   | 3.96614173 |
|                    | $1 \times 10^{-1}$ | 20, 17       |  |            |
|                    | $1 \times 10^{-2}$ | 2, 2         |  |            |
| Dark treated 3     | $1 \times 10^0$    | 75, 83       | 7900   | 3.89762709 |
|                    | $1 \times 10^{-1}$ | 6, 11        |  |            |
|                    | $1 \times 10^{-2}$ | 1, 0         |  |            |

Test requirement fulfilment validation

1.  $5.56-5.43/5.50 = 0.023$

**Requirement is fulfilled**

2. Logarithmic value of bacteria after inoculation must be within  $1 \times 10^5$  and  $4 \times 10^5$  range

**Requirement is fulfilled**

3. The viable bacteria in non-treated specimens following light exposure is greater than  $1 \times 10^3$  cells for all three specimens

**Requirement is fulfilled**

4. The viability of bacteria from non-treated specimens after being kept in a dark place is greater than  $1 \times 10^3$  cells for all three specimens

**Requirement is fulfilled**

Photocatalyst antibacterial activity value calculation

$$R_L = \log[21000/0] = 4.32$$

$$\begin{aligned}\Delta R &= \log[21000/0] - \log[90250/11216] \\ &= \log[21000] - \log[8] \\ &= 4.32 - 0.90 \\ &= \underline{3.42}\end{aligned}$$

## 2. Plastic

| Sample description   | Dilution           | Colony count | Number of viable bacteria recovered per specimen | Log values |
|----------------------|--------------------|--------------|--|------------|
| Untreated 1<br>t = 0 | $1 \times 10^{-1}$ | TNTC         | 102500   | 5.01072387 |
|                      | $1 \times 10^{-2}$ | 22, 19       |  |            |
|                      | $1 \times 10^{-3}$ | 1, 3         |  |            |
| Untreated 2<br>t=0   | $1 \times 10^{-1}$ | TNTC         | 102500   | 5.01072387 |
|                      | $1 \times 10^{-2}$ | 18, 23       |  |            |
|                      | $1 \times 10^{-3}$ | 3, 1         |  |            |
| Untreated 3<br>t=0   | $1 \times 10^{-1}$ | TNTC         | 160000   | 5.20411998 |
|                      | $1 \times 10^{-2}$ | 37, 27       |  |            |
|                      | $1 \times 10^{-3}$ | 2, 2         |  |            |
| Light untreated 1    | $1 \times 10^0$    | TNTC         | 8750   | 3.94200805 |
|                      | $1 \times 10^{-1}$ | 17, 18       |  |            |
|                      | $1 \times 10^{-2}$ | 2, 1         |  |            |
| Light untreated 2    | $1 \times 10^0$    | TNTC         | 70110  | 4.84577997 |
|                      | $1 \times 10^{-1}$ | 110, 140     |  |            |
|                      | $1 \times 10^{-2}$ | 11, 15       |  |            |
| Light untreated 3    | $1 \times 10^0$    | 150, 160     | 7750   | 3.8893017  |
|                      | $1 \times 10^{-1}$ | 14, 15       |  |            |
|                      | $1 \times 10^{-2}$ | 2, 3         |  |            |
| Light treated 1      | $1 \times 10^0$    | 1, 0         | 50   | 1.69897    |
|                      | $1 \times 10^{-1}$ | 0, 0         |  |            |
|                      | $1 \times 10^{-2}$ | 0, 0         |  |            |
| Light treated 2      | $1 \times 10^0$    | 1, 3         | 100  | 2          |
|                      | $1 \times 10^{-1}$ | 0, 0         |  |            |
|                      | $1 \times 10^{-2}$ | 0, 0         |  |            |
| Light treated 3      | $1 \times 10^0$    | 1, 0         | 50   | 1.69897    |
|                      | $1 \times 10^{-1}$ | 0, 0         |  |            |
|                      | $1 \times 10^{-2}$ | 0, 0         |  |            |



| Sample description | Dilution           | Colony count | Number of viable bacteria recovered per specimen | Log values |
|--------------------|--------------------|--------------|--|------------|
| Dark untreated 1   | $1 \times 10^0$    | TNTC         | 6250   | 3.79588002 |
|                    | $1 \times 10^{-1}$ | 14, 11       |  |            |
|                    | $1 \times 10^{-2}$ | 1, 1         |  |            |
| Dark untreated 2   | $1 \times 10^0$    | TNTC         | 25000  | 4.39794001 |
|                    | $1 \times 10^{-1}$ | 50, 50       |  |            |
|                    | $1 \times 10^{-2}$ | 3, 0         |  |            |
| Dark untreated 3   | $1 \times 10^0$    | TNTC         | 9000   | 3.95424251 |
|                    | $1 \times 10^{-1}$ | 21, 15       |  |            |
|                    | $1 \times 10^{-2}$ | 2, 1         |  |            |
| Dark treated 1     | $1 \times 10^0$    | 65, 63       | 3200   | 3.50514998 |
|                    | $1 \times 10^{-1}$ | 9, 7         |  |            |
|                    | $1 \times 10^{-2}$ | 1, 0         |  |            |
| Dark treated 2     | $1 \times 10^0$    | 61, 82       | 3575   | 3.55327605 |
|                    | $1 \times 10^{-1}$ | 9, 6         |  |            |
|                    | $1 \times 10^{-2}$ | 2, 0         |  |            |
| Dark treated 3     | $1 \times 10^0$    | 54, 47       | 2525   | 3.40226138 |
|                    | $1 \times 10^{-1}$ | 3, 3         |  |            |
|                    | $1 \times 10^{-2}$ | 2, 0         |  |            |

*Test requirement fulfilment validation*

1.  $5.204 - 5.010 / 5.074 = 0.038$

**Requirement is fulfilled**

2. Logarithmic value of bacteria after inoculation must be within  $1 \times 10^5$  and  $4 \times 10^5$  range

**Requirement is fulfilled**

3. The viable bacteria in non-treated specimens following light exposure is greater than  $1 \times 10^3$  cells for all three specimens

**Requirement is fulfilled**

4. The viability of bacteria from non-treated specimens after being kept in a dark place is greater than  $1 \times 10^3$  cells for all three specimens

**Requirement is fulfilled**

#### Photocatalyst antibacterial activity value calculation

$$\begin{aligned} R_L &= \log[28870/66.66] = 433.09 \\ &= \log[433.09] \\ &= 2.63 \end{aligned}$$

$$\begin{aligned} \Delta R &= \log[28870/66.66] - \log[13416/3100] \\ &= \log[433.09] - \log[4.32] \\ &= 2.63 - 0.63 \\ &= \underline{2} \end{aligned}$$

### 3. Textile

| Sample description   | Dilution             | Colony count | Number of viable bacteria recovered per specimen | Log values |
|----------------------|----------------------|--------------|--|------------|
| Untreated 1<br>t = 0 | 1 x 10 <sup>0</sup>  | 96, 69       | 3300   | 3.51851394 |
|                      | 1 x 10 <sup>-1</sup> | 10, 7        |  |            |
| Untreated 2<br>t=0   | 1 x 10 <sup>0</sup>  | 97, 93       | 3800   | 3.5797836  |
|                      | 1 x 10 <sup>-1</sup> | 9, 9         |  |            |
| Untreated 3<br>t=0   | 1 x 10 <sup>0</sup>  | 113, 99      | 4240   | 3.62736586 |
|                      | 1 x 10 <sup>-1</sup> | 5, 11        |  |            |
| Light untreated 1    | 1 x 10 <sup>0</sup>  | 106, 96      | 4040   | 3.60638137 |
|                      | 1 x 10 <sup>-1</sup> | 10, 8        |  |            |
| Light untreated 2    | 1 x 10 <sup>0</sup>  | 130, 136     | 5320   | 3.72591163 |
|                      | 1 x 10 <sup>-1</sup> | 3, 3         |  |            |
| Light untreated 3    | 1 x 10 <sup>0</sup>  | 118, 118     | 4720   | 3.673942   |
|                      | 1 x 10 <sup>-1</sup> | 3, 1         |  |            |
| Light treated 1      | 1 x 10 <sup>0</sup>  | 5, 1         | 120  | 2.07918125 |
|                      | 1 x 10 <sup>-1</sup> | 0, 0         |  |            |
| Light treated 2      | 1 x 10 <sup>0</sup>  | 2, 2         | 80   | 1.90308999 |
|                      | 1 x 10 <sup>-1</sup> | 0, 0         |  |            |
| Light treated 3      | 1 x 10 <sup>0</sup>  | 0, 0         | 0  | 0          |
|                      | 1 x 10 <sup>-1</sup> | 0, 0         |  |            |
| Dark untreated 1     | 1 x 10 <sup>0</sup>  | 183, 191     | 7480   | 3.8739016  |
|                      | 1 x 10 <sup>-1</sup> | 9, 4         |  |            |
| Dark untreated 2     | 1 x 10 <sup>0</sup>  | 173, 169     | 6840   | 3.8350561  |
|                      | 1 x 10 <sup>-1</sup> | 4, 4         |  |            |
| Dark untreated 3     | 1 x 10 <sup>0</sup>  | 138, 151     | 5780   | 3.76192784 |
|                      | 1 x 10 <sup>-1</sup> | 40, 22       |  |            |
| Dark treated 1       | 1 x 10 <sup>0</sup>  | 77, 83       | 3200   | 3.50514998 |
|                      | 1 x 10 <sup>-1</sup> | 5, 5         |  |            |



| Sample description | Dilution           | Colony count | Number of viable bacteria recovered per specimen | Log values |
|--------------------|--------------------|--------------|--|------------|
| Dark treated 2     | $1 \times 10^0$    | 126, 104     | 4600   | 3.66275783 |
|                    | $1 \times 10^{-1}$ | 13, 12       |  |            |
| Dark treated 3     | $1 \times 10^0$    | 126, 93      | 4380   | 3.64147411 |
|                    | $1 \times 10^{-1}$ | 10, 9        |  |            |

*Test requirement fulfilment validation*

1.  $F_{BL} = 3.668 - 3.574 = 0.094$

$F_{BL}$  is greater than 0 therefore parameter is validated

2.  $F_{BD} = 3.823 - 3.574 = 0.249$

$F_{BD}$  is greater than 0 therefore parameter is validated

$S_L = 3.668 - 1.32 = 2.348$

$\Delta S = (3.668 - 1.32) - (3.823 - 3.602)$

$= 2.348 - 0.221$

$= \underline{2.217}$

## Discussion and Conclusion

In accordance with the wishes of the client the ISO 22447:2009 procedure was modified slightly as the surfaces to be tested were stainless steel, hard plastic, and textile samples.

In understanding the data it must be noted that  $R_L$  and  $S_L$  values account for the reduction of viability caused by the exposure of the treated surfaces to light on hard surfaces and textiles respectively. In contrast  $\Delta R$  and  $\Delta S$  values address the reduction of bacterial viability caused by the coating becoming light activated while accounting for the reduction in viability caused by the same coatings in a dark environment. From the data presented here it is clear that in all cases the coatings resulted in a reduction in bacterial viability even when stored in a dark place.

The coatings have a significant photocatalyst antibacterial activity value against *S. aureus*, with activity values of 3.42, 2 and 2.217 on stainless steel, clear plastic and textile samples respectively. In each of these cases the conditions for a valid test were satisfied.

In conclusion the coatings appear to be very effective at reducing the viability of *S. aureus* when exposed to light.

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Report Compiled by:

**John  
Fallon**

Digitally signed by John Fallon  
DN: cn=John Fallon, o=ou,  
email=jfallon@airmidhealthgroup.  
com, c=IE  
Reason: I am the author of this  
document  
Date: 2012.07.05 11:59:22 +01'00'

John Fallon PhD  
Senior Scientific Officer

Report Reviewed by:

**Maire  
Fox**

Digitally signed by Maire Fox  
DN: cn=Maire Fox, o=ou,  
email=mfox@airmidhealthgroup.  
com, c=IE  
Reason: I have reviewed this  
document  
Date: 2012.07.05 12:04:38 +01'00'

Máire Fox MSc  
Laboratory Manger

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