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Report on:

Assessing the efficacy of two Vikan microfibre cloths for removal of micro-organisms

Work performed by Campden BRI (Chipping Campden) Limited
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2 SUMMARY

Campden BRI (CC) was requested to determine, the efficacy of two different Vikan microfibre cloths at removing *Methicillin-resistant staphylococcus aureus* (MRSA), *Clostridium difficile* (in it's spore form) and *Escherichia coli* from furniture laminate surface materials (see Appendix 1 for details).

3 BACKGROUND

Vikan plans to replace its existing cloth with a new cloth and wants to ensure that the new cloth will be as good as the previous one. As background, the previous Vikan project (PN 111367) was used to determine the experimental conditions. The previous experiment was published in the Journal of Hospital Infection in article 78(2011) 182-186 “Assessing the efficacy of different microfibre cloths at remaining surface micro-organisms associated with healthcare-associated infections”.

4 SAMPLES/MATERIALS

Furniture laminate surfaces were cut to a size of 15 by 60cm.

Micro fibre cloths were obtained from Vikan (washed once and 75 times).

Media and cultures were prepared. Surface soiling, sponge swabs and brush were purchased (see Appendix 1).

5 METHODS

Twenty nine new laminate surfaces of 15 cm by 60 cm size, were cleaned using a sponge, with detergent (Fairy Liquid) and hot water to remove any residues. These were rinsed with clean, de-ionised water and allowed to air dry. Prior to use, the surfaces were disinfected with 70% alcohol. Alcohol was allowed to evaporate completely before inoculating the surfaces.

The surfaces were inoculated with Browne’s soil solution.

Browne’s soil solution prepared using 20 ml of 10^5/ml *C. difficile* spore suspension and 10 ml each of an *E.Coli* and *MRSA* 10^8/ml suspension and mixed using a vortex until visually mixed.

Application method: 2 ml of Browne’s soil solution were pipetted as a liquid and evenly spread over the surface using a sterile 2 inch paint brush.

The inoculated surface was placed into a purpose built, automated cleaning rig fitted with a new microfibre cloth sample (see Appendix 1).

The plate was cleaned using the rig under controlled conditions of speed, pressure, stroke rate and direction.

The following operational parameters were determined as representative of manual cleaning and consistently achievable for all sampling:

- Starting position (of the cloth within the rig, i.e. at one end of the surface) = 50.
- Stopping position (of the cloth within the rig, i.e. at the other end of the surface) = 570.
- X speed (speed at which the cloth travels across the surface) = 20.
- Z position = 214 mm (the position to which the cloth is lowered so that it is in contact with the surface. This produced a consistent pressure).
- X position = 7 mm.
- Brush push = 10 mm.
- Up at stop position = Off (so that cloth is not removed from the surface at the far end of the plate and returned to the front of the plate but moves forwards and backwards over the plate surface).
- Strokes = 2 (1 stroke = cloth moved backward and forward across the surface).
- Constant run = Off (so that the cloth is only used for 2 strokes).

The entire area of the cleaned surface was swabbed using a damp sponge swab using a standardised method (see Appendix 1). The swab was then placed into the sterile bag in which it came.

The samples were placed in a stomacher for 30 seconds to recover the organisms.

The recovery medium (Maximum recovery diluents - MRD, LABM: LAB103) was serially diluted and plated out in duplicate, using organism appropriate agar (see Table 1 for details). Samples were incubated in appropriate conditions times and temperatures shown in Table 1. Sterility checks were also carried out.

Table 1: Agar and incubation conditions used to facilitate the growth of *C. difficile, E. coli and MRSA.*

Microorganism	Agar	Incubation conditions
C. difficile	1ml pour plate on Reinforced Clostridial Agar (RCA, Oxoid CM0151)	anaerobically for 3 days at 37°C;
E. coli	1 ml pour plate on Violet Red Bile Agar (VRBA, LabM Lab031)	24 hours at 37°C;
MRSA	0.5ml spread plate on Baird Parker agar (BP, Oxoid CM0275)	48 hours at 37°C

The results were expressed as cfu's per sponge.

6 RESULTS

The results for the study are shown in Tables 2 to 4. Preliminary tests showed that the controls were suitable for the study (Table 2). The inoculation levels were also as desired for the study (Table 3). The results obtained for all 4 cloths after treatment and mean of 5 replicas are shown in Table 4. Log reductions were calculated as the differences between logarithms of mean positive controls (Table 2) and logarithms of counts after treatment (Table 4). Statistical analysis was carried out using one-way ANOVA for Log reductions versus Type of cloth. The log reductions for each cloth type were compared for each wash condition (washed once and washed 75 times) for each bacteria strain (*C. difficile, E. coli* and MRSA). Figure 1 illustrates the statistical comparison of the log reduction results obtained for all 3 microorganisms on the new and old cloths used once or 75 times. More statistical data is provided in Appendix 2. It was observed that all residuals were distributed Normally (Example of residual plot for log reduction of *C. difficile* on cloths washed once shown in Figure 3; Appendix 2).

Table 2: **Negative and positive control results obtained in triplicate. Mean of the 3 replicates are also shown.**

Control type	Microorganism	Count cfu/sponge			
Negative Control; surface, no Browne's		**1**	**2**	**3**	**Mean**
	C. difficile	<50	50	<50	<50
	E. coli	<50	<50	<50	<50
	MRSA	<100	<100	<100	<100
Negative Control; surface with Browne's					
	C. difficile	<50	<50	<50	<50
	E. coli	<50	<50	<50	<50
	MRSA	100	<100	<100	<100
Positive Control; surface with Brown's plus microorganisms					
	C. difficile	1.2×10^8	1.4×10^8	1.5×10^8	1.4×10^8
	E. coli	4.0×10^7	4.4×10^7	2.9×10^7	3.8×10^7
	MRSA	1.4×10^8	7.1×10^7	6.5×10^7	9.2×10^7

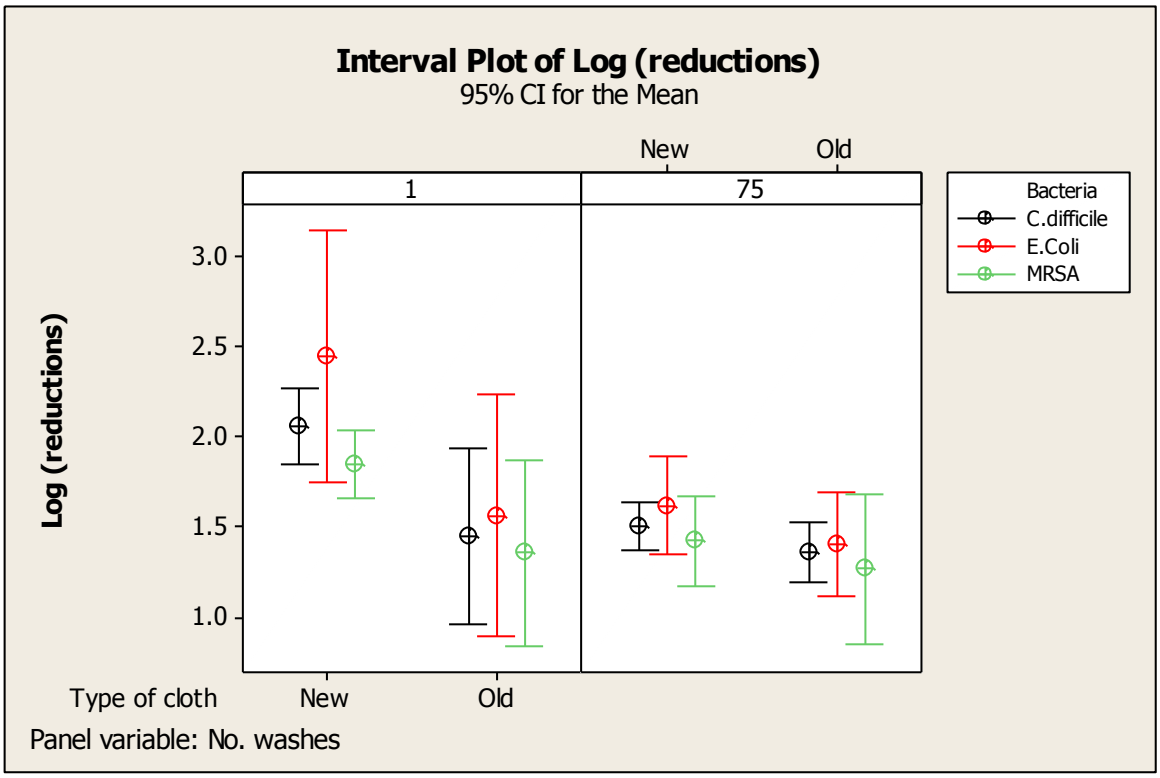
Table 3: Inoculation level (cfu/ml); Browne's plus microorganisms.

Microorganism	Count cfu/ml
C. difficile	6.8×10^9
E. coli	3.6×10^9
MRSA	$>1.0 \times 10^9$

Table 4: Test results obtained for all 4 cloths after treatment. Mean of the 5 replicates are also shown.

Test Type	Microorganism	Count cfu/sponge					
		1	2	3	4	5	Mean
Old Cloth, 1 Wash	*C. difficile*	1.2×10^6	4.8×10^6	6.4×10^6	4.7×10^6	1.5×10^7	**6.4×10^6**
	E. coli	1.5×10^5	8.5×10^5	2.1×10^6	9.0×10^5	4.2×10^6	**1.6×10^6**
	MRSA	8.2×10^5	3.3×10^6	5.8×10^6	4.5×10^6	1.1×10^7	**5.1×10^6**
Old Cloth, 75 Washes							
	C. difficile	7.2×10^6	7.5×10^6	3.7×10^6	5.0×10^6	7.3×10^6	**6.4×10^6**
	E. coli	1.3×10^6	1.3×10^6	7.6×10^5	1.5×10^6	3.3×10^6	**1.6×10^6**
	MRSA	5.3×10^6	8.2×10^6	5.6×10^6	7.1×10^6	1.2×10^6	**5.5×10^6**
New Cloth, 1 Wash	*C. difficile*	1.3×10^6	1.4×10^6	8.9×10^5	2.0×10^6	7.4×10^5	**1.3×10^6**
	E. coli	1.9×10^5	1.5×10^5	1.5×10^4	4.9×10^5	1.9×10^5	**2.1×10^5**
	MRSA	1.3×10^6	1.4×10^6	1.1×10^6	1.9×10^6	7.3×10^5	**1.3×10^6**
New Cloth 75 Washes	*C. difficile*	2.8×10^6	4.1×10^6	4.5×10^6	5.3×10^6	4.9×10^6	**4.3×10^6**
	E. coli	4.0×10^5	1.0×10^6	8.4×10^5	1.0×10^6	1.6×10^6	**9.7×10^5**
	MRSA	1.7×10^6	2.9×10^6	3.0×10^6	4.2×10^6	5.8×10^6	**3.6×10^6**

Figure 1: Interval plot of log reductions obtained with new and old cloths washed once or 75 times.



7 DISCUSSION/CONCLUSION

All analysis was carried out using one-way ANOVA for Log reductions versus Type of cloth. The log reductions for each cloth type were compared for each wash condition (washed once and washed 75 times) for each bacteria strain (*C. difficile, E. coli* and MRSA).

For cloths washed once the log reduction for new cloths was significantly better then for old cloths for all bacteria.

For cloths washed seventy five times there was no significant difference detected between old or new cloths, however the general trend has shown that new cloth appeared to have higher mean log reductions for all of the bacteria.

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8 APPENDICES

Appendix 1

Methods and media used by Campden BRI

Details of test surfaces

Furniture laminate - Formica fundamental laminate, white, shell finish. Supplied by C L S Fabrication Ltd., Unit 1, Caswell Road, Leamington Spa, Warwickshire. CV31 1QD.

Surface soiling

Browne's – Isopharm Ltd

Soil application method

Pipetted and evenly spread over surface using a sterile 2 inch paint brush.

8.1 Preparation of cultures and inoculation solutions.

Master culture preparation

MRSA and *E. coli*:

- For each organism, inoculate a Tryptone Soya Agar slope with a bead of the library strain of the organism, taken from the -80^{o}C freezer.
- Incubate this slope for 24 hours at 37^{o}C to give a master culture. Use within 6 weeks of initial inoculation.
- Conduct a purity check by streaking the organisms out to single colonies.

Cl. difficile:

- Take a bead of the library strain of the organism from a -80^{o}C freezer.
- Place the bead into 10 ml of cooked meat media
- Incubate at 37ºC for 2x 24h
- Transfer culture to 1000ml CMM
- Incubate for 7 days at 37ºC
- The culture will then be filtered through sterile glass wool in a sterile glass funnel
- Centrifuge (4000rpm/40min).
- Decant supernatant and add sterile distilled water to the pellet to re-suspend it. Repeat this process 3 times
- Pour suspension into a sterile container and store until use at 4ºC.

8.2 Inoculation solution preparation

MRSA and *E. coli*:

- From the master cultures, make working sub-cultures by taking a loop of cells from the master cultures and inoculating other TSA slopes, and then incubating these for ~24 hours at 37^{o}C.
- Growth on the sub-culture slope will be removed by adding 5g of sterile glass beads and 10 ml of sterile Maximum recovery diluent (MRD) and shaking them gently together to re-suspend the culture.
- the suspension will be enumerated by use of a spectrophotometer using a calibration curve of concentration vs absorbance. The sub-culture solution will be adjusted to give a 10^{8} organisms per ml concentration.

Cl. Difficile:

For enumeration of *Cl. diff* spores.

- Take a sample of the prepared suspension to enumerate TVC's on pre-heated and post-heated stock.
- A ten-fold serial dilution of the spore suspension using MRD.
- Pour plate the dilutions in duplicate (1 ml) using Reinforced Clostridial Agar (RCA).
- Place plates into anaerobic boxes.
- Incubate plates for 48 hours at 37°C.

Having enumerated the spore solution make dilutions as required to give the necessary concentration for inoculation of the surfaces.

8.3 Surface swabbing

Media & equipment:

- Sponge swabs (Dry-Sponge with Glove, 3M, BP-237-SPG, LOT 2018-06) packaged in sterile plastic bags.
- 100 ml volumes of Maximum recovery diluent (MRD, LABM: LAB103) in sterile plastic, screw top container (150 ml universal containers, Bibby Sterilin: 128A), equilibrated to the sampling temperature.

Swabbing procedure:

- Wearing plastic disposable gloves, open the sterile bag containing the sponge swab using the tabs provided and wet the sponge with approximately 10 ml of diluent from a 100ml volume.
- Remove excess fluid from sponge by gently squeezing the outside of the bag.
- Remove the damp sponge from the bag and rub the sponge vertically, horizontally, and diagonally across the whole area of the surface to be sampled.
- Replace the sponge into the bag from which it came and add the remaining 90 ml of diluent.
- Stomach the sponge in the bag for 30 seconds to remove the organisms from the sponge.
- Use a single, new swab for each new surface.
- Keep the samples chilled (~5°C) until processed. Time to processing should be as short as possible and should be consistent.

Swab processing: Enumerate specific organisms using the methods given below:

- Stomach the samples for 30 seconds to recover the organisms.
- Serially dilute the recovery medium (Maximum recovery diluents - MRD, LABM: LAB103) and plate out 1/0.5ml, in duplicate, using organism appropriate agar.
- Incubate in appropriate conditions, at appropriate temperatures for appropriate times and then record the colony forming units (cfu's) [*C. difficile*: 1ml pour plate on Reinforced Clostridial Agar (RCA, Oxoid CM0151) and incubate anaerobically for 3 days at 37°C; *E. coli* 1 ml pour plate on Violet Red Bile Agar (VRBA, LabM Lab031) and incubate for 24 hours at 37°C; *MRSA* 0.5ml spread plate on Baird Parker agar (BP, Oxoid CM0275) and incubated for 48 hours at 37°C].
- At the same time sterility checks should be undertaken.
- The results express as cfu's per sponge.

8.4 Experimental lay out

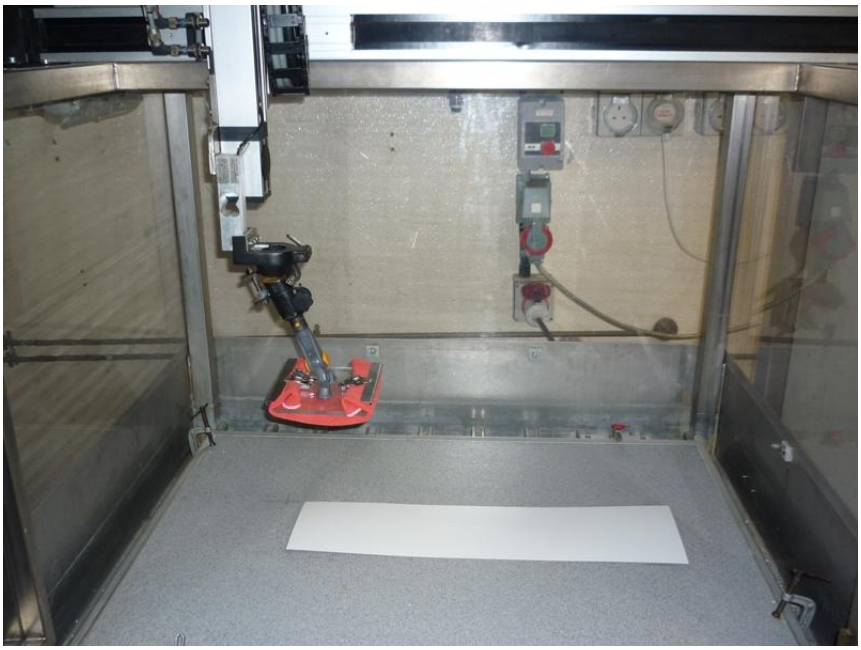

Figure 2: The automated cleaning rig fitted with a micro fibre cloth and placed furniture laminate surface.

Appendix 2

8.5 Statistical data *C.difficile*

8.5.1 One-way ANOVA log reductions for *C.difficile* on cloths washed once:

Results for: All data(Bacteria = *C.difficile*)(No. washes = 1)

One-way ANOVA: Log reductions versus Type of cloth

```
Source          DF      SS      MS     F      P
Type of cloth    1  0.9212  0.9212  9.97  0.013
Error            8  0.7388  0.0924
Total            9  1.6600

S = 0.3039   R-Sq = 55.49%   R-Sq(adj) = 49.93%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean   StDev  --------+---------+---------+---------+-
New    5  2.0579  0.1701                    (--------*--------)
Old    5  1.4508  0.3947  (--------*--------)
                          --------+---------+---------+---------+-
                                1.40      1.75      2.10      2.45

Pooled StDev = 0.3039
```

8.5.2 One-way ANOVA log reductions for *C.difficile* on cloths washed 75 times:

Results for: All data(Bacteria = *C.difficile*)(No. washes = 75)

One-way ANOVA: Log reductions versus Type of cloth

```
Source          DF      SS      MS     F      P
Type of cloth    1  0.0541  0.0541  3.61  0.094
Error            8  0.1198  0.0150
Total            9  0.1738

S = 0.1224   R-Sq = 31.10%   R-Sq(adj) = 22.48%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean   StDev  ------+---------+---------+---------+---
New    5  1.5083  0.1080               (------------*-----------)
Old    5  1.3612  0.1352  (-----------*------------)
                          ------+---------+---------+---------+---
                              1.30      1.40      1.50      1.60

Pooled StDev = 0.1224
```

8.6 Statistical data: *E. coli*

8.6.1 One-way ANOVA log reductions for *E. coli* on cloths washed once:

Results for: All data(Bacteria = *E.Coli*)(No. washes = 1)

One-way ANOVA: Log reductions versus Type of cloth

```
Source          DF     SS     MS     F      P
Type of cloth    1  1.941  1.941  6.34  0.036
Error            8  2.448  0.306
Total            9  4.388

S = 0.5532   R-Sq = 44.22%   R-Sq(adj) = 37.25%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean   StDev  ---+---------+---------+---------+------
New    5  2.4493  0.5634               (---------*--------)
Old    5  1.5683  0.5427  (--------*---------)
                          ---+---------+---------+---------+------
                           1.20      1.80      2.40      3.00

Pooled StDev = 0.5532
```

8.6.2 One-way ANOVA log reductions for *E. coli* on cloths washed 75 times:

Results for: All data(Bacteria = *E.Coli*)(No. washes = 75)

One-way ANOVA: Log reductions versus Type of cloth

```
Source          DF      SS      MS     F      P
Type of cloth    1  0.1151  0.1151  2.29  0.169
Error            8  0.4022  0.0503
Total            9  0.5173

S = 0.2242   R-Sq = 22.25%   R-Sq(adj) = 12.53%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean   StDev   -+---------+---------+---------+--------
New    5  1.6232  0.2186              (----------*-----------)
Old    5  1.4086  0.2297   (----------*-----------)
                           -+---------+---------+---------+--------
                          1.20      1.40      1.60      1.80

Pooled StDev = 0.2242
```

8.7 Statistical data: *MRSA*

8.7.1 One-way ANOVA log reductions for *MRSA* on cloths washed once:

Results for: All data(Bacteria = *MRSA*)(No. washes = 1)

One-way ANOVA: Log reductions versus Type of cloth

```
Source          DF      SS      MS     F      P
Type of cloth    1  0.5987  0.5987  6.04  0.039
Error            8  0.7925  0.0991
Total            9  1.3912

S = 0.3148   R-Sq = 43.03%   R-Sq(adj) = 35.91%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean   StDev  ------+---------+---------+---------+---
New    5  1.8481  0.1526                   (----------*---------)
Old    5  1.3587  0.4182  (----------*----------)
                          ------+---------+---------+---------+---
                              1.20      1.50      1.80      2.10

Pooled StDev = 0.3148
```

8.7.2 One-way ANOVA log reductions for *MRSA* on cloths washed 75 times:

Results for: All data (Bacteria = *MRSA*)(No. washes = 75)

One-way ANOVA: Log reductions versus Type of cloth

```
Source          DF      SS      MS     F      P
Type of cloth    1  0.0578  0.0578  0.76  0.409
Error            8  0.6087  0.0761
Total            9  0.6665

S = 0.2758   R-Sq = 8.67%   R-Sq(adj) = 0.00%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean   StDev   -+---------+---------+---------+--------
New    5  1.4254  0.1991           (-------------*-------------)
Old    5  1.2734  0.3355   (-------------*-------------)
                           -+---------+---------+---------+--------
                          1.00      1.20      1.40      1.60

Pooled StDev = 0.2758
```

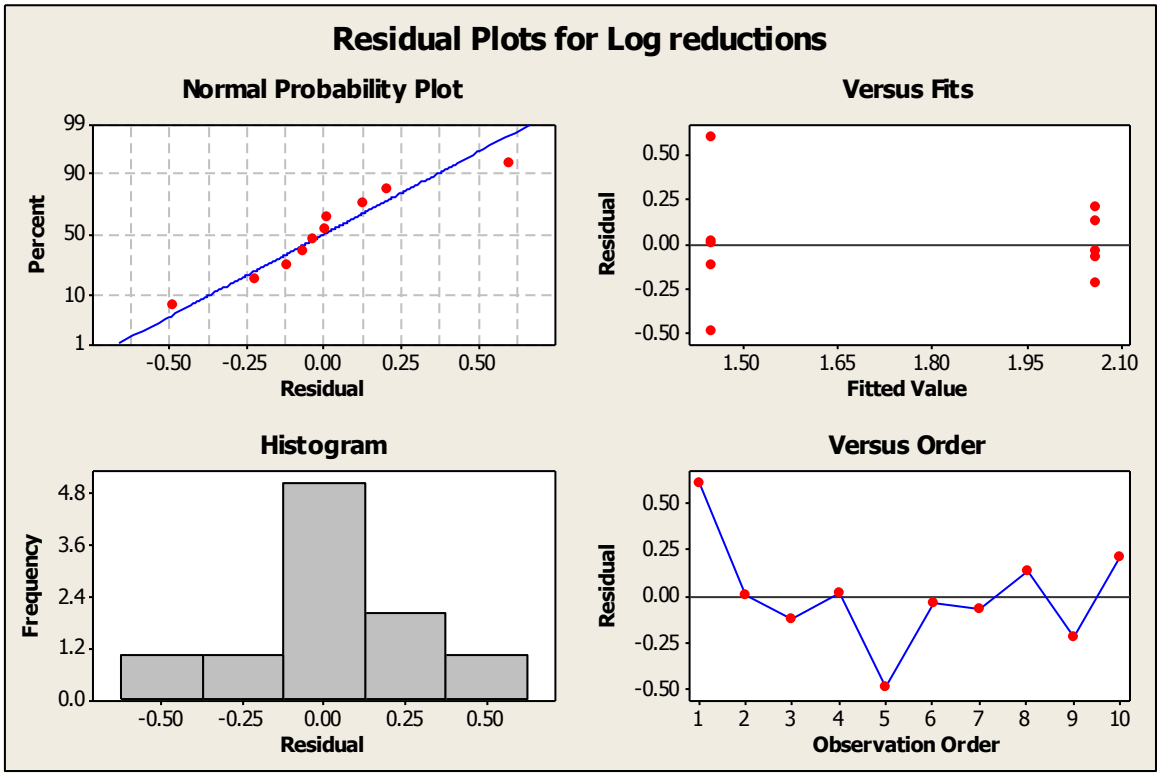

Figure 3: Example of the residual plot for log reduction of *C. difficile* on cloths washed once.