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## INTRODUCTION

The role of the environment in the transmission of important pathogens in the hospital is increasingly recognized. This study uses a unique approach that combines several platforms to assess BOTH the thoroughness AND the effectiveness of room cleaning in a newly constructed and opened hospital – Fort Belvoir Community Hospital (FBCH). The former is accomplished using CDC developed protocols and an invisible dye- ultraviolet light system for sampling 17 high-touch surfaces (i.e. call buttons, bathroom hand rails, IV pumps/poles) before and after terminal room cleaning. This is incorporated into a web-based reporting and feedback system. Effectiveness of cleaning is accomplished using BOTH standard culture- based techniques AND molecular techniques - a real-time, species specific multiplex assay we have developed. Results and feedback are relayed to staff in the form of educational sessions. Post intervention surveillance is then continued.

## BODY

Major achievements to date include the garnering of protocol approval by the IRB, and a specific letter of support from the commander of FBCH, optimization of the molecular assay, use of the BROOM software to collect, barcode and store swab samples, and the collection of pre-opening baseline data, along with having collected 11 months of surveillance data that includes over 2500 samples from over 160 rooms in the new hospital.

Additionally, two sets of species-specific RT-PCR primers were designed for each of six clinically important bacteria, including *Acinetobacter baumannii*, *Clostridium difficile*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and methicillin resistant *Staphylococcus aureus* (MRSA). Primer specificity and sensitivity was assessed using a panel of 42 bacterial species. Primers were subsequently used to detect the presence of bacteria from environmental and clinical swabs taken from both a primary and tertiary-care hospital. All swabs were directly treated with a rapid lysis buffer that facilitated maximum amplification. All twelve primer pairs were capable of detecting 10-50 gene copies with a high degree of specificity from pure bacterial cultures. The procedure was also capable of detecting bacterial contamination of clinical and laboratory work surfaces from pre-moistened swabs, comparable to results obtained from culture-based techniques. Subsequent analysis of the melting curves provided accurate identification of the contaminating species, both from control and test swabs. One manuscript is at press (Plos1) and another has been submitted.

In our original proposal and protocol we intended, and therefore budgeted only for, a nonspecific 16s bacterial DNA indicator. The success of the species specific assay led us to incorporate it into the surveillance. Reagents are significantly more expensive and more than 6x the originally anticipated volume of master mix is required. Additionally, the Dazo and Encompass systems and the 17-surface CDC protocol were not available when the original proposal was submitted in 2008. These enhancements have increased the projected cost of the study. We would also like to continue surveillance as long as possible after opening to reduce threats to validity such as seasonal fluctuations or asymmetric sample sizes- this means for a minimum of 12 months following the opening in October 2011.

## KEY RESEARCH ACCOMPLISHMENTS

- Garnered protocol approval by the IRB, and a specific letter of support from the commander of Fort Belvoir Community Hospital
- Optimized and integrated the molecular assay, with use of the BROOM software to collect, barcode and store swab samples, and with the Dazo and Encompass surveillance and reporting system
- Collected pre-opening baseline data in WRNMMC and FBCH
- Collected 11 months of surveillance data that includes over 2500 samples from over 160 rooms in the new hospital
- Delivered mid-point intervention in the form of feedback of results to the cleaning and infection control teams.
- Developed RT-PCR assay capable of detecting important nosocomial pathogens; (manuscript at press)
- Incorporation of Dazo/Encompass system provided important feedback about the thoroughness of cleaning to hospital staff – at no cost to the hospital. (Most hospitals pay for this because of its usefulness to the cleaning staff)

## REPORTABLE OUTCOMES

All findings and outcomes relating to room cleanliness and contamination must be approved by the WRAIR and FBCH PAO before release or reporting because of their potentially sensitive nature. Some reportable outcomes- if permitted by PAO and OPSEC- will be:

- 1) The rates and trajectory of hospital contamination with multidrug-resistant organisms and *C. difficile*.
- 2) The percentages of surfaces cleaned before and after educational interventions
- 3) How cleaning correlated with rates of MDRO contamination
- 4) How molecular detection assays correlated with culture based assays.
- 5) If any MDRO isolated from the hospital surfaces were later isolated from patients wound or other infections

Potentially reportable findings for which we currently have data include:

- percentage of surfaces cleaned
- percentage of surfaces w/ culture-able bacterial before and after terminal cleaning
- percentage of surfaces w/ molecular evidence of bacteria before and after terminal cleaning
- if and how the above changed following the educational intervention
- concordance of culture results with molecular results
- type, amount, and location of multidrug-resistant organisms found

## CONCLUSION

When finished, this study will have important- possibly landmark implications- for the role of standard of care and state of the art methods for assessing the thoroughness and effectiveness of cleaning in the hospital environment. It will be the first to have combined all three systems plus an educational intervention in a brand new hospital. Currently we are still collecting data in the 11<sup>th</sup> month in what is the planned minimum of at least 12 months of data collection following the hospital opening.

To date, very few MDRO have been isolated from the high-touch surfaces in the hospital. In December, we will stop collecting surveillance samples and begin the first major round of data analysis. At that time, a full report will follow.

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Clifford RJ, Milillo M, Prestwood J, Quintero R, Zurawski DV, Kwak YI, Waterman PE, Lesho EP, McGann P. Species-specific real-time PCR primers for the detection of clinical pathogens and bacterial 16S rRNA. *Plos One*. At press

APPENDICES

1 **Species-specific real-time PCR primers for the detection of clinical pathogens and bacterial *I6S***

2 **rRNA**

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11 Running Title: Detection of clinical bacteria by real-time PCR

12

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21

22 **Abstract**

23 Within the paradigm of clinical infectious disease research, *Acinetobacter baumannii*, *Escherichia coli*,  
24 *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* represent the four most clinically relevant, and  
25 hence most extensively studied bacteria. Current culture-based methods for identifying these organisms  
26 are slow and cumbersome, and there is increasing need for more rapid and accurate molecular detection  
27 methods.

28 Using bioinformatic tools, 962,279 bacterial *16S* rRNA gene sequences were aligned, and regions of  
29 homology were selected to generate a set of real-time PCR primers that target 93.6% of all bacterial *16S*  
30 rRNA sequences published to date. A set of four species-specific real-time PCR primer pairs were also  
31 designed, capable of detecting less than 100 genome copies of *A. baumannii*, *E. coli*, *K. pneumoniae*,  
32 and *P. aeruginosa*. All primers were tested for specificity *in vitro* against 50 species of Gram-positive  
33 and –negative bacteria. Additionally, the species-specific primers were tested against a panel of 200  
34 clinical isolates of each species, randomly selected from a large repository of clinical isolates from  
35 diverse areas and sources.

36 A comparison of culture and real-time PCR demonstrated 100% concordance. The primers were  
37 incorporated into a rapid assay capable of positive identification from plate or broth cultures in less than  
38 90 minutes. Furthermore, our data demonstrate that current targets, such as the *uidA* gene in *E.coli*, are  
39 not suitable as species-specific genes due to sequence variation. The assay described herein is rapid,  
40 cost-effective and accurate, and can be easily incorporated into any research laboratory capable of real-  
41 time PCR.

42



43 **Introduction**

44 In clinical microbiology laboratories, traditional culture based techniques remain the primary  
45 methodology used for identifying bacterial isolates. These methods are time-consuming, can involve  
46 multiple biochemical tests, and can be expensive, particularly for fastidious organisms. In recent years,  
47 the introduction of automated identification instruments has resulted in greater reliability, but  
48 discrepancies between the various platforms have been reported [1-3]. Furthermore, many research  
49 laboratories lack the funds necessary to invest in these instruments, and often rely on minimal  
50 biochemical characterization or *16S* rRNA sequencing to confirm species type.

51 Despite significant advances in molecular biology, molecular methods for species identification have  
52 not achieved widespread use, with the possible exception of the Biodefense community [4]. Factors  
53 such as allelic variation within target genes, cross-reaction with other species [5] and the lack of  
54 experienced molecular technicians have all contributed to their relative scarcity. However, despite these  
55 limitations, molecular methods can provide significant advantages over phenotypic-based methods,  
56 including rapid turnaround time, scalability, and high sensitivity [5]. Furthermore, using appropriate  
57 bioinformatic tools and careful primer design, many of the limitations outlined above can be mitigated  
58 considerably.

59 Recent global calls [6-8] to help combat the spread of carbapenem-resistant *Enterobacteriaceae* (CRE),  
60 multi-drug resistant *A. baumannii* and *P.aeruginosa*, and methicillin-resistant *Staphylococcus aureus*  
61 (MRSA) has resulted in a plethora of research focused on these pathogens. A number of conventional  
62 PCR and real-time PCR assays have been developed targeting these organisms [9-13], but a  
63 comprehensive panel encompassing all four species is lacking.

64 We recently published a highly sensitive and specific real-time PCR assay for the detection of MRSA  
65 [14]. This report adds an additional four real-time PCR primers, designed using a combination of *in*

66 silico and *in vitro* methodologies that are highly specific to the four most common nosocomial  
67 pathogens. We also describe a novel bacterial *16S* rRNA real-time PCR primer designed from the  
68 alignment of 962,279 bacterial *16S* rRNA gene sequences, which will amplify a product from the *16S*  
69 rRNA gene in 93.6% of all bacterial *16S* rRNA genes published to date.

70 **Material and Methods**

71 **Primer Design**

72 A complete list of the final primer panel and their primary characteristics is presented in Table 1. Unless  
73 noted otherwise, all primers were designed using Primer Express version 2.0 (Applied Biosystems,  
74 Carlsbad, CA). All primers were designed to have an optimal annealing temperature of 56°C, and real-  
75 time PCR reactions were performed in 20 µl volumes with 200 nM primers. Where appropriate,  
76 alignment of individual gene sequences was performed using MegAlign version 10.0.1 (DNASar Inc,  
77 Madison, WI).

78 **16S real-time PCR primers**

79 *16S* rRNA real-time PCR primers were designed manually from a consensus sequence based on an  
80 alignment of 962,279 bacterial *16S* rRNA gene sequences obtained from the Ribosomal Database  
81 Project release 10 [15]. To derive the consensus sequence, nucleotide frequencies were determined at  
82 each position in the alignment using a custom Perl script; positions where the majority of sequences had  
83 a gap were excluded. Regions of the consensus sequence where the majority nucleotide was present in  
84 >90% of the sequences were used for primer design. A synopsis of each nucleotide frequency is  
85 presented as supplemental material (Supplemental Table S1).

86 ***E. coli* and *A. baumannii* – specific primers.**

87 To identify genes specific to *E. coli* and *A. baumannii*, annotated complete genome sequences for 38 *E.*  
88 *coli* strains, and 8 completed and 52 draft *A. baumannii* genomes were selected (Supplemental Table S2  
89 and S3). In addition, the two draft genomes of *A. nosocomialis* (RUH 2624 and NCTC 8102) and the  
90 four draft genomes of *A. pittii* (SH024, D499, DSM 21653, and DSM 9306) were used to ensure that all  
91 *Acinetobacter*-specific primers would also amplify targets from these two species also. For each protein  
92 in a strain, BLASTP [16] was used to find the best match to a protein family in the HOGENOM release

93 5 database [17]; orthologous proteins from different strains show the best match to the same  
94 HOGENOM protein family. Genes that are not single copy (that is, two or more proteins from the same  
95 strain are members of the same HOGENOM protein family) were excluded from further analysis. In  
96 addition genes for which the nucleotide length of any ortholog varies from the mean gene length by  
97 more than 5% were excluded in order to restrict the analysis to those genes that were highly conserved.  
98 For the genes that met these criteria and had the fewest variable positions, BLASTN analysis was  
99 performed against the NCBI non-redundant nucleotide database using a gene sequence randomly  
100 selected from the final species-specific gene targets.

101 Four genes showed no significant match to non-*Escherichia* species: HBG518163 (*hdeA*), HBG636731  
102 (Predicted secreted protein, function unknown, *ynfB*), HBG473849 (conserved protein, function  
103 unknown, *yccT*), and HBG758393 (Function unknown, DUF1418 family, *ybjC*). Nineteen genes showed  
104 no significant match to non-*Acinetobacter* species (Supplemental Table S4), and the top three  
105 candidates, based on total gene length and number of mismatches between all genomes; HBG594899  
106 (*rpiN*, 50S ribosomal protein L14), HBG701403 (*secE*, preprotein translocase, SecE subunit), and  
107 HBG752450 (*scpB*, segregation and condensation protein B) were selected for further analysis.  
108 Starting with a multiple sequence alignment, a custom Perl script was used to identify positions within  
109 each gene that are invariant among all strains. With *E. coli*, two of the four genes (*hdeA* and *yccA*)  
110 contained regions > 100bp that were suitable for primer design. All three genes from the *Acinetobacter*  
111 analysis contained extensively conserved regions suitable for primer design.

#### 112 ***K. pneumoniae* and *P. aeruginosa* – specific primers**

113 The limited number of published genome sequences available for *K. pneumoniae* and *P. aeruginosa*  
114 precluded extensive bioinformatic analysis. Therefore, the top two candidate genes from both species

115 (*K. pneumoniae*; *gltA* and *khe*; *P. aeruginosa*; *ecfX* and 23S) were selected based on conserved gene  
116 sequences.

### 117 **Validation of real-time PCR primers**

118 All primer sets were tested for sensitivity, optimal annealing temperature and primer efficiency as  
119 previously described [18,19]. Primers were tested using two different instruments, the Roche Light  
120 Cycler 480 II (LC 480 II) with SYBR Green I Master Mix (Roche Applied Sciences, Indianapolis, IN),  
121 and the BioRad CFX96 with SsoAdvanced SYBR Green supermix (Bio-Rad Laboratories, Hercules,  
122 CA). Each primer was tested for specificity by two methods. First, the primers were tested against  
123 genomic DNA extracted from a panel of American Type Culture Collection (ATCC, Manassas, VA) and  
124 clinical isolates representing fifty different bacterial species, including closely related members from the  
125 same genus (Supplemental Table S5). Secondly, primers were tested against 200 clinical isolates of each  
126 species, identified to the species level using three automated identification systems; the Vitek 2  
127 (bioMerieux, Durham, NC ), the BD Pheonix (Diagnostics Systems, Sparks, MD), and the Microscan  
128 Walkway (Siemens Healthcare Diagnostics Inc, Deerfield, IL), selected from a large repository of  
129 isolates (>10,000 strains) collected between 2002 to 2012 from 23 different facilities in the United  
130 States of America, Europe, Asia and the Middle East. Pulsed-field Gel Electrophoresis (PFGE) indicated  
131 that the selected clinical isolates represented a wide variety of different pulse-types (Unpublished  
132 results).

### 133 **High throughput testing of clinical isolates**

134 The primers were incorporated into a 96-well plate assay to allow high throughput testing of multiple  
135 clinical isolates. Single colonies of an overnight culture of each isolate were re-suspended in 200 µl of  
136 sterile, ultra-pure water and mixed by vortexing. 10 µl of the resulting suspension (or 10 µl taken  
137 directly from an overnight broth culture) was added to 20 µl of Lyse-and-go reagent (Thermo Scientific,

138 Waltham, MA) in 96-well plates, and run in a thermal cycler using the manufacturer's protocol for the  
139 isolation of total genomic DNA. Isolates were held at 80°C for 15 minutes at the end of the program to  
140 maximize lysis, and 2 µl of the resulting lysate was used directly for real-time PCR. Leftover bacterial  
141 DNA in lyse-and-go reagent was stored at -20°C, and no reduction in real-time PCR amplification was  
142 evidenced after 9 months (Data not shown). Appropriate positive (ATCC Type strains for each species),  
143 negative (Two ATCC type strains from species other than the target organism), and no template controls  
144 (Ultra-pure water) were incorporated onto every plate. Cycling parameters were 95°C for 5 minutes,  
145 followed by 40 cycles of 95°C for 10 seconds and 56°C for 10 seconds. A melting curve analysis was  
146 included at the end of every program to assist in data analysis. Quantification cycle (Cq; CFX96) and  
147 crossing threshold (Ct; LC 480 II) values were calculated automatically using instrument software.

148

149 **Results and Discussion**

150 ***16S* rRNA real-time PCR primers**

151 Analysis of the alignment of 962,279 bacterial *16S* rRNA gene sequences revealed considerable  
152 nucleotide variation between species (Supplemental Table S1). However, two regions were identified  
153 that were suitable for primer design, where the nucleotide sequence was highly conserved in >90% of all  
154 rRNA sequences. Forward and Reverse primers were manually designed, with the nucleotide sequence  
155 of the forward primer being conserved in 98.5% of *16S* rRNA sequences, and the nucleotide sequence  
156 in the reverse primer being conserved in 93.8% (Supplemental Table S1).

157 Primers were tested against serial dilutions of genomic DNA from *A. baumannii* ATCC 19606, *E. coli*  
158 ATCC 35218, and *P. aeruginosa* ATCC 27853. The primers were reproducibly capable of detecting  
159 <100 copies of genomic DNA, and primer efficiency was 96.4%, 98.2% and 97.9% respectively, with an  
160  $R^2 >99\%$ . The primers successfully amplified a product from the 50 ATCC and clinical strains  
161 (Supplemental Table S5), providing *in vitro* support for the *in silico* primer design method employed.

162 The *16S* rRNA gene is a frequent target for many assays, and universal PCR primers are routinely used  
163 for species identification [20]. A number of real-time primers for this gene have also been developed,  
164 but they either contain degenerate nucleotides [21], or produce long amplicons that are not suitable for  
165 optimal real-time PCR [22]. The primer set described here overcomes both these limitations, and has  
166 proved a valuable tool in detecting bacterial contamination of environmental swabs (manuscript in  
167 preparation), and as a positive control for bacterial lysis.

168 **Species-specific real-time PCR primers**

169 Five sets of primers were tested for sensitivity and specificity for *A. baumannii*; three primers were  
170 generated using the genome alignment algorithm (*rpiN*, *secE* and *scpB*), one primer was designed in-  
171 house from an alignment of 54 *ompA* sequences, and the fifth primer, also targeting the *ompA* gene, has

172 been published previously [11]. *OmpA* was included as a potential target as this gene has been used as a  
173 target for *A. baumannii*-specific primers in previous publications [11]. However, though *ompA* was  
174 identified as a potential target using our algorithm, sequence variation among *A. baumannii*, *A.*  
175 *nosocomialis* and *A. pitii*, as well as variation in the protein length, resulted in this gene being flagged as  
176 unsuitable as an *Acinetobacter*-specific target. All five primers did not cross-react with other bacterial  
177 species, including *A. lwoffii* and *A. hemolyticus*. Primer efficiency at 56°C, based on serial dilutions of *A.*  
178 *baumannii* AB0057 and *A. baumannii* ACICU, ranged from 97.2% (*rpiN*) to 99.4% (*secE*), with R<sup>2</sup>  
179 >99%, and all primers were reproducibly capable of detecting < 100 genome copies of DNA.  
180 Furthermore, all five primers successfully amplified products from the 200 clinical test isolates. The  
181 primer pair targeting the *secE* gene consistently provided greater sensitivity than all other primers at an  
182 annealing temperature of 56°C, and was therefore selected as the optimal candidate for identifying *A.*  
183 *baumannii*. *In silico* analysis of the *secE* primer with the draft *A. nosocomialis* and *A. pitii* genomes  
184 demonstrated that these primers would also amplify a product from these two species.  
185 Four sets of primers were tested for sensitivity and specificity for *E.coli*; two primers were generated  
186 using the genome alignment algorithm (*hdeA* and *yccT*), one primer was designed from an alignment of  
187 38 *uidA* genes (Supplemental Table S2), and a fourth primer, also targeting the *uidA* gene, has been  
188 described previously [7,18]. *UidA* was included as this gene has been used as a species-specific target in  
189 a number of previous publications [10,12,23-25], but it did not meet the criteria demanded by the  
190 species-specific algorithm in this study. This was confirmed by both *in silico* and *in vitro* testing. An  
191 alignment of 38 *E.coli* genomes indicated that published *uidA* primer sequences [10,12] have 3  
192 nucleotide variations in the forward primer and 1 nucleotide variation in the reverse primer when  
193 aligned with some *E.coli uidA* gene sequences. When tested against the 200 clinical isolates, the primers  
194 failed to amplify any product from 8 strains (Figure 1a). A second set of *uidA* primers was designed,



195 targeting a conserved region of the 38 *uidA* gene sequences. However, no amplification was again  
196 observed from 7 of the same 8 strains that were *uidA* negative from the first primer pair. One of these 7  
197 isolates amplified a product after 28 cycles, indicative of inefficient primer annealing, most likely due to  
198 primer/target mismatches within the *uidA* gene.

199 The final two primers, targeting the *yccT* and *hdeA* genes, were identified as the most promising  
200 candidates based on the algorithm used in this study. Primer efficiency was 98.1% and 97.4%  
201 respectively, with  $R^2 > 99\%$ . However, 9 of the 200 clinical isolates failed to amplify a product with the  
202 *hdeA* primer, including 3 of the 8 *uidA*-negative isolates. In contrast, the *yccT* primer successfully  
203 amplified a product from all 200 isolates, and was therefore selected for as the final *E. coli*-specific  
204 primer set (Figure 1b). As expected, due to the very high homology between *E. coli* and *Shigella* strains  
205 [26], the *yccT* primer set also amplified a product from *Shigella flexneri* ATCC 12022, but did not  
206 cross-react with any other species.

207 Despite 38 sequenced genomes of *E. coli*, developing an *E. coli*-specific primer pair is challenging. We  
208 have shown that current targets, such as the *uidA* gene are not suitable due to considerably allelic  
209 variation, particularly among *E. coli* clinical isolates from diverse regions. The remarkable diversity  
210 among *E. coli* strains is also highlighted in the bioinformatic approach that we used, where just 2  
211 candidate genes passed all of the criteria employed, and just a single gene, *yccT*, was eventually  
212 successful *in vitro*.

213 The paucity of completed genomic sequences for both *K. pneumoniae* and *P. aeruginosa* limited the  
214 application of our algorithm to just a single genome for *P. aeruginosa* with an additional 4 whole  
215 genome shotgun sequences, and 5 genomes for *K. pneumoniae*.

216 BLAST analysis suggested a potential cross-reaction between the *K. pneumoniae* *khe* primer set and  
217 *Citrobacter freundii*, and this was confirmed *in vitro*. However, the *gltA* primer set demonstrated no

218 cross-reactivity with other species, a primer efficiency of 97.1%, and successfully amplified a product  
219 from all 200 clinical isolates of *K. pneumoniae*.

220 Both sets of *P. aeruginosa* primers demonstrated no cross-reactivity with other species, including *P.*  
221 *fluorescens*, *P. stutzeri* and *P. putida*. However, when tested against a panel of 200 clinical isolates of *P.*  
222 *aeruginosa* only the *ecfX* primer successfully amplified a product from all strains, with 12 strains failing  
223 to generate a product with the 23S rRNA primer set. The *ecfX* primer set demonstrated a primer  
224 efficiency of 93.8% with an R<sup>2</sup> value of 99.1%.

225 Species-specific primers targeting the *ecfX* gene in *P. aeruginosa* have been published for both  
226 conventional [27,28] and real-time PCR assays [9,29]. Our results support the continued use of this gene  
227 as a target for *P. aeruginosa*-specific primers, at least until further full genome sequences of this strain  
228 become available. In contrast, species-specific primers for *K. pneumoniae* are less well described, and  
229 primarily involve detecting targets that are specific to certain strains, such as the ST258 clone [30], or  
230 those with the hypermucoviscosity phenotype [31]. In addition, a number of conventional PCR assays to  
231 detect *K. pneumoniae* have been described [13,32], though to our knowledge, no assay described to date  
232 uses *gltA* as the target gene.

## 233 **Conclusion**

234 We describe a set of real-time PCR primers, designed to have the same optimal annealing temperature,  
235 and displaying high specificity for four clinically important pathogens. The primers are well suited for  
236 high-throughput testing of isolates, with results available in less than 90 minutes from bacterial colonies  
237 or overnight broth cultures. We also demonstrate the power of bioinformatics in designing optimal  
238 primer sequences, and provide a novel 16S rRNA real-time PCR primer designed from the alignment of

239 over 960,000 bacterial *16S* rRNA sequences. The primers described herein have been an invaluable  
240 addition to our surveillance network, and have demonstrated a 100% concordance with traditional  
241 phenotypic identification systems.

242

**Table 1 Primer characteristics**

Name <sup>1</sup>	Gene	Target species	Sequence (5' to 3')	Eff (%) <sup>3</sup>	Length <sup>4</sup>
U16SRT-F	16S	variable	ACTCCTACGGGAGGCAGCAGT	>96.4%	140
U16SRT-F			TATTACCGCGGCTGCTGGC		
secERT-F		<i>A. baumannii</i>	GTTGTGGCTTTAGGTTTATTATACG		
secERT-R	secE	<i>A. nosocomialis</i>	AAGTTACTCGACGCAATTCG	99.4	94
secERT-Probe		<i>A. pitii</i>	ACCCATCAAGGTAAAGGCTTCGTTTCG		
yccTRT-F		<i>Escherichia coli</i>	GCATCGTGACCACCTTGA		
yccTRT-R	yccT	<i>Shigella</i> spp.	CAGCGTGGTGGCAAAA	98.1	59
yccTRT-Probe			TGCATTATGTTTGCCGGTATCCG		
gltART-F	gltA	<i>K. pneumoniae</i>	ACGGCCGAATATGACGAATTC	97.1	68
gltART-R			AGAGTGATCTGCTCATGAA		
ecfXRT-F	ecfX	<i>P. aeruginosa</i>	AGCGTTCGTCCTGCACAAGT	93.8	81
ecfXRT-R			TCCACCATGCTCAGGGAGAT		

244 <sup>1</sup> Probe sequences were generated for the *secE* and *yccT* real-time PCR primers, but no *in vitro* testing was  
 245 performed. The probe sequences are provided here for the benefit of researchers wishing to perform multiplex  
 246 real-time PCR reactions using these primers. All primers have an optimal annealing temperature of 56°C.

247 <sup>2</sup> Efficiency was calculated from the slope and intercept of the trendline produced following amplification  
 248 with serial dilutions of genomic DNA from the ATCC strains of each species, as described previously [14].

249 <sup>3</sup> Amplicon size in base pairs

250 **FIGURE LEGENDS**

251 **Figure 1. (A)** Amplification curves of four *E. coli* isolates (MRSN 1628 – Red line, MRSN 1681 – Blue  
252 Line, MRSN 7544 – Green Line, and MRSN 7851 – Yellow Line) with *uidA* and *yccT* primers. MRSN  
253 1628, 1681, and 7544 were negative for *uidA* but positive for *yccT*. MRSN 7851 was positive for both  
254 *uidA* and *yccT*. *E.coli* ATCC 35218 (Black line) was used as a positive control and *K. pneumoniae*  
255 ATCC 1706 (Grey Line) was used a negative control. Amplification curve is one sample from  
256 quintuplicate experiments. **(B)** Melting curve analysis of the amplicons produced by the *ycc* primer pair  
257 from 30 clinical isolates of *E. coli* demonstrating a highly conserved sequence in all strains. Melting  
258 curve of the *yccT* amplicon from *E. coli* ATCC 35218 is shown in red. All 30 isolates represented  
259 diverse pulse-types as determined by PFGE.

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SUPPORTING DATA

BELVOIR Bacteria

Bacteria	SampleGroup	RoomNumber	Surface
<i>Shigella flexneri</i>	Pediatric Ward	06.248	COMPOSITE 2
<i>Pantoea agglomerans</i>	Surgical Ward	06.342	Call box
<i>Leclercia adecarboxylata</i>	Surgical Ward	06.342	Bedside table
<i>Pseudomonas putida</i>	Surgical Ward	06.342	Bedside table
<i>Pseudomonas luteola</i>	Surgical Ward	06.342	COMPOSITE 1
<i>Escherichia vulneris</i>	Pediatric Ward	06.244	COMPOSITE 2
<i>Pseudomonas putida</i>	Pediatric Ward	06.244	COMPOSITE 2
<i>Acinetobacter lwoffii</i>	Pediatric Ward	06.244	COMPOSITE 1
<i>Klebsiella pneumoniae ssp ozaenae</i>	Pediatric Ward	06.244	COMPOSITE 1
<i>Pantoea agglomerans</i>	Pediatric Ward	06.244	Telephone
<i>Pantoea agglomerans</i>	Pediatric Ward	06.244	Toilet seat
<i>Pantoea agglomerans</i>	Pediatric Ward	06.244	Sink top
<i>Shigella flexneri</i>	Pediatric Ward	06.244	Sink top
<i>Leclercia adecarboxylata</i>	Pediatric Ward	06.244	Bedside table
<i>Tatumella ptyseos</i>	Pediatric Ward	06.248	Bathroom door closer
<i>Enterobacter sakazakii</i>	Pediatric Ward	06.248	COMPOSITE 2
<i>Pantoea agglomerans</i>	Medical Telemetry	07.334	Call box
<i>Enterobacter sakzakii</i>	Medical Telemetry	07.334	COMPOSITE 1
<i>Pantoea agglomerans</i>	Medical Telemetry	07.334	COMPOSITE 2
<i>Pantoea agglomerans</i>	Medical Telemetry	07.334	Sink top
<i>Pseudomonas putida</i>	Medical Telemetry	07.334	Sink top
<i>Pantoea agglomerans</i>	Medical Telemetry	07.334	Bathroom door closer
<i>Pantoea agglomerans</i>	Medical Telemetry	07.334	Telephone
<i>Pantoea agglomerans</i>	Medical Telemetry	07.334	Tray table
CDC group EO-2	Medical Telemetry	07.304	Room door closer
<i>Enterobacter cloacae</i>	Medical Telemetry	07.304	Sink top

<b>Bacteria</b>	<b>SampleGroup</b>	<b>RoomNumber</b>	<b>Surface</b>
<i>Pseudomonas putida</i>	Medical Telemetry	07.304	PT chair
<i>Pantoea agglomerans</i>	Medical Telemetry	07.328	Toilet rail
<i>Shigella flexneri</i>	Medical Telemetry	07.328	Toilet seat
<i>Pantoea agglomerans</i>	Medical Telemetry	07.328	Telephone
<i>Pantoea agglomerans</i>	Medical Telemetry	07.328	PT chair
<i>Enterobacter sakazakii</i>	Medical Telemetry	07.328	COMPOSITE 1
<i>Pseudomonas oryzihabitans</i>	Medical Telemetry	07.328	Bedpan cleaner
<i>Shigella flexneri</i>	Pediatric Ward	06.248	COMPOSITE 1
<i>Rhizobium radiobacter</i>	Medical Telemetry	07.334	Sink top
<i>Staphylococcus saprophyticus</i>	Medical Telemetry	07.334	Toilet rail
<i>Enterobacter cloacae</i>	Maternity Ward	05.312	COMPOSITE 1
<i>Citrobacter koseri</i>	Maternity Ward	05.312	Room door closer
<i>Enterobacter cloacae</i>	Maternity Ward	05.312	Sink top
<i>Micrococcus luteus</i>	Maternity Ward	05.312	Telephone
<i>Pantoea agglomerans</i>	Maternity Ward	05.312	Toilet seat
<i>Enterobacter cloacae</i>	Maternity Ward	05.312	COMPOSITE 2
<i>Stenotrophomonas maltophilia</i>	Maternity Ward	05.312	COMPOSITE 2
<i>Pseudomonas putida</i>	Maternity Ward	05.312	Bedside table
<i>Stenotrophomonas maltophilia</i>	Pediatric Ward	06.244	Sink top
<i>Pantoea agglomerans</i>	Pediatric Ward	06.244	COMPOSITE 2
<i>Acinetobacter lwoffii/haemolyticus</i>	ICU	02.122	Call box
<i>Staphylococcus saprophyticus</i>	ICU	02.123	COMPOSITE 2
<i>Pantoea agglomerans</i>	ICU	02.123	Bedside table
<i>Staphylococcus saprophyticus</i>	ICU	02.123	Tray table
<i>Staphylococcus saprophyticus</i>	Surgical Ward	06.342	PT chair
<i>Pseudomonas putida</i>	Surgical Ward	06.342	Sink top
<i>Aerococcus viridans</i>	Surgical Ward	06.342	Call box
<i>Rhizobium radiobacter</i>	Surgical Ward	06.342	Bedside table
<i>Staphylococcus warneri</i>	Surgical Ward	06.342	Telephone
<i>Staphylococcus saprophyticus</i>	Surgical Ward	06.342	COMPOSITE 2
<i>Aerococcus viridans</i>	Surgical Ward	06.342	Tray table

<b>Bacteria</b>	<b>SampleGroup</b>	<b>RoomNumber</b>	<b>Surface</b>
<i>Pseudomonas putida</i>	Surgical Ward	06.342	COMPOSITE 1
<i>Enterobacter cloacae</i>	Maternity Ward	05.312	COMPOSITE 1
<i>Pseudomonas putida</i>	Maternity Ward	05.312	COMPOSITE 1
<i>Pseudomonas putida</i>	Maternity Ward	05.312	Sink top
<i>Pseudomonas putida</i>	Maternity Ward	05.312	PT chair
<i>Pseudomonas putida</i>	Maternity Ward	05.312	Tray table
<i>Acinetobacter lwoffii</i>	Maternity Ward	05.312	Bedside table
<i>Acinetobacter lwoffii/haemolyticus</i>	Maternity Ward	05.312	Telephone
<i>Escherichia coli</i>	Maternity Ward	05.312	Toilet seat
<i>Enterobacter cloacae</i>	Maternity Ward	05.312	COMPOSITE 2
<i>Pseudomonas putida</i>	Maternity Ward	05.312	Toilet handle
<i>Moraxella species</i>	Surgical Ward	06.338	Sink top
<i>Pseudomonas putida</i>	Surgical Ward	06.338	Telephone
<i>Delftia acidovorans</i>	Medical Telemetry	07.328	COMPOSITE 1
<i>Staphylococcus saprophyticus</i>	Medical Telemetry	07.328	Toilet rail
<i>Escherichia coli</i>	ICU	02.122	Toilet seat
<i>Acinetobacter baumannii/calcoaceticus complex</i>	ICU	02.122	Call box
<i>Acinetobacter lwoffii</i>	ICU	02.122	Tray table
<i>Citrobacter freundii</i>	Surgical Ward	06.338	Toilet rail
<i>Klebsiella pneumoniae ssp ozaenae</i>	Surgical Ward	06.338	COMPOSITE 1
<i>Pantoea agglomerans</i>	Medical Telemetry	07.304	COMPOSITE 2
<i>Pantoea agglomerans</i>	Medical Telemetry	07.304	Sink top
<i>Pantoea agglomerans</i>	Medical Telemetry	07.304	COMPOSITE 1
<i>Staphylococcus saprophyticus</i>	Medical Telemetry	07.304	Toilet seat
<i>Acinetobacter baumannii/calcoaceticus complex</i>	Medical Telemetry	07.342	Toilet handle
<i>Acinetobacter baumannii/calcoaceticus complex</i>	Medical Telemetry	07.342	Sink top
<i>Acinetobacter baumannii/calcoaceticus complex</i>	Medical Telemetry	07.342	Toilet seat
<i>Acinetobacter baumannii</i>	Medical Telemetry	07.342	Call box
<i>Acinetobacter baumannii/calcoaceticus complex</i>	Medical Telemetry	07.342	Toilet rail

<b>Bacteria</b>	<b>SampleGroup</b>	<b>RoomNumber</b>	<b>Surface</b>
Acinetobacter baumannii	Medical Telemetry	07.342	Bathroom door closer
Acinetobacter baumannii/calcoaceticus complex	Medical Telemetry	07.342	Telephone
Acinetobacter baumannii	Medical Telemetry	07.342	Room Sink
CDC Group EF-4b	Medical Telemetry	07.342	Chair
Escherichia coli	Medical Ward	07.244	Toilet seat
Acinetobacter lwoffii/haemolyticus	Surgical Ward	06.316	Tray table
Acinetobacter baumannii	Surgical Ward	06.316	Room Sink
Acinetobacter lwoffii/haemolyticus	Surgical Ward	06.316	Bedpan cleaner
Serratia marcescens	Maternity Ward	05.322	Sink top
Acinetobacter lwoffii	Maternity Ward	05.322	Bedside table
Ochrobactrum anthropi	Maternity Ward	05.322	Chair
Acinetobacter baumannii/cacoaceticus complex	Surgical Ward	06.316	Toilet rail
Acinetobacter baumannii	Surgical Ward	06.316	IV Pole
Pseudomonas putida	Surgical Ward	06.316	Bedside table
Pseudomonas luteola	Surgical Ward	06.316	Chair
Acinetobacter baumannii/cacoaceticus complex	Surgical Ward	06.316	Tray table
Acinetobacter baumannii/calcoaceticus complex	Surgical Ward	06.316	Room Sink
Acinetobacter species	Surgical Ward	06.316	Room Sink
Staphylococcus schleiferi ssp coagulans	Surgical Ward	06.316	Room Sink
Acinetobacter baumannii	Surgical Ward	06.316	Toilet seat
Acinetobacter baumannii	Surgical Ward	06.316	Call box
Acinetobacter baumannii/calcoaceticus complex	Surgical Ward	06.316	Sink top
Micrococcus luteus	Medical Ward	07.216	Sink top
Micrococcus luteus	Medical Ward	07.216	Room Sink
Staphylococcus sciuri	ICU	02.123	Toilet seat
Staphylococcus sciuri	ICU	02.123	Toilet rail
Aerococcus viridans	ICU	02.123	Toilet handle
Staphylococcus sciuri	ICU	02.123	Toilet handle
Enterococcus casseliflavus/gallinarum	ICU	02.123	Bedside table
Micrococcus luteus	ICU	02.123	Telephone

Bacteria	SampleGroup	RoomNumber	Surface
Staphylococcus saprophyticus	ICU	02.123	Chair
Aerococcus viridans	ICU	02.123	Tray table
Pantoea agglomerans	ICU	02.123	Room Sink
Micrococcus luteus	ICU	02.123	Room door closer
Citrobacter koseri	ICU	02.123	Sink top
Klebsiella pneumoniae ssp pneumoniae	ICU	02.123	Sink top
Moraxella species	Medical Ward	07.242	Sink top
Klebsiella pneumoniae ssp pneumoniae	Medical Ward	07.242	Chair
Acinetobacter lwoffii/haemolyticus	Surgical Ward	06.312	Toilet rail
Micrococcus lylae	Surgical Ward	06.312	IV Pole
Pseudomonas aeruginosa	Maternity Ward	05.334	Sink top
Stenotrophomonas maltophilia	Maternity Ward	05.334	Chair
Escherichia coli	Maternity Ward	05.334	Toilet seat
Pantoea agglomerans	Maternity Ward	05.334	Toilet seat
Micrococcus luteus	Maternity Ward	05.334	Room door closer
Pantoea agglomerans	Medical Telemetry	07.336	Room Sink
Shigella dysenteriae	Medical Telemetry	07.336	Room Sink
Micrococcus luteus	Medical Telemetry	07.336	Call box
Micrococcus luteus	Medical Telemetry	07.336	Toilet seat
Micrococcus luteus	Medical Telemetry	07.336	Bathroom door closer
Acinetobacter lwoffii	Medical Telemetry	07.336	Tray table
Pantoea agglomerans	Medical Telemetry	07.336	Tray table
Leclercia adecarboxylata	Medical Telemetry	07.336	Chair
Sphingomonas paucimobilis	Medical Telemetry	07.336	Room Sink
Micrococcus luteus	Medical Ward	07.216	Room door closer
Micrococcus luteus	ICU	02.123	Toilet seat
Pseudomonas putida	ICU	02.123	Room Sink

<b>Bacteria</b>	<b>SampleGroup</b>	<b>RoomNumber</b>	<b>Surface</b>
Acinetobacter lwoffii	Maternity Ward	05.334	Toilet rail
Pseudomonas putida	Maternity Ward	05.334	Sink top
Pantoea agglomerans	Surgical Ward	06.312	Bathroom door closer
Pantoea agglomerans	Surgical Ward	06.312	Chair
Micrococcus luteus	Surgical Ward	06.312	Bedside table
Staphylococcus haemolyticus	ICU	02.114	Sink top
Staphylococcus hominis	ICU	02.114	Chair
Staphylococcus epidermis	ICU	02.114	Room Lightswitch
Staphylococcus hominis	ICU	02.114	Toilet handle
Staphylococcus hominis	ICU	02.114	Bathroom door closer
Enterococcus faecium	ICU	02.116	Toilet handle
Enterococcus faecium	ICU	02.116	Toilet handle
Staphylococcus hominis	ICU	02.116	Call box
Staphylococcus epidermis	ICU	02.116	Bedside table
Staphylococcus capitis	ICU	02.114	Tray Table
Staphylococcus haemolyticus	ICU	02.114	Chair
Staphylococcus haemolyticus	ICU	02.116	Bedside table
Staphylococcus aureus	Pediatric Ward	06.218	Side rail
Staphylococcus epidermis	Pediatric Ward	06.218	Bedside table
Staphylococcus epidermis	Pediatric Ward	06.218	Room Chair
Staphylococcus hominis	Pediatric Ward	06.218	Room Sink
Staphylococcus simulans	Pediatric Ward	06.218	Sink top
Staphylococcus simulans	Pediatric Ward	06.218	Toilet seat
Staphylococcus epidermis	Pediatric Ward	06.218	Room door closer
Staphylococcus haemolyticus	Maternity Ward	05.330	Toilet rail
Staphylococcus aureus	Maternity Ward	05.330	Sink top
Staphylococcus haemolyticus	Maternity Ward	05.314	Toilet rail
Klebsiella oxytoca	Maternity Ward	05.314	Sink top
Staphylococcus haemolyticus	Maternity Ward	05.314	Sink top
Staphylococcus warneri	Maternity Ward	05.314	Tray table
Staphylococcus capitis ssp ureolyticus	Maternity Ward	05.314	Room door closer
Staphylococcus epidermis	Maternity Ward	05.314	Bathroom door closer
Staphylococcus warneri	Maternity Ward	05.314	Bathroom door closer
Staphylococcus haemolyticus	Maternity Ward	05.314	Bedside table

<b>Bacteria</b>	<b>SampleGroup</b>	<b>RoomNumber</b>	<b>Surface</b>
Staphylococcus haemolyticus	Maternity Ward	05.314	IV Pole
Staphylococcus haemolyticus	Maternity Ward	05.314	Room Chair
Staphylococcus haemolyticus	Medical Telemetry	07.314	Telephone
Staphylococcus haemolyticus	Medical Telemetry	07.314	Room Chair
Staphylococcus haemolyticus	Medical Telemetry	07.314	Tray table
Staphylococcus haemolyticus	Medical Telemetry	07.314	Sink top
Staphylococcus haemolyticus	Medical Telemetry	07.314	Bedpan cleaner
Staphylococcus cohnii ssp cohnii	Medical Telemetry	07.314	Room Sink
Staphylococcus haemolyticus	Medical Telemetry	07.314	Toilet seat
Staphylococcus simulans	Medical Telemetry	07.314	IV Pole
Staphylococcus haemolyticus	Medical Telemetry	07.314	Bedside table
Staphylococcus haemolyticus	Medical Telemetry	07.334	Room Sink
Staphylococcus epidermis	Medical Telemetry	07.334	Toilet seat
Staphylococcus haemolyticus	Medical Telemetry	07.334	Toilet rail
Staphylococcus acidominimus	Medical Telemetry	07.334	Room door closer
Staphylococcus aureus	Medical Telemetry	07.334	Telephone
Staphylococcus aureus	Medical Ward	07.230	Side rail
Staphylococcus hominis	Medical Ward	07.230	Room Sink
Micrococcus luteus	Medical Telemetry	07.314	Bathroom lightswitch
Stenotrophomonas maltophilia	Medical Telemetry	07.314	Sink top
Acinetobacter baumannii/calcoaceticus complex	Medical Telemetry	07.334	Sink top
Micrococcus luteus	Medical Telemetry	07.334	Bedside table

Bacteria	SampleGroup	RoomNumber	Surface
Staphylococcus saprophyticus	Medical Telemetry	07.334	Bedside table
Staphylococcus aureus	Medical Telemetry	07.334	Call box
Micrococcus lylae	Medical Telemetry	07.334	Toilet seat
Staphylococcus aureus	Medical Telemetry	07.334	Toilet rail
Klebsiella oxytoca	Pediatric Ward	06.218	Tray table
Leclercia adecarboxylata	Pediatric Ward	06.218	Tray table
Serratia plymuthica	Pediatric Ward	06.218	Tray table
Escherichia vulneris	Pediatric Ward	06.218	Sink top
Acinetobacter baumannii/calcoaceticus complex	Pediatric Ward	06.218	Room Sink
Pseudomonas putida	Pediatric Ward	06.218	Room Sink
Micrococcus lylae	Pediatric Ward	06.218	Toilet seat
Staphylococcus capitis	Maternity Ward	05.314	Call box
Micrococcus luteus	Maternity Ward	05.314	Bedside table
Staphyococcus saprophyticus	Maternity Ward	05.314	Bedside table
Staphylococcus epidermidis	Maternity Ward	05.314	Bedpan cleaner
Pseudomonas putida	Maternity Ward	05.314	Sink top
Staphylococcus saprophyticus	Maternity Ward	05.314	Telephone
Pseudomonas fluorescens	Maternity Ward	05.330	Room Chair
Staphyococcus saprophyticus	Maternity Ward	05.330	Bedside table
Pseudomonas orzihabitans	Medical Ward	07.208	Room Chair
Klebsiella pneumoniae ssp ozaenae	Medical Ward	07.208	Sink top
Pseudomonas putida	Medical Ward	07.208	Sink top
Serratia liquefaciens	Medical Ward	07.208	Sink top
Staphylococcus warneri	Medical Ward	07.230	Toilet rail
Acinetobacter baumannii/calcoaceticus complex	Medical Ward	07.230	Sink top
Pseudomonas fluorescens	Medical Ward	07.230	Sink top
Enterococcus faecium	Medical Ward	07.230	Bedpan cleaner
Acinetobacter baumannii/cacloaceticus complex	Medical Ward	07.230	Room Sink
Pseudomonas putida	Pediatric Ward	06.216	Sink top
Acinetobacter baumannii	Pediatric Ward	06.218	Sink top
Enterobacter cloacae	Maternity Ward	05.340	Sink top
Pseudomonas putida	Maternity Ward	05.340	Sink top



<b>Bacteria</b>	<b>SampleGroup</b>	<b>RoomNumber</b>	<b>Surface</b>
Micrococcus lylae	Pediatric Ward	06.212	Side rail
Acinetobacter baumannii/calcoaceticus complex	Pediatric Ward	06.212	Toilet rail
Staphylococcus aureus	Surgical Ward	06.316	Room Chair
Pseudomonas aeruginosa	Surgical Ward	06.316	Sink top
Serratia marcescens	ICU	02.123	Tray table
Enterobacter cloacae	ICU	02.123	Toilet seat
Escherichia coli	ICU	02.123	Toilet seat
Klebsiella oxytoca	ICU	02.123	Toilet seat
Citrobacter werkmanii	ICU	02.123	Sink top
Moraxella species	ICU	02.123	Bathroom door closer
Pangoea agglomerans	ICU	02.123	IV Pole
Pantoea agglomerans	ICU	02.123	IV Pole
Pantoea agglomerans	ICU	02.123	Tray table
Serratia odorifera 1	Medical Ward	07.234	Sink top
Moraxella species	Medical Telemetry	07.314	Telephone
Aerococcus viridans	Medical Ward	07.236	Toilet rail
Enterobacter aerogenes	Medical Ward	07.236	Sink top
Escherichia coli	Medical Ward	07.236	Sink top
Staphylococcus aureus	Medical Ward	07.236	Sink top
Staphylococcus aureus	Medical Ward	07.236	IV Pole
Staphylococcus aureus	Medical Ward	07.236	Bathroom lightswitch
Acinetobacter lwoffii	Pediatric Ward	06.212	Bathroom door closer
Pantoea agglomerans	Pediatric Ward	06.212	Telephone
Pantoea agglomerans	Surgical Ward	06.314	Sink top
Klebsiella pneumoniae	Maternity Ward	05.332	Sink top
Acinetobacter lwoffii	Maternity Ward	05.338	Sink top
Acinetobacter lwoffii	Maternity Ward	05.338	Room Chair
Stenotrophomonas maltophilia	Maternity Ward	05.338	Toilet rail
Acinetobacter lwoffii	Maternity Ward	05.338	Bedpan cleaner
Acinetobacter lwoffii	Maternity Ward	05.338	Bedside table
Acinetobacter lwoffii	Maternity Ward	05.338	Toilet seat
Staphylococcus hyicus	Surgical Ward	06.314	Call box
Arcanobacterium pyogenes	Surgical Ward	06.314	IV Pole
Arcanobacterium pyogenes	Surgical Ward	06.314	Bathroom door closer

<b>Bacteria</b>	<b>SampleGroup</b>	<b>RoomNumber</b>	<b>Surface</b>
Arcanobacterium pyogenes	Surgical Ward	06.314	Toilet rail
Pseudomonas oryzihabitans	Medical Telemetry	07.314	Toilet seat
Staphylococcus haemolyticus	Medical Telemetry	07.314	IV Pole
Acinetobacter lwoffii	Maternity Ward	05.338	Toilet seat
Staphylococcus hominis	Maternity Ward	05.338	IV Pole
Pantoea agglomerans	Maternity Ward	05.338	Toilet rail
Acinetobacter lwoffii	Maternity Ward	05.338	Room Chair
CDC group EO-2	Maternity Ward	05.338	Telephone
Staphylococcus lugdunensis	Medical Ward	07.236	Bedside table
Enterobacter cloacae	Medical Ward	07.236	Sink top
Klebsiella oxytoca	Medical Ward	07.236	Sink top
Suttonella indologenes	Medical Ward	07.236	Sink top
Pantoea agglomerans	Medical Ward	07.236	Bathroom lightswitch
Staphylococcus epidermidis	Maternity Ward	05.332	Bedside table
Staphylococcus hominis	Maternity Ward	05.332	Telephone
Pantoea agglomerans	Maternity Ward	05.332	Tray table
Pseudomonas fluorescens	Maternity Ward	05.332	Toilet handle
Staphylococcus epidermidis	Maternity Ward	05.332	Sink top
Pseudomonas fluorescens	Pediatric Ward	06.212	Room Lightswitch
Pantoea agglomerans	Pediatric Ward	06.212	Toilet rail
Staphylococcus haemolyticus	Maternity Ward	05.338	Bedside table
Dermacoccus nishinomiyaensis	Maternity Ward	05.338	Telephone
Staphylococcus capitis ssp ureolyticus	Maternity Ward	05.338	Telephone
Staphylococcus haemolyticus	Maternity Ward	05.338	Tray table
Enterobacter cloacae	Maternity Ward	05.338	Room Sink
Staphylococcus haemolyticus	Maternity Ward	05.338	IV Pole
Staphylococcus epidermidis	Maternity Ward	05.338	Toilet seat
Staphylococcus haemolyticus	Maternity Ward	05.338	Toilet rail
Enterobacter cloacae	Maternity Ward	05.338	Sink top
Pseudomonas putida	Maternity Ward	05.338	Sink top
Stenotrophomonas maltophilia	Surgical Ward	06.344	Toilet rail
Staphylococcus hominis	Surgical Ward	06.344	Telephone
Staphylococcus epidermidis	Surgical Ward	06.344	Bedside table
Staphylococcus epidermidis	Medical Telemetry	07.328	Toilet seat

Bacteria	SampleGroup	RoomNumber	Surface
Staphylococcus equorum	Medical Telemetry	07.328	Bedside table
Staphylococcus haemolyticus	Medical Telemetry	07.328	Toilet rail
Pseudomonas putida	Medical Telemetry	07.328	Sink top
Acinetobacter baumannii/calcoaceticus complex	Medical Telemetry	07.328	Room Lightswitch
Acinetobacter baumannii/calcoaceticus complex	Medical Telemetry	07.328	Room Chair
Staphylococcus haemolyticus	Medical Telemetry	07.328	Room Chair
Staphylococcus haemolyticus	Medical Telemetry	07.328	Call box
Pseudomonas fluorescens	Medical Telemetry	07.328	Tray table
Klebsiella pneumoniae ssp pneumoniae	Medical Telemetry	07.328	Telephone
Acinetobacter baumannii/calcoaceticus complex	Medical Telemetry	07.328	Side rail
Staphylococcus haemolyticus	Medical Telemetry	07.328	Room Sink
Staphylococcus haemolyticus	Maternity Ward	05.336	Bathroom door closer
Staphylococcus haemolyticus	Maternity Ward	05.336	Tray table
Staphylococcus haemolyticus	Maternity Ward	05.336	Toilet rail
Staphylococcus haemolyticus	Maternity Ward	05.336	Toilet handle
Klebsiella oxytoca	Maternity Ward	05.336	Sink top
Pseudomonas putida	Maternity Ward	05.336	Sink top
Staphylococcus haemolyticus	Maternity Ward	05.336	Toilet seat
Staphylococcus simulans	Surgical Ward	06.344	IV Pole
Streptococcus acidominimus	Surgical Ward	06.344	Sink top
Micrococcus luteus	Surgical Ward	06.344	Call box
Micrococcus luteus	Maternity Ward	05.336	Telephone
Staphylococcus haemolyticus	Maternity Ward	05.336	IV Pole
Pantoea agglomerans	Maternity Ward	05.336	Toilet rail
Pseudomonas oryzae	Maternity Ward	05.336	Tray table
Moraxella (Branhamella) catarrhalis	Maternity Ward	05.338	Room Chair
Acinetobacter lwoffii	Maternity Ward	05.338	Toilet rail
Pseudomonas luteola	Maternity Ward	05.338	Telephone
Dermacoccus nishinomiyaensis	Maternity Ward	05.338	IV Pole

<b>Bacteria</b>	<b>SampleGroup</b>	<b>RoomNumber</b>	<b>Surface</b>
Enterobacter cloacae	Maternity Ward	05.338	Room Sink
Esherichia vulneris	Maternity Ward	05.338	Room Sink
Acinetobacter species	Maternity Ward	05.338	Sink top
Aerococcus viridans	Medical Ward	07.236	Tray table
Staphylococcus saprophyticus	ICU	02.121	Telephone
Bacillus megaterium	Maternity Ward	05.326	Call box
Pantoea agglomerans	Maternity Ward	05.326	Toilet rail
Staphylococcus aureus	Maternity Ward	05.326	Toilet rail
Staphylococcus haemolyticus	ICU	02.121	Bedside table
Staphylococcus aureus	ICU	02.121	Sink top
Staphylococcus saprophyticus	ICU	02.121	Room Chair
Staphylococcus haemolyticus	ICU	02.121	Toilet rail
Staphylococcus epidermidis	ICU	02.121	Room Sink
Staphylococcus saprophyticus	ICU	02.121	IV Pole
Pseudomonas species	Maternity Ward	05.326	Room Sink
Staphylococcus hominis	Maternity Ward	05.326	Bedside table
Citrobacter freundii	Maternity Ward	05.326	Sink top
Pseudomonas aeruginosa	Maternity Ward	05.326	Sink top
Pseudomonas putida	Maternity Ward	05.326	Sink top
Staphylococcus haemolyticus	Maternity Ward	05.326	Toilet rail
Staphylococcus haemolyticus	Medical Telemetry	07.332	Room Sink
Staphylococcus hominis	Medical Telemetry	07.332	Bathroom lightswitch
Staphylococcus hominis	Medical Telemetry	07.332	Bedside table
Staphylococcus haemolyticus	Medical Telemetry	07.332	IV Pole
Staphylococcus capitis	Medical Telemetry	07.332	Toilet rail
Staphylococcus hominis	Medical Telemetry	07.332	Toilet seat
Staphylococcus haemolyticus	Medical Telemetry	07.332	Sink top
Corynebacterium amycolatum/ minutissimum	Medical Ward	07.236	Sink top
Aerococcus viridans	Medical Ward	07.236	Room Sink
Acinetobacter lwoffii	Medical Ward	07.236	Tray table
Staphylococcus haemolyticus	Medical Telemetry	07.332	Toilet seat

Bacteria	SampleGroup	RoomNumber	Surface
Aerococcus viridans	Medical Telemetry	07.342	Toilet handle
Staphylococcus epidermidis	Medical Telemetry	07.342	Toilet seat
Staphylococcus haemolyticus	Medical Telemetry	07.342	Sink top
Aerococcus viridans	Medical Telemetry	07.342	IV Pole
Staphylococcus hominis	Medical Telemetry	07.342	Toilet rail
Staphylococcus capitis ssp capitis	Medical Ward	07.242	Room Sink
Enterobacter aerogenes	Medical Ward	07.242	Toilet rail
Enterobacter aerogenes	Medical Ward	07.242	Toilet handle
Pseudomonas aeruginosa	Medical Ward	07.242	Sink top
Staphylococcus epidermidis	Medical Ward	07.242	Tray table
Staphylococcus capitis	Medical Ward	07.242	Bathroom door closer
Staphylococcus gallinarum	Medical Ward	07.242	Room Chair
Enterobacter aerogenes	Medical Ward	07.242	Toilet seat
Escherichia coli	Medical Ward	07.242	Toilet seat
Staphylococcus aureus	Medical Ward	07.242	Toilet seat
Aerococcus viridans	Surgical Ward	06.344	Side rail
Staphylococcus epidermidis	Surgical Ward	06.344	Bedside table
Staphylococcus capitis ssp ureolyticus	Surgical Ward	06.344	Sink top
Pantoea agglomerans	Surgical Ward	06.344	Room Chair
Staphylococcus simulans	Surgical Ward	06.344	Room Chair
Corynebacterium urealyticum	Surgical Ward	06.344	Telephone
Pantoea agglomerans	Surgical Ward	06.344	Toilet rail
Pseudomonas putida	Surgical Ward	06.344	Toilet rail
Staphylococcus carnosus	Surgical Ward	06.344	Toilet rail
Alcaligenes faecalis	Maternity Ward	05.336	Bedside table
Dermacoccus nishinomiyaensis	Maternity Ward	05.336	Bedside table
Kocuria varians	Maternity Ward	05.336	Room Chair
Moraxella species	Maternity Ward	05.336	Tray table
Kytococcus sedentarius	Maternity Ward	05.336	Telephone
Acinetobacter baumannii	Maternity Ward	05.336	Toilet rail
Kocuria varians	Maternity Ward	05.336	Toilet rail
Acinetobacter lwoffii/haemolyticus	Maternity Ward	05.336	Toilet seat
Acinetobacter lwoffii	Maternity Ward	05.336	Room Sink
Alcaligenes faecalis	Maternity Ward	05.336	IV Pole

<b>Bacteria</b>	<b>SampleGroup</b>	<b>RoomNumber</b>	<b>Surface</b>
Leclercia adecarboxylata	Maternity Ward	05.336	Call box
Pseudomonas putida	Maternity Ward	05.336	Call box
Klebsiella oxytoca	Maternity Ward	05.336	Sink top
Oligella urethralis	Maternity Ward	05.336	Sink top
Kocuria rosea	Medical Ward	07.242	Room Chair
Staphylococcus haemolyticus	Medical Ward	07.242	Bedside table
Enterobacter cloacae	Medical Ward	07.242	Room Sink
Staphylococcus hominis	Medical Ward	07.242	Toilet rail
Staphylococcus warneri	Medical Ward	07.242	Toilet seat
Enterococcus faecalis	Medical Ward	07.242	Tray table
Staphylococcus hominis	Medical Telemetry	07.342	Toilet rail
Staphylococcus warneri	Maternity Ward	05.336	Room Sink
Staphylococcus haemolyticus	Maternity Ward	05.336	Call box
Staphylococcus epidermidis	Maternity Ward	05.336	Telephone
Staphylococcus capitis	Maternity Ward	05.336	Toilet handle
Pseudomonas putida	Maternity Ward	05.336	Bathroom lightswitch
Staphylococcus chromogenes	Maternity Ward	05.336	Bathroom door closer
Staphylococcus hominis	Maternity Ward	05.336	Room Chair
Staphylococcus warneri	Maternity Ward	05.336	Bedpan cleaner
Citrobacter amalonaticus	Maternity Ward	05.336	Sink top
Klebsiella oxytoca	Maternity Ward	05.336	Sink top
Pseudomonas putida	Maternity Ward	05.336	Sink top
Acinetobacter lwoffii	Maternity Ward	05.336	Toilet rail
Kocuria varians	Maternity Ward	05.336	Toilet rail
Staphylococcus epidermidis	Maternity Ward	05.336	Room door closer

### BELVOIR Molecular Before-After Comparison

RoomNumber	SampleGroup	1-SampleID	1-16S	2-SampleID	2-16S
02.116	ICU	1238	+	1268	-
02.122	ICU	661	+	750	+
02.122	ICU	662	+	760	-
02.122	ICU	660	-	753	+
02.122	ICU	658	+		
02.122	ICU	657	+	754	+
02.122	ICU	656	+	762	+
02.122	ICU	654	+	761	-
02.122	ICU	650	+	759	+
02.123	ICU	670	+		
02.123	ICU	1026	+	1165	+
02.123	ICU	1031	+	1169	+
02.123	ICU	1029	+	1163	+
02.123	ICU	1028	+	1159	-
02.123	ICU	1027	-	1170	+
02.123	ICU	1032	+	1166	+
02.123	ICU	1024	+	1164	+
02.123	ICU	1021	+	1168	+
02.123	ICU	1034	+	1156	+
02.123	ICU	675	+		
02.123	ICU	680	+		
02.123	ICU	669	+		
02.123	ICU	676	+		
05.312	Maternity Ward	626	+	712	-
05.312	Maternity Ward	625	+	715	+
05.312	Maternity Ward	624	+	714	+
05.312	Maternity Ward	622	+	704	+
05.312	Maternity Ward	621	+	716	+
05.312	Maternity Ward	620	+	711	-
05.312	Maternity Ward	619	+	703	+
05.312	Maternity Ward	618	+	710	-

RoomNumber	SampleGroup	1-SampleID	1-16S	2-SampleID	2-16S
05.312	Maternity Ward	617	+	706	-
05.312	Maternity Ward	631	+	708	+
05.322	Maternity Ward	888	+	816	-
05.322	Maternity Ward	889	+	810	+
05.322	Maternity Ward	892	+	806	+
05.322	Maternity Ward	893	-	804	+
05.322	Maternity Ward	894	+	815	-
05.322	Maternity Ward	899	+	802	+
05.334	Maternity Ward	1096	+	1174	-
05.334	Maternity Ward	1093	+	1178	+
05.334	Maternity Ward	1099	+	1176	-
05.334	Maternity Ward	1098	+	1179	+
05.334	Maternity Ward	1102	+	1186	+
05.334	Maternity Ward	1100	+	1180	-
05.334	Maternity Ward	1092	+	1187	+
05.334	Maternity Ward	1088	+	1181	+
05.334	Maternity Ward	1086	+	1182	+
05.334	Maternity Ward	1097	+	1173	+
06.244	Pediatric Ward	430	+	639	-
06.244	Pediatric Ward	424	+	644	-



RoomNumber	SampleGroup	1-SampleID	1-16S	2-SampleID	2-16S
06.244	Pediatric Ward	427	+	645	-
06.244	Pediatric Ward	428	+	648	+
06.244	Pediatric Ward	426	+	638	-
06.244	Pediatric Ward	417	+	636	+
06.244	Pediatric Ward	418	+	633	+
06.244	Pediatric Ward	419	+	637	-
06.244	Pediatric Ward	420	+	643	+
06.244	Pediatric Ward	422	+	635	+
06.244	Pediatric Ward	425	+	646	-
06.244	Pediatric Ward	423	+	641	+
06.244	Pediatric Ward	431	+	634	+
06.244	Pediatric Ward	432	+		
06.244	Pediatric Ward	429	+	640	-
06.248	Pediatric Ward	448	-	558	+
06.248	Pediatric Ward	447	+	542	-
06.248	Pediatric Ward	445	+	476	+
06.248	Pediatric Ward	443	+	550	+
06.248	Pediatric Ward	434	+	513	-
06.248	Pediatric Ward	442	+	581	+
06.248	Pediatric Ward	433	+	361	+

RoomNumber	SampleGroup	1-SampleID	1-16S	2-SampleID	2-16S
06.248	Pediatric Ward	435	+	597	+
06.248	Pediatric Ward	436	+	490	+
06.248	Pediatric Ward	437	+	510	-
06.248	Pediatric Ward	438	+	354	+
06.248	Pediatric Ward	439	+	598	+
06.248	Pediatric Ward	440	+	357	+
06.248	Pediatric Ward	441	+	514	-
06.312	Surgical Ward	1083	+	1199	+
06.312	Surgical Ward	1070	+	1189	+
06.312	Surgical Ward	1075	-	1195	+
06.312	Surgical Ward	1076	+	1192	+
06.312	Surgical Ward	1078	+	1204	+
06.312	Surgical Ward	1079	+	1201	+
06.312	Surgical Ward	1080	-	1194	+
06.312	Surgical Ward	1081	+	1190	+
06.312	Surgical Ward	1082	-	1202	+
06.312	Surgical Ward	1085	-	1197	+
06.316	Surgical Ward	921	+	870	+
06.316	Surgical Ward	920	+	875	-
06.316	Surgical Ward	931	+	879	+
06.316	Surgical Ward	930	+	869	-
06.316	Surgical Ward	929	-	883	+
06.316	Surgical Ward	926	+	873	-
06.316	Surgical Ward	925	+	885	-
06.316	Surgical Ward	923	+	874	+
06.316	Surgical Ward	919	+	882	-
06.316	Surgical Ward	922	+	877	-
06.316	Surgical Ward	918	+	871	-
06.316	Surgical Ward	917	+	872	+
06.316	Surgical Ward	916	+	881	+
06.338	Surgical Ward	717	-	768	+
06.338	Surgical Ward	719	+	771	-
06.338	Surgical Ward	723	+	780	+
06.338	Surgical Ward	729	+	772	+

RoomNumber	SampleGroup	1-SampleID	1-16S	2-SampleID	2-16S
06.338	Surgical Ward	730	+	775	+
06.342	Surgical Ward	402	+	690	-
06.342	Surgical Ward	403	+	682	+
06.342	Surgical Ward	414	-	695	+
06.342	Surgical Ward	416	-	689	+
06.342	Surgical Ward	406	+	686	+
06.342	Surgical Ward	404	+	681	-
06.342	Surgical Ward	407	+	684	-
06.342	Surgical Ward	408	+	687	-
06.342	Surgical Ward	409	+	691	+
06.342	Surgical Ward	411	+	692	+
06.342	Surgical Ward	405	+	685	-
06.342	Surgical Ward	410	+	693	+
07.216	Medical Ward	1013	-	1153	+
07.216	Medical Ward	1012	+	1148	+
07.216	Medical Ward	1014	+	1144	+
07.216	Medical Ward	1002	+	1146	-
07.216	Medical Ward	1006	+	1147	+
07.216	Medical Ward	1011	+	1138	+
07.216	Medical Ward	1015	+	1141	-
07.216	Medical Ward	1005	+	1149	+
07.242	Medical Ward	1053	+	1039	+
07.242	Medical Ward	1065	-	1043	+
07.242	Medical Ward	1062	+	1045	+
07.242	Medical Ward	1059	+	1048	+
07.242	Medical Ward	1057	+	1049	+
07.242	Medical Ward	1055	-	1035	+
07.242	Medical Ward	1066	-	1042	+
07.242	Medical Ward	1056	+	1051	+
07.244	Medical Ward	830	+	855	+
07.244	Medical Ward	827	+	853	-
07.244	Medical Ward	831	+	858	+
07.244	Medical Ward	828	+	864	-
07.244	Medical Ward	825	-	852	+
07.244	Medical Ward	819	+	861	+
07.304	Medical Telemetry	475	+	787	+
07.304	Medical Telemetry	468	+	791	-

RoomNumber	SampleGroup	1-SampleID	1-16S	2-SampleID	2-16S
07.304	Medical Telemetry	469	+	790	-
07.304	Medical Telemetry	473	+	782	+
07.304	Medical Telemetry	465	+	795	+
07.304	Medical Telemetry	478	+	785	+
07.304	Medical Telemetry	479	+	788	-
07.304	Medical Telemetry	480	+	794	+
07.304	Medical Telemetry	467	+	792	-
07.304	Medical Telemetry	471	+	796	+
07.328	Medical Telemetry	485	+	747	-
07.328	Medical Telemetry	495	+	737	-
07.328	Medical Telemetry	494	+	739	+
07.328	Medical Telemetry	493	+	734	-
07.328	Medical Telemetry	492	+	744	-
07.328	Medical Telemetry	491	+	733	-
07.328	Medical Telemetry	489	+	735	-
07.328	Medical Telemetry	488	+	746	-
07.328	Medical Telemetry	486	+	742	+
07.328	Medical Telemetry	484	+	738	+
07.328	Medical Telemetry	487	+	740	-
07.334	Medical Telemetry	449	+	614	-

RoomNumber	SampleGroup	1-SampleID	1-16S	2-SampleID	2-16S
07.334	Medical Telemetry	452	+	602	-
07.334	Medical Telemetry	453	+	608	-
07.334	Medical Telemetry	455	+	613	-
07.334	Medical Telemetry	456	+	605	-
07.334	Medical Telemetry	457	+	611	-
07.334	Medical Telemetry	458	+	606	+
07.334	Medical Telemetry	459	+	610	-
07.334	Medical Telemetry	460	+	615	+
07.334	Medical Telemetry	461	+	612	-
07.334	Medical Telemetry	464	+	603	-
07.334	Medical Telemetry	463	+	604	-
07.336	Medical Telemetry	1103	+	1133	+
07.336	Medical Telemetry	1105	-	1130	+
07.336	Medical Telemetry	1106	+	1123	+
07.336	Medical Telemetry	1115	+	1127	+
07.336	Medical Telemetry	1113	+	1121	+
07.336	Medical Telemetry	1111	+	1137	-
07.336	Medical Telemetry	1109	-	1135	+
07.336	Medical Telemetry	1108	+	1125	-
07.336	Medical Telemetry	1104	-	1134	+

RoomNumber	SampleGroup	1-SampleID	1-16S	2-SampleID	2-16S
07.336	Medical Telemetry	1110	+	1120	+
07.342	Medical Telemetry	848	+	781	-
07.342	Medical Telemetry	847	+	901	-
07.342	Medical Telemetry	846	+	902	-
07.342	Medical Telemetry	844	+	903	-
07.342	Medical Telemetry	843	+	907	+
07.342	Medical Telemetry	839	+	905	+
07.342	Medical Telemetry	837	+	911	-
07.342	Medical Telemetry	841	+	912	+
07.342	Medical Telemetry	835	+	910	-
07.342	Medical Telemetry	836	+	914	+

BELVOIR Acinetobacter

RoomNumber	SampleGroup	Surface	1-SampleID	1-ACB	2-SampleID	2-ACB
06.342	Surgical Ward	Sink top	403	-	682	x
06.342	Surgical Ward	Call box	405	x	685	
06.342	Surgical Ward	Bedside table	406	x	686	-
06.342	Surgical Ward	Room door closer	407	x	684	
06.342	Surgical Ward	Toilet rail	409	-	691	+
06.342	Surgical Ward	COMPOSITE 1	410	-	693	+
06.342	Surgical Ward	Tray table	411	-	692	x
06.342	Surgical Ward	Toilet handle	414		695	x
06.342	Surgical Ward	COMPOSITE 2	416		689	+
06.244	Pediatric Ward	Tray table	422	-	635	x
06.248	Pediatric Ward	COMPOSITE 1	435	x	597	+
06.248	Pediatric Ward	Toilet rail	436	-	490	x
06.248	Pediatric Ward	Toilet seat	439	-	598	+
06.248	Pediatric Ward	Room door closer	445	-	476	+
07.334	Medical Telemetry	Call box	452	x	602	
07.334	Medical Telemetry	COMPOSITE 1	453	+	608	
07.334	Medical Telemetry	Sink top	459	x	610	
07.304	Medical Telemetry	Toilet seat	465	-	795	x
07.304	Medical Telemetry	PT chair	471	-	796	x
07.304	Medical Telemetry	Tray table	473	-	782	+
07.304	Medical Telemetry	Telephone	475	-	787	x
07.304	Medical Telemetry	COMPOSITE 2	478	x	785	x
07.304	Medical Telemetry	COMPOSITE 1	480	-	794	x

RoomNumber	SampleGroup	Surface	1-SampleID	1-ACB	2-SampleID	2-ACB
07.328	Medical Telemetry	Tray table	484	x	738	-
07.328	Medical Telemetry	Toilet seat	488	x	746	
05.312	Maternity Ward	COMPOSITE 1	619	-	703	+
05.312	Maternity Ward	Toilet handle	621	-	716	x
05.312	Maternity Ward	Toilet seat	624	-	714	x
05.312	Maternity Ward	COMPOSITE 2	625	-	715	x
05.312	Maternity Ward	PT chair	631	-	708	x
02.122	ICU	COMPOSITE 2	650	-	759	x
02.122	ICU	Tray table	656	-	762	+
02.122	ICU	COMPOSITE 1	661	-	750	+
02.123	ICU	COMPOSITE 1	680	x		
06.338	Surgical Ward	COMPOSITE 2	717		768	+
06.338	Surgical Ward	Sink top	719	x	771	
06.338	Surgical Ward	COMPOSITE 1	723	x	780	-
06.338	Surgical Ward	Tray table	729	-	772	x
06.338	Surgical Ward	Toilet rail	730	+	775	-
06.338	Surgical Ward	Toilet handle	731	+	779	
07.244	Medical Ward	Room sink	831	-	858	x
07.342	Medical Telemetry	Toilet handle	835	x	910	
07.342	Medical Telemetry	Sink top	836	x	914	-
07.342	Medical Telemetry	Toilet seat	837	x	911	
07.342	Medical Telemetry	Toilet rail	839	x	905	x
07.342	Medical Telemetry	Telephone	844	x	903	
07.342	Medical Telemetry	Room Sink	846	x	902	
07.342	Medical Telemetry	Chair	847	x	901	
05.322	Maternity Ward	Bedside table	892	-	806	x



RoomNumber	SampleGroup	Surface	1-SampleID	1-ACB	2-SampleID	2-ACB
05.322	Maternity Ward	IV Pole	893		804	x
06.316	Surgical Ward	Toilet rail	916	+	881	-
06.316	Surgical Ward	IV Pole	917	+	872	-
06.316	Surgical Ward	Bedside table	918	+	871	
06.316	Surgical Ward	Chair	919	+	882	
06.316	Surgical Ward	Tray table	921	+	870	-
06.316	Surgical Ward	Room Sink	923	+	874	+
06.316	Surgical Ward	Toilet seat	925	+	885	
06.316	Surgical Ward	Call box	926	+	873	

1 **A Comparison of Culture and Real-Time PCR for Detecting Bacteria Directly from**  
2 **Environmental Swabs**

3 Patrick Mc Gann\*, Michael Milillo, Eve Hosford, Yuanzheng Si, Paige Waterman, and Emil Lesho

4 Running Title: Detection of nosocomial pathogens by RT-PCR

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23 **ABSTRACT**

24 Despite acknowledged limitations, swabbing with subsequent culturing remains the principal method for  
25 sampling environmental surfaces. Alternative, molecular techniques, such as real-time PCR, offer  
26 important advantages over culture dependent techniques, but concerns about specificity, accuracy and  
27 cost have hampered their widespread application.

28 318 environmental swabs were collected over a 5 month period from high touch surfaces in publicly  
29 accessible locations in three referral and teaching hospitals and 2 community hospitals in the National  
30 Capitol and Baltimore, Maryland regions. Swabs were plated onto Blood and Mac Conkey Agar plates  
31 and simultaneously tested for bacterial *16S* rRNA using real-time PCR. *16S* rRNA positive swabs were  
32 subsequently tested by real-time PCR for the presence of six clinically important nosocomial bacteria  
33 using a set of species-specific primers. Bacterial colonies from BAP and MAC plates were identified to  
34 the species level using the BD Phoenix Automated Microbiology System and compared to real-time  
35 PCR.

36 Bacterial growth and detection of *16S* rRNA were strongly correlated ( $P < 0.001$ ; Chi-squared). Four of  
37 the target organisms were isolated from 20 swabs using culture-based techniques, whereas 33 swabs,  
38 including all 20 culture-positive swabs, were positive by real-time PCR for five of target organisms,  
39 including *C. difficile*.

40 We demonstrate that real-time PCR can be successfully employed to detect clinically important bacteria  
41 from environmental swabs, and is comparable, and in some instances superior to culture-based  
42 techniques.

43

## 44 INTRODUCTION

45 Though initially believed to play an insignificant role (REF), environmental contamination is  
46 increasingly implicated in the transmission of hospital acquired infections (HAIs) (Otter 2011).

47 However, ascertaining a definitive correlation between an environmentally contaminated surface and  
48 subsequent HAIs can be difficult, though studies have suggested that the extent of patient-to-patient  
49 transmission is directly proportional to the level of environmental contamination (REF). The inherent  
50 challenge in proving direct transmission via environmental contamination is further exacerbated by  
51 current methodologies for identifying and isolating bacteria species from surfaces. These techniques are  
52 essentially limited to culture-based methodologies, which suffer from multiple deficiencies, including  
53 labor- intensive culturing methods, complicated growth requirements for some bacterial species, and, in  
54 particular, the ability to separate bacterial species of interest from a mixed bacterial flora, particularly  
55 when the target organism is present in low concentrations.

56 In recent years, efforts to develop rapid and accurate molecular detection assays have been proposed  
57 (REF). Molecular methods can provide significant advantages over traditional techniques, including  
58 high sensitivity, rapid turnaround time, and greater flexibility in detecting new targets. However, no  
59 assay is perfect, and the limitations of molecular methods, including cross-reactivity with other species  
60 and a lack of molecularly trained technicians (REF, Maurer2011), have contributed to their sluggish  
61 implementation. This is particularly relevant when dealing with environmental surfaces, where multiple  
62 species of varying concentrations can be present (REF).

63 Bacterial *16S* rRNA is a desirable primary target, primarily due to its evolutionary conservation among  
64 disparate bacterial species, and has been used in multiple assays for detecting bacteria (REF). Its broad  
65 range makes it unsuitable for species identification, but other assays targeting clinically relevant bacteria  
66 have also been developed (REF). A number of these assays, most notably for the detection of

67 Methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococci* (VRE), and  
68 toxic *Clostridium difficile* have been translated into the clinical setting, usually via automated  
69 instrumentation, but they are costly to maintain and have very specific targets.

70 We recently developed and validated a set of species specific primers for MRSA (Mc Gann ICHE),  
71 *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, as well  
72 as a novel bacterial *16S* rRNA real-time PCR primer which will amplify a product from 93.8% of all  
73 bacterial *16S* rRNA sequences published to date (REF Clifford 2012). In this report, we take this assay  
74 into the field in an effort to directly compare culture and real-time PCR for the detection of bacterial  
75 pathogens of clinical importance directly from environmental swabs. The study was performed over a  
76 five-month period, using environmental swabs collected from high-tough surfaces in 5 major healthcare  
77 facilities in the Washington DC and Baltimore Area. Our data demonstrate that real-time PCR was not  
78 only directly comparable to culture-based techniques in detecting the presence of potentially pathogenic  
79 bacteria, but it was also capable of detecting organisms missed by culture-based techniques.

80 **MATERIALS AND METHODS**

81 **Real-time PCR primers**

82 All real-time PCR primers, with the exception of those targeting *C. difficile* have been described and  
83 validated previously (REF). The lack of complete genome sequences and the difficulty in isolating  
84 sufficient quantities of different strains of *C. difficile* for validation made design of a specific primer set  
85 challenging. Therefore, we used the primers targeting the toxin gene A (*tcdA*) designed by Knetsch and  
86 colleagues (REF). The primers were tested for specificity against a panel of American Type Culture  
87 Collection strains (ATCC, Manassas, VA) and clinical isolates representing fifty different bacterial  
88 species as described previously (REF). All reactions were performed in 20 µl volumes with 200 nM  
89 primers using a BioRad CFX96 with SsoAdvanced SYBR Green supermix (Bio-Rad Laboratories,  
90 Hercules, CA).

91 **Optimization of swabbing protocol**

92 Initial optimization studies were conducted under controlled conditions in the laboratory using spiked  
93 environmental surfaces. Serial dilutions of target organisms were spread evenly onto sterilized surfaces  
94 of defined sizes ranging from one square inch to four square inches and allowed to dry. A variety of  
95 swab types were tested for bacterial recovery, including standard sterile cotton swabs, pre-moistened  
96 sterile cotton swabs, large-sized 3M Sponge-Stick swabs (3M Center, St. Paul, MN), and liquid Amies  
97 Medium transport swabs (Copan Diagnostics Inc, Murrieta, CA) that support bacterial preservation  
98 during extended transport. Swabbing times were varied from 10 seconds to 30 seconds.

99 **Isolation and identification of bacterial species by culture**

100 Swabs were streaked directly onto Blood (BAP) and Mac Conkey (MAC) agar plates without any broth  
101 enrichment of the swabs (see Results) and incubated at 37°C for 48 hours. All BAP and MAC plates  
102 were examined for growth by two microbiologists with clinical microbiology experience. Individual

103 colonies were isolated, purified and identified to the species level using the BD Phoenix Automated  
104 Microbiology System (Diagnostics Systems, Sparks, MD).

### 105 **Identification of bacterial species by real-time PCR**

106 Immediately after streaking, the swab heads were aseptically removed and added to 300 µl of either  
107 sterile phosphate buffered saline (PBS) or ultra-pure water (See Results) and vortexed vigorously for 1  
108 minute. A 20 µl aliquot was removed and used directly for DNA preparation using Lyse-and-go as  
109 described previously (Mc Gann NDM plasmid). Briefly, the 20 µl aliquot was added to 20 µl of Lyse-  
110 and-go reagent (Thermo Scientific, Waltham, MA) in 96-well plates, and run in a thermal cycler using  
111 the manufacturer's protocol for the isolation of total genomic DNA. Isolates were held at 80°C for 15  
112 minutes at the end of the program to maximize lysis, and 2 µl of the resulting lysate was used directly  
113 for real-time PCR. Appropriate positive, negative, and no template controls (Ultra-pure water) were  
114 incorporated onto every plate. Cycling parameters were 95°C for 5 minutes, followed by 40 cycles of  
115 95°C for 10 seconds and 56°C for 10 seconds. A melting curve analysis was included at the end of every  
116 program to assist in data analysis. Quantification cycle (Cq) was calculated automatically using  
117 instrument software.

### 118 **Collection and processing of environmental swabs**

119 Environmental swabs were collected from July 2011 through November 2011 from a variety of  
120 publically accessible areas, such as restrooms and waiting areas, within healthcare centers in the  
121 National Capitol and Baltimore, MD regions. (supplemental Table SX). Based on CDC  
122 recommendations for environmental sampling, seventeen high-touch surfaces with corresponding  
123 clinical significance were selected, including door handles, light switches, bathroom rails, sink faucets,  
124 hand rests, and telephones. Where clinical surrogates were not readily obvious, surfaces with similar  
125 functions were selected instead, such as elevator buttons in place of call buttons (Supplemental Table

126 S1). Each swab collection protocol was conducted over two days, with the same seventeen sites  
127 swabbed on both days at different times of the day to account for cleaning schedules. Swabs were  
128 collected from each healthcare center on two separate collection periods over the 5 month period. Swabs  
129 were immediately transported to the research laboratory and streaked onto BAP and MAC plates, and  
130 tested for *16S* rRNA and the target organisms by real-time PCR as described above.

### 131 **Sequencing of *16S* rRNA gene by PCR**

132 Where appropriate, a 1,465bp sequence of *16S* rRNA was amplified by conventional PCR using a  
133 universal primer pair described by Weisburg and co-workers (Weisburg 1992). Amplicons were  
134 sequenced by MacroGen Corp (Rockville, MD, USA) and assembled using SeqMan and aligned using  
135 MegAlign (DNASTar Inc., Madison, WI, USA).

### 136 **Statistical analyses**

137 Where appropriate, statistical analyses were performed using Sigma Plot 12.0 (Systat Software, Inc, San  
138 Jose, CA).



139 **RESULTS**

140 **Optimal swabbing parameters**

141 Swab type, swabbing technique, and choice of diluents had significant effects on experimental outcome.

142

143 Both the pre-moistened and the Amies Medium transport swabs demonstrated comparable results in the  
144 recovery of bacteria from contaminated surfaces (data not shown), but the greater versatility in transport  
145 time offered by the Amies Medium transport swabs proved to be the deciding factor in choosing the  
146 optimal swab. As expected, dry cotton swabs were the least effective in collecting bacterial samples.

147 Similarly, the sized 3M Sponge-Stick were too large, requiring submersion in over 20 mL of diluent to  
148 adequately cover the entire swabs, which resulted in a concomitant decrease in the sensitivity of real-  
149 time PCR to detect bacterial *16S rRNA* (data not shown).

150 Swabbing time was varied from 10 to 30 seconds, and initial optimization studies using spiked benches  
151 indicated that swabbing for 20 or 30 seconds consistently resulted in 4-8 fold higher yields of amplified  
152 product (as determined by Cq) compared to swabbing for just 10 seconds (Figure 1a). In an effort to  
153 reduce the number of swabs required for the study, single composite swabs were used to swab all  
154 surfaces both before and after individual swab use. However, culture data indicated that the composite  
155 swab was consistently less efficient at collecting a representative sample of the microbial flora when  
156 compared to individual swabs. Based on these results, use of the composite swab was discontinued prior  
157 to the commencement of the study.

158 Finally, detection of bacterial DNA was conducted from swabs immersed in both PBS and water.

159 However, validation studies demonstrated that real-time PCR was adversely affected by the higher salt  
160 concentration in PBS, which resulted in a 2 to 3 log reduction in signal, even after the sample was  
161 diluted in the lyse-and-go buffer (Figure 1b).

162 The final swabbing protocol required firmly swabbing an area no less than 5 square centimeters for 20  
163 seconds with pre-moistened cotton swabs from up to 17 high touch surfaces in publically accessible  
164 areas. Swabs were rolled during the 20 second sampling to ensure maximal contact area of the swab tip,  
165 streaked onto BAP and MAC plates, and incubated for up to 48 hours before colony isolation and  
166 subsequent identification. Immediately after streaking, swab heads were aseptically removed and added  
167 to 300 µl of sterile, ultra-pure water and vortexed vigorously for 45 seconds.

#### 168 **Optimized real-time PCR conditions for use with environmental swabs**

169 All primer sets were capable of reproducibly detecting as few as  $3 \times 10^2$  genome copies from  
170 purified genomic DNA (Clifford 2012). This capability was unaffected by the use of mixed bacterial  
171 cultures, including a cocktail prepared from the serial dilution of a mix of *A. baumannii*, *E. coli*,  
172 *Klebsiella pneumoniae*, and *P. aeruginosa*.

173 Despite the unavoidable dilution factor, real-time PCR consistently detected bacterial *16S rRNA* from  
174 spiked surfaces, even when bacterial load was as low as  $1 \times 10^3$  organisms/mL. Consequently, all swabs  
175 were immersed in water after culturing and stored at  $-20^\circ\text{C}$ . Monthly, swab samples were removed,  
176 thawed and retested with both the *16S rRNA* primer set and the corresponding species-specific primer  
177 set where appropriate. No appreciable degradation in real-time PCR signal has been observed from any  
178 sample to date.

#### 179 **Bacterial contamination of environmental surfaces using culture-based techniques**

180 Three hundred and eighteen swabs were collected over the five month sampling period, and growth on  
181 BAP was observed with 217 (68.2%). Individual colonies were selected from 89 swabs, including 5  
182 swabs where  $>1$  colonies were chosen. Ninety-five individual isolates, spanning 15 genera where  
183 identified (Table 2). Twenty isolates from the five target bacterial species were identified, with *A.*  
184 *baumannii*/*A. baumannii/calcoaceticus* complex predominating (55%), followed by *P. aeruginosa*

185 (30%), *E. coli* (10%) and *K. pneumoniae* spp. *pneumoniae* (5%). Two isolates of *S. aureus* were also  
186 isolated, but were methicillin-sensitive by oxacillin screen (REF).

### 187 **Bacterial contamination of environmental surfaces using real-time PCR**

188 217 swabs were both culture and *16S rRNA* positive and bacterial growth was strongly concordant with  
189 *16S rRNA* gene detection ( $P < 0.001$ ; Chi-square with Yates correction). Growth on BAP or MAC  
190 without a corresponding detection of *16S rRNA* signal was observed from just five swabs (supplemental  
191 Table S1), and subsequent culture and microscopic analyses demonstrated that this growth was due to  
192 fungi and molds. A positive *16S rRNA* signal, without corresponding growth on either BAP or MAC,  
193 was detected from eight swabs. To determine whether this was due to the presence of viable, but non-  
194 culturable bacteria (REF), full *16S rRNA* sequencing was performed using the universal conventional  
195 PCR primer set designed by Wurziel and colleagues (REF). Three of the eight sequences could not be  
196 assembled as they consisted of overlapping *16S rRNA* sequences, indicating the presence of multiple  
197 bacterial species. The remaining amplicons had *16S rRNA* sequences that were 100% identical to  
198 *Pseudomonas putida* (4 swabs) and *Stenotrophomonas maltocida* (1 swab). As both these species can be  
199 isolated using the growth conditions employed in this study, the absence of a corresponding culture from  
200 these swabs could be the result of a number of factors, including very low numbers of bacteria in the  
201 swabs, non-viable bacterial cells, or technical errors.

202 Real-time PCR detected DNA from five of the six target organisms on 35 swabs, compared to  
203 just 20 from culture. *A. baumannii*/*A. baumannii/calcoaceticus* complex was the most prevalent  
204 (42.4%), followed by *P. aeruginosa* (39%), *E. coli* (12.1%) and *K. pneumoniae* spp. *pneumoniae* (6%).  
205 *S. aureus* was detected on two swabs, but positive amplification was only detected from *femA*, and not  
206 with *mecA*, indicating the presence of methicillin-sensitive *S. aureus* (MSSA) (REF Mc Gann ICHE).  
207 Two of the swabs that were positive for *A. baumannii* were also positive for *C. difficile*, but due to the

208 fastidious growth requirements for this bacterium, these results could not be confirmed by culture-based  
209 methods. Crucially, every swab that was culture-positive for the target organisms was also positive for  
210 the same organism by real-time PCR, resulting in a 100% agreement between culture and real-time PCR  
211 detection methods. However, a further 13 swabs were positive by real-time PCR for one or more of the  
212 target organisms without a corresponding culture, including 7 *P. aeruginosa*, 3 *A. baumannii*, 2 *E. coli*,  
213 and 1 *K. pneumoniae* (Supplemental table X). These results were confirmed by melting curve analysis,  
214 which unambiguously detected amplicons with the same melting curve profile as the control strains.

215 **DISCUSSION**

216

217

218

219 Turnaround time from swab processing to results was less than 4 hours. This was greatly  
220 enhanced by first testing all swabs with a novel bacterial *16S* rRNA real-time primer, capable fo  
221 detecting >93% of all bacterial *16S* rRNA sequences published to date (Clifford 2012). A direct  
222 comparison between real-time PCR and culture-based techniques demonstrated almost 100%  
223 concordance, though real-time PCR did amplify products from 21 swabs without a corresponding  
224 culture.

225

226 To our knowledge, this is the first comprehensive report comparing culture and real-time PCR  
227 techniques in detecting bacterial contamination directly from environmental swabs, and demonstrates  
228 that real-time PCR has broad applicability with environmental surveillance.

229

230

231

232 As bacterial *16S rRNA* copy number can vary from just a single copy up to fifteen copies  
233 depending on the bacterial species (klappenbach 2001), the use of this target provides additional  
234 advantages in detecting the presence of bacteria.

235

236 application has been hampered by concerns about specificity (REF), and costs (REF).

237

238

239 Another limitation of our study is that we did not perform broth enrichment for MRSA.

240

241

242 Culture dependent methods for assessing the level of contamination of healthcare  
243 surfaces with MDRO are relatively slow and laborious. Culture based methods for  
244 detecting C. diff are especially laborious, and the current cost of molecular assays  
245 for C. diff make high volume screening of environmental surfaces difficult or  
246 impossible in cost-constrained settings.

247

248

249

250

251 **Human error**

252 A variety of factors can influence the efficacy and reproducibility of environmental swabbing, including  
253 choice of swab, swabbing time, swabbing technique, growth media for bacterial recovery, and the  
254 selection of appropriate target surfaces.

255

256 Detection of bacterial DNA from environmental swabs after using the swabs for direct culturing creates  
257 some technical challenges. Bacterial load would inevitably be lowered due to the culturing, and  
258 submersion of the swab heads in diluent would further dilute the number of available bacteria for  
259 detection.

260

261 melting curve analysis,

262

263 ). For example, Huang and colleagues demonstrated that among patients whose prior room occupant  
264 was methicillin-resistant Staphylococcus aureus (MRSA) positive, a significantly higher number

265 (P=0.4) acquired MRSA compared to patients whose prior room occupant was MRSA-negative (REF).  
266 Similarly, [Huang et al, and](#) Nseir and colleagues have recently shown that admission to an ICU room  
267 previously occupied by a patient with [c diff , or](#) MDR *P. aeruginosa* or *A. baumannii* is an independent  
268 risk factor for acquisition of these bacteria by subsequent room occupants (REF).

269 **REFERENCES**

270 . Nadkarni MA, Martin FE, Jacques NA, Hunter N (2002) Determination of bacterial load by real-time PCR using a  
271 broad-range (universal) probe and primers set. Microbiology 148: 257-266.



Verified. Measured. Delivered.

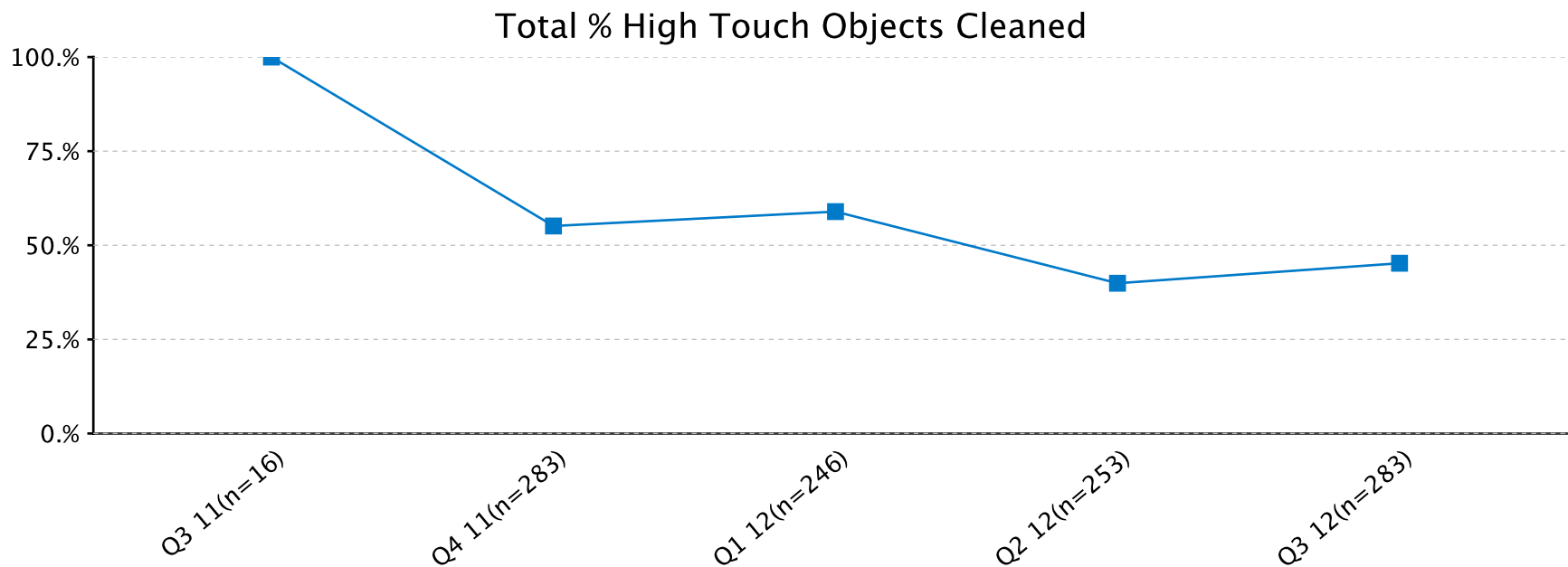


Dewitt Army Community Hospital

# EnCompass™ Environmental Hygiene Results



# Total % High Touch Objects Cleaned



Include Baseline Results? yes, Dates between:2011-01-01 and 2012-09-26 Isolation Rooms?:All

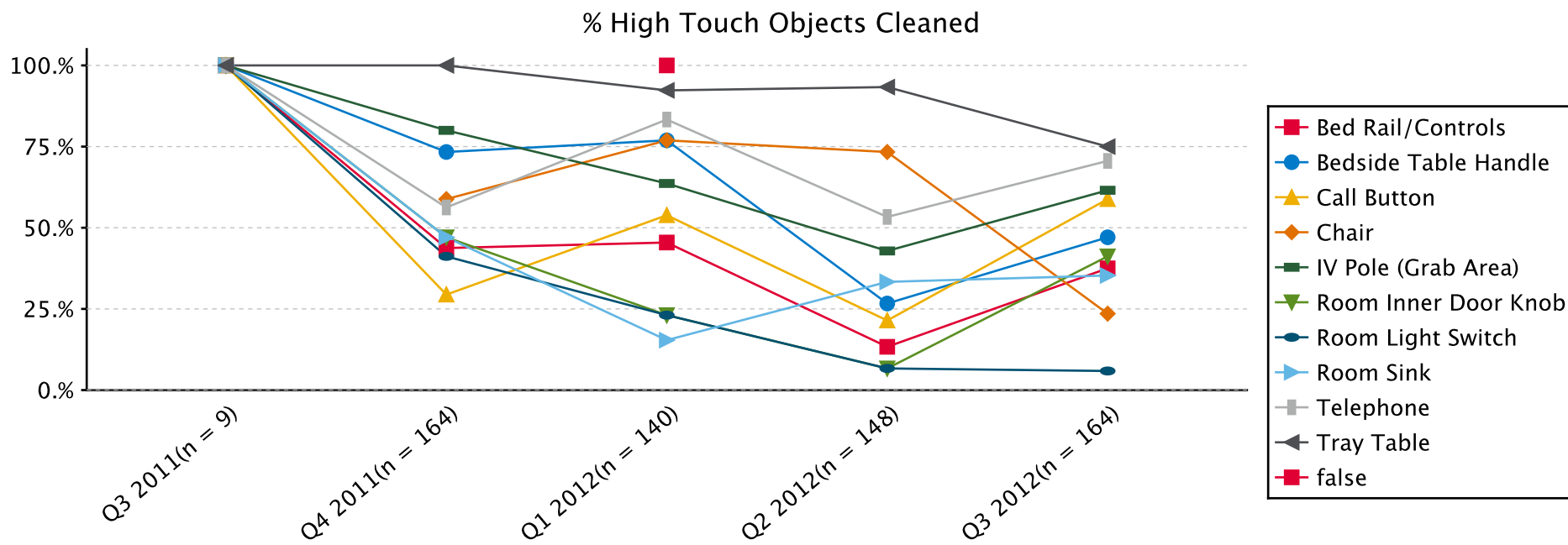
# Focus Areas Report

Top 3 HTOs	% Cleaned
Toilet Seat	92 %
Tray Table	90 %
Telephone	66 %

Bottom 3 HTOs	% Cleaned
Room Light Switch	21 %
Bathroom Handrail by Toilet	22 %
Bathroom Light Switch	30 %

Include Baseline Results? yes, Dates between:2011-01-01 and 2012-09-26 Isolation Rooms?:All

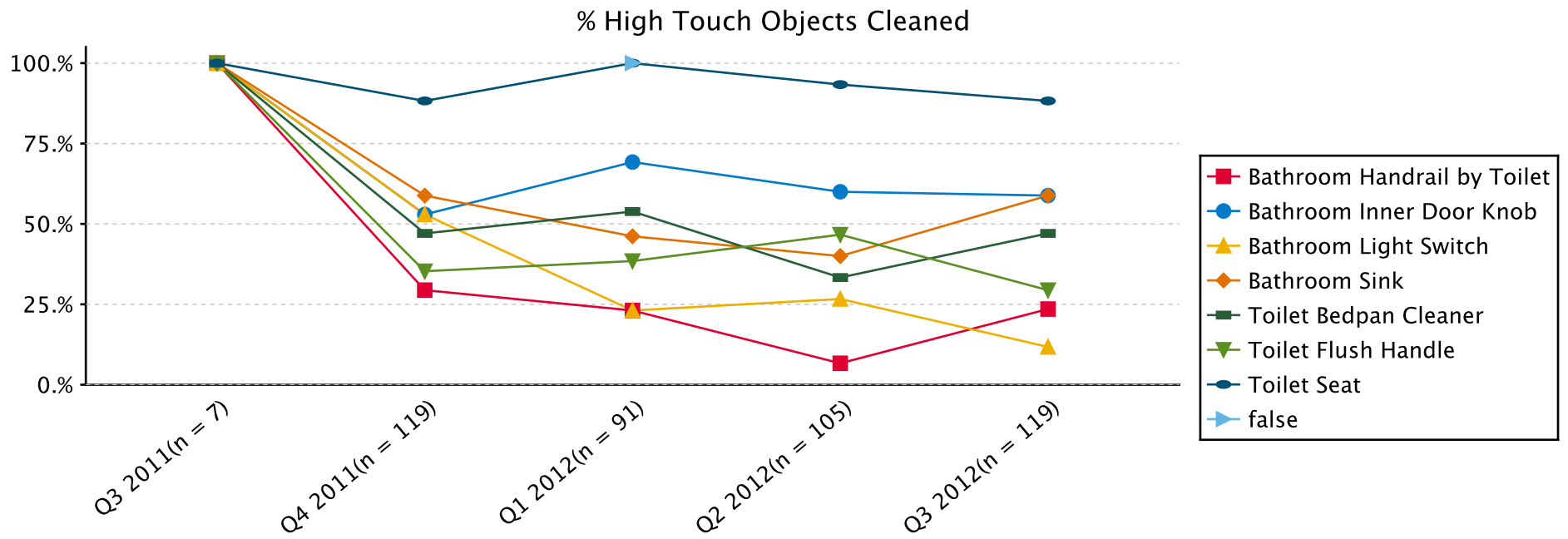
# High Touch Object



Quarter	Bed Rail/Controls	Bedside Table Handle	Call Button	Chair	IV Pole (Grab Area)	Room Inner Door Knob	Room Light Switch	Room Sink	Telephone	Tray Table			
Q3 2011	100 %	100 %	100 %		100 %	100 %	100 %	100 %	100 %	100 %			
Q4 2011	44 %	73 %	29 %	59 %	80 %	47 %	41 %	47 %	56 %	100 %			
Q1 2012	45 %	77 %	54 %	77 %	64 %	23 %	23 %	15 %	83 %	92 %			
Q2 2012	13 %	27 %	21 %	73 %	43 %	7 %	7 %	33 %	53 %	93 %			

Include Baseline Results? yes, Dates between:2011-01-01 and 2012-09-26 Isolation Rooms?:All

Q3 2012	38 %	47 %	59 %	24 %	62 %	41 %	6 %	35 %	71 %	75 %			
---------	------	------	------	------	------	------	-----	------	------	------	--	--	--



Quarter	Bathroom Handrail by Toilet	Bathroom Inner Door Knob	Bathroom Light Switch	Bathroom Sink	Toilet Bedpan Cleaner	Toilet Flush Handle	Toilet Seat			
Q3 2011	100 %	100 %	100 %	100 %	100 %	100 %	100 %			
Q4 2011	29 %	53 %	53 %	59 %	47 %	35 %	88 %			
Q1 2012	23 %	69 %	23 %	46 %	54 %	38 %	100 %			

Include Baseline Results? yes, Dates between:2011-01-01 and 2012-09-26 Isolation Rooms?:All

Q2 2012	7 %	60 %	27 %	40 %	33 %	47 %	93 %			
Q3 2012	24 %	59 %	12 %	59 %	47 %	29 %	88 %			

Include Baseline Results? yes, Dates between:2011-01-01 and 2012-09-26 Isolation Rooms?:All

# Percent High Touch Object Cleaned

High Touch Objects	Baseline	Q2 2012	Q3 2012	Net Improvement (Over Baseline)	Trend (Over Last Period)
Bathroom Handrail by Toilet		7 %	24 %	24 %	Up
Bathroom Inner Door Knob		60 %	59 %	59 %	Down
Bathroom Light Switch		27 %	12 %	12 %	Down
Bathroom Sink		40 %	59 %	59 %	Up
Bed Rail/Controls		13 %	38 %	38 %	Up
Bedside Table Handle		27 %	47 %	47 %	Up
Call Button		21 %	59 %	59 %	Up
Chair		73 %	24 %	24 %	Down
IV Pole (Grab Area)		43 %	62 %	62 %	Up
Room Inner Door Knob		7 %	41 %	41 %	Up
Room Light Switch		7 %	6 %	6 %	Down
Room Sink		33 %	35 %	35 %	Up
Telephone		53 %	71 %	71 %	Up
Toilet Bedpan Cleaner		33 %	47 %	47 %	Up
Toilet Flush Handle		47 %	29 %	29 %	Down
Toilet Seat		93 %	88 %	88 %	Down
Tray Table		93 %	75 %	75 %	Down
<b>Total Patient Bathroom</b>					
		44 %	45 %	45 %	Up
<b>Total Patient Room</b>					
		37 %	45 %	45 %	Up
<b>Grand Total</b>					
		40 %	45 %	45 %	Up
n=(# of objects evaluated)					
		253	283		

	< 70%
	70% - 80%
	> 80%

Include Baseline Results? yes, Dates between:2011-01-01 and 2012-09-26 Isolation Rooms?:All