

COMMERCIAL IN CONFIDENCE

# ZOONO<sup>®</sup>

**TRIALS AND DEVELOPMENT TEAM**



---

**LONDON UNDERGROUND - ZOONO ANTIMICROBIAL TREATMENTS  
TRIAL REPORT**

---

Report Number: 02/15

21 October 2015

COMMERCIAL IN CONFIDENCE

COMMERCIAL IN CONFIDENCE

**CONDITIONS FOR RELEASE**

Applicable to copies supplied with Zoono Holdings Limited approval to Commercial Organisations and Governments for official purposes.

1. This information is released by Zoono Holdings Limited to the recipient for Commercial purposes only.
2. This information must be accorded the same degree of commercial protection as that accorded by the commercial agreements between the recipient and Zoono Holdings Limited.
3. This information may be disclosed only with the express permission of Zoono Holdings Limited.

**SECURITY WARNING NOTICE**

THIS DOCUMENT IS THE PROPERTY OF ZOONO HOLDINGS LIMITED AND 'THE CLIENT' and is issued for the information of such persons only as need to know its contents in the course of their official duties. Any person finding this document should forward it to ZOONO HOLDINGS LIMITED, UNIT 15 Bunting Road, Bury St Edmunds, Suffolk, IP32 7BX with the particulars of how and where found.

COMMERCIAL IN CONFIDENCE

COMMERCIAL IN CONFIDENCE

**ZOONO HOLDINGS LIMITED**

Civilian Telephone: 0800 583 7 583

Mobile Telephone: 07852 922214

E-mail: james.milnes@zoono.com

Website: www.zoono.co.uk

---

**INTELLECTUAL PROPERTY RIGHTS**

This document is the property of Zoono Holdings Limited and 'The Client'. If any company or organisation wishes to use any information, drawings, design or concept described in it that company or organisation must, in the first instance, apply for the appropriate licence from Zoono Holdings Limited through:

**Mr James AJ Milnes**

**Zoono Holdings Limited, Unit 15 Bunting Road**

**Bury St Edmunds, Suffolk, IP32 7BX**

COMMERCIAL IN CONFIDENCE

**LONDON UNDERGROUND ZOONO ANTIMICROBIAL TREATMENTS TRIAL**

**Zoono Holdings Ltd TRIAL REPORT No: 02/15**

**CONCLUSIONS AND RECOMMENDATIONS**

Conclusions and recommendations of a practical nature in this report reflect the views of the Zoono Holdings Ltd. Recommendations regarding acceptance into service or scales of issue, however, reflect only the views of the Zoono Holdings Ltd Trials and Development Unit, and are subject to confirmation by the Trial Sponsor.

TRIALS OFFICER	:	Mr James Milnes
TRIALS STAFF	:	Mr James Milnes Mr Stuart Sullivan
TRIALS UNIT	:	Zoono Holdings Ltd
SPONSOR	:	London Underground Ltd
AUTHORITY	:	London Underground
DATES	:	27 August – 21 September 2015
LOCATION	:	Central Line Depot

Trials Officer:

James AJ Milnes  
Director  
Zoono Holdings Ltd

Date: 21 October 2015

COMMERCIAL IN CONFIDENCE

Distribution:

London Underground\*

COMMERCIAL IN CONFIDENCE

**CONTENTS**

1. Executive Summary	1
2. Aim of report	2
3. Overview of Zoono	2
4. Trial Operating process	2
5. The Equipment used in the trial	2
6. Trial staff	3
7. Climate conditions	3
8. Limitations	3
9. Locations	3
10. Trial Conduct	4
11. Baseline test	4
a) Results	4
b) Comments	4
12. 21 Day Trial point	5
a) Result	5
b) Comments	5
13. Conclusions and Recommendations	6
Annex A – Detailed test results	7-9

## 1. EXECUTIVE SUMMARY

Even with the population of London passing 8.6million the TfL's network has been increased along with improved reliability. In a recent statement by Boris Johnson he stated that:

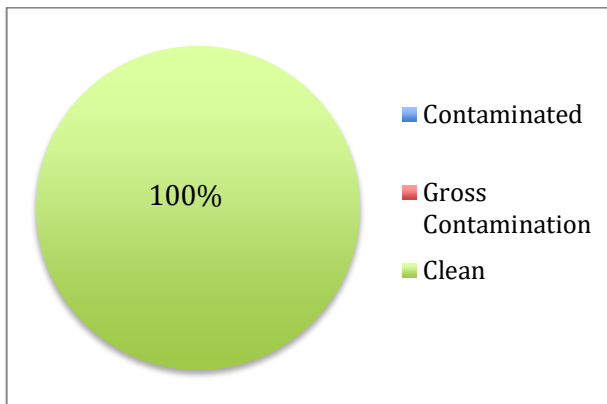
***"TfL has not been content just to do the same old job on a bigger scale. It is adapting and innovating to support a very different London".***

Whilst London underground maintains best practice for cleaning, high levels of bacteria and viruses, through normal usage, can still be present in between cleaning cycles.

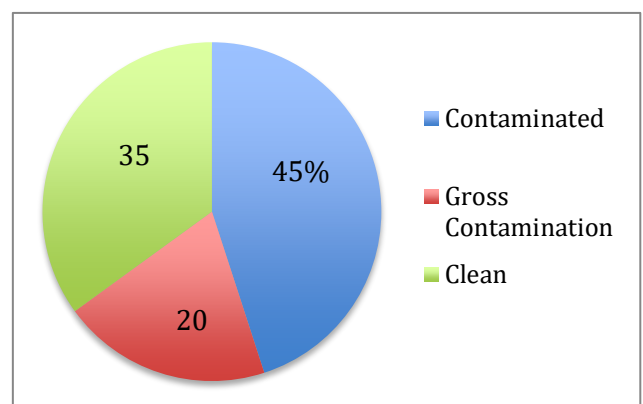
To mitigate its existing risks and to prepare for new and varied challenges, such as a predicted, long overdue, pandemic in the UK, the London underground wishes to explore new innovations that can add a further layer of protection to its existing cleaning programme.

The London underground invited Zoono Holdings Ltd to conduct 'live' trials of Zoono Microbe Shield. Zoono unlike other sanitizing products, creates an invisible coating that continuously kills bacteria for up to 30 days. The purpose of the test was to provide accurate and tangible results in order to assess its effectiveness as an antimicrobial treatment for potential use on the London Underground System.

Samples were taken and sent to an independent laboratory, Eurofins Wolverhampton Microbiology and Chemistry laboratory (UKAS registration number 0342), for analysis. The results of the test concluded that Zoono reduced the presence of bacteria by 100% over a 25 day period compared to untreated areas.



**Zoono treated – Results after 25 days**



**Non Zoono treated – Results after 25 days**

As well as being successfully tested in numerous accredited laboratories around the world Zoono has received the green light to supply Boeing/airbus and the NHS. It has just finished a successful trial within the Intensive care unit in a USA East Coast hospital (reduces pathogens by 80% over 24 hours) and a large corporate office (reducing absenteeism by 46%).

## LONDON UNDERGROUND – ZOONO ANTIMICROBIAL TREATMENT TRIAL

### Zoono Holdings Ltd TRIAL REPORT No: 02/15

#### MAIN REPORT

#### 2. Aim of trial

The aim of the trial was to assess the performance of the Zoono Antimicrobial capability against the current cleaning and sanitisation protocols. This would provide tangible results, which could be used to assess the applicability of the Zoono capability for protecting staff and customers utilising the London Underground.

#### 3. Zoono Overview

Zoono Antimicrobial Treatments represent the next generation in surface and hand sanitisers providing protection for up to 28 days on surfaces and 24 hours on the skin. According to the UK National Security Risk Register a (long overdue) Influenza Pandemic is one of the biggest threats to the United Kingdom. Coupled with the emerging threat of antibacterial resistant bacteria the importance of capabilities such a Zoono cannot be overstated enough. The unique way in which Zoono works ensures lasting protection whilst killing by lysis thereby mitigating the threat of bacteria becoming resistant to it.

#### 4. Operating Process. There are three functional areas where the Zoono Capability could be employed:

- a. Trains.
- b. Staff and Welfare.
- c. Infrastructure.

#### 5. The equipment used in the trial was as follows:

- a. **Zoono Microbe Shield.** Zoono Microbe Shield is a food safe, water based surface sanitiser that bonds to the surface to kill 99.99% of all common pathogens. Zoono Microbe Shield is easy to apply, is environmentally friendly and is safe to use around people, plants and pets alike.

Zoono Microbe Shield has been tested in numerous, accredited laboratories around the world and proven to be highly effective against a variety of pathogens including E.coli, Staph, Pseudomonas, H1N1, Salmonella, Norovirus and even the Hospital Superbugs MRSA, VRE and C-Diff. In addition, it has been proven to be highly effective against yeast, fungi and mould spores including the black mould (Stachybotrys). Zoono Group has over 100 Test Results on file confirming the efficacy and durability of Zoono products. Zoono Microbe Shield is easy to apply via a trigger pack and one application will normally provide durable surface protection for up to 30 days.



Zoono bonds to the target surface (both hard and fabric surfaces). It can be likened to millions of road spikes that attract and kill bacteria. Because it does not kill by toxicity



or poisoning, the organisms cannot adapt to it = No mutation = No superbugs. The result is an antimicrobial that cannot diminish in strength, cannot leave the surface to which it is applied and cannot encourage the organisms that it targets to become used to it.

- b. **London Underground trains.** The train stock used on the Central Line are of 1992 stock vintage. The dimensions of the carriages are: Length 16.25m, Width 2.62m, Height 2.87m.



- c. **Swab testing** - EU Legislation states “Sampling of the environment can be a useful tool to identify and prevent the presence of pathogenic microorganisms”. The environment can be contaminated with a variety of micro-organisms derived from various sources. Estimation of the overall numbers of bacteria (Aerobic Colony Count) present can provide useful information when assessing general hygiene. Environmental monitoring can also be used as part of routine inspections of premises or in an investigation of a suspected viral outbreak where surfaces are thought to be likely vehicles of cross-contamination.

Determination of the number of aerobic viable micro-organisms and the number of Enterobacteriaceae on a specified area of a surface can provide an indication of the whether a surface is contaminated.

The results will be expressed as colony forming units:

Ser	CFU Count	Result
1	<10	Clean
2	10-160	Contaminated
3	>160	Gross Contamination

6. **Trial Staff.** The trials staffs were drawn from Zoono Holdings Ltd Technical Expert Support Team (TEST) pool.

7. **Climatic Conditions.**

London Central Line Depot. The User Trial phase was conducted over 21 days from 27 August – 21 Sept 2015. Weather information was gathered from Zoono Holdings Ltd Metrological equipment. Temperatures ranged from 14°C to 27°C during the day and at night highs of 14°C and lows of 6°C.

8. **Limitations**

There were no limitations to the trial environments.

9. **Locations.**

The locations/touch points for the Trial were chosen in order that a varied yet fair representation of the rolling stock was tested. Moreover, the locations identified were those most likely to be touched by both staff and travellers.

## 10. Trial Conduct

Method/Concept. The method employed was to split the trial into two parts

### a) Baseline Tests

The plan was to take 40 samples in total from two trains (with two carriages each – drivers cab and driver motor carriage plus the adjacent non driving motor carriage) prior to cleaning in order to establish a baseline. Once tested all four carriages would then be cleaned by the normal cleaning staff. The Driver's cab and Driving Motor carriage would be treated with Zoono and the adjacent Non Driving Motor carriage would be left untreated.



### b) Follow up tests 21 days later

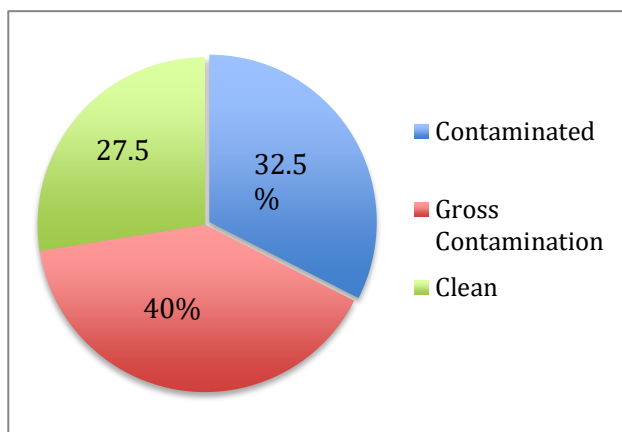
40 samples were to be taken overall, 20 from the Zoono carriages and 20 from the non-treated Zoono carriages. The purpose of which was to demonstrate the effectiveness or not of the Zoono product.

## 11. Baseline Test. (Prior to the application of Zoono)

This phase was conducted by Zoono Holdings Ltd's trials team at the London Underground Central Line depot. Two 'double-cab' carriages were selected by the London underground staff. Prior to the regular cleaning process the team identified 'touch points'/sampling locations within each cab. A total of 40 samples were recorded in accordance with the data sheets.

The samples were then sent to an independent laboratory, Eurofins Wolverhampton Microbiology and Chemistry laboratory (UKAS registration number 0342), for for analysis.

### a) Results



27 August - Overall Conatmination

### b) Comment.

The surfaces swabbed are not considered as surfaces that would naturally harbour bacteria and therefore any positive results mean that bacteria has been 'placed' on the surface by contact and cross contamination.

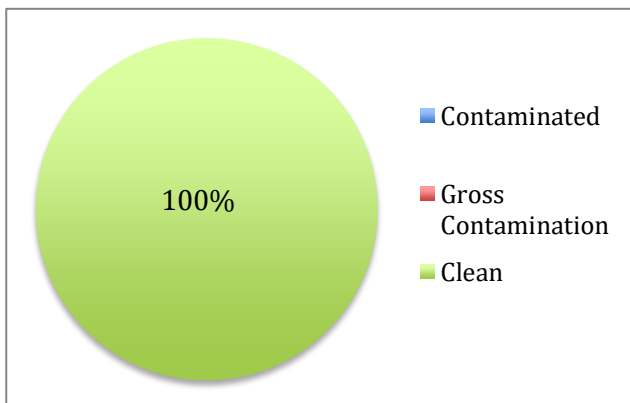
**12. 25-Day Trial Point.**

This phase was planned to be conducted at the 21-day point, as the carriages were returned to the depot as part of the cleaning cycle. However, due to circumstances outside of reasonable control the carriages could not be returned to the depot at the 21-day point. This portion of the Trial was subsequently undertaken at the 25-day point. Each of the 40 identified sampling locations were sampled again and the samples sent to Eurofins Wolverhampton Microbiology and Chemistry laboratory (UKAS registration number 0342)

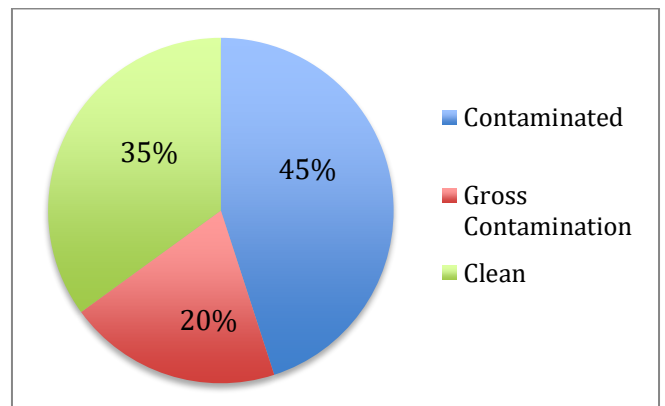


**a) 25-day results**

Data was collected, collated and assessed and then each data set was compared in order to see the effectiveness of Zoono.



**Zoono treated – Results after 25 days**



**Non Zoono treated – Results after 25 days**

**b) Comment.**

The results showed that the touch points treated with Zoono were kept clean for 25 days compared to the non-Zoono touch points. Whilst there were nil results on the presence of Coagulase Positive Staphylococcus and Enterobacteriaceae the high results which showed 27.5% of the touch points were clean and 72.5% were contaminated (32.5% were contaminated and 40% were Grossly Contaminated) suggesting that there is a very high probability of other pathogenic bacteria being present.

**13. Conclusion and comments**

The evaluation allows the following conclusions to be reached

- a. The Underground maintains best practice for cleaning.
- b. High levels of bacteria and viruses were present throughout the undergrounds existing cleaning cycle, during normal usage.
- c. The results of the test concluded that Zoono reduced the presence of bacteria by 100% over a 25 day period compared to untreated areas.
- d. Zoono can complement existing cleaning cycles and has shown to reduce staff absenteeism (for sickness) by up to 46%
- e. As well as being successfully tested in numerous accredited laboratories around the world, Zoono has received the green light to supply Boeing/airbus and the NHS.
- f. The Zoono product has just finished a successful trial within the Intensive care unit of a major USA East Coast hospital.
- g. The reduction in bacteria achieved on the interior of trains are of a similar order to those achieved in the Zoono treated intensive care unit of hospitals.

**DETAILED TEST RESULTS – 27 AUGUST 2015**

Analytical Report Code	Lab. Sample Number	Client code	Client Reference	Aerobic Plate Count	Coagulase Positive Staphylococcus	Enterobacteriaceae
AR-15-UD-301084-01	400-2015-60236300	1.1.1	HORIZONTAL GRAB RAIL	1500	<10	<10
AR-15-UD-301090-01	400-2015-60236309	1.10.1	TELEPHONE	<10	<10	<10
AR-15-UD-301091-01	400-2015-60236310	1.11.1	BUTTON LEFT DRIVER CAB	27	<10	<10
AR-15-UD-302475-01	400-2015-60236311	1.12.1	BIG RED BUTTON	11000	<10	<10
AR-15-UD-302472-01	400-2015-60236301	1.2.1	VERTICAL GRAB RAIL	82	<10	<10
AR-15-UD-301085-01	400-2015-60236302	1.3.1	GLASS SCREEN	100	<10	<10
AR-15-UD-301086-01	400-2015-60236303	1.4.1	DOOR - OPEN BUTTON	<10	<10	<10
AR-15-UD-301087-01	400-2015-60236304	1.5.1	HORIZONTAL GRAB RAIL	18	<10	<10
AR-15-UD-301088-01	400-2015-60236305	1.6.1	VERTICAL GRAB RAIL	<10	<10	<10
AR-15-UD-302473-01	400-2015-60236306	1.7.1	GLASS SCREEN	180	<10	<10
AR-15-UD-302474-01	400-2015-60236307	1.8.1	DOOR - OPEN BUTTON	<10	<10	<10
AR-15-UD-301089-01	400-2015-60236308	1.9.1	DEAD MANS HANDLE	<10	<10	<10
AR-15-UD-302476-01	400-2015-60236312	2.1.1	HORIZONTAL GRAB RAIL	<10	<10	<10
AR-15-UD-301092-01	400-2015-60236313	2.2.1	VERTICAL GRAB RAIL	18	<10	<10
AR-15-UD-302477-01	400-2015-60236314	2.3.1	GLASS SCREEN	210	<10	<10
AR-15-UD-302478-01	400-2015-60236315	2.4.1	DOOR - OPEN BUTTON	>30000	<10	<10
AR-15-UD-302479-01	400-2015-60236316	2.5.1	HORIZONTAL GRAB RAIL	220	<10	<10
AR-15-UD-302480-01	400-2015-60236317	2.6.1	VERTICAL GRAB RAIL	370	<10	<10
AR-15-UD-301093-01	400-2015-60236318	2.7.1	GLASS SCREEN	>30000	<10	<10
AR-15-UD-301094-01	400-2015-60236319	2.8.1	DOOR - OPEN BUTTON	<10	<10	<10
AR-15-UD-302482-01	400-2015-60236329	3.10.1	TELEPHONE	45	<10	<10
AR-15-UD-301095-01	400-2015-60236320	3.1.1	HORIZONTAL GRAB RAIL	45	<10	<10
AR-15-UD-302540-01	400-2015-60236330	3.11.1	BIG RED BUTTON	840	<10	<10
AR-15-UD-301103-01	400-2015-60236331	3.12.1	BUTTON CLOSE SALOON	<10	<10	<10
AR-15-UD-301096-01	400-2015-60236321	3.2.1	VERTICAL GRAB RAIL	<10	<10	<10
AR-15-UD-301097-01	400-2015-60236322	3.3.1	GLASS SCREEN	210	<10	<10
AR-15-UD-301098-01	400-2015-60236323	3.4.1	DOOR - OPEN BUTTON	27	<10	<10
AR-15-UD-301099-01	400-2015-60236324	3.5.1	GREY VENT	140	<10	<10
AR-15-UD-301100-01	400-2015-60236325	3.6.1	EDGE OF SEAT	190	<10	<10
AR-15-UD-302481-01	400-2015-60236326	3.7.1	GLASS SCREEN	27	<10	<10
AR-15-UD-301101-01	400-2015-60236327	3.8.1	OPEN DOOR BUTTON	190	<10	<10
AR-15-UD-301102-01	400-2015-60236328	3.9.1	DEAD MANS HANDLE	18	<10	<10
AR-15-UD-302483-01	400-2015-60236332	4.1.1	VERTICAL DOOR GRAB	91	<10	<10
AR-15-UD-302484-01	400-2015-60236333	4.2.1	HORIZONTAL GRAB RAIL	170	<10	<10
AR-15-UD-301104-01	400-2015-60236334	4.3.1	GLASS SCREEN	<10	<10	<10
AR-15-UD-301105-01	400-2015-60236335	4.4.1	OPEN BUTTON	2800	<10	<10
AR-15-UD-302485-01	400-2015-60236336	4.5.1	HORIZONTAL GRAB RAIL	110	<10	<10
AR-15-UD-301106-01	400-2015-60236337	4.6.1	VERTICAL GRAB RAIL	420	<10	<10
AR-15-UD-302486-01	400-2015-60236338	4.7.1	GLASS SCREEN	690	<10	<10
AR-15-UD-302487-01	400-2015-60236339	4.8.1	OPEN BUTTON	<10	<10	<10

**DETAILED TEST RESULTS – NON-ZOONO – 21 SEPTEMBER 2015**

Analytical Report Code	Lab. Sample Number	Client code	Client Reference	Aerobic Plate Count	Coagulase Positive Staphylococcus	Enterobacteriaceae
PR-15-UD-001899-01	400-2015-60257183	01	GLASS PANEL	30000	<10	<10
PR-15-UD-001900-01	400-2015-60257184	02	MIDDLE VERTICAL GRAB RAIL	<10	<10	<10
PR-15-UD-001901-01	400-2015-60257185	03	GREY HOT GRAB RAIL	<10	<10	<10
PR-15-UD-001902-01	400-2015-60257186	04	MIDDLE OPEN BUTTON	45	<10	<10
PR-15-UD-001903-01	400-2015-60257187	05	VERTICAL GRAB RAIL	27	<10	<10
PR-15-UD-001904-01	400-2015-60257188	06	HORIZONTAL GRAB RAIL	220	<10	<10
PR-15-UD-001905-01	400-2015-60257189	07	SIDE OPEN BUTTON	550	<10	<10
PR-15-UD-001906-01	400-2015-60257190	08	HORIZONTAL GRAB RAIL	45	<10	<10
PR-15-UD-001907-01	400-2015-60257191	09	VERTICAL GRAB RAIL	73	<10	<10
PR-15-UD-001908-01	400-2015-60257192	10	VERTICAL GRAB RAIL	<10	<10	<10
PR-15-UD-001909-01	400-2015-60257193	11	GLASS PANEL	<10	<10	<10
PR-15-UD-001910-01	400-2015-60257194	12	VERTICAL GRAB RAIL	5200	<10	<10
PR-15-UD-001911-01	400-2015-60257195	13	GLASS PANEL	3300	<10	<10
PR-15-UD-001912-01	400-2015-60257196	14	HORIZONTAL GRAB RAIL	82	<10	<10
PR-15-UD-001913-01	400-2015-60257197	15	VERTICAL GRAB RAIL	100	<10	<10
PR-15-UD-001914-01	400-2015-60257198	16	GLASS PANEL	<10	<10	<10
PR-15-UD-001915-01	400-2015-60257199	17	MIDDLE VERTICAL GRAB RAIL	<10	<10	<10
PR-15-UD-001916-01	400-2015-60257200	18	VERTICAL GRAB RAIL	110	<10	<10
PR-15-UD-001917-01	400-2015-60257201	19	HORIZONTAL GRAB RAIL	45	<10	<10
PR-15-UD-001918-01	400-2015-60257202	20	GREY HORIZONTAL GRAB RAIL	55	<10	<10

**DETAILED TEST RESULTS – ZOONO TREATED – 21 SEPTEMBER 2015**

Analytical Report Code	Lab. Sample Number	Client code	Client Reference	Aerobic Plate Count	Coagulase Positive Staphylococcus	Enterobacteriaceae
PR-15-UD-001919-01	400-2015-60257203	Z1	DEAD MANS HANDLE	<10	<10	<10
PR-15-UD-001920-01	400-2015-60257204	Z2	DRIVER SIDE BUTTON	<10	<10	<10
PR-15-UD-001921-01	400-2015-60257205	Z3	BIG RED BUTTON	<10	<10	<10
PR-15-UD-001922-01	400-2015-60257206	Z4	TELEPHONE	<10	<10	<10
PR-15-UD-001923-01	400-2015-60257207	Z5	HORIZONTAL GRAB RAIL	<10	<10	<10
PR-15-UD-001924-01	400-2015-60257208	Z6	VERTICAL GRAB RAIL	<10	<10	<10
PR-15-UD-001925-01	400-2015-60257209	Z7	GLASS PANEL	<10	<10	<10
PR-15-UD-001926-01	400-2015-60257210	Z8	OPEN BUTTON	<10	<10	<10
PR-15-UD-001927-01	400-2015-60257211	Z9	HORIZONTAL GRAB RAIL	<10	<10	<10
PR-15-UD-001928-01	400-2015-60257212	Z10	MIDDLE OPEN BUTTON	<10	<10	<10
PR-15-UD-001929-01	400-2015-60257213	Z11	GLASS PANEL	<10	<10	<10
PR-15-UD-001930-01	400-2015-60257214	Z12	MIDDLE VERTICAL GRAB RAIL	<10	<10	<10
PR-15-UD-001931-01	400-2015-60257215	Z13	HORIZONTAL GRAB RAIL	<10	<10	<10
PR-15-UD-001932-01	400-2015-60257216	Z14	VERTICAL GRAB RAIL	<10	<10	<10
PR-15-UD-001933-01	400-2015-60257217	Z15	MIDDLE VERTICAL GRAB RAIL	<10	<10	<10
PR-15-UD-001934-01	400-2015-60257218	Z16	GLASS PANEL	<10	<10	<10
PR-15-UD-001935-01	400-2015-60257219	Z17	MIDDLE OPEN BUTTON	<10	<10	<10
PR-15-UD-001936-01	400-2015-60257220	Z18	VERTICAL GRAB RAIL	<10	<10	<10
PR-15-UD-001937-01	400-2015-60257221	Z19	GLASS PANEL	<10	<10	<10
PR-15-UD-001938-01	400-2015-60257222	Z20	VERTICAL GRAB RAIL	<10	<10	<10