Microbiological AD evaluations for SEM

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Foreword

The Ambulance Services have become increasingly aware of the expectations of the general public and the responsibilities placed on them for the maintenance of standards related to surface hygiene. In addition, the environment within vehicles must remain free of potentially infectious pathogens that can affect patients and paramedic medical staff.

The Ambulance Services are a clear example of ongoing perseverance in maintaining standards of hygiene and disinfection inside their vehicles. When in service, ambulances are continually on the move, transporting many patients in any one day. They are clearly susceptible and potentially exposed to a high concentration of airborne and surface based pathogens from various sources, including the external environment itself and the transference of infectious patients. This demands a consistent and effective hygiene protocol that provides the highest levels of disinfection of the ambulance interior, specifically all areas of instrumentation, on an ongoing daily basis.

The (AD) Air Disinfection System supplied by Inov8 Science Limited reduces the airborne and surface microbial load to undetectable levels. The unit can be operational continuously without paramedics or patients having to evacuate the vehicle. The AD unit measures 40 x 20 cm. and it constantly emits a controlled flow of hydroxyl radicals which act on all bacteria, viruses and fungi in suspension, neutralizing and de-activating them and thus preventing cross infection.

About Inov8 Science

Inov8 is a dynamic company with a strong vision driving it forward. Inov8 is focused upon its unique understanding of atmospheric chemistry in general and the Hydroxyl Radical, in particular, to deliver products and services that ensure air disinfection and reduction in the risk of disease.

Inov8 embraces a unique blend of skills and resources, from a comprehensive understanding of microbiology and complex atmospheric chemistry through to key product design and engineering skills.

Dedicated scientists and engineers continue their work in this field and today, Inov8 has a range of disinfection applications and solutions tailored for different markets.

Inov8 Air and Surface Microbial Testing Services Solutions (ITD)

Inov8 Air and Surface Microbial Testing Solutions are tailored to meet all specific testing demands, providing the market with accurate confirmative, microbial counts.

Using state of the art equipment and the latest available technologies, ITD provides a wide range of indoor Microbial Air Quality testing. ITD focus on air testing in critical production areas, hospital surgical suites, pharmacies and critical patient care areas.

The Range of Services Include:

- Evaluation of Indoor Air Bioburden (TVC and Y&M)
- Assessment of Microbial Air Quality
- Assessment of Microbial Surface Contamination
- Specified Confirmed Test Results
- Expert Interpretation of Results

These services are especially targeted for environments where there is a need to:

- Assess decontamination and cleaning procedures.
- Conduct hygiene quality monitoring (Air, Surface and Hand).
- Conduct out-break/incident investigations (Air and Surface).
- Commission and carry out routine testing of operating theatres (HTM2025).

The Inov8 Microbial Testing Solutions are amongst the leading solutions available on the market and you can have complete confidence in our flexible, confidential and comprehensive air and surface solutions.

More information available at http://www.inov8.com

About Laboratorio de análisis Dr. Echevarne

The Laboratorio de análisis Dr. Echevarne was founded in 1958. Technical improvements and customer adaptability have been two important constants throughout their history, a fact that has made Laboratorios Dr. Echevarne one of the main and most advanced laboratories in Europe.

The first reference analyses in the history of the laboratory were hormonal detections, then an analytical novelty, and carried on from there to include other fields such as cytogenetics and toxicology, as complements to the classical laboratory areas of biochemistry, haematology, Immunology, and microbiology.

Echevarne has developed new departments such as Industry and Veterinary Medicine, with the aim to provide an integral service to companies and centers for which health control checks in employees were already being performed and which required further testing such as water, food or environmental analyses.

Quality Assurance is a priority of the laboratory, and a special unit, Quality Management was created for this purpose, managing the preparation of audits and certifications and the encouragement of a Total Quality policy throughout the institution.

In 1998, Laboratorio de análisis Dr. Echevarne was certified compliance with GLP's and EN ISO 9001:1994. Since then, the laboratory has offered its customers a service based on quality management.

Nowadays, the compliance with society and customer driven quality standards has generated a change in the managerial style. A Quality Management System is crucial to improve performance beyond the aims attained so far. The operational platform in which this system stands is Process management, based on a dynamic cycle which includes planning-doing-verifying-acting, and a Customer satisfaction oriented approach, which enables the laboratory to ascertain whether the service provided satisfies, and to what extent, the customer's requirements.

This new approach makes it possible from the Quality Management to provide the board of directors with the information needed to establish a solid base that considers the needs and interests of the different organizations and customers.

More information available at: http://www.echevarne.com

Objective

In cooperation with *Sistema d'Emergències Mèdiques S.A.* (SEM) and *Oxidoc Exclusivas S.L.*, the objective of the evaluation is to assess the effect of the Inov8 Air Disinfection (AD) Unit on the environmental microbial load in an in-service SEM advanced ambulance during the course of a typical day's use. Both air samples and surface samples have been taken in the assessment process, which involved three advanced ambulances having tests carried out in them over a period of 3 days. The analysis of both air samples and surface samples has been carried out at *Laboratorio de Análisis Dr. Echevarne*, an accredited laboratory within the EU. The analysis certificates are kept on file at Inov8 Science Ltd. and may be inspected upon request.

The Study Protocol

Location

SEM S.A. facilities in Hospitalet de Llobregat, Spain

Microbial Sampling

For each ambulance there were 9 sampling events, the sampling regime for each sampling event consisted of the following:

Air Sampling: Taken using a Spin Air sampler and 90mm pre-poured media (Blood Agar, SDA & Chloramphenicol Agar)

The uncertainty level with air sampling for microbial bioburden is very high and to increase the accuracy of the measurements each sampling event consisted of four samples of air (500 L, 200 L, 200 L, and 100 L) to highlight any inaccurate result(s) and to maintain consistent, meaningful measurements.

1. Sample 500L of Air on Blood Agar and again on SDA+Chm.
2. Sample 200L of Air on Blood Agar and again on SDA+Chm.
3. Sample another 200L of Air on Blood Agar and again on SDA+Chm.
4. Sample 100L of Air on Blood Agar and again on SDA+Chm.

Surface sampling: Using Rodac contact plates (TSA and SDA) sample from the same location.

1. Sample from a vertical surface - the wall adjacent driver hatch. (see appendix II)
2. Sample from a horizontal surface - the shelf above the AD. (see appendix II)

Sampling Schedule

Air and surface sampling was taken in line with the following schedule:

Day 1	Description	Day 2	Description	Day 3	Description
S1 – AD OFF	Taken immediately before ambulance going out on service for normal activities – no AD installed or running.	**S1 – AD OFF**	Taken immediately before ambulance going out on service for normal activities. AD units switched on immediately after for the day's service.	**S1 – AD OFF**	Taken immediately before ambulance going out on service for normal activities. Ad units switched on immediately after for the day's service.
S2 – AD OFF	Taken on ambulance's return from (12 hrs) service on Day 1 - no AD installed or running.	**S2 – AD ON**	Taken on ambulance's return from (12 hrs) service on Day 2, with AD installed and running.	**S2 – AD ON**	Taken on ambulance's return from (12 hrs) service on Day 3, with AD installed and running.
S3 – AD OFF	Taken after the ambulance has remained running in stationary ambulance for 60-90 minutes prior to normal overnight cleaning being carried out – no AD installed or running.	**S3 – AD ON**	Taken after AD has been running in stationary ambulance for a further 60-90 minutes after Sample S2, and prior to normal overnight cleaning being carried out. AD turned off overnight.	**S3 – AD ON**	Taken after AD has been running in stationary ambulance for a further 60-90 minutes after Sample S2, and prior to normal overnight cleaning being carried out. AD turned off overnight.

Results Data

The Laboratorio de análisis Dr. Echevarne have supplied the analysis for the environmental samples taken and the results for each sample. The number of samples for Air totalled 108 samples and for Surface samples totalled 54 samples. Each result was logged individually in a spread sheet, however due to the length and size of the spread sheet it is attached as an appendix to this document.

A summary table is provided overleaf. In order to produce this summary table some analysis has been carried out by Inov8 Science and based on our experience, some results have disregarded on the basis that some were outliers and outside of the normal range expected. Results have also been averaged where appropriate in order to provide more meaningful results.

Summary of Results

Ambulance Y05 (YI17)					
Total Viable Count (T.V.C)					
SAMPLE -AD STATUS	Day (1)	SAMPLE -AD STATUS	Day (2)	SAMPLE -AD STATUS	Day (3)
S1 START - AD OFF		**S1 START - AD OFF**		**S1 START - AD OFF**	
AIR (cfu/m³)	198	AIR (cfu/m³)	68	AIR (cfu/m³)	30
SURFACE (cfu/100 cm²)	58	SURFACE (cfu/100 cm²)	46	SURFACE (cfu/100 cm²)	2
S2 After 12 Hrs -AD OFF		**S2 After 12 Hrs w/ AD ON**		**S2 After 12 Hrs w/ AD ON**	
AIR (cfu/m³)	130	AIR (cfu/m³)	168	AIR (cfu/m³)	200
SURFACE (cfu/100 cm²)	52	SURFACE (cfu/100 cm²)	4	SURFACE (cfu/100 cm²)	16
S3 After 90 mins -AD OFF		**S2 After 90 min w/ AD ON**		**S2 After 90 min w/ AD ON**	
AIR (cfu/m³)	111	AIR (cfu/m³)	33	AIR (cfu/m³)	98
SURFACE (cfu/100 cm²)	34	SURFACE (cfu/100 cm²)	0*	SURFACE (cfu/100 cm²)	12
Below detection limits					

Ambulance Y01 (YM6)					
Total Viable Count (T.V.C)					
SAMPLE -AD STATUS	Day (1)	SAMPLE -AD STATUS	Day (2)	SAMPLE -AD STATUS	Day (3)
S1 START - AD OFF		**S1 START - AD OFF**		**S1 START - AD OFF**	
AIR (cfu/m³)	194	**AIR** (cfu/m³)	44	AIR (cfu/m³)	62
SURFACE (cfu/100 cm²)	54	SURFACE (cfu/100 cm²)	*	SURFACE (cfu/100 cm²)	20
S2 After 12 Hrs -AD OFF		**S2 After 12 Hrs w/ AD ON**		**S2 After 12 Hrs w/ AD ON**	
AIR (cfu/m³)	215	**AIR** (cfu/m³)	223	AIR (cfu/m³)	142
SURFACE (cfu/100 cm²)	124	SURFACE (cfu/100 cm²)	4	SURFACE (cfu/100 cm²)	110
S3 After 90 mins -AD OFF		**S2 After 90 min w/ AD ON**		**S2 After 90 min w/ AD ON**	
AIR (cfu/m³)	64	**AIR** (cfu/m³)	74	AIR (cfu/m³)	18
SURFACE (cfu/100 cm²)	76	SURFACE (cfu/100 cm²)	4	SURFACE (cfu/100 cm²)	34
Result disregarded as Horizontal sample was uncountable and vertical sample was non presentable					

Ambulance Y07 (YM8)					
Total Viable Count (T.V.C)					
SAMPLE -AD STATUS	Day (1)	SAMPLE -AD STATUS	Day (2)	SAMPLE -AD STATUS	Day (3)
S1 START - AD OFF		**S1 START - AD OFF**		**S1 START - AD OFF**	
AIR (cfu/m³)	241	AIR (cfu/m³)	57	AIR (cfu/m³)	13
SURFACE (cfu/100 cm²)	26	SURFACE (cfu/100 cm²)	78	SURFACE (cfu/100 cm²)	12
S2 After 12 Hrs -AD OFF		**S2 After 12 Hrs w/ AD ON**		**S2 After 12 Hrs w/ AD ON**	
AIR (cfu/m³)	172	AIR (cfu/m³)	113	AIR (cfu/m³)	139
SURFACE (cfu/100 cm²)	102	SURFACE (cfu/100 cm²)	4	SURFACE (cfu/100 cm²)	106
S3 After 90 mins -AD OFF		**S2 After 90 min w/ AD ON**		**S2 After 90 min w/ AD ON**	
AIR (cfu/m³)	78	AIR (cfu/m³)	50	AIR (cfu/m³)	76
SURFACE (cfu/100 cm²)	76	SURFACE (cfu/100 cm²)	4	SURFACE (cfu/100 cm²)	106

On a number of occasions during days 2 & 3 the crews took actions that may have affected the results of samples that were subsequently taken. Any such User Interventions will be referred to in the text, please see appendix I.

The Analysis of the Results

1. Comparison of Total Viable Counts (TVC) at end of Service with and without AD operating:

Day 1: No AD running:

	T.V.C	
	Air	**Surface**
AD **OFF:** Vehicle on return from Service	172	93

- Reading averaged from all ambulances on return from service on Day 1

Day 2: AD Operating throughout Service, Ambulance Y07 (YM8):

	T.V.C	
	Air	**Surface**
AD **ON:** Vehicle on return from Service	113	4

% Reduction	T.V.C	
	Air	**Surface**
	34%	95%

- Only on Day 2, Ambulance Y07 (YM8) had no user interventions (action by crew member that may have affected sampling result). This case was taken as a reference for an ideal condition.

2. Comparison of TVCs on return from service and after a further 90 minutes of AD operating in stationary vehicle:

Air		On return from service	168	200	223	142	139	113
		AD On - After 90 mins further operation	33	98	74	18	76	50
	%	**Reduction**	**80%**	**51%**	**67%**	**87%**	**45%**	**56%**
Surface		On Return from service	4	16	4	110	106	4
		AD On - After 90 mins further operation	0	12	4	34	106	4
	%	**Reduction**	**100%**	**25%**	**N/A**	**69%**	**N/A**	**N/A**

- Actual readings from ALL ambulances at close of service and from second sample taken 90 minute later during the operation of the AD (Please refer to Summary of Results table). Results from surfaces are compared to a low bioburden baseline. The low bioburden was achieved by the AD during 12 hours service operation.

3. Comparison of bioburden at start of sampling - AD OFF (morning of Day 1, Day 2 and Day 3)

	T.V.C	
Day 1	**Air**	**Surface**
Vehicle before start of Service	211	46
Day 2		
Vehicle before start of service	**56**	**62**
Day 3		
Vehicle before start of Service	35	11
% Reduction (overall Day 1 to 3)	**84%**	**46%**
% Reduction due to the AD	**38%**	**82%**

- Averages taken from all Air and Surface readings for each Day 1, 2 & 3. Reductions due to the AD are related to the operation of the AD during the previous night (with and without user interventions).

Conclusions

1. Comparing the Total Viable Counts of airborne pathogens (TVCs) at the end of Service with and without an AD operating shows an overall reduction in Air TVCs of 34% and of surface TVCs of 95%. This indicates that the Inov8 AD has a beneficial effect on both airborne pathogens and those found on surfaces within an ambulance during the normal daily service.

2. Comparing the TVCs for each ambulance on return from Service with those after a further 90 minutes of operation of the AD shows significant reductions in the airborne bioburden as well as reductions in levels of surface contamination. The maximum reduction of Air microbial load after the 90 minutes period was 87% and 69% for surfaces.

3. Comparing the TVCs for at the beginning of each day shows an incremental decrease in the levels of both airborne and surface contamination. The overall reduction from Day 1 to Day 3 was 84% for air and 46% for surfaces. However, the actual reduction during the use of the AD in day 2 shows 38% for air and 82% for surfaces.

   It should be noted that during this period, normal overnight cleaning of the ambulances was not done because of the timing of the sampling process, yet the contamination levels have been reduced to well below those found after normal cleaning.

Discussion

The Ambulance Service provides for an extremely dynamic environment and there are many variables which may influence the results of analysing the microbial load in such an environment. The use of 3 separate ambulances over a 2 day period gave us the opportunity to 6 sets of data from which to take data.

Whilst the result obtained and which are summarised in the conclusions above, the reductions obtained when comparing the Total Viable Counts of airborne micro-organisms (TVCs) at the end of Service with and without an AD operating were, at 34%, lower than expected. Unfortunately, because of the odours reported by the crews, particularly on the 3rd day, the results of samples were compromised.

The period of 90 minutes of AD operation, after the return of the ambulance, was more controlled. Therefore, the results achieved were able to show an over all reduction of Microbial load up to 87%.

For reference, Inov8 Science have previously carried out trials in the Ambulance environment with the Northern Ireland Ambulance Services (NIAS) in Belfast. The trial was aimed to measure the Microbial load after four hours of continuous operation of the AD. The results showed that, after the introduction of the Inov8 AD, the suspended microbial load inside the Ambulance had been reduced by 91%. As a comparison, this is in line with the results achieved in these trials after shorter exposure periods (90 minutes) of AD operation (i.e., 87%).

Notes:

Note 1:	TVCs (total viable counts) are measured in cfu/m^3 for air samples and cfu/100cm^2 for surface samples
Note 2:	Results from experiments with user interventions at Ambulances Y05 (YI17), Y01 (YM6) and Y07 (YM8) on Day 2 and Day 3 have been disregarded (i.e., opening windows and turning off the AD).
Note 3:	The three Ambulances were not cleaned throughout the 3 days period (cleaning normally takes place overnight)
Note 4:	We are aware that some concerns were raised by the ambulance crews during the Trials, and steps are being taken to deal with these. The results of this exercise will Be communicated in a separate report.

Appendix I:

Recorded events which occurred during the 3 day trial period and their potential effects:

Day 1

- None noted

Day 2

- Y05 (YI17): Window opened during service. AD turned off 40 min before returning to the base.
  AD turned on and left it running for 30 min before taking sample S2
- Y01(YM6): Staff opened the side door window throughout the 12hr service
- Y07(YM8): No user interventions reported

Day 3

- Y05(YI17):: Staff left the side door window open during service
- Y01(YM6):: Staff turned the AD off 1hr after leaving the base.
  AD turned on and left it running for 30 min before taking sample S2
- Y07(YM8): Staff kept the side door window open throughout the day

Notes:

- An open side window in the vehicle during service is likely to allow microbe-carrying particles to enter the Ambulance and affect the level of bioburden, distorting the results of the sampling

Appendix II:

Location of Surface sampling: