RESEARCH REPORT
PUBLIC HEALTH/WORKER SAFETY

Title: Evaluation of the electrostatic particle ionization technology to decrease the risk of zoonotic infections. Identification **NPB# 13-025**

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Industry Summary:

Influenza A virus (IAV) and *Staphylococcus aureus* (S. aureus) are important swine pathogens able to transmit via aerosol and with potential to affect human health. The electrostatic particle ionization system (EPI) is a technology able to reduce particles from the air and, as a result, it improves air quality and potentially the risk of pathogen spread. The objective of this study was to determine the effect of the EPI system on IAV and S. aureus in aerosols generated under experimental conditions. We also evaluated placing the EPI line at various distances to the ground and the effect of relative humidity (RH) on the system particle removal efficiency. Aerosols were generated artificially and sampled using both a cyclonic air sampler and an Andersen cascade impactor able to separate particles into different sizes. Air samples were collected with the EPI system "off" and "on" for 30 minutes with the EPI line connected at 1, 2 and 3 meters from the ground. Our results indicate that the EPI system was effective at reducing the levels of IAV and S. aureus found in the air. Under the conditions of this study, relative humidity did not affect the efficiency of the EPI system and reduction levels were greater for both pathogens when the EPI line was installed at 3 m from the ground which also corresponded to the closest distance to the aerosol source. In summary, the EPI system has

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tential to reduce exposure of zoonotic agents to producers and swine workers and improve the overall health and well being of pigs and people.

Keywords: Influenza virus, *Staphylococcus aureus,* aerosols, air sanitation, electrostatic particle ionization, EPI

Scientific Abstract:

Influenza A virus (IAV) and *Staphylococcus aureus* (S. aureus) are important swine pathogens able to transmit via aerosols. Both of them are considered important zoonotic agents of important public health concern. The electrostatic particle ionization system (EPI) is a technology able to reduce airborne particles because of its ability to clump and settle the particles. As a result it improves the air quality and has the potential to decrease the risk of disseminating pathogens. The objectives of this study were to determine under controlled conditions the effect of the EPI system on the quantity and viability of IAV and S. aureus in experimentally generated aerosols. We also assessed whether the distance to the source of ions and the relative air humidity had an effect on EPI's pathogen removal efficiency. The EPI system, consisting of a line of stainless steel corona points attached to a stainless steel cable (30KV), was installed at 3 different levels (1, 2 and 3 meters) along the length of a 35.1 m^3 isolation unit at the University of Minnesota. Aerosols were generated using a Collison nebulizer and sampled using both a cyclonic air sampler and two Andersen cascade impactors able to separate particles as a function of size. Air samples were collected with the EPI system "off" and "on" for 30 minutes. Three replicates were performed with the EPI line connected at 1, 2 and 3 meters from the ground. Samples were analyzed quantitatively by quantitative RRT-PCRs in the case of IAV, and bacterial culture (colonies forming units (CFUs)) in the case of S. aureus. Difference in the quantity of pathogens with the system "off" and "on" and the removal efficiency by particle size were calculated for both agents during the study. The effect of RH was tested at 30% and 70% in an environmentally controlled chamber. Both IAV and S. aureus could be found associated to all particle size ranges

measured in the study, which included ranges of 0.3 to 10 microns for IAV, and 0.7 to and 9 microns for S. aureus. Overall, reduction levels were greater for both pathogens when the EPI line was located at 3 m from the ground, which also corresponded to closer proximity to the aerosol source. Reduction levels between the system “off” and “on” ranged, between 0.56 and 2.58 logs per m3 of air for IAV, and 0.62 and 1.35 logs CFUs/m3 of air for S. aureus. There were no differences in the EPI removal efficiency of IAV at 30% and 70% relative humidity. In summary, our results indicated that the EPI system was effective at reducing IAV and S. aureus in aerosols generated experimentally and that the level of reduction was influenced by the location of the EPI line, the type of pathogen but not the conditions of relative humidity. In summary, the EPI system has potential to reduce exposure of zoonotic agents to producers and swine workers and improve the overall health and well being of pigs and people.

Introduction:

Zoonotic infections are a continuous threat to public health, and a more specific concern for people working with animals. Both, influenza A virus (IAV) and *Staphylococcus aureus* (S. aureus) are important zoonotic agents transmitted by aerosols, and among all the routes of transmission, preventing or mitigating aerosol transmission is by far the most difficult. Although the risk of aerosol exposure to people is poorly understood, it is self evident that if there is a high concentration of zoonotic agents in the air of work environments such as pig farms, the risk of transmission to personnel will be significantly increased.

In the case of IAV, the airborne route has been shown to play a role in viral spread [1, 2]. Aerosol transmission of IAV has been reported in humans [3, 4], mice, guinea pigs, ferrets and chickens [5-9]. More recently, IAV was detected in aerosols generated from infected pigs vaccinated for IAV [10], pigs with passive immunity [11] and IAV was isolated from the inside and outside air of commercial swine farms [12]. Overall, evidence is building on the risk of IAV aerosol transmission in pigs.

Recognition that domestic animals can act as reservoirs of MRSA has occurred concurrently with a rise in incidence of community acquired MRSA infections, prompting concerns about the

importance of non-human species in the changing epidemiology of MRSA in people. Since the initial implication of pigs in MRSA cases in Holland, studies from several countries have shown the MRSA occurs commonly in the nasal cavities of pigs and people who are occupationally exposed to pigs [13-15]. It is also reported that S. aureus is the predominant bacterial species in bioaerosols from swine barns [16]. Although the true zoonotic potential of S. aureus of swine origin is still uncertain, the common presence of this organism in commercial barns and farm workers, and their potential zoonotic significance, make S. aureus an appropriate model organism for evaluating air sanitation technologies.

The concept of sanitizing the air to decrease the risk of infections is not new. Air sanitation technologies have been applied in human hospitals, households, agricultural industries and in other environments to decrease the pressure of infection and the risk of pathogen dissemination [17-19]. More recently the EPI system (Electrostatic Particle Ionization) initially pioneered by the USDA and developed commercially and patented by Baumgartner Environics Inc, Olivia, Minnesota, USA has been installed in some swine facilities to improve the air quality and production performance. This technology is based on the principle of negative air ionization which transfers strong negative electrostatic charge to dust and microorganisms. As a result the charged particles settle on surfaces and are removed from the air. By removing airborne dust, it is reasonable to expect a potential reduction of airborne pathogens such as IAV and S. aureus.

Objectives:

The overall goal of this project was to evaluate a commercially available air sanitation technology, the EPI system, as a novel tool to decrease the risk of two important airborne zoonotic agents, IAV and S. aureus.

The specific objectives were:

1. To determine, under controlled conditions, the effect of the EPI system on the quantity and viability of influenza virus in experimentally generated aerosols

2. To determine the effect of the EPI system on the quantity and viability of *S. aureus* in experimentally generated aerosols.

Materials & Methods:

i. System set up and testing protocol

The study was performed at the University of Minnesota BSL-2 research animal units, St. Paul, MN. The isolation room was filtered, equipped with mechanical negative ventilation system of 0.11 inches of water, and had a total air space of 35.1 m^3. Environmental conditions of relative humidity and temperature were monitored at all times.

Aerosols were generated in the isolation room as described below and air was sampled for 30 minutes with two air samplers (cyclonic air collector and Andersen cascade impactor) with the EPI system "off" and "on". Measurements of ion concentrations generated by the EPI system were also taken by an ionizer performance analyzer at the point of air sample collection.

The efficacy of the EPI system was tested in a range of operating conditions including 3 distances of 1, 2 and 3 meters to the ground and 2 different relative humidity levels (30% vs 70% RH). This latter measurement was carried out in an environmental controlled chamber, where environmental conditions of temperature and humidity were controlled and recorded throughout the sampling.

To determine the viability of the air samples containing IAV, a bioassay consisting of inoculating susceptible piglets with the air samples was performed.

ii. Influenza virus and *S. aureus* aerosolization

A 10^6 tissue culture infective dose per ml (TCID50/ml) of isolate A/Swine/Iowa/00239/2004 H1N1 previously used in transmission studies [11] and known to be excreted in aerosols by infected pigs was used to generate aerosols. In order to create a bacterial aerosol, a 10^8 CFU/ml of a S. aureus strain recovered from pigs and of similar genotype to a strain recovered simultaneously from the nasal cavity of personnel working with pigs as part of a NIOSH study by Davies et al., was cultured using agar blood plates. In both cases, the aerosols were generated continuously using a 6-jet

Collison nebulizer (CN60, BGI, Inc) at a constant rate throughout the duration of each replica for a total of 60 minutes per replica approximately. The nebulizer was located on a wooden platform attached to the north wall of the room, beside the air inlet at 2.8 m height from the ground. Aerosols were produced at a rate of 1.1 ml/min and, according to the nebulizer manufacturer specifications creating aerosol particles with a median aerodynamic diameter of 1 to 3 μm [20].

iii. EPI system

The EPI system, consisting of stainless steel corona points attached to a stainless steel cable, was installed along the length of the room. Corona points were suspended by a wooden frame at 1, 2 and 3 meters from the floor and energized with about 30,000 volts of electricity at a low current of 2mA by a specially designed corrosion resistant power supply. The power supply was mounted inside the room and operated on common 110 volt, 50-60 Hz electric service. The system was connected and disconnected ("on" and "off") releasing while connected a large quantity of negative ions that interacted with airborne particles making them cluster and forcing them to settle onto surfaces.

iv. Sampling procedures

Aerosols were collected with the system "off" and "on", as previously described, using both a liquid cyclonic collector (Midwest Micro-tek, Brookings, SD, USA)[21] and an Anderson Cascade Impactor (ACI; Thermo Electron Corporation, Waltham, MA, USA)[22] able to capture size-selective samples of aerosolized virus or bacteria. The 2 air samplers were located 20 cm raised from the floor level. Both air collectors were separated 90 cm apart.

Sample collection using the cyclonic air collector, which was capable to process 200 l of air per minute, was carried out for 30 min. Ten milliliters of MEM (minimum essential media) supplemented with 4 % of bovine serum albumin were used as collection media. After collection, an average of 4 ml of sample was recovered, divided into 2 aliquots, and stored at -80 ℃. The collector was then disinfected with 70 % ethanol, rinsed with distilled water and dried with paper towels.

After disinfection, the collection vessel and the turbine were swabbed, and samples were processed and stored at -80℃ until analysis.

Influenza samples with the ACI (able to sample air at 28.3 l/min and separate particles into 8 stages with particle size diameter cut-points of 9.0, 5.6, 4.7, 3.3, 2.1, 1.1, 0.7, and 0.4 micrometer) were collected for 30 minutes. As described by Appert et al. (2011), samples from this device were eluted from every plate stage using a cell scraper and 1 ml of MEM. All samples were transferred into 1.5 ml sterile plastic tubes, placed on ice and stored at -80℃ until testing.

Air samples for S. aureus were taken using a 6 staged viable ACI that replaced the aluminum plates for Columbia-CNA agar plates able to capture bacteria associated to particles with size diameter cut-points of 5.6, 4.7, 3.3, 2.1, 1.1 and 0.7 micrometer. After each sampling, plates were removed, inverted in their covers, incubated for a minimum of 12 hours (12-18 hours) at 37℃ and colony forming units (CFUs) were counted as previously described [23, 24]. After each sampling, the ACI was dissembled and plates and stages were scrubbed and disinfected with alkyl dimethyl benzyl ammonium chloride soap (Lysol, Reckitt Benckiser) and finally rinsed and dried with paper towels.

v. Relative humidity (RH) test

For the purpose of evaluating the effect of RH on the EPI system performance, an environmentally controlled chamber located at Mechanical Engineering Department of the University of Minnesota was utilized [25]. The chamber consisted of a 1.95 m (width) by 1.95 m (depth) by 1.45 m (height) environmental chamber. Equipped with a frontal acrylic door and Hypalon gloves, instruments were safe to manipulate and retrieve from the chamber during and after each of the tests. To control the temperature inside the chamber, walls and floor were constructed using plate heat exchangers attached to aluminum plates. A water heater and a water chiller were connected to adjust temperature of the working fluid. Completely insulated and HEPA filtered, air flow, negative pressure, temperature and relative humidity were monitored and recorded at all times. In order to generate IAV aerosols, a 6-jet Collison nebulizer was utilized as previously described and aerosols

were sampled using an ACI for 15 minutes with the EPI system “off” and “on”. RH conditions were set at 30% and 70% and there were three replicates per each of the RHs.

vi. Viability procedures

To assess the infectivity of the air samples for IAV, a bioassay consisting of inoculating susceptible piglets with the air samples containing IAV was performed. Briefly, six 10-day-old pigs from an influenza-negative farm were purchased and each pig allocated to a separate isolation room. On arrival pigs were bled, nasal swabbed and confirmed negative by IAV RT-PCR. Inoculation material per pig consisted of a pool of 3 air samples containing the collection media of the cyclonic air collector with the system “off” and another pool of samples with the system “on”. Each pig was intranasally and intratraqueally inoculated with 1 ml of the air sample solution. Pigs were bled and nasal swabs collected 2 and 4 days post inoculation. All pigs were euthanized 4 days post-exposure by injection of 2 ml of pentobarbital (Fatal-Plus®, 100 mg/kg IV) into the external jugular vein. Viability of S. aureus was assessed by culture as described below.

vii. Laboratory tests

Air and nasal swab samples from the IAV trial were tested for quantitative IAV RRT-PCR as previously described [21, 26]. RRT-PCR Ct values <35 were considered positive, Ct between 35 and 40 were considered suspect, and Ct > 40 were considered negative. Columbia-CNA agar plates were incubated for a minimum of 12 hours (12-18 hours) at 37℃ for S. aureus growth and colony forming units (CFUs) were then counted as previously described [23, 24].

viii. Statistical analysis

Data from the PCR results, number of CFUs, type of air sampler, replicate, distance of the EPI system to the ground and type of pathogen were consolidated in a spreadsheet (Microsoft EXCEL; Microsoft Corporation, Redmond, Washington, USA) and organized for analysis. Means, standard deviations, minimum and maximum values for quantitative variables, and frequency counts and

percentages for qualitative variables were calculated for descriptive analysis. Differences in the quantity of pathogens per volume of air sampled (m^3) with and without the EPI system associated to the different particle sizes measured by the ACI were assessed for significance using a regression model in SAS 9.3 (SAS Institute, Cary, North Carolina, USA). Removal efficiency of IAV and *S. aureus* by the EPI system was calculate for each of the agents. Removal efficiency was defined as initial concentration of aerosolized virus or bacteria with the EPI system off minus final concentration with EPI system on divided by initial concentration.

Results:

Objective 1. Effect of the EPI system on the quantity and viability of influenza virus in experimentally generated aerosols.

i. Virus challenge and concentration of IAV with the EPI system "off" and "on"

A total of 144 ACI air samples and 18 cyclonic collector air samples were analyzed by IAV RRT-PCR. The estimated mean RNA copies per m^3 of air measured by the ACI ranged between $1.33x10^2$ to $1.21x10^4$ with the EPI system "off" and from 0 to $3.0x10^2$ with the EPI system "on". The estimated mean RNA copies per m^3 of air measured by the cyclonic collector ranged between $6.7x10^3$ to $2.6x10^4$ with the EPI system "off" and from $7.8x10^3$ to $3.0x10^4$ with the system "on". Negative controls tested negative. Viral solution used in the nebulizer had an average mean copy of $1.53x10^8$ RNA copies IAV/m^3 (positive control).

Removal efficiency of the EPI system by distance of the EPI line to the ground and measured by the ACI can be seen in Figure 1 (green bars). Removal efficiency was greater when the EPI line was located at 3 meters from the ground and closer to the source of aerosols.

Effect of the EPI system on the quantity of IAV for each of the particle sizes analyzed is shown in Figure 2. Results are shown in RNA copies/m^3 of air with the system "on" (red bars) and system "off" (blue bars). IAV could be found in all particle size ranges with the system "off" and in most of the size ranges with the system "on" except for particle sizes of 5.8 to 10μm at 2m distance and for particle

sizes of 4.7-5.8 and 9.0-10.0 μm at 3m distance. There was a reduction in the number of RNA copies/m^3 with the system "on" and this reduction was greater at 3 m distance of the EPI line to the ground. The predicted reduction difference per stage between the system "off" and "on" by distance and using the data from the ACI collector is shown in Figure 3. The results indicated a total reduction between 0.56 logs (size of particles between 2.1 and 3.3 μm) and 2.58 logs (size of particles between 3.3 and 4.7μm) at 3 m distance of the EPI line to the ground.

ii. Relative humidity test

Figure 4 summarizes the effect of RH on IAV RRT-PCR results with the EPI system "off" and "on". Results are shown in RNA copies/m^3 of air with the system "on" (red bars) and "off" (blue bars). There was a decrease in the number of RNA copies/m^3 with the system "on" under the 2 different RH scenarios. A greater reduction was observed under 70% RH. However, this removal efficiency was not statistically significant between the 2 RH levels as shown in Figure 5.

iii. Viability procedures in aerosol samples

Nasal swabs' and serum samples' PCR results from the bioassay procedure were negative for cyclonic collector air samples with the system "off" and "on". Despite these results, further studies are needed and additional virus isolation is in progress.

S. aureus was cultured from samples collected with the EPI system "on" as described below. These results indicated that the EPI system was not effective at completely eliminating S. aureus from the air.

Objective 2. Effect of the EPI system on the quantity and viability of *S. aureus* in experimentally generated aerosols.

A total of 108 plates collected using the viable ACI were incubated and analyzed. The estimated mean of CFUs per m^3 of air measured by the ACI ranged between $1.88x10^1$ and $2.87x10^3$ with the EPI system "off" and from 0 to $1.43x10^3$ with the system "on". Plates incubated with the air samples collected with the cyclonic collector had too many CFUs to count and results are not reported. Negative controls tested negative.

Removal efficiency of the EPI system on S. aureus by distance of the EPI line to the ground measured by the ACI can be seen in Figure 1 (blue bars). Removal efficiency was greater when the EPI line was located at 3 meters from the ground and closer to the source of aerosols.

Effect of the EPI system on the quantity of bacteria for each of the different particle sizes analyzed is shown in Figure 6. Results are shown in CFUs/m^3 of air with the system "on" (red bars) and system "off" (blue bars). S. aureus could be found in all particle size ranges with both the system "off" and "on". There was a reduction in the number of CFUs/m^3 with the system "on" and this reduction was greater at 3 m distance of the EPI line to the ground. The predicted reduction difference per stage between the system "off" and "on" by distance using the data from the ACI collector is shown in Figure 7. The results indicated a total reduction of 0.62 logs (size of particles between 0.7 and 1.1 μm) to 1.35 logs (size of particles between 4.7 and 5.8 μm) at 3 m distance of the EPI line to the ground.

Discussion:

Our results indicate that the EPI system was effective at reducing the levels of IAV and S. aureus found in the air. Under the conditions of this study, reduction levels were greater for both pathogens when the EPI line was located at 3 m from the ground, which also corresponded to closer proximity to the aerosol source. Reduction levels or concentration differences between the system "off" and "on" ranged between 0.56 and 2.58 logs per m^3 for IAV, and 0.62 and 1.35 logs of CFUs for S. aureus. Both IAV and S. aureus could be found in all particle size ranges measured in the study which included ranges of 0.3 to 10 microns for IAV, and 0.7 to 9 microns for S. aureus. The EPI system was able to reduce IAV and S. aureus in all particle size ranges but this reduction was only significant when the EPI system was located at 3 m from the ground.

Under the conditions of this study we did not find a significant effect of humidity on the effectiveness of the EPI system at reducing IAV.

In summary, our results indicate that the EPI system is effective at reducing IAV and S. aureus aerosols but the level of reduction vary based on the location of the EPI line and the type of pathogen. Thus, the EPI system has potential to reduce exposure of zoonotic agents to producers and swine workers and improve the overall health and well being of pigs and people.

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Figures

Figure 1. Total removal efficiency of the EPI system for IAV and S. aureus based on distance of the EPI line to the ground. Results are the average of efficiencies from 3 replicates measured with the ACI sampler with error bars representing ± the standard error of the mean.

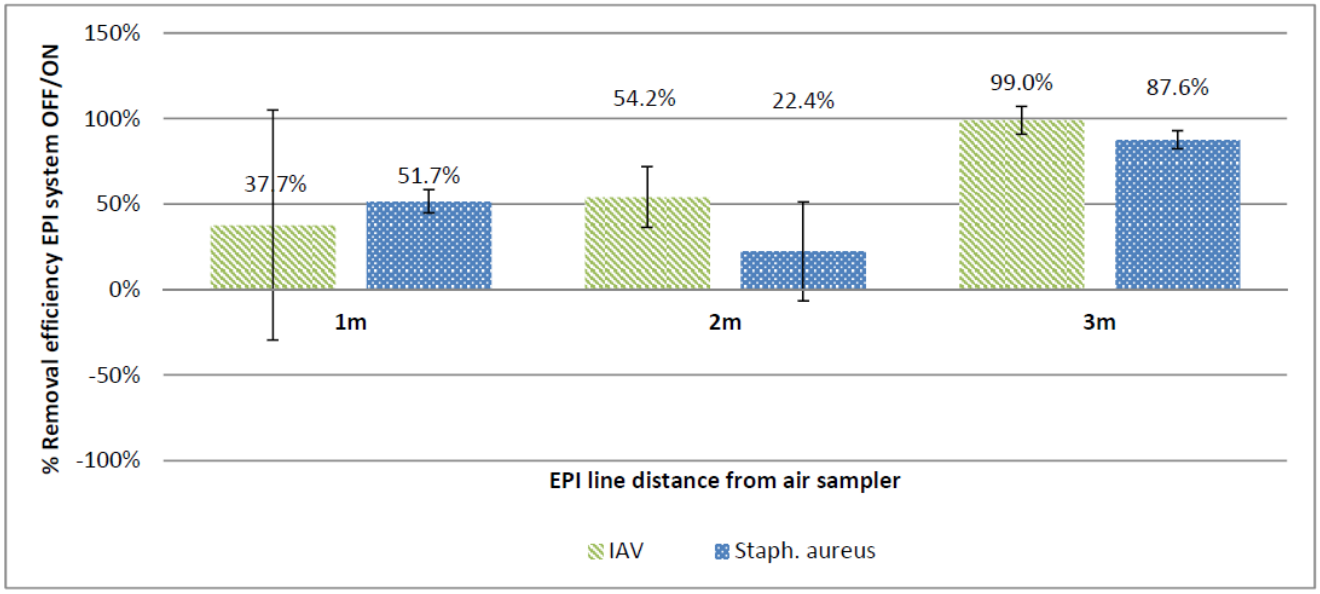


Figure 2. Concentration of RNA copies of influenza per m^3 of air with the EPI system 'off' and 'on' as a function of particle size and distance of the EPI line to the ground.

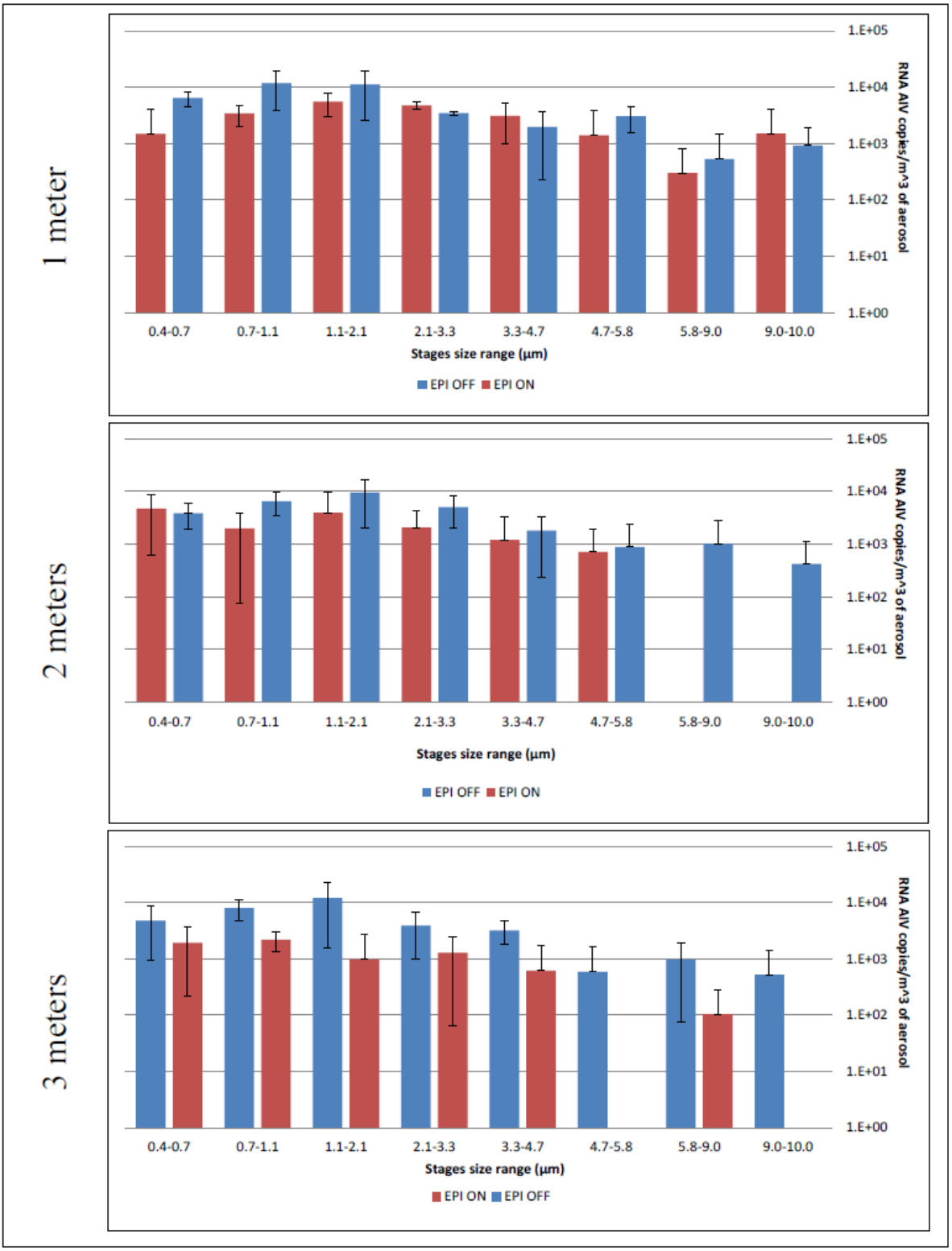
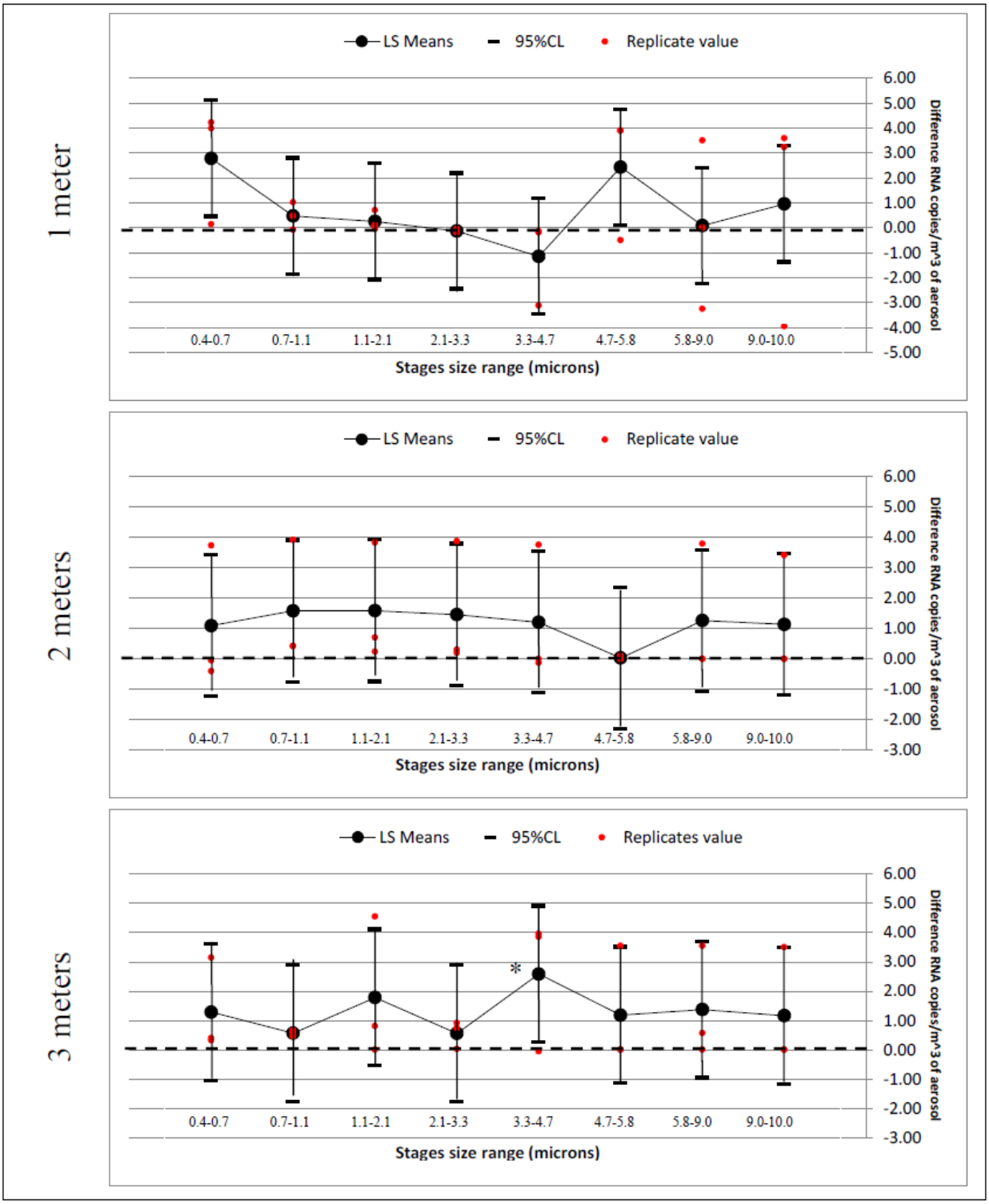


Figure 3. Linear regression modelling for total reduction of IAV aerosols with the EPI system "on". LS Means of concentration difference of virus copies per m^3 of aerosol by particle size measured with the ACI sampler at different distances from the EPI line to the ground. Dashed line indicates the null value.



* 95% CL does not include the null value

Figure 4. Concentration of RNA copies of influenza per m^3 of air with the EPI system 'off' and 'on' at 30% and 70% relative humidity. Results represent the 3 replicates average of the sum of total particles captured by the different stages of the ACI with error bars representing ± the standard error of the mean.

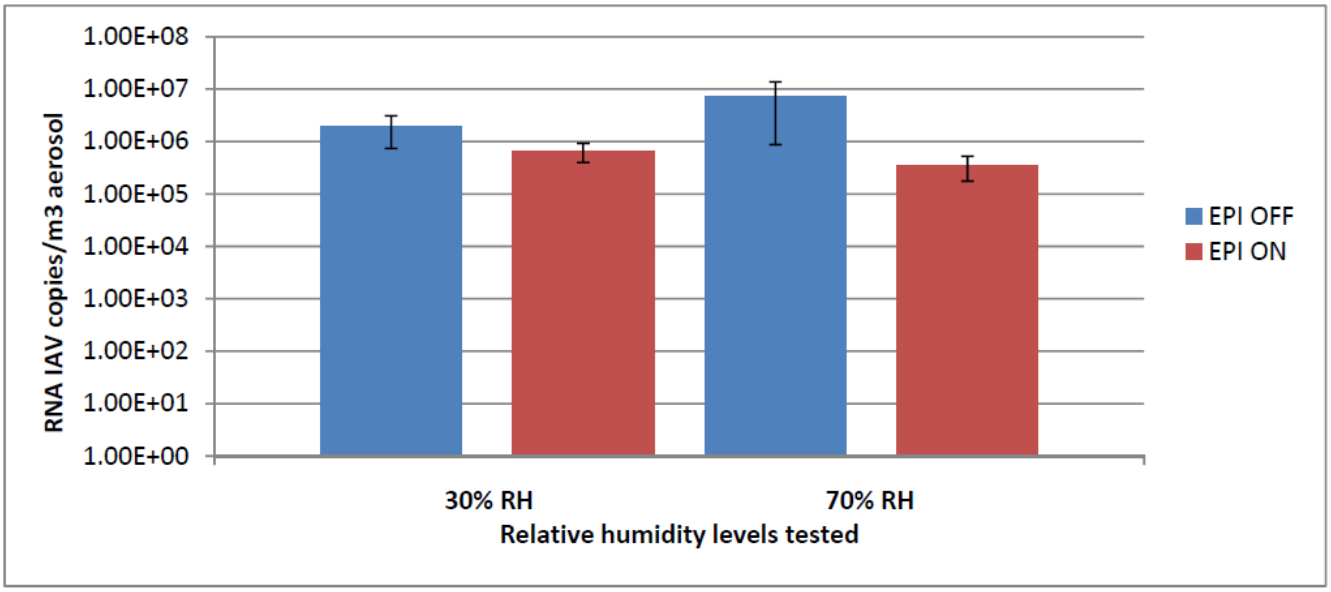


Figure 5. Removal efficiency of the EPI system for IAV at 30% and 70% relative humidity levels. Results are average of efficiencies measured with the ACI with error bars representing ± the standard error of the mean.

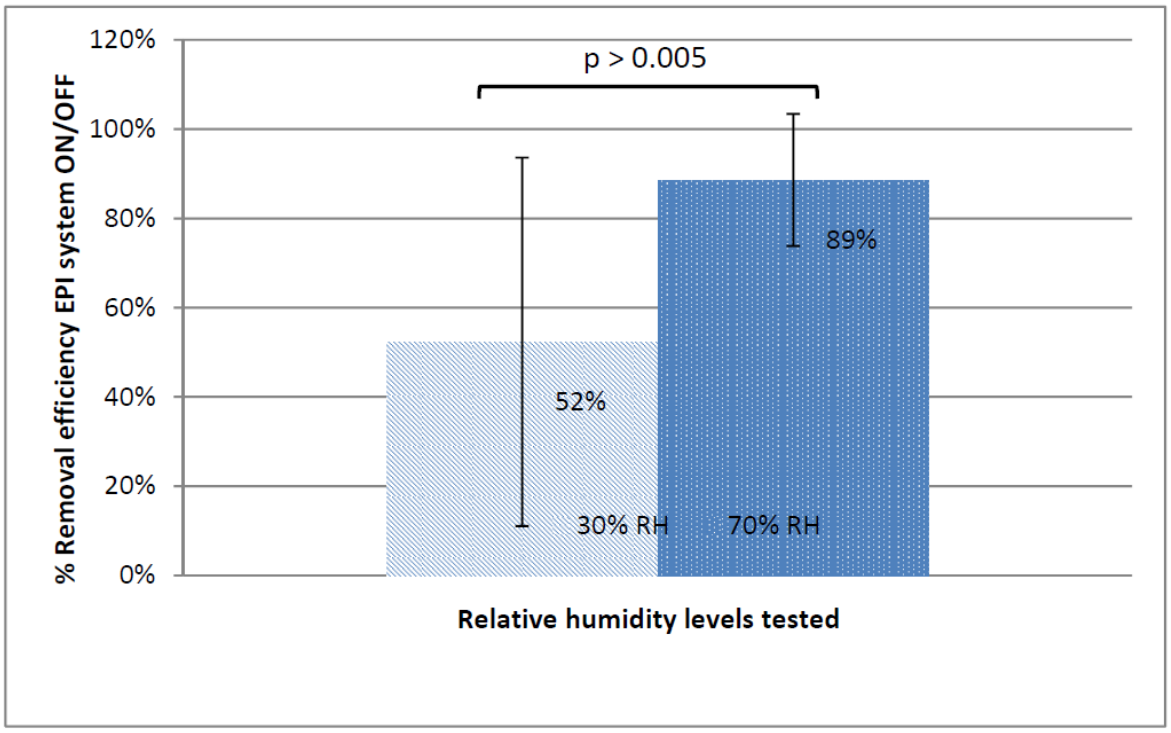


Figure 6. Concentration of *Staphylococcus aureus* in air (CFU/per m^3 of air) with the EPI system "off" and "on" as a function of particle size and distance of the EPI line to the ground.

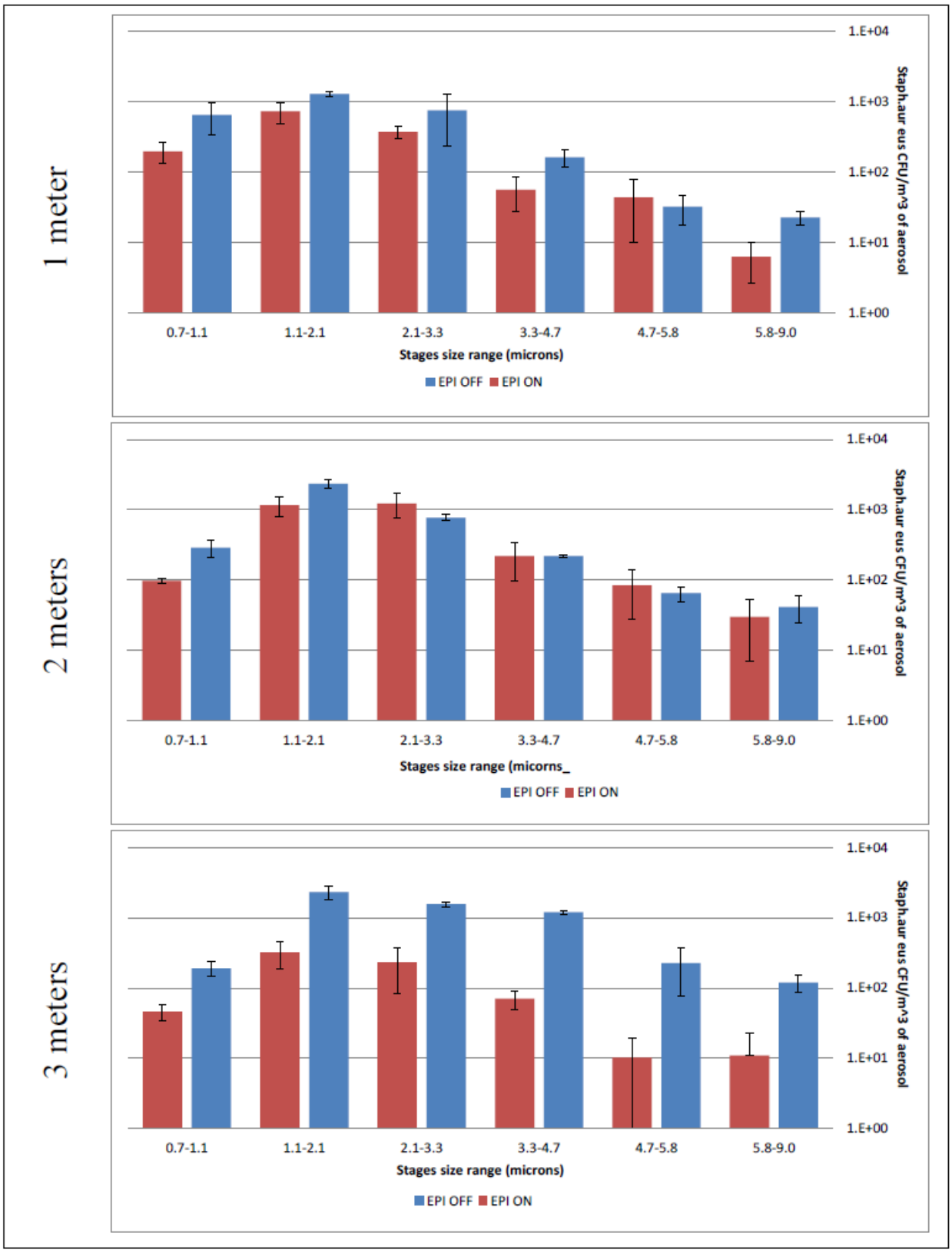
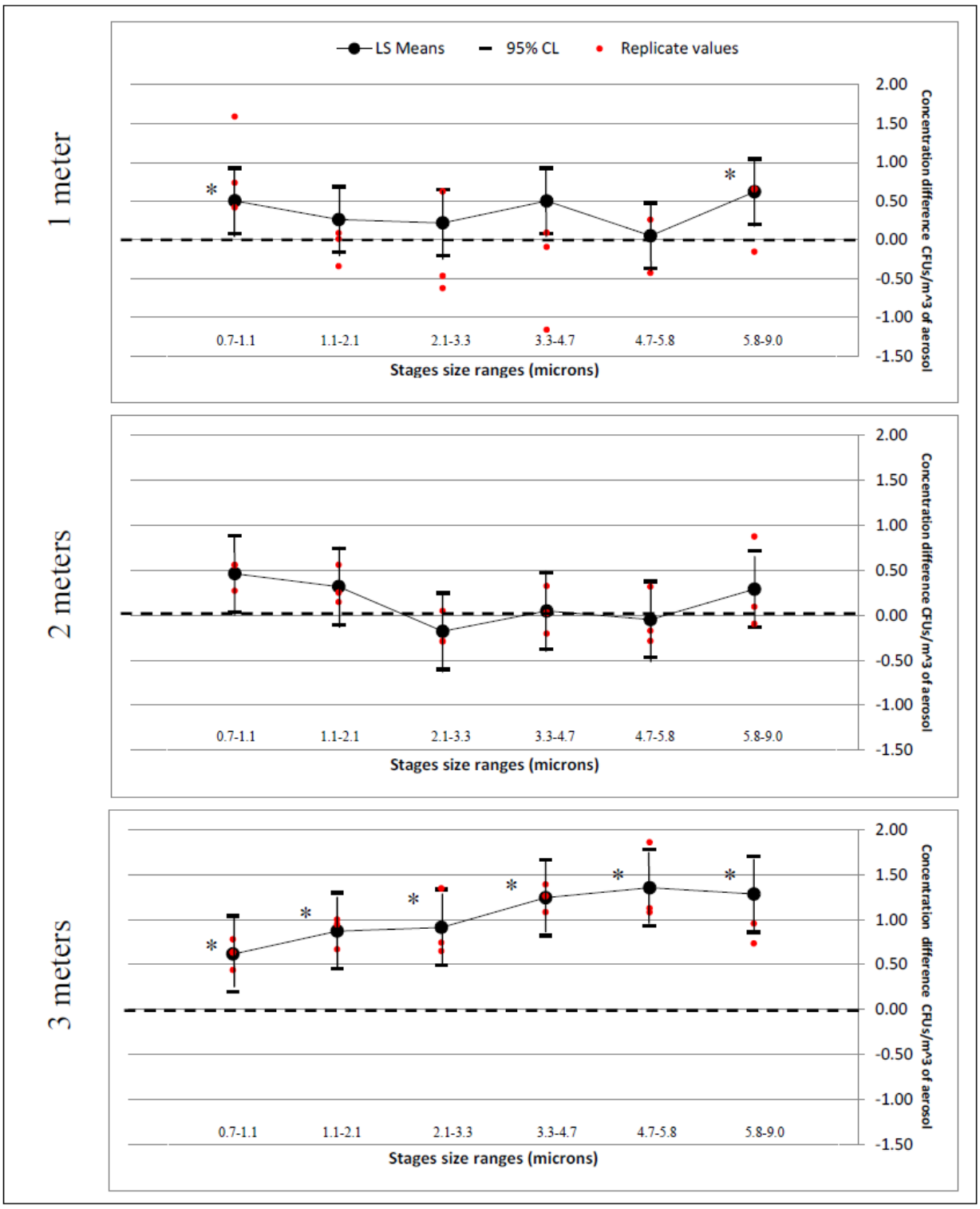


Figure 7. Linear regression modelling for total reduction of *Staphylococcus aureus* with the EPI system "on". LS Means of concentration difference of CFUs per m^3 of aerosol by particle size measured with the ACI sampler at different distances from the EPI lines to the ground. Dashed line indicates the null value.



* 95% CL does not include the null value