

Two Year Evaluation of Nonthermal Plasma Technology to Reduce Microbial Contamination and Improve Air Quality at a Large Casino

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DOI: <https://doi.org/10.52403/ijrr.20250315>

ABSTRACT

Bacteria and viruses are an increasing health challenge, particularly for casinos, given the density of occupation in common and gaming areas. Guests may interact with gaming equipment for extended periods of time with high levels of touch on common surfaces. Several government agencies agree on the importance of antimicrobial mitigation, including The White House. At their recent event, 'Let's Clear the Air on COVID', it was emphasized that improving indoor air should be a high priority to prepare for existing and future pandemic threats. This study evaluates the efficacy of nonthermal plasma technology in reducing surface and airborne microbial contamination in a 400,000 sq ft active casino gaming space. Traditional air filtration and disinfection efforts of casinos result in substantial labor, material, and energy costs. After an initial pretreatment testing on July 2, 2020, the Casino Property was treated with gas-phase H₂O₂ generators utilizing a nonthermal plasma technology provided by AIRPHX. All areas tested showed significant reductions in airborne and surface microbes. Substantially lower CFU counts were achieved in the post-treatment samples compared to those of the exterior samples and the pretreatment samples. As documented in this study, the technology tested appears to offer proactive disinfection in large, highly trafficked facilities. The long-term, real-world nature of this study

supports its relevance as a disinfectant in indoor environments and adds additional validation to already published independent studies in controlled lab settings.

Keywords: nonthermal plasma, indoor air quality, air disinfection, gas-phase H₂O₂ generator, air sanitization

INTRODUCTION

This study evaluates the effectiveness of a nonthermal plasma technology developed to reduce airborne and surface microbes throughout an active, 400,000 sq ft casino gaming area including common areas, administrative facilities, event/convention facilities, restaurants, and gaming floor. The large number of active inhabitants frequently interacting with one another and with high-touch surfaces create a high-risk environment for microbial contamination and dissemination.

Gas-phase H₂O₂ generator technology has been documented in this study to offer proactive sanitization of a large and heavily trafficked casino facility in a real-world setting over a two-year time period. The National Institutes of Health states: "Potential pandemic pathogens are bacteria, viruses, and other microorganisms that are likely highly transmissible and capable of wide, uncontrollable spread in human populations and highly virulent, making them likely to cause significant morbidity and/or mortality in humans" [1]. Viruses are not susceptible to antibiotics or many

common chemical disinfectants. This makes them a more likely perpetrator of widespread or pandemic threat. Within the classes of virus, RNA viruses are of special concern due to their higher mutability when compared to DNA viruses.

The recent SARS-CoV-2 pandemic and the emergence of new infectious diseases with pandemic potential have triggered a host of state and federal incentives and programs to address microbial threats. These include the White House event, 'Let's Clear the Air on COVID', the Environmental Protection Agency (EPA) Clean Air in Buildings Challenge to assist building owners and operators in ensuring better indoor air quality by (1) increasing air exchanges (bringing more outdoor air in), and (2) installing higher rated air filtration.

Although there are benefits in increasing the number of air exchanges, there are significant drawbacks to that approach [2]. Although generally free of pathogens, outdoor air often has high levels of ozone compared to indoor air according to California Air Resources Board [3]. Airborne mold spores and fungi can also be carried indoors via air exchanges [4]. Dielectric barrier discharge nonthermal plasma devices have been evaluated and effectively inactivate aerosolized microbes including bacteria (*E. coli*, *P. fluorescens*, *B. subtilis*, etc) [5,6], fungi including *Candida auris* [7], and viruses including MS2 bacteriophage, porcine reproductive and respiratory syndrome virus (PRRSV), and Newcastle disease virus (NDV) [8,9,10].

Independent lab Scientific Air Solutions has studied pre and post-AIRPHX treatment in over 75 locations including healthcare and hotel environments [11,12]. These evaluations demonstrated high counts of fungi, including mold-forming fungi, present in outdoor air and the resulting mitigation of indoor air contamination by over 90%. According to the EPA [13], there is ample documentation on the serious adverse health effects to humans caused by high counts of fungi, mold spores, and ozone. The increased energy cost resulting from heating and cooling outdoor

air exchanges is substantial, making them environmentally and economically unsustainable for building owners, says Allen [14]. In addition, increased air exchanges result in increased wear on air handling equipment, says researchers [15, 16]. Effective and energy-efficient air treatment systems are valuable in achieving improved indoor air quality.

Traditional air filtration and disinfection of casinos include enhancing sanitizing procedures using (i) electrostatic sprayers and chemicals, (ii) active disinfection products like ionizers (including bipolar and needlepoint types) and photocatalytic oxidation (PCO) technologies sometimes referred to as dry hydrogen peroxide and claim antimicrobial efficacy throughout a facility instead of disinfecting only within the device), and (iii) use of passive filtration devices that capture and or disinfect airborne particulates and microbes as they travel through the filter. These include ultraviolet light products, high-efficiency particulate air (HEPA) filters, and electromagnetic filters. Although laboratory testing may support claims of high efficacy against microbes, these solutions may have limited efficacy and potential increased costs (Cortiços N.D., 2021) in real-world applications.

Enhancing disinfection protocols with electrostatic sprayers and chemicals results in increased labor and consumables costs. The efficacy of this approach is limited to addressing surface contamination and is dependent on staff compliance. Active disinfecting technologies, including ionizers and PCO devices, while proven effective antimicrobials in controlled laboratory tests, have limited coverage areas. Scaling these to effectively treat a large facility may be cost prohibitive. Air filtration, whether deployed in air handlers or in stand-alone devices, treats air that passes through the device, but is unable to disinfect ambient air and surfaces. These methods for addressing microbial contamination are inadequate or impractical for long-term deployment in a casino property due to expense, non-scalability, and/or lack of evidence of

efficacy in large indoor environments.

Gas-phase H₂O₂ Air Treatment Technology
The technology evaluated in this study generates antimicrobial reactive molecules with nonthermal plasma technology. The plasma field is created with electricity. When combined with ambient air, oxidizing molecules are created without the need for catalysts or added chemicals. The oxygen present in the ambient air produces these oxidizing molecules without the need for added chemicals or catalysts. These oxidizing molecules include oxygen ions, free radicals, and peroxides.

Figure 1 Plasma Chamber



Within the plasma chamber, a non-collapsing plasma field is created without increasing ambient temperature (Figure 1). Some oxidation systems may generate significant temperature increases due to inefficient plasma-field production. Hence, these devices produce what is considered nonthermal plasma.

Measurable levels of gaseous hydrogen peroxide, ozone, and other types of oxidizing molecules are produced within the gas-phase H₂O₂ generator chamber. Oxidizing molecules are created within the chamber. These include singlet oxygen (O₂ with displaced electron), hydroxyl radicals and superoxide (O²⁻ and atomic oxygen (O).

MATERIAL AND METHODS

Long-term tests were conducted in a full-size regional destination casino operated by a nationally recognized casino operator (the "Casino Property"). The tests began on July 2, 2020 with three H₂O₂-generating devices installed in the 1st floor administrative areas and the poker room, six devices installed in the 2nd floor administrative areas, and five devices installed in other areas of the facility primarily on the 1st floor. At the time of

pretreatment testing, management indicated that the 1st floor gaming area was being treated with a bipolar ionizer product installed in 2014, and therefore there were no devices installed for direct treatment in the 1st floor gaming area. Direct treatment areas included the 1st floor administrative area, the 1st floor poker room, and the 2nd floor administrative, training, and events areas. Pretreatment testing was conducted on July 2, 2020. After the installation of the H₂O₂-generating devices, in-treatment testing was conducted on April 5, 2021 and December 13, 2022.

The Casino Property Facility Management staff monitored the sampling on site for each round of sampling and were able to confirm that material changes had not been made to the HVAC/air handling systems or to regular sanitation processes at the Casino Property during the test period.

Volumetric Air Sampling

Air sampling (30 liters of air per sample) was drawn using a MicroBio MB1 volumetric air sampler, Cantium Scientific, Clarendon Gardens, Dartford UK. Scientific Air Solutions is the North American Distributor of the MB1 and MB2 volumetric air samplers.

Air samples were impinged on 15x100mm potato dextrose agar plates acquired from Hardy Diagnostics, Santa Maria, California. The morphology and enumeration of the air sample was completed by Scientific Air Solutions, Turlock, California. The recorded results are normalized to colony-forming units per cubic meter of air, CFU/m³. A colony-forming unit or CFU is a unit used in microbiology to estimate the number of bacteria or fungal cells in a sample size that are viable and / or capable of multiplying. Counting with colony-forming units requires culturing microbes and counts only viable cells, in contrast to microscopic examination that counts all cells, living or dead.

The air quality scale for workplaces, public buildings (including casinos), schools, and homes are as follows:

- < 100 CFU/m³ is considered **clean and acceptable**.
- 100 to 300 CFU/m³ is **marginal**.
- 300 CFU/m³ is **not acceptable** and needs corrective action.

Surface Testing

Surface testing included surface swabs acquired from Solar Biologicals, Inc., Ontario, Canada. A uniform six inch by six-inch square surface area is swabbed for each sample, with swab sponges forwarded to Scientific Air Solutions for enumeration. All swab samples were examined for the number of organisms and recorded as colony-forming units per square centimeter, CFU/cm².

Contact surface quality scale for workplaces, public buildings (including casinos), schools, and homes are as follows:

- < 5 CFU/cm² is considered **clean and acceptable**.
- 5 to 10 CFU/cm² is considered **marginal**.
- 10 CFU/cm² is considered **not acceptable** and needs corrective action.

Treatment

For all areas of the casino, we mapped locations to be sampled via with air sampling or surface swabbing. After completing the pretreatment samples, the gas-phase H₂O₂ generators were placed in those mapped locations and activated. For the purpose of this study, these devices were then in operation without pause from July 2, 2020 to December 13, 2022. In-treatment volumetric air samples and surface swabbing were taken at the same locations as the pretreatment sampling on the dates above. Air samples were taken from outside the building as well, to evaluate the influence of outdoor air brought in via air exchanges to the test locations. See results from testing dates in Table 1.

Air and surface sampling and enumeration followed the methods of Rick Falkenberg et al. 2023. [11]

STATISTICAL METHODS

Purpose: To determine if there is a significant effect or relationship between variables. Common Tests that we use:

- t-tests: Compare the means of two groups.
- Chi-square tests: Assess the association between categorical variables.
- ANOVA: Compares the means among three or more groups.

Mean (Average)

Purpose: Summarizes the central tendency of a data set.

- Calculation: Sum of all data points divided by the number of data points.
- Usefulness: Gives you a quick glimpse of the overall data.

High Range and Low Range

Purpose: Shows the spread and extremes in a data set.

- Calculation: Highest value (high range) and lowest value (low range) in the data set.
- Usefulness: Highlights the variability and potential outliers.

Standard Deviation

Purpose: Measures the average distance of data points from the mean.

- Calculation: Square root of the variance (average of the squared differences from the mean).
- Usefulness: Indicates how spread out the values are; a low standard deviation means data points are close to the mean, while a high standard deviation means they are more spread out.

These concepts are fundamental in statistical analysis to describe and infer properties of data.

RESULTS

The total volume of treatment space was measured prior to the treatment and was recorded as several million cubic feet. Based on the volume of the treatment space, fourteen (14) devices were deployed. Test samples were taken between 9:00 am and

11:00 am for each test report to maintain consistency.

Treatment areas on the 1st floor

The 1st floor was serviced with eight (8) devices located throughout the administrative and poker room spaces. The direct treatment spaces on the 1st floor included employee spaces, the poker room, the VIP bar/high roller gaming area, the

loading dock, and administrative areas of the first floor. The 1st floor gaming area did not receive direct treatment, but was nonetheless tested in the same manner as the direct treatment spaces. Based on the test results, it was apparent that the gaming floor was indirectly benefiting from the technology in adjacent spaces. A total of 23 air samples and ten 10 surface swabs were taken on the 1st floor (see Figure 2).

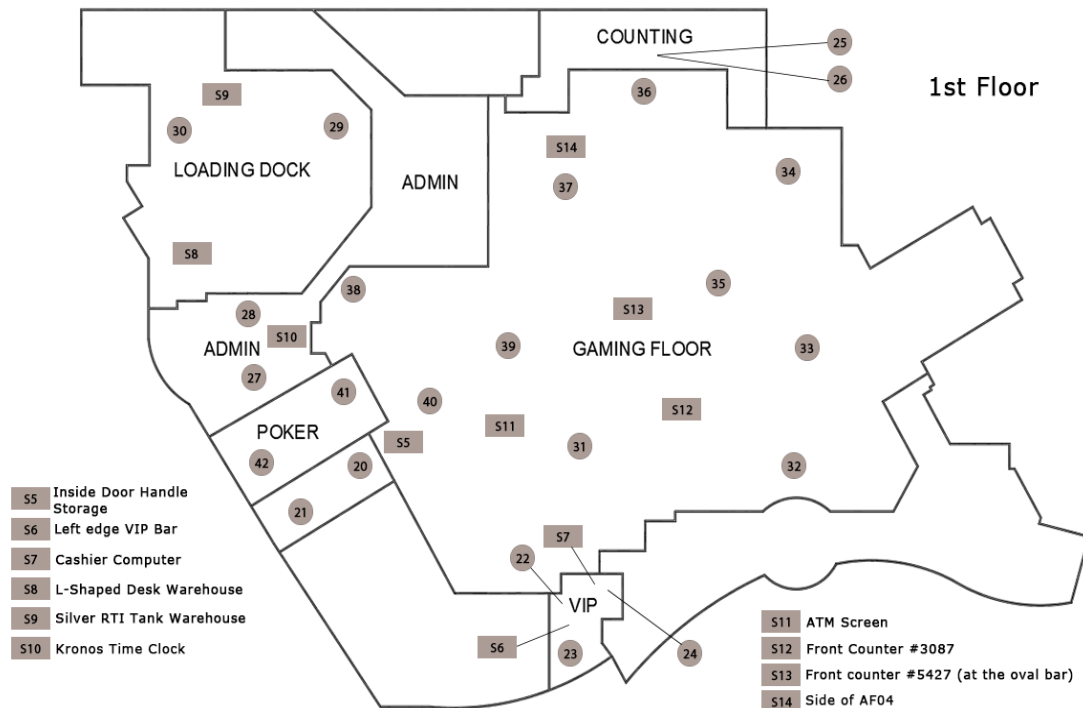


Figure 2. Subject Casino – 1st Floor

Treatment areas on the 2nd floor

The 2nd floor was serviced with six PA2400 devices located in the administrative areas and the event area of the property. The 2nd floor treatment areas include the administrative facilities, the event space, the restaurant and common area hallway, the banquet space, and the training facility.

Three devices were deployed in the long hallway serving the administrative facilities and the remaining devices were deployed elsewhere on the 2nd floor. Therefore, all spaces on the second floor received direct treatment from the devices. A total of nineteen (19) air samples and four (4) surface swabs were taken (see Figure 3).

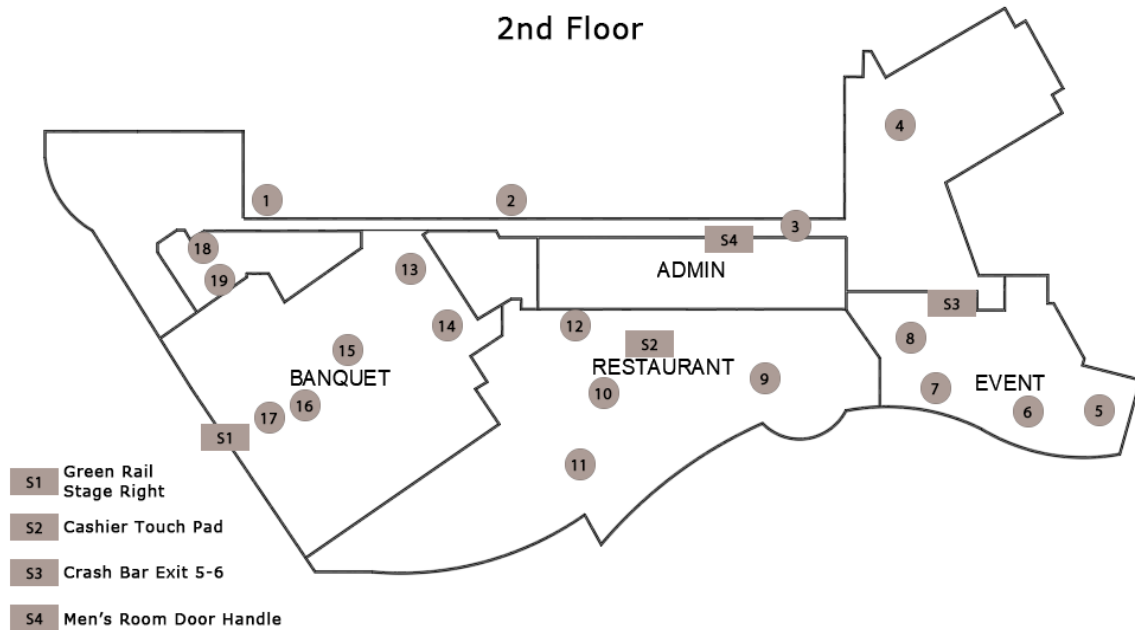


Figure 3. Subject Casino – 2nd Floor Direct Treatment Area

Testing results

Pretreatment sampling was done on July 2, 2020. The devices were activated after that date. The initial in-treatment sampling was conducted on April 5, 2021. Additional in-treatment sampling was conducted most recently on December 13, 2020, with the Casino Property at normal occupancy. The test results are grouped by location and reported as of each testing date (see Table 1). Results are summarized as follows.

Volumetric Air Samples

1. **Pretreatment** air samples were recorded to contain a range of microbial presence 311 to 590 CFU/m³ with an **average of 478 CFU/m³**
2. **Initial in-treatment** were recorded to contain a range of microbial presence from 17 to 44 CFU/m³, or an **average of 23 CFU/m³**. **This represents a reduction of 95%** and shows the nonthermal plasma treatment was an effective antimicrobial within the treated areas.
3. **Additional in-treatment results** including sampling on December 13, 2022 show results ranged from 11 to 20 CFU/m³, an **average of 15 CFU/m³** and **a reduction of 97%**, showing the nonthermal plasma treatment was

effective when the Casino Property was at full occupancy.

4. **The 1st Floor gaming area** did not receive direct treatment, but exhibited similar results as spaces receiving direct treatment, with air samples reducing from **an average of 590 CFU/m³ to 20 CFU/m³** and **a reduction of 97 %** with the casino at normal occupancy levels.

Surface Contact ‘Swabs’

1. **Pretreatment** surface swab results were **18.4 CFU/cm²**.
2. **Initial In-treatment** surface testing results revealed an average count of **1.8 CFU/cm²**, yielding a **90% reduction** during the initial treatment phase.
3. **Additional in-treatment results** and surface testing results on December 13, 2022 revealed an average count of **1.8 CFU/cm²**, yielding the same **90% reduction** as the initial in-treatment tests and showing that the device continued to be effective even with the Casino Property at full occupancy.
4. **1st Floor Gaming Area** exhibited similar results as spaces receiving direct treatment with surface samples reducing from **an average of 17.5 CFU/cm² to 1.6 CFU/cm²** and **a reduction of 91%** with the casino operating at full occupancy.

Consistency of Testing Results

When grouped by location, the test results were consistent throughout the casino facility, with similar levels of CFU reductions in both air and surface samples realized throughout the casino facility. This result is achieved because the gas-phase H₂O₂ produced by the device, due to its long half-life, appears to be effectively circulating throughout the casino's air handling equipment – achieving consistent CFU

reductions in areas receiving both direct treatment and indirect treatment. The largest indirect treatment area, the 1st floor gaming area, in fact realized similar CFU reductions as other areas in the casino – suggesting that direct treatment was not necessary to reduce CFU counts. Rather, it may be sufficient to locate the devices in proximity to spaces where the treated air is collected and circulated through the facility's air handling systems.

Table 1: Summary of testing results.

Samples	Pre-Treatment		In-Treatment	
	7/2/2020	4/5/2021	12/13/2022 (2)	
AIR TESTING (CFU/M3)				
1st Floor (other than Gaming Area)	11	491	24	12
1st Floor Poker	2	567	33	17
1st Floor Gaming (Indirect Treatment)(1)	10	590	13	20
2nd Floor Admin	14	433	24	14
2nd Floor Events Spaces	3	311	44	11
2nd Floor Training Area	2	333	17	17
Totals/Averages	<u>42</u>	<u>478</u>	<u>23</u>	<u>15</u>
Percent reduction		n/a	95%	97%
Exterior Samples				
Totals/Averages	4	3,042	2,567	1,967
Percent reduction			16%	35%
SURFACE TESTING (CFU/CM2)				
1st Floor (various)	5	17.8	1.9	1.9
1st Floor Gaming (Indirect Treatment)(1)	4	17.5	1.7	1.6
2nd Floor (various)	5	19.8	1.9	1.8
Totals/Averages	<u>14</u>	<u>18.4</u>	<u>1.8</u>	<u>1.8</u>
Percent reduction			90%	90%

- (1) Management indicated that at each testing date the gaming floor was being treated by a bi-polar ionizer technology. Based on pre-treatment sampling, it appears that the bi-polar ionizer technology was not effective in reducing CFU counts. The reductions in CFU counts appear to be the result of the indirect effect of AIRPHX technology operating in adjacent spaces. The slight increase in airborne CFUs at the December 2022 testing date appear to reflect full occupancy.
- (2) Property at full occupancy.

DISCUSSION

After the initial in-treatment testing on July 2, 2020, the Casino Property was being treated with fourteen (14) H₂O₂-generating devices. The results were consistent throughout the casino, with direct treatment spaces on the 1st floor and 2nd floor achieving significant reductions in airborne and surface organisms. Evaluation of all test data, including air and surface microbial counts,

showed that CFU count was lower in post-treatment samples than in both the pretreatment samples and exterior samples. This indicates that the gas-phase H₂O₂ generator devices evaluated were effective in reducing existing high microbial contamination from the outside environment and mitigating ongoing indoor microbial populations in the Casino Property.

Although the 1st floor gaming area did not receive direct treatment, the CFU counts were dramatically reduced at a level consistent with the direct treatment spaces. This suggests that gas-phase H₂O₂-generating technology can achieve microbial reductions in the environment, throughout the facility, even in areas that are not subject to direct treatment. Additionally, although the Casino Property was implementing enhanced sanitation procedures prior to the pretreatment sampling (owing to the COVID-19 pandemic), the gas-phase H₂O₂ generating technology resulted in improved disinfection results while avoiding additional labor and chemical costs.

CONCLUSION

It appears that the technology evaluated here offers a proactive solution, without added labor burden and cost, to reduce microbial contamination in a large and heavily trafficked casino. Based on these test results, and other studies on the efficacy of dielectric barrier discharge nonthermal plasma disinfection [17], the scalable and easily deployed nature of the gas-phase H₂O₂ generating devices may be of value to high-trafficked facilities similar to those in the casino industry.

Data Availability

The pretreatment and in-treatment and reduction (all) data used to support the findings of this study are included within the article.

Declaration by Authors

Acknowledgement: The author would like to acknowledge the writing, editing, and review assistance given by Cordell Price and Jennifer McNary Smith.

Source of Funding: None

Conflict of Interest: The author declares the following potential conflicts of interest with respect to the publication of this article.

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How to cite this article: Rick Falkenberg. Two Year evaluation of nonthermal plasma technology to reduce microbial contamination and improve air quality at a large casino. *International Journal of Research and Review*. 2025; 12(3): 103-111. DOI: <https://doi.org/10.52403/ijrr.20250315>
