

Healthcare

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## Bactericidal Performance Testing of Indigo-Clean Upon Bacterial Species

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### ABSTRACT

Susceptibility of a variety of medically-relevant Gram positive and Gram negative vegetative bacteria to 405 nm visible light was investigated. Bacteria, deposited onto agar surface and stainless steel coupons, were exposed to a high-intensity 405-nm visible light generated from a light-emitting diode array. The degree of bacterial inactivation was calculated by determining the number of surviving CFUs after exposure to light at a pre-determined distance and exposure times when compared to the controls that were not exposed to the bactericidal light. The studies showed broad-spectrum activity of light against Gram positive bacteria such as *Staphylococcus aureus* and Gram negative bacteria such as *Enterobacter aerogenes*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* when seeded onto an agar surface. The bactericidal light was least effective against *Enterococcus faecalis* (Gram positive organism) and *Acinetobacter baumannii* (Gram negative organism). When tested on stainless steel surface, the significant light-dependent inactivation of population was observed with *E. aerogenes*, *S. aureus*, and *P. aeruginosa* whereas least inactivation was observed with *K. pneumoniae*, *A. baumannii*, and *E. faecalis*. The results of the study showing effective inactivation of medically important bacteria offers the potential to provide continuous decontamination technology in a clinical setting and other industries such as pharmaceutical and food manufacturing.



## INTRODUCTION

Healthcare Acquired Infections (HAI) are a substantial concern for healthcare providers with approximately 1.7M infections, 99,000 deaths and ~\$96B-147B in excess costs each year<sup>1</sup>. In an effort to improve this situation, the Affordable Care Act has created a series of reimbursements and penalties directly related to provider's performance in reducing the number of Healthcare Acquired Infections.

As part of this effort, healthcare providers are turning to a variety of solutions such as improved handwashing, antimicrobial stewardship, isolation precautions, patient and staff education, and better policies and procedures. The risk of pathogenic transmission via the environment is frequently overlooked or given far less attention than any of the other modalities. Given the rise of antibiotic resistant bacteria, improving environmental hygiene to prevent the bacteria from getting into the body will take on an increasing role.

Healthcare providers routinely clean the environment, typically on a daily basis (or episodically). The limitation of this approach is that immediately after cleaning, bacteria begin to repopulate the space. This suggests a need for an environmental disinfection system that operates continuously and which allows people to work in the space while it's in use.

The use of 405nm visible light as a potential method for reduction or inactivation of bacteria in the environment has been the subject of academic interest since 2001<sup>2</sup> and has recently been introduced commercially under the brand name Indigo-Clean. It is deployed clinically in an overhead light fixture operating in one of two modes depending upon whether or not tasks are being performed in the room as shown below:



***White Disinfection Mode =  
Ambient White Light + Disinfection***

***Indigo Disinfection Mode =  
Increased Disinfection Only***

Visible light disinfection constantly emits a narrow spectrum of visible light at 405 nm to kill harmful bacteria in the environment (air, hard and soft surfaces) safely, automatically and continuously. Specifically, the light is first absorbed by porphyrin molecules inside the bacteria creating toxic and biocidal reactive oxygen species (ROS) which inactivates the pathogen. Because it uses visible light, the room can be in use while the disinfection is occurring. This technology has been recently commercialized and the capability of an Indigo-Clean™ light unit was evaluated in the laboratory to assess the inactivation of variety of medically important bacteria (ESKAPE organisms) on surfaces wherein the disinfection efficiency was calculated. The ESKAPE group of organisms have become highly relevant in hospital settings due to the rise in antibiotic resistance and the

fact that they are responsible for ~90% of nosocomial infections. This group includes *Enterobacter aerogenes*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis*.

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Regardless of approach, all of these measures are ultimately graded by their ability to prevent infections. This outcome data is highly sought after but can be very difficult and time consuming to collect due to the generally low infection rates in US healthcare institutions (~1 in 25) and the number of variables to control throughout the study. This becomes even more challenging for environmental disinfection technologies whose clinical benefit is often obscured by larger effects such as handwashing compliance.

Therefore, the performance of environmental disinfection technologies is typically measured by its ability to reduce bacteria on surfaces (or in the air) within a clinical setting. While clinical measurements such as this are generally regarded as the most effective means of characterization, laboratory performance measurements offer a faster alternative by which to make an initial assessment. Additionally, laboratory measurements offer a more controlled environment in which the bactericidal performance can be measured against specific organisms on various surfaces using known amounts of disinfectant.

With this in mind, the capability of an Indigo-Clean™ light unit was evaluated in the laboratory to assess the inactivation of a variety of medically important bacteria (known as the ESKAPE organisms) on surfaces wherein the disinfection efficiency was calculated.

## MATERIALS AND METHODS

### Bacteria

The organisms used in this study were: *Enterobacter aerogenes* ATCC 13048, *Staphylococcus aureus* ATCC 6538, *Klebsiella pneumoniae* ATCC 13883, *Acinetobacter baumannii* ATCC 19606, *Pseudomonas aeruginosa* ATCC 9027, and *Enterococcus faecalis* ATCC 19433 were used in the study. Dilutions of each microorganism suspension were prepared in phosphate buffer and were titered concurrent to test inoculations.

### LED Light Source

An Indigo-Clean™ fixture (Manufacturer Part No: M4SEDIC-24-150I/100L) from Kenall Manufacturing was used to create a uniform distribution of disinfecting light at a task plane 1.5m beneath the fixture. This irradiance was measured at various points across the plane to be 0.498 mW/cm<sup>2</sup> to 0.558 mW/cm<sup>2</sup> using a NIST-calibrated, Ocean Optics portable spectrometer optimized for short wavelength measurements (Manufacturer Part No: USB4000). All measurements were made over the spectral range 400-420nm consistent with other published works on this subject. The average irradiance was calculated to be 0.525 mW/cm<sup>2</sup> and was used for all calculations during the experiment. This level of irradiance is typically used in a clinical setting. Agar plates (100 mm diameter) and stainless steel coupons (1 in x 3 in) were exposed to an average irradiance of 0.525 mW/cm<sup>2</sup>.

### Experimental Design

**Light Exposure of Bacteria Seeded onto Agar Surfaces.** Dilutions of each bacteria were prepared in phosphate buffer (PB) such that 100 µL contained approximately 200-300 CFU. Tryptic Soy Agar plates (BBL, Franklin Lakes, NJ, USA) were inoculated with 100 µL of bacteria and spread over the surface of the agar using a sterile spreader and then lids placed on the plates. The test plates were exposed to the light at a distance of 1.5 m for duration of 24 hours. Non-exposed control plates were prepared for each light-exposed sample. At 24 hours, test plates were removed and test plates and non-exposed control plates were incubated at 30-35 °C for at least 48 hours. Upon incubation, the plates were enumerated and results reported as CFU per plate. For varying exposure time study, the seeded plates were exposed to the light for 2, 6, 12, and 24 hours whereas *E. faecalis* was exposed for 24, 48, 72 and 96 hours. Figure 1 shows an example of plates being exposed to the visible light generated from 405-nm light emitting diodes (LEDs).

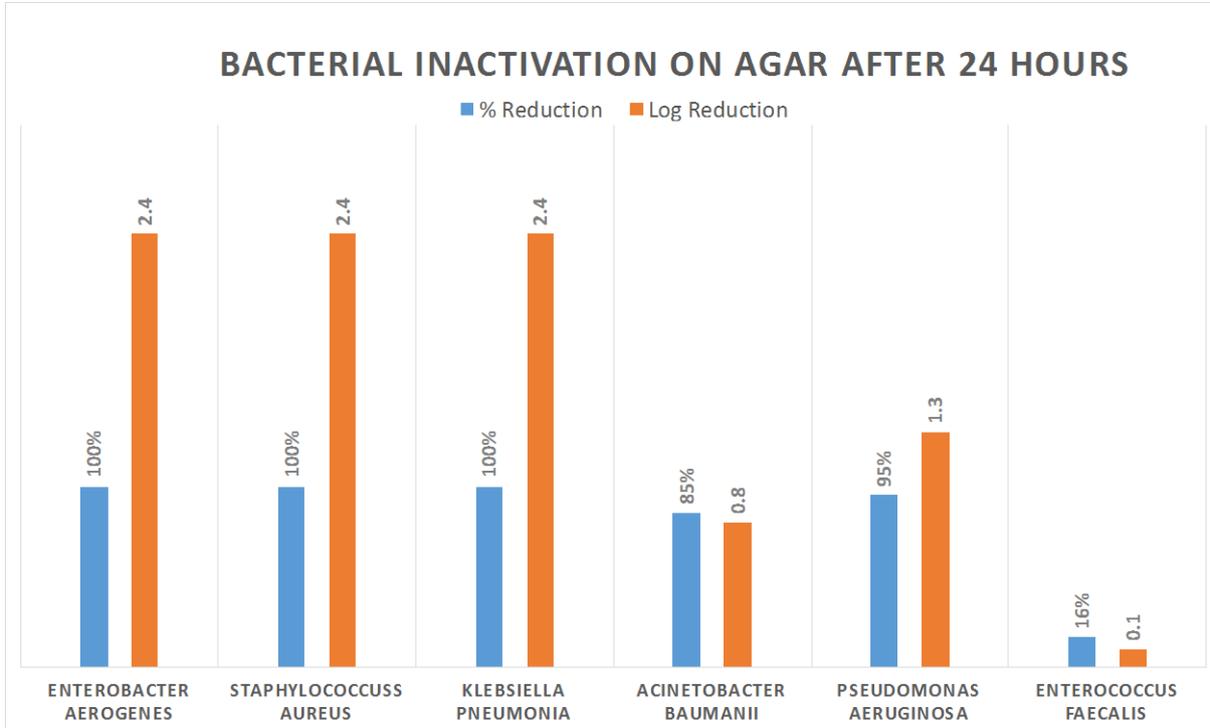
**Light Exposure of Bacteria Seeded onto Stainless Steel Coupons.** Dilutions of each bacteria were prepared in phosphate buffer (PB) such that 100 µL contained approximately 1E07 CFU. Stainless steel coupons were inoculated with 100 µL of bacteria. The test coupons were exposed to the light at a distance of 1.5 m for durations of 4 hours and/or 24 hours. Non-exposed control coupons were prepared for each light-exposed sample. Following exposure at 4 hours and 24 hours, coupons were suspended in Fluid D and vortexed to recover bacteria from the coupons. Serial dilutions were prepared and plated using Tryptic Soy Agar. The test plates and non-exposed control plates were incubated at 30-35 °C for at least 48 hours. Upon incubation, the plates were enumerated and results reported as CFU per coupon.



**Figure 1.** Indigo-Clean™ light fixture and experimental set up for light exposure of bacteria seeded onto agar surfaces.

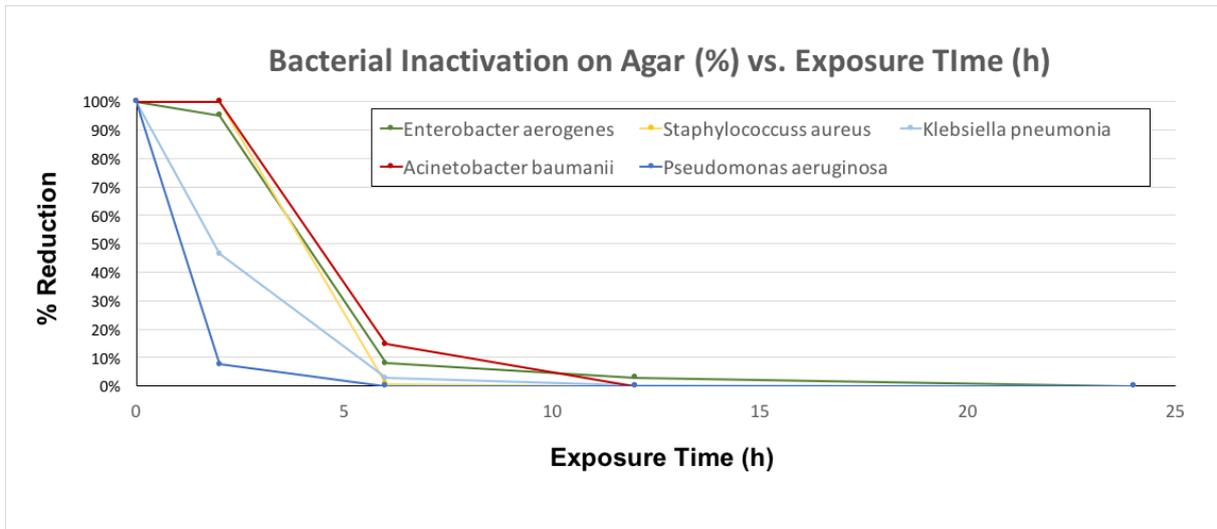
## RESULTS

Figure 2 shows the bactericidal effect of visible light onto an agar surface seeded with 6 different ESKAPE pathogens after an exposure period of 24 hours.



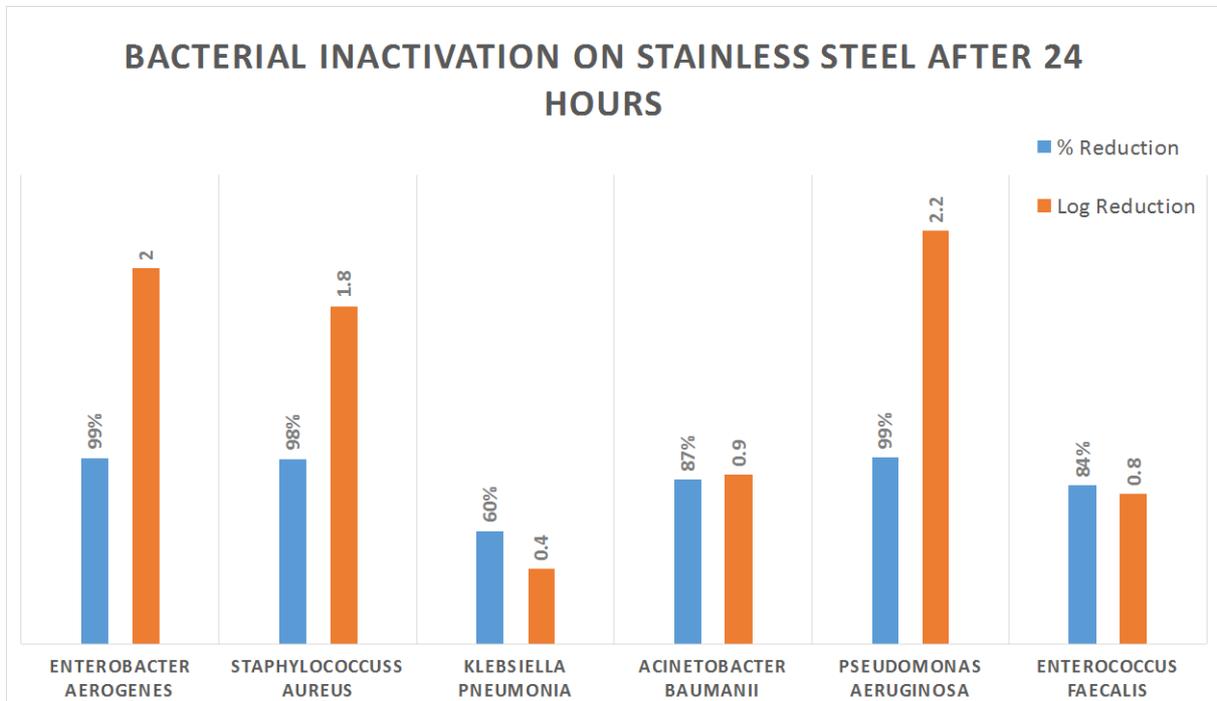
**Figure 2.** Inactivation of various ESKAPE pathogens in a nutritious media after 24 hours of exposure to Indigo-Clean. This test showed the potential level of disinfection that could be achieved in a clinical setting and was used to determine the sampling interval for subsequent measurements.

In order to understand the inactivation for shorter (or longer) time periods, a more detailed investigation included the exposure of agar plates seeded with 5 of the 6 ESKAPE pathogens for 2-24 hours as shown in Figure 3. A separate investigation of the *enterococcus faecalis* organism was performed due to the low level of inactivation achieved during the 24-hour exposure.



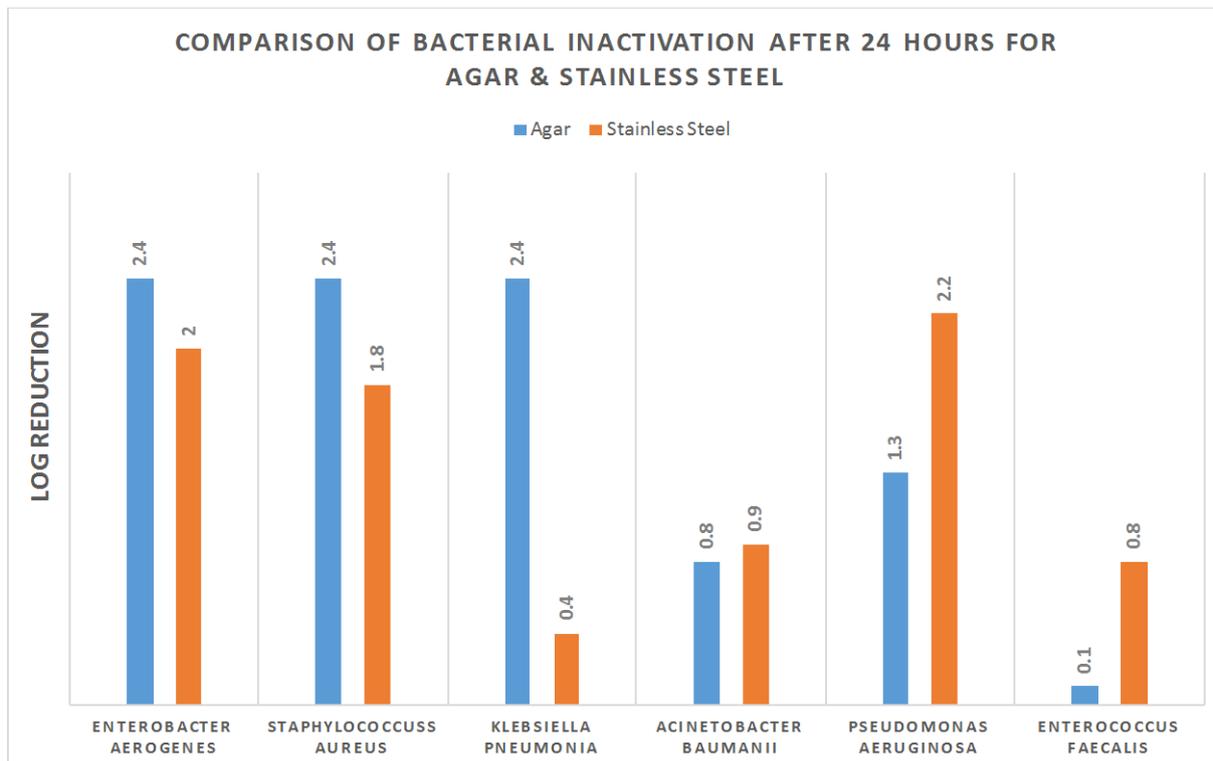
**Figure 3.** Inactivation of various ESKAPE pathogens in a nutritious media as a function of time. Note that 5 of the 6 organisms show a greater than 90% reduction after 6 hours of exposure to an irradiance typically found in a clinical setting.

While these measurements are instructive, they do not represent the full range of clinical conditions than an organism could experience. While is impossible to re-create all of these conditions in a laboratory setting, one can get a better indication of how Indigo-Clean would perform in a clinical environment by seeding bacteria onto surface types found in a clinical environment and exposing them to the disinfecting light. One example of this is stainless steel. Figure 4 below shows the results of this effort, similar to the measurements shown in Figure 2.



**Figure 4.** Inactivation of various ESKAPE pathogens on a stainless steel surface after 24 hours of exposure to Indigo-Clean. This test showed the potential level of disinfection that could be achieved in a clinical setting.

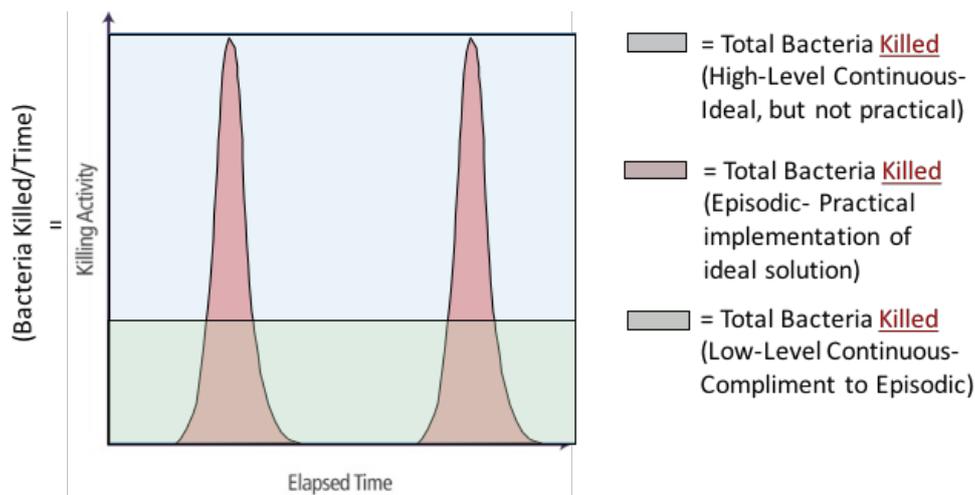
To better illustrate the effect that the surface has upon the disinfection, Figure 5 compares the inactivation of the bacteria studied in agar and on a stainless steel surface.



**Figure 5.** Comparison of inactivation achieved for various organisms seeded onto agar and stainless steel after 24 hours of exposure to Indigo-Clean

## DISCUSSION

This study demonstrated the bactericidal effect of 405 nm visible light on the variety of Gram positive and Gram negative organisms when seeded onto different surfaces. *E. aerogenes*, *S. aureus*, and *K. pneumoniae* showed a greater than 99% reduction on the agar surface while *A. baumannii* and *P. aeruginosa* showed an approximately 90% reduction. *E. faecalis* was the most resistant of the group showing negligible or least reduction on the agar surface. Comparison of these results with those for a normal disinfectant are challenging because narrow-spectrum light (Indigo-Clean) operates continuously, 24/7. As shown in Figure 6 below, a normal disinfectant operates episodically, killing a high proportion of bacteria, but only during the time it is applied. After cleaning, bacteria begin to quickly repopulate the space. Thus, a more objective frame of reference in which to compare the two solutions is the total amount of bacteria killed between routine cleanings (approx. 24 hours).



**Figure 6.** Comparison of continuous and episodic disinfection. Note that the best method of comparison between these two types of disinfectants is the total number of bacteria killed over a normal, daily cycle.

High levels of inactivation (> 95%) were obtained on stainless steel for *E. aerogenes*, *S. aureus*, *P. aeruginosa*. *A. baumannii* and *E. faecalis* showed substantial inactivation (> 70%) on this surface as well. *K. pneumoniae* showed the lowest level of inactivation on this surface (~40%).

In general, neither the Gram-positive bacteria nor the Gram-negative bacteria showed any preferential level of inactivation on either agar or stainless steel. Any comparison is complicated by the natural die-off of organisms on the stainless steel surface itself, forcing a much higher inoculum to be used. This could, in turn, create a self-shielding effect from the narrow-spectrum light (or any disinfectant for that matter) leading to differences in observed inactivation. The differences in activation for the various organisms on each surface type could be explained by this hypothesis and additionally, the differences in how each organism reacts to the difference between a nutritious media and a clinical surface.

In addition to bactericidal effect against vegetative cells, the effect of light-inducing inactivation of *Bacillus* and *Clostridium* spores has been investigated<sup>3,4</sup> and showed that although bacterial spores were sensitive to light inactivation, such reduction in spore population required a higher dose of light compared to that required for inactivation of vegetative cells. In other studies, inactivation of yeasts and molds due to lethal effects of light at 405-nm has also been investigated<sup>5</sup> with similarly positive results.

## CONCLUSIONS

Indigo-Clean can be used to achieve significant, continuous inactivation of pathogenic organisms on both nutritious media and clinically relevant surfaces. These results are consistent with those previously obtained and suggest that the clinical performance of the product would be consistent with that found in previous clinical studies<sup>6,7</sup>.

## ACKNOWLEDGEMENTS

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