

**Density of Bacteria on Fluorescent and Non-Fluorescent Surfaces
of Interest to Public Health**

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ABSTRACT:

In recent years, public health agencies have relied on UV light to detect unsanitary conditions in hotels, public restrooms, and other public areas. This detection of bacteria and other filth depends on the fluorescence of many organic compounds, especially those common in body fluids which enable both the inspector and consumer to find and destroy bacteria contaminating contact surfaces. This study reveals that using fluorescence to determine cleanliness in areas of interest to public health is undependable. Results from this study indicate that average bacterial density in non-fluorescing areas is significantly higher than in fluorescing areas when both were sampled using contact films to recover and grow bacteria.

A new practice in the public health arena involves the detection of unsanitary areas by using ultraviolet light (UV) to detect fluorescence. Fluorescence is the emission of light caused by a molecule as it absorbs radiation. The molecule is stimulated to a higher energy level, and as it drops back down to its ground state, it releases the gained energy as visible light (1). This light can either be fluorescent, meaning the material will stop glowing the instant the stimulus is removed, or phosphorescent, meaning the material will continue to glow after the stimulus is removed(1).

Some natural fluids and organic matter fluoresce; for example, semen, urine, mineral oil, earwax, perspiration, and many more (1). UV light is used commercially by the food industry in canning processes to detect bacteria like *E.coli* on machinery and in the cans themselves (2, 3). The dairy industry uses UV light to detect milkstones (3). Most pest control companies use UV light to detect rat feces and urine that fluoresce (3). Due to increasing cases of antibiotic resistant infection caused by mutated strains of bacteria like *Staphylococcus aureus*, many hospitals have installed UV-sterilized air and water systems (3, 4, 5, 6, 7). The main purpose in using UV light in these areas is to sterilize unsanitary areas, air, or water. It has long been proven that UV-C light is antimicrobial when bacteria are exposed to it for various lengths of time (3, 11). UV-C light extends from 180nm to 280 nm and exposes the bacteria to harmful rays which mutate the bacterium DNA by the formation of pyrimidine dimers. These dimers cause an inaccurate DNA template, which prevents many bacteria from reproducing. At 254 nm, which is required for 99.9% destruction of various

microorganisms, the UV energy in microwatt-seconds per cm² necessary to cause cell death ranges from 2500 to 36000 (19).

Many companies like RestAssured, Mobility Solutions, and Natural Solutions Environmental, Inc. sell a variety of UV lights so that customers can detect contamination via fluorescence. One company even claims that their handheld UV light, the “Degerminator”, will help the customer to detect and destroy bacteria (8, 9, 10). The publicity these companies have received highlights the need for quantitative data to support their claims. There is no clear-cut evidence that the appearance of fluorescence when a surface is illuminated with UV light coincides with an increased level of bacterial density. While food industries regularly swab for contamination to catch any bacteria that may be present, this study focuses on whether fluorescent areas exhibit higher bacterial density than non-fluorescing areas. This angle has been ignored, even though many commercial companies sell products geared toward using fluorescence produced under ultraviolet light to find areas of bacterial contamination.

MATERIALS AND METHODS

There are several different methods of surface sampling recommended by the American Public Health Association: the cellulose sponge technique, swab procedure, or Rodac[®] plate, as well as the developed methods using agar

sausage technique, adhesive tape, Petrifilm™ plates, contact-transfer and contact slides (12,15,16). The Food and Drug Administration (FDA) has proposed general guidelines for hygienic food equipment surfaces and utensils; a standard of no more than 100 colonies (nonspecified) per utensil or surface area of equipment swabbed is allowed, and CFU (colony forming unit) counts not exceeding 100 per 8 in². (i.e. averaging 12.5 colonies per sq in. of surface) (16). Due to the areas tested and the nature of the time constraints inherent in this study, a quick and easy method was desired. The 3M Petrifilm™ Aerobic Count Plate (3M Corporation) was the logical choice as it had minimal requirements of preparation and handling. Both the American Public Health Association and the Health Protection Branch of the Compendium of Analytical Method in Canada have approved the Petrifilm™ for environmental sampling (14).

Preparation of Medium

Petrifilm™ allows the rapid sampling of surface contact areas. Petrifilm™ contains Standard Methods nutrients, a cold-water-soluble gelling agent, and a tetrazolium indicator to facilitate enumeration. This most closely approximates agar pour plates. This type of sampling by surface contact is the best choice for enumerating particulates containing viable aerobic microorganisms, as well as more convenient in terms of time and effort (13, 15, 17). Preparation of the film prior to sampling the surfaces involved pipetting 1 mL of boiled distilled water onto the bottom film surface. The top portion of the film was then replaced, and a circular template pressed onto the film to create a round area of agar ($\approx 20 \text{ cm}^2$).

The agar was allowed a minimum of one minute to solidify. The film was then used to test surface contamination.

Selection of Surfaces to be Tested, Fluorescence Detection, and Surface Sampling

The four locations studied were public bathrooms, doctor's waiting rooms, public nurseries, and baby changing stations. One hundred fluorescing surfaces were sampled using 3M Petrifilm™. Fifty of these films were randomly selected to be compared with fifty samples of completely non-fluorescing surfaces in the same location (See Table 3).

A portable UV-A light (Model B-160, 6 watt, 365 nm longwave) manufactured by Spectronics Corporation was used to illuminate various surfaces of interest to public health, namely: walls, fixtures and countertops in public restrooms, nurseries, doctor's waiting rooms, baby changing stations, and hotel rooms. The light was held approximately one foot away from the various surfaces. The rooms being sampled were not required to be completely dark, however, darker rooms were easier to sample because the fluorescence was more easily observed. Fluorescence detection involved darkening a given room to improve visual observation, and scanning the area with the UV light. Fluorescing areas began to glow, and samples were collected.

Surface sampling was completed by locating a fluorescing surface, pressing the top half of the film to the surface, and marking the surface's borders using a sharp edged pen. Location of the sample, date sampled, and the

fluorescence color of the sample was recorded. The samples were then incubated at room temperature (25-27°C) to imitate natural surface conditions for a period of five days. Bacterial presence was confirmed by the appearance of red colonies, each one representing the growth of a bacterium removed from the surface into a visible colony forming unit, CFU. The colonies appear red due to the tetrazolium indicator. This indicator changes color from clear to red when electrons from the bacterium cytochromes are bound to the tetrazolium. The numbers of CFU present within the experimental unit containing a fluorescing area were counted and recorded as CFU/cm². A control sample of the population was taken in the same method described above using completely non-fluorescing areas. The samples were incubated for a period of 5 days at room temperature. The numbers of CFU present were recorded as CFU/cm² and compared to the samples containing fluorescing areas.

Statistical Analyses

A paired t-test was used to compare the average bacterial density between fluorescing and non-fluorescing areas paired by location. The rest of the report was purely descriptive in nature.

RESULTS

The average bacterial density in fluorescing areas (1.39 CFU/cm²) was significantly less ($p=0.0085$) than the average bacterial density in non-fluorescing areas (2.80 CFU/cm²). Bacterial densities for these locations in both fluorescing and non-fluorescing areas are listed in Table 3 in the Appendix. Of these locations, the highest mean viable bacterial density (CFU/cm²) in fluorescing areas were found to occur in doctors' waiting rooms, while the lowest bacterial densities were in public bathrooms. The highest densities were in non-fluorescing areas found to occur in public bathrooms, while the lowest occurred in doctors' waiting rooms.

A second avenue of study was the relationship of fluorescence color to bacterial density. Four detected colors were studied. They occurred in these percentages blue/white (33.0%), dark green (10.0%), yellow/green (39.0%), and pink/orange (18.0%). See Table 2 in the Appendix. A comparison of average bacterial density between fluorescing and non-fluorescing surfaces by color of fluorescence is found in the bar graph in Figure 5. As seen, the pink/orange fluorescent color had the highest mean bacterial density, while the blue/white fluorescent color had the lowest mean bacterial density. Blue/white fluorescing surfaces appear predominantly in doctors' offices. Yellow/green fluorescing areas were found predominantly in public restrooms and on baby changing stations (see Table 5). The substances that emit colors of fluorescence are listed in Table 4.

DISCUSSION

The results of this study show that fluorescing areas cannot generally be considered unclean when compared with their immediate non-fluorescing surroundings. In the locations sampled for this study, colony densities were significantly higher in non-fluorescing areas versus fluorescing areas. The only caveat to this statement was found in doctors' offices, where CFU densities were slightly higher in fluorescing areas (See Table 3). The statistical analysis of the samples using a paired t-test revealed that the mean bacterial density present in the fluorescing surfaces and their immediate surroundings (within the 20cm² template) was less than the mean bacterial density present in completely non-fluorescing surfaces from the same location. Practically, this means that fluorescent surfaces are more sanitary than most non-fluorescent surfaces.

As for specific relationships between location and viable bacteria density counts, the highest densities in doctors' offices were expected due to the high traffic of sick children and adults in those areas. The most interesting observation is that in areas that public health agencies are sensitive to, such as sites like public restrooms and especially sites relating to possible risks associated with childrens' health, there are more colonies in non-fluorescing surfaces than in the fluorescing surfaces.

The color of fluorescence is important to consider because it may determine the level of sanitation necessary to properly clean a room. Regardless of overall CFU densities in non-fluorescing surfaces, areas containing mostly pink/orange fluorescing surfaces had more bacterial density than areas exhibiting mostly dark green or blue/white fluorescing surfaces. This may be due to the

nature of the substance that is causing that particular color of fluorescence. For example, pink/orange spots caused by grease or oil may provide a better source of nutrients and resist desiccation longer than the other substances causing the other colors of fluorescence. Therefore, if an industry or health agency chooses to continue inspection by UV light, then further exploration along this line of study could begin to develop a scale of cleanliness based solely on color of fluorescence. If an agency knew that certain colors of fluorescence generally harbor more bacterium than others, then a more stringent disinfection policy might be used for that color.

The scope of this study does not explain why the fluorescing surfaces have lower bacterial density than non-fluorescing surfaces. However, there are several possible explanations for this phenomenon. The most likely is that the Petrifilm aerobic count plates are not indicating every CFU present. Perhaps the fluorescent material causing the surface to glow includes an inhibitor for certain strains of bacteria. Also, the fluorescing material may be masking or covering up some colonies. Another factor that may affect bacterial densities is the age of the fluorescing material. The density of bacteria in any one area may be influenced by the amount of fresh nutrients present in the fluorescing surfaces, and as the surface ages, the nutrients could be exhausted leading to bacteriostasis and eventually cell death. This could signify resistance to desiccation and potential long-term viability of bacteria in these areas. These are all factors that can be studied in future experimentation. How does this affect the arena of Public Health? The companies that sell products designed to capitalize

on the theory that fluorescing areas denote decreased sanitation are incorrect- not because these areas do not contain bacteria and so can be called sanitary, but in that they are more sanitary than the non-glowing areas containing bacteria that are not fluorescing when excited by UV light.

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Appendix

Table 1. Locations tested for microbial distribution in Fluorescing and Non-fluorescing Areas.

Location	Frequency	Percent
Baby station	6	6.0
Public bathrooms	55	55.0
Doctor's waiting rooms	27	27.0
Nursery	12	12.0
Total	100	100.0

Table 2. Colors of Fluorescence detected in a study of surface cleanliness at a variety of locations

	Frequency (no. samples of each color)	Percent samples per total number of samples
Blue/white	33	33.0
Yellow/green	39	39.0
Dark green	10	10.0
Pink/Orange	18	18.0
Total	100	100.0

Table 3. Average Surface counts of viable and recoverable bacteria from fluorescing and non-fluorescing areas by location (CFU/cm²)

LOCATION	Number of surfaces sampled	Fluorescing Area	Non-Fluorescing Area	Standard Deviation	Standard Error of Mean
Baby changing station/ and Nurseries (4 sites)	18	1.98	2.50	0.368	0.240
Public bathrooms (4 sites)	55	1.32	2.89	1.110	0.040
Doctor's waiting rooms (2 sites)	27	2.22	1.00	0.862	0.294
Mean		1.39	2.80	4.073	0.570

Table 4. Colors of fluorescence commonly emitted by various substances

Substance	Color emitted
Urine (new)	Yellow/green in humans, Blue/white in rodents
Urine (old)	Pale yellow/green in humans, yellow/white in rodents
Semen	Bright green when fresh, blue-white when older
Ear wax	Bright blue to blue/green, yellow when older
Feces	Dark green
Greases/oils/fats	Pinks, oranges, and dark yellows
Detergents	Bright purple white
Nylons	Bright white
Aflatoxins in corn/maize	Green-yellow
Chlorophylls	Reds

Table 5. Frequency of color fluorescence by location

Fluorescence Color	Doctors' offices	Public Bathrooms	Nurseries	Baby changing stations
Blue/white	13	13	5	2
Yellow/green	8	23	4	4
Dark green	5	5	3	0
Pink/orange	6	9	0	0

Figure 1. Comparative average densities of viable bacteria from fluorescing surfaces and nearby non-fluorescing surfaces by color of fluorescence



