Opportunities and Methods for Using Fluorescent Gel as a Proxy for Pathogen Transfer in Biosecurity Research

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HIGHLIGHTS

- While fluorescing gel may evaporate from a surface, luminance of the surface does not change.
- Fluorescing gel exhibits thresholds beyond which additional gel density does not increase luminance.
- Fluorescing gel only transfers between surfaces when it is wet.
- There are limits to relating luminance and mass transfer.
- Fluorescent material is a useful proxy for contamination transfer demonstration and research.

ABSTRACT. Glo Germ fluorescing material is a popular tool for teaching and researching contaminant transfer in and out of agriculture. The objectives of this paper were to: (1) quantify relationships between gel area density (mass per unit area) on a surface and its luminance, and (2) identify factors important in measuring Glo Germ gel transfer from one surface to another. Varying densities of Glo Germ gel were applied to paper, plastic, and rubber surfaces; each combination was replicated three times. Digital images collected over one hour were analyzed for luminance (the average gray value per unit area) under ultraviolet light. Changes in mass were also measured. For the gel transfer objective, a fixed weight was placed over varying wet and dried fluorescent material densities on paper and plastic surfaces. Gel masses were weighed, and images of the surface and receptor were taken before and after transfer. Evaporation was significantly faster ($p = 0.0019$) on the paper surface compared to the plastic surface. The luminance did not change as the gel evaporated from either surface. For each material, luminance initially increased with increasing density until a threshold, after which additional fluorescing gel density did not change luminance. The thresholds for paper, plastic, and rubber surfaces...
were 0.018, 0.014, and 0.041 g cm$^{-2}$, respectively. Wet gel transfer test results suggest that transfer is easier to quantify on the receptor than the source. The dried gel did not exhibit measurable transfer. This research found limitations in equating mass transfer and luminance, but luminance threshold values can inform maximum Glo Germ application for imaging purposes. These research results support continued research and outreach with fluorescent material to reduce and prevent the spread of disease or other harmful contaminants in food and animal production.

**Keywords.** Biosecurity, Fluorescence, Luminance, Mass transfer.

In the livestock industry, disease outbreaks threaten the health and welfare of animal herds and flocks, agricultural workers, and the continuity of business for a region or country. A pathogen can be transmitted to a new host through direct contact with an infected agent or its secretions or indirect contact via a fomite contaminated with or by an infected agent (Martin, 2017). Many pathogens can spread through contact transmission, including Hepatitis A and B in humans (Gray Davis et al., 1989; Nelson et al., 2020), the Porcine Epidemic Diarrhea virus (Jung et al., 2020), the Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) in swine (Dee et al., 2002), and the Highly Pathogenic Avian Influenza among poultry (Van der Goot et al., 2003). The focus is often on interspecies transmission, but there are zoonotic pathogens that transfer from animals to humans and vice versa (i.e., Peiris et al., 2007; Dale and Brown, 2013). On farms, notable mechanical transmission pathways are animal-to-animal contact, shared equipment uses, human habits and traffic patterns, and movement of live animals and mortalities (CFSPH, ISU, 2013; USDA APHIS, 2017). While the risk of infection depends on pathogen characteristics, dose, host susceptibility, and other factors, contact characteristics impact transmission rates for multiple pathogens.

Biosecurity is the suite of steps taken to reduce and prevent the spread of disease. In the absence of a specific disease outbreak or for the protection of the affected industry, models or proxies enable the demonstration and quantification of transmission and exposure risks. Dee et al. (2002) demonstrated potentially significant modes of mechanical transmission of a field strain of PRRSV inoculated into carriers like snow and water by replicating common on-farm human behaviors within a field-scale model of a farm. Where they exist, modified live vaccines facilitate viral transfer rate measurements in place of viral strains (e.g., Dee et al., 2006). In the agricultural food production sector, Fenske et al. (2002) used the fluorescent tracer Calcofluor RWP to investigate the performance of chemical protective clothing as a method of exposure mitigation to pesticide application.

Another fluorescing product has several applications in agricultural and industrial research settings. Glo Germ (Glo Germ Co., Moab, UT) is an ultraviolet (UV) fluorescent product available in powder, gel, or aerosol form and marketed to demonstrate contamination. Maitland et al. (2013) examined cross-contamination at a mock retail deli market, wherein a trained sensory panel ranked fluorescent gel presence in UV light pictures based on coverage and intensity. Conover and Gibson (2017) compared the effectiveness of foaming versus gel-based soap during handwashing. The metric for comparison was the absorbance of 370 nm light by any residual fluorescent compound in a pooled sample of swabs extracted in ethanol. Call et al. (2017) investigated hazardous drug (HD) preparation and handling methods by simulating HD spread onto downstream surfaces using powdered fluorescent powder placed on exterior vial surfaces. They quantified spread based on the
number of fluoresced pixels in vial images collected before and after handling. Anderson et al. (2018) used fluorescent powder to compare the effectiveness of showers and bench entry (physical barrier) protocols to no bench (no physical barrier) entries in commercial swine facilities. Barn workers picked up fluorescent powder from the floor surface in a barn entryway, and researchers used a grid system and the presence or absence of fluorescence to quantify contamination following the post-entry protocol.

These previous studies demonstrated creative approaches to measuring pathogen (or other harmful substance) transfer. The four Glo Germ studies accommodated the potential variability in fluorescent material application, transfer, and detection. The methods generated quantitative data to facilitate treatment comparisons; however, there is a lack of information regarding the potential to quantify actual mass transfer from the fluorescent substance. For example, hypothetically, if the contaminant concentration on a surface is known and the mass transfer of surficial material is available, the contact transmission rate can be quantified and compared to other transmission pathways like those via air. Halvorson and Hueston (2006) use the (log) mass of the contaminant and the percent available for transmission in the calculation of a pathogen exposure risk index in biosecurity programming. Contact surfaces vary, but the mass transfer is theoretically very minute and difficult to measure in field-scale situations. Thus, this research set out to answer the question of whether fluorescing material traits relate to mass and subsequent mass transfer between two surfaces. The objectives of this study were to: (1) quantify the relationship between the area density (mass per unit area) of Glo Germ gel applied to a surface and its resulting luminance and determine whether the relationship depended on time and surface type; and (2) identify important factors affecting the ability to measure Glo Germ gel transfer from an application surface to a receptor. This study considered the traits of Glo Germ gel specifically and recognizes that the product is also available in powder and aerosol formats. Understanding fluorescent material traits supports future research and outreach methods for assessing contact transmission pathways and, ultimately, protecting humans and animals in food and livestock production.

**Materials and Methods**

Each experiment required fluorescent material, an initial application surface, an ultraviolet light source, background light conditioning, a camera, and image processing software. Glo Germ UV fluorescent gel (Glo Germ Co., Moab, UT) was used in all tests. A UV flashlight (UVL 1006, Glo Germ Co., Moab, UT) illuminated the Glo Germ. A custom-built curtained table (20 x 20 x 20 cm; fig. A1) eliminated surrounding light exposure, which preliminary tests deemed an important variable for image analysis. All images were taken using a Digital Single Lens Reflex (DSLR) camera (Canon EOS Rebel SL1, Canon U.S.A. Inc., Melville, NY) with camera settings detailed in table A1. This camera was chosen to maintain consistency within the laboratory (versus, i.e., multiple, personal cell phone cameras) while providing high-quality images.

ImageJ, an image processing and analysis software package, was used to quantify both fluorescent luminance and the area containing fluoresced pixels (Schindelin et al., 2012). Fluorescent luminance is a measurement that quantifies how much light is reflected off a surface. After importing an image file into ImageJ, the image was converted to an 8-bit grayscale image, assigning an intensity number (or “gray value”) between 0 and 255 to each pixel to define its brightness. On this scale, 0 equated to a pure black pixel, and
was a pure white pixel. To aid in analysis, a threshold was set to select only pixels above a specified gray value (155) to separate the Glo Germ gel area from the rest of the image. After the gel pixels were isolated, the area and mean gray value (MGV) were measured for both the fluoresced area and the surrounding image area. MGV is a luminance measurement found by taking an average of gray values for every selected pixel and outputting a single number between 0 and 255. The luminance density (mass per unit area) of the fluoresced area of an image could then be found by dividing the MGV by the image area, in units of cm$^{-2}$. Example images associated with the various steps are available in figure A2 (located in the appendix).

Three different application surfaces were used in this study. They were:
- Paper (sections of blank, white, continuous form paper);
- Plastic (sections of white polystyrene antistatic weighing dishes); and
- Rubber (sections of blue nitrile powder-free disposable gloves).

These three application surfaces were chosen because of their availability. They also represent potential transfer surfaces related to humans found in a livestock barn environment (i.e., record sheets, plastic markers, and gloves).

**Test Series 1 Mass over Time**

The first experiment evaluated two gel masses (0.5 and 1.0 g), two circular surface diameters (2 and 6 cm), and two surfaces (paper and plastic) in a factorial design (eight combinations) replicated three times ($n = 24$) to measure changes in mass (i.e., evaporation) of Glo Germ gel up to 60 minutes post application. All experimental units were prepared by drawing the appropriate circle size (i.e., 2 and 6 cm) in the center of the surface and labeling by surface type, circle diameter, and gel mass. Initial surface masses were recorded, and the desired gel mass was squeezed directly from its tube-shaped container and spread evenly across the circular area with a small, flat spatula while the mass was monitored on the precision scale. All application masses were measured on a precision balance with an uncertainty of ±0.02 g (PGW 2502i, Adam Equipment Inc., Oxford, CT), allowing ten seconds to equilibrate for each mass reading. Experimental unit masses were recorded every two minutes for the first ten minutes, then every ten minutes for the remaining 50 minutes. Gel masses were found by subtracting initial surface masses from each experimental unit mass.

The average evaporation rates for each treatment combination over the hour-long test were compiled into JMP Pro 14® (SAS Institute Inc., Cary, NC) to run a mixed model evaluating evaporation rates with material, circle diameter, initial mass, and interactions as fixed effects, a time block as a random effect, and a first-order autoregressive structure.

**Test Series 2 Luminance over Time**

Tests to determine whether gel evaporation changed the measured luminance density were done using the same eight combinations used in Test Series 1, replicated three times. After the desired gel mass was spread over the specified area and recorded, an image was taken of the surface. Experimental unit masses (i.e., the mass of both the surface and the gel) and images were recorded every ten minutes for one hour. All masses were measured on a precision balance (PGW 2502i), allowing ten seconds to equilibrate for each mass reading. All images were analyzed in ImageJ to measure the surface area (in cm$^2$) and MGV for both the gel and the surrounding area. The difference between those measurements was used to calculate corrected MGV per unit area (luminance density) for each
image, and all values were compiled into JMP Pro 14® to run a mixed model evaluating luminance density with material, circle diameter, initial mass, and interactions as fixed effects, time block as a random effect, and an AR(1) repeated structure.

**Test Series 3 Luminance Density Threshold**

Preliminary experiments suggested there was a threshold beyond which additional gel mass per unit area would not increase luminance measurements. This test used ten gel densities between 0.01 and 0.1 g (in 0.01 g increments) spread over a 2 cm diameter circle on three source materials (paper, plastic, and rubber) in a factorial design (30 combinations), and three replicates of each combination. For the rubber source material, two additional gel masses (0.2 and 0.5 g) were also used (32 total combinations) to further investigate whether previous observations of luminance density plateaus applied to greater masses. For each combination, the surfaces were prepared in the same manner as described in Test Series 2, and the appropriate gel mass was spread over the indicated area, measured on an electric microbalance with an uncertainty of ±0.002 mg (Sartorius M5P, Sartorius AG, Bohemia, NY), allowing ten seconds to equilibrate for each mass reading. An image of each test surface was taken and analyzed in ImageJ to measure MGV. Luminance densities were calculated by dividing the corrected MGV by the area of a 2-cm diameter circle.

Luminance densities, with corresponding gel masses and source materials, were compiled into JMP® Pro 14 to run a fit least squares model analyzing luminance densities by mass, material, and interactions. Nonlinear curve fitting was also performed for each material using exponential curves (3P; eq. 1). To force the curves to intercept at approximately the origin, coordinates of (0, 0) were included in each replicate.

\[
y = a + b e^{c \text{mass}}
\]  

where
- \(y\) = luminance density
- \(cm^{-2}\); \(a, b, c\) = regression coefficients
- \(\text{mass}\) = mass of gel, g
- \(e\) = exponential constant.

**Test Series 4 Transferability of Wet Gel**

Test Series 4 documented how the fluorescent gel transferred from one surface to another. Two initial gel masses (0.1 and 0.2 g), two circular surface diameters (2 and 4 cm), and two surfaces (paper and plastic) were used in a factorial design (eight combinations), replicated three times. The experimental units were prepared in the same manner as described in Test Series 1, and initial experimental unit masses and images were recorded. All masses were measured on an electronic microbalance for finer measurements (Sartorius M5P, Sartorius AG, Bohemia, NY).

To control the process of transferring from one surface (the source) to another (the receptor), a fixed, prepared transfer surface was used. The transfer surface was 218 g in weight, with nitrile material (a section of a glove) stretched across the flat, 2.735 cm diameter bottom surface and secured to the sides of the weight. The transfer surface was set on top of the test surface for 30 seconds. The final mass and luminance measurements of the test surface and the final luminance of the transfer surface followed the previous methods.

Each image was analyzed in ImageJ to measure area and MGV to calculate luminance density values. The differences between initial and final mass and initial and final
luminance densities were calculated and compiled into JMP® Pro 14 to run a mixed model analyzing the effects of material, circle diameter, initial mass, and interactions on the changes in mass and luminance density.

To evaluate the luminance densities present on the receptor after transfer from each experimental unit (i.e., paper or plastic), all calculated luminance density values were compiled into JMP® Pro 14. A mixed model was run to analyze the effects of material, circle diameter, initial mass, and interactions on the change in luminance density, as well as the change in gel mass and the mass transferred onto the fixed weight.

**Test Series 5 Transferability of Dried Gel**

This test was like test 4, except that the fluorescent gel was allowed to dry before attempting transfer from the initial surface to the transfer surface. This test considered two gel masses (0.5 and 1.0 g), two circular surface diameters (2 and 4 cm), and two surfaces (paper and plastic) in a factorial design (eight combinations), replicated three times. The experimental units were prepared in the same manner as described in Test Series 1. All 24 surfaces were left to dry for two days at room temperature, with conditions consistent for all surfaces. Two days ensured enough time for surfaces to dry completely, based on observations from the researchers. After drying, the masses of each test surface were measured, and images of each were taken.

Each image was analyzed in ImageJ to measure area and MGV. Those measurements were used to calculate luminance density values for each image. The same statistical analysis methods described in Test Series 4 were used to evaluate the change in luminance and mass at the source after transfer and to evaluate the luminance densities on the receptor.

**Results**

**Test Series 1 Mass over Time**

Circle diameter (p < 0.0001), surface material (p < 0.0001), and the interaction between circle diameter and surface material (p = 0.0462) significantly affected evaporation rates. There was no significant difference between 2 cm diameter circles on paper or plastic surfaces. The least squares mean estimate for paper with a diameter of 2 cm was 0.00153 ± 0.00015 g min⁻¹, and it was 0.00137 ± 0.00015 g min⁻¹ for plastic with a diameter of 2 cm. All other pairwise differences were significant (fig. 1). The evaporation rate increased overall for larger diameters. The estimate for paper with a diameter of 6 cm was 0.00467 g min⁻¹, which was significantly higher than 0.00405 g min⁻¹ for plastic with a diameter of 6 cm. This implies that gel spread over a larger surface area will have a higher evaporation rate regardless of the material, but there is more variability in evaporation rates between surface materials at larger surface areas.

Initial gel mass also significantly influenced evaporation rates (p < 0.0001). The least squares mean estimate for an initial mass of 0.5 g was 0.00261 g min⁻¹ and increased to 0.00320 g min⁻¹ for a greater initial mass of 1.0 g. This result was expected because it is assumed that experimental units with a higher initial mass density will have a higher evaporation rate.

**Test Series 2 Luminance over Time**

Luminance was significantly affected by surface material (p = 0.0004), circle diameter (p < 0.0001), and interactions between the surface material and circle diameter (p = 0.0016), but luminance was not affected by time (p = 0.2883) or initial mass (p = 0.4788).
Figure 2 shows the difference in luminance between paper and plastic with a 2 cm diameter, while they do not differ at a 4 cm diameter. This suggests surface material is more influential on luminance for smaller coverage areas. Additionally, while luminance decreases as the circle diameter increases for each material, it does not decrease at the same rate; it decreases more rapidly on a paper surface than on a plastic surface. The reason for this difference is unknown, but it may come from the differences in reflectivity between paper and plastic. Since the paper has a lower reflectivity, measured luminance may be more subject to change with coverage area because it is less influenced by background reflectance.

The insignificance of time on luminance suggests the fluorescent substance within the gel is not evaporating over time. Since luminance measurements are not sensitive to
different masses (neither initially nor over time), the potential to estimate gel mass based exclusively on luminance is limited. Additionally, the insignificance of initial mass on luminance suggests there may be a luminance threshold, based on gel area density, after which the luminance no longer increases with the addition of more gel.

**Test Series 3 Luminance Density Threshold**

Luminance was significantly influenced by surface material ($p < 0.0001$) and mass ($p = 0.0103$), but not by interactions between the surface material and mass ($p = 0.1313$). Figure 3 shows the average luminance density values as a function of average mass for each material; it also includes the exponential 3P curves fit for each material.

Figure 3 suggests there is a threshold after which adding more gel would not change the luminance density for a given mass density of Glo Germ. This maximum value is different for each surface material, most likely due to the different reflectivity of the materials, like that seen in test series 2. Since plastic reflects the greatest light, its background MGV is the largest, followed by rubber, then paper. This corresponds to paper having the largest corrected MGV, followed by rubber, and then plastic.

The asymptote parameter estimates represent the luminance density threshold for each surface material, after which increasing mass density would not increase luminance density. Although the maximum values differ between materials, the luminance density values reach that value at a consistently low gel mass. The luminance density reached within 0.1% of the asymptote value at 0.056 g of gel for paper, 0.043 g for plastic, and 0.130 g for rubber. Considering the 2 cm diameter circle area, this equates to mass densities of 0.018, 0.014, and 0.041 g cm$^{-2}$, respectively.

Figure 3. Average luminance density values versus mass for each material ($n=3$), along with fitted curves for each material. Average corrected luminance densities of 38.4 cm$^{-2}$ and 34.8 cm$^{-2}$ for 0.2 g and 0.5 g, respectively for rubber surface were excluded to highlight results from 0 to 0.1 g. Horizontal and vertical bars indicate standard error for each material and mass combination.
Test Series 4 Transferability of Wet Gel

Analyzing the changes in the source surface luminance before and after transfer found significance for the initial mass (p = 0.0021), circle diameter (p < 0.0001), and interactions between the two (p = 0.0060), while the material was insignificant (p = 0.8450). There was no significant difference with 4 cm diameter circles for the two initial masses. The change in luminance ranged from -0.1002 to 0.3642 cm\(^2\) for 4 cm circles. Least square means estimates indicate that the luminance on the source surface did not change after mass transfer to the weight for a 4 cm circle diameter. This is likely because the diameter of the fixed weight was less than 4 cm and the mass densities in both cases (0.25 g cm\(^{-2}\) and 0.5 g cm\(^{-2}\)) were greater than the thresholds found for paper and plastic surfaces in Test Series 3. A significant change in luminance was seen for the 2 cm circle diameter combinations, likely because the diameter of the fixed mass was greater than 2 cm, causing the final gel area on the source to increase. Since luminance is directly dependent on the coverage area, the increase in the area after the transfer caused the final luminance to decrease. All other pairwise differences were significant (fig. 4).

A mixed model to determine the significance of each factor and their interactions on the change in mass of the source surface found significance in material (p < 0.0001), initial mass (p < 0.0001), circle diameter (p < 0.0001), and the interactions between material and initial mass (p = 0.0124), material and circle diameter (p = 0.0440), and initial mass and circle diameter (p = 0.0066). Least square means estimates for material and circle diameter combinations showed no significant difference between Paper with a gel diameter of 2 cm (0.0602 ± 0.0035 g) and Plastic with a gel diameter of 4 cm (0.0724 ± 0.0035 g). All other pairwise differences were significantly different (fig. 5a).

The results suggest combinations with a larger initial mass had a larger change in mass at the source, which was expected, but the source material influenced the degree of mass change (fig. 5b). In general, there is a greater change in mass for a plastic source surface when compared to paper, meaning the gel tends to transfer more easily off plastic compared to paper surfaces.

![Figure 4. Change in luminance least square mean estimates for each initial mass and circle diameter combination. Vertical standard error bars are included, and letters above bars denote significant differences.](image-url)
The significant interaction between initial mass and circle diameter shows that sources with a larger initial mass had a larger change in mass, but the degree of change was influenced by the area it was spread across (fig. 5c). Combinations with the 4 cm circle diameter had a smaller change in mass than those with the 2 cm diameter circle, likely due to their gel area in relation to the receptor transfer area. The same gel mass spread across the 4 cm diameter circle created a thinner layer of gel than that found across the 2 cm diameter circle, so the receptor came into contact with less gel during transfer.

A mixed model to determine the significance of each factor and its interactions on the luminance transferred to the fixed weight found the source material to be significant (p = 0.0303). The least square mean for paper was 23.4 ± 1.3 cm⁻², and it was 19.3 ± 1.3 cm⁻² for plastic. This suggests the gel transfers off a paper surface more easily than a plastic surface, which is not consistent with the results from the changes in mass at the source. Overall, since there are so many factors influencing the changes in luminance and mass on the surface, namely the relative sizes of the source and receptor, it is preferable to focus results on changes in mass and luminance to the receptor as opposed to the source.

**Test Series 5 Transferability of Dried Gel**

Analyzing the change in luminance before and after the transfer of the sources with dried gel found no significant factors, indicating there was no significant transfer between the source and receptor. Observations during testing supported this conclusion, as the dried gel remained fixed to the initial surface with little to no visible transfer onto the fixed weight. A second mixed model to evaluate the change in mass at the source found material (p < 0.0001), initial mass (p = 0.0015), and circle diameter (p = 0.0020) significant, but all interactions were insignificant. Least square means estimates suggested plastic (0.000946 ± 0.000059 g) had a significantly larger change in mass when compared to paper.
(0.000373 ± 0.000059 g), implying the dried gel sticks better to a paper surface versus plastic. Additionally, an initial mass of 1.0 g had a least square mean of 0.000848 ± 0.000059 g, while an initial mass of 0.5 g had a value of 0.000471 ± 0.000059 g, suggesting that a greater mass of dried gel transfers off a surface when the initial mass is greater. A circle diameter of 2 cm (0.000842 ± 0.000059 g) had a significantly greater change in mass than a circle of 4 cm (0.000477 ± 0.000059 g). This is likely because of the size of the transfer surface relative to the area of the source. Since the 2 cm source area is smaller than the transfer surface, there is more opportunity for the weight to fully stick to the dried gel, as opposed to the 4 cm source area, which is larger than the transfer surface.

A mixed model considering the significance of each factor and interactions on the luminance transferred to the fixed weight found material (p = 0.0046) and circle diameter (p < 0.0001) as significant factors. The least square means showed that transfer from a paper source (14.8 ± 0.6 cm−2) was greater than that from a plastic source (10.9 ± 0.6 cm−2). Although the least square means from the change in mass on the source would suggest the opposite would occur, this result exemplifies the difficulty present in relying on the change in the source gel mass or luminance as a way to quantify transfer. Additionally, the least square means showed that transfer from a 2 cm diameter circle (20.1 ± 0.6 cm−2) was greater than from a 4 cm diameter circle (5.61 ± 0.63 cm−2). This, again, is likely due to the relative sizes of the source areas compared to the transfer surface.

**Discussion**

This research demonstrated that evaporation affects scenarios when using gel as a proxy for contamination. Source material, surface area, and initial mass influence gel evaporation rates. Gel evaporation can change the gel’s properties and performance, most notably its ability to simulate transfer from one surface to another. As the gel evaporates, the luminance does not change, suggesting it would be very difficult to accurately estimate mass transfer based solely on measured luminance. However, the lack of change in luminance with evaporation allows researchers to collect luminance measurements for a longer period of time in an experiment where demonstrating contamination is more important than mass transfer. If luminance is the measurement of choice, a longer stability period eases data collection in large spaces (i.e., barns), and over time. Fenske et al. (2002) and Anderson et al. (2018) comment on waiting periods or cleaning periods between trials necessary to eliminate residual tracer material between tests, with protocols dependent on the use scenario. The gel cannot be used to simulate contamination by transfer after it is dry because it tends to stick to its source material after it dries. If wet gel is used to simulate transfer, it is best to focus on quantifying gel transferred to the receptor rather than the difference before and after transfer from the source. Traits of other Glo Germ formats, like the dry powder, are likely less affected by evaporation over time for transfer tests. Evaporation must also be considered in outreach demonstration planning, considering the time between application to a surface and detection, and if a transfer is part of the demonstration.

In experimental research including mass transfer, it seems necessary to keep gel mass densities below threshold values to use luminance as an indicator of mass density. Since the mass density threshold is different for different materials, calibration must be done to determine the threshold for a specific material before beginning each experimental data collection. The procedure laid out in Test Series 3 provides a calibration method that can be used by researchers to fit the specific experimental designs, which include varying surface areas and materials. Other surfaces that could be explored to better represent the
agricultural industry include various metals, different wooden surfaces, and glass. Since this series of tests was done at a lab scale, more research needs to be done to ensure the mass density thresholds persist for larger surface areas. More considerations when scaling up this procedure include guaranteeing a method of light control is in place and large enough for the test area and keeping all camera settings consistent across images. In mock or field-scale settings, several researchers comment that fluorescent material is more valuable as a qualitative tool than a quantitative tool for research, considering the variability in the test subjects and surfaces (Conover and Gibson, 2017; Harrison et al., 2021).

Any proxy has benefits and limitations in its representation of something else. This research furthers the understanding of where and how fluorescent gel material characteristics influence luminance and mass density measurements. Fluorescent substances continue to be a valuable tool in agriculture for investigating and demonstrating modes and rates of pathogen or contaminant transfer without having to inoculate surfaces with these potentially harmful substances, using many creative methods described by previous researchers. Quantification data still seems limited to luminance, versus mass metrics, but this research provides guidance for dosing to ensure consistent luminance data.

**Conclusion**

A series of tests with Glo Germ gel identified opportunities and limitations for its use in research settings in the agricultural industry. Gel evaporated over time, causing the mass of Glo Germ on a surface to change. However, the luminance did not change during evaporation. There was a linear relationship between luminance and mass up to specific gel mass density values for paper, plastic, and rubber source materials (0.018, 0.014, and 0.041 g cm⁻², respectively). Beyond the mass density thresholds, the luminance no longer changed. The gel showed no significant transfer after being allowed to dry for two days on any surface, but there was a significant transfer when the gel was still wet. Quantifying the transfer of wet gel based on the changes to the source was influenced by several other factors, including the source material, initial gel mass, and the circle diameter size in relation to the receptor, suggesting focusing on quantifying the gel transferred is preferable to quantifying change in gel on the receptor. Glo Germ gel is a safer approach in agricultural settings to simulate surface transfer without having to use harmful substances like pathogens or chemicals. Surface transfer can be quantified to better understand areas of concern and the effectiveness of potential transfer reduction strategies.

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**References**


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Appendix

Table A1. Camera settings used for Canon EOS Rebel SL1 in Test Series 1-5.

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<td></td>
<td>F11 for plastic and rubber</td>
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Figure A1. Curtained table model constructed from wood (a) without and (b) with curtains made from blackout fabric lining. Fabric was secured using Velcro strips. Smaller hole was used for UV light and larger hole for camera lens.

Figure A2. Image analysis process to quantify fluorescent luminance and area containing fluoresced pixels for circle of Glo Germ gel on paper, using ImageJ; method adapted from Schindelin et al. (2012).