A proposed “Protocol for the Determination of Fluence for Medium Pressure (MP) Lamps” has been developed by Jim Bolton, Karl Linden, and Ian Mayor-Smith. The protocol is designed to standardize MP fluence quantification for disinfection and advanced oxidation research so that research undertaken in different laboratories can apply consistent and comparable methods. The goal of this proposed Task Force is to facilitate the process of third-party reviews of this protocol, with the aim of eventually gaining IUVA endorsement.

Scope

The protocol will be reviewed in the context of water and wastewater treatment, using a collimated beam or quasi collimated beam equipped with a medium pressure UV lamp as the test apparatus. Although the emission spectrum of MP lamp extends from about 180 nm to over 800 nm, the current version only covers the range from 200 nm to 400 nm, which is the range that is most relevant to water treatment practice.

Process

The draft proposal will be reviewed (Stream 1) and experimentally validated (Stream 2).

Stream 1: The protocol will be published as a draft protocol on the IUVA website to allow comments to be submitted to either Dr. Chengjin Wang (chengjin.wang@utoronto.ca) or Dr. Jim Bolton (jbolton@boltonuv.com), proposed Co-Chairs of this Task Force. Emails will be sent out to all IUVA members to invite their input. The Co-Chairs will also actively seek comments from non-IUVA members as supplemental contributors, if necessary.

Stream 2: The protocol will be experimentally validated at several participating labs. These participating labs (ideally >3) will follow the protocol to (1) measure the UV fluence needed to degrade a certain percentage of methylene blue color in pure water (e.g., 20% absorbance at 664 nm) using an MP quasi collimated beam reactor, and to (2) measure and calculate the UV fluence needed to achieve 4 log inactivation of T1 coliphage using an MP quasi collimated beam reactor and a low pressure (LP) quasi collimated beam reactor, respectively. The required MP and LP fluence should be the same within experimental error if the proposed protocol is correct. Each lab will conduct these tests by at least two people independently. The results will be used to identify any potential uncertainties associated with the protocol.

The task force will revise the protocol according to the feedback and will, if necessary, return it to the previous reviewers for comments. Once all the comments are addressed, the task force will submit the protocol to the IUVA Board for final approval.
Deliverables

The final version of the protocol will be published in UV Solutions and posted on the IUVA website. A journal article based on the experimental validation data is recommended but optional.

Schedule

<table>
<thead>
<tr>
<th>Time</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan 31, 2020</td>
<td>Finalize the Task Force proposal</td>
</tr>
<tr>
<td>Feb 7, 2020</td>
<td>Obtain support from two Board Members</td>
</tr>
<tr>
<td>Feb 28, 2020</td>
<td>Obtain EOC approval on the Task Force proposal</td>
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<tr>
<td>Feb 28-May 29, 2020</td>
<td>Stream 1 Review</td>
</tr>
<tr>
<td>Feb 28, 2020</td>
<td>Finalize the Round Robin Test protocol</td>
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<tr>
<td>Mar 2-May 29, 2020</td>
<td>Stream 2 Experimental Validation</td>
</tr>
<tr>
<td>Jun 1-Jun 30, 2020</td>
<td>Protocol Revision</td>
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<tr>
<td>Jul. 1, 2020</td>
<td>Submit the final version to all Board Members for approval</td>
</tr>
<tr>
<td>Aug. 1, 2020</td>
<td>Submit the approved draft for publication in UV Solutions</td>
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</tbody>
</table>

Note: the dates are approximate and will likely change as the review/validation proceeds.

Participating members

All IUVA members are welcome to comment on the protocol. Non-members may also be invited to comment by the Co-Chairs.

Proposed Task Force Members:

Jim Bolton

Chengjin Wang (University of Toronto)

Karl Linden (University of Colorado Boulder)

Ian Mayor-Smith (University of Brighton)

Keith Bircher (Calgon Carbon)

Ted Mao (Trojan)

Ron Hofmann (University of Toronto)

Task Force Chairs

Chengjin Wang, Ph.D.

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Jim Bolton, Ph.D.

Bolton Photosciences Inc.
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Tel: 780-439-4709 Email: jbolton@boltonuv.com

Resource requirement
At least three laboratories to participate in the experimental validation of the protocol.
Appendix 1

New Task Force / Project Board Member Evaluation Checklist—
Please email completed form to info@iuva.org

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Yes</th>
<th>No</th>
<th>Comments</th>
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</thead>
<tbody>
<tr>
<td>Does the Project align with the IUVA Mission?</td>
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<td>Is there a carefully defined problem to guide the project?</td>
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<td>Does the project have specifically stated and achievable goals?</td>
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<td>Are the elements of the problem specified and prioritized?</td>
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<td>Is there a specified work plan that aligns with the goals?</td>
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<td>Does the work plan incorporate existing best practices, if any?</td>
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<td>Are the proposed solutions achievable and significant?</td>
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<td>Does the solution hold promise for new IUVA Membership?</td>
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<td>Are there existing resources to support project?</td>
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<td>Do the proposed Task Force members reflect an inclusive industry cross-</td>
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<td>section?</td>
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<td>Is there reasonable expectation for new resource development?</td>
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<td>Is specified project infrastructure sufficient to support project?</td>
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<td>Is there a strong leader and membership to achieve project goals?</td>
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<td>Are roles and responsibilities of implementation team specified?</td>
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<td>Are the expectations for IUVA involvement reasonable?</td>
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<td>Are there stated reasonable and measurable outcomes?</td>
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<td>Is the timeline appropriated for scope of project?</td>
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<td>Does the project have potential to “achieve best practice” status?</td>
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<td>Will project have regional, national, global impact, if successful?</td>
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<td>Is there a communication strategy for sharing results?</td>
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</table>
Appendix 2

Protocol for the determination of fluence (UV dose) using a medium-pressure UV lamp in bench-scale collimated beam ultraviolet experiments

James R. Bolton,1 Karl G. Linden2 and Ian Mayor-Smith3

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1. Introduction

This Protocol is an extension of the “Protocol for the determination of fluence (UV dose) using a low-pressure or low-pressure high output UV lamp in bench-scale collimated beam ultraviolet experiments” (Bolton et al., 2015a) to accommodate a medium-pressure (MP) UV lamp. It is based on the paper by Bolton et al. (2015b), but it is set out in a step-by-step manner to make it easier to follow for experimental measurements. The reader should first read the Bolton et al. (2015b) paper to understand the background for this Protocol. Also, convenient Excel spreadsheets are available either from the authors or in the Member Zone of the web site of the International Ultraviolet Association (www.iuva.org).

2. Background

Medium-pressure UV lamps differ from low-pressure or low-pressure high output UV lamps (which emit primarily at 253.7 nm) in that the emission is ‘broad-band’ extending from about 180 nm to over 800 nm. This necessitates some additional procedures.

It is assumed that the user has available a quasi-collimated beam apparatus set up according to the instructions in Bolton and Linden (2003), a spectroradiometer calibrated within the past 12 months, a Petri dish to contain the suspension of microorganisms, a small Teflon® coated stir bar and a stirring motor mounted on a platform that can be adjusted vertically.

3. Terms and units

Absorbance, A(λ) (unitless)

Logarithm to the base 10 (linear absorbance) of the incident (prior to absorption) spectral radiant power \( P_0 \) divided by the transmitted spectral radiant power \( P_\lambda \). In practice, the absorbance, as determined in a spectrophotometer, is based on the logarithm to the base 10 of
the ratio of the detector signal with the sample cell containing pure (e.g., Milli-Q) water to that with the sample cell containing the solution of interest.

\textit{Absorption coefficient, } \alpha(\lambda) \textit{ (cm}\textsuperscript{-1})

Absorbance \(A(\lambda)\) divided by the optical path length, \(l\) (cm).

\textit{Einstein}

One mole (\(N_A\) \(6.02214 \times 10^{23}\)) of photons. All photon-based units are expressed in amount basis, that is, as einsteins, rather than as photons.

\textit{Irradiance, } \(E\) (W m\(^{-2}\))

The total radiant power \textit{incident} from all upward directions \textit{on} an infinitesimal element of \textit{surface} of area \(dS\) containing the point under consideration divided by \(dS\).

\textit{Fluence Rate, } \(E_o\) (W m\(^{-2}\))

The total radiant power incident from all directions through an infinitesimally small sphere of cross-sectional area \(dA\), divided by \(dA\).

Note that in a collimated beam the irradiance and the fluence rate are virtually the same.

\textit{Fluence, } \(F_o\) (J m\(^{-2}\)) \textit{(also called UV dose)}

The time integral of the fluence rate and is defined as the total radiant energy of all wavelengths passing from \textit{all} directions through an infinitesimally small sphere of cross-sectional area \(dA\), divided by \(dA\). If the fluence rate (\(\approx\) irradiance) is constant in time, the fluence (UV dose) is given by \(E \times t\), where \(t\) is the exposure time in seconds.

In North America, it is common to use the units mW cm\(^{-2}\) for fluence rate or irradiance and mJ cm\(^{-2}\) for fluence (UV dose). Note that 1 mW cm\(^{-2}\) = 10 W m\(^{-2}\) and 1 mJ cm\(^{-2}\) = 10 J m\(^{-2}\).

\textit{Photon fluence, } \(F_{p,o}\) (einstein m\(^{-2}\))

Total number of moles of photons (einsteins) incident from all directions on a small sphere over time divided by the cross-sectional area of the sphere.

\textit{Photon fluence rate, } \(E_{p,o}\) (einstein m\(^{-2}\) s\(^{-1}\))

Rate of \textit{photon fluence}. Total number of moles of photons (einsteins) incident from all directions on a small sphere per time interval divided by the cross-sectional area of the sphere.

\textit{Photon flux, } \(q_p\) (einstein s\(^{-1}\))

Number of moles of photons (einsteins) passing through a specified plane per time interval.
Photon irradiance, $E_p$ (einstein m$^{-2}$ s$^{-1}$)

Number of moles of photons (einstein) per time interval (photon flux) $q_p$ incident from all upward directions on a small element of surface containing the point under consideration divided by the area of the element.

Spectral fluence, $F_{\lambda,o}$ (J m$^{-2}$ nm$^{-1}$) $^1$

Derivative of fluence, $F_o$, with respect to wavelength $\lambda$.

Spectral fluence rate, $E_{\lambda,o}$ (W m$^{-2}$ nm$^{-1}$)

Derivative of fluence rate $E_o$ with respect to wavelength $\lambda$.

Spectral irradiance, $E_\lambda$ (W m$^{-2}$ nm$^{-1}$)

Derivative of irradiance $E$ with respect to wavelength $\lambda$.

Spectral photon flux, $q_{p,\lambda}$ (einstein s$^{-1}$ nm$^{-1}$)

Derivative of the photon flux $q_p$ with respect to wavelength $\lambda$.

Spectral photon fluence rate, $E_{p,o,\lambda}$ (einstein s$^{-1}$ m$^{-2}$ nm$^{-1}$)

Derivative of the photon fluence rate $E_{p,o}$ with respect to wavelength $\lambda$.

Spectral photon irradiance, $E_{p,\lambda}$ (einstein s$^{-1}$ m$^{-2}$ nm$^{-1}$)

Derivative of the photon irradiance $E_p$ with respect to wavelength $\lambda$.

Note that there are two kinds of wavelength dependent parameters: (1) those that have functional dependence on wavelength {e.g., the molar absorption coefficient [ ( )]}, and (2) those that are the result of differentiation with respect to the wavelength [e.g., the spectral irradiance ($E_\lambda$)]. The wavelength dependence of the former will be designated with ( ) at the end of the symbol, and the wavelength dependence of the latter will be designated with a subscript (e.g., $E_o$). One can always tell which parameters are of type 2 – they will have nm$^{-1}$ as part of the units.

For polychromatic UV light sources, such as a medium-pressure UV lamp, the emission spectrum should be split up into wavelength intervals (preferably 1 nm). The overall effect of the polychromatic UV light source is then determined by a numerical integration over the relevant wavelength range.

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$^1$ Strictly speaking, the SI units would be J m$^{-3}$; however, we retain the units of nm for clarity. Similar statements apply to the other spectral quantities.
4. **Importance of using the spectral photon irradiance in the calculations**

As noted by Bolton et al. (2015b), photobiological processes proceed at a rate that is proportional to the absorbed photon flux. Thus it is not appropriate to weight calculations by the spectral irradiance (W m\(^{-2}\) nm\(^{-1}\)) because this is a power-based quantity. Instead one should weight the calculations by the spectral photon irradiance (einstein m\(^{-2}\) s\(^{-1}\) nm\(^{-1}\)). The calculations allow one to determine the effective average photon irradiance at 254 nm. One can then convert back to the average irradiance at 254 nm and from there determine a fluence (or UV dose), which can then be compared with a fluence (UV dose) determined with a low-pressure lamp.

5. **Procedure when a medium pressure UV lamp is used**

In the Protocol below, reference is made to the Excel spreadsheet “Fluence – MP.xls”.

5.1. **Germicidally weighted or unweighted worksheets**

If the medium-pressure collimated beam apparatus is being used to determine the fluence – response curve for a specific microorganism, the “Germicidal Fluence Calculations” worksheet should be used. If germicidal weighting is not required, the “Unweighted Fluence Calculations” worksheet should be used. The default values of the germicidal factors (GFs) in the spreadsheet are the relative absorbance of DNA from *Salmonella typhimurium* LT2 (Chen, 2007), where the GF is set to 1.000 at 254 nm. If an action spectrum is available for the microorganism of interest, the action spectrum (normalized to 1.000 at 254 nm) should be used instead of the DNA GFs.

5.2. **Determination of the relative medium-pressure UV lamp emission**

The spectral emission of a medium pressure mercury lamp is determined by its individual design (e.g., quartz envelop selected, internal mercury pressure), the lamp driver selected and the environmental conditions in which it is run (i.e., predominantly temperature). Depending on these factors, the spectral output may be wholly or in part changed (e.g., in a particular direction), hence it is necessary for the spectral irradiance to be measured (e.g., in units of W cm\(^{-2}\) nm\(^{-1}\)) at the plane of exposure. This should be carried out using a calibrated spectroradiometer (although care must be taken to select the appropriate equipment and methodology employed). Ideally, the spectral irradiance spectrum should be measured before and after a set of exposures.

Once determined the average absolute spectral irradiance values should be inserted into cells B50 to B150 in either the *Germicidal Fluence Calculations* or the *Unweighted Fluence Calculations* worksheet of the Excel spreadsheet.

5.3. **Preliminary setup**

- Choose a sample volume for the suspension of microorganisms and enter this volume into cell I36 in the *Fluence Calculations* worksheet of the Excel spreadsheet. This volume, plus the volume of the stir bar, is used to calculate the water path length (I40). Alternatively, the user may choose to measure the sample depth directly and put this value into cell I40.
• Enter the volume of the stir bar into cell I37 in the *Fluence Calculations* worksheet of the Excel spreadsheet. This volume can be determined from caliper measurements of the length and diameter of the stir bar or by displacement using at least 10 stir bars of equal size.

5.4. *Determination of the Petri Factor (PF)*

• Determine the inner diameter (cm) of the Petri dish using a caliper and enter it into cell I38 of the *Petri Factor* worksheet of the Excel spreadsheet.

• On a light-colored cardboard, draw two perpendicular lines that cross at the center of the cardboard. Draw ticks every 0.5 cm from the center intersection and label the ticks according to the distance from the center.

• Place the light-colored cardboard on the stir motor and the radiometer detector on top of the cardboard. Adjust the level so that the calibration plane of the radiometer detector is at the level that the top surface of the water in the Petri dish will be during exposure.

• Center the stir motor so that the center of the light-colored cardboard is at the center of the light circle projected by the collimated beam.

• Allow the UV lamp to warm up until the output stabilizes, typically for at least 10 min.

• Place the radiometer detector so that the center of the detector is at the center of the light-colored cardboard; record the irradiance and the position of the radiometer detector.

• Progressively move the detector in 0.5 cm steps in the +x, −x, +y, and −y directions, recording the irradiance at each position. Go out far enough so that the distance is greater than the inner radius of the Petri dish.

• Enter the radiometer detector data into columns Y and AD of the *Germicidal Fluence Calculations* or the *Unweighted Fluence Calculations* worksheet of the Excel spreadsheet.

• Note the value of the Petri Factor in cell I43 or DC151. If the Petri Factor is less than 0.90, use a Petri dish with a smaller diameter or a larger diameter collimator. For best practice, the Petri Factor should not be less than 0.90.

5.5. *Determination of the Water Factors (WF)*

• Place a sample of the suspension of microorganisms into a synthetic quartz spectrophotometer cell and determine the absorbance spectrum from 200 to 300 nm in 1 nm steps. If the absorbance in a certain wavelength range is less than 0.1, use a cell with a longer path length (e.g., 5 cm or 10 cm). If the absorbance in a certain wavelength range is greater than 1.5, use a cell with a shorter path length (e.g., 0.5 cm or 0.1 cm) or quantitatively dilute the sample.

• The absorption coefficient \( a \) is given by \( A/l \), where \( A \) is the absorbance and \( l \) is the path length (cm).

• Average the absorption coefficients over each 1 nm wavelength band and enter them into cells E50 to E150 in either of the *Fluence Calculations* worksheets.

• The Water Factor is displayed in cells F50 to F150 in either of the *Fluence Calculations* worksheets.
5.6. **Determination of the Divergence Factor (DF)**

- Measure the distance (cm) from the top surface of the Petri dish to the center of the UV lamp. Enter this value into cell I41 in either of the *Fluence Calculations* worksheets of the Excel spreadsheet.

5.7. **Reflection Factor (RF)**

- Assuming that water is the medium, the Reflection Factors [calculated from data in Daimon and Masumura (2007)] are wavelength dependent (they are in cells G50 to G150 in either of the *Fluence Calculations* worksheets.

5.8. **Determination of the true irradiance in each wavelength band**

The true irradiance in each wavelength band can be determined using a calibrated spectroradiometer to determine the true average irradiance at the level of the top of the solution in each 1 nm wavelength band. These numbers should then be inserted into cells B50 to B150 in the either of the *Fluence Calculations* worksheets. In this process, one must be careful that the spectroradiometer does not saturate. Saturation is indicated when the peaks have flat tops.

The calibration of the spectroradiometer can be checked using the following procedure:

1. Mount a low pressure lamp in place of the medium pressure lamp in the CB apparatus.
2. Take a scan of the spectral irradiance using the calibrated spectroradiometer. Be careful not to saturate the detectors.
3. Integrate the scan from 250 to 258 nm to obtain the total spectroradiometer irradiance (W m$^{-2}$) – call this SR1.
4. Mount a NIST-calibrated radiometer so that its calibration plane is at the same vertical distance as that used for the spectroradiometer.
5. Record the radiometer irradiance as R1.
6. The ratio R1/SR1 is a correction factor that should be applied to all of the spectroradiometer readings.

5.9. **Determination of the volume averaged irradiance in the cell suspension**

In both the *Germicidal Fluence Calculations* and the *Unweighted Fluence Calculations* worksheets, the volume averaged photon irradiance in the cell suspension is determined by a numerical integration in rows 50 to 150 to yield the final result in cell K152. Because of the weighting applied during the calculation of this result, this value can be multiplied by the energy per einstein at 253.7 nm to obtain the *equivalent* average germicidal (or unweighted) irradiance at 254 nm in the water, which is displayed in cell K153.

5.10. **Determination of the exposure times for the target fluences (UV doses)**

- Enter the target fluences (UV doses) into cells G157 to G163 in the either of the *Fluence Calculations* worksheets of the Excel spreadsheet.
- The exposure times for the target fluences (UV doses) are the given in cells K157 to M163 in either of the *Fluence Calculations* worksheets.
• Note that ideally, the exposure times should be at least 30 seconds. If the calculated exposure times are less than 30 seconds, move the platform to a greater distance from the UV lamp to decrease the irradiance.

5.11. Determination of fluence (UV dose) – Response Curves

The following is an example of a suggested protocol for determining fluence (UV dose) response curves. Experienced users will use best practice and standard operating procedures to establish their own protocol.

• Prepare a set of culture plates sufficient in number to handle the exposures planned.
• Choose a set (at least five including zero) of target fluences (UV doses) that cover a range in which the log inactivation is at least 4. For example, the set might be 0, 15, 30, 45, 60, 75 mJ cm$^{-2}$.
• Randomize the set. Assuming that triplicate determinations are made, a possible sequence of exposures and corresponding target fluences (UV doses) could be 60, 15, 75, 45, 0, 30, 75, 30, 0, 15, 45, 60, 15, 45, 0, 30, 75, 60.
• The ‘0’ samples in the above set should be placed into position in the collimated beam apparatus and left there stirring for a period equal to the longest exposure times used, but with the shutter closed.
• After the samples have been exposed, carry out 10-fold dilutions according to the expected viable cell count levels. Use at least two dilutions that bracket the expected count levels.
• Assay at least two (preferably three) culture plates with each diluted suspension. Be very careful to maintain a sterile environment during the assays.
• Culture the plates for the specified time at the specified temperature and then count the colonies or plaques on each plate.
• For each dilution, average the counts and, using the dilution factor, determine the viable count level in the original suspension.
• Average the suspension count levels from different dilutions if the plates are countable. Here it may be necessary to calculate a weighted average, since the reliability of the counts may be different for different dilutions.
• Determine the average log inactivation for each fluence (UV dose) from log\[N_0/N\], where $N$ is the count level for a given fluence (UV dose) and $N_0$ is the count level for the ‘0’ control samples.
• The Fluence (UV dose) – Response Curve is a plot of log\[N_0/N\] as a function of the fluence (UV dose).

References


