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Drinking water treatment with ultraviolet light for travelers — Evaluation of a mobile lightweight system

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Summary
Background: The SteriPEN® is a handheld device for disinfecting water with ultraviolet (UV) radiation. The manufacturer claims a reduction of at least 99.9% of bacteria, viruses, and protozoa. The present study intends to verify the general effectiveness of the device. Furthermore, the influence of bottle geometry and water movement is examined and the issue of user safety with regard to UV-C radiation is addressed.
Methods: The device was applied on water containing a known number of microorganisms (*Escherichia coli*, *Staphylococcus aureus*, and the spore of *Geobacillus stearothermophilus*) and the survival rate was examined. Three different types of bottles commonly used among travelers served as test containers. All tests were conducted with and without agitating the water during irradiation. Furthermore, a spectral analysis was performed on the light of the device.
Results: The SteriPEN® reached a mean reduction of more than 99.99% of bacteria and 99.57% of the spores when applied correctly. However, the results of the trials without agitating the water only yielded a 94.98% germ reduction. The device's maximal radiation intensity lies at 254 nm which is the wavelength most efficient in inactivating bacteria. The UV-C fraction is filtered out completely by common bottle materials. However, when applied in larger containers a portion of the UV-C rays exits the water surface.
Conclusions: If applied according to the instructions the device manages a satisfactory inactivation of bacteria. However, it bears the danger of user errors relevant to health. Therefore, education on the risks of incorrect application should be included in the travel

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medical consultation. Also there are still aspects that need to be subject to further independent research.

1. Introduction

Drinking water hygiene is one of the major topics in a travel medical consultation before visiting countries with poor hygienic conditions. For the prevention of travelers' diarrhea and other diseases transmitted by waterborne pathogens travelers are advised to choose an adequate method for personally treating the local water [1,2]. Among the well-established techniques for drinking water disinfection are chemical (e.g. chlorine, iodine) and physical methods (e.g. boiling, filtration) [3].

A relatively recent development on the market for personal water treatment equipment is the SteriPEN®. It is a handheld battery-powered device for disinfecting water by means of ultraviolet (UV) radiation, marketed by its manufacturer Hydro-Photon Inc. since 1999 (Fig. 1). While in the first years the SteriPEN® was not very widely spread it nowadays enjoys increasing popularity among travelers in regions where there is limited access to clean drinking water. A questionnaire based study conducted 2011 in the Everest region, Nepal, showed that the SteriPEN® was just as frequently used as ceramic filters and iodine drops/tablets, each method represented with around 7% of the participants (compared to around 33% chlorine, 24% boiling, 17% bought, and 5% none/other) [4].

The principle of UV-disinfection is not new. It has been used for the treatment of communal water supplies since the middle of the 20th century [5]. Also, methods do exist for using part of the sunlight's UV-fraction to disinfect water stored in PET bottles by placing them on a reflecting surface for several hours ("SODIS") [6].

Figure 1 The SteriPEN® (Photo: L. Timmermann).

The SteriPEN® constitutes the first commercially available UV-application for personal use. Compared to the conventional chemical and physical methods of drinking water disinfection the SteriPEN® has some appealing advantages but also some disadvantages (Table 1).

The SteriPEN® has been tested by several laboratories in the USA and Canada, contracted by the manufacturer, and found to meet the requirements of the U.S. Environmental Protection Agency. Most of these studies work with the MS-2 coliphage, a very UV resistant virus that infects and replicates in the bacterium *E. coli*, as a surrogate for a wide range of waterborne human pathogens. Correct application of the SteriPEN® on one liter of clear water showed a reduction of the coliphage between 99.6806% (log 2.51) [7] and 99.9641% (log 3.45) [8]. This is considered to be equivalent to a reduction of at least 99.9999% of bacteria and 99.99% of viruses [9]. Furthermore, the SteriPEN®'s efficacy has been evaluated against Klebsiella, Cryptosporidium, poliovirus type 1 and rotavirus SA-11 with good results respectively [10–12]. The studies described above are published on the SteriPEN®'s website [13].

However, papers published by independent, peer-reviewed journals are scarce. There currently is no search result on the term "Steripen" in PubMed (October 2015). This gave reason to conduct the present study which intends to verify the general effectiveness of the device. Furthermore, the radiation geometry of the SteriPEN®'s bulb suggests that it might be less effective in slim long bottles with a narrow bottle mouth than in shorter wide-mouthed containers (Fig. 2). Thus, the influence of bottle shape and the importance of water movement during irradiation were investigated. Finally, a spectral analysis was performed on the radiation emitted by the device.

1.1. Functional principle of UV-disinfection

Ultraviolet light is electromagnetic radiation of wavelengths just below the spectrum of visible light (400–780 nm). It is subdivided into three groups: UV-A with a wavelength of 315–400 nm, UV-B with 280–315 nm, and UV-C with 100–280 nm (Fig. 3) [14]. The smaller the wavelength the more energetic is the radiation. Next to its visible spectrum the sun also emits UV light. However, in contrast to the UV-A and -B rays the UV-C fraction is virtually completely absorbed by the atmosphere [14]. This is why microorganisms did not have the opportunity to develop proper mechanisms of resistance against UV-C. Therefore, the part of UV radiation most effective in destroying these organisms is UV-C with a peak of inactivation at 254 nm for bacteria [15].

The damage to microorganisms caused by UV radiation occurs directly on DNA. UV irradiation of the DNA molecule causes thymine bases to form dimers [15,16]. Thus, the

Table 1 Advantages and disadvantages of the SteriPEN®.

Advantages
- lighter than ceramic filters (156 g vs. 425 g)
- no chemical by-products which cause an irritating smell or taste
- less time consuming than chemical treatment (residence time of chemicals between 30 min and 2 h vs. 90 s of UV-irradiation)
- less time/material consuming than boiling → cool drinking water immediately after application

Disadvantages
- fragile light bulb → backup method necessary
- lifetime of batteries limited (100 cycles with four AA lithium batteries) → extra set of batteries necessary on longer trips
- does not remove toxins or heavy metals (common to all non-filtrating methods)
- does not conserve the water (common to all non-chemical methods) → re-contamination possible
- water needs to be clear because any turbidity weakens the UV radiation → it may be necessary to pre-filtrate the water

enzymes responsible for unwinding and copying the DNA during replication are not able to function anymore. This renders the microorganism unable to reproduce and cause an infection. Thereby, UV light has a bacteriostatic effect, it is not primarily bactericidal [15]. The water treated with the SteriPEN® is disinfected but not sterile.

All waterborne enteric pathogens can be inactivated by ultraviolet light, provided a sufficient dose is administered [6]. Different microorganisms show different sensitivities to UV radiation. The published data on the exact doses required to inactivate different species vary substantially – depending among other things on the germ strain and UV source. In general, bacterial spores (e.g. *Bacillus subtilis*) and viruses (e.g. Adeno-, Polio-, or Hepatitis A virus) have a relatively high resistance while most bacteria are inactivated by significantly lower UV doses [6,17,18]. Within the realm of bacteria gram-negative organisms such as Salmonella, Campylobacter, and *Vibrio cholerae* are more susceptible to the damaging radiation than gram-positive ones (e.g. Staphylo- and Enterococci) [5,19]. Protozoan cysts like Cryptosporidium parvum and Giardia lamblia have been found to be even weaker against UV light than bacteria [5,6,17,20].

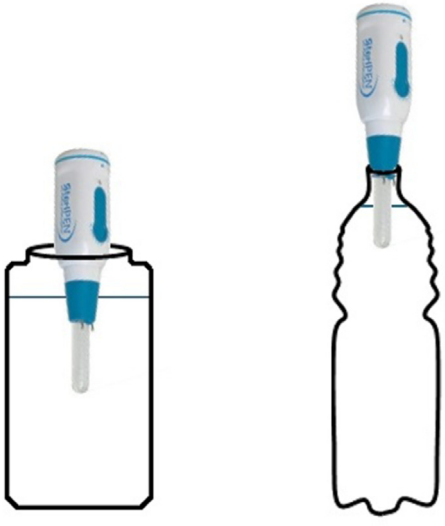

Figure 2 Position of the SteriPEN® in relation to the geometry of different bottle types.

2. Material & methods

There are a number of different SteriPEN® models on the market, differing in aspects like size, energy source, or the presence of a rubber plug which allows for turning a narrow mouth bottle upside down (Fig. 4). The present study was conducted using the model SteriPEN® *Classic*. As an energy source it requires four lithium AA batteries lasting for around 100 cycles of disinfecting one liter of water.

2.1. Operation of the SteriPEN®

The SteriPEN® offers two different timing functions, one for treating 1 L (90 s, default setting) and another for treating 0.5 L of water (48 s). For the safety of the user the SteriPEN® is equipped with a water sensor which allows the light bulb to turn on only when submerged in water. To indicate its proper functioning the bulb not only emits ultraviolet but also visible light. During irradiation the user is instructed to agitate the water by stirring with the SteriPEN® or swaying the bottle. After the time cycle is complete the SteriPEN® is switched off automatically [21].

2.2. Test organisms

Three different microorganisms were chosen to serve as test organisms:

- *Escherichia coli* (ATCC 25922): gram-negative rod bacterium, common component of water containing fecal pollution [22].
- *Staphylococcus aureus* (ATCC 25923): gram-positive coccus, more environmentally resistant than *E. coli* due to a thick cell wall [23].
- *Spore of Geobacillus stearothermophilus* (ATCC 7953, spore suspension): spore forming gram-positive rod bacterium, extremely heat resistant, serves as a test germ

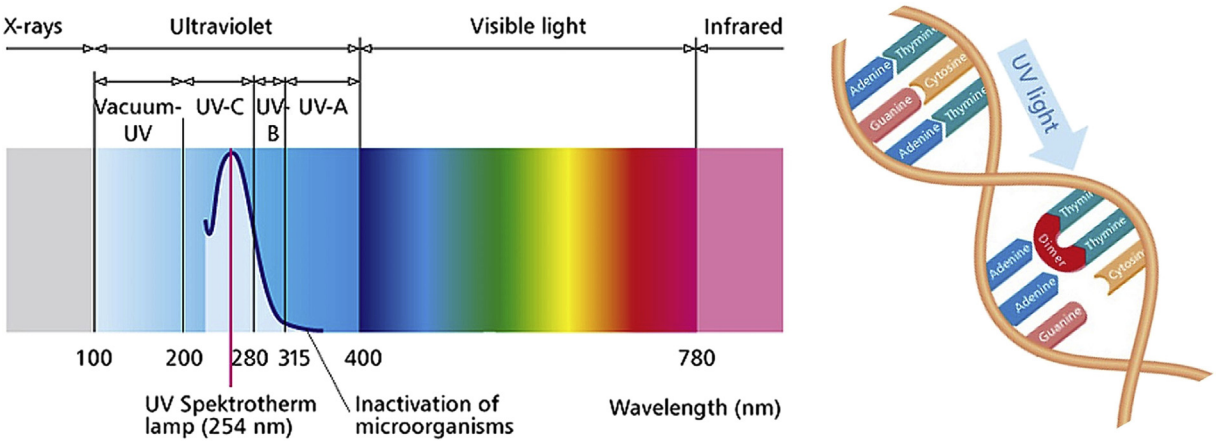

Figure 3 Electromagnetic spectrum and wavelength dependent inactivation of microorganisms (left), mechanism of DNA damage (right) [16].

for thermal sterilization processes, not human pathogenic [24].

The test germs were cultivated on blood agar (PSASB). The spectrum of germs used in this study was limited to bacteria and bacterial spores since viruses and protozoa require a much more elaborate test setup and different laboratory conditions. The three types of microorganisms described above were chosen to represent three different levels of UV resistance (see chapter 1.1.).

Figure 4 Use of the rubber plug, SteriPEN® model "Classic" (Photo: L. Timmermann).

2.3. Test bottles

To create a setting as close as possible to outdoor conditions three of the most widely used types of bottles among trekkers were chosen as test containers: a Nalgene® wide mouth plastic bottle, a SIGG™ aluminum bottle, and a disposable mineral water PET bottle (Fig. 5). Each of these bottles had a capacity of one liter.

2.4. Test medium and serial dilution of the germ suspension

As test medium regular mineral water (Volvic®) was chosen. It contains a natural mineral and salt composition thus representing real conditions.

To examine the SteriPEN®'s effect on different concentrations of microorganisms a serial dilution was prepared. A standardized germ concentration was achieved by determining the optical density of the germ solution in a spectrophotometer. The stock solution was then diluted in steps of 1:10 which resulted in four different test concentrations (dilution level 10^0-10^{-3}, i.e. dilution level 10^0 = stock solution, dilution level 10^{-3} = stock solution diluted 1:1000).

The test bottles were filled with mineral water and the respective test solution in a relation of 1:500, i.e. 0.998 L of water were supplemented with 2 ml of the germ solution. Subsequently, the bottles were shaken thoroughly to achieve an equal distribution of the germs.

2.5. Test procedure

Immediately after filling the bottle a sample of 100 µl was taken as initial value. After 90 s of UV irradiation with the SteriPEN® three additional samples were taken: 1) directly underneath the water surface where the UV bulb had been situated ("TOP"), 2) from the bottom of the bottle ("BOTTOM"), and 3) after stirring the water thoroughly with a sterile plastic rod ("MIXED"). The samples were then streaked on petri dishes containing blood agar. After incubation the viable germs were counted as colony forming units (CFU) [25].

Figure 5 Test bottles.

The tests regarding the general effectiveness of the SteriPEN® were performed using the Nalgene® bottle. The SteriPEN® was applied according to the instructions of the user guide.

For testing the importance of correct application and the effects of the SteriPEN®'s radiation geometry all tests were conducted with and without agitating the water (Fig. 6). Water movement was achieved by stirring with the SteriPEN® itself (Nalgene® wide mouth bottle) or by using the function of the rubber plug to turn the bottle upside down and sway it (SIGG™, PET bottle).

Some users with narrow mouth bottles merely stir with the SteriPEN® inside the bottle neck while the bottle itself is standing still. To test whether this manner of agitating the water is sufficient another trial was conducted simulating this situation.

All tests regarding the influence of bottle type and water movement were conducted using *E. coli* as a test organism.

2.6. Spectral analysis

In order to determine the SteriPEN®'s intensity distribution a spectral analysis was performed using the spectrometer module Ocean Optics Jaz (model *Jaz-EL200-XR1*). Since the sensing unit is not suitable for underwater use, the measurements were conducted without submerging the SteriPEN® in water.

To demonstrate the user safety claimed by the SteriPEN®'s manufacturer the spectrum was recorded again covering the bulb with a disposable PET bottle and a regular drinking glass, respectively. When applied in larger containers the light of the SteriPEN® is not shielded from the user's eyes except for the interface between air and water which reflects some of the radiation back into the container. In order to test whether there is UV-C radiation exiting the water surface the device was applied in a regular cooking pot made of stainless steel (diameter: 16 cm, height: 8 cm). The spectrum of the radiation passing through the water surface was detected at three different angles (25°, 45°, and 90° to the water surface).

3. Results

3.1. Effectiveness of the UV irradiation depending on germ concentration and species

Table 2 shows the results of the tests regarding the general effectiveness of the SteriPEN®. The reduction of viable germs is presented as a percentage of the initial concentration. Additionally the reduction factor (log reduction) was calculated to illustrate the magnitude of change in germ concentration.

Up to the dilution level 10^{-1} (i.e. prior germ concentration of around 3×10^3 CFU/ml) the counts of *E. coli* and *S. aureus* were effectively brought below the detection limit. Treatment of the highest germ concentration (i.e. $10^0 \triangleq$ initial concentration of around 3×10^4 CFU/ml) resulted in a small remainder of proliferative bacteria. The reduction of the spore was slightly less efficient.

3.2. Influence of bottle type and importance of water movement

This series of tests was conducted with *E. coli* in all three bottle types. As an example the results of dilution level 10^{-1} are presented in Table 3. The results of the other concentrations tested correspond to the results in Table 2.

Again only the highest germ concentration (10^0) showed a small count of proliferative bacteria after irradiation. Taking the average of all bottles and all concentrations the SteriPEN® reached a reduction of more than 99.99% (log 4.64) when applied correctly.

In contrast to this, not agitating the water during irradiation resulted in an average germ reduction of only 94.98% (average of all trials conducted). Directly underneath the water surface where the bulb had been situated no proliferative bacteria were detectable after irradiation. However, the counts at the bottom were still remarkably high. Surprisingly, in the case of the disposable bottle the number of proliferative germs was higher after mixing the water than in the bottom sample before mixing.

The same research design was conducted with *S. aureus* as well (not shown here). The results (including the phenomenon of the PET bottle) were comparable to those of *E. coli*.

Finally, the effectiveness of stirring with the SteriPEN® inside the neck of a narrow-mouthed bottle (SIGG™) was investigated. During this procedure the bottle itself was standing still. On average this method of agitating the water only yielded a germ reduction of 88.93%.

3.3. Spectrum of the SteriPEN®'s radiation and user safety

The spectral analysis showed that the maximum intensity of the SteriPEN®'s radiation lies within the UV-C spectrum between 253 and 255 nm, with a peak at 254 nm (Fig. 7). Next to this there are several less intense peaks in the UV-A and -B range (around 297, 313, and 365 nm) as well as in the spectrum of visible light (around 405, 436, 546, and 578 nm).

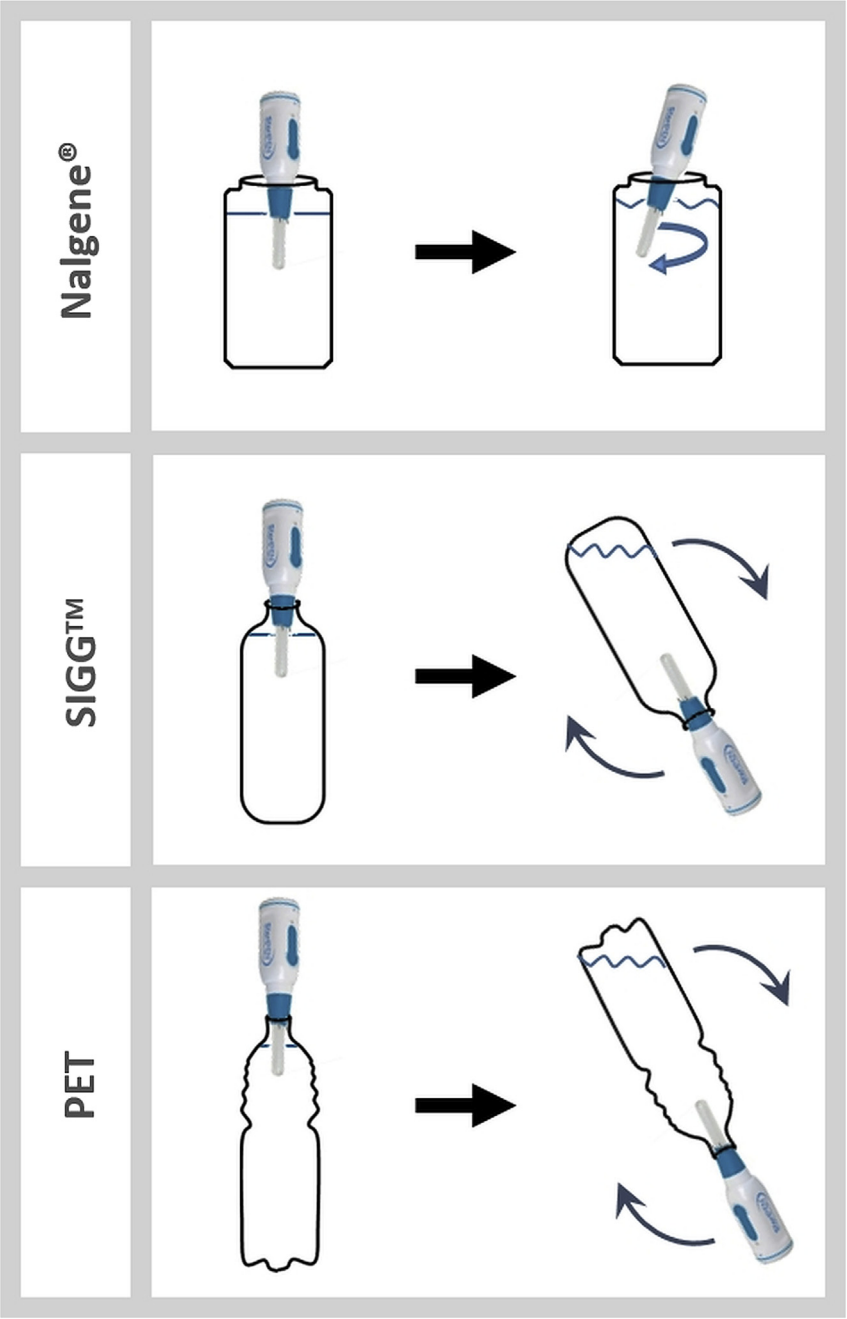

Figure 6 Tests conducted with and without agitating the water during irradiation.

When covering the SteriPEN®'s bulb with a PET bottle or a drinking glass, the peaks in the UV-B and -C range disappear. Only visible light as well as a small part of the UV-A fraction passes through the material. This effect could be reproduced by placing a regular pair of eyeglasses in front of the detection unit. Also in this case the UV-B and -C rays were filtered out completely.

When applying the SteriPEN® in a cooking pot it could be demonstrated that besides the weakened rays of the visible spectrum also a part of the UV-B and -C fraction exits the water surface. With a diminishing angle to the surface the relative intensity of this radiation increases.

4. Discussion

On average, the SteriPEN® reached a reduction of more than 99.99% of *E. coli* and *S. aureus* when applied correctly. Only at the highest concentration of germ test solutions – which corresponds to the maximal pathogen concentration that can be found in optically clear natural water [26] – were there any viable pathogens remaining. Even here a reduction of more than 99.97% of *E. coli* and *S. aureus* was achieved. Also the majority of spores of *G. stearothermophilus* become inactivated but the reduction of viable germs is not as efficient as with the other two bacteria. This can

Table 2 Germ counts before and after correct application of the SteriPEN® in a Nalgene® wide mouth bottle.

Degree of dilution	Microorganism	Before SP [CFU/100 µl]	After SP [CFU/100 µl]	Reduction
10^0	*E. coli*	25 000	4	99.9840% (log 3.80)
	S. aureus	33 400	9	99.9731% (log 3.57)
	G. stearothermophilus	15 700	77	99.5096% (log 2.31)
10^{-1}	*E. coli*	2 500	0	100%
	S. aureus	3 340	0	100%
	G. Stearothermophilus	1 570	10	99.3631% (log 2.20)
10^{-2}	*E. coli*	246	0	100%
	S. aureus	328	0	100%
	G. Stearothermophilus	165	1	99.3939% (log 2.22)
10^{-3}	*E. coli*	24	0	100%
	S. aureus	32	0	100%
	G. Stearothermophilus	15	0	100%
Average *E. coli*				99.9960% (log 4.40)
Average *S. aureus*				99.9933% (log 4.17)
Average *G. stearothermophilus*				99.5667% (log 2.36)

be explained by the extreme radiation resistance of spores [18]. The fact that the SteriPEN® reaches its limit with the spores is unlikely to be of any practical relevance since generally spores are not primary pathogens relevant to drinking water hygiene [27]. There are however some types of viruses whose UV resistance is comparable to that of bacterial spores (e.g. some strains of Adeno- and Rotavirus [18]). It therefore seems necessary to conduct further independent research on the device's effectiveness against such viruses.

However, if the water was not agitated the SteriPEN® did not accomplish a satisfactory disinfection (average: 94.98%). This outcome occurred independently of the bottle type, thus showing that the disinfecting effect is not merely a function of the distance of the light bulb to the bottom of the bottle. The results from the bottom of the long slim PET bottle were even slightly better than the ones of the short wide-mouthed Nalgene® bottle. This phenomenon might be attributable to a specific pattern of reflection inside the bottle resulting in an uneven distribution of radiation intensity. Attempts to agitate the water by stirring within the neck of a narrow mouth bottle are not sufficient to guarantee safe drinking water.

The results of the tests conducted in the PET bottle without agitating the water pose further questions. Following irradiation there tended to be more viable germs present after mixing the water than at the bottom of the bottle before mixing. This may indicate that there is an area within the bottle where the microorganisms are protected from the damaging radiation. The reason and relevance of this phenomenon is yet to be evaluated.

The spectral analysis of the SteriPEN®'s light yielded a maximal intensity of radiation at 254 nm. This corresponds exactly to the wavelength most efficient in inactivating bacterial DNA [15]. Furthermore, it was demonstrated that common bottle materials such as PET and glass filter out the

Table 3 Results of *E.coli*, dilution level 10^{-1}. Incorrect ("Water calm") and correct ("Water agitated") application of the SteriPEN® in all three bottle types. The reduction percentage was calculated from the "MIXED"-values.

Bottle	Mode of application	Before SP [CFU/100 µl]	After SP [CFU/100 µl]			Reduction
			Top	Bottom	Mixed	
Nalgene®	Water calm	2 500	0	231	95	96.2000% (log 1.42)
	Water agitated	2 500	0	0	0	100%
SIGG™	Water calm	3 230	0	467	359	88,8854% (log 0.95)
	Water agitated	3 230	0	0	0	100%
PET	Water calm	3 360	0	23	82	97.5595% (log 1.61)
	Water agitated	3 360	0	0	0	100%
Average - Water calm						**94.2150% (log 1.24)**
Average - Water agitated						**100%**

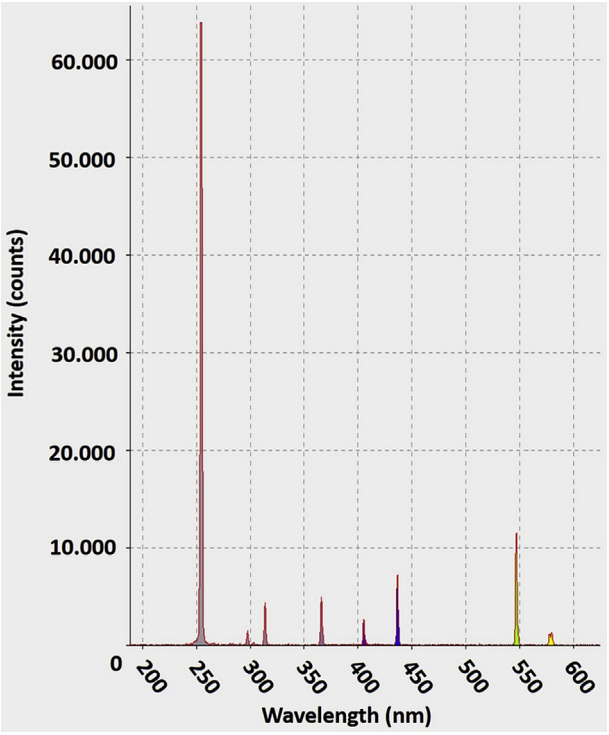

Figure 7 Spectral analysis.

entire UV-B and -C fraction of the spectrum and the UV-A fraction is weakened substantially. It can thus be concluded, that there is no danger to the eyes when watching the SteriPEN®'s bulb through the wall of a plastic or glass bottle during irradiation.

When used in a larger container like a cooking pot a fraction of the UV-B and -C radiation exits the water surface. The intensity of this radiation depends on the angle of vision. The applied measuring technique, however, only yields information on the relative intensity of the different wavelengths. Therefore, on the basis of these data it is impossible to draw a conclusion concerning a potential threat to the user. For this purpose it is suggested to conduct further research determining the absolute intensity of the exiting radiation.

5. Conclusion

When applied correctly according to the user guide the SteriPEN® constitutes an alternative to the classical methods of drinking water disinfection for travelers. However, the device bears the danger of user errors relevant to health. Because the number of travelers who are using the SteriPEN® is increasing it is advisable to include the education on potential risks into the travel medical consultation. The following aspects should be addressed in this context:

- It is essential to agitate the water during irradiation. In narrow mouth bottles it does not suffice to carry out stirring motions with the SteriPEN®. Consequently, for this type of bottle SteriPEN® models without a rubber plug are not suitable.
- Droplets present in the bottle cap or neck are not disinfected when using the SteriPEN® and are therefore a potential source of recontamination. The manufacturer recommends to dry any such water remnants with a clean towel. Alternatively, the neck and cap can be flushed out with some of the freshly irradiated water. However, this procedure only reduces the extent of recontamination but does not eliminate the risk. It is thus advisable not to store the water for longer periods of time before consumption.
- According to the manufacturer the SteriPEN® only yields proper disinfection results if the water is clear. Thus, turbid water needs to be filtered before application of the device.
- Watching the SteriPEN®'s light through the wall of a plastic or glass bottle can be considered nonhazardous. However, it is yet unclear whether the UV-C radiation exiting the water surface when applying the SteriPEN® in larger containers (e.g. cooking pots) poses a health risk for users and bystanders. Until clarification of this issue the user should be advised not to look directly into the light emitted by the SteriPEN® through a water surface or protect his or her eyes with a pair of glasses.

There are further aspects concerning the SteriPEN®'s effectiveness that were not dealt with in this paper and have only been tested by the manufacturer so far. Examples are the application of the device in hydration bladders and containers with a capacity of more than 1 L, the influence of turbidity, or the effectiveness against other types of microorganisms like viruses and protozoa. It is suggested that such issues are addressed by further independent research.

While this paper is dealing with drinking water hygiene other methods concerning the prevention of travelers' diarrhea are of course equally important. There are e.g. preliminary reports on the use of an alcohol based hand gel sanitizer which show at least a partial efficacy in preventing gastrointestinal infections [28]. Such new preventive methods including the use of the SteriPEN® or hand gel sanitizer are promising and have the potential of increasing popularity among travelers. They should be further evaluated in the context of the prevention of traveler's diarrhea.

Conflict of interests

The authors state that they have no conflicts of interest. The tests were conducted without knowledge or support of the manufacturer of the SteriPEN®.

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