Relationship between lumen diameter and length sterilized in the 125L ozone sterilizer

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Background: A safe alternative to ethylene oxide for reprocessing heat-sensitive lumen medical devices, such as endoscopes, is needed. The effectiveness of a new, safe, low-cost, and environmentally friendly low-temperature sterilization process using ozone was assessed.

Method: Rigid lumen devices were used to assess the maximum length of lumens of different internal diameters that can be sterilized in the TSO_3 model 125L ozone sterilizer. Two inoculation techniques were used. An inoculated wire was placed inside lumens with internal diameters of 0.8 mm and larger, whereas lumens with an internal diameter of 0.5 mm were inoculated directly.

Results: Lumens with internal diameters of 0.5 mm, 1 mm, 2 mm, 3 mm, and 4 mm with lengths varying between 45 and 70 cm can be sterilized with ozone. Calculation of the log reduction value for each size demonstrated the achievement of a sterility assurance level of 10^{-6}. Experimental results demonstrated a linear relationship (with $r^2 = 0.990$) between the length of lumen that can be sterilized in the 125L ozone sterilizer and its internal diameter. Effective sterilization of an ACMI ureteroscope that is more challenging in terms of sterilant penetration in a small lumen (0.8 mm) compared with the stated lumen claims confirms that the relationship can conservatively be used to predict the length of a lumen device that can be sterilized in the 125L ozone sterilizer for a given diameter.

Conclusion: Intermediate sizes of lumen devices that can be sterilized in the 125L ozone sterilizer can be interpolated from the linear relationship between diameter and length found in the present study. (Am J Infect Control 2008;36:291-7.)

FDA reprocessing requirements for reusable medical devices are related to the Centers for Disease Control and Prevention Guidelines published in 1985.[1] These guidelines define devices intended for contact with normally sterile body areas, such as minimally invasive surgery (MIS) devices, as critical reusable devices. Given the increasing use of invasive procedures with MIS devices, such as endoscopes with lumens of extended lengths and small diameters, there is a need for appropriate testing methods to validate the technology for sterilizing these instruments.

The validation of a sterilization process for a critical medical device is generally done using a biological indicator (BI). In USP 29, a BI is defined as ''a characterized preparation of a specific microorganism that provides a defined and stable challenge to a specific sterilization process.''[2] Three types of biological indicators are defined in USP 29; the first form includes spores that are added to a carrier, the second form is a spore suspension that is inoculated on or into a product, and the third form is a self-contained indicator.[3]

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Selecting the proper biological indicator depends on the sterilizing agent. Guidance for the selection, use, and interpretation of biological indicators for sterilization of health care products has been published by the International Organization for Standards.[4] Small channels in lumen devices cannot accommodate traditional biological indicators, such as spore strips and self-contained BIs; thus, inoculated carriers and a spore suspension inoculated into the lumen device channel were selected. Each method has pros and cons. On one hand, inoculation of carriers, such as threads, wires, and sutures, often is more accurate and reproducible for spore distribution and recovery than the inoculation of a spore solution into the channel of a device. On the other hand, an inoculated carrier inserted into the channel of a lumen can obstruct gas penetration and create a challenge not truly representing the product.

The resistance of the inoculated product will be influenced by the clumping of spores, surface phenomena, and/or other environmental factors.[5,6] In addition, the percentage recovery obtained using a particular technique is a factor in choosing the optimum inoculation and recovery methods. The recovery percentage generally should be above 50%, although reaching that level is not possible in some circumstances.[5]

The effectiveness of repeated sterilization cycles on critical devices must be demonstrated using a sterilization process cleared for use in health care facilities by the Food and Drug Administration. Some medical

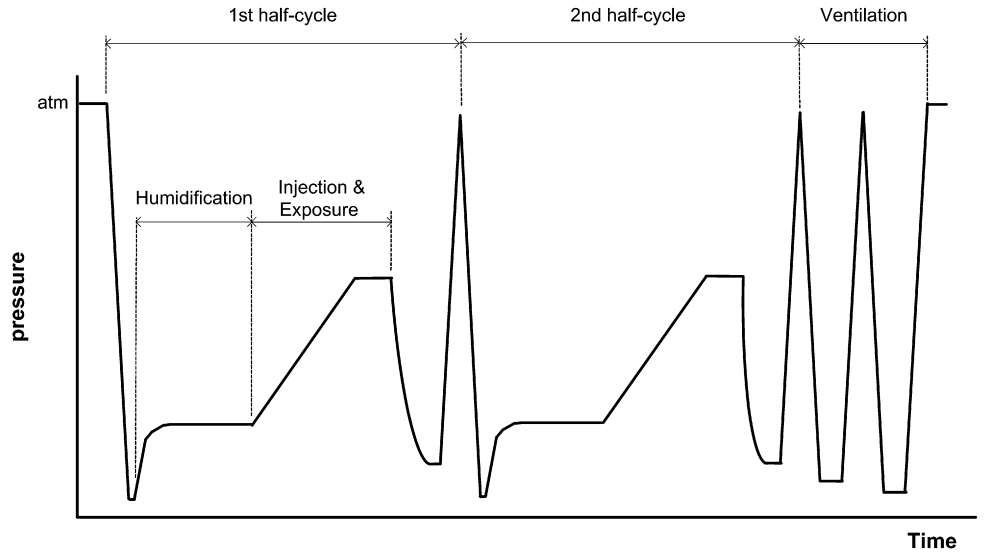

Fig 1. The 125L ozone sterilizer cycle.

devices with lumens may be damaged by autoclaving (ie, application of moist heat under pressure) due to their materials of construction. Thus, low-temperature sterilization processes are essential for reprocessing these medical devices. Guidelines for the reduction of ethylene oxide emissions from sterilization facilities are limiting ethylene oxide use in Canada[7] and the United States.[8] Ethylene oxide residues on devices are also regulated.[9] Penetration capabilities or the number of lumen devices that can be processed in one cycle is limited in some gaseous hydrogen peroxide processes.[10,11]

A new environmentally friendly sterilization process using ozone is a safe alternative for the sterilization of MIS devices, particularly those with lumens.

METHODS

Sterilizer

The TSO_3 (Québec, Canada) model 125L ozone sterilizer was used for this study.[12] The sterilization cycle comprises 2 identical half-cycles (Fig 1). After the door is closed, a vacuum is drawn, followed by a humidification step. Ozone is then injected into the chamber, and the sterilization process begins. When the half-cycle has been reached, the steps, from the vacuum to the ozone injection phases, are repeated, followed by a final ventilation phase to remove ozone from the chamber.

Bacterial strain

Spore suspensions of *Geobacillus stearothermophilus* ATCC 7953 (batch: AR323; population: 2.0×10^6 colony-forming units [cfu]/10 μL; D-value$_{121°C}$: 1.9 min), used to prepare the inoculation solution, were obtained from STS duoTEK Inc (Rochester, NY). The spore suspensions were verified and adjusted to achieve a final concentration of 1.0×10^6 to 1.5×10^6 cfu/10 μL.

Lumen devices and carrier inoculation

Stainless steel tubing used in the fabrication of hypodermic needles (Popper & Sons Inc, New Hyde Park, NY) was used for the lumen devices. Devices with internal diameters of 0.5 mm, 1.0 mm, 2.0 mm, 3.0 mm, and 4.0 mm were tested.

The 0.5-mm lumen devices were inoculated with a 10-μL volume of the inoculation suspension and left to dry in the laboratory for a minimum of 14 hours. For the lumen devices with an internal diameter $>$ 0.5 mm, the microbial challenge was created by placing an inoculated wire inside the channel of the devices. Stainless steel wires (Air Liquide Canada Inc, Québec, Québec, Canada) were inoculated with 10 μL of *G stearothermophilus* inoculation solution as a biological indicator (1.38×10^6 cfu/10 μL). The stainless steel wires were cut 5 cm longer than the lumens to be sterilized. The inoculation was done in the central portion of the wire. After a minimum of 14 hours of drying at room temperature, the inoculated wires were placed inside the lumens, and the ends were bent at 2.5 cm to keep the inoculated section in the middle of the lumens (Fig 2).

Sterilization

After the lumens for each length and diameter had been prepared, each was placed in a sealed TSO_3

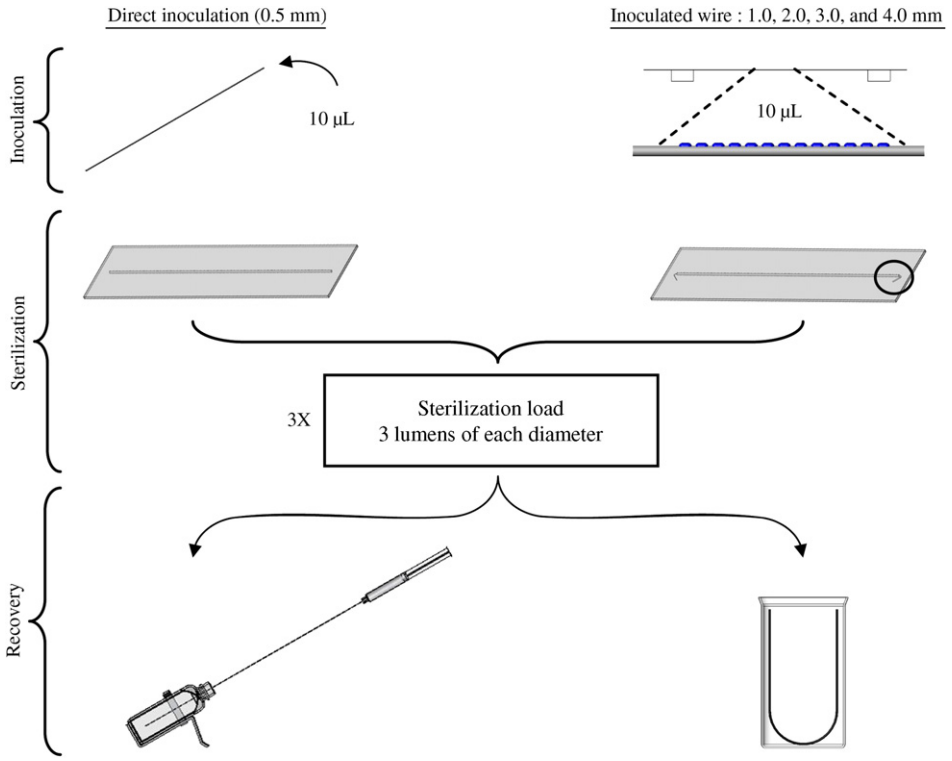

Fig 2. Summary of the manipulation step involved in the lumen device testing: inoculation, sterilization and recovery.

sterilization pouch. Three samples of each lumen were placed on the loading carriage shelves and then loaded into the sterilizer. Each load was exposed to a half-cycle in the 125L ozone sterilizer. The tests were repeated until 9 lumens (3 lumens per load in 3 different cycles) for each tubing configuration were processed (Fig 2).

Recovery

The directly inoculated lumens (0.5 mm) were transferred under a laminar flow hood and flushed with an adequate volume (100 times the internal lumen volume) of recovery buffer (Na_2HPO_4, $NaH_2PO_4H_2O$, NaCl, Tween) (Fig 2). For the inoculated wire method, the wires were aseptically removed from the lumens and transferred to a sterile beaker containing 200 mL of recovery buffer, then covered with aluminum foil before being transferred to the ultrasound bath. They were then sonicated for 20 minutes. For both methods, the recovery buffer was added to the same volume of TSB broth (2 ×). The mediums were incubated at 50°C to 60°C for 14 days. Observation of growth was recorded as a nonsterile sample (ie, positive).

Controls

High-level recovery validation was done using 3 lumens of 0.5 mm diameter and on 3 wires. These negative controls were tested after being subjected to the same manipulation as the processed lumen samples, but without the sterilization step. A serial dilution of the recovery buffer was performed to result in the formation of 30 to 300 cfu per agar plate. The inoculation solution population was also estimated by a serial dilution in sterile distilled water. Then the recovered population was compared with the spore solution population used to inoculate each lumen configuration. Low-level recovery validation was performed by direct site inoculation with $\leq$ 100 cfu of *G stearothermophilus*. Three 0.5 mm lumens and 3 wires were tested.

Determination of lumen length that can be sterilized

The maximum lumen lengths that could be physically loaded into the sterilization chamber due to chamber dimension restrictions were processed in the 125L ozone sterilizer. Lumen devices demonstrating positive results were shortened by 2.5 to 5 cm until sterility was observed at the half-cycle (0 or 1 positive out of 9 samples, or attainment of a log reduction value of 7). The ozone sterilization cycle was demonstrated to be effective in sterilizing devices of 0.5 mm internal diameter and 45 cm long, 1.0 mm internal diameter and 50 cm long, 2.0 mm internal diameter and 57.5 cm long, 3.0 mm internal diameter and 65 cm long, and 4.0 mm internal diameter and 70 cm long.

Sterilization of challenging medical devices

An ACMI semirigid ureteroscope (Gyrus ACMI, Southboro, MA) was used to confirm the use of hypodermic needle stainless steel tubing as a valid carrier for conducting lumen device sterilization assessment studies. The ACMI ureteroscope was chosen because it is one of the longest and smallest-lumen ACMI endoscopes available. It has 2 working channels, 2.3 Fr (0.8 mm) and 3.4 Fr (1.1 mm), and the channel length is 50.5 cm from the side-by-side ports to the triangular end. The inoculated wire method described above was used to test the effectiveness of sterilization. Because the channel is not straight, the wire was inoculated on 1 of its extremities and inserted until the inoculated part was set in the middle of the channel (Fig 3). The other end was bent as for the lumen devices. The ureteroscope was packaged and sterilized, and recovery was performed as for the lumen devices of $\geq$ 1 mm.

Calculation of the log reduction value

Effectiveness of lumen device sterilization was analyzed using the fraction-negative method.[13] The log reduction value (LRV) was calculated by

$$\mathrm{LRV} = \mathrm{Log}N_0 - \mathrm{Log}\,N_1 \text{ and } N_1 = \ln\,(n/r),$$

where N_0 is the initial spore population on the sample before sterilization and N_1 is the average number of spores surviving after sterilization. In fraction-negative analysis, N_1 is estimated using the most probable number (MPN) of surviving spores by testing replicate units, ln (n/r), where n is the number of negative samples and r is the number of replicates.

RESULTS

Two inoculation methods were used to determine the maximum lumen length and smallest lumen diameter that could be processed in the 125L ozone sterilizer. The inoculated wire method is preferred for lumens with an inside diameter of $\geq$ 1 mm, because it allows for greater repeatability compared with direct inoculation. The direct inoculation method was used for the 0.5-mm-diameter lumen devices due to the obstruction of these lumens by the wire.

The maximum lumen lengths validated in this study were determined by sterilizing longer lumens. For example, for the 0.5-mm lumen device, 3 50-cm-long lumens were inoculated and sterilized in a half-cycle. Two of these 3 samples demonstrated growth. Then 5 cm was removed, and the test was repeated. Sterility was achieved with the 45-cm-long lumen; therefore, that length was selected for validation. Each lumen length studied was determined using this method.

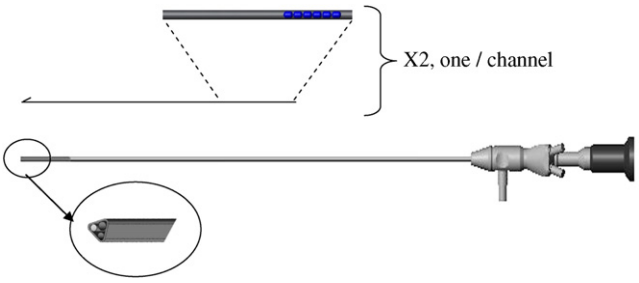

Fig 3. ACMI ureteroscope inoculation.

Half-cycle validation testing was performed in triplicate for all lumen sizes, for a total of 9 lumens per diameter and length. Results for the sterilization at the half-cycle for all lumen sizes packaged in pouches are given in Table 1.

Only a single 1-mm lumen demonstrated growth after 14 days of incubation. It turned positive after 9 days of incubation. No growth was observed after 14 days of incubation at 50°C to 60°C for all of the other lumen devices. The results demonstrate that the acceptable lengths for lumens with diameters of 0.5 mm, 1 mm, 2 mm, 3 mm, and 4 mm varied between 45 and 70 cm.

The potential of the 125L ozone sterilizer to sterilize medical instruments with lumens was confirmed using an actual medical device. The ACMI ureteroscope was chosen because it is one of the longest and smallest-lumen ACMI endoscopes and is claimed by the manufacturer to be one of the most difficult to sterilize medical devices. No growth was observed after 14 days of incubation for the 3 tests performed. Sterilization of the ACMI ureteroscope demonstrated the efficacy of the 125L ozone sterilizer in sterilizing medical devices that are very challenging in terms of sterilant penetration.

After inoculation with a suspension of *G stearothermophilus* spores containing 1.38×10^6 cfu/10 μL, high-level recovery on negative controls showed average initial populations of 1.05×10^6 cfu on wires and 1.40×10^6 cfu inside the 0.5-mm-diameter lumens. The low-level recovery percentage was 25% for the direct inoculation method and $>$ 76% for the wire technique when carriers were inoculated with $<$ 100 cfu of *G stearothermophilus* spores.

DISCUSSION

Sterilization is a process through which an object or product is freed of microorganisms. However, there is no level of lethal stress at which absolute assurance is provided that all microorganisms are dead. One or

Table 1. Half-cycle sterilization results for inoculated lumens of different internal diameters

Internal diameter	Length	Results for 3 lumens (Nb+/3)
0.5 mm	45 cm	0+/3
		0+/3
		0+/3
1 mm	50 cm	1+/3
		0+/3
		0+/3
2 mm	57.5 cm	0+/3
		0+/3
		0+/3
3 mm	65 cm	0+/3
		0+/3
		0+/3
4 mm	70 cm	0+/3
		0+/3
		0+/3

fewer nonsterile products per million (or a sterility assurance level [SAL] of 10^{-6}) is the endpoint recognized by most regulatory agencies in establishing the specification for terminally sterilized products. The half-cycle approach[13-15] is a practical and commonly accepted method of qualifying a gas sterilization process and demonstrating achievement of a SAL of 10^{-6}. In the first half-cycle, the microbial kill shows a 6-log reduction from 10^6 to 10^0. If the survivor curve is linear, then extrapolation predicts that another 6 logs of microorganisms will be killed, from 10^0 to 10^{-6} in the second half-cycle. When the initial level of contamination is 10^6, a SAL^{-6} is equivalent to a 12-log reduction.

Spores of *G. stearothermophilus* have proven to be the spore species most resistant to the 125L ozone sterilization process,[12] as they are for other oxidative sterilizing agents, such as vapor-phase hydrogen peroxide.[3] It was previously shown that the microbial kill kinetic of *G. stearothermophilus* spores in the 125L ozone sterilization process is linear in relation to the ozone dose injected into the sterilization chamber.[10]

A sterilization process is considered linear[16] when the coefficient of determination (r^2) is not < 0.8. Therefore, the 125L ozone sterilization process can be considered linear, because the coefficient of determination of the survivor curve is > 0.9 for *G stearothermophilus* spores in different conditions.

Sterility of direct inoculated 0.5-mm lumen devices was evaluated using an indirect recovery method, because it was impossible to fully immerse this device in broth. The same recovery and incubation methods were used for the inoculated wires, even though it would have been possible to cut the wires and immerse them in broth. This decision was made to maximize comparability of the results for analysis of the relationships among the various lumen lengths sterilized. Although this indirect recovery method has limitations, with the possibility that some spores could be left inside the lumens or on the wire after recovery, this recovery method was validated by performing population recoveries. SALs were based on the recovered populations.

The number of spores recovered from the unexposed lumen devices (negative control) was $> 1.0 \times 10^6$ cfu, demonstrating that the inoculated wire provides a sufficient challenge in assessing the effectiveness of a sterilization process. Analysis of the fraction-negative results for lumen devices sterilized in the 125L revealed an LRV of > 6 logs at the half-cycle (Table 2), even though 1 of the samples demonstrated growth. Because the lethality was demonstrated to be linear in the 125L ozone sterilization process,[12] this shows that more than a 12-log reduction or SAL-6 was achieved at the full cycle.

Moreover, the maximum lumen length selected in this study is the length at which $< 15\%$ of the positive samples (1+/9 samples) were observed at the half-cycle. Based on the half-cycle theory,[4] the percentage of positive samples could reach 63% (5+/9 samples) and still demonstrate a 6-log reduction value if the initial population was $> 1.0 \times 10^6$ cfu. For the 0.5-mm lumen devices, 66% (2+/3 samples) were observed for a lumen length of 50 cm. Selecting lumen lengths for which an LRV of almost 7 is achieved at the half-cycle adds a level of safety to the process.

The diameters and lengths of the lumen devices listed in Table 2 were plotted on a graph (Fig 4). Each point on this graph was verified on 9 replicates. The lumen length that could be sterilized based on a standard interpretation of the results is represented by the histogram included in the graph. However, a linear relationship was discovered between the length of lumen that can be sterilized in the 125L sterilizer and its internal diameter (Fig 4). The r^2 of 0.990 demonstrates a very strong linear relationship between the 2 parameters. Because the linear regression was based on 5 points determined by experimentation, and each point was confirmed by performing 9 replicates, it is scientifically sound to state that the lumen length that could be sterilized in the 125L device for lumens with internal diameters in between the internal diameters tested, from 0.5 mm (1.5 Fr) to 4 mm (12 Fr), can be predicted using the linear relationship found. All points below, or under, the inclined line illustrated in Figure 4 yield an inner diameter and associated length combination that can be expected to be sterilized; for example, according to the graph, a lumen of 1.5 mm internal diameter and up to 53.75 cm long can be sterilized.

Interpolation of the results is possible based on the added safety level when evaluating the maximum

Table 2. Summary of sterilization results and LRV values for lumen devices exposed to the half-cycle

Internal diameter (mm)	Length (cm)	Sterile samples (Nb-/9)	Log reduction value (log)
0.5	45	9-/9	>6.98*
1	50	8-/9	6.93
2	57.5	9-/9	>6.98*
3	65	9-/9	>6.98*
4	70	9-/9	>6.98*

*Because it is impossible to evaluate the LRV when there is no positive sample, the LRV value was estimated for a result of 9 sterile samples in 10 (log 0.9 = -0.98).

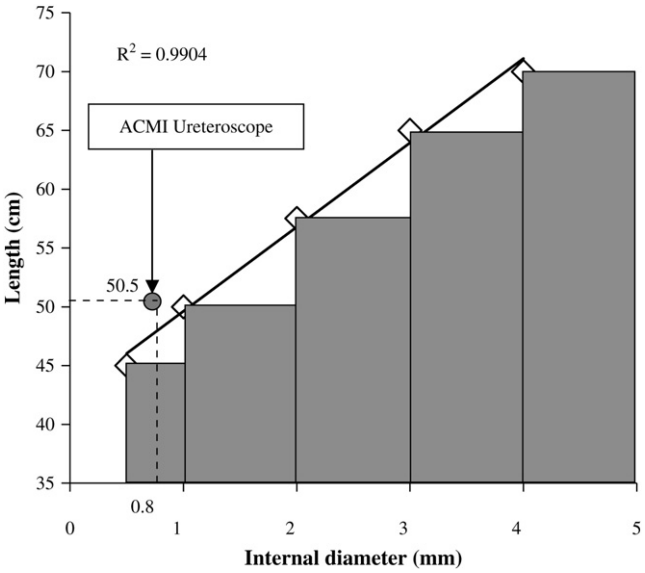

Fig 4. Relationship between lumen diameter and maximum respective length sterilized in the 125L ozone sterilizer.

lumen length that can be sterilized in the 125L sterilizer. Simulated use performed on the smallest channel in the ACMI ureteroscope, which have an inner diameter and associated length combination that place it over the line (• in Figure 4) in the zone in which it should show > 15% positive tests, demonstrated the safety level of the interpolation of the line by its sterility when exposed to the half-cycle (0+/3 tests). It confirms that the lumen devices used to test for maximum length and diameter in the 125L ozone sterilizer are representative and even more resistant to the ozone sterilization process than real lumen medical devices, such as MIS instruments.

In conclusion, the TSO_3 model 125L ozone sterilizer has been shown to effectively sterilize medical devices with long, narrow rigid lumens. Moreover, a linear relationship was found between the length of lumens that can be sterilized and their internal diameters. Consequently, intermediate sizes for diameters and lengths of medical devices that can be sterilized in the 125L ozone sterilizer can be interpolated from the linear relationship found between these 2 parameters. Thus, rigid medical devices with lumens compatible with the 125L ozone sterilizer have the following lengths:

Internal diameter (mm)	Internal diameter (Fr)	Maximum length (mm)
0.5	1.5	450
	2	472
1	3	500
	4	520
	5	543
2	6	575
	7	591
	8	615
3	9	650
	10	663
	11	687
4	12	700

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