

## ARTICLE: ATP BIOLUMINESCENCE AS A RAPID DETECTION

### EXCERPT FROM: RAPID DETECTION OF MICROBIAL CONTAMINATION/ACTIVITY

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#### ***Bioluminescent ATP assay***

ATP (adenosine triphosphate) is the chemical compound in which energy is stored in all living cells. In the ATP-luminometric test the firefly enzyme (luciferase) in the presence of its substrate, luciferin, oxygen and magnesium ions catalyzes conversion of chemical energy of ATP into light through oxidation-reduction reaction (Figure 1). The quantity of light generated is directly proportional to the amount of ATP present, thus, the light units can be used to estimate the biomass of cells in a sample. With state of the art equipment, and highly purified reagents, it is possible to detect amounts of ATP corresponding to approximately 100 bacterial cells, although in practice it is usually nearer to 10<sup>3</sup>-10<sup>4</sup>. Quantification of intracellular microbial ATP can be conveniently accomplished using rapid and simplified extraction and assay procedures. The light emitted by this process can be monitored by a variety of luminometers. Supplying companies provide customers with test kits with all necessary reagents. The reagents are injected into the instruments and readout is reported in relative light units (RLUs). By knowing the number of microorganisms responsible for generating known RLUs, one can estimate the number of microorganisms in the (food) sample. The ATP method has been used to evaluate microbial loads in e.g. meat, milk, water, fruit juice samples in a winery and a brewery, and sweeteners and syrups (FUNG, 1997). Much interest has been developed also in using ATP estimation not for total viable cell counts but as a sanitation check including also the verification of somatic cells presence on a surface.

This version of the ATP bioluminescence method based on detecting all ATP on a surface provides an indication of cleanliness detecting also ATP of somatic origin that the traditional plate count method does not detect, instead of only of microbial origin (CHEN, 2000; ILLSLEY et al., 2000; QUINN et al., 2002; PAEZ et al., 2003). Samples for assessing surface hygiene by this method can be obtained by swabbing the surface, or by taking aliquots of the rinse water (KISS et al., 1999). Reading of the bio-luminometers may be assessed as „acceptable” or „unacceptable”. The procedure can be easily performed by almost anyone, with little training. The preparation and measuring time takes only several minutes. Portable luminometer reading units test swabs with pre-packaged reagents. The user swabs the surface to be tested, activates the swab by placing it into the solution of reagents then inserts it into the chamber of the luminometer to obtain the measurement.

The ATP bioluminescence methods can be effective because of their very rapid assessment of hygiene and sanitation efficacy.

## ARTICLE: ATP BIOLUMINESCENCE AS A RAPID DETECTION

### RESEARCH ARTICLE: ATP vs. TRADITIONAL METHODS

#### ***Traditional Methods***

The traditional aerobic plate count is a time consuming process requiring a level of skill to prepare plates, take samples, and read results. Aerobic plate counts take at least two days to complete, with the potential result that food processed has reached the consumer before the test results are known. Traditional methods for cleanliness testing include visual inspection and the aerobic plate count. While visual inspection is inherently subjective, qualitative, and limited to the detection of only visible contamination, it does offer the advantage of instant, "real time" inspection. For quantitative detection, most quality assurance programs rely on 100 year old methodology, the traditional agar-based aerobic plate count. While other areas of the production process have been modernized and automated, many manufacturers continue to use traditional aerobic plate counts for cleanliness testing - a process introduced in the 19th century. As a cleaning test the aerobic plate count is limited in capabilities, for the test cannot detect the presence of organic residues which may not have been removed in an effective cleaning program; the aerobic plate count can only detect a limited range of micro-organisms. While aerobic plate counts can detect actual microbial contamination on production surfaces, this technology cannot detect the potential for microbial contamination hours later due to invisible organic food residues on production surfaces.

#### ***ATP Bioluminescence***

The significant limitations of traditional methods, the heightened concern regarding food safety, and the need for better and more timely cleanliness testing have caused usage of ATP bioluminescence testing systems to increase significantly in recent years. Although ATP bioluminescence technology has been commercially available since the 1970's, only recently have advances in portable instrumentation and time stable reagent chemistry been achieved. With these advances, ATP bioluminescence has gained widespread acceptance in the food industry as processors find the technology provides significant benefits over traditional methods of cleanliness testing.

Bioluminescence is best known as the flash of light from the firefly's ail, which occurs when the enzyme luciferase comes into contact with the molecule ATP (adenosine triphosphate), which is present in the cells of all living organisms. The amount of light produced is directly proportional to the amount of ATP present, and is measured by sensitive light meters (luminometers) and usually expressed in terms of Relative light Units (RLU).

The measurement of total ATP allows real time measurement of total biological residue, both microbial and food residue, on production surfaces to determine the potential for microbial contamination from surfaces that have not been properly cleaned. The entire test typically takes less than a minute to complete, thus becoming a "real time" test and allowing for immediate corrective action.

EXCERPT FROM: Hy-lite, Corp., A Portable Hygiene Monitor – USA

**ARTICLE: INFECTION CONTROL - HEALTHCARE - MEDICAL RESEARCH**

## **Compliance with New USP-NF Chapter on Compounding Sterile Preparations**

### **JCAHO Requirement**

Manual: AMCAH

Effective Date: immediately

### **Joint Commission to Survey Compliance with New USP-NF Chapter on Compounding Sterile Preparations**

Effective immediately, the Joint Commission is surveying compliance with a new chapter in the 2004 United States Pharmacopeia—National Formulary (USP-NF) entitled “USP Tests and Assays Chapter 797, Pharmaceutical Compounding, Sterile Preparations.” This new chapter deals with new requirements for the compounding, preparation, and labeling of sterile drug preparations and applies to health care institutions, pharmacies, physician practices, and other facilities that prepare or compound sterile preparations. The requirement extends to any health care practitioner involved in the preparation and compounding of sterile products and goes beyond the pharmacy to address compounding and preparation by nurses and physicians in patient care areas of the organization. This will impact hospitals, including critical access hospitals, and can impact ambulatory care, behavioral health care, home care, and long term care organizations in which medications are prepared.

“The new requirements match extremely well with our 2004 standards, but are more detailed,” explains Darryl Rich, Pharm.D., associate director for surveyor management and development at the Joint Commission. “Compliance with the USP-NF Chapter 797 will certainly help organizations comply with our standards in the area of sterile medication preparation and infection control.”

The Federal Food, Drug and Cosmetic Act recognizes the USP-NF as an “official compendia” of drug standards. Under the act, if a drug product that appears in the USP-NF fails to meet the standards for strength, quality, purity, preparing, packaging, or labeling contained in the USP-NF, the drug may be deemed “misbranded” or “adulterated” by the Food and Drug Administration under the act. USP-NF standards in the chapter on compounding sterile preparations contain requirements related to product standards and additional good pharmacy practices. As such, the Joint Commission requires compliance with them.

The new USP-NF chapter addresses the following issues:

- \* Responsibilities of all compounding personnel
- \* Classification of IV products into three risk levels, with quality assurance practices specific to each
- \* Verification of compounding accuracy and sterilization
- \* Personnel training and competence assessment in aseptic manipulation skills
- \* Environmental quality and control
- \* Equipment used in the preparation of compounded sterile products
- \* Verification of automated compounding devices for parenteral nutrition
- \* Finished product release checks and tests
- \* Storage and beyond-use (expiration) dating
- \* Maintaining product quality and control once the product leaves the compounding facility
- \* Packing, handling, storage, and transport of compounded sterile products
- \* Patient or caregiver training
- \* Patient monitoring and adverse events reporting
- \* A quality assurance program for compounded sterile products

Starting July 1, 2004, Joint Commission surveyors will address these requirements during surveys of organizations that compound sterile products (for example, intravenous solutions, eye drops) in the following ways:

- \* Surveyors will help organizations become aware of these new requirements (if unaware)
- \* While USP-NF Chapter 797 is not the focus of surveyors during an on-site survey, noncompliance with the major requirements in this USP-NF chapter that relate to Joint Commission standards will be scored at the appropriate element of performance effective immediately using the appropriate track record for compliance with the new requirements. Since the requirements were first released by the USP in November 2003, implementation

of plans of action to comply with them will be considered an acceptable approach in 2004 towards meeting the requirements.

To comply with these requirements, organizations should immediately evaluate their current practices in the light of the new USP-NF chapter and make appropriate changes. Based on a national survey in 2003, only 5.2% of hospitals were in compliance with similar guidelines.

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## ARTICLE: INFECTION CONTROL - HEALTHCARE - MEDICAL RESEARCH

### Research Article:

### ATP measurement as method to monitor the quality of reprocessing flexible endoscopes

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German Medical Science 2004;2:Doc04

### Abstracts

Insufficient performance of cleaning and disinfection of flexible endoscopes can pose an infection risk to patients. Actually quality of reprocessing is checked by performing microbiological cultures. Unfortunately, their results are not available on the same day so that more rapid methods are desirable. We compared the ATP (adenosine triphosphate) bioluminescence for hygiene checking of the reprocessing procedures of 108 flexible endoscopes with routine microbiological culture technics.

Sensitivity and specificity of ATP bioluminescence was calculated. 28 endoscopes showed bacterial growth of at least one sample. Depending on the applied threshold of bioluminescence between 67 and 28 endoscopes were positive. Sensitivity varied between 0.46 and 0.75 and specificity between 0.43 and 0.81. ATP bioluminescence does not replace routine microbiologic methods but it can indicate the need of immediate check of reprocessing.

### Introduction

Infections by endoscopes have been described as a consequence of insufficient cleaning and disinfection [1], [2]. Reports regard duodenoscopes [3], colonoscopes [4], and bronchoscopes [5], [6] and concern exists regarding the possible transmission of infective agents like hepatitis B-/C- virus, HIV, Mycobacterium tuberculosis and Helicobacter pylori [7]. An investigation in southern Germany has shown that 50% of endoscopes were still contaminated by bacteria after reprocessing [8] American [9], [10], [11] and European [7], [12], [13], [14], [15] recommendations for their processing of endoscopes have been published. Whereas in Germany and various other countries control of cleaning and disinfection by microbiological methods is recommended the use of microbiological cultures to routinely check the reprocessing process is discussed controversially in USA [7], [16]. Unfortunately, there are many disadvantages of microbiological cultures: Getting results lasts for days, so the endoscopes are used with other patients. Additionally, viruses, Helicobacter and M. tuberculosis are not at all included and slowly growing organisms only if the time of incubation is long enough. So a more rapid method for checking the reprocessing of endoscopes is needed. ATP (adenosine triphosphate) measurement is used as indicator of cleaning control [17], [18] in food and kitchen hygiene. ATP is as well an indicator of organic as of microbiological contamination. It is a simple method measuring the amount of light which is emitted when the enzyme luciferase comes into contact with molecular ATP and which is directly proportional to the amount of ATP [19]. ATP measurement may be a suitable method to control the quality of endoscope reprocessing as it is measuring cleaning effectiveness which may indicate the reduction of infection risk.

We did an investigation to compare the ATP bioluminescence for hygiene checking of reprocessing with routine microbiological cultures.

### Methods

Between January and December 2003 we examined 108 endoscopes (40 gastroscopes, 18 colonoscopes, 8 duodenoscopes and 42 bronchoscopes) after reprocessing. Sterile swabs were moistened with sterile 0.9% NaCl. We took swabs of distal end and rinsing valve and rinsed the operating channel with 20 ml sterile 0.9% NaCl. We did not use neutralizers. Swabs and 0.5 ml of rinsing fluid were inoculated on blood agar, MacConkey agar and Sabouraud agar and incubated for 48 hours at 37° C and 7 days at 22° C respectively. Bacterial species identification followed routine microbial laboratory proceedings (API biomerieux). Every bacterial growth was considered microbiological positive regardless of species or number of cfu (colony forming unit). ATP and AMP (adenosine monophosphate) bioluminescence was determined using Lumitester PD 10 (Scil Diagnostics). The

assay was carried out according to the manufacturer's instructions immediately after swabbing.

Reagent blanks were obtained using sterile swabs moistened with sterile 0.9% NaCl, instrument disinfectant and endoscope cleaner used for endoscope reprocessing. Bioluminescence readings were expressed as relative light units (RLU). Thresholds for bioluminescence were chosen between 30 and 100 RLU according to manufacturer's personal recommendation and after determining RLU of disinfectant and cleaner below 10 RLU. Sensitivity and specificity of ATP bioluminescence compared with microbiological culture as a gold standard were calculated for all types of examined endoscopes together and displayed in a ROC curve for various threshold values. The area under the ROC curve, which is usually chosen as the summary measure of diagnostic accuracy, was also computed.

## Results

Results of microbiological culture and ATP bioluminescence are shown in Table 1. Microbiological cultures of 28 endoscopes (26%) showed bacterial growth. 13% of checked duodenoscopes, 28% of coloscopes, 23% of gastroscopes and 31% of bronchoscopes were bacterially contaminated. The detected organisms were *Pseudomonas aeruginosa*, other non fermenting gramnegative rods, Enterobacteriaceae, *Staphylococcus aureus*, koagulase negative *Staphylococci*, *Corynebacteriae*, Bacilli, *Candida* and moulds. Dependent on the chosen threshold between 28 (26%) and 67 (62%) Hansen et al. ATP measurement as method to monitor the quality of reprocessing flexible endoscopes.

Endoscopes were positive for ATP bioluminescence. ATP bioluminescence of 75% of duodenoscopes, 67% of coloscopes, 63% of gastroscopes and 57% of bronchoscopes was above a threshold of 30 RLU. There were still 25% of duodenoscopes, 50% of coloscopes, 30% of gastroscopes and 24% of bronchoscopes above a threshold of 100 RLU. ATP bioluminescence of 5 bronchoscopes and 2 gastroscopes was below 30 RLU despite being microbiologically contaminated. Choosing 30 RLU as threshold 21 endoscopes (8 bronchoscopes, 7 gastroscopes, 5 coloscopes and 1 duodenoscope) were positive for ATP bioluminescence and microbiological culture and 34 endoscopes (13 bronchoscopes, 13 gastroscopes, 6 coloscopes and 2 duodenoscopes) were negative. 46 endoscopes (16 bronchoscopes, 18 gastroscopes, 7 coloscopes and 5 duodenoscopes) had an ATP bioluminescence above 30 RLU despite negative microbiological result. Above a threshold of 100 RLU 65 endoscopes (24 bronchoscopes, 23 gastroscopes, 12 coloscopes and 6 duodenoscopes) had concordant negative and 13 endoscopes (5 bronchoscopes, 4 gastroscopes, 3 coloscopes and 1 duodenoscope) concordant positive results. Microbiological cultures of 15 endoscopes (5 bronchoscopes, 8 gastroscopes, 1 coloscope and 1 duodenoscope) were negative despite ATP bioluminescence above 100 RLU. The ROC curve of sensitivity and specificity for thresholds between 30 and 100 RLU is presented in Figure 1. The area under the ROC curve is 0.63. Compared with microbiological culture as a gold standard sensitivity of bioluminescence varied between 0.75 for a threshold of 30 RLU (95% confidence interval 0.55-0.89) and 0.46 for 100 RLU (95% confidence interval 0.28-0.66) and specificity between 0.43 (95% confidence interval 0.32-0.54) and 0.81 (95% confidence interval 0.71-0.89) respectively (Tab. 2).

## Discussion

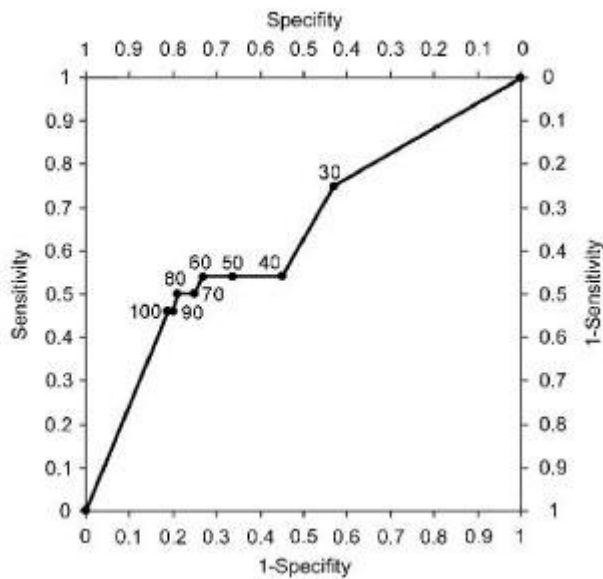
In our study, 26% of tested endoscopes showed microbiological contamination. The number of contaminated endoscopes in our investigation is in accordance with the results of Moses and Lee [20], who found between 12% and 24% positive cultures during a 10-year study period. It is much lower than that of the HYGEA study [8]. Moses and Lee examined only endoscopes used in a clinical institution and reprocessed in an automated washer whereas in half of endoscopy facilities of the HYGEA study endoscopes were reprocessed manually. In our study 92 endoscopes (85%) were reprocessed in an automated washer. 3 Dependent on the chosen threshold between 62% and 26% of the tested endoscopes had a positive bioluminescence result indicating possible organic contamination. In order to calculate sensitivity and specificity of ATP bioluminescence there must be another method which truly indicates contamination of reprocessed endoscopes. The only established method for checking endoscope reprocessing is microbiological culture. Microbiological culture may fail in indicating all contaminated endoscopes. There may be non viable organisms or organisms which cannot be cultured on conventional culture medium and other than bacterial contaminations are possible.

Because of the absence of other methods for checking endoscopes we calculated sensitivity and specificity of ATP bioluminescence compared to microbiological culture as gold standard. Sensitivity and specificity of bioluminescence



differ dependent on the chosen threshold. In our study sensitivity was only 0.75 even when the chosen threshold of RLU was low. The ROC curve of ATP bioluminescence presented in Figure 1 with an area under the curve of 0.63 indicates that there is no strong concordance between ATP bioluminescence and microbiological culture. Our results are similar to those found by Murphy et al. [18] for testing food contact surfaces. Murphy et al. suspected that conventional microbiology is more sensitive than ATP bioluminescence when total ATP is low [18]. Bacterial ATP content may be below the limit of ATP bioluminescence. Different bacterial specimen can contain different amounts of ATP and the amount of ATP also depends on the metabolism of the organisms [19]. ATP bioluminescence may also be influenced by the number of viable bacteria present. We did not differ between kind of specimen and number of cfu cultured. The number of cfu found on most swabs was very low and this may explain the low sensitivity of ATP bioluminescence compared to routine microbiology in our study. Additionally the low specificity of ATP bioluminescence may be explained by the fact, that not only viable bacteria but also other organic contamination is detected. Similar to our study Poulis et al. [21] could not find a clear relationship between ATP bioluminescence measurements and number of cfu on surface plates under practical conditions on surfaces in a factory. Alfa et al. [22] reported that the presence of high residual soil (protein, carbohydrate, hemoglobin and endotoxin) did not correlate with microbiological contamination of reprocessed endoscopes. Thus measurable ATP bioluminescence may indicate contamination of endoscopes without presence of cultivable microorganisms. Reprocessed endoscopes should be clean. A clean endoscope should not only show a less amount of viable organisms but also a less amount of all organic contamination and ATP sources. The presence of any ATP source may indicate an infectious risk for consecutively examined patients and should be avoided irrespective of cultivable bacteria.

We conclude that ATP bioluminescence does not replace routine microbiologic methods but it should be applied additionally to check endoscope reprocessing. In contrast to microbiologic methods results of ATP bioluminescence are available at once and can indicate the need for checking the reprocessing practice immediately.



**Figure 1:** ROC curve of bioluminescence compared with microbiological culture as gold standard for thresholds between 30 and 100 RLU

Kind of endoscope	Microbiological culture	Threshold (RLU)							
		>30	>40	>50	>60	>70	>80	>90	>100
bronchoscope n=42	positive n=13	8	5	5	5	5	5	5	5
	negative n=29	16	15	8	6	5	5	5	5
gastroscope n=40	positive n=9	7	5	5	5	5	5	4	4
	negative n=31	18	14	12	10	9	8	8	8
coloscope n=18	positive n=5	5	4	4	4	3	3	3	3
	negative n=13	7	4	4	3	3	2	2	1
duodenoscope n=8	positive n=1	1	1	1	1	1	1	1	1
	negative n=7	5	3	3	3	3	2	1	1
all endoscopes n=108	positive n=28	21	15	15	15	14	14	13	13
	negative n=80	46	36	27	22	20	17	16	15

**Table 1:** Number of endoscopes with a bioluminescence value above the threshold compared with microbiological culture Threshold (RLU) Sensitivity.

Threshold (RLU)	Sensitivity (95% confidence interval)	Specificity (95% confidence interval)
30	0.75 (0.55-0.89)	0.43 (0.32-0.54)
40	0.54 (0.34-0.72)	0.55 (0.47-0.66)
50	0.54 (0.34-0.72)	0.66 (0.54-0.76)
60	0.54 (0.34-0.72)	0.73 (0.61-0.82)
70	0.50 (0.31-0.69)	0.75 (0.64-0.84)
80	0.50 (0.31-0.69)	0.79 (0.68-0.87)
90	0.46 (0.28-0.66)	0.80 (0.70-0.88)
100	0.46 (0.28-0.66)	0.81 (0.71-0.89)

**Table 2:** Sensitivity and specificity of bioluminescence as compared with microbiological culture and 95% confidence interval

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## ARTICLE: INFECTION CONTROL - HEALTHCARE - MEDICAL RESEARCH

Hospital Infection 2000 May;45(1):19-28.

### **An evaluation of hospital cleaning regimes and standards using ATP bioluminescence**

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#### **Abstract:**

A four-part study assessing cleanliness in up to 113 environmental surfaces in an operating theatre and a hospital ward was reported. Surfaces were assessed visually, using microbiological methods and ATP bioluminescence. Results from a preliminary random survey indicated variability in cleanliness. These results were then used to select sites for monitoring before and after routine cleaning, over a 14-day period. Using published microbiological and ATP specifications 70 and 76% of these sites were unacceptable after cleaning. Visual assessment was a poor indicator of cleaning efficacy with only 18% considered unacceptable. Sites most likely to fail in the ward were in the toilet and kitchen, areas which are frequently implicated in the spread of infectious intestinal disease. Operating theatre sites had lower ATP results but 61% of sites would be considered unacceptable. There was no significant difference in general microbiological or ATP results overall before and after routine cleaning. Although some important hand contact sites showed no significant difference, overall there was a significant decrease in staphylococcal and enterobacteria counts in the ward but not in the operating theatre after cleaning. The routine cleaning programmes used did not include a biocide and cleaning using a hypochlorite based sanitizer gave much lower values. The results are discussed in relation to infection control, cleaning audits and cleaning schedules: an integrated cleaning monitoring programme using ATP bioluminescence in conjunction with visual and microbiological assessments is recommended. Copyright 2000 The Hospital Infection Society.

PMID: 10833340 [PubMed - indexed for MEDLINE]

## ARTICLE: INFECTION CONTROL - HEALTHCARE - MEDICAL RESEARCH

Immunol Methods. 1993 Mar 15;160(1):81-8.

### **The use of ATP bioluminescence as a measure of cell proliferation and cytotoxicity**

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Adenosine triphosphate (ATP) bioluminescence was used to determine whether there was a linear relationship between cultured cell number and measured luminescence using the luciferin-luciferase reaction. In all the cells tested including peripheral blood mononuclear cells (MNC), MOLT-4, HL-60, TF-1, NFS-60 and L-929 cell lines there was a significant correlation as determined by Spearman's rank correlation coefficient ( $p > 0.00001$ ). These observations were then used to determine whether ATP bioluminescence could be used as a suitable substitute for tritiated thymidine uptake as a measure of cell proliferation. The cell lines MOLT-4, HL-60, TF-1 and NFS-60 showed a strong correlation between thymidine uptake and ATP bioluminescence ( $p > 0.00001$  for all cell types). Additionally the ATP method could detect the cytokine dependent proliferation on TF-1 and NFS-60 cells by granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) respectively. The tumour necrosis factor alpha (TNF)-induced cytotoxic effect on L-929 cells could also be accurately detected using this method. It would therefore appear to be possible to use ATP bioluminescence in the detection of cytokine activity in a number of different bioassays.

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