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Impact of the Microbial Biofilms Formation in the Pasteurized Cow's Milk Production Line on the Quality Assurance of the Product, Taif, KSA

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Abstract: *This paper work was done for the impact of the microbial biofilms formation in the pasteurized cow's milk production line on the quality assurance (QA) of the product, Taif, KSA. The mean incidence of microscopical examination quality of microorganisms/High Power Field (MOs/HPF) presented in control group (CG) were (00, 00 and 00%) but in tested group (TG) were (100, 41.7 and 25%) within the specimens from (raw milk (RM), production line biofilm (PLB) and pasteurized milk (PM)) respectively. The mean incidence of MOs growth rate in percentage, it was determined as (00, 00 and 00%) from CG and (38.3, 24 and 8.3%) from TG for the specimens of (RM, PLB and PM) respectively. The mean incidence of colony forming unites/ml (CFUs/ml) for MOs, it observed the CG were (00, 00 and 00CFUs/ml) and the TG were (1.3, 0.08 and 0.004X10^{4}CFUs/ml) from (RM, PLB and PM) respectively. The present of MOs in RM as a sources which attached to the production line and uncleaning mechanisms which helped to reach that MOs to the PM even with low numbers. It was revealed that PM with low-qualify grades, it is in-need of follow-up the production line maintenance and hygienic measures to keep the PM product in the high-quality grade.*

Keywords: *Quality Assurance (QA), Microorganisms/High Power Field (MOs/HPF), Control Group (CG), Tested Group (TG), Raw Milk (RM), Production Line Biofilm (PLB), Pasteurized Milk (PM), Colony Forming Unites/ml (CFUs/ml), Total Bacterial Counts (TBCs), Total Microbial Counts (TMCs), Microbial growth (MG).*

I. Introduction

The sanitary quality of milk is to estimate its TBCs was varies from animal to animal and even from different quarters of the same animal. Aseptically drawn milk from healthy udders contains (50-100CFUs/mL). High initial counts (>10^{4}CFUs/mL) is evidence of poor production hygiene[1]. RM of dairy product shops had mean TBCs were (0.69, 0.54 and 0.68CFUs/mL). These high TMCs indicated the importance of MOs contamination[2]. MOs populations were increased during refrigeration, reaching after 72hrs, values as (8.0, 6.5 and up to 4.0CFUs/ml)[3]. TBCs showed (4.5×10^5, 8.3×10^7 and 1.7×10^{9}CFUs/ml) among the RM samples[4]. Pasteurization process of cow's milk, is the reason for milk's extended shelf life. High-temperature and short-time (HTST), PM typically has a refrigerated shelf life of (2-3) weeks, whereas ultra-PM (UHT) can last much longer, sometimes (2-3) months. It can even be stored unrefrigerated for up to 9 months. Pasteurization is used to kill harmful MOs by heating the milk for a short time and then immediately cooling. HTST process produce a 99.999% reduction in the number of MOs in PM, it safe to drink for up to 3 weeks if continually refrigerated[5]. UHT is one approach to do this but consumers, particularly young children, clearly do not like the heat induced off-flavors associated with high heat treatments and would prefer HTST milk[6]. Milk pasteurized at 85°C and milk heated to boiling temperature had revealed TBCs as (<1-3X10^{4}CFUs/ml)[7]. Milk is labeled by pasteurization method[8]. Pasteurization aims to reduce the number of viable MOs so they are unlikely to cause disease[9]. RM is forced between metal plates or through pipes heated on the outside by hot water, and is heated to 72°C for 15 seconds. UHT processing holds the milk at a temperature of 138°C for a minimum of 2 seconds then cooling it to 4°C to ensure any harmful MOs are destroyed [10, 12]. The hygienic packaging of milk would result in decline of milk MOs contamination[11]. Pasteurization methods are usually standardized and controlled by national food safety agencies (USDA in USA and FSA in UK), which legally requires that it ensure any harmful MOs are destroyed[12]. Biofilm formation in the cow's PM production line, if the MOs from food-contact surfaces are not completely removed, they can lead to mature biofilm formation and so increase the bio-transfer potential. Examples of the pay particular attention to the possibility of cross-contamination are the milk industry[13]. In the dairy industry, equipment surfaces were recognized to be a major source of contamination of processed milk with pathogenic MOs. Adhered MOs caught detach and contaminate the product as it passed the surfaces. It was resistance to heat treatments and to antimicrobial agents, biofilms were developed on dairy processing lines were also difficult to remove even with acceptable cleaning and disinfecting procedures[14]. It

had known to threaten the quality and safety of dairy products and to significantly reduce their shelf-life[15]. PM were collected before and after cleaning-in-place (CIP) systems from different segments of pasteurization lines. QA were showed little reduction of the TBCs after CIP as (5.6×10^3, 1.2×10^4, 5.1×10^4, 2.5×10^5 and 9.7×10^7CFUs/cm^2) respectively in the different units. This study emphasized the importance of aerobic spore-forming bacteria in dairy-processing equipment as they were able to build recalcitrant biofilms on the inside equipment surfaces with subsequent resistance to conventional CIP system and potential transfer to PM. Therefore, in order to reduce the contamination levels of MOs and improve the quality and shelf life of the product, these dairies must be besides improvement in the hygienic status of the plant equipment, also to monitor either the pasteurization process or the contamination from raw material[16]. *Bacillus cereus* was able to reach up to (5.5-6.4CFUs/cm^{-2}) when the initial inoculate were (0.3-0.6CFUs/ml) respectively, it was adhesion to stainless steel surface under conditions assessed and to assess the adhesion was under a range of conditions to which this can be exposed during either milk processing or cleaning procedures[17]. It formed biofilms within milking pipelines and on surfaces of equipment in the dairy industry which represent a continuous hygiene problem and can lead to serious economic losses due to food spoilage and equipment impairment, which were known to contaminate milk. Milk triggered the formation of biofilm-related structures, were termed bundles[18]. The presence of undesirable biofilms on food processing contact surfaces may lead to: transmission of diseases, food spoilage, shortened time between cleaning events, contamination of product by nonstarter bacteria, metal corrosion in pipelines and tanks and reduced heat transfer efficacy or even obstruction of the heat equipment. Despite the significant problems caused by biofilms in the food industry. Although it was understood that cell attachment and biofilm formation were influenced by several factors, including type of strain, chemical–physical properties of the surface, temperature, growth media and the presence of other MOs[19]. PM ordinance (PMO) requires that TBCs in Grade "A", RM leaving the farm must be in (<1X10^{5}CFUs/ml) and that the total TBCs in commingled milk at the processing plant must be in (<3X10^{5}CFUs/ml). Most milk has much lower counts than these requirements. The PMO requirements for maximum bacteria must be ensure public health and is not intended as dairy product quality standards[20]. Milk quality payment incentive programs typically had multiple criteria, TBCs were (<2.5X10^{4}CFUs/ml), laboratory PM count (<0.5X10^{3}CFUs/ml)[21]. It was not uncommon for TBCs of RM to be (<1X10^{4}CFUs/ml). When starting with RM that had a low TBCs, and in the absence of MG in PM, and produce off-flavors[22]. TBCs were found as (6.5X10^5-<6.5X10^{14}CFUs/ml), psychrophilic bacteria were (6.5X10^7-<6.5X10^{14}CFUs/ml), CCs revealed (6.5X10^{12}-6.5X10^{14}CFUs/ml) and *E. coli* were (0-<6.5X10^{11}CFUs/ml). The results showed that storage conditions had effects on bacterial counts[23]. A recurrent problem in the dairy industry was the MOs quality of PM. This product was exposed to middle heat treatments that do not ensure complete destruction of both spoilage and pathogenic bacteria. Despite improvement in the dairy technology, contamination of PM especially with aerobic spore-forming bacteria remains a specific biological barrier that limits shelf life and quality of the product[24]. Different potential contamination sources of PM were reported: RM equipment surfaces and packaging materials. Temperatures used for the pasteurization processes were also reported to affect processed milk shelf life as well as the somatic cell count of RM. Pasteurization process appeared to be a key step with regard to spore-forming bacteria because the role of temperature on spore activation. Temperature affected the microbial population of PM in terms of the amount and type of MOs present following pasteurization, with higher bacterial number in milk processed at higher temperatures[25]. Imported organic milk from the mainland were prepackaged and shipped by air freight. At 5 day before the expiration date, 70% of the mainland samples and 62% of the local samples had aerobic bacteria count exceeding the regulatory limit of (2X10^{4}CFUs/ml) for grade "A" PM set by the United States FDA[26]. Consequently, the operational costs soar and profit decreased. The MOs *Spp*., in question might not pose a threat to health but represented a continuing problem of spoilage and production of out-of-specification products. The results indicated a significant reduction in the growth rate of a thermophiles[27].

Aim of the work: It was follow-up the biofilm consisting by identification the present of MOs load in RM, PLB and PM. It was clarify the source and the effectiveness of the MOs biofilms formation in the production line which affect the QA of PM and caught lead to serious economic losses due to milk spoilage and equipment impairment.

II. Materials And Methods

Specimens collection and transportation: Specimens were collected from two dairy plants located at Taif, KSA. It had been got the agreements from plant owners with explanation the aim of research work and that for research without any memorization or trading of their plant dairy products, also without any mention of personal information according this condition they had been agreed to collect our research specimens. RM specimens were in No=3 as control specimens, from washed cow's udder by Pot. Permanganate and the others specimens were in No=12 from un-washed cow's udder. PLB specimens were in No=3 as control specimen, from washed production line by 2% Na OH at 70°C/5 min, and rinse with distilled water, the other specimens were in No=12

from un-washed production line. PM specimens were in No=3 from washed production line, and the others were in No=12 from un-washed production line. The specimens were collected in No=9 as CG in classification of 3 from each (RM, PLB and PM), and in No=36 as TG in classification of 12 from each (RM, PLB and PM). The total specimens were equal to No=45 specimens. All specimens were transported in ice box with aseptic condition to Micro. Lab. within 1hr.

Microscopical examinations: It was done for each specimen separately to detect the quality of MOs cells in grades, that were used (Bi-nuclear Microscope) with high power. The microbial populations were counted in each specimen as number / microscopic fields present in $1cm^2$ square prescribed area of microscope glass slides and were recorded as MOs/HPF[28].

MOs growth rate and CFUs/ml: Each specimens was transferred to 10ml normal saline with 0.1% peptone (Merck, Germany) and10fold dilutions were performed, then were spread on patient media, the plates were incubated at 30°C for 72hr. The growth rates and CFUs/ml were counted and the colonies morphology were noted[29].

Data analysis: The data recorded during the study period were entered into Microsoft excel sheet for analysis and graphs production[30].

III. Results And Discussion

Table and graph 1: The mean incidence of microscopical examination quality of *MOs/*HPF presented

Item	*No	*RM	*PLB	*PM
*CG	1			
	2			
	3			
Total	3	00/3=00%	00/3=00%	00/3=00%
*TG	1	*++		
	2	*+++	*+	*+
	3	*++	*+	
	4	*+		
	5	*++		
	6	*+		
	7	*+++	*+	
	8	*+		
	9	*+++	*+	*+
	10	*+		
	11	*+++	*+	*+
	12	*+		
Total	12	12/12=100%	5/12=41.7%	3/12=25%

**MOs: Microorganisms, *PHF: High Power Field, *No: Number, *RM: Raw Milk, *PLB: Production Line Biofilm, *PM: Pasteurized Milk, *CG: Control Group, *TG: Test Group, *+:Less than 5microbial cells/HPF, *++: 5-10microbial cells/HPF, *+++: More than 10microbial cells/HPF*

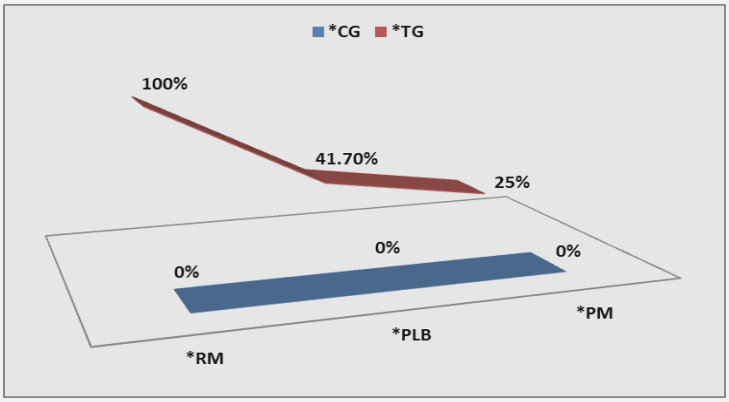

Table and graph 1 showed the mean incidence of microscopical examination quality of MOs/HPF presented, it was determined by grades in (+, ++ and +++). It was revealed the mean in CG were (00, 00 and 00%) but in TG were (100, 41.7 and 25%) within the specimens from (RM, PLB and PM) respectively. The results indicated the present of MOs biofilms in the pasteurization production lines with RM as started sources,

it was lead to increase the MOs load in PM which produced from the same production line and decrease the QA of PM. The MOs cells were examined in RM and it was decreased to about 1/2 in PLB and to the 1/4 in PM.

Table and graph 2: The mean incidence of *MOs growth rate in percentage

Item	*No.	*RM	*PLB	*PM
*CG	1			
	2			
	3			
Mean	3	00/3=00%	00/3=00%	00/3=00%
*TG	1	30%		
	2	60%	25%	10%
	3	50%	20%	
	4	20%		
	5	40%		
	6	20%		
	7	50%	20%	
	8	30%		
	9	60%	30%	10%
	10	20%		
	11	60%	25%	5%
	12	20%		
Mean	12	460/12=38.3%	120/5=24%	25/3=8.3%

**MOs: Microorganisms, *No: Number, *RM: Raw Milk, *PLB: Production Line Biofilm, *PM: Pasteurized Milk, *CG: Control Group, *TG: Test Group*

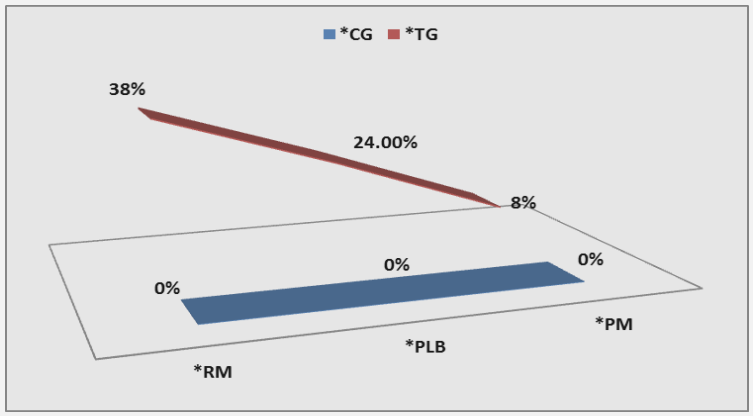

Table and graph 2 showed the mean incidence of MOs growth rate in percentage, it was determined as (00, 00 and 00%) from CG and (38.3, 24 and 8.3%) from TG from the specimens of (RM, PLB and PM) respectively. This results were confirmed the present of live MOs cells were showed by microscopical examination and the indication of the MOs biofilm sources which were the most reasons for contamination of PM and lead to low quality in production grading and QA of PM. The growth were in RM decreased to about 2/3 in PLB and to about 1/5 in PM. High initial MOS counts are evidence of poor production hygiene[1]. These high TMCs indicated the importance of MOs contamination[2]. It was increased during refrigeration, reaching after 72hrs[3]. Pasteurization process of the cow's milk produced a 99.999% reduction in the number of MOs in milk[5]. Pasteurization aims to reduce the number of viable MOs so they are unlikely to cause disease[9]. The hygienic packaging of milk would result in decline of milk contamination[11]. Pasteurization methods are usually standardized and controlled by national food safety agencies (USDA in USA and FSA in UK). Which legally requires that it ensure any harmful bacteria are destroyed[12]. Biofilm formation in the cow's milk pasteurization production line, if the MOs from food-contact surfaces were not completely removed, they can lead to mature biofilm formation and so increased the bio-transfer potential. Examples of the food sectors that pay particular attention to the possibility of cross-contamination are the milk industry[13]. In the dairy industry, equipment surfaces were recognized to be a major source of contamination of processed milk with both spoilage and pathogenic MOs. Adhered bacteria can detached and contaminated the product as it passes the surfaces. Due to their resistance to heat treatments and to antimicrobial agents, biofilms developed on dairy processing lines were also difficult to remove even with acceptable cleaning and disinfecting procedures[14]. Biofilms were known to threaten the quality and safety of dairy products and to significantly reduced their shelf-life[15]. QA showed little reduction of the TBCs after CIP were presented in the different units. That indicated the importance of aerobic spore-forming bacteria in dairy-processing equipment as they are able to build recalcitrant biofilms on the inside equipment surfaces with subsequent resistance to conventional CIP system and potential transfer to PM. The

presence of undesirable biofilms on food processing contact surfaces may lead to: transmission of diseases, food spoilage, shortened time between cleaning events, contamination of product by nonstarter bacteria, metal corrosion in pipelines and tanks and reduced heat transfer efficacy or even obstruction of the heat equipment. Despite the significant problems caused by biofilms in the food industry[19]. When starting with RM that had a low TBCs, and in the absence of MG in PM, and produce off-flavors[22]. A recurrent problem in the dairy industry was the microbial quality of PM. This product was exposed to middle heat treatments that do not ensure complete destruction of both spoilage and pathogenic bacteria. Despite improvement in the dairy technology, contamination of PM especially with aerobic spore-forming bacteria remained a specific biological barrier that limits shelf life and quality of the product[24].

Table and graph 3: The mean incidence of *CFUs/ml for *MOs

Item	*No.	*RM	*PLB	*PM
*CG	1			
	2			
	3			
Mean	3	(00CFUs/ml)	(00CFUs/ml)	(00CFUs/ml)
*TG	1	0.4 X 10^2		
	2	3 X 10^4	1 X10^3	0.6 X 10^2
	3	5 X 10^3	0.8 X 10^2	
	4	0.06 X 10^2		
	5	5 X 10^2		
	6	0.01 X 10^2		
	7	4 X 10^3	0.7 X 10^2	
	8	0.8 X 10^2		
	9	7 X10^4	0.9 X 10^3	0.4 X 10^2
	10	0.09 X 10^2		
	11	5 X 10^4	1 X 10^3	0.2 X 10^2
	12	0.05 X 10^2		
Mean	12	1.3X10^4CFUs/ml (13300CFUs/ml)	0.08X10^4CFUs/ml (840CFUs/ml)	0.004X10^4CFUs/ml (40CFUs/ml)

**CFUs/ml: Colony Forming Units/ml, *MOs: Microorganisms, *No: Number, *RM: Raw Milk, *PLB: Production Line Biofilm, *PM: Pasteurized Milk, *CG: Control Group, *TG: Test Group*

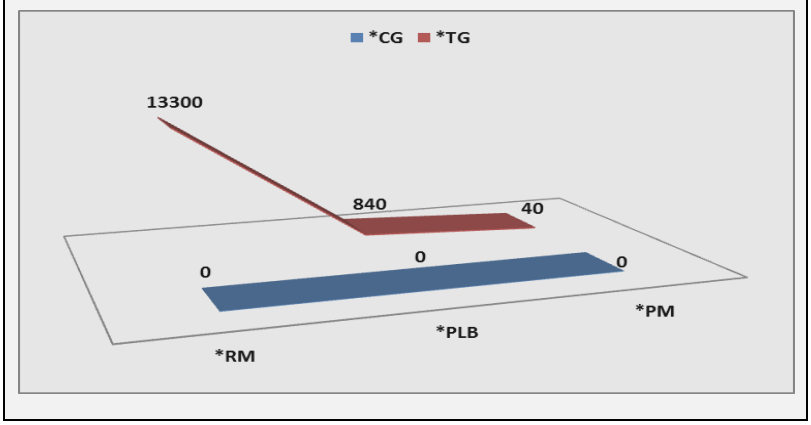

Table and graph 3 showed the mean incidence of CFUs/ml for MOs, it observed the CG were as (00, 00 and 00CFUs/ml) and the TG were as (1.3, 0.08 and 0.004X10^4CFUs/ml = 13300, 840 and 40/ml) from (RM, PLB and PM) respectively. That obviously were indicated the present of MOs in RM as a sources which attached to the production line and uncleaning mechanisms helped to reach that MOs to the PM even with low numbers. It was revealed that some of PM qualify as low-grades but it in-need of follow-up the production line maintenance and hygienic measures to keep the product in quality of high-grade. The number of CFUs/ml were in RM decreased to about 1/15 in PLB and then to 1/333 in PM. Aseptically drawn milk from healthy udders contains (50-100CFUs/mL). High initial counts (>10^4CFUs/mL) are evidence of poor production hygiene[1]. RM of dairy product shops had TBCs were (0.69, 0.54 and 0.68CFUs/mL). These high TMCs indicated the importance of MOs contamination[2]. The result of TBCs showed the variation (4.5×10^5, 8.3×10^7 and 1.7×10^9CFUs/ml) among the RM samples[4]. The standard HTST process produced a 99.999% reduction of MOs in milk[5]. Milk pasteurized at 85°C and milk heated to boiling temperature had revealed TBCs (<1-3X10^4CFUs/ml)[7]. TBCs after CIP were (5.6×10^3, 1.2×10^4, 5.1×10^4, 2.5×10^5 and 9.7×10^7CFUs/cm^2) respectively in the different units. This study emphasized the importance of aerobic spore-forming bacteria in dairy-processing equipment as they were able to build recalcitrant biofilms on the inside equipment surfaces

with subsequent resistance to conventional CIP system and potential transfer to PM [16]. *Bacillus cereus* was able to reach up to (5.5 and 6.4CFUs/cm^{-2}) when the initial inoculate were (0.3 and 0.6CFUs/ml) respectively, it was adhesion to stainless steel surface under conditions assessed and to assess the adhesion was under a range of conditions to which this MO can be exposed during either milk processing or cleaning procedures[17]. It formed biofilms within milking pipelines and on surfaces of equipment in the dairy industry which represent a continuous hygiene problem and can lead to serious economic losses due to food spoilage and equipment impairment, which were known to contaminate milk. Milk triggers the formation of biofilm-related structures, were termed bundles[18]. PMO requires that TBCs in Grade "A" milk leaving the farm was (<1X10^{5}CFUs/ml) and that the total TBCs in commingled milk at the processing plant was (<3X10^{5}CFUs/ml). Most milk has much lower counts than these requirements. The PMO requirements for maximum bacteria was to ensure public health and were not intended as dairy product quality standards[20]. Milk quality payment incentive programs typically TBCs were (<2.5X10^{4}CFUs/ml), laboratory PM count (<0.5X10^{3}CFUs/ml)[21]. It was not uncommon for TBCs of RM to be (<1X10^{4}CFUs/ml). When starting with RM that had a low TBCs, and in the absence of MG in PM, and produced off-flavors[22]. TBCs were found as (6.5X10^5-<6.5X10^{14}CFUs/ml), psychrophilic bacteria were (6.5X10^7-<6.5X10^{14}CFUs/ml), CCs revealed (6.5X10^{12}-6.5X10^{14}CFUs/ml) and *E. coli* were (0-<6.5X10^{11}CFUs/ml)[23]. Contamination of PM especially with aerobic spore-forming bacteria remains a specific biological barrier that limits shelf life and quality of the product[24]. Different potential contamination sources of PM are reported, RM equipment surfaces and packaging materials. Temperatures used for the pasteurization processes are also reported to affect processed milk shelf life. Nevertheless, among these limiting factors, pasteurization process appears to be a key step with regard to spore-forming bacteria because the role of temperature on spore activation. Temperature affects the microbial population of PM in terms of the amount and type of MOs present following pasteurization, with higher bacterial number in milk processed at higher temperatures[25]. Imported organic milk from the mainland were prepackaged and shipped by air freight. At 5day before the expiration date, 70% of the mainland samples and 62% of the local samples had aerobic bacteria count exceeding the regulatory limit of (2X10^{4}CFUs/ml) for grade "A" PM set by the United States FDA[26]. Consequently, the operational costs soar and profit decreases. The MOs *Spp*., in question may not pose a threat to health but represent a continuing problem of spoilage and production of out-of-specification products. The results indicated a significant reduction in the growth rate of a thermophiles[27].

IV. Conclusion

Therefore, in order to reduce the contamination levels of PM from the biofilm forming MOs, improve the QA and shelf life time of PM. Dairies plants must have besides improvement in the hygienic status of the equipment, also monitor either the pasteurization process or the contamination from RM.

V. Acknowledgment

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VI. References

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