# Comparison of the Copan eSwab System with an Agar Swab Transport System for Maintenance of Fastidious Anaerobic Bacteria Viability

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## ABSTRACT

We compared the eSwab system to a swab with an anaerobic transport semi-solid agar system for their capacities to maintain viability of 20 species of fastidious anaerobes inoculated on the bench and held at ambient and refrigerator temperature for 24 and 48h. On average, both systems maintained similar viability among analogous groups of organisms at both temperatures although there were quantitative differences among some species.

Suitable specimen transport from collection to the laboratory is essential for accurate laboratory diagnosis. Given increasing laboratory centralization, transport times have increased as well, requiring systems to be robust enough to ensure sufficient organism collection, viability and release. Specimens with anaerobic organisms have the added requirement of anaerobiasis for at least 48 hours. The eSwab (Copan Diagnostics, Inc., Murrieta, CA) is a relatively new system compared to conventional gel-tube systems and lends itself to automation. The eSwab consists of a nylon-flocked swab, which provides better capillary action and strong hydraulic uptake of liquids compared to spun-fiber nylon or rayon swabs (1) and a screw-top tube containing liquid modified-Amies medium. After specimen collection, the swab is inserted into the tube and the scored shaft of the swab is easily broken to the length of the tube. A swab capture system in the cap locks the broken shaft into the lid of the tube after it is fully closed. Favorable release studies comparing the flocked swab to convention rayon or Dacron swabs have been performed (2), as well as other studies comparing viability of aerobic and a small number of anaerobic organisms (1, 3-7). The recommended CLSI standard control strains have been shown in a previous study (1) to meet the requirements of the M40-A recommendations for transport systems (8). This is the first study comparing numerous fastidious anaerobic bacteria. We compare the eSwab with Anaerobic Transport Medium (ATM) (Anaerobe Systems, Morgan Hill, CA), both of which use modified-Amies medium in liquid and gel form respectively, for the release and recovery of fastidious anaerobic bacteria from the swabs after 24 and 48h at 4°C and room temperature (RT).



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**MATERIALS AND METHODS.** Twenty fastidious anaerobes, nine Gram-positive and 11 Gram-negative, from various sources were selected for study (Table 1). The organisms were identified by standard (9, 10) or molecular methods. This feasibility study of the recovery of various fastidious anaerobic bacteria was based on the CLSI (NCCLS) document M40-A (8) the approved standard for quality control of transport media. A 24–48h subculture of each organism was suspended in saline in the anaerobe chamber to a turbidity of 0.5 McFarland (~$1.5x10^8$ CFU/ml). To mimic clinical settings, the inoculation suspension was transferred to room air and 0.1 ml aliquots were pipetted into microcentrifuge tubes to inoculate eSwabs and rayon swabs for the ATM system. Each system was set up for recovery testing at room temperature and 4°C; each temperature had separate tubes set up for subculture at t=0, 24 and 48h. At each sampling time a suspension was made from each tube. The eSwab tube was vortexed for 5 sec, whereas the rayon swabs were removed from the ATM, the tip placed in 0.9 ml saline and vortexed for 5 sec. Each suspension was serially diluted, plated onto Brucella agar, incubated in an anaerobic chamber for 24–72h at 37°C and colony counts determined. The inoculum suspension was also serially diluted and colony counts were performed. Although the CLSI M40A quality control standard recommends dilutions in triplicate and platings in duplicate, because this was a performance study of each transport system and not a quantitative quality control analysis, each organism was studied once and each dilution was plated once. If, however, the colony counts from the serial dilutions were inconsistent, the organism was repeated. In addition, *C. difficile* the dilutions were also plated onto CCFA-HT in order to better recover spores, which germinate better in the presence of taurocholate (11).



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**RELEASE OF SAMPLE FROM SWABS.** The eSwabs released more organisms than did the rayon swabs although, on average, the difference was minor (Table 2). There were some exceptions (Figure 1a, 1b). In the Gram-negative group the eSwabs and rayon swabs retained 1.5 and 1.9 $\log_{10}$ CFU/ml on average, respectively. All *Fusobacterium* spp. were retained ~1 $\log_{10}$ CFU/ml more than the Gram-negative group average by both swab systems. In the Gram-positive group, the eSwabs and rayon swabs retained 1.5 and 1.6 $\log_{10}$ CFU/ml on average, respectively. *Finegoldia magna* was retained by both swab systems ~1.5 $\log_{10}$ CFU/ml more than the Gram-positive group average. In the *Clostridium* spp. group, the eSwabs and rayon swabs were retained 1.4 and 2.1 $\log_{10}$ CFU/ml on average, respectively. *C. ramosum* was retained by 0.7 $\log_{10}$ CFU/ml more with the eSwab and 1.6 $\log_{10}$ CFU/ml more than the rayon swab compared to the *Clostridium* spp. group average.

**RECOVERY OF SAMPLE.** All organisms were recovered at room temperature and 4°C at t = 0, 24 and 48h (Figures 2–4). Overall, both Gram-positive and Gram-negative organisms maintained similar average viability in both systems at room temperature (RT) and 4°C (Table 2); however, there were some exceptions.

In the Gram-negative group (Figures 2a, 2b), the best recovery of all organisms over $t_{0-24}$ and $t_{24-48}$ at 4°C and RT were *Bacteroides* spp. and *Bilophila wadsworthia*, with an average loss of only 0.1 $\log_{10}$ CFU/ml over 48h.

At 24h, *F. necrophorum* lost 0.9 $\log_{10}$ CFU/ml in ATM at 4°C and RT but had almost no loss in the eSwab. At 48 h, there was 0.8 $\log_{10}$ CFU/ml loss in ATM at 4°C and RT, however, in the eSwab there was a loss of 1.4 $\log_{10}$ CFU/ml at RT but only 0.3 $\log_{10}$



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CFU/ml at 4°C. Best performance for *F. necrophorum* was the eSwab at 4°C. The two *F. nucleatum* species had mixed results. One strain lost >1 $\log_{10}$ CFU/ml at 24h in both systems and temperatures; the loss was less at 48h for the eSwab at RT and the ATM at 4°C and RT, but the eSwab lost >1 $\log_{10}$ CFU/ml at 4°C. The other *F. nucleatum* strain lost an average of 0.5 $\log_{10}$ CFU/ml in the eSwab at RT and ATM at 4°C and RT but lost 2.2 $\log_{10}$ CFU/ml in the eSwab at 4°C. Fusobacteria had the most loss in the Gram-negative group in both systems.

*P. asaccharolytica* and *P. gingivalis* had <1 $\log_{10}$ CFU/ml loss in both systems and temperatures over 48 h despite their very fastidious nature.

On average, the *Prevotella* species lost most during the first 24h, 0.9 $\log_{10}$ CFU/ml $t_{0-24}$ and 0.5 $\log_{10}$ CFU/ml $t_{24-48}$. After 48h, *P. buccae* decreased only 0.5 $\log_{10}$ on average at 4°C and RT in the eSwab, but in the ATM lost 2.5 $\log_{10}$ CFU/ml at RT and 1.1 $\log_{10}$ CFU/ml at 4°C. *P. melaninogenica* lost 2.2 and 1.0 $\log_{10}$ CFU/ml in the ATM at RT and 4°C; the eSwab loss was 1.3 and 0.7 $\log_{10}$ CFU/ml at RT and 4°C. *P. intermedia* performed similarly to *P. melaninogenica* except the eSwab loss at RT was 3.3 $\log_{10}$ CFU/ml. The best performance for all *Prevotella* species was the eSwab at 4°C.

In the Gram-positive group (Figure 3), the average loss was 0.3 and 0.2 $\log_{10}$ CFU/ml at $t_{0-24h}$ and $t_{24-48h}$ respectively. Both systems performed similarly on average at RT and 4°C with the exception of *P. anaerobius*, which lost 2.2 and 1.9 $\log_{10}$ CFU/ml at RT and 4°C over 48h.

*Clostridium* spp. varied considerably (Figure 4), those strains known for producing more spores (e.g. *C. difficile*, ribotype 027) lost less in the first 24h than in

the second. The average loss of sample of *C. clostridioforme* and *C. ramosum* was 1.1 $\log_{10}$ CFU/ml at $t_{0-24h}$ and no average loss at $t_{24-48h}$. The average loss of sample of *C. difficile* ribotype 027 was greater at $t_{24-48h}$ than at $t_{0-24h}$. *C. clostridioforme* did not perform as well in the eSwab system at $t_{0-24h}$ and $t_{24-48h}$.

All counts were higher on HT than Brucella with the exception of the 027 ribotype of *C. difficile*, indicating more organism recovery from HT than suggested by Brucella (results not shown).

The eSwab is an all-in-one collection device that was shown to provide equal or superior release, viability and recovery performance for 48h at both room temperature and 4°C with most fastidious anaerobic bacteria compared to the conventional anaerobic transport system consisting of a rayon swab and an anaerobic transport tube. In addition, the eSwab provides the added ability to be used in automated specimen plating devices.

## ACKNOWLEDGEMENTS

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176 **Table 1.** Specimen sources of fastidious anaerobic bacteria tested, by groups.

| Group | Organisms | Source |
|---|---|---|
| Gram-negative | *Bacteroides fragilis* | Appendix |
| | *Bacteroides thetaiotaomicron* | Gluteal abscess |
| | *Bilophila wadsworthia* | Appendix |
| | *Fusobacterium necrophorum* | Tonsillar abscess |
| | *Fusobacterium nucleatum* (1) | Facial lesion |
| | *Fusobacterium nucleatum* (2) | Appendix |
| | *Porphyromonas asaccharolytica* | Diabetic foot |
| | *Porphyromonas gingivalis* | Tongue |
| | *Prevotella buccae* | Abdominal abscess |
| | *Prevotella intermedia* | Respiratory, sinus |
| | *Prevotella melaninogenica* | Sputum |
| Gram-positive | *Finegoldia magna* | Respiratory, sinus |
| | *Parvimonas micra* | Respiratory, sinus |
| | *Peptostreptococcus anaerobius* | Unknown |
| | *Eggerthella lenta* | Peri-rectal abscess |
| | *Propionibacterium acnes* | Facial acne |
| *Clostridium* spp. | *Clostridium clostridioforme* | Gluteal abscess |
| | *Clostridium difficile* (1), nontoxigenic | Stool |
| | *Clostridium difficile* (2), ribotype BI | Stool |
| | *Clostridium ramosum* | Blood |

177 **Table 2.** Aggregate change in CFU/ml ($log_{10}$).

| | | eSwab | | | ATM | | |
|---|---|---|---|---|---|---|---|
| **Group (no.)** | **Time (hr)** | **Inoculum[a]** | **RT[b]** | **4°C** | **Inoculum** | **RT** | **4°C** |
| **Gram-negative (11)** | 0 | -1.5 | | | -1.9 | | |
| | 0–24 | | -0.4 | -0.6 | | -0.9 | -0.6 |
| | 24–48 | | -0.7 | -0.2 | | -0.4 | -0.3 |
| **Gram-positive (5)** | 0 | -1.5 | | | -1.6 | | |
| | 0–24 | | 0.1 | 0.1 | | -0.8 | -0.5 |
| | 24–48 | | -0.1 | -0.3 | | -0.2 | -0.3 |
| ***Clostridium* spp. (4)** | 0 | -1.4 | | | -2.1 | | |
| | 0–24 | | -1.2 | -1.2 | | -1.0 | -1.2 |
| | 24–48 | | -0.3 | -0.4 | | 0.2 | -0.1 |

178 [a] Inoculum loss by organism retention of swab; [b] RT, ambient temperature

179 **Figure 1a, 1b.** Release of inoculum by the eSwab and ATM systems.

180 **Figure 2a, 2b.** Recovery of sample at t = 0, 24 and 48hr (CFU/ml $\log_{10}$), Gram-

181 negative group.

182 **Figure 3.** Recovery of sample at t = 0, 24 and 48hr (CFU/ml $\log_{10}$), Gram-positive

183 group.

184 **Figure 4.** Recovery of sample at t = 0, 24 and 48hr (CFU/ml $\log_{10}$), *Clostridium* group.



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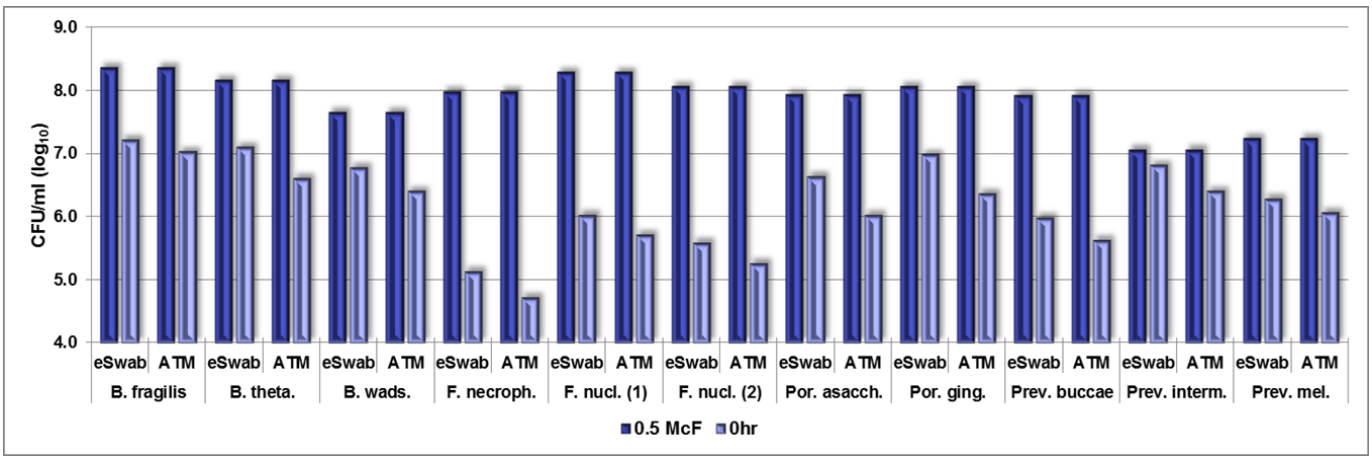

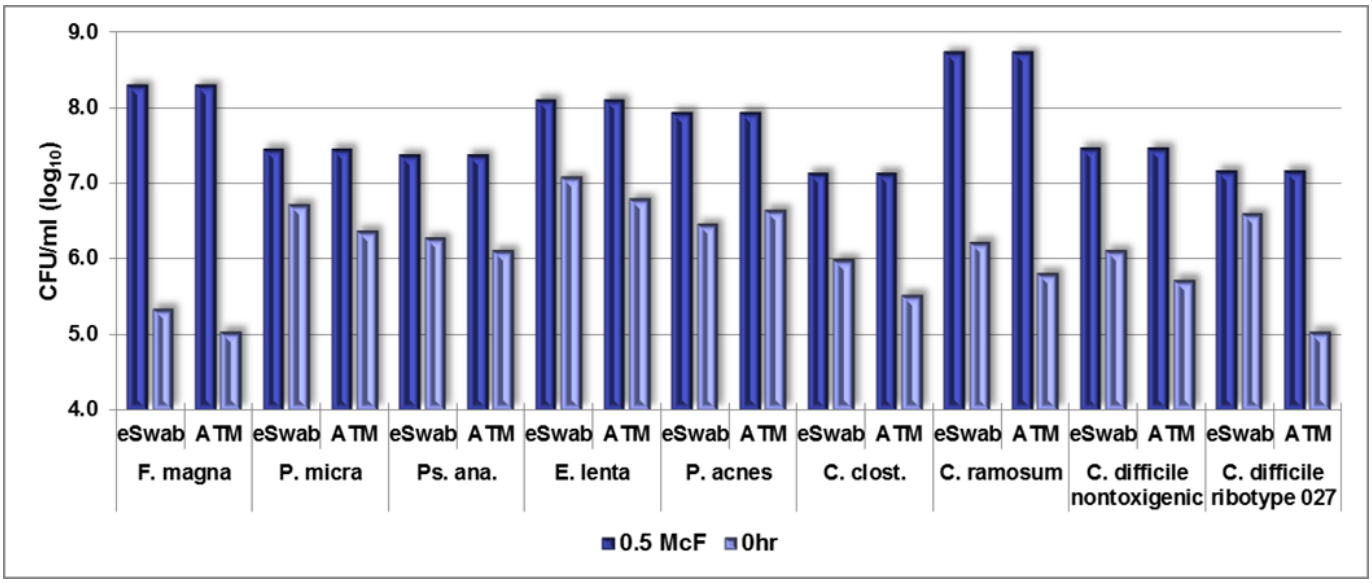

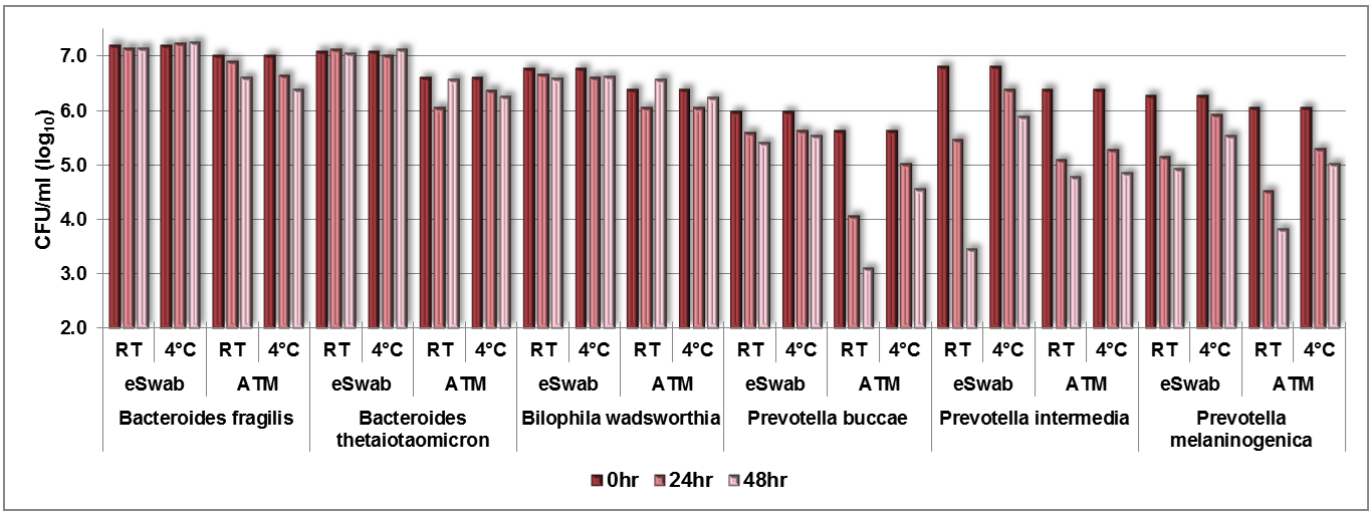

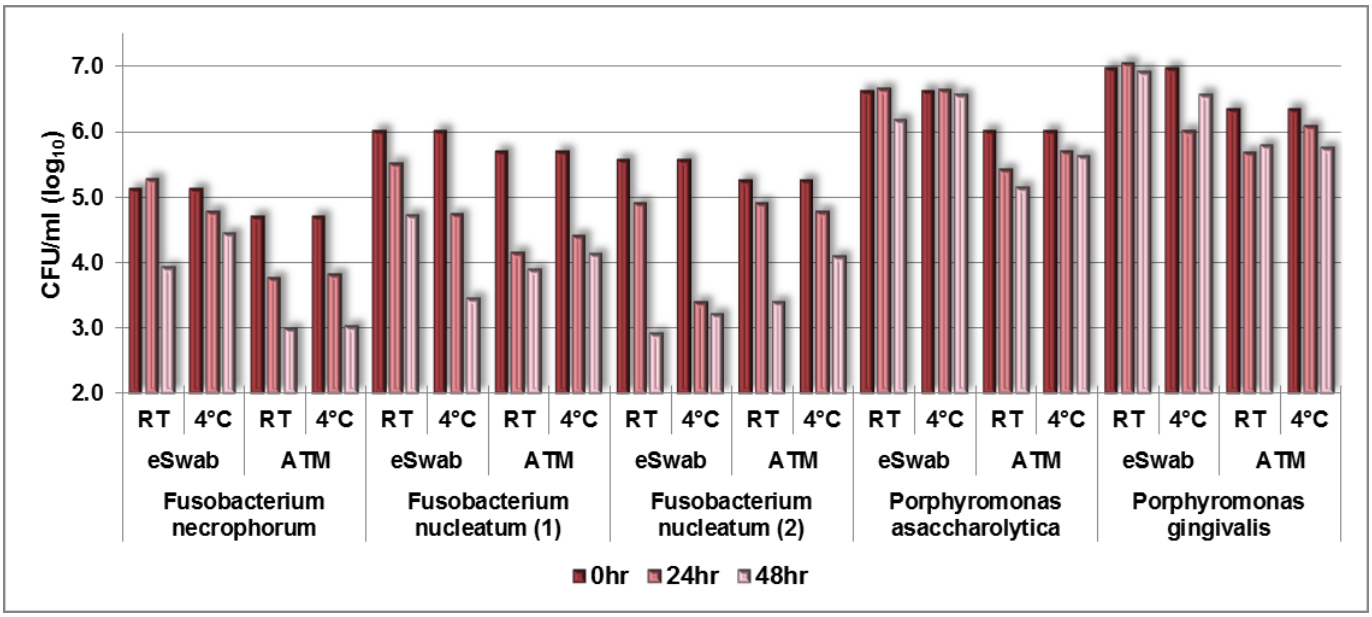

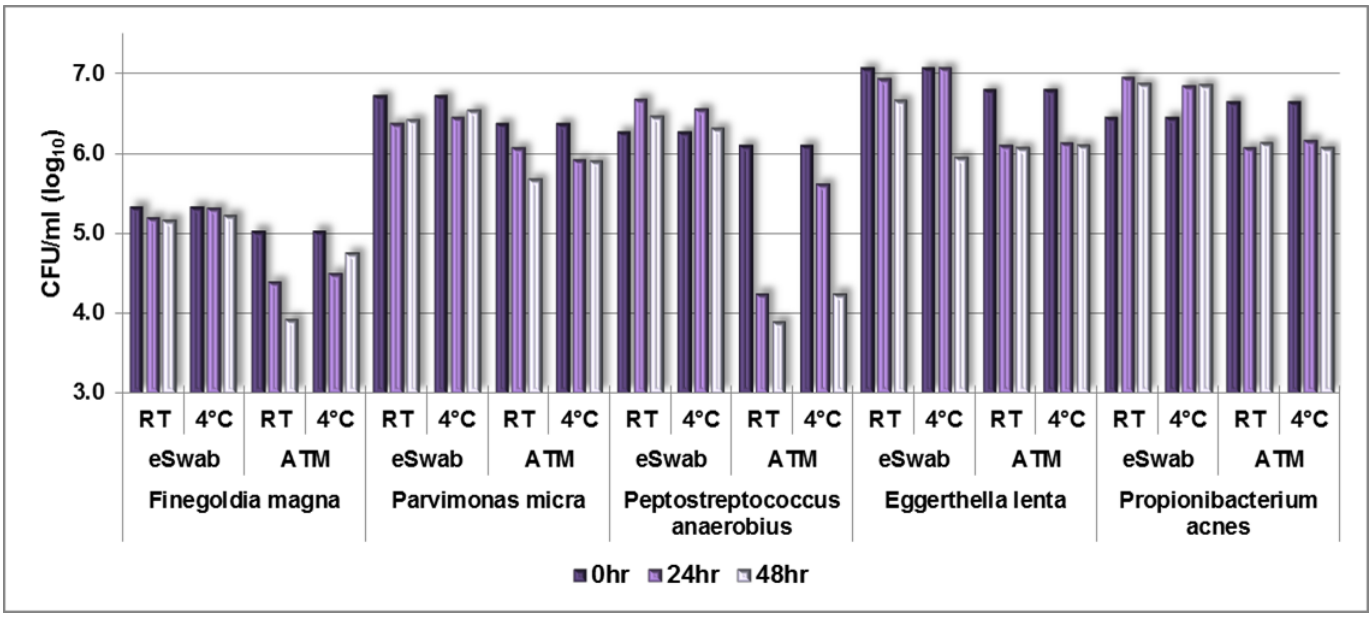






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