

Antibacterial and antifungal activity of MTA-based root canal sealer versus epoxy resin-based and methacrylate resin-based sealers

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Abstract

Aim: The purpose of this study was to compare the antibacterial and antifungal activity of MTA Fillapex with AH 26, AH Plus, and RealSeal root canal sealers. *S. aureus*, *E. faecalis*, and *C. albicans* were used as test microorganisms with the agar-diffusion test (ADT) and the direct contact test (DCT).

Methodology: For the ADT, 48 Mueller-Hinton plates were divided into 3 groups according to the microorganism used. Each group was then divided into 4 subgroups according to root canal sealer. Mueller-Hinton and Sabouraud agar mediums were preferred, and inhibition zones were measured to determine the antimicrobial efficacy at designated intervals. In the DCT, 96-well microtiter plates were used. For each microorganism and each sealer, 8 consecutive wells were prepared vertically on the plate. Microbial suspensions were allowed to directly contact the sealers in each well for 1 hour at 37°C. Subsequently, microbial growth was spectrophotometrically measured at set intervals for the freshly mixed and set forms.

Results: A statistically significant difference was found between the tested root canal sealers for antimicrobial effectiveness ($p < 0.05$). According to the ADT results, all sealers had antimicrobial activity against the tested microorganisms. MTA Fillapex demonstrated satisfying results in the ADT against all microorganisms. In the DCT, MTA Fillapex inhibited bacterial and fungal growth in all freshly mixed and set forms. However, the set forms of AH 26 and AH Plus began to lose their antimicrobial activity on the tested microorganisms after a while.

Conclusion: The results showed that the MTA-based root canal sealer MTA Fillapex may be a favorable alternative sealer against bacterial and/or fungal species in clinical practice.

Keywords: Antifungal, antimicrobial, MTA Fillapex, RealSeal, root canal sealer.

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Introduction

Endodontic therapy aims to eliminate residual pulp, microorganisms, and by-products, followed by a hermetic obturation to prevent reinfection inside the root canal system (1). Root canal sealers can provide

an impermeable sealing as a filler for irregularities and between the root canal wall and core material. Sealing ability, biocompatibility, and antimicrobial activity are important properties for root canal sealers. The antimicrobial activity of a sealer might help to reduce or inhibit the growth of the remaining microorganisms

when pulpal necrosis and apical periodontitis are present (2).

Staphylococcus aureus, *Enterococcus faecalis*, and *Candida albicans* are considered the most resistant facultative microorganism species in the oral cavity and can cause unsuccessful treatment results (3). *S. aureus* can be isolated from acute dentoalveolar infections, such as dental abscesses (4). *E. faecalis* is the most commonly isolated pathogen from endodontic infections, ranging from 24 to 77% (5), and is associated with persistent periradicular lesions after endodontic treatment (6). Similarly, *C. albicans* is the most commonly detected fungal species in the oral cavity of both healthy and medically supported individuals (7); it has been reported in primary, persistent, and secondary endodontic infections (8,9).

AH Plus (Dentsply, Konstanz, Germany) is an epoxy resin-based sealer that has the same physical and chemical properties as AH 26. In a study, it is reported that both AH Plus and AH 26 have antimicrobial activity on various microorganisms (10). On the other hand, these root canal sealers have been shown to have highly, moderately, or slightly toxic effects in different studies (11,12). RealSeal (SybronEndo, Orange, CA, USA) is a methacrylate resin-based root canal sealer. It has a Resilon ‘monoblock’ system introduced as an alternative obturation against an AH Plus/gutta percha combination. However, there are few studies in the literature about its antimicrobial activity.

MTA Fillapex (Angelus, Londrina, Brazil) is a recently developed endodontic sealer that claims to retain the biological and physical properties of mineral trioxide aggregate (MTA) (13). In this way, it may utilize the advantages of MTA as a permanent root canal sealer (14). According to the manufacturer, MTA Fillapex has a sufficient working time, high radiopacity, and is easy to handle. MTA Fillapex has a high pH, solubility, and calcium ion release (15). Alkaline pH and solubility may benefit the antibacterial activity of MTA Fillapex.

The antimicrobial activity of root canal sealers can be evaluated using different methods. The agar diffusion test (ADT) is a method that is still used, though it has limitations (16). The direct contact test (DCT) investigates the effect of direct and close contact on microorganisms and tested materials. The DCT can also be used to test the endodontic sealers in various setting forms (17).

The aim of this in vitro study was to compare both the antibacterial and antifungal activity of the MTA Fillapex sealer against epoxy resin-based and methacrylate-based sealers.

Materials and Methods

The compositions of the sealers that were tested in the study are listed in Table 1. All sealers were prepared according to the manufacturers' advice.

Test Microorganisms and Growth Conditions

Antimicrobial activity of the root canal sealers was evaluated against *S. aureus* (ATCC 6538), *E. faecalis* (ATCC 29212), and *C. albicans* (10231; RSHSE, Ankara, Turkey). Microorganisms from frozen stock cultures were grown aerobically to a late logarithmic or early stationary phase in fresh brain heart infusion (BHI) broth.

Agar Diffusion Test (ADT)

In the ADT, root canal sealers were used only as a freshly mixed form. Fifty-one Mueller-Hinton plates were used for the ADT. Forty-eight plates were divided into 3 groups of 16, according to the microorganism used. Each group was then divided into 4 subgroups for the 4 different root canal sealers. The remaining 3 positive control plates were streaked with 3 different bacteria or fungi, but no sealer was used. Mueller-Hinton agar was preferred as a medium for *S. aureus* and *E. faecalis*; Sabouraud agar was used for *C. albicans*. Three wells (6 mm in diameter) were formed in each agar plate. The 200 µL of microbial suspension (approximately 10⁶ CFU/ml of each test microorganism) were spread on the agar plates. Using a cavity liner applicator, 0.1 ml of sealer was spread in the wells. Only one type of microorganism and root canal sealer was set to each agar plate. In total, 12 observation wells were used for each type of microorganism and root canal sealer. The plates were preserved at 37°C in a humid environment.

The plates were analyzed under ×2.5 magnification loupes and evaluated for zones of inhibition, as evidenced by a lack of bacterial colonization around each well. The diameters of the established inhibition zones were measured in millimeters in two perpendicular locations for each sample and periodically recorded at 24 hours, 48 hours, 7 days, and 10 days. The samples were returned to incubation after each measurement.

Direct Contact Test (DCT)

In the DCT, both the freshly mixed forms and the set forms of the root canal sealers were tested separately on different plates. Microtiter plates with 96 wells were used. According to group, a cavity liner applicator was used to coat the wells with freshly mixed root canal sealers. According to the recommendations made by Cobankara et al, 10 µL of microbial suspension (approximately 10⁶ CFU/ml) were added to the root canal sealer surface about 20 minutes later (18). The prepared plates were stored at 37°C in a humid environment for 1 hour and it was ensured that there was direct contact between the test materials and microorganisms. Then fresh BHI broth (245 µL) was added to each well and gently mixed for 2 minutes. Fifteen µL of the mixture was taken from each well of the first microtiter plate, this was then added to the second microtiter plate in an adjacent set of 8 wells

containing fresh broth (215 µm) and mixed. The second plate was used for optical density determinations. In a different microplate, microorganisms were cultivated without root canal sealers and served as a positive control group. To determine the optical density, the microplate spectrophotometer (BioTek, PowerWave XS2) was used for 16 different evaluation times within 24 hours. The kinetics of the outgrowth in each well was observed at 620 nm. Before each reading, automixing was performed for each homogeneous microorganism suspension. In the set form group, the root canal sealers were mixed and left for 24 hours to completely set at 37°C and 100% humidity before the microorganism suspension was placed in contact with them. Other processes were similarly applied with the freshly mixed form group. The entire experiment was carried out under aseptic conditions.

Statistical Analysis

The statistical software SPSS 25.0 (IBM Corp. New York, NY, USA) was used to analyze the data. For the ADT, the Kolmogorov-Smirnov test was used to test the normality of distribution. The data in a normal distribution were analyzed using the analysis of variance (ANOVA) test and post-hoc comparisons were made with the Tukey test for ADT.

Results

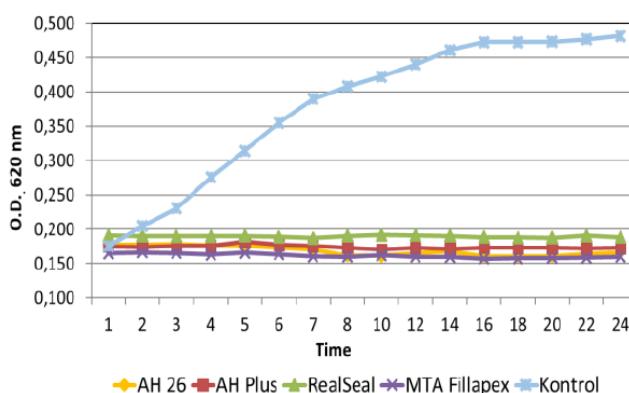
ADT

The results of the ADT are shown in Table 2. According to the ADT results, all sealers had antimicrobial activity against the tested microorganisms ($p < 0.05$) and no statistically significant correlation was found among antimicrobial effectiveness of the tested sealers and different time

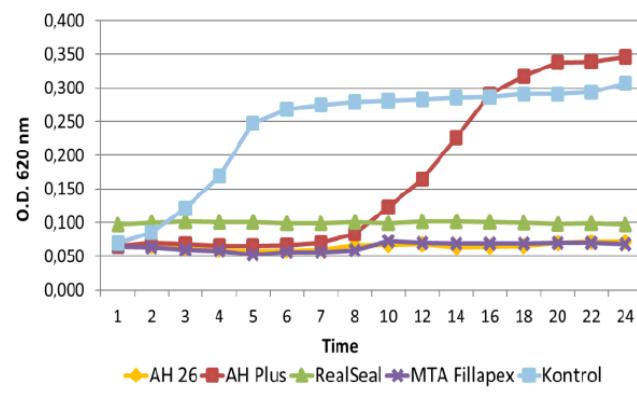
intervals ($p > 0.05$) except for an MTA Fillapex and *E. faecalis* correlation which increased with time. Against *S. aureus*, there was no significant difference between AH 26 and RealSeal; nor was there a significant difference between AH Plus and RealSeal ($p > 0.05$). These sealers showed a significant difference to MTA Fillapex sealers ($p < 0.05$). Against *E. faecalis*, AH 26 had the highest antibacterial activity ($p < 0.05$). The antibacterial activity of MTA Fillapex increased with time. There was no significant difference between RealSeal and MTA Fillapex ($p > 0.05$) and AH plus had the lowest antibacterial activity. Against *C. albicans*, AH 26 had the largest inhibition zone and showed the highest antifungal activity. AH Plus showed the lowest antifungal activity ($p < 0.05$). There was no significant difference between the RealSeal and MTA Fillapex sealers ($p > 0.05$). The three positive control plates showed bacterial or fungi growth.

DCT

The results of the DCT with the freshly mixed sealers are presented in Figures 1a, 2a, and 3a. Each curve includes 16 measurements taken within 24 hours. All sealers showed similar effectiveness for freshly mixed forms against all microorganisms. Freshly mixed samples of sealers showed complete inhibition of *S. aureus*, *E. faecalis*, and *C. albicans*. The results of the DCT with the 24-hour set forms of the sealers are presented in Figures 1b, 2b, and 3b. AH Plus has a similar antibacterial activity as the other sealers in the first 8 hours; after this time it began to lose its activity against *S. aureus* and *E. faecalis*. Additionally, both AH 26 and AH Plus could not inhibit fungal growth after 8 hours and microbial growth continued to increase. The 24-hour set samples of MTA Fillapex and RealSeal exhibited more stable antibacterial and antifungal activity against all microorganisms than the other sealers.

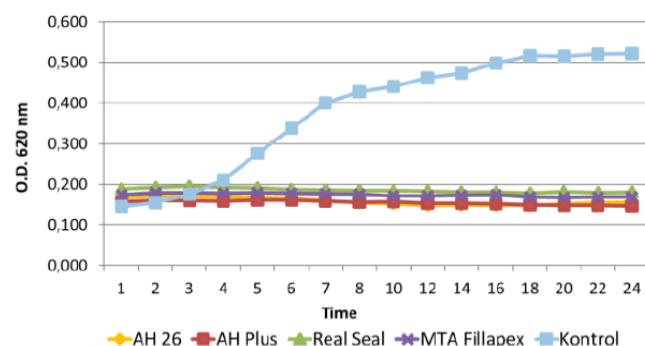


(a)

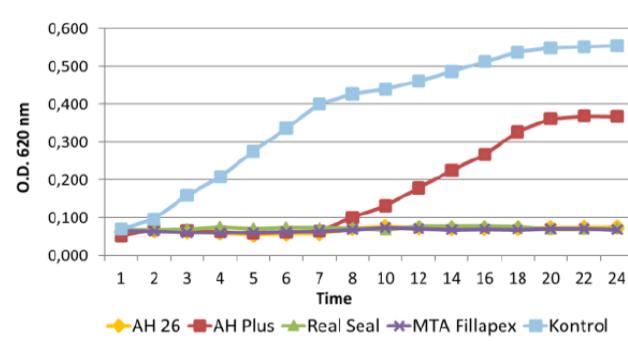


(b)

Figure 1. Line graphs showing microbial growth curve of *S. aureus* after direct contact with fresh mixed sealers (a) and 24 h set sealers (b). Each point on the curve is the average optical density (OD₆₂₀) on a logarithmic scale measured in 8 separate wells at the same time.

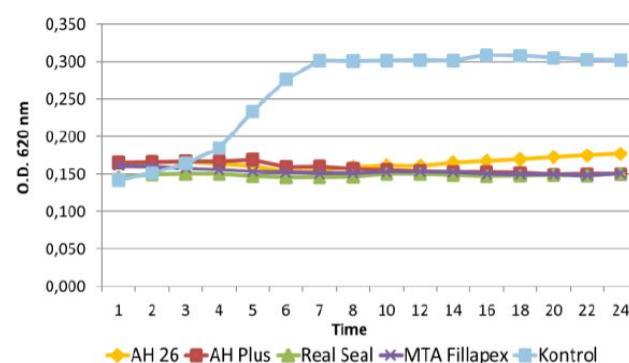


(a)

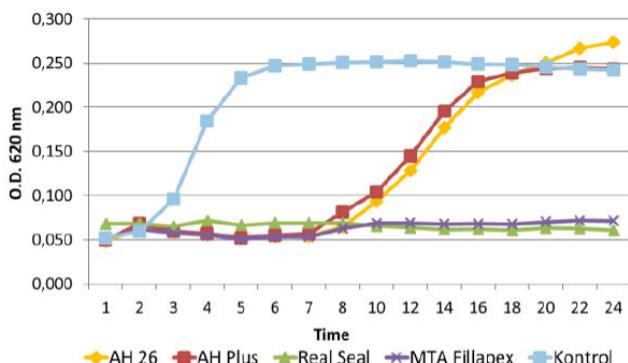


(b)

Figure 2. Line graphs showing microbial growth curve of *E. faecalis* after direct contact with fresh mixed sealers (a) and 24 h set sealers (b). Each point on the curve is the average optical density (OD_{620}) on a logarithmic scale measured in 8 separate wells at the same time.



(a)



(b)

Figure 3. Line graphs showing microbial growth curve of *C. albicans* after direct contact with fresh mixed sealers (a) and 24 h set sealers (b). Each point on the curve is the average optical density (OD_{620}) on a logarithmic scale measured in 8 separate wells at the same time.

Table 1. Composition of sealers

Sealers	Compositions	Manufacturer
AH 26	Paste A: Bisphenol-A epoxy resin, Bisphenol-F epoxy resin, calcium tungstate, silica, zirconium oxide, iron oxide pigments Paste B: Dibenzidiamin, aminoadamantane, trisiklodekan-diamine, calcium tungstate, zirconium oxide, silica, silicon oil.	Dentsply, De Trey, Konstanz, Germany
AH Plus	Paste A: Diglycidil-bisphenol-A-ether, calcium tungsten, zirconium oxide, aerosol, iron oxide. Paste B: amina-1-adamantane, N,N-dibenyl-5- oxanonandiamine-1,9, TCD-di-amine, calcium tungsten, zirconium oxide, silicone oxide.	Dentsply, De Trey, Konstanz, Germany
RealSeal	Bis GMA, ethoxylated Bis GMA, urethane dimethacrylate, methacrylate, calcium hydroxide, barium sulfate, barium glass, bismuth oxychloride, silica.	SybronEndo, Orange, USA
MTA Fillapex	Salicylate resin, diluting resin, natural resin, bismuth trioxide, nanoparticulated silica, MTA, pigments.	Angelus, Londrina, PR, Brasil

Table 2. Inhibition zone measurement of microbial growth of sealers in periods (mm)

	AH 26	AH Plus	RealSeal	MTA Fillapex
<i>S. aureus</i>				
24 h	20.3±2.6 ^a	17.1±0.7 ^b	17.8±1.2 ^b	13.2±1.0 ^c
48 h	20.1±2.1 ^a	17.1±0.7 ^b	18.7±2.1 ^{a,b}	13.1±1.0 ^c
7 days	19.8±2.0 ^a	17.3±0.6 ^b	18.8±2.0 ^{a,b}	13.3±0.9 ^c
10 days	19.8±1.8 ^a	17.2±0.6 ^b	19.5±1.3 ^a	13.1±1.0 ^c
<i>E. faecalis</i>				
24 h	20.3±0.9 ^a	12.8±1.1 ^c	18.3±2.2 ^b	13.6±0.9 ^c
48 h	19.9±1.3 ^a	12.7±1.2 ^d	17.3±0.9 ^b	15.0±0.8 ^c
7 days	20.9±0.7 ^a	13.2±1.0 ^c	17.7±1.0 ^b	17.7±1.3 ^b
10 days	21.0±1.1 ^a	13.1±1.0 ^c	17.9±0.9 ^b	17.2±1.2 ^b
<i>C. albicans</i>				
24 h	39.5±1.5 ^a	12.4±0.6 ^c	17.6±0.6 ^b	18.4±2.2 ^b
48 h	41.1±2.4 ^a	12.3±0.4 ^d	17.3±0.6 ^c	19.3±1.9 ^b
7 days	39.4±1.6 ^a	12.6±0.7 ^d	17.6±0.8 ^c	19.4±1.6 ^b
10 days	40.2±1.3 ^a	12.6±0.7 ^c	17.6±0.7 ^b	18.9±1.8 ^b

Discussion

Root canal treatment reduces microorganisms in the root canal, but it does not eliminate all of them. Microorganisms can persist in the isthmus, dentinal tubules, lateral canals and apical ramifications after root canal treatments have been informed (19). Microorganisms may persist in these areas and may reproduce. This results in a complete failure of the root canal treatment. Sealers that exhibit both excellent sealing and antibacterial properties can be used to improve the success rate of endodontic treatment. Using sealers with antimicrobial properties can be advantageous against persistent residual infection and bacteria reentering from the oral cavity (20).

This study investigated the antibacterial and antifungal properties of a new generation of root canal sealer against widely used or accepted root canal sealers.

S. aureus is one of the standard microorganisms that is used in antimicrobial activity tests of endodontic materials (21). *E. faecalis* has a high prevalence in persistent endodontic infections. It has a collagen-binding protein that allows it to bond to root canal dentine (22), so it is difficult to remove. Peciuliene et al. showed the isolation of *E. faecalis* and *C. albicans* in root-filled teeth with persistent apical periodontitis (23). *C. albicans* can form a biofilm similar to *E. faecalis*. In a study that used the polymerase chain reaction (PCR) technique, the presence of *C. albicans* was reported in 5 of 24 isolations from the root canals in primary infections (8). In secondary infections, it was identified in 6 of 33 samples; 3 of these 6 cases were identified with *E. faecalis* (23). Therefore, it should be

noted that determining the antifungal activity of root canal sealers is important in order to enhance the treatment's success. Consequently, because of their common pathogenic disaster, these 3 microorganisms were preferred to be used for the tests.

In the ADT, freshly mixed root canal sealers were used. The DCT was performed to analyze both fresh and 24-hour set forms of root canal sealers. The ADT assays have been a semi-quantitative technique to determine the antimicrobial activities of dental materials. The ADT has some limitations: results are highly influenced by the solubility and diffusion ability of the material, and also do not distinguish between bacteriostatic or bactericidal properties of the tested materials (24). Nawal et al. (25) indicate that, when new materials are tested, more than 1 method should be used. Some studies about antimicrobial activity have preferred to use ADT together with DCT to overcome or minimize its disadvantages or limitations (16, 26).

The DCT is based on evaluating the effect of direct and close contact between the tested materials and microorganisms on microbial outgrowth; it also provides significant advantages including reproducibility, simultaneous testing of samples, quantitative assay, and continuous measurements of bacterial outgrowth (17). It is independent of the diffusion and solubility properties of the media and tested material and the effect of sealers at different levels of setting reaction can be evaluated (27). The increase in the optical density value in DCT indicates that the number of bacteria in the environment increases. An increase in the number of bacteria is interpreted as a decrease in the antimicrobial activity of the tested material.

In the ADT, AH 26 showed the highest antimicrobial activity on all tested microorganisms. AH 26 contains formaldehyde and it is thought that high antimicrobial activity occurs when this substance is released (28). MTA Fillapex showed higher antibacterial and antifungal effects than AH Plus against *E. faecalis* and *C. albicans* respectively. Similarly, Poggio et al. (29) reported that AH Plus showed a low antibacterial effect against *E. faecalis* and *C. albicans* in the ADT. When MTA Fillapex was compared with RealSeal, it had a lower antibacterial effect against only *S. aureus* and showed a similar effect against the others. These results are important because of the inadequate published articles comparing a methacrylate resin sealer with MTA-based sealer.

Pizzo et al. (27) reported that, in a DCT, only fresh AH Plus showed antibacterial activity and 24-hour and 7-day set samples did not show effectiveness against *E. faecalis*. Similarly, in the present study, AH 26 and AH Plus set forms were not effective against some microorganisms over longer periods. Eldeniz et al. reported that 3-day aging of root-end filling materials had a variable antibacterial activity and emphasized that, when the specimens were aged in a 100% humidity environment at 37°C, this allowed continued release of antibacterial components (30).

In this study, freshly mixed and 24-hour set form samples of MTA Fillapex and RealSeal exhibited greater antibacterial activity against all microorganisms than the other sealers. MTA consists of 50-75% calcium oxide by weight. When water is added, the cement hydrates to silicate hydrate gel and calcium hydroxide (31). It can be supposed that MTA and calcium hydroxide may have a similar degree of activity. The release of hydroxyl ions from calcium hydroxide (Ca(OH)_2) to the hydrous environment creates antimicrobial activity. Hydroxyl ions are highly oxidant free radicals that exhibit severe reactivity with several biomolecules (2). They play an important role in the elimination of bacteria, causing an increase in pH. A pH greater than 9 may reversibly or irreversibly influence the cellular membrane enzymes of microorganisms and results in the loss of biological activity (32).

MTA Fillapex has a high solubility, pH, and calcium ion release (33). Faria-Junior et al. compared the pH and solubility of MTA Fillapex with several root canal sealers and reported a pH rise to 10.06 after 15 hours of immersion in deionized water (34). Similarly, it was shown that the pH value of MTA after mixing was 10.2 which increased to 12.5 after 3 hours (35). In a study, MTA Fillapex showed antibacterial effect over 7 days and the pH value was between 10.14 and 10.50 (16). It is thought that this high pH and solubility may contribute to the antibacterial activity of MTA Fillapex.

The physicochemical properties of a root canal sealer were combined with the biological advantages of MTA and MTA Fillapex was produced. The composition of MTA Fillapex contains mineral trioxide aggregate, salicylate resin, natural resin, bismuth, and silica. MTA Fillapex has an antimicrobial mechanism due to its MTA substance.

In root canal therapy, moisture remaining in the tubules and on the root dentin surface after root canal

irrigation affects the sealing ability of traditional hydrophobic sealers (33). This problem can be solved using a hydrophilic sealer. MTA Fillapex is a hydrophilic material similar to MTA (2). Hydrophilic materials can increase sealing ability and support antimicrobial activity (36).

Conclusions

The results of the present study showed that all tested sealers had antibacterial and antifungal activity on the tested microorganisms in the ADT. But in the DCT, 24-hour set forms of AH 26 and AH Plus lost their antimicrobial activity over time. This can be attributed to a diminished release of the antimicrobial component from the tested sealers. MTA Fillapex showed suitable antimicrobial properties to be used as an endodontic sealer.

Ethical Approval: Ethics committee approval was not obtained because no material or radiographs of human or animal origin were studied.

Peer-review: Externally peer-reviewed.

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