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# An *In Vitro* Comparative Evaluation of Apical Leakage Using Different Root Canal Sealers

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Statistical Analysis C  
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**Background:** Successful endodontic therapy requires a fluid-tight apical seal. The aim of this *in vitro* study was to evaluate and compare the apical sealing ability of 3 root canal sealers: Apexit Plus, AH Plus, and Resilon/Epiphany sealers.

**Material/Methods:** Samples of 152 single-rooted teeth were divided into 2 test groups, and each group was divided into 3 sub-groups based on the preparation and obturation technique. After preparation with the step-back technique, the first group of root canals were obturated with lateral gutta-percha compaction in combination with AH Plus sealer or Apexit Plus or with the Resilon/Epiphany system alone. In the second group, preparation was done with the crown-down technique and root canals were then obturated with Thermafil obturator in combination with AH Plus or Apexit Plus or with Resilon/Epiphany sealer alone. Apical leakage was determined using a dye leakage test observed with a stereomicroscope. The degree of dye leakage was assessed using the *t* test to comparing the arithmetic averages of the groups.

**Results:** In the groups prepared with the step-back technique, the average dye leakage was lower in samples obturated with Resilon/Epiphany, than in those filled with AH Plus/lateral gutta-percha compaction or Apexit Plus/lateral gutta-percha compaction. In groups prepared with the crown-down technique and obturated with the Resilon/Epiphany system, the average dye leakage was lower than in those filled with AH Plus/Thermafil obturation and those filled with Apexit Plus/Thermafil obturation.

**Conclusions:** Although all preparation and obturation techniques showed dye penetration, the crown-down technique paired with the Resilon/Epiphany system showed the least leakage.

**MeSH Keywords:** **Endodontics • Gutta-Percha • Root Canal Filling Materials**

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## Background

The purpose of an endodontic root canal treatment is to provide a hermetic seal after adequate cleaning and shaping to ensure 3-dimensional obturation of the root canal system [1,2]. The obturation procedure should provide suitable lateral and apical sealing of the endodontic space by the sealer, thus preventing communication of the root canal contents with periapical tissues [3–5]. Therefore, it is clinically important to choose a high-quality endodontic sealer because it contributes to the long-term success of nonsurgical root canal treatment [6].

Commercial endodontic sealers are typically a thin tacky paste that serves as both a lubricant and a luting agent during the obturation procedure, enabling the core obturation material to slide in and become fixed in the root canal [7,8].

The most commonly used core material is gutta-percha. It occupies the majority of the endodontic space in combination with pastes that cement it to the canal wall and fill irregularities and gaps between root canal walls and the cones of gutta-percha [9,10]. Other obturating materials are also currently in use because gutta-percha paired with sealers does not provide a fluid-tight seal [11]. New improved products such as Resilon™ (Epiphany, Pentron Clinical Technologies, Wallingford, CT, USA), a thermoplastic synthetic polymer-based endodontic material, have been used as an alternative to gutta-percha in combination with Epiphany sealer, which bonds to the root canal sealer and dentin [12]. In addition, as an alternative to the lateral cold-compaction gutta-percha technique, thermo-plasticized gutta-percha has been introduced to improve 3-dimensional filling of root canals [13].

Despite the obturating materials mentioned above, endodontic failure can still occur. Apical microleakage may occur because of the physical and chemical properties of sealers, root canal preparation and obturation techniques, and smear layer presence [10,14,15]. Various methods are used to detect the apical sealing ability of root canal materials, such as dye leakage, bacterial penetration, radioisotope techniques, electrochemical techniques, and fluid filtration techniques [16–20]. Dye is the most commonly used agent for this purpose [21,22].

The aim of the current *in vitro* study was to determine and compare the apical sealing ability of root canals filled with gutta-percha and the root canal sealer Apexit Plus or AH Plus and those filled using the Resilon/Epiphany system, after root canal preparation with a step-back technique. A second set of root canals were prepared with a crown-down technique and filled with Thermafil in combination with Apexit Plus or AH Plus or filled using the Resilon/Epiphany system.

## Material and Methods

This study was approved by the Ethics Commission (Nr. 269/2014).

A total of 152 human upper central incisors and canines with fully formed apices were taken from a pool of extracted teeth that had been removed for periodontal reasons and preserved in normal saline. Prior to the study, the teeth were placed in 1% sodium hypochlorite for 2 h. The crowns were then removed at the cement-enamel junction with a diamond disk under water coolant. All preparation and obturation procedures were performed by the same operator.

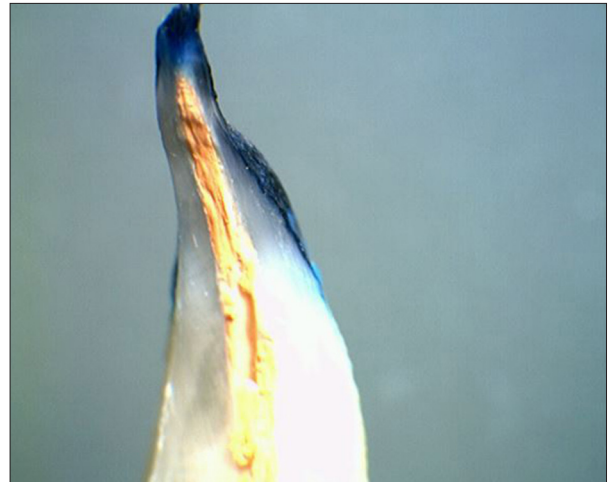
The apical patency of the root canal was established with a no. 15 K-file (Dentsply Maillefer, Ballaigues, Switzerland) until the tip was visible at the apical foramen. The working length was set at 1 mm shorter than the apical foramen. The root canals were divided in 2 experimental groups, and each group was subdivided into 3 subgroups (n=20 each) based on the root canal preparation and obturation technique.

The root canals for the first 3 subgroups were prepared with K-files (Dentsply Maillefer) using a step-back technique. Apical enlargement was made with instrument size up to no. 30. The root canals were disinfected with 2 mL of 5.25% sodium hypochlorite (Chloraxid, CerkaMed, Stalowa Wola, Poland). Next, 5 mL of 17% EDTA (Chelaton III dihydrate p.a., Lach-Ner, Czech Republic) was prepared by adding NaOH to pH 7.4. After irrigation with EDTA, root canals were dried with paper points and obturated with cold lateral compaction of gutta-percha and AH Plus (Dentsply DeTrey GmbH, Konstanz, Germany) or Apexit Plus (Ivoclar Vivadent, Lichtenstein), or with Epiphany SE Resilon (Pentron, Wallingford, CT, USA). In the third subgroup, in which the root canal was obturated with Epiphany sealer and a 0.04 Resilon cone according to the manufacturer's instructions, the final irrigation was completed with a physiological solution, because NaOCl would inhibit polymerization of the filling material. The bonding material was applied with a brush into the root canals. After this, Resilon master cones were coated with Epiphany sealer and applied into the canals, followed by light curing for 40 s.

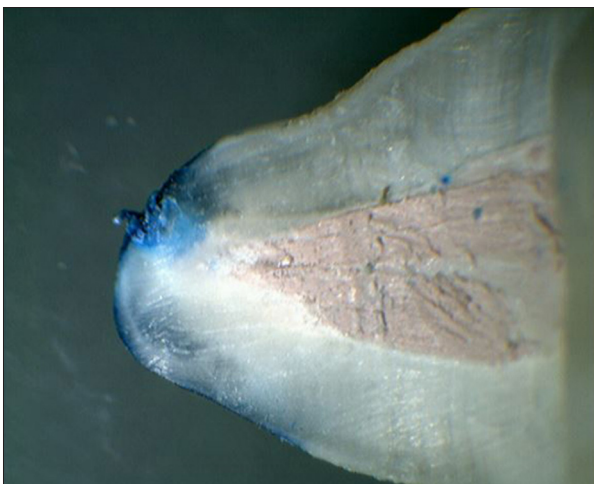
The root canals of the second experimental group (subdivided in 3 subgroups) were prepared with the ProTaper rotary system (Dentsply Maillefer) using a crown-down technique. The canals were prepared with an Sx instrument to shape the coronal sector of root canal, followed by S1, S2, F1, F2, and F3 instruments to prepare the middle and apical sectors of the canal to the working length. An X-Smart endodontic motor (Dentsply Maillefer) was used at a rotation of 300 rpm, with passive introduction of the instruments into the root canal. The root canals were filled with Thermafil obturator (Dentsply Maillefer),



**Figure 1.** Apical dye leakage after the step-back technique and obturation with AH Plus and lateral gutta-percha compaction.



**Figure 2.** Apical dye leakage after the step-back technique and filling with Apexit Plus and lateral gutta-percha compaction.



**Figure 3.** Apical dye leakage after the step-back technique and filling with Resilon/Epiphany system.



**Figure 4.** Apical dye leakage after the crown-down preparation technique and filling with AH Plus and Thermanfil obturator.

which was heated in the Thermo-Prep Plus apparatus (Dentsply Maillefer). The obturator was then introduced into the root canal in combination with AH Plus or Apexit sealer in the first and second subgroups; the root canals of the third subgroup were obturated with Epiphany sealer and a 0.04 Resilon cone.

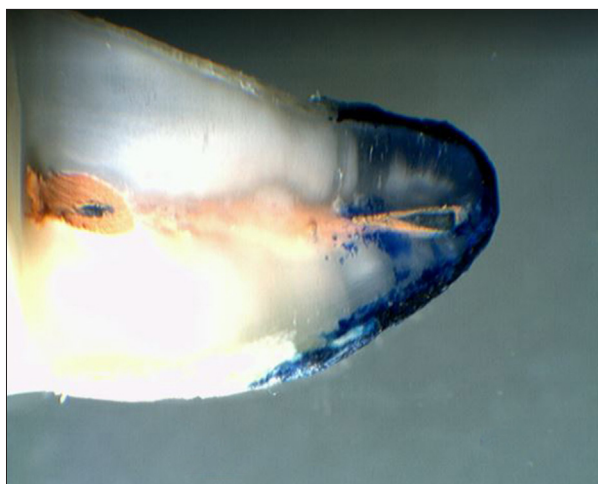
The root canals of the positive control group (n=16) were filled with gutta-percha only, while the root canals of the negative control group (n=16) were obturated with gutta-percha and sealer.

After placement of the root canal material and removal of any excess, a temporary filling Cavit (3M ESPE, Germany) was placed.

After the procedure was completed, the samples were stored in an incubator for 7 days. The entire surface of the roots was

coated with 2 layers of nail varnish, except for the apical 2 mm. Teeth from the positive control group were not coated with nail polish, while teeth from the negative control group were completely coated.

Apical leakage was estimated using a dye penetration test. All samples were submerged in 2% methylene blue solution for 48 h, and then washed with water and dried. A diamond disk was used to longitudinally section the root in a bucco-lingual direction. Apical leakage was measured from the apex of the root to the coronal extent of the methylene blue dye penetration (Figures 1–6). The linear measurement of the methylene blue was evaluated using a stereomicroscope (Brunel Microscope MX6T, Wiltshire, UK) with  $\times 20$  and  $\times 30$  magnification.



**Figure 5.** Apical dye leakage after the crown-down preparation technique and filling with Apexit Plus and Thermafil obturator.



**Figure 6.** Apical dye leakage after the crown-down preparation technique and filling with the Resilon/Epiphany system.

**Table 1.** Apical dye leakage according to sealers after the step-back preparation technique.

Tested group	Degree	SD	SE	CI of Mean	Max	Min	Median
SB-Ap+	0.38	0.31	0.07	0.15	1.14	0.12	0.25
SB-AH+	0.29	0.23	0.05	0.11	0.89	0.00	0.22
SB-E-se-R	0.06	0.08	0.02	0.04	0.27	0.00	0.04

SD – standard deviation; SE – standard error; CI – confidence interval; SB-Ap+ – step-back preparation and Apexit Plus; SB-AH+ – step-back preparation and AH plus; SB-E-se-R – step-back preparation and Resilon/Epiphany.

Statistics were processed through descriptive analysis, and the arithmetic mean, standard deviation and standard error, and confidence interval with 95% reliability (95% CI) were determined. The degree of color indicating micro-permeability was assessed by using the *t* test to compare the arithmetic averages of groups. The criterion for determining statistical significance was  $\alpha=0.05$ .

## Results

We found dye leakage along the entire root canal in the positive control group, whereas the negative control group did not show any dye leakage.

The mean values and standard deviations of apical dye leakage for each sealer after the step-back preparation technique are shown in Table 1. In the experimental groups prepared with the step-back technique, the average dye leakage was lower in roots filled with Epiphany SE Resilon (0.06 mm) compared with the roots obturated with AH Plus and lateral gutta-percha compaction (0.29 mm) and roots filled with Apexit Plus and lateral gutta-percha compaction (0.38 mm).

Table 2 shows differences in the average dye leakage, which was significantly higher in roots filled with Epiphany SE Resilon compared with roots filled with AH Plus (average difference=0.23 mm, 95% CI 0.12 to 0.34 mm,  $P<0.001$ ). Differences in average dye leakage were significantly higher in roots filled with Epiphany SE Resilon than in roots filled with Apexit Plus (average difference=0.325 mm, 95% CI 0.18 to 0.47 mm,  $P<0.001$ ), but there was no significant difference between roots filled with AH Plus and those filled with Apexit Plus (average difference=0.097 mm, 95% CI -0.08 to 0.27 mm,  $P=0.3$ ) (Table 2).

Table 3 shows the mean values and standard deviations for the apical dye leakage of sealers after the crown-down technique. In experimental groups prepared with the crown-down technique and filled with Epiphany SE Resilon, the mean value of dye leakage was lower (0.015 mm) than in the roots filled with AH Plus in combination with Thermafil obturation (0.07 mm) and those filled with Apexit Plus in combination with Thermafil obturation (0.15 mm).

Table 4 shows the differences in average dye leakage after the crown-down preparation technique. This difference was greater in the groups filled with Apexit Plus in combination



**Table 2.** Differences in the average dye leakage according to sealers.

Tested group	Degree	SD	Comparative group	Degree	SD	Difference	95% CI	P
SB-Ap+	0.38	0.31	SB-AH+	0.29	0.23	0.097	-0.08 to 0.27	0.3
			SB-E-se-R	0.06	0.08	0.325	0.18 to 0.47	<0.001

CI – confidence interval; SD – standard deviation; SB-Ap+ – step-back preparation and Apexit plus; SB-AH+ – step-back preparation and AH plus; SB-E-se-R – step-back preparation and Resilon/Epiphany.

**Table 3.** Apical dye leakage according to sealers after the crown-down preparation technique.

Tested group	Degree	SD	SE	CI of mean	Max	Min	Median
CD-Ap+	0.17	0.13	0.03	0.06	0.55	0	0.15
CD-AH+	0.07	0.04	0.01	0.02	0.14	0	0.07
CD-E-se-R	0.03	0.03	0.01	0.01	0.08	0	0.015

SD – standard deviation; SE – standard error; CI – confidence interval; CD-Ap+ – crown-down preparation and Apexit plus; CD-AH+ – crown-down preparation and AH plus; CD-E-se-R – crown-down preparation and Resilon/Epiphany.

**Table 4.** Differences in the average dye leakage based on sealers after the crown-down preparation technique.

Tested group	Degree	SD	Comparative group	Degree	SD	Difference	95% CI	P
CD-Ap+	0.17	0.13	CD-AH+	0.07	0.04	0.101	0.04 to 0.16	0.002
			CD-E-se-R	0.03	0.03	0.144	0.08 to 0.21	<0.001

CI – confidence interval; SD – standard deviation; CD-Ap+ – crown-down preparation and Apexit plus; CD-AH+ – crown-down preparation and AH plus; CD-E-se-R – crown-down preparation and Resilon/Epiphany.

with Thermafil obturation than in those filled with Epiphany SE Resilon (average difference=0.144 mm, 95% CI 0.08 to 0.21 mm,  $P<0.001$ ), and was also greater in the groups filled with Apexit Plus compared with those filled with AH Plus (average difference=0.101 mm, 95% CI 0.04 to 0.16 mm,  $P=0.002$ ). The difference was smallest in the groups obturated with AH Plus and Epiphany SE Resilon (average difference=0.02 to 0.07,  $P<0.001$ ).

Among all the sealers and root canal preparation techniques, the mean dye leakage values were significantly higher in groups prepared with the step-back technique and obturated with Apexit Plus sealer in combination with lateral gutta-percha compaction compared with other tested materials that were used for experimental groups prepared with the crown-down technique ( $P<0.001$ ) (Figure 7).

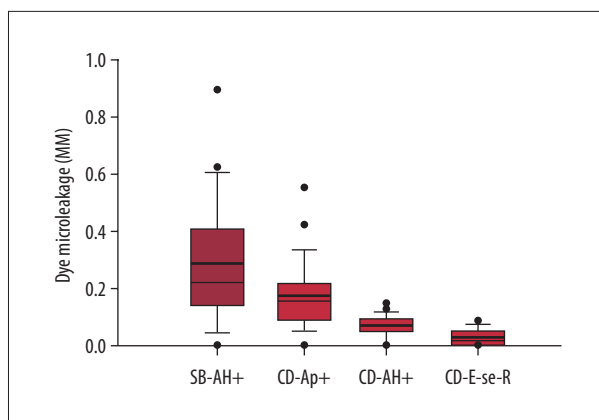
The experimental group prepared with the step-back technique and obturated with AH Plus in combination with lateral gutta-percha compaction exhibited significantly higher mean

values for dye penetration than the group with roots filled with Epiphany SE Resilon sealer after preparation with the crown-down technique ( $P<0.001$ ), but there was no significant difference in the mean values for dye leakage with Apexit Plus sealer (crown-down technique) ( $p=0.06$ ) (Figure 8).

The mean dye leakage values were lower in the groups prepared with the step-back technique and filled with Epiphany SE Resilon than in the groups filled with the same sealer after the crown-down technique ( $P<0.001$ ).

## Discussion

Microleakage in the root canal system is one of the causes of endodontic therapy failure. Hermetic seal of the root canal system prevents reinfection and provides favorable biological conditions for healing [23]. Therefore, a fluid-tight apical seal is important for the success of any endodontically treated teeth [24]. Dye leakage studies are mostly used to evaluate



**Figure 7.** Comparison of apical dye leakage of AH Plus after the step-back preparation technique with other groups.

sealing ability of root canals sealers because they are simple and do not require complicated conditions and materials [25].

The efficacy of the dye leakage method has been validated [26], so we chose this method to measure the apical microleakage of root canals. We used methylene blue as the dye in our study because its molecular size is similar to that of bacterial byproducts (e.g., butyric acid) that can leak from infected root canals and irritate the periapical tissue [27].

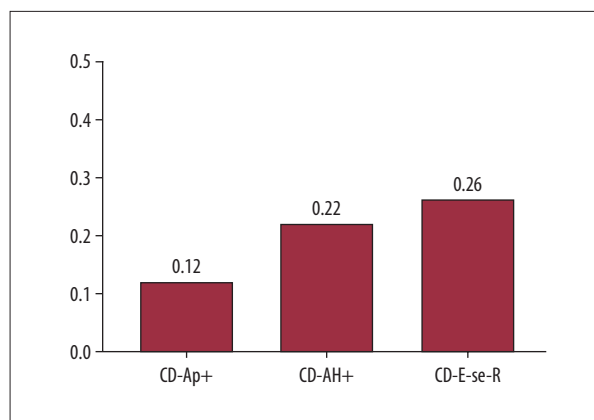
Gutta-percha lateral condensation is a commonly technique, but studies have reported that it does not ensure a fluid-tight seal [28]. Gutta-percha also exhibits deficient rigidity and adhesiveness [23].

The Thermafil technique uses a central carrier coated with alpha-phase gutta-percha. Despite the short time required to perform the obturation and the ability of the alpha phase to replicate canal irregularities, Gutman et al. [29] found that Thermafil can be stripped from the carrier after cooling, resulting in microleakage.

New thermoplastic polymer fillers, such as the Resilon/Epiphany system have been developed to provide a monoblock effect, which ensures radiopacity, tissue compatibility, and minimal shrinkage [30]; however, microleakage may still occur.

Skidmore et al. [31] reported that the compressive strength of teeth that were endodontically treated and filled with Resilon possessed significantly higher compressive strength than those obturated with gutta-percha.

Our dye leakage results confirmed that the Resilon/Epiphany system appears to be superior to the Thermafil system paired with AH Plus. This finding is consistent with reports from Punia et al. [23] and Bhandi et al. [32], who noted the higher sealing ability of the Resilon/Epiphany system compared with Thermafil



**Figure 8.** Comparison of apical dye leakage of sealers after the crown-down preparation technique.

in their dye penetration studies. This finding may be explained by the higher thermoplasticity of Resilon compared with conventional and thermoplastic gutta-percha [33,34].

Our study indicated that the Resilon/Epiphany system showed more effective sealing ability than lateral gutta-percha compaction with AH Plus or Apexit Plus sealer, similar to the findings of Pawar et al. [35] and Sultana et al. [36]. These results may be explained by the gutta-percha filling leaking rapidly and not providing a dependable long-term seal [37,38]. Additionally, Koçak et al. [39] found that gutta-percha did not chemically bond to AH Plus sealer, confirming the findings of Bodrumlu et al. [40], who reported that no chemical bond occurred between the gutta-percha and AH Plus sealer, although the sealer was chemically bonded to the dentin walls. Additionally, it has been reported that the silicone oils in AH Plus can affect the sealing ability of this material [41].

The comparison of the AH Plus and Apexit Plus sealers in our study showed less dye leakage in specimens filled with AH Plus, but the difference was not significant. Similar to our results, Ballullaya et al. [3] found no statistical significance between Apexit Plus and AH Plus sealers in their dye leakage study. A study of McMichen et al. [42] demonstrated that the solubility values for Apexit Plus were 200 times greater than those for AH Plus. AH Plus sealer as an epoxy-resin-based sealer has shown good adhesiveness to dentin walls and it can penetrate up to 80  $\mu\text{m}$  into the dentin tubules [43]. In contrast, Salz et al. [44] reported that the sealing ability of Apexit Plus was more effective than that of AH Plus.

With regard to sealers, the highest value of dye leakage (1.14 mm) in our study was in the group prepared with the step-back technique and filled with Apexit Plus. This outcome may be explained by poor adaptation to canal walls because after this calcium hydroxide paste is in contact with moisture, its setting time of may decrease [45]. The confidence interval for

Apexit Plus in our study was 1.14 to 0.12 mm (Table 1). The confidence intervals were used to have standardized statistical methodologies and provide more accurate results [46].

In relation to the smear layer, preparation techniques, and obturation techniques, Fraunhofer et al. [17] reported that microleakage was reduced after smear layer removal and vertical gutta-percha condensation. They also found that microleakage was lower in root canals prepared with rotary instruments than in those prepared with manual instruments. These results are consistent with ours in which the crown-down technique with the ProTaper system resulted in less dye leakage than the step-back technique. In addition, Koçak et al. [47] reported that root canal preparation with endomotor-driven NiTi files produced less dye leakage in root canals than stainless steel manual files. According to the same authors, NiTi instruments produced better cleaning than stainless steel instruments, especially in the apical region, and this better shaping produced better adaptation, which consequently resulted in less leakage.

As for the sealing ability of the various gutta-percha techniques, results are contradictory. Sinhal et al. [48] reported that a thermoplasticized obturating technique showed less dye penetration leakage than cold lateral condensation. In another study of obturation techniques, Lone et al. [49] reported that cold lateral condensation showed better sealing than thermoplasticized gutta-percha. These results were explained by the better length control of the obturation material as indicated by the “tug back” felt when the gutta-percha point was inserted to the established working length [50]. However, according to Olczak et al. [51] the lateral compaction of cold of

gutta-percha can provide approximately the same results as can be achieved by using thermoplasticized methods [51].

Although *in vitro* leakage studies may not replicate clinical conditions, they can provide a simple means of evaluating and comparing endodontic materials and preparation and obturation techniques. Further studies are needed to determine the exact materials and the best preparation and obturation techniques to achieve optimal endodontic treatment.

## Conclusions

The results of this study showed dye penetration leakage was associated with both preparation techniques and all obturation techniques; however, the crown-down technique had less microleakage than the step-back technique. In addition, Epiphany sealer in combination with Resilon showed less leakage compared with AH Plus and Apexit Plus sealer in combination with lateral gutta-percha condensation or Thermafil obturation.

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## Conflict of interest

None.

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