# Assessment of Self Disinfecting Capacity and Dimensional Stability of Irreversible Hydrocolloid Impression Material Mixed With Different Concentrations of Chlorhexidine Solution-An Invitro Study

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## Abstract

**Introduction:** Impression materials are used to record the anatomical topography of oral tissues. Irreversible hydrocolloid is an impression material routinely used in dentistry. However, it retains bacteria, 2 to 3 times higher than other impression material. Spraying and immersion technique has been employed for disinfection of irreversible hydrocolloid with varying degree of success. Thus the need of impression material that has self-disinfecting capacity seems to be very important to have infection control in dentistry.

**Materials and Methods**: This invitro study was done in two parts. Assessment of self-disinfecting capacity of irreversible hydrocolloid impression material mixed with different concentrations of chlorhexidine solution and dimensional stability of irreversible hydrocolloid impression material mixed with different concentrations of chlorhexidine solution.

For assessment of self-disinfecting capacity, irreversible hydrocolloid was mixed with various concentrations of chlorhexidine (0.05%, 0.1%, 0.2%) and distilled water. Total 64 culture plates were prepared, n=16 for each group of microorganisms (E. coli, S. aureus, Pseudomonas, Klebsiella). Inhibition zones were measured for each microorganism.

For dimensional stability, the specimens were divided into four groups similar to specimen for self-disinfecting ability. Total 56 samples were prepared, n=14 for each group. Interpretation (IP), Mesiodistal (MD), Buccolingual (BL), Occlusogingival (OG) dimensions were measured by digital Vernier caliper.

One-way analysis of variance was done for assessment of self-disinfecting capacity and for dimensional stability. Dunnett comparison test was performed to test the significance between test and control. P value was calculated under the predetermined level of significance (0.05).

**Results:** Zones of inhibition were observed around test specimens (groups 0.05, 0.1, 0.2), but not around control specimen (Group d/w). There was a significant difference in the mean diameters of the inhibition zones between test groups and control which was statistically highly significant ($p < 0.001$). In the test for dimensional stability, no significant differences were detected among groups for all the measured dimensions ($p > 0.05$).

**Conclusion:** Irreversible hydrocolloid impression material mixed with chlorhexidine exhibits varying degrees of self-disinfecting activity without influencing the dimensional stability of set material.

**Key words:** Irreversible hydrocolloid. Chlorhexidine, Self-disinfecting capacity, Dimensional stability.

*Conflict of Interest: No*

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## Introduction

Dental impressions are an integral part of patient management from diagnosis to treatment. Irreversible hydrocolloid is an impression material routinely used in dentistry

for some of its advantages like easy handling, low cost, capability of reproducing details and high comfort for the patient. During the process of impression making these impression materials readily comes in contact with saliva and blood which may harbor different types of pathogenic organisms. The risk of infections to dental practitioners, patients, and laboratory personnel transmitted by saliva and blood is considered a potential occupational hazard in dentistry.[1, 2] It has been reported that washing the impression materials with water alone removes only 40% of bacteria.[3]

In general practice, impressions are either sprayed or immersed in suitable disinfectant for a particular length of time for disinfection. Aqueous solutions of alcohols, aldehydes, chlorine compounds, phenolic, biguanides, iodine compounds, and quaternary ammonium compounds are used as disinfectant.[4] Immersion of the impression in the disinfectant solution has been considered superior to spraying disinfectant over the impression. Immersion is more effective in reaching all parts of the impression, including undercuts, which may harbor a significant number of microorganisms. However, immersion also leads to more distortion of the impression compared with spraying. The difficulties associated with disinfecting irreversible hydrocolloid material have resulted in the development of self-disinfecting irreversible hydrocolloid impression materials. Materials such as didecyldimethyl ammonium chloride, chlorhexidine(CHX), quaternary ammonium compounds, magnesium oxide, fluoride etc., have been used as disinfectant additives.[5,6]

However, disinfectant is impregnated into the powder of impression material for most of all self-disinfecting irreversible hydrocolloid and few attempts have been made to add disinfectants into mixing liquid. Hence in this study various concentrations of chlorohexidine gluconate have been used to mix irreversible hydrocolloid impression material powder. The objective of this study was to evaluate the self-disinfecting effect and dimensional stability of irreversible hydrocolloid impression material by adding different concentrations of Chlorhexidine.

## Materials and Methods

This cross sectional comparative study was conducted at Department of Dental Surgery, Prosthodontic Unit and Department of Microbiology, National Academy of Medical Sciences, Bir Hospital after ethical clearance from Institutional Review Board (IRB) of National Academy of Medical Sciences. Convenience sampling technique was used to calculate the sample size. The various concentrations of chlorhexidine were prepared by diluting 2% of Chlorhexidine with distilled water.

This invitro study was done in two parts.

1. Assessment of self-disinfecting capacity of irreversible hydrocolloid impression material mixed with different concentrations of chlorhexidine solution
2. Dimensional stability of irreversible hydrocolloid impression material mixed with different concentrations of chlorhexidine solution

### Assessment of self-disinfecting capacity

For assessment of self-disinfecting capacity of irreversible hydrocolloid impression material mixed with different concentrations of chlorhexidine solution

Disc Diffusion technique was used to assess the antibacterial activity of the specimens.[17] Strains of the following microorganisms were used: Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and klebsiella as per the requirement of the study.

The specimens were divided into four groups in each test: specimens mixed with (0.05%, 0.1%

and 0.2%) of chlorhexidine solution served as test groups and distilled water as control group. The irreversible hydrocolloid impression material was mixed according to the powder/ liquid ratio (10 g/23 mL) recommended by the manufacturer.[7] Immediately after mixing, the mixture was placed between two glass slab and kept under slight pressure (1 kg) for 1 minute. Then impression disks, 6 mm in diameter by 2 mm in thickness were prepared with the help of sterile needle caps till marking of 2mm in the needle caps.[8] Nutrient agar was selected as media in this study. The areas for the disk placement were marked in the base of culture plates as per the concentration of Chlorhexidine. The impressions disk was then placed on their respective mark. Each organism (n=16) were inoculated in separate nutrient agar under sterile conditions. On each culture plate, four discs of irreversible hydrocolloid were placed containing distilled water as control and different CHX concentrations (0.05%,0.1% and 0.2%) as test groups. Finally, all the culture plates were incubated in the appropriate aerobic environment for 24 at 37-degree C. After incubation, clear zones or inhibitory areas were observed in the culture plates around the specimens (Figure 1-4) and measured with plastic scale to evaluate the antibacterial effect.

**Measurement of Dimensional Accuracy**

Dimensional Accuracy was evaluated by using master die of known dimensions. According to American National Standards Institute (ANSI)/American Dental Association (ADA) specification brass master model was machined that consisted of two base simulating a three-unit fixed denture.[5] The Dimensions of occluso-gingival length of abutments of master model was 8 mm, width was 6.25 mm and the base was 2 mm in height. Cross grooves; Buccolingual (BL), Mesiodistal (MD), Interpretation Point (IP) and Occluso gingival (OG) lines were inscribed on master model. The distance between two dies was 21.5 mm. Perforated brass trays were fabricated maintaining a space of 7 mm and holes of 2 mm in diameter for mechanical retention of the impression material. (Figure 5). Impressions of the model were taken using perforated brass tray (Figure 6).

Type IV gypsum product (Die Stone) was used to pour the impression material mixed with various concentration of chlorhexidine and the test specimens were made as per the requirement of study. The die stone was hand mixed to wet the powder then mechanically spatulated with an automatic vacuum mixing and stirring instrument for 15 sec. Water/powder ratio of 22 mL water to 100 g powder was used for each mix.

The specimens were divided into five groups in each test as follows:

1) Specimens made from irreversible hydrocolloid impression mixed with 0.05 % chlorhexidine solution (Group 0.05)
2) Specimens made from irreversible hydrocolloid impression mixed with 0.1 % chlorhexidine solution (Group 0.1)
3) Specimens made from irreversible hydrocolloid impression mixed with 0.2 % chlorhexidine solution (Group 0.2)
4) Specimens made from irreversible hydrocolloid impression material mixed with distilled water (Group D/W)

The stone casts were allowed to set for 2 hours before separation and were dried at room temperature for at least 24 hours before being measured. Finally, measurements of four dimensions were recorded for the recovered stone casts to indirectly assess the three-dimensional accuracy. The dimensions measured included

- Interpretation (IP)
- Mesiodistal (MD)
- Buccolingual (BL)
- Occlusogingival (OG)

Three test groups and one control group were tested with 14 replications of each group in total of 56 samples for each dimension (n =14). Measurements of the metal master die and stone casts were recorded using a digital Vernier caliper placing the Vernier Caliper parallel with horizontal (Figure 7-8).

## Results

Well-defined zones of inhibition were apparent after incubation period, and consistent measuring of inhibitory fields was done. Mean diameters of inhibited zones for each microorganism are presented in Table 1.The results demonstrated zones of inhibition around the test specimens on all plates. No zones of inhibited growth were observed around the control wells on agar plates. Inhibition zone was evident only at concentration 0.2% in Pseudomonas Species. Test groups showed larger inhibition zone in S.Aureus than other microorganisms. One-way ANOVA and Dunnett comparison test revealed that the inhibition zones tested became significantly larger ($P$<0.001) for each microorganism when the concentrations of chlorhexidine solution were raised from 0.05% to 0.2%.

The means and standard deviations of the dimensional changes measured at four different dimensions are presented in Table 2. No significant differences ($P$ >0.05) were identified between groups for all dimensions (IP,MD, OG, and BL). Control specimens showed greater dimensional accuracy in comparison with test groups. However, there was no significant difference in dimensional accuracy between impression materials mixed with different concentrations of disinfectant solution among groups in relation to each other. The discrepancies between the master die and stone casts in the OG dimensions were positive for each group, which indicated that the stone casts were larger in these dimensions than the metal master die. However, the discrepancies in the IP, BL, MD dimensions were negative, which indicated that the stone casts were smaller in these dimensions.

**Table 1:** Mean diameter of inhibition zone (mm) and standard deviation for bacterial species among test groups (0.05, 0.1, 0.2) and control group (D/W)

| Species | 0.05 | 0.1 | 0.2 | D/W |
|---|---|---|---|---|
| S. aureus | 14.4±0.646 | 15.6±0.756 | 16.8±0.802 | 0 |
| E. coli | 9.21±1.67 | 11.5±0.519 | 12.6±0.49 | 0 |
| Klebsiella | 11.4±0.745 | 12.5±0.519 | 13.9±0.616 | 0 |
| Pseudomonas | 0 | 0 | 9±0.877 | 0 |

**Table 2:** Mean ± Standard deviation and percent of dimensional change between master die and stone casts (mm) for each group).

| Group | MD<br>Mean±sd | BL<br>Mean±sd | OG<br>Mean±sd | IP<br>Mean± sd |
|---|---|---|---|---|
| **Distilled water** | 6.25±0.0112 | 6.25±0.0399 | 5.05±0.11 | 21.2±0.0117 |
| 0.05 | 6.25±0.0173 | 6.25±0.0181 | 4.97±0.113 | 21.3±0.0252 |
| 0.1 | 6.25±0.0365 | 6.25±0.0242 | 5.01±0.0645 | 21.2±0.0391 |
| 0.2 | 6.25±0.0177 | 6.25±0.0324 | 5.03±0.0091 | 21.2±0.0638 |

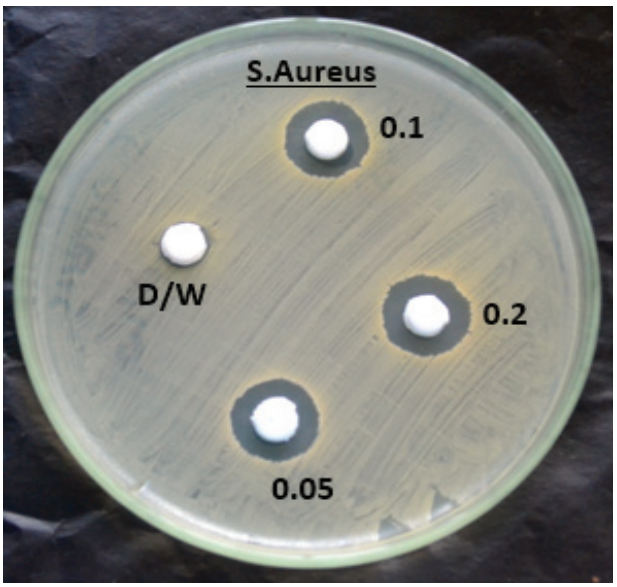


*Figure 1: Inhibition Zone of S. aureus with different concentrations of CHX*

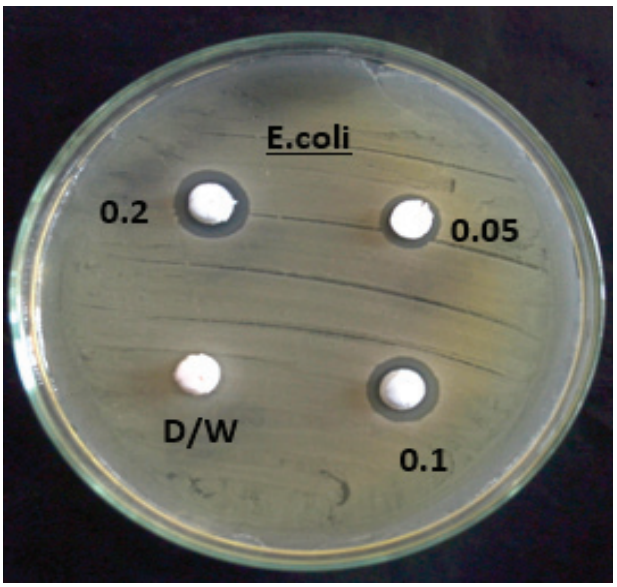


*Figure 2: Inhibition Zone of E. coli with different concentrations of CHX*

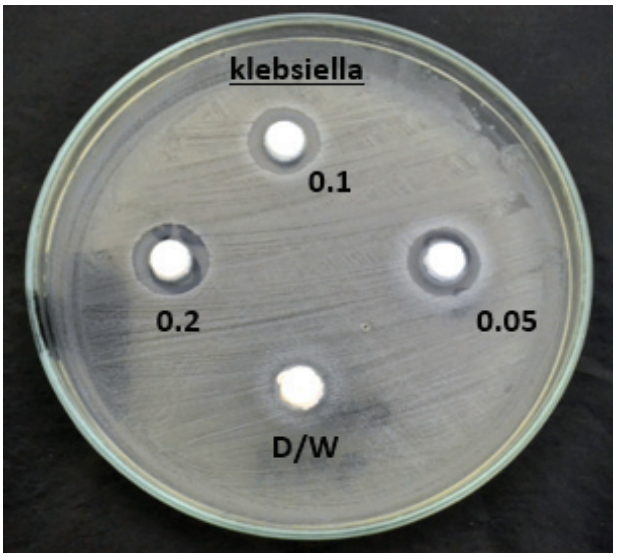


*Figure 3: Inhibition Zone of Klebsiella with different concentrations of CHX*

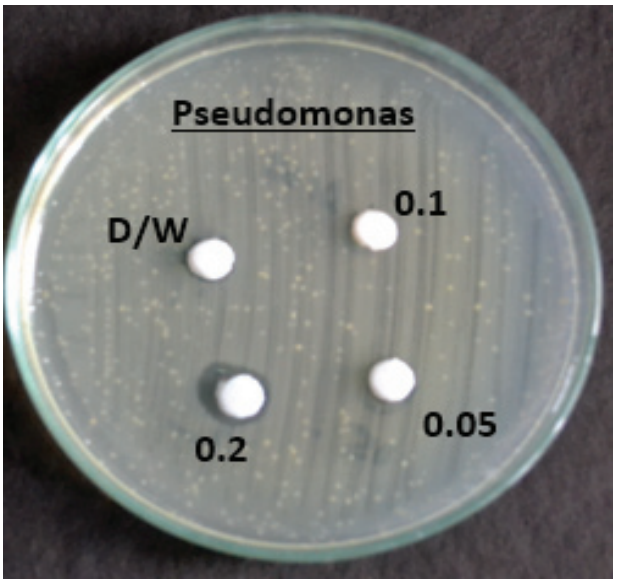


*Figure 4: Inhibition Zone of Pseudomonas with different concentrations of CHX*

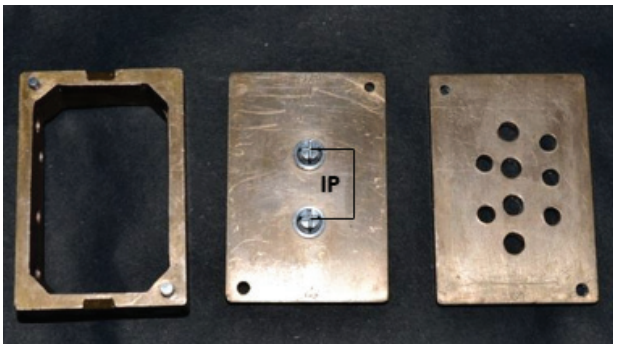


*Figure 5: Master model*

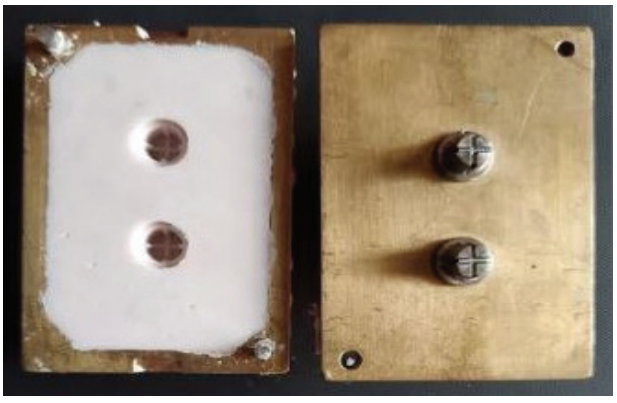

*Figure 6: Impression of Master model taken with irreversible hydrocolloid*


*Figure 7: Measurement of interpretation dimension (IP) with digital Vernier caliper*


*Figure 8: Measurement of mesio distal (MD) dimension with digital Vernier caliper*

## Discussion

During the dental practice, dentists and dental staff are exposed to a large number of microorganisms which are potentially harmful. The fundamental routes of spreading harmful microorganisms in a dental procedures are: blood-borne, saliva-droplet, direct contact with a patient and with infected equipment, and water-droplet infections.[9] Disinfection of impression material is very important to prevent cross infection in dentistry among dental clinics and dental labs. According to Miller's study, a saliva droplet contains more than 50,000 bacteria. Unfortunately, these pathogens can be easily spread through impressions sent to the laboratories. Similarly, other studies also indicate that the surface of impressions taken out of the mouth is polluted with bacteria. It has also been demonstrated by studies that microorganisms on and/or in impression materials can be transferred to stone casts and remain viable (Huizing et al., 1994; Schutt, 1989; Leung and Schonfeld, 1983).[10]

In this study, alginate, an irreversible hydrocolloid was taken as impression material as its use is most popular in dental clinics because of its low cost and easy use. However, it transmits more bacteria than silicon and elastomeric impression. Alginate impressions are hydrophilic material that take up water thus lose their dimensional accuracy in an antiseptic solution while disinfecting it by immersion technique.[11]

Several methods of disinfection for alginate impression materials were proposed. Spray and immersion methods are the two most widely used techniques in clinical practice. It is recommended that a disinfectant spray be used while the impression is placed in a plastic bag for 10 minutes.[12,13] These conventional strategies present several disadvantages like loss of surface detail and dimensional inaccuracy of the impression (Wood PR, 1992). Moreover, immersing or spraying rinsed impressions provide only surface disinfection while the self-disinfecting impression would cause disinfection throughout the material and not just on the surface. Because this area of research is of great importance, hence self-disinfecting of irreversible hydrocolloid is sought for in this study. Studies have shown that self-disinfecting impression technique demonstrated better dimensional stability than spray and immersion techniques, and saved disinfection time (Touyz LZ and Rosen M, 1991; Rosen M and Touyz LZ, 1991; Poulos JG and Anton off LR, 1997).

As impressions and occlusal records cannot be sterilized by heat, chemical disinfection

is still the common practical method to eradicate microorganisms. Chlorhexidine, a cationic bisbiguanide [1,6-DI (4-chlorophenyl-diguanido) hexane] agent with a broad antibacterial spectrum (Gram negative and Gram positive), some anti-viral and antifungal activities, with low mammalian toxicity was first described in1954. Chlorhexidine is also a broad-spectrum disinfectant, i.e., an effective antiplaque and anti-gingivitis agent.[14] Chlorhexidine has been studied in several mouth rinse formulations and is proved to be safe and effective. Most commonly used concentrations of chlorhexidine solutions as mouth rinse are 0.12 and 0.2%; these concentrations are proved to be effective against most of the microbes. Thus, maximum concentration of Chlorhexidine used in our study was 0.2%.In this study, the inhibitory effect of CHX on cell growth was enhanced by increasing CHX concentration to 0.2% which was in accordance with studies of Wang(2007)[5], Benakatti (2017).[15]

In order to be indicated as a substitute for water, the solution used for the mix must be biocompatible and must not change either the physical or the mechanical properties of the irreversible hydrocolloid.[16] Thus, 0.2% aqueous chlorhexidine digluconate solution was selected in this research because it fulfills those requirements. Besides, it is the same antimicrobial agent present in the antimicrobial irreversible hydrocolloid (Greengel).[10] It also provides scope of future studies to assess which form of the disinfectant is the best, powder or liquid.

Agar diffusion test (Kirby Baeur test) was used in this study for the assessment of antimicrobial effect of irreversible hydrocolloid as described by Wallhauser and Fischer (1970).[17] Agar diffusion test used in this study was also in accordance with the antimicrobial test used by Casmero et al(2007),[18] Alwahab (2012). Disinfection method used in this study was shown to be effective against S. aureus, which was in accordance with a study conducted by Casemiro et al[18]and Benakatti et al.[15]

The least antibacterial activity of chlorhexidine digluconate was observed against Pseudomonas aeruginosa. This might be due to the fact that chlorhexidine is moderately active against Pseudomonas aeruginosa, which is considered to have resistance to antibacterial agents.[20] This result coincides with the results obtained by Casemiro et al and Wang et al.The effect of chlorhexidine on Pseudomonas was seen at 0.1% in the study done by Wang et al (2007) but in our study 0.2% concentration of Chlorhexidine only inhibited the growth of Pseudomonas. This difference in the observation may be attributed to the differences in the methodology employed in both studies. In the present study, the test organism pseudomonas was isolated from patient's saliva sample but Wang et al had used standardized ATCC (American Type Culture Collection Pseudomonas aeruginosa ATCC 25314 for his study.

Acceptable methods for measuring dimensional accuracy of casts include measuring microscopes, micrometers, dial gauges, and calipers.[19] Digital Vernier caliper was used in the study with good reproducibility between repeat readings of each measurement.

In the assessment of dimensional stability of the test specimens, the $p > .05$ value was obtained for the measured dimensions interpretation (IP), mesiodistal (MD), Buccolingual (BL), Occlusogingival (OG) which was statistically insignificant. This result was in agreement with results of the study conducted by Wang et al (2007), Ramer et al (1993) who evaluated the accuracy of alginate impressions made with water to which iodine or chlorhexidine was added. the cast obtained in our study were smaller in the MD, BL and IP dimensions than the metal die and larger in the OG dimensions which was similar to the study by wang et al

(2007). Although there is no evident reason, it is speculated that it may be related to the tray design.[5]

Some limitations of this study were **:**

1. It is an in-vitro study which is different from clinical and in-vivo studies.
2. Self-disinfecting ability and dimensional stability of irreversible hydrocolloid were only considered in our study while other factors flowability, setting time, surface details were not considered.
3. Standard strains (ATCC) was not available for the study.
4. We were not able to include variety of microorganisms e.g. opportunistic microorganisms like Candida and viruses eg. Hbs Ag, HIV due to inability of microorganisms in our research set-up .

Therefore further studies, with larger sample size are needed.

## Conclusions

1. Irreversible hydrocolloid mixed with different concentrations (0.05%,0.1%,0.2%) of chlorhexidine was able to exhibit self-disinfecting capacity of varying degrees. This disinfection method was found to be effective in the elimination of S. aureus, E. coli, Klebsiella and Pseudomonas. Based on the findings of this study, 0.2% is recommended concentration for chlorhexidine solution to produce the self-disinfecting impression material.
2. The dimensional change between master die and stone casts for test groups and control group was insignificant that suggests chlorhexidine can be used as mixing liquid for irreversible hydrocolloid, without influencing the dimensional stability of set material.

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