

Chlorhexidine-assisted cleaning increases staining of PETG clear aligners exposed to common beverages: an in vitro study

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Abstract

Background

Clear aligner therapy is increasingly popular due to its esthetic appearance, comfort, and removability. Successful outcomes rely on patient compliance, requiring 20–22 hours of daily wear. Exposure to dietary chromogens and inadequate cleaning can cause discoloration, compromising appliance transparency and hygiene. Since commercial cleaning systems are often inaccessible or costly in some settings, clinicians may suggest affordable alternatives, such as brushing or using common antibacterial agents like chlorhexidine (CHX) mouthwash. This study aimed to evaluate the esthetic impact of CHX-assisted cleaning by investigating the color stability of two polyethylene terephthalate glycol (PETG) aligner materials (Taglus Premium and Taglus Standard) during exposure to common staining beverages.

Methods

In this in vitro experimental study, 240 thermoformed specimens (120 per material) were fabricated. Samples were assigned to four environments (tea, coffee, cola, and dry control) and two daily cleaning protocols: brushing under running water or brushing following a one-minute immersion in 0.2% CHX mouthwash. Color measurements were recorded using a spectrophotometer at baseline (T0), after 7 hours of staining without cleaning (T1), and after 14 days of staining combined with daily cleaning (T2).

Results

No baseline color differences were observed. At T1, Taglus Premium exhibited significantly greater discoloration than Taglus Standard in tea and control groups ($p < 0.05$), with tea producing the highest ΔE . At T2, both materials exhibited marked cumulative discoloration. Staining solution, cleaning protocol, and their interaction significantly influenced ΔE ($p < 0.01$). Tea induced the greatest color change across both materials, while coffee and cola produced smaller effects. CHX-assisted cleaning resulted in significantly higher discoloration than brushing alone. After 14 days, the highest ΔE values occurred in the tea + CHX groups.

Conclusions

Color stability of PETG aligners was significantly affected by staining environment, cleaning protocol, exposure duration, and material type. Tea was identified as the most potent chromogenic agent, and the use of 0.2% CHX mouthwash markedly exacerbated staining. Clinicians should advise patients to remove aligners during consumption of chromogenic beverages and exercise caution in recommending

CHX as a routine cleaning method. Further in vivo research is warranted to confirm these findings and provide evidence-based hygiene recommendations.

Trial registration

Clinical trial number: Not applicable.

Background

Clear aligner therapy has emerged as one of the most rapidly expanding treatment modalities in contemporary orthodontics, primarily due to its esthetic appeal, comfort, and removability, which facilitate improved oral hygiene compared to fixed appliances (1). Following Kesling's foundational concept of the tooth-positioning appliance (2), advances in material science and digital technologies have enabled aligners to effectively manage a range of malocclusions. Several clinical studies and systematic reviews report that clear aligners can achieve outcomes comparable to conventional fixed appliances for many types of orthodontic movement (3, 4, 5, 6).

Despite these advantages, clinical success of aligner therapy is heavily dependent on patient compliance, particularly adherence to recommended wear times of 20–22 hours per day (7, 8). Given the esthetic nature of the treatment and its role in patient motivation for choosing this treatment modality (9, 10), esthetic properties of aligner therapy are central to patient acceptance. Even though patients are instructed to remove the aligners before consuming almost any type of food and beverages, the current evidence shows that some patients do not comply with this instruction (11). However, exposure to dietary chromogens such as tea and coffee, as well as inadequate cleaning protocols, can cause discoloration that compromises transparency and reduces patient satisfaction (12, 13, 14). Color changes beyond clinically perceptible thresholds compromise appliance esthetics and may adversely influence patient satisfaction and compliance, with potential implications for treatment outcomes (15).

The color stability of aligner materials is influenced by factors such as their composition, forming method, surface roughness, and exposure to dietary substances (16, 17). Studies investigating aligner discoloration identify high-consumption beverages such as tea as producing a great staining effect among commonly consumed beverages (12, 14). Given the high consumption of chromogenic foods in many populations, understanding aligner behavior in such environments is clinically important.

Maintaining oral hygiene during aligner therapy is essential, as biofilm accumulation on appliance surfaces can predispose patients to gingival inflammation and halitosis (15, 18). Various mechanical and chemical cleaning methods have been proposed, including brushing, effervescent cleansing tablets, and disinfectant solutions (11, 19, 20, 21). However, specialized aligner-cleaning products are not always readily available in all regions, or carry a high financial burden in low-income countries, leaving the patients to rely on more accessible alternatives.

Chlorhexidine (CHX) mouthwash is widely used in orthodontics as an antimicrobial agent (22, 23, 24); however, it is also known to cause staining of teeth and restorative materials (24, 25). These staining mechanisms—primarily related to the cationic properties of CHX and its interaction with dietary chromogens—raise concerns regarding discoloration when CHX is used as an adjunctive cleaning method for clear aligners. Although aligner discoloration and color stability have been investigated in previous studies (12, 17, 26), and CHX exposure has been evaluated for its mechanical or chemical effects on clear aligners (27), evidence remains limited regarding the combined effect of CHX-assisted cleaning and simultaneous exposure to common staining beverages on contemporary polyethylene terephthalate glycol (PETG)-based aligner materials. In particular, Taglus Premium and Taglus Standard are widely used in clinical practice, yet their color stability under conditions reflective of typical patient behavior has not been adequately evaluated.

Therefore, this in vitro study aimed to evaluate the effect of common staining beverages (tea, coffee, and cola) and chlorhexidine-assisted cleaning on the color stability of two PETG-based clear aligner materials (Taglus Premium and Taglus Standard).

Methods

Study Design

This in vitro experimental study compares the effects of three commonly consumed staining agents (tea, coffee, and cola) and two cleaning protocols (brushing under running water and after submergence in 0.2% CHX mouthwash) on the color stability of two PETG-based clear aligner materials (Taglus Premium and Taglus Standard; Taglus®, India). The primary outcome measure, color change (ΔE), was assessed at two time points: 7 hours (T1) and 14 days (T2).

Specimen Preparation

A dental arch model consisting of 15 A2 colored acrylic maxillary first premolars was fabricated and digitized using CAD/CAM technology. A 3-D printed reference model was fabricated using digital light processing and used to thermoform uniform specimens from 0.8-mm PETG sheets (Taglus®, India) for both Taglus Premium and Taglus Standard materials. A total of 120 specimens per material were fabricated in eight arches and allocated to experimental groups for color stability assessment.

Staining and Cleaning Protocol

The staining solutions consisted of tea (Twinings, London, UK), coffee (Nescafé, Nestlé), and cola (Coca-Cola®, Iran), with a dry condition serving as the control environment. Tea was prepared by steeping two tea bags in 400 mL of water at 90°C for four minutes. The coffee solution was prepared by dissolving 4.8 g of instant coffee in 400 mL of water at 90°C. All staining solutions were maintained at 37°C in an incubator to simulate intraoral temperature. Cleaning procedures included either brushing the specimens

for two minutes using a soft-bristled toothbrush (Oral-B, Boston, USA) under running water or brushing for two minutes following a one-minute immersion in 0.2% CHX gluconate mouthwash (Cobalt, Iran).

Study Phases

The study was conducted in two phases. In the first phase, specimens were immersed for 7 hours in their assigned staining solutions without cleaning, then rinsed with running water and air-dried prior to color measurement (T1). Before immersion, arches were numbered, and the arch selected for T1 analysis in each staining environment was determined using a pre-defined sequence to minimize operator selection bias. As cleaning protocols had not yet been initiated at this stage, one arch per material per staining environment (n = 15 specimens) was analyzed at T1.

The 7-hour staining period at T1 was designed to simulate cumulative intraoral exposure during a 14-day aligner wear cycle. Studies quantifying daily mastication time report averages of 35 min/day across human (28). Assuming 20–30 minutes per day of total food and beverage contact during aligner wear, this corresponds to approximately 4.7–7 hours cumulative over a 14-days wear cycle. Accordingly, a 7-hour immersion period at T1 was selected to represent a worst-case cumulative exposure within a single aligner lifespan.

In the second phase, specimens underwent 14 consecutive days of staining exposure for 22 hours per day, combined with their designated daily cleaning protocol. Specimens were then air-dried and kept out of the staining solution for 2 hours per day to complete a 24-hour cycle, after which they were returned to fresh staining solutions. Color measurements were recorded again at the end of this period (T2).

Color analysis was performed using a portable spectrophotometer (DS-700C-2, CHNSpec, China) at baseline (T0), T1, and T2. The A2 acrylic arch served as a standardized background for all color measurements. The arch model consisted of maxillary first premolars, allowing the buccal height of contour to be used as a reproducible reference point for color measurement. Measurements were performed using the Commission Internationale de l'Éclairage (CIE) L*a*b* color space. The system consists of three orthogonal axes: L*, a*, and b*. In this system, L* represents lightness (ranging from 0 = black to 100 = white), a* indicates the red–green axis (positive values for red; negative values for green), and b* represents the yellow–blue axis (positive values for yellow; negative values for blue). The total color difference (ΔE^*_{ab}) between time points was calculated using the CIE 1976 formula:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where ΔL^* , Δa^* , and Δb^* represent the changes in the respective axes between the two time intervals. The ΔE value provides a single, comprehensive metric of overall color change. Higher ΔE values reflect greater color alteration. ΔE values were modified to National Bureau of Standards System (NBS) units to facilitate clinical interpretation. NBS units were interpreted as follows: 0.0–0.5 = 'Trace', 0.5–1.5 = 'Slight', 1.5–3.0 = 'Noticeable', 3.0–6.0 = 'Appreciable', 6.0–12.0 = 'Much', and > 12.0 = 'Very much' color change.

$$\text{NBS} = \Delta E \times 0.92$$

The design enabled comparison of color changes between aligner materials, staining agents, and cleaning methods, as well as assessment of the interaction between these factors.

Statistical analyses

Normality was assessed using the Shapiro-Wilk test. ΔE values for the T1-T0 interval showed a non-normal distribution ($p < 0.05$), whereas ΔE values for the T2-T0 interval were normally distributed ($p = 0.34$). Consequently, data for T1-T0 are presented as median and interquartile range (IQR), whereas T2-T0 data are presented as mean and standard deviation (SD). For the T1-T0 interval, differences among staining environments were evaluated using the Kruskal-Wallis test, followed by pairwise Mann-Whitney U test with Bonferroni correction when significant differences were identified. Pairwise comparisons between aligner materials within each staining environment at T1 were performed using the Mann-Whitney U test. For the T2-T0 interval, a two-way ANOVA was performed to evaluate the effects of staining environment, cleaning method, and their interaction. Post hoc comparisons were performed using Tukey's HSD for the staining environment factor when significant differences were detected. A significance level of $\alpha = 0.05$ was adopted for all tests. Statistical analyses were performed using IBM SPSS Statistics, version 27 (Armonk: NY: USA).

Results

A total of 240 specimens were fabricated. For the T1-T0 analysis, one arch per material per staining environment was measured ($n = 120$ total). For the T2-T0 analysis, all specimens were included ($n = 240$ total). Baseline color values (L^* , a^* , b^*) demonstrated no statistically significant differences among groups, confirming uniformity prior to intervention.

After 7 hours of immersion without cleaning, Taglus Premium specimens exhibited significant differences in color change among staining solutions ($p = 0.001$), with tea producing the highest ΔE (median ≈ 2.38 ; IQR 0.83). In contrast, Taglus Standard specimens did not show significant differences across staining environments at this stage ($p = 0.320$), with median ΔE values distributed narrowly among groups (approximately 1.35–1.82), indicating lower short-term staining susceptibility. A direct comparison between materials showed that Premium aligner specimens exhibited significantly greater color change than Standard aligner specimens in the tea ($p = 0.026$) and control ($p = 0.044$) groups.

Following 14 days of daily staining and cleaning, a pronounced increase in color change was observed for both materials. Two-way ANOVA revealed significant effects of staining environment ($p < 0.01$), and cleaning method ($p < 0.01$), as well as a significant interaction between these factors ($p < 0.01$). Tea produced the greatest color change in both materials, and CHX-assisted cleaning consistently resulted in higher ΔE values than brushing under running water. For Taglus Premium aligner specimens, the highest ΔE values were observed in samples stained with tea and cleaned using CHX solution ($\Delta E = 15.66 \pm 2.20$), while Taglus Standard aligner specimens showed even greater color change under the same

condition ($\Delta E = 19.98 \pm 3.84$), representing the highest overall values recorded in the study. Compared with tea, cola and coffee produced smaller color changes. Comparison between materials at T2 showed that although Taglus Premium was more susceptible to early staining, Taglus Standard demonstrated greater cumulative discoloration after prolonged exposure, particularly in highly chromogenic environments.

Within-material comparisons demonstrated that ΔE values increased significantly from T1 to T2 across all staining environments for both aligner materials ($p < 0.05$), indicating a time-dependent cumulative staining effect.

Clinical significance was assessed by converting ΔE values to NBS units. At T1, the majority of specimens immersed in coffee and cola remained within NBS categories below 'Noticeable'. Specifically, 80% of coffee-immersed and 60.0% of cola-immersed Taglus Premium specimens remained within the 'Slight' category. In contrast, tea-immersed specimens showed a marked shift, with 73.3% of the Taglus Premium and 53.3% of Taglus Standard specimens progressing to the 'Noticeable' category. Notably, 13.3% of Taglus Premium specimens in the tea group reached the 'Appreciable' category within this 7-hour period. Figure 1 illustrates the distribution of NBS color change categories across staining beverages and aligner materials at T1.

At T2, NBS classifications shifted as cumulative staining and cleaning interactions accumulated. The impact of cleaning was most pronounced in the tea-immersed groups. While tea-immersed specimens cleaned with running water were predominantly categorized as 'Appreciable', the introduction of CHX increased staining severity. Specifically, 86.7% of Taglus Premium and 100% of Taglus Standard specimens in the tea + CHX group exhibited 'Very Much' change. Unlike at T1, the cola- and coffee-immersed specimens also exhibited clinically relevant shifts by T2, with the majority of specimens moving into the 'Much' category regardless of cleaning protocol.

The control group also exhibited changes over time. At T1, 73.3% of Taglus Premium specimens remained within the 'Slight' category, whereas 53.3% of Taglus Standard Specimens progressed to the 'Noticeable' or 'Appreciable' categories. By T2, despite the absence of staining agents, the majority of control specimens for both materials shifted into the 'Much' category. Figure 2 illustrates the distribution of NBS color change categories across staining beverages, cleaning protocols, and aligner materials at T2.

Discussion

The findings demonstrated that both materials exhibited discoloration over time, with the extent of staining influenced by the type of chromogenic solution, exposure duration, cleaning protocol, and the material type.

Several studies have investigated the effect of various staining agents. In the present study, tea produced the highest color changes in both materials at both evaluation points. Bernard et al. (12)

similarly reported tea to produce the greatest color change among the tested beverages. Other studies have reported coffee to produce a relatively stronger effect (14, 29). In this study, coffee-immersed specimens showed limited color change at T1; however, by T2 the majority shifted into the 'Much' NBS category. Olteanu et al. also reported significant staining of Taglus Premium specimens following coffee exposure and attributed this effect to the slow-release and low-polarity nature of coffee pigments (30).

An important observation of this study was the distinct staining pattern between the two materials. Taglus Premium exhibited greater susceptibility to early (T1) color changes, whereas Taglus Standard showed higher cumulative ΔE values after prolonged exposure. This material-dependent variation may be related to differences in polymer composition, surface energy, or microstructural characteristics, all of which influence the absorption and adsorption of pigments. Although both products are PETG-based, proprietary differences in formulation or manufacturing processes may contribute to variable staining behavior over time.

The effect of cleaning method was also significant. Specimens cleaned with 0.2% CHX experienced substantially higher color changes than those cleaned under running water, regardless of material type and staining solution. This supports the existing research identifying CHX as a staining agent for teeth and restorative materials. The presence of cationic molecules in CHX is known to facilitate the precipitation of chromogens, which may explain the considerable increase in ΔE values when combined with staining beverages (31, 32, 33). The current findings therefore support caution in recommending CHX when esthetic transparency is critical, as in clear aligner therapy.

From a clinical standpoint, the findings have meaningful implications. Discoloration of clear aligners may negatively impact patient satisfaction, compliance, and perception of treatment quality. Because thermoplastic aligners are worn for extended daily durations, patients are particularly concerned with maintaining their esthetic appearance. The significant staining observed in tea-exposed samples—especially in combination with CHX—highlights the need for clinicians to provide clear guidance regarding beverage consumption and hygiene practices. Although this study was conducted in vitro, the magnitude of color change observed over realistic exposure periods suggests that esthetic degradation may become clinically evident under comparable intraoral conditions.

The control group, maintained in a dry environment at 37°C, exhibited distinct behavior between the two time points, despite the absence of extrinsic pigments, significant ΔE values were observed at T2. This finding suggests that the observed color difference in the control group may primarily reflect alterations in surface properties rather than chromogenic staining. The daily mechanical brushing protocol may have increased surface roughness, contributing to increased opacity. This could highlight that 'discoloration', as measured by total ΔE , may reflect physical surface changes in addition to chemical staining.

This study used controlled experimental design, standardized specimen fabrication, the CIE L*a*b* color system for objective measurements of color changes, and the comparison of two clinically available aligner materials under identical conditions. The dual-timepoint assessment (T1 and T2) enabled

evaluation of both immediate and cumulative discoloration effects, providing a more comprehensive understanding of staining dynamics. However, some limitations should be considered when interpreting the results. First, the study was conducted in vitro and therefore cannot fully reproduce intraoral conditions such as saliva flow, temperature fluctuations, masticatory forces, enzymatic activity, and intermittent rather than continuous exposure to staining solutions. These variables may influence pigment deposition and color stability differently in vivo. Second, thermoplastic aligner surfaces in the oral cavity may undergo mechanical wear, plaque accumulation, or chemical modification, which could alter their optical behavior over time. Finally, only two cleaning methods were tested, and clinical patients may use a range of commercial cleaning products with differing effects on color stability. Future research should focus on in-vivo confirmation of these findings, evaluation of additional aligner materials and commercial cleaning agents, and assessment of surface morphology changes associated with staining. Investigating patient-related factors—such as compliance with removal during drinking, variability in hygiene practices, and dietary behaviors—may also help refine clinical recommendations. Understanding the interplay between material properties and environmental exposures may support the development of more stain-resistant aligners and inform evidence-based clinical guidance.

Conclusions

This in-vitro study demonstrates that both staining environment and cleaning method significantly influence the color stability of Taglus Premium and Taglus Standard PETG-based clear aligners, with tea exerting the greatest effect and 0.2% CHX mouthwash exacerbating staining. Taglus Premium exhibited higher short-term susceptibility to discoloration, particularly with tea, while Taglus Standard showed greater cumulative staining after prolonged exposure. These findings underscore the importance of advising patients to remove aligners during consumption of chromogenic beverages and exercising caution in recommending 0.2% CHX as a routine cleaning method, particularly for tea consumers. Although the controlled design provides objective insights into staining dynamics, the in vitro nature limits direct intraoral applicability. Future in vivo studies should validate these findings and evaluate additional cleaning agents under clinical conditions.

Abbreviations

CHX, chlorhexidine; PETG, polyethylene terephthalate glycol-modified; CIE, Commission Internationale de l'Éclairage; NBS, National Bureau of Standards; IQR, interquartile range; SD, standard deviation.

Declarations

Ethics Approval and consent to participate

Not applicable. The study protocol was reviewed and approved by the Ethics Committee of Shahid Sadoughi University of Medical Sciences (approval code **IR.SSU.DENTISTRY.REC.1404.024**).

Consent for publication

Not applicable.

Trial registration

Clinical trial number: Not applicable.

Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no competing interests.

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The authors received no specific funding for this work.

Authors' Contributions

MJM contributed to project administration, resources, supervision, methodology, and writing-review and editing. MAF contributed to supervision and writing-review and editing. EA contributed to formal analysis, investigation, methodology, writing-original draft and writing-review and editing. KM contributed to resources and writing-review and editing. All authors read and approved the final manuscript

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Figures

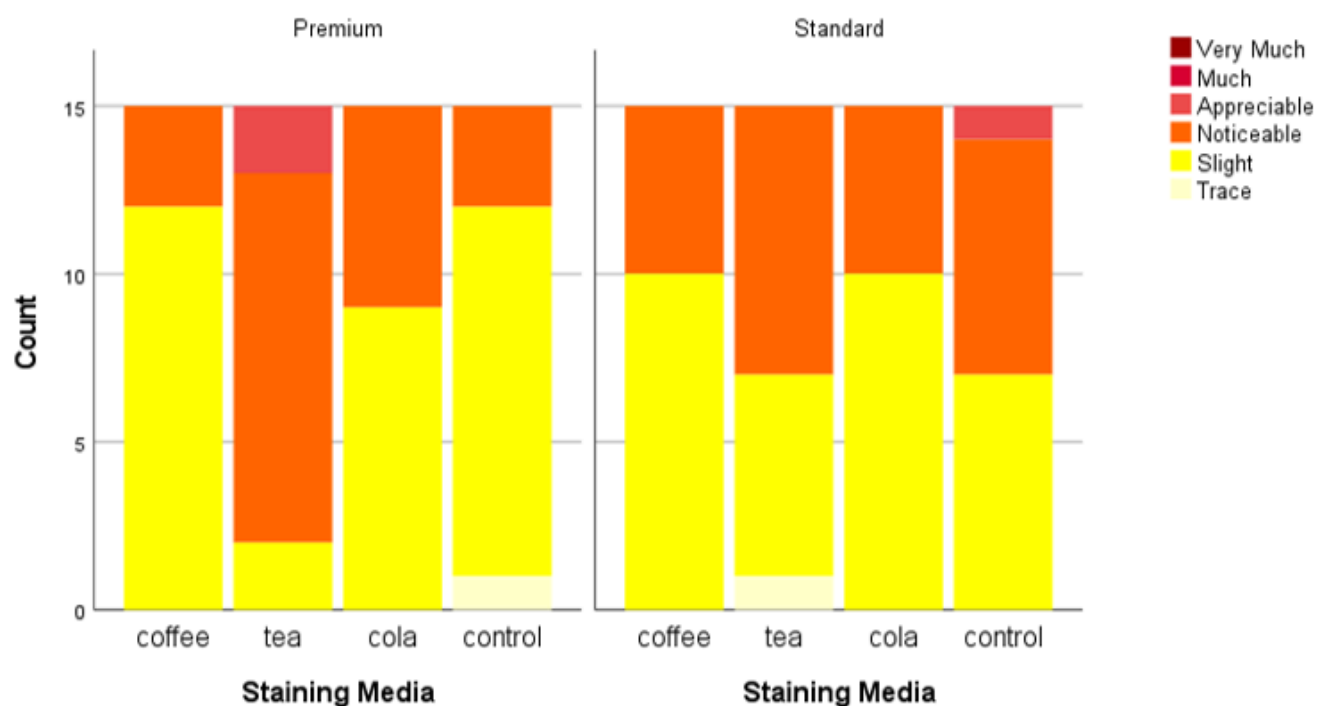


Figure 1

NBS categories at T1.

Legend: Distribution of NBS color change categories across staining beverages and aligner materials after 7 hours (T1).

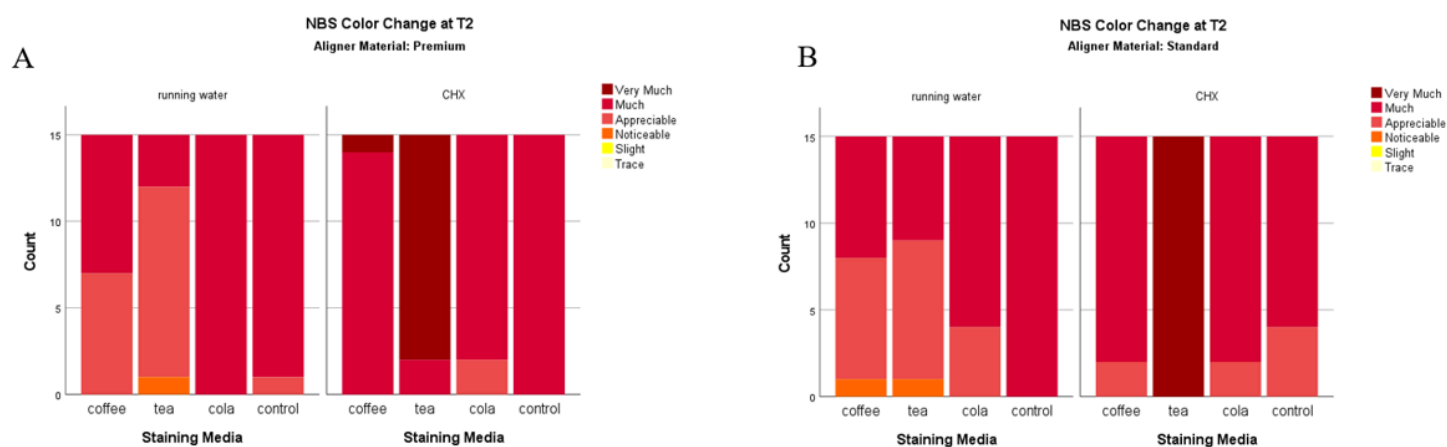


Figure 2

NBS Categories at T2 by cleaning protocol

Legend: Distribution of NBS color change categories across staining beverages and cleaning protocols for Taglus Premium (A) and Taglus Standard (B) after 14 days (T2).