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HOW TO SELECT STUDY DESIGNS AND PARAMETERS TO INVESTIGATE THE EFFECT OF MOUTHRINSES? PART I: RATIONALE AND BACKGROUND

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In the literature and in the daily study routine, a wide range of study designs exists to test the efficacy of mouthrinses. Within these various designs, a considerable number of parameters is applied. Due to the different protocols currently used the outcome of these investigations is hardly to compare. Therefore, it was the aim of the present investigation to assess suitable study designs of different durations, to select and compare parameters, and to find suitable study participants in order to finally recommend more standardized protocols for future studies on oral antiseptics. Eight-hour substantivity studies, four-day plaque re-growth studies, 21-day experimental gingivitis studies, and six-month home-use studies were performed either cross-over or parallel. The clinical studies were randomized, controlled, and investigator-blinded. From most often applied clinical and laboratory parameters, two plaque indices, bacterial vitality, three gingivitis indices, gingival crevicular fluid, colony forming units, and the discoloration index were selected to assess plaque inhibition, gingivitis development, tooth staining, and possible bacterial shifts in the oral cavity. Four or five, respectively, treatment groups were formed that rinsed with the following solutions: chlorhexidine digluconate 0.06%, 0.12%, 0.20%, amine fluoride/stannous fluoride, and a negative control. In addition, all study designs were tested on two different study populations. Population α consisted of persons who had a gingival index <0.8 while population β participants presented a mean gingival index of ≥0.8. Pearson correlation coefficients were calculated between parameters and between subsets of teeth and full-mouth recording. T-tests were applied to compare between populations α and β.

Key words: *substantivity studies, plaque re-growth studies, experimental gingivitis studies, home-use studies, plaque, gingivitis*

INTRODUCTION

Design of clinical investigations

Among clinical investigations in periodontology, clinical studies that test the effectiveness of chemotherapeutic agents are of special interest. These studies are aimed to test agents that inhibit plaque formation, influence plaque removal, and prevent or reduce gingivitis and calculus (1). The design of these studies was a matter of discussion in the past in order to find consensus about important details of the protocol that can effect the outcome (2-4). Agreement was established in many areas and guidelines could be accomplished (5-7). However, some of the open questions still remain unsolved. Due to the various designs and protocols currently used, the outcome of studies is hardly to compare. This is considered as a shortcoming of these studies in the relatively small field of antiseptic testing (1, 8). Almost every study uses a specific protocol or modifications of formerly described protocols. Study selection is mainly based on the aim or hypothesis under investigation (9) but also on the individual preferences of the investigator (1). Parameters vary between an abundance of clinical and laboratory variables and are combined in many ways to answer the research question. New technologies evolve and lead to the implementation of new parameters. Participants are recruited among different populations having diverse ethnic, social, and genetic background. Subject-related factors can increase the variability of certain study designs and therefore influence the outcome. Smoking for example had been shown to alter various immunological reactions in different regions of the body (10). In smokers, reduced gingival bleeding was found. It was suggested to exclude smokers from experimental gingivitis trials (11, 12). The study by Loe *et al*. already demonstrated that the time in that participants develop a plaque-induced gingivitis differs tremendously (13). Therefore, experimental gingivitis studies should take at least three weeks in duration to ensure that there is enough time to develop a plaque-induced gingivitis in every participant. The tendency to accumulate plaque in experimental plaque studies also varies between individuals. A combination of individual variables like the extent of pre-existing gingivitis, dietary habits, salivary flow and composition, existing plaque retention sites, and the composition of the pellicle is assumed to influence plaque development (14). Interactions between the oral condition and general health or disease, respectively, are increasingly investigated (15). Therefore, more standardization in design, parameter, and participant selection would lead to improved quality and comparability in studies on oral antiseptics.

Taking these considerations into account, it was the aim of the present investigation on mouthrinses to assess:

- short-term and long-term study designs,
- suitable parameters,
- suitable study participants,

and enable researchers to perform more standardized procedures in order to enhance the comparability of future studies on oral antiseptics. It was not the aim to repeat studies that have been done before.

MATERIALS AND METHODS

Overall study design

These randomized, controlled, investigator-blind, clinical studies were performed according to GCP/ICH requirements. Ethical approval was obtained from the local Ethics Committee of the Medical Faculty, University of Technology, Dresden, Germany. The studies were then performed between 2001 and 2004.

Each study commenced with a recruiting period 14 days before the treatment started. Participants were selected according to the inclusion and exclusion criteria and received a dental prophylaxis. They gave their written consent after the study was extensively explained to them. At the start of the treatment period, another dental prophylaxis was performed. The dental prophylaxis included scaling and polishing of the entire dentition until any supragingival and subgingival deposits and stain were removed. A visit took about one hour. At any visit, adverse events were recorded. Interference with the inclusion or exclusion criteria led to study exclusion. At the end of every single study, a dental prophylaxis concluded the final visit.

Depending on the design, the participants were randomly allocated to 4 or 5 treatment groups, respectively (*Table 1*). The mouthrinses used to test the hypotheses included chlorhexidine 0.06%, 0.12%, 0.20%, amine fluoride/stannous fluoride, and a negative control (*Table 2*). The participants were informed about the use of the rinses by a study nurse. In addition, they received a written description. The first self-administered product use in design II, III, and IV was conducted at the evening of day 0. At the following days, the study products were administered according to the instructions of the manufacturers. For design I, the single rinse was done under the supervision of the study nurse in the clinical setting.

Selected study designs

As study designs the eight-hour substantivity study, the four-day plaque re-growth model, the three-week experimental gingivitis model, and the six-month home-use trial were selected. These designs are the most often applied models found in the literature (16). They can be named as the classical methods to test oral antiseptics and have previously shown their reliability, validity, responsiveness, and interpretability. Every design is aimed to answer specific research questions at certain stages of product evaluation. A combined flow chart for all designs and parameters used is presented in *Table 3*.

The design of eight-hour substantivity studies was first used and described by Bonesvoll *et al*. and afterwards applied in many studies (17-22). As short term test, the aim of substantivity studies is to determine whether or not and if, for how long a formulation performs a persisting effect *in vivo*. This substantivity depends on the ability of a substance of physical and chemical bonding to a surface as well as its resistance against removal or inactivation, as long as it remains biologically active. As the first test stage, failure of a formulation to show substantivity would prove an inappropriate effect on the inhibition of plaque development. If there is an effect then according to the obtained efficacy profile an optimal frequency of use for any product can be recommended (23-25). The substantivity of an agent determines the rinsing frequency needed, however, practically the rinsing frequency is limited to 2 or 3 times a day. After application of a single rinse on pre-existing plaque, plaque and saliva bacteria are studied for the following eight hours while the participants cease oral hygiene measures. A cross-over design was applied to test all mouthrinses in the same individuals.

Plaque re-growth studies were first implemented and afterwards most frequently performed by Addy and coworkers (26). The model is aimed to study the plaque inhibitory effect of a formulation *in vivo* while any oral hygiene is stopped during the test phase. Primarily plaque-free teeth undergo a four-day period with no oral hygiene measures except the rinsing with the allocated formulation. A final plaque assessment shows whether or not the mouthrinse *per se* is able to depress plaque development and to what extent. If no plaque inhibition can be shown in this type of study no further effect of the rinsing solution can be expected in studies when oral hygiene is performed (27). Therefore, this model seems to be the second

Table 1. Study designs, duration, and treatment groups.

Steps	Design	Type	Brushing	Duration	Populations	Mouthrinses
I	Eight-hour substantivity study	Cross over	No	8 hours	α, β	A, B, C, D, E
II	Four-day-plaque-regrowth study	Cross over	No	4 days	α, β	A, B, C, D, E
III	21-day experimental gingivitis study	Parallel	No	21 days	α, β	A, C, D, E
IV	Home use study	Parallel	Yes	6 months	α, β	A, B, D, E

α participants who have a mean gingival index <0.8; *β* participants who have a mean gingival index ≥0.8; A chlorhexidine mouthrinse (CHX) 0.06%; *B* CHX 0.12%; *C* CHX 0.20%; *D* amine fluoride/stannous fluoride mouthrinse; *E* placebo mouthrinse

Table 2. Treatments.

	Mouthrinse formulation	Trade name
A	0.06% chlorhexidine digluconate	Corsodyl Zahnfleisch-Fluid®, GlaxoSmithKline, Bühl, Germany
B	0.12% chlorhexidine digluconate	Oral B®, Gillette, Kronberg, Germany
C	0.20% chlorhexidine digluconate (positive control)	Corsodyl®, GlaxoSmithKline, Bühl, Germany
D	Amine fluoride/stannous fluoride	meridol®, GABA, Münchenstein, Switzerland
E	Placebo (negative control)	meridol® without amine fluoride/stannous fluoride, GABA, Münchenstein, Switzerland

Table 3. Flow chart for all designs, visits, parameters used (+).

Visit	PlI	M-QHI	PlA	BV	GI	M-GI	BOP	GCF	DI	CFU	PTC
Design I (Eight-hour substantivity study)											
Day -14	+				+	+	+				+
1 (0 h)				+						+	
2 (2 h)				+						+	
3 (4 h)				+						+	
4 (6 h)				+						+	
5 (8 h)				+						+	+
Design II (Four-day plaque re-growth study)											
Day - 14	+				+	+	+				+
1 (day 0)	+	+		+	+	+	+	+		+	
2 (day 4)	+	+	+	+	+	+	+	+		+	+
Design III (21-day experimental gingivitis study)											
Day - 14	+	+		+	+	+	+	+	+	+	+
1 (day 0)	+	+		+	+	+	+	+	+	+	+
2 (day 7)	+			+	+	+	+	+	+	+	
3 (day 14)	+			+	+	+	+	+	+	+	
4 ((day 21)	+	+	+	+	+	+	+	+	+	+	+
Design IV (6-month home use study)											
Day - 14	+	+			+	+	+	+	+	+	+
1 (day 0)	+	+			+	+	+	+	+	+	+
2 (day 21)	+	+			+	+	+	+	+	+	
3 (month 3)	+	+			+	+	+	+	+	+	
4 (month 6)	+	+			+	+	+	+	+	+	+

PlI plaque index (42); M-QHI plaque index (44); GI gingival index (65); M-GI modified gingival index (59); BOP bleeding on probing (66); BV bacterial vitality (48); GCF gingival crevicular fluid; DI discoloration index (72); CFU colony forming units; PTC professional tooth cleaning.

stage in product testing. A cross-over approach was chosen for this study.

Experimental gingivitis studies date back to the experiments by Loe *et al*. (13) to prove the etiological role of plaque in the development of gingivitis. Since then, the model was adopted to investigate the influence of a compound on the development of plaque and gingivitis in the absence of mechanical oral hygiene (28-33). Experimental gingivitis studies over three weeks are short-term studies. A shorter investigation time less than 21 days was not taken into consideration because the original studies of Loe *et al*. (13) had already shown that some individuals need a longer time than 14 days to develop plaque-induced gingivitis. During 21 days, the participants stop their habitual oral hygiene. Instead, they rinse with the allocated mouthrinse once or twice a day. The ability of a formulation to inhibit gingivitis and plaque is assessed weekly. Once a test agent has shown its potential to inhibit plaque this model elucidates whether the plaque inhibiting effect also affects the development of gingivitis. Therefore, the experimental gingivitis study is considered as a third stage during product evaluation. To avoid forcing individuals to undergo more than one experimental gingivitis and to ensure good compliance, this study was conducted in parallel groups.

Home-use studies are long-term studies to test the efficacy of anti-plaque and anti-gingivitis agents under almost real-life circumstances. This model refers to the FDA requirements that ask for safety records for oral hygiene products as well. The study was performed in parallel groups. In addition to the rinsings, mechanical oral hygiene was part of the protocol. Chlorhexidine 0.20% was not used in this study because it is not designed for long-term application (34).

Selected parameters

There is a variety of parameters available (35, 36) to be applied in the above mentioned study designs. Parameters for these investigations were selected according to their appearance and importance in the literature about clinical studies on mouthrinses. Both clinical and laboratory parameters were chosen to find out whether the more subjective clinical indices or the more objective laboratory parameters are superior and therefore have to be preferred. Parameters were recorded during the whole course of studies by two clinical investigators and two laboratory assistants. Plaque indices were recorded by one investigator (GB) while gingivitis parameters were assessed by the other (KL) as was bacterial vitality (SH) and colony forming units each by one of the laboratory assistants (JR, AT). In *Table 3*, parameters are listed as appropriately assigned to the corresponding design.

Plaque parameters

Dental plaque can be assessed in two different ways: qualitatively and quantitatively. For use in clinical trials on oral hygiene products quantitative methods like indices (37), gravimetry (38), or planimetry (39) are utilized. Among these, the indices reached a significant importance and are frequently employed. They are used to assess plaque by estimating its extent over the tooth area or its thickness and are described as being very sensitive in determining slight differences in the effectiveness of agents being tested in a study (40). Especially indices that estimate plaque adjacent to the gingival margin were found to be appropriate for evaluating chemotherapeutic agents in clinical trials (41). Disadvantages are seen in the high expenditure of time in administering those indices (40). In addition, they tend to be highly subjective and should be only applied after careful calibration of the examiners (41). Binary indices that only distinguish between the presence or absence of plaque were not found to be suitable for the evaluation of oral hygiene products (40).

For this study, two different indices were selected. The plaque index (PlI) (42) differentiates between absence of plaque

and plaque that is either detectable by a dental probe or visible by the naked eye in different extent around the gingival margin. Plaque is partially destroyed by the dental probe that is run along the gingival crevice and therefore further plaque assessment can be impaired. For this investigation plaque was dried by air. In sites where plaque was not visible a dental probe was run along the gingival margin to distinguish between score 0 and 1. Scores 2 and 3 were estimated according to the original description by the naked eye. The distal, median, and mesial sites of the vestibular surface and the oral surface of all teeth were scored. In contrast, for modified plaque index recordings (43, 44) plaque is disclosed and the extent of plaque covered tooth area at both buccal and oral sites at all teeth is scored. While disclosed plaque is easier to evaluate and therefore the scoring is more objective, this method is not suitable in certain study designs like for in-between visits during an experimental gingivitis. When used in these studies, all teeth were dyed with erythrosine solution (Mira-2-Tone, Hager & Werken GmbH, Duisburg, Germany), the participants rinsed until the rinsing water was colorless, and then plaque was scored.

Another quantitative method are plaque area (PlA) measurements. These planimetric indices can include several numbers of teeth (45, 46). The percentage of the plaque covered surface of these teeth is recorded after staining with erythrosine, digital standardized photographing, and computer-based calculation. For the present studies only the upper right central incisor was selected. Orthoradial photographs were taken in order to assess the tooth surface in its full extent. The stained buccal surface was highlighted on the photograph using the drawing tool of Adobe Photoshop 7.0 software and then the number of pixels within this area was calculated. In addition, the circumference of the whole tooth surface was also highlighted and numbers of pixels within this area calculated. The relation between the plaque covered area (number of pixels) to the total vestibular tooth surface (number of pixels) gave the percentage of existing plaque. The advantage of these photographs is that they are permanent records that can be re-evaluated at any point of time and transferred into other index scores (47).

Bacterial vitality within the dental plaque is a qualitative measurement of the antibacterial effect. It is a laboratory parameter and measured by using the vital fluorescence technique (48). Plaque samples were collected at the vestibular sites of the selected teeth. Selected teeth were the first and second premolars of both upper and lower jaw. The samples were wiped on slides and disclosed for 3 minutes with 5 µl ethidium bromide and fluoresceine diacetate solution. Vital bacteria cells were dyed green while dead bacteria were dyed red. Under a fluorescence microscope with 250 fold magnification (Jenamed 2 histology fluorescence, Carl Zeiss Jena GmbH, Jena, Germany) a 5x5 square grid was applied in 5 different areas of the plaque sample. The percentage of vital cells in a plaque sample was estimated. According to Weiger *et al*. (49), it was distinguished between 100%, 90%, 70%, 50%, 30%, 10%, and 0% vital plaque in the sample. The mean percentage of all 125 squares represented the overall vitality and was used as a measurement of the antibacterial effect of the treatment.

Gingivitis parameters

Assessment of plaque-associated gingivitis in studies on mouthrinses is an essential part of the protocol but was always a matter of discussion. Beside the recording of indices, inflammatory markers of the gingival crevicular fluid can be analyzed (50).

Gingival indices are based on clinical symptoms of inflammation like gingival color, contour, bleeding, extent of gingival involvement and crevicular fluid flow (51). These clinical features can be assessed non-invasively (*e.g.*, color, contour, spontaneous bleeding) and/or invasively (*e.g.*, bleeding on provocation). With regard to selecting the most suitable index in clinical trials, the advantages and disadvantages of invasive versus non-invasive indices have been discussed (41, 52). Some authors prefer invasive indices because they believe that bleeding is the most objective sign of inflammation and seems to be a more sensitive symptom than visual changes (53, 54). However, not all visual inflamed sites bleed. Also, bleeding depends on a variety of approaches to elicit the bleeding and can therefore vary a lot between studies. Influencing factors include the time lapse between provocation and bleeding, the depth of sulcular insertion of the probe, the probing technique, the angle of insertion, and the probing force (55). There are further challenges that have to be considered before an index containing a bleeding component is selected. Different studies have shown that bleeding on probing can cause gingival trauma and increased bleeding after provocation (56). Concerns have arisen that bacteremia following invasive procedures can represent a risk for certain patients. A calibration of examiners or assessment of the reliability of single examiners using the same site is not feasible (57). Furthermore, bleeding sites can be obscured by blood oozing from previously probed areas to adjacent tooth surfaces that makes the assessment more difficult (58, 59).

There is no doubt that indices containing a bleeding component can successfully used in clinical trials. On the other hand, there is no evidence that invasive indices are truly objective (52). Utilizing a pure visual index in assessing gingivitis is an alternative to the invasive index (60). For the present studies, two gingival indices were applied - a pure visually based index and a invasive index containing a bleeding component.

The gingival index (GI) developed by Loe & Silness (61) has gained general acceptance for use in clinical trials (62-64). However, in a later publication the method to provoke the bleeding was described more specifically (65). Therefore, in this study the index described by Loe in 1967 was used. Bleeding is provoked by running a periodontal probe along the entrance of the gingival crevice. While scores 0 and 1 only represent visual changes, the bleeding component is included in scores 2 and 3. The distal, median, and mesial surfaces of the vestibular part of each tooth and the whole oral part of each tooth are areas to be scored.

Introducing a modification of the Loe & Silness index, Lobene *et al*. (59) eliminated the bleeding component from this modified gingival index (M-GI) and increased the sensitivity at the low-end of the scoring scale. Scores 1 and 2 both represent mild inflammation but distinguish between any portion of the gingival unit (score 1) and the entire gingival unit (score 2) in order to detect more subtle visual changes in inflammation. Score 3 corresponds with score 2 of the gingival index by Loe (65) and score 4 with score 3 of Loe's index. As an advantage, when utilizing this index then calibration of examiners is unlimited. The index highly correlates with indices that include bleeding on provocation. These results indicate that gingivitis can be assessed with a pure visual index (60).

A pure bleeding index that was included into these investigations is the gingival bleeding index (BOP) as described by Ainamo & Bay (66). Bleeding is provoked with a periodontal probe inserted at the orifice of the gingival crevice and recorded 10 seconds after provocation. It should not be mixed up with bleeding upon probing that refers to probing of periodontal pockets. Therefore, quadrant wise bleeding was provoked at one distal, median, and mesial site of both vestibular and oral aspects of each tooth. As suggested, the bleeding was recorded after 10 seconds as a yes/no decision. The proportion of bleeding sites compared to all examined sites gave the percentage of bleeding per participant. A pressure calibrated periodontal probe was used to standardize probing force (Paro Audio-Probe, Esro AG, Thalwil, Switzerland).

Gingival crevicular fluid (GCF) is a serum transudate from clinically normal periodontal tissues which becomes an inflammatory exsudate when the disease is clinically detectable (67). It was considered as an objective parameter compared to subjective clinical indices for inflammation (68). Some studies found a positive correlation between GCF and clinical signs of inflammation (68) and others did not (69). After the gingiva was dried with air, two paper strips (PerioPaper Strips; Oraflow, New York, USA) were inserted in the gingival crevice. One was applied at the distal and the other was applied at the mesial aspect of the vestibular surface of the upper right canine. After 40 seconds the paper strips were transferred to the Periotron 8000® (Oraflow Inc., Babylon, New York, USA) to measure the volume of GCF. The obtained scores were converted into volumes (µl) using a software. The device was calibrated with human serum before the studies started. Before each reading, the device was adjusted to zero.

Discoloration index

Discoloration is the most frequent side effect that occurs when mouthrinses are applied for a longer period of time. The majority of studies only counts the numbers of side effects. The discoloration index however, provides an opportunity to estimate the extent of discoloration per tooth. The esthetic component plays an important role when patients' compliance of mouthrinse use is considered. This is especially true for long-term use of these formulations. Among a few available parameters that measure the discoloration of teeth (26, 70, 71), the discoloration index by Brecx and co-workers was selected (72). The index includes color, intensity, and distribution of extrinsic stains among vestibular and lingual surfaces and seems to be a simple and practical method compared to the other approaches.

Colony forming units (CFU)

The oral cavity is equipped with a natural flora that colonizes the soft and hard tissues. This flora exists of about 700 to 800 species that coexist in a specific equilibrium in each individual. Since antimicrobial agents are aimed to suppress these microbiota, it has to be proven whether meaningful shifts occur within this microbiological balance and to what extent. A method to determine the bacterial load is to measure colony forming units in saliva or in plaque. This parameter was chosen to document changes in the amount of especially anaerobic micro-organisms caused by mouthrinse use in both short-term and long-term studies.

Study populations

Participants for clinical studies are usually recruited among two different groups. Some investigators recruit students who are easily available within the dental or medical schools. Other investigators include persons who are the potential users of the product. However, no study exists so far that investigated whether the one or the other group is more suitable or whether there is no difference. Therefore, for each of the designs I to IV, persons from two separate study populations (α and β) were recruited. The majority of these individuals participated in all four designs. Population α consisted of dental and medical students of Dresden University, Germany, who were intended to have very good oral hygiene. The gingival index should not exceed 0.8. In contrast, population β consisted of participants having average oral hygiene from a local community in Dresden, Germany. Their gingival index was above 0.8. The students were recruited during the lectures and by means of advertisements throughout the campus whereas the participants of the local population and non-academic staff by advertisements in the community and word of mouth publicity.

Systemically healthy persons between 18 and 50 years who had at least 20 teeth excluding third molars were recruited. Persons were excluded from study participation when at least one of the following criteria existed: (i) systemic diseases, (ii) history of severe oral diseases or surgery with a possible interference on the use of mouthrinses to be tested, (iii) history of periodontitis, (iv) pregnancy or breast feeding, (v) untreated caries, (vi) wearing of dentures and orthodontic appliances, (vii) heavy smokers >30 packyears, (viii) treatment with test formulations 2 weeks before and during recruitment, (ix) treatment with antibiotics, antiphlogistics, immunostimulants, immunosuppressive drugs, antimitotic drugs, drugs which influenced salivary flow 3 months prior inclusion, (x) treatment with topical medication that interferes with the study products. All participants were informed about the studies by oral and written explanations of the protocol. Any discrepancies were clarified and questions were answered before they gave their written consent. Participants were free to drop out at any time without a need to declare reasons for study interruption.

Calibration of examiners

Before starting the study, intra-individual and inter-individual agreement was assessed for all indices used except for BOP. A group of 10 staff members of the dental school was ask to participate in the calibration sessions. A training took place where index scores were discussed and discrepancies in the index judgment were resolved. Full-mouth indices were recorded twice per subject by each investigator to calculate the intra-examiner and inter-examiner reproducibility. The mean κ coefficients ranged from 0.59±0.06 to 0.72±0.05 for all indices. Examiners for bacterial vitality and CFU were trained prior to study start by an experienced laboratory assistant who had extensive routine with the processing and evaluation of the parameters under test.

Sample size calculation, randomization, statistical analysis

The sample size calculation for each study design was based on mean differences and standard deviations between placebo and CHX 0.2% for the primary parameters. Anticipated drop-out rates, normal distribution in the groups, a significance level $\alpha=0.05$ and a power of 80% were also included in the sample size calculation. For design I, II, and III, a sample size of 20 subjects per group was calculated while for design IV 25 participants per group had to be included.

Participants were randomized for each of the studies and stratified according to age, sex, plaque index, and gingivitis index. The randomization list was created by the statistician using a special software. In the study center, knowledge of the randomization list was limited to the study nurse who was responsible for the treatment distribution.

Descriptive summary statistics including means, standard errors of the means, standard deviations of the variable, medians, quartiles, minimums and maximums were computed for all study parameters documented in the case report form (CRF). For all indices, means per subject were calculated and represented the unit of measurement for the statistical analyses. Depending on the distribution of the data (Kolmogorov-Smirnov tests), either parametric variance analysis models (t-tests in pair wise comparisons) or non-parametric methods (Wilcoxon rank sum test) were applied to test the outcome for all parameters at all time points. In addition, correlations in-between the plaque and in-between the gingivitis parameters were evaluated as well as between full-mouth and partial-mouth recording of selected indices (Pearson correlation coefficient). To compare parameters between population α and β, t-tests were applied. All calculations were

based on the per protocol analysis set on a significance level of 0.05 and a power of 80%. The data were entered in the electronic databases twice. All statistical analyses were computed by means of Statistical Package of Social Science (SPSS), version 11.5.

The studies were aimed to test correlations between all parameters, between whole-mouth and partial-mouth approach when plaque, gingivitis, and discoloration indices are used, and to compare the outcome between participants with good oral hygiene and average oral hygiene. The designs and test products used were only the vehicle to answer these questions. It was not the intention to repeat study results that had been obtained before by several other studies. Therefore, these papers do not focus on test results of the products but primarily on comparisons of designs, parameters, and populations. Results and conclusions are aimed in helping of the selection of these to answer future questions on oral antiseptics.

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