CONFIDENTIAL

Study Report

Study Protocol Code: STAB 1
Internal Project Code: CCI [redacted]
EudraCT Number: 2006-005953-31

Inhibition of Dental Plaque Regrowth by an Ethanol-Free 0.2% Chlorhexidine Mouth Rinsing Solution

1. Title Page

Development Phase of Study:

Phase IV

Name of Investigational Products

Chlorhexamed® alkoholfrei

Indication Studied:
Inhibition of Bacterial Plaque Regrowth on Teeth in the Absence of Mechanical Plaque Control in healthy volunteers.

Study Description:
In a monocentric, prospective, randomized, examiner-blinded, 3 arms, cross-over, placebo controlled test design the inhibitory effect of an ethanol-free 0.2% chlorhexidine mouth rinsing solution on bacterial plaque regrowth on teeth was evaluated in 42 healthy volunteers over a time course of 4 days without mechanical tooth cleaning. An ethanol-containing 0.2% chlorhexidine mouth rinsing solution and a placebo mouth rinsing solution served as positive and negative controls respectively.

First-Subject-In:
May 8 th, 2007

Study Completion Date:
August 24 th, 2007

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Compliance with GCP

The present study was performed in compliance with Good Clinical Practices (GCP) including the archiving of essential documents

Signature Page

STUDY TITLE:

Inhibition of Dental Plaque Regrowth by an Ethanol-Free 0.2 % Chlorhexidine Mouth Rinsing Solution

AUTHOR(S) OF REPORT:

PPD

I have read this report and confirm that to the best of my knowledge it accurately describes the conduct and results of the study

PPD

Signature of the Princ PPD vestigator pursuant to § 40 AMG
Prof. Dr. med. dent. PPD

PPD

Date

PPD

Signature of the responsible project leader of the sponsor
Dr. PPD

PPD

Date PPD

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Signature of the sponsor's contact person
Dr. PPD

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2. Synopsis

Title of the study:

Inhibition of Plaque Regrowth by an Ethanol-Free 0.2 % Chlorhexidine Mouth Rinsing Solution

Investigators: *Monocentre trial, conducted by:*	*Principal investigator:* Prof. Dr. med. PPD *Co-Investigators*: Dr. med. dent. PPD Dr. med. dent. PPD Dr. med. dent. PPD Dr. med. dent. PPD Mr. PPD, Dentist

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Publication (reference): none

Studied period (years):	**Clinical Phase:**
Date of first enrolment: 8th of May, 2007 Date of last completed: 24th of August 2007	Phase IV

Objectives:
The objective of this clinical trial was to evaluate the inhibitory effect of an ethanol-free 0.2% chlorhexidine digluconate mouth rinsing solution, newly formulated by GSK (CHX,ef; Trade name: Chlorhexamed alkoholfrei®) on bacterial regrowth on teeth in the absence of mechanical tooth cleaning over a period of 4 days in healthy volunteers. A placebo solution (PCB,ef) and a commercially available ethanol-containing 0.2% chlorhexidine digluconate mouthrinse (CHX,ec; Trade name: Chlorhexamed Forte® 0.2 %) served as negative and positive control respectively.

Methodology:
Monocentric, prospective, randomized, investigator-blinded, three-armed, cross-over, placebo-controlled, including healthy volunteers, age 18-50, of both genders with good oral health and regular effective oral hygiene participated in the study.

Number of subjects (planned and analysed):
Calculation of Sample Size
Regarding the Quigley Hein Plaque Index as the primary endpoint, to have 95% power to detect a minimal clinical important effect size δ(QHI) = 0.68 using a paired t-test with a 0.05 one-sided significance level, a (per-protocol) sample size of approximately 32 test persons will be needed. By assumption of a drop-out rate of 25 % the overall sample was increased to about 39 subjects. Because of the cross-over design with six different sequences of experimental conditions, a multiple of six was chosen resulting in the final sample size of 42 subjects.

Analysed:

Safety/Intent-to-treat (ITT): N=42; as three subjects dropped out, 4 rinsing periods failed: CHX,ef: N=40; CHX,ec: N=42; PCB,ef (Placebo): N=40

12 men; 30 women with a mean age of 25.6 (SD = 3.3) years in men and 25.8 (SD = 6.6) years in women.

Per-protocol (PP): n=33 due to 3 drop-outs and 6 subjects with major protocol violations (4x non-compliant, 2x non-permitted comedication):

9 men, 24 women with a mean age 25.8 (SD = 3.8) years in men and 26.1 (SD = 7.00), years in women.

Diagnosis and criteria for inclusion:
Healthy men and women aged 20-50 years with a minimum of 20 natural teeth, healthy mucosa and no regular use of drugs interfering with bacterial plaque growth or the generation of gingival inflammation.
Subjects with known sensitivity to the ingredients of the study medication and subjects using locally or systemically active antibiotics ot other substances which likely influence bacterial growth in the oral cavity were excluded.

Test product, dose, mode of administration, batch No.: CSC283
Test Solution 1: 0.2 % ethanol-free CHX formulation = CHX ef Batch No CSC283
Active pharmaceutical ingredient: 100mL solution containing 0.2g chlorhexidine digluconate
Other ingredients: glycerol, macrogolglycerolhydroxystearate; D-Glucitol, purified water, peppermint flavour.

Product Dose and Mode of Administration:
Rinsing of the oral cavity with 10 ml of the test solution postprandial in the morning and the evening postprandial for 60 seconds each and subsequent removal by spitting. Each solution was used for a period over 4 days, while no mechanical or any other dental care at home was applied.

Duration of treatment:
Total study duration per subject lasted 42 days with 3 treatment periods of 4 days, each and 3 run-in/wash-out-periods of 10 days, each.

Reference therapy, dose, mode of administration, batch No.:
Test Solution 2: 0.2 % CHX ethanol-containing formulation (Chlorhexamed® Forte 0.2%) = CHX ec; Batch No: CSC283
Active pharmaceutical ingredient: 100mL solution containing 0.2 g chlorhexidine digluconate
Other ingredients: ethanol 96%, macrogolglycerolhydroxystearate, d-glucitol, purified water, peppermint oil

Test Solution 3: CHX-free, ethanol-free mouth-rinse solution (placebo) = PCB ef; Batch No CSC283
Ingredients: glycerol, macrogolglycerolhydroxystearate, purified water, peppermint flavour

Criteria for evaluation:
The main target criterion of the study was the extent of plaque regrowth on the previously professionally cleaned teeth (equal to initial value QHI=0) when using the CHX-rinsing formulations without mechanical or any other dental care at home, after 4 days. The extent

of bacterial plaque regrowth was evaluated using the Turesky modification of the Quigley-Hein plaque index (QHI), determined on the vestibular surfaces of the so-called Ramfjord teeth (Teeth 16,21,24,36,42, 44).
The secondary target criterion was the eventual development of gingival inflammation induced by the growth of adjacent bacterial plaque, monitored on the vestibular surfaces of the Ramfjord teeth according to the criteria of the Gingival Index of Löe and Silness (GI).

Statistical Analysis

Due to the analyst's change of job during the study, he did not have access to the software mentioned in the study protocol. Therefore the computation of the hypothesis tests and confidence intervals could not be performed as one-sided (cf. study protocol) but as two-sided as these procedures will only be available SAS, Version 9.2 (and higher). However, these changes can only affect the inferential statistical results in a conservative way.
The difference of the effect of ethanol-free chlorhexidine mouthrinse solution vs. ethanol-free placebo was analysed using a two-sided t-test for dependent samples on the pre-post-differences of the mean Quigley-Hein Index of all six examined teeth as the primary endpoint. The pre-post-differences of the QHI were computed within the pooled sample receiving ethanol-free chlorhexidine mouthrinse solution and compared with the pre-post-differences of QHI of the pooled sample applying placebo. As the QH index was set to zero before each treatment period the measurements after each treatment are considered as pre-post-differences. Statistical inference was evaluated using a 95% confidence interval and the p-value of the effect. Furthermore, a standardized effect size, also known as standardized response mean (SRM), was calculated.

The Gingival Index (GI) as the secondary endpoint was only analysed on a descriptive level similar to the primary endpoint. Furthermore, safety variables have been measured.

Results:

Intention-to-treat population (ITT-Population)

The analysis of the ITT-population (N = 42) including all randomized subjects yielded the following results:

At study start in the presence of regular mechanical oral hygiene the average QHI score was 0.91 (SD=0.66). Before each treatment period the QHI was set to zero by the professional tooth cleaning procedure.

After 4 days of repeated rinsing and in the absence of mechanical tooth cleaning the recorded average QHI score was 0.78 (SD=0.55) for the ethanol-free CHX mouth rinsing solutions compared to QHI 2.93 (SD=0.89) for the placebo solution ($p < 0.00001$). The average QHI score recorded for the ethanol-containing CHX mouth rinsing solution was QHI 0.78 (SD=0.47).

The distributional assumption of normality of the treatment differences has been examined graphically using Quantil-Quantil (QQ) plots. They showed that the tested treatment differences can be considered as normally distributed and, therefore, parametric tests, such as paired t-tests, can be regarded as valid statistical procedures.

The mean QHI difference after treatment between ethanol-free chlorhexidine and ethanol-free placebo was d(QHI) = 2.14 (SD = 0.84) with a two-sided confidence interval CI(CHX,ef – PCB,ef, 95%) = [1.87;2.42]; $p < 0.0001$). This difference can be regarded as

statistical significant (df = 38, t = 16.01,) The standardized effect size, d(QHI, CHX,ef – PCB,ef) = 2.14/0.84 = 2.55 can be regarded as very large on the basis of the rule[1] of Cohen for the evaluation of effects.

Similar results were observed for the ethanol-containing CHX mouth rinsing solution serving as positive control. The difference between ethanol-containing chlorhexidine and the ethanol-free placebo solution was d(QHI) = 2.14 (SD = 0.72) with a two-sided confidence interval CI(CHX,ec – PCB,ef) = [1.91;2.37]. Therefore, this difference can be regarded as statistical significant (df = 39, t = 18.72, p < 0.0001) and is incompatible with the assumption of an equal effect in both treatments. The standardized effect size d(QHI, CHX,ec - PCB,ef 2.14/0.72 = 2.97 is located in a similar range, but has a higher value compared to ethanol-free chlorhexidine, because of reduced variability in the change scores.

The results on a descriptive level show a very similar inhibitory effect on plaque growth by both chlorhexidine solutions. The according standardized effect size of 0.05 can be considered as negligible in clinical terms.

Regarding the secondary outcome, the Gingival Index (GI), the average value in the ITT-population was below 0.01 (SD ≤ 0.05) after all treatments (incl. ethanol-free placebo and baseline), which is considered as not clinically relevant.

Per-protocol Population (PP-Population)

The analysis of the PP-population (N = 33) yielded the following results:
At study start, in the presence of regular mechanical oral hygiene the average QHI score was 0.96 (SD=0.65). Before each treatment period the QHI was set to zero by the professional tooth cleaning procedure.

After 4 days of repeated rinsing and in the absence of mechanical tooth cleaning the recorded average QHI score was 0.79 (SD=0.55) for the ethanol-free CHX mouth rinsing solutions compared to QHI 2.97 (SD=0.69) for the placebo solution (p < 0.00001). The average QHI score recorded for the ethanol-containing CHX mouth rinsing solution was QHI 0.81 (SD=0.44).

QQ-Plots showed that the tested treatment differences can be considered as normally distributed and, therefore, parametric tests, such as paired t-tests, can be regarded as valid statistical procedures.

The mean QHI difference between ethanol-free chlorhexidine and ethanol-free placebo was d(QHI) = 2.19 (SD = 0.68) with a two-sided confidence interval CI(CHX,ef - PCB,ef, 95%) = [1.94;2.42]. This difference can be regarded as statistical significant (df = 32, t = 18.50, p < 0.0001). The standardized effect size, d(QHI, CHX,ef - PCB,ef) = 2.19/0.68 = 3.22 can be regarded as very large on the basis of the rule[1] of Cohen for the evaluation of effects.

Similar results were observed for the ethanol-containing CHX mouth rinsing solution serving as positive control. The difference between the ethanol-containing CHX rinsing solution and the ethanol-free placebo was d(QHI) = 2.16 (SD = 0.46) with a two-sided confidence interval CI(CHX,ec - PCB,ef) = [2.00;2.32]. Therefore, this difference can be regarded as statistical significant (df = 32, t = 27.04, p < 0.0001) and is incompatible with the assumption of an equal effect in both treatments. The standardized effect size d(QHI, CHX,ec - PCB,ef) = 2.16/0.46 = 4.70 is located in an even higher, extreme range, because of very low variability in the change scores.

[1] d = 0.20 as small, d = 0.50 as medium, d = 0.80 as large effect size

The results on a descriptive level show a very similar effect on plaque growth. The according standardized effect size of 0.04 can be considered as negligible in clinical terms.

Regarding the secondary outcome, the Gingival Index (GI), the average value in the intention-to-treat-population was below 0.01 (SD ≤ 0.06) after all treatments (incl. ethanol-free placebo and baseline), which is considered as not clinically relevant.

Safety Results

No serious adverse events were observed within the study population during the observed time course of the study.

Overall 16 adverse reactions have been reported in the study by 13 subjects, with 6 symptoms during the treatment period with the ethanol-free chlorhexidine mouth rinse solution and 10 symptoms when the ethanol-containing chlorhexidine mouth rinse solution was applied. Overall irritation of taste was reported nine times (4x CHX,ef ; 5x CHX, ec), bitter taste was described twice during CHX, ef-treatment, and brown tongue twice during CHX;ec-treatment. Other symptoms like discoloration of teeth, unclean feeling, and sensitive teeth each were only reported once during CHX,ec-treatment.

Conclusions

Twice daily repeated rinsing with the ethanol-free 0.2% CHX mouth rinsing solution in the absence of mechanical tooth cleaning over a period of 4 consecutive days in a study population of healthy volunteers significantly inhibited bacterial regrowth on teeth compared to a CHX-free placebo. Average QHI plaque scores for the ethanol-free CHX solution were clincially equivalent to those recorded for a commerical ethanol-containing CHX mouth rinsing solution (Chlorhexamed® Forte 0.2 %).

Initiation of gingival inflammation expressed as a significant change of the recorded GI scores before and after the 4 day rinsing period was not observed for any of the three experimental mouth rinsing solutions in a study population mostly lacking clinical signs of gingival inflammation at baseline.

Observed adverse reactions were all identical to those known to be typical for the use of ethanol-containing CHX mouth rinsing solutions.

3. Table of Contents

4. List of Abbreviations

AE	Adverse event
AMG	German Drug Law (“Arzneimittelgesetz”)
BfArM	Federal Institute for Drugs and Medical Devices (Bundesinstitut für Arzneimittel und Medizinprodukte)
CHX	Chlorhexidine digluconate [= Chlorhexidinbis(D-gluconat)]
CHX, ec	Ethanol-containing Chlorhexidine mouth rinsing solution
CHX, ef	Ethanol-free Chlorhexidine mouth rinsing solution
CRF	Case Report Form
EC	Ethics Committee
ec	Ethanol-containing
ef	Ethanol-free
GCP	Good Clinical Practice
GI	Gingival Index of Löe and Silness 1963
GSK	GlaxoSmithKline Consumer Healthcare GmbH & Co. KG
ICH	International Conference of Harmonization
PCB	Placebo
PCB, ef	Ethanol-free placebo mouth rinsing solution
QHI	Quigley Hein Plaque Index (Modification of Turesky et al. 1972)
SAE	Serious adverse event

5. Ethics

The design of this study has been approved by a vote of the ethics committee of the University of Wuerzburg, Germany and the Competent Authority according to the German Drug Law (BfArM), prior to the start of the study.
All participating subjects were provided with an Informed Consent Form describing this study and giving sufficient information to make an informed decision about the participation in this study. The consent form was approved by the above mentioned ethics committee. All included subjects gave their written informed consent regarding their participation in the study, prior to their individual study start.
The clinical study was conducted in accordance with applicable German law, the Helsinki Declaration in its version of 1996, and the principles of Good Clinical Practice.

6. Investigators and Administrative Structure of Study

Prof. Dr. med. dent. PPD was the principal investigator PPD of this clinical trial. GlaxoSmithKline Consumer Healthcare GmbH & Co. KG was the sponsor of the study, with Dr. PPD being the responsible project leader. Responsible contact persons of the sponsor were Dr. PPD and Mrs. PPD Mr. PPD was the consultative biometrician and statistician responsible for the data analysis of the trial data.
Study Monitor of the sponsor was Mrs. PPD. The study physicians involved in the practical examinations and data collections were Dr. PPD, Dr. PPD, Dr. PPD, Dr. PPD and Mr. PPD.

7. Introduction

Chlorhexidine digluconate (CHX) containing mouth rinsing solutions are the established gold standard of chemotherapeutic anti-plaque/anti-gingivitis treatment. The efficacy and safety of the use of chlorhexidine in the oral cavity has been comprehensively documented by a wealth of experimental studies.
Typical examples for registered applications of mouthrinses are:
Therapy of gingivitis, disinfection of the oral cavity after interventions of oral surgery, suppression of the oral microflora in the course of a systematic periodontal therapy, substitution of mechanical plaque control at home after jaw fractures or intermaxillary fixation.
The typical application period of mouthrinses containing CHX ranges from a few days to several weeks or months.

Due to the unpleasantly bitter taste of chlorhexidine digluconate taste improving flavouring ingredients, mostly essential oils and solvents such as ethanol are added to commercial chlorhexidine mouth rinsing formulations. There are however some patient groups who may not be exposed to ethanol (e.g. children, alcohol addicts, etc.). Therefore ethanol-free formulations of CHX containing mouth rinsing solutions have been developed. Clinical studies however revealed that the alternatively used solvent

could interfere with the antibacterial efficacy of chlorhexidine resulting in a clinically impaired suppression of the oral microflora when compared to ethanol-containing CHX mouth rinsing solutions.

The established standard to assess mouthrinses in their inhibitory action against the formation of bacterial biofilms on dental surfaces consists in the standardized longitudinal registration of bacterial plaque growth after preceding professional tooth cleaning and in the absence of any kind of dental care. Among these evaluation standards, the Turesky modification of the plaque index according to Quigley and Hein is one which is most commonly used. The accretion of bacterial biofilms is frequently evaluated on the vestibular surfaces of so-called Ramfjord teeth (teeth 16, 21, 24, 36, 42, 44), because numerous studies have demonstrated that they are representative of bacterial population of human dentition (Turesky 1972; Silness 1988; Rams 1993; Kozlovsky 1996; Mumghamba 2004)

8. Study Objective

The purpose of this study was to evaluate the inhibitory effect of a newly formulated ethanol-free 0.2% CHX mouth rinsing solution on plaque regrowth after professional tooth cleaning and in the absence of mechanical plaque control in a population of healthy volunteers.
An ethanol-free placebo solution and an ethanol-containing 0.2% CHX mouth rinsing solution (Chlorhexamed®Forte 0.2 %) served as negative and positive control respectively. Furthermore as a secondary end point of this study the influence of the experimental mouth rinsing solutions on gingival health was evaluated.

9. Investigational Plan

9.1 Overall Study Design and Plan Description

9.1.1 Study Design

For the evaluation of the inhibitory effect of antiseptic oral mouth rinses on microbial growth on teeth a test protocol was chosen, which had been successfully applied in a comparable manner in other preceding clinical studies.
The inhibitory effect of an ethanol-free 0.2% chlorhexidine digluconate mouth rinsing solution, newly formulated by GSK (CHX,ef; Trade name: Chlorhexamed alkoholfrei®) on bacterial regrowth on teeth was assessed in the absence of mechanical tooth cleaning over a period of 4 days in healthy volunteers. A placebo solution (PCB,ef) and a commercially available ethanol-containing 0.2% chlorhexidine digluconate mouthrinse (CHX,ec; Trade name: Chlorhexamed Forte® 0.2 %) served as negative and positive control respectively.

After a 10-day run-in or 10-day wash-out period, the trial subjects used the randomly allocated solutions twice daily (in the morning and in the evening) over four days. The study physician and all persons involved in the study were blinded (single-investigator-blind).

9.1.2 Time Schedule of the Study

The time course of the study design was as follows (see also Table1):

Visit 1 (recruitment):
Recruitment of appropriate volunteers, provision of comprehensive information about the objectives and risks of the clinical study, undersigning the declaration of consent, and scheduling the visiting appointments (Visit 2 to Visit 8).

Visit 2: Day -9 (initial examination):
Ten days prior to the onset of the first treatment (Period A), dental findings were registered and the QHI value as well as the GI value of the Ramfjord teeth was determined after staining the dental plaque with Mira2Ton plaque-staining-solution in all subjects who were found to be eligible to participate in the study and who, after having received comprehensive oral and written information, gave their informed written consent. Afterward, thorough professional cleaning of all teeth was performed by applying an air abrasion tool (EMS Airflow 1; EMS, Nyon, Switzerland) and the ClinPro® cleaning powder containing crystalline glycine (3M Espe, Seefeld, Germany). In addition, unwaxed dental floss and fluoridated „*Dr. Best Multiaktiv*“ toothpaste was handed out to all study participants. Both were to be used for dental care at home during the run-in period and the wash-out periods (10 days each). Moreover, the test subjects received a *„Dr. Best flex plus medium“* toothbrush to be used during the run-in period and the wash-out periods.

Run-In: Day -9 to Day 0 (= 10 days):
Domestic, standardized oral hygiene, twice daily, using only the oral hygiene products provided.

Visit 3: Day 1 (Baseline of Treatment Period A):
The QHI value and the GI value were determined again and professional dental cleaning was repeated (Baseline: QHI=0). Afterwards, the next higher subject number was allocated to each subject (randomization) and the first rinsing solution (labeled with “Period A” and the respective subject number) was handed out.
The participants were instructed to refrain from all personal mechanical dental cleaning in the ensuing 4-day period. Instead, 10mL of the provided rinsing solution (1 bottle cap filled up to the mark) were applied twice daily in the morning and in the evening postprandial (after meals) for the duration of 1 minute each and were then expectorated. The subjects’ first rinsing was monitored and instructions on correct usage were given, when necessary.
The subjects were instructed to report adverse events immediately to the responsible study physician who was assigned to them.

Treatment Period A: Day 1 to Day 4 (= 4 days):

Domestic application of the provided rinsing solution: rinsing with 10mL solution twice daily for 1 minute in the morning and the evening after meals, without mechanical or any other dental care at home.

Visit 4: Day 5 (Evaluation after Treatment Period A):

After the 4-day treatment period, the QHI and GI values of the test subjects' Ramfjord teeth was determined. In addition, any occurrence of adverse events and the intake of co-medication was surveyed and documented.
Residual (unused) study medication was withdrawn.
Subsequently, the trial subjects were requested to resume their regular dental care routines at home (without application of antibacterial mouth-rinsing solutions) in the following 10-day wash-out period. For this purpose, the test subjects received a new *„Dr. Best flex plus medium"* toothbrush.

Wash-Out: Day 5 to Day 14 (= 10 days):

Standardized oral hygiene twice daily using the provided oral hygiene products as during the run-in stage.

Visit 5: Tag 15 (Baseline of Treatment Period B):

Same procedure as in Visit 3 with issuance of the second externally blinded mouth-rinsing solution, which was labeled with the subject number and "Period B".

Treatment Period B: Day 15 to Day 18 (= 4 days):

Application of the second rinsing solution (rinsing with 10mL of the solution, 2x daily for 1 minute, in the morning and in the evening postprandial, no mechanical or any other dental care at home).

Visit 6: Day 19 (Evaluation after Treatment Period B):

Same procedure as in Visit 4 with issuance of a new „Dr. Best flex plus medium" toothbrush.

Wash-Out-: Day 19 to Day 28 (= 10 days):

Standardized oral hygiene twice daily, with the provided oral hygiene products as during the run-in period.

Visit 7: Day 29 (Baseline of Treatment Period C):

Same procedure as in Visit 3 with issuance of the third externally blinded mouth-rinsing solution, which was labeled with the trial subject number and "Period C".

Treatment Period C: Day 29 to Day 32 (= 4 days):

Application of the rinsing solution (rinsing with 10mL of the solution, 2x daily for 1 minute, in the mornings and in the evenings postprandial, no mechanical or any other dental care to proceed at home).

Visit 8 (final visit): Tag 33 (Evaluation after Treatment Period C):

Same procedure as in Visit 4 with ultimate registration of any adverse events that might have occurred, termination of clinical documentation.
A schematic summary of the time table of the study is found in Table 1.

9.1.3 Documentation and Reporting of Adverse events

The safety of 0.2% chlorhexidine digluconate containing antiseptic mouth rinsing solutions has been comprehensively evaluated and documented. Amongst the known adverse reactions accompanying the use of 0.2% chlorhexidine digluconate in mouth rinsing solutions only allergic reactions may have a severe impact on the health of the study subjects. During recruitment of study subjects known allergic or adverse reactions towards the ingredients of the experimental rinsing solutions was an exclusion criterion.

An adverse event may be any unfavorable or unintended reaction (including deviations of clinically relevant laboratory values), any symptom or disease which stand in a time-related connection with the administration of a study medication, regardless whether a causal relationship to the test products exists or not.

All study subjects were instructed to report any adverse events accompanying the use of the experimental rinsing solutions immediately to the responsible study physician. All adverse events had to be documented by the study physicians.

Adverse and serious adverse events being observed during this trial were documented as of the beginning of randomization (Visit 3, Day 1) till the subject's last application of the experimental rinsing solution (Visit 8, Day 33).

All serious adverse events had to be reported within 24 hours to the Qualified Person for Pharmacovigilance (PPD) of the sponsor even if the study medication is not causally related to the event.

All other adverse events were documented on the form sheet in the CRF and will remain in the CRF until the clinical study was finished.

9.1.4 Monitoring

In accordance with the sponsor's requirements, the responsible study monitor visited the trial site during conduction of the study at appropriate times according to the progress of the ongoing study. The monitor verified, that the correct informed consent forms have been signed and dated by all parties prior to beginning of study procedures. Due to the fact, that only healthy volunteers participated in the study historical patients' records were not available. Therefore a full check of the case report forms and available

subjects' data was done. After completion of the treatment phase, the monitoring person visited the trial site in order to verify the source data and to review the completeness of the CRFs before they were used for data entry.

9.2 Discussion of Study Design including choice of control groups

The main purpose of antiseptic oral rinses is the control of plaque-related inflammations in the oral cavity. Amongst those gingivitis is by far the most common disease entitiy, induced by the overgrowth of bacterial biofilm on the tooth surfaces adjacent to the gingival tissues. Basis therapy for gingivitis is the establishment of proper oral hygiene at home by mechanical tooth brushing using a tooth brush, interdental cleaning devices and a dentifrice.
In cases where mechanical tooth cleaning is not possible for the patient or not recommended (e.g. after periodontal surgery) antiseptic oral rinses are used as a substitute. This study was therefore designed to evaluate the plaque accumulation on teeth surfaces adjacent to gingival tissues in a healthy study population of volunteers with established good oral hygiene and little or missing gingival inflammation. Professional tooth cleaning assured comparable baseline conditions for each treatment. A four day rinsing period in the absence of mechanical tooth brushing has been successfully applied in preceding studies on the evaluation of the plaque-inhibiting properties of oral rinses (Arweiler et al. 2006). A frequency of two daily rinses has been established as being sufficient for CHX containing mouth rinses to suppress the formation of biofilms (Brecx et al. 1990). Besides an ethanol- and CHX-free placebo solution with miminal presumed inhibitory effect on plaque growth Chlorhexamed Forte® a commerical 0.2% CHX mouth rinsing was chosen as a positive control. The inhibitory effect of Chlorhexamed Forte® on the growth of oral mico-organisms has been thoroughly documented before. Besides the placebo control Chlorhexamed Forte® therefore served as a reference for the inhibitory potency of the newly formulated ethanol-free CHX mouth rinse.

9.3 Study Outcome Criteria

Primary Outcome Criterion

The main target criterion of the study was the extent of plaque regrowth on the previously professionally cleaned teeth (equal to initial value QHI=0) after a 4 day use of the CHX-rinsing formulations without any additional mechanical or other measures of dental home care. The extent of bacterial plaque regrowth was evaluated using the Turesky modification of the Quigley-Hein plaque index (QHI). The QHI was determined on the vestibular surfaces of the so-called Ramfjord teeth (teeth 16, 21, 24, 36, 42, 44) being representative for the whole dentition. The QHI scores range from 0 to 5 (0 = no plaque, 5 = abundant plaque, covering at least 2 thirds of the crown surface)[2].

[2] QHI scaling options: 0="No plaque", 1="Separate flecks of plaque at the cervical margin of the tooth", 2="A thin continuous band of plaque (up to one mm) at the cervical margin of the tooth", 3="A band of plaque wider than one mm but covering less than one third of the crown of the tooth", 4="Plaque covering at least one-third but less than two thirds of the crown of the tooth", 5="Plaque covering two-thirds or more of the crown of the tooth" (Turesky, Golmore, & Glickman, 1972).

Secondary Outcome Criterion

The secondary target criterion was the eventual development of gingival inflammation associated with the growth of bacterial plaque. The status of gingival health on the vestibular surfaces of the Ramfjord teeth was monitored at baseline visits for each treatment sequence and after the 4-day rinsing periods using the Gingival Index by Löe and Silness, 1963

9.4 Selection of the Study Population

In this study only healthy volunteers aged 18-50 of both genders with good regular oral home care and little if any clinical signs of gingival inflammation were included. The choice was made for the following reasons:
The etiology of periodontal disease and severe gingival inflammations is multifactorial. While for many years bacterial plaque was thought to be the central and exclusive cause of the disease, recent findings have proven, that the varying individual fitness of the host response decisively modifies the inflammatory processes accompanying bacterial biofilms. The increased flow of the protein-rich sulcular fluid provides an important nutritional source for the growth of periodontitis-associated proteolytic micro-organisms. Plaque growth in the absence of individual oral hygiene is significantly higher in patients suffering from periodontal disease than in periodontally healthy controls (Ramberg, et al. 1995). As most of the preceding studies on the inhibitory effect of oral mouth rinsing solutions evaluated periodontally healthy subjects with good general health it was decided to choose healthy volunteers to make the results of this investigation comparable with the findings of previous studies. Furthermore it is established, that the inhibition of bacterial growth in healthy volunteers indicate the efficacy of mouth rinse solution for their intended therapeutic use.

9.4.1 Inclusion Criteria

Volunteers were included in this clinical study only if all of the following inclusion criteria were met:

- the subject's written declaration of informed consent has been given
- the subject possesses the mental capability to understand the study
- the subject is able to comply to the appointment schedule
- the subject is in a good general condition of health
- minimum of 20 natural teeth in the mouth,
- age ≥ 20 ≤ 50 years
- regular dental care at home (tooth brushing twice daily)
- healthy, clinically inconspicuous oral mucosa with the exception of possible gingivitis simplex and/or periodontal recessions

9.4.2 Exclusion Criteria

Volunteers were not eligible for participation in this clinical study if one or several of the following exclusion criteria were applicable:

- participation in a clinical trial within the last 30 days or subject has already participated in this clinical study

- all types of known allergies
- known hypersensitivity to the ingredients of the study medication
- regular use of substances which are likely to influence bacterial growth in the oral cavity and/or the development of gingivitis (4 weeks before and during recruitment):
- locally in the mouth or systemically active antibiotics
- steroidal and und non-steroidal antiphlogistics
- drugs which influence salivation (e.g. antihistaminics, anticholinergics, drugs against hypertension, psychotropics)
- necessity of antibiotic protection prior to dental treatment
- current orthodontic therapy or extended restorations
- Gingival Index score >2
- subject currently or in the past addicted to alcohol
- subject was unable to fully comply with the time table of the study protocol
- scheduled surgical intervention or hospitalization within the next 6 weeks from baseline.

In case of female subjects:

- pregnant or breast-feeding
- woman capable of bearing children AND not applying an adequate method of contraception

9.4.3 Permitted co-medications during the study

- Any medication that does not affect the growth of plaque and/or promote the development of gingivitis.

9.4.4 Non-permitted co-medications during the study

- Any medication that influences the growth of plaques and/or promotes the development of gingivitis, such as:
- Antibiotics acting locally in the mouth or systemically
- Steroidal and und non-steroidal antiphlogistics
- Drugs which influence salivation (e.g. antihistaminics, anticholinergics, drugs against hypertension, psychotropics)
- Commercial oral rinses
- Candy or chewing gums containing essential oils

9.5 Description of the Study Medication

9.5.1 Test solutions

The following mouthrinse solutions were tested:

Test Solution 1: 0.2 % ethanol-free CHX formulation (CHX,ef)

Active pharmaceutical ingredient: 100mL solution containing 0.2g chlorhexidine digluconate
Other ingredients: glycerol, macrogolglycerolhydroxystearate; D-Glucitol, purified water, peppermint flavour

Test Solution 2: 0.2 % CHX ethanol-containing formulation (commercial product: *Chlorhexamed® Forte 0.2%; CHX,ec)*

Active pharmaceutical ingredient: 100mL solution containing 0.2g chlorhexidine digluconate
Other ingredients: ethanol 96%, macrogolglycerolhydroxystearate, D-glucitol, purified water, peppermint oil

Test Solution 3: CHX-free, ethanol-free mouth-rinse solution (placebo; PCB,ef)

Ingredients: glycerol, macrogolglycerolhydroxystearate, purified water, peppermint flavour

Chlorhexidine digluconate (CHX) in a concentration of 0.2% was the active antibacterial ingredient of the ethanol-free oral rinse as well as of the ethanol-containing positive control (Chlorhexamed Forte®). The placebo mouth rinsing solution was free of CHX as well as of ethanol. All experimental rinsing solutions contained macrogolglycerol-hydroxystearate, and peppermint flavour in comparable concentrations.
D-Glucitol was added to the ethanol-free as well as to the ethanol-containing CHX mouth rinse but not to the placebo solution. Glycerol was added to the ethanol-free CHX mouth rinse as well as to the ethanol-free placebo mouth rinse but not to the ethanol-containing CHX mouth rinse.

All three experimental mouth rinsing solutions were colorless and indistinguishable by their appearance. The investigational products were packed in neutral cardboard boxes and labeled according to the random list with the trial subject number and the treatment period A, B, or C as applied to the subject number involving the respective solution. The subject number labeled on the investigational products corresponded with the randomization numbers 1-42.

The lettering on the immediate container (bottle) and the outer packaging (cardboard box) was consistent with the regulations of the German Medicines Act (AMG).

9.5.2 Selected Treatment Dose

The CHX concentration in the ethanol-free CHX mouth rinsing solution as well as in the positive control (Chlorhexamed® Forte 0.2%) was 0.2%. The volume for each of the two daily rinsing episodes was set to 10 mL, being the established standard dosage for the positive control as preceding studies proved that the twice daily repeated administration of 20 mg of chlorhexidine digluconate in an aqueous ethanol-containing mouth rinsing solution results in a sustained suppression of the growth of the oral microflora even in the absence of mechanical tooth brushing (Brecx et al 1990).

9.5.3 Dispatching and reception of study medication

All experimental oral rinses including the bottling, labeling and packaging of the bottles were provided by the sponsor.
Dispatching the investigational products to the trial site by GSK proceeded after the ethics committee of the University of Wuerzburg had stated its approval, the federal superior authority (BfArM) had given its permission, and all documents required according applicable provisions and internal guidelines of the company had been received.
After reception of the study medication, the investigator or an authorized associate took inventory and sent an undersigned note of receipt to GSK. The principal investigator kept a copy of this receipt.

9.5.4 Storage of Study Medication

The study medication was permantly kept under lock and key and out of the reach of unauthorized persons.

9.5.5 Administration of Treatment

Prior to the onset of each treatment period, a sufficient amount of the randomly allocated test solution was issued to each patient. The patients were instructed to return the residual (unused) medication after termination of each treatment stage.
All experimental solutions were self-administered by the study participants after an initial demonstration and supervision by a study physician. An aliquot of 10 mL of the experimental solution was measured using an indicator line in the screw cap of the bottle containing the experimental solution. Subsequently the experimental solution was brought into the oral cavity. After 60 seconds of permanent rinsing the mouth the solution was spitted out again.

9.5.6 Random assignment of the experimental rinsing solutions

As a consequence of the cross-over design each subject received all 3 experimental rinsing solutions for application. In case of this study, randomization relates to the sequence in which the experimental solutions were applied.

Treatment Order	Treatment Period A	Treatment Period B	Treatment Period C
1.	Test solution 1	Test solution 2	Test solution 3
2.	Test solution 1	Test solution 3	Test solution 2
3.	Test solution 2	Test solution 1	Test solution 3
4.	Test solution 2	Test solution 3	Test solution 1
5.	Test solution 3	Test solution 1	Test solution 2
6.	Test solution 3	Test solution 2	Test solution 1

Test solution 1 = CHX, ef; Test solution 2= CHX,ec; Test solution 3=PCB,ef

One of these 6 treatment orders was allocated to each subject number. The allocation proceeded randomly according to a scheme (random list) previously computer-generated by the Internet based program "Research Randomizer" (Urbaniak and Plous, 2007).

On visit 3 (start of treatment period A) each trial subject was randomly assigned by allocation of the consecutive subject number not yet used and the study medication labeled period "A" was handed out.
Before the two ensuing treatment periods the subject received the solution which was labeled with the subject number allocated to him/her and labeled with the respective treatment period ("Period B" or "Period C").

9.5.7 Control of Study Medication

The responsible study physicians recorded all medication issued to the patients in a provided form-sheet.

9.5.8 Return of Study Medication

After completion of the clinical study, the responsible study monitor and the investigator took inventory and all the residual and unused study medication was sent back to the sponsor.

9.6 Data Quality Assurance

All data resulting from this clinical study were documented on case report forms (CRFs) indicating the number and type of measurements or examinations due to for a particular trial subject at any given time point in the course of the trial.
All data were entered immediately after they had been obtained. Without reason, no part of the CRF should be without an entry.

The data were entered and processed without unblinding which subject received which treatment in which study period. Unblinding for the statistical analyses was done after decision on study populations.
Data entry into the computer was performed twice by two independent persons eliminating possible transferring errors.

9.7 Statistical Methods

9.7.1 Determination of Sample Size

The primary research question of this study addressed the superiority of the ethanol-free CHX mouth rinsing solution (= test solution 1) compared to placebo regarding the Quigley-Hein-Plaque Index scores (QHI, modified by Turesky et al. 1972) as the primary endpoint. To have 95% of power to detect a minimal clinically important effect size ∂(QHI) =0,68 using a paired t-test with a 0.05 one-sided level of significance, a (per protocol) sample size of 25 subjects was calculated to be necessary. This effect size compares to a difference in means of d(QHI) = 0.85 of plaque growth with e.g. a placebo effect m_1 = 2.90 and a verum effect m_2 = 2.05 assuming a standard deviation of growth differences sd_{diff} = 1.25.

To allow for changes of the statistical analysis procedures depending on the features of the data (e.g. distribution characteristics) this sample size was increased by a factor of 1.25 to allow for loss of power if non-parametric tests (e.g. Wilcoxon test for dependent samples) had to be applied, yielding a sample size of approximately 32 persons. Furthermore a drop-out rate of 25% was taken into account increasing the overall sample to about 39 subjects. Because of the cross-over design with six different sequences of experimental conditions, a multiple of six was chosen, resulting in the final sample size of 42 participating subjects.

9.7.2 Methods against Bias

Possible biases which might interfere with the validity of the trial were baseline differences, carry over effects and observer biases. To avoid baseline differences the initial assignment of the oral rinses were strictly random after the run-in phase of 10 days. To avoid effects of sequence a cross-over design was applied with a balanced order of experimental conditions. Additionally wash-out periods of 10 days duration before the 3 rinsing periods assured that no carry-over effects took place. Furthermore clinical examiners were blinded regarding the content of the rinsing solutions.

9.7.3 Populations for Analysis

The “safety” and "intent-to-treat" population (ITT population) comprised all trial subjects that were randomized and received at least one dose of the study medication. The values of the completed treatment stages were used in the evaluation, irrespective of whether a test subject completed all treatment periods, or only two or one treatment period.

The "per-protocol" -population (PPT population) consisted of all patients of the ITT population without any relevant violations of the study protocol i.e. those who had properly undergone all 3 treatment periods and had not taken any non-permitted comedications during the study, that may have influenced the QHI.

9.7.4 Statistical Plan

Question 1: Superiority of CHX oral rinse compared to placebo

a) Ethanol-free CHX oral rinse compared to placebo:
The difference of effects of the ethanol-free CHX mouthrinse solution (=test solution 1) vs. placebo will be analysed by applying a one-sided *t*-test for dependent samples on the pre-post-differences of the QH-Index as the primary endpoint. The pre-post-differences of the QHI are computed within the pooled sample receiving ethanol-free CHX-mouthrinse solution and compared with the pre-post-differences of the QHI of the pooled sample applying placebo. Statistical significance will be evaluated using the 95% confidence interval and the *p*-value of the effect (Kazdin, 2002). Furthermore, the standardized effect size ($\delta = m_{verum} - m_{placebo} / s_{diff}$) is estimated as a descriptive index (Cohen, 1988).

Parametric tests are only applied if the distribution requirements, e. g. normal distribution, are met. The distribution characteristics of the QH-Index will be evaluated visually within each sample by histograms and Q-Q plots. In all other cases, these two groups are compared by using the non-parametric Wilcoxon-test ($\alpha = 0.05$, one-sided) for dependent samples.

Furthermore, the gingival index (GI) as a secondary endpoint will be analysed with descriptive statistics and standardized effect sizes.

b) Ethanol-containing CHX oral rinse compared to placebo:

The difference of effects of the ethanolic CHX mouthrinse solution (=test solution 2) vs. placebo will be analysed similarly to Question 1. a).

Both tests will be performed with a type I error of $\alpha = 0.05$ as these questions are regarded as inferentially distinct (Proschan & Waclawiw, 2000).

Question 2: Non-Inferiority of ethanol-free vs. ethanolic CHX mouthrinse solution

The size of the difference of the plaque-inhibiting effects of these two CHX-containing solutions is analysed by descriptive statistics and standardized effect sizes, which are used to evaluate its clinical significance.

Statistical procedures are primarily applied to the intention-to-treat population and secondarily to the per-protocol-population such as defined above. Missing data will be treated according to the available case approach.

9.8 Changes in the Conduct of Study of Planned Analyses

As the analyst is located in a different working environment than specified in the study protocol two modifications from the study protocol have been necessary, which, however, do not bias the results in a progressive way.

Instead of the software package IDV (Datenanalyse und Versuchsplanung, Gauting, Germany) the software package SAS (Version 9.1, SAS Institute Inc. Cary, NC, USA) (SAS Institute Inc., 2007) has been used, as the analyst did not have access to the software mentioned in the study protocol. The second modification refers to the computation of the hypothesis tests and confidence intervals which could not be performed as one-sided (cf. study protocol) but as two-sided as these procedures will only be available SAS, Version 9.2 (and higher) (Castelloe & Tobias, 2006). However, these changes can only affect the inferential statistical results in a conservative way.

10. Results

10.1 Study populations

In total 44 subjects were enroled in the study. There were two screening failures, as one subject did not fullfil the elegibility criteria at Visit 1 and one subject finished the study before randomisation (Visit 3) due to sudden time problem making it impossible for the trial subject to follow the time schedule.
42 subjects were randomised and treated and were assigned to the ITT-Population.

The PP-Population consisted of 33 individuals due to 3 Drop-outs and 6 major protocol violations with possible influence on the main efficacy criterion QH-plaque index. The exclusion of individuals from PP-analysis was decided by the study responsibles Prof. Dr. PPD and Dr. PPD before unblinding.

Disposition of study subjects is presented in Figure 1.

Drop-outs (n=3):

- One subject dropped out, during treatment period A due to resumed tooth brushing induced by feeling of uncleanliness in the mouth, and therefore had not received CHX,ef and PCB (PPD).
- Two subjects lost to follow up after treatment period B (PPD). One did not receive CHX,ef, the other did not receive the PCB solution.

Therefore 39 individuals had received all three test solutions, 1 subjects had received both CHX solutions, but no placebo rinse, another subject had received the ethanol-containg CHX solution and the placebo rinse, but not the ethanol-free CHX solution. The third drop-out subject had only used the CHX, ec- solution (see Figure 1).

Major protocol violations (n=6):

- In one case the subject's traveling bag with the study medication of period C in it was stolen. Therefore the volunteer could not complete the treatment period (PPD).
- One subject took Cetirizin against hay fever. Due to the common side effect xerostomia of this substance, the antibacterial effect of saliva may be reduced and therefore the bacterial plaque formation being increased (PPD).

- Two subjects were not compliant with at least one rinsing less during one treatment period. A medication pause of longer than 12 hours may enhance bacterial growth and therefore non-compliance influences the QHI (PPD).
- One subject used azithromycine from wash-out-day 1 to wash-out-day 3 before treatment period B. As this macrolid has a long elimination period influence on bacterial plaque development can not be excluded (PPD).
- One subject brushed her teeth immediately before examination of the QHI after treatment period A, and this resulted in a misleading low QHI (PPD).

Minor protocol violations:

In several cases the non-permitted drugs ASS or Ibuprofen were used during wash-out or rinsing periods by the study volunteers. As the use of steroidal and und non-steroidal antiphlogistics was excluded only because of a possible influence on the secondary criterion gingiva index, these cases were judged as minor violations without any impact on the main efficacy criterion QHI.

Other deviations from the original protocol were confined to variations in the time frame between baseline and the actual time of recording the QHI and GI scores in some trial subjects. For unforseeable appointment problems these study participants could not be examined after 4 days = 4x 24 h = 96 h. Instead the actual time frame varied in some subjects between 92 h and 100 h, a difference too small to induce a major shift in the recorded QHI or GI scores.

10.2 Demographics

42 dentistry students and employees of the dental school participated in this study (intention-to-treat population). The 12 (29%) male and the 30 (71%) female subjects were of similar age with a mean age of 25.6 (SD = 3.3) years in men and 25.8 (SD = 6.6) years in women (Min(age)= 20, Max(age) = 50 in the overall sample). At the start of the run-in-period the mean initial Quigley-Hein Index score of the study population was 0.91 (SD = 0.66), the mean initial Gingival Index (GI) score was 0.01 (SD = 0.05) .

The per-protocol population consisted of 33 individuals with 9 men (27%) and 24 (73%) women. Mean age of men, 25.8 (SD = 3.8) years, and women, 26.1 (SD = 7.00), were similar to the values recorded for the overall sample (ITT). At the start of the run-in-period the mean initial Quigley-Hein Index score of the PP population was 0.96 (SD = 0.65) , the mean initial Gingival Index (GI) score was 0.01 (SD = 0.06) .

After a professional tooth cleaning the volunteers had to carry out standardized oral hygiene at home during the 10-day run-in/wash-out periods prior to each treatment sequence by twice daily using the provided oral hygiene products .

The QHI-scores after wash-out and before the different treatment sequences are presented in table 2 and table 3, for both study populations.

10.3 Efficacy Results

10.3.1 ITT-Analysis

The intention-to-treat population (N = 42) included all randomized subjects. The QHI results for all treatments are presented in table 4.

The analysis yielded the following results: The distributional assumption of normality of the treatment differences has been examined graphically using Quantil-Quantil (QQ) plots such as recommended by Field (Field, 2005). They showed that the tested treatment differences can be considered as normally distributed and, therefore, parametric tests, such as paired t-tests, can be regarded as valid statistical procedures.

The mean QHI difference between ethanol-free chlorhexidine and ethanol-free placebo was d(QHI) = 2.14 (SD = 0.84) with a two-sided confidence interval CI(CHXEF - PCBEF, 95%) = [1.87;2.42]. This difference can be regarded statistically significant (df = 38, t = 16.01, p < 0.0001) and, therefore, is incompatible with the assumption of an equal effect in both treatments. The standardized effect size, d(QHI, CHXEF - PCBEF) = 2.14/0.84 = 2.55 can be regarded as very large on the basis of the rule[3] of Cohen (Cohen, 1988) for the evaluation of effects (Figure 2, Table 5).

Similar results were observed for the positive control solution (chlorhexidine, ethanol-containing). The difference between ethanol-containing chlorhexidine and the ethanol-free placebo solution was d(QHI) = 2.14 (SD = 0.72) with a two-sided confidence interval CI(CHXEC - PCBEF) = [1.91;2.37]. Therefore, this difference can be regarded as statistical significant (df = 39, t = 18.72, p < 0.0001) and is incompatible with the assumption of an equal effect in both treatments. The standardized effect size d(QHI, CHXEC - PCBEF) = 2.14/0.72 = 2.97 is located in a similar range, but has a higher value compared to ethanol-free chlorhexidine, because of reduced variability in the change scores (Figure 2, Table 5).

The equivalence of both chlorhexidine-based mouth rinsing solutions has not been evaluated in terms of hypothesis testing. On a descriptive level the results show a similar effect on plaque growth d(QHI, CHXEC-CHXEF) = 0.021 (SD = 0.47). The pertinent standardized effect size d(QHI, CHXEC, CHXEF) = 0.021/0.47 = 0.05 can be considered as negligible in clinical terms, although a final proof of statistical significance of equivalence can not be given within the scope of this study (Figure 2, Table 5).

Regarding the secondary outcome, the Gingival Index (GI), the average value in the intention-to-treat-population was below 0.01 (SD ≤ 0.05) after all treatments (incl. ethanol-free placebo and baseline), which is considered as not clinically relevant.

10.3.2 PP-Analysis

The per-protocol population (N = 33) included all subjects without any major protocol violation (see chapter 10.1. “study populations”). The QHI results for all treatments are presented in Table 6.

[3] d = 0.20 as small, d = 0.50 as medium, d = 0.80 as large effect size

The analysis yielded the following results: QQ-Plots showed that the observed treatment differences can be considered as normally distributed and, therefore, parametric tests, such as paired t-tests, may be used for a valid data analysis.

The mean QHI difference between ethanol-free chlorhexidine and ethanol-free placebo was d(QHI) = 2.19 (SD = 0.68) with a two-sided confidence interval CI(CHXEF - PCBEF, 95%) = [1.94;2.42]. This difference can be regarded as statistical significant (df = 32, t = 18.50, p < 0.0001) and, therefore, is incompatible with the assumption of an equal effect in both treatments. The standardized effect size, d(QHI, CHXEF - PCBEF) = 2.19/0.68 = 3.22 can be regarded as very large on the basis of the rule[4] of Cohen (Cohen, 1988) for the evaluation of effects (Figure 3, Table 7).

Similar results were observed for the positive control (chlorhexidine, ethanol-containing). The difference between ethanol-containing chlorhexidine and the ethanol-free placebo solution was d(QHI) = 2.16 (SD = 0.46) with a two-sided confidence interval CI(CHXEC - PCBEF) = [2.00;2.32]. Therefore, this difference can be regarded as statistical significant (df = 32, t = 27.04, p < 0.0001) and is incompatible with the assumption of an equal effect in both treatments. The standardized effect size d(QHI, CHXEC - PCBEF) = 2.16/0.46 = 4.70 is located in an even higher, extreme range, because of very low variability in the change scores (Figure 3, Table 7).

The equivalence of both chlorhexidine-based mouth rinsing solutions has not been evaluated in terms of hypothesis testing. On a descriptive level the results show a similar effect on plaque growth d(QHI, CHXEC-CHXEF) = 0.020 (SD = 0.52). The according standardized effect size d(QHI, CHXEC, CHXEF) = 0.020/0.52 = 0.04 can be considered as negligible in clinical terms, although a final proof of statistical significance of equivalence can not be given within the scope of this study (Figure 3,Table 7).

Regarding the secondary outcome, the Gingival Index (GI), the average value in the intention-to-treat-population was below 0.01 (SD ≤ 0.06) after all treatments (incl. ethanol-free placebo and baseline), which is considered as not clinically relevant.

10.3.3 Safety Results

No serious adverse events were observed in the course of the clinical trial.

During the washout periods 20 adverse events were reported by 16 individuals (Table 2). 19 subjects reported about 24 adverse events during the treatment periods, with 16 of them being judged as related to the study medication (Tables 8 and 9).

Overall 16 adverse reactions have been reported in the study by 13 subjects, with 6 symptoms occurring during the treatment period with ethanol-free chlorhexidine mouth rinse solution and 10 symptoms occurring when ethanol-containing chlorhexidine mouth rinse solution was applied (Table 10). Overall taste irritation was reported nine times (4x CHX,ef ; 5x CHX, ec), bitter taste was described twice during CHX, ef-treatment, and the development of a brown tongue twice during CHX,ec-treatment. Other symptoms like discoloration of teeth, sensitive teeth and unclean feeling occurred each only once during CHX,ec-treatment, whereas the latter may have been caused rather by the study procedure.

The observed reactions for both CHX solutions were transiently and not serious and did not diverge from the known risks of chlorhexidine-containing preparations.

[4] d = 0.20 as small, d = 0.50 as medium, d = 0.80 as large effect size

11. Discussion and overall conclusions

The purpose of this study was to examine the inhibitory effect of an ethanol-free 0.2% chlorhexidine digluconate (CHX) mouthrinse on bacterial plaque growth after professional tooth cleaning in the absence of domestic oral hygiene, in comparison to the established clinical standard of an ethanol-containing commercial CHX mouth rinsing solution (Chlorhexamed® Forte 0.2%) as well as an ethanol-free placebo solution. The 0.2% ethanol-free CHX mouthrinse to be tested merely differs from the ethanol-containing reference product Chlorhexamed® Forte 0.2% in the composition of its excipients.

Within the confines of the applied study protocol the use of the newly formulated ethanol-free 0.2% CHX mouth rinsing solution resulted in a significant inhibition of plaque regrowth in the absence of mechanical tooth cleaning compared to the placebo solution.
A statistically proven equivalence of both tested CHX-solutions was not in the scope of this study. But data analysis revealed only marginal, if any, differences in the observed mean QHI scores. Therefore, from a clinical viewpoint both solution may be regarded as equally effective and the ethanol-free CHX mouth rinsing solution therefore may be used for the same indications as the ethanol-containing CHX formulation. Additionally the lack of ethanol allows the administration in patient groups not compatible with the administration of ethanol-containing drugs (e.g. alcohol addicts...)
Observed adverse events with a causal relationship to the use of the ethanol-free formulation as judged by the investigator were all identical to those known to be typical for the use of ethanol-containing CHX mouth rinsing solutions.
In conclusion the evaluated ethanol-free 0.2 %- CHX mouth rinsing solution may effectively substitute the use of the proven ethanol-containing 0.2% CHX formulation without limitations for effectively inhibiting bacterial biofilms in the oral cavity and the treatment of plaque-induced gingivitis.

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13. Tables and Figures

	Visit 1	Visit 2	Run-in	Visit 3	Treatment A	Visit 4	Wash-out	Visit 5	Treatment B	Visit 6	Wash-out	Visit 7	Treatment C	Visit 8 (final visit)
Days of study	**up to-9**	**-9**	**-9 to 0**	**1**	**1-4**	**5**	**5-14**	**15**	**15-18**	**19**	**19-28**	**29**	**29-32**	**33**
Informed consent	x													
Eligibility criteria (=randomization criteria at Visit 3)	x			x										
Demographical data	x													
Dentistry findings		x												
Determination of QHI value		x		x		x		x		x		x		x
Determination of GI value	x	x		x		x		x		x		x		x
Professional tooth cleaning		x		x				x				x		
Issuance of material for dental care at home (dental floss, toothpaste)		x												
Issuance of a toothbrush		x				x				x				
Regular dental care at home			x				x				x			
Mouth-rinsing instructions				x				x				x		
Domestic treatment (mouth rinsing with assigned test solution 2x daily) without applying any other dental care					**x**				x				x	
AE registration						x		x		x		x		x
Registration of co-medication						x		x		x		x		x
Issuance of study medication				x				x				x		
Receipt of residual study medication						x				x				x

Table 1: Study Flow Chart

	N	Mean	Std Dev	Median	Minimum	Maximum
CHX, ef	40	0.81	0.56	0.67	0.17	3.16
CHX, ec	42	0.77	0.56	0.50	0.00	2.50
PCB, ef	40	0.67	0.44	0.58	0.00	2.00

Table 2: Quigley Hein plaque index (QHI) after wash-out periods with standardized domestic dental care (ITT-Population). CHX,ef=Chlorhexidine, ethanol-free; CHX,ec=Chlorhexidine, ethanol-containing; PCB, ef=Placebo,ethanol-free

	N	Mean	Std Dev	Median	Minimum	Maximum
CHX, ef	33	0.82	0.60	0.67	0.17	3.17
CHX, ec	33	0.80	0.59	0.67	0.00	2.50
PCB, ef	33	0.70	0.47	0.67	0.00	2.00

Table 3: Quigley Hein plaque index after wash-out periods with standardized domestic dental care (PP-Population). CHX,ef=Chlorhexidine, ethanol-free; CHX,ec=Chlorhexidine, ethanol-containing; PCB, ef=Placebo,ethanol-free

	N	Mean	Std Dev	Median	Minimum	Maximum
CHX, ef	40	0.78	0.55	0.67	0.17	2.33
CHX, ec	41*	0.78	0.47	0.83	0.00	1.67
PCB, ef	40	2.93	0.89	3.00	0.00	4.33

Table 4: Quigley Hein plaque index after a 4-day rinsing sequence without any further oral hygiene measures. (ITT-Population). CHX,ef=Chlorhexidine, ethanol-free; CHX,ec=Chlorhexidine, ethanol-containing; PCB, ef=Placebo, ethanol-free (see figure 2).

* 42 subjects had been randomized to the CHX, ec-treatment, but one subject dropped out during period A and the QHI could not be evaluated.

	N	Mean	Std Dev	Median	Minimum	Maximum
PCB, ef - CHX,ef p < 0.0001	39	2.14	0.84	2.33	-0.50	3.67
PCB, ef - CHX,ec p < 0.0001	40	2.14	0.72	2.25	-0.50	3.50
CHX,ef - CHX, ec	40	-0.02	0.47	0.00	-1.17	1.00

Table 5: Differences of the primary criterion Quigley Hein plaque index after a 4-day rinsing sequence without any further oral hygiene measures. (ITT-Population). CHX,ef=Chlorhexidine, ethanol-free; CHX,ec=Chlorhexidine, ethanol-containing; PCB, ef=Placebo,ethanol-free.

	N	Mean	Std Dev	Median	Minimum	Maximum
CHX, ef	33	0.79	0.55	0.67	0.00	2.33
CHX, ec	33	0.81	0.44	0.83	0.00	1.67
PCB, ef	33	2.97	0.69	3.00	0.00	4.33

Table 6: Quigley Hein plaque index after a 4-day rinsing sequence without any further oral hygiene measures. (PP-Population). CHX,ef=Chlorhexidine, ethanol-free; CHX,ec=Chlorhexidine, ethanol-containing; PCB, ef=Placebo,ethanol-free (see figure 3).

	N	Mean	Std Dev	Median	Minimum	Maximum
PCB, ef - CHX,ef p < 0.0001	33	2.19	0.68	2.33	0.83	3.67
PCB, ef - CHX,ec p < 0.0001	33	2.16	0.46	2.17	1.50	3.17
CHX,ef - CHX, ec	33	-0.02	0.52	0.00	-1.17	1.00

Table 7: Differences of the primary criterion Quigley Hein plaque index after a 4-day rinsing sequence without any furhter oral hygiene measures (PP-Population). CHX,ef=Chlorhexidine, ethanol-free; CHX,ec= Chlorhexidine, ethanol-containing; PCB, ef=Placebo,ethanol-free.

Adverse Events	1st wash-out period	2nd wash-out period	TOTAL
Headache	1	6	7
Common cold	4	1	5
Migraine	1	1	2
Cold	1	0	1
Fever	1	0	1
Aphthous ulcers	1	0	1
Herpes labialis	1	0	1
Oral lesion after brushing	1	0	1
Swelling of tongue papilles	1	0	1
TOTAL	12	8	20

Table 8: Adverse events (AEs) during wash-out periods have been reported by 16 subjects, without any causal relationship to the study or the study medications.

Adverse Event	CHX, ef	CHX, ec	PCB, ef	TOTAL
Headache	1	2	1	4
Common cold	0	1	0	1
Allergic rhinitis	0	1	0	1
Back pain	0	0	1	1
Fracture of filling tooth 36	0	1	0	1
TOTAL	1	5	2	8

Table 9: Adverse events (AE) during the treatments, without any causal relationsship to the study medications. Note: CHX = chlorhexidine, ef = ethanol-free, ec = ethanol-containing, PCB = Placebo.

The 8 events were reported by 7 subjects: PPD reported "back pain" during the placebo treatment period and "headache" during the CHX, ef - treatment period.

Adverse Reaction	CHX, ef	CHX, ec	PCB, ef	TOTAL
irritation of taste	4	5	0	9
bitter taste	2	0	0	2
discoloration of teeth	0	1	0	1
brown tongue	0	2	0	2
unclean feeling	0	1	0	1
sensitive teeth	0	1	0	1
TOTAL	6	10	0	16

Table 10: Adverse reactions (ADR) during the treatments with causal relationship to the study medication, as judged by the investigator. Note: CHX = chlorhexidine, ef = ethanol-free, ec = ethanol-containing, PCB = Placebo.

The 16 drug reactions were reported by 13 subjects: PPD reported "irritation of taste" during both active treatments; two subjects reported two events during the CHX, ec - treatment period: PPD: "irritation of taste" and "sensitive teeth" and PPD: "discoloration of teeth" and "brown tongue" .

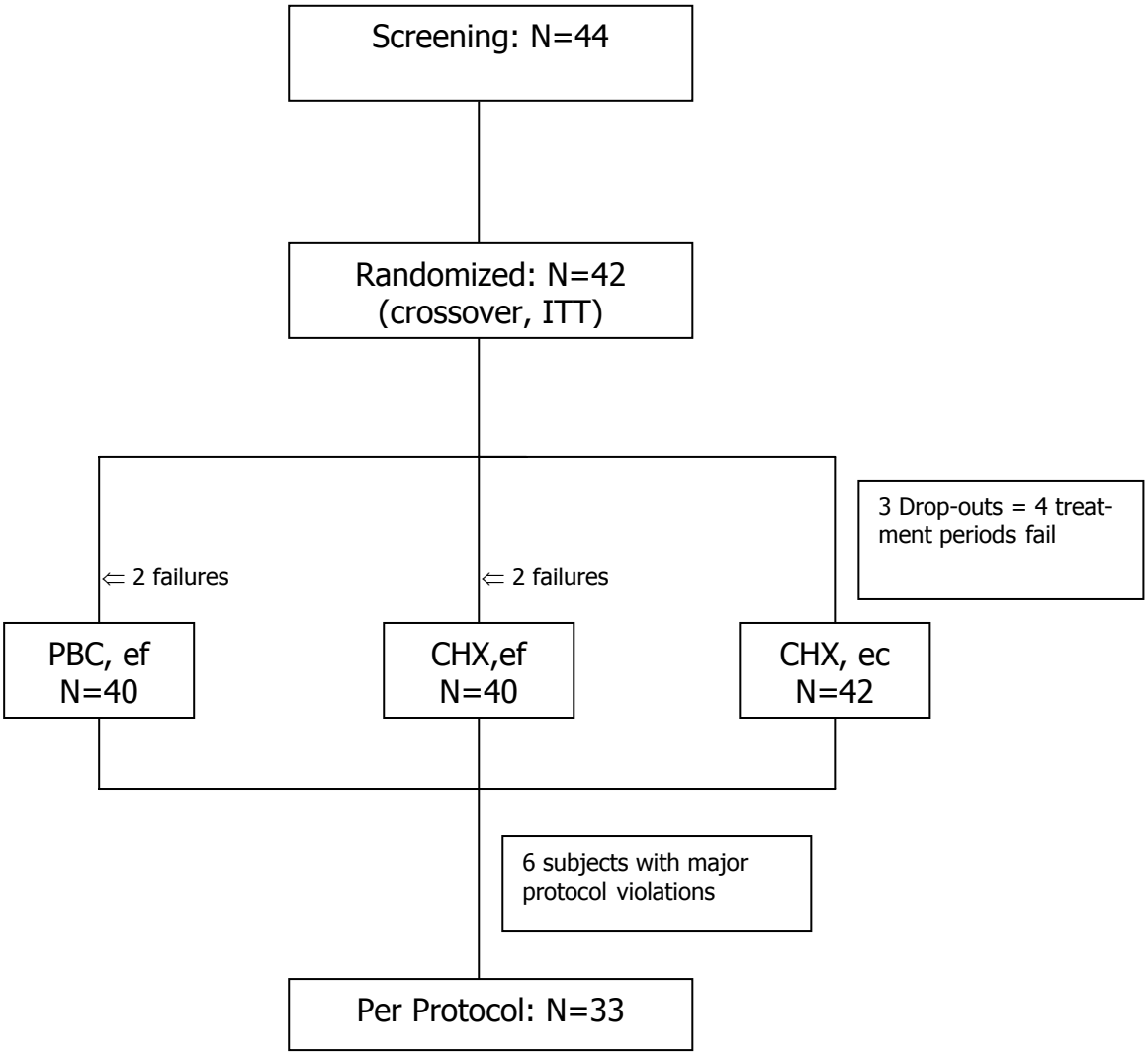

Figure 1: Disposition of Study Subjects

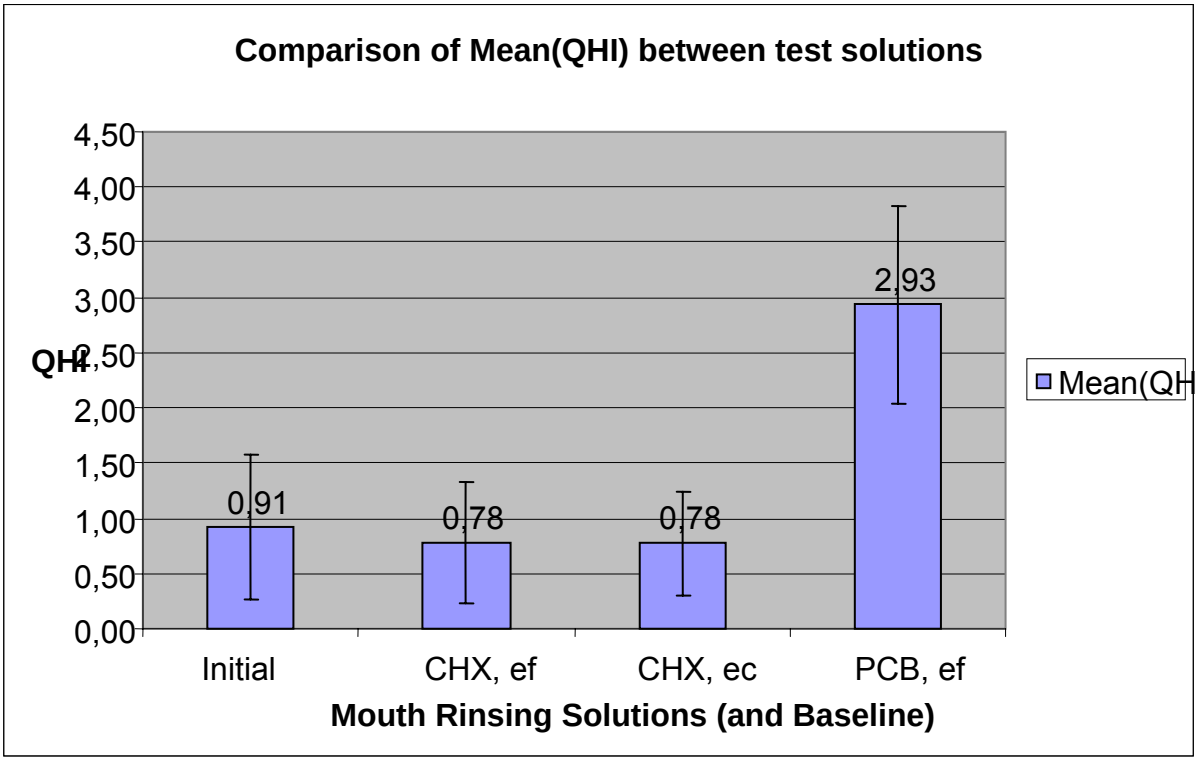

Figure 2: Mean Quigley-Hein Index (QHI) scores before the start of the run-in phase (initial) and after the use of the different mouthrinse solutions (intention-to-treat population); Note: CHX = chlorhexidine, ef = ethanol-free, ec = ethanol-containing, PCB = Placebo; The bars indicate the standard deviations.

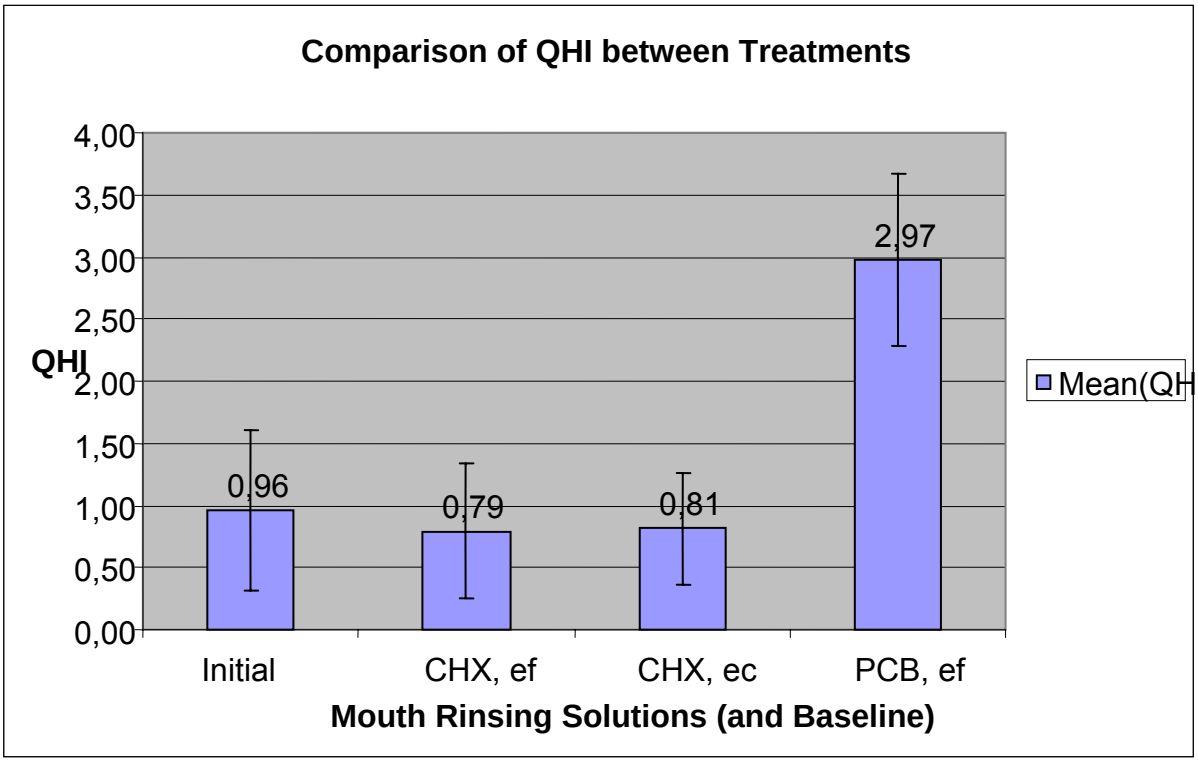

Figure 3: Mean Quigley-Hein Index (QHI) scores before the run-in phase (Initial) and after application of the different mouthrinse solutions (per-protocol population); Note: CHX = chlorhexidine, ef = ethanol-free, ec = ethanol-containing, PCB = Placebo; The bars indicate the standard deviations.