
PGRM - SnF₂ and CPC

2.D.1. The Plaque Glycolysis and Regrowth Model (PGRM) Performance Test (Appendix 1)

2.D.1.1. Scientific Basis of the PGRM Test

The *ex vivo* Plaque Glycolysis and Regrowth Model (PGRM) was initially developed as an R&D screening test which was previously described to the Subcommittee during its deliberations. Based on the Plaque Subcommittee recommendation, Procter & Gamble has optimized the testing procedures to establish the PGRM as a performance test for monograph purposes.

PGRM is a model to assess the *in vivo* therapeutic biological activity of antiplaque and antigingivitis agents with broad spectrum antimicrobial activity that includes generalized actions on glycolysis response of overnight *de novo* plaque biofilms.²⁷ The model is uniquely designed in that it ensures that topical treatment of plaque occur *in vivo*, hence plaque is treated *de novo*, as in the clinical situation as an intraoral biofilm. The model permits the sampling of treated plaques at timed intervals, following dentifrice or rinse exposure, thereby permitting an assessment of the retained activity of antimicrobials post-treatment. Lastly, the model uses non-treated plaque samples taken from subjects to serve as internal control for treatment comparisons. The model is classified as '*ex vivo*' because treated and untreated plaques are sampled and then examined *in vitro* under standardized conditions for comparative evaluations of *in vivo* treatment effects. A critical feature of the model involves the normalized testing of *in vivo* developed and vital dental plaque biofilms for antibacterial effects of dentifrice and mouthrinse formulations applied *in situ*.

²⁷ White, D.J. et al.: A New Plaque Glycolysis and Regrowth Method (PGRM) for the *In Vivo* Determination of Antimicrobial Dentifrice/Rinse Efficacy Towards the Inhibition of Plaque Growth and Metabolism—Method Development, Validation and Initial Activity Screens. *J. Clin. Dent.* 6 (Special Issue), pp. 59-70, 1995.

The method in principle allows for multiple analytical characterization of *in situ* antiplaque/antibacterial effects of topical formulations including assessments of live/dead bacterial populations, regrowth or matrix reproduction capabilities of treated biofilms and metabolic activity of treated biofilms. The glycolysis portion of the test assesses the ability of treated biofilm bacteria to uptake and metabolize dietary sugar to produce acidic end products which are easily assayed either as pH reduction in media buffer or by assessments of acids produced. The acid portion of the test offers a convenient, specific and sensitive target for evaluating the formulation activity, which is of primary interest in establishing equivalence of formulation variations, and thus verifying clinical effectiveness. Importantly, the assay has been shown to correlate strongly with the clinical gingivitis and bleeding scores of several products that have similar clinical outcomes.

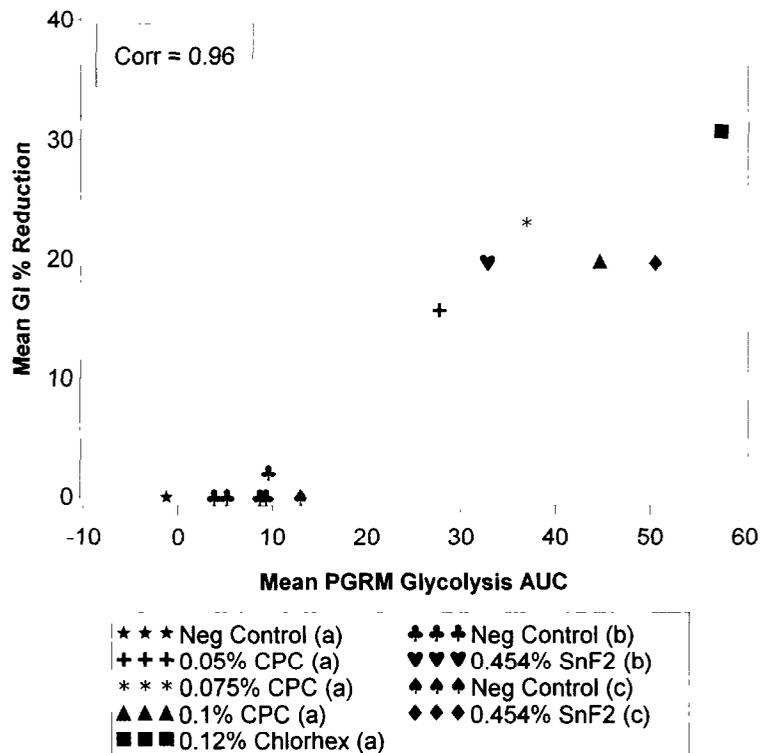
Obviously to use the PGRM acid reduction portion as a bioequivalence marker, it is important that the antimicrobial exhibit strong properties in this regard, which is true for stannous fluoride and CPC. Alternate efficacy endpoints such as microbial composition, bacterial regrowth activity, exopolysaccharide synthesis, volatile sulfur generation, peptide catabolism can also be applied in PGRM testing although the generic acid metabolic activity of the assay is most easily suited to generic formulation screening for stannous fluoride and CPC and is thus recommended herein.

2.D.1.2 Statistical Methods for the PGRM Test

With respect to the statistical interpretation of PGRM performance data, the approach should be one that passes effective antigingivitis products and fails ineffective products. The high positive correlation between clinical gingivitis reduction and PGRM results (Figure 3) suggests that SnF₂ dentifrice and CPC rinse test products that demonstrate positive PGRM glycolysis results are an excellent predictor of a clinical gingivitis benefit. The specific pass/fail performance rules for

SnF₂ (and CPC) test products can be established using existing statistical methodologies for non-inferiority and equivalence.

Figure 3 Percent gingivitis reductions as a function of PGRM glycolysis AUC. Treatments include a 0.12% chlorhexidine rinse, 0.05% - 0.1% CPC rinses, stabilized 0.454% SnF₂ dentifrices, and relevant negative controls.



(a) rinse treatments, (b) dentifrice treatments – PGRM brushing protocol, and (c) dentifrice treatments – PGRM slurry protocol

For clinical equivalence testing, often a placebo will have a small response relative to the positive control. As such, a placebo will often be included in the research. Equivalence can then be determined by demonstrating that a test formulation mean is within a pre-specified range from the positive control reference formulation. That range is often based on the difference between the reference and placebo means. If the objective of the research is to determine true equivalence then the test is two-sided. If the objective of the research is to determine non-inferiority of the test product to the

reference product then the test is one-sided and the acceptable range is termed the “non-inferiority margin”. This approach is not uncommon in the healthcare research industry, including the asthma and cardiac therapeutic areas.^{28,29,30}

For clinical non-inferiority testing, we recommend an approach that sets a meaningful non-inferiority margin by directly incorporating the positive and negative control means in the test. This recommendation is consistent with that of the Non-inferiority Fluoride Test (NIFT) in the recent CHPA/CTFA Anticaries Task Group response to the FDA call for data³¹ supporting the Intra-Oral Appliance (IOA) models as a substitute for the animal caries reduction (“rat caries models”) biological testing currently required as a performance test by the OTC Anticaries monograph. This approach has three treatments in a single study that includes a test product, a reference product (positive control), and a negative control. Each of these requirements can be assessed using an appropriate analysis of variance. Since all of the following criteria must be simultaneously met, no multiple comparison adjustments are required.

1. The reference product mean must be statistically superior to that of the negative control (two-sided 5% type I error rate)
2. The test product mean must be statistically superior to that of the negative control product (two-sided 5% type I error rate)

²⁸ R. B. D’Agostino Sr., J. M. Massaro and L. M. Sullivan (2003). “Non-inferiority trails: design concepts and issues – the encounters of academic consultants in statistics” *Statist. Med.* 22, 169-186.

²⁹ G. Gupta, H. Hsu, T. Ng, J. Tiwari and C. Wang (1999). “Statistical Review Experiences in Equivalence Testing at FDA/CBER” *American Statistical Association Proceedings of the Biopharmaceutical Section* 220-223.

³⁰ A. F. Ebbutt and L. Frith (1998). “Practical Issues in Equivalence Trials” *Statist. Med.* 17, 1691-1701.

³¹ *Federal Register*: 66, October 15, 2001, pp.52418-52420.

3. The test product mean must be demonstrated to be statistically superior to the average of the negative control mean and the reference product mean (one-sided 5% type I error rate).

The first criteria validates the study by requiring the statistical superiority of the positive control compared to the negative control. The second criteria ensures that the test product to be statistically more effective than the negative control. The third criteria ensures that the test product is significantly more similar to the positive control than the negative control.

As the sponsor of the PGRM performance test, Procter & Gamble proposes that the statistical approach taken for NIFT be reapplied to evaluate PGRM performance of SnF₂ dentifrice products. As described in Section 2.D, glycolysis and regrowth inhibition will be used as primary and secondary endpoints, respectively, for establishing bioactivity of SnF₂. As with NIFT, no multiple comparison adjustments are required because all of the following criteria must be simultaneously satisfied.

2.D.1.3. Proposed Effectiveness Criteria for PGRM Testing

The *ex vivo* Plaque Glycolysis and Regrowth Model (PGRM) is a predictive model to assess the biological activity of anti-plaque and anti-gingivitis agents, particularly SnF₂ and CPC. This model involves the measurement of the effectiveness of topical antimicrobials in reducing the metabolism and regrowth of dental plaque following *in situ* treatment of biofilms and timed sampling and fermentation of dispersed planktonic suspensions of the treated biofilms. The glycolysis assay basically provides a simple and sensitive measurement endpoint - the change in media pH - associated with activity of treated plaques to metabolize dietary sugars to acids. In addition, the model also provides data on regrowth inhibition of the plaque mass and has been adapted for the assay of other susceptible pathways for plaque inhibition. It is our experience that for antimicrobials with broad anti-metabolic potential, the glycolysis pathway offers

the simplest target for evaluating antimicrobial activity owing to the preponderance of organisms exhibiting this activity in dental plaque.

Although both glycolysis and regrowth are correlated with gingivitis and gingival bleeding, glycolysis demonstrates the stronger correlation. This is expected given that bacterial regrowth depends upon coordination of multiple cellular systems. This inherently increases the potential for greater variability in bacterial regrowth when compared to metabolic pathways, such as glycolysis, which are dependent upon far fewer cellular processes.

Because glycolysis is subject to less variation and is more strongly correlated with gingivitis efficacy, we recommend that PGRM performance testing place greater emphasis on the primary mechanism glycolysis than on the secondary mechanism regrowth. Consistent with this approach, we recommend that the following criteria be established for PGRM performance testing:

Proposed Glycolysis Activity Requirements (Primary Measure)

1. The positive control treatment must exhibit statistically greater mean inhibition than the negative control treatment (two-sided 5% type I error rate).
2. The test treatment must exhibit statistically greater mean inhibition than the negative control treatment (two-sided 5% type I error rate).
3. The test treatment must exhibit statistically greater mean inhibition than the average of the reference and negative control treatment mean inhibition (one-sided 5% type I error rate)*.

* In practice this criteria is satisfied if the 95% lower confidence bound on $\mu_{\text{test}} - 1/2(\mu_{\text{pc}} + \mu_{\text{nc}})$ is greater than zero, where μ_{test} , μ_{pc} , and μ_{nc} are the true means for the respective test, positive control, and negative control treatments.

Proposed Regrowth Activity Requirements (Secondary Measure)

1. The positive control treatment must exhibit statistically greater mean inhibition than the negative control treatment (two-sided 5% type I error rate).
2. The test treatment must exhibit statistically greater mean inhibition than the negative control treatment (two-sided 5% type I error rate).

Each of these requirements can be assessed using analysis of variance appropriate for the study design followed by treatment contrasts. Test SnF₂ treatments are dentifrices; the positive control treatment is a stabilized SnF₂ 0.454% dentifrice and the negative control treatment is a regular anticaries dentifrice. Test CPC treatments are rinses; the positive control treatment is a 0.036% CPC in deionized water (360ppm bioavailable CPC rinse), i.e. a 0.05% CPC rinse that is 72% bioavailable, and the negative control treatment is deionized water.

2.D.1.4. Reference Product for PGRM Performance Testing

Irrespective of the test procedure used, in order for a final formulation to be accepted as effective, it must be demonstrated that it is non-inferior to an adequate positive control as well as superior to a negative control. It is therefore important that supplies of well characterized positive control products, substantially identical to the clinically tested products used to secure Category I status, be made generally available. Procter & Gamble is willing to work with U.S. Pharmacopoeia representatives to establish and supply finished product reference standards needed for PGRM performance testing of SnF₂ dentifrices under the OTC Antigingivitis/Antiplaque Monograph.