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Evaluation of Chair-Side Assays in High Microbiological Caries-Risk Subjects

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The aim of this study was to evaluate the commercial chair-side assays Saliva-Check Mutans and Clinpro™ Cario L-Pop™ in high microbiological caries-risk dental students compared with conventional semi-quantitative colony counting culture-based technique as the reference method. Saliva samples from 93 subjects of both sexes aged 18-26 years were seeded (Köhler and Bratthall method) on plates containing SB-20M culture medium method and 12 subjects with high caries risk were selected. These 12 individuals were subjected to determination of caries risk using two commercial rapid detection chair-side assays (Saliva-Check Mutans and Clinpro™ Cario L-Pop™) according to the manufacturers' instructions. The results were analyzed by the Kappa correlation test using SAS statistical software. There was a perfect agreement (Kappa=1) among the three caries risk evaluation methods - chair-side assays and semi-quantitative CFU count (control) - in all subjects. The results suggest that the commercial chair-side assays evaluated in this study may be practical and useful to identify high microbiological caries-risk subjects.

Key Words: *Streptococcus mutans*, caries risk assessment, Saliva-Check Mutans, Clinpro™ Cario L-Pop™

Introduction

According to the Susan Fisher-Owens conceptual model, oral health is influenced by several layers of factors that act together and interact in a dynamic and complex way. Basically, this multilevel conception includes 5 major fields: genetics and biology; social environment; physical environment; health-influencing behaviors; and medical care; in individual, family and community levels, interacting across time and space (1).

Considering the biological determinants, microorganisms may cause an imbalance between tooth and plaque and play an important role in the development of dental caries. It is proposed that various species of microorganisms, such as mutans streptococci, lactobacilli, aciduric strains of non-mutans streptococci, *Actinomyces*, bifidobacteria and yeasts are involved in caries progression (2).

Mutans streptococci (MS) have been identified as the major pathogens of dental caries (2, 3) because they are frequently present in caries lesions, are acidogenic and aciduric species (4), and are able to adhere to the tooth surface and other microorganisms (4).

Although the complexity of the contemporary concepts on dental caries and its manifestations hinders the establishment of a risk assessment model, an effective caries control and management requires the early identification of susceptible individuals for timely intervention.

Streptococcus mutans is an important biomarker of caries risk (5) because it has a key role in the development of dental caries. Detection of *S. mutans* in dental biofilm and saliva has been used as a manner to predict microbiological caries risk and monitor caries activity (6). The prevalence of *S. mutans* has been traditionally determined by microbial culture-based techniques (7-10), but there are also commercially available chair-side assay kits for this purpose (5, 11).

Saliva-Check Mutans (GC America, Alsip, IL, USA) immunoassay detection system was introduced to promote a rapid detection of *S. mutans* levels in saliva. According to the manufacturer, it uses a very specific immunochromatography process with two monoclonal antibodies that selectively detect only *S. mutans*. A positive result of the antigen-antibody reaction means that the subject has *S. mutans* levels equal to or above 500,000 colony forming units (CFU) per mL of saliva (12), which indicates risk for caries disease. With sensitivity and specificity above 90%, Saliva-Check Mutans has been considered as a promising tool for caries risk assessment in the community and clinical settings (5) and its efficacy either alone or combined with other chair-side assays has been demonstrated (5,11,13,14). Except from a recent study (11) that evaluated the combined use of Saliva-Check Mutans and Saliva-Check IgA Mutans to assess caries risk in comparison with Cariogram computer-based program, there are no published studies evaluating Saliva-Check Mutans in its latest version, in comparison with traditional culture techniques.

Clinpro™ Cario L-Pop™ (3M ESPE Dental Products, St. Paul, MN, USA) is a biochemical commercial rapid chair-side assay for clinical assessment caries risk that measures the production of lactic acid by metabolically active cariogenic

bacteria (15). The reaction is based on the enzymatic degradation of lactic acid by lactate dehydrogenase and coupled to a cascade of redox indicators that generate a color signal, which is analyzed using a semi-quantitative scale. Clinpro™ Cario L-Pop™ has been previously evaluated (16-19) and, according to Schiffner and Torres-Quintero (16), it can indicate changes in the oral microflora and thus could be used to monitor the effect of interventions.

An advantage of using commercial kits for assessing microbiological caries risk is that these assays produce results shortly after the collection of saliva samples. In addition, the kits are easy to use in the daily clinical practice, without the need of laboratory procedures (11). Commercial chair-side assays can also be used to obtain information in community caries prevention/control studies. When traditional culture-based detection tests are used, results are normally obtained after 2 to 4 days because of the need of incubation and CFU counting procedures, which can be an inconvenient for patients, clinicians and public health workers (5).

The aim of this study was to evaluate the commercial chair-side assays Saliva-Check Mutans and Clinpro™ Cario L-Pop™ in high microbiological caries-risk subjects compared with conventional semi-quantitative colony counting culture-based technique as the reference method.

Material and Methods

Ninety-three 18-26-year-old undergraduate dental students of both sexes from the Dental School of the Universidad Nacional de Tucumán, San Miguel de Tucumán (Argentina) who did not use antimicrobial agents or antibiotics in the previous 3 months were invited to participate in the study. The study was approved by the Ethics Research Committee of the Medical School of the Universidad Nacional de Tucumán (Argentina). Approximately 2 h after toothbrushing and without any food ingestion, non-stimulated saliva samples were collected with a wooden spatula during 1 min, according to the Köhler and Bratthall (20) method and seeded on the surface of plates containing SB-20M culture medium selective for mutans streptococci (9,10). The plates were incubated for 3 days at 37 °C in microaerophilia using the candle jar technique for determination of the salivary levels of mutans streptococci.

The number of colony forming units (CFU) *per* milliliter of saliva was counted by a single calibrated examiner (Kappa>0.8) using a stereomicroscope (Nikon, Tokyo, Japan) with reflected light at 20x magnification. During examination, the plates were maintained against a dark background in order to highlight the characteristics of the colonies and facilitate identification. *S. mutans* colonies have a granular surface resembling ground glass, with or without a polysaccharide drop on the surface. Colonies with the typical morphology of *S. mutans* were randomly selected and transferred to tubes containing thioglycollate broth without glucose or indicator, which were incubated at 37 °C for 24 h for biochemical identification. The broth cultures were then used as inoculum to test the capacity of each isolate to ferment mannitol (with and without bacitracin), sorbitol, raffinose and melibiose (21) and to produce hydrogen peroxide (22).

After this initial screening (sample 1), the 12 students with salivary *S. mutans* levels greater than 100 CFU/mL, indicating high microbiological caries-risk according to Köhler and Bratthall (20) classification, were enrolled in the study and formed a convenience sample. After that, the participants received verbal explanation about the study purposes and procedures. The 12 individuals were subjected to caries risk assessment using the commercial chair-side assay kits Saliva-Check Mutans (GC America) and Clinpro™ Cario L-Pop™ (3M ESPE) and the results were compared with those obtained with the conventional semi-quantitative *S. mutans* colony counting culture-based technique (reference microbiological method). Both commercial kits were used according to the manufacturers' instructions.

For microbiological caries risk assessment with Saliva-Check Mutans assay, a paraffin-stimulated whole-saliva sample was collected from each individual (sample 2) and one drop of #1 reagent was added. The container was tap 15 times and then four drops #2 reagent were added. Saliva was shaken until it turned green. Three drops of the saliva with reagents were dispensed in the sample window of the test device, with a 15-minute waiting time. Positive result was obtained if either a faint or clear red line appeared, indicating the presence of over 500,000 cfu/mL of *S. mutans*.

For microbiological caries risk assessment with Clinpro™ Cario L-Pop™ test, the subjects were first asked to brush their teeth for 1 min with the toothbrush and dentifrice used routinely. Five minutes later, a cotton-tipped applicator provided by the manufacturer was rubbed four times on the dorsum of the tongue using rotating movements to soak the applicator with saliva (sample 3). Next, the applicator was introduced into a device containing specific reagents that produce enzymatic degradation of the lactic acid present in saliva, and left in contact 2 min. After this time, the color change in the applicator tip was compared and matched with a specific color scale that comes in the kit. The results of this assay were validated by a control test made with a black applicator supplied by the manufacturer. Each color grade in the scale is related to scores from 1 to 9. Scores 1 to 3 indicate low lactic acid production; scores 4 to 6 indicate a moderate production, and scores 7 to 9 indicate

high lactic acid production, and indicate low, medium and high caries risk, respectively.

The results obtained in the assays were analyzed by the Kappa correlation test using the SAS (Statistical Analysis System) statistical software for Windows v. 9.1.3 (SAS Institute Inc., Cary, NC, USA).

Results

Table 1 presents the mean results of the analysis of three saliva samples obtained from of each of the 12 subjects with high caries risk, determined by counting more than 100 CFU/mL of saliva in the semi-quantitative colony counting culture-based technique. Positive reaction was observed in all subjects (100%) after application of the Saliva-Check Mutans assay, confirming the presence of anti-mutans antibodies and the presence of over 500,000 *S. mutans* CFUs per mL of saliva. Scores between 7 and 9 were obtained with the Clinpro™ Cario L-Pop™ assay. Score 7 was observed in 8.3% of the cases, score 8 in 50% and score 9 in 41.7%, which means that all subjects presented high levels of lactic acid production and should therefore be classified as having high microbiological risk for dental caries.

There was a perfect agreement (Kappa=1) among the three methods of microbiological caries risk assessment - chair-side assays and conventional semi-quantitative colony counting culture-based technique (reference method) - in all subjects with high caries risk (100% of the cases).

Table 1. Results obtained from application of two chair-side assays (Saliva-Check Mutans and ClinproTM Cario L-PopTM) in patients with high microbiological caries risk*, using conventional culture-based assay as reference

Subjects	Sample 1 (Conventional microbial culture-based technique)*	Sample 2 (Saliva-Check Mutans immunoassay) detection system)	Sample 3 (Clinpro™ Cario L-Pop™)
1	>100 CFU	Positive	score 9
2	>100 CFU	Positive	score 9
3	>100 CFU	Positive	score 8
4	>100 CFU	Positive	score 8
5	>100 CFU	Positive	score 8
6	>100 CFU	Positive	score 8
7	>100 CFU	Positive	score 8
8	>100 CFU	Positive	score 7
9	>100 CFU	Positive	score 9
10	>100 CFU	Positive	score 8
11	>100 CFU	Positive	score 9
12	>100 CFU	Positive	score 9

CFU: colony-forming units. *Köhler and Bratthall method (20).

Discussion

Due to the polarized distribution of dental caries among a high-caries risk minority, 20-25% of children account for over 50% of all caries lesions (23). In this way, targeting prevention and interventional measures to high caries risk populations is a rational approach for achieving a cost-effective dental caries control (24). Although it is known that caries disease involves a complex, multilevel and multifactorial concept, the main focus of this study was high microbiological caries risk. For this reason, this study was performed only with individuals considered to be at high microbiological risk for caries.

In this study, the participants were selected using the wooden spatula method proposed by Köhler and Bratthall (20) (conventional microbial culture technique), according to which individuals with more than 100 *S. mutans* CFUs per saliva mL are considered at high microbiological risk for dental caries.

The objective of this study was to evaluate if the results of two widely used commercial chair-side assays - Saliva-Check Mutans and Clinpro™ Cario L-Pop™ - agree with those of the conventional detection of microbiological caries risk (semi-quantitative colony counting culture-based technique) in highly infected individuals by *S. mutans*.

Using commercial kits allows detecting the microbiological caries risk in the daily clinical practice in a practical and fast way, without the need of laboratory procedures (11).

The introduction of the Saliva-Check Mutans assay allowed for bringing a molecular detection method into practical applications at chair-side since the dentist can perform all the procedures. Culture, additional apparatus or special facilities are not required and there are no expenses for laboratory costs. Because of its high correlation with polymerase chain reaction-confirmed CFU/mL of *S. mutans* and its compatibility with other commercial products, Saliva-Check Mutans appears to be suitable for use in a dental clinic, easy to use and rapid in providing results. It could be used, for example, for screening children with high *S. mutans* levels at primary schools (13). According to Gao et al. (5), the monoclonal antibody-based immunoassay accurately and rapidly determines abundant *S. mutans* salivary levels, and is appropriate for microbiological chair-side caries risk assessment.

Wennerholm and Emilson (11) evaluated the combination of two rapid semi-quantitative detection kits (Saliva-Check Mutans and Saliva-Check IgA Mutans) to assess caries risk compared with the Cariogram computer-based program.

In view of the good agreement with the Cariogram, those authors concluded that these commercial kits could be used for microbiological caries risk assessment. The results agree with those of the present study, in which Saliva-Check Mutans test was able to adequately classify microbiological high-caries-risk patients, using conventional microbial culture technique as the reference method.

Clinpro™ Cario L-Pop™ evaluates microbiological caries risk in an indirect manner, since it does not enumerate CFUs, but rather detects the presence of a bacterial product - lactic acid. The darkness of the color signal reflects the ability of dental plaque to produce acids and thus promote the caries process. The higher the metabolic activity of the acidogenic bacteria, the higher their potential to promote caries development (15). It is not, therefore, a specific test for *S. mutans*, since it detects lactic acid production by all bacteria from the oral microbiota that can produce lactate. However, the results with the use of Clinpro™ Cario L-Pop™ in this study showed an association with high salivary levels of *S. mutans*. In a clinical trial, Gerardu et al. (2006) (17) concluded that Clinpro™ Cario L-Pop™ can be used to monitor and motivate compliance to oral hygiene. In the present study, the results obtained with Clinpro™ Cario L-Pop™ confirmed those obtained with the conventional culture-based method in all cases, showing a perfect agreement. Further studies are required, including longitudinal investigations and studies that have a larger sample size and assess the influence of other aspects of caries risk than microbiological ones.

Within the limitations of this study it may be concluded that Saliva-Check Mutans and Clinpro™ Cario L-Pop™ assays showed perfect agreement in comparison with the conventional semi-quantitative colony counting culture-based technique as the reference method. The results indicate that both commercial chair-side assays may be practical and useful to identify high microbiological caries risk subjects.

Resumo

O objetivo do estudo foi avaliar os testes comerciais de consultório Saliva-Check Mutans e Clinpro™ Cario L-Pop™, em estudantes de Odontologia de alto risco à cárie, comparado à técnica convencional semi-quantitativa baseada em contagem de colônias, como método de referência. Amostras de saliva de 93 estudantes de ambos os sexos, entre 18 e 26 anos de idade, foram semeadas em placas contendo meio de cultura SB-20M e 12 pacientes de alto risco à cárie foram selecionados. Estes 12 indivíduos foram submetidos à determinação de risco à cárie por dois testes comerciais de rápida detecção (Saliva-Check Mutans e Clinpro™ Cario L-Pop™), seguindo as instruções dos fabricantes. Os resultados foram analisados pelo teste de correlação Kappa, por meio do software estatístico SAS. Houve uma concordância perfeita (Kappa=1) entre os três métodos de avaliação de risco á cárie - testes comerciais e contagem semi-quantitativa de UFC (controle) - em todos os pacientes. O resultado sugere que os testes comerciais de consultório avaliados neste estudo podem ser práticos e úteis para identificar indivíduos de alto risco microbiológico à cárie.

References

1. Fisher-Owens SA, Gansky SA, Platt LJ, Weintraub JA, Soobader M-J, Bramlett MD, et al.. Influences on children's oral health: a conceptual model. Pediatrics 2007;120:e510-e520.
2. Takahashi N, Nyvad B. The role of bacteria in the caries process: ecological perspectives. J Dent Res 2011;90:294-303.
3. Tanzer JM, Livingston J, Thompson AM. The microbiology of primary dental caries in humans. J Dent Educ 2001;65:1028-1037.
4. Hamada S, Slade HD. Biology, immunology, and cariogenicity of *Streptococcus mutans*. Microbiol Rev 1980;44:331-384.
5. Gao XL, Seneviratne CJ, Lo EC, Chu CH, Samaranayake LP. Novel and conventional assays in determining abundance of *Streptococcus mutans* in saliva. Int J Paediatr Dent 2012;22:363-368.
6. Fontana M, Zero DT. Assessing patients' caries risk. J Am Dent Assoc 2006;137:1231-1239.
7. Gold OG, Jordan HV, Van Houte J. A selective medium for *Streptococcus mutans*. Arch Oral Biol 1973;18:1357-1364.
8. Emilson CG, Bratthall D. Growth of *Streptococcus mutans* on various selective media. J Clin Microbiol 1976;4:95-98.
9. Saravia ME, Nelson-Filho P, Ito IY, da Silva LA, da Silva RA, Emilson CG. Morphological differentiation between *S. mutans* and *S. sobrinus* on modified SB-20 culture medium. Microbiol Res 2011;166:63-67.
10. Saravia ME, Nelson-Filho P, Silva RA, De Rossi A, Faria G, Silva LA, Emilson CG. Recovery of mutans streptococci on MSB, SB-20 and SB-20M agar media. Arch Oral Biol 2013;58:311-316.
11. Wennerholm K, Emilson CG. Comparison of Saliva-Check Mutans and Saliva-Check IgA Mutans with the Cariogram for caries risk assessment. Eur J Oral Sci 2013;121:389-393.
12. GC America Inc. Brochure of Saliva-Check Mutans. Alsip (IL): GC America Inc; 2009.
13. Matsumoto Y, Sugihara N, Koseki M, Maki Y. A rapid and quantitative detection system for *Streptococcus mutans* in saliva using monoclonal antibodies. Caries Res 2006;40:15-19.
14. Senpuku H, Miyazaki H, Yoneda S, Yoshihara A, Tada A. A quick statistically accurate diagnosis for caries risk in the elderly. Clin Lab 2010;56:505-512.
15. Bretz WA, Corby PM, Costa S, Quadros M, Tavares VS, Moreira G, et al.. Microbial acid production (Clinpro Cario L-Pop) and dental caries in infants and children. Quintessence Int 2007;38:e213-217.
16. Schiffner U, Torres-Quintero A. Reproducibility of a new caries risk test under different oral conditions. Clin Oral Investig 2005;9:187-191.
17. Gerardu V, Heijnsbroek M, Buijs M, van der Weijden F, Ten Cate B, van Loveren C. Comparison of Clinpro Cario L-Pop estimates with CIA lactic acid estimates of the oral microflora. Eur J Oral Sci 2006;114:128-132.
18. Hahnel S, Mühlbauer G, Hoffmann J, Ionescu A, Bürgers R, Rosentritt M, et al.. *Streptococcus mutans* and Streptococcus sobrinus biofilm formation and metabolic activity on dental materials. Acta Odontol Scand 2012;70:114-121.
19. Mannaa A, Carlén A, Zaura E, Buijs MJ, Bukhary S, Lingström P. Effects of high-fluoride dentifrice (5,000-ppm) on caries-related plaque and salivary variables. Clin Oral Investig 2014;18:1419-1426.
20. Köhler B, Bratthall D. Practical method to facilitate estimation of *Streptococcus mutans* levels in saliva. J Clin Microbiol 1979;9:584-588.
21. Shklair IL, Keene HJA. Biomechanical scheme for the separation of the five varieties of *Streptococcus mutans*. Arch Oral Biol 1974;19:1079-1081.
22. Whittenbury R. Hydrogen peroxide formation and catalase activity in the lactic acid bacteria. J Gen Microbiol 1964;35:13-26.
23. Winter GB. Epidemiology of dental caries. Arch Oral Biol 1990;35 Suppl:1S-7S.
24. Beck JD, Weintraub JA, Disney JA, Graves RC, Stamm JW, Kaste LM, et al. University of North Carolina caries risk assessment study: comparisons of high risk prediction, any risk prediction, and any risk etiologic models. Community Dent Oral Epidemiol 1992;20:313-321.

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