Comparison of Chairside Microbiological Screening Systems and Conventional Selective Media in Children With and Without Visible Dental Caries

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Abstract

Purpose: The purpose of this study was to compare the results of commercial chairside microbial tests (CT) and conventional selective media (GS, gold standards) for mutans streptococci (MS) and lactobacilli (LB) using oral specimens from young children with and without visible dental caries.

Methods: Using cotton swabs to collect oral microbial specimens from children 10 to 36 months old, microbial counts of CT and GS were compared with caries experience of the subjects. Contamination levels by non-MS or non-LB isolates on CT and GS were also determined. The CT employed were: (1) CRT bacteria for MS and LB; (2) CarioCheck Plus for MS and LB; and (3) Dentocult SM and Mucount for MS.

Results: All CT and GS for MS represented caries status of the participants ($P<.001$, Fisher exact test; $P<.015$ linear regression), whereas only GS for LB showed significant association with caries status ($P<.001$, Fisher exact test; $P<.001$, linear regression). Non-MS or non-LB isolates were observed on most media, and CT usually exhibited higher contaminant levels than GS. Dentocult SM and Mucount did not harbor contaminants.

Conclusions: Despite contamination, CT and GS for MS and GS for LB exhibited satisfactory outcomes based on cross-sectional caries experience of infants and toddlers. (Pediatr Dent 2006;28:363-368)

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Microbial tests for caries risk assessment have been employed since the 1940s, when Snyder developed methods to approximate the quantity of lactobacilli (LB) in saliva[1]. During the past 40 years, the emphasis has been on salivary or plaque levels of mutans streptococci (MS) detected with selective agar media or commercial chairside microbiological screening tests (reviewed by van Houte, 1993, and Krasse, 1988).[2,3] Several chairside tests (CT) are commercially available and have been extensively utilized:

1. in clinical research;
2. in public health evaluations to determine the need for services;
3. for patient/parent dental education; or
4. to assess caries risk with individual patients.

A limitation of some microbial screening systems when applied to children younger than 3 years old is the requirement for stimulated salivary specimens, which are often difficult to obtain. This has been remedied by direct inoculation of agar media by tongue depressors used to collect salivary specimens from the tongue (tongue blade/Rodac plate assessment method)[4,5] Another limitation is selectivity of the CT. Assuming that most dental practitioners will interpret colony forming units (CFU) which appear on a CT as target bacteria (ie, either MS or LB), presence of non-MS or non-LB contaminants may lead to misinterpretation of the results and possibly misdiagnoses. Although CT evaluations or comparisons have appeared in the scientific literature,[6-15] some of the products examined are no longer available or widely utilized and few, if any, inter-test comparisons or evaluations of selectivity have been reported. In

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a companion study, CT counts of MS from young children showed significant correlation with MS counts on conventional selective media for MS (GS, gold standards) despite presence of contaminants. The CT utilized for LB, however, did not correlate with GS counts for LB due to presence of contaminants.[16]

The purpose of the present study was to examine the association between visible cavitated dental lesions in infants and toddlers vs CT counts (with or without microbial contaminants) and GS counts. Associations were appraised by sensitivity and specificity using threshold microbial counts to signify presence or absence of cavitated lesions,[17] and linear regression analysis.

Table 1. Quantitative Data of Microbiological Systems Relative to Caries Experience (Presence or Absence of Cavitated Lesions)

	Total subjects mean±(SD*) n=50	Subjects with visible cavitated lesions mean±(SD) n=25	Subjects with no visible cavitated lesions mean±(SD) n=25
MS systems			
MSKB (total)	43±94†	61±82	26±103
Dentocult (total)	67±159	81±102	52±200
CarioCheck (total)	74±105	107±100	43±102
CRT (total)	72±105	102±100	44±102
Mucount (total)	58±143	60±43	56±200
MSKB (MS)	43±93	60±81	26±102
Dentocult (MS)	67±159	81±102	52±200
CarioCheck (MS)	59±82	98±95	20±41
CRT (MS)	58±83	93±98	24±44
Mucount (MS)	58±143	60±43	56±200
LB systems			
Rogosa (total)	54±202‡	108±282	1.2±2.9
CarioCheck (total)	267±398	417±447	126±282
CRT (total)	222±388	427±479	29±66
Rogosa (LB)	33±161	66±229	1.2±5.9
CarioCheck (LB)	62±242	126±339	1.4±2.0
CRT (LB)	44±200	87±282	2.6±10

*SD=standard deviation
†Microbial counts for MS systems x 10^4
‡Microbial counts for LB systems x 10^3

Methods

Population and sampling procedure

A convenience sample of 50 subjects was recruited from children entering the Pediatric Dentistry clinic at the University of Maryland Dental School, Baltimore, Md. The sample inclusion criteria were: (1) dentate children between 10 and 36 months old; and (2) parental consent. Exclusion criteria were:

1. children older than 36 months;
2. systemic antibiotics within 14 days; and
3. professional fluoride treatment within 48 hours of the microbial specimen collection.

The investigation received approval from the Institutional Review Board of the University of Maryland, Baltimore, Md. Caries was diagnosed without radiographs using a mouth mirror and air and recorded if visible cavitated lesions were present. A cotton swab specimen of all dental surfaces was collected from each child. Clinical data, consisting of number and location of teeth present and dmfs scores,[18] was also recorded. The swab was dispersed in 1.0 ml of sterile saline,[17] which was employed as the microbial specimen.

Microbiological procedures

Each CT was inoculated with a uniform aliquot of dispersed fluid from the sample-collection vial of the subject. The following CT were employed:

1. CRT bacteria (CRT; Ivoclar Vivadent Inc, Amherst, NY) containing 1 culture surface for MS and 1 for LB;
2. CarioCheck Plus (Hain Diagnostika, Nehren, Germany) containing 1 culture surface for MS and 1 for LB;
3. Dentocult SM (Orion Diagnostica, Espoo, Finland) for MS only; and
4. Mucount (Showa Yakuhin Kako Ltd, Toyko, Japan) for MS only.

CT were processed according to manufacturers' directions, with the exception that culture surfaces were inoculated with the dispersed microbial specimen instead of saliva, and were incubated at 37°C in air for 72 hours. The GS employed were:

1. Mitis Salivarius Kanamycin Bacitracin agar (MSKB)[19] for MS; and
2. Rogosa SL agar (Difco, Detroit, Mich) for LB.

With the latter, the dispersed specimens were serially diluted 1:10 in normal saline prior to plating and the plates were incubated at 37°C in air containing 5.0% carbon dioxide (CO_2) for 72 hours.

On all media except Mucount, wherein colonies were not accessible to isolation, representative colony-forming units

Table 2. The Ability of Mutans Streptococci (MS) Counts to Signify Presence or Absence of Cavitated Lesions Using A Threshold Risk Value For MS Counts ($1x10^5$/ml)

	Visible carious lesions present	Visible carious lesions absent	Sensitivity	Specificity	Fisher exact test (P)	Odds ratio	CIs* for odds ratio
MSKB+ (total)†	15‡	2	0.60	0.92	.001	17.3	3.3-90.0
MSKB- (total)§	10	23					
MSKB+ (MS)	15	2	0.60	0.92	.001	17.3	3.3-89.0
MSKB- (MS)	10	23					
Dentocult+ (MS)‖	19	5	0.79	0.80	.001	15.2	3.8-61.0
Dentocult- (MS)	5	20					
CarioCheck+ (total)	22	9	0.71	0.89	.001	24.4	4.7-127.0
CarioCheck- (total)	2	20					
CarioCheck+ (MS)	22	6	0.79	0.91	.001	36.7	6.6-203.0
CarioCheck- (MS)	2	20					
CRT+ (total)	22	8	0.73	0.89	.001	23.4	4.4-124.6
CRT- (total)	2	17					
CRT+ (MS)	19	6	0.76	0.79	.001	12.0	3.1-46.3
CRT- (MS)	5	19					
Mucount+ (MS)‖	19	6	0.76	0.79	.001	12.0	3.1-46.3
Mucount- (MS)	5	19					

*CIs=95% confidence intervals
†+=counts≥$1x10^5$/ml
‡No. of samples
§-=counts<$1x10^5$/ml
‖MS count=total count

(CFUs) of all colony types which constituted greater than 5% of CFUs were picked with sterile wire needles or loops and streaked for purification on CDC Anaerobic Blood agar (Becton-Dickinson, Sparks, Md). After incubation for 48 hours at 37°C in air containing 5.0% CO_2, isolates were identified with Becton-Dickinson (BD) BBL Crystal Identification Systems (Becton-Dickinson, Sparks, Md). Yeasts were tentatively identified by Gram stain and cultivation on CHROMagar (Becton Dickinson; Sparks, Md). Mucount colonies, which were attached to interior surfaces of small glass vials, were examined with a stereomicroscope, but were not identified by Gram stain or biochemical tests.

Data analysis

CT CFUs were enumerated from density charts furnished with the systems and recorded as:

1. total counts (ie, target bacteria [MS or LB] plus contaminants); and
2. counts of only the target bacteria.

GS were also recorded as total and as MS or LB counts. Using $1x10^5$ as a threshold value for microbial counts of MS systems and $1x10^3$ as the threshold value for LB systems,[17] sensitivity and specificity of both total and MS or LB counts vs presence or absence of visible carious lesions, were determined. The latter findings were further evaluated using the Fisher exact test and odds ratios with a 95% confidence interval (CI). Associations between cross-sectional visible carious lesions (dmfs scores, dependent variable) and microbial counts (independent variables) were evaluated by linear regression. Statistical evaluations were conducted using the Sigma Stat 2.0 statistics program (Jandel Corporation, San Rafael, Calif).

Results

The mean age of the population was 26.5 (±6.5 SD) months, and the average number of teeth present was 17.6 (±3.4). Twenty-five children presented with dmfs=0 and 25 with dmfs≥1.0. The mean dmfs score of subjects with caries was 17.9 (±11.9), represented by 15.7 (±11.8) unrestored carious, 1.2 (±4.4) extracted, and 1.0 (±4.0) filled surfaces. Mean counts of CT and GS are presented in Table 1. All media recovered non-MS or non-LB contaminants except Dentocult and Mucount systems.

Table 3. The Ability of Total Lactobacilli (LB) Counts to Signify Presence or Absence of Cavitated Lesions Using A Threshold Risk Value For LB Counts ($1x10^3$/ml)

	Caries+	Caries–	Sensitivity	Specificity	Fisher exact test (P)	Odds ratio	CIs* for odds ratio
Rogosa+ (total)†	13‡	4	0.76	0.66	.008	6.2	1.6-24.0
Rogosa- (total)§	10	19					
Rogosa+ (LB)	7	1	0.88	0.61	.019	11.0	1.2-99.3
Rogosa- (LB)	14	22					
CarioCheck+ (total)	21	20	0.51	0.63	.700	1.75	.37-8.3
CarioCheck- (total)	3	5					
CarioCheck+ (LB)	4	1	0.80	0.56	.180	5.05	.52-49.0
CarioCheck- (LB)	19	24					
CRT+ (total)	19	9	0.68	0.76	.004	6.76	1.9-24.3
CRT- (total)	5	16					
CRT+ (LB)	7	3	0.70	0.67	.171	3.02	.68-13.4
CRT- (LB)	17	22					

*CIs=95% confidence intervals
†+=counts≥$1x10^3$/ml
‡No. of samples
§-=counts<$1x10^3$/ml

Association of microbial counts with caries status

With the sensitivity and specificity determinations (Table 2), counts greater than or equal to the designated threshold value signified a presence of visible carious lesions. Counts below the value, meanwhile, signified absence of visible carious lesions. All media (CT and GS) for MS demonstrated statistically significant outcomes (P<.001, Fisher exact test). Sensitivity ranged from 0.60 for MSKB to 0.79 for Dentocult. Specificity ranged from 0.89 for CarioCheck (total) and CRT (total) to 0.92 for MSKB. Odds ratios ranged from 12 for Mucount (total and MS; CI=3.1, 46.3) and 12 for CRT (MS; CI=3.1, 46.3) to 36.7 for CarioCheck (MS; CI=6.6, 203). With LB systems, only Rogosa (P<.019, Fisher exact test) and CRT total (P<.004, Fisher exact test) showed significant association with caries status (Table 3). Sensitivities ranged from 0.51 for CarioCheck (total) to 0.80 for CarioCheck (LB), and specificities ranged from 0.56

Table 4. Linear Regression Analysis of Counts of Mutans Streptococci (MS) or Lactobacilli (LB) Vs Presence or Absence of Cavitated Lesions

	R^2±(SE)	ß coefficient	Probability value
MS systems			
MSKB (total)*	0.28±0.66	2.87	.001
Dentocult (total)	0.20±0.62	2.11	.001
CarioCheck (total)	0.24±0.62	2.37	.001
CRT (total)	0.22±0.65	2.36	.001
Mucount (total)	0.12±0.71	1.78	.015
MSKB (MS)†	0.32±0.62	2.13	.001
Dentocult (MS)	0.20±0.62	2.11	.001
CarioCheck (MS)	0.36±0.51	2.63	.001
CRT (MS)	0.29±0.52	2.26	.001
Mucount (MS)	0.12±0.71	1.78	.015
LB Systems			
Rogosa (total)‡	0.27±0.75	2.95	.001
CarioCheck (total)	0.11±0.94	2.28	.019
CRT (total)	0.27±0.65	2.68	.001
Rogosa (LB)	0.36±0.89	4.42	.001
CarioCheck (LB)	0.23±0.99	3.72	.001
CRT (LB)	0.07±0.99	1.83	.070

*Total=counts of MS (MS media) plus non-MS contaminants
†MS=counts of only MS
‡Total=counts of LB (LB media) plus non-LB contaminants

for CarioCheck (LB) to 0.76 for CRT (total). Odds ratios ranged from 1.75 for CarioCheck total (CI=0.37, 8.3) to 11 for Rogosa LB (CI=1.2, 99.3).

Associations between cross-sectional caries status (dmfs scores, dependent variable) and microbial counts (independent variables) were evaluated by linear regression (Table 4). Counts of all media for MS (MS and total) yielded significant association with dmfs ($P<.016$). The same test applied to counts of media for LB (total and LB) also yielded significance ($P<.02$) for all evaluations except CRT (LB), which was not significant.

Selectivity of CT and GS media

Details of microbial contamination of the CT has been reported.[16] In summary, contaminants appeared on 36%, 34%, and 22% of specimens using CRT (for MS), CarioCheck (for MS), and MSKB, respectively. Dentocult and Mucount specimens did not harbor detectable contaminants. On LB systems, contaminants were recovered on 82% of CarioCheck, 50% of CRT, and 40% of Rogosa specimens. The mean percentage of contaminants relative to TVC of the medium was highest for CarioCheck (for LB), followed in order by CRT (for LB), Rogosa SL, CRT (for MS), CarioCheck (for MS), and MSKB. On MS systems, prominent contaminants were *Candida* spp (mainly *Candida albicans* and *Candida parapsilosis)* followed by *Staphylococcus epidermidis* and *Klebsiella pneumoniae.* On LB systems, *Candida* spp followed by *Streptococcus* and *S epidermidis* were the major contaminants. Although identifications of MS colonies on MS systems were mainly intended to verify that they were not contaminants, *Streptococcus mutans* isolates were found to exceed *Streptococcus sobrinus.*

Discussion

Since timing of MS colonization in infants and toddlers may influence the onset of future caries in the child,[20,21] microbial screening of young children is highly recommended. The CT offers a convenient approach to screening, but when salivary specimens are required, sampling is often difficult with young children. As an alternative, cotton swab collection of specimens from teeth is a simple and atraumatic method and increases the chance that cariogenic bacteria will be recovered from the mouth if low levels are present.[17] The present investigation addressed comparison of commonly used CT and GS using cross-sectional caries experience of infants and toddlers as a parametric tool (ie, dmfs scores vs microbial counts).[17,22] Longitudinal trials would be the ideal experimental method, however, since prediction of future caries risk is the usual objective of CT.

Two variables which might affect microbial counts vs dmfs scores—since both may influence MS colonization—especially in young children, are: (1) age of subjects; and (2) number of teeth present. These variables were not addressed in this investigation, however, since 90% of the children were older than 19 months and presented with more than 16 teeth. In addition, correlations between both age and number of teeth vs MS counts on all MS media were insignificant when presence of dental caries was controlled. This investigation, furthermore, did not include precarious white spot lesions as part of the caries scores. The rationale for this was that carious and noncarious white spots could not be definitively differentiated by visible means.[23] While most white spots were observed in children with caries, some subjects with no visible carious lesions did exhibit white spots. If the latter were precarious, the evaluations may have been affected.

Despite the relatively high level of contaminants on the agar-based systems (GS, CRT, CarioCheck), statistical tests showed significant associations between visible carious lesions and counts of MS on all media (Tables 2, 3, and 4). The latter finding is interesting in that the contaminants (primarily *Candida* species) may have been positively associated with caries status along with the MS counts, as has been found in previous studies.[24,25] The less-than-satisfactory outcomes of LB media counts vs dmfs scores may have been due to high contamination by oral yeasts, which exceeded that of MS media. Other reasons may be that LB is not as strong a predictor of caries risk as MS in this age population[17] or that the low pH of the LB systems stimulated yeast growth.

The relatively high levels of contaminants on all media, except Dentocult and Mucount, may also reflect the particular population utilized in the study. High yeast recovery appears to be age related, since both Hannula et al (1999)[26] and Mattos-Graner et al (2001)[27] reported abundant yeast colonization in infant and toddler mouths. Hannula et al found *C parapsilosis* to be the predominant yeast isolate, and both research teams implicated pacifiers as a related factor. Nonindigenous bacteria also are often found in oral cavities of young children and even newborns.[28] Common isolates have been *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and noncoagulase-positive staphylococcus (ie, *S epidermidis*). Reasons offered for this finding are that they may be transient colonizers that can withstand stresses of the early oral biofilms, or that they survive due to an underdeveloped immune system.[29] Makhoul et al (2002)[30] found *S epidermidis* frequently in the mouths of neonates who received antibiotics. They suggested that *S epidermidis* may be transmitted from mothers, possibly via birth canals during delivery, and become enriched during antibiotic treatments.

In summary, Dentocult and Mucount appeared to be the systems of choice for MS since they did not recover contaminants (that the authors were able to detect) and appeared to adequately represent the subjects' caries status. Although Rogosa was the best media for LB, clinicians should be aware that non-LB colonies appear on it and are often difficult to differentiate from streptococcal isolates by colonial morphology.

Conclusions

Based on this study's results, the authors conclude that all media for mutans streptococci (MS) exhibited satisfactory outcomes (even with contaminants). Based on the clinical parameters used in this research, this MS media may be useful for microbial screening of infants and toddlers. Media for lactobacilli were not as effective, due in part to a high recovery of yeast contaminants.

References

1. Snyder ML. A simple colorimetric method for the estimation of relative numbers of lactobacilli in the saliva. J Dent Res 1940;19:349-355.
2. van Houte J. Microbiological predictors of caries risk. Adv Dent Res 1993;7:87-96.
3. Krasse B. Biological factors as indicators of future caries. Int Dent J 1988;38,219-225.
4. Weinberger SJ, Wright GZ. Variables influencing Streptococcus mutans testing. Pediatr Dent 1990;12:312-315.
5. Weinberger SJ, Wright GZ. A comparison of S mutans clinical assessment methods. Pediatr Dent 1990;12:375-379.
6. Larmas M. A new dip-slide method for the counting of salivary lactobacilli. Proc Finn Dent Soc 1975;71:31-35.
7. Birkhed D, Edwardsson S, Andersson H. Comparison among a dip-slide test (Dentocult), plate count, and Snyder test for estimating number of lactobacilli in human saliva. J Dent Res 1981;60:1832-1841.
8. Emilson CG, Krasse B. Comparison between a dip-slide test and plate count for determination of Streptococcus mutans infection. Scand J Dent Res 1986;94:500-506.
9. Jordan HV, Laraway R, Snirch R, Marmel M. A simplified diagnostic system for cultural detection and enumeration of Streptococcus mutans. J Dent Res 1987;66:57-61.
10. Jensen B, Bratthall D. A new method for the estimation of mutans streptococci in human saliva. J Dent Res 1989;68:468-471.
11. Davenport ES, Day S, Hardie JM, Smith JM. A comparison between commercial kits and conventional methods for enumeration of salivary mutans streptococci and lactobacilli. Community Dent Health 1992;9:261-271.
12. Wallman C, Krasse B. A simple method for monitoring mutans streptococci in margins of restorations. J Dent 1993;21:216-219.
13. Shi S, Deng Q, Hayashi Y, Yakushiji M, Machida Y, Liang Q. A follow-up study on three caries activity tests. J Clin Pediatr Dent 2003;27:359-364.
14. Karjalainen S, Soderling E, Pienihäkkinen K. Validation and inter-examiner agreement of mutans streptococci levels in plaque and saliva of 10-year-old children using simple chair-side tests. Acta Odontol Scand 2004;62:153-157.
15. Edelstein B, Tinanoff N. Screening preschool children for dental caries using a microbial test. Pediatr Dent 1989;11:129-132.
16. Park JH, Tanabe Y, Tinanoff N, Turng BF, Lilli H, Minah GE. Evaluation of microbiological screening systems using oral specimens from young children aged 6 to 36 months. Caries Res. In press.
17. Wunsch PB, Park JH, Watson MR, Tinanoff N, Minah GE. Microbiological screening for cariogenic bacteria in children 6 to 36 months of age: An evaluation of clinical and laboratory procedures. Pediatr Dent 2004;26:231-239.
18. Burt BA. Measuring dental caries. In: Burt BA and Eklund SA , ed. Dentistry, Dental Practice, and the Community. 5th ed. Philadelphia, Pa: WB Saunders Company; 1999:178-184.
19. Kimmel L, Tinanoff N. A modified mitis salivarius medium for a caries diagnostic test. Oral Microbiol Immunol 1991;6:275-279.
20. Alaluusua S, Renkonen O-V. Streptococcus mutans establishment and dental caries experience in children from 2 to 4 years old. Scand J Dent Res 1983;91:453-457.
21. Kohler B, Andreen I, Jonsson B. The earlier the colonization by mutans streptococci, the higher the caries prevalence at 4 years of age. Oral Microbiol Immunol 1988;3:14-17.
22. Thibodeau EA, O'Sullivan DM, Tinanoff N. Mutans streptococci and caries prevalence in preschool children. Community Dent Oral Epidemiol 1993;21:288-291.
23. Ismail AI, Sohn W. A systematic review of clinical diagnostic criteria of early childhood caries. J Public Health Dent 1999;59:171-191.
24. Marchant S, Brailsford SR, Twomey AC, Roberts GJ, Beighton D. The predominant microflora of nursing caries lesions. Caries Res 2001;35:397-406.
25. Radford JR, Ballantyne HM, Nugent Z, et al. Caries-associated micro-organisms in infants from different socioeconomic backgrounds in Scotland. J Dent 2000;28:307-312.
26. Hannula J, Saarela M, Jousimies-Somer H, et al. Age-related acquisition of oral and nasopharyngeal yeast species and stability of colonization in young children. Oral Microbiol Immunol 1999;14:176-182.
27. Mattos-Graner RO, de Moraes AB, Rontani RM, Birman EG. Relation of oral yeast infection in Brazilian infants and use of a pacifier. J Dent Child 2001;68:33-36.
28. Hedge S, Munshi AK. Influence of the maternal vaginal microbiota on the oral microbiota of the newborn. J Clin Pediatr Dent 1998;22:317-321.
29. Kononen E. Development of oral bacterial flora in young children. Ann Med 2000;32:107-112.
30. Makhoul IR, Sujov P, Ardekian L, et al. Factors influencing oral colonization in premature infants. Israel Med Assoc J 2002;4:98-102.