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Validation Studies of the CARE Test for Deciduous Teeth (CT/D) by Oligosaccharide Pattern Analysis

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Background

The algorithm for the CARE test for Deciduous Teeth (CT/D) is based on a correlation of the presence or absence of caries in deciduous teeth in children ages 10-13 with specific oligosaccharide patterns in saliva. CT/D is hoped to be prognostic when it is applied to younger children before they experience caries. The prognostic potential of CT/D continues to be validated in two ways:

- A traditional prospective study: young children, already tested with CT/D, are tracked by future claims records as they return for routine dental visits. The accruing records of these children will assess the accuracy of the CT/D prediction either when the child experiences a restoration or when they reach age 10.
- The method using oligosaccharide pattern analysis for CT/D prognostic validation relies on the permanence of blood types, such as A, B, O, and is not prospective by nature. A comparison of different age groups will show whether the oligosaccharide patterns, like blood types, are quantitatively consistent over time.

This presentation focuses on the second validation method.

Blood Type = Sugar and Linkage = Lectin Affinity

- There are more than 26 blood group families, with ABO the best known
- Illustration: the ABO Family:

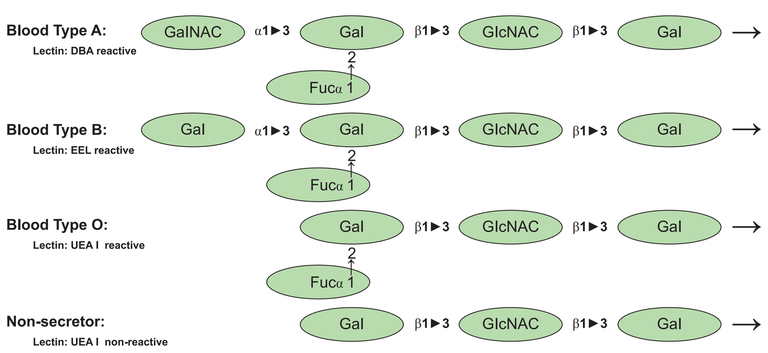
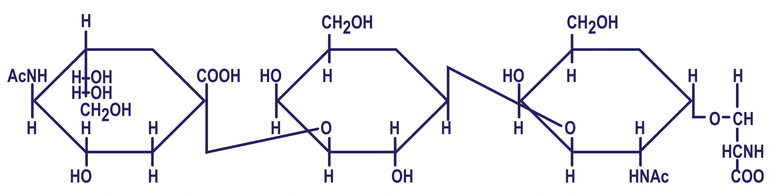


Illustration of Oligosaccharide on MUC7 Mucin that Interacts with Planktonic Bacteria in Saliva*

*Property is Lost when Terminal Sialic Acid is Removed or Missing
Murray, P.A., Levine, M.J. *et al. Infect. Immun.* 53:359-365 (1986).

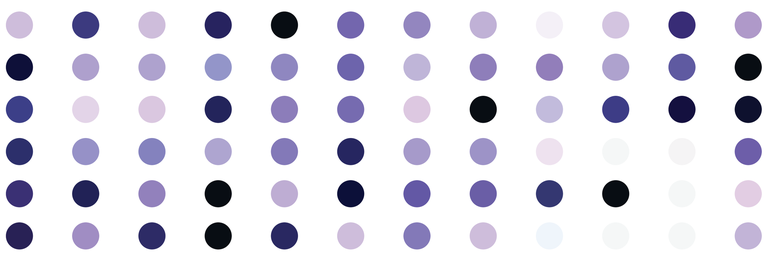


Sialic Acid α-2,3 Galactose β-1,3 N-Acetylgalactosamine

Lectin: MAL II (Affinity: Sialic Acid α-2,3 Galactose)

Lectin: BPL (Affinity:Galactose β-1,3 N-Acetylgalactosamine)
[Partially Inhibited by terminal Sialic Acid]

Dot Blot System for Quantitating Individual Amounts of Inherent Sugar Chain Configurations in Saliva



Assay of SAα-2,3Gal, using MAL II, in dried spots of saliva from a group of young adults (18-24 years).

ABSTRACT

Objective

To determine if the proportion of oligosaccharide patterns associated with "caries" and "no caries" remains consistent over the age range of the test population.

Methods

1. Resting saliva is collected and immediately frozen.
2. A battery of lectins is employed to quantitate levels of selected oligosaccharide motifs in spots of dried saliva using a dot blot technology.
3. From the lectins employed, an algorithm was derived from multiple regression and neural net mathematics to describe the relevant oligosaccharide patterns.
4. Each pattern was subsequently identified by an alpha-numeric label.
   - This oligosaccharide pattern identification system allows observation of changes (or absence thereof) in patterns that might occur in childhood during growth and maturation.
   - For study purposes, each label becomes a tracking device for determining accuracy of the pattern, for identifying algorithm "learning" opportunities, and for assessing stability of the pattern throughout the age range of the test.
   - The claim-derived caries history of each child was matched with the corresponding pattern identification.
5. The number and identity of lectins, composition of the algorithm, and number of oligosaccharide patterns in the algorithm are proprietary.

Results

- The distributions of children with caries-associated and non-associated oligosaccharide patterns is very similar between age groups 6-9 and 10-13.
- The slight difference between groups was explored further by examining for a correlation based on percentage of each group of patterns at each incremental age.
- The lack of correlation and the general similarity of pattern distributions between groups is support for consistency of oligosaccharide patterns over time.

Conclusion

While not fully resolved, oligosaccharide patterns within the age-range of the test suggest consistency. As the number of subjects continues to accrue, we expect this approach to provide a valuable assessment of the prognostic potential of CT/D.

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Sample of Data Associated with Alpha-Numeric Labels Representing Oligosaccharide Patterns

Pattern	SL[2]	Number[3] (mismatches)[4]			NH[6] (ages)	sC[7] (ages)
			age 6-9[5]	age 10-13[5]		
A						
A1						
A1a	1	6 (1)	5	1	2 (6,10)	4 (6,7,8,8)
A1b	2	1	1	0	1 (8)	0
A2	2	2	2	0	1 (6)	1 (6)
A3	2	2	2	0	0	2 (7,7)
A4	2	5	2	3	5 (7,7,10,11,11)	0
B						
B1	2	2	2	0	1 (8)	1 (7)
B2	1	2	1	1	1 (9)	1 (10)
B3	1	5	2	3	4 (6,8,11,13)	1 (10)
B4	2	5	3	2	4 (6,8,8,10)	1 (10)

[1] Example of alpha-numeric identification system applied to oligosaccharide patterns
[2] Pattern associated caries susceptibility level: (1) no caries by age 10, (2) caries by age 10 or earlier
[3] Total subjects representing pattern ID to date
[4] One subject in this pattern group has caries. If mismatch is validated with additional subjects, the algorithm may be "trained" to recognize additional corrective oligosaccharide patterns
[5] Total subjects in age group to date
[6] Total subjects and ages in New Hampshire study to date
[7] Total subjects and ages in southern California study to date

Analysis

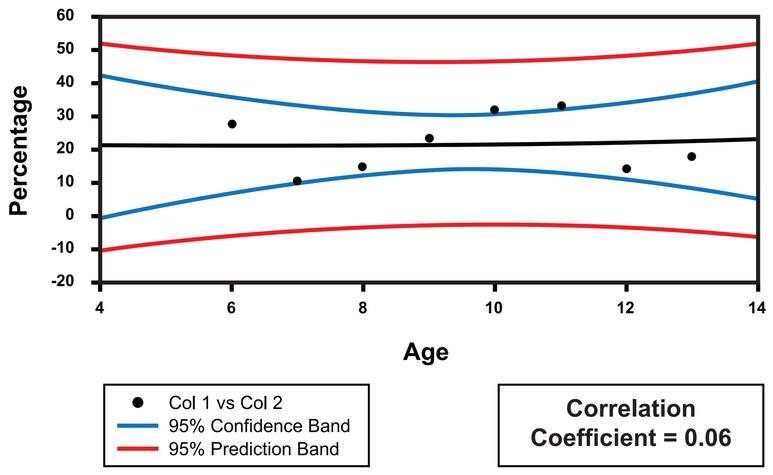
- Ideally, a standard for the consistency of each oligosaccharide pattern would be applied to the current study at all ages, 6-13 years. However, there are not yet enough subjects in the study to complete this type of evaluation.
- Thus, analysis has been conducted to provide an early assessment which would still be expected to show that consistency of oligosaccharide patterns is independent of age.
- Younger children ages 6–9 are combined and compared to older children ages 10–13. If there is an age-related shift in oligosaccharide patterns it should be apparent in this type of age-banded cohort comparison.

Comparison of Oligosaccharide Patterns between Younger and Older Children

Age group	Caries patterns	Non-caries patterns	Total subjects
6 to 9	81.00%	19.00%	104
10 to 13	75.00%**	25.00%	65

**Includes two pattern mismatches

Test for Correlation between Ages and Percentage of Children with Non-caries Patterns



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- Denny PC, Denny PA, Takashima J, Si Y, Navazesh M, Galligan JM (2006). A novel saliva test for caries risk assessment. J Calif Dent Assoc 34(4):287-90, 292-4.
- Denny PC, Denny PA, Takashima J, Galligan J, Navazesh M (2007). A novel caries risk test. Ann N Y Acad Sci 1098 (204-215).