

ARIC DENTAL PROCEDURES MANUAL

Baseline Examination

1996 - 1999

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daricman.doc

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I. SEQUENCE OF PROCEDURES AND CHECKLIST

(This form should be filled out for each participant and placed in the ARIC record at the completion of the dental exam)

Participant ID _____ Date __ / __ / __ Examiner ID _____

- 1) **Review Computerized Health History to Verify Eligibility**
 See Procedure Manual, Page _____
 Not Eligible () Send participant to next station.
 Eligible () continue
- 2) **ARIC Dental Questionnaire Completed** ()
 See Procedure Manual, Page _____
- 3) **Informed Consent Completed?** ()
 See Procedure Manual, Page _____
- 4) **Complete Soft Tissue Screening** ()
 See Procedure Manual, Page _____
- 5) **Record DMFS score and Plaque Score**
 per protocol for each tooth sequentially ()
 See Procedure Manual, Page _____
- 6) **Collect GCF samples per protocol** ()
 See Procedure Manual, Page _____
- 7) **Collect plaque samples per protocol and store** ()
 See Procedure Manual, Page _____
- 8) **Record Gingival Index per protocol for**
 for each tooth sequentially ()
 See Procedure Manual, Page _____
- 9) **Record CEJ Measurement and Probing Depth**
 per protocol for each site sequentially, while
 recording BOP prior to moving to new
 quadrant See Procedure Manual, Page ()
- 10) **Print recommendation sheet and**
 go over with participant to conclude dental portion. ()
 See Procedure Manual, Page _____

II. Participant Screening Protocol

A. Telephone Screening Form

When the participant arrives at the dental station, he/she should have a folder that contains a telephone screening form. The participant has been enrolled in the dental study based on information provided over the telephone. Review answers on the form to verify eligibility. If ineligible, note reason on the form and send to next station.

B. Consent Form

There also should be a consent form for the dental examination. Make sure that this form is properly filled out and signed. When completed, indicate on checklist.

C. Dental Interview Form

A dental interview also is part of the protocol. This interview should have been completed prior to the dental examination station and the data entered into the computer. Verify that the dental interview has been completed and indicate on checklist prior to completing the examination.

Examples of telephone screening form, consent form and interview form go here.

DENTAL SCREENING FORM (DSRA Screen 2 of 3)

10. Have you had a heart, kidney, or other organ transplant? Yes Y — Exclude. No N — Go to Item 15. Unknown U
11. Do you have a surgically implanted heart valve, stent, shunt or artificial joint? Yes Y — Exclude. No N — Go to Item 15. Unknown U
12. Are you on kidney dialysis? Yes Y — Exclude. No N — Go to Item 15. Unknown U
13. Have you had major surgery, radiation, or chemotherapy for cancer within the last 2 months? Yes Y — Exclude. No N — Go to Item 15. Unknown U
14. Are you taking prednisone or an immunosuppressive medication? Yes Y — Exclude. No N — Go to Item 15. Unknown U

15. The participant meets an exclusion criterion from the screening interview?

Yes Y ----> EXCLUDE, read exclusion statement

No N ----> RECRUIT, read recruitment statement

EXCLUSION STATEMENT:

Because you (SELECT THE RELEVANT STATEMENT BELOW):

-- do not have any of your natural teeth

-- have been told by a dentist that you need to take antibiotics before every dental visit

-- have a medical condition that might require you to have antibiotics before a dental examination

it may not be useful or safe for you to participate in this portion of the study. However, we will be asking you some questions about your dental history as part of the ARIC visit. [GO TO ITEM 17.]

DENTAL SCREENING FORM (DSRA Screen 3 of 3)

RECRUITMENT STATEMENT:

You are eligible to take part in this new study on the effect of infections on heart disease. Read DESCRIPTION OF DENTAL EXAM. Do you have any questions?

[CONTINUE WITH ITEM 16.]

16. May I schedule you for the dental exam? Yes Y ---> READ REMINDER
No N ---> EXCLUDE

17. Date of telephone interview: ___/___/___

18. Code Number of person completing telephone interview: ___

DESCRIPTION OF DENTAL EXAM for ITEM 16.

This portion of the study includes a simple examination of your mouth to see if there are any cavities, gum disease or spaces between your teeth and gums. We will also collect a little plaque and pick up some fluid from around your teeth. Most people find these procedures quite comfortable.

REMINDER FOR PARTICIPANTS SCHEDULED FOR DENTAL EXAM.

The usual procedure in ARIC is to send your doctor a copy of your results reports. If you would like us to send your dentist a copy of your dental exam report, please bring his or her name and address with you when you come for your appointment.

DENTAL HISTORY FORM

ID NUMBER:

CONTACT YEAR: 1 0

FORM CODE: D H S

VERSION: A 07/24/96

LAST NAME:

INITIALS:

Public reporting burden for this collection of information is estimated to average 3 minutes per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to: PHS Reports Clearance Officer, Rm. 737-F, Humphrey Building, 200 Independence Ave., SW, Washington, D.C. 20201, ATTN: PRA (0925-0281). Do not return the completed form to this address.

INSTRUCTIONS: This form is completed during the participant's visit. ID Number, Contact Year and Name must be entered above. Whenever numerical responses are required, enter the number so that the last digit appears in the rightmost box. Enter leading zeroes where necessary to fill all boxes. On the paper form, if a number is entered incorrectly, mark through the incorrect entry with an "X". Code the correct entry clearly above the incorrect entry. For "multiple choice" questions, circle the letter corresponding to the most appropriate response. If a letter is circled incorrectly, mark through it with an "X" and circle the correct response.

DENTAL HISTORY FORM (DHSA screen 1 of 4)

1. Have you lost any of your natural teeth?Yes Y

Go to Item 5, Screen 1. ————— No N

2. Did you lose any teeth because of:

	Yes	No	Unknown
a. Cavities	Y	N	U
b. Gum disease	Y	N	U
c. Accident	Y	N	U
d. Wisdom teeth pulled	Y	N	U
e. Extracted because of overcrowding	Y	N	U
f. Other	Y	N	U

3. Do you have false teeth? Yes Y

Go to Item 5, Screen 1. ————— No N

4. How old were you when you got your first false teeth?

age

5. Have you ever noticed any of your teeth were loose? Do not include the times when you lost your baby teeth, had braces, or had a tooth hit and made loose..... Yes Y

No N

Unknown U

DENTAL HISTORY FORM (DHSA screen 2 of 4)

6.a. Have you ever had a root canal done? Yes Y

Go to Item 7.

No N

Unknown U

b. Did you have a root canal done on more than one tooth? Yes Y

No N

Unknown U

7. Have you ever had a dental implant? Yes Y

No N

8. How often did you brush your teeth yesterday?

Not at all A

One time B

Two times C

Three times or more D

9. How often did you use dental floss last week?

Not at all A

One time B

Two times C

Three times or more D

DENTAL HISTORY FORM (DHSA screen 3 of 4)

10. When was the last time you went to the dentist for any reason?

Within the last 6 months A

6 months to less than 1 year ago ... B

1 to less than 2 years ago C

2 to less than 3 years ago D

3 to less than 5 years ago E

5 or more years ago F

11. Would you say that you use a dentist on a regular basis, or do you only go when you are in discomfort or when you need something fixed?

Regular basis A

Only when in discomfort B

When something needs to be fixed C

Don't go to the dentist D

Other E

12. Do you have a dentist? Yes Y

No N

DENTAL HISTORY FORM (DHSA Screen 4 of 4)

13. Date of collection:

		/			/		
--	--	---	--	--	---	--	--

14. Method of data collection: Computer
Paper

C
P

15. Code Number of person
completing this form:

--	--	--

III. Clinic Preparation

A. Computer set-up:

B. In preparation for the clinical exam and sample collection the following instruments and supplies should be set up in the operatory

1. Instrument Kits

Each study center will be provided with 8 instrument trays. One will be used for each patient and should be sterilized and set up in the operatory prior to the patient arrival. These kits include the following instruments:

- Dental Mirror
- UNC-15 Periodontal Probe
- Explorer
- 1 4R/4L Curettes
- 2 Cotton Forceps

2. Supplies and Equipment On Site

Each study center will have the following supplies and equipment made available to them and kept at the study center. Each of the following will need to be used at each patients visit:

- Hamilton Syringe (*for weekly Periotron calibration*)
- Periotron (*Harco*)
- Dewars Flask
- Liquid Nitrogen
- Periopaper Holder (2)
- Instrument Kits (8)
- Autoclave
- Self-sealing autoclave bags
- 0.5M NaOH in bottle with dispenser
- cotton rolls
- gauze 2x2
- holding rack for sample vials

3. Patient Kits

Each study center will be sent one kit per patient. Each kit will be prelabelled and will include:

GCF Vials (4)
 Conical Bottom Tubes (4) (*sterile w/ TE buffer*)
 Sterile Aluminum Foil Strips (4) (*fingerprint-free*)
 Periopaper (1 *set*)
 Sample requisition form to be completed

4. Sample Requisition Forms

Each form will have a preprinted patient number with initials and anniversary date. One form will be in each patient sample kit and needs to be filled out at during the visit. It includes patient information, sample information, and preprinted accession numbers corresponding to each sample to be collected. Vials will be prelabelled. Each vial (and each sample) has a unique number according to the following system:

Digits 1 and 2 = Study Designation (two letters)

Digits 3 through 9 = Patient ID number (one letter, 6 numbers)

Digit 10 = Sample Type - 1 = GCF

2 = Plaque

3 = Serum

Digit 11 and 12 = Sample Number 1-16 = GCF (vial label shows every fourth sample

17-20 = Plaque

21-24 = Serum

For example the number : ARW126423217

would designate the following sample:

The ARIC study (“AR” in the first two digits)

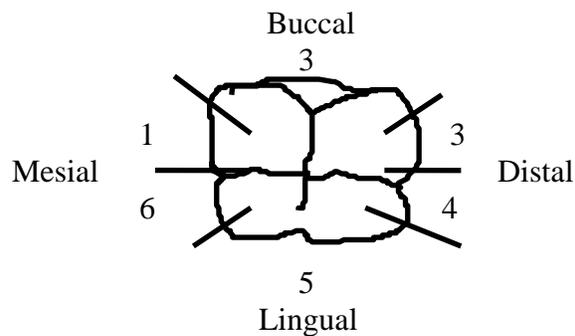
Patient number W126423 (from Winston-Salem site)

A plaque sample (“2” in the tenth digit)

Sample number “17”(the first plaque sample)

III. QTS SYSTEM

The new chart utilizes the QTS System for site nomenclature. QTS (*Quadrant - Tooth - Site*) utilizes a 3-digit code for charting purposes. The first digit of this code corresponds to the quadrant with the UR = #1) UL = #2, LL = #3 and LR = #4. The second digit in this code corresponds to the tooth. Teeth are numbered 1-8 beginning at the midline and counting distally. The third digit corresponds to site designation and is numbered 1-6 beginning at the mesio-buccal site of each tooth, numbering the sites distally and peripherally around each tooth, and ending with site #6 at the mesio-lingual site



IV. CLINICAL EXAM AND SAMPLE COLLECTION

A. *Soft Tissue Screening*

This screening is done as a service to the participant. We are looking for any suspicious lesions or conditions that should be referred to a dentist. If any such conditions are seen, an entry of “1” should be made for “Oral Lesions”. If nothing is noted, an entry of “0” is made. A “1” entry will then be noted by the computer in the Patient Summary and Recommendations form.

This screening examination is performed with a tongue blade and mirror and should cover the following areas:

- Upper and lower lips, including the Vermillion border
- labial mucosa
- buccal mucosa from the maxillary vestibule to the mandibular vestibule
- palpate cheeks
- mucosa of hard and soft palate
- uvula, tonsillar pillars, mandibular retromolar pad, and posterior pharyngeal wall
- dorsal surface of tongue
- ventral surface of tongue and anterior floor of mouth

MS DENTRY.EXE

Dental Clinic Entry System

ID: [REDACTED]
DATE: [REDACTED] / [REDACTED] / [REDACTED]
Examiner: [REDACTED]
Recorder: [REDACTED]
Visit #: [REDACTED]

[Validate ID]
[REDACTED]

[Oral Lesions: [REDACTED]]
0 = No
1 = Yes

(You must complete all fields before you can advance.)

3:55:56 PM

DENTRY.EXE

ID ABC000001 Missing Teeth

Quadrant I

Quadrant II

Quadrant III

Quadrant IV

0 = Missing
1 = Present

3:57:10 PM

Quadrant	Teeth	Status
I	3	0
	2	1
	1	1
	1	1
	2	1
	3	0
	5	0
	4	0
	8	1
	7	1
II	4	0
	5	0
	6	1
	7	1
	8	1
	6	1
	7	1
	8	1
	4	1
	5	1
III	4	1
	5	1
IV	3	0
	2	1
	1	1
	1	1
	2	1
	3	0

III. CLINICAL EXAM AND SAMPLE COLLECTION

B. *Caries Status and Plaque Score*

1. Criteria for coronal caries (Radike)

Caries Status - Caries status is recorded using criteria described by Radike. Caries is recorded in two phases, tooth status and number of surfaces affected. Incisors are scored as having 4 coronal surfaces while all other teeth are assumed to have 5 coronal surfaces. Four surfaces are scored for all root lesions.

Frank lesions: when cavitation is present, caries is scored. Cavitation is defined as a discontinuity of the enamel surface caused by the loss of tooth substance. Must be distinguished from fractures, erosion, and abrasion.

Lesions not showing frank cavitation:

Detection of PIT AND FISSURE lesions

1. area is carious when the explorer "catches" or resists removal after insertion into a pit or fissure with moderate pressure, **AND** when this is accompanied by one or more of the following signs of caries-
 - a. a softness at the base of the area
 - b. opacity adjacent to the pit or fissure as evidence of undermining or demineralization.
 - c. softened enamel adjacent to the pit or fissure that can scraped away with an explorer.
2. area is carious if there is loss of the normal translucency of enamel around a pit or fissure, even if the explorer does not catch or penetrate the pit or fissure.

Detection of SMOOTH SURFACE lesions on BUCCAL & LINGUAL:

1. area is carious if surface is etched or there is a white spot, **AND** if the area is found to be soft by:
 - a. penetration with an explorer
 - b. scraping away enamel with explorer
2. area is sound if there is no softness.

Detection of lesion on PROXIMAL SURFACES:

1. for areas exposed to direct visual or tactile examination, use the criteria in B above.
2. for areas not exposed to direct examination:

- a. visual examination -- if the marginal ridge shows opacity as evidence of undermined enamel, the proximal surface is carious.
- b. tactile examination -- any discontinuity of the enamel in which an explorer will enter is carious, IF it also shows other evidence of decay, such as softness, shadow by illumination, or loss of translucency.
- c. transillumination -- (for anterior teeth mainly) -- a loss of translucency producing a characteristic shadow in a calculusfree and stain-free proximal surface is adequate evidence of caries.

Additional notes:

1. stain and pigmentation are not regarded as evidence of caries, since they occur in sound teeth.
2. erosion, abrasion, hypoplasia, attrition, fractures, mottled enamel, and enamel opacities on exposed hard surfaces are not classified as carious.

Criteria for scoring multiple surfaces:

Anterior teeth	Coronal	D or F	1/3 rule
	Root	D or F	1/3 rule
Posterior teeth	Coronal	D or F	line angle
	Root	D or F	1/3 rule

2. Criteria for root caries

- Root caries is recorded as present if there is a discrete, well-defined, and discolored cavitation on the root surface and the explorer enters easily.
- Root caries, rather than coronal caries, is scored if at least half of the lesion or restoration extends apical to the cemento-enamel junction.
- Since the examinations are conducted in the subjects' homes, no radiographs will be taken, the teeth will not be dried, and calculus will not be removed.
- Root restorations due to caries must be distinguished from those due to cervical abrasion. Restorations due to caries are rounded. Those due to abrasion are angular. Also check adjacent and contralateral teeth for unfilled abrasion.
- Gingival recession is recorded if the exposed root surface is sound.
- All teeth, including third molars, are evaluated.

- Broken restorations and lost restorations are recorded as filled surfaces.
- If less than 1/4 of the crown is retained, score it as a root fragment.

3. Recording tooth status, coronal caries, root caries

STATUS	CORONAL CARIES	ROOT CARIES
1= sound		
2= decayed or filled	if a "2 or 4" code, record # of surfaces decayed and # of surfaces filled in the appropriate boxes. If surface has recurrent or new lesion on same surface with restoration then record as D	
3= missing,		
4= restored with crown		
5= decayed root fragment		
6= sound root fragment (overdenture abutment)		
7= implant		

Start in Quadrant 1, tooth 8 and proceed to tooth 1. Then move to Quadrant 2, tooth 1 and continue to tooth 8, followed by Quadrant 3, tooth 8 - 1; and then Quadrant 4, tooth 1 - 8.

For each tooth, if Tooth Status is a "2", the cursor will allow calls for DS, FS, DRS, and FRS. Simply record the number of surfaces affected by each condition. If surface is both D and F, record as D. If Tooth Status is a "4", then root caries is a possible call. Record any surfaces affected by root decay (DRS). If there is a root filling, record as FRS if there are no signs that it was filled due to abrasion. Then go on to record the plaques score for that tooth. For any other Tooth Status calls, the go directly to record the plaque score for that tooth.

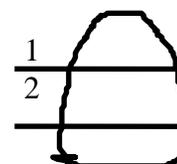
4. Plaque Score (PS)

Plaque scores for the buccal surface of each tooth are determined on a 0 - 3 scale. The tooth surface to be scored should be air dried and not disclosed. The plaque score is recorded in the appropriate box for each tooth on the computer screen

0 = absence of plaque or stain of the clinical crown

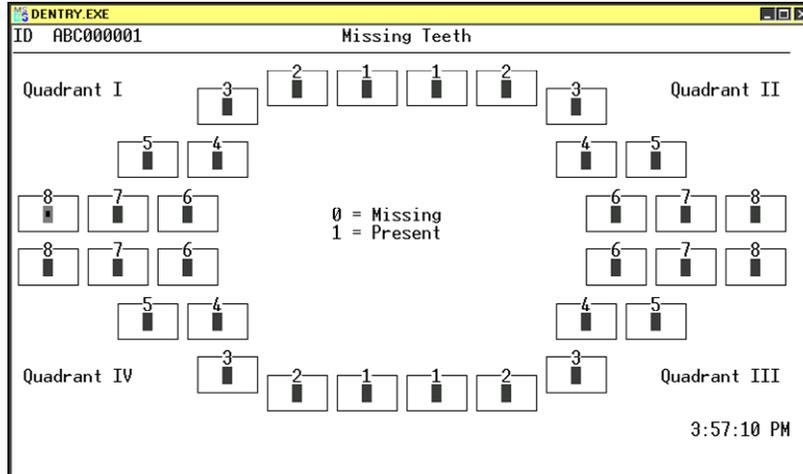
1 = deposits covering less than one-third of the surface

2 = deposits covering less than 2/3 of the surface



3 = deposits covering more than 2/3 of the surface

3



MS DENTRY.EXE			
ID	ABC0000	GCF	
Site	QTS	Periotron Reading	
Site 1:	161	[Bar]	Vial #1
Site 2:	261		
Site 3:	361		
Site 4:	461		
Site 5:	163	[Bar]	Vial #2
Site 6:	263		
Site 7:	363		
Site 8:	463		
Site 9:	171	[Bar]	Vial #3
Site 10:	271		
Site 11:	371		
Site 12:	471		
Site 13:	173	[Bar]	Vial #4
Site 14:	273		
Site 15:	373		
Site 16:	473		

XXX = Could not perform test

4:05:21 PM

C. Collection and Storage of Crevicular Fluid

This protocol describes the methods for the collection of crevicular fluid, volume quantitation, crevicular fluid storage and PGE, RIA.

Overview

Crevicular Fluid is collected using Harco Periopaper prior to any probing measurements. A site (identification described below) is first isolated with cotton rolls and air dried. The periopaper strip is placed gently into the crevice and left in place until the strip is visibly dampened. The fluid volume is determined with a calibrated Harco Periotron. This volume is used to compute the final mediator concentration. Each GCF sample is assayed independently for a chosen inflammatory mediator. This enables the determination of a site-specific mediator level as well as an overall patient mean % standard error for statistical testing. Periopaper strips will be wrapped in aluminum foil, sealed in screwtop vials, catalogued and stored in liquid nitrogen.

1. Techniques for GCF Samples & Storage

The site to be sampled is identified from the data entry screen, then **isolated with cotton rolls and air dried**. Make sure there is not saliva pooling at the gingival margin. The examiner and the assistant wear rubber gloves when collecting samples; do not touch periopaper or aluminum foil with fingers as fingerprints cause false positives in RIA. A periopaper strip is placed into the crevice of the tooth with cotton pliers and left in place until the strip is visibly dampened, but not saturated to the orange saturation mark on the strip. The strip is gently placed until it is held by the gingival margin without attempting to push the strip down into the pocket. This is referred to as an orifice sampling method. It is then removed with pliers and measured on a calibrated Harco Periotron. The reading from the Periotron should be **between 30 and 180**, as readings outside this range are less reliable by linear extrapolation. If the reading is not in this range the sample should be taken again with a new strip. The Periotron reading is recorded on the sample requisition form with the corresponding QTS # (see example) and into the computer screen along with the QTS location. The filter paper strip is then placed onto a strip of aluminum foil and the foil folded over to cover the strip. Be sure to wrap each strip once by folding before placing the next strip. For this purpose use the precut aluminum foil. Always wear gloves when handling foil

so as to avoid GCF contamination with fingerprints. The next filter paper strip is then placed on the aluminum foil and the foil is folded over to make sure that the filter papers do not touch. The aluminum foil strip is rolled until all 4 samples are taken, and then folded completely. 4 strips will be placed into each of 4 vials for a total of 16 GCF samples. The last filter paper strip put onto the aluminum foil is, by necessity, the first strip removed. Thus, as the foil is unfolded, the samples are uncovered in the reverse order from which they were taken. As soon as 4 samples have been collected into the aluminum foil, the foil is placed into a 1.5 ml. screw top cryovial and placed immediately into liquid nitrogen chairside. Prostaglandins deteriorate rapidly at room temperature in the presence of oxygen, thus it is very important to get the samples into liquid nitrogen as soon as possible. The small dewar flask containing liquid nitrogen at chairside is used for this purpose.

It is absolutely critical since more than one mediator is to be analyzed that there be only one piece of foil with the GCF strips grouped for that mediator. Thus, one vial should contain only one foil piece which has GCF strips to be analyzed for only one mediator. Since 4 mediators are to be analyzed there are 4 vials. Sampling should proceed one mediator at a time, 1 GCF from each quadrant (as shown on the computer screen). After 4 GCF samples are collected, the vial is put into liquid nitrogen and then 4 more samples are taken for the next mediator, until all 16 GCF samples have been collected. Again remember to include at least one full turn of foil between subsequent strips, otherwise they can fall together during unwrapping.

If a GCF sample is dropped, contaminated with saliva, lost or has a questionable reading, discard the sample and take another GCF sample from the same site and proceed as before.

2. Gingival Crevicular Fluid Sampling Sequence

Gingival crevicular fluid is collected and placed into liquid nitrogen for storage prior to mediator assay by radioimmunoassay or ELISA. Four sites in each quadrant will be sampled to provide enough GCF samples for analyses of four mediators per patient. A total of 16 GCF samples are collected per patient using periopaper (10-30 sec) and recording the volume with the Periotron 6000. 16 samples will be collected - 4 per foil strip, 4 strips - one into each of 4 vials.

3. Records

The computer screen displays the 16 sample sites to be used and those sites are identified by the computer. The QTS sites are recorded on the Sample Requisition Form. After each sample is read in the Periotron, that reading must be recorded for the appropriate QTS on the requisition form. After all 16 of the Periotron readings have been recorded on the form, the readings are then transferred to the computer screen. Be sure to double check that the number on the vial matches that on the sample requisition form. All GCF vials must be transferred to liquid nitrogen tank for storage at the end of the day.

4. Quick Reference sheet for Crevicular Fluid Collection

- 1). Have operatory prepared as described previously in the Preparation Section. Have vials set up in row within the rack such that there are 4 vials in each of 3 rows. The first row will be for the GCF samples, the second row for the plaque and the third row for the serum.
- 2). Site to be sampled is identified from the data entry screen. The 16 QTS numbers are recorded on to the Sample Requisition Form.
- 3). Site is isolated and dried. Periopaper is placed into site until visibly dampened.
- 4). Examiner places periopaper into Periotron and closes the sensor on it.
- 5). Examiner returns to step 2) to sample the next site
- 6). Assistant records the final reading on the Periotron on the Sample Requisition Form, removes the periopaper and places it on the foil strip.
- 7). Assistant folds foil over the periopaper at least twice using cotton pliers.
- 8). After every fourth periopaper, the entire foil strip is folded at ends and placed in a cryovial. The number on the cryovial is checked with the appropriate accession number in sequence from the Sample Requisition Form. The cryovial is stored chairside in the flask of liquid nitrogen.
- 9). Assistant returns to step 6) until sixteenth strip is completed.
- 10). Transfer the Periotron readings from the form to the data entry screen according to site number. Place GCF vials in larger liquid nitrogen storage tank.

III. CLINICAL EXAM AND SAMPLE COLLECTION

D. Microbial Sampling and Analysis of Plaque Samples

1. Preparation

For supplies, each site will be provided with 4 plaque sampling vials that are prelabelled, color coded and in the patient sample kit. Each tube contains 100 μ L of sterile 1 x TE buffer. These vials are now stable for several years if the caps are snug. To tighten caps, screw until just barely tight. DO NOT OVER-TIGHTEN.

2. Plaque Sampling

Plaque samples are obtained from the mesial surface of all 4 first molars using a sterile curette. These samples are each placed in a separate tube for a total of 4 samples. The 4 areas to be sampled will be the mesial buccal of all 4 first molars. If this tooth is missing go to the nearest most posterior adjacent tooth.. The site is isolated with a cotton roll and removed of gross supragingival plaque or debris if present and gently dried with sterile gauze or air stream. GCF's should be taken prior to plaque sampling. Plaque samples will be taken by placing a sterile curette to the base of each pocket and, with a single stroke, pressing against the tooth at the depth of the pocket, removing a sample for transfer to the storage buffer. Do not attempt to sample the grossly adherent plaque which appears supragingivally.. After collecting one plaque sample, the tip is wiped with an alcohol gauze before taking the next sample. The plaque sample will be placed into the tube containing 0.1 mL sterile TE buffer (premade in the Patient Kit provided). Place the tip of the curette into the TE buffer, and swirl the curette between your fingers to remove plaque. If a particularly heavy deposit adheres to the curette, it may be removed by scrapping onto the upper lip of the tube, and then pushing the sample down into the buffer. However, in general, sufficient bacteria will be transferred to the buffer for DNA analysis, even if not visible by eye. This procedure is repeated for the remaining 3 molar sites.

Following sample collection, 0.1 mL of 0.5 M NaOH (sterile) will be added using the plunger-style dispenser and mixed well by closing the lid tightly and shaking. At the beginning of each day the plunger pump is primed by pumping solution into the sink twice. Check the

prelabelled vial to assure that the appropriate accession number matches with the sample requisition form. Samples are stored at room temperature until a sufficient quantity has been obtained to be shipped. Storage may be at room temperature, refrigerator or freezer temperature, as convenient, since they are quite stable(see Jurassic Park).

When plaque samples have been collected and stored, enter a “y” in the designated box on the computer screen in order to move to the next screen.

Quick Reference Sheet for Plaque Sample Collection

- 1). Have operatory prepared as described previously and vials in 3x4 rows.
- 2). The sequence of sites to be sampled are as follows:
 - Four samples (mesial of each first molar) in 4 separate tubes
 - Use a wiped end of a curette for each site sampled.
 - Adjacent, most posterior tooth may be substituted if first molars are not present.
- 3). Areas to be sampled are cleared of gross supragingival plaque and dried with gauze.
- 4). Obtain the sample by placing the curette at the base of the pocket and removing the sample with a single stroke.
- 5). Place the curette end into the tube (pre-prepared and provided in the Patient Kit). Place below the buffer level and rotate gently.
- 6). Recap tube and repeat with the next sample.
- 7). The prelabelled tube number is checked against the Sample Requisition Form..
- 8) Sodium hydroxide buffer placed in each vial using pump dispenser, capped shaken and stored.

III. CLINICAL EXAM AND SAMPLE COLLECTION

E. Gingival Index: Loe & Silness

This is a tooth-based score. Mesio Buccal to distobuccal surfaces are examined. Periodontal probe is placed under the gingival margin at the mesio Buccal site and swept along the buccal surface to the distobuccal site and bleeding is noted.

0 = Normal gingiva

1 = Mild inflammation: slight change in color, slight edema. No bleeding on probing.

2 = Moderate inflammation: redness, edema, and glazing. Bleeding on probing.

3 = Severe inflammation: marked redness and edema. Ulceration. Tendency to spontaneous bleeding.

Sequence of Exam.

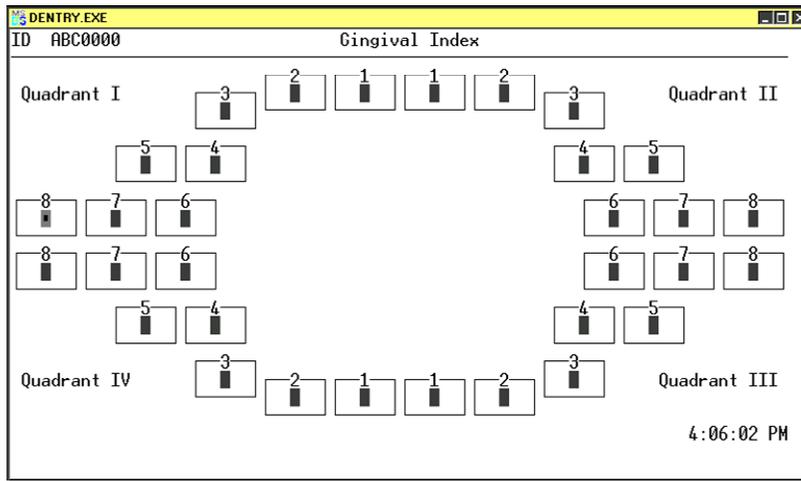
The sequence is the same as for caries:

Quadrant 1: teeth 8 - 1

Quadrant 2: teeth 1 - 8

Quadrant 3: teeth 8 - 1

Quadrant 4: teeth 1 - 8.



III. CLINICAL EXAM AND SAMPLE COLLECTION

F. *Probing Depth, CEJ, and Bleeding on Probing*

Probing depth is measured first and recorded on the computer screen for the site. For the same site, the CEJ measurement is made and then the cursor moves to the next site. The measurements start in Quadrant 1, tooth 8 and moves from sites 3 (distobuccal) to 1 (mesiobuccal) on each tooth. At the end of quadrant 1, bleeding for the sites just probed is recorded. Measures then move to Quadrant 2, tooth 1, and moves from sites 1 (mesiobuccal) to 3 (distobuccal). Bleeding for the sites just probed in Quadrant 2 are recorded. Lingual sites for Quadrant 2, teeth 8 to 1 are then measured for sites 4 (distolingual) to 6 (mesiolingual). Bleeding is then recorded for sites just measured. Quadrant 1, teeth 1 to 8, sites 6 (mesiolingual) to 4 (distolingual) are then measured and bleeding recorded. The same procedure is then repeated for Quadrants 3 and 4. The cursor on the screen moves in the appropriate sequence.

Examination Sequence

Quadrant	Teeth	Sites	
1	8 to 1	Db to Mb	Record BOP
2	1 to 8	Mb to Db	Record BOP
2	8 to 1	Dl to Ml	Record BOP
1	1 to 8	Ml to Dl	Record BOP
3	8 to 1	Db to Mb	Record BOP
4	1 to 8	Mb to Db	Record BOP
4	8 to 1	Dl to Ml	Record BOP
3	1 to 8	Ml to Dl	Record BOP

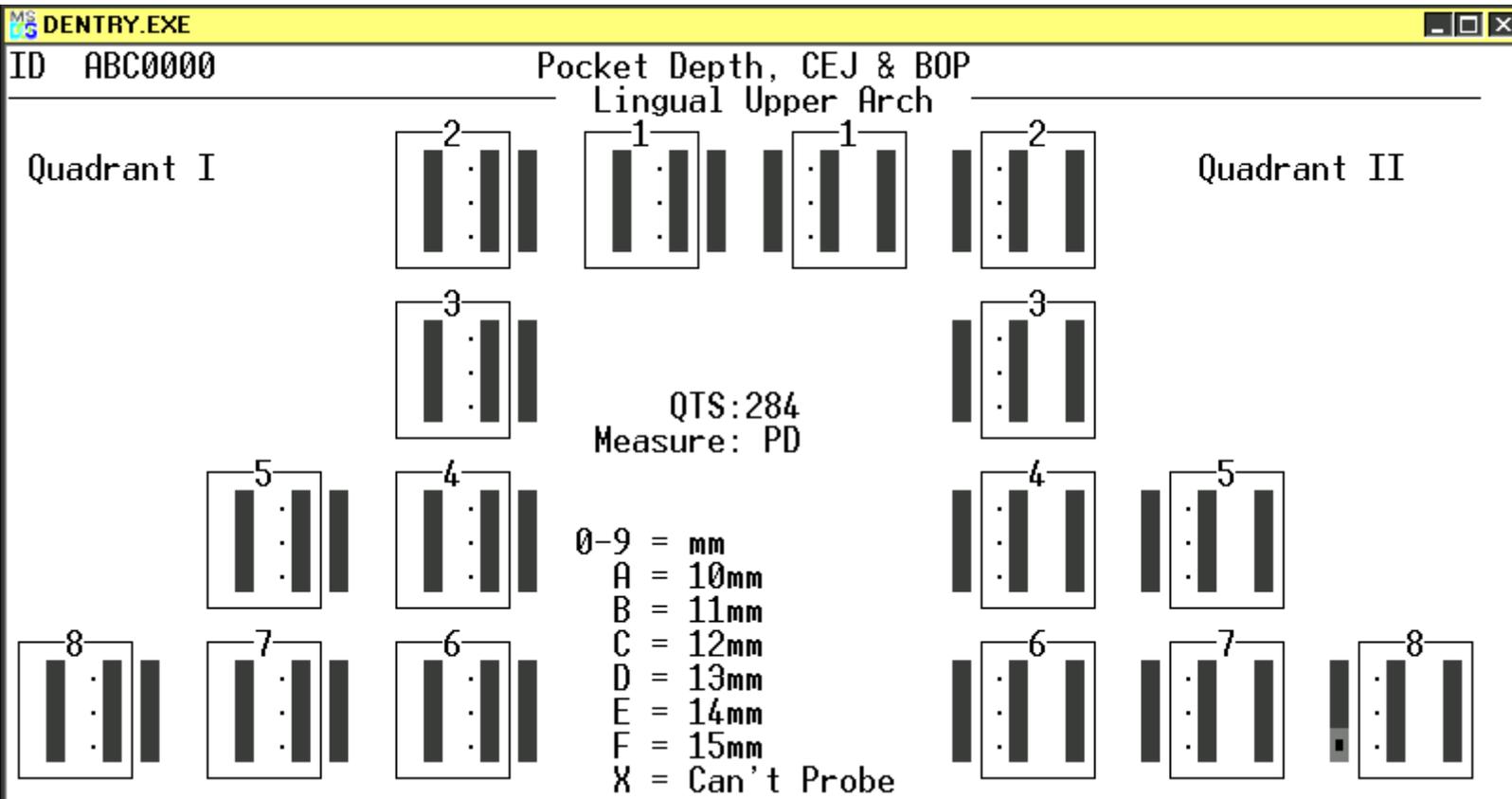
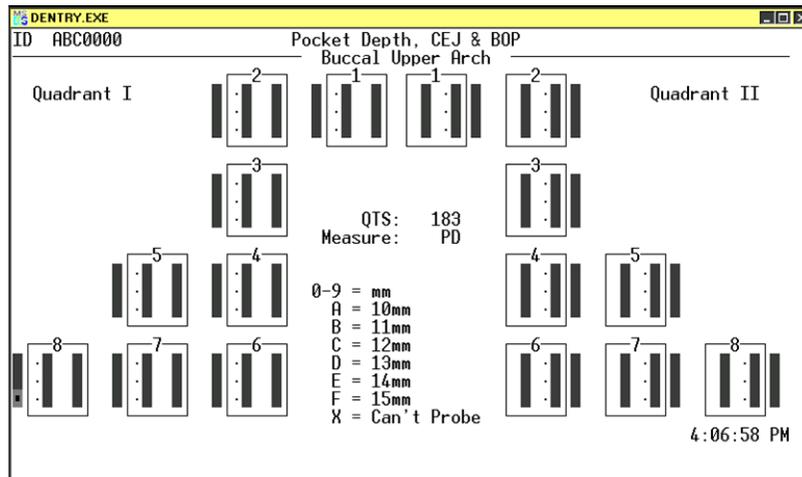
Probing Depth (PD) - Periodontal pocket depth is determined with a UNC-15 periodontal probe at six sites per tooth rounded to the next lower whole mm. The probing depth reading is recorded on the computer screen labeled ***PD*** for each tooth.

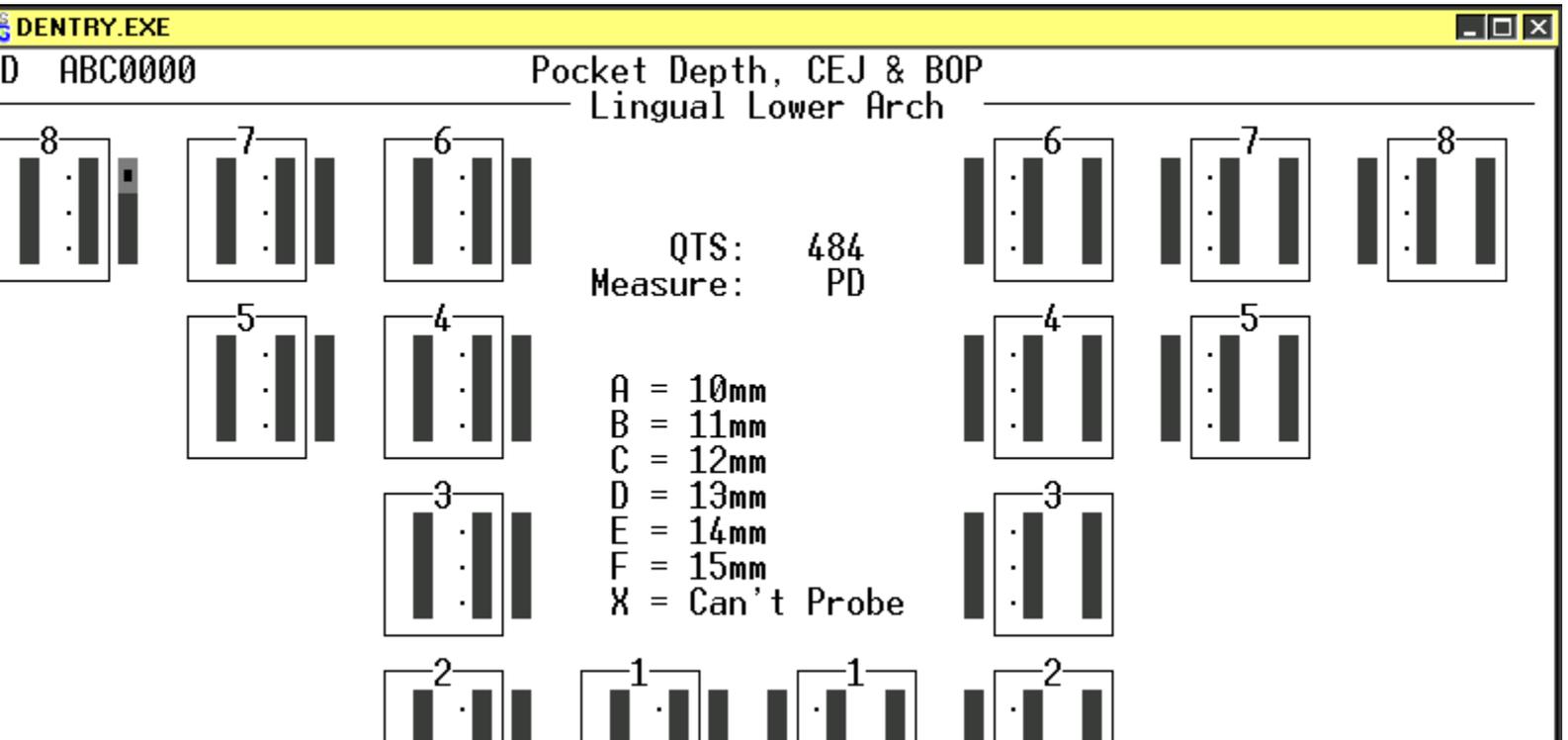
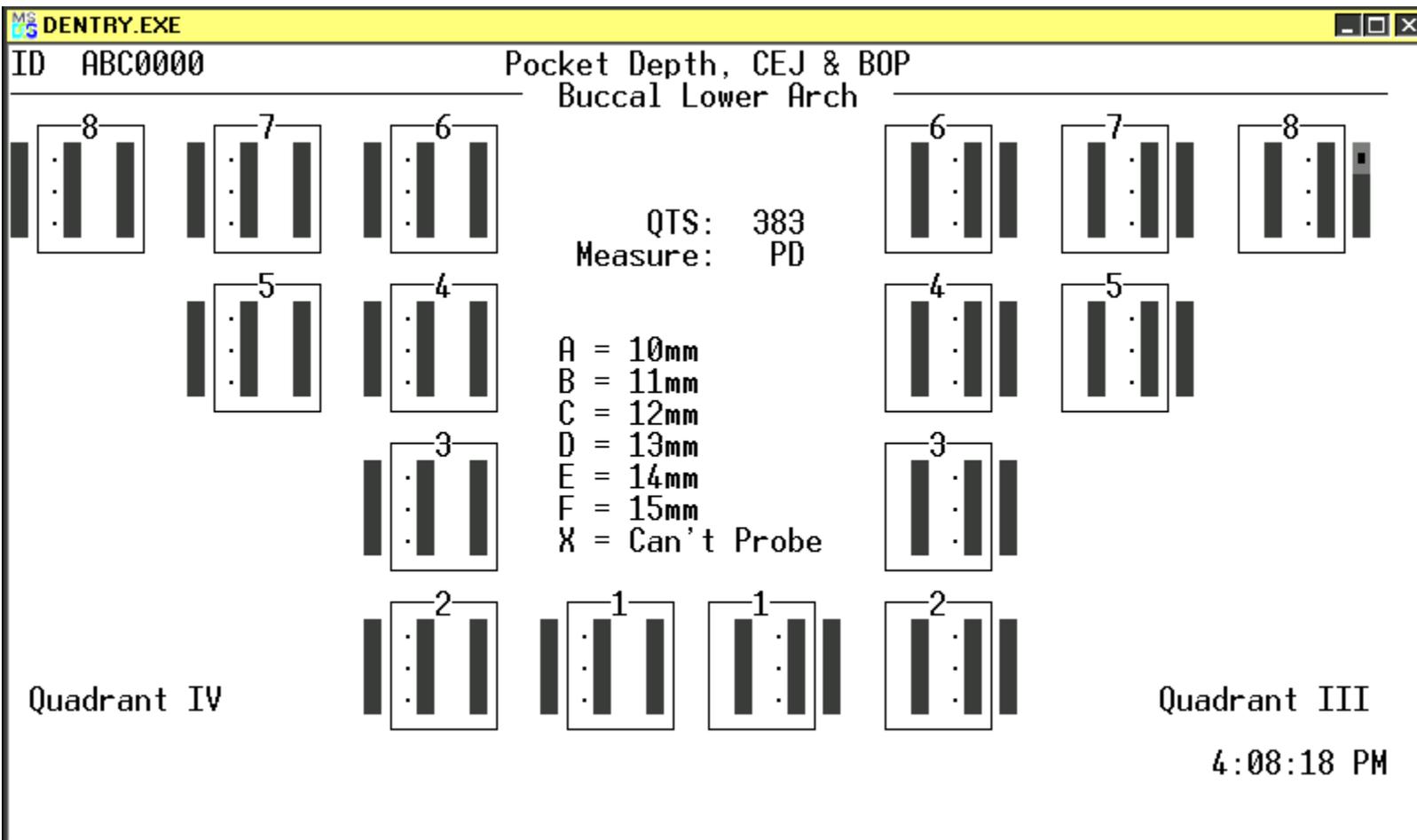
Cemento-enamel Junction (CEJ) - CEJ levels are measured with a periodontal probe at six sites per tooth. The distance from the CEJ to the gingival margin is measured and recorded in millimeters rounded to the next lower mm. Millimeters of recession are recorded as negative numbers, otherwise the number is positive.

Bleeding on Probing (BOP) - Bleeding on probing is assessed after probing each quadrant on the buccal (surfaces 1-3) and on the lingual surfaces (surfaces 4-6). A dichotomous scoring system is used at six sites per tooth using one (1) and zero (0) for presence or absence, respectively.

0 = absence of bleeding

1 = bleeding present





PATIENT SUMMARY AND RECOMMENDATIONS

At the end of the periodontal examination, the Patient Summary and Recommendation screen will appear on the computer (see next page). The computer automatically fills out this form based on data generated by the examination and places an “x” in the appropriate box.

One or more surfaces of your teeth that may need to be checked by your dentist to determine if there are cavities.

(The above box is marked if the caries examination finds one or more DS calls)

One or more areas of your gums that appear to be red and inflamed. These areas should be checked by your dentist.

(The above box is marked if one or more teeth have a GI score of 2 or more)

One or more deep pockets between your teeth and gums that could indicate a periodontal condition exists. These areas should be checked by your dentist.

(The above box is marked if one or more probing depth measures is 4+ mm in depth)

An area in your mouth that should be evaluated by your dentist as soon as possible.

(The above box is marked if the examiner recorded a “1” score after the soft tissue examination. This box may be marked for a range of conditions that necessitate a dental visit, e.g. candida infection, denture stomatitis, suspicious lesion.)

Print the form, write in the participant's name, and review the recommendations with them.

THE ARIC STUDY PERIODONTAL (GUM DISEASE) EXAMINATION
RECOMMENDATIONS

PARTICIPANT NAME: _____ ID: _____

EXAMINER: _____ DATE: _____

Our dental hygienist has found the following:

- One or more surfaces of your teeth that may need to be checked by your dentist to determine if there are cavities.
- One or more areas of your gums that appear to be red and inflamed. These areas should be checked by your dentist.
- One or more deep pockets between your teeth and gums that could indicate a periodontal condition exists. These areas should be checked by your dentist.
- An area in your mouth that should be evaluated by your dentist as soon as possible.

This screening dental examination was conducted as part of the ARIC Study. It is not a substitute for the complete examination given to people who go to a dentist seeking dental care, since neither a complete dental history, caries examination, soft tissue examination, nor x-rays were taken. Thus, our evaluation of your dental condition may not necessarily agree with the results of an examination by your dentist. However, the recommendation of our hygienist is provided to supplement your knowledge of your dental health. -We would like to take this opportunity to thank you for participating in this important study.

c:\axic\visit4\planning\dentrpt.002.3/14/96:ccp

VII. PERIOTRON:

A. CALIBRATION AND GENERAL INFORMATION

Detailed documentation of calibrations of the Periotron must be kept in the study notebook. Calibration of the Periotron involves obtaining readings on a series of measured amounts of fluids. There are two different procedures used for calibration. A comprehensive calibration is performed at the beginning of the study and at any point during the study when an interim calibration is out of range. A simpler, three point calibration procedure is completed each week before using the Periotron.

INSTRUCTIONS FOR CALIBRATING THE PERIOTRON

COMPREHENSIVE CALIBRATION

The Purpose of a comprehensive calibration is to establish the relationship between the Periotron readings and the standard volumes of water. The steps for a comprehensive calibration are as follows:

1. Distilled Water is used for calibrating. Operators should always wear latex gloves. A small amount of water kept in a cryovial is easier to access for measurement. The Hamilton Microliter Syringe is used for measuring the water. It is very important that the instructions for use of the Hamilton Microliter Syringe be followed carefully.
2. Initially, turn on the Periotron and allow it to warm up for at least 10 minutes. Open a pack of Periopapers and secure it to the Periopaper holder. Clean the sensors with an alcohol wipe and dry with cotton gauze.

(NEVER TOUCH THE WHITE PAPER STRIP WITH YOUR GLOVED FINGERS)

3. Place a dry Periopaper on the lower sensor aligning the edge of the sensor with the black margin on the strip. Close the upper sensor on the dry strip and move hands away from the sensor. Adjust the Zero Adjustment knob until “0” appears on the digital readout.

4. Repeat the test for “zero” two additional times using clean, dry Periopaper each time. A slight drift of values after adjusting the zero knob is acceptable up to a reading of “2”. A reading producing a blank line is not acceptable.
5. Using locking cotton pliers, remove one Periopaper from the holder; and, being careful not to let the white strip contact another surface, place the cotton pliers on a flat surface. Set the Hamilton Microliter Syringe to the first volume indicated on the comprehensive calibration document form. Load the pipette with this indicated amount of distilled water from the vial and empty the water from the pipette onto the Periopaper. It is best to empty the water onto the lower half of the Periopaper close to the tip.
6. Without delay, place the Periopaper on the sensor of the Periotron being careful to line up the edge of the black mark on the strip with the edge of the sensor (do not have the sensor touching the black line on the strip). The periopaper is “positive sensitive” so each operator should always place Periopaper on the sensor in the same position for each Periotron. After placing the Periopaper on the sensor, always move your hand away from the instrument. Wait approximately 15 seconds until the “11” lights up on the Periotron indicating that the reading is final. Record this reading on the calibration form.
7. After each reading, the sensors of the Periotron unit should be wiped with a prepackaged, sterile alcohol wipe and allowed to dry for 15 seconds; or you may dry the alcohol with a gauze pad.
8. Procedures 5 through 7 should be repeated for each volume indicated on the calibration form. Each volume will be repeated three times before the calibration is completed. The volumes are given on the calibration form in a randomized order. Calibration forms will be provided.
9. A copy of the calibration documentation form is kept in the Investigator’s study notebook.

INTERIM

The interim calibration is a 3-point calibration intended to validate sampling values to the comprehensive calibration. This procedure should be completed weekly before the Periotron is used. Repeat the steps given previously recording three readings each for 0.1 microliters, 0.4 microliters, and 0.7 microliters of water. Record the three readings on the interim calibration documentation form. A copy of the calibration should be kept in the Investigator’s notebook on site.

Compare the interim data to the comprehensive calibration ranges for each individual Periotron. In the event that the weekly calibration values deviate more than the range provided, complete a comprehensive calibration. It is very important that the same individual complete the comprehensive calibration and the interim calibrations.

GENERAL PERIOTRON 6000 INFORMATION

- Keep hands away from the sensors during measurement.
- Never close the Periotron jaws on anything thicker than paper.
- Turn the power switch off if you experience voltage fluctuations or electrical storms.
- When not in use, leave a Periopaper strip between the closed jaws.

1. Comprehensive Periotron Calibration Form

Protocol #: _____ Site#: _____ Date of Calibration: _____ Periotron Serial #: _____ Person Calibrating: _____	
Did Periotron warm-up for at least 10 minutes? (circle one) Yes No Was Periotron adjusted to "zero" three times (circle one) Yes No	
Randomized Volumes of Distilled Water* (in microliters, complete calibration in the order given)	Periotron Reading

0.05	
0.05	
0.05	
0.8	
0.8	
0.8	
1.0	
1.0	
1.0	
1.2	
1.2	
1.2	
0.2	
0.2	
0.2	
0.6	
0.6	
0.6	
0.5	
0.5	
0.5	
0.4	
0.4	
0.4	
0.9	
0.9	
0.9	
0.3	
0.3	
0.3	

0.7	
0.7	
0.7	
0.1	
0.1	
0.1	

* Water is measured in microliters with a Hamilton Microliter Syringe

2. Interim Periotron Calibration Form

Protocol #: _____ Site #: _____ Date of Calibration: _____ Periotron Serial #: _____ Person Calibrating: _____ Did Periotron warm-up for at least 10 minutes? (circle one) Yes No Was Peritron adjusted to "zero" three times? (circle one) Yes No	
Randomized Volumes of Distilled Water* (in microliters, complete calibration in the order given)	Periotron Reading
0.1	
0.1	
0.1	
0.4	
0.4	
0.4	
0.7	
0.7	
0.7	
*Water is measured in microliters with a Hamilton Microliter Syringe The median Periotron reading for each of the above volumes is compared to the comprehensive calibration range provided.	

VIII. PATIENT VISIT SUPPLIES

THE FOLLOWING IS AN OUTLINE OF THE SUPPLIES THAT ARE NEEDED FOR CLINICAL SAMPLING PER PATIENT AT EXTRAMURAL SITES.

INSTRUMENTS

Dental Mirror
 UNC - 15 Probe
 Tongue Blade
 3 Universal Curettes (double-ended i.e. Columbia 4R/4L)
 3 Cotton Forceps

DISPOSABLES & STORAGE

FOR BLOOD ONLY

21 Gauge Needle with Vacutainer Holder
 Appropriate Vacutainer Tubes
 Alcohol Swabs
 Tourniquet
 2x2 Gauze
 Bandage

OR

Winged Infusion Set 21gauge
 3 Syringes (60cc) with 1cc Heparin Each
 Heparin
 Alcohol Swabs
 Tourniquet
 2x2 Gauze
 Bandage

FOR GCF ONLY

Cotton Rolls
 2 Sets of Perio Paper
 4 GCF Cryovials with Colored Tops Designating the Mediator
 4 Sterile Aluminum Foil Strips
 4 Index Cards
 Perio Paper Holder
 Dewar Flask with Liquid Nitrogen
 Calibrated Periotron (Harco®)

FOR MICROBIAL SAMPLING ONLY

5 Conical Bottom Tubes (2.0ml) with:

- 0.1ml Sterile TE Buffer Before Sample
- 0.1ml Sterile 0.5M NaOH Buffer After Sample

Cotton Rolls

ADDITIONAL EQUIPMENT

Hamilton Syringe (for Periotron Calibration)

100ml Micropipette with Sterile Tip

Canned air

FORMS

Consent Form

Medical History

Patient Demographics and Interview

Procedure Checklist

Perio Data

Assay Recording (GCF Cards, Accession Number Log, etc.)

IX. SHIPPING INSTRUCTIONS