



CariFree Caries Bacterial Detection and Treatment Protocol

Patent Pending

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Protocol

Program Steps and Decision Tree:

There are several steps and decisions to be made in implementing the CariFree complete caries solution. They are as follows:

Step 1 - Screen for Caries Risk Factors:

All patients are screened with both the CariScreen Caries Susceptibility Test and a Caries Risk Assessment Form to determine and identify moderate to high risk patients and also identify specific risk factors. Patients that score low in RLU's (relative light units) on the CariScreen Test and low risk on the Caries Risk Assessment Form should be educated on the importance of risk factors and are rescheduled for a CariScreen Test again in about one year. Patients with moderate to high CariScreen scores, go to Step 2.

Step 2 – Confirm Bacterial Load

Patients with moderate to high CariScreen scores and/or score moderate to high risk on the Caries Risk Assessment Form should have their saliva cultured with the CariCult oral bacterial detection system to determine the actual CFU's of Mutans streptococci present.

Low Test Results - Patients with CFU's in the range of less than 10^5 CFU/ml are considered to be low risk to get dental caries. They are educated on the importance of risk factors and are rescheduled for Caries screening again in about one year.

Moderate to High Test Results - Patients with CFU's in the range of 10^5 CFU/ml are diagnosed moderate risk for dental caries. Patients with CFU's greater than 10^5 CFU/ml of saliva are diagnosed as high risk for dental caries. Both patients receive the diagnosis of dental caries.

Step 3 - Treatment for Dental Caries:

Patients with a diagnosis of dental caries with moderate or high risk should be treated with the CariFree™ Treatment Rinse, a potent antimicrobial rinse, used to reduce the cariogenic bacterial infection in the oral biofilm. This therapy is followed with a daily regimen of the CariFree™ Maintenance Rinse for an ongoing period of time.

Coinciding with the rinse therapy, all risk factors should be discussed and other supportive measures and behavior modification reinforced. At this time all cavitations should be restored with a fluoride releasing glass ionomer material as indicated.

Step 4 - Monitor Results

Thirty days after the initiation of antimicrobial therapy with the CariFree™ Treatment Rinse, and Maintenance Rinse, the patient should be retested to determine the results of the antimicrobial therapy. Patients that now exhibit low numbers of CFU's, less than 10^5 CFU/ml can proceed with definitive restorative treatment and then placed into normal recare scheduling.

Patients that still have elevated CFU's, should be re-cycled into the CariFree™ Treatment Rinse and CariFree™ Maintenance Rinse regimen. The patient should be tested again 30 days after initiation of that rinse therapy. This cycle of antimicrobial rinse therapy and subsequent testing should continue until the patient exhibits a low CFU score. All moderate and high risk patients should continue with the CariFree Maintenance Rinse on an ongoing daily basis.

Specific Directions for Use:

1. CariScreen Caries Susceptibility Test:

Preparing the Patient

This simple screening test requires only a couple of minutes to perform. The patient should have a mouth at rest, which in general terms means no mechanical activity, brushing, flossing, swishing, coughing, chewing, eating etc for a period of 60 minutes prior to the test. **Always perform this test as the first thing during a patient visit.**

The CariScreen Caries Susceptibility Test is being used to measure bacterial ATP present on the teeth surfaces, as an indication of levels of cariogenic bacteria in the oral biofilm. Excessive mechanical activity of the mouth may produce increased levels of somatic ATP from the gingival and mucosa and can result in incorrect high numbers or false positive results.

Taking Swab Samples

When collecting a sample make sure to use aseptic techniques. Do not touch the swab or the inside of the sampling device with fingers.

Holding the swab tube, twist and pull the top of the swab out of the swab tube. The swab tip comes pre-moistened for collection. Condensation may be visible on the inside of the swab tube, this is normal. Carefully swab the buccal surface of a maxillary molar in the gingival third **without touching the gingiva** with the swab tip. Swab the tip across the tooth surface **three times** (not two and not four) from mesial to distal. Use the maxillary right first molar if it is present; if not present select the second molar or premolar to swab

instead. Using the same swab, carefully swab a maxillary incisor in the gingival third with the same protocol: swab **three times** (not two and not four) across the surface from mesial to distal **being sure not to contact the gingiva**. Use the maxillary right central incisor if it is present; if not present, select another incisor and swab it with the same protocol. After swabbing the desired test area, place swab back in swab tube. The sample can be left for up to 4 hours on the swab bud before activating the device, however once activated the sample must be read in luminometer within 60 seconds. This test is technique sensitive, so for best results, follow the directions carefully, develop good sampling techniques, and be consistent with your sampling methodology. Consistency in your sampling technique will yield the most reliable results over time.

Reading the Results

To activate the device hold the swab tube firmly, use the thumb and forefinger to break the Snap Valve by bending the bulb forward and backward. Squeeze the bulb twice, expelling all liquid down the swab shaft. Bathe the swab bud in liquid by gently shaking for 5 – 10 seconds.

Insert the CariScreen Swab device into the CariScreen luminometer, close lid and read the results by pressing “OK”. A 15-second sequence will then commence. Results should be read within one minute of activation of the CariScreen Swab device. Please refer to the CariScreen instrument manual for operating instructions.

At the end of this count-down, the RLU will appear on the screen in a value of 1-9999. Record this number for the patient on the Data Collection Form. Values established on the first clinical trial indicate the following.

<u>Caries Risk Level</u>	<u>RLU Values</u>	<u>Test Result</u>
Low Risk	Below 1,500	Negative
Moderate Risk	1,500 to 3,500	Positive
High Risk	3,500 to 9,999	Positive

2. Caries Risk Assessment Form:

The Caries Risk Assessment Form is a straightforward questionnaire that identifies risk factors. The form is designed with the risk factors on the left vertical column and then in order across the page to the right, the vertical columns identify high risk, moderate and low risk responses. Fill in the form using your data on the patient and ask questions where indicated. Place a check alongside the risk factor in the appropriate column.

The highest risk indicator is the presence of an existing lesion. A patient with an existing smooth surface cavitated lesion should be considered potentially high risk. Upon completion of filling out the form, look at the risk column answers. The purpose for the risk assessment for is not to confirm a diagnosis, but rather to identify specific risks for

individual patients, and to screen for potentially moderate to high risk patients who should be cultured to confirm a diagnosis.

Designate the patient high, moderate or low risk based on the general number of responses in the columns. For low risk patients who also had low ATP Bioluminescence RLU scores, they should be educated about risk factors and how they may change over time, and placed in normal Recare/restorative schedules. For moderate and high risk patients, the patient should have a saliva culture using the CariCult for Mutans streptococci to confirm diagnoses.

3. CariCult *Oral Bacterial Detection System:*

The CariCult Oral Bacteria Detection System and incubator are a straightforward in-office culture system to quickly and accurately determine the patient's specific Mutans streptococci CFU's in their saliva.

In preparation, the incubator should be heated and maintained at body temperature or 37 degree Centigrade. See incubator manual for specific instruction on set up and maintenance.

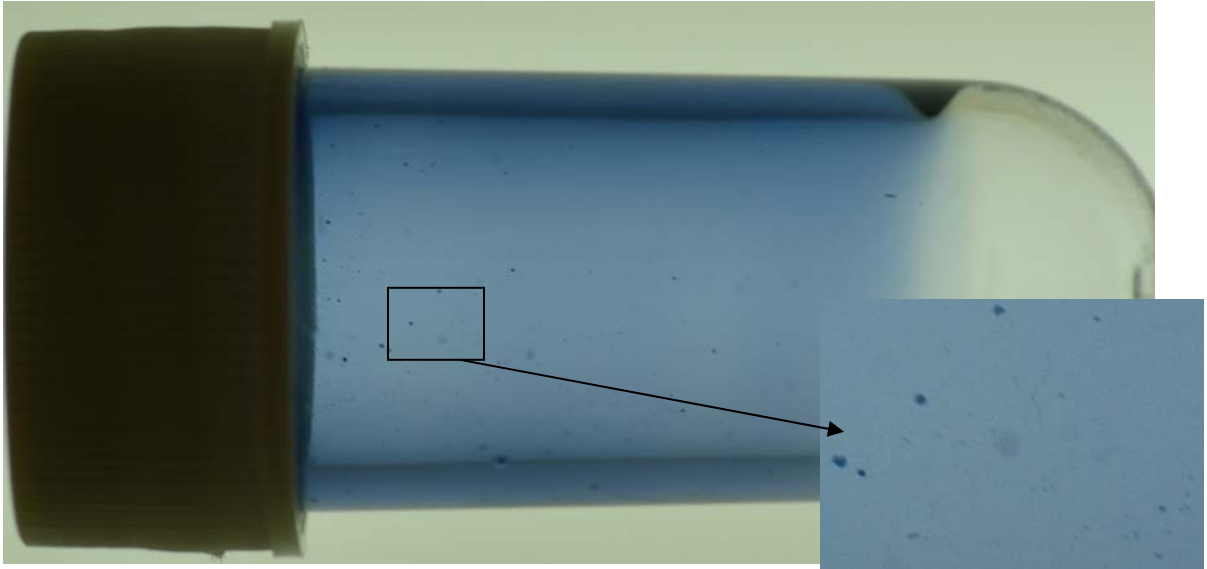
To perform the test, use a sterile Falcon 15 ml tube with the blue-cap, and pour about 2 ml of transfer media into the tube. Then take a blue colored Microbrush from the package, utilizing sterile techniques, that is to say be careful not to contaminate the brush with bacteria other than from the teeth. **Firmly scrub** (inadequate scrubbing during this step can cause a false negative culture result) the brush bristles across the lower anterior teeth back and forth 5 times on the buccal and then 5 times on the lingual surfaces to the mandibular incisors, or 4 alternate teeth.

Next place the brush into the Falcon tube and agitate the swab tip with the transfer media liquid. Tap the bottom of the tube with your finger (plunking) about 30-40 times to get the bacteria into the solution, or shake the tube back and forth about 20 times,

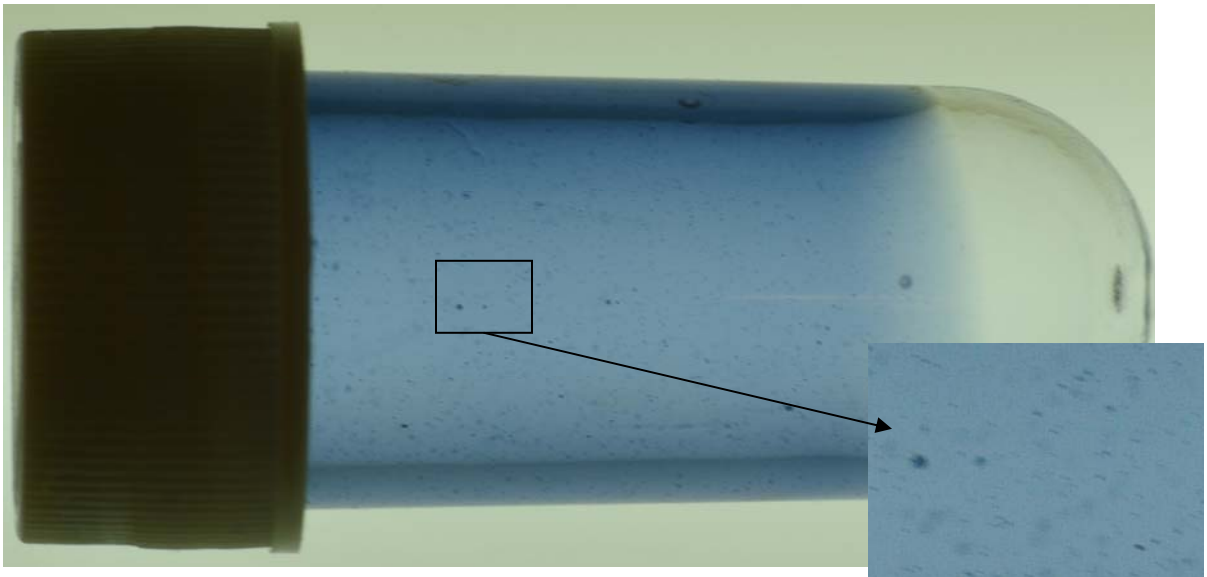
Next open the Falcon tube and carefully pour all of the liquid into the CariCult agar tube. With care you can leave the blue Microbrush in the Falcon tube and just pour the inoculated transfer media liquid into the agar tube. Place the cap back on the Falcon tube and discard. Place the cap back on to the CariCult tube and carefully invert **two times** (do not shake the culture tube, shaking can cause the agar to separate from the tube) and then pour out and completely discard the excess solution from the CariCult tube. Place the cap back on the CariCult tube and mark the cap with the patient's name or ID number. Incubate the tube in the inverted (cap down) position at 37 C overnight.

After about 24 hours of incubation, check the culture growth in the CariCult tube. Use the following photographs to help you identify Low, Moderate, or High risk:

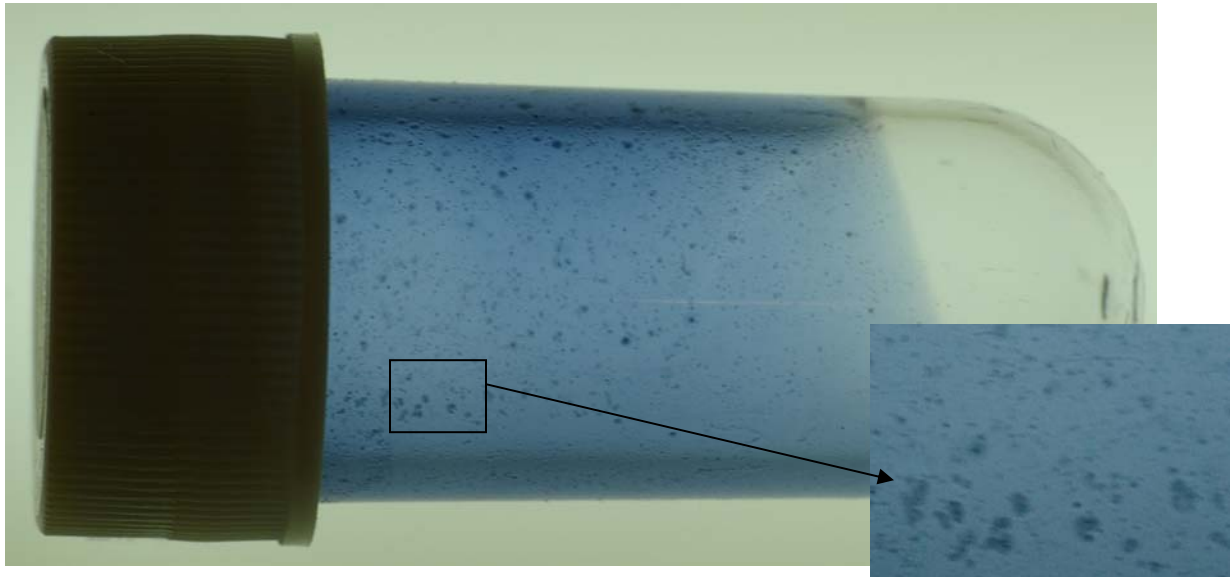
LOW RISK SAMPLE



MODERATE RISK SAMPLES



HIGH RISK SAMPLES



4. CariFree *Treatment Rinse:*

Patients that demonstrate a moderate or high risk level of Mutans streptococci in their oral biofilm culture should undergo antimicrobial therapy in addition to behavior management of specific risk factors.

The CariFree™ Treatment Rinse is a two component rinse that is mixed in single use amounts at the time of each use. The antimicrobial agents used in this rinse are not stable in a single combined solution, so they must be mixed together immediately prior to each use. This results in a very effective antimicrobial rinse.

The patient should mix approximately 5 ml of component A and 5 ml of component B into the supplied cup, for a total of 10 ml of mixed rinse. They should swish vigorously with the rinse for 1 minute, and then expectorate. **It is important not to swallow the antimicrobial rinse.**

It is best to rinse after brushing and flossing. This regimen should be followed for 7-10 days. The rinse is supplied in 4 oz. bottles, which should provide the patient enough material to use for a minimum of 7 days. Encourage the patient to mix and use **until it is all gone.**

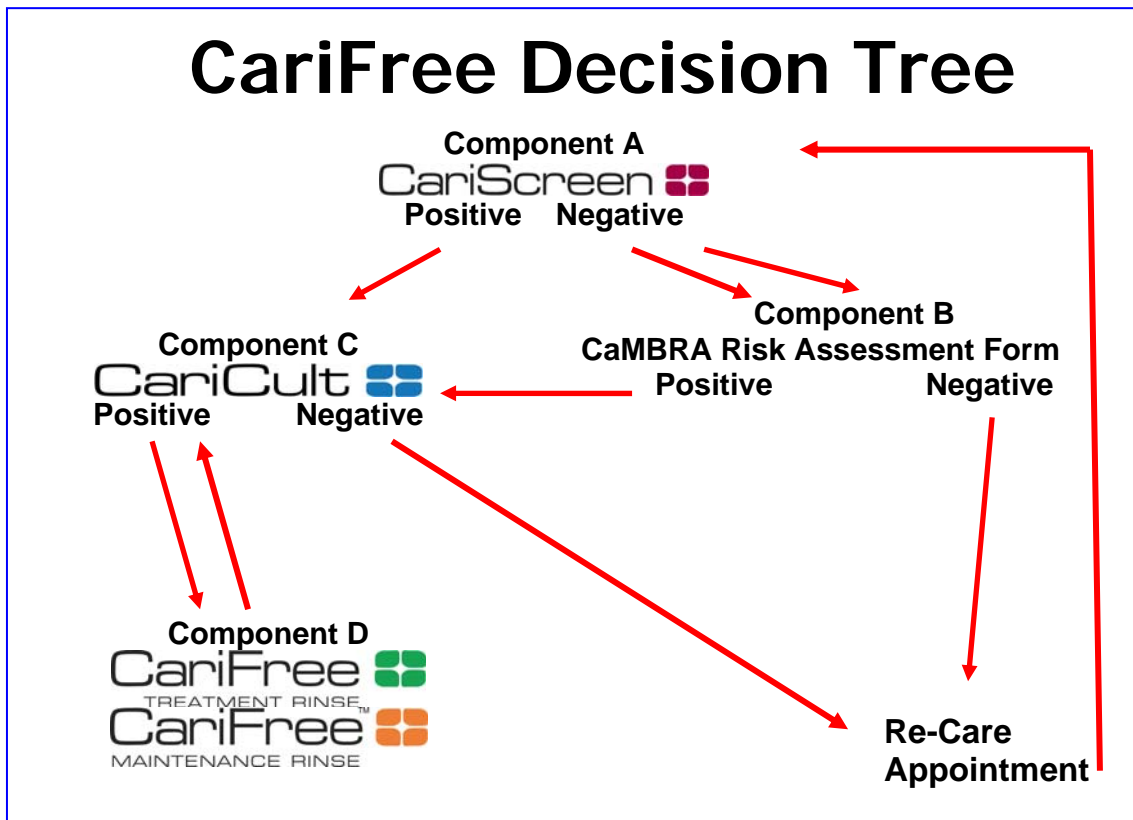
5. CariFree™ Maintenance Rinse.

Following completion of the therapeutic antimicrobial rinse therapy, the patient should be placed on the maintenance rinse. This rinse is a single component rinse designed to help the patient maintain low cariogenic bacterial levels in their oral biofilm and aid in remineralization.

This rinse has a long and stable shelf life until opened, once opened the product should be used daily until the product is completely gone.

The patient may elect (and it is suggested) to use the CariFree™ Maintenance rinse indefinitely once per day to help maintain their health and prevent the infection of Mutans streptococci that results in dental caries. Thirty days from the initiation of the rinse therapy with the CariFree™ Treatment Rinse, and while still using the CariFree™ Maintenance Rinse, they should be re-cultured to determine progress of the antimicrobial therapy. The patient should be treated based on previous recommendations in the Decision Tree section.

Quick Reference Decision Tree.



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