

BANA

BANA-Zyme Reagent Strips

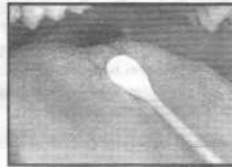
Directions For Use

The BANA-Zyme Test is a rapid, 5-minute, Chairside Test for the detection of an enzyme(s) in tongue coatings and plaque samples, that hydrolyzes the synthetic peptide, Benzoyl-DL-arginine-B-naphthylamide (BANA). This enzyme is possessed by three anaerobic, periodontopathic species, i.e., *Porphyromonas gingivalis*, *Treponema denticola* and *Tommerella forsythia* (formerly known as *Bacteroides forsythus*), that *in vitro* produce copious amounts of malodorous compounds. Plaque samples that are BANA-zyme positive invariably have one or more of these species present. Tongue -samples may have these and other malodorous bacterial species present.

Tongue Samples

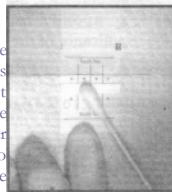
1.

Remove a BANA-Zyme test strip from the bottle just prior to use. The BANA-Zyme test reagents are sensitive to light and humidity so that only the strip to be used should be removed from the bottle and then the bottle cap should be replaced and hand tightened. Record the patient's name and date in the spaces provided on the strip.



2.

Take a cotton tip swab and wipe it on the posterior dorsum of the tongue, removing as much coating material as is possible without the subject gagging. Wipe that portion of the swab that contains the coating onto the lower reagent strip of the BANA card. Try to concentrate the wipe in a small area on the reagent strip. Proceed to step 3.



Plaque Samples

There are two ways to remove plaque for the BANA test. The toothpick method or the curette method.

Toothpick Method

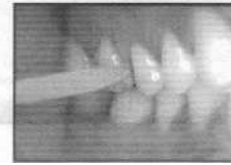
1.

Remove a Bana test strip from the bottle just prior to use. The BANA test reagents are sensitive to light and humidity, so that only the strip to be used should be removed from the bottle, and then the bottle cap should be replaced and hand tightened. Record the patient's name and date in the spaces provided on the strip.

2.

Use a soft wooded toothpick such as the STIM-U-DENT Insert the toothpick interproximally between the teeth. Chose dental papilla

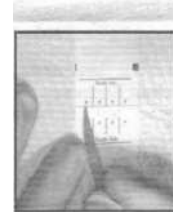
that appear to be inflamed. Remove the toothpick and record whether there is any bleeding about the papilla. Take the toothpick and wipe each of its sides onto the same spot on the lower reagent strip. Take a new toothpick and sample dental papilla in another quadrant. Again wipe both sides of the toothpick on a new location on the lower reagent strip. Repeat this procedure, so as to sample at least one inflamed papilla in each of the quadrants. Discard the toothpicks in a manner appropriate for bacterially contaminated material. Proceed to step 3.



Curette Method

1.

Remove a BANA test strip from the bottle just prior to use. The BANA-Zyme test reagents are sensitive to light and humidity, so that only the strip to be used should be removed from the bottle, and then the bottle cap should be replaced and tightened. Record the patients name and date in the spaces provided.



2.

Remove supragingival plaque prior to sampling. Apply the subgingival plaque specimens using a curette onto the raised reagent matrix affixed to the lower portion of the test strip. Before taking another specimen, wipe the curette on a clean piece of cotton or other suitable wipe to prevent carry-over of plaque. Repeat this

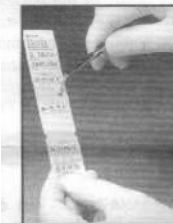


Running the Test

procedure on the other inflamed papilla so that at least one site is sampled in each quadrant.

3.

After all desired sites have been sampled, moisten the upper test strip (salmon colour) with distilled water using a cotton swab. (Do not use tap water, as there has been a report of tap water being inhibitory for the BANA-Zyme test). It is important to moisten and not overwet at this stage. Too much water will dilute the color over a larger area and may give a false negative result.



4.

Fold BANA-Zyme test strip at the crease mark so that the lower and upper reagent strips meet with each other.



5.

Place the BANA-Zyme⁴ test strip into either of the slots on the top of the processor. The heating element of the processor will start automatically when the strip is inserted to the bottom of the slot, as indicated by the flashing light. When the indicator light remains on, the heating element has reached 55°C and will stay on for 5 minutes. The BANA-Zyme test strip's color development will be complete when the indicator light goes off and the bell rings. In some instances the BANA-Zyme test will turn positive if incubated at room temperature for about 30 minutes.



A second BANA-Zyme test strip can be placed into the other slot. Each slot has its own heater and timer and is independent of the events in the other slot. After completion of a cycle it is best to allow at least 10 minutes cool down time between insertion of a new BANA-Zyme test strip into either slot.

6.

Remove the BANA-Zyme test from the processor and discard the lower reagent strip that had been inoculated with plaque in a manner appropriate for contaminated material. (Caution: this lower portion may contain traces of pathogenic bacteria, viruses, and B-naphthylamine, a product of BANA hydrolysis which is believed to be a carcinogen.)



7.

Examine the upper reagent strip for the presence of any blue color. If a blue color is detected, mark the site as either weak positive or positive. (See color intensities on illustration on plastic bottle label)



8.

Record the results for each sampled site as negative, weak positive or positive. Record the result in the patient's chart and/or preserve the BANA Test strip by sealing the upper buff strip with clear, non-porous tape and attach to the patient's chart for future reference. The blue color is permanent.

The BANA-Zyme test detects the presence of an enzyme(s), an arginine hydrolase, in the oral sample. This enzyme is found in the malodorous species *Theponema denticola*, *Porphyromonas gingivitis*, *Tannerella forsythia*, and *Stomatococcus mucilaginus*, and organism found on the tongue. The presence of this enzyme(s) does not indicate malodor or pathology, as these species, when present in low numbers can be considered as normal inhabitants of the oral cavity. Dental professionals have to use their clinical judgement, and interpret the test results within the context of the patient's complaint of malodor, the actual level of malodor as evaluated by an experienced individual, the extent of gingivitis and/or periodontitis, the overall oral hygiene, and overall general health.

Negative Reaction

No blue colour is detected. This indicates that the BANA-Zyme

positive organisms, if present in the sample, are below the detection threshold of the BANA-Zyme⁴ test, i.e., below the range of 1,000 to 5,000 CFU's at the site of sampling.

Tongue: If a tongue sample is BANA-Zyme – negative, and the organoleptic score is also negative the BANA-Zyme negative score would be helpful in assuring the individual that she does not have malodor.

If a tongue sample is BANA-Zyme - negative, and the organoleptic score is positive, this would suggest that the malodor is not associated with those anaerobic organisms measured by the BANA-Zyme – test. But because the tongue surface is so large and because the organisms contributing to the malodor may be buried within the crypts of the tongue surface, there is a possibility that the sample did not contain the bacterial species responsible for the malodor. In this case, a second sample could be taken for the BANA-Zyme test from another area on the back of the tongue. Also, the examiner might consider sampling the subgingival plaque for the BANA positive species.

Plaque: If a subject has 3 or more negative plaque samples out of 4 sampled plaques, then it is unlikely that periodontal disease is contributing to the malodor.

Weak Positive Reaction

If a faint blue color can be detected anywhere on the BANA-Zyme strip this is considered as a weak positive reaction. Use the colour guide on the bottle label to assess this faint color. Generally, a weak positive result indicates that low levels of the BANA-Zyme positive species are present in the sample.

Tongue: In a tongue sample a weak positive reaction indicates the presence of proteolytic organisms in the sample. As many of these proteolytic organisms are anaerobes, such a finding would support the usage of oxidizing mouthrinses, if the subject also had a detectable malodor.

Plaque: Statistical analysis shows that a weak positive reaction is more like a positive reaction than a negative reaction when evaluating the significance of the BANA-Zyme test in periodontal disease. If a subject has 3 or more weak positive reactions, then s/he may have periodontal disease, and this periodontal problem could be contributing to the patients' malodor.

Positive Reaction

A positive reaction appears as a distinct blue colour over a very small area or over the entire area of contact with the sample. Because the BANA-Zyme⁴ positive bacteria may exist as microcolonies in only part of the plaque sample, the clinician should pay attention to the intensity of the colour as well as the area of the colour reaction. A positive reaction indicates that the BANA positive species are present in high levels in the sample.

Tongue: In a tongue sample a positive BANA-Zyme test would indicate that the proteolytic species are prominent in the sample and would recommend the usage of mouthrinses that contain either oxidizing agents or antimicrobial agents. A positive BANA-Zyme score would provide objective confirmation of an organoleptic score >3.

Plaque: If a subject has 3 or more positive reactions, then s/he may have an anaerobic periodontal infection, and this periodontal problem could be contributing to the patients' malodor. In such a situation the periodontal condition may need to be treated in conjunction with other treatments aimed at the tongue.