



BANA TEST: A REVIEW

Periodontology

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ABSTRACT

Many diagnostic tools are available today for the detection and assessment of the periodontal status before and during the periodontal treatment. The microbial-enzymatic BANA test is one of the modern alternatives to bacterial cultures. It detects the presence of three periodontal pathogens in the subgingival plaque (*T. denticola*, *P. gingivalis*, and *B. forsythus*). BANA test is a simple chair side diagnostic assay, which enables the clinician to monitor the potential sites of active periodontal disease and even predict future attachment loss. BANA test is a simple chair side test which can be used in dental office as well as large epidemiologic studies.

KEYWORDS

BANA test, Periodontal disease, Micro-organisms, Plaque, Peptide.

INTRODUCTION

The microbial etiology of periodontal disease is well known. Most forms of periodontal disease are associated with the presence or overgrowth of anaerobic species that could include *Treponema denticola*, *Porphyromonas gingivalis*, and *Bacteroides forsythus* among others. The gram negative anaerobic species developing in subgingival area being more aggressive for the periodontal structures.[1] These micro-organisms lead to the destruction of the periodontal ligament and alveolar bone that surrounds the teeth thus causing loss of attachment to the tooth. When these micro-organisms are attached on the tooth surface in microbial colonies, they form a layer, known as dental plaque or microbial biofilm. Dental plaque has been defined as "a specific but highly variable structural entity consisting of micro-organisms and their products embedded in a highly organized intercellular matrix."

Gram-negative anaerobes which, in vivo, have an enzyme capable of hydrolyzing the synthetic trypsin substrate, N-benzoyl-DL-arginine-2-naphthylamide (BANA). Based on this enzymatic profile, Loesche et al. (1990) described BANA test, which uses a chromophore added to a synthetic peptidase as a substrate (Benzoyl-DL-Arginine-Naphthylamide).[2] In 1994, Socransky et al. (1994) described the Checkerboard DNA-DNA hybridization microbiological test.[3]

Currently, there are many methods directed at identifying periodontal pathogenic species: microbial cultures, DNA probes, polymerase chain reaction. The latest two methods can detect uncultivable species, but they require good laboratory equipment and they cannot be used as routine tests.[4]

BANA TEST:

The BANA test (Figure 1) was developed by Dr. Walter L oesche and coworkers at Michigan University, being the result of more than 15 years of research. Of the 60 bacterial species studied in the subgingival microbiota, only the anaerobic bacteria *Porphyromonas gingivalis*, *Bacteroides forsythus* and *Treponema denticola* possess a trypsin-like enzyme, which hydrolyzes the synthetic peptide benzoyl-DL-arginine-naphthylamide or BANA.

BANA-Enzymatic test™ kit (Ora Tec Corporation, Manassas, USA) (Figure 1) is a rapid and reliable chair side diagnostic test, which can be performed in about 15 min time, that can give information about the presence of three anaerobic bacteria in subgingival plaque samples, namely,



FIGURE 1

Porphyromonas gingivalis, *Treponema denticola* and *Bacteroides forsythus*. The test can detect the presence of these three anaerobic species, without being able to differentiate them.[5]

PRINCIPLE OF BANA TEST:

Peptidases of *T. denticola*, *P. gingivalis*, and *B. forsythus* can hydrolyze the peptide analog N-benzoyl-DL-arginine-2 naphthylamide (BANA). The BANA reagent strips are plastic cards (Figure 2) to which separate reagent containing matrices are affixed. The lower white reagent matrix is impregnated with BANA. Subgingival plaque samples are applied to this lower matrix. The upper reagent matrix contains a chromogenic diazo reagent (fast black K). Peptidase in certain anaerobic micro-organisms associated with periodontal diseases can hydrolyze the peptide analog BANA. The upper reagent matrix, reacts with one of the hydrolytic products of the enzyme reaction, forming a permanent blue color. The blue color appears in the upper reagent matrix and is permanent. Blood and saliva do not interfere with the test.[6]



FIGURE 2

METHOD TO USE BANA TEST KIT:

To obtain specimens for testing, sites should be cleared of supragingival plaque. A Gracey curette may be used to obtain subgingival plaque specimens, which are placed on the lower matrix. Four teeth should be sampled in each subject. Before taking another specimen, wipe the curette with a clean piece of cotton or other suitable wipe to prevent carry-over of plaque. Then the upper matrix is moistened with saline solution and the strip is folded at the given mark so as the two matrices come in contact. It is incubated for 5 minutes at 55°C temperature. If there is no reaction the strip can be incubated for 10 min or 15 min at 55°C. After interpretation of the results, the strips should be sealed with non-porous transparent tape and stored individually.

INTERPRETATION OF RESULTS:

A permanent blue coloration on the upper matrix is found if BANA

positive species are present when the test matrix is opened after incubation. If the concentration of bacterial species is higher, the blue colour will be darker. According to the result, the test can be positive, weak positive, or negative. A BANA positive result indicates the presence of (more than 10,000 colony-forming units) of bacterial species in each plaque sample.[7,8] A BANA negative result indicates the absence of the three pathogenic species, or if present, in reduced numbers (less than 10,000 colony forming units) in each plaque sample.

The results obtained by the BANA test can be categorized as:

1. Strongly Positive – Blue Colour
2. Weakly Positive – Faint Blue Colour
3. Negative – No Colour

CONCLUSIONS:

Microscopy, culture, immunoassays, enzyme tests, and DNA-based techniques have been introduced for the detection of periodontopathogens.[9] The ability of BANA test to detect a particular threshold of anaerobic periodontopathic bacteria can be a valuable diagnostic tool for screening the individuals at risk for anaerobic infection. The findings can be used to identify those sites in individuals who might require treatment to reduce their pathogenic microflora as well as can enable the clinician to monitor the adequacy of treatment procedures. BANA test can also determine whether initial treatment has been adequate or whether additional modalities are called for.

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