AMA DENT key performance data

Scientific validity

The species spectrum of the oral microbiome is considered to be the key factor in the development and progression of periodontal inflammatory disorders. The "red complex" including *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola* has the highest pathogenic potential. Proteolytic trypsin-like enzymes are established to be the virulence factors of "red complex" bacteria; therefore, evaluation their enzymatic activity allows for the prediction of inflammation in periodontal tissues at an early stage.

Periodontal disorders may be asymptomatic in the early stages of the disease, and patients usually ask for medical help because of pronounced signs of the periodontal disorders: bleeding, swelling of the gums, dental mobility or tooth loss. It is important to timely detect and stop the inflammatory process in the dentogingival junction (gingivitis) that otherwise would spread and affect the epithelial and connective tissues of the dentogingival junction.

Analytical Performance

The development of the AMA DENT test and the evaluation of the analytical performance was carried out by AMA Co Ltd research chemists in the R&D laboratory.

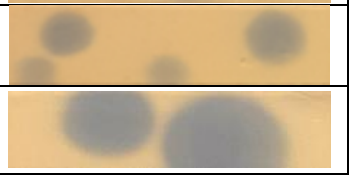
Verification was conducted on each batch that was randomly selected for analytical performance evaluation. Standard matching trypsin solution were used. Standard solution (SS) concentrations were prepared from trypsin obtained by bovine pancreas (Chemical Abstracts Service (CAS): 9002-07-7): SS^{5000+}, SS^{25+}, $SS^{12.5+}$ and distilled water SS^{-}.

Limit of Detection: 0.0125mg/ml (5mg/ml) for trypsin obtained by bovine pancreas

Analytical specificity: 99-100%

Interfering agents that can affect the reaction: *substances that inhibit protease activity; mouthwashes containing plant components with antibacterial properties (e.g. essential oils).*

Analytical sensitivity of the AMA DENT express test to trypsin concentration by laboratory solutions SS^{5000+}, SS^{25+}, $SS^{12.5+}$ and distilled water SS^{-}:

Standard solution	Standard solution concentration	AMA DENT reaction
SS^{-}	Does not contain trypsin (distilled water)	
$SS^{12,5+}$	Trypsin enzyme concentration 0.0125 mg/ml	
SS^{25+}	Trypsin enzyme concentration 0.025 mg/ml	
SS^{5000+}	Trypsin enzyme concentration 5 mg/ml	

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Reproducibility: 99-100%

Reproducibility between 3 batches			*Intra-batch reproducibility (for 100 tests)*		
Laboratory solution	**Number of tests in one batch**	**Coefficient of variation, %**	**Laboratory solution**	**Number of tests**	**Coefficient of variation, %**
$SS^{12.5+}$	25	0.15	$SS^{12.5+}$	25	0.09
SS^{25+}	25	0.07	SS^{25+}	25	0.05
SS^{5000+}	25	0.05	SS^{5000+}	25	0.03
SS^{-}	25	No changes in interpretation	SS^{-}	25	No changes in interpretation

Clinical Performance

Clinical trials were conducted at the clinical and educational base of the Department of Therapeutic Stomatology of St. Petersburg State University, in the clinic for the prevention of dental diseases in adult patients and children, called "Classica". High level certificate physician who conducted the trial has a Doctorate degree in Medicine and more than 30 years of experience as a dentist-therapist, surgeon and pediatric dentist. The studies involved patients of both genders, aged 18 to 65 years, with different conditions of teeth and gums. All trail participants were informed and gave their permission to participate in the study and to publish the results. Real time polymerase chain reaction (PCR) analysis and the "Dentoscreen" (*"Liteh" manufacturer, Russia, One-Step-PB-60*) kit were used as a reference method.

Limit of Detection: $1*10^4$ GE/ml ($1*10^8$ GE/ml) of "red complex" bacterial load

Clinical Characteristics	
Number of patients, qty	115
Sensitivity	90%
Specificity	97%
Positive predictive value	99%
Negative predictive value	80%

AMA DENT results in the study of biological materials with different "red complex" bacterial loads:

AMA DENT	**PCR results, GE/ml**
	*P. gingivalis – 0 T. forsythia – 0 T. denticola – 0*
	*P. gingivalis – $8,33*10^4$ T. forsythia – $4,05*10^3$ T. denticola – $3,75*10^3$*
	*P. gingivalis – $2,51*10^4$ T. forsythia – 0 T. denticola – $1,21*10^4$*
	*P. gingivalis – $5,62*10^5$ T. forsythia - $5,32*10^4$ T. denticola – 0*
	*P. gingivalis – $6,42*10^5$ T. forsythia – $3,98*10^3$ T. denticola – $3,24*10^5$*
	*P. gingivalis – $8,25*10^5$ T. forsythia – $6,8*10^4$ T. denticola – $2,04*10^6$*
	*P. gingivalis – $1,25*10^7$ T. forsythia – $2,05*10^5$ T. denticola – $3,38*10^6$*

The presence of endogenous and exogenous substances in a biomaterial sample can affect the reaction of AMA DENT test.

The main endogenous interfering factors include the following: metabolites formed in pathological conditions such as diabetes mellitus, multiple myeloma, cholestatic hepatitis. For example, the presence of α2-macroglobulin, namely blood plasma, when collecting the sample will contribute to obtaining false positive results.

Exogenous factors include: drugs and their metabolites, parenteral nutrition, plasma expanders, anticoagulants; substances taken by the patient, such as alcohol, drugs, nutritional supplements, etc.; substances added during sample preparation, such as anticoagulants, preservatives, stabilizers.

AMA DENT test is valid and relevant for general safety and performance requirements, has good analytical and diagnostic characteristics for invasive diagnostics and practical application in medical institutions.

References:

1. Aronova, E.; Dmitrienko, M.; Ivanova, A.; Gaykova, Y.; Kurochkina, A.; Blinova, A.; Bazarnova, J.; Paponova, E. Express Diagnostics of Proteolytic Activity of Periodontopathogens—Methodological Approach. Dent. J. 2022, 10, 217.
2. Hajishengallis, G.; Lamont, R.J. Beyond the red complex and into more complexity: The polymicrobial synergy and dysbiosis (PSD) model of periodontal disease etiology. Mol. Oral Microbiol. 2012, 27, 409–419.
3. Caselli, E.; Fabbri, C.; D'Accolti, M.; Soffritti, I.; Bassi, C.; Mazzacane, S.; Franchi, M. Defining the oral microbiome by wholegenome sequencing and resistome analysis: The complexity of the healthy picture. BMC Microbiol. 2020, 20, 120.
4. Kinane, D.F.; Stathopoulou, P.G.; Papapanou, P.N. Periodontal diseases. Nat. Rev. Dis. Prim. 2017, 3, 17038.
5. Saidath, K.; Mohanty, R.; Asopa, S.J.; Joseph, M.D.; Singh, B.; Rajguru, J.P.; Sharma, U. Red complex: Polymicrobial conglomerate in oral flora: A review. J. Fam. Med. Prim. Care 2019, 8, 3480–3486.
6. Sinev, I.I.; Nesterov, A.M.; Sadykov, M.I.; Khaikin, M.B. Modern view on integrated treatment of patients with chronic localized periodontitis of medium severity (a literature review). Aspir. Vestnik Povolzhiya 2020, 20, 108–121.
7. Könönen, E.; Gursoy, M.; Gursoy, U. Periodontitis: A Multifaceted Disease of Tooth-Supporting Tissues. J. Clin. Med. 2019, 8, 1135–1165.
8. Schenkein, H.A. Host responses in maintaining periodontal health and determining periodontal disease. Periodontology 2000. 2006, 40, 77–93.
9. Travis, J.; Potempa, J. The Biphasic Virulence Activities of Gingipains: Activation and Inactivation of Host Proteins. Curr. Protein Pept. Sci. 2003, 4, 443–450.
10. Suetenkov, D.; Akulovich, A.; Gritsenko, E. Qualitative and quantitative assessment of oral periodontopathic microflora with BANA-test. Periodontology 2012, 2, 66–70.