COMPARISON OF REAL-TIME PCR AND DNA-STRIP TECHNOLOGY IN DETECTION OF PERIODONTOPATHOGENS

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Objective

Periodontopathogenic bacteria, among them *Aggregatibacter actinomycetem-comitans*, *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola* and *Prevotella intermedia*, play a major role in pathogenesis of periodontitis. Further other pathogens, *e.g. Prevotella intermedia*, *Parvimonas micra*, *Eubacterium nodatum*, *Eikenella corrodens Fusobacterium nucleatum*, *F. periodonticum*, *Campylobacter rectus* and capnocytophaga sp. (*C. gingivalis*, *C. ochracea*, *C. sputigena*) support development and maintenance of disease. The load of those periodontopathogenic bacteria within subgingival plaque is an important indicator of disease activity and success of periodontal treatment.

In the last years, nucleic-acid based methods replaced cultivation as the "gold standard" in microbiological analysis. Moreover, quantitative test systems have been introduced. The purpose of the study was to compare a commercially available semiquantitative test (microIdent® including microIdent®plus) detecting the species mentioned above with quantitative in-house real-time PCR.

Material and Methods

Subjects

- **27 patients with severe chronic periodontitis (age: 31-55 years)**
- **308 subgingival plaque samples obtained from different time-points during treatment**

Obtaining of subgingival plaque by means of paper points in each three pockets

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DNA extraction

Real-time PCR (In-house)

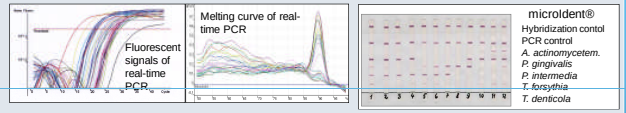
- *A. actinomycetemcomitans*[1]
- *P. gingivalis*[2]
- *T. forsythia*[2]
- *T. denticola*[2]
- *P. intermedia*[2]
- *P. micra* (accession AF542231)
- *F. nucleatum*[3]
- *C. rectus*[2]
- *E. nodatum* (accession U134041)
- *E. corrodens*[2]
- *C. gingivalis*[4] (and accession AF543295)
- *C. ochracea*[4] (and accession U41350)
- *C. sputigena*[4] (and accession L14636)

(Primers: [1]Rudney et al., 2001; [2]Ashimoto et. al, 1996; [3]Fouad et al., 2002; [1]Hayashi et al., 2001)

micro-IDent® incl. microIdent®plus

PCR
Reverse hybridization
Scanning of the strips
Quantification by means of densitometry

- *A. actinomycetemcomitans*
- *P. gingivalis*
- *T. forsythia*
- *T. denticola*
- *P. intermedia*
- *P. micra*
- *F. nucleatum/periodonticum*
- *C. rectus*
- *E. nodatum*
- *E. corrodens*
- Capnocytophaga sp.



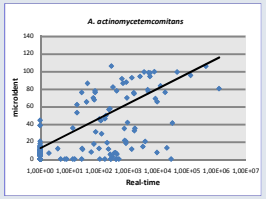
Semiquantification of real-time results and the microIdent® incl. microIdent®plus test

Level	microIdent (plus) Densitometry (% of conjugate (positive) control	Real-time Numbers of bacteria
0	0.00	1 (no detection)
1	0.01 – 9.99	2 - 999
2	10 – 39.99	1 000 – 9 999
3	40 – 69.99	10 000 – 99 999
4	≥ 70.00	100 000 – 999 999
5		≥ 1 000 000

Statistical analysis by means of the Spearman test using SPSS 15.0

Results

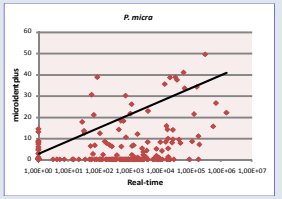
Comparison quantitative



Comparison semi-quantitative

A. actino-mycetem.	microIdent 0	1	2	3	4	Total
Real-time 0	170	35	17	1	0	223
1	13	7	14	13	10	57
2	0	1	5	2	11	19
3	1	0	1	1	3	6
4	0	0	0	0	2	2
5	0	0	0	0	1	1
Total	184	43	37	17	27	308

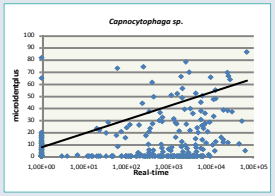
Comparison quantitative

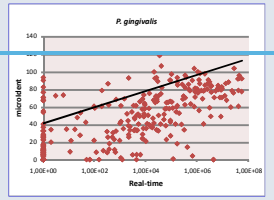


Comparison semi-quantitative

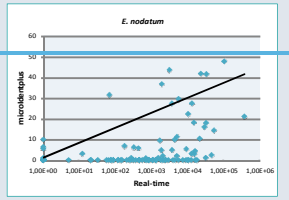
P. micra	microIdent 0	1	2	3	4	Total
Real-time 0	141	11	2	0	0	154
1	47	10	12	0	0	69
2	28	16	4	0	0	48
3	9	9	8	1	0	27
4	1	2	5	1	0	9
5	0	0	1	0	0	1
Total	226	48	32	2	0	308

Comparison quantitative





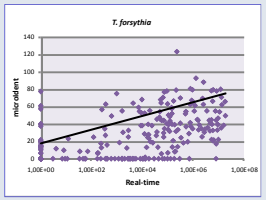
P. gingivalis	microIdent 0	1	2	3	4	Total
Real-time 0	25	15	26	5	6	97
1	4	4	18	9	4	39
2	2	4	15	24	2	47
3	0	0	10	21	15	46
4	0	2	3	12	19	36
5	2	0	0	9	42	53
Total	43	25	72	80	88	308



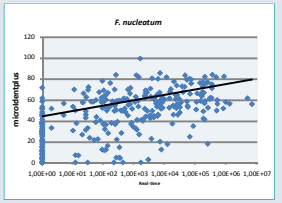
E. nodatum	microIdent 0	1	2	3	4	Total
Real-time 0	214	6	0	0	0	220
1	29	6	1	0	0	36
2	19	8	4	1	0	32
3	4	5	7	2	0	18
4	0	0	1	1	0	2
5	0	0	0	0	0	0
Total	266	25	13	4	0	308

Comparison semi-quantitative

Capnocyto-phaga sp.	microIdent 0	1	2	3	4	Total
Real-time 0	58	12	12	1	1	84
1	57	14	19	3	2	95
2	57	15	25	10	1	108
3	0	3	11	6	1	21
4	0	0	0	0	0	0
5	0	0	0	0	0	0
Total	172	44	67	20	5	308



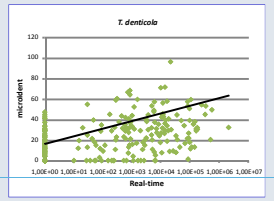
T. forsythia	microIdent 0	1	2	3	4	Total
Real-time 0	96	2	14	3	1	116
1	15	5	8	3	1	32
2	15	0	7	6	0	28
3	9	3	16	12	3	43
4	4	2	17	15	4	42
5	1	0	21	18	7	47
Total	140	12	83	57	16	308



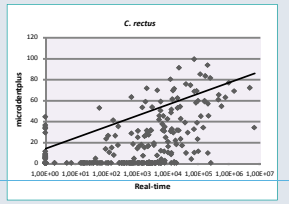
F. nucleatum	microIdent 0	1	2	3	4	Total
Real-time 0	14	10	38	11	1	74
1	4	5	27	57	5	98
2	1	1	7	44	7	60
3	0	0	4	22	10	36
4	0	0	1	22	14	37
5	0	0	0	3	0	3
Total	19	16	77	159	37	308

Correlation quantitative

	Spearman R	P
A. actinomycetemcomitans	0.678	<0.001
P. gingivalis	0.705	<0.001
P. intermedia	0.640	<0.001
T. forsythia	0.691	<0.001
T. denticola	0.622	<0.001
P. micra	0.481	<0.001
F. nucleatum	0.594	<0.001
C. rectus	0.630	<0.001
E. corrodons	0.590	<0.001
E. nodatum	0.570	<0.001
Capnocytophaga sp.	0.331	<0.001



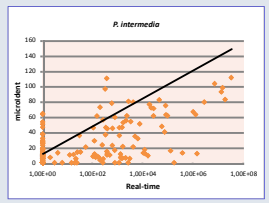
T. denticola	microIdent 0	1	2	3	4	Total
Real-time 0	101	12	35	5	0	153
1	12	3	33	11	0	59
2	6	3	23	8	1	41
3	0	5	21	12	2	40
4	0	0	9	5	0	14
5	0	0	1	0	0	1
Total	119	23	122	41	3	308



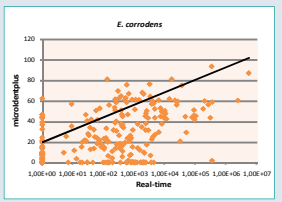
C. rectus	microIdent 0	1	2	3	4	Total
Real-time 0	82	12	5	1	0	100
1	49	4	11	3	0	67
2	36	6	25	10	1	78
3	3	6	16	12	6	43
4	1	1	1	9	5	17
5	0	0	1	1	1	3
Total	171	29	59	36	13	308

Correlation semi-quantitative

	Spearman R	P
A. actinomycetemcomitans	0.669	<0.001
P. gingivalis	0.697	<0.001
P. intermedia	0.635	<0.001
T. forsythia	0.662	<0.001
T. denticola	0.594	<0.001
P. micra	0.484	<0.001
F. nucleatum	0.611	<0.001
C. rectus	0.613	<0.001
E. corrodons	0.585	<0.001
E. nodatum	0.567	<0.001
Capnocytophaga sp.	0.290	<0.001



P. inter-media	microIdent 0	1	2	3	4	Total
Real-time 0	152	29	31	5	0	217
1	7	8	17	7	4	43
2	2	5	6	7	2	22
3	0	1	3	5	5	14
4	1	0	2	1	0	4
5	0	0	1	1	6	8
Total	162	43	60	26	17	308



E. corrodens	microIdent 0	1	2	3	4	Total
Real-time 0	82	20	12	6	0	120
1	21	9	53	24	2	109
2	14	3	13	23	1	54
3	0	0	2	11	2	15
4	0	1	0	6	1	8
5	0	0	0	1	1	2
Total	117	33	80	71	7	308

Summary and Discussion

In general, in-house real-time PCR was more accident-sensitive than the micro-IDent® test, so negative results obtained by real-time PCR should be confirmed by a second run. The results for all 11 bacterial species correlated significantly between the two methods. Higher R-values were obtained for the major pathogens. The numbers of negative samples by one method and a high load by the other method was always below 2%. Nevertheless micro-IDent® might be more sensitive for *P. gingivalis*, *T.denticola*, *P. intermedia* and *F. nucleatum l periodonticum*, real-time PCR detected more samples positive for *T. forsythia*, *P. micra*, *C. rectus*, *E. nodatum* and capnocytophaga sp.; which is to discuss in relation to the toxicity of SybrGreen used in real-time PCR and the selected cut-off and the multiplex PCR in micro-IDent® as well as the regions of rDNA where the primers bind to.

In-house real-time PCR is a cheap useful method for large studies. Semi-quantitative DNA-strip technology can be recommended for diagnosis, and control of treatment in dental practice and also in clinical trials.