

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

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Objective

Periodontopathogenic bacteria, among them *Aggregatibacter actinomycetemcomitans*, *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 semi-quantitative 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

DNA extraction

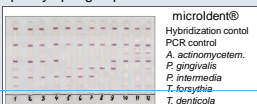
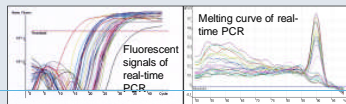
Real-time PCR (In-house)

- *A. actinomycetemcomitans*¹
- *P. gingivalis*²
- *T. forsythia*²
- *T. denticola*²
- *P. intermedia*²
- *P. micra* (accession AF542231)
- *F. nucleatum*³
- *C. rectus*³
- *E. nodatum* (accession U134041)
- *E. corrodens*²
- *C. gingivalis*⁴ (and accession AF542235)
- *C. ochracea*⁴ (and accession U41350)
- *C. sputigena*⁴ (and accession L14636)

(Primers: ¹Rudney et al., 2001; ²Ashimoto et al., 1996; ³Fouad et al., 2002; ⁴Hayashi 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.



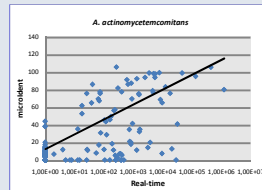
Semi-quantification of real-time results and the microIdent® incl. microIdent®plus test

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

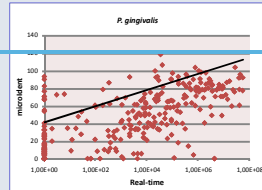
Results

Comparison quantitative

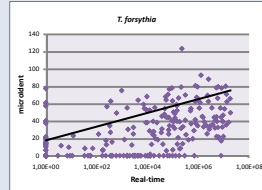


Real-time 0	microIdent					Total
	0	1	2	3	4	
1	13	7	14	13	10	57
2	0	1	5	2	11	19
3	1	0	1	1	3	6
4	0	0	0	0	2	2
5	0	0	0	0	1	1
Total	184	43	37	17	27	308

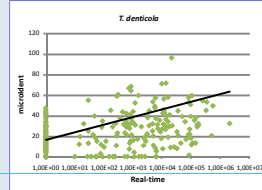
Comparison semi-quantitative



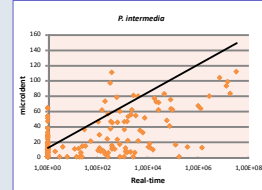
Real-time 0	microIdent					Total
	0	1	2	3	4	
1	4	4	18	9	4	39
2	2	4	15	24	2	47
3	0	0	10	21	15	46
4	0	2	3	12	19	36
5	2	0	0	9	42	53
Total	43	25	72	80	88	308



Real-time 0	microIdent					Total
	0	1	2	3	4	
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

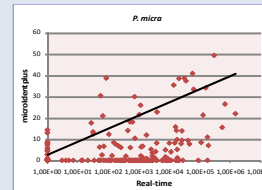


Real-time 0	microIdent					Total
	0	1	2	3	4	
1	12	3	33	11	0	59
2	6	3	23	8	1	41
3	0	5	21	12	2	40
4	0	0	9	5	0	14
5	0	0	1	0	0	1
Total	119	23	122	41	3	308

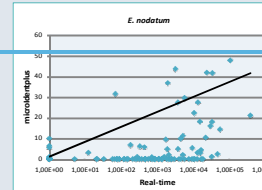


Real-time 0	microIdent					Total
	0	1	2	3	4	
1	7	8	17	7	4	43
2	2	5	6	7	2	22
3	0	1	3	5	5	14
4	1	0	2	1	0	4
5	0	0	1	1	6	8
Total	162	43	60	26	17	308

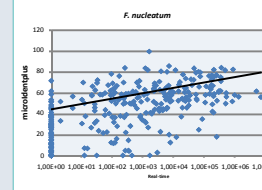
Comparison quantitative



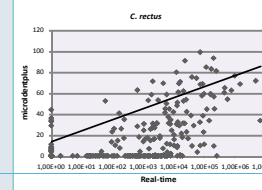
Real-time 0	microIdent					Total
	0	1	2	3	4	
1	141	11	2	0	0	154
2	47	10	12	0	0	69
3	28	16	4	0	0	48
4	9	9	8	1	0	27
5	4	2	5	1	0	9
6	5	0	0	1	0	1
Total	226	48	32	2	0	308



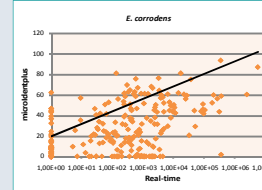
Real-time 0	microIdent					Total
	0	1	2	3	4	
1	29	6	1	0	0	36
2	19	8	4	1	0	32
3	4	5	7	2	0	18
4	0	0	1	1	0	2
5	0	0	0	0	0	0
Total	266	25	13	4	0	308



Real-time 0	microIdent					Total
	0	1	2	3	4	
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

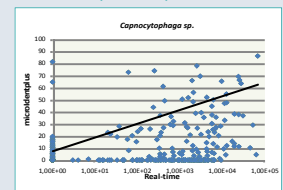


Real-time 0	microIdent					Total
	0	1	2	3	4	
1	49	4	11	3	0	67
2	36	6	25	10	1	78
3	3	6	16	12	6	43
4	1	1	1	9	5	17
5	0	0	1	1	1	3
Total	171	29	59	36	13	308



Real-time 0	microIdent					Total
	0	1	2	3	4	
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

Comparison quantitative



Real-time 0	microIdent					Total
	0	1	2	3	4	
1	57	14	19	3	2	95
2	57	15	25	10	1	108
3	3	0	3	11	6	21
4	0	0	0	0	0	0
5	0	0	0	0	0	0
Total	172	44	67	20	5	308

Comparison semi-quantitative

Correlation quantitative

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

Correlation semi-quantitative

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

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 / 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.