Point-of-care Analysis for Non-invasive Diagnosis of Oral cancer (PANDORA): a technology-development proof of concept diagnostic accuracy study of dielectrophoresis in patients with oral squamous cell carcinoma and dysplasia.

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Acknowledgements

Key Words

ABSTRACT

Background: Delays in the identification and referral of oral cancer remain frequent. An accurate and non-invasive diagnostic test to be performed in primary care may help identifying oral cancer at an early stage and reduce mortality. PANDORA was a proof-of-concept prospective diagnostic accuracy study aimed at advancing the development of a dielectrophoresis-based diagnostic platform for oral squamous cell carcinoma (OSCC) and epithelial dysplasia (OED) using a novel automated DEPtech 3DEP analyser.

Methods: the aim of PANDORA was to identify the set-up of the DEPtech 3DEP analyser associated with the highest diagnostic accuracy in identifying OSCC and OED from non-invasive brush biopsy samples, as compared to the gold standard test (histopathology). Measures of accuracy included sensitivity, specificity, positive and negative predictive value. Brush biopsies were collected from individuals with histologically-proven OSCC and OED, histologically-proven benign mucosal disease, and healthy mucosa (standard test), and analysed via dielectrophoresis (index test).

Results: 40 individuals with OSCC/OED and 79 with benign oral mucosal disease/healthy mucosa were recruited. Sensitivity and specificity of the index test was 86.8% (95% CI, 71.9%-95.6%) and 83.6% (95% CI, 73.0%-91.2%). Analysing OSCC samples separately led to higher diagnostic accuracy, with 92.0% (95% CI, 74.0%-99.0%) sensitivity and 94.5% (95% CI, 86.6%-98.5%) specificity.

Conclusion: The DEPtech 3DEP analyser has the potential to identify OSCC and OED with notable diagnostic accuracy and warrants further investigation as a potential triage test in the primary care setting for patients who may need to progress along the diagnostic pathway and be offered a surgical biopsy.

INTRODUCTION

Approximately 470,000 individuals were diagnosed with oral cancer (lip, oral cavity and oropharynx) worldwide in 2020.[1] Oral cancer is associated with multiple risk factors including tobacco, alcohol and human papillomavirus infection,[2] and is characterised by a poor prognosis, with current data suggesting an overall mortality of approximately 50% (~225,000 deaths in 2020).[1] Advanced cancer stage at time of diagnosis is one of the main factors accounting for the high mortality and morbidity of oral cancer.[3-4] Despite the accessibility of the oral mucosa to clinical inspection, a significant majority of patients present with locally advanced disease at diagnosis, with relevant survival rates reducing significantly with respect to early disease.[5] Available evidence suggests that delays in the identification and referral of patients with oral cancer remain frequent, and that the early stages of disease development (premalignant epithelial dysplasia to early invasive carcinoma) often remain overlooked and undiagnosed for several months.[6] Research attempts to address the delayed diagnosis of oral cancer have included the development and testing of a number of non-invasive diagnostic aids. A 2021 systematic review of 63 diagnostic studies concluded that in the majority of cases the overall quality of the studies was poor (high risk of bias), with no robust evidence of diagnostic accuracy for any of the tested diagnostic aids.[7] One other major limitation of available studies is their design, as it is often unclear whether the proposed index test is aimed at replacing the current standard test (reference standard), should be used in addition to the reference standard (add-on test), or represents a triage test to be performed in primary care in order to identify which patients should be referred to secondary care and subsequently receive the reference standard test.[8] As a consequence, none of the diagnostic aids investigated so far can be recommended in clinical practice, especially in primary care.[7] The latter seems to be a particularly relevant unmet need, as there is evidence that out of all patients referred to oral cancer specialist units for further investigation of a suspected malignancy, only a small portion (< 10%) are eventually diagnosed with oral cancer.[9]

In the quest for a non- or minimally-invasive, accurate and ideally fast diagnostic tool for oral cancer and precancer, our work has involved the use of an electrostatic phenomenon (dielectrophoresis: DEP) to detect oral squamous cell carcinoma (OSCC) and oral epithelial dysplasia (OED) cells. DEP is used to measure the physical response of cells to an applied electric field, presenting an "electrophysiological fingerprint" which can be used to discriminate between cells of different types.[10] Observing the response of the cells in the electric field over a range of frequencies allows the measurement of these properties.[11] The technique is fast, simple and relatively inexpensive, and can rapidly detect cellular characteristics without the need for fluorescent dyes or immune-reagents. DEP has been used in a wide range of oncology settings, including isolation of circulating tumour cells [12-14] and rapid monitoring of drug efficacy.[15] Our pre-clinical *in vitro* DEP studies investigated electrophysiological phenotype of OSCC cell lines in 2D culture,[16] cell tumourogenicity [17] and 3D organotypic models,[18] and demonstrated that there are substantial differences between OSCC and non-cancerous epithelial cell lines. This was followed by an initial preliminary clinical study where samples were taken non-invasively from histopathology-confirmed oral cancer lesions and healthy controls using a painless soft brush (brush-biopsy), stored in a liquid medium, and transferred to a central laboratory where relevant cells were re-suspended in an iso-osmotic low-conductivity medium and analysed using a prototype DEP cell analyser[19]. The prototype DEP system required an operator to take measurements manually, requiring typically 2h per sample followed by significant user analysis and interpretation. Although the study demonstrated significant differences in the dielectric fingerprint between OSCC and normal mucosal cells,[19] the potential for further research at that time was limited by the early stage of DEP technology development, which was heavily operator-dependent. More recently, an advanced commercially available DEP analyser (DEPtech 3DEP, Deparator, Uckfield UK) has been introduced, which allows automated analysis of cell dielectric fingerprint in a few seconds.[20-22]

In this paper, we report the results of PANDORA study (Point-of-care Analysis for Non-invasive Diagnosis of Oral cancer), the next step in the development of a DEP-based

diagnostic platform for oral cancer and precancer using the novel automated DEPtech 3DEP analyser. The aim of PANDORA was to identify, in a proof of concept study, the set-up (or a range of set-ups) of the DEPtech 3DEP analyser associated with the highest diagnostic accuracy in identifying OSCC and OED from non-invasive brush biopsy samples, as compared to the gold standard test (histopathology, which necessitates a surgical biopsy). Our long-term research strategy is for PANDORA to inform further development of the DEPtech 3DEP diagnostic technology, as well as paving the way of further clinical testing and validation. With respect to the future intended use and clinical positioning of the test, we anticipate that the DEP-based diagnostic platform could primarily represent a triage test for patients who present in the primary care setting with visible oral mucosa lesions suspicious for OSCC or OED and may need to progress along the diagnostic pathway and be offered the standard test (surgical biopsy with histopathology), typically via an urgent referral to a secondary care specialist unit. The study is reported according to the STARD 2015 guidelines for reporting diagnostic accuracy studies.[23]

METHODS

Study design and participant recruitment

PANDORA was a technology-development prospective proof of concept case-control multicentre study recruiting four groups of participants: individuals with histologically-proven oral squamous cell carcinoma [OSCC] (A) and oral epithelial dysplasia [OED] (B), individuals with histologically-proven benign mucosal disease (C), and individuals with no history of mucosal disease and no visible abnormality of the oral mucosa (D). Potential participants were identified in the Oral Medicine and Head & Neck cancer clinics of University College London Hospital, Bradford Teaching Hospital, and the Royal Marsden Hospital (secondary care setting). Participants formed a consecutive series of patients presenting to the above study sites with the conditions of interest and were recruited between August 2013 and March 2015. Individuals were considered eligible for inclusion if they had a histologically-proven OSCC or OED (groups A and B respectively) that was clearly visible and easily accessible for brush

biopsy and waiting for treatment or under surveillance (typically reviewed 2-3 weeks from biopsy/diagnosis), or a histologically-proven benign mucosal disease (group C) within two months from biopsy/diagnosis that was clearly visible and easily accessible for brush biopsy, or if they were individuals with no history of mucosal disease and no visible abnormality of the oral mucosa (group D). The complete list of inclusion criteria is presented in table 1.

Sample size calculation

The samples size calculation was based on the nomogram approach of Carley et al:[24] an overall sample size of 100 subjects would have an alpha of 5% (P=0.05) for a 0.1 confidence interval range around a hypothesised sensitivity of 0.9 assuming a specificity of 0.8, and an overall true positive prevalence of 50% among those tested. We therefore set a recruitment target of a minimum of 100 participants.

Standard test

Individuals in Groups A, B and C had a history of surgical biopsy with histopathology as standard test for their oral mucosa disease before enrolment in the study. Following surgical biopsy, sample storage, staining, and the associated histopathology reports were performed by an experienced Oral & Maxillofacial Pathology service at each study site as part of the patients' standard care pathway, in keeping with standard practice and widely accepted diagnostic criteria of dysplastic, malignant and non-malignant disease of the oral mucosa.[25] Individuals of group D had no abnormality of the oral mucosa and no history of mucosal disease and received an accurate visual inspection of the oral mucosa, which was considered an acceptable standard test surrogate, as taking a surgical biopsy from healthy mucosa in individuals with no history of mucosal disease was deemed not ethically appropriate.

Index test: sample collection, packaging and shipment

As this was a proof-of-concept study, the index test (analysis of the dielectric fingerprint of tissue samples collected through non-invasive brush biopsy) in groups A-C was performed after the standard tests (surgical biopsy with histopathology). Individuals of group D received the index test following comprehensive and accurate visual inspection of the oral mucosa

(standard test surrogate). Details of the sample packaging and shipment are provided in the Appendix

Index test: DEP analysis

Details of the sample preparation and DEP analysis as per previously published protocol [20,26,27] are provided in the Appendix.

Data collection and statistical analysis

Patient demographics and data relevant to the anatomical site of the study sample collection (groups A, B, C and D) and the histopathology results of prior standard of care biopsy (Groups A-C) were recorded on a pre-defined case report form. With respect to DEP analysis (index test), following data processing, values were analysed using Prism (GraphPad Software, San Diego, USA). ROC curve analysis was used to identify optimal positivity cut-off of the index test associated with diagnostic accuracy. Sensitivity and specificity, positive and negative predictive value (PPV and NPV) with 95% confidence interval of the index test were determined by assessing the following groups:

- All OSCC/OED patients vs all patients without OSCC/OED (Groups A+B vs C+D)
- All OSCC patients vs all patients without OSCC/OED (Group A vs C+D)
- All OED patients vs all patients without OSCC/OED (Group B vs C+D)

Where needed, analyses were performed stratifying results according to site of sample collection in order to maximise diagnostic accuracy. Analyses were initially performed for all samples and subsequently repeated after excluding samples with flat/absent DEP spectrum due to low cell count, defined as a sum of MDV below 0.37, as per the method outlined by Hoque et al.[27]

Funding and Ethics Committee favourable opinion

Details provided in the Appendix

RESULTS

Between August 2013 and March 2015, 185 patients attending the Oral Medicine and Head & Neck cancer clinics of University College London Hospital, Bradford Teaching Hospitals, and the Royal Marsden Hospital were identified as potentially eligible after reviewing their clinical notes and approached for potential study participation. A total of one hundred and nineteen patients met the inclusion criteria and agreed to participate, including forty individuals with histopathologically-confirmed OSCC (group A, n=26) and OED (group B, n=14), twenty-two patients with histopathologically-confirmed benign oral mucosal disease (group C), and fifty-seven individuals with no history of mucosal disease and no evidence of oral mucosa abnormality (group D). Gender and ethnicity details were missing for 11 and 18 participants respectively. There were 56 confirmed females (47.1%), with 61.3% (n=73) and 16% (n=19) of participants being of Caucasian and Asian ethnicity respectively (Table 2). Site of lesions for the standard tests and related index test samples were tongue/gingivae (n=54, 36.9%) and buccal mucosa (n=65, 51.7%). The index test was performed in all 119 participants after collecting brush biopsy samples as per protocol (Figure 1). The interval between the reference standard and the subsequent index test for group A and B was ≤3 weeks, and ≤2 months for group C. The index test in group D was taken at the same time of the standard test (clinical examination).

Diagnostic accuracy of the index test in OSCC/OED patients vs patients without OSCC/OED (group A+B vs C+D)

The set-up (MDV cut-off) and related diagnostic performance of the index test in terms of true/false positive and negative and the related estimates of diagnostic accuracy (sensitivity, specificity, and positive and negative predictive value with 95% CI) after stratifying OSCC/OED samples by site into two groups (tongue/gingivae and buccal mucosa) and combined all together is reported in table 3 and 4.

The index test identified as positive (true positive) 35 of the 40 biopsy-proven OSCC/OED (group A + B) and 17 (false positive) of the 79 benign oral mucosal disease/normal mucosa samples (group C + D). The index test correctly identified as negative (true negative) 62 of the

79 benign oral mucosal disease/normal mucosa samples (group C + D), whereas 5 of the 40 biopsy-proven OSCC/OED (group A + B) were also identified as negative (false negative). After excluding samples (n=8) with flat/absent DEP spectrum due to low cell count (sum of MDV < 0.37) the true positive were 33 of 38 (86.8%) OSCC/OED and true negative were 61 of 73 (83.6%) benign oral mucosal disease/normal mucosa samples (Table 3).

Sensitivity, specificity, PPV and NPV for all samples combined were 86.8% (CI 95%: 71.9%-95.6%), 83.6% (73.0%-91.2%), 73.3% (58.1%-85.4%), and 92.4% (83.2%-97.5%) respectively (Table 4).

Diagnostic accuracy of the index test in *OSCC patients vs patients without OSCC/OED (group A vs C+D)*

The set-up (MDV cut-off) and related diagnostic performance of the index test in terms of true/false positive and negative and the related estimates of diagnostic accuracy (sensitivity, specificity, PPV and NPV with 95% CI) after stratifying OSCC samples by site into two groups (tongue/gingivae and buccal mucosa) and combined all together is reported in table 3 and 4. Results are presented for all samples and after removing the samples with flat/absent DEP spectrum due to low cell count. Sensitivity, specificity, PPV and NPV for all samples combined were 92.0% (CI 95%: 74.0%-99.0%), 94.5% (86.6%-98.5%), 85.2% (66.3%-95.8%), and 97.2% (90.2%-99.7%) respectively (Table 4).

Diagnostic accuracy of the index test in *OED patients vs patients without OSCC/OED (group A vs C+D)*

The set-up (MDV cut-off) and related diagnostic performance of the index test in terms of true/false positive and negative and the related estimates of diagnostic accuracy (sensitivity, specificity, and positive and negative predictive value with 95% CI) after stratifying OED samples by site into two groups (tongue/gingivae and buccal mucosa) and combined all together is reported in table 3 and 4. Results are presented for all samples and after removing the samples with flat/absent DEP spectrum due to low cell count. Sensitivity, specificity, PPV and NPV for all samples combined were 84.6% (CI 95%: 54.6%-98.1%), 84.7% (74.3%-92.1%), 50% (28.2%-71.8%), and 96.8% (89.0%-99.6%) respectively (Table 4).

Adverse events of the index test

No adverse event occurred as result of the brush biopsy used to take samples for the index test.

DISCUSSION

PANDORA was a proof-of-concept study designed to the identify the set-up, or a range of set-ups, of the DEPtech 3DEP analyser maximising diagnostic accuracy, with a view to paving the way for further clinical validation studies. Our results suggest that the 3DEP has the potential to identify OSCC and OED with significant and clinically beneficial diagnostic accuracy, and warrant further investigation as a potential triage test for patients who present in the primary and secondary care settings with visible oral mucosa lesions suspicious for OSCC/OED. The index test performed better when samples with low cell count were removed from the analysis (table 3 and 4), which suggests that future validation studies should be designed so that the results of the index test would screening for inconclusive reading due to low cell count. For these cases a repeat sampling protocol could be incorporated. When analysis was performed stratifying samples by site, diagnostic thresholds of index test were associated with higher accuracy for tongue/gingival samples and lower accuracy for the buccal mucosa samples (table 3 and 4). It remains unclear why the diagnostic performance of the index test varied according to the oral mucosal site where the OSCC/OED samples were collected. Interestingly, anatomic differences at different sites of the oral mucosa (e.g. degree of mucosal keratinization) have been associated with different electric properties,[28] which may in part explain the differences in dielectric properties observed in our study between OSCC and OED cells collected at different sites. These findings suggest that the index test may require different set-ups based on the location of the disease and related site of sample collection in order to maximise accuracy. However, when results were combined across all samples using a threshold range, the sensitivity and specificity of the index test remained well above 80% for the OSCC/OED and OED group, and above 90% for OSCC, therefore

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suggesting that high diagnostic accuracy may be maintained with a simpler single set-up of the DEP reader independent of the site of sample collection.

In order to assess whether the diagnostic outcomes of the index test were disproportionally influenced by one of the two groups of interest (OSCC vs OED), we tested the diagnostic performance separately in each of the group: OSCC (A) against benign disease/normal oral mucosa (C+D) and OED (B) against benign disease/normal oral mucosa (C+D). Interestingly the diagnostic performance of the index test improved for the OSCC group (without the OED samples) and reduced for the OED group (Table 3 and 4). The above findings seem to suggest that the index test may be less accurate at identifying OED. A triage test with high sensitivity means that people who test negative are very unlikely to have the target condition and therefore can be confidently ruled out from needing to proceed with the subsequent confirmatory standard test.[29] Accordingly, a test with high sensitivity for OSCC and slightly lower sensitivity for OED can still be very useful in clinical practice, as the false negative would mostly occur in the group of people with less severe disease (OED), whereas individuals with more severe disease (OSCC) would be unlikely to be classified as negative.

This study has a number of limitations. PANDORA was a proof-of-concept study with the primary aim of identifying the index test set-up maximising its diagnostic accuracy. Therefore the accuracy results presented in this paper are to be considered preliminary. Further clinical testing would be required to fully validate its diagnostic performance. Furthermore, the PPV and NPV are well known to be notably affected by the prevalence of the disease and can differ from one setting to another for the same diagnostic test.[29] In PANDORA 33.6% of participants had the conditions of interest (OSCC or OED), whereas the prevalence of OSCC and OED in individuals attending a dental primary care setting is known to be as low as 3.3%.[30] Further clinical studies would therefore be needed to validate the PPV and NPV of the index test in a real-world population.

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FIGURE LEGENDS

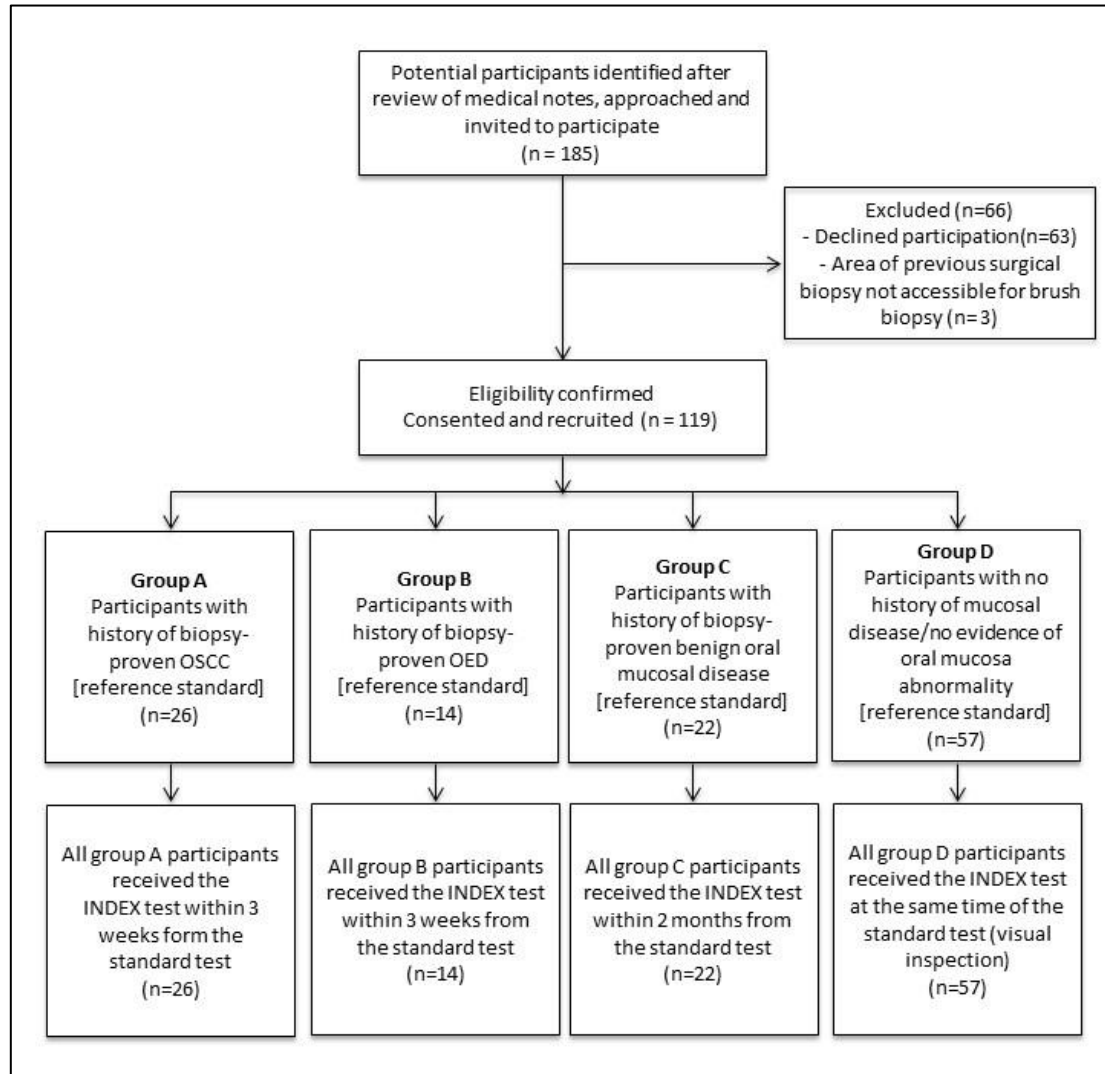**Figure 1. Flow chart of study participants**

Table 1: Inclusion criteria

Groups A and B	Group C	Group D
• ≥18 years of age. • Histological evidence * of oral squamous cell carcinoma (group A) or epithelial dysplasia (group B) (≤3 weeks from biopsy) • Area of previous surgical biopsy to be easily accessible for brush biopsy.	• ≥18 years of age. • Histological evidence of non-malignant disease of the oral mucosa (≤2 months from biopsy) • Area of previous surgical biopsy to be easily accessible for brush biopsy.	• ≥18 years of age. • No history of oral mucosal disease • No visible mucosal abnormality
* As per El-Naggar AK, Chan JKC, Grandis JR, Takata T, Slootweg PJ. WHO Classification of Head and Neck Tumours. WHO Classification of Tumours, 4th Edition, Volume 9, 4th Edition, IARC Press, Lyon; 2015. 109-112 p. [25]		

Table 2: participants' demographics and sample data

	Group A	Group B	Group C	Group D	All participant
Disease of interest					
OSCC n	26	-	-	-	**26**
OED n	-	14	-	-	**14**
Benign disease n	-	-	22	-	**22**
Normal mucosa n	-	-	-	57	**57**
Total	-	-	-	-	**119**
Gender					
Males n (%)	12 (46.2%)	5 (35.7%)	10 (45.5%)	25 (43.9%)	**52 (43.7%)**
Females n (%)	11 (42.3%)	9 (64.3%)	11 (50%)	25 (43.9%)	**56 (47.1%)**
Missing info n (%)	3 (11.5%)	0	1 (4.5%)	7 (12.2%)	**11 (9.2%)**
Ethnicity					
Caucasian n (%)	18 (69.2%)	5 (35.7%)	13 (59.1%)	37 (64.9%)	**73 (61.3%)**
Black n (%)	0 (0%)	1 (7.1%)	0 (0%)	3 (5.3%)	**4 (3.4%)**
Asian n (%)	2 (7.7%)	6 (42.9%)	4 (18.2%)	7 (12.3%)	**19 (16.0%)**
Other n (%)	0 (0%)	0 (0%)	2 (9.1%)	3 (5.3%)	**5 (4.2%)**
Missing info n (%)	6 (23.1)	2 (14.3%)	3 (13.6%)	7 (12.3%)	**18 (15.1%)**
Site					
Tongue/gingivae n(%)	21 (80.8%)	7 (50.0%)	9 (40.9%)	17 (29.8%)	**54 (36.9%)**
Buccal mucosa n(%)	5 (19.2%)	7 (50.0%)	13 (59.1%)	40 (70.2%)	**65 (51.7%)**

TABLE 3: Cross tabulation of index test results against reference standard results – all and stratified by site, before and after removing samples with very low cell count. A= biopsy-proven OSCC, B= biopsy-proven dysplasia, C= biopsy-proven benign disease, D= normal oral mucosa.

		ALL SAMPLES			AFTER LOW CELL COUNT SAMPLE REMOVAL *		
		Reference standard test			Reference standard test		
	DEP analysis (index test)	Positive (Cancer and dysplasia)	Negative (Benign disease and normal mucosa)	Total	Positive (Cancer and dysplasia)	Negative (Benign disease and normal mucosa)	Total
Group A+B vs C+D	*All*						
	Positive	35	17	52	33	12	45
	Negative	5	62	67	5	61	66
	Stratified per site						
	Tongue/gingivae - Positive	25	7	32	23	2	25
	Tongue/gingivae - Negative	3	19	22	3	18	21
	Buccal Mucosa - Positive	10	10	20	10	10	20
	Buccal Mucosa - Negative	2	43	45	2	43	45
	Total	**40**	**79**	**119**	**38**	**73**	**111**
Group A vs C+D	*All*						
	Positive	23	8	31	23	4	27
	Negative	3	71	74	2	69	71
	Stratified per site						
	Tongue/gingivae - Positive	19	7	26	19	3	22
	Tongue/gingivae - Negative	2	19	21	1	17	18
	Buccal Mucosa - Positive	4	1	5	4	1	5
	Buccal Mucosa - Negative	1	52	53	1	52	53
	All	**26**	**79**	**105**	**25**	**73**	**98**
Group B vs C+D	*All*						
	Positive	12	16	28	11	11	22
	Negative	2	62	64	2	61	63
	Stratified per site						
	Tongue/gingivae - Positive	6	7	13	5	2	7
	Tongue/gingivae - Negative	1	19	20	1	18	19
	Buccal Mucosa - Positive	6	9	15	6	9	15
	Buccal Mucosa - Negative	1	43	44	1	43	44
	All	**14**	**79**	**93**	**13**	**72**	**85**

* n=8 samples were removed from the analysis due to very low cell count (sum of MDV below 0.37), all in the tongue/gingivae group.

Table 4: Diagnostic accuracy (sensitivity and specificity with CI) of the index test

			ALL SAMPLES				*AFTER LOW CELL COUNT SAMPLE REMOVAL* *				
		MDV Threshold	**Sensitivity (95% CI)**	**Specificity (95% CI)**	**PPV (95% CI)**	**NPV (95% CI)**	**MDV Threshold**	**Sensitivity (95% CI)**	**Specificity (95% CI)**	**PPV (95% CI)**	**NPV (95% CI)**
Group A+B vs C+D	**Tongue/ gingivae**	> 0.8305	89.3% (71.92-97.7)	73.1% (52.2-88.4)	78.1% (60.0-90.7)	86.4% (65.1-97.1)	> 0.8305	88.5% (69.8-97.6)	90.0% (68.3-98.8)	92.0% (74.0-99.0)	85.7% (63.7-97.0)
	Buccal mucosa	> 0.8891	83.3% (51.6-97.9)	81.1% (68.0-90.6)	50.0% (27.2-72.8)	95.6% (84.9-99.4)	> 0.8891	83.3% (51.6-97.9)	81.1% (68.0-90.6)	50.0% (27.2-72.8)	95.6% (84.9-99.5)
	Combined	0.8305 -0.8891	87.5% (73.2-95.8)	78.5% (67.8-86.9)	67.3% (52.9-79.7)	92.5% (83.4-97.5)	0.8305 -0.8891	86.8% (71.9-95.6)	83.6% (73.0-91.2)	73.3% (58.1-85.4)	92.4% (83.2-97.5)
Group A vs C+D	**Tongue/ gingivae**	> 0.6982	90.5% (69.6-98.8)	73.1% (52.2-88.4)	73.1% (52.2-88.4)	90.5% (69.2-98.8)	> 0.6961	95.0% (75.1-99.9)	85.0% (62.1-96.8)	86.4% (65.1-97.1)	94.4% (72.7-99.9)
	Buccal mucosa	> 0.9280	80.0% (28.4-99.4)	98.1% (89.9-100)	80.0% (28.4-99.5)	98.1% (89.9-100)	> 0.9280	80.0% (28.4-99.5)	98.1% (89.9-100)	80% (28.4-99.5)	98.1% (89.9-100)
	Combined	0.6982 - 0.9280	88.5% (69.8-97.6)	89.9% (81.0-95.5)	74.2% (55.4-88.1)	95.9% (88.6-99.2)	0.6982 - 0.9280	92.0% (74.0-99.0)	94.5% (86.6-98.5)	85.2% (66.3-95.8)	97.2% (90.2-99.7)
Group B vs C+D	**Tongue/ gingivae**	> 0.8582	85.7% (42.1-99.6)	73.1% (52.2-88.4)	46.2% (19.2-74.9)	95.0% (75.1-99.9)	> 0.7533	83.3% (35.9-99.6)	90.0% (68.3-98.8)	71.4% (29.0-96.3)	94.7% (74.0-99.9)
	Buccal mucosa	< 0.1311	85.7% (42.1-99.6)	82.7% (69.7-91.8)	40.0% (16.3-67.7)	97.7% (88.0-99.9)	< 0.1311	85.7% (42.1-99.6)	82.7% (69.7-91.8)	40.0% (16.3-67.7)	97.7% (88.0-99.9)
	Combined	0.1311- 0.8582	85.7% (57.2-98.2)	79.5% (68.8-87.8)	42.9% (24.5-62.8)	96.9% (89.2-99.6)	0.1311- 0.7533	84.6% (54.6-98.1)	84.7% (74.3-92.1)	50% (28.2-71.8)	96.8% (89.0-99.6)

* n=8 samples were removed from the analysis due to very low cell count (sum of MDV below 0.37), all in the tongue/gingivae group.