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Critical Evaluation of Diagnostic Aids for the Detection of Oral Cancer

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Abstract

Historically, the screening of patients for signs of oral cancer and precancerous lesions has relied upon the conventional oral examination. A variety of commercial diagnostic aids and adjunctive techniques are available to potentially assist in the screening of healthy patients for evidence of otherwise occult cancerous change or to assess the biologic potential of clinically abnormal mucosal lesions. This manuscript systematically and critically examines the literature associated with current oral cancer screening and case-finding aids or adjuncts such as toluidine blue, brush cytology, tissue chemiluminescence and autofluorescence. The characteristics of an ideal screening test are outlined and the authors pose several questions for clinicians and scientists to consider in the evaluation of current and future studies of oral cancer detection and diagnosis. Although the increased public awareness of oral cancer made possible by the marketing of recently-introduced screening adjuncts is commendable, the tantalizing implication that such technologies may improve detection of oral cancers and precancers beyond conventional oral examination alone has yet to be rigorously confirmed.

Keywords

Oral Cancer; premalignancy; screening; diagnosis

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I. INTRODUCTION

Oral cancer is traditionally defined as squamous cell carcinoma of the lip, oral cavity and oropharynx. At current rates, approximately 30,000 cases in the United States and more than 400,000 cases worldwide will be diagnosed in 2006, making it the sixth most common malignancy in the world [1, 2]. Despite numerous advances in treatment, the 5-year survival has remained approximately 50% for the last 50 years [3]. This poor prognosis is likely due to several factors. First, oral cancer is frequently associated with the development of multiple primary tumors. The rate of second primary tumors in these patients, 3–7% per year, is higher than for any other malignancy[4]. This characteristic led Slaughter to propose that multiple individual primary tumors develop independently in the upper aerodigestive tract as a result of chronic exposure of the lining mucosal epithelium to carcinogens, a theory known as "field cancerization"[5]. Although this theory is not accepted by all authorities, oral cancer patients who live five years after their initial primary disease is diagnosed and treated have up to a 35% chance of developing at least one new primary tumor during that time. To underscore the significance of this complication, the most common cause of treatment failure and death in oral cancer patients is their second primary tumor [6]. Second, poor survival among oral cancer patients can also be attributed to the advanced extent of the disease at the time of diagnosis, with over 60% of patients presenting in stages III and IV. Such dismal statistics seem perverse since the disease primarily arises in the surface oral epithelium that is readily accessible to direct visual and tactile examination. The conclusion that at least some lesions are ignored or missed by patients, health care professionals or both is inescapable. In part, this may be due to an incomplete understanding or awareness that even small asymptomatic lesions can have significant malignant potential.

One approach to this problem would be to improve the ability of oral health care professionals to detect relevant potentially malignant lesions or cancerous lesions at their earliest or most incipient stage. Such a goal could be achieved by increasing public awareness about the importance of regular oral screening or case finding examinations to identify small, otherwise asymptomatic cancers and precancers (secondary prevention). Another strategy would be the development and use of diagnostic aids that could help the general dentist or dental specialist more readily identify or assess persistent oral lesions of uncertain biologic significance. This paper will examine the role of screening examinations in oral cancer and evaluate the literature regarding currently available diagnostic tests or techniques that are purported to aid in the detection and diagnosis of cancerous and precancerous lesions.

II. SCREENING

Screening for disease has a precise definition and implies an ongoing, structured health care intervention designed to detect disease at an asymptomatic stage when its natural course can be readily interrupted if not cured. It has been defined as: '*the application of a test or tests to people who are apparently free from the disease in question in order to sort out those who probably have the disease from those who probably do not*' [7]. The important factor is that screening involves checking for the presence of disease in a person who is symptom-free.

A number of established cancer screening programs been shown to significantly reduce patient morbidity and mortality. Well-known examples such as the Pap test for cervical cancer and mammography for breast cancer are readily available in virtually any health care setting and are conducted as national screening programs (National Cancer Institute, National Health Service).

In contrast to screening, case-finding is defined as a diagnostic test or method that is applied to a patient who has abnormal signs or symptoms in order to establish a diagnosis and bring the patient to treatment. In the past, screening (detection) and case-finding (diagnosis) have

often been mistakenly used interchangeably in epidemiological studies designed to determine the prevalence of a given disease in a particular population. In this paper, the term screening will be used to denote a method or test applied to asymptomatic persons to detect disease and case-finding will refer to the application of a diagnostic test or procedure to a patient with an identified lesion.

III. CRITERIA FOR SCREENING AND FOR SCREENING TESTS

Because of the cost implications and the potential for over-diagnosis (false positive result), strict criteria are needed to evaluate screening programs and to determine their appropriateness. In the UK for example, the National Screening Committee lists 22 criteria that should be met before a screening program is introduced [8]. These were originally taken from the work of Wilson and Jungner [7], and are summarized in Table 1. Since oral cancer meets at least three of these criteria, screening measures for this condition would seem warranted. It is unlikely, though, that oral cancer screening programs will be implemented without more scientific support of their efficacy. In addition, there are a number of characteristics that should be considered in the development of an ideal screening test (Table 2).

When assessing an individual publication with respect to the efficacy of a particular screening/diagnostic test, a number of important questions should be considered (Table 3) [9, 10]. First, are the results of the study valid? One important criterion in assessing the validity of a test would be to determine whether or not it was compared to an accepted “gold standard”. For screening purposes, the standard may consist of examination and clinical evaluation by an expert clinician trained in diagnosis such as an oral and maxillofacial pathologist or oral medicine specialist. For case-finding or diagnostic purposes, the acknowledged gold standard is the scalpel biopsy [11]. Second, were both the new test or technology and the gold standard assessed in every subject and in an independent and blinded fashion? Third, does the study population represent an appropriate spectrum of patients to whom the diagnostic test will be applied in clinical practice? Fourth, was the study accomplished by the practitioners most likely to perform the screening test in a practice or for whom the diagnostic aid was designed? Most authorities would agree that results obtained by a group of specialists would probably differ, possibly significantly, from results obtained by generalists or dental auxiliaries. Fifth, can the screening/diagnostic test distinguish between the disease of interest (in this case: oral dysplasia and/or oral cancer) and other noncancerous conditions? The importance of this criterion can be appreciated by recalling the development of carcinoembryonic antigen (CEA) as a potential screening tool for colon cancer. CEA was found to be markedly elevated in the majority of patients with late stage colon cancer [12]. Conversely, lower or undetectable levels were found in patients without colon cancer, suggesting that CEA might be a successful biomarker for this malignancy. Unfortunately, subsequent studies determined that patients with earlier stage disease did not express increased levels of CEA. Thus initial studies suffered from “spectrum bias”, i.e., the patients evaluated in those studies were not representative of the entire population of interest [13]. Furthermore, increased levels of CEA could also be found in nonmalignant diseases of the gastrointestinal tract [14], and CEA was abandoned as a colon cancer screening biomarker. Finally, are the methods of the test or technology described in sufficient detail to permit replication of the study by others? This latter question is critically important in order to determine the feasibility and reproducibility of the test. At a minimum, the report should include an adequate description of the cohort of patients that were studied, a description of how the screening exam or test was performed and a detailed explanation of how the test was analyzed and interpreted.

IV. CURRENT ORAL CANCER SCREENING OR CASE-FINDING TESTS

Among the screening tests or diagnostic aids now available for oral cancer, some have been used and studied for many years while others have recently become commercially available (Table 4). Screening or case-finding tests should always be evaluated with respect to their sensitivity, specificity and predictive values (Fig 1). Such analysis requires that the test outcome from a sample of subjects be compared to the results of an appropriate gold standard on the same population. The gold standard is used to classify subjects as to their true state of disease (present or absent). The *sensitivity* measures the proportion of subjects with the disease who test positive, while the *specificity* determines the proportion without the disease who test negative. The *predictive values* determine the proportion of subjects with positive or negative test results that either do or do not have the disease. There are no defined values for the ideal screening test, but in general it is desirable to have both high specificity (few false positives) and high sensitivity (few false negatives). The acceptable trade off between sensitivity and specificity will depend upon the consequences of failing to detect the disease versus the costs, anxieties and other added burdens associated with false positive tests. Another relevant issue is the overall prevalence of the disease in question. If the disease is rare, even tests with very high sensitivity and specificity will yield many false positive results.

A. Oral Examination

A conventional oral examination (COE), using normal (incandescent) light, has long been the standard method for oral cancer screening. Conventional visual cancer screenings for some anatomic locations can be highly successful. For example, visual inspection of skin lesions can be an effective screening method for melanoma, with sensitivity and specificity rates as high as 98 percent [15, 16]. However, while COE has traditionally been the mainstay of oral cancer screenings for decades, its utility remains controversial. A number of publications have suggested that COE may have limited value as a method for detecting pre-cancerous or early cancerous lesions [17–19]. Conversely, other studies have reported a relatively high degree of sensitivity, specificity and positive predictive value of COE.

The largest study in the West consisted of two pilot oral cancer-screening programs involving over 2300 individuals who were examined for the presence or absence of relevant oral mucosal lesions (red or white lesions or ulcers of greater than 2 weeks duration) [20, 21]. A general dentist examined each subject and the presence of relevant lesions was recorded. All subjects were also independently examined by a specialist in oral medicine who assigned the expert or “true” clinical diagnosis, thus providing a gold standard. Although arguably a “soft” standard, the routine use of confirmatory biopsy to provide the “hard” diagnostic gold standard in patients who screen negative by COE (clinically normal) has been deemed inappropriate and an ethically questionable practice [22]. The sensitivity and specificity of the oral examination using the “soft standard” was 0.74 and 0.99 respectively, indicating that a visual oral examination can detect relevant lesions with a sensitivity and specificity similar to that found in other screening programs. In a recent systematic review [22] only five other studies have determined the sensitivity and specificity of an oral examination [23–27]. Four were conducted in developing countries using health care auxiliaries as screeners and one was undertaken in Japan utilizing general dentists. In these studies, the lowest specificity was 0.75 [23] but all other studies had values over 0.94. Sensitivities ranged from 0.60 to 0.97. A meta-analysis of this data showed an overall sensitivity of 0.85 (95% CI 0.73, 0.92) and specificity of 0.97 (95% CI 0.93, 0.98) indicating a satisfactory test performance for an oral examination [22]. Furthermore, an analysis of heterogeneity indicated no differences between studies suggesting that trained auxiliaries are able to screen with a degree of accuracy similar to dental practitioners.

Importantly, while these studies evaluated the performance and reproducibility of oral cancer screening, they did not assess the *effectiveness* of screening programs based on oral

examination alone. A recent systematic review identified 100 potentially relevant publications but could only find one randomized controlled trial (RCT) [28]. This RCT was carried out in India by the 'Kerala' group [29, 30]. Initiated in 1995, this study involved over 130,000 individuals randomized into two groups (screening or control), with early results presented at 3 [30] and 6 [29] years. Surrogate markers of improved outcome including 3-year survival, stage of presentation and yield were all significantly greater in the intervention group compared to the control group. There was, however, no evidence of reduced mortality with regard to oral cancer. Since disease-related mortality is the gold standard outcome for effectiveness, Kujan *et al*. [28] concluded that there was no evidence to support or refute the use of a visual examination as a method of screening for oral cancer. It was thought that the early reports of improved survival might be due to lead-time bias. Subsequently, the Kerala group reported their results at 9 years [31]. Although no increase in survival was observed for the overall population, a significant increase in survival was seen among males with high-risk habits, such as tobacco use. This was the first clear evidence to support the efficacy of an oral cancer screening program, as measured by reduced mortality. It prompted others to call for the broader use of oral screening measures throughout the world [32]. Recent studies using simulation modelling have shown that opportunistic high-risk screening in dental practice may be feasible and cost-effective[33, 34].

Although COE may be effective as a screening test, there are still many problems with this approach. First, approximately 5–15% of the general population have oral mucosal abnormalities [35–37]. Without question, the vast majority of these lesions are clinically/biologically benign. Second, the classic clinical presentation of an oral malignancy or premalignant lesion: a red patch, white patch or persistent ulcer that cannot be diagnosed as any other condition, is well recognized. In reality, most lesions are white patches or plaques, also known as true leukoplakias. The problem, however, is that only a small percentage of leukoplakias are progressive or become malignant and a COE cannot discriminate between these lesions and their non-progressive counterparts. Furthermore, while COE may detect a number of clinical lesions and a small percentage of those may exhibit histological features of premalignancy, recent data suggests that some precancerous lesions may be lurking within mucosa that appears clinically normal by COE alone. This concept is supported by the work of Thomson, who found that 9/26 consecutive patients (36%) with a newly diagnosed HNSCC had histologic evidence of dysplasia or microinvasive cancer in a biopsy from clinically normal mucosa from the corresponding, contralateral anatomic site [38].

Therefore, while COE may be useful in the discovery of some oral lesions, it does not identify all potentially premalignant lesions, nor does it accurately detect the small proportion of biologically relevant lesions that are likely to progress to cancer.

B. Brush Cytology

The Brush Biopsy (CDx Laboratories, Suffren, NY) was introduced as a potential oral cancer case-finding device in 1999. It was designed for the interrogation of clinical lesions that would otherwise not be subjected to biopsy because the level of suspicion for carcinoma, based upon clinical features, was low [39–41]. When an abnormal result is reported (*atypical* or *positive*), the clinician must follow-up with a scalpel biopsy of the lesion, as the use of brush cytology does not provide a definitive diagnosis.

Several studies have shown encouraging results with oral brush cytology for evaluation of oral precancerous lesions. The study by Scuibba et al [42] was a prospective, multicenter study to determine the sensitivity and specificity of oral brush biopsy (OralCDx) for the detection of pre-cancerous and cancerous lesions of the oral mucosa. Brush biopsy results were recorded as "positive", "atypical", or "negative". Patients with clinically suspicious lesions (Class I) underwent both the OralCDx and the "gold standard" scalpel biopsy (n=298). The remaining

patients, whose lesions were judged to be innocuous (Class II), only underwent OralCDx testing (n=647). The only exception was for a small number of cases with abnormal OralCDx results that underwent subsequent scalpel biopsy at the investigator's discretion (n=29). Using a combination of Class I and Class II lesions, a 100% sensitivity with 100% specificity was reported if *positive* test results were deemed indicative of cancer and 92.9% specificity if *atypical* or positive results were considered indicative of cancer. The strengths of the study include its multicenter nature, enhancing generalizability of the results and the large sample size. Another positive feature is the fact that the brush and scalpel biopsies were analyzed independently by different pathologists (the brush biopsies at OralScan Laboratories, the scalpel biopsies at the participating sites) who were blinded to each other's results.

The major weakness of the study, however, is that the gold standard (scalpel biopsy) was not performed in the majority of the Class II patients, a cardinal rule for the evaluation of diagnostic tests [9]. By omitting a scalpel biopsy in nearly all patients from Class II, information critical to the assessment of the brush technique was lost in this study. Of particular relevance, Class II lesions (those lesions that appear innocuous and would otherwise not be biopsied) are the target lesions of this technology in the routine dental practice setting [39–41]. Thus, while this report appears to indicate that the brush biopsy technique uncovered cases that might otherwise have gone unsampled by scalpel biopsy, no information was provided regarding the true sensitivity and specificity of the test within the Class II patients. Furthermore, it is inappropriate to cite the Class I results in this regard, since this would suffer from the well known problem of *spectrum bias*, alluded to above, i.e., the sensitivity and specificity in Class II may well be different from that in Class I. In summary, the Scuibba study provides evidence that brush biopsy may be a useful diagnostic device for the testing of potential oral cancer or precancerous lesions. While the results are encouraging, the data most pertinent to its target patient population (Class II subjects) is lacking.

In another study of 298 patients, Svirsky et al. [43] analyzed scalpel biopsies with test requisition forms that either were accompanied by an oral brush biopsy report or contained the findings of an oral brush biopsy report. Of the 298 patients so identified, 243 (82%) had abnormal brush biopsies, strongly suggesting that, once again, many patients with negative brush biopsies were excluded from the evaluation because a subsequent scalpel biopsy was not performed. This is important to consider in the calculations of sensitivity, specificity and positive predictive values presented for the study. Among the 243 abnormal brush biopsies, 93 showed dysplasia or cancer upon histological evaluation, yielding a calculated PPV of 38%. Among the 55 cases that were brush biopsy negative, 51 had a negative and 4 had positive scalpel biopsies. A comparison of 80 patients who had both brush cytology and a scalpel biopsy found that the brush technique had a sensitivity of 92% and a specificity of 94% for both *positive* and *atypical* results in detecting dysplasia and oral cancer. An important strength of this study is that the patients evaluated were derived from general dentists who performed the initial examination and brush biopsy. This aspect is important because the study design better reflects the actual use of this device in the community. Conversely, a major weakness of this study in addition to that noted above is that insufficient information is provided regarding Class designation for each of the 298 patients. This is a critical issue because the inclusion of an inordinate number of Class I (suspicious lesion) patients would obviously skew the calculations of sensitivity, specificity and, most importantly, positive predictive value. Therefore, the lack of apparent control as well as documentation with respect to the manner in which the sample was selected limits the utility of this study.

Poate et al performed a retrospective study of 112 patients referred to an Oral Medicine Unit due to clinical findings suggestive of malignancy [44]. As a result, most of the patients in this cohort would almost certainly be classified as Class I. All patients with positive brush biopsy results were offered scalpel biopsies, but those with negative brush biopsies were only offered

scalpel biopsy if the Oral Medicine specialists judged the lesion to be clinically suspicious. In the end, only 15 of the 75 lesions that were brush biopsy negative underwent a scalpel biopsy. A sensitivity of 71%, specificity of 32% and a positive predictive value of 44% were reported. Importantly, the authors did find 6 of 15 negative brush biopsy cases to have dysplasia or carcinoma present in the scalpel biopsy, underscoring the potential for false negative results that have been reported by others [43, 45, 46]. There are a number of weaknesses associated with this study. First, the patient cohort in this study (Class I) does not appear to be consistent with the type of patients for which the technology was intended, namely Class II patients. Second, the gold standard was not performed in all subjects, in particular the Class II patients. Therefore, the statistics reported for sensitivity, specificity, and positive predictive value are biased.

Christian investigated the utility of the brush biopsy by screening a presumably low risk population of dentists and dental hygienists that were attending an annual ADA meeting [47]. Participants with clinically abnormal oral epithelial lesions (of which “nearly all” were asymptomatic and innocuous) underwent a brush biopsy, suggesting that at least the majority of the lesions were Class II. Of the 930 individuals screened, 89 were found to have one or more epithelial lesions, for a total of 93 lesions. Seven of these lesions were abnormal by brush biopsy and four of the seven abnormal lesions had a scalpel biopsy. Three of the four scalpel biopsies demonstrated dysplasia. Strengths of this study include a large sample size and a study population that appeared to mainly consist of the type of lesions for which this technology was intended (Class II). However, this study suffers from the same problem as in Sciubba et al. [42], namely, the failure to perform scalpel biopsies on all subjects. As a consequence, it is impossible to calculate sensitivity and specificity in this study population. We do learn that out of 4 cases with an abnormal brush biopsy, 3 were precancerous. But the positive predictive value for the study cannot be calculated because only four of the seven atypical lesions underwent the gold standard scalpel biopsy. The author states that the three precancerous lesions found using the brush biopsy would not have been identified unless scalpel biopsies were performed on all 93 lesions. It is further argued that by using the brush biopsy, only seven of the 93 lesions required incisional biopsies. This argument is unsatisfactory, however, since the total number of the 86 negatives that might have been precancerous or cancerous (false negative results) is left unknown.

In a study by Scheifele et al, 96 oral lesions from 80 patients with a clinical diagnosis of oral leukoplakia (OL, n=49), oral lichen planus (OLP, n=18), or squamous cell carcinoma (OSCC, n=13) that underwent scalpel biopsy within one month of the brush biopsy [48]. Assuming the cases are in fact consecutive, we obtain valid estimates of sensitivity and specificity, which are 92.3% and 94.3%, respectively. A major strength of this study is that this appears to be the only study in which both brush and scalpel biopsies were performed on all subjects. It is also the only one to report likelihood ratios, which are useful summary statistics for diagnostic tests [9]. However, a key weakness associated with the study is whether the lesions tested were Class I or Class II. Based upon the clinical diagnosis, it would appear to be a mixture of both. This would of course have an effect on the reported sensitivities and specificities. More importantly, a major weakness of the study is that two of the clinical diagnostic categories (lichen planus and squamous cell carcinoma) should not have been evaluated using the brush biopsy. Lichen planus is an autoimmune disease in which the patient’s T cells attack the basal cells of the oral mucosa. As such, the cytologic and morphologic changes observed in cell dissociates derived from a lesion of lichen planus will invariably be seen out of context, often resulting in an “atypical” Oral CDx result. Furthermore, as previously discussed, the brush biopsy is intended for innocuous (Class II) lesions that would not otherwise be biopsied [39–41]. Therefore, the cases assigned a clinical diagnosis of squamous cell carcinoma should not have been included in the study.

In summary, based on the evidence thus far, the oral brush biopsy technique shows promise. Before any firm conclusions can be reached, however, a study needs to be conducted in a sufficient cohort of Class II subjects where both brush biopsy and scalpel biopsy are performed on each participant. That this has generally not been done in the studies reported in the literature to date is unfortunate. Despite these limitations, one can already envision two current clinical scenarios where this technology may prove useful. First, it may be beneficial in the patient with multiple lesions throughout their oral cavity. If a patient, in particular those with no history of oral cancer, present with four or five unique areas of concern, it is unlikely that he or she would readily consent to multiple scalpel biopsies. Similarly, this technique may be useful in the non-compliant patient who is unlikely to come back for a follow-up exam or accept an immediate referral to an oral surgeon. Despite the overall uncertainty of this particular technology as an oral cancer diagnostic or case-finding aid, the judicious use of the brush cytology in these scenarios may be clinically useful.

C. Toluidine blue staining

Toluidine blue (also known as tolonium chloride) is a vital dye that may stain nucleic acids and abnormal tissues. It has been used for decades as an aid to the identification of mucosal abnormalities of the cervix as well as in the oral cavity. It has been valued by surgeons as a useful way of demarcating the extent of a lesion prior to excision. While not currently approved by the FDA for use as a oral cancer screening technique in the United States, toluidine blue has been championed in other parts of the world for several decades as a means of identifying clinically occult lesions in patients whose oral mucosa may otherwise be normal – that is, as a screening test or adjunct [49].

Overall, there appears to be some evidence that toluidine blue can stain oral lesions and that it is useful as an adjunct to a clinical examination for the identification of potentially premalignant lesions. To date, however, it has only been evaluated in a secondary care environment in the hands of specialists. The literature on toluidine blue is large and a recent systematic review [50] identified 77 publications. However, only 14 of these evaluated the ability of the dye to identify oral cancers that would not otherwise have been diagnosed by unaided clinical examination. Unfortunately, these studies are of limited relevance to the use of the dye as a screening test, because none were randomized controlled trials, none were conducted in a primary care setting, and most studies were case series conducted by specialists on high-risk populations, often with known lesions. Overall, the sensitivity of toluidine blue staining for the detection of oral cancers has ranged from 0.78 to 1.00 and the specificity from 0.31 to 1.00.

Only one study properly evaluated the use of toluidine blue to detect lesions that had not been detected by visual examination [51]. This was a complex study carried out in a specialist clinic on patients with a past history of oral cancer and who had been seen regularly in follow up. Patients were entered into a screening program where they were re-examined and screened with toluidine blue rinse. Of 235 people screened, 50 visible lesions were detected clinically and a further 32 patients had areas that retained dye in the absence of a visible lesion. All 82 lesions were biopsied and 6 of the 32 clinically undetected, but toluidine blue-positive, lesions proved to be carcinomas. Of the 50 clinically visible lesions, 20 stained positively with toluidine blue and 6 of these were carcinomas. One of the clinically detected carcinomas was dye-negative. Therefore, out of 82 patients screened with the aid of toluidine blue and biopsied, 6 cancers were identified that would have been otherwise undetected. The overall sensitivity and specificity was 0.92 and 0.42 respectively. In these circumstances the test was quite sensitive but specificity was low –forty patients (58%) had a false positive toluidine blue stain.

Most studies have shown a high sensitivity for the detection of oral carcinomas. Warnakulasuriya and Johnson [52] for example, stained the oral mucosa of 102 patients with clinically suspicious lesions. Eighteen patients proved to have oral carcinomas and all of their

respective malignant lesions stained with toluidine blue (sensitivity = 100% for detection of oral cancer). However the overall specificity was low at 0.62 and since lesions besides squamous cell carcinoma were also examined, the results of this study raise several issues. First, the dye-positive carcinomas were all clinically visible and would have been subjected to biopsy in the absence of toluidine blue staining. Second, of the 39 lesions that proved on biopsy to be dysplastic, only 29 (74%) stained positively. Third, 18 cases (50%) of oral lichen planus stained positively. Thus the overall sensitivity and specificity of the test for the detection of potentially malignant lesions (dysplasia) was only 0.74 and 0.66 respectively. Despite these limitations, the authors also reported 12 oral sites with no clinical evidence of abnormality that were dye-positive and five of these sites showed dysplasia on biopsy. In a similar study, Onofre et al [53] found that all carcinomas stained positively, but only 50% of dysplasias were positive and that 13 of 37 (35%) benign lesions also stained. The overall sensitivity and specificity was 0.77 and 0.67 respectively. Finally, Martin et al [54] stained a series of resection specimens to correlate stain uptake to histological areas of carcinoma or dysplasia. All sites of carcinoma were positive, but only 17 of 40 (42%) areas of dysplasia were positive.

The main problematic issues associated with studies of toluidine blue are listed in Table 5. Over the years, mixed results have been a persistent feature of these studies and although several authors have expressed reservations with the technique, further research may be warranted. Overall, toluidine blue appears to be good at detecting carcinomas but is positive in only ~50% of lesions with dysplasia. In addition, it also frequently stains common, benign conditions such as non-specific ulcers. In their systematic review, Gray et al [50] concluded that there is no evidence that toluidine blue is effective as a screening test in a primary care setting. The high rate of false positive stains and the low specificity in staining dysplasia likely outweigh the potential benefits of any additional cancers detected at this time. This does not however preclude its usefulness as an adjunct to clinical examination and case-finding, even in primary care. In experienced hands toluidine blue staining may be useful in the evaluation of oral lesions and as an adjunct in the surveillance of high-risk individuals, such as patients at risk for a second primary lesion. Furthermore, a recent publication demonstrated that toluidine blue might be useful in determining which clinically evident oral lesion is more likely to progress to oral cancer (Zhang *et al*., 2005). This publication demonstrated that toluidine blue preferentially stained lesions that exhibited high risk clinical features, preferentially stained lesions with higher degrees of dysplasia, recognized lesions with high risk molecular patterns and correlated with outcome. Importantly, it predicted risk and outcome of visible oral lesions with little to no microscopic evidence of dysplasia. These findings underscore the potential utility of toluidine blue in a case-finding setting. To date, however, these studies have not been extended to determine whether toluidine blue screening can help identify and predict the risk of progression for lesions that cannot be seen with the naked eye. Given the possibility that positive toluidine blue staining has a relatively high correlation with high-risk molecular patterns, it would be worthwhile to investigate a potential correlation of these findings with screening for lesions that cannot be seen by COE alone. With regard to the criteria for a positive result, there has been much debate about the intensity of staining and whether or not pale blue staining should be regarded as positive. A recent study [55] suggests that only dark royal blue staining should be regarded as positive. All carcinomas stained dark royal blue and on histology showed nuclear staining. Benign lesions had no nuclear staining and were more often pale blue in color. These findings may be helpful to clinicians but the data requires confirmation since dysplastic lesions, either clinically visible or not, were not included and the number of cases was small.

V. LIGHT-BASED DETECTION SYSTEMS

A. Chemiluminescence (reflective tissue fluorescence)

Chemiluminescence has been used for many years as an adjunct in the examination of the cervical mucosa for “acetowhite” premalignant and malignant lesions. Recently, this technology has been adapted for use in the oral cavity and is currently marketed under the names ViziLite Plus and MicroLux DL. These products are intended to enhance the identification of oral mucosal abnormalities. With both systems, the patient must first rinse with a 1% acetic acid solution followed by direct visual examination of the oral cavity using a blue-white light source. ViziLite Plus uses a disposable chemiluminescent light packet, while the MicroLux unit offers a reusable, battery-powered light source. The 1% acetic acid wash is used to help remove surface debris and may increase the visibility of epithelial cell nuclei, possibly as a result of mild cellular dehydration. Under blue-white illumination, normal epithelium appears lightly bluish while abnormal epithelium appears distinctly white (acetowhite). ViziLite Plus also provides a tolonium chloride solution (TBlue), which is intended to aid in the marking of an acetowhite lesion for subsequent biopsy once the light source is removed.

Several studies have examined chemiluminescence as an oral cancer screening aid. Although none have been published with MicroLux DL, the similarities in emission characteristics between the two technologies make it unlikely that their results would be significantly different. In a clinical survey of 150 patients, ViziLite was used to examine a variety of oral lesions, including linea alba, leukoedema, hairy tongue, leukoplakia, traumatic ulcer, fibroma, amalgam tattoo, tori, and frictional keratosis [56]. While most of the clinically innocuous lesions were negative, all cases (n=32) of leukoedema were positive (acetowhite). Since leukoedema is well-recognized as a benign mucosal alteration, the cases represent false positive screens. In addition, 2 of 14 frictional keratoses were found to be acetowhite. These two lesions were subsequently brush biopsied, and found to be cytologically *normal*. Unfortunately, scalpel biopsies were not performed on any lesion judged clinically to be frictional keratosis so definitive diagnostic information was unavailable. Three lesions categorized as leukoplakia were found to be acetowhite. Two of these were found to be cytologically *atypical*. One of these two lesions (a speckled leukoplakia) underwent subsequent scalpel biopsy with a final diagnosis of nonspecific ulcer. The third leukoplakia was biopsied immediately and diagnosed as hyperplasia/hyperkeratosis. Importantly, a single acetowhite lesion was identified with ViziLite illumination that was not clinically apparent by COE.

Strengths of the study include the large sample size as well as the fact that 150 consecutive patients were evaluated, thereby providing insight into the incidence of lesion detection using the device. Weaknesses include the limited number of cases with histopathologic correlation. As mentioned previously, sensitivity, specificity or positive predictive value of the device cannot be accurately assessed in the absence of a comparison to the gold standard diagnostic test (scalpel biopsy). In addition, while 150 patients took part in the study, only 17 presented with clinical lesions (14 frictional keratoses and 3 leukoplakias) that might warrant evaluation to exclude oral premalignancy. Although one lesion was detected with ViziLite that was not observed using incandescent light alone, the finding that all cases of the leukoedema were acetowhite suggests that while the sensitivity of ViziLite may be relatively high, its specificity and PPV are probably low.

In a second study, forty patients with a previous history of oral cancer or premalignancy were examined by ViziLite [57]. Out of a total of 46 acetowhite lesions, 31 received scalpel biopsy. The sensitivity and specificity was reported as 100% and 14% respectively. One of the weaknesses of this study is the small sample size. In addition, the majority of the lesions examined appear to be of the Class I type, rather than Class II lesions for which the technology

would have the greatest potential benefit. Finally, since 1/3 of the lesions did not undergo the gold standard test (scalpel biopsy), sensitivity, specificity and positive predictive value cannot be accurately determined.

Recently, a multi-center ViziLite study involving 134 patients was reported[58]. All patients in the study had a history of previously-detected oral mucosal lesions. A total of 138 lesions were identified by COE and most (89%) were described clinically as leukoplakia. Three of these lesions were not enhanced using ViziLite. Two were described as "red lesions…not suspicious for malignancy" while the other was a gingival leukoplakia later diagnosed by biopsy as lichen planus. ViziLite examination detected two previously occult lesions in separate patients with a prior history of squamous cell carcinoma. On biopsy, one case was found to represent recurrent carcinoma while the other was reported as "benign". This study included a subjective comparison of the brightness, sharpness, texture and size of lesions examined by COE compared to ViziLite. Significant improvements were reported with all of these characteristics except for lesion size, which was statistically unchanged. Unfortunately, these comparisons are admittedly subjective in nature and the authors concluded that ViziLite examination did not significantly improve lesion detection compared to COE. As with previous reports, this study suffered from the lack of standardized correlation of clinical findings to histopathologic diagnosis of the lesional tissue.

In another similar descriptive study, a total of 501 consecutive patients over the age of 40 years and with a positive history of tobacco use were examined by COE followed by ViziLite chemiluminescence [59]. A total of 490 oral lesions were identified in 270 of the patients examined by COE. Of these, a total of 127 were classified as "suspicious" while 363 were "non-suspicious". Among the suspicious lesions, 77 (61%) were enhanced by ViziLite examination, while only 21 (5.8%) non-suspicious lesions were ViziLite positive. Six lesions were initially detected using the ViziLite unit; however, the authors noted that all six could be visualized as homogenous areas of leukoplakia upon retrospective COE. As with the previous study, a descriptive comparison of lesion characteristics indicated that only sharpness was significantly improved by the ViziLite technique.

Once again, a major weakness of this study is the lack of diagnostic correlation with biopsy findings. The findings in the current study also diverge notably from the work of others. For example, no cases of leukoedema were identified in 501 patients yet this relatively common mucosal variation was found to be uniformly ViziLite positive in 32 out of 150 patients in the report by Huber and others [56]. Similarly, all four cases of traumatic ulcer in the current study were ViziLite positive. In comparison, a single previous case of traumatic ulcer was reportedly ViziLite negative, while a subsequent study found one non-specific ulcer to be ViziLite positive [56, 60]. Another noteworthy feature of this study was the detection of 127 "suspicious" lesions. The remarkable nature of this result is made clear by the authors themselves, who chose to examine 501 patients in the hopes of identifying a minimum of 15 "suspicious" lesions, based upon a reported 3% prevalence of leukoplakia in the general population. Despite the markedly higher prevalence within this study population (25%), the authors provided no explanation. Finally, while the discovery of six lesions by ViziLite that were undetected by COE is intriguing, its significance cannot be determined due to the lack of histopathologic correlation.

ViziLite examination was used in a study of 55 patients referred to an oral medicine specialist for assessment of oral white lesions [60]. Intra-oral examination with standard operatory lighting was repeated with the ViziLite system. In both cases, lesions were assessed as to clinical diagnosis, relative visibility and border sharpness. All lesions were then subjected to incisional scalpel biopsy and subsequently interpreted by a separate, uninvolved oral pathologist. Besides the 55 sentinel lesions, 25 satellite lesions were identified by COE. ViziLite examination reportedly enhanced lesional visibility in 26 of the sentinel abnormalities (47%). However, this

difference was not statistically significant. Likewise, no significant improvement in border distinctness was observed. All 55 lesions were positive by ViziLite. The histopathologic diagnoses of these lesions included: (hyper)keratosis, fibroepithelial hyperplasia, lichenoid mucositis, epithelial dysplasia, squamous cell carcinoma and non-specific ulceration. In one case, ViziLite examination revealed a satellite lesion that had not been detected by COE, but this did not change the clinical diagnosis or biopsy procedure. Correlation of ViziLite findings to the histopathologic diagnosis resulted in 10 true positives, zero true negatives, 45 false positives and zero false negatives for a sensitivity of 100%, specificity of 0% and an accuracy of 18.2%. The authors concluded that the technique provided little benefit to lesion detection (as a screening device) or diagnosis (as a case-finding device) beyond COE alone. Limitations of the study include the fact that two diagnostic specialists performed all examinations rather than generalists and that the data presentation makes it difficult to directly compare the clinical diagnosis and histopathologic diagnosis for a given lesion.

Most recently, a series of 100 consecutive patients who presented for dental screening examinations was reported [61]. The patients were examined by routine incandescent lighting before and after a one-minute rinse with 1% acetic acid. Patients were then examined with ViziLite chemiluminescence. Soft tissue abnormalities were recorded under each condition. Any lesion with an uncertain clinical diagnosis was subjected to brush cytology sampling and, if necessary, scalpel biopsy. A total of 95 lesions were detected in 63 of the 100 patients and the majority (90%) of these were detected by the initial examination with incandescent light alone. Among the 86 lesions, 29 were considered to have an uncertain diagnosis by clinical examination. Following the acetic acid rinse, an additional six diagnosable lesions (linea alba) were noted as well as three lesions of uncertain clinical diagnosis. A total of 32 lesions were subjected to brush cytology and 2 “atypical” results were returned. Follow-up scalpel biopsy failed to detect evidence of premalignant or malignant change in either case.

A strong point of this study is that the random patient sample is appropriate to a screening protocol. A single examiner was responsible for each study patient, but it is unclear whether the same practitioner examined every patient in the study. Although not stated explicitly, the examinations were likely performed by a dental specialist trained in oral and maxillofacial surgery. Finally, the sparse data set provides no details about location or other physical characteristics of the lesions. The authors concluded that the use of an acetic acid pre-rinse might be of value for purposes of oral screening examinations. Chemiluminescent lighting, however, provided no additional benefit. The authors further commented that distracting highlights produced by the ViziLite system made tissue examination more difficult than with normal operatory lighting.

In summary, evidence that supports the use of reflective tissue fluorescence systems to aid in the detection of oral premalignant lesions is currently quite sparse. The published studies to date suffer from numerous experimental design issues, especially the critical comparison to the diagnostic gold standard (scalpel biopsy) in all cases. Furthermore, based upon the current suggested usage for these devices, it is unclear what added benefit they would provide to the practicing clinician. If a clinician is able to clinically identify a lesion, they are obligated to obtain a definitive diagnosis in order to direct the treatment of the patient’s lesion. Thus, subjective improvement of one’s ability to see a lesion would provide minimal diagnostic advantage to the practicing dentist or the patient, unless the test can also discriminate indolent lesions from those that are more biologically worrisome. On the other hand, some reports hint that this technique may help identify lesions that cannot be seen with incandescent light [56, 58, 59]. Well-controlled clinical trials are needed that specifically investigate the ability of these devices to detect precancerous lesions that are invisible by COE alone. If such discrimination can be confirmed, it would support the use of this technology as a true screening device.

B. VELscope (narrow-emission tissue fluorescence)

Approximately 30 years ago, it was observed that the autofluorescence of tissues (tissue fluorescence) could potentially be used for cancer detection. As such, there has been considerable interest in the technologies of both fluorescence imaging and spectroscopy in cancer screening for a number of anatomic sites including the oral cavity [62–81]. Fluorescence spectroscopy involves the exposure of tissues to various excitation wavelengths so that subtle differences between normal and abnormal tissues can be identified. Conversely, fluorescence imaging involves the exposure of tissue to a rather specific wavelength of light, which results in the autofluorescence of cellular fluorophores after excitation. The presence of cellular alterations will change the concentrations of fluorophores, which will affect the scattering and absorption of light in the tissue, thus resulting in changes in color that can be observed visually. A recent review highlighted the strengths and weaknesses of both fluorescence imaging and spectroscopy for oral cancer detection [82]. Based upon the available data, it found that both imaging and spectroscopy were excellent at distinguishing between normal and malignant tissue (case-finding). However, it found that imaging was likely to be far more useful in the detection of new lesions (screening) than spectroscopy because it was not feasible to scan the entire oral cavity using the small optical fibers required for spectroscopy.

The VELscope is a portable device that allows for direct visualization of the oral cavity and is being marketed for use in oral cancer screening. Under intense blue excitation light (400 to 460 nm) provided by the unit, normal oral mucosa emits a pale green autofluorescence when viewed through the selective (narrow-band) filter incorporated within the instrument handpiece. Proper filtration is critical, as the intensity of the reflected blue-white light makes it otherwise impossible to visualize the narrow autofluorescent signal. In contrast, abnormal or suspicious tissue exhibits decreased levels of normal autofluorescence and appears dark by comparison to the surrounding healthy tissue. Using this device, Lane et al, investigated the ability of the VELscope to identify precancerous or cancer lesions [83]. The study consisted of 44 patients who had a history of oral dysplasia or HNSCC. Following a COE, the oral cavity was screened using the VELscope to identify areas that demonstrated loss of autofluorescence. In addition, biopsies of the lesions were also obtained. Using histology as the gold standard, the device demonstrated a 98% sensitivity and a 100% specificity for discriminating dysplasia and cancers from normal oral mucosa. However, it should be noted that all of the dysplasias and/or carcinomas were observed using incandescent light alone. A major strength of this study is that the device was directly compared to the appropriate gold standard (scalpel biopsy) and the high degree of sensitivity and specificity is also encouraging. But the study has a number of weaknesses as well. First, the sample size (n=44) is relatively small. Importantly, the majority of the lesions included in the study appear to be Class I (suspicious) lesions.

A second study reported three non-consecutive representative cases in which clinically non evident lesions were identified using the VELscope [84]. Each of the cases reported demonstrated a potentially different use for this technology based upon the clinical setting: initial diagnosis of dysplasia, recurrent cancer, and second primary tumor. The major strength of this preliminary report is the demonstration that the VELscope may be capable of identifying lesions that cannot be seen using normal (incandescent) light. As previously discussed, the demonstration of this capability by any technology would be perceived as a major improvement in oral cancer screening. Another important strength of this study is that all three lesions appear to be Class II in nature. A major weakness of this report is that rather than being data reported from a controlled clinical trial, in which a certain number of consecutive patients are screened, the individual cases represent anecdotal observations.

A third study investigated the role of fluorescence visualization for the detection of surgical tumor margins for oral cancer when used in the operating room [85]. Twenty consecutive patients undergoing surgical excision for a previously diagnosed oral cancer were evaluated in the

operating room with the VELscope in order to document potential areas of loss of autofluorescence that might be indicative of cytologic and/or molecular changes indicative of premalignancy. Nineteen of twenty tumors demonstrated loss of autofluorescence that extended as much as 25 mm beyond the clinically evident tumors. Eighty-nine percent (32/36) of the biopsies taken from these areas demonstrated either carcinoma or dysplasia. Furthermore, molecular analysis using Loss of Heterozygosity (LOH) studies found loss of 3p and/or 9p, two markers that have been shown to be predictive of cancer progression, were present in 63% (12/19) of the lesions that had lost autofluorescence. The results of this small study suggest that VELscope may be useful in a true oral cancer screening mode by identifying lesions that cannot be seen by COE alone. However, a limitation of this study is that the Class II lesions identified within this work were found within the background of obvious Class I lesions. As such, we are unable to determine if VELscope is able to identify de novo Class II lesions.

In summary, while the preliminary results are promising, information regarding the ability of the VELscope to identify premalignant regions within Class II (innocuous) lesions or to reveal lesions otherwise visually undetectable is limited. Additional well-designed clinical trials are necessary to address the utility of this device in those settings.

VI. SUMMARY

Screening and early detection in populations at risk have been proposed to decrease both the morbidity and mortality associated with oral cancer [17, 18]. However, the visual detection of premalignant oral lesions has remained problematic throughout the world. This is in stark contrast to skin lesions such as melanoma, where visual screening has been shown to have sensitivity and specificity rates of 93 and 98 percent [15, 16]. One explanation for this discrepancy is that early lesions of oral cancer and precancer are often subtle and rarely demonstrate the clinical characteristics observed in advanced cases: ulceration, induration, pain, or associated cervical lymphadenopathy [86]. Besides their clinical subtlety, premalignant lesions are highly heterogeneous in their presentation and may mimic a variety of common benign or reactive conditions. Furthermore, there is a growing realization that some premalignant and early cancerous lesions are not readily detectable to the naked eye [38]. As such, additional screening aids for oral cancer are desperately needed.

Fortunately, there has been a dramatic increase in the development of potential oral cancer screening or case-finding tools in the last decade. Each of them may hold promise in selected clinical settings. Unfortunately, no technique or technology to date has provided definitive evidence to suggest that it improves the sensitivity or specificity of oral cancer screening beyond COE alone. As discussed above, many of the studies have design flaws. Many studies that have been performed using these diagnostic devices also suffer from the fact that they are being employed in a “case-finding” fashion, rather than as true screening tools. That is to say, they are being used to aid in the diagnosis of a lesion that has already been identified by the naked eye. Several of the technologies (ViziLite Plus, MicroLux DL, toluidine blue and VELscope) may be useful in a true screening fashion. Yet, there is currently no hard data to support the contention that these technologies can help the clinician to identify premalignant lesions before they are detectable by COE alone. Nevertheless, studies to determine their utility in this setting are anticipated in the near future.

Regardless of the outcome of these studies, new technology and even its attendant marketing has clearly made a positive impact on the field of dentistry by encouraging clinicians to more routinely perform thorough oral cancer exams. Until recently, surveys had consistently demonstrated a limited understanding of proper oral cancer screening and diagnosis among the dental community [87–91]. Preliminary results from a recent oral cancer awareness campaign

in the United States, however, suggest that intensive, well-designed and prolonged attempts to educate the dental community as well as their patients may increase overall awareness about the disease [92]. To capitalize on this increasing awareness, well-designed clinical studies are needed to help dental scientists and clinicians assess the various new and evolving diagnostic aids for oral cancer and precancerous lesions. Scientific journals and their readers must look to ensure that issues of validity, comparison to the gold standard of histopathologic analysis, appropriateness of patient population, use of proper study clinicians, specificity and potential for replication are satisfied. Improving oral cancer detection and diagnosis have long been major challenges facing both dental and medical providers around the globe. Combined with an increased public awareness of oral cancer in general, robust diagnostic aids that allow clinicians to detect lesions unseen by conventional examination techniques should help more affected patients become long-term survivors of this challenging disease.

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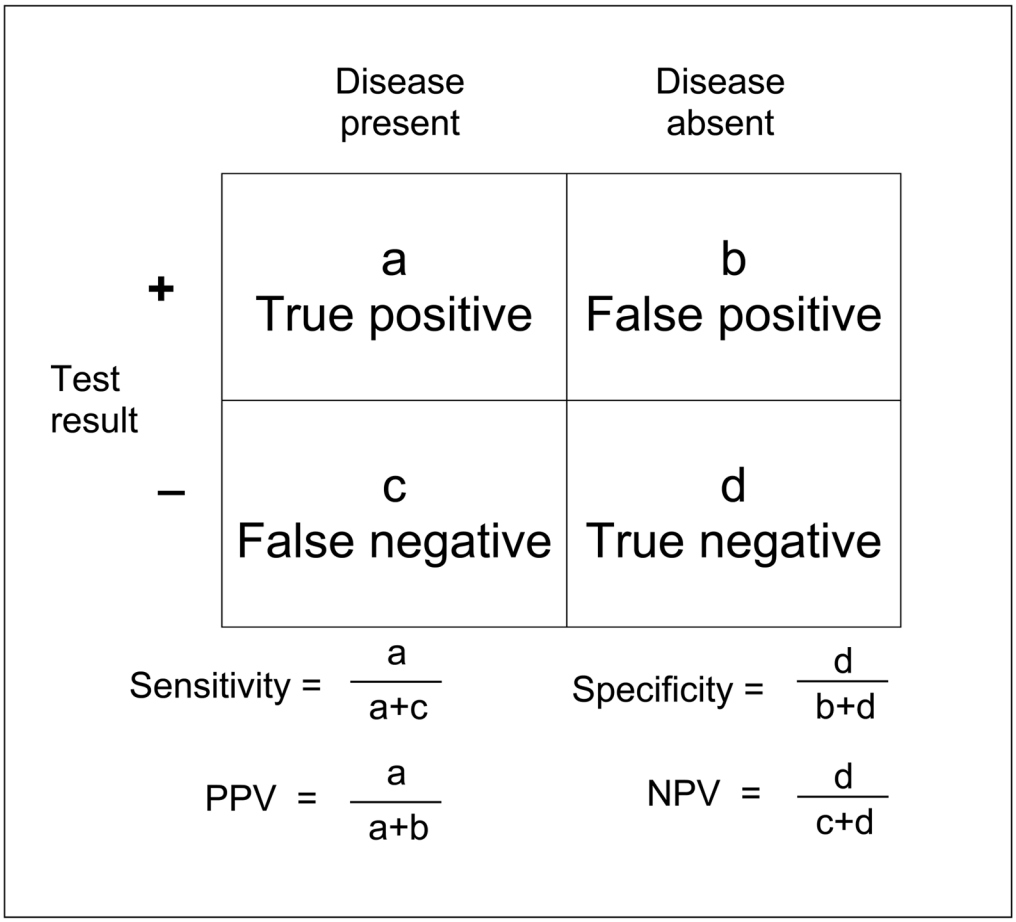

Figure 1.
A standard 2×2 table for the calculation of sensitivity and specificity

Table 1

Criteria for the implementation of a screening programme[7]

- The disease must be an important health problem
- An accepted treatment must be available for patients with recognised disease
- Facilities for diagnosis and treatment must be available
- There must be a recognisable latent or early symptomatic stage
- A suitable test must be available
- The test should be acceptable to the population
- The natural history of the condition should be adequately understood
- There should be an agreed policy on whom to treat as patients
- The screening programme should be (cost)-effective
- The screening process should be a continuing process and not a ‘once and for all’ project

Table 2

Characteristics of a good screening test

A screening test should:	
1	be simple, safe and acceptable to the public
2	detect disease early in its natural history
3	preferentially detect those lesions which are likely to progress
4	detect lesions which are treatable or where an intervention will prevent progression
5	have a high positive predictive value and low false negatives (high sensitivity)

Table 3

Questions for assessing studies of oral cancer screening or diagnostic tests

Does the study:	
1	provide a valid basis of comparison (use of appropriate gold standard)?
2	provide consistent, blinded test comparison to the appropriate gold standard?
3	examine a patient population appropriate to the purpose of the test?
4	use examiners representative of the target or primary clinical providers of the test?
5	show evidence that the test can distinguish cancer/precancer from other conditions (specificity)?
6	provide sufficient detail about the test, its performance and patient cohort to permit replication by others?

Table 4

Screening and case-finding aids to diagnosis of oral cancer and precancer

Standard screening test

- Conventional oral examination (COE)

Established diagnostic adjuncts

- Oral cytology
- Toluidine Blue (tolonium chloride)

Recently available light detection systems

- ViziLite, ViziLite Plus
- MicroLux DL
- VELscope

Table 5

Problems with studies of toluidine blue

- No studies carried out in a primary care environment
- Data from studies in secondary care are not necessarily applicable to general population
- No randomized controlled trials
- Some studies only include carcinomas or dysplasia and some include both
- Histological diagnosis is rarely used as a gold standard
- Methods vary – single rinse, double rinse, ‘painting’
- Confusion over inclusion of equivocal (pale) staining as positive or negative.