

# Sensitivity and specificity of OraScan® toluidine blue mouthrinse in the detection of oral cancer and precancer

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The efficacy of 1% toluidine blue in the identification of oral malignancies and potentially malignant oral lesions was evaluated among a group of Asian patients ( $n=102$ ) with undiagnosed oral mucosal lesions and conditions ( $n=145$ ). The trial, utilising a ready-to-use kit, was controlled by histopathologic evaluation of a total of 87 dye-retained or dye-negative lesions. Eighteen oral carcinomas all retained the dye and there were no false negatives, yielding a test sensitivity of 100%. Eight of 39 oral epithelial dysplasias were toluidine blue-negative, giving a false negative rate of 20.5% and a sensitivity of 79.5% for oral epithelial dysplasias. The specificity of the technique was low (62%). Five dysplastic lesions were detected solely by the kit and this suggests that the method is valuable for surveillance of high-risk subjects in addition to its remarkable sensitivity in the detection of invasive carcinoma.

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During the 1960s, the suggestion was made that toluidine blue O (TB) may stain malignant epithelia of mucous membranes *in vivo*, while normal tissues fail to retain the dye (1). Since then TB has been used in a number of clinical studies designed to differentiate neoplastic, dysplastic and benign lesions of the oral cavity (2–18) and of the uterine cervix (19). Vital staining of the oral epithelium has also been suggested as a means of surveillance in patients who are at risk of developing oral cancer (20) and for those who have had a confirmed neoplasm in other parts of the aerodigestive tract (11). The results and accuracy of these studies have been variable, with differing false-positive and false-negative rates (21).

Although the precise mechanism whereby TB preferentially stains neoplasms has not been elucidated, it is likely that the technique detects relative rather than absolute differences between normal and malignant cells and tissues (22). Most studies for the

detection of oral malignancies in the past have used TB as a 1% or 2% oral rinse or an application either in aqueous form, as a weak acid solution, or of undefined formulation. Only about 5% of the dye by weight is retained in the oral cavity following expectoration.

Both positive and negative results have been reported using TB in the Ames bacterial test with reference to its genotoxicity (23–25). *In vivo* studies on hamster cheek pouch (26) suggest that TB was not itself carcinogenic in this model, nor did it act as a co-carcinogen or promoter when administered with DMBA. On the basis of critical analysis of toxicological, pharmacodynamic, pharmacokinetic and clinical data of TB preparations, the present formulation has been approved by regulatory bodies in the UK, Canada and Australia, at least, to our current knowledge. The dye is considered nontoxic to humans at these levels.

Extensive studies conducted in the

United States (2, 4, 15–17) on the evaluation of TB as a diagnostic adjunct in the detection of oral lesions have primarily related to invasive oral carcinomas, carcinoma *in situ* or asymptomatic early oral carcinomas. It is said that TB indicates the site most likely to reflect serious pathological changes (23). The major objective of the present study was to test the reliability of 1% TB in differentiating invasive and dysplastic lesions from benign keratoses in the oral cavity and thereby assess the sensitivity and specificity of the test separately for oral malignancies and dysplastic lesions. We also undertook microscopic evaluation of negatively and positively staining zones of the same oral lesion on discrete biopsies of such areas following *in vivo* staining. Toluidine blue is now commercially available in a ready-to-use kit (OraScan®; Germiphene, Ontario, Canada) as flavoured solutions comprising 1% TB as the staining rinse and 1% acetic acid for use as both pre- and post-rinses. Ora-

Table 1. The method of using OraScan®

The oral cavity is examined and the location, size, morphology and surface characteristics of suspect lesions are recorded and photographed. The patient rinses mouth for 20 s with pre/post-rinse solution followed by mouthrinse with water. Patient rinses mouth and gargles with 10 ml of staining rinse solution (toluidine blue O) for 1 min after which it is expectorated. Mouth rinsed for 20 s with pre/post-rinse solution followed by a further water rinse. Oral cavity is examined and the location, size, morphology and surface characteristics of sites stained or unstained recorded and photographed. (The purpose of the pre-rinse is to remove excess saliva and provide a consistent oral environment. The post-rinse reduces the overall background level of staining and facilitates identification of suspect lesions).

Table 2. Clinical characteristics of oral lesions and the concordance of the dye result

Clinical finding	Toluidine blue			
	n	+	-	+/-
Clinical carcinoma	11	11	0	0
White patches	29	9	16	4
Red/speckled/nodular	66	37	20	9
Lichen planus (keratotic)	6	0	5	1
Lichen planus (ulcerative)	12	7	2	3
Other benign	21	7	9	5
Total	145	71	52	22

Scan® was used as the test substance in the present study.

## Material and methods

The study was conducted in August 1993 in 7 centres in Asia among a population of Sri Lankan and Pakistani subjects who consented to participate in the programme. The consultant dental surgeon in each centre approved the appropriateness of each included case.

All patients had been referred to, or had attended, the specialist centres with unconfirmed oral mucosal lesions. 102 patients (73 men; 29 women; mean age  $60 \pm 15$  years) with white/red/nodular/exophytic/atrophic and ulcerative oral lesions were enlisted. 84 subjects were regular betel quid chewers and 49 included tobacco in their quid mixture. 28 smoked tobacco in some form and 4 reported Niswari use. 13 subjects were known to misuse alcohol. None had previously received regular dental care.

Following a systematic oral examination, clinically visible oral lesions were comprehensively charted using WHO criteria (28) with both examiners agreeing on the location, morphology and the visual clinical extent of the spread of the lesion. All lesions were photographed to scale. The rinse protocol using OraScan® was as per manufacturer's instructions (Table 1) except that the study was limited to a single TB rinse per person and not repeated 10-

14 days later. Sites stained by the dye were charted and re-photographed. 86 clinically detected lesions, dye-retained or not, were biopsied. Ten patients had two biopsies taken from separate areas of their lesions corresponding to the dye result, one from a stain-positive area and another from a stain-negative site.

Tissues were bisected so that one portion of the biopsy could be fixed in buffered formal saline and the other in 70% alcohol; specimens were then transported to London. Following processing in wax, 6-μm sections were cut at two levels and stained with H&E and PAS. Microscopy diagnosis and, where relevant, degree of dysplasia were recorded independently by two experienced histopathologists blinded to the dye results. Dysplasias were graded as mild, moderate or severe, using the intensity of the signs specified by SMITH & PRINDBORG (29). When there was disagreement, concordance was reached following consultation.

## Results

Among the 102 patients enrolled, 145 oral mucosal lesions were clinically identified and photographed. The classification of oral lesions entered to the trial is given in Table 2. Thirteen subjects were detected on clinical criteria (30) to have concurrent oral submucous fibrosis as an underlying premalignant condition. Seventy-one oral lesions and

or conditions stained positive, 52 did not retain the dye and in 22 the staining was equivocal. None of the subjects reported any undesirable side effects during or following the rinse programme. Eleven clinically suspected overt carcinomas stained dark royal blue. An example of a palatal ulcer is illustrated in Fig. 1. In all carcinomas the entire lesion that was clinically evident stained solidly with clear margination. White/red/nodular lesions stained variably with a higher proportion (37/66) of red/nodular lesions retaining the stain compared to homogenous white lesions (9/29) (Fig. 2-4). The staining appearance of these white/red mucosal lesions varied from dark royal blue to a patchy/stippled appearance (Fig. 3) and, at times, to a uniform pale stain, which seemed to affect deeper tissues visible through translucent mucosa. Of the 18 oral lichen planus cases included in the study, half stained positive, all with ulcerative components within the lesion. Seven of 21 other benign mucosal lesions (predominantly fibro-epithelial polyps) unrelated to the natural history of oral cancer/precancer also retained the dye. None of these benign lesions, however, developed the dark blue stain exhibited by other and more sinister lesions.

In 12 patients, clinically "normal" sites that had no macroscopical evidence of any morphological alteration retained the dye. Several of these patients had underlying oral submucous fibrosis or oral lichen planus, suggesting that the dye was demarcating areas of "field change" in these patients (Fig. 5).

Eighty-seven suspicious lesions, dye-retained or not, were subjected to biopsy evaluation. Eleven clinically obvious cancers and 7 other distinctive white/red lesions that were not clinically overtly malignant were confirmed by microscopy to be invasive carcinomas. All these histopathologically confirmed malignancies ( $n=18$ ) demonstrated stain uptake, indicating that the rinse procedure was 100% sensitive for the detection of invasive carcinoma. There were no false negatives among the carcinomas in this series (Table 3). There were no invasive lesions in any of the stain-negative biopsies examined by histology ( $n=28$ ).

Among the 95 white/red lesions subjected to biopsy, microscopic evaluation revealed 39 lesions to exhibit dysplastic features. Assessment of clinical charts supported by photographic records showed that 29/39 of these lesions were

Table 3. Staining characteristics of oral lesions by histology

	Toluidine blue			
	n	+	-	+/-
Invasive carcinoma	18	18	0	0
Dysplasia	39	29	8	2
Keratoses/hyperplasia	29	11	13	5
Total	86	58	21	7

Sensitivity: Carcinoma=100%; Dysplasia=79.5%

Specificity: Precancer=62%

stain-positive. Unlike cases with carcinomas, these 29 positive lesions had a variable stain uptake ranging from solid blue to speckled appearances. Eight lesions with dysplasia were stained negative (Fig. 4). Excluding the two equivocal cases, the false-negative rate for oral epithelial dysplasias was 22%; the sensitivity of the dye test in this group was therefore 78%. A further 29 cases in the series clinically presented as white lesions or small growths with no histological dysplastic features and were entirely benign or reactive on microscopic evaluation; 11 of these lesions were stain-positive, giving a false-positive rate of 38% for this mixed group of oral lesions.

In 12 patients the dye was retained in locations that did not show any discrete morphological alterations, indicating absence of any overt mucosal lesion, though 4 of these patients had underlying oral submucous fibrosis and mucosal atrophy. Five of these biopsies demonstrated microscopic dysplasia to a varying degree (1 severe; 3 moderate; 1 mild).

Most of the white/red lesions which were large and distributed over a wide area of the oral cavity resulted, when rinsed with OraScan®, in a mixture of stain characteristics; some areas retained the dye, while adjacent morphologically altered sites decolorised with the post-rinse. In 10 such cases biopsies were taken from both a stain-positive and a stain-negative site. The distribution of dysplastic features in the pair of biopsies from the same lesion was recorded blind and compared with staining characteristics. The presence or absence of dysplasia in each pair of biopsies was similar irrespective of surface-staining characteristics. In 5 pairs no dysplasia was noted in either biopsy, 4 had dysplasia in both pairs, one was a carcinoma in the biopsy from the stained area while the adjoining stain-negative area was dysplastic.

## Discussion

We report here the diagnostic potential of a simple *in vivo* procedure – rinsing with toluidine blue dye – to delineate areas suspicious of malignancy or dysplasia. The dye is now commercially available as a ready-to-use kit. This is the first report to evaluate the use of OraScan® in its present formulation. The study was conducted on subjects with unconfirmed suspicious mucosal lesions, in a prospective trial to test its diagnostic accuracy. Seventeen clinical trials have so far evaluated TB as a diagnostic adjunct for suspicious mucosal lesions in the lip vermillion or intraoral sites (2–18). These studies have shown that TB has a high sensitivity in the detection of oral malignancies. In general, a well-designed study for any diagnostic test should include normal and abnormal subjects to allow assessment of the probability of the test to generate a positive or a negative result in both the presence or absence of the disease. Well-defined objective criteria are needed to score a positive test result and to eliminate equivocal results. Furthermore, during trials the test result should be validated by an established method such as biopsy. Critical review of the studies conducted so far suggests several faults in their study design. Some of these earlier investigations have not been properly controlled in that the negative stain result of a lesion has not been validated by biopsy (2, 4–6, 8, 11, 14). Most trials have been conducted on preselected subjects by inclusion of large groups with clinically suspected malignancy and a few cases with precancer. So far no studies are reported which include a wide range of oral lesions important in the differential diagnosis such as oral keratosis, lichen planus and oral submucous fibrosis.

The present study was controlled with biopsy of the majority (87/145) of the lesions, including at least one signifi-

cant site from most subjects, following *in vivo* staining and with histopathological evaluation conducted under blind conditions by two observers agreeing on both clinical and microscopical assessments. It was not possible to randomise the study by including normal subjects, as it would not be ethical to perform confirmatory biopsies in negatively stained normal mucosa. We therefore assessed the specificity of the test by including benign oral lesions ( $n=21$ ) and also subjecting to biopsy all dye-negative oral lesions and any equivocal sites to assess the false-negative rate of the test result. Such a trial design enables assessment of the technique as a screening procedure.

## Sensitivity

All 11 clinically suspected carcinomas (Table 2) and 7 other unsuspected cancers confirmed by histopathology (Table 3; Total  $n=18$ ) were stain-positive. There were no false-negative results among clinically suspected malignancies nor were there invasive features in stain-negative sites examined by biopsy. The sensitivity of the test result was truly 100%. We note that 18 cases we examined is far too small to generalize this finding, particularly to other populations outside the Indian subcontinent. In studies reported so far, the sensitivity of the TB test for the detection of a malignant lesion ranges from 86% to 100%, except in one study (7) which recorded a high proportion of false negatives (3/5). The reasons why some cancers did not take up the stain in their study must be examined in detail. However, it is worthwhile to note that the latter study used only 5 ml of the dye for rinsing, and this may not have reached all intraoral sites. The early studies (2, 3) documented that carcinomas and carcinomas *in situ* retain a dark blue stain following rinsing with TB. Our observations confirm this. The clinical impression gained was that a dark royal blue coloration of the mucosa which is resistant to decolorisation by 1% acetic acid post-rinse is strongly indicative of neoplastic disease and warrants immediate further investigation. Some of these, however, on histopathology may prove to be noninvasive lesions, but the majority demonstrate dysplasia; thus false-positives are likely to occur if this test interpretation is exclusively used for the detection of malignancy.

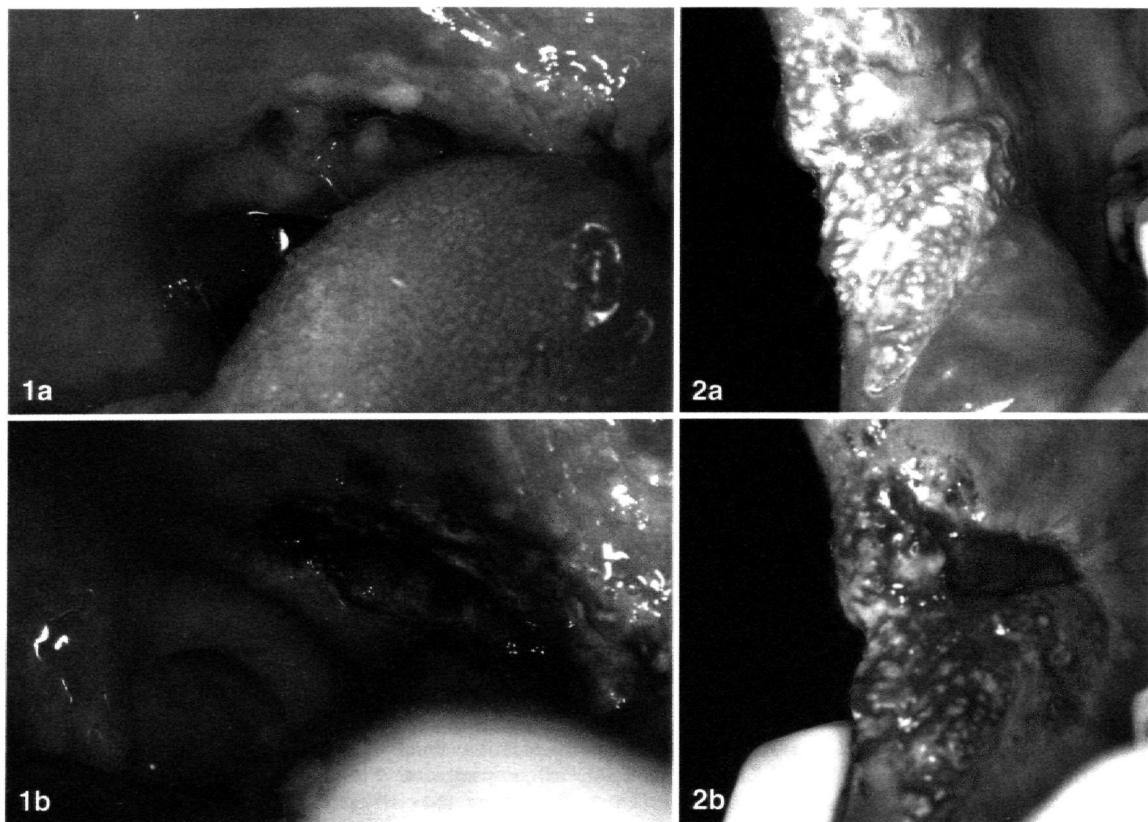


Fig. 1. Carcinoma of soft palate, pre- (A) and post- (B) staining with toluidine blue. Note clear margination of the neoplasm by the dye.

Fig. 2. A nodular elevated commissural lesion (A). This is well demarcated by the stain (B), with the ulcerated zone taking a deeper blue stain. Histology - microinvasive carcinoma.

Almost all cancers detected in this study showed sharp margination of stain (Fig. 1b). We only performed incisional biopsies to establish diagnoses, not to examine representative margins, and therefore cannot comment on the value of the rinse in delineating the full extent of the neoplasms. Morphologically normal mucosa with infiltrating epithelium underneath may not retain the dye, and the use of this technique to determine excision margins may be unreliable. A well-designed study using excision specimens is required to confirm the role of TB in determining the excision margins of oral cancer.

The variable ability of the TB test to identify premalignant lesions is clear from previous studies. Unlike malignan-

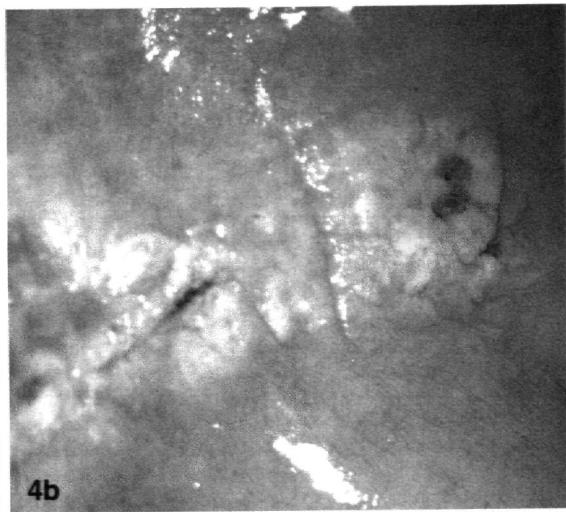
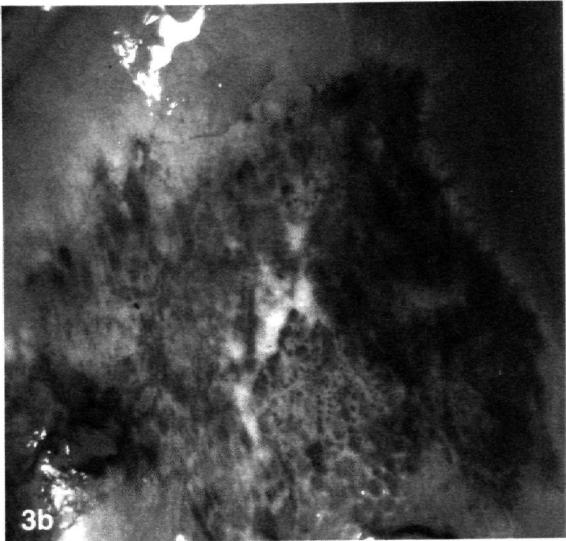
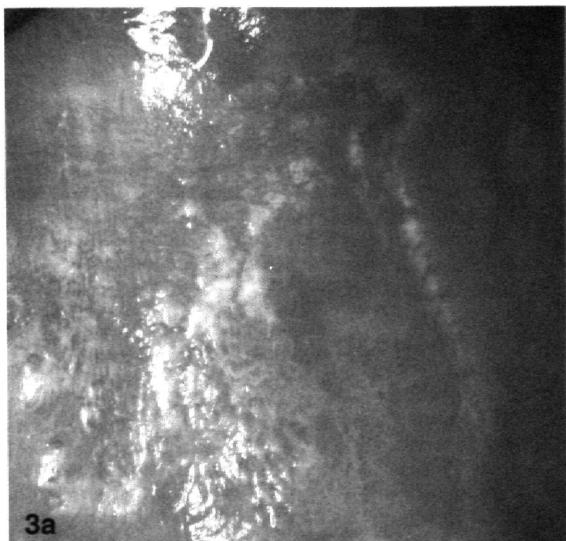
cies, precancerous lesions yield a variety of colour reactions following staining. Well-defined evaluation criteria are not yet established. For example, REDDY *et al.* (9) arbitrarily defined a positive result as an area staining greater than 2 mm diameter. In 160/1190 patients investigated by VAHIDY *et al.* (8) the test result was reported to be doubtful. In our study among the lesions extensive in size, confluent staining was not observed over the whole range of the morphologically altered mucosa.

Only 9/29 white patches and 37/66 red/speckled/nodular lesions retained the dye, confirming that the dye result was inconsistent with the clinical judgement. This may be peculiar to the type of keratoses associated with the tobacco/betel quid chewing habits among Asians. Many of these lesions were not dysplastic. Hyperkeratotic lesions among Asian subjects are reported by others to reject the stain (8). It is noteworthy that, in two earlier studies on white Caucasian patients, all premalignant and dysplastic lesions retained the dye, giving a 100% sensitivity (10, 12).

Among the lesions that had microscopic dysplasia in our series, 29/39 (74%) had stained positive and two had a doubtful result. By TB test alone, 8 dysplasias would have been missed. MASHBERG (17), following extensive studies in the United States claimed that lesions with limited dysplasia or atypia do not stain with TB consistently. Our results are in agreement with this.

Fig. 3-4. Pre- (A) and post- (B) staining of white and red oral lesions. Fig. 3 A-B. An erythroleukoplakia of left commissure and buccal mucosa. Patchy stippled appearance following staining; erythroplakic zone has taken a deeper stain. Fig. 4 A-B. A leukoplakia that did not retain the dye; histology showed mild dysplasia.

Fig. 5. Oral lichen planus. Most keratotic striae have not retained the dye. Clinically "normal" mucosa below the lichenoid zone shows dye uptake.



### Specificity

Among the 18 lichen planus cases studied, 7 cases, all with ulcerative components, retained the dye. This is consistent with earlier observations that ulcerated lesions will stain positively (5). It has been reported previously that keratotic lichen planus does not retain dye (12).

Twenty-nine benign keratoses, hyperplastic growths and ulcerative lesions, none of which had dysplasia, were included in this study. Of these, 11 retained the dye, yielding a specificity of 62%. Previous studies have recorded a range of values for specificity of the dye to differentiate benign lesions, and this may result from the wide range of clinical lesions included in this category in the trial designs. The lowest specificity recorded so far is 50% (7). Other studies report a range from 63%–94% following a single rinse or application of the TB. Trials that include a second rinse or application, allowing a period of days or weeks for resolution of transient inflammatory lesions, generally record a higher specificity (88%–99%) (14–18).

For practical reasons, we did not repeat the dye test at these centres. Previous studies in South Asia have shown poor compliance for attendance at follow-up of asymptomatic oral disease (31). Had the rinse been repeated 2 weeks later, as recommended by the OraScan manufacturer, the specificity of the test result may have improved. On the other hand many erosions/ulcerations in this population are due to chronic trauma and would not resolve in this time. Nevertheless, a specificity of 62% as reported here should not inhibit the recommendation of TB as a diagnostic test in experienced hands. Several other diagnostic tests routinely used in screening (for example, alpha fetoprotein test for neural tube defects) are known to yield a specificity close to that found in the present study.

It is claimed that the dye test can assist in the selection of the optimal site for a diagnostic biopsy (13, 27). We attempted to assess this by taking a pair of biopsies from positively and negatively stained zones of the same lesion. Ten lesions were examined and the results were equivocal. Further studies are required.

Review of all the trials of oral TB conducted so far (2–18) reveals a small number of carcinomas which were detected by stain uptake in the absence of any clinical signs in non-diseased in-

dividuals. One of these was a lip lesion among a random group of 265 patients presenting for routine dental care (14) and others were intraoral neoplasms. Five were reported by MASHBERG (17) among 179 subjects in the United States and 3 in a randomly selected Indian population (6). In our study, 12 patients in whom we did not visually observe a distinct oral lesion retained the dye in a discrete fashion and on biopsy evaluation 5 of these demonstrated dysplasia. This finding alone may not justify stain-testing healthy people with clinically normal oral mucosa, particularly in low incidence countries. The low specificity of the test further dampens its reliability. It may, however, have some merit in application to known high-risk populations to enable earlier detection of asymptomatic disease. Future trials are justified to test the feasibility of mouthrinsing with TB to detect dysplasia/malignancy at follow-up/regular examinations among patients with known mucosal conditions or those at risk.

We confirm that oral squamous cell carcinomas in this population can be detected with a 100% sensitivity using the OraScan® rinse with 1% TB as the active ingredient. Development of a dark royal blue stain following rinsing arouses a strong suspicion that one is dealing with a malignant lesion and a biopsy is mandatory for confirmation. Some oral premalignant lesions with dysplasia may, however, be missed by the dye test alone (false-negative rate 20.5%). This may be due to lack of objective criteria for evaluation of the stain uptake. The exact mechanisms by which the dye differentially stains malignant or dysplastic tissues remains unknown. Until these issues are resolved, routine staining of dysplastic lesions is of questionable value and, if undertaken, should be followed up with a second rinse to reduce false-positive results. OraScan may be used as an initial screening test for "high-risk countries" in which personnel may not be available for clinical detection of oral cancer by mass examinations. The feasibility of the technique as a screening tool among such populations needs further evaluation. In experienced hands, detection of oral malignancies can, however, be achieved with good marginal demarcation using OraScan® enabling intervention methods to be adopted earlier for this disease which carries a high rate of morbidity and mortality.

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