The clinical effectiveness of toluidine blue dye as an adjunct to oral cancer screening in general dental practice

A West Midlands Development and Evaluation Service Report

Authors: Margaret Gray[1], Lisa Gold[2], Amanda Burls[3] & Karen Elley[4]

[1]Walsall Health Authority
27-31 Lichfield Street
Walsall
WS1 1TE

[2]Health Services Management Centre
[3]Department of Public Health and Epidemiology
University of Birmingham
Edgbaston
Birmingham B15 2TT

[4]Sandwell Health Authority
Kingston House
438 High Street
West Bromwich
B70 9LD

Correspondence to: Margaret Gray
3, Sycamore Way
Market Bosworth
Nuneaton
Warwickshire
CV13 0LU

ISBN No. 0704421755

Question addressed by this review:

How effective and cost-effective is using toluidine blue dye as an adjunct to oral cancer screening in primary care?

Conclusion

Currently there is no evidence to suggest that toluidine blue is a cost-effective method of picking up oral cancers in a primary care setting. Given the large number of people that will have false positive rates for a first positive test and even a double positive test, the harm of using it in terms of anxiety could well outweigh the benefits in terms of additional cancers detected. Further research in the use of toluidine blue as an adjunct to visual examination in general practice is unlikely to be an efficient use of resources.

In primary care, however, the findings suggest there may be some benefit from the use of toluidine blue as an adjunct to clinical examination in the detection of oral cancer in high-risk populations. These results were not generalisable to general dental practice. The primary care study was undertaken to assess the acceptability of toluidine blue to the patient and was too small to detect any significant effect of the use of toluidine blue as an adjunct to oral cancer screening in general dental practice.

EXPIRY DATE: 2005

West Midlands Development & Evaluation Service

The West Midlands Development and Evaluation Service (DES) produce rapid systematic reviews about the effectiveness of healthcare interventions and technologies, in response to requests from West Midlands Health Authorities or the HTA programme. Reviews usually take 3-6 months and aim to give a timely and accurate analysis of the quality, strength and direction of the available evidence, generating an economic analysis (where possible a cost-utility analysis) of the intervention.

About InterTASC

West Midlands DES is a member of InterTASC which is a national collaboration with three other units who do rapid reviews: the Trent Working Group on Acute Purchasing; the Wessex Institute for Health Research and Development; York Centre for Reviews and Dissemination. The aim of InterTASC is to share the work on reviewing the effectiveness and cost-effectiveness of health care interventions in order to avoid unnecessary duplication and improve the peer reviewing and quality control of reports.

Contribution of Authors

Margaret Gray undertook the collection and collation of evidence for this review. Lisa Gold and Amanda Burls gave advice on the formulation of the question and overall process of the review, helped with some of the writing and structuring of the report and read and commented on the draft report. Karen Elley provided duplicate data extraction and support.

Conflicts of Interest

This work has been undertaken by people funded by the NHS. The authors have received no funding from any sponsor in this work.

West Midlands Regional Evaluation Panel Recommendation:

The recommendation for how effective and cost-effective the use of toluidine blue dye is as an adjunct to oral cancer screening in primary care is:

Not recommended

Anticipated expiry date: 2005

- This report was completed in December 2000
- The searches were completed in January 1999
- There are no known studies or clinical trials in progress of toluidine blue dye as a screening test for oral cancer in a general practice setting. Further research is not recommended. It is unlikely that the conclusions of this report will alter during the next five years. This review should be updated in five years time if toluidine blue is still being marketed as a screening tool for oral cancer in primary care.

CONTENTS

Abbreviations

WHO	World Health Organisation
ICD	International classification of diseases
FDI	Federation Dentaire International
BDA	British Dental Association
SCC	Squamous cell carcinoma
TB	Toluidine Blue dye

Definition of terms

Leukoplakia

A white patch or plague that cannot be rubbed off and cannot be characterised clinically or histologically as any other disease.

Erythroplakia

A red patch that cannot be characterised clinically or histologically as any other disease.

Keratosis

Keratosis is characterised by erosion and white and brown crusting of the vermilion border of the lower lip. It is caused by exposure to ultra-violet light particularly from strong sunlight.

Lichen Planus

Lichen Planus appears as atrophy, erosion or superficial ulceration of the oral mucosa in association with white papules and keratotic striae.

Dysplasia

Disorderly maturation and disturbed cell proliferation in the oral mucosa.

Apthous ulceration

Small benign ulcers that normally heal within two weeks.

Squamous cell carcinoma

A malignant neoplasm of the oral mucosa which can invade and destroy adjacent structures and can spread to nearby lymph nodes and distant sites.

Summary

Objective

To review the usefulness of toluidine blue dye as a screening tool for the detection of oral cancer in primary care.

Methods

The literature on the use of toluidine blue dye as a screening tool to detect oral cancer was sought using structured searches on the internet, Medline and Pub-Med databases, and by scrutiny of the references of all identified studies. All study designs were included provided that the diagnosis of oral cancer was confirmed using the gold standard test of biopsy and histological examination. Papers which only reviewed other studies, described the technique, offered only expert opinion, or described interim results of included studies were excluded. The quality of these studies was assessed and relevant data extracted.

Results of searches

Studies found:
Seventy-five papers were initially identified, the majority of these (64) did not satisfy the inclusion criteria and were excluded. No studies of the use of toluidine blue in primary care were found in the published literature, one unpublished study was found which assessed its acceptability but not its effectiveness. Case series reports of the use of toluidine blue in secondary care show variable results in terms of the sensitivity and specificity of the test.

Quality:
In general, the quality of these included studies is poor. Only one study reported the main outcome of interest: the detection of cancerous lesions not apparent on visual examination. One further study noted that some cancers were detected this way but gave no numerical information.

Effectiveness:
In primary care, however, the findings suggest there may be some benefit from the use of toluidine blue as an adjunct to clinical examination in the detection of oral cancer in high-risk populations. These results were not generalisable to general dental practice. The primary care study was undertaken to assess the acceptability of toluidine blue to the patient and was too small to detect any significant effect of the use of toluidine blue as an adjunct to oral cancer screening in general dental practice.

Conclusion

There is no evidence to suggest that toluidine blue is a cost-effective method of picking up oral cancers in a primary care setting.

Given the large number of people that will have false positive rates for a first positive test and even a double positive test, the harm of using it in terms of anxiety could well outweigh the benefits in terms

of additional cancers detected. Further research in the use of toluidine blue as an adjunct to visual examination in general practice is unlikely to be an efficient use of resources.

1 Aim of the Review

To review systematically the literature on the clinical effectiveness of toluidine blue dye (tolonium chloride) as an adjunct to oral cancer screening in general dental practice and to determine its cost effectiveness. The review addresses the following specific question:

How effective and cost effective is the use of toluidine blue dye in increasing the detection rate of oral squamous cell carcinoma in general dental practice?

2 Background

2.1 Description of the underlying health problem

2.1.1 Oral Cancer

The definition of 'oral cancer' used in this report is that defined by the British Dental Association using the WHO International Classification of Diseases (ICD).[1] It includes cancers of the lip (code 141), tongue (code 142), gum (code 143), floor of mouth (code 144), other unspecified parts of the mouth (code 145), oropharynx (code 146), hypopharynx (code 148) and other and ill-defined sites within lip, oral cavity and pharynx (code 149), but excludes cancers of the salivary glands and the nasopharynx.

Cancers of the mouth and lips, if undetected and untreated, can invade and destroy adjacent structures and spread to lymph nodes in the neck and to distant sites. Metastasis is via the lymphatics and the blood stream. Approximately 95% of oral cancers are squamous cell carcinomas.[2] The most common sites of cancer in the oral cavity are the lower lip, the lateral margin of the tongue and the floor of the mouth. Cancers of the mouth can present as ulcers, as red or white patches, as lumps or as fissuring; they may develop in an area of previously healthy epithelium or from a precancerous lesion.[3]

Other lesions which occur in the mouth are either benign or are thought to be precancerous. Included in the benign lesions are keratosis, apthous ulceration, lichen planus,[4] traumatic ulceration[5] and fibroepithelial polyps.[6] The main precancerous lesions are erythroplasias (red velvety lesions) and leukoplakias (white patches). Erythroplasias are rare, however, around 75-90% are reported to be carcinoma, carcinoma in situ or severe dysplasia on biopsy.[3] The prevalence of leukoplakias is less than 3-4% in the general population, clinically they fall into two main groups, the majority are uniformly white patches (homogenous leukoplakias) which have a low potential for malignant change. The more sinister leukoplakias are the nodular or speckled leukoplakias. Overall it is reported that over 10 years 3-6% of leukoplakias undergo malignant transformation and about 15% regress clinically.[3]

2.1.2 Prevalence and incidence of oral cancer

World wide there are considerable variations in the incidence of oral cancer.[7] In the UK, around 2000 new cases are reported to cancer registries each year and around 900 deaths,[8] giving an incidence rate of approximately 3.4 per 100,000 population.[5] It is, however, likely that the true incidence of the condition in the UK is higher than this as cancer registration in the UK is voluntary and the methods of registration, completeness and accuracy of registration vary between registries.[9] In an oral cancer screening programme undertaken in a primary care setting in the UK the detection rate of malignant or potentially malignant lesions was 0.5%.

Table 1 - Results of the oral cancer screening programme[38]

	Clinical examination
Population	Staff aged over 40 years working for Marks & Spencer plc.
Number of people screened	11,970
Number of visually observable lesions detected at initial screen	160
Number of patients with persistent lesion at second screen (referred to secondary care)	116
Number of lesions confirmed as malignant or potentially malignant	64

In the UK, both the incidence rate and the mortality rate for oral cancer are higher in Scotland than in England and Wales. Within England and Wales there is a north-south gradient for oral cancer with the higher rates in the north.[9] Approximately twice as many reported cases of oral cancer are in males than in females. The majority of cases (85%) of oral cancer are in people aged over 50 years.[9] The incidence of and mortality from oral cancer fell from the turn of the century until about 1970, particularly in males. Since 1970, the incidence of cancers of the tongue, floor of mouth and other ill defined oropharyngeal sites has been rising for both males and females.[9] The same trend is seen across Western Europe and North America.[10]

The high risk factors associated with oral cancer among white Caucasian populations are primarily tobacco and alcohol.[11] The risk is increased with increasing frequency of smoking and is proportional to the amount of alcohol consumed.[12] In Western Europe the majority of tobacco-related oral cancers are due to smoking, however, globally and particularly in high risk countries of Southern Asia chewing tobacco is responsible for the majority of cases. Betel-quid chewing has been linked with oral cancer in the past, however, the addition of tobacco is now considered to be the critical factor, ingredients in betel-quid other than tobacco are yet to be proven as risk factors.[9] Excess consumption of all types of alcohol raises the risk status for oral cancer.[13] The combination of tobacco use and alcohol consumption is multiplicative.[9]

An increased consumption of fruit and vegetables has been linked to a lower overall risk of oral cancer whilst a high fat consumption in the diet is linked to increased risk.[9] There is also an increased risk of developing oral cancers in people with a compromised immune system.[14]

2.1.3 Significance in terms of ill-health

Early detection and treatment of oral cancer is claimed to increase five year survival rates from 50% to 80%.[15] On average the five year survival rate for persons with oral cancer in England and Wales is 50%.[8]

2.1.4 Current service provision

Screening for oral cancer is conducted by General Dental Practitioners on an opportunistic basis as part of routine dental care. In May 1998 the British Dental Association (BDA) first published guidelines for the early detection of oral cancer.1 These guidelines were updated in April 2000[16]. The guidelines were widely distributed to all British Dental Association members as a supplement to the BDA newsletter 'BDA news' in May 1998, a further distribution of the revised guidelines is expected to be circulated in May 2000. The extent to which the guidelines have been adopted in General Dental Practice has not been evaluated. The guidelines recommended routine visual examination of the oral mucosa following a set pattern. The recommended approach was a general appraisal of the well-being of the patient followed by an extra oral examination of the face and lips, removal of any dentures and an intra-oral examination. The original guidelines were sponsored by Stafford-Miller, the updated version are sponsored by Zila inc, both include a description of the use of toluidine blue dye as an aid to the early detection of oral cancer. Toluidine blue dye application is not available as an item of service payment under General Dental Service (GDS) regulations. Application of the dye is currently provided under private contract, with the cost to the patient decided by the individual clinician.

The approach recommended for the diagnosis and assessment of oral cancer by the Welsh Health Planning forum in 1992 was history, visual examination of soft tissue, palpation, biopsy, radiography, and computerised tomography (CT scan).[17]

Both the BDA guidelines and the Welsh Health Planning forum recommend the referral of all patients with oral lesions suspected of malignancy to oral or maxillo-facial surgeons for further assessment and biopsy.

The gold standard for the diagnosis of oral cancer is biopsy and histological examination.

Treatment of oral cancer includes surgery, radiotherapy, chemotherapy and re-constructive surgery.[17] Rehabilitation and continuing care for patients includes the use of implants, implant supported prostheses, restorative dentistry, psychological support, speech therapy, smoking cessation and regular follow-up examinations.[18]

2.2 Description of the new intervention

The use of toluidine blue dye has been suggested as an adjunct to visual examination in the identification and management of oral cancer since the 1960s. The first preliminary report describing its use in the early detection of oral squamous cell carcinoma was published in 1962.[19] It is marketed and recommended as a way of picking up cancers that may not be detected by visual inspection alone. It is not suggested that the failure to stain of a visually observable lesion should rule out the need to biopsy such a lesion.

Toluidine blue is cationic metachromatic dye that selectively binds to free anionic groups such as sulphate, phosphate and carboxylate radicals of large molecules. It has been used for decades as an in vitro nuclear stain, binding the phosphate groups of nucleic acids. In vivo, malignant lesions stain a brilliant deep blue. The precise mechanism of action of the stain has not been fully elucidated, however an accumulation of the dye between tumour cells has been reported, thus the stain penetrates the enlarged intracellular spaces that are a feature of carcinomatous epithelium.[20]

Since the publication of the BDA guidelines on the detection of oral cancer in general dental practice,[1] toluidine blue dye oral cancer screening kits have been marketed to General Dental Practitioners in the UK first under the trade name 'OraScreen' by distributors Stafford-Miller Ltd., and since March 2000 by Zila Europe under the trade name 'Oratest ' The product licence holders are Zila Inc., a multinational pharmaceutical company. In the United States and elsewhere Zila Inc. are also marketing toluidine blue dye under the trade name 'OraTest'. Zila Inc. claim that the use of toluidine blue (OraTest) for the detection and management of oral cancer has been formally endorsed by the World Dental Congress (FDI) at their congress in Barcelona in October 1998. The FDI issued a statement which is quoted in part in press releases from Zila Inc.:[21]

> *"The use of Toluidine Blue in expert and experienced hands is recommended:*
>
> - *in the monitoring of suspicious lesions over time*
> - *in the screening for oral mucosal malignancy and potential malignant lesions in high risk individuals and populations*
> - *in the follow-up of patients already treated for upper aerodigestive tract cancer*
> - *in helping to determine an optimal site for biopsy when suspicious lesion or condition is present; and*
> - *intra-operatively during surgery of upper aerodigestive tract malignancy"*
>
> *"To clinicians in primary care settings, specific training is needed for correct application of the test and correct interpretation of the results"*
>
> *"Toluidine blue shall not be considered a replacement for a visual and digital examination, but as an extra tool for the identification of patients who should be referred to specialist centres experienced in the diagnosis and treatment of oral cancer and potentially malignant lesions or conditions"*

Concern has been expressed recently in the dental press that the general use of toluidine blue without correct training may result in false positive and false negative results that will be detrimental to public health.[22] Stafford-Miller are provide training, however when contacted in October 1998, Dental Advisers were not available to conduct training in all areas. Since this time the training provided by Stafford-Miller has been changed. A training pack consisting of a video and supporting papers is now distributed to practices for self-training. Confirmation that this training has taken place is required by the company before the product can be ordered. Where support is required by practices, Stafford-Miller offer this on a one-to-one basis (Personal communication January 2000). Since March 2000 the training and marketing have been taken over by Zila Europe.

At the end of January 2000 Stafford Miller had a record of approximately 1,500 practices who had expressed an interest in using toluidine blue, of these about half had received training and about 30 practices were routinely using toluidine blue for oral cancer screening (Personal communication January 2000).

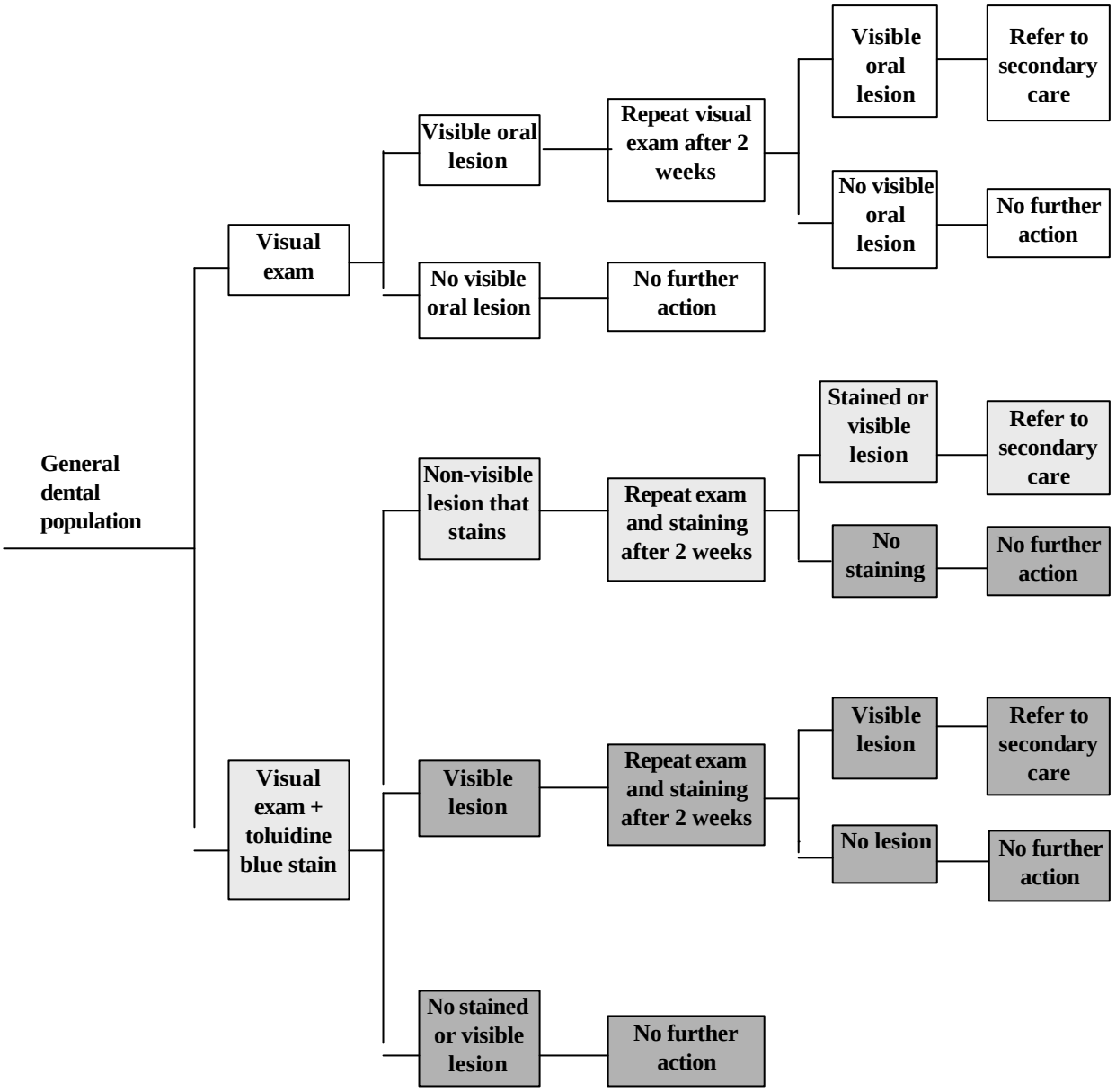
2.2.1 Anticipated Costs

Toluidine blue dye application is not available as an item of service payment under General Dental Service (GDS) regulations. Application of toluidine blue dye is currently provided under private contract, the cost to the patient being decided by the individual clinician. The purchase cost of a single application kit is currently £16.20, the cost of the materials for the two-stage application recommended by the manufacturer in the event of an initial positive test result is therefore £32.40. Other resources involved in the use of toluidine blue as an adjunct to oral cancer screening in general dental practice include the staff time involved in test administration and associated practice overheads and patient time. The cost of this additional resource use will depend on the additional time required for the administration of toluidine blue as part of an opportunistic screening process. Costs fall on patients - anecdotal reports suggest that patients are being charged up to £85 for the test.

2.2.2 Decision tree

The decision tree shown in Figure 1 below compares oral cancer screening in general dental practice with and without the use of toluidine blue as an adjunct to clinical examination (the current recommended approach). It is the basic model we used to examine the overall effectiveness and cost effectiveness of toluidine blue. It is a simplification in that it only extends as far as diagnosis; some of those patients with a diagnosis of dysplasia or pre-cancer may pass through the decision tree on more than one occasion, others may receive treatment. The shaded boxes represent the critical path which would represent additional patients being diagnosed early with oral cancer.

Figure 1 - Decision tree



3 Effectiveness

3.1 Methods for reviewing effectiveness

The review was undertaken following the methods laid down in a protocol. The protocol was developed using literature identified through a scoping review and included the strategies for the literature search, inclusion and exclusion criteria and quality assessment criteria.

3.1.1 Search strategy

1. The Cochrane Library was used to identify any recent systematic review of the use of toluidine blue, none were recorded
2. The Cochrane Library, Medline and Pub-Med databases on the Internet were searched for all references to the use of toluidine blue dye or tolonium chloride between 1955 and 1999
3. The references to all identified studies were scrutinised for futher relevant studies

Searches were conducted in January 1999. No language restrictions were applied. Details of the search strategy are shown in Appendix 1.

3.1.2 Inclusion criteria

Study design

An early scoping review suggested that evidence on this topic would be scarce so all study designs were included. This included randomised controlled trials, controlled trials and case series; expert opinion only was excluded. Literature reviews were used to inform the background section of this report.

No language restrictions were applied in the search strategy.

Study Population

The question of this review relates to screening in the general dental population. However, as the scoping review suggested scarce evidence on this topic, studies were included if they related either to the general population or to high risk groups (such as patients with a previous history of oral cancer or those with risk factors for oral cancer, e.g. people over 50 years who smoke and drink).

Types of intervention

Included studies assessed the use of toluidine blue as an adjunct to diagnosis of oral cancer. All methods of application were considered, i.e.:

- Single applications, both directly to a visible lesion e.g. using a swab and also using a toluidine blue mouth rinse

- A two stage application where the initial application either direct or rinse was followed up after 10-14 days with a further application

3.1.3 Outcome measures

Primary outcome

Toluidine blue is not promoted as a diagnostic test - its proponents do not suggest that the fact that a visual lesion does not stain mean that the lesion is not cancerous or precludes the need to undertake a biopsy (biopsy and histology are recognised as the standard diagnostic test). Rather it is promoted as a screening test – its proponents suggest that it is useful for detecting oral cancers that would not otherwise have been picked up. Accordingly, the primary outcome of interest for this report was defined as the number of oral cancers detected that would not have been picked up by visual examination alone, together with the number of false positives.

Secondary outcomes

The test performance in terms of sensitivity and specificity, as measured by the recognised reference standard of histological examination following biopsy of lesions, was also recorded.

Information on resource use and patient outcomes was noted if presented.

3.1.4 Exclusion criteria

Studies were excluded if they:

- only provided background information and gave expert opinion on the use of toluidine blue
- only described the techniques for staining lesions with toluidine blue dye
- did not utilise the gold standard of biopsy on the majority (90%) of lesions
- report studies but do not present any results
- only report partial results of studies
- report on the interim results of included studies at earlier stages

3.2 Quality assessment strategy

The quality of the papers were assessed against the following pre-defined criteria:

Study design:

- Randomised Controlled Trials (RCT)
- Case Series (CS)
- Cohort study (CT)

Was the study blind and the histology of the lesion unknown to the examiner?

Were examiners calibrated or intra-examiner reproducibility tested?

Was the histological assessment blind to the results of the screening?

Was the study conducted in a primary care setting?

Quality assessment was conducted independently by a second reviewer on a randomly selected sample of one paper in ten.

3.3 Data Extraction Strategy

A data extraction form (Appendix 2) was used to evaluate studies and extract data. Data extraction was conducted independently by two reviewers.

4 Results

4.1 Quantity and quality of research available

4.1.1 Number of studies identified

A total of 77 articles were identified from the literature. No existing systematic reviews of the use of toluidine blue was in the Cochrane Library. Electronic searches identified 77 possible articles. No further relevant articles were identified through scrutiny of the references to included studies.

Of the 77 articles, fourteen met the inclusion criteria. There were also four literature reviews which offered no additional or new data.

In addition to the published research, an unpublished study was made known to the lead author through contact with a specialist in this clinical field.

4.1.2 Previous reviews

There are four reviews in the literature of the use of toluidine blue dye in the detection of oral cancer.[23] [24] [25] [26] All of the research appraised in these reviews is included in the articles identified by the search strategy of this review. As such, these reviews provide no additional data for inclusion, but are useful for informing the background section of this report.

The earliest review, by Wong in 1982[23] is a narrative review which provides background information on some of the early studies. The second review conducted by Vercellino et al[24] was only available in English in abstract form; this reviews six of the studies identified here and surveys expert opinion. A meta-analysis conducted by Rosenberg and Cretin[25] included twelve studies of suspected oral cancerous lesions that had been referred to secondary care, all of which are considered in this review. The most recent review by Johnson[26] does not include a systematic search or discriminate between studies on quality grounds, although it provides useful background information and a summary of most of the trials and reports of case series studies up to 1997.

4.1.3 Included studies

There were fourteen published studies that met the inclusion criteria. The study design for each study was case series. None were undertaken in a primary care - **all** were conducted in secondary care settings on high-risk population groups. Thirteen used a single rinse technique, and one study used an additional, follow up rinse. Only one of these studies systematically reported the primary outcome defined in this report of the performance of toluidine blue for detecting lesions not picked up on visual inspections.[31]

A further prospective cohort study using a two-rinse procedure and conducted in a primary care setting was included in this review. This study addressed the question of acceptability to patients rather than effectiveness.[39]

A list of included studies is given in Table 2 below. A brief description of these studies can be found in Appendix 1 page 23. The characteristics of these studies and an assessment of their quality against the criteria listed above are summarised in Table 3, page 12.

Table 2 - Included studies

Warnakulasuriya K A A S, Johnson N W, Sensitivity and specificity of OraScan toluidine blue mouthrinse in the detection of oral cancer and precancer J Oral Pathol Med 1996; 25:97-103[6]
Epstein J B, Oakley C, Millner A, Emerton S, van der Meij E, The utility of Toluidine Blue as a diagnostic aid in patients previously treated for upper oropharyngeal carcinoma Oral surg Oral med Oral Pathol Oral Radiol Endod, 1997 May 83:5 537-547[27]
Silverman S Jnr, Migliorati C, Barbosa J, Toluidine blue staining in the detection of oral precancerous and malignant lesions, Oral surg Oral med Oral Pathol 1984 Apr 57:4, 379-382[(4)]
Rosen I B, Cornish M Edelson J, Detection of early oral cancer by toluidine blue, J Can Dent Assoc (1971) Sep 37(9) 347-349[28]
Mashberg A, Re-evaluation of Toluidine blue application as a diagnostic adjunct in the detection of asymptomatic oral squamous carcinoma, Cancer (1980) 46:758-763[29]
Menasse J and Reychler H, Value of toluidine blue in the diagnosis of tumors of the oral cavity, Acta Stomatol Belg 1984 Sep 81(2) 145-151[30]
Epstein J.B., Scully C., Spinelli J.J., Toluidine blue and Lugol's iodine application in the assessment of oral malignant disease and lesions at risk of malignancy, J Orol Pathol Med (1992) 21 160-16332
Barellier P, Babin E, Louis M Y, and Meunier-Guttin A The use of toluidine blue in the diagnosis of neoplastic lesions of the oral cavity Rev Stomatol Chir Maxillofac (1993) 94(1) 51-54[31]
Silverman S., Oral Cancer Semin Dermatol 1994 Jun 13(2) 132-13733
Myers E.N. The toluidine blue Test in Lesions of the Oral Cavity CA Cancer J Clin (1970) May-Jun 20(3) 134-139[5]
Vahidy N A., Zaidi S H, and Jafarey Toluidine blue test for detection of carcinoma of the oral cavity: an evaluation J Surg Oncol (1972) 4(5) 434-43834
Sigurdson A. and Willen R., In vivo färgning med Toluidine blue som hjälpmedel för lokalisation av provexcisionsställe vid misstänkt skivepitelcancer I munhålan, Swed Dent J (1975) 65 117-12635
Reddy C R., Ramulu C, Sundaseshwar B Raju M V, Gopal R, and Sharma R Toluidine blue staining of oral cancer and precancerous lesions Indian J Med Res (1973) Aug 61(8) 1161-116436
Mashberg A., Final evaluation of tolonium chloride rinse for screening of high-risk patients with asymptomatic squamous carcinoma. JADA (1983) 106 319-32337
Feaver G.P. Report of the Marks and Spencer working group on screening for oral cancer and pre-cancer March 1998, and update on this study by personal communication dated June 8th 1999[38] Feaver G.P., Morrison T., Humphris G. A study to determine the acceptability in patients and dentists of toluidine blue in screening for oral cancer, Primary Dental Care (1999) 6(2) 45-50[39]

4.1.4 Excluded Studies

Details of the excluded studies and the reasons for exclusion are given in Appendix 4, page 32. The majority of studies and papers that were identified were excluded from the meta-analysis for a number of reasons as follows:

- twenty-six articles provided background information and expert opinion but no additional data
- sixteen papers described a technique for staining and gave expert opinion but no additional data
- three studies only performed biopsy on lesions with a positive staining result
- one study only provided partial results
- four papers provided early reports of other assessed publications
- one study was a duplicate of an assessed article in a different journal
- three studies were of the use of toluidine blue dye to determine biopsy site not for oral cancer screening.
- one study was an in vitro study of the staining properties of toluidine blue
- four studies were unobtainable in a format sufficient for assessment (one study unobtainable in the UK and three which we were unable to get translated).

4.1.5 Characteristics and quality of included studies

Table 3 summarises the characteristics of included studies and presents an assessment of their quality. There was total agreement between reviewers on the quality of the 10% sample of papers that were doubly assessed. In general, the quality of included studies is poor, with only five studies assessed as fair or good. Only one study was conducted in a primary care setting.[38,39] A fuller description of these studies can be found in Appendix 3, page 28.

Table 3 - Characteristics of included studies

Study	Study design	Technique (single or two stage)	Number of patients	Number of lesions	Histology unknown to examiner?	Examiner calibration?	Histological assessment blind?	Primary care setting?	Study population	Quality
Published studies										
Warnakulasuriya[6]	CS	Single	102	145	Y	Y	Y	N	People referred with oral lesions	good
Epstein[27]	CS	Single	46	81	Y	Unknown	Y	N	People with a history of oral malignancy	fair
Silverman[4]	CS	Single	132	132	Y	Unknown	Y	N	People suspected of having oral cancer or pre-cancerous (dysplastic) lesions	fair
Rosen[28]	CS	Single	45	45	Y	Unknown	Unknown	N	People referred with oral lesions for private consultation and inpatients of an alcohol & drug dependency unit	poor
Mashberg[29]	CS	Single	178	235	Y	Unknown	Unknown	N	People referred with oral lesions	poor
Menasse[30]	CS	Single	66	66	Y	Unknown	Unknown	N	People referred with oral lesions	poor
Epstein[32]	CS	Single	59	59	Y	Unknown	Y	N	People with a history of oral cancer (26) or suspected oral carcinoma (33)	fair
Barrellier[31]	CS	Single	235	82	Y	Unknown	Unknown	N	People with previous history of cancer of pharynx or oesophagus	poor
Silverman[33]	CS	Unknown	Unknown	169	Unknown	Unknown	Unknown	N	People referred with lesions	poor
Myers[5]	CS	Single	70	70	Y	Unknown	Unknown	N	People with a history of oral cancer	poor
Vahidy[34]	CS	Single	1190	1190	Y	N	Unknown	N	People attending cancer clinic	poor
Sigurdson[35]	CS	Single	51	54	Y	Unknown	Unknown	N	People referred with lesions	poor
Reddy[36]	CS	Single	430	430	N	Unknown	Unknown	N	People with oral cancer (420) or with pigmented areas of the palate (10)	poor
Mashberg[37]	CS	Two stage	134	179	Y	Unknown	Unknown	N	People referred with oral lesions	poor
Unpublished studies										
Feaver[38]	CT	Two stage	140	1	Y	Y	N	Y	Marks & Spencer's staff aged over 40	good

Unknown = insufficient information provided in publication

4.1.6 Assessment of effectiveness

There was complete agreement on the data extraction by the two independent reviewers.

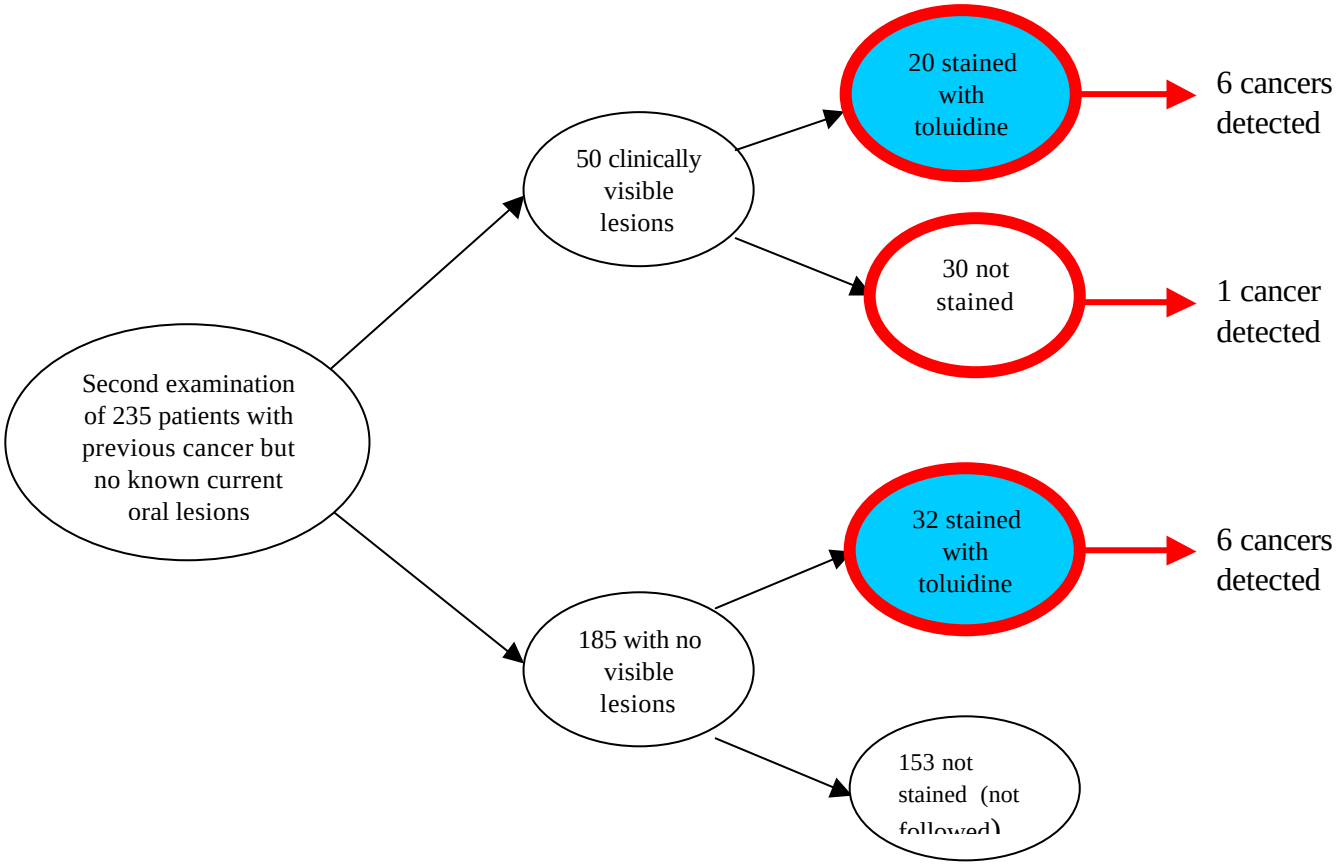
4.2 Primary Outcome – detection of lesions not otherwise picked up

Only one study, Barrellier [31], reported the main outcome of interest - the detection of malignant lesions that were not detected visually, the rest simply reported the sensitivity and specificity of toluidine blue in all lesions compared to the reference standard of biopsy and histology.

In this study, 235 people with a past history of oral cancer and who had subsequently been regularly followed up and had been assessed as not having an oral lesion were entered into a screening programme where they were re-examined and screened with toluidine blue rinse. Eighty-two lesions were sent for biopsy: 50 were visible lesions detected by the visual inspection; 32 were areas where there was no visible lesion but which stained positive with toluidine blue. No biopsies were undertaken from the 153 subjects who had negative results on visual inspection and rinsing.

The results are given in figure 1 below. Thirty-two people stained positive with toluidine where there was no visible lesion. Six of these "lesions" were confirmed as malignant on biopsy. Thus out of 235 patients screened with toluidine blue 6 cancers which would not otherwise have been detected were picked up (2.5%) and 26 people had biopsies and the anxiety associated with a false positive result and biopsy (11%).

Figure 2 - Results of screening for Barrellier study



The patients in the ovals with the red outline were biopsied.

This was a high risk population and thus these figures are not generalisable to general practice because the probability of a patient having a malignant lesion is much lower in general practice. The generalisability of the proportion of visually-detected to staining-detected lesions to other high risk populations is also not possible because this was a high risk group that had **already been** examined and categorised as having no visible lesions rather than simply being the follow up of a high risk population. Four people had a false positive result for every additional cancer detected. Again the false positive to true positive ratio is likely to be much higher in general practice where there is a very low prevalence of cancer. The cancer rate in this group was 5%, despite them already have been screened negative for lesions. This compares with the incidence rate of only 0.0034% from routine cancer registrations (3.4/100,000) or a rate of 0.5% for malignant or potentially malignant lesions found during screening in a primary care setting.[38]

One further study, Mashberg[29], mentions the detection of cancerous lesions that were not visually detected as an incidental finding but gives no numerical details.

4.2.1 Secondary results – sensitivity and specificity

The results for the secondary care studies are tabulated in table 2. The calculations of sensitivity and specificity for these studies are shown in table 3. There is considerable variation in the findings of the studies in terms of the sensitivity and specificity of toluidine blue as a screening test for oral cancer in high risk populations. Reported sensitivity varies from 1.0 (Warnakulasuriya[6] and Epstein[27]) to 0.4 (Rosen[28]). Since it is not proposed that toluidine blue screening be used to exclude the requirement to biopsy a visible lesion, the sensitivity of the test is not relevant (otherwise a negative result in a highly sensitive test could have been used exclude the need for biopsy). The reported specificity of toluidine blue varies from 0.31 (Silverman[4]) to 0.92 (Mashberg[29]).

The results of six of the studies included some equivocal results, i.e. toluidine blue stains were interpreted as positive, negative or equivocal rather than as either positive or negative. The calculations of sensitivity and specificity in table 3 are presented firstly with equivocal results included with positive results, and secondly with equivocal results included with negative results. As shown, the impact of equivocal results on estimates of test characteristics for the individual studies can be significant, but the impact on overall estimates is marginal.

The results of the only study undertaken in the general dental populations are shown in Table 4. This study can be interpreted as a cohort study comparing oral cancer screening in general dental practice with and without the use of toluidine blue as an adjunct to clinical examination.

Table 4 - Results of Marks and Spencer Toluidine blue study

	Clinical examination plus toluidine blue
Population	Staff aged over 40 years working for Marks & Spencer plc.
Number of people screened	140
Number of visually observable lesions detected at initial screen	Not mentioned
Number of lesions detected at initial toluidine blue screen	19 (not detected visually)
Number of patients with persistent lesion at second screen (referred to secondary care)	1 (visually observable and stained with TB)
Number of lesions confirmed as malignant or potentially malignant	0 (lesion no longer visually observable and therefore biopsy not taken)

No additional cancerous lesions were detected in the 140 patients screened that were not detectable visually. However, the only patient who tested positive twice did not go on to have the standard diagnostic test of biopsy and histology. However, the small size of the cohort on which toluidine blue has been used means it did not have the power to detect a effect of the additional test. In addition, follow-up data is not available for people screened as negative.

This study raises a major problem of oral cancer screening trials in primary care, in that large study populations are required to enable a study to detect a clinically significant difference in test characteristics should one exist. The potential cost of such a study, or of continuing this study until a sufficient sample size is reached, will be high, as will the cost of detecting an additional cancer should it be implemented.

Table 5 - Results of the included studies

First Author	Year	Number of lesions	Number receiving gold standard test	Number with confirmed SCC	SCC and positive TB stain	SCC and negative TB stain	SCC and equivocal TB stain	Non malignant lesion	Non malignant lesion positive TB stain	Non malignant lesion negative TB stain	Non malignant lesion equivocal TB stain
Single application or rinse											
Warnakulasuriya[6]	1996	145	86	18	18	0	0	68	40	21	7
Epstein[34]	1997	81	81	27	22	0	5	54	11	28	15
Silverman[4]	1984	132	132	57	55	2	0	75	52	23	0
Rosen[35]	1971	45	45	5	2	3	0	40	21	19	0
Mashberg[36]	1980	235	235	105	97	7	1	130	10	119	1
Menasse[37]	1984	66	66	15	13	2	0	50	27	23	0
Epstein[28]§	1992	59	59	40	37	3	0	19	7	12	0
Barellier[38]	1993	82	82	13	12	1	0	69	40	29	0
Silverman[29]	1994	169	169	62	58	4	0	107	17	90	0
Myers[5]	1970	70	70	47	47	0	0	19	0	10	9
Vahidy[30]	1972	1190	1190	535	415	66	54	655	131	418	106
Sigurdson[31]	1975	54	54	11	11	0	0	43	20	23	0
Reddy[32]	1973	430	430	420	420	0	0	10	2	8	0
Two stage application or rinse											
Mashberg[33]	1983	179	179	81	65	9	7	98	8	89	1

Totals do not sum due to:

Menasse[37] Results only refer to patients with Squamous cell carcinoma and non malignant lesions 1 patient with sarcoma has been excluded from the results.

Myers[5] Results only refer to patients with Squamous cell carcinoma and non malignant lesions 2 patients with malignant melanoma, one with fibrosarcoma and one with lymphocarcoma have been excluded

§Epstein32: SCC results include dysplasia as cannot be separated in published results

Table 6 - Sensitivity and specificity of screening in high-risk populations

First Author	Year	Number of lesions	Sensitivity for SCC when equivocal results included as positive	Specificity for SCC when equivocal results included as positive	Sensitivity for SCC when equivocal results included as negative	Specificity for SCC when equivocal results included as negative
Single application or rinse						
Warnakulasuriya[6]	1996	145	1.00	0.31	1.00	0.41
Epstein[34]	1997	81	1.00	0.52	0.81	0.80
Silverman[4]	1984	132	0.96	0.31	0.96	0.31
Rosen[35]	1971	45	0.40	0.48	0.40	0.48
Mashberg[36]	1980	235	0.93	0.92	0.92	0.92
Menasse[37]	1984	66	0.87	0.46	0.87	0.46
Epstein[28]	1992	59	0.93	0.63	0.93	0.63
Barellier[38]	1993	82	0.92	0.42	0.92	0.42
Silverman[29]	1994	169	0.94	0.84	0.94	0.84
Myers[5]	1970	70	1.00	0.53	1.00	1.00
Vahidy[30]	1972	1190	0.88	0.64	0.78	0.80
Sigurdson[31]	1975	54	1.00	0.53	1.00	0.53
Reddy[32]	1973	430	1.00	0.80	1.00	0.80
Two stage application or rinse						
Mashberg[33]	1983	179	0.89	0.91	0.80	0.92

5 Summary about effectiveness data

No published studies have evaluated the use of toluidine blue dye in the general population. The one study in primary care had 140 patients and did not have the power to demonstrate any effect. There is no evidence that toluidine blue is effective in screening for oral cancer in primary care.

Case series reports of the use of toluidine blue in secondary care show variable results in terms of the sensitivity and specificity of the test. In general, the quality of these included studies is poor. One study demonstrates additional cancers detected when toluidine blue was used as an adjunct to clinical examination. Toluidine blue may, therefore, be a useful adjunct to clinical examination for the detection of oral cancer in high-risk populations and in people referred to secondary care with oral lesions when used by trained and experienced clinicians. The 14 studies in secondary care were all of people at high risk of oral cancer and all but one of the studies concerned people referred to secondary care **with** oral lesions. Thus, the results of the included studies relate to the test characteristics of toluidine blue as a screening test for oral cancer in people with detected oral lesions and are not generalisable to primary care.

6 Economic Analysis

6.1 Resource use and costs associated with the use of toluidine blue as an adjunct to oral cancer screening in general dental practice

Toluidine blue dye is not currently available as an item of service under General Dental Services. The cost of the test is therefore currently born entirely by the patient. The current price of a single application pack of OraScreen is £16.20. In the event of an initial test giving a positive result the manufacturers recommend repeat application after 10-14 days. In addition to the cost of the materials, patients are likely to be charged for the dental practice time to conduct the test. We estimate the consultation time taken for the first screening is up to 5 minutes, which we have costed at £5.60 for time and overheads. A second consultation for re screening should take between 10-15 minutes. This has been costed at £17, giving a total cost for a two-stage screen of £55. The cost of a referral to secondary care and biopsy and histology is £75 (data from Walsall & Wolverhampton Health Authorities 1999-2000).

To screen a population of 100,000 people with one rinse it will cost £2,080,000 (£16.2+£5.60/person). If rates of positive staining are the same as in the Marks and Spencer population, then 13.6% of the population will stain positive and require a further smear. So 13,600 people will have a second smear at a cost of £451,500 (£16.20+£17/person). One in 140 people will stain twice and require a biopsy at a cost of £75 which is £53,400. The total monetary cost of screening 1000 people would thus be £2,585,100. The cost to the population would also include 13,600 people who are made anxious by having a positive staining result.

If the annual incidence rate of cancer from national statistics were applied to these figures (almost certainly an underestimate), **and** *we assume that all cancers could be detected early in this way* (undoubtedly a massive overestimate), then we could expect to pick up 3.4 cancers if the screening were performed annually **and** *there were no interval cancers*. The cost per case detected would

be £861,700. Since early detection increases the five year survival from 50% to 80% . Thus *using five year survival as a proxy measure of the number of people cured* it would cost £2,872,333/person cured. This is probably an underestimate of cost as most of the assumptions made (in italics) are very optimistic and the calculation does not include treatment costs. Moreover there would be 45,000 people who were made anxious per person cured.

7 Implications for further research

Although we have found no evidence of the effectiveness of toluidine blue screening in general dental practice as an adjunct to visual and digital examination, it is also true that the absence of evidence is not evidence of absence. Given the evidence from secondary care of additional cancers detected, should we be undertaking research into the possible benefits of screening in a general practice population? We believe that such research would be difficult to justify ethically and financially, as there is little to suggest that the benefits would outweigh the harms and costs.

8 Acknowledgements

This report was completed when the lead author was a participant on a systematic review course run by the West Midlands Development and Evaluation Service. I am grateful for the instruction and support given by the DES team and the staff of the Department of Public Health and Epidemiology of the University of Birmingham.

I am also grateful to Derek Richards and others who have peer reviewed this report.

9 Appendices

Appendix 1 - Search Strategy for Medline and Pub-Med databases

MeSH heading	Toluidine
MeSH Heading	Tolonium
Textword	Toluidine
Textword	Tolonium

Combine 2.1 or 2.2 or 2.3 or 2.4

MeSH heading	Cancer
MeSH heading	Malignan*
MeSH heading	Neoplasm
MeSH heading	Carcinoma
Textword	Cancer
Textword	Malignan*
Textword	Neoplasm
Textword	Carcinoma

Combine 2.6 or 2.7 or 2.8 or 2.9 or 2.10 or 2.11 or 2.12 or 2.13

MeSH heading	Oral
MeSH heading	Mouth
MeSH heading	Lip
MeSH heading	Tongue
Textword	Oral
Textword	Mouth
Textword	Lip
Textword	Tongue

Combine 2.15 or 2.16 or 2.17 or 2.18 or 2.19 or 2.20 or 2.21 or 2.22

Combine 2.5 and 2.14 and 2.23

Appendix 2 - Data extraction form

Data Extraction Form

Using toluidine blue (TB) as an adjunct to clinical examination in the diagnosis of oral cancer

Reference No. ☐

Type of study	Case Series	☐
	Clinical Trial	☐
	Other (please specify…….)	☐
Number included in study		☐
Description of sample	general population	☐
	high risk group eg smokers	☐
	referred for oral lesions	☐
	previous history of oral cancer	☐
	being treated for oral cancer at time of study	☐
	other (please specify……..)	☐
Where study conducted	Primary care/field trial	☐
	Secondary care hospital/specialised clinic	☐
Age of subjects	Range	☐
	Mean	☐
	Median	☐
Technique used	Single direct application eg with swab	☐
	Two stage direct application	☐
	Single rinse	☐
	Two stage rinse	☐
Lesions or patients counted		☐
Diagnosis confirmed by biopsy/histology		☐
Number of lesions or patients with oral carcinomas inc CA in situ		☐
Number with oral carcinomas who were screened +ve with TB		☐
Number with oral carcinomas who were screened +ve without TB		☐
Number with oral carcinomas who were screened -ve with TB		☐

Number with oral carcinomas who were screened -ve without TB

Number with dysplasia	
Number with dysplasias who were screened +ve with TB	
Number with dysplasias who were screened +ve without TB	
Number with dysplasias who were screened -ve with TB	
Number with dysplasias who were screened -ve without TB	
Number with no disease	
Number with no disease who were screened +ve with TB	
Number with no disease who were screened +ve without TB	
Number with no disease who were screened -ve with TB	
Number with no disease who were screened -ve without TB	
Number included in original sample not counted in results	

Number identified with carcinoma or dysplasia where the lesions had not been identified in a clinical examination eg where a second primary carcinoma was unexpectedly found

carcinomas	
dysplasias	

General comments

Appendix 3 - Included studies

Warnakulasuriya[6]

This study was conducted on patients who had been referred with unconfirmed oral mucosal lesions to seven specialist centres in Asia. All clinically visible lesions were charted using WHO criteria and photographed, patients were then given a toluidine blue rinse. Of the 145 lesions only 86 suspicious lesions, dye retained or not, were subjected to biopsy. Whilst recognising the need for biopsy to confirm negative results the authors felt that it would not be ethical to perform biopsies on negative stained normal mucosa. As such, although the trial claims to be a study of subjects with a wide range of oral lesions, the results relate to a population with suspicious lesions.

Epstein[27]

The patients included in this small case series were all referred for diagnosis of oral mucosal changes. Patients included in the study were those in whom biopsies had been completed.

Silverman[4]

This study is set in an oral medicine clinic and concerns people suspected of having cancer or pre-cancerous lesions. One hundred and thirty two (132) consecutive cases are reported. All patients underwent biopsy.

Rosen[28]

This study was conducted on patients with oral mucosal lesions. There is no indication of any blinding in the histological examination, or of examiner training and calibration.

Mashberg[29]

The subjects included in this study were initially examined, then re-examined after a 10 to 14-day waiting period to allow inflammatory lesions to heal. Only those subjects with persistent lesions were stained. There is no indication of examiner training. All lesions were subjected to biopsy. This study reports an incidental finding of second primary cancers in the mouth and oropharynx which were missed on clinical examination and identified when stain flowed into these areas. The number of second primaries and the patients on whom they were found are not, however, described.

Menasse[30]

This study is a small case series of patients with oral lesions. There is no indication of examiner training or of blinded histological examination. All lesions were subjected to biopsy. One patient with cancer other than squamous cell carcinoma is excluded from the results below.

Epstein[32]

This is a prospective case series which focuses on the use of toluidine blue in combination with Lugol's iodine. Results for dysplasia and Squamous cell carcinoma cannot be separated.

Barrellier[31]

In this study, 235 people with a past history of oral cancer and who had subsequently been regularly followed up and had been assessed as not having an oral lesion were entered into a screening programme where they were re-examined and screened with toluidine blue rinse. Eighty-two lesions were sent for biopsy: 50 were visible lesions detected by the visual inspection; 32 were areas where there was no visible lesion but which stained positive with toluidine blue. No biopsies were undertaken from the 153 subjects who had negative results on visual inspection and rinsing.

Silverman[33]

The available paper by this author presents only a summary table, no methodology is given. The characteristics of the patients included in the study are not known, nor is it clear that all patients had their diagnosis confirmed by biopsy.

Myers[5]

All the subjects in this study had a previous history of oral cancer. It seems likely that many of the subjects had cancers at an advanced stage. Four patients with cancers other than squamous cell carcinoma are excluded from the results below.

Vahidy[34]

This was a large study with a high turnover of clinicians. The results given in the paper are the interpretations of the examiners doing the initial examinations, all cases were later re-examined by more senior examiners but results of the re-examination are not given. Punch biopsies were taken in all cases where it was clinically warranted, where the dye test was equivocal or positive, or where a cytology report was suggestive of malignancy. Follow up, however, provides final diagnosis in all cases.

Sigurdson[35]

This is a consecutive case series.

Reddy[36]

This paper presents the results of toluidine blue staining on five groups. Two groups are included here: 420 people with squamous cell carcinoma and ten people with pigmented areas of the palate. The other three groups are excluded: one was a study of the use of toluidine blue dye to detect dysplastic lesions; one was a field study of 9,400 individuals in which there was no confirmation of the diagnosis; and the third was a description of the staining pattern in ten normal people.

Mashberg[37]

This study compares direct application of toluidine blue dye with toluidine dye rinse as a method of administration. The report suggested the use of a two stage rinse as a screening agent and the data extracted below are those from the results of the rinse. This is the only included study to use the two-stage rinse approach currently recommended by the manufacturer.

Feaver[38,39]

In addition to the studies reported in the literature, data has been made available on the results of an oral cancer screening programme for staff aged over 40 years working for Marks and Spencer PLC. The screening is undertaken by dental practitioners working to a common screening protocol. Screening has been undertaken since 1993. Between 1993 and 1996, 11,970 patients were visually screened without the additional use of toluidine blue dye. Following this, a study has been conducted into the acceptability of using toluidine blue dye as an adjunct to screening based on 140 staff.[39] This showed that 83% of patients found the screen to be "a comfortable experience", although 31% indicated they were anxious about receiving the Orascreen.[40] There was no attempt to measure the anxiety in those who screened positive on the first rinse (13.6% of those screened). Unpublished data made available to us revealed that one patient had a visually observable lesion that stained on two occasions and was referred to secondary care. The lesion was diagnosed as "tobacco related lesion" and resolved before a biopsy was taken (Personal communication from Gerald Feaver, Chief Dental Officer, Marks and Spencer PLC). The small size of the toluidine blue cohort means the study it does not have the power to detect any statistically significant effect of the use of toluidine blue as an adjunct to oral cancer screening in general dental practice.

Appendix 4 - Excluded studies

Author	Reference	Reason for exclusion
Warnakulasuriya K.A.A.S. and Johnson N.W[7]	International Dental Journal (1996)46 (Suppl 1)245-250	Background information and expert opinion with no additional data
Johnson N.W.[41]	FDI World (1997) 6(3)19-21	Background information and expert opinion with no additional data
Johnson N W[42]	FDI World (1997) 6(4) 7-11	Background information and expert opinion with no additional data
Johnson N W[43]	FDI World (1997) 6(5) 7-13	Background information and expert opinion with no additional data
Johnson N W[44]	FDI World (1997) 6(6) 10-16	Background information and expert opinion with no additional data
Johnson N W[45]	FDI World (1998) 7(1)14-19	Background information and expert opinion with no additional data
Chamberlain J[46]	Community Dental Health (1993)10 (Suppl 1) 5-10	Background information and expert opinion with no additional data
Johnson N W and Warnakulasuriya K A A S [9]	Community Dental Health (1993) 10 (Suppl 1) 13-19	Background information and expert opinion with no additional data
Speight P M and Morgan P R[47]	Community Dental Health (1993) 10 (Suppl 1) 31-41	Background information and expert opinion with no additional data
Scully C[3]	Community Dental Health (1993) 10 (Suppl 1) 43-52	Background information and expert opinion with no additional data
Downer M C and Speight P M[48]	Community Dental Health (1993)10 (Suppl 1) 71-78	Background information and expert opinion with no additional data
Scully C[49]	Oral oncology. European Journal of Cancer (1995);31B(1):16-26	Background information and expert opinion with no additional data
Frame P S and Rosati C [50]	Guide to Clinical Preventive Services, (16. Screening for oral cancer) Draft chapter update Ch 16	Background information and expert opinion with no additional data
Porter S R and Scully C[51]	Br Dent J 1998 Jul 25th 185:2 72-73	Background information and expert opinion with no

		additional data
Thomas J E and Bell W A[52]	Gen Dent 1985 Nov-Dec 33:6, 492-493	Background information and expert opinion with no additional data
Ligthelm A J, Weber A, van Niekerk P J ,and van Heerden W F[53]	J Dent Assoc S Afr 1989 Mar (Suppl 1) 2-5	Background information and expert opinion with no additional data
Miller R L Simms B W, and Gould A R[54]	J Oral Pathol 1988 Feb 16:2, 73-78	Background information and expert opinion with no additional data
Portugal L G, Wilson K M, Biddinger P W and Gluckman J L[55]	Arch otolaryngol Head Neck Surg (1996) May 122(5) 517-519	Background information and expert opinion with no additional data
Epstein J B and Scully C[56]	Spec Care dentist (1997) Jul-Aug 17(4) 120-128	Background information and expert opinion with no additional data
Mashberg A and Samit A M[57]	Cancer journal for clinicians (1989) 39(2) 67-88	Background information and expert opinion with no additional data
Silverman S and Greenspan D[58]	CDA J (1985) May 13(5) 29-33	Background information and expert opinion with no additional data
Allen C M[59]	Oral surg Oral med Oral Pathol oral pathol Oral radiol Endod (1998) Sep 86(3)255	Background information and expert opinion with no additional data
Sugarman J, Hamilton R, Graham W P et al[60]	Arch Surg (1970) 100: 240-243	Background information and expert opinion with no additional data
Boden E[61]	J of Oral Surg and Oral Path(1967) 23: ?	Background information and expert opinion with no additional data
Helsper J, Sharp G S, and Bullock W K[62]	Am J Surg (1963) 106: 802-806	Background information and expert opinion with no additional data
Matthews D C, Banting D W, and Bohay R N[63]	J Can Dent Assoc. (1995) Sep, 61:9, 785-791	Background information and expert opinion with no additional data
Srivastava Y C and Mathur M[64]	N J Indian Dent Assoc (1971) May 43(5) 98-101	Duplicate of assessed publication
Pizer M E,[65]	Va Dent J (1975) Aug 52(4) 53-54	Early report of study assessed in later publication
Mashberg A,[66]	JAMA (1981) 245:2408-2410	Early report of study assessed in later publication
Barrellier P, Rame J P, Chasle J, and Souquieres Y,[67]	Rev Stomatol Chir Maxillofac (1980) 81 (6) 364-367	Early report of study assessed in later publication

Barellier P, Rame P, Chasle J, Souquieres Y, and Lecacheux B,[68]	Actual odontostomatol (Paris) (1982) 36(137) 87-92	Early report of study assessed in later publication
Moyer G N, Taybos G M, Pelleu G B Jnr,[69]	J Oral Med 1986 Apr-Jun, 41:2 111-113	Only positive stain results were biopsied
Pizer M E, and Dubois D D,[70]	Va Med (1979) Nov 106 (11) 860-862	Only positive stain results were biopsied
Srivastava Y C and Mathur M N,[71]	Dent dig (1971)Jul 77(7) 400-403	Only positive stain results were biopsied
Niebel H H and Chomet[72]	JADA (1964) 68:801-806	Results only given for 11 out of 20 cases staining characteristics of the benign 'control' lesions is not given
Martin I C, Kerawala C J, Reed M[73]	Oral surg Oral med Oral Pathol Oral Radiol Endod, 1998 Apr, 85:4, 444-446	Study compared the staining properties in vitro not the utility of toluidine blue in screening
Szmeja Z, Szyfter W and Zielinski L[74]	Czas Stomatol (1984) Feb 37(2) 115-117	Study describes a technique and gives expert opinion
Economopoulou P and Papanicolaou S[75]	Odontostomatol proodos (1983) Jul-Aug 37(4) 197-202	Study describes a technique and gives expert opinion but no additional data
Israel H[76]	Compend Contin Ed Dent, 1986 Oct, 7-9, 616-618, 620, 622 passim	Study describes a technique and gives expert opinion but no additional data
Mashberg A and Samit A M[57]	Cancer journal for clinicians (1989) 39(2) 67-88	Study describes a technique and gives expert opinion but no additional data
Strong M S, Vaughan C W, and Incze J S[19]	Arch Otolaryngol (1968) 87:527-531	Study describes a technique and gives expert opinion but no additional data
Chuden H[77]	Med Welt (1969) Aug 30,35:1871-1872	Study describes a technique and gives expert opinion but no additional data
Russell T E[78]	J Acad Gen Dent (1974) Mar-Apr 22(2) 27	Study describes a technique and gives expert opinion but no additional data
Kaugars G E and Mehailescu W L[79]	Gen Dent 1989 Sep-Oct 37(5) 404-407	Study describes a technique and gives expert opinion but no additional data
Helsper J T[80]	CA Cancer L Clin (1972) May-Jun 22(3): 172-175	Study describes a technique and gives expert opinion but no additional data
Vaughan C W[81]	Otolaryngol Clin North Am (1972) Jun 5(2) 301-302	Study describes a technique and gives expert opinion but no additional data

Wong P N[23]	P N G Med J (1982) Dec 25(4) 278-280	Study describes a technique and gives expert opinion but no additional data
Perrin C, Vermelin, M., and Mullerr, Ch[82]	J Fr Otorhinolaryngol (1976) 25(2): 160-164	Study describes a technique and gives expert opinion but no additional data
Thomas J E and Bell W A[52]	Gen Dent 1985 Nov-Dec 33:6, 492-493	Study describes a technique and gives expert opinion but no additional data
Kovesi G[83]	Fogorv Sz (1998) Apr 91(4) 107-116	Study describes a technique and gives expert opinion but no additional data
Porter S R and Scully C[51]	Br Dent J 1998 Jul 25th 185:2 72-73	Study describes a technique and gives expert opinion but no additional data
Mela F[84]	Minerva stomatol (1967) Jan 16(1):35-39	Study describes a technique and gives expert opinion but no additional data
Amorin C and Caffarena M P[185]	An Fac Odontol (1990) Dec (26)21-26	Study is of use of toluidine blue to determine biopsy site not oral cancer screening
Shedd P S, Hukill P B, and Bahn S,[86]	Am J Surg (1965) 110: 631-634	Study is of use of toluidine blue to determine biopsy site not oral cancer screening
Sigurdson A[87]	Acta Otolaryngol (Stockh) (1973) Apr 75(4) 308	Study is of use of toluidine blue to determine biopsy site not oral cancer screening
Wiersma A C van der Waal I[88]	Ned Tijdschr Tandheelkd (1990) Oct 97(10) 431-432	Unobtainable
Gomez S M T Toranzo F J M, Martinez B J[89]	Rev ADM (1989) Feb 46(1) 29-35	Unobtainable
Iwano T[90]	Shikwa Gakuho (1965) Aug 65(8) 66-69	Unobtainable in translated format
Silverman S Jnr., Migliorati C[91]	Iowa Dent J 1992 Apr 78:2, 15-16	Unobtainable, but results appear to be included in later publication

10 References

[1] British Dental Association. Oral Cancer - Guidelines for early detection. *BDA Occasional Paper* 1998; 5.

[2] Jullien et al. Evaluation of a screening test for the early detection of oral cancer and pre-cancer. *Community Dental Health* 1995; **12**:3-7.

[3] Scully C. Clinical diagnostic methods for the detection of premalignant and early malignant oral lesions. *Community Dental Health* 1993; **10**(Suppl 1):43-52.

[4] Silverman S, Migliorati C, Barbosa J. Toluidine blue staining in the detection of oral precancerous and malignant lesions. *Oral Surg* 1984; **57**:379-382.

[5] Myers E N. The toluidine blue test in lesions of the oral cavity. *CA Cancer J Clin* 1970; **20**(3):134-139.

[6] Warnakulasuriya K A A S, Johnson N W. Sensitivity and specificity of OraScan toluidine blue mouthrinse in the detection of oral cancer and precancer. *J Oral Pathol Med* 1996; **25**:97-103.

[7] Warnakulasuriya K A A S, Johnson N W. Epidemiology and risk factors for oral cancer: Rising trends in Europe and possible effects of migration. *Int Dent J* 1996; **46**(Suppl 1):245-250.

[8] Cancer Research Campaign. Oral Cancer. *Factsheet* 1993; 14.1-14.3.

[9] Johnson N W, Warnakulasuriya K A A S. Epidemiology and aetiology of oral cancer in the United Kingdom *Community Dental Health* 1993; **10**(Suppl 1):13-29.

[10] Johnson N W. Orofacial Neoplasms: global epidemiology, risk factors and recommendations for research. *International Dental Journal* 1991; **41**:365-375.

[11] Blot W J, McLaughlin J N, Winn D M. et al Smoking and drinking in relation to oral and oropharyngeal cancer. *Cancer Res* 1988; **48**:3282-3287.

[12] Boffetta P, Mashberg A, Winklemann R. Carcinogenic effect of tobacco smoking and alcohol drinking on anatomical sites of the oral cavity and the oral pharynx. *Int J Cancer* 1992; **52**:530-533.

[13] IARC Alcoholic Beverages. IARC Monographs on the Evaluation of Carcinogenic risk of chemicals to humans. Vol. 44, pp153-259. Lyon: IARC, 1988.

[14] Scully C, Prime S S. Immunology of oral cancer. *In* Prabhu S R, Wilson D S, Daftary D K, Johnson N W (eds) *Oral diseases in the tropics*. Oxford: Oxford University Press, 1992.

[15] Silverman S. Early diagnosis of oral cancer. *Cancer* 1988; **62**(112):1796-1799.

[16] British Dental Association. Oral Cancer - Guidelines for early detection. *BDA Occasional Paper* 2000; 6

[17] Welsh Health Planning Forum. Oral Health Protocol for Investment in Health Gain Cardiff: Welsh Office NHS Directorate November 1992.

[18] Finlay P M, Dawson F, Robertson A G, Soutar D S. An evaluation of functional outcome after surgery and radiotherapy for oral cancer. *British Journal of Oral and Maxillofacial Surgery* 1992; **30**:14-17.

[19] Strong S, Vaughn C W, Incze J S. Toluidine blue in the management of carcinoma of the oral cavity. *Arch Otolaryngol* 1968; **87**:527-531.

[20] Stafford Miller Ltd, OraScreen Summary of Product Characteristics, February 1997.

[21] Zila Inc. Oral Cancer Detection System Endorsed by FDI World Dental Federation. Zila Inc Company Press Release www.zila.com accessed October 9th 1998.

[22] Richards D, Lawrence A, Thomas D. Toluidine blue. *BDA News* 1998; **11**(7):4.

[23] Wong P N C. In Vivo toluidine blue staining for the detection of oral cancer and pre-cancer. *Papua New Guinea Medical Journal* 1982; **25**(4):278-280.

[24] Vercellino V, Gandolfo S, Camoletto D, Griffa B, Mori C. Toluidine blue (tolonium chloride) in the early diagnosis of dysplasias and carcinomas of the oral mucosa. *Minerva Stomatol* 1985; **2**:257-261.

[25] Rosenberg D, Cretin S. Use of meta-analysis to evaluate tolonium chloride in oral cancer screening. *Oral Surg Oral Med Oral Pathol* 1989; **67**:621-627.

[26] Johnson N. Diagnosing oral cancer: can toluidine blue mouthwash help? *FDI World* 1998; **7**(2):22-26.

[27] Epstein J B, Oakley C, Millner A, Emerton S, van der Meij E. The utility of toluidine blue as a diagnostic aid in patients previously treated for upper oropharyngeal carcinoma. *Oral surg Oral med Oral Pathol Oral Radiol Endod* 1997; **83**(5):537-547.

[28] N Y Rosen I B, Cornish M, Edelson J. Detection of early oral cancer by toluidine blue. *J Can Dent Assoc* 1971; **37**(9):347-349.

[29] Mashberg A. Re-evaluation of toluidine blue application as a diagnostic adjunct in the detection of asymptomatic oral squamous carcinoma. *Cancer* 1980; **46**:758-763.

[30] Menasse J, Reychler H. Value of toluidine blue in the diagnosis of tumors of the oral cavity. *cta Stomatol Belg* 1984; **81**(2):145-151.

[31] Barellier P, Babin E, Louis M Y, Meunier-Guttin A. The use of toluidine blue in the diagnosis of neoplastic lesions of the oral cavity. *Rev Stomatol Chir Maxillofac* 1993; **94**(1):51-54.

[32] Epstein J B, Scully C, Spinelli J J. Toluidine blue and Lugol's iodine application in the assessment of oral malignant disease and lesions at risk of malignancy. *J Orol Pathol Med* 1992; **21**:160-163.

[33] Silverman S. Oral cancer. *Semin Dermatol* 1994; **13**(2):132-137.

[34] Vahidy N A, Zaidi S H, Jafarey. Toluidine blue test for detection of carcinoma of the oral cavity: an evaluation. *J Surg Oncol* 1972; **4**(5):434-438.

[35] Sigurdson A, Willen R. In vivo färgning med toluidine blue som hjälpmedel för lokalisation av provexcisionsställe vid misstänkt skivepitelcancer i munhålan. *Swed Dent J* 1975; **65**:117-126.

[36] Reddy C R, Ramulu C, Sundaseshwar B, Raju M V, Gopal R, Sharma R. Toluidine blue staining of oral cancer and precancerous lesions. *Indian J Med Res* 1973; **61**(8):1161-1164.

[37] Mashberg A. Final evaluation of tolonium chloride rinse for screening of high-risk patients with asymptomatic squamous carcinoma. *JADA* 1983; **106**:319-323.

[38] Feaver G P. Screening for oral cancer and precancer – The industrial experience. *Oral Oncology V Proceedings of 5th IOCC* 1997; 69-72.

[39] Feaver G P, Morrison T, Humphris G. A study to determine the acceptability in patients and dentists of toluidine blue in screening for oral cancer. *Primary Dental Care* 1999; **6**(2):45-50.

[40] Lerman,CE, Rimer BK. Psycosocial impact of cancer screening. (review) (53 refs). *Oncology* 1993;**7**(4):67-72.

[41] Johnson N W. Oral cancer - A worldwide problem. *FDI World* 1997; **6**(3):19-21.

[42] Johnson N W. What causes oral cancer. *FDI World* 1997; **6**(4):7-11.

[43] Johnson N W. How do we recognise and treat oral cancer and potentially malignant lesions. *FDI World* 1997; **6**(5):7-13.

[44] Johnson N W. Practical prevention. *FDI World* 1997; **6**(6):10-16.

[45] Johnson N W. Oral cancer screening. *FDI World* 1998; **7**(1):14-19.

[46] Chamberlain J. Evaluation of screening for cancer. *Community Dental Health* 1993; **10**(Suppl 1):5-10.

[47] Speight P M, Morgan P R. The natural history and pathology of oral cancer and precancer. *Community Dental Health* 1993; **10**(Suppl 1):31-41.

[48] Downer M C, Speight P M. Cost and value considerations in screening for oral cancer and precancer. *Community Dental Health* 1993; **10**(Suppl 1):71-78.

[49] Scully C. Oral pre-cancer: preventive and medical approaches to management. *Oral oncology. European Journal of Cancer* 1995; **31B**(1):16-26.

[50] Frame P S, Rosati C. Screening for oral cancer. Guide to Clinical Preventive Services, (16. Screening for oral cancer) Draft chapter update Ch 16.

[51] Porter S R, Scully C. Early detection of oral cancer in the practice. *Br Dent J* 1998; **185**(2):72-73.

[52] Thomas J E, Bell W A. Clinical aids for the detection of oral cancer in the elderly. *Gen Dent* 1985; **33**(6):492-493.

[53] Ligthelm A J, Weber A, van Niekerk P J, van Heerden W F. Diagnosis of oral cancer and precancer. *J Dent Assoc S Afr* 1989; Mar(Suppl 1):2-5.

[54] Miller R L, Simms B W, Gould A R, Toluidine blue staining for detection of oral premalignant lesions and carcinomas. *J Oral Pathol* 1988; **16**(2):73-78.

[55] Portugal L G, Wilson K M, Biddinger P W, Gluckman J L. The role of toluidine blue in assessing margin status after resection of squamous cell carcinomas of the upper aerodigestive system. *Arch otolaryngol Head Neck Surg* 1996; **122**(5):517-519.

[56] Epstein J B, Scully C. Assessing the patient at risk for squamous cell carcinoma. *Spec Care dentist* 1997; **17**(4):120-128.

[57] Mashberg A, Samit A M. Early detection, diagnosis and management of oral and oropharyngeal cancer. *Cancer journal for clinicians* 1989; **39**(2):67-88.

[58] Silverman S, Greenspan D. Early detection and diagnosis of oral cancer. *CDA J* 1985; **13**(5):29-33.

[59] Allen C M. Toluidine blue - proceed with caution? *Oral surg Oral med Oral Pathol oral pathol Oral radiol Endod* 1998; **86**(3):255.

[60] Sugarman J, Hamilton R, Graham W P et al. Diagnostic staining of neoplastic lesions with toluidine blue. *Arch Surg* 1970; **100**:240-243.

[61] Boden E. Early detection of oral cancer by application of four methods. *J of Oral Surg and Oral Path* 1967; **23**: 811-814.

[62] Helsper J, Sharp G S, Bullock W K. The mouthwash technic. A method of screening for intra-oral carcinoma. *Am J Surg* 1963; **106**:802-806.

[63] Matthews D C, Banting D W, Bohay R N. The use of diagnostic tests to aid clinical diagnosis. *J Can Dent Assoc* 1995; **61**(9):785-791.

[64] Srivastava Y C, Mathur M N. Toluidine blue staining test for early detection of oral cancer. *J Indian Dent Assoc* 1971; **43**(5):98-101.

[65] Pizer M E. Toluidine blue and lip lesions. *Va Dent J* 1975; **52**(4):53-54.

[66] Mashberg A. Tolonium (toluidine blue) rinse-a screening method for recognition of squamous carcinoma. *JAMA* 1981; **245**:2408-2410.

[67] Barrellier P, Rame J P, Chasle J, Souquieres Y. Detection of buccal cavity cancers with toluidine blue. *Rev Stomatol Chir Maxillofac* 1980; **81**(6):364-367.

[68] Barellier P, Rame P, Chasle J, Souquieres Y, Lecacheux B. Detection of cancer of the oral cavity using toluidine blue. *Actual odontostomatol (Paris)* 1982; **36**(137):87-92.

[69] Moyer G N, Taybos G M, Pelleu G B Jnr. Toluidine blue rinse: potential for benign lesions in detection of oral neoplasms. *J Oral Med* 1986; **41**(2):111-113.

[70] Pizer M E, Dubois D D. An assessment of toluidine blue for the diagnosis of lip lesions. *Va Med* 1979; **106**(11):860-862.

[71]Srivastava Y C, Mathur M N. Toluidine blue staining test for early detection of oral cancer: an adjunct to biopsy. *Dent dig* 1971; **77**(7):400-403.

[72] Niebel H H, Chomet B. In Vivo test for delineation of oral intraepithelial neoplastic change – preliminary report. *JADA* 1964; **68**:801-806.

[73] Martin I C, Kerawala C J, Reed M. The application of toluidine blue as a diagnostic adjunct in the detecton of epithelial dysplasia. *Oral surg Oral med Oral Pathol Oral Radiol Endod* 1998; **85**(4):444-446.

[74] Szmeja Z, Szyfter W, Zielinski L. The use of toluidine blue in the diagnosis of oral carcinoma. *Czas Stomatol* 1984; **37**(2):115-117.

[75] Economopoulou P, Papanicolaou S. The use of toluidine blue stain in the early diagnosis of oral cancer. *Odontostomatol proodos* 1983; **37**(4):197-202.

[76] Israel H. Early squamous cell carcinoma of the mouth - part II: diagnosis and management. *Compend Contin Ed Dent* 1986; **7-9**:616-622.

[77] Chuden H. Diagnosis of carcinoma within the oral cavity using a dye test. *Med Welt* 1969; **35**:1871-1872.

[78] Russell T E. Toluidine blue - its role in oral cancer detection. *J Acad Gen Dent* 1974; (2): 27.

[79] Kaugars G E, Mehailescu W L. Toluidine blue: its significance and application in dentistry. *Gen Dent* 1989; **37**(5):404-407.

[80] Helsper J T. Staining techniques: screening tests for oral cancer. *CA Cancer L Clin* 1972; **22**(3):172-175.

[81] Vaughan C W. Supravital staining for early diagnosis of carcinoma. *Otolaryngol Clin North Am* 1972; **5**(2):301-302.

[82] Perrin C, Vermelin M, Muller C. Epreuve au bleu de toluidine en carcinologie. *O R L J Fr Otorhinolaryngol* 1976; **25**(2):160-164.

[83] Kovesi G. OraTest. A new method for differentiating between benign and malignant oral tumors. *Fogorv Sz* 1998; **91**(4):107-116.

[84] Mela F. Technique for clinical delimitation of neoplastic changes in oral epithelium: in vivo staining with toluidine blue. *Minerva stomatol* 1967; **16**(1):35-39.

[85] Amorin C, Caffarena M P. White lesions of the oral mucosa - auxiliary diagnostic methods. *An Fac Odontol* 1990; **26**:21-26.

[86] Shedd P S, Hukill P B, Bahn S. In vivo staining properties of oral cancer. *Am J Surg* 1965; **110**:631-634.

[87] Sigurdson A. Toluidine staining as a malignancy test. *Acta Otolaryngol (Stockh)* 1973; **75**(4):308.

[88] Wiersma A C, van der Waal I. Toluidine blue staining for the detection of oral squamous carcinoma. *Ned Tijdschr Tandheelkd* 1990; **97**(10):431-432.

[89] Gomez S M T, Toranzo F J M, Martinez B J. Toluidine blue solution as a diagnostic aid in oropharyngeal cancer. *Rev ADM* 1989; **46**(1):29-35.

[90] Iwano T. An in-vivo staining test, using toluidine blue for oral cytodiagnosis. *Shikwa Gakuho* 1965; **65**(8):66-69.

[91] Silverman S Jnr, Migliorati C. Toluidine blue staining and early detection of oral precancerous and malignant lesions. *Iowa Dent J* 1992; **78**(2):15-16.