A separate copy of this form must accompany each policy submitted for review. Policies submitted without this form will not be considered for review.

<table>
<thead>
<tr>
<th>Plan: Aetna Better Health</th>
<th>Submission Date: 11/01/2019</th>
</tr>
</thead>
<tbody>
<tr>
<td>Policy Number: 0686</td>
<td>Effective Date: 10/02/2018</td>
</tr>
<tr>
<td>Policy Name: Oral and Esophageal Brush Biopsy</td>
<td></td>
</tr>
</tbody>
</table>

**Type of Submission – Check all that apply:**

- [ ] New Policy
- [X] Revised Policy*
- [ ] Annual Review – NoRevisions
- [ ] Statewide PDL

*All revisions to the policy must be highlighted using track changes throughout the document.

Please provide any clarifying information for the policy below:

CPB 0686 Oral and Esophageal Brush Biopsy

Clinical content was last revised on 10/02/2018. No additional non-clinical updates were made by Corporate since the last PARP submission.

Name of Authorized Individual (Please type or print): Dr. Bernard Lewin, M.D.

Signature of Authorized Individual: [Signature]

Revised July 22, 2019
Oral and Esophageal Brush Biopsy

Policy

*Please see amendment for Pennsylvania Medicaid

at the end of this CPB.

Aetna considers oral brush biopsy (OralCDx Brush Test), with or without melanoma-associated antigens A (MAGE-A) staining or glucose transporter (GLUT)-1 staining experimental and investigational for screening, diagnosis or surveillance of cancerous or pre-cancerous oral lesions because of insufficient evidence.

Aetna considers esophageal brush biopsy (WATS3D, formerly known as EndoCDx) experimental and investigational for screening, diagnosis or surveillance of cancerous or pre-cancerous esophageal lesions because of insufficient evidence.

Aetna considers DNA-image cytometry of brush biopsies for early detection of oral malignancy experimental and investigational because of insufficient evidence.

Aetna considers RNA-based oral brush biopsy for the detection and prognosis of oral malignancy experimental and investigational because of insufficient evidence.

See also

CPB 0760 - Oral Screening and Lesion Identification Systems (.//700_799/0760.html).

Background
Oral Brush Biopsy

The most definitive, accurate, and reliable method for diagnosing oral mucosal lesions is the scalpel biopsy. The oral brush biopsy coupled with computer-assisted analysis (OralCDx, OralScan Laboratories, Inc., Suffern, NY) has been developed as a technique for evaluating unexplained clinically detectable alterations of the surface epithelium of the oral mucosa whether cancer or pre-cancer is suspected (Sciubba et al, 2003). The goal of the oral brush biopsy is to provide a highly sensitive and specific technique that is less painful and simpler to perform than a scalpel or punch biopsy.

The oral brush biopsy, using a specially designed circular bristled brush, has been designed to access and sample all epithelial layers, including the basal cell layer and the most superficial aspects of the lamina propria (Sciubba et al, 2003). Thus, the cellular material obtained should include all epithelial layers in a disaggregated form spread over the surface of a glass slide.

The argument for oral brush biopsy raises 2 questions: (i) whether indications for oral mucosal biopsy should be expanded to include certain “benign-appearing” lesions, either in high-risk patients (e.g., current or former smokers, heavy drinkers), or in all persons regardless of risk, and (ii) what is the most effective and efficient method of biopsy of oral mucosal lesions.

Only 1 large-scale study has been published about the use of this technique in the oral cavity (Sciubba et al, 1999). The study reported that in 945 patients with oral mucosal lesions, the OralCDx had 100 % sensitivity and a zero false negative rate. The analysis, however, must be considered incomplete, as 618 of 945 brush samples, including 517 of the 699 negative brush samples (73.9 %), were not followed with definitive incisional biopsy for diagnostic confirmation. In addition, the investigators reported that 7 % of oral brush biopsy specimens were non-diagnostic (Sciubba et al, 1999), which is a much higher incidence than commonly seen with scalpel biopsy (Potter et al, 2002).

A smaller study by Svirsky et al (2002) compared oral brush biopsy results with scalpel biopsy and histology to determine the positive-predictive value (PPV) of an abnormal brush biopsy finding. Of 243 patients with abnormal brush biopsies, 93 proved positive for dysplasia (n = 79) or carcinoma (n = 14) and 150 were negative for either dysplasia or carcinoma. Therefore, the PPV of an abnormal brush biopsy was 38 % (93/243). This smaller study suffers from the same major weakness as the OralCDx Multicenter Study cited above (Sciubba et al, 1999), in that it does not adequately define the negative-predictive value (NPV) of the oral brush biopsy, because only a small proportion of normal brush biopsy results were followed by scalpel biopsy.
Christian (2002) reported on the results of oral brush biopsy in 930 dentists and oral hygienists who were screened for oral cancer while attending the American Dental Association annual session. Eighty-nine subjects (9.7 %) with 93 oral epithelial lesions were identified and evaluated by brush biopsy. Seven of the 93 oral lesions -- all benign appearing -- were determined to be “atypical” or “positive” on oral brush biopsy. Of these, three were diagnosed as precancerous by scalpel biopsy and histological evaluation. The study by Christian (2002) is of much weaker methodology than the OralCDx Multicenter study cited above. The study reported by Christian (2002) suffers from the same methodological weaknesses as the OralCDx Multicenter Study, as only some of the positive oral brush biopsy results and none of the negative results were followed by scalpel biopsy. In addition, the study by Christian (2002) was not a multi-center study, and the subjects (dentists and dental hygienists who volunteered for testing) is not representative of the patient group to which this test is directed (patients seen by their dentist for periodic check-ups and routine cleaning); this raises questions about the generalizability of the findings of this study.

There are some reports of significant rates of false negatives from brush biopsy. Potter et al (2003) examined all diagnoses of oral squamous cell carcinoma from a university oral pathology service over a 2-year period to determine if any had previously undergone brush biopsy reported to be “negative for epithelial abnormality”. Those cases identified were further investigated to determine the time lapse between brush biopsy and definitive tissue diagnosis. Potter et al (2003) found 4 of 115 squamous cell carcinomas that were reported to be negative on brush biopsy, a false negative rate of 3.5 %. The authors noted that, because not all 115 squamous cell carcinomas were preceded by a brush biopsy, that the false negative rate for the oral brush biopsy technique may actually be greater.

Although the study by Potter et al (2003) is limited by its retrospective design, it is informative in that it helps define the false negative rate of oral brush biopsy and the clinical consequences of falsely negative results.

Potter et al (2003) stated that the false-negative rate for the oral brush biopsy may be unacceptably high for a diagnostic test. These researchers explained: "It has been argued that a 3.5 % false-negative rate may be acceptable, particularly if one compares this result with a screening modality like mammography, which has a false-negative rate that varies from approximately 6 % to 25 %. This analogy, however, is flawed. Mammography is a screening test directed toward at-risk populations without known disease. The brush biopsy technique, on the other hand, is directed toward clinically obvious pathologic change, and thus a comparison of false-negative rates with mammography or other screening tests is inappropriate".
Potter et al (2003) went on to explain why the falsely negative results are unlikely to have resulted from an inaccuracy of the data reported by the authors. They concluded that “[i]t seems that the most likely probability may be that the technique may not be of adequate sensitivity to detect all clinically dysplastic or malignant lesions.” Furthermore, this study highlights the need to adequately evaluate the rate of false negatives with oral brush biopsy and its consequences.

Although it has been argued that the oral brush biopsy may provide earlier diagnosis of oral cancers and pre-malignant lesions, there are no clinical studies demonstrating this. The oral brush biopsy has been criticized for adding time and cost to the diagnosis of oral lesions without additional benefit to the patient. Because the brush biopsy detects only cellular atypia, positive oral brush biopsy results must be confirmed with a scalpel biopsy for definitive diagnosis. This results in the need for two procedures, rather than one, to establish a diagnosis. The need to perform two procedures may significantly delay diagnosis. In the study described above, Potter et al (2003) reported an “undeniably unacceptable” average delay in diagnosis of squamous cell carcinoma between brush and scalpel biopsies. In the 4 false negative oral brush biopsies identified, the average delay in diagnosis between negative oral brush biopsy and positive scalpel biopsy was 117.2 days (range of 5 to 292 days). The investigators stated that this delay “can be potentially disastrous.”

The oral brush biopsy technique may also delay diagnosis if the results are negative. If oral brush biopsy results are negative, no diagnosis is rendered, making it difficult to determine appropriate treatment or anticipate whether an additional procedure is necessary (Potter et al, 2003).

There is insufficient evidence to support the use of oral brush biopsy as a general screening technique for oral cancer or pre-malignant lesions. A Cochrane evidence review found that there is no evidence from prospective clinical trials that screening with brush biopsy reduces mortality (Kujan et al, 2005; Kujan et al, 2006). The investigators concluded that “no robust evidence exists” to suggest that brush biopsy for screening of oral cancer or potentially pre-malignant lesions is either beneficial or harmful.

Although the oral brush biopsy technique has been promoted as “painless” (OralScan Laboratories, 2001), there are no studies examining the pain elicited by oral brush biopsy or comparing this pain with that elicited by scalpel biopsy preceded by administration of local anesthetic (Potter et al, 2002). Given that an adequate oral brush biopsy sample should include all epithelial layers, the contention has been questioned that oral brush biopsy is completely “painless” or that it is substantially less painful than scalpel biopsy with local anesthetic.
The oral brush biopsy technique has also been promoted as easier to perform than scalpel biopsy, such that dentists who are unskilled at performing scalpel biopsy may be able to perform oral brush biopsy (Sciubba et al, 2003). However, Potter et al (2002) stated that “[f]ear of performing a scalpel biopsy, or inadequate training in its performance, should not be construed as an indication to perform other tests that will further delay completion of the definitive diagnostic test.”

The National Cancer Institute (2004) and the U.S. Preventive Services Task Force (2004) have not recommended routine screening for oral cancer. Although the American Dental Association has issued a contrary recommendation regarding oral cancer screening, the ADA recommendation does not endorse any specific biopsy method (ADA, 2003; Engber, 2002; Potter et al, 2002; Oral cancer campaign: Editors note, 2002). The OralCDx Oral Brush Biopsy has gained a seal of acceptance by the American Dental Association’s Council on Dental Therapeutics (ADA, 2001); however, the opinions and evaluations of medical professional organizations are considered according to the scientific quality of the evidence and supporting rationale. An American Dental Association Expert Panel on Screening for Oral Cancers (Rethman, et al., 2010) has concluded that, although transepithelial cytology has validity in identifying disaggregated dysplastic cells, the panel suggests surgical biopsy for definitive diagnosis.

Poate and colleagues (2004) reported that the sensitivity of detection of oral epithelial dysplasia or squamous cell carcinoma of the oral brush biopsy system was 71.4 %; while the specificity was 32 %. The PPV of an abnormal brush biopsy result (positive or atypical) was 44.1 %; while the NPV was 60 % (n = 112). These investigators concluded that not all potentially malignant disease is detected with this non-invasive investigative procedure. Scheifele et al (2004) concluded that further trials on OralCDx (computerized analysis of brush biopsies) as a screening tool of oral lesions are still necessary. van der Waal (2005) stated that the value of cytological examination, whether obtained by exfoliation of cells or by a brush technique, is somewhat questionable.

Furthermore, in a systematic review on the effectiveness of oral cancer screening, Kujan et al (2006) stated that no robust evidence exists that indicates whether other screening methods including toluidine blue, fluorescence imaging, or brush biopsy are either beneficial or harmful. These authors noted that further high-quality studies are needed to evaluate the effectiveness of these methods in screening oral cancer.

Driemel and colleagues (2008) assessed the performance of oral brush biopsies using standard morphological analysis and hematoxylin and eosin (HE) staining for detecting oral squamous cell carcinomas and their respective precursor lesions. Brush biopsies were obtained in 169
consecutive patients who underwent routine biopsies and histological examination for clinically suspicious oral lesions. Air-dried smears were processed by acetone fixation and HE staining. Cytological assessment used well-established criteria of atypia to classify the specimen as either tumor-negative (no signs of atypia, no malignant cells) or tumor-positive (malignant cells, any sign of atypia or doubtful cells). Despite a sufficient number of cells, a definite cytological diagnosis could not be established in 6 cases. According to the criteria specified above, these specimens were classified as tumor-positive. The cytological analysis identified 49 out of 62 oral malignancies (sensitivity 79 %). Seven out of 107 benign lesions were classified as false-positive (specificity 93 %). The positive and negative predictive values were each 88 %. The authors concluded that oral brush biopsies will identify only about 80 % of oral malignancies when the smears are processed by routine HE stains and are analyzed via standard morphological criteria. Thus, this technique should not be used for diagnostic proof or to exclude malignant cells in a lesion suspicious for cancer.

In a systematic review on adjunctive techniques for oral cancer examination and lesion diagnosis, Patton et al (2008) evaluated the effectiveness of toluidine blue (TB), ViziLite Plus with TBlue, ViziLite, Microlux DL, Orascoptic DK, VELscope and OralCDx brush biopsy. These investigators abstracted data relating to study design, sampling and characteristics of the study group, interventions, reported outcomes and diagnostic accuracy of adjunctive aids from 23 articles meeting inclusion and exclusion criteria, including availability of histological outcomes. The largest evidence base was for TB. A limited number of studies was available for ViziLite, ViziLite Plus with TBlue and OralCDx. Studies of VELscope have been conducted primarily to assess the margins of lesions in known oral pre-malignant and malignant lesions. The authors identified no studies of Microlux DL or Orascoptic DK. Study designs had various limitations in applicability to the general practice setting, including use of higher-risk populations and expert examiners. The authors concluded that there is evidence that TB is effective as a diagnostic adjunct for use in high-risk populations and suspicious mucosal lesions. OralCDx is useful in assessment of dysplastic changes in clinically suspicious lesions; however, there are insufficient data meeting the inclusion criteria to assess usefulness in innocuous mucosal lesions. Overall, there is insufficient evidence to support or refute the use of visually based examination adjuncts. Given the lack of evidence on the effectiveness of adjunctive cancer detection techniques in general dental practice settings, clinicians must rely on a thorough oral mucosal examination supported by specialty referral and/or tissue biopsy for oral pre-malignant and malignant lesions diagnosis.

In a cross-sectional study, Bhoopathi (2009) assessed the effectiveness of the oral brush biopsy technique as a diagnostic tool in detecting dysplastic oral lesions. Pathologic reports (n = 152) from the scalpel biopsies (tissue samples) in patients who previously tested either "positive" (n = 3) or "atypical" (n = 149) for dysplasia by brush biopsy (OralCDx) were evaluated. Information
on the age and sex of the patient, the site of the lesion, the brush biopsy results, and the histopathological diagnosis of the scalpel biopsy was collected. The positive predictive values (PPVs) for "abnormal," "atypical," and "positive" brush biopsies were determined. Overall, the PPV of an abnormal brush biopsy was only 7.9 % (95 % confidence interval [CI]: 4.2 % to 13.4 %), and the PPV of an "atypical" brush biopsy was 7.4 % (95 % CI: 3.7 % to 12.8 %). Of the 3 positive brush biopsies, only 1 was identified as dysplastic. The proportion of false-positive biopsy results was as high as 92.1 % (95 % CI: 86.6 % to 95.9 %). The author concluded that the OralCDx technique over-estimated dysplastic lesions and produced a high number of false-positive results. An evidence review by Juber and Huber (2012) noted limitations of this study, including: the "suspiciousness" of the lesions evaluated was not mentioned; the time frame between OralCDx brush test and scalpel biopsy was not defined; and the investigators were only able to calculate the PPV, because surgical biopsy was only used to evaluate lesions that had previously tested positive with the OralCDx test.

In a prospective, randomized, controlled study, Hohlweg-Majert and associates (2009) evaluated the advantage of computer-assisted analysis of the oral brush biopsy compared with synchronous scalpel biopsy in the early detection of oral lesions. Brush and scalpel biopsies were performed on 75 patients; 6 patients had to be excluded due to inadequate results, and 43 were shown to have dysplastic epithelium, 15 carcinoma, and 11 suspicious lesions. Thus, the sensitivity for the detection of abnormal cells by means of OralCDx was 52 %, specificity 29 %, and the PPV 63 %. According to these findings, the use of oral brush biopsy as a standardized, minimally invasive method of screening oral lesions should be re-considered.

Fedele (2009) stated that the World Health Organization has clearly indentified prevention and early detection as major objectives in the control of the oral cancer burden worldwide. At the present time, screening of oral cancer and its pre-invasive intra-epithelial stages, as well as its early detection, is still largely based on visual examination of the mouth. There is strong available evidence to suggest that visual inspection of the oral mucosa is effective in reducing mortality from oral cancer in individuals exposed to risk factors. Simple visual examination, however, is well known to be limited by subjective interpretation and by the potential, albeit rare, occurrence of dysplasia and early oral squamous cell carcinoma (OSCC) within areas of normal-looking oral mucosa. As a consequence, adjunctive techniques have been used to increase the ability to differentiate between benign abnormalities and dysplastic/malignant changes as well as to identify areas of dysplasia/early OSCC that are invisible to the naked eye. These include TB, brush biopsy, chemi-luminescence and tissue auto-fluorescence. The author reviewed the evidence supporting the efficacy of the afore-mentioned techniques in improving the identification of dysplastic/malignant changes of the oral mucosa; and concluded that available
studies have shown promising results, but strong evidence to support the use of oral cancer diagnostic aids is still lacking. The author stated that further research with clear objectives, well-defined population cohorts, and sound methodology is strongly needed.

Trullenque-Eriksson and colleagues (2009) analyzed publications related to examination techniques that might improve the visualization of suspicious lesions of the oral mucosa (ViziLite system and VELscope system) or that might facilitate the cytological identification of suspicious lesions (OralCDx). A literature search was performed, using the PubMed database and the key words "brush biopsy", "OralCDx", "ViziLite" and "Velscope", limiting the search to papers in English or Spanish published from 2002 to 2008. According to the results of studies identified, the ViziLite system has a sensitivity of 100 % and specificity ranging from 0 to 14.2 %, the VELscope system has a sensitivity of 98 to 100 % and specificity of 94 to 100 % and the Oral CDx system has a sensitivity of 71.4 to 100 % and specificity of 32 to 100 %. The authors concluded that clinical examination and histopathological confirmation with biopsy remain the gold standard for the detection of oral cancer. Moreover, they noted that more randomized controlled studies are needed to confirm the positive cost-benefit relationship and the true usefulness of these "new diagnostic methods" in oral mucosal pathology.

Toyoshima et al (2009) determined the detection of cytokeratin (CK) mRNA in OSCC cells and evaluated the CK relevance for OSCC diagnosis in a brush biopsy test. A total fo 52 pairs of OSCC cells and normal oral mucosal cells were obtained by brush biopsy from OSCC patients. Messenger RNA was extracted from cell pellets for real-time quantitative reverse transcriptase polymerase chain reaction (RT-qPCR). The over-expression levels of CK 17, CK 19 and CK 20 mRNA in OSCC cells were examined by SYBR green real-time RT-qPCR. Compared to normal mucosal cells, the over-expression of CK 17 mRNA was detectable in 40 OSCC cells (76.9 %), that of CK 19 mRNA in 19 (36.5 %), while that of CK 20 mRNA was not detectable. Compared with CK 19, the mean value of CK 17 mRNA expression level was significantly higher in all 52 patients (p < 0.02). Moreover, the value of CK 17 was significantly higher in T1 and T2 OSCC patients (p < 0.03, respectively), in patients without metastases of neck lymph nodes (p < 0.04), in stage I and stage II patients (p < 0.03 and p < 0.05, respectively) and in well-differentiated OSCC patients (p < 0.05). The authors concluded that brush biopsy properly serves for detection of CK mRNA using real-time RT-qPCR. This preliminary study demonstrated the CK 17 possibility for application; however, pivotal studies are needed to confirm CK 17 as a diagnostic marker of OSCC in a brush biopsy test.

Seoane Lestón and Diz Dios (2010) noted that conventional oral exploration (visual and palpation examination) constitutes the current gold standard for oral cancer screening, while biopsy and histopathological examination represents the indispensable study for the detection of cases in patients with an identified lesion. Imaging techniques (DPT, CT, and MRI) are
frequently used to supplement the clinical evaluation and staging of the primary tumor and regional lymph nodes. There are also a number of techniques that may contribute to the diagnosis of oral cancer: toluidine blue test has been used as a diagnostic aid for the detection of oral cancer over decades. Recently developed light-based detection systems have progressively improved in sensitivity and specificity, but multi-center controlled studies conducted by general dental practitioners must be designed in order to justify their application. The oral brush biopsy appears to over-estimate dysplastic lesions and produces a high number of false-positive results.

Remmerbach and colleagues (2011) stated that OSCCs often present as advanced tumors requiring aggressive local and regional therapy and result in significant functional impairment. The objective is to develop pre-symptomatic screening detection of OSCC by a brush biopsy method that is less invasive than the conventional biopsy for histology. Given the molecular heterogeneity of oral cancer, it is unlikely that even a panel of tumor markers would provide accurate diagnosis. Thus, approaches such as the matrix-assisted-laser-desorption/ionisation-time-of-flight-mass-spectrometry (MALDI-TOF-MS) allow several biomarkers or peptide profile patterns to be simultaneously assessed. Brush biopsies from 27 patients with histology-proven OSCCs plus 40 biopsies from 10 healthy controls were collected. MALDI-TOF-MS profiling was performed and additional statistical analysis of the data was used to classify the disease status according to the biological behaviour of the lesion. For classification a support vector machine algorithm was trained using spectra of brush biopsy samples to distinguish healthy control patients from patients with histology-proven OSCC. MALDI-TOF-MS was able to distinguish between healthy patients and OSCC patients with a sensitivity of 100 % and specificity of 93 %. The authors concluded that MALDI-TOF-MS in combination with sophisticated bioinformatic methods can distinguish OSCC patients from non-cancer controls with excellent sensitivity and specificity. Moreover, they stated that further improvement and validation of this approach is needed to determine its feasibility in aiding pre-symptomatic detection of head and neck cancer screening in routine daily practice.

In a cross-sectional study, Bhoopathi and Mascarenhas (2011) evaluated oral surgeons' effectiveness in diagnosing oral dysplastic lesions and compared it to OralCDx brush biopsy. In this study, the oral surgeon's ability to diagnose dysplasia among 152 consecutive cases (tissue samples) that had previously tested either "positive" (n = 3) or "atypical" (n = 149) for dysplasia by OralCDx brush biopsy was determined by calculating sensitivity, specificity, PPV, and negative predictive value using the scalpel biopsy as the gold standard. The PPV for oral surgeons and atypical brush biopsy was compared stratified by age, gender, and lesion site. The PPV, negative predictive value, sensitivity, and specificity for oral surgeons were 10.3 %, 100 %, 100 %, and 23.5 %, respectively. After controlling for age, gender, and lesion site, oral surgeons were 19 % to 58 % more likely to diagnose a dysplastic lesion compared to OralCDx brush...
biopsy. The authors concluded that oral surgeons’ effectiveness in diagnosing oral dysplastic lesions was slightly better than the OralCDx brush biopsy; hence, it is recommended that patients be referred to an oral surgeon for evaluation.

Mehrotra et al (2011) performed oral brush biopsies and scalpel biopsies in 85 consecutive patients presenting with an oral lesion deemed to be "minimally suspicious" by clinical examination. Of 79 patients with adequate brush biopsy samples with matching scalpel biopsies, 27 revealed histopathologic evidence of dysplasia or carcinoma, 26 of which were independently identified with the oral brush biopsy, with a sensitivity of 96.3 % (95 % CI: 87 % to 100 %). Fifty-two oral lesions did not reveal any histopathologic evidence of dysplasia or carcinoma and of these, brush biopsy reported 47 as "negative" and 5 as "atypical". The authors reported that the specificity of a "positive" brush biopsy result was 100 % (95 % CI: 93 % to 100 %); the specificity for "atypical" brush biopsy result was 90.4 % (95 % CI: 82 % to 97 %). The authors found that the positive predictive value of an abnormal oral brush biopsy was 84 % and the negative predictive value was 98 %. An evidence review (Juber and Huber, 2012) noted limitations of the study by Mehrota, including the fact that the study population has a higher tobacco use and higher prevalence of oral cancer compared to the United States; the definition "minimally suspicious" is open to interpretation; and the location of just under half the lesions was the buccal mucosa, which is considered a low risk area.

Balevi (2011) evaluated the performance of the OralCDx, the VELscope, and toluidine blue staining as clinical adjunctive diagnostic procedures in routine screening for oral cancer in dental practice. The author obtained the sensitivity and specificity for each device from a review of the literature. The author calculated the PPV and false positive rate, based on three clinical screening scenario, using Bayes’ Theorem. The author found that, under three clinical scenarios (screening the general population, screening only adults (40 years or older) and screening adults that present with intraoral visible lesions), The author found that the VELscope produced the highest PPV’s of 1.27%, 2.53% and 8.11%, respectively, indicating a false positive rate of between 91.89% and 98.73%. The author concluded that the VELscope, OralCDx and toluidine blue staining have high false positive rates when they are used to screen routinely for oral cancer.

Seijas-Naya and colleagues (2012) evaluated the effectiveness of the brush biopsy technique using OralCDx (OralScan Laboratories Inc., Suffern, NY) as a new method for early diagnosis and control of a "potentially malignant disorder" such as oral leukoplakia. These investigators performed a study in which samples were taken using OralCDx on 24 patients who visited the Master of Oral Medicine, Oral Surgery and Implantology of the University of Santiago de Compostela between February 2009 and May 2010. These patients presented clinical and histological lesions that were consistent with oral leukoplakia. These researchers evaluated the
relationship between the keratinization degree of the lesions and cell representation; the diagnosis obtained through OralCDx and biopsies; and sensitivity, specificity, PPV and NPV. The average age of patients was 62.38 years and 50% of them were men. The kappa coefficient relating keratinization of lesions and cell representation was 0.33, the OralCDx - biopsy diagnostic rate reached a kappa value of 0.66, recording 72.7% sensitivity and 92.3% specificity, PPV was 88.8%, while NPV reached 80%. The authors concluded that cytology sampling with OralCDx showed high sensitivity and specificity values, which make it a good tool for monitoring oral leukoplakia, but nowadays the most reliable method that allows confirmation of the exact diagnosis of the lesions and their anatomical and pathological characteristics is still conventional biopsy using a surgical scalpel.

Huber (2012) stated that during the past 10 years, several adjunctive aids have been introduced to the marketplace with the promoted goal of improving the dental practitioner's ability to screen for and identify oral premalignant and malignant lesions (OPMLs). These products include the OralCDx Brush Test, ViziLite Plus with TBlue, Microlux, VELscope Vx, Sapphire Plus, Identafi, and the DOE Oral Exam System. They are all marketed as aids for the clinician to use in addition to, not in lieu of, the accomplishment of a conventional oral examination (COE). Studies addressing the effectiveness of these products when used in the general practice setting to screen for OPMLs are limited and conflicting. The ability to discriminate between truly dangerous OPML against the milieu of benign mucosal lesions remains a concern and further research is needed to ascertain the true value of these products as marketed to the general practitioner. The attainment of a complete history and the accomplishment of a thorough and disciplined COE remains the foundation upon which the practitioner evaluates the patient for OPMLs. Findings deemed suspicious or equivocal should be referred to an expert for further assessment or undergo immediate biopsy, while findings deemed innocuous should be re-evaluated within 2 weeks and referred to an expert for further assessment or undergo biopsy if still present.

An evidence review by Juber and Huber (2012) concluded: “The OralCDx brush test may have the ability to detect dysplasia in innocuous looking oral lesions, but recently published reports continue to have contrasting results pertaining to its overall clinical applicability. Surgical biopsy is still considered the gold standard and should be used to achieve the correct detection and diagnosis”.

In a Cochrane review, Brocklehurst et al (2010) evaluated the effectiveness of current screening methods in decreasing oral cancer mortality. The following electronic databases were searched: the Cochrane Oral Health Group Trials Register (to May 20, 2010), the Cochrane Central Register of Controlled Trials (CENTRAL) (The Cochrane Library 2010, Issue 2), MEDLINE via OVID (1950 to May 20, 2010), EMBASE via OVID (1980 to May 20, 2010) and CANCERLIT via
PubMed (1950 to May 20, 2010). There were no restrictions regarding language or date of publication. Randomized controlled trials (RCTs) of screening for oral cancer or potentially malignant disorders using visual examination, toluidine blue, fluorescence imaging or brush biopsy were selected for review. The original review identified 1,389 citations and this update identified an additional 330 studies, highlighting 1,719 studies for consideration. Only 1 study met the inclusion criteria and validity assessment, data extraction and statistics evaluation were undertaken by 6 independent review authors. One 9-year RCT has been included (n = 13 clusters: 191,873 participants). There was no statistically significant difference in the age-standardized oral cancer mortality rates for the screened group (16.4/100,000 person-years) and the control group (20.7/100,000 person-years). A 43 % reduction in mortality was reported between the intervention cohort (29.9/100,000 person-years) and the control arm (45.4/100,000) for high-risk individuals who used tobacco or alcohol or both, which was statistically significant. However, this study had a number of methodological weaknesses and the associated risk of bias was high. The authors concluded that although there is evidence that a visual examination as part of a population based screening program reduced the mortality rate of oral cancer in high-risk individuals, while producing a stage shift and improvement in survival rates across the population as a whole, the evidence is limited to 1 study and was associated with a high risk of bias. This was compounded by the fact that the effect of cluster randomization was not accounted for in the analysis. Furthermore, no robust evidence was identified to support the use of other adjunctive technologies like toluidine blue, brush biopsy or fluorescence imaging within a primary care environment. The authors concluded that further RCTs are recommended to assess the efficacy, effectiveness and cost-effectiveness of a visual examination as part of a population based screening program.

The HealthPartners Dental Group and Clinics’ (Minneapolis, MN) oral cancer guideline (2012) stated that “The brush "biopsy", an exfoliative cytologic technique was developed as a means of harvesting a transepithelial sample of cells from an oral surface lesion without having to anesthetize and remove an actual tissue sample (i.e., biopsy specimen) with the scalpel. This, too, is simply a screening tool similar to one that has been used in gynecology for a number of years and is known as a Papanicolaou ("Pap") smear. Many dysplastic lesions are first identified by histopathologically evident changes in the morphology of cells in the epithelial basal cell layer. Therefore, in order to be of use, the brush must obtain cells from this layer. This test can be used as a preliminary tool in helping to confirm a clinician's suspicion regarding an oral lesion. It must be emphasized that a brush "biopsy" sample analysis does not and cannot provide a definitive diagnosis for oral cancer. A tissue biopsy must be obtained to confirm the diagnosis".
In a Cochrane review, Brocklehurst et al (2013) evaluated the effectiveness of current screening methods in decreasing oral cancer mortality. These investigators searched the following electronic databases: the Cochrane Oral Health Group’s Trials Register (to July 22, 2013), the Cochrane Central Register of Controlled Trials (CENTRAL) (The Cochrane Library 2013, Issue 6), MEDLINE via OVID (1946 to July 22, 2013), EMBASE via OVID (1980 to July 22, 2013) and CANCERLIT via PubMed (1950 to July 22, 2013). There were no restrictions on language in the search of the electronic databases. Randomized controlled trials of screening for oral cancer or potentially malignant disorders using visual examination, toluidine blue, fluorescence imaging or brush biopsy were selected for analysis. Two review authors screened the results of the searches against inclusion criteria, extracted data and assessed risk of bias independently and in duplicate. They used mean differences (MDs) and 95 % CIs for continuous data and risk ratios (RRs) with 95 % CIs for dichotomous data. Meta-analyses would have been undertaken using a random-effects model if the number of studies had exceeded a minimum of 3. Study authors were contacted where possible and where deemed necessary for missing information. A total of 3,239 citations were identified through the searches. Only 1 RCT, with 15-year follow-up met the inclusion criteria (n = 13 clusters: 191,873 participants). There was no statistically significant difference in the oral cancer mortality rates for the screened group (15.4/100,000 person-years) and the control group (17.1/100,000 person-years), with a RR of 0.88 (95 % CI: 0.69 to 1.2). A 24 % reduction in mortality was reported between the screening group (30/100,000 person-years) and the control group (39.0/100,000) for high-risk individuals who used tobacco or alcohol or both, which was statistically significant (RR 0.76; 95 % CI: 0.60 to 0.97). No statistically significant differences were found for incidence rates. A statistically significant reduction in the number of individuals diagnosed with stage III or worse oral cancer was found for those in the screening group (RR 0.81; 95 % CI: 0.70 to 0.93). No harms were reported. The study was assessed as at high-risk of bias. The authors concluded that there is evidence that a visual examination as part of a population-based screening program reduces the mortality rate of oral cancer in high-risk individuals. In addition, there is a stage shift and improvement in survival rates across the population as a whole. However, the evidence is limited to 1 study, which has a high-risk of bias and did not account for the effect of cluster randomization in the analysis. They stated that there was no evidence to support the use of adjunctive technologies like toluidine blue, brush biopsy or fluorescence imaging as a screening tool to reduce oral cancer mortality; further RCTs are recommended to evaluate the efficacy and cost-effectiveness of a visual examination as part of a population-based screening program in low-, middle- and high-income countries.

Omar (2015) examined the validity of using advanced non-invasive technologies in diagnosis of OSCC by identifying and evaluating relevant published reports. MEDLINE, EMBASE, and CINAHL were searched to identify clinical trials and other information published between 1990 and June 10, 2014; the searches of MEDLINE and EMBASE were updated to November 2014.
Studies of non-invasive methods of diagnosing OSCC, including oral brush biopsy, optical biopsy, saliva-based oral cancer diagnosis, and others were included. Data were abstracted and evaluated in duplicate for possible relevance on 2 occasions at an interval of 2 months before being included or excluded. This study identified 163 studies of non-invasive methods for diagnosing OSCC that met the inclusion criteria. These included six studies of oral brush biopsy, 42 of saliva-based oral diagnosis, and 115 of optical biopsy. Sixty nine of these studies were assessed by the modified version of the QUADAS instrument. Saliva-based oral cancer diagnosis and optical biopsy were found to be promising non-invasive methods for diagnosing OSCC. The authors concluded that it is clear that screening for and early detection of cancer and pre-cancerous lesions have the potential to reduce the morbidity and mortality of this disease. Advances in technologies for saliva-based oral diagnosis and optical biopsy are promising pathways for the future development of more effective non-invasive methods for diagnosing OSCC that are easy to perform clinically in primary care settings.

In a Cochrane review, Macey and colleagues (2015) estimated the diagnostic accuracy of index tests for the detection of oral cancer and potentially malignant disorders (PMD) of the lip and oral cavity, in people presenting with clinically evident lesions. They also estimated the relative accuracy of the different index tests. The electronic databases were searched on April 30, 2013. These investigators searched Medline (OVID) (1946 to April 2013) and 4 other electronic databases (the Cochrane Diagnostic Test Accuracy Studies Register, the Cochrane Oral Health Group's Trials Register, Embase (OVID) and Medion (Ovid)). There were no restrictions on language in the searches of the electronic databases. They conducted citation searches and screened reference lists of included studies for additional references. These researchers selected studies that reported the diagnostic test accuracy of the following index tests when used as an adjunct to conventional oral examination in detecting PMD or oral squamous cell carcinoma of the lip or oral cavity: blood or saliva analysis (which test for the presence of biomarkers in blood or saliva), light-based detection and oral spectroscopy, oral cytology, and vital staining. Two review authors independently screened titles and abstracts for relevance. Eligibility, data extraction and quality assessment were carried out by at least 2 authors, independently and in duplicate. Studies were assessed for methodological quality using QUADAS-2. Meta-analysis was used to combine the results of studies for each index test using the bivariate approach to estimate the expected values of sensitivity and specificity. The authors included 41 studies, recruiting 4,002 participants, in this review. These studies evaluated the diagnostic accuracy of conventional oral examination with: light-based detection or oral spectroscopy (13 studies), oral cytology (13 studies), and vital staining (14 studies). Six studies assessed 2 combined index tests. There were no eligible diagnostic accuracy studies evaluating blood or salivary sample analysis. The summary estimates for vital staining obtained from the meta-analysis were sensitivity of 0.84 (95 % CI: 0.74 to 0.90) with specificity of 0.70 (0.59 to 0.79), with 14 studies were included in the meta-analysis. For cytology, sensitivity was 0.91
(0.81 to 0.96) and specificity was 0.91 (0.81 to 0.95) with 12 studies included in the meta-analysis. For light-based detection, sensitivity was 0.91 (0.77 to 0.97) and specificity was 0.58 (0.22 to 0.87) with 11 studies included in the meta-analysis. The relative test accuracy was assessed by adding covariates to the bivariate analysis, no difference in model fit was observed. The authors concluded that the overall quality of the included studies was poor. None of the adjunctive tests can be recommended as a replacement for the currently used standard of a scalp biopsy and histological assessment. They stated that given the relatively high values of the summary estimates of sensitivity and specificity for cytology, this would appear to offer the most potential. Moreover, they stated that combined adjunctive tests involving cytology warrant further investigation.

Kujan and associates (2018) examined the applicability of liquid-based cytology (LBC) using an innovative oral brush, Orcellex. A total of 50 healthy volunteers were recruited. From each subject, 4 samples were collected using "Orcellex" from apparently normal oral mucosal sites. A plastic spatula was also used to obtain an additional sample. Data on the tolerability and acceptability of the Orcellex were collected from the subjects, together with assessments of the adequacy of LBC slide preparations for cellularity, preparation quality, and the types of cells observed. The Orcellex brush was well-accepted by the volunteers, who reported relatively little pain. Orcellex brush LBC preparations were of good quality in terms of cell morphology and staining, with a clean background. Only 2 smears (2/200; 1%) were found to be inadequate due to low cellularity. All of the plastic spatula LBC preparations were inadequate. Representative cells from all layers of the different oral epithelia examined were documented. The authors concluded that oral liquid-based cytology using the Orcellex brush may have considerable potential for early detection of oral cancer and potentially malignant disorders. Moreover, these investigators stated that further research is needed to compare the yield obtained using the Orcellex brush with other collection tools and devices.

In a systematic review, H Alsarraf and colleagues (2018) analyzed the published evidence for the use of oral brush cytology for the early detection of oral cancer and oral potentially malignant disorders (OPMDs). Literature was systematically searched through several databases: Medline, Embase, PubMed, SCOPUS, Cochrane Library, and Web of Science. Additional review was performed through cross-checks on the bibliographies of selected articles. The inclusion criteria involved studies assessing the utility of oral brush cytology on human tissues and its applications in the diagnosis, screening, or surveillance of oral cancer or OPMDs. The search strategy resulted in 343 abstracts or full-text articles, of which 36 met the inclusion criteria. The year of publication ranged from 1994 to 2017, and a total of 4,302 samples from OPMDs, OSSC, and healthy controls have been investigated. Baby toothbrush, cytobrush, OralCDx, and Orcellex are the brushes that were used to obtain trans-epithelial mucosal samples for conventional and liquid-based cytology evaluation. The authors concluded that the findings from

www.aetna.com/cpb/medical/data/600_699/0686.html

Proprietary 15/33
this study indicated that meaningful evidence-based recommendations for the implementation of a minimally invasive technique to be utilized as an adjunctive tool for screening and early detection of oral cancer and OPMDs are complicated from the reported studies in the literature. They stated that there is need for well-designed clinical studies to evaluate the accuracy of oral brush cytology utilizing validated cytological assessment criteria for the diagnosis and prediction of OPMDs.

**Melanoma-Associated Antigens A (MAGE-A) Staining:**

Hartmann and colleagues (2015) stated that in oral cancer and in other tumor entities, melanoma-associated antigens are present. These antigens contribute to tumor progression and poor prognosis, and reduce the cytotoxicity of antineoplastic drugs. These researchers examined the diagnostic potential of these antigens in combination with oral brush biopsies. They analyzed 72 oral brush biopsy specimens for melanoma-associated antigens A (MAGE-A) expression by immunocytologic staining with the MAGE-A 57B antibody. A total of 24 healthy specimens, 15 lichen ruber cases, 18 leukoplakia cases, and 15 invasive carcinomas were studied. Incisional biopsy served as the gold standard. In total, 66 of 72 specimens (91.6 %) could be assessed; 12 of 15 (80 %) carcinomas stained positive for MAGE-A. MAGE-A staining was detected in 4 of 51 non-malignant specimens, resulting in a false-positive rate of 7.8 %. However, MAGE-A positive staining was significantly correlated with oral squamous cell carcinoma (p < 0.0005). Sensitivity and specificity for MAGE-A staining and carcinoma were 80 % and 92.2 %, respectively. The diagnostic accuracy was 89.4 %. The authors concluded that the findings of this study indicated that oral brush biopsy with MAGE-A staining serves as an additional tool for use in oral cancer diagnosis. They stated that these findings might help to facilitate an easier and more representative surveillance of the mucosa, particularly for large areas of altered mucosa.

**Glucose Transporter (GLUT)-1 Staining:**

Brands and colleagues (2017) evaluated the sensitivity and specificity of an oral brush biopsy in combination with glucose transporter (GLUT)-1 staining in identifying pre-malignant and malignant lesions. A total of 72 patients were included in the study; they were divided into 4 diagnostic subgroups (24 healthy, 15 carcinoma, 18 leukoplakia, 15 oral lichen planus). Oral brush biopsies were taken and analyzed for GLUT-1 expression by immunocytologic staining. Incisional biopsy served as the gold standard; 12 (80 %) of the 15 carcinomas, 9 (50 %) of the 18 leukoplakia, 9 (60 %) of the 15 oral lichen planus, and none of the healthy specimens stained positive for GLUT-1. This resulted in a sensitivity rate of 80 % and a specificity rate of 68.42 %. Diagnostic accuracy was 70.83 % based on the correct diagnoses in 51 of 72 patients. The authors concluded that oral brush biopsy can easily be performed throughout the entire oral
cavity, is non-invasive, and showed high sensitivity and specificity rates with conventional
cytology or computer-assisted analysis. Moreover, they noted that the significance of GLUT-1-
specific staining with an oral brush biopsy is more limited than expected, but could be used as an
additional tool in detecting malignant transformation in the oral cavity. This was a relatively small
study (n = 48 for carcinomas/leukoplakia/oral lichen planus); and the sensitivity, specificity, and
diagnostic accuracy of oral brush biopsy with GLUT-1 staining were 80 %, 68.42 %, and 70.83
%, respectively.

Esophageal Brush Biopsy (WATS3D, formerly known as EndoCDx)

WATS3D, formerly known as EndoCDx, is a computer-assisted biopsy adjunct to standard
forceps biopsy of the esophagus which increases the tissue area sampled and therefore,
increases the yield of patients identified with abnormality in the esophagus. Unlike standard
cytology brushes that are typically soft and primarily designed to gently remove spontaneously
exfoliated squamous cells in the esophagus, the WATS3D Biopsy is specifically designed to
consistently sample deeper layers of the more firmly attached glandular epithelium found in
Barrett's esophagus.

Evidence for esophageal brush biopsy in Barrett's esophagus screening or surveillance is limited
to diagnostic yield. However, whether this increase in diagnostic yield leads to improved patient
outcomes is unclear.

Johanson et al (2011) conducted a study to evaluate the diagnostic yield of computer assisted
analysis of an abrasive, transepithelial brush biopsy as an adjunct to forceps biopsy to increase
detection of Barret's esophagus and esophageal dysplasia. This was a multi-center prospective
trial of patients being screened for Barrett's esophagus and esophageal dysplasia. Each patient
had 2 brush biopsies and then random 4-quadrant forceps biopsies every 1 to 2 cm of the
esophagus. All brush biopsies were examined with computer assistance by pathologists at CDx
Laboratories, and all forceps biopsies were examined by the investigators' local pathologists. Of
1,266 patients enrolled, 363 were diagnosed with Barrett's esophagus by forceps biopsy alone
and 146 additional cases of Barrett's esophagus were identified by adding brush biopsy. Among
a subset of 848 patients with gastroesophageal reflux disease and no prior history of Barrett's
esophagus, 150 were diagnosed with forceps biopsy and an additional 105 patients were
diagnosed with Barrett's esophagus with brush biopsy. Dysplasia was diagnosed in 16 patients
by forceps biopsy (FB) alone, with an additional 14 cases detected by adding BB. The authors
concluded that these results suggested that adjunctive computer-assisted analysis of an
abrasive brush biopsy has the potential to substantially improve the detection of Barrett's
esophagus and dysplasia in screening populations.
Anandasabapathy et al (2011) studied whether the detection of dysplasia is improved by adding computer-assisted brush biopsy (EndoCDx) to 4-quadrant biopsy protocol. Patients with a history of Barrett's esophagus with dysplasia scheduled for endoscopic surveillance were recruited from 4 academic medical centers. Patients underwent brush biopsy followed by 4-quadrant biopsy every 1 to 2 cm. The results from brush and forceps biopsy were reviewed independently by pathologists blinded to the other's results. Among 151 patients enrolled (124 men, 27 women; mean age of 65 years), 117 (77.5%) had forceps and brush-biopsy specimens adequate for interpretation. The mean number of forceps biopsies was 11.9 (median of 10, range of 2 to 40) and brush biopsies was 2.0 (median of 2, range of 1 to 4). The overall yield of forceps alone was 38 positive cases. Brush biopsy added an additional 16 positive cases. There were no significant differences in results among different centers, between standard versus jumbo forceps, or between forceps biopsies taken every 1 cm versus every 2 cm. The authors concluded that these data suggested that computer-assisted brush biopsy is a useful adjunct to standard endoscopic surveillance regimens for the identification of dysplasia in Barrett's esophagus.

Current guidelines on Barrett's esophagus from leading medical professional organizations have no recommendation for brush biopsy for screening or surveillance (AGA, 2011; ASGE, 2012; Fitzgerald et al, 2014; Tham, 2015; Whiteman et al, 2015).

Kern and colleagues (2016) noted that patients with eosinophilic esophagitis (EoE) undergo multiple endoscopies with biopsy for both diagnosis and assessment of treatment response, which is inconvenient and costly. Brush cytology has been examined in Barrett's esophagus (BE) to reduce the need for repeated endoscopic biopsies. In a prospective study, these researchers evaluated the ability of brush cytology to detect mucosal eosinophilia in patients with EoE. This study included adults with untreated and treated esophageal eosinophilia undergoing endoscopy at a tertiary-care center. Participants received paired brushings and biopsies at the proximal and distal esophagus. A blinded pathologist quantified the number of eosinophils and epithelial cells per high-power field (hpf) on the cytology slides. The ratio of eosinophils/epithelial cells was used to normalize the cytology specimens for density of cells collected. The main outcome measures were sensitivity and specificity of brush cytology, and correlation between cytology and histology. A total of 28 patients were enrolled in this trial. The average age of the cohort was 37.7 ± 10.4 years; 75% of subjects were male. The sensitivity of cytology was 67 to 69% at the proximal esophagus and 70 to 72% at the distal esophagus. The specificity was 61 to 67% proximally and 70 to 75% distally. Histology was not significantly correlated with the max ratio of eosinophils/epithelial cells per hpf or the absolute number of eosinophils on cytology slides. The authors concluded that cytology using esophageal brushing has limited sensitivity and specificity for the detection of esophageal mucosal eosinophilia. The presence of exudates on endoscopy increased the detection of eosinophilia, which could make cytology useful in
pediatric EoE, which often has a more exudative presentation. Moreover, they stated that diagnostic yield may improve with alternative acquisition techniques or the incorporation of eosinophil degranulation proteins.

Furthermore, National Comprehensive Cancer Network’s clinical practice guideline on “Esophageal and esophagogastric junction cancers” (Version 1.2017) does not mention computer-assisted brush-biopsy analysis as a management tool.

Vennalaganti and co-workers (2015) examined inter-observer agreement among pathologists in the diagnosis of Barrett’s esophagus (BE)-associated dysplasia using the wide-area transepithelial sampling (WATS) computer-assisted analysis technique; WATS slides with varying degrees of BE dysplasia were randomly selected and distributed to 4 pathologists. Each pathologist graded the slides as non-dysplastic BE (NDBE), low-grade dysplasia (LGD), or high-grade dysplasia/esophageal adenocarcinoma (HGD/EAC) and completed a standardized case report form (CRF) for each slide. A total of 149 BE slides were evaluated in a blinded manner by 4 pathologists. The slides included the following: ND (n = 109), LGD, and HGD/EAC (n = 40). The overall mean kappa value for all 3 diagnoses for the 4 observers was calculated at 0.86 (95 % confidence interval [CI]: 0.75 to 0.97). The kappa values (95 % CI) for HGD/EAC, indefinite for dysplasia (IND)/LGD, and NDBE were 0.95 (0.88 to 0.99), 0.74 (0.61 to 0.85), and 0.88 (0.81 to 0.94), respectively. The authors concluded that diagnosis of BE and associated dysplasia using the WATS technique had very high inter-observer agreement. This appeared to be significantly higher as compared with previously published data using standard histopathology.

Vennalaganti and colleagues (2018) noted that WATS with computer-assisted 3-D analysis is a sampling technique that combines abrasive brushing of the BE mucosa followed by neural network analysis to high-light abnormal-appearing cells. These researchers performed a randomized trial of referred BE patients undergoing surveillance at 16 medical centers. Subjects received either biopsy sampling followed by WATS or WATS followed by biopsy sampling. The primary outcome was rate of detection of HGD/EAC using WATS in conjunction with biopsy sampling compared with biopsy sampling alone using standard histopathologic criteria. Secondary aims included evaluating neoplasia detection rates based on the procedure order (WATS versus biopsy sampling first), of each procedure separately, and the additional time required for WATS. A total of 160 patients (mean age of 63.4 years; 76 % men; 95 % white) completed the trial. The median circumferential and maximal BE extents were 1.0 cm (inter-quartile range [IQR]: 0.0 to 5.0) and 4.0 cm (IQR, 2.0 to 8.0), respectively. The diagnostic yield for biopsy sampling alone was as follows: HGD/EAC, 7 (4.4 %); LGD, 28 (17.5 NDBE, 106 (66.25 %); and no BE, 19 (11.9 %). The addition of WATS to biopsy sampling yielded an additional 23 cases of HGD/EAC (absolute increase, 14.4 %; 95 % CI: 7.5 % to 21.2 %). Among these 23 patients, 11 were classified by biopsy sampling as NDBE and 12 as LGD/IND; 14
received biopsy sampling first and 9 WATS first (not significant) and most (n = 21; 91.7 %) had a prior dysplasia history; WATS added an average of 4.5 mins to the procedure. The authors concluded that results of this multi-center, prospective, randomized trial demonstrated that the use of WATS in a referral BE population increased the detection of HGD/EAC.

In a multi-center, prospective trial, Gross and associates (2018) examined if WATS with 3-D computer-assisted analysis used adjunctively with forceps biopsy (FB) can increase the detection of BE and esophageal dysplasia (ED). Patients screened for suspected BE and those with known BE undergoing surveillance were enrolled. Patients at 25 community-based practices underwent WATS adjunctively to targeted FB and random 4-quadrant FB. Of 4,203 patients, 594 were diagnosed with BE by FB alone, and 493 additional cases were detected by adding WATS, increasing the overall detection of BE by 83 % (493/594, 95 % CI: 74 % to 93 %); LGD was diagnosed in 26 patients by FB alone, and 23 additional cases were detected by adding WATS, increasing the detection of LGD by 88.5 % (23/26, 95 % CI: 48 % to 160 %). The authors concluded that adjunctive use of WATS to FB significantly improved the detection of both BE and ED. Sampling error, an inherent limitation associated with screening and surveillance, could be improved with WATS allowing better informed decisions to be made about the management and subsequent treatment of these patients.

The authors stated that this study had several drawbacks. The order of WATS and FB was not randomized. All the FB samples were obtained after WATS sampling. However, the order of performing FB and WATS has been demonstrated in one study to not have any impact on the final results. All specimens in this study were analyzed by 2 different sets of pathologists (WATS and FB) who were blinded from each other’s findings. Although this analysis was conducted at a single-central laboratory, the data were comparable to studies in which WATS was analyzed at one laboratory and FB at another. Although all investigators were instructed to sample only the tubular esophagus, this requirement was not independently monitored. Furthermore, it may be difficult for gastroenterologists to definitively identify where a biopsy specimen may have come from in relation to the gastro-esophageal junction (GEJ) as there are no universally accepted validated landmarks that demarcate the distal extent of the esophagus. Studies in BE patients have used the proximal extent of the gastric folds as the landmark for the GEJ despite the fact that it is a dynamic structure whose position changes with respiration, gut motor activity, and the degree of distention of the esophagus and stomach. Thus, given these limitations, it was likely that some patients in this study diagnosed with BE, especially those with ultra-short BE, had in fact intestinal metaplasia of the cardia, which was thought to carry significantly less risk of cancer development than traditional BE. These drawbacks may be overcome by ensuring gastroenterologists follow published guidelines for sampling suspected BE, and accurately assessing the extent of BE on endoscopy. Measuring both the circumferential extent (C) and the maximum extent (M) (Prague C and M criteria) increased the accuracy in determining the length
of intestinal metaplasia in the columnar-lined esophagus and helped identify those at greater risk for cancer. The authors believed that the increased detection of disease will allow better informed decisions to be made about the management and subsequent treatment of these patients.

Furthermore, there is a clinical trial on “The Analysis of WATS3D (Wide Area Transepithelial Sample Biopsy With 3-Dimensional Computer-Assisted Analysis) Increased Yield of Barrett's Esophagus and Esophageal Dysplasia” that is currently recruiting patients (last updated January 25, 2018).

DNA-Image Cytometry of Brush Biopsies

Siebers and colleagues (2013) stated that despite advances in surgical and other treatment modalities, the prognosis of patients with OSCC remains poor. It is well-known that the early detection of OSCC and recurrent lesions is of utmost importance in obtaining disease control. Besides difficulty in visually differentiating between benign and (pre) cancerous lesions, the detection is hampered by the fact that scalpel biopsy is an invasive activity with potential morbidity. It is therefore not recommended to biopsy lesions on a regular basis. An easily performed, non-invasive screening method suitable to monitor lesions over time is urgently needed. In addition, the current histopathological examination suffers from inter- and intra-observer variability. In a previous study these researchers showed the prognostic value of DNA image cytometry (DNA-ICM). The objective of the present study is assessment of the possibility to determine ploidy status using an oral brush. This prospective study included 85 (23 pre-malignancies, 27 stage T1 and 31 stage T2 OSCC) patients with a suspected (pre) malignant lesion of the oral cavity. Newly discovered lesions as well as suspected recurrent OSCC were included. Both a brush and biopsy specimen were obtained for each patient. All specimens were analyzed using ICM according to a well-established procedure. A significant difference was observed between brushes from diploid biopsies and brushes from non-diploid biopsies ($p < 0.01$). The brush was able to significantly differentiate between diploid and non-diploid lesions. This technique was able to correctly identify 50% of the non-diploid lesions with a corresponding specificity of 80%. The authors concluded that the oral brush biopsy is not suitable to replace the conventional surgical biopsy, however it may be of additional value in monitoring (pre) malignant lesions over time.

Kammerer et al (2013) stated that adjunctive techniques like DNA-ICM, a non-invasive method, have been attributed to enhance the diagnostic performance of oral brush biopsies. The aim of the study was an evaluation of brush biopsies, analyzed according to morphological criteria and by DNA-ICM versus histological findings in a blinded prospective trial. A total of 88 brush biopsies of 70 patients were sampled. Only clinical suspicious but not evident malignant oral
lesions were included. Clinical diagnosis was leukoplakia (n = 36), lichen planus (n = 18), verruciform erythroplakia (n = 12), erythroleukoplakia (n = 9), erosion (n = 7) and induration (n = 6). Evaluation was conducted via histology, cytology and DNA-ICM. Histological diagnosis revealed 8 cases of squamous intraepithelial dysplasia (SIN 1 n = 6, SIN 2 n = 2), 4 cases of carcinoma-in situ and 25 cases of oral T1-cancer. Remaining cases were leukoplakia (n = 28), lichen planus (n = 15) and local inflammation (n = 8). Brush biopsy detected malignant lesions including SIN 1 with a sensitivity of 55 % and a specificity of 100 %. DNA-ICM had a sensitivity of 70 % and a specificity of 100 %. The combination of both methods showed a sensitivity of 76 % and a specificity of 100 %. The predominant reason for false negative results was sampling errors with insufficient cells (86 % in brush biopsy and 100 % in DNA-ICM). The authors concluded that DNA-ICM has the potential to substantially improve the sensitivity of a pure morphological interpretation of oral brush biopsies. Method inherent sampling errors may be accountable for a lower sensitivity compared to conventional histological diagnosis. Therefore, DNA-ICM should not be used to rule out malignancy, when lesions are already clinically suspicious for oral cancer.

Ma and colleagues (2014) estimated the diagnostic accuracy of brush biopsy with DNA-ICM for potentially malignant oral disorders compared with tissue biopsy pathology in China. Exfoliative cells were obtained using a cytobrush cell collector from oral mucosa of 52 subjects, followed by scalpel biopsy from the same region. Nuclear DNA contents (ploidy) were measured after Feulgen re-staining, using an automated DNA image cytometer. Exfoliative cytology with DNA-ICM and histopathological diagnosis were performed separately at different institutions. Histological investigation was considered the gold standard. These researchers reported that the sensitivity of DNA aneuploidy for the detection of cancer cells in potentially malignant oral disorders was 86.36 %; its specificity was 90.00 %, its PPV was 86.36 %, and its NPV was 90.00 %. The authors concluded that brush biopsy with DNA-ICM is a useful method for monitoring potentially malignant oral disorders. The findings of this small, uncontrolled study need to be validated by well-designed studies.

The U.S. Preventive Services Task Force (USPSTF)’s guideline on “Screening for oral cancer” (USPSTF, 2014) stated that “Additional tests proposed as adjuncts to the oral cancer screening examination include toluidine blue dye staining, chemiluminescent and autofluorescent lighting devices, and brush cytopathology. These screening and adjunct tests have not been adequately tested in primary care non dental settings. Although there is interest in screening for oral HPV infection, medical and dental organizations do not recommend it. Currently, no screening test for oral HPV infection has been approved by the U.S. Food and Drug Administration (FDA)".
Ye and colleagues (2015) conducted a metaanalysis to compare the accuracy between the OralCDx brush biopsy and DNA-image cytometry in diagnosing both conditions. The authors systematically searched bibliographic databases for original relevant studies on the early diagnosis of oral precancer and oral cancer. They evaluated study characteristics to determine the accuracy of the two screening strategies. They identified 13 studies (8 of OralCDx brush biopsy and 5 of DNA-image cytometry) reporting on 1,981 oral mucosa lesions. The meta-analysis found that the area under the summary receiver operating characteristic curves of the OralCDx brush biopsy and DNA-image cytometry were 0.8879 and 0.9885, respectively. The pooled sensitivity, specificity, and diagnostic odds ratio (OR) of the OralCDx brush biopsy were 86 % (95% CI: 81 to 90), 81 % (95% CI: 78 to 85), and 20.36 (95% CI: 2.72 to 152.67), respectively, while these modalities of DNA-image cytometry were 89 % (95% CI: 83 to 94), 99 % (95% CI: 97 to 100), and 446.08 (95% CI: 73.36 to 2712.43), respectively. Results of a pairwise comparison between each modality demonstrated that specificity, area under the curve (AUC), and Q(∗) index of DNA-image cytometry was significantly higher than that of the OralCDx brush biopsy (Z = 2.821, p < 0.05; Z = 1.711, p < 0.05; Z = 1.727, p < 0.05), but no significant difference in sensitivity was found (Z = 1.520, p > 0.05). The authors concluded that the metaanalysis of the published studies indicated that DNA-image cytometry is more accurate than the OralCDx brush biopsy in diagnosing oral precancer and oral cancer.

In a meta-analysis, Abt (2015) examined the clinical value of DNA-image cytometry for detecting oral cancer. Medline, Embase, PubMed, Elsevier and Web of Science databases and the reference lists of known primary and review papers were scanned for relevant citations. Prospective and retrospective studies evaluating brush cytology were considered. Only computer-assisted methods that included histologically confirmed disease positive status were included. Data were extracted using a standardized form. Study quality was assessed by 1 reviewer using the quality assessment of diagnostic accuracy studies (QUADAS) checklist. Pooled sensitivity and specificity were calculated with 95% CIs separately for each study. Likelihood and diagnostic odds ratios were also calculated along with a summary receiver-operating characteristic (SROC) curve analysis. A total of 13 studies (8 of OralCDx brush biopsy and 5 of DNA-image cytometry) reporting on 1,981 oral mucosa lesions were included. OralCDx brush biopsy had a pooled sensitivity of 86 % (95% CI: 81 to 90) and pooled specificity of 81 % (95% CI: 78 to 85). The pooled sensitivity and specificity of DNA-image cytometry were 89 % (95% CI: 83 to 94) and 99 % (95% CI: 97 to 100). Diagnostic OR estimates for OralCDx brush biopsy and DNA-image cytometry were 20.36 (95% CI: 2.72 to 152.67) and 446.08 (95% CI: 73.36 to 2712.43), respectively. Study size was found to be closely related to heterogeneity among studies and analysis suggested publication bias in relation to OralCDx brush biopsy. The authors concluded that these findings suggested that DNA-image cytometry had a highly significant potential over OralCDx brush biopsy as an accurate and simple diagnostic tool for clinically suspected oral pre-cancer and oral cancer.
Adami and colleagues (2017) stated that RNA-based diagnosis and prognosis of SCC has been slow to come to the clinic. Improvements in RNA measurement, statistical evaluation, and sample preservation, along with increased sample numbers, have not made these methods reproducible enough to be used clinically. These researchers proposed that, in the case of OSCC, a chief source of variability is sample dissection, which leads to variable amounts of stroma mixed in with tumor epithelium. This heterogeneity of the samples, which requires great care to avoid, makes it difficult to see changes in RNA levels specific to tumor cells. An evaluation of the data suggested that, paradoxically, brush biopsy samples of oral lesions may provide a more reproducible method than surgical acquisition of samples for microRNA (miRNA) measurement. The evidence also indicated that body fluid samples can show similar changes in miRNAs with OSCC as those seen in tumor brush biopsy samples, suggesting much of the miRNA in these samples is coming from the same source -- tumor epithelium. The authors concluded that brush biopsy or body fluid samples may be superior to surgical samples in allowing miRNA-based diagnosis and prognosis of OSCC in that they feature a rapid method to obtain homogeneous tumor cells and/or RNA.

Zhang and co-workers (2017) noted that OSCC is one of the most malignant tumors with high mortality rate worldwide. Biomarker discovery is critical for early diagnosis and precision treatment of this disease. MicroRNAs are small non-coding RNA molecules, which often regulate essential biological processes and are good candidates for biomarkers. By integrative analysis of both the cancer-associated gene expression data and microRNA-mRNA network, miR-148b-3p, miR-629-3p, miR-27a-3p, and miR-142-3p were screened as novel diagnostic biomarkers for OSCC based on their unique regulatory abilities in the network structure of the conditional microRNA-mRNA network and their important functions. These researchers identified 5 microRNAs that could be putative biomarkers for OSCC. Among them, 1 has been reported as biomarker and 2 are reported as associated microRNAs. The other 2 are the novel finding microRNA biomarkers. As a result, 4 novel biomarker microRNAs (i.e., miR-148b-3p, miR-629-3p, miR-27a-3p, and miR-142-3p) are discovered. The authors concluded that further experimental verification and clinical testing were suggested for these putative OSCC microRNA biomarkers.

Yan and colleagues (2017) stated that several studies have been shown that miRNA play important roles during the progression of OSCC. However, the results varied largely in different studies due to different platforms and sample sizes. These investigators systematically evaluated a large scale of miRNA profiles from current qualified OSCC samples, and further examined the functions of genes regulated by these key miRNAs as well as the signaling pathways through which these miRNA effect carcinogenesis. A total of 7 key miRNAs were
identified, and of which 3 were significantly up-regulated, including hsa-miR-21, hsa-miR-31, hsa-miR-338, and 4 were down-regulated, namely hsa-miR-125b, hsa-miR-133a, hsa-miR-133b, and hsa-miR-139. The function enrichment analysis revealed that target genes of up-regulated miRNAs were associated with cellular protein metabolic process, macromolecule metabolic process, and TGF-beta pathway, while the targets of down-regulated miRNAs were enriched in negative regulation of macromolecule biosynthetic process and gene expression, and p53, long-term potentiation and adherens junction pathways. Transcription factor analysis revealed that there were 67 (51.1 %) transcription factors influenced by both up- and down-regulated miRNAs. The authors concluded that 7 key miRNAs were found to play essential role in progression of OSCC, as well as the target genes and transcription factors of these miRNAs. They stated that the potential functions of these target genes identified in this study may be useful in the diagnosis and prognostic prediction of OSCC as biomarkers.

In a systematic review, El-Sakka and associates (2018) evaluated current findings on altered expression of miRNAs in OPMDs and whether they can be used as risk stratification biomarkers. Studies were collated after searching 3 different electronic databases: PubMed, Embase, Medline. Additional searches were carried out through cross-checking the bibliographies of selected articles. After a thorough selection process made by 2 of the authors, 40 articles met the inclusion criteria and were included in the review. Studies were assessed and analyzed in terms of how the candidate miRNA biomarkers were differentially expressed and validated. The included studies examined the expression of miRNAs from human specimens (blood serum/plasma, saliva, tissue) as diagnostic or prognostic biomarkers in patients with OPMDs, some of which have been utilized as risk stratification biomarkers for malignant transformation and have showed promising findings. The authors concluded that the current evidence to support or refute the prognostic utility of miRNAs in predicting cancer progression in OPMDs is equivocal. They stated that further well-designed longitudinal prospective studies are needed.

Furthermore, an UpToDate review on “Overview of the diagnosis and staging of head and neck cancer” (Poon and Stenson, 2018) does not mention RNA-based methods as a diagnostic tool.

**CPT Codes / HCPCS Codes / ICD-10 Codes**

Information in the [brackets] below has been added for clarification purposes. Codes requiring a 7th character are represented by "+".

<table>
<thead>
<tr>
<th>Code</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPT codes not covered for indications listed in the CPB:</td>
<td></td>
</tr>
<tr>
<td>RNA based oral brush biopsy-no specific code:</td>
<td></td>
</tr>
<tr>
<td>Code</td>
<td>Code Description</td>
</tr>
<tr>
<td>---------</td>
<td>-----------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>88104</td>
<td>Cytopathology, fluids, washings or brushings, except cervical or vaginal; smears with interpretation</td>
</tr>
<tr>
<td>99000</td>
<td>Handling and/or conveyance of specimen for transfer from the physician's office to a laboratory</td>
</tr>
</tbody>
</table>

Other CPT codes related to the CPB:

<table>
<thead>
<tr>
<th>Code</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>40808</td>
<td>Biopsy, vestibule of mouth</td>
</tr>
<tr>
<td>41108</td>
<td>Biopsy of floor of mouth</td>
</tr>
<tr>
<td>42800</td>
<td>Biopsy; oropharynx</td>
</tr>
<tr>
<td>42804</td>
<td>nasopharynx, visible lesion, simple</td>
</tr>
<tr>
<td>42806</td>
<td>nasopharynx, survey for unknown primary lesion</td>
</tr>
<tr>
<td>43191</td>
<td>Esophagoscopy, rigid, transoral; diagnostic, including collection of specimen(s) by brushing or washing when performed (separate procedure)</td>
</tr>
<tr>
<td>43200</td>
<td>Esophagoscopy, flexible, transoral; diagnostic, including collection of specimen(s) by brushing or washing, when performed (separate procedure)</td>
</tr>
<tr>
<td>88160</td>
<td>Cytopathology smears, any other source; screening and interpretation</td>
</tr>
</tbody>
</table>

HCPCS codes not covered for indications listed in the CPB:

<table>
<thead>
<tr>
<th>Code</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>D0486</td>
<td>Laboratory accession of transepithelial cytologic sample, microscopic examination, preparation and transmission of written report</td>
</tr>
<tr>
<td>D7288</td>
<td>Brush biopsy - transepithelial sample collection</td>
</tr>
</tbody>
</table>

Other HCPCS codes related to the CPB:

<table>
<thead>
<tr>
<th>Code</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>D7287</td>
<td>Exfoliative cytological sample collection</td>
</tr>
</tbody>
</table>

ICD-10 codes not covered for indications listed in the CPB (not all-inclusive):

<table>
<thead>
<tr>
<th>Code</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>D00.00  - D00.08</td>
<td>Carcinoma in situ of lip, oral cavity, and pharynx</td>
</tr>
<tr>
<td>D10.0  - D11.9</td>
<td>Benign neoplasm of mouth, pharynx and major salivary glands</td>
</tr>
<tr>
<td>D37.01  - D37.02</td>
<td>Neoplasm of uncertain behavior of lip, oral cavity, and pharynx</td>
</tr>
<tr>
<td>D37.04  - D37.09</td>
<td>Neoplasm of uncertain behavior of lip, oral cavity, and pharynx</td>
</tr>
<tr>
<td>K09.1  - K09.9</td>
<td>Cysts of oral region, not elsewhere classified</td>
</tr>
<tr>
<td>K12.0  - K13.79</td>
<td>Stomatitis and related lesions and other diseases of lip and oral mucosa</td>
</tr>
<tr>
<td>K14.0  - K14.9</td>
<td>Diseases of tongue</td>
</tr>
<tr>
<td>Z12.81</td>
<td>Encounter for screening for malignant neoplasm of oral cavity</td>
</tr>
</tbody>
</table>
The above policy is based on the following references:


43. Cook D, Huber MA. In the primary care setting, the value of adjunctive aids for oral cancer examinations remains unanswered. Critically Appraised Topics (CATS). ID# 265. San Antonio, TX: Oral Health Evidence-based Practice Program, Dental School, University of Texas Health Science Center at San Antonio; revised December 14, 2011.
44. Juber S, Huber M. OralCDx Brush Test should not replace surgical biopsy in oral cancer examinations. Critically Appraised Topics (CATS). ID# 2194. San Antonio, TX: Oral Health Evidence-based Practice Program, Dental School, University of Texas Health Science Center at San Antonio; April 5, 2012.


78. Poon CS, Stenson KM. Overview of the diagnosis and staging of head and neck cancer. UpToDate Inc., Waltham, MA. Last reviewed May 2018.

AETNA BETTER HEALTH® OF PENNSYLVANIA

Amendment to
Aetna Clinical Policy Bulletin Number: 0686 Oral and Esophageal Brush Biopsy

There are no amendments for Medicaid.

www.aetnabetterhealth.com/pennsylvania  annual 11/01/2019