Establishment of a Standardized Biological Sample Collection Protocol for OPMDs

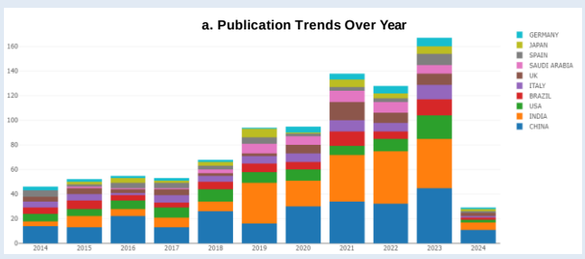
Yu Jiaao[1], Carina Ladeira[2], Vera van Dis[3], Daniela Elena Costea[4], Stephanie Villar[1], Zisis Kozlakidis[1]

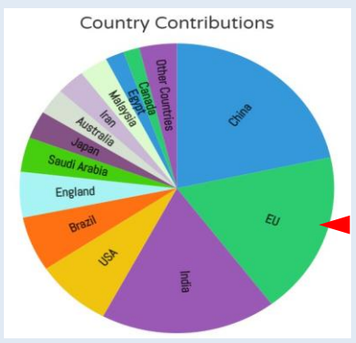
Affiliation: 1. International Agency for Research on Cancer/World Health Organization; 2. Escola Superior de Tecnologia da Saúde de Lisboa; 3. Erasmus MC University Medical Centre; 4. Haukeland University Hospital, University of Bergen.

INTRODUCTION

There has been a growing number of clinical studies on OPMDs in recent years, reflecting increasing interest in this field (**S1a**).

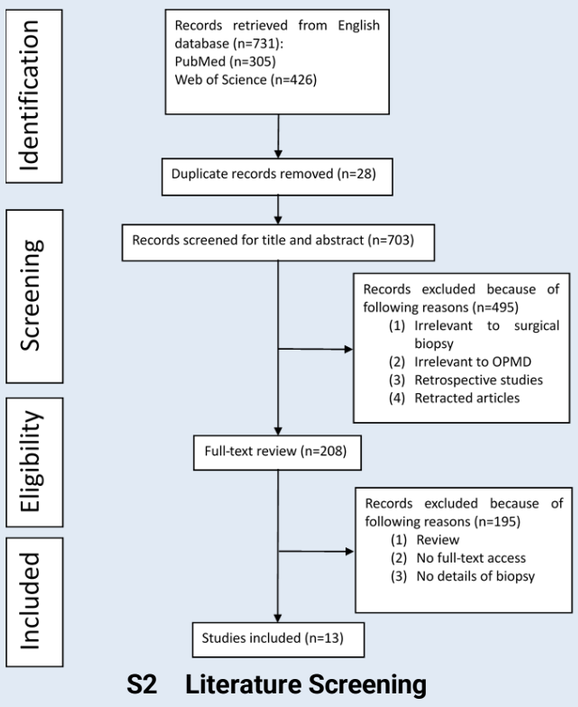
Among these studies, EU countries have the second highest number of publications after China (**S1b**). According to a meta-analysis, the prevalence of OPMDs is approximately 3.07%. While surgical diagnosis remains the gold standard, there is currently no standardized protocol for the surgical biopsy of oral lesions.





METHODS

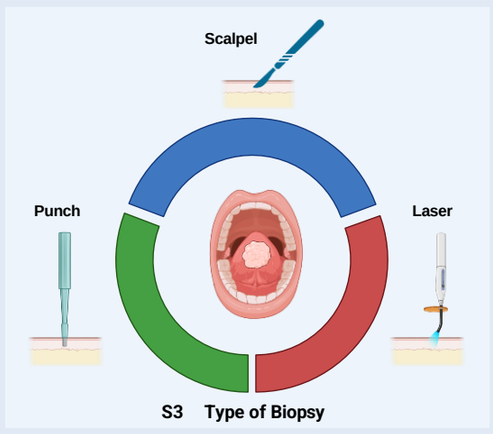
In order to reach a protocol compendium for the collection of oral biological samples, a literature review on clinical studies with detailed methotologies of surgical biopsy of OPMDs in clinical settings was performed.
Databases in English (Pubmed and Web of Science) in recent 10 years were searched using following keywords: OPMD (including Oral Potentially Malignant Disorders, Oral Precancerous Lesions, Oral Leukoplakia, Oral Erythroplakia, Oral Lichen Planus, Oral Submucous Fibrosis, and Oral Dysplasia); Biopsy; Pathological Diagnosis.



S2 Literature Screening

As a result, 731 publications were extracted and 28 duplicates were excluded, leaving 703 articles. Then 495 articles were excluded because of being: (1) Irrelevant to surgical biopsy; (2) Irrelevant to OPMD; (3) Retrospective studies. 208 articles were reviewed by full text. 195 studies were then excluded because of following reasons: (1) Review; (2) No full-text access; (3) No details of biopsy. In the end, 13 articles were selected for further analysis. Grey literatures such as guidelines and protocols were reviewed (S2).

RESULTS

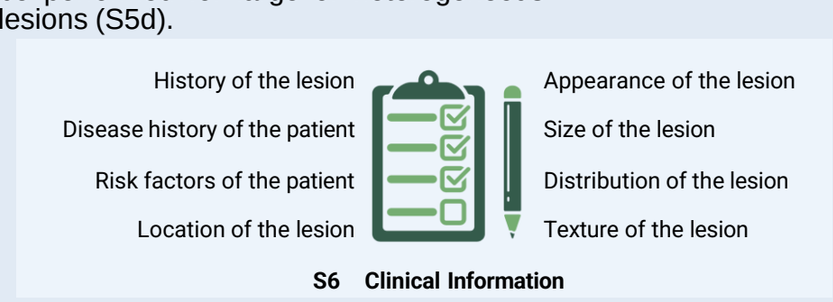


During a biopsy, the clinician chooses between incision and excision (S5a). An incisional biopsy involves partial removal of the lesion and is often used for large or potentially malignant lesions. An excisional biopsy involves complete removal of the lesion and is generally avoided in malignant diseases to prevent damaging the edges of the malignancy.

There is no consensus on the optimal specimen diameter (S5b). Some guidelines recommend that the diameter of a punch biopsy should be at least 3 mm. For scalpel biopsies, an elliptical incision with a length greater than 10 mm is recommended.

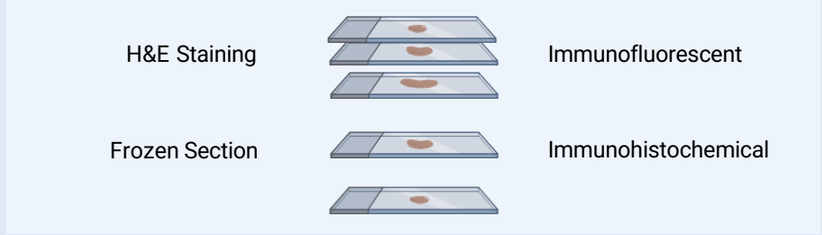
The biopsy should reach an adequate depth for accurate pathological diagnosis (S5c). Some guidelines suggest that the biopsy should extend to the lamina propria.

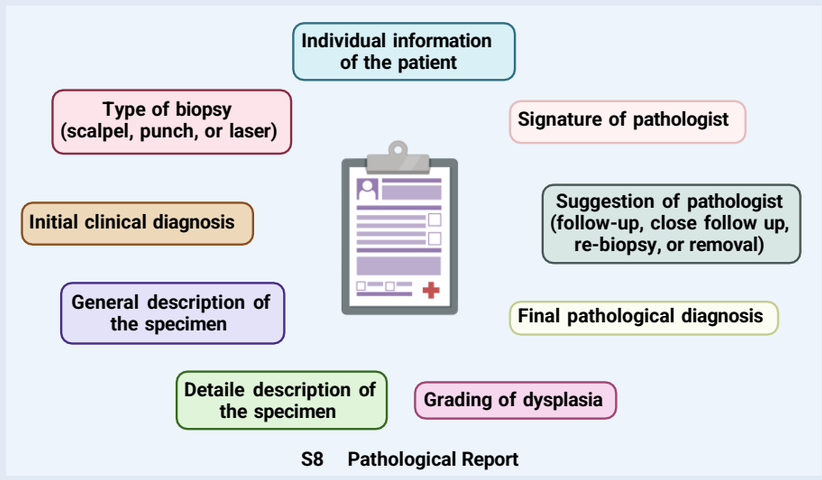
While a single biopsy carries a risk of underdiagnosis, multiple biopsies should be performed for large or heterogeneous lesions (S5d).



S6 Clinical Information

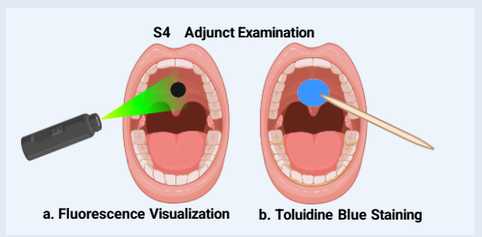
S7 Pathological Examination





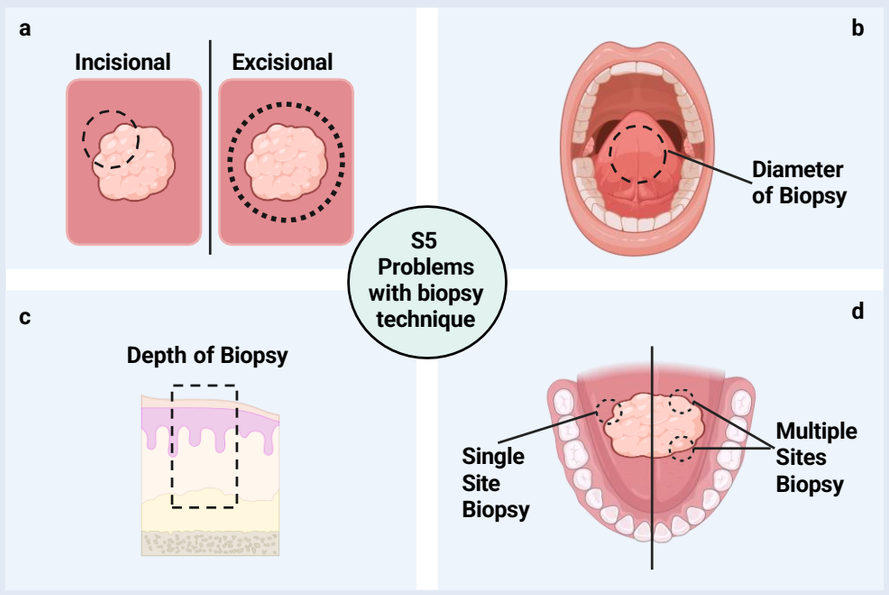
S8 Pathological Report

Given that histopathological examination is subjective, a standardized form for pathological reports should be established (S8). In addition to the diagnosis, the pathologist should also provide suggestions for the patient's future treatment, such as follow-up, close follow-up, re-biopsy, or complete removal of the lesion.



Fluorescence visualization (S4a) or toluidine blue (S4b) help clinicians identify the most severe or significant changes for biopsy.
A loss of green autofluorescence or strong toluidine staining (deep blue) typically indicates abnormal tissue.

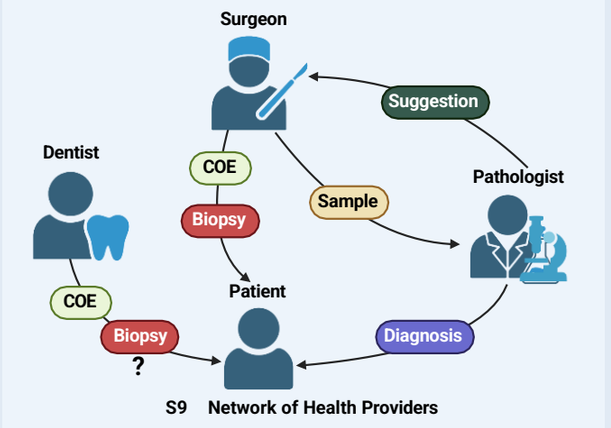
Scalpel, punch, and laser are the most frequently used instruments (S3). Scalpel can be used throughout the oral cavity including hard tissues such as gingiva. It allows precise control over the depth and width of the incision but requires skill and experience. Punch instruments have fixed diameters and depths, which make it easy to standardize. However, punch cannot be performed in certain areas of the oral cavity where access and visibility are limited. Laser has benefits such as minimal tissue damage, reduced bleeding, and improved post-operative recover but the heat damage from laser may cause unwanted artifacts in tissue samples, which might complicate histopathological evaluation.



Clinical information is essential for the accurate diagnosis by pathologists (S6). Clinicians provide adequate medical data along with the biological specimen. If possible, a photograph of the oral lesion before the biopsy should also be collected.

For pathological diagnosis (S7) Hematoxylin and eosin (H&E) staining is the standard initial stain for examining tissue morphology.

Frozen sections are typically used for intraoperative margin assessment of malignancies. Immunofluorescence is employed in the diagnosis of autoimmune and inflammatory disorders, such as lichen planus. Immunohistochemistry (IHC) uses antibodies to detect genetic mutations associated with malignancies.



S9 Network of Health Providers

The early detection of OPMDs requires collaboration of dentists, specialized surgeons, and pathologists (S9).

Dentists and surgeons perform conventional oral examinations, screening for suspicious oral lesions during routine check-ups. When a suspicious lesion is identified, surgeons should perform the biopsy. Pathologists then analyze the biopsy samples, providing the final diagnosis and recommendations for further treatment, such as follow-up or removal surgery.

CONCLUSION

The biopsy methods of oral potentially malignant diseases (OMPDs) can influence the quality of biological specimens, potentially leading to underdiagnosis. Therefore, the selection of biopsy instruments, the depth and width of the sample, and the number of biopsies should be standardized as much as that is practical and feasible, as well as the associated clinical information of the biological sample. Establishing a standardized form for pathological reports is also crucial for many countries.
The INTERCEPTOR network aims to review the literature, collect expert opinions via a survey (currently conducted) and promote a harmonized approach towards the tissue collection methods, clinical data requirements and biobanking.