Chapter

Methods of Collection and Transport of Materials to Laboratory from Oral and Dental Tissue Lesions

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Abstract

The oral pathology laboratory is the most resourceful place for the diagnosis of oral lesions. Most clinicians err on the collection and transport of oral and associated tissues to the laboratory. Oral tissue examination includes a wide range such as oral biopsy (for routine formalin fixed and fresh tissue), saliva, swabs, cytology smears and fine needle-aspirated, cystic fluid. This in turn adversely affects the final diagnosis of the disease. Thus, it is high time to appreciate and acknowledge the role of collection containers, fixing reagents and transport media as an adjunct for successful diagnosis.

Keywords: oral, biopsy, saliva, cytology, laboratory

1. Introduction

The role of the general as well as oral pathology and microbiology laboratory is essential to the successful provision of patient care. Appropriate, professional and knowledgeable interaction with the dental or head and neck surgeon can benefit the patients by achieving accurate diagnosis as well as effective treatment approaches. Acquiring proper laboratory data allows the dental practitioner to arrive at a definitive diagnosis for further referral in a timely manner as oral cavity often presents the first signs of a systemic illness. The pathologist plays a valuable role in education and documentation of the learned information for future cases with similar presentation for he/she presents the final verdict.

The oral pathology laboratory is the most resourceful place for the diagnosis of oral lesions. A multitude of lesions are encountered in the oral and maxillofacial regions that need a sound knowledge of how to approach their diagnosis, and it begins with a good clinical history and examination. The basic requirements of a useful diagnostic technique are ease of use, patient acceptance and sufficient specimen collection. The ideal diagnostic procedure should also be highly sensitive and specific, simple, and not time-consuming and have a potential for automation [1]. Oral tissue examination includes a wide range such as oral biopsy (for routine formalin fixed and fresh tissue), saliva, swabs, cytology smears and fine needle-aspirated, cystic fluid and microbiology.

2. Biopsy: rationale

When a patient with a particular lesion is seen, a list of differential diagnosis is formulated, and biopsy is useful at arriving at a definitive diagnosis or to confirm the clinical diagnosis. Oral biopsy was and still is the gold standard for oral diagnostic procedures. It is an invasive procedure with procedural limitations and a psychological effect on patients. It is important that the biopsy specimen be a true representation of the entire lesion. A carefully selected area involving normal as well as pathologic areas can produce good diagnostic specimen.

2.1 Dos and Don'ts for sample collection

1. Take tissue specimen, and put it into a wide-mouth container with 10% formalin at least 20 times the volume of the surgical specimen. Care should be taken to be sure that the tissue has not become lodged on the wall of the container above the level of the formalin.

2. Incisional biopsy specimens should be taken from each area showing different characteristics. Even if the lesion clinically looks uniform, it is still wise to sample different areas of a large lesion. Multiple samples must be adequately labeled.

3. Vigorous manipulation of lesion should be avoided if it is suspected to be tumor as it can increase the tumor cell emboli in venous drainage.

4. The tissue should never be put on gauze, cotton or paper, as it can lead to dehydration of the tissue specimen.

5. If culture is desired, take the material for bacteriologic study before fixing the specimen.

6. On the other hand, if the lesion is a large one with variations in its clinical appearance multiple biopsies could be planned for better sampling.

7. Even though the pathologist would like to receive the biggest specimen possible, the minimum size of the biopsy should not be less than 5 mm in diameter, to enable the pathologist to obtain well-prepared slides.

8. In majority of cases, the most active part of the lesion and, therefore, the most representative are located peripherally. If the biopsy specimen is taken from a necrotic part of the tumor, the diagnosis rendered by the oral pathologist can be only the "necrotic tissue." Therefore, as a rule, it is unwise to biopsy the center of a lesion, which is probably its least active part [2, 3].

3. Tissue fixation

It is extremely important to place the biopsy specimen into proper fixative immediately after removal from the patient. Ten percent formalin is the standard fixative used to prevent autolysis, distortion and destruction of the tissues. Most oral pathology laboratories will provide mailing containers, specimen bottles filled with 10% formalin, and history-biopsy request sheet. **Table 1** is a list of fixatives with reference to oral tissue.

	Name	Composition	Comments
For routine histopathology	Phosphate buffered formalin	40% formaldehyde with Sodium dihydrogen phosphate monohydrate & disodium hydrogen phosphate anhydrous. pH - 6.8 Fixation time: 12 – 24 hours	Antigen retrival of successful IHC
	Formal calcium	40% formaldehyde & Calcium chloride Fixation time: 12 – 24 hours	for the preservation of lipids especially phospholipids.
	Formal saline	40% formaldehyde & Sodium chloride Fixation time: 12 – 24 hours	Widely used for routine histopathology prior to the introduction of phosphate buffered formalin. Often produces formalin pigment.
	Zinc formalin (unbuffered)	Zinc sulphate: 1 g 40% formaldehyde Fixation time: 4 – 8 hours	Alternatives to mercuric chloride formulations. Improved results with IHC
	Zenker's fixative	Mercuric chloride: 50 g Potassium dichromate: 25 g Glacial acetic acid: 50 ml in 950mL distilled water. Fixation time: 4 – 24 hours	Good nuclear preservation but lyses red blood cells (recommended for congested specimens) Gives good results with PTAH and trichrome staining. Produces mercury pigment
			which should be removed from sections prior to staining and can produce chrome pigment if tissue is not washed in water prior to processing. Is an intolerant agent so, after water washing, tissue should be stored in 70% ethanol.
	Helly's fixative	Potassium dichromate: 25 g Sodium sulphate: 10 g Mercuric chloride: 50 g Immediately before use add – 40% formaldehyde Fixation time: 4 – 24 hours	
	B-5 fixative	B-5 fixative prepare immediately before use Sodium sulphate: 10 g Mercuric chloride: 50 g Immediately before use add – 40% formaldehyde: 50 ml Fixation time: 4 – 24 hours	Fixation of haematopoietic and lymphoid tissue. It produces excellent nuclear detail, provides good results with many special stains and is recommended for IHC.
	Carnoy's solution	Ethanol absolute: 60 ml Chloroform: 30 ml Acetic acid glacial: 10 ml Fixation time: 1 – 4 hours	Is rapid acting, gives good nuclear preservation and retains glycogen

Table 1.
List of fixatives with reference to oral tissue.

3.1 Precautions

a. If the fixative in the mailing container has evaporated, leaving a white powder residue, it cannot be reconstituted by adding water.

b. Another container must be used or the fixative prepared by getting formalin (formaldehyde 37–40%) and mixing 10 parts of formalin solution with 100 parts of tap water.

c. The biopsy specimen must not dry out on the bracket table while one is finding the fixative, bottles and the like.

d. Everything should be ready in advance so that the tissues can be properly fixed and a diagnosis can be rendered and to avoid the statement "improper fixation, unable to render diagnosis."

e. The biopsy specimen should never be submitted in normal saline or water, as the tissues become completely degenerated by autolysis.

f. In an emergency, when 10% formalin cannot be obtained, 70% alcohol may be used. Alcohol causes hardening of the specimen to the degree that cutting may be difficult, so on the biopsy request form it should be noted that the specimen is submitted in alcohol. The histology technician can then transfer the specimen to 10% formalin, hopefully before 48 h, so that the tissue will not become too hard to cut.

g. Preserving the specimen. The specimen—a tooth, piece of bone, or soft tissue—is at once placed in a bottle containing a fixing solution, such as 10% formalin, Zenker's solution, or Carnoy's solution. Most pathologists prefer 10% formalin solution.

h. It should be promptly sent to a pathologist for examination. The latter should be given all the information gained by clinical study and X-ray examination, or other laboratory tests, as this will facilitate diagnosis in difficult cases.

i. A consultation between the pathologist and the dentist will be particularly helpful and will also give the opportunity for discussing the method of procedure in the treatment of the patient.

j. It is also very important that the completed history form be sent with the biopsy specimen [3].

3.1.1 Precautions during transport: freezing

During winter months in climates where the temperature drops to freezing or below, there is a danger that the biopsy specimens dropped in a mailbox may freeze. The freezing of the tissue forms ice crystals within the cells. These crystals disrupt cell membranes and cause great distortion and introduction of artifacts into the specimen. Thus interpretation of the tissue specimen becomes very tenuous.

Before mailing biopsy specimens during cold weather, one must make sure that they are fixed in 10% formalin at room temperature for at least 2 h before mailing.

3.1.2 Adjunct techniques: electrocautery

Biopsy specimens can display tissue changes that might interfere with the accurate diagnosis by the use of electrocautery. The frying action of the electric current generating high temperatures in the tissues results in changes.

In the case of a biopsy of an oral mucosa lesion, an eosinophilic homogenization of the fibrous tissue can be seen histologically. Thus, it is especially important not to use electrocautery for excision of small lesions. It is preferable to use a surgical scalpel to remove the biopsy specimen followed by the use of electrocautery to control bleeding [3].

3.2 Oral exfoliative cytology

Oral exfoliative cytology was developed as a potential diagnostic tool for early detection of malignant lesion. It is relatively simple, easy to master and least invasive and has high patient acceptance [1]. Though it has been always used as an adjunct to oral biopsy in oral cancer diagnostics, it holds potential in diagnosis of oral dermatosis and certain microbial infections. The specimen obtained can be used for cytomorphometry, DNA cytometry and immunocytochemical studies [1].

3.3 Technique: collection of smear

a. Toluidine blue should be used as a supravital stain before the site selection and smear preparation.

b. Label one end of the slide with patient's name, date, and area from which material is to be obtained. Wipe the slide clean.

c. Use a clean cotton tip applicator or wooden spatula for the collection of the smear. If the area to be scraped is dry, moistened the applicator or spatula.

d. Collect the material using a slight rolling motion or scraping of the lesion. (Inadequate slides may be obtained if there is a pseudomembrane, thick saliva, no moisture or excess bleeding).

e. Immediately apply the scraping to the center area of the slide previously marked.

3.3.1 Fixation of smear

a. Alcohol (70%) is adequate for fixation. Equal parts of ether and 95% ethyl alcohol give superior staining qualities.

b. Immediately immerse the slide in fixative or put the fixative on the slide with a dropper. Do not allow any drying of the smear before fixation.

c. Keep the slide in fixative for a minimum of 30 min.

d. At this point, the slides can be air dried and sent for staining and screening, or it can be left in the fixative.

Pt's name Site Date		C-

3.3.2 Advantages

a. Very good, easy, rapid, painless and bloodless procedure.

b. Adjunct to biopsy, better to take cytology first, and then if necessary advice biopsy.

c. Creates less psychological trauma and fear.

d. Useful in follow-up after radio- and chemotherapy.

e. Recurrence can be known very easily without taking biopsy.

f. Mainly used for ulcerative epithelial lesion; intact epithelium gives false negative result.

g. Also used for dermatological condition like pemphigus vulgaris, Darier's disease, and viral infections like herpes simplex as well as aphthous ulcers, etc.

Deeper lesions are not identified by this technique, and for that another technique called fine needle aspiration cytology (FNAC) is used.

4. Fine needle aspiration cytology

Fine needle aspiration biopsy or cytology is an effective tool in evaluating and diagnosing suspect lumps or masses. The name indicates this biopsy technique uses aspiration to obtain cells or fluid from a superficial or deep palpable mass. A quick diagnosis means that tumor is detected early, or benign lumps are diagnosed without the need for multiple surgeries [4]. The success of perfect FNAC depends on the technique for collection and preparation of samples along with a detailed clinical history and clinical impression. If an infectious process is suspected, often a portion of the specimen is submitted for microbiology in an appropriate sterile medium or transport container.

FNAC is indicated in head and neck lesions, which include salivary gland lesions, thyroid and parathyroid lesions, cervical lymph nodes and intraosseous lesions.

4.1 Collection: preparation for FNAC

Alcohol wipe; 4 × 4-inch gauze pads; 10-ml plastic syringes; 25-gauge 1 1/2-inch stiff, noncutting, bevel-edged needles; glass slides; alcohol bottles; pistol-grip mechanical syringe holder.

Procedure:

1. Explanation of procedure to the patient before doing FNAC ensures the patient's cooperation.

2. For head and neck biopsies, a chair with head rest is essential.

3. Prebiopsy sedation is usually not required, except in the deep aspirations in very anxious patients or for deep biopsies.

4. The patient is placed in a comfortable position—with mass readily palpable and easily graspable.

5. The lesion is grasped with one hand usually between two fingers with an attempt to determine the location and surrounding tissue.

6. The syringe pistol with attached needle is laid against the surface of the lesion at determined puncture site and angle.

7. The needle is inserted quickly and advanced into the mass.

8. The suction is applied to syringe, about one third the length of syringe barrel observing the junction of the hub and needle for appearance of any specimen.

9. Multiple short 5-mm "in-and-out" motions are made till tissue material is seen coming into the hub of the needle.

10. At first appearance of any sample at junction, the syringe pistol is released, letting the vacuum equate to normal, and the needle is withdrawn slowly.

11. Pressure is applied to the puncture site with sterile gauze pad.

12. The above procedure can be repeated again using a clean needle for a second pass (and more passes if needed).

4.2 Preparing the aspirate: making direct smears

The needle is removed quickly from the syringe. Five milliliters of air is aspirated into the syringe, and the needle is placed back on the syringe. With the needle bevel facing down, 1–2 drop of aspirated material is expelled onto each of several marked glass slides.

Preparation of smear: A drop of aspirate is placed at the center of plain glass slide. A second slide is inverted over the drop, and the slides are gently pulled apart vertically or horizontally once. Fix immediately in 95% ethyl alcohol for a wet smear. For studying and evaluating cytoplasmic features or background elements of smear, air drying is preferred. Until all the material in the needle is used, continue making more slides. Or a drop is placed near the frosted end of slide held in the left hand; the bloody material is spread along the edge of the slide held in the right hand. The material is pulled gently down the slide in a manner of making a blood smear.

Alternatively the expressed specimen is sent directly to the laboratory in a 50% ethyl alcohol fixative or Hank's balanced salt solution for slide preparation. If a cyst is aspirated, the laboratory will have to spin the specimen for concentration.

Modifications:

1. Often needle biopsy without aspiration based on capillary pressure in a fine needle is sufficient to keep the scraped cells inside the lumen. A 25-gauge needle is held directly with finger tips and is inserted into the target lesion and is moved back and forth in various directions. This procedure offers the advantage of better feel of tissue consistency and less admixture of blood and is valuable for tiny lymph nodes.

2. Cell block preparation: It refers to formalin fixing and paraffin embedding of aspiration biopsy that help reinforce some tissue patterns that may be seen on smears—aids in specific diagnosis especially for immunoperoxidase cytology staining.

3. Flow cytometry, electron microscopy and molecular diagnostic studies such as ELISA and FISH can be performed on FNAC specimens.

Storage instructions: Refrigerate in a fixative if there is delay.

Causes for rejection: Improper labeling, improper fixation and air-drying artifact.

5. Collection of specimen from cervical lymph nodes

The cervical and supraclavicular lymph nodes of the neck are in the drainage path of many infectious and malignant diseases; an examination should be made by

FNAC. FNAC of cervical lymph nodes is a well-accepted diagnostic test of choice in both adult and pediatric patients for reliably distinguishing between benign/reactive and malignant processes and guiding patient management with simple observation or antimicrobial therapy for infections, chemotherapy and radiation therapy or the need for more sample tissue like core biopsies or excisional biopsy of the lymph node itself [5].

Thus FNAC is recommended as a safe, quick and inexpensive tool in the diagnosis of head and neck lesions.

5.1 Saliva

Collection of human saliva offers a noninvasive method for monitoring the disposition of unbound (free) drugs and many endogenous biomarkers. Human genomic DNA extracted from buccal epithelial cells and white blood cells found in saliva can be used in various applications in diagnostics. The correlations between blood and saliva biomolecule/biomarker concentrations range from good to excellent. Methods of collecting saliva range from simply spitting into a collection cup or using absorbent pads or swabs or the trademarked collecting devices. Freeze-thaw techniques are often employed to help break up the mucin protein that is responsible for the sticky, foamy saliva. There are few inherent drawbacks for using saliva as an ideal biofluid. In some cases, the drug, metabolites or other compounds being assayed may bind to absorbent materials, thus reducing recovery or giving a misleading result [6].

Saliva is useful for testing:

a. As an index to metabolic processes

b. For caries activity tests

c. For detection of various metabolites in smokers

5.2 Collection of specimens

Whole saliva is commonly collected by draining, spitting, suction, and swab or absorbent method. Common stimuli used are chewing on paraffin wax and chewing gum at a fixed rate. As described by Wong D, the proposal for standardized collection of whole and glandular saliva can be followed for saliva sample collection.

1. Collect saliva samples at the same time of the day between 9:00 and 11:00 a.m.

2. Patient should refrain from eating and drinking at least 90 min before advised collection.

3. If present, drug usage should be stopped that might affect salivary secretion for at least 1 day.

4. Rinse mouth with preferably deionized water prior to the saliva collection.

5. Collect saliva for 10 min [6].

Collection of saliva into ice-cooled vials is recommended to slow down the activity of hydrolytic enzymes present in saliva in air-cooled preset environment. Proprietary collection vials contain a cocktail of protease inhibitors and

bacteriostatic chemicals. Bacteria and cellular debris have to be removed directly after collection by centrifugating for 5 min at 10,000 g, or 20 min at 3000 g or by non-cotton-based filtration or vortexing (2 min, maximal speed).

6. Storage and transport

After collection, salivary samples must be snapped frozen in liquid nitrogen. In the absence of liquid nitrogen, freezing in dry ice is a practical choice when samples are collected. For a prolonged storage, −80°C temperature is preferred over storage at−20°C. The salivary samples can be diluted with glycerol (1:1) before storage. For immunochemical analysis such as ELISA, the saliva can be stored frozen after dilution (e.g., 1:100) in the assay buffer, usually PBS—0.5% Tween-20. To maintain the integrity of the proteins, before testing, the deep-frozen samples must be thawed as quickly as possible. Analysis of pH (H^+ and HCO_4^-) and viscoelastic properties is best analyzed in fresh saliva samples. Storage of salivary DNA and RNA is similar to that of a salivary protein sample [6].

7. Culture and sensitivity tests

Rationale: The idea of culturing microorganisms is not new or foreign to the practice of dentistry. There are situations in dentistry where it is not only important to know whether microorganisms are present in a lesion but also the type of microorganism. It follows from this that to best treat these infections the dentist needs to know what antibiotic would be most effective against the particular organism. Thus the culture and sensitivity test for bacterial organism is indispensable [7]. The biggest challenge today will be the transition from culture-based microbiological testing to molecular-based testing.

7.1 Collection of specimen

a. The success of oral bacteriologic/fungal identification procedures depends to a great extent on the manner in which the specimen is collected.

b. Lack of care and faulty methods of collection and handling of the specimen make the laboratory procedures valueless.

c. Because most of the microorganisms encountered in dental or head and neck infections are caused by facultative anaerobic or obligate anaerobic bacteria, the laboratory may prefer the use of a reducing transport media.

7.2 Tissue specimen for culture

a. The biopsy for culture is similar to the surgical biopsy for tissue diagnosis. A 5 × 5 × 5-mm piece of tissue is excised from the lesion with aseptic technique (here, it is undesirable to biopsy the normal tissue border, as there will not be any organisms in the normal tissues).

b. The biopsy specimen is placed in a sterile test tube containing sterile physiologic saline as the transport media (formalin is not used). The cap is secured and the specimen sent to the laboratory, along with the completed history sheet request form.

c. At the laboratory, using the sterile tissue grinders, the specimen is ground into suspension, which is left to settle. The supernatant fluid is then used for the inoculation of appropriate media [8].

8. Oral bacteriologic examination

8.1 Oral bacterial smears

For examination of surface lesions or exudates from a fistula or cyst, a specimen is procured by the means of a sterile platinum wire or a sterile exploring point. A sample of deposit or plaque on a tooth is gathered by the platinum loop, by exploring point or by a pipette. Root canal specimens may be taken with a sterile point. The specimen is then smeared on a glass slide. It is allowed to dry in the air and is fixed on the slide by drawing it three times through a Bunsen flame, when it is ready to be stained. For the proper collection of specimens, the precautions are:

a. Do not use antiseptic or disinfectants for cleaning the site from which the culture specimen is taken; just dry the site with sterile gauze.

b. The sterile swab moistened with sterile saline should be introduced into the wound or lesion and removed without touching the adjacent tissues.

c. The sterile swab should be immediately put into the sterile test tube and the part of the swab handle touched by the fingers and hand in grasping should be broken off and discarded. Some commercials have plastic cap that covers the end of the swab handle and also serves as a cap for the test tube. Two cultures should be taken from each site so that one can be grown under aerobic conditions and the other under anaerobic conditions.

d. The cover of the test tube should then be screwed on tightly and the tube titled to saturate the swab with the transport media.

e. Sterile swabs in empty tubes. These are not to be used as they may cause death of organisms and alteration of flora [9].

8.2 Examination of exudates from inflammatory lesions: gingival pockets

Discharge from gingival abscess shows various types of pyogenic bacteria (*Staphylococcus*, *Streptococcus pyogenes* and pneumococcus species). Discharge from fistulae and gingival pockets may in addition show a large number of leukocytes. Appleton gives the following method of obtaining a specimen from the gingival pockets:

a. Isolate the area with sterile cotton rolls.

b. Stroke the gingival wall of the pocket to milk out the grosser quantity of microorganisms. With a sterile cotton pledget, wipe away the exudates.

c. Paint the gingival margin with tincture of iodine 1 part, acetone 1.5 parts and glycerin 0.5 part.

d. With a sterile flat platinum needle, collect material from the very depth of the pocket or draw a bit of the material into the capillary pipet.

e. Examine the specimen on a slide or else inoculate a number of deep test tubes of semi-liquid medium as ascitic fluid or ordinary nutrient agar plus a piece of fresh, sterile rabbit kidney tissue. This is satisfactory for the cultivation of many anaerobes [10, 11].

8.3 Bacteriologic tests of pulp canals

The technique for culture from pulp canals is:

a. Drying the canal with two or three sterile absorbent points.

b. Inserting a fresh sterile absorbent point and leaving it until the tip is moistened with the exudate (usually 1 min).

c. Removing the point with cotton pliers and, with one hand, opening the test tube of the medium held in the other hand.

d. Flaming the test tube lip, dropping the point into the tube, and plugging or covering. Make certain that the point is in the medium.

e. Incubating for 48 h.

f. If the medium is clear after the incubation, it is assumed that there is no growth of organism although a smear may be taken as a check. If the medium is cloudy or a precipitate is seen, assume that infection is still present. In questionable cases a second culture is taken [10, 11].

9. Blood cultures

These are used if bacteremia or septicemia is suspected. *Streptococci*, *Staphylococci* or *Pneumococci* may be found. In patients with multiple osteomyelitis, with extreme lesions in mandible, blood cultures showed that streptococcal septicemia was the cause. In chronic cases at least 15–30 cc. of blood should be taken for the test; in acute cases, 5 cc. is sufficient.

9.1 Examination of exudates for actinomycosis

In actinomycosis the discharge pressed out from the fistula contains the so-called sulfur granules. When soft, these granules can be pressed between two slides and examined without staining. The granules appear as rosette-like masses with dense centers and a network of mycelium. Bulbous clubs or rays extend from the periphery.

9.2 Examination for *Candida albicans*

Where thrush is suspected, moist preparations may be used, placing scrapings from the suspected lesion directly on a microscopic slide. A 10% solution of potassium hydroxide is added, and the slide is heated slightly and inspected under the microscope. The slide is examined for the branching mycelia and for spores of ***Candida albicans*** [11].

9.3 Examination of cystic fluid

Cysts contain a fluid or semi-fluid material, which for diagnostic purposes is aspirated by the means of glass syringe and hypodermic needle. The anesthetized place where the needle is to be inserted or the bone perforated with a sterilizing agent is prepared; the area with sterile gauze should be isolated. The cystic content is then aspirated and examined on a slide or cultured [10].

9.4 Transportation of diagnostic specimens

With the increasing specimen loads and collection centers now at the remotest parts of the country, transportation of the pathology specimen plays a crucial role in timely diagnosis. Thus specimen transport needs special care and attention to detail and appropriately filled laboratory requisition form guidelines that are usually issued by the national authorities, e.g., Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM) or Indian Council for Medical Research (ICMR) and the World Health Organization (WHO), are to be strictly followed.

For any hand-carried specimen that is transported over a short distance, the specimen needs to be placed upright in appropriate bottles with sufficient fixatives in appropriate racks. For long-distance cross country or different countries, the triple packing system has been advocated specially for infectious substances [12].

The triple packing system contains three layers as (1) primary container/receptacle that has the specimen and is leak proof with a screw cap and (2) secondary container that is durable, waterproof and made of metal/plastic with a screw cap. It contains absorptive material, and details of the specimen are pasted on the outside of the container. (3) The outer packing or tertiary container is made of wood or card-box and withstands the transportation shocks. Dry ice is normally kept between the outer two containers with provision for carbon dioxide gas release vents. A biohazard label is a must [12].

9.5 Basic criteria for rejection of specimens

A laboratory should always consider strict rules on the basis of which an oral specimen could be rejected. This important decision must be taken with full conviction of doing the right thing to save time and laboratory resources. Such decision should be taken when the following criteria are not fulfilled:

1. Inappropriate test requests (incomplete, duplicate, missing or inconsistent information)

2. Errors in transport and handling (light exposure, delayed transport time or broken sample bottles)

3. Misidentification of specimen (unlabeled or mismatched)

4. Improper or wrong container

5. Insufficient specimen quantity for the quantity of preservative or insufficient quantity for the test requested transport media

6. Contamination of specimen

7. Incorrect storage

8. Hemolyzed sample [13]

10. Conclusions

Thus a continuous effort must be made in order to ensure proper collection and transportation of clinical specimens by all involved. A sound understanding of contemporary principles and practices of various methods of collection and transport of specimens is of critical importance to the clinician dealing with oral and maxillofacial infections.

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Conflict of interest

The authors declare no conflict of interest.

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