Contaminants and Mimickers in Cytopathology

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Abstract

Context: Many contaminants are routinely encountered in cytopathology practice. However, because of lack of familiarity and experience with them, many are unnoticed, neglected, or confused with other structures of major relevance. Aims: The purpose of this study was to intentionally introduce contaminants into the smears and to provide distinctive morphological criteria required for the microscopist to identify them confidently to avoid possible confusion. Settings and Design: Prospective cross-sectional study. Methods and Material: This study included smears prepared from the buccal mucosa of healthy volunteers. Common contaminants were deliberately introduced into the smears, fixed using 90% ethanol, and stained with Hematoxylin and Eosin stain (H and E) and Papanicolaou stain (PAP). The study also included smears from leftover cerebrospinal fluid (CSF) and wet mount preparations. The morphology of these contaminants was studied and the results were tabulated. Statistical Analysis Used: Nil. Results: The vivid morphological appearance of these commonly encountered contaminants were described and many of these mimicked structures of major relevance. Conclusions: Contaminants and mimickers can make the evaluation of cytologic specimens challenging and may necessitate secondary review by another pathologist or further workup. Knowledge and familiarity of these commonly encountered extraneous substances will help to prevent misinterpretation.

Keywords: Contaminants, cytopathology, mimickers

INTRODUCTION

Contaminants are objects or phenomena influencing the appearance of smear and obstructing or even preventing proper analysis of important diagnostic factors of the cytological sample. Contaminants and mimickers in cytoptic smears have been considered as a potential diagnostic pitfall in cytodagnosis. Many contaminants are routinely encountered in cytopathology practice. Several of them are considered as inherent and trivial but some, because of lack of familiarity and experience with them, are either unnoticed or neglected or confused with other structures of major relevance. These contaminants cannot be completely eliminated but may only be minimized. When these are encountered, their significance and ways to avoid them should be determined. These have been meticulously projected at the tissue level but not so well at the cytological level except for some isolated thematic studies.

Aim and Objectives

The purpose of this study was to provide distinctive morphological criteria of contaminants required for the microscopists to identify them confidently to avoid possible confusion.

METHODOLOGY

It was a prospective cross-sectional study. Study included smears prepared from the buccal mucosa of healthy volunteers. Common contaminants were deliberately introduced into these smears which were fixed with 95% ethyl alcohol and stained with haematoxylin and eosin stain and Papanicolaou stain. The study also included smears from leftover cerebrospinal fluid (CSF). Wet mount preparations using tap water were used to study glove powder, pollen grains, and plant origin contaminants. Cytological artifacts pertaining to fixation, drying, and smearing were excluded from the study.

A single blind study was conducted in which the smears were viewed by two cytopathologists for identification and...
morphological description of the contaminant using light microscope Labomed L × 400 and later these were subjected to polarizing microscopy. Their effect on interpretation of smears was noted. Buccal smear sampling from the volunteers included an informed consent along with preserving the anonymity. Ethical clearance was obtained from institutional ethical committee.

Many of the contaminants presented in this paper are considered as either intrinsic (sample contaminants derived from patient) or extrinsic contaminants (contaminants of cytological smears incorporated during their processing). Common contaminants introduced included:

**First category** (iatrogenic contaminants)- Procedure-related contaminants like lubricants, ultrasonography gel (USG gel), fibers (natural/synthetic), glove powder, and blood.

**Second category**- Extraneous substances that may get introduced during slide preparation and processing like air bubbles, dust particles, exfoliated squamous cells, plant matter (pollens, trichome, etc.), bacterial and fungal growths, airborne (spores) and waterborne contaminants (algae), stain precipitates, human hair, etc.

**Third category**- Contaminants derived from the patient like normal flora, fibrin strands, skeletal muscle fragments, RBCs, food contaminants, etc.

**Results**

The vivid morphological appearance of these commonly encountered contaminants was studied and described. Many of these mimicked structures of major relevance. The morphological appearances of contaminants with their possible mimickers are listed in Table 1 and Figures 1-5.

Some of the contaminants like glove powder, plant cell wall, dust particles, insect parts, cotton fibers, and synthetic fibers showed positive birefringence under polarized microscopy. Glove powder showed Maltese cross birefringence [Figure 2]. Pollen, algae, vegetable cells, USG gel, lubricants, and fungus were negatively birefringent. Air and trichomes showed partial birefringence.

Pollen grains and plant trichomes chosen for the study belonged to various species of commonly seen flowering plants around our laboratory, particularly parthenium, grass pollen, and *Chloris barbata* [Figure 3].

Commonly encountered airborne fungal spores, which included *Aspergillus*, *Penicillium*, and *Alternaria*, were introduced into the smears from culture tubes and bread molds. Either entire conidiophores or isolated conidia were found on smears. Spores of *Aspergillus* and *Penicillium* were of same size and shape. *Alternaria* appeared as racket-shaped macroconidia with longitudinal and transversal support. Algae samples commonly grown on the surface of stagnant water were chosen to study their varied morphology as depicted in Figure 4.

**Figure 1:** (a) Albumenized slide, PAP × 10. (b) Desquamated squamous cells in CSF, PAP × 10. (c) Dust particles, PAP × 10. (d) Stain precipitate, H and E × 10. (e) Hair, PAP × 4. (f) Lubricant gel, (xylocaine) H and E × 10. (g) Medication gel (candid V), PAP × 40. (h) USG gel, PAP × 10

**Figure 2:** (a, b) Cotton fiber, PAP × 10, Polarized microscopy × 40. (c, d) Gauze fiber, PAP × 40, Polarizer × 40. (e, f) Synthetic fiber (tampon fiber), PAP × 40, Polarizer × 40. (g-i) Glove powder, PAP × 4, wet mount × 40, Polarizer × 40

**Figure 3:** (a, b) Pollen of parthenium, large round to oval in shape with speculated exine, H and E × 40, wet mount × 40. (c, d) Plant trichome, PAP × 4, wet mount × 40. (e-h) Grass pollen wet mount × 40

Various cooked vegetable particles were introduced on to the smears as contaminants. Vegetable cells appeared in clusters...
and in singles. Individual cells of green chilli were polygonal with large hyperchromatic nucleus resembling malignant cells. Garlic cell clusters were of polygonal/ballooned cells with central small round nuclei, resembling squamous cells. Ginger cell, clusters of round cells, mimicked a fatty tissue fragment. Onion cells appeared as compactly arranged sheets of polygonal cells with small dark nucleus. Meat skeletal muscle fibers showed prominent striations [Figure 5].

**Discussion**

A great variety of exotic contaminants may pollute the cytology smears. These may be intrinsic (from patient) or extrinsic which generally arise from surgeon’s chair or pathologist’s laboratory.[4] Few contaminants can be trivial but some of them can result in false diagnosis or misclassification of the lesion.[1] Polarization is a useful procedure to help recognize these foreign bodies since many are birefringent.[5] Many of the potential common and uncommon contaminants that are encountered in cytology practice are discussed hereunder.

Hasty cover slipping or areas of thick unevenly distributed cytological material result in air bubbles and mounting of wet slide gives rise to water bubbles.[3] Air trapping under coverslip is referred to as cornflakes or brown cell artefact.[6] Stain precipitates form as staining solutions age. Precipitates can also form if a saturated solution of stain is allowed to evaporate or dry up on the smear.[1]

Lubricating gel contamination compromises the adequacy and causes serious interpretation errors in both conventional and liquid based preparations of cervical smears.[3,6] Lubricant contamination can also occur in endoscopy and cystoscopy guided specimens.[1] Albumenized slides and lubricant gels morphologically simulate mucin but lack of columnar cells, inflammation, and debris can be a differentiating feature. Ultrasound gel results from guided aspirations and from stain build up.[3]

Desquamated Squamous epithelial cells usually represent contaminants from patient’s oral cavity or upper respiratory tract in respiratory cytology. Exfoliated cells from hands and from sneeze droplets can also act as source.[3]

Glove powder typically used to facilitate donning in surgical gloves is modified corn starch. It is a well-recognized potentially confusing common contaminant of cytological specimens.[6,9]

Normal bacterial flora and candida elements are common contaminants in vaginal, oropharyngeal, and urine samples.[2] Unsterile instruments, unsterile stains, time delay to reach laboratory, and poor sample preservation lead to overgrowth of microbes.[2] Contamination of smears by bacterial and fungal spores can mimic true infection. Positive clinical findings and the presence of significant inflammation in the smears aid in differentiating contamination from true infection.[10,11] Some

![Figure 4: Algae](image_url)

**Figure 4: Algae** (a-c, e, f, h) - Round and pennate diatoms (Silicated cell wall), PAP × 40. (d) - Blue filamentous algae PAP × 40. (g) - Green Ulothrix, unbranched filamentous algae with single row of identical cells and thick cell walls

![Figure 5: Food particles](image_url)

**Figure 5: Food particles** (a) - Green chilli, H and E × 10. (b) - Garlic, PAP × 10. (c) - Ginger, H and E × 10. (d) - Onion, PAP × 10. (e) - Skeletal muscle fiber, PAP × 40. (f) - Carrot - H and E × 10. (g) - Coriander, H and E × 10
Table 1: Morphological appearances and possible mimics of commonly encountered contaminants

| Contaminants                  | Morphological appearance                                      | Possible Mimics / misdiagnosis                        |
|------------------------------|----------------------------------------------------------------|-------------------------------------------------------|
| USG Gel                      | Amorphous granular deposits                                    | Bacteria, platelets                                    |
| Lubricant                    | Homogenous eosinophilic material, Lacks granularity             | Mucin                                                 |
| Medication gel               | Amorphous granular deposits                                    | Bacteria, Clue cells                                   |
| Albuminized slides           | Homogenous eosinophilic material, Lacks granularity             | Mucin, colloid                                        |
| Glove powder                 | Refractile, polygonal shaped, 5-20 µm, with central dark striation or Y-shaped structures | Epithelial cells, parasite ova, fungal yeasts         |
| Dust                         | Unevenly light/ dark brown to black in color. 8-10 µm in diameter, angular and irregularly polygonal with sharp edges | -                                                     |
| Human hair                   | Long, cylindrical, relatively thick dark brown in color, dark central medulla with light outer cortex in deep focusing plane | -                                                     |
| Air bubble                   | Thick circular dark border with a bright center                 | -                                                     |
| Stain precipitate            | Highly amorphous and granular precipitate                       | Bacteria                                              |
| Fungal molds                 | Isolated conidia or entire conidiophore                         | True infection                                         |
| Cotton fiber                 | Long thin filamentous, spirally twisted with series of lines forming a fringe along the entire length | Fungal hyphae, parasite larva                          |
| Synthetic fiber (rayon)      | Numerous fine dots covering the entire surface and the two ends terminate abruptly | Larvae                                                |
| Gauze fiber                  | Linear filamentous, absence of internal anatomical structure. Ends terminate abruptly | -                                                     |
| Plant trichome               | Linear branching structures, with thick walls. One end is rounded, other end is tapering. Internal refractile elongated core may be visible. Lack septae and budding | Fungal hyphae                                         |
| Pollen grains                | Spherical structure, 6-100 µm, Inner wall-thin and delicate, Outer wall may be smooth or rough with warts, grains, troughs, etc. | Parasitic ova, atypical cells, psammoma bodies, algae, protozoa cysts, etc. |
| Fibrin strands and meshwork  | Variable in size and shape, lack of structure and thin fibrillary morphology | Fungal pseudo hyphae, necrosis                        |
| Algae                        | Unicellular or multicellular, assume different morphologies. Round, pennate, spheroid, filamentous, etc. | Parasite worms, pollen, fungi, filamentous bacilli, Charcot Leyden crystals |
| Vegetable cells              | Singles or in loose clusters. Have thick cell wall, basophilic cytoplasm and dark nuclei | Normal human cells, Malignant cells                    |
| Skeletal muscle fiber        | Large diameter, lacks internal structure.                      | Parasite                                              |

Intrinsic endogenous structures which act as pseudo microbes include RBCs, platelets, mucus, tissue fibers (skeletal, elastic), fibrin ciliocytophthoria, Liesegang rings, and psammoma bodies.\cite{2,12}

A variety of pollen grains and airborne fungal spores (especially Alternaria, Aspergillus, Penicillium, Fusarium) are ubiquitous in the atmosphere and hence gain entry onto glass slide from contamination of collection materials or laboratory equipments.\cite{2,13} Fungal spores may also be inhaled and then deposited in various parts of respiratory tract and act as intrinsic contaminants.\cite{14} Air spores are well studied as allergens and their concentration varies according to seasonal periodicity.\cite{15,16}

Pollen grains are round or ovoid in shape, 6–100 µm with a double wall, inner wall being thin and delicate. Outer wall is smooth or rough with warts, grains, troughs, etc.\cite{15} In a study by Accorsi et al., smears with pollen content higher than 15 grains per slide were recorded in poliosisnosis patients. In case of intrinsic contamination, stained pollen grains are at the same depth of focus as the cytological material.\cite{17} Pollens and plant cells in stool may be confused for a variety of helminth eggs especially of taenia species and *Trichuris trichiura* eggs. Features like small size, lack of hooked oncospheres, and embryo wall help in distinguishing these from parasite ova.\cite{18}

Human tissue fibers (skeletal muscle or elastic fibers), suture material, strands of mucus (Curschmann’s spirals), and plant matter (plant hairs) mimic parasite worms. Their existence in unusual shapes like sharp kinks, lack of external (mouth, hooks, etc.) and internal (digestive or reproductive tract) noticeable anatomical structures are often vital in differentiating contaminants from real worms.\cite{2,3}

The sources of fiber contaminants include cotton swabs, gauze, hair, cytobrush bristles, commercial tampons, etc.\cite{19} In the study by Van Hoeven and Bertolini, fibers were identified in 178 of 1368 cervical smears with cotton and rayon being the most common ones.\cite{19} The surface of natural fibers like cotton shows a series of lines forming a fringe along the whole of its length, whereas the surface of synthetic fibers shows dotted pattern.\cite{1,2} Features such as geometric shape, absence of nuclei, and lack of septation assist in differentiating these contaminants from real findings.\cite{2,3}
Presence of microalgae in cytology smears can be from intrinsic contamination or from tap water. They may be unicellular or multicellular, assume different morphologies. Diatoms range from 2–200 μm, either circular, elliptical or triangular in shape contained within silicated cell wall. Their presence indicates faulty water purification system. Algae in body fluids and viscera may be used in the investigation of death by drowning. Algae have been confused with fibers of vegetable origin, synthetic fibers, and pollen.

Food contaminants can originate from brushings of the GIT, anal pap smears, urinary diversion specimens, or as an aspirated contaminant in respiratory specimens. In respiratory cytology specimens, vegetable contaminants may be mistaken for squamous cell carcinoma. Identification of refractile cell wall is the key to avoid this pitfall.

Martinez-Giron et al. noticed both freshwater and terrestrial arthropods in routine Papanicolaou smears and considered them as due to accidental contamination of samples.

Some of the important factors that differentiate contaminants from genuine findings include their presence on any one of the slide, location of structure at the edge of slide, and its placement in a different focal plane. Contaminants can be minimized by following simple measures like proper patient preparation before sample collection, strict adherence to standard operating protocol in terms of sample collection, preservation, and processing, proper preparation and maintenance of stains and fixatives, maintaining clean working area and with good clinical correlation. Ours is a unique study because it is a first comprehensive catalogue illustrating and describing the cytological contaminants to bring awareness among pathologists.

It was a modest attempt at providing morphological appearances of a variety of familiar and unfamiliar contaminants acting as potential mimickers. They may pose difficulty to the unwary and may cause a potential “wild goose chase” that can result in wasted valuable time and resources. Our basic concept was to bring awareness among Pathologists because “what the mind does not know eyes cannot see.”

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**Declaration of patient consent**

The authors certify that they have obtained all appropriate patient consent forms. In the form, the participants have given their consent for their images and other clinical information to be reported in the journal. The participants understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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**Conflicts of interest**

There are no conflicts of interest.

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