Pre-Analytical Phase in Mycology

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ABSTRACT

Fungal infections remain a frequent cause for medical consultation, representing a prevalence of 20-25%. Mycology laboratory represents the key to the management of fungal diseases, whose objective lies not only in providing accurate analysis results, as well as completing them within a satisfactory time, maintaining traceability for all laboratory procedures, in accordance with ethical principles, while ensuring the safety of both patients and staff. The pre-analytical phase is the most complex part of the biological process, and some steps may escape the attention of biologists, since this phase involves several external factors beyond the laboratory, which requires the management of a massive flow of information. or this reason, the implementation of a quality system allows the detection and minimization of errors occurred during each stage of this phase, in particular the ISO15189 standard and the Guide of proper execution of analysis. Through this work, we have reviewed the main stages of the pre-analytical phase in mycology, from prescription to sample preservation, focusing on the requirements of this process, as well as the primary investigations to be conducted for each type of mycological specimen.

Keywords: pre-analytical phase, mycology laboratory, samples, steps.

RESUME

Les infections mycosiques demeurent un motif fréquent de consultation médicale avec une prévalence de 20 à 25 %. Le laboratoire de mycologie représente la clé de la prise en charge des maladies mycologiques dont leur objectif ne réside pas seulement dans la fourniture de résultats d'analyses exactes, mais également dans le fait de les réaliser dans un délai satisfaisant, en assurant une traçabilité de toutes les procédures du laboratoire, dans le respect de l'éthique, en assurant à la fois la sécurité des patients et du personnel. La phase pré-analytique est la phase la plus complexe du processus biologique, certaines étapes peuvent échapper aux biologistes puisque cette phase fait intervenir plusieurs acteurs extérieurs au laboratoire et nécessite la gestion d'un flux énorme d'informations. Pour cela la mise en place d'un système qualité permet de reconnaître et de minimiser les erreurs produites lors de chaque étape de cette phase notamment la norme ISO15189 et le Guide de bonne exécution des analyses (GBEA). A travers ce travail, nous avons rappelé les principales étapes de la phase pré-analytique en mycologie avec une mise au point sur les exigences de ce processus, ainsi que les principales recherches à effectuer sur chaque type de prélèvement mycologique.

MOTS CLES : Phase pré-analytique, laboratoire mycologie, prélèvements, étapes.

INTRODUCTION

Fungal infections remain a frequent cause for medical consultation. Superficial fungal infections of the skin and nails, as an example, have a high prevalence of 20 to 25% of the world population, that suffers from fungal diseases, while the most cases remain benign [1].

The mycology laboratory assumes a crucial role in the management of fungal diseases, by the identification of the mycosic agent of the infection [2]. The laboratory's objective lies not only in providing accurate analysis results, but also in completing them within a satisfactory period of time, ensuring traceability of all laboratory procedures, respecting ethical standards and ensuring the safety of both patients and staff. Therefore, every medical analysis laboratory must have appropriate procedures to ensure the quality of the results delivered. However, biologists still face discrepancies between results and clinical practice [3]. These inaccuracies, which account approximately to 93% of errors detected during the biological diagnosis process, are mainly caused by the absence of standardization of pre-analytical procedures [4].

The pre-analytical phase includes all steps from the medical prescription to the receiving of the sample in the laboratory sampling, routing, reception, non-conformity management, recording, pre-processing, and preservation [5,6]. The pre-analytical phase is the most complex part of the biological process, and some steps may escape the attention of biologists, since this phase involves several external factors beyond the laboratory, which requires the management of a massive flow of information [7,8,9].

The implementation of a quality system allows the detection and minimization of errors occurred during each stage of this phase. Various international standards have been established to optimize error control in biological analyses and standardize practices across different laboratories, notably ISO 15189 and the Guide of Proper Execution of Analysis (**GPEA**). The **GPEA** represents the first regulatory reference concerning quality in Morocco [10]. It focuses on the general standards for managing biological samples regarding sampling, identification, and preservation (Chapter II) [11]. The latest states that every laboratory must have procedures for the pre-analytical phase to ensure the quality of the results provided [9,12]. Furthermore, the advent of ISO 15189 as a mandatory reference for medical biology laboratories has led to a significant improvement in the quality of the pre-analytical phase (Chapter 4.7 and 5.4) [13,14]. For this reason, various requirements should be considered to control all the stages of this process [10].

Through this work, we have reviewed the main stages of the pre-analytical phase in mycology, from prescription to sample preservation, focusing on the requirements of this process, as well as the primary investigations to be conducted for each type of mycological specimen.

REQUIREMENTS OF THE PRE-ANALYTICAL PROCESS IN MYCOLOGY

- **Analysis request:**

This sheet must include all the information required for accurate identification of the patient and the prescriber, particularly the identity of the patient, the prescriber, and the sampler. This last mention is required by the **GPEA** and is part of the care traceability process. A specific case should be provided forthis purpose on the transmission sheet. To provide credible results, the information concerning the sampleshould be mentioned [15], such as the specimen type, sampling site, the nature of prescribed analyses, aswell as the date and, if necessary, the time of sampling. Certain clinical data relating to the patient areessential for interpreting and processing the samples. Such as a notion of travel to foreign country or contactwith animals, for example, contact with cattle, should be investigated in cases of suspected dermatophytes infection of *Trichophyton verrucosum* [16]. The notion of immunodeficiency is also crucial for interpreting results in mycology, especially to differentiate colonization from infection. An antifungigram is more likely

to be performed for fungi that would have been considered simple contaminants in an immunocompetent subject. All this information must be stated clearly and precisely to minimize possible errors. Finally, the samples should be ideally taken prior any antifungal treatment that may inhibit the in vitro growth of the fungus [17,18]. The intake of antifungals, such as for nails infection, must be mentioned on the prescription sheet.

- **Sampling procedure:**
  - ***Sample collection:***

Sampling is a decisive step in establishing a mycological diagnosis [2]. Therefore, mycological examination must be carried out at a distance from any application of antifungal treatment [17,18]. For a nail examination a therapeutic time window of approximately 15 days must be respected after prescribing a filmogenic solution or a nail polish with ciclopirox as the active substance, and at least 3 months after taking antifungal oral medication [17]. In the case of tinea corporis or tinea capitis a window of 4 weeks after applying an antifungal treatment must be respected [15]. A toilet with a PH neutral soap is recommended before sampling [15]. Cosmetic nail polish should be avoided [15]. As well as Avoid applying cream or other substances: before taking any samples from the nails and skin, those substances will interfere with the direct examination. Most of the samples must be taken inside a laboratory by an expert biologist, such as in the case of dermatophytes research, and some samples must be taken as soon as possible if the prognosis is life-threatening, as example, the research cryptococcosis infection [19].

  - ***Identification of sample:*** The sample should be identified by the patient's first and last name, date of birth, and patient ID [4]. For skin samples and biopsies, the sampling site must also be indicated.
  - ***Sampling methods:*** Detailed methods for mycological sampling are summarized in tables **(Tables I and II).**

To standardize practices, it is essential to provide prescribers with a documented sampling manual. This will entail general recommendations for patient preparation, sample identification, sampling steps, sample type and quality, and the time of sampling [4,9]. This meets the requirement of ISO 15189 in chapter 5.4.4: "The specimen collection manual must be part of the document control system".

Material: The quality of mycological sampling is essential for the quality of the results obtained during the examination. This condition is particularly important for skin samples, for which many false-negative results are due to improper execution of the sampling. A high mycological quality of sample cannot be taken without a minimum of equipment, including sterile Brocq curette or sterile Vidal scrapers.

The choice of equipment will depend on the location of the lesion. Similarly, a preliminary examination under a Wood's lamp is necessary to identify lesions of pityriasis versicolor and differentiate them from erythrasma, while it is rarely used to localize tinea capitis, thus differentiate microsporosis from trichophytosis and favus tinea capitis [2,17,20]. Although they have little influence on the quality of the analysis, sterile Petri dishes made of glass are preferred instead polystyrene Petri dishes for the collection of epidermal scales [21]. Similarly, in the case of onychosis to use scissors to trim the upper nail plate, which is typically not very contributory for dermatophytes investigation [2]. And it's essential to always remember that universal guidelines recommend wearing gloves for every sampling procedure [22].

- **Sample routing:**

The transport of samples to the laboratory must respect the time and temperature appropriate to each type of analysis in the sampling manual [4]. In this regard, the prescription sheet, which includes the date and time

of sampling, ensures traceability to the laboratory and thus confirms the absence of any non-conformity [4]. Each non-conform sample to the recommendations is managed in a special way, both the sampler and the prescriber are informed through a non-conformity form. Furthermore, most samples do not present any particular constraints for their routing to the laboratory. A maximum delay of 24 hours at room temperature is conceivable for most samples. It can even be extended if the sample is stored at 4°C [2,12,15]. Finally, some types of samples should be carried in a specific way [23].

- **Sample reception:**

The received samples must be recorded and examined by authorized staff. In addition, samples must be checked for conformity. The laboratory is required to have a documented procedure with acceptance and rejection criteria. If a non-conformity sample is accepted, they must specify the actions taken to correct the problem, and the persons responsible for accepting the sample. The final report should specify the nature of the non-conformity as well as any reservations concerning interpretation of the result [4,9]. Samples must be stored for a specific period determined by the manual for each type of sampling. Most mycological samples are stored at a temperature of 4°C [2,12,15].

MYCOLOGY SAMPLE COLLECTION PROTOCOL AND PRESERVATION

We have summarized all mycological analyses in tables form (**Table I**). These tables are presented according to the context or location of the sampling. They contain the necessary information on conditioning, routing times, preservation of samples in the event of delayed processing, and the investigations that can be carried out on each type of sample. Furthermore, **Table II** corresponds to the protocols used in our laboratory for conducting mycological sampling.

CONCLUSION

The pre-analytical stage is the main step in the mycological examination process and must be mastered by biologists in order to provide the most reliable results. To this end, everyone involved in this phase must be aware of the importance of the pre-analytical stage, of the risks posed by making errors, and their potential consequences for the patient. Thus, the implementation, respect, and exact application of the procedures and the quality improvement systems for this stage are necessary to avoid all errors that may occur during all mycological examinations conducted.

TABLES

Table I : mycological samples [2,15,24]

Nature of the sample		Conditioning and/or required materials	Routing	Preservation if treatment delayed	Performed analysis
Blood sampling	Serum	Dry tube (s)	24h	4°C	-Fungal serology:
					Aspergillosis, candidiasis, scedosporiosis,
					Coccidioidomycosis, histoplasmosis, blastomycosis,

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					- Antigen detection :
					Aspergillosis
	Hemocultures	Specific systems for hemocultures	Adapt to manufacturer's specific instructions		Pathogenic fungi
Samples of urogenital origin	Total morning urine (Do not drink after 10 p.m. the day before sampling) / First morning urine stream	Sterile plastic vial	24h	4°C	Pathogenic fungi
	Vaginal/urethral sampling	Moistened sterile swab	24h	4°C	Yeasts
Intestinal samples	Feces (low fiber diet 3 days prior to sampling)	Sterile vial	24h	4°C	Standard mycological analysis
	Anal sampling	Sterile swab	24h	4° C	Pathogenic fungi
	sputum	Sterile vial	24 h	4° C	Aspergillus spp and other Pathogenic fungi
	Bronchoalveolar lavage	Sterile vial of 20 ml	24 h	4° C	Cryptococcus spp, Pneumocystis jirovecii, Aspergillus spp and other Pathogenic fungi
	Pleural fluid	Sterile vial	24 h	4° C	Pathogenic fungi
	Bronchial/tracheal suction	Sterile conical vial	24h	4° C	Aspergillus spp and other Pathogenic fungi
Ocular samples	Conjunctivitis	Sterile swab	24h	4° C	Pathogenic fungi
	Cornea (scraping)	Swab	2h	Immediate treatement	Pathogenic fungi
	Contact lenses	Sterile vial, add sterile physiological water to cover the lenses. obtain the lenses case	2h	Immediate treatment	Pathogenic fungi
	Eyelashes	Sterile bottle, with sterile physiological water	24h	4° C	Pathogenic fungi

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Skin samples	Tinea capitis	Wood's lamp, sterile Vidal scraper, tweezers, petri dish, sterile swab, sterile distilled water or physiological water	Immediate hair examination (contagious risk)		Dermatophytes
	Pytiriasis vesicolor	Wood's lamp, blade, sterile Vidal scraper, transparent adhesive cellophane, petri dish		Ambient temperature	Malassezia furfur
	Onychosis	Sterile Vidal scraper, sterile glass Petri dish, sterile swab, sterile distilled water or physiological water, compress.		Ambient temperature	Dermatophytes and yeasts
	Paronychia				yeasts (Candida spp)
	Tinea cruris, tinea corporis	Sterile Vidal scraper, petri dish, sterile swab, sterile distilled water or physiological water, compress.		Ambient temperature	Dermatophytes and yeasts
Natural cavity sampling	External auditory canal	Swab Or use a transport medium	24h	4° C	Pathogenic fungi
	Throat		24h	4° C	Pathogenic fungi
	Nose		24h	4° C	Pathogenic fungi
	Sinus		24h	4° C	Pathogenic fungi
	Mouth		24h	4° C	Pathogenic fungi
	Saliva	Sterile vial	24h	4° C	Pathogenic fungi
Foreign material sampling	blade	Sterile vial	24h	4° C	Pathogenic fungi
	Catheters (last 5 cm from distal tip)				
	Urinary catheterization				
	Implantable material	Sterile vial/ sterile packaging			

Table II: protocol used in our laboratory for mycological sampling.

Tinea capitis	- Use the Wood's lamp and see the existence of a fluorescence.
	- Sampling within the alopecia patch and around the periphery of the broken hair using tweezers.
	- Scrape the lesion with the curette and collect the scales and broken hair stuck in the scales in the Petri dish.
	- use a sterile swab on the scraped area.

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Onyxis	Clean the nail plate with alcohol.
* Superficial leukonychia	Scrape the entire lesion with a curette and collect the nail fragments in a Petri dish.
* Proximal onyxis	Scrape carefully the lesion along the lateral edge of the nail with a curette and collect the nail fragments in the Petri dish. Swab up serous material using a sterile swab previously moistened with sterile distilled water. If an associated Perionyxis.
* Distal or total onyxis	Cut the distal part of the nail.
	Scrape the underside of the nail at the junction of the affected/healthy area using a curette and collect the nail fragments in the Petri dish.
Perionyxis	Wipe the lesion with a sterile compress, scrape the periungual pad with the curette and collect the scales in the Petri dish.
	Swab the scraped area with a previously moistened sterile swab.
	After sampling, disinfect the lesion.
Tinea cruris, Epidermophytosis	Wipe the lesion with a sterile compress.
	Scrape the periphery of the lesion with the curette and collect the scales in the Petri dish.
	Swab the scraped area with a sterile swab
Tineas corporis, eczema	Wipe the lesion with a sterile compress.
	Scrape the periphery of the lesion with a curette and collect the scales in the Petri dish.
	Swab the scraped area with a sterile swab.
Pytiriasis versicolor:	Examine the skin with a Wood's lamp and mark the suspected area with a felt-tip pen.
* Squamous lesions	Apply a piece of adhesive cellophane to the lesions.
* Less squamous lesions	Apply pressure for 3 to 5 seconds, then remove the cellophane and apply it to the object plate.
	Scrape the lesion using a vaccinostyle/curette, then apply adhesive cellophane to the lesion.
Malassezia furfur folliculitis / sampling for Malassezia furfur culture	Scrape several suspected lesions with a vaccinostyle/curette.
	Apply an adhesive cellophane, then swab vigorously with a sterile swab previously moistened.
Folliculitis and sycosis	Pluck hairs from the inflamed area.
	Open folliculitis pustules with a curette.
	Swab the scraped area using a sterile swab previously moistened.
Vulvo-vaginitis, balanoposthitis	Apply two sterile swabs moistened with distilled water on the area to sample (one for direct examination, the other for culture).
Aspergillus testing	The samples must be taken under aseptic conditions.
	use a dry swab.
	Serology using a blood test collected in a dry tube

Declaration of Interest

The authors declare to have no interest in relation to this article

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