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(REVIEW ARTICLE)

The pre-analytical phase in parasitology

O. NASSIRI [1, 2, *], F. BOUSNINA [1, 2], B. EL RAFFOULI [1, 2], H. EZZAIDI [1, 2], I. MAHI [1, 2], S. LAGHZAOUI [1, 2], O. KHARKHACH [1, 2], A. RHOUBI [1, 2] and A. HAMI [1, 2]

[1] *Faculty of Medicine and Pharmacy of Oujda, Mohammed First University, Morocco.*
[2] *Central Laboratory, Mohammed VI University Hospital of Oujda, Morocco.*

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Abstract

Parasites are among the main agents of the diseases from which mankind suffers. The parasitology laboratory plays an important role in the diagnosis of parasitic disease. One of its main challenges is to achieve, maintain and improve the accuracy, reliability and speed of its analyses. However, only good quality management in the laboratory will enable these objectives to be achieved and reliable results to be delivered. The pre-analytical phase covers all stages from the medical prescription, through sampling, routing, reception, non-conformity analysis, recording, pre-treatment and storage, right up to the analysis of the sample in the laboratory [3]. A number of requirements need to be taken into account in order to master all the stages of this process, over which the biologist has little control, as a significant proportion takes place outside the laboratory.

In the present work, we have highlighted the importance and complexity of the pre-analytical phase in parasitology. We have identified and described the various sub-processes involved in this phase, namely: prescription, timing of sampling, its stages, routing, reception and storage. We have also described the main investigations that can be carried out on each type of sample in the various parasitology laboratories in general, and in our laboratory in particular.

Keywords: Pre-analytical phase; Parasitology; Quality; Results

1. Introduction

Parasites are among the main agents of the diseases from which mankind suffers. The incidence of parasitic diseases remains fairly high. According to the latest report from the World Health Organization (WHO), there are 247 million cases of malaria in the world. Also, the estimated number of deaths attributable to malaria has risen from 625,000 in 2020 to 619,000 in 2021 [1].

The parasitology laboratory plays an important role in the diagnosis of parasitic disease. One of its main challenges is to achieve, maintain and improve the accuracy, reliability and speed of its analyses. However, only good quality management in the laboratory will enable these objectives to be achieved and reliable results to be delivered.

Biologists are constantly confronted with discrepancies between results and clinical findings. These inconsistencies are often due to a lack of standardization in pre-analytical procedures. They can account for up to 93% of errors encountered during the biological diagnostic process [2].

The pre-analytical phase covers all stages from the medical prescription, through sampling, routing, reception, non-conformity analysis, recording, pre-treatment and storage, right up to the analysis of the sample in the laboratory [3]. The Moroccan Guide to the correct performance of analyses (GBEA) provides general rules for the management of

* Corresponding author: O. NASSIRI

biological samples in terms of sampling, identity-vigilance, identification and storage (Chapter II) [4]. The latter requires each laboratory to draw up procedures for this stage, in order to ensure the quality of results delivered [5]. ISO 15189 also has a chapter entirely dedicated to the management of the pre-analytical phase (Chapter 4.7 and 5.4). However, a number of requirements need to be taken into account in order to control the whole of the pre-analytical phase.

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In the present work, we have highlighted the importance and complexity of the pre-analytical phase in parasitology. We have identified and described the various sub-processes involved in this phase, namely: prescription, timing of sampling, its stages, routing, reception and storage. We have also described the main investigations that can be carried out on each type of sample in the various parasitology laboratories in general, and in our laboratory in particular.

2. Pre-analytical process requirements in parasitology

2.1. Request for analysis

The prescription form must enable the laboratory to access all the information required for unambiguous identification of the patient and the prescriber. It must also include all the administrative information required for the laboratory's billing process. In this way, the biologist will have access to the information needed to interpret the results, such as the date and time of sampling [2]. This type of information can be useful for urgent samples (malaria…) or for microfilaria research having a periodicity. The type of sample, the sampling site, clinical information about the patient, and in particular any notions of a stay abroad, particularly in malaria-risk countries (place, duration and date of stay, chemoprophylaxis followed) or immunodepression (chemotherapy, AIDS...) must be mentioned, and thus guide the search for certain tropical or opportunistic parasites (Cryptosporidiosis). Contact with animals or high-risk professions (bird breeders, etc.) may also be useful to the biologist. If treatment is in progress, this must be mentioned. In parasitology, clinical, epidemiological and biological information is essential for good analysis, correct interpretation and advice.

2.2. Sampling

2.2.1. Time of sampling

- Immediately, without delay if there is a notion of urgency (vital prognosis at stake).
- Sampling must be carried out before any antiparasitic treatment; otherwise, the treatment must be mentioned on the prescription sheet.
- Depending on the periodicity of the parasite, daytime or night-time sampling: for microfilariae (blood sample taken at 12 p.m. or 10 p.m., depending on periodicity).
- In the morning, before toileting and bowel movements, for anal scotch testing or anal sampling [6].
- For parasitological examination of stools: three parasitological examinations of stools on three different days after a residue-free diet and cessation of certain medicinal substances (kerosene oil, charcoal, laxatives, intestinal dressings, barium) are required to improve the sensitivity of the examination [7].
- When testing for amoebae, stool exoneration must be performed in the laboratory (fresh stool test).

2.2.2. Sample identification

The sample must be identified by the patient's first and last name, date of birth and identifier.

2.2.3. Sampling procedures

Detailed procedures for parasitological sampling are summarized in tables (Tables I, II and III). In order to standardize practices, it is essential to provide prescribers with a documented sampling manual. This should detail general recommendations for patient preparation, sample identification procedures, sampling steps, sample type and quality, and timing of sampling [2]. This meets the requirement of ISO 15189 in chapter 5.4.4: "The specimen collection manual should be part of the document control system" [8].

2.3. Sample transmission

The laboratory must ensure that samples are transported within the time limits appropriate to each type of analysis, and at the temperature specified in its sampling manual. In this respect, the prescription sheet containing the time of sampling will enable the laboratory to trace the time of receipt and thus ensure the absence of any non-conformity [2].

In addition, when sending samples to the laboratory, a very short turnaround time is essential in certain urgent situations, for example, when testing for Plasmodium in a blood smear/thick drop. The same applies to fragile parasites, such as amoebae in stool parasitology. Samples must be taken in the laboratory to preserve the viability and mobility of vegetative forms. For other samples, a maximum delay of 24 h at room temperature is conceivable. This can even be increased if the sample is stored at 4°C. Finally, transport media are required for certain types of samples.

2.4. Receipt of samples

Samples received must be recorded. Prescription sheets and samples must be examined by authorized personnel. Urgent requests must be sorted and prioritized. Sample identification by the laboratory is carried out in the sampling room when the patient is sampled in the laboratory, or in the sorting room when the sample comes from outside the laboratory. Samples must be checked for conformity. The laboratory must have a documented procedure specifying sample acceptance and rejection criteria. In the event of acceptance of a sample that does not meet the criteria, a waiver must specify the actions taken to correct the anomaly, and the persons responsible for accepting the sample. The final report should indicate the nature of the problem encountered, as well as any reservations regarding the interpretation of the result [2].

3. Sample management and procedures

We have summarized all parasitological analyses in the form of tables (Tables I and II). These tables are presented according to the context or location of the sample. They contain the necessary information on packaging, delivery times, preservation of samples in the event of delayed processing, and the investigations that can be carried out on each type of sample. Table III shows the methodologies used in our laboratory for parasitological sampling.

Table 1 Parasitological samples

Type of sample		**Packaging and/or equipment required**	**Routing**	**Preservation if processing deferred**	**Analyses performed**
Blood sampling	Blood smear/ thick drop	Blades, slides	15 min Immediate treatment Plasmodium, microfilaria	Immediate treatment	Plasmodium, microfilariae
	Whole blood	EDTA tube	30 min	Immediate processing,	Detection of specific antigens of P. falciparum, blood microfilariae,
	serum	Dry tube(s)	24h	4°C	Parasitic serologies Toxoplasmosis, amoebosis, leishmaniasis, filariasis, toxocariasis, anisakiasis, alveolar echinococcosis, hydatidosis, cysticercosis, distomatosis, schistosomiasis, hypodermosis.
Urogenital samples	All morning urine (no drinking after 10 p.m. the day before sampling)	2 L bottle	2h	Analysis must be performed on the same day	Schistosomes, microfilaria after treatment or during chyluria
	First morning urine	Steril plastic bottle	1h	Immediate treatment	Trichomonas vaginalis
	Vaginal/urethral swab	Sterile swab discharged in a transport medium	24h	Ambient temperature	Trichomonas vaginalis
		Without transport media		Immediate treatment	Trichomonas vaginalis
Intestinal samples	Feces (residue-free diet 3 days prior to sampling)	Sterile jar filled 3/4 full	24h	4° C	Standard parasitological analysis: eggs of nematodes, cestodes, trematodes, cysts and vegetative forms of flagellates and amoebae.

	Liquid, soft feces, bloody feces		1h	Immediate treatment	Standard parasitological examination and specific investigations: Amoebae, anguillules, hookworms, flukes, schistosomes, microsporidia, cryptosporidia Antigen detection (Giardia and Cryptospridium)
			24h	4° C	
	Duodenal aspiration fluid	Sterile bottle	1h	Immediate treatment	Anguillules, staves, Giardia
	Scotch anal test	Transparent Adhesive cellophane	24h	Room temperature	Pinworms, Taenia
Respiratory samples	Sputum	Sterile bottle	24 h	4° C	lung flukes
	Bronchoalveolar lavage	20 ml sterile bottle	24 h	4° C	Toxoplasma
	Pleural fluid	Sterile bottle	24 h	4° C	Parasites
Ocular samples	Aqueous humor	Syringe or other container (minimum volume 50 µl)	24h	4° C	Anti-toxocara antibodies Toxoplasmosis-specific antibodies (for immune load, enclose serum) a serum)
		Collect the sample in physiological water	24h	Immediate treatment	Free amoebae
	Cornea (scraping)	Swab	2h	Immediate treatment	Free amoebae
	Contact lenses	Sterile bottle, add sterile physiological water water to cover lenses.	2h	Immediate treatment	Free amoebae
	Passage of an adult worm	Sterile bottle, with sterile physiological water	24h	4° C	Identification of adult filariae
Skin samples	Scabies	Vaccinostyles, slides, chloral-lactophenol, India ink		Immediate treatment	Sarcoptes scabiei (adults, larvae, eggs)
	Cutaneous leishmaniasis	Vaccinostyles, blades, forceps, sterile compresses, dressing, disinfectant		Immediate treatment	Leishmanias

Table 2 Specimens to be taken in the event of toxoplasma seroconversion in pregnant women and newborns

Type of sample	**Sample Packaging and/or material Required**	**Routing**	**Preservation if processing delayed**	**Analyses performed**
Antenatal : amniotic fluid	2 x 10 ml sterile vials	24h	Immediate treatment	Inoculation to mice
In the mother delivery : blood	Dry tube	24h	4° C	toxoplasmosis serology
Placenta	Plastic pot 1 kg : minimum 200g without formaldehyde	24h	4° C	Inoculation to mice
In the newborn : Cord blood	Gel-free dry tube	24h	4° C	Inoculation to mice, Toxoplasmosis serology.
	EDTA Tube	72h	-20°C	PCR toxoplasmosis
Infant blood	Dry tube	24h	4° C	Toxoplasmosis serology
CSF	Sterile bottle	24h	-20°C	PCR toxoplasmosis

Table 3 Methods used in our laboratory for parasitological sampling

Parasitological examination of stools	Prepare the patient for the examination: Avoid drugs containing charcoal, kerosene oil, laxatives, intestinal dressings and barium. Avoid all drugs containing quinolines or imidazoles. Avoid foods rich in residues: fruits with undigested cuticles (peaches, tomatoes), seeded fruits (figs), rosaceous fruits (apples and especially pears). Pulses. Seeds with husks (beans, lentils). Perform 3 parasitological stool tests 2-3 days apart, as some parasites emit intermittently. Single-use gloves, first stool of the morning, 30 to 50 grams of stool not mixed with urine. Transparent, clean jar with wide lid and hermetic seal. Label with patient's name. Homogenize well using a wooden tongue depressor, Rapid delivery to the laboratory, within 2 hours. Maximum time allowed is 24 h at room temperature.
Scab test	Carefully scrape the stratum corneum around the lesion with a sterile feather. Apply a drop of chloral-lactophenol to the scales and cover with a coverslip.
Testing for leishmaniasis	Disinfect the sampling site. Use forceps to lift crust, avoiding bleeding as much as possible (if bleeding occurs, blot with bleeding, blot with compresses). Scrape the edges and bottom of the lesion with sterile vaccinostyl. Take the thinnest possible smears on the slides
Scotch anal test	To be applied in the morning before washing or defecation Apply a piece of adhesive cellophane along the anal margin Smooth out the piece of adhesive cellophane, then remove it with a quick tug and spread it out on the blade without wrinkles
Blood smear/drop Thick	Capillary sampling : Place equipment close to the patient. Disinfect fingertip Press on the pulp to make the blood flow. Never prick the thumb, and in children, prick at the heel.

	Prick the finger with a vaccinostyle or capillary puncture pen. Venous sampling :Place a tourniquet around the patient's upper arm and look for a large vein with little mobility. Disinfect the injection site with alcohol and allow to air dry. Insert needle and gradually draw bloodRelease the tourniquet, remove the needle and press firmly on the puncture site with dry absorbent cotton Transfer the blood to the EDTA-containing tube and mix gently by inverting the tube six times Take 3 to 4 smears and one thick drop
For blood smears: Emergency diagnosis of Malaria	Place a small drop of blood at the end of the slide Place a clean slide at a 45° angle to the support slide, allowing the blood to diffuse along the slide by capillary action. slide by capillary action and spread the blood evenly over the slide with a sharp, even stroke. Air dry the slide.
For the thick drop	Collect a large drop of blood in the middle of a slide. Stop the bleeding by applying pressure to the puncture site with a compress. Rapidly defibrillate the drop of blood by rotating it regularly for 1 to 3 min using the tipof a vaccinostyle.
Ear swab	Patient in supine position, head immobilized Direct illumination: lamp, ear speculum if necessary Clean the ear canal with a dry swab soaked in sterile water Gently swab the ear canal Return swab to case Rapid transfer to laboratory
Eye swab	Pull the eyelid tight Take 2 swabs from the inner edge of the conjunctiva, passing over the inner corner of the eye Rapid transfer to laboratory
Oropharyngeal and nasal swab and nasal swabs	Tilt the user's head back at an angle of approximately 70°. Depress the back of the tongue with the tongue depressor and ask the user to vocalize user to vocalize "A" sounds. Insert the swab, avoiding touching the lips, teeth, tongue, cheeks or uvula cheeks or uvula, and quickly rub the swab over the posterior pharynx and tonsils usingthe swab Gently insert the same swab 1 to 2 cm into an anterior nostril until resistance is resistance at the turbinates and rotate against the nasal mucosa for 3 seconds.
Anal swab	Insert the swab about 3 to 5 cm into the anal canal Gently rotate the swab clockwise for approx. 5 to 10 seconds while rubbing against the rectal walls. If the sample is grossly contaminated with faeces, discard it and repeat the sampling procedure. Carefully remove the swab. Send promptly to the laboratory.
Vaginal swab	Inserting the sterile swab into the vagina Rotate swab Removal and return of swab to sheath Rapid transfer to laboratory
Skin swab	Clean lesion Rinse with sterile saline solution Cure active edge

	Swab Rapid transfer to laboratory
CSF	Lumbar puncture is performed after rigorous asepsis of the puncture site. The average quantity of CSF required is 2 to 5 drops in a sterile bottle Rapid transfer to laboratory
Bronchoalveolar fluid	Isotonic saline solution is injected into a segmental or sub-segmental bronchus solution (NaCl 0.9%) through a flexible fiberscope. The fluid should then be re-aspirated. Samples should be sent to the laboratory in a sterile vial as soon as possible.
PERITONEAL Liquid	Patient lying down After skin disinfection A catheter connected to a suction system collects the liquid Transfer to a sterile vial in the laboratory within the hour Treat immediately
Pleural fluid	Introduce a needle into the pleural space to remove pleural fluid Quickly transfer to a sterile vial for laboratory use

4. Conclusion

The pre-analytical phase determines the quality of results. In the hospital environment, this phase is difficult to manage and is often beyond the biologist's control. For this reason, dialogue between clinicians and biologists must be strengthened to ensure more reliable results.

Compliance with ethical standards

Disclosure of Conflict of Interest

The author has no conflict of interest in this research.

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