

PEARLS OF LABORATORY MEDICINE

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TITLE: Diagnosis of Dermatophytes

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Slide 1: Title slide

Hello, my name is Kristen Smith. I am a technical director of Routine and Esoteric Microbiology at the Laboratory Corporation of American. Welcome to this Pearl of Laboratory Medicine on "Diagnosis of Dermatophytes"

Slide 2: So what is a dermatophyte

A dermatophyte is a name for a pathogen that causes an infection of tissue that contains keratin. These primarily include hair, skin, and nails. These types of infections may also be called superficial mycoses. For the purpose of this discussion, we will focus on human infectious, but they are able to cause infections in a range of other animals as well. Dermatophytes can readily spread in a population, either by direct contact with the infected tissue such as during physical interactions during sporting events like wrestling, or through objects that have come into contact with infected tissue such as nail clippers or a hair brush. While anyone can get these types of infections, as noted, there are certain risk factors. There is a positive correlation with age and with participation in sports. Certain behaviors such as sharing equipment or personal hygiene products that are not properly disinfected can also lead to these types of fungal infections. But in general, these are common infections that affect a significant portion of the population and are likely under-diagnosed due to over the counter remedies and the lack of presentation to clinics.

A dermatophyte is a fungi, which is a eukaryotic organism that contains a cell wall, has a true nucleus, and undergoes cellular division. Most dermatophytes belong to one of three genera: Epidermophyton, Microsporum, and Trichophyton. Today we are going to discuss what signs are suggestive of an infection with one of these organisms, how to properly obtain a specimen for testing, and how the lab identifies the microorganism.

Slide 3: When do you suspect a dermatophyte

So now that you know a little bit about what a dermatophyte is, when should you expect to see these organisms show up in your patients and what are some associated symptoms? As previously mentioned, there are some risk factors for dermatophytes, but they also can be seasonal. Fungi like warm, moist environments and so we see a rise in their numbers in the hotter, summer months. There may also be more in the summer because people are paying more attention to their nails and are going for more manicures and pedicures, which can spread infections if tools are not adequately disinfected! Dermatophytes infect the hair, skin, and nails, so what do these infected areas look like and what do we call them? As with most infections, there is a range in severity which can sometimes be attributed to the immune system of the patient. Dermatophyte infections follow a naming convention based on location of the infection and they begin with the word "tinea". Thus, an infection of the skin would be Tinea Corporis. There is a table on the next slide that summarizes the names, corresponding sites of infection, and common organisms that cause the infection. Dermatophytic infections also have common names such as ringworm (general skin infection with a characteristic ring lesion), athlete's foot (also known as tinea pedis), and jock itch (or tinea cruris). In general, symptoms include dry, flaky or scaly skin in a 'ring' formation (hence the name ringworm, even though there are no 'worms'). Infected hair could break more easily than normal or fall out entirely causing temporary balding. Itching is perhaps the most common symptom for the hair and skin infections. Onychomycosis, or infected nails, can appear yellow or white, be very brittle, or be much thicker than normal. Additionally, nails may totally lose integrity and fall off. Unfortunately, I do not have any images to present, but a quick internet search will provide a plethora of cases of these common infections.

Slide 4: Common Dermatophyte Infections

As mentioned earlier, here is a slide of the naming conventions for dermatophyte infections based on location. Common causative organisms are also listed, but this is not meant to be an exhaustive list.

Slide 5: How do you test for a dermatophyte

The first step for testing for dermatophytes is to first investigate whether an antifungal agent has already been used to treat the patient. Since an important step in testing for a dermatophyte is culturing the organism, it is useful to know if treatment may preclude growth of the organism. If a topical antifungal has been utilized, it is suggested to wait 14 days until trying to culture. The next step is to prepare the sampling area by disinfecting the region that you will be sampling with 70% ethanol. For hair samples, it is best to use sterile forceps to pull out 10 to 12 hairs near the root such that the follicle can be tested. Select hairs that appear damaged or utilize a Wood's lamp to identify fluorescing hairs as these can indicate infection. Scrape skin with a scalpel or glass slide at the leading edge, or the area that looks most recently affected. The centers likely only contain nonviable organisms and are not useful for culture. For nails, clip or scrape the underside of the nail, getting near as possible to the nail bed. All collected material should be placed in a sterile envelope or container. These specimen types are stable at room temperature for several days. Do not refrigerate, because dermatophytes are sensitive to cold temperatures.

Slide 6: How does the lab identify a dermatophyte - Microscopy

The first tool that microbiologists can utilize to ask the question, "Is this a fungal infection?", is by direct visualization of the microorganism through microscopy. Samples must first be processed in order to digest keratinized and proteinaceous material that prevents visualization and staining of fungal elements. This is typically done with Potassium Hydroxide or KOH, but sodium hydroxide can also be used. Depending on the specimen, this step can be done at room temperature or by slightly heating to enhance the digestive process. The time is also variable, for instance, it takes less time to digest skin than nails. Next, a stain is used to enhance visualization. There are several options available, but the most common is calcofluor white, examples of this fluorescent stain are below. This stain binds preferentially to chitin, a component of fungal cell walls. The slides are viewed under a fluorescent microscope and scanned for evidence of a fungal infection. The images below show a positive KOH from a skin scraping and you can see several examples of hyphal elements fluorescing. Septations, or the divisional lines between fungal cells, are visible, and the white arrow is pointing to an area of branching. Since moulds can take several weeks to grow, this quick tool is very useful to the clinician and could aid in quickly guiding treatment. However, it is not possible to identify the microorganism to the genus and species level through this method of examination.

Slide 7: How does the lab identify a dermatophyte – Culture

Culture is the better approach if a more definitive diagnosis is required or if susceptibility testing is desired in cases of treatment failure. The specimen is inoculated into medium and allowed to grow for several weeks. The types of media can vary between labs, but typically more than one medium is used. This is because the specimens required (hair, skin and nails) are colonized by commensal organisms, or our normal bacterial flora, and so an inhibitory media containing antibacterials such as chloramphenicol and cycloheximide is used to prevent these organisms from over-growing the mould. Remember that bacterial growth can be much faster, taking a day to become visible on a plate, and moulds can take up to 3 or 4 weeks! A non-inhibitory medium is also used, the most common of which is SDA or Sabourand's Dextrose Agar, as some moulds may be sensitive to antimicrobials as well. Imbedding the specimen into the agar is best to ensure that the moulds come into contact with the media. An incubation temperature of 30C is used versus 37C which is routinely used in other areas of the microbiology lab. This is because dermatophytes grow ON the human body, and not IN it. They also do not handle cooler temperatures well, so specimens should never be placed in the refrigerator. It is typical for most dermatophytes to growth within 3 weeks, so a common practice is to check the plates weekly with the plates being discarded if no mould is present after 3 weeks.

So what does the lab do if a mould grows? The first step is to document the morphology or visually describe what the mould looks like on a plate and to determine that there is only one mould present. If there are colonies that appear different, the moulds must be sub-cultured to try and isolate a single organism. This can be very difficult because mould spores are easily disturbed and dispersed (a trait that is very useful in the environment!). Once there is a single mould present, the mycologist can attempt to view the microscopic structures of the organism. This is done by a tape prep – whereby a piece of clear tape is touched to the surface of the mould and then placed on top of a drop of lactophenol cotton blue on a slide. The stain has two functions – to inactivate the mould via the phenol and to stain the structures present. Most dermatophytes have distinct morphology and can be identified through the colony or macroscopic morphology and the microscopic morphology. However, sometimes the organisms don't sporulate, or produce identifying structures and so another method must be employed. Analysis of the protein structures through Matrix-Assisted Laser Desorption/Ionization Time of Flight or MALDI-TOF is a relatively new method that can be useful in obtaining identification to the genus and species level. Sanger sequencing of the Internal transcribed spacer or ITS region can also be employed. Keep in mind that identification can take several weeks as well, especially in labs that send out their isolates for identification. That means a positive mould culture can easily take over 5 weeks for an identification.

Slide 8: Epidermophyton floccosum

This organism is able to cause infections of the skin and nails, but not hair. The macroscopic morphology is initially flat and granular on solid media, but then turns fluffy white and eventually sandy to green-brown. The image on the left is a traditional lactophenol cotton blue slide showing the macroconidia (conidia are fungal spores). You can see that these are club-shaped and have cells or compartments. Epidermophyton floccosum should not have more than 6 cells in their macroconidia. The image on the right uses the same staining approach, but with phase-contrast instead of typical brightfield microscopy. This is to help with visualization of the cells. All lactophenol cotton blue stained images in this presentation were taken by Eileen Rojas.

Slide 9: Microsporum

While there are more members of the microsporum genus than the two are shown here, *Microsporum gypseum* and *Microsporum canis* are good examples to demonstrate differences in the conidia. *M. gypseum* is commonly isolated from soil and has a light colored macroscopic morphology that is granular in appearance when cultured. Depending on the isolate, the coloration can range from rosy to cinnamon. The macroconidia are ellipsoidal in shape with tapered ends and have 4-6 cells. The walls of the macroconidia are also smooth, which may not be obvious to the untrained eye. Compare the walls of *M. canis*, which is very similar in appearance to *M. gypseum*, but note the roughened walls of *M. canis*. The walls of *M. canis* are also thick, which again is different than *M. gypseum*, and can have 5-15 cells. For these small details, it often helps the mycologist to adjust the fine focus to get a better 3D appreciation for these structures. Macroscopically, *M. canis* has a more velvety appearance ranging from pale to yellow as shown on the far right.

Slide 10: Trichophyton

The last member of the dermatophytes is *Trichophyton*. Members of this genus are able to produce a variety of conidia. To show the differences between the selected species, the microscopic images are on this slide and the colony morphologies are shown on the next slide. Once again, these organisms are stained using lactophenol cotton blue. *Trichophyton tonsurans*

is exhibiting both microconidia (smaller) and macroconidia (larger) in Figure A. A pencil-shaped macroconidia is identified by a number 1 in the top right image. Figure B is a higher magnification to better show the details of the conidia. *Trichophyton mentagrophytes* (Figure C) has many microconidia that are typically round or teardrop shaped and this isolate also exhibited spiral hyphae (number 2), which isn't always observed. Lastly, *Trichophyton rubrum* is shown in Figure D, where we can see numerous teardrop shaped microconidia still connected to the hyphae. We also see characteristic alternating of conidia at arrow 3. So while these organisms are all from the same genus, they will look very different microscopically. *T. tonsurans* can produce many different types of conidia, both the larger macroconidia that can have a pencil or club-shaped appearance, to the smaller, more abundant microconidia that look more like teardrops or matchsticks. *T. mentagrophytes* infrequently produces macroconidia, but it does have many microconidia that are spherical or teardrop-shaped. This organism also can produce spiral hyphae. And lastly, *T. rubrum* will typically only produce microconidia that once again have a teardrop appearance that alternate on the hyphae.

Slide 11: *Trychophyton*

As we discussed with *Microsporum*, the gross or colony morphology can also be very helpful with identification of *Trychophyton* species. On the left, we will once again begin with *T. tonsurans*. This mould can present as powdery or velvety with a white to yellow coloration. As with the microscopic presentation, there can be large variations with the gross morphology as well. Some colonies can have a raised center, as we see here, or can appear to be folded or have radial grooves. Next we move on to *T. rubrum*. The meaning of *rubrum* in Latin is red, so this organism was aptly named – note the deep ruby red in the top image of *T. rubrum* and the more red-brown hues in the bottom image. These colonies can range from more cottony (above) to velvety (below). *T. mentagrophytes* appears more powdery and sometimes cottony with a yellow-cream surface as shown on the far right image. It may also be helpful on to look at the back of the plate, or the reverse, to note the colorations. If you have the opportunity, I would suggest visiting your microbiology lab to look at moulds in person in order to appreciate their diverse morphologies.

In summary, dermatophytes are common fungal pathogens that frequently cause hair, skin, and nail infections. They can infect a variety of hosts and can be identified to the genus and species level in clinical mycology laboratories. Thank you for your time and attention, I hope you find this Pearl on the Diagnosis of Dermatophytes to be useful.

