

Evaluation of False Negative Mycological Results in Superficial Fungal Infections

Thesis

Submitted for partial fulfillment of Master degree of
Dermatology, Venereology and Andrology

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2011

Acknowledgement

First and foremost, thanks to “GOD” who granted me the ability to accomplish this work.

*I would like to express my sincere appreciation and my deep gratitude to **Prof. Dr. Mahira Hamdy El-Sayed**, Professor of Dermatology, Venereology and Andrology, Faculty of Medicine, Ain Shams University, for her faithful supervision and guidance and her overwhelming kindness that has been of great help throughout this work.*

*I am also deeply indebted to **Dr. Ghada Fathy Mohamed**, Assistant Professor of Dermatology, Venereology and Andrology, Faculty of Medicine, Ain Shams University, for her sincere support and great effort helping me throughout this work, and for that I am proud to express my deepest thanks*

*I owe special thanks to **Dr. Sahar El-Sayed Ahmed**, Assistant Professor of Dermatology, Venereology and Andrology, Faculty of Medicine, Ain Shams University, for her patience, unfailing help, advice and strict supervision. Without her guidance, this work would have never come to light.*

Finally, to my Parents, for the love and support they gave me throughout my life, I dedicate this work.

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Abbreviations

BCP-MS-G.....	Bromocresol purple-milk solids glucose medium
CAC.....	CHROM agar Candida
CMA.....	Corn meal agar
DMSO.....	Dimethylsulphoxide
DTM.....	Dermatophyte Test Medium
EDM.....	Enriched Dermatophyte Medium
E.floccosum.....	Epidermophyton floccosum
E.stockdaleae.....	Epidermophyton stockdaleae
KOH.....	Potassium hydroxide
M.amazonicum.....	Microsporum amazonicum
M.audouinii.....	Microsporum audouinii
M.boullardii.....	Microsporum boullardii
M.canis.....	Microsporum canis
M.cookie.....	Microsporum cookie
M.equinum.....	Microsporum equinum
M.ferrugineum.....	Microsporum ferrugineum
M.gallinae.....	Microsporum gallinae
M.gypseum.....	Microsporum gypseum
M.nanum.....	Microsporum nanum
M.persicolor.....	Microsporum persicolor
M.praecox.....	Microsporum praecox
M.racemosum.....	Microsporum racemosum
M.ripariae.....	Microsporum ripariae
M.vanbreuseghemii.....	Microsporum vanbreuseghemii
PAS.....	Periodic acid-Schiff
PDA.....	Potato dextrose agar
SDA.....	Sabouraud dextrose agar
T.Ajelloi.....	Trichophyton ajelloi
T.concentricum.....	Trichophyton concentricum
T.equinum.....	Trichophyton equinum
T.flavescens.....	Trichophyton flavescens
T.gloriae.....	Trichophyton gloriae
T.gourvilii.....	Trichophyton gourvilii
T.interdigitale.....	Trichophyton interdigitale
T.kanei.....	Trichophyton kanei

T.longifusum.....	Trichophyton longifusum
T.megninii.....	Trichophyton megninii
T.mentagrophytes.....	Trichophyton mentagrophytes
T.phaseoliforme.....	Trichophyton phaseoliforme
T.tonsurans.....	Trichophyton tonsurans
T.raubitschekii.....	Trichophyton raubitschekii
T.rubrum.....	Trichophyton rubrum
T.sarkisorii.....	Trichophyton sarkisorii
T.schoenleinii.....	Trichophyton schoenleinii
T.simii.....	Trichophyton simii
T.Soudanense.....	Trichophyton soudanense
T.terrestre.....	Trichophyton terrestre
T.vanbreuseghemi.....	Trichophyton vanbreuseghemi
T.verrucosum.....	Trichophyton verrucosum
T.violaceum.....	Trichophyton violaceum
T.yaoundei.....	Trichophyton yaoundei

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Introduction and Aim of Work

Over the last decade, there have been changes in the epidemiology of fungal infections as well as dramatic improvements in the antifungal agents. Many epidemiological studies have investigated the prevalence of fungi-causing superficial mycoses in different parts of the world. The relative occurrence of the etiologic agents of these infections varies from country to country and from one climatic region to another (**Korstanje and Staats, 1995; Hay et al., 2001**).

Cutaneous fungal infections are divided into three major groups: superficial, subcutaneous, and deep or systemic fungal infections. Superficial mycoses are due to fungi that only invade fully keratinized tissues, i.e. stratum corneum, hair and nails. Subcutaneous mycoses are due to a large group of organisms that cause disease when implanted or introduced into the dermis or subcutis. Deep mycoses involves deep structures and have the propensity to disseminate usually via the bloodstream from the original focus of infection. They are divided into true and opportunistic pathogens (**Hay, 1996; Martin et al., 1999**).

Superficial mycoses can be further subdivided into those that induce minimal, if any, inflammatory response, e.g. pityriasis versicolor, and those that do lead to cutaneous inflammation, e.g. dermatophytoses, candidiasis and saprophytes (**Martin and Kobayashi, 1999**).

Dermatophytoses are fungal infections that have the unique ability to invade and multiply within keratinized tissue. They are caused by three genera of fungi *Microsporum*, *Trichophyton*, and *Epidermophyton*. Dermatophytoses occur most frequently in postpubertal hosts. One major exception is *Tinea capitis* which occurs primarily in prepubertal children. Risk factors for its development in prepubertal population includes contaminated hats, brushes and barber instruments. While both sexes are affected, men tend to more frequently have *tinea pedis* and *tinea cruris*. After

puberty, men also acquire Tinea unguis more readily than do women (**Martin et al., 1999; Rinaldi, 2000**).

Superficial Candidiasis is an infection of the skin and mucous membranes caused by yeast of the genus *Candida*. Most frequently these infections are due to *Candida albicans* but other species with increasing frequency cause human disease. The most common cutaneous pattern of *Candida* infection is candidal intertrigo. The genitocrural and gluteal folds, the submammary region, and the interdigital spaces of the hands and feet are usually affected (**Pereyo and Leshner 1997; Pappas and Ray, 1998**).

Onychomycosis is a chronic fungal infection of fingernails and/or toenails, caused by dermatophytes, yeasts and moulds leading to gradual destruction of the nail plate. *Trichophyton rubrum* is the cause of most onychomycosis cases followed by *T. mentagrophytes* and *T. tonsurans*. Some yeasts, such as *Candida*, *Trichosporon* and *Malassezia* species, are also able to cause ungual infection. *Candida albicans* predominates in most yeast-caused onychomycosis cases. However, other *Candida* species, have also been isolated in infected nail (**Brilhante et al., 2005; Hay, 2005; Gupta and Ricci, 2006**).

Saprophytic fungi are moulds normally found in the soil, air and some plants; they are considered opportunistic fungi. *Scopulariopsis* species, *Fusarium* species, and *Aspergillus* species, for example, have been identified in nails as primary pathogen of onychomycosis (**Gupta et al., 2003; Godoy et al., 2004**).

Fungal infections are more common in the immunocompromised patients suffering malnutrition, some infections (e.g. AIDS), diabetes mellitus, tumors of the immune system (e.g. leukemia, lymphoma), prolonged administration of corticosteroids, immunosuppressive therapy, splenectomy or autoimmune diseases. As the number of immunocompromised patients increases, there has been a concomitant increase in patient morbidity and mortality due to fungi. The etiologic microorganisms vary depending on the type of immune dysfunction. Patients with malignancies and chemotherapy-induced neutropenia are commonly infected with

Candida and Aspergillus (Wolfson et al., 1985; Stein and Sugar, 1989).

Diagnosis of fungal infections is made by direct microscopic examination and fungal culture. However, when only one sample is analyzed, the frequency of false-negative results is very high. Repeated collections should always be considered in cases of suspected dermatophytosis with negative laboratory reports **(Hull et al., 1998).**

For a laboratory diagnosis, clinicians should be aware of the need to generate an adequate amount of suitable clinical material. Unfortunately many specimens submitted are either of an inadequate amount or are not appropriate to make a definitive diagnosis. The laboratory needs enough specimen to perform both microscopy and culture. It must be stressed that up to 30% of suspicious material collected from nail specimens may be negative by either direct microscopy or culture. A positive microscopy result showing fungal hyphae and/or arthroconidia is generally sufficient for the diagnosis of dermatophytosis, but gives no indication as to the species of fungus involved. Culture is often more reliable and permits the species of fungus involved to be accurately identified **(Kwon-Chung and Bennett, 1992; Richardson and Warnock, 1993).**

Aim of the Work

The aim of this study is to investigate whether serial repetition of routine direct microscopy examination and fungal culture improves diagnosis efficacy of fungal infections and reduces the possibility of false negative results or not.

Review of Literature

History

Fungi were once believed to be descended from plants, it was recognized over 20 years ago that they represented a distinct kingdom, and that certain features of their biochemistry, such as the pathway of lysine synthesis, were quite different from those found in bacteria and plants. With the use of molecular techniques, it is now recognized that the organisms originally included in this kingdom of Fungi contained at least three phylogenetically distinct groups, and some phyla have now been reclassified in the kingdoms Protozoa and Chromista (**Hawkesworth and Kirk, 1995**).

In **1835**, **Robert Remak** a Polish physician on the medical faculty of Berlin University first observed peculiar microscopic structures appearing as rods and buds in crusts from favic lesions. He never published his observations, but he permitted those observations to be cited in a doctoral dissertation by **Xavier Hube** in **1837**. Remak claimed that he did not recognize the structures as fungal and credited this recognition to **Schoenlein**, who described their mycotic nature in **1839**. However, Remak established that the etiologic agent of favus was infectious, cultured it on apple slices, and validly described it as *Achorion schoenleinii*, in honor of his mentor and his initial discovery (**Seeliger, 1985; Weitzman and Summerbell 1995**).

David Gruby, a Hungarian physician practising in Paris was the real founder of dermatomycology on the basis of his discoveries during **1841 to 1844**, his communications to the French Academy of Science, and his publications during this period. Independently, and unaware of the work of Remak and Schoenlein, he described the causative agent of favus, both clinically and in microscopic details of the crusts, and established the contagious nature of the disease. He also described **ectothrix invasion** of the beard and scalp, discovered and named the genus **Microsporum** in **1843** and described **M. audouinii** on the basis of the appearance of the fungus in clinical materials. **Trichophyton**, the second genus, was

described in 1845 by **Per Hendrik Malmsten**, the Swedish investigator (**Ajello, 1974; Weitzman and Summerbell 1995**).

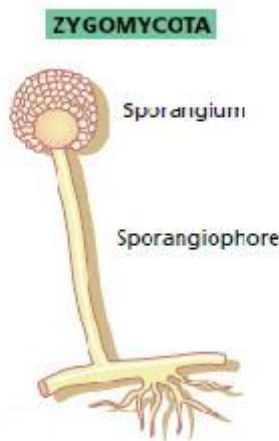
Raimond Sabouraud, one of the best known and most influential of the early medical mycologists, began his scientific studies of the dermatophytes around 1890, culminating in the publication of his classic volume, *Les Teignes*, in 1910. Sabouraud's contributions included his studies on the taxonomy, morphology, and methods of culturing the dermatophytes and the therapy of the dermatophytoses. He classified the dermatophytes into four genera, *Achorion*, *Epidermophyton*, *Microsporum*, and *Trichophyton*, primarily on the basis of the clinical aspects of the disease, combined with cultural and microscopic observations. The medium that he developed is in use today for culturing fungi (although the ingredients are modified) and is named in his honor, Sabouraud glucose (dextrose) agar (**Odds, 1991; Weitzman and Summerbell 1995**).

In 1934, **Chester Emmons** modernized the taxonomic scheme of Sabouraud and others and established the current classification of the dermatophytes on the bases of spore morphology and accessory organs. He eliminated the genus *Achorion* and recognized only the three genera *Microsporum*, *Trichophyton*, and *Epidermophyton* on the basis of mycological principles. Nutritional and physiological studies of the dermatophytes pioneered at Columbia University by Rhoda Benham and Margarita Silva and at the Center for Disease Control, in Georgia, by Libero Ajello, Lucille K. Georg, and coworkers simplified the identification of dermatophytes and led to reduction of the number of species and varieties (**Weitzman and Summerbell 1995**).

Classification of Fungi

The formal classification of fungi is based upon the features of the sexual phase of growth. This involves the fusion of two nuclei followed by meiotic division. Three groups of sexually reproducing fungi, Zygomycota, Ascomycota and Basidiomycota, contain human pathogens. A fourth, completely artificial group, the Deuteromycota or Fungi Imperfecti, was originally created to include those fungi with no known sexual phase, and the asexual phases of the Ascomycota and Basidiomycota. These asexual mitosporic species can be placed within the recognized sexual groups, and it is possible that at some time in the future the artificial form-phyllum Deuteromycota will disappear from the literature. (Hay and Moore, 2004).

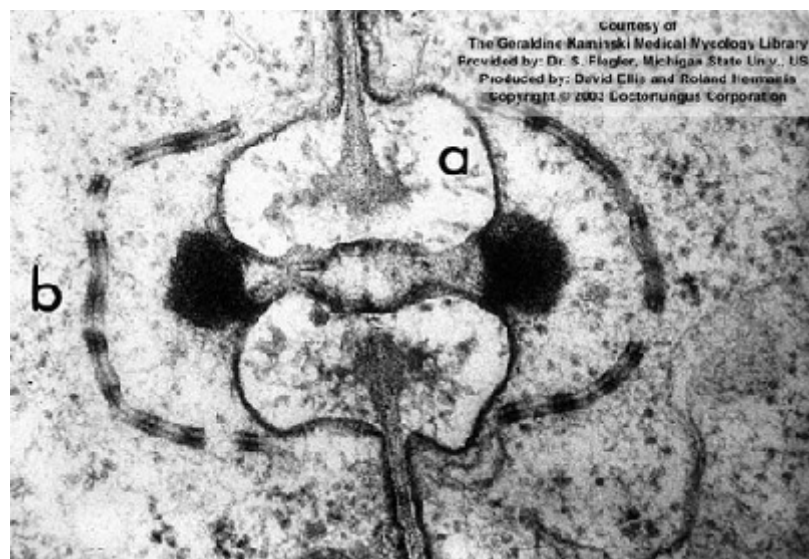
1. The Zygomycota: are all moulds, they reproduce sexually by the formation of thick-walled zygospores following the fusion of two outgrowths arising from the hyphae. The hyphae of the Zygomycota are very characteristic, being both extremely wide and often sparsely septate. Asexual reproduction is usually by means of sporangiospores formed by the cleavage of cytoplasm within a sporangium. This is a sac-like structure, which is supported by a specialized hypha, the sporangiophore (Figure 1)(Raven et al., 2005).



Figure(1): Asexual reproduction in fungi. Zygomycota (Hay and Moore, 2004).

2. The Ascomycota: contains both moulds and yeasts, like *Aspergillus* species and *Penicillium* species. Sexual reproduction is by the production of ascospores, formed within a sac-like ascus. The hyphae of the Ascomycota have a lamellate cell wall, with a thin electron-dense outer layer and a thicker electron-transparent inner layer. The hyphae are narrow and regularly septate, but passage of cytoplasm and even nuclei from one cell to the next is made possible by the presence of a pore in the centre of each septum (**Hay and Moore, 2004**).

3. The Basidiomycota: Sexual reproduction is the production of basidiospores formed externally on sterigmata protruding from a club-shaped basidium. The cell walls of the Basidiomycota are lamellate and electron dense. When present, the hyphae are narrow and septate. e.g. basidiomycetous yeasts (Figure 2)(**Swann et al., 2007**).



Figure(2): Transmission electron micrograph of a section through a "complex" septal pore, called a dolipore, of a basidiomycetous fungus (**Ellis and Hermanis, 2003**).