

# GMS is superior to PAS for diagnosis of onychomycosis

**Background:** Onychomycosis is a common cause of deformed nails. Periodic acid-Schiff (PAS) stains are more sensitive than fungal cultures for diagnosing onychomycosis. We performed a retrospective study comparing the use of PAS and Gomori methenamine silver (GMS) stains for histopathologic diagnosis of onychomycosis.

**Methods:** GMS stains were performed on 20 PAS-positive and 51 PAS-negative cases from nail biopsies with a clinical diagnosis of onychomycosis. The PAS stained slides and GMS stained slides were evaluated for the presence of fungal hyphae. The results were analyzed with McNemar's test.

**Results:** All 20 PAS-positive cases were also positive with GMS stains. Of the 51 PAS-negative cases, GMS stains detected an additional five cases with fungal hyphae. GMS stains were quantitatively superior ( $p < 0.0253$ ). GMS stains were also qualitatively superior. More fungal hyphae were highlighted and fungal hyphae were more easily recognized on low or medium power magnification.

**Conclusions:** GMS stains are superior to PAS stains for the routine diagnosis of onychomycosis.

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Zandra D'Hue<sup>1</sup>, Susan M. Perkins<sup>2</sup> and Steven D. Billings<sup>1,3</sup>

<sup>1</sup>Department of Pathology and Laboratory Medicine,

<sup>2</sup>Department of Medicine and

<sup>3</sup>Department of Dermatology, Indiana University School of Medicine, Indianapolis, IN, USA

Steven D. Billings, MD, Cleveland Clinic 9500 Euclid Avenue, L25 Cleveland, OH 44195, USA  
Tel: +216 444 2826  
Fax: +216 445 3707  
e-mail: billins@ccf.org

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Onychomycosis is a common reason for deformed nails. The prevalence of onychomycosis is estimated to be 0.1–8.7%.<sup>1</sup> Onychomycosis can cause severe pain and discomfort leading to physical limitations. Dermatophytes are the most common cause of onychomycosis. The most common pathogen is *Trichophyton rubrum*, followed by *Trichophyton mentagrophytes* and *Epidermophyton floccosum*.<sup>2</sup>

Routine histopathological examination of nail clippings with standard hematoxylin and eosin stained sections is insufficiently sensitive for the diagnosis of onychomycosis. It has been documented that the periodic acid-Schiff (PAS) stain is a sensitive method superior to culture and potassium hydroxide preparation for the diagnosis of onychomycosis.<sup>3–5</sup> PAS stains have become the *de facto* gold standard for the diagnosis of onychomycosis. However, none of these previous reports compared the PAS stain with the Gomori methenamine silver (GMS) stain. The

GMS stain is a histochemical stain used to stain fungi, basement membranes and carbohydrates. GMS stains are considered the most sensitive histochemical stain for the detection of fungi in deep fungal infections. We hypothesized that GMS stains might be more useful than PAS stains in the diagnosis of dermatophyte infections of the nail.

## Material and methods

From the dermatopathology files of Indiana University School of Medicine, 20 cases with PAS-confirmed onychomycosis and 51 cases of possible onychomycosis that were PAS-negative were retrieved. All cases were stained with GMS stains using standard reagents and an automated stainer (Dakocytomation, Carpinteria, CA, USA). For the GMS stain tissue is pretreated with chromic acid and sodium bisulfite, stained with a methenamine-silver nitrate

solution with gold chloride and sodium thiosulfate and then counterstained with a light green solution.<sup>6</sup> The fungi appeared black-brown with a pale green background. The original PAS stains from all 71 cases and the GMS stains were reviewed by the authors (S. D. B., Z. D.). Appropriate positive controls were used for both the PAS stains and GMS stains. Cases negative for fungal hyphae by both methodologies were assumed to represent true negatives. Statistical analysis using McNemar's test was performed.

The institutional review board of the Indiana University School of Medicine approved this study.

### Results

GMS highlighted fungal hyphae in 20/20 PAS-positive cases and 5/51 PAS-negative cases. In one of the PAS-negative cases, the GMS stain highlighted numerous fungal hyphae (Fig. 1). GMS stains detected significantly more cases of onychomycosis than PAS stains (35% vs. 28%,  $p < 0.0253$ ). The

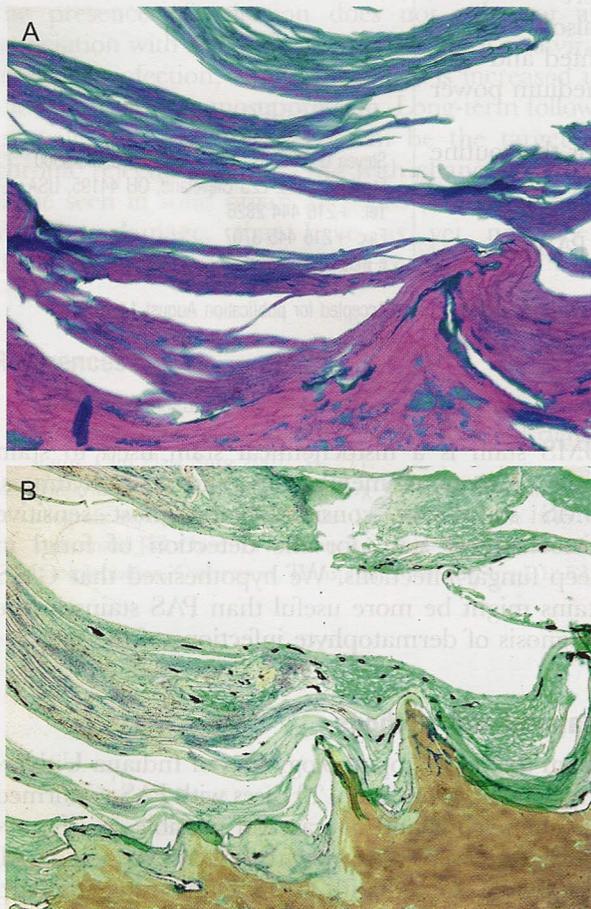


Fig. 1. A) In five cases the periodic acid-Schiff (PAS) stains were negative for fungal hyphae (400 $\times$ ). B) In one of the five PAS-negative cases, numerous fungal hyphae were evident in the GMS stained section (400 $\times$ ).

GMS stains were also qualitatively superior. In the PAS stains, the presence of fungal hyphae was sometimes subtle, requiring close examination under high power magnification (40 $\times$  objective) to establish the diagnosis of onychomycosis in these cases (Fig. 2). In contrast the GMS stains detected more fungal hyphae than were visualized with the PAS

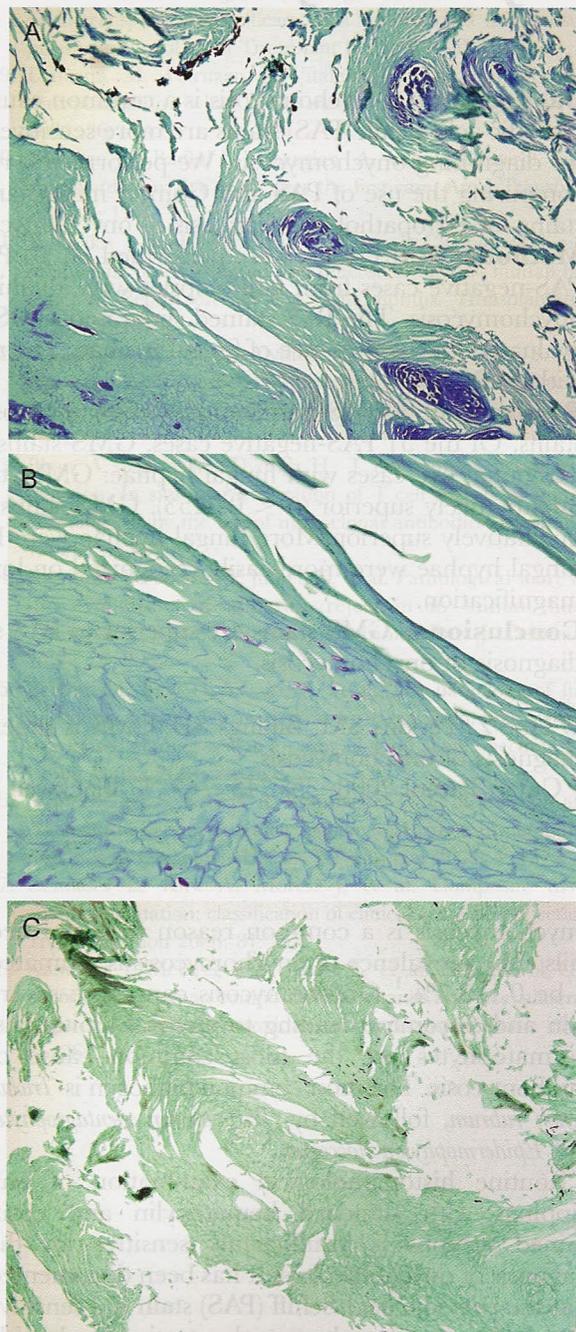


Fig. 2. A) In this periodic acid-Schiff (PAS)-positive case, fungal hyphae were inconspicuous at low or intermediate power with the PAS stain (PAS, 100 $\times$ ). B) Identification of fungal hyphae required microscopic examination at high power (PAS, 400 $\times$ ). C) With the GMS stain fungal hyphae were readily appreciated at low or intermediate power (GMS, 100 $\times$ ).

stain. The combination of increased numbers of fungal hyphae visualized and the increased contrast of the GMS stain in comparison to the light green counterstain allowed for the detection of the fungal hyphae on low (4× objective) to intermediate (10× objective) power in the majority of cases (Fig. 2). This was less frequently observed with the corresponding PAS stains.

**Discussion**

Dermatophyte infection leading to onychomycosis is a common reason for deformed nails. There are four major clinical presentations: distal subungual (most common), proximal subungual (most common in patients with human immunodeficiency virus infection), and superficial and total dystrophic onychomycosis.<sup>1,2,5,7</sup> In our practice, nail clippings for suspected onychomycosis are a common specimen. Nails often appear negative for onychomycosis when routinely stained with hematoxylin and eosin. Therefore, we had routinely performed PAS stains for confirmation of the diagnosis of onychomycosis as has been suggested by previous studies as the standard of practice in our laboratory.<sup>5,8,9</sup> We had noted that, while PAS stains allowed for detection of fungal hyphae not seen in routine hematoxylin and eosin stains, their presence could be subtle requiring close examination under high magnification.

In 1946, George Gomori described a staining method using methenamine silver to detect fungi. Today, we use a modification of this stain described by Grocott.<sup>6</sup> The GMS stain is considered the most sensitive histochemical method for the detection of fungal organisms in deep infections. We were surprised that, to our knowledge, there have been no published studies on the use of GMS stains for the diagnosis of onychomycosis. We hypothesized that GMS stains may be more useful for the diagnosis of onychomycosis.

Our study confirmed our hypothesis. GMS stains detected five additional cases of onychomycosis in 51

**GMS Superior to PAS for Onychomycosis**

PAS-negative cases. Surprisingly, in one PAS-negative case, numerous fungal hyphae were evident in the GMS stain. Although we are not certain of the explanation of this staining pattern, we suspect it is because of species related differences. It is well known that fungi may stain differently with PAS and GMS stains.

The GMS stain was also qualitatively superior, as it allowed easier detection of the fungal hyphae on low and intermediate power microscopic examination. In contradistinction, detection of fungal hyphae by PAS stains frequently required more time consuming examination of the specimen on high magnification before detecting the fungal hyphae. In our practice we now preferentially use the GMS stain for the diagnosis of onychomycosis and suggest that the GMS stain may represent the new gold standard for the diagnosis of onychomycosis.

**References**

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Case	Age (years)	Sex	Anatomic site
1	60	Male	Right
2	67	Female	Left
3	67	Female	Left
4	67	Female	Left
5	67	Female	Left