Developing best practice for fungal specimen management: audit of UK microbiology laboratories

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ABSTRACT

This study represents an audit of microbiology laboratories in the UK to ascertain whether they are aware of, or follow, the Health Protection Agency (HPA) National Standard Methods Standard Operating Procedure (NSM SOP) for the investigation of dermatological specimens for superficial mycoses, or use a locally adapted version. A questionnaire audit was distributed to 179 NHS microbiology laboratories throughout England, Wales, Scotland and Northern Ireland. The NSM SOP was followed by 92% of laboratories for the microscopy of dermatological samples; light microscopy/ KOH digestion was used by 63% and fluorescence microscopy/KOH digestion by 29% of laboratories. Preliminary reports post-microscopy were issued by 98% of laboratories, with 93% issuing reports within 48 hours. Adherence to the NSM SOP guidelines for culture was low; only 34% of laboratories incubated microscopy-negative specimens for the recommended 14 days, while approximately 60% incubated microscopy-positive specimens for 21 days. The culture medium recommended by the NSM SOP was used in 82% of laboratories. Comments were added to culture reports by 51% of laboratories; most were added manually and comments varied between laboratories. Nail samples were the most common sample received from primary care, followed by skin and hair. These results show no significant difference in the rate of microscopy positives versus culture positives. Microscopy and culture are the easiest and cheapest methods available to UK laboratories for the investigation of suspected superficial fungal infections. Although most laboratories included in this audit claimed to follow the NSM SOP for microscopy and culture, these results show that the techniques used vary throughout the UK. To maximise the service provided to primary care, UK laboratories should use standardise methods based on the NSM SOP.

KEY WORDS: Audit.
Hair.
Mycoses.
Nail.
Skin.

Introduction

Roberts *et al*. reported the results of an omnibus survey of nearly 10,000 people which suggested the prevalence of onychomycosis in the United Kingdom is 2.7%. Current UK primary care guidance advises general practitioners to confirm the diagnosis of fungal nail, skin or hair infections by sending specimens to microbiology laboratories for investigation.[1–3] With only 45% of dermatology specimens received by microbiology laboratories positive for fungal infection,[4] GPs are encouraged always to send a sample before starting lengthy treatments.[3]

There is a range of techniques available to laboratories for establishing a definitive diagnosis of fungal infection, including rapid polymerase chain reaction (PCR) methods, direct light microscopy, fluorescence microscopy and culture. The Health Protection Agency (HPA) National Standards Methods (NSM) has developed evidence-based Standard Operating Procedures (SOP) for the investigation of nail, skin and hair specimens for the definitive diagnosis of superficial mycoses.[5] This guidance document was developed in conjunction with the British Society for Medical Mycology, UK Clinical Mycology Network (UKCMN), Association of Medical Microbiologists, Institute of Biomedical Science, Association of Clinical Microbiologists, Scottish Microbiology Association and the Welsh Microbiology Association and presents a good minimum standard of practice.[5] The NSM SOPs were developed to "promote high-quality laboratory practices and to assure the comparability of diagnostic information obtained in different laboratories"; however, adherence to these guidelines is not mandatory for UK laboratories and it is not known how many laboratories in the UK follow this guidance or use locally adapted versions.[5]

This study reports an audit to determine the SOPs used by UK laboratories for the investigation of superficial fungal infection and whether or not these techniques correspond with the NSM SOP. The audit also aims to examine the current and agreed best reporting practice of fungal specimen reports by microbiology laboratories to primary care. It also investigates the type and number of samples submitted to microbiology laboratories from primary care, and overall positivity rates.

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Materials and methods

Questionnaire audit

A questionnaire was developed by members of the HPA GP Microbiology Laboratory Use Group; questions were based on the guidance from the NSM SOP and through collaboration with the Mycology Reference Laboratory, Bristol. The questionnaire was piloted in March 2009 in 12 HPA collaborating laboratories in the south-west of England. Questionnaires asked each laboratory about its microscopy and culture techniques and whether current procedures were based on the NSM SOP or a locally modified version. Laboratories were asked whether or not preliminary reports were sent after microscopy, and whether or not comments were added to preliminary or final reports. The questionnaire also requested the number of samples, sample types and positivity rates of samples received from primary care. To audit actual reporting policy, 50 consecutive anonymised mycology reports were requested from each responding laboratory.

Hard copies of the final questionnaire were posted by the HPA Primary Care Unit (PCU) in August 2009 to consultant medical microbiologists at 179 NHS microbiology laboratories throughout England, Wales, Scotland and Northern Ireland (available online) All non-responding laboratories were contacted via telephone in October and December 2009 and a senior biomedical scientist was identified and emailed a copy of the questionnaire. All respondents were asked to return questionnaires using Freepost envelopes provided or via email.

Data analysis

Two researchers agreed categories of response to the open-ended questions asked in the questionnaire. These categories were then used to develop an Epidata database (version 3.1). One researcher input all questionnaires into this database; a second researcher then checked for inconsistencies by double data entering 10% of the questionnaires. No significant disagreements were found.

Statistical analysis was performed using Epidata analysis (Version 2.1) and Stata software (Version 11). Descriptive statistics were calculated as simple tabulations of frequencies and percentages.

Ethical approval

The Local Research Ethics Committee (Taunton LREC) confirmed that ethical approval was not required as the survey was considered audit and service evaluation; this is in line with definitions provided by the National Patient Safety Agency.[6]

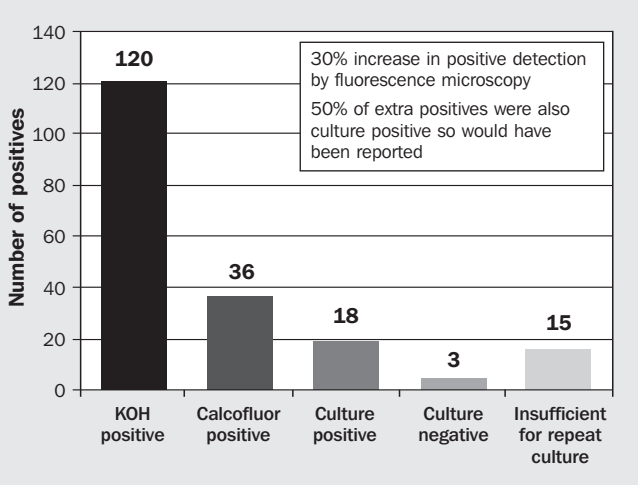

Fig. 1. Calcofluor staining/fluorescence microscopy versus KOH/light microscopy for nail specimens (Mycology Reference Laboratory, Bristol).

Results

Completed questionnaires were received from 120 (67%) laboratories. A little over half (65/120) of these laboratories provided information on the total number of samples received from general practice, while only 22% (26/120) of laboratories returned copies of laboratory reports issued between 1 April 2007 and 31 March 2008.

Use of NSM SOP for microscopy

The audit showed that 67% (81/120) of laboratories reported following the SOP for the microscopy of dermatological samples for superficial mycoses, 10% (12/120) used a modified version of the NSM SOP, and 22.5% (27/120) used a local SOP. However, when specifically asked about the microscopy technique used, 92% (111/120) of laboratories adhered to the NSM SOP, which recommends the use of potassium hydroxide (KOH) for the digestion of nail, hair and skin samples prior to light or fluorescence microscopy; 63% (76/120) reported using KOH digestion and light microscopy; 29% (35/120) KOH and fluorescence microscopy; 3% (4/120) KOH, ink stain and light microscopy; 3% (4/120) KOH and phase contrast microscopy; and 1% (1/120) used sodium hydroxide and light microscopy. This disparity would indicate that some of the laboratories audited were unaware of the NSM SOP.

Preliminary microscopy results reporting to primary care

As antifungal treatment can be started on a microscopy

Table 1. Duration of incubation for fungal culture specimens, depending on microscopy results. Technique reported by audited microbiology laboratories in the UK.

Sample	*n*	DAYS incubated if POSITIVE microscopy				DAYS incubated if NEGATIVE microscopy			
		Up to 14	Up to 21*	Up to 28	Up to 42	Up to 14*	Up to 21	Up to 28	Up to 30
Nail	113	23 (20%)	68 (60%)	19 (17%)	3 (3%)	38 (34%)	62 (55%)	12 (10%)	1 (1%)
Skin	113	22 (19%)	70 (62%)	18 (16%)	3 (3%)	38 (34%)	62 (55%)	12 (10%)	1 (1%)
Hair	113	23 (20%)	69 (61%)	18 (16%)	3 (3%)	38 (34%)	62 (55%)	12 (10%)	1 (1%)

*Recommended incubation time stated in the National Standard Methods Standard Operating Procedure.

result alone, the NSM SOP advises that laboratories should issue a report detailing the microscopy results within 24-48 hours. Almost all laboratories (98%; 119/120) provided a preliminary microbiology report to primary care, while 2% (3/119) only provided reports on positive specimens; 78% (93/119) sent reports directly after microscopy; and 15% (18/119) issued reports within 48 hours. The remaining 8% of laboratories did not adhere to the NSM SOP. Nineteen laboratories (16%) reported adding comments in response to the clinical details provided by GPs on specimen requests forms.

It is possible the GPs do not fully understand the significance of microscopy results and the NSM SOP does not provide laboratories with clear guidance on the type of comments appropriate for preliminary reports issued to primary care.[4] Some 59% (70/119) of laboratories included in this audit added comments to the preliminary microscopy: 41% (29/70) comments, where appropriate, stating "microscopy suggestive of *Malassezia furfur*"; 29% (20/70) included "culture to follow"; 7% (5/70) "fungal elements seen", 6% (4/70) "sample too small for culture", 13% (9/70) added a variety of comments and 4% (3/70) provided no comments. Most laboratories added comments manually to each report, with only 14% (17/119) using automatically generated comments.

Fungal culture and adherence to the NSM SOP

The NSM SOP recommends that laboratories incubate all specimens at 26–30°C for seven days; after this time culture-negative samples should be reported and all plates re-incubated for an additional seven days. If growth is obtained at 14 days an amended report can be issued. For microscopy-positive but culture-negative specimens a preliminary report is recommended at seven days, but samples should be recultured using supplementary media, and the original plates should be re-incubated for an additional 14 days. Of the 120 responders, only nine (7.5%) laboratories reported following the NSM SOP for culture; however, when specifically asked about the methodology used it was found that the number of laboratories adhering to the national SOP was higher. Seven (6%) laboratories did not provide information on incubation periods. Table 1 shows the culture incubation methods used by the responding 113 laboratories. For negative microscopy specimens, only 34% (38/113) of laboratories followed the NSM SOP and incubated specimens for up to 14 days. For microscopy-positive specimens, adherence to the NSM SOP depended on the specimen type, with nail, hair and skin samples incubated for up to 21 days by 60%, 61% and 62% of laboratories, respectively. Approximately 20% of laboratories incubated positive microscopy samples for up to 14 days, which is less than is recommended by the NSM SOP.

Culture media used in UK laboratories

Sabouraud medium (SAB) is recommended in the NSM SOP as the best medium for routine fungal isolation.[5] The SOP states that the "addition of chloramphenicol to this medium is essential to prevent bacterial overgrowth and the addition of cycloheximide (also known as Actidione) prevents overgrowth of non-dermatophyte moulds, although the addition of Actidione should not be used when infection with a non-dermatophyte mould is suspected".[4] Table 2 confirms that 80% (92/114) of laboratories followed the NSM SOP guidelines, using SAB medium with chloramphenicol (SABC) and Actidione (SABA). It shows that the other 20% of laboratories used various media. Table 3 shows which manufacturers were preferred by audited laboratories for SAB media; Oxoid was the most popular, supplying 68% of laboratories.

Table 2. Culture media used for routine fungal isolation in UK microbiology laboratories.

Media Type	Frequency (n=114)
SABC and SABA*	92 (80%)
SAB only	9 (8%)
SABC only	7 (6%)
SAB and SABC	3 (3%)
SAB, SABA and SABC	2 (2%)
SAB and Malt agar plates	1 (1%)

*Recommended media in the National Standard Methods Standard Operating procedure.

Table 3. Manufacturers of culture media used for routine fungal isolation in UK microbiology laboratories.

Manufacturer	Frequency (n=114)
Oxoid	70 (61%)
Oxoid/Bioconnection	3 (3%)
Oxoid/Southern Group	1 (1%)
Oxoid/bioMérieux	3 (3%)
bioMérieux	8 (7%)
E&O	27 (23%)
E&O/bioMérieux	2 (2%)
Becton Dickinson	1 (1%)

Table 4. Total number of samples received by audited microbiology laboratories from general practice (1 April 2007 – 31 March 2008).

Variable	No. Labs	Median	95% Confidence Interval
Nail Total	65	1174	1002–1323
Nail Micro	64	353	305–495
Nail Culture	65	357	261–398
Skin Total	64	234	185–298
Skin Micro	63	32	24–43
Skin Culture	64	38	30–54
Hair Total	64	5	3–7
Hair Micro	63	Not estimable	Not estimable
Hair Culture	65	Not estimable	Not estimable

Reporting nail culture results to primary care

The NSM SOP recommends issuing a culture result at one, two or three weeks stating, as appropriate, that a further report will be issued.[5] Other than these recommendations, the NSM SOP does not provide guidance on what information should be included on culture reports issued to

primary care. Of the 120 laboratories included in this audit, 51% (61/120) included comments on fungal nail culture reports but only 15% (9/61) added comments automatically. Box 1 shows the range of comments used by laboratories; 67% (41/61) reported adding various comments to reports depending on the isolate, clinical details or sample.

Audit of laboratory reporting procedure

In total, 22% (26/120) of laboratories returned copies of 50 consecutive specimen reports issued between 1 April 2007 and 31 March 2008. These reports were used to audit each laboratory's reporting procedure against the information provided in the questionnaire. These reports were used to audit microscopy results, culture results and laboratory comments routinely reported to primary care. Review of these reports showed that all 26 responding laboratories provided accurate information in the questionnaire about their reporting practice.

Samples received from primary care

Sixty-five (54%) laboratories provided the total number of samples received from general practice during the period 1 April 2007 to 31 March 2008. The distribution of these results was skewed and therefore the median and 95% confidence intervals (CI) were calculated (Table 4). Overall, there was a significantly larger number of nail samples (95% CI 1002–1323) than skin samples (95% CI 185–298), which in turn form a significantly larger number than hair samples (95% CI 3–7). The number of microscopy-positive nail samples (95% CI 305–495) was not significantly different to the number of culture-positive nail samples (95% CI 261–398). Likewise, the difference between microscopy-positive (95% CI 24–43) and culture-positive skin samples (95% CI 30–54) was not significant, nor was the difference between the number of microscopy-positive and culture-positive hair samples.

Box 1. Comments attached to fungal nail culture reports issued by UK microbiology laboratories to primary care.

Comments	Frequency (%)
Non-dermatophyte isolated, significance of isolate questionable	11 (18%)
Suggest repeat to establish significance	6 (10%)
Microscopy positive but culture negative, suggest repeat sample	3 (5%)
Various, depending on isolate, clinical details and sample:	41 (67%)
• Culture positive – Terbinafine treatment recommended for nail disease	
• At discretion of medical microbiologist.	
• As reported from reference laboratory.	
• Non-dermatophyte isolated, please repeat	
• As per IPF publication.	
• *Scopulariopsis brevicaulis* is usually resistant to Griseofulvin	
• Significant numbers of microscopy positive nail specimens are negative on culture	
• Unequivocal evidence of infection despite negative culture	
• Dermatophyte isolated, contact the medical microbiologist for advice	
• Non-significant contaminants	
• May be significant from fingernails, unlikely to be significant from toenails	

Discussion

The NSM SOP was followed by 92% of laboratories for the microscopy of dermatological samples. Digestion of samples with KOH prior to direct light or fluorescence microscopy was recommended in the NSM SOP and used by 63% and 29% of laboratories, respectively. Preliminary reports were issued after microscopy by 98% of laboratories, and 93% issued reports within 48 hours; this is in line with the NSM SOP. Over half of laboratories added comments to preliminary microscopy reports and a wide variation in comments was identified. Adherence to the NSM SOP for culture was low; only 34% of laboratories incubated microscopy-negative specimens for the recommended 14 days, while approximately 60% incubated microscopy-positive specimens for 21 days. The SABC and SABA media were used by 82% of laboratories overall, but media was obtained from various commercial sources. Comments were added to fungal nail culture reports issued to primary care by 51% of laboratories, with an array of comments used depending on the isolated organism, clinical details or sample type. Nail samples were the most common sample received from primary care, followed by skin and hair.

Strengths and weaknesses

To the authors' knowledge, this is the first large UK audit to investigate laboratory management of suspected superficial fungal infections in comparison with the NSM SOP, with an emphasis on reporting to primary care. The results were obtained from 67% of laboratories undertaking mycology culture and included NHS and HPA collaborating laboratories throughout England, Wales, Scotland and Northern Ireland.

Although the questionnaire was designed to be quick to complete, many of the questions asked respondents to answer with free text. This design meant that respondents were not led by categorical answers, thus allowing a variety of themes to emerge. However, this methodology did require categorisation during data analysis, and handwriting was sometimes difficult to interpret.

The amount of specimen 'seeded' on an agar plate is key to ensuring that an accurate result is obtained from fungal culture. The NSM SOP recommends placing approximately 20 fragments on the surface of the culture medium to maximise pathogen isolation.[5] Unfortunately, this aspect of the culture process was not audited and therefore it is not possible to say whether UK laboratories routinely culture this amount of specimen or indeed primary care routinely provides sufficient specimen (ideally multiple fragments) to facilitate accurate culture results. It may be worth investigating this in future audits and issuing a reminder to UK GPs about how to collect an optimal sample to facilitate accurate fungal microscopy and culture.

Microscopy

Direct microscopy examination of specimens provides clinicians with the relevant information needed to commence treatment, while culture results ensure treatment

Box 2. Comments suggested for inclusion on fungal microscopy and culture reports issued to primary care.

MICROSCOPY COMMENTS	
Negative microscopy	Fungal microscopy: No fungus seen. Culture result to follow
Positive microscopy (fungal hyphae)	Fungal microscopy: Fungal elements seen. This positive microscopy is diagnostic for fungal infection – Culture result to follow
Positive microscopy *Malassezia*	Microscopy: *Malassezia furfur* seen – causative organism of pityriasis versicolor
Positive microscopy yeasts. Nail samples only	Fungal Microscopy: Yeast seen. Culture result to follow
CULTURE COMMENTS	
If microscopy is either positive or negative and a dermatophyte or *Scytilidium* grown	Just the name of the isolate is reported
If microscopy is positive and a pure culture (>4 colonies) of an *Aspergillus* sp., *Acremonium* sp., *Fusarium* sp., or *Scopulariopsis* sp.	The name of the organism is reported along with the comment: This mould is a recognised cause of nail infection
If microscopy is negative and either a pure culture (>4 colonies) of an *Aspergillus* sp., *Acremonium* sp., *Fusarium* sp., or *Scopulariopsis* sp.	The name of the organism is reported along with the comment: Significance of result unclear in absence of positive microscopy Please repeat for confirmation if clinically indicated

is targeted. The first stage of the microscopy process requires the dissociation of cellular material and background keratin to ensure fungal hyphae and arthrospores are visible during microscopy examination, and this is routinely achieved by treating specimens with clearing reagents. One of the simplest, cheapest and most commonly used clearing agents is 10–30% KOH, which allows immediate examination of digested specimens and was the primary choice for 99% of audited laboratories.[5,7] After digestion, the visualisation of fungal elements via direct light microscopy is often difficult and requires an experienced eye. False-negative results have been reported in 5–15% of direct microscopy examinations, depending on the skill of the laboratory staff and sample quality.[8] Nevertheless, direct light microscopy is still considered highly efficient as a primary screening method and the present results show that 63% of UK laboratories use this method.[5,9]

To increase the sensitivity of direct microscopy, 3% of audited laboratories reported using ink stains to highlight fungal elements. There is a wide variety of stains available to enhance light microscopy, including Chlorazol black E, permanent blue-black inks (Parker Quink), lactophenol cotton blue, periodic acid-Schiff, 1% crystal violet and Congo red.[5,7,11] However, the most sensitive staining method available uses fluorochromes such as Calcofluor white. A study by Borman *et al*. found 91% of positive samples at the Bristol Mycology Reference Laboratory (MRL) were detected by fluorescence microscopy following KOH digestion.[4] Other work at the MRL has shown that Calcofluor fluorescence microscopy increases the detection rate of fungal nail samples by 30% compared to direct light microscopy (M. Palmer, personal communication). However, a fluorescence microscope with appropriate filters is required for this method and this may explain why less than a third (29%) of UK laboratories have adopted this technique.

A previous UK audit found that 78% of GPs always waited for laboratory results from suspected fungal infection specimens before initiating treatment.[3] In the current audit, 93% of laboratories followed the NSM SOP and issued a preliminary microscopy report within 48 hours of sample receipt. Comments were added to microscopy results by over half of the audited laboratories, and 76% of these added comments manually. These results suggest that there is too much variation in the comments reported to primary care, and the authors suggest that the NSM SOP should include a set of standardised comments to rationalise reporting to primary care (Box 2). A useful algorithm has been produced by Leeds Mycology Reference Centre, HPA and UKCMN, and lists a range of comments depending on the specimen type, microscopy results and culture findings.[11]

Culture

Culture is invaluable for the isolation and identification of an infecting pathogen to the species level, and this can be important when choosing appropriate treatment. Culture is also known to be more specific than microscopy, yet culture is more time-consuming, has a lower sensitivity and higher risk of sample contamination.[10] False-negative cultures (i.e., samples that are positive on microscopy and negative on culture) can arise due to a variety of reasons, such as insufficient material collected for culture, poor specimen, inappropriate incubation conditions, prior antifungal treatment, specimen contamination or insufficient period of incubation when patients are infected with a slow-growing organism.[12] Of the laboratories included in this audit only 34% adhered to the NSM SOP and incubated negative microscopy samples for the recommended 14 days, while approximately 60% of laboratories incubated samples for longer than this (Table 1). Prolonging the incubation period for longer than 14 days is unwarranted and may increase the likelihood of environmental contamination. Although approximately 60% of audited laboratories followed the NSM SOP for positive microscopy samples and incubated specimens up to 21 days, 20% reported incubating positive microscopy samples for only 14 days.

Comments were added to final culture reports by 51% of laboratories, and 67% of comments were unique between laboratories. This high level of variation may cause confusion in the interpretation of results or the management and treatment of patients. A standardised template could be designed for all laboratory fungal report comments. The comments outlined in Box 2 were agreed by the HPA SW GP Laboratory Use Group and the authors. This standardisation

for all UK laboratories has the potential to reduce workload, speed up reporting and provide primary care with a standardised message.

Sabouraud's agar is recommended in the NSM SOP and was the most popular choice for all laboratories audited (Table 2). There are now many preprepared SAB agar plates commercially available; however, these are often made using different formulas that can vary in pH, composition, additives and thickness. To the authors' knowledge there has been no UK validation study that directly compares the performance of all commercially available media, and therefore laboratories should be aware that colony morphology, pigmentation and growth rate may vary on media from different manufacturers. Indeed, the NSM SOP notes that laboratories should be aware of this effect and recommends that "laboratories become familiar with the appearance of different species on their own agar".[5] □

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