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# Comparison of test methods for mould growth in buildings

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**Abstract**
*The purpose of this work is to compare a range of test methods and kits for assessing whether a building structure is infested by mould fungi. A further purpose of this work is to evaluate whether air-based methods for sampling fungal emissions provide information qualifying decisions concerning renovation needs. This is of importance when hidden surface testing would require destructive measures and subsequent renovation. After identifying available methods on the Danish market for assessing mould growth in dwellings, a case study was conducted to test the usefulness of the methods in four dwellings of different typology and with or without known mould infestations. In each dwelling seven methods were used in parallel. The criteria for choosing the different methods were that they had to be non-destructive, relatively quick and easy, and frequently used by building professionals. The chosen methods measure different aspects relating to mould growth and vary in selectivity and precision. The two types of air samples indicated low levels of mould growth, even where the results of the other methods indicated high to moderate growth. With methods based on culture and DNA testing some differences in the species that each identified were apparent. In conclusion we found visual and olfactory inspection to be quite indicative of mould growth while none of the surface tests gave the complete representation as stand-alone tests. The air sampling methods seemed only to react to very comprehensive infestation with fungi.*

***Keywords - indoor climate, building moulds, test methods***

## 1. Introduction

A survey in 2011 with 13000 Danish respondents showed that 25% reported some extent of mould growth in their home while 5% reported more than 0.25 $m^2$ of visible moulds [1]. Knowledge about prevalence of hidden mould growth among respondents is limited. Most of the reported infestations are small and only of concern because they could grow into more comprehensive infestations with impact on occupant health. Still there is a need for better mould prevention through improved building quality and maintenance, and better targeted behaviour among occupants. Mould handling in existing buildings could be much improved by increased awareness, willingness to act, and quick and simple methods for objectively assessing whether a building structure is infested.

## 2. Background

Previously, mould fungi growth was often considered a technical and aesthetic problem with discoloration of material surfaces and odour annoyance as the main issues. Based on knowledge gathered trough the last 4 decades the WHO have however concluded that there is sufficient scientific proof that inhabitants of humid or mould infested buildings have an increased risk of respiratory problems, respiratory disease and worsening of asthma [2].

It is estimated that mould growth is a problem in 20-50% of Northern European and North American homes [3][4]. In most countries, including Denmark, people spend 60-90% of their time indoor [5][6]. The long exposure time aggravates the risk associated with indoor exposure to moulds, in particular the risk of becoming sensitised and the risk of suffering among people sensitised to moulds.

To validate suspicions of mould growth and for quality control of cleaning measures there is a need for methods for quickly quantifying the extent of the mould growth and in some cases obtaining qualitative information including the mould species. By using quick test methods that precisely and inexpensively describe the extent and spread of the mould growth it would in many cases be possible to limit the renovation or avoid eventually having to remove supporting structures. The methods may also improve collaboration between building management and building occupants by objective measurements of suspected mould infestation.

Mould growth may be visible on inner surfaces. It may however also be hidden on the back side of wall paper, below wooden floors, inside composite walls with cavities, and in cold attics etc. Applying a surface-based method to quantify such mould growth is not simple because access to the hidden surface requires destruction that will need efforts to renovate regardless of the findings. Some methods have tried to overcome this obstacle by sampling fungal emissions in room air.

## 3. Purpose

The purpose of this work is to compare a range of test methods and kits available in Denmark, to aid in reaching a common understanding of assessment of mould growth in buildings. A further purpose of this work is to evaluate whether air-based methods provide information qualifying decisions concerning renovation needs.

## 4. Methods

A survey identifying available methods on the Danish market for assessing mould growth in dwellings showed us that several different methods

are used. Among those we chose seven methods for our case study. The criteria for choosing the 7 methods were that they had to be non-destructive, relatively quick and easy, and frequently used by building professionals. In this project we compare the results of mould tests performed in parallel. Usability, price and the timespan for obtaining results are compared and variations between results of parallel samples are examined. In each of 4 dwellings the seven methods were used in parallel.

Figures 1 and 2 show a comparison of the methods concerning unit of measurement, evaluation scale, price and time for obtaining results.

| Method | Culture methods | DNA | MycoMeter surface | ProClean |
|---|---|---|---|---|
| Measures | CFU/species quantity/quality | Species quantity/quality | Fungi biomass quantity | Protein quantity |
| Unit | Number of CFU/species* | Spore equivalents of 20 species** | MycoMeter value (NAHA activity) | Peptide bonds |
| Eval. scale | High (>50), mod., low (<10) or none | Very low (a) to very high (e) | <25=low (a) to >450= high (c) | High to low |
| Price/ sample | 750 DKK | 1500 DKK | 800 DKK | 120 DKK |
| Results | One week | Two days | Two days | Few minutes |

* One lab only reported total CFU and species, the two other also reported CFU/species
** The evaluation scaled is based on the interrelationship between indicator and non-indicator species and descibes the risk of hidden mould growth

Fig. 1 Summary of characteristics of the different surface based methods

| Method | Impaction (air) | MycoMeter air | | Overall evaluation |
|---|---|---|---|---|
| Measures | CFU/species quantity/quality | Fungi biomass quantity | | General inspection and measurements |
| Unit | CFU/$m^3$ air | MycoMeter value per 300 l. air | | Various |
| Eval. scale | <200 = harmless | Low (a) to high (c)* | | Written report |
| Price / sample | Min.1200 DKK | Min. 1500 DKK | | 10000-18000 DKK/ 120$m^2$ house |
| Results | One week | Two days | | 1-2 weeks |

* Passive a=<350, c=>450 Agressive a=<900, c=>1700

Fig. 2 Summary of characteristics of the different air based methods and an overall evaluation

The scientific basis and use of each method and some comments on usability and reliability is briefly summarised below.

**Inspection** – A visual and olfactory walk-through performed by a building physics expert often including humidity measurements and resident interviews. It is described as a common method which is often sufficient in cases of visible mould growth but limited by the experience and thoroughness of the investigator [7, 5].

**Surface testing with culturing on V8-agar plates** - This method has for many years been the most frequently used in Denmark. An agar plate is pressed against a surface with suspected mould growth and cultured in a laboratory for approximately 1 week. The number of CFU (colony forming units) is then counted by microscopy and some detection of species is performed. It is described as a standard approach to mould testing, relying much on the expertise of the laboratory technician performing the analysis [7, 8]. Research point out that the culturability of fungi samples might vary from <1% to 100% [7-9] depending on factors such as organism, species, substrate for culturing etc. We found that the agar plates were sensitive to cross-contamination and required some experience to handle.

**MycoMeter suface swabs** - Based on quantification of the mould specific enzyme (β-N-acetylhexosaminidase (NAHA)) [10] a small surface area (app. 4x4cm) with suspected mould growth is swabbed. In the lab a fluorogenic enzyme substrate solution is added to the swab and after 30 minutes of incubation the fluorescence is measured by a fluorometer, adjusted for temperature this measure is termed the MycoMeter value [11] We found the method very easy to use with no requirements for expertise and the results took much shorter time than with culturing or DNA.

**DNA testing of surface swabs** - The DNA-array methods using qPCR (quantitative polymerase chain reaction) is gaining popularity in Denmark. A similar method was developed by the EPA in USA and called MSQPCR (Mold specific qPCR) [7]. The Danish version of the test has an array of 20 species, 10 described as indicative of water damage and 10 described as normally occurring in indoor and outdoor environments [HouseTest® www.housetest.com/the-dna-analysis/glossary]. The test is marketed mainly as a way to detect hidden moulds. It is based on surface dust analysis and requires the sampled surface to have had at least one month since last cleaning. Several researchers describes qPCR as a very accurate method, detecting a larger variety and quantity than traditional CFU counting and visual determination of species, its main limitation is whether the DNA-array accurately matches the present fungal flora [7-9]. The test can be performed without any expertise, as long as the guidelines for the condition of the dust are followed.

**ProClean surface test** – A cheap test kit based on biuret testing of protein concentration, initially developed as a hygiene test for the food industry [Hygiena® http://www.hygiena.com/pro-clean-food-and-beverage.html].

ProClean reacts to all proteins but is not specific when it comes to biomass of living and dead mould fungi. It does not differentiate between any sources of proteins. The test is not frequently used by qualified consulting firms, but it is nevertheless marketed as a mould fungi test and can be bought

online and in many hardware stores. It was included since it was the only test method with instant results and no requirement of equipment or laboratory.

**Air sampling with culturing** - Instead of pressing an agar plate against a surface, air testing is performed by using an air sampler to impact a certain amount of room air on a filter or directly on agar. Culturing is then performed in the same manner as with the agar plates for surface testing. The method requires well-calibrated equipment and is by many considered unreliable due to short sampling times and many factors being able to affect the amount of airborne spores [2-4, 8, 12].

**MycoMeter air** – A filter cassette is attached to an air sampling device and 300 l. of air is sampled through the filter [13]. In the lab the same procedure is performed as with MycoMeter surface swabs. Taking into consideration that many factors affect the amount of airborne fungal matter the MycoMeter air sampling should be performed in an aggressive way, where the room before sampling begins is primed with a leaf blower. Sampling requires a certification course and the most extensive on-site equipment of the methods we have tried.

## 5. Cases

The study consisted of 4 cases in the greater Copenhagen area and Northern Zealand. The cases were chosen because of their availability and their diversity in regard to age and style of the building, suspicion of mould growth, and perceived symptoms of the inhabitants.

Building characteristics of the four case dwellings are summarised in Fig.3 and a short resume of the condition of each buildings regarding symptoms of mould growth at time of testing follows below.

| | Typology | Year of construction /renovation | Heating | Ventilation | Roof | External walls |
|---|---|---|---|---|---|---|
| C1 | Single-family house in Dragør | 1962/1965 | Central heating with natural gas | Natural vent. + exhaust valves kitchen & bath | Flat composite roof | Bricks |
| C2 | Apartment at Islands Brygge | 1910/2001 (total rebuilding) | District heating - radiators | Mechanical vent. | Sloped w. asphalt roofing | Bricks - well insulated |
| C3 | Vacation house in Dronning-mølle | 2000/none | Electrical radiators | Natural vent. + exhaust valves kitchen & bath | Tile roof | Bricks - well insulated |
| C4 | Apartment in Holte | 1970/1993 | District heating - radiators | Natural vent. + exhaust valves kitchen & bath | Tile roof | Bricks |

Fig. 3 Building characteristics of the four cases

**Case 1 (C1)** - The house had visible mould growth and was scheduled to undergo comprehensive renovation. When living in the house the inhabitants reported symptoms such as worsening of asthma symptoms in one child, headaches, itching, eye and nasal irritation.

Fig. 4 External and internal photo of case 1

**Case 2 (C2)** - The apartment presented no humidity problems or visible mould growth. The inhabitants neither complained nor displayed symptoms.

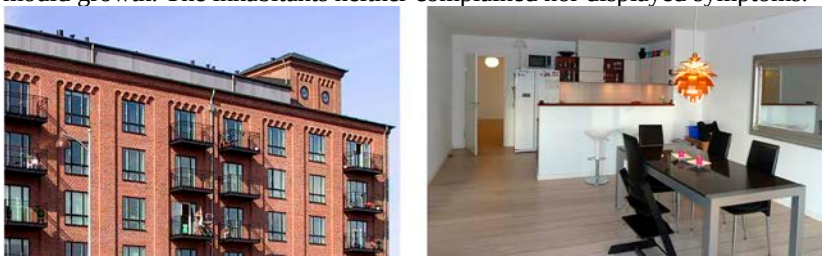

Fig. 5 External and internal photo of case 2

**Case 3 (C3)** – At the time of inspection, the house had been closed down for the winter. It presented a damp smell but no visible mould growth. The inhabitants report no symptoms and have no complaints in the summer period when they occupy the house.

Fig. 6 External and internal photo of case 3

**Case 4 (C4)** - The inhabitants of the apartment suspected hidden mould growth and complained of headaches, itching, eye and nasal irritation.

Fig. 7 External and internal photo of case 4

## 6. Results

The results are summarised in figures 8 and 9 and each case is described below.

| | Inspection & interviews(SBi) | | | Culture methods | | | DNA* | | MycoMeter surface* | | ProClean | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | visual | odour | sympt. | lab 1 | lab 2 | lab 3 | 1 | 2 | 1 | 2 | norm. | contam. |
| **C1** | yes | yes | yes | >50 | >303 | >300 | B | B | 462 | 378 | high | high |
| **C2** | no | no | no | 0 | 11 | 0 | C | A | 0 | 2 | low | high |
| **C3** | no | yes | no | >50 | 36 | 35 | E | E | 47 | 70 | high | high |
| **C4** | no | yes | yes | 10-50 | 11 | 18 | A | B | 6 | 3 | high | high |

* Parallel samples for each method examined by the same lab

Fig. 8 Summary of results – see fig. 1 and 2 for the scales each method uses.

| | Impaction - air | | | MycoMeter air | | Overall evaluation | |
|---|---|---|---|---|---|---|---|
| | lab 1 | lab 2 | lab 3 | passive | agressive | firm 1 | firm 2 |
| **C1** | 137 | 64 | 487 | 53 | 260 | - | - |
| **C2** | 7 | 3 | 21 | 163 | 220 | - | - |
| **C3** | 13 | 4 | 3 | 50 | 83 | No | Yes |
| **C4** | 4 | 2 | - | 147 | 228 | - | - |

Fig. 9 Summary of results continued – see fig. 1 and 2 for the scales each method uses.

**General remarks:** Due to limited space a complete review of speciation is not performed, but in general the cultures and the DNA-tests showed large differences among themselves and between the two methods.

**Case 1:** All cultures confirmed the presence of building moulds especially penicillium, but with different quantifications and determination of other species. MycoMeter surface showed moderate to high quantity. The DNA tests showed a larger variety of species than the cultures, but low risk of hidden moulds which could be attributed to the dust samples being mostly new dust from the ongoing renovation. All instances of air sampling by impaction showed high levels of mould growth, while both the passive and aggressive MycoMeter air samples showed low values.

**Case 2:** Only one lab culture showed signs of mould growth, mainly unspecified yeasts. The two parallel DNA samples gave low but markedly different evaluation scores although the species found and quantified are quite similar. Both air sampling by impaction on agar and the MycoMeter air tests had results in the low range which corresponds with most of the surface samples.

**Case 3:** The variation between results were larger than in the previous cases, one of the cultured agar plates showed very high levels of mould growth, the DNA method showed very high risk of hidden moulds, while two cultured agar plates and the MycoMeter test showed low levels of mould growth. Both air sampling by impaction on agar and the MycoMeter air tests had results in the low range which in this case does not correspond with the surface tests.

**Case 4:** The cultured agar plates showed a moderate mould growth, while DNA and MycoMeter results both indicated low growth or risk thereof. Mould growth was later found by visual inspection by an independent consulting firm. Both air sampling by impaction on agar and the MycoMeter air tests had results in the low range.

**Phase 2 – re-testing of Case 3:** Two independent consulting firms were hired to do an overall evaluation, they were asked to use their own preferred methods. Firm 1 used visual inspection and the MycoMeter method and found no indication of mould growth. Firm 2 used visual inspection and the culture method. Visible mould growth was found under the kitchen sink.

## 7. Discussion

Before performing the different sampling methods, the inhabitants were interviewed and a visual and olfactory inspection was performed by the researchers. Especially the olfactory inspection seemed to be indicative of mould growth which is in accordance with research [8, 10] concluding that mouldy odour is an important characteristic that should be included in building health assessments.

In correlation with previously mentioned research [7-9] we found that the identified species of moulds differed substantially between cultured agar plates and the DNA method, but also within instances of the same method.

The MycoMeter surface method was very easy to use in cases where a quantitative measure of moulds is sufficient. When both a qualitative and a quantitative measure are needed the DNA method seems a fast and precise method but since it has been developed for detecting hidden mould, it is difficult to compare the results directly to the other tests.

The ProClean method reacts to all proteins not just moulds. In a parallel test where swabs touched human skin before use the swabs all gave positive results, even when the uncontaminated swabs did not. To use the ProClean for testing for mould growth seems very unreliable, but they could be used after a renovation to test the cleaning effort.

In relation to the possibility of detecting hidden mould growth with air sampling, we found that both types of air samples showed low levels of mould growth, even in a situation where the results of the other methods indicated high to moderate growth.

## 8. Conclusion

It seems difficult to use any of the studied sampling methods as stand-alone tests, as they are liable to produce both false positives and false negatives as we saw in case 4. When combined with a visual and olfactory overall inspection performed by a professional consultant, correctly performed surface samples analysed by either culturing, DNA methods, or MycoMeter do seem to give a clear image of the density of mould infestation. Hiring an independent consulting firm to do an overall evaluation of mould growth might therefore be preferred, but possesses the risk of a large variation in their thoroughness. It is also a very high price per visit for a private homeowner or tenant.

In relation to the usefulness of air sampling methods for detecting hidden mould, we did not find that they could supply reliable information regarding renovation needs.

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