National Collaborating Centre for Environmental Health

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NCCEH Mould Investigation Toolkit

Interpretation of Microbial Laboratory Reports

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This document is one of several in the NCCEH Mould Investigation Toolkit. *Production of this document has been made possible through a financial contribution from the Public Health Agency of Canada.*

Introduction

This document summarizes the information typically contained or that should be found in the following types of microbial analytical reports:

Direct Microscopic Examination Microbial Report

- Fungal Spore Air Samples
- Tape, Bulk and Swab Samples

Cultured Samples Microbial Report

- Impactor Air Samples (e.g., Andersen, RCS, etc.)
- Bulk and Swab Samples
- Filter Samples (for Dust)

Examples of the laboratory reports for the analyses types listed above are referenced at the end of this document. These reports are examples only. Report styles and information reported between labs will vary.

The information provided in this document is intended to assist Public Health Inspectors to understand how to read microbial analytical reports and to gain a basic understanding of the results. Sampling is not often necessary and is supplemental if evidence of mould is suspected but areas of concern are unclear due to lack of observable indicators. If air samples are taken, using culturable (viable) and non-culturable (non-viable) methods are recommended to obtain better characterization of indoor moulds.

NOTE: Laboratory reports must be interpreted in context with qualitative observations, building/occupant history, and other sources of information to inform microbial investigations and decision-making. Some basic interpretation guidance is provided in this document; however, interpretation of microbial analytical results is complicated and a qualified person with the necessary education, experience, and/or certification in microbial assessments and remediations should be consulted for assistance with interpretation of sample results.

For an electronic copy of the reports referenced in this document, please contact us at contact@ncceh.ca

Generic Information – All Microbial Laboratory Reports

The following is a summary of information that should be present on all microbial reports:

- Lab Name and Location
- Lab Accreditation Details – Verify if the lab is accredited by the American Industrial Hygiene Association or other appropriate accrediting body and the accreditation number.
- Analyst Name
- Report Reviewer/Approver – Reports should be signed by the report reviewer.
- Site Description / Site Identifier – Identifying where the samples were collected.
- Date samples collected, received by laboratory, and analyzed by laboratory – There are no specific guidelines on acceptable times between sample collection and sample analysis; however, these times should be relatively close together and may be specified for culture-based analysis (e.g., within 24 hours after sampling).
- Condition of Samples Comments – Report should be reviewed for comments about the condition of samples when they were received by the laboratory. Comments should be kept in mind when reviewing the sample results. For example, if the lab commented that the samples were shipped inappropriately and that this may impact the sample results, the results reported should be viewed with caution, and repeating the sampling may be desirable.
- Specific Analyses Conducted – The report should specify the specific analyses performed and the analytical methods utilized.
- Analytical Sensitivity – The limit of detection/quantification of the analysis method, or the sensitivity of the analysis method.
- Request for Analysis Form – The request for analysis form should be included with the laboratory report. Confirm that all of the sample results are present in the report, the sample information (sample number, description, volume of air, area, etc.) are correct and that chain of custody practices were followed with respect to the samples (record of everyone who had custody of the samples from time of collection to receipt at the laboratory).

Direct Examination Methods

Fungal Spore Samples

Fungal spore samples analyzed by direct microscopic examination should report the following in addition to the general information above.[1,2]

- The sample number.
- The laboratory sample number.
- Sample description where provided by sample collector.
- Comments about the individual samples.

- The sample volume in litres/cubic metres of air.
- A comment about the amount of background debris. Commonly the words “light”, “moderate", and “heavy” are used to describe the amount of background debris.[1] Alternatively a numerical scale may be used (1 [low] to 5 [high]).[2] Heavy amounts of background debris may interfere with the analyst’s ability to see or identify microbial growth present.
- The raw slide count indicating the number of individual spore types identified, number of spores unable to identify (e.g., other) and the total number of spores counted. In some cases, spore counts for groups of fungal spores are reported, as it is not possible to distinguish between spore types (e.g., *Aspergillus / Penicillium*).
- The spore types by percentage of the total number of spores counted.
- The concentration of each spore type in air in spores per cubic metre of air (spores/m^3) and the total spore concentration in air in spores/m^3.
- The concentration of hyphal fragments, fibrous particulates, insect fragments, and pollen in air (reporting varies by laboratory and analyses requested). Hyphal fragments are components of fungi (similar to the roots and branches of a tree). A large quantity of hyphal fragments indoors suggests an active fungal colony may be present in the building.
- Interpretation of Guidelines: Some reports provide a specific section for the interpretation of the samples results; other labs provide this information in the additional comments section. In EMSL’s Spore Trap Assessment Report, the lab indicates how the concentration measured compares to background with coloured symbols.[2] In addition, they indicate if the spores are normally found growing inside, and if the spores can cause allergies, produce mycotoxins, and/or are considered indicators of water damage using coloured symbols.
- Additional comments. The analyst’s comments about the samples, information on how to interpret the sample results, and information on the laboratory limit of detection are provided in this section. Specific comments about individual samples may also be included in this section.

When reviewing fungal spore sample reports, the following types of samples should be present. A greater number of samples will increase the confidence of results (e.g., duplicate samples):

- Sample(s) from the area(s) of concern inside the building.
- Control sample(s) from area(s) where there are no concerns (e.g., no water staining, standing water, visible suspect microbial contamination, or musty odours) inside the building (where possible).
- Outdoor reference sample(s).
- Field blank sample(s) – Samples submitted for quality control purposes to confirm there is no background contamination on the sample cassettes due to manufacturing, handling, or shipping processes.

In addition, while usually not provided with the analytical report, the sample collector’s notes or a report, detailing the time of day the samples were collected, the results of the site inspection, the locations where the samples were collected, the environmental conditions at the time the

samples were collected, and any suspect microbial growth occuring in the vicinity of where the samples were collected, should be provided to the report reviewer. This information will assist in the interpretation of the sample results.

Interpretation of Spore Trap Assessment Report Results

Some laboratories offer more detailed fungal spore reports that show results graphically, provide information on typical outside concentrations of spores for North America, compare the sample data statistically (indoor to outdoor/control, between suspect area and non-suspect area, etc.), and provide information on the probability of the spores in ambient air originating from inside the building. Four example reports are referenced at the end of this document[3-6] and are discussed below. Not all laboratories offer these types of reports, and report styles differ between laboratories. The information provided in these more detailed reports is useful in interpreting the sampling results.

Where detailed reports are not provided, the reviewer should as a minimum conduct a comparison of results themselves similar to that presented in the EMSL Analytical Inc. Expanded Fungal Report [3] or equivalent. The reviewer should note the following:

1. Total spores/m^3 of each sample and for each fungi: High concentration in indoor air, as compared to outdoor air, may indicate growth.

2. The rank order and type of fungi: Note which fungi are present/absent and predominant; drastic differences in diversity of fungi in indoor air compared to outdoor air may indicate problems. Note any “water damage” or hydrophilic indicator fungi found indoor (See NCCEH Mould Investigation Toolkit “Overview of Typical Fungi”); hydrophilic fungi require high moisture environments to grow and their presence in indoor samples may indicate dampness and growth. A comparison is then made between indoor samples and outdoor/control samples to determine whether or not the the results indicate a fungal spore amplification source in the area of concern.

In the EMSL Analytical Inc. Expanded Fungal Report, [3] the spore trap results are in table format and, in addition, are provided graphically for easy visual comparison for each sample individually, and then together for comparison with background/outside samples collected. This report also includes a section at the back on how to interpret the sample results and information about each genera of fungi identified (information from laboratory fungal library).

In the MoldRANGE™: Extended Outdoor Comparison Report,[4] the concentration of fungal spores measured outside are compared to typical outdoor concentrations in the nearest location for the month the samples were collected in, and the yearly average. In addition, the report details which fungal spores are capable of growing inside and which spores rarely grow inside.

In the MoldSCORE™: Spore Trap Report,[5] the concentration of fungal spores for each sample analyzed are grouped by whether or not they typically grow inside / seldom grow inside and are displayed graphically to allow for an easy visual comparison of results. For samples collected indoors, a MoldSCORE™ is provided for each type of fungal spore, which indicates the probability that the fungal spores originated from inside the building, rather than through entrainment into the building from outside. Details on how to interpret the MoldSCORE™ are provided on the last page of the report.

The MoldSTAT™: Supplementary Statistical Spore Trap Report [6] presents the results of the samples collected graphically for easy visual comparison of results. In addition, this report provides the "MoldSCORE™" discussed above. This report also compares the indoor samples to one another and the indoor samples to the outdoor samples statistically, as follows:

- Comparison of Indoor Spore Counts: Friedman chi-square test conducted. The null hypothesis (H0) is that there is no meaningful difference in the data for all indoor locations. The alternative hypothesis (used if the test disproves the null hypothesis) is that there is a difference between the indoor locations. The null hypothesis is rejected when the result of the test is greater than the critical value. The critical value that is displayed is based on the degrees of freedom (dF) of the test and a significance level of 0.05.
- Comparison of Indoor and Outdoor Spore Counts: Reports an agreement ratio that assesses the similarity of two samples (in this case the indoor sample and the outdoor summary) based on the spore types present. A score of one indicates that the types detected in one location are the same as that in the other location. A score of zero indicates that none of the types detected indoors are present outdoors. Typically, an agreement of 0.8 or higher is considered high agreement.
- Comparison of Indoor and Outdoor Spore Counts: Spearman rank correlation test conducted. The null hypothesis (H0) being tested is that the indoor and outdoor samples are unrelated. The alternative hypothesis (used if the test disproves the null hypothesis) is that the samples are similar. The null hypothesis is rejected when the result of the test is greater than the critical value. The critical value that is displayed is based on the degrees of freedom (dF) of the test and a significance level of 0.05.

In the example report referenced,[6] the Friedman chi-square test results indicate that there is no meaningful difference between the samples collected at location 2 and 3 (both inside samples). The agreement ratio indicates that the inside and outside samples do not have a high level of agreement but are not completely different. The Spearman rank correlation test results indicate that the the indoor and outdoor samples are similar and do not substantially differ from one another. The MoldSCORE is also listed as 271, which is considered "HIGH"; a score over 300 indicates that there is high likelihood that the spores originated from inside rather than outside (i.e., there is a mould source indoors).

Swab/Tape/Bulk Samples

Swab/tape/bulk samples analyzed by direct microscopic examination should report the following in addition to the general information above.[7,8]

- The sample number.
- The laboratory sample number.
- The sample description where provided by the sample collector.
- A comment about the amount of background debris. Commonly, the words light, moderate, and heavy are used to describe the amount of background debris. Heavy amounts of background debris may interfere with the analyst's ability to see or identify microbial growth present.
- A description of the material being analyzed for bulk samples (e.g., wallboard).
- Identify semi-quantitatively the total amount of fungal spores identified.
- Identify semi-quantitatively the amount of specific fungi or fungal spores identified. Normally, the number of fungi/spores is quantified using percentages, a scale (e.g., 0 – 4 in "EMLab P&K. Lab report 4645524. Direct microscopic examination report")[7] where the higher the number the more dominant that fungi/spore type, or categories (e.g., rare, low, or medium high in "EMLab P&K. Microscopic examination of fungal spores, fungal structures, hyphae, and other particulates from tape samples".[8]
- Additional comments and general impressions. The analyst's general comments/impressions about the samples are noted in this column.
- Footnotes. Additional information to assist in the interpretation of the sample results, or overall comments about the samples, is frequently included in the footnotes.

While not included in the example report referenced, reports should contain information on how swab and bulk samples were prepared and analyzed. For swab samples, the analyst will analyze the swab for areas of discolouration and then take small amounts of the swab materials and mount the fibres onto a microscope slide. For bulk samples the analyst typically will collect tape samples fron the surface of the material submitted for analysis.

Interpretation of Swab/Tape/Bulk Direct Examination Report Results

The results of swab, tape, and bulk samples help to confirm whether there is microbial growth in areas in which suspect visible microbial growth or water damage were observed on surfaces. Caution must be taken with interpreting direct examination results of samples that were collected from surfaces without suspect visible microbial growth as the spores detected may be only settled spores. Hyphal fragments and other growth structures can help identify if there is actual growth for those samples collected on stained surfaces where visual observations are ambiguous.

Cultured Samples

Air Samples – Impactor

Air samples collected onto agar media and analyzed for microbial organisms should report the following information in addition to the general information above.[9-13]

- The sample number.
- The laboratory sample number.
- Sample description where provided by sample collector.
- Comments about the individual samples.
- The sample volume in litres/cubic metres of air.
- The sampling media used (e.g., malt extract agar – MEA) – The type of agar used is normally abbreviated.
- The incubation temperature(s) – What temperatures were the agar plates/strips incubated at before analysis (e.g., room temperature or body temperature).
- The raw plate count indicating the number of colonies of each type of fungi/yeast/bacteria identified.[9-12]Reports can present information to the genera level[9]or to the species level.[10] As a preliminary screen, some reports only present the total counts for bacteria, fungi, and yeast[13] and carry out more detailed analyses based on the results of the total counts (e.g., the investigator may choose to identify and enumerate bacteria and fungi instead of just fungi based on results).
- The concentration of each type of fungi/yeast/bacteria in the air in colony forming units per cubic metre of air (CFU/m^3) and the concentration of fungi/yeast/bacteria in air in CFUs/m^3.
- Additional comments. The analyst's comments about the samples, information on how to interpret the sample results, and information on the laboratory limit of detection are provided in this section. Specific comments about individual samples may also be included in this section.

When reviewing reports for air samples collected with an impaction device, the following types of samples should be present. A greater number of samples will increase the confidence of results (e.g., duplicate samples):

- Sample(s) from the area(s) of concern inside the building.
- Control sample(s) from area(s) where there are no concerns (e.g., no visible water staining, standing water, visible suspect microbial growth, or musty odours) inside the building (where possible).
- Outdoor reference sample(s).
- Field blank sample(s) – Samples submitted for quality control purposes to confirm there is no background contamination on the sample cassettes due to manufacturing, handling, or shipping processes.

In addition, while usually not provided with the analytical report, the sample collector's notes or a report detailing the time of day the samples were collected, the results of the site inspection, the locations where the samples were collected, the environmental conditions at the time the samples were collected, and any suspect microbial growth occuring in the vicinity of where the samples were collected should be provided to the report reviewer. This information will assist in the interpretation of the sample results.

In addition to the information described above, some labs offer more detailed reports comparing the concentrations measured outside with typical outdoor concentrations.[14]

Interpretation of Cultured Air Sample Results

Similar to the interpretation of spore trap assessment reports, a comparison between indoor and outdoor/control samples can be made. The reviewer should note the following:

1. Total CFU/m^3 of each sample and for each fungi: High concentration in indoor air, as compared to outdoor air, may indicate growth.

2. The rank order and type of fungi: Note which fungi are present/absent and predominant; drastic differences in diversity of fungi in indoor air compared to outdoor air may indicate problems. Note any "water damage" or hydrophilic indicator fungi found indoor (see NCCEH Mould Investigation Toolkit "Overview of Typical Fungi"); hydrophilic fungi require high moisture environments to grow, and their presence in indoor samples may indicate dampness and growth. A comparison is then made between indoor samples and outdoor/control samples to determine whether or not the the results indicate a fungal amplification source in the area of concern.

Speciation of Culturable Air Samples

Speciation from culturable sampling can assist with identifying whether or not there are different species indoors versus outdoors where spore analysis and regular culture analysis can only resolve to the genus level (e.g., cannot distinguish between *Penicillium/Aspergillus*). Additional guidance on the interpretation of culturable sample results can be found in Table 1 of the NCCEH Mould Assessment in Indoor Environments – Review of Guidelines and Evidence (March 2014).[15]

EXAMPLE 1: Speciation vs. Spore Trap Only Monitoring

In EMLab's Spore Trap Analysis Sample Report,[1] if Sample 3 was compared only to the Outdoor Reference Sample 1, one might conclude that the Sample 3 location was unaffected by airborne fungal contamination as the total spores/m^3 and individual fungal grouping of spores is lower in Sample 3 than Outdoor Reference Sample 1, and the rank order of fungi are not very different. However, with more information, such as that provided by the EMLab's Culturable Air Full Speciation Fungi Report,[10] a reviewer can see that *Aspergillus versicolor* (an indicator fungi) is predominant in Sample 3 and there are different *Penicillium* species found indoors compared to outdoors (Sample 1). These differences are not obvious in the Spore Trap Report[1] as *Penicillium* and *Aspergillus* were grouped together and could not be differentiated from each other. Results of speciation[10] indicate that there may be a fungal amplication source in the Sample 3 location.

Bulk/Swab/Dust Samples

Swab/bulk/dust samples analyzed following culturing should report the following in addition to the general information above.[16-18]

- The sample number.
- The laboratory sample number.
- The sample description where provided by the sample collector.
- A description of the material being analyzed for bulk samples (e.g., wallboard).
- The sampling media used (e.g., malt extract agar – MEA).
- The incubation temperature(s) – What temperatures were the agar plates/strips incubated at before analysis (e.g., room temperature, or body temperature).
- The area is swabbed / surface area vacuumed where appropriate.
- Identify quantitatively the total amount of colony forming units (CFU) of bacteria / fungi / yeast identified per unit (CFU/gram, CFU/swab or CFU/area [e.g. 100 cm^2])
- Identify quantitatively the amount of individual genera or species of bacteria/fungi/yeast identified depending on the specific analyses requested in CFU/unit. The results, as a percentage of the total count, may also be presented to allow for easier comparison between samples.
- Additional comments. The analyst's general comments about the samples are noted in this section. Additional information to assist in the interpretation of the sample results may also be included in this section.

While not included in the example reports referenced in this document, reports should contain information on how swab, bulk, and dust samples were prepared and analyzed.

In addition, while usually not provided with the analytical report, the sample collector's notes or a report, detailing the time of day the samples were collected, the results of the site inspection, the locations where the samples were collected, the environmental conditions at the time the samples were collected, and any suspect microbial growth occurring in the vicinity of where the samples were collected should be provided to the report reviewer. This information will assist in the interpretation of the sample results.

Interpretation of Swab/Tape/Bulk Surface Culture Report Results

Culture analysis of swab/tape/bulk samples in conjunction with direct examination analysis can help determine whether the spores in the direct examination are culturable and, if speciation is conducted, the species of microorganisms present. The bulk culture analytical results can be compared to the indoor air to determine whether or not the microorganisms from these surfaces are contaminating the air. Sometimes, the predominant species in the surface culture results are not the predominant species in air, which may indicate that there is another source of microbial growth or that the predominant species on the surface do not become airborne easily.

Surface Sampling for Post-Remediation Clearance

If only surface samples are conducted (no air sampling) during the investigation, surface sample results can also be used for comparison with the results of any final clearance air samples or settled spore samples collected.

EXAMPLE 2: Using Pre-Remediation Surface Sample Results to Assist in Determining Post-Remediation Clearance

If certain species are predominant in the surface samples such as *Aspergillus versicolor*, and the clearance sampling indicates either higher than outdoor airborne concentrations of *A. versicolor* or many *A. versicolor* settled spores on surfaces after remediation, then one can deduce that that the cleaning may not have been adequate if all visible sources of the mould have been removed. Additional cleaning may be recommended, and the clearance sampling repeated. However, if all pre-remediation surface sampling results showed mainly *A. versicolor*, but post-remediation air sampling indicates predominantly *Penicillium Brevicompactum* at concentrations higher than outside/control areas, one might suspect that there is a source of *P. Brevicompactum* that was not discovered during the initial inspection. If after cleaning surfaces again, *P. Brevicompactum* is still impacting the air, then a more detailed inspection may be required to try to determine the source.

References

1. EMLab P&K. Lab report 4647821. Spore trap report: non-viable methodology [sample report]. San Bruno, CA: EMLab P&K. Available from: https://www.emlab.com/app/report/SampleReportGenerator.po?reportId=4647821.
2. Environmental Molecular Sciences Laboratory. Spore trap assessment report. EMSL Order ID: 991200015. North Cinnaminson, NJ: EMSL Analytical Inc; 2012. Available from: http://www.emsl.com/PDFDocuments/Marketing/SporeTrapASSESSMENTReporttmUSA.pdf.
3. Environmental Molecular Sciences Laboratory. Expanded fungal report. EMSL Order: 990700063. Westmont, NJ: EMSL Analytical Inc; 2012 May. Available from: http://moldremovalalbany.com/wp-content/uploads/2012/11/Click-for-a-Sample-Mold-Laboratory-Report.pdf.
4. EMLab P&K. Lab report 4647809. MoldRANGE™: Extended outdoor comparison EML ID 1014146 [sample report]. San Bruno, CA: EMLab P&K. Available from: https://www.emlab.com/app/report/SampleReportGenerator.po?reportId=4647809.
5. EMLab P&K. Lab report 4647811. MoldSCORE™: Spore trap report [sample report]. San Bruno, CA: EMLab P&K. Available from: https://www.emlab.com/app/report/SampleReportGenerator.po?reportId=4647811.
6. EMLab P&K. Lab report 4647812. MoldSTAT™: Supplementary statistical spore trap report [sample report]. San Bruno, CA: EMLab P&K. Available from: https://www.emlab.com/app/report/SampleReportGenerator.po?reportId=4647812.
7. EMLab P&K. Lab report 4645524. Direct microscopic examination report [sample report]. San Bruno, CA: EMLab P&K. Available from: https://www.emlab.com/app/report/SampleReportGenerator.po?reportId=4645524.
8. EMLab P&K. Microscopic examination of fungal spores, fungal structures, hyphae, and other particulates from tape samples (EMSL Method: M041) [sample report]. Westmont, NJ: EMLab P&K. Available from: http://www.certifiedmoldsolutions.org/wp-content/uploads/2014/06/Mold_Solutions_Example_Lab_Report.pdf.
9. EMLab P&K. Lab report 4693920. Culturable air fungi report [sample report]. San Bruno, CA: EMLab P&K. Available from: https://www.emlab.com/app/report/SampleReportGenerator.po?reportId=4693920.
10. EMLab P&K. Lab report 4694078. Culturable air fungi report [sample report]. San Bruno, CA: EMLab P&K. Available from: https://www.emlab.com/app/report/SampleReportGenerator.po?reportId=4694078.
11. Environmental Molecular Sciences Laboratory. Identification and enumeration of culturable bacteria by air (three most prominent types (EMSL Method M009)) [sample report]. Westmont, NJ: EMSL Test Lab. Available from: http://www.emsl.com/index.cfm?nav=Services&action=show&ServiceID=110.
12. Environmental Molecular Sciences Laboratory. Viable fungi identification and enumeration (Genus Level ID from plate and strip impactors (EMSL Method M005)) [sample report]. Westmont, NJ: EMSL Test Lab. Available from: http://www.emsl.com/PDFDocuments/Culturable%20Fungi%20M005%20Sample%20Report.pdf .
13. MB Laboratories Ltd. Total fungi, bacteria and yeast enumeration report W861. Sidney, BC: MB Laboratories Ltd.
14. EMLab P&K. Lab report 4693939. MoldRANGE™ CFA: extended outdoor comparison [sample report]. San Bruno, CA: EMLab P&K. Available from: https://www.emlab.com/app/report/SampleReportGenerator.po?reportId=4693939.
15. Palaty C, Shum M. Mould assessment recommendations. Vancouver, BC: National Collaborating Centre for Environmental Health; 2014 Mar. Available from: http://www.ncceh.ca/documents/guide/mould-assessment-recommendations-revised.

16. EMLab P&K. Lab report 4723582. Surface culture report [sample report]. San Bruno, CA: EMLab P&K. Available from: https://www.emlab.com/app/report/SampleReportGenerator.po?reportId=4723582.
17. EMLab P&K. Culturable surface bacteria report EML ID 170455. San Bruno, CA: EMLab P&K. Available from: https://www.emlab.com/app/report/SampleReportGenerator.po?reportId=170455.
18. MB Laboratories Ltd. Carpet dust samples report W854. Sidney, BC: MB Laboratories Ltd.