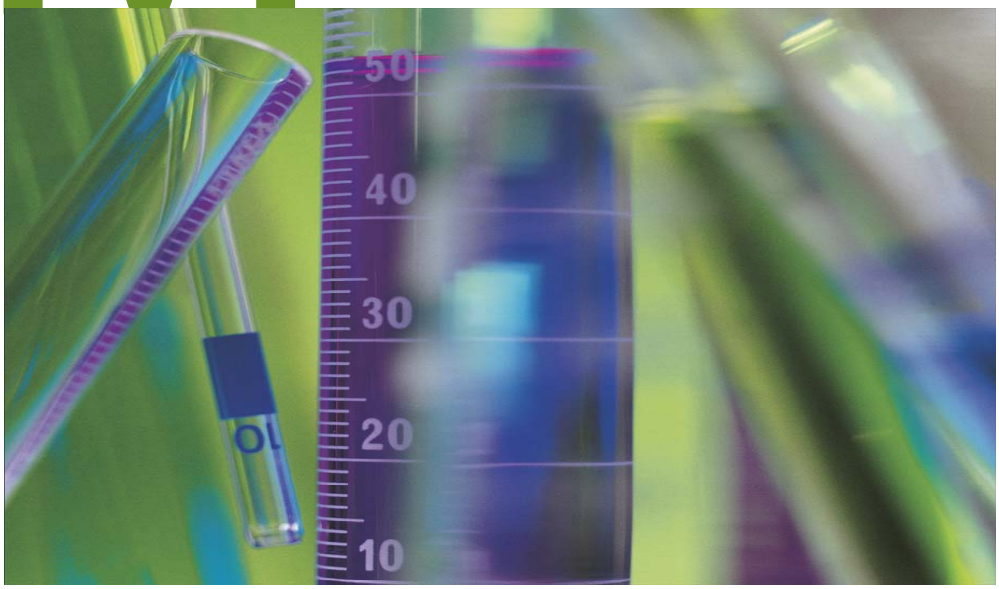
Analytical Method

Evaluation of mycological structures by microscopic examination

(Revision No 2)

ANALYTICAL METHOD **360**



Applicability
This method is used for the semi-quantitative evaluation of mycological structures.

Standard
No standard.

Sampling system
Autoadhesive cover slip, sterile sponge, and sterile container.

Recommended sampling volume and flow rate
N.A.

Analysis
Transmitted light microscopy.

Minimum reported value (MRV)
N.A.

Range of application
N.A.

Reliability
3.6% replicability; 6.4% repeatability.

Analytical Uncertainty (CV_A)
N.A.

Established in Québec since 1980, the Institut de recherche Robert-Sauvé en santé et en sécurité du travail (IRSST) is a scientific research organization known for the quality of its work and the expertise of its personnel.

OUR RESEARCH is *working* for you !

Mission

To contribute, through research, to the prevention of industrial accidents and occupational diseases as well as to the rehabilitation of affected workers.

To offer the laboratory services and expertise necessary for the activities of the public occupational health and safety prevention network.

To disseminate knowledge, and to act as scientific benchmark and expert.

Funded by the Commission de la santé et de la sécurité du travail, the IRSST has a board of directors made up of an equal number of employer and worker representatives.

To find out more

Visit our Web site for complete up-to-date information about the IRSST. All our publications can be downloaded at no charge.
www.irsst.qc.ca

To obtain the latest information on the research carried out or funded by the IRSST, subscribe to Prévention au travail, the free magazine published jointly by the IRSST and the CSST.
Subscription: 1-877-221-7046

Legal Deposit
Bibliothèque et Archives nationales
2009
ISBN: 978-2-89631-381-5 (PDF)
ISSN: 0820-8395

IRSST – Communications Division
505, De Maisonneuve Blvd West
Montréal (Québec)
H3A 3C2
Phone: 514 288-1551
Fax: 514 288-7636
sac.labo@irsst.qc.ca
www.irsst.qc.ca

Analytical Method

Evaluation of mycological structures by microscopic examination

(Revision No 2)

ANALYTICAL METHOD **360**

Disclaimer

The IRSST makes no guarantee regarding the accuracy, reliability or completeness of the information contained in this document.

In no case shall the IRSST be held responsible for any physical or psychological injury or material damage resulting from the use of this information.

Note that the content of the documents is protected by Canadian intellectual property legislation.

Analytical or calibration methods are the methods developed or chosen by the IRSST to carry out its different mandates. They may require the use of hazardous materials, operations or equipment. These methods do not mention all of the safety problems related to their use. It is the user's responsibility to establish the appropriate health and safety practices. Use of the data included in these methods is at the user's own risk: the IRSST is not responsible in any way for any errors or damage that may result from such use and application. The hyperlinks that appear in this document were validated at the time of publication.

Technical person in charge of the method

Geneviève Marchand, Ph.D., microbiologist,
Laboratory Services and Expertise Department, IRSST

Approval

Geneviève Marchand, Ph.D., microbiologist,
Marie-Claude Barrette, M.Sc. chemist, QA coordinator,
Jacques Lesage, M.Sc. chemist, director.

Laboratory Services and Expertise Department, IRSST

Authorization for publication

Marie Larue, M.Sc. President - CEO,
Executive office

Clic Research
www.irsst.qc.ca

This publication is available free of charge on the Web site.

This study was financed by the IRSST. The conclusions and recommendations are those of the authors.

TABLE OF CONTENTS

Preamble

The goal of the Act respecting occupational health and safety in Québec is to eliminate, at the source, dangers to the health, safety and physical well-being of workers. Permissible exposure values (PEVs) for chemical substances have been established in Schedule I of the Regulation respecting occupational health and safety (ROHS). Section 44 of this regulation entitled "*Methods*" specifies that:

> *"… These dusts, gases, fumes, vapours and mists found in the workplace environment shall be sampled and analyzed to obtain an accuracy equivalent to that obtained by applying the methods described in the Sampling Guide for Air Contaminants published by the Institut de recherche Robert-Sauvé en santé et en sécurité du travail du Québec…."*.

To achieve these objectives, analytical methods for quantifying the workers' degree of exposure are developed and written to establish appropriate means of control. In order to help health and safety professionals in workplaces, the IRSST publishes, periodically revises, and disseminates the *Sampling Guide for Air Contaminants in the Workplace*, and the Laboratory Services and Expertise Department publishes contaminant analysis methods.

These methods must be used in combination with the following regulatory and normative references:

- ✓ *Act respecting occupational health and safety. R.S.Q.,* chapter S-2.1. Éditeur officiel du Québec, (August 1st, 2007). http://www2.publicationsduquebec.gouv.qc.ca/dynamicSearch/telecharge.php?type=2&file=/S_2_1/S2_1.html
- ✓ *Regulation respecting occupational health and safety*. S-2.1, r.19.01, O.C. 885-2001. Éditeur officiel du Québec (July 25, 2007). http://www2.publicationsduquebec.gouv.qc.ca/dynamicSearch/telecharge.php?type=2&file=%2F%2FS_2_1%2FS2_1R19_01_A.htm
- ✓ *Sampling Guide for Air Contaminants in the Workplace*. Operations Division, IRSST, T-15 Guide technique, Montréal, Québec, (March 2005) http://www.irsst.qc.ca/files/documents/PubIRSST/T-15.pdf
- ✓ NIOSH, National Institute for Occupational Safety and Health.
- ✓ ISO Guide 30, Terms and definitions used in connection with reference materials, 2nd edition, 1992.
- ✓ ISO, International vocabulary of basic and general terms in metrology (VIM), 2nd edition, 1993.
- ✓ American Industrial Hygiene Association (AIHA), organization that accredits the IRSST laboratory in the field of workplace chemical contaminant analysis and microbiological environmental analysis.

Furthermore, all the terminology used in this method is described in work instruction "I-G-014" of the document management system associated with the IRSST's quality system.

1. PRINCIPLE OF THE METHOD

The surface suspected of being contaminated by moulds is sampled. The sample thus collected is subjected to microscopic examination.

This method is limited to confirmation of visible mould growth.

2. INTERFERENCES

The laboratory analysis method is transmitted light microscopy examination. The mycological density, as well as the opacity of the sample, can affect the mycological-structure detection capacity. Sampling of a significant amount of dust or material can mask the presence of mycological structures and result in a false negative or in a sample that cannot be analyzed.

An evaluation of the density of debris is recorded in the laboratory notebook. It can be noted in the following way:

0: No debris.
1: Small quantity of debris ⇒ No interference.
2: Large amount of debris ⇒ Possible interference. Interpret with care.
3: Too much debris ⇒ Analysis impossible. Unsuitable sample.

3. MATERIAL

- ✓ Phase contrast transmitted light microscope
- ✓ 12.5X eyepiece
- ✓ 40X objective
- ✓ 100X objective
- ✓ Slide and slip cover
- ✓ Sterile 50 mL plastic centrifuge tube
- ✓ 20X-120X stereo microscope
- ✓ Fibre optic light source
- ✓ Maxi-Vortex
- ✓ Tabletop centrifuge
- ✓ Refrigerator
- ✓ Manuel counter
- ✓ Tweezers that can be sterilized
- ✓ Sterilized disposable pipettes
- ✓ Sterile pipette and tip
- ✓ Autoclave

4. REAGENTS

- ✓ Sterile 0.01% peptone water (BBL 299111)

- ✓ Tween 20
- ✓ Lactic acid with cotton blue

5. SAMPLING

The surface suspected of being contaminated by moulds is sampled either:

1. with an autoadhesive cover slip that is applied directly on the target region;
2. with a sterile sponge, used to sample the target region;
3. by sampling part of the target region with tools disinfected with alcohol (process sample).

It is not necessary to provide a control sample.

Avoid sampling friable surfaces with autoadhesive cover slips because they must retain a certain transparency.

Use clean containers to transport samples (ex.: jar used for urine samples, sandwich bag).

For more details on the preparation of sampling material, calibration and the strategy used, refer to the IRSST's Sampling Guide[1].

6. ANALYTICAL PROTOCOL

6.1 Preparation of solutions

- ✓ Extraction solution for sterile sponge and process samples:

  Prepare a sterile 0.01% peptone water solution (BBL) with Tween 20 added at a final concentration of 0.05%. This solution promotes the break-up of the spore aggregates that could be present in the sample.

6.2 Preparation of samples

For samples on slides, perform a wet mount with lactic acid and cotton blue directly on the sample.

For sponges, add a volume of 10 to 100 mL of the sterile 0.01% peptone water solution containing Tween 20 directly into the sample transport container.

For the process samples, transfer a fraction of the sample into a sterile 50 mL centrifuge tube and add 5 to 20 mL of the sterile 0.01% peptone water solution containing Tween 20.

6.3 Analysis

6.3.1 Semi-quantitative examination of autoadhesive slip covers:

6.3.1.1 For each slide, observation at low magnification (100X-200X) is done in order to identify the regions of the slide that contain mycological material.

6.3.1.2 The analysis of the slide at 400X-600X is concentrated on the region or regions identified as having material present. If no region can be identified, the complete slide is scanned for the analysis.

6.3.1.3 Each field containing a mycological structure is counted as being positive.

6.3.1.4 A percentage of positive fields is counted in relation to the total number of fields up to a maximum of 100 fields in total OR 31 positive fields.

6.3.1.5 The semi-quantitative results are reported according to the following criteria:

RESULTS PRODUCED	PERCENTAGE
Negligible	0 to 5%
+	6 to 30%
++	31 to 60%
+++	61 to 100%

6.3.2 Examination of the microorganisms contained in a sterile sponge

When possible, the first wet mount between slide and slip cover is examined directly from the sample. If the result is negative, an extraction is done. The microorganisms contained in a sterile sponge are extracted using peptone water. The container in which the sponge is transported serves as the container for the extraction. From 10 mL to 100 mL of the sterile 0.01% peptone water solution containing 0.05% Tween 20 is added to the bag according to the estimated microbial load of the sample and an extraction by a stomacher is performed. The suspension obtained is placed between the slide and slip cover and is examined by microscope in order to determine the presence of mycological structures. Before recording the sample as not having any mycological structures (i.e., negative suspension), at least 5 mounts between slide and slip cover are performed.

6.3.3 Examination of the microorganisms contained in a solid matrix (process samples)

When possible, the first wet mount between slide and slip cover is examined directly from the sample. If the result is negative, an extraction is done. The microorganisms contained in a solid matrix are extracted using peptone water. If the matrix allows it, the solid matter is transferred to sterile bottles containing 5 to 20 mL of the sterile 0.01% peptone water solution containing 0.05% Tween 20. The bottles are shaken on the maxi-vortex for 20 minutes. The suspension obtained is placed between the slide and slip cover and is examined by microscope in order to determine the presence of mycological structures. Before recording the sample as not having any mycological structures (i.e., negative suspension), at least 5 mounts between slide and slip cover are performed.

7. APPLICATION PARAMETERS

7.1 Limit of detection and limit of quantification

Not evaluated.

7.2 Reliability

The repeatability of the semi-quantitative method was evaluated at 6.4%. The first evaluation was done using 9 slides from the field (4 concentration levels, 6 replicates per slide), by one analyst. The second evaluation was done by analyzing 45 field slides for 4 different levels, by 3 analysts. The reported value of 6.4% corresponds to the mean of the two evaluations. Furthermore, replicability was evaluated at 3.6%.

7.3 Accuracy

To be evaluated.

The laboratory performs an intra-laboratory quality control. At least 5% of the samples are analyzed in duplicate in order to calculate the RDP (relative difference percentage).

7.4 Measurement uncertainty

Not evaluated.

8. REFERENCES

1 *Sampling Guide for Air Contaminants in the Workplace*. Operations Division, IRSST, T-15 Guide technique, Montréal, Québec, (February 2005). http://www.irsst.qc.ca/files/documents/PubIRSST/T-15.pdf

9. BIBLIOGRAPHY

ACGIH. *Assessing Bioaerosols in Indoor Environment*, Sponsored by the University of Michigan and ACGIH, Oct. 4-8, 1988, Ann Arbor, Michigan.

COLLINS, C.H. *Microbiological Methods*, Sixth Edition, Butterworths, London, 1989, 409 p.

HURST, C.J., et al., *Manual of Environmental Microbiology*, ASM Press, 1997, 894 p.