



## **Rozšíření výuky v laboratořích TZB o mikrobiální hodnocení kvality vnitřního vzduchu**

### **Řešitel:**

Ing. Hana Kabrhelová, Ph.D., [Hana.kabrhelova@fsv.cvut.cz](mailto:Hana.kabrhelova@fsv.cvut.cz)

Katedra Technických zařízení budov

### **Projekt:**

Finanční podpora inovačních projektů akademických pracovníků ČVUT zaměřených na podporu výuky a vzdělávání pro rok 2024

### **Výstupy projektu:**

<b>Typ výstupu</b>	<b>počet</b>
Podklady pro přednášky / semináře / cvičení v CZ/EN (včetně elektronických)	2
Elektronická učebnice / skripta / studijní materiály / dokumentace pro podporu výuky v CZ/EN	1
Vzdělávací / inovativní opory pro zefektivnění a inovaci různých forem výuky (audio/video nahrávky, VR/AR apod.) v CZ/EN	1

Podklady pro přednášky / semináře / cvičení v CZ/EN (včetně elektronických):

Dokumentace pro podporu výuky v EN část 2.

## **Laboratory Exercise - Quantitative Assessment of Microbial Indoor Air Quality**

### **Part 2: Collection and cultivation of microbial samples**

The exercise is used to determine microbial contamination of air and surfaces, bacterial and fungal contamination will be examined in more detail. Students will first learn about microbiological sampling methods, then take samples from the air and surfaces of the area under investigation, and finally prepare samples for culture. In a subsequent exercise, they will be introduced to and practice quantitative assessment of the samples collected.

#### **Necessary equipment and aids:**

- Protective equipment (lab coat and disposable gloves)
- Incubator
- Petri dishes with colonies (air aspiration, fallout and swab samples)
- Manual colony counter (Figure 1)
- Marking pen for marking the counted colonies
- Information on the volume of air aspirated (e.g. litres, cubic metres, l, m<sup>3</sup>)
- Record sheet and measurement reports
- Autoclave
- Disinfection - denatured alcohol in spray bottle and Savo



*Figure 1 – Manual colony counter*

Theoretical part:

For quantitative assessment, the microorganisms on the media collected need to be cultured in an incubator at the set temperature required for cultivation for a precise period of time, depending on whether it is moulds or bacteria that are being assessed.

For quantitative evaluation, a special instrument, the colony counter, is used. The counter can be automatic or manual. For teaching purposes, a manual colony counter is used.

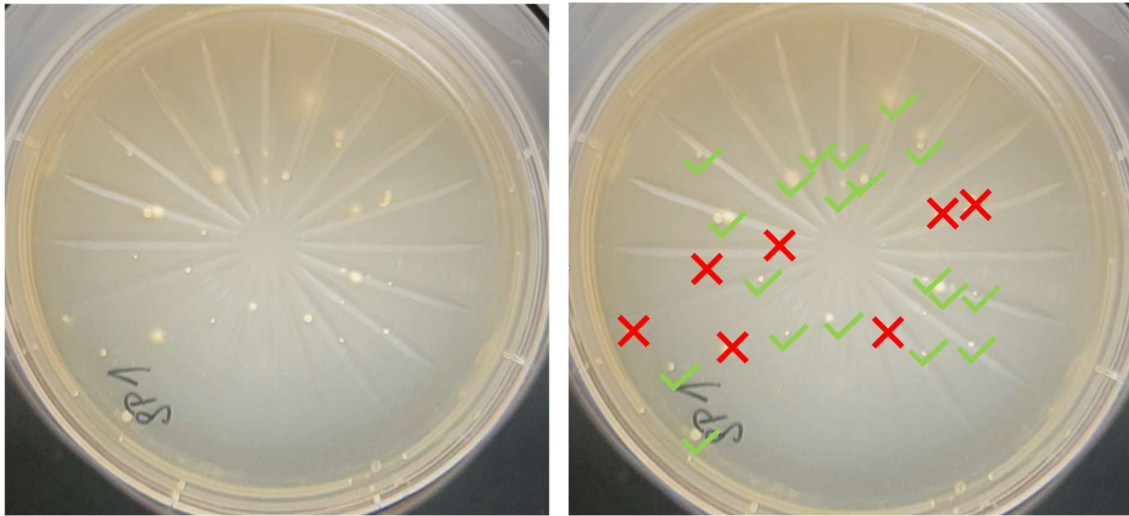
Petri dishes with multiplied colonies from active air aspiration with an aeroscope must be evaluated separately and a positive finding and a false positive finding must be distinguished. The impactor of the aeroscope has a special "star" (Figs. 2, 3) through which air is drawn onto the petri dish. Thus, colonies grown at the site of this "star" are a positive finding. Colonies grown outside this 'star' are false positives and are not included in the assessment. An example of positive and false positive results is shown in Figure 4.



*Figure 2 – The impactor of the aeroscope and its "star"*



*Figure 3 – „Star" on a petri dish*



*Figure 4 - Example of a petri dish with colonies (left) and a petri dish with labeled positive and false positive results (right))*

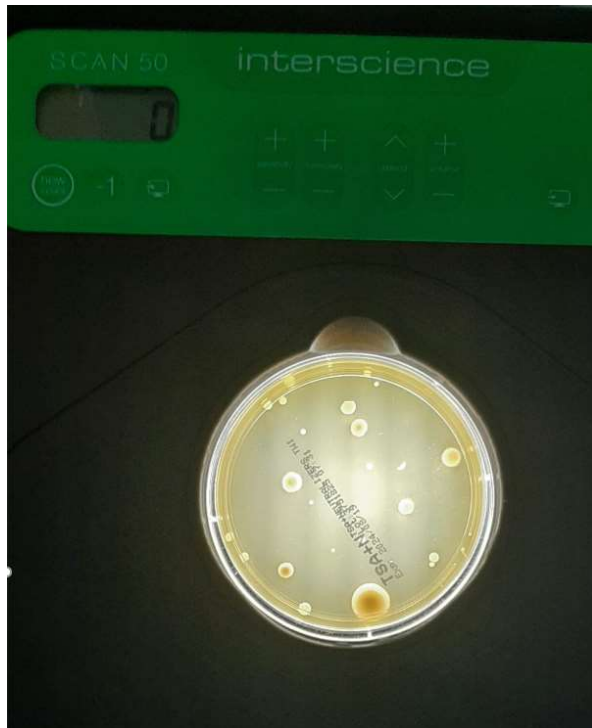
The number of colony forming units (CFU) for both bacteria and fungi are counted on petri dishes. The number of CFUs on the plates is then evaluated. In particular, the number of colonies will be compared between passive collection and active collection with an aeroscope. From the petri dishes collected with the aeroscope, FCU/m<sup>3</sup> of air will be determined based on the amount of air used during collection.

At present, according to Decree No 6/2003 Coll., which establishes hygienic limits for chemical, physical and biological indicators for the indoor environment of living rooms of certain buildings, we have set requirements where the limit is met if the concentration of microorganisms does not exceed 500 and moulds 500 colony-forming units per 1 m<sup>3</sup> of air by active air intake using an aeroscope (FCU/m<sup>3</sup>).

*Simplified procedure:*

- 1) Removing the petri dishes from the incubator (wearing gloves)
- 2) Place the petri dishes individually in the designated location in the manual colony counter
- 3) Manual colony counting - mark each colony with a marking pen, when the Petri dish is touched the colony counter automatically counts the colony, confirms it with an audible signal and displays it on the screen, do not open the dishes with cultured colonies! (Fig. 5)
- 4) Calculate the CTUs on all petri dishes one by one
- 5) Record the counts of fungi and bacteria in the sheet for each sample

- 6) Compare the amount of FCU between passive collection and active collection with the aeroscope
- 7) Describe color, shape, estimate how many different types of microorganisms grew
- 8) Sterilise used petri dishes in an autoclave (at 134°C)
- 9) Disinfect the colony counter and all surfaces in the workstation
- 10) Disposal of petri dishes using disinfectant



*Figure 5 - Petri dish placed in the colony counter*

Experimental part:

Laboratory procedure: calculation of the number of colonies on petri dishes

**1. Preparation of the working area:**

- Arrange the petri dishes removed from the incubators and check their labels.
- Dividing the petri dishes into three groups: active collection, passive collection and swab samples.

**2. Visualisation of the colonies:**

- Place the petri dish under the magnifying glass of the manual colony counter.
- Adjust the intensity of the backlight
- Evaluate the number of colony types in terms of shape, color and size

### 3. Counting colonies:

- Count colonies manually using the colony counter. Count each colony with one click of the counter.
- For ease of checking, mark the already counted colonies on the bottom of the petri dish with a marker.

### 4. Record the results:

- Once all colonies have been counted on the Petri dish, record the total number of colonies on the recording sheet and the measurement report.

### 5. Calculate the number of colonies in air CFU/m<sup>3</sup>:

- Use the following formula to calculate the concentration of microorganisms in the air:

$$\text{CFU/m}^3 = \frac{\text{Number of colonies (CFU)}}{\text{Volume of sampled air (m}^3\text{)}}$$

Where:

- Colony count (CFU) = number of colonies counted on the petri dish.
- Volume of air aspirated (m<sup>3</sup>) = volume of air aspirated and cultured on the Petri dish (e.g. 0.5 m<sup>3</sup>, 1 m<sup>3</sup>, etc.).

**Example:** if you have aspirated 1 m<sup>3</sup> of air and 100 colonies have grown on the petri dish:

$$\text{CFU/m}^3 = \frac{100}{1} = 100 \text{ CFU/m}^3$$

This result means that there were 100 colonies per cubic meter of air.

### 6. Repeat the procedure for other samples:

- If you have multiple petri dishes with different volumes of air drawn, repeat the calculation for each sample separately.

### 7. Conclusion:

- Check that all results have been correctly recorded and calculations performed for all dishes.

**Notes:**

- CFU/m<sup>3</sup> (colony forming units per cubic meter of air) is the standard unit for expressing the concentration of microorganisms in the intake air.
- If too small a volume of air has been aspirated and few colonies have grown on the plate, the results may be inaccurate - in this case, a larger volume of air should be aspirated.
- If the number of colonies is too high and it is not possible to count them accurately, a smaller volume of air may need to be sampled.

***For the measurement protocol see Annexes 1 and 2***

## Annex 1 - Indoor air microbial quality assessment protocol

**Date of measurement:** [to be added]

**Place of measurement:** [add]

**Equipment used: Aeroscope** [type of equipment, e.g. BioCapt® Single-Use]

**Air volume:** [add] number of litres per sampling

**Total number of samplings:** [add] samplings ([add] to TSA, [add] to Sabourad soil)

**CFU (colony forming unit) limit:** 500 CFU/m<sup>3</sup>

### **Measurement description**

Measurements were made in an enclosed room of approximately [fill in] m<sup>2</sup> with [fill in ventilation method, number of persons, etc.]. In order to determine the microbial purity of the air, two different culture soils were used: Trypticase Soy Agar (TSA) and Sabourad Glucose Soil.

Parameters and measurement procedure:

#### Active sampling by aeroscope:

##### **1. Sampling No. 1a:**

- **Soil used:** TSA
- **Sampling location:** [to be added]
- **Air volume:** [add]
- **Result:** [add number of CFU/m<sup>3</sup>]

##### **2. Sample No. 1b:**

- **Soil used:** Sabourad soil
- **Sampling location:** [to be added]
- **Air volume:** [add]
- **Result:** [add number of CFU/m<sup>3</sup>]

##### **3. Sampling No. 2a:**

- **Soil used:** TSA
- **Sampling location:** [to be added]
- **Air volume:** [add]
- **Result:** [add number of CFU/m<sup>3</sup>]

##### **4. Sample No. 2b:**

- **Soil used:** Sabourad soil
- **Sampling location:** [to be added]
- **Air volume:** [add]

- **Result:** [add number of CFU/m<sup>3</sup>]

#### Passive sampling

##### **5. Sampling No. 3a:**

- **Soil used:** TSA
- **Sampling location:** [to be added]
- **Result:** [add number of CFU/dish]

##### **6. Sample No. 3b:**

- **Soil used:** Sabourad soil
- **Sampling location:** [to be added]
- **Result:** [add number of CFU/ dish]

##### **7. Sampling No. 4a:**

- **Soil used:** TSA
- **Sampling location:** [to be added]
- **Result:** [add number of CFU/ dish]

##### **8. Sample No. 4b:**

- **Soil used:** Sabourad soil
- **Sampling location:** [to be added]
- **Result:** [add number of CFU/ dish]

#### **Evaluation of results:**

The limit for microbial air contamination is 500 CFU/m<sup>3</sup>. If this limit is exceeded, further investigation is required and measures must be taken to remove the source of contamination.

- **Sample 1a** (TSA): [add result CFU/m<sup>3</sup> and whether it is above/below the limit]
- **Sample 1b** (Sabourad soil): [add CFU/m<sup>3</sup> result and whether it is above/below the limit]
- **Sample 2a** (TSA): [add CFU/m<sup>3</sup> result and whether it is above/below the limit]
- **Sample 2b** (Sabourad soil): [add CFU/m<sup>3</sup> result and whether it is above/below the limit]

- **Sample 3a** (TSA): [add CFU result/bowl]
- **Sample 3b** (Sabourad soil): [add CFU/bowl result]

And compare with active abstraction whether the amount of CFU is higher/lower

- **Sample 4a** (TSA): [add CFU result/bowl]
- **Sample 4b** (Sabourad soil): [add CFU result/bowl]

And compare with active sampling whether the amount of CFU is higher/lower

[add description of other abstractions]

### **Conclusion:**

On the basis of the results of the sampling [final assessment, e.g. "the measurement results are within the standard and no further action is required" or "the limit has been exceeded, corrective action is required"]. Evaluation and discussion of active and passive sampling results.

### **Signature of the person taking the measurements:**

[add names]

## Annex 2 - Protocol for the assessment of the microbial quality of surfaces

**Date of measurement:** [to be added]

**Place of measurement:** [add]

**Total removals:** [add] removals ([add] to TSA, [add] to Sabourad's land)

### **Measurement description**

Measurements were taken by ..... In order to determine microbial purity, two different culture media were used: Trypticase Soy Agar (TSA) and Sabourad's glucose soil.

Parameters and measurement procedure:

#### **1. Sampling No. 1a:**

- a. **Soil used:** TSA
- b. **Sampling location:** [add]
- c. **Result:** [add number of CFU]

#### **2. Sampling No. 1b:**

- a. **Soil used:** Sabourad soil
- b. **Sampling location:** [add]
- c. **Result:** [add number of CFU]

[add description of other abstractions]

#### **3. Sampling No. 2a:**

- a. **Soil used:** TSA
- b. **Sampling location:** [add]
- c. **Result:** [add number of CFU]

#### **4. Sampling No. 2b:**

- a. **Soil used:** Sabourad soil
- b. **Sampling location:** [add]
- c. **Result:** [add number of CFU]

[add description of other abstractions]

#### Evaluation of results:

The measured values cannot be compared with Decree No. 6/2003 Coll., as they are swabs and the values obtained are the number of moulds or CFU (colony forming units) per swab, i.e. the data obtained are about microorganisms living on the surface.

- Sampling No. 1a(**TSA**): [add result of CFU]
- Sampling No. **1b (Sabourad soil)**: [add result of CFU]
- Sampling No. **2a (TSA)**: [add result of CFU]
- Sampling No. **2b (Sabourad soil)**: [add result of CFU]

[add description of other abstractions]

#### **Conclusion:**

The results are an indicator of the contamination of the surface in question and these results can still be discussed.

#### **Signature of the person taking the measurements:**

[add names]