

Faster Evaluation of Contaminated Surfaces for Mould Inspections by Tape Sampling

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Abstract

Taking a tape-lift sample is one of the main practices used by indoor environmental quality investigators for detecting whether mould structures (for example, spores and hyphae) have either settled onto or colonized the surface. Despite the popularity of the method, there can be significant inconsistency in how tape lifts are collected.

The common ASTM standard D7910, Practice for the Collection of Fungal Material from Surfaces by Tape Lift, describes the correct way to collect a tape-lift sample. Using ASTM D7910 semi-quantitative results (scale 0 up to 5) are only available.

In case histories or for mould removal control, the total cell count is needed. This cannot be realized by the ASTM method. Therefore, an innovative method is needed to combine the quickness of taping and the precision of total cell count. Our research team developed two methods to quickly and fully quantify the tape samples. Regarding the assessment criteria, the user can decide to operate with the 3-LINE method to achieve the highest precision or use the faster 3-STEP method for even better results.

1 Introduction

In case of moist damage and for measuring the success of a mould remediation, a diagnosing method is needed for a fast, simple, and yet an accurate determination of the mycelia growth and contamination by spores, hyphae, and other fungal structures. The method should determine the concentration of cells and cell structures to evaluate case histories or whether the biomass has been sufficiently reduced.

Cultivating samples can deliver results with sufficient precision concerning different species and genus. However, this process also has its limitations, as it requires a lengthy standard incubation and the only organisms that will grow are the ones that are ready to germinate. These factors are further influenced by the types of culture medium and the incubation environment.

The question is: how to analyze the mould biomass within a shorter timeframe and independently of cultivation and cell vitality?

Section 3.2.1 of the draft of the WTA leaflet E-4-12 edition: 03.2020/D '*Ziele und Kontrolle von Schimmelpilzschadensanierungen in Innenräumen*' (*Aims and control of mould damage remediation of indoor spaces*) deals with the 'material sample analysis as a method for controlling mould remediation'. In it, one would find this note (1):

For the direct determination of a successful mould remediation, the microscopic examination of surfaces in the form of adhesive tape contact samples or in the form of microscopic examination of material samples is preferable to complex and indirect methods.

Further, Point 8, 'Cleaning control of outsourced inventory', states:

In the case of adhesive film contact samples...due to the ratio of the sample surface to the total surface, however, has a very low statistical significance. The representation of the result increases with the number of samples taken. A smooth, closed surface should have a small number of mould components after cleaning. In practice, the following values of the evaluation from Table 2 have proved their worth:

Table 1
Suggestions for target values of purified inventory
(according to [1])

	Hyphae /cm ²	Spores/cm ²
Smooth surfaces	< 50	< 150

Using the standard method, ASTM standard D7910, it is not possible to get a result conforming to the WTA or other guidelines demanding a total cell count value. Therefore, the quantifying microscopic analysis of surface tape samples is brought into a standardization process. However, in order to obtain reproducible results for the evaluation Table 2, an appropriate approach and standardization of the methodology is required. For the past two years, the team associated with this paper has been working on the validation of the adhesive film methodology. The results show several methods that allow a reliable assessment of adhesive films (2). In this paper we would like to examine whether this task can be carried out with a microscopy method using an adhesive tape preparation.

The starting point of the investigation was a definition of the task ahead that allows us to derive different procedures having the necessary quality (3).

2 Materials And Method

To evaluate a new method of analysis, 37 commercial tape samples from case histories were counted fully in 24,000 fields of view, and later in 100, 300 and 900 fields of view by 3 analysts of two independent laboratories. In the end, up to 200 tape sample enumerations were used for a statistical analysis.

Basically, adhesive film analysis concerns the detection of biomass. The detection is differentiated into quantity and quality of the biomass. The first option means determining an amount or a partial amount, using a reference basis, e.g. cm². The second option is the detection of the composition, and also of the condition or properties (distinctions, infestation/contamination/intact, defective). The two different types of analysis enable equally wide-ranging grades of compatible measurements. The information from these methods can range from yes/no to the type of species and number of species per cm².

In applying the quantifying adhesive tape analysis, the aim is to record the biomass per cm². This should be executed by adopting a procedure that would yield comparable results, using the same requirements regardless of the type of microscope used. Further, the qualitative method was simplified, and the

countable fractions were specified. The question whether the moulds were growing on the surface or were a contamination had no relevance to the methodology, and the matter was, therefore, examined further.

In addition, a cancellation criterion was defined to avoid 'meaningless counting' especially when there are clearly interpretable results. One must always remember, especially in the case of 'non-microscopic' readings, that if a semi-quantitative adhesive tape is clearly evaluable, trying to achieve a full quantification is clearly a waste of time.

2.1 Microscopy Techniques

Microscopes are used to map objects that are smaller than the resolution limit of the human eye. Being able to look at images of the smallest objects is no longer limited to light microscopes. All wavelengths, or electromagnetic fields of any kind, can be used as an imaging medium or lenses. As a result, electron beam microscopes are found in today's laboratories, as well as tunnel, force, Raman, and even acoustic microscopes.

Light microscopes are limited in their resolution by the wavelength of the light. In addition, the image mode and the quality of the preparation (transparent, opaque) have an influence on the resulting image. Here, we distinguish between incident (also called reflected) and transmitted light microscopes. Usually, the resolution of a reflected light microscope is limited and barely reaches magnifications above 50 times. The reflected microscope uses the back scattered light of the illuminated surface. Thus, layers of mould can be identified as such, but spores or bacteria remain out of the range. The transmitted light microscope achieves a significantly higher resolution. Here, the light rays that radiate through the object are used for imaging, whereby the object either changes the phase or the amplitude of the light. In this way, magnifications of up to 1,000 times can be achieved, enabling images of incredibly small organisms like, for example, bacteria to be seen. With the magnification, the field depth decreases. Many spores and hyphae appear translucent, indicating that, in order to make even the smallest biological structures visible, one has to use special dyeing or contrast methods.

A disadvantage of translucent microscopy is that the resolution capacity required for the detection of microorganisms depends on the transmission capability of the affected substrate. However, plasters, wallpaper, wood or even EPS insulating materials only allow light to pass through up to 50 microns of layer thickness.

The problem could be solved with microtome sections, but this is rarely feasible in practice in case of a mould investigation.

The invention of the adhesive film preparations solved this problem, at least in part. In this context, examining an adhesive tape is not a 'real' direct microscopy (3).

The adhesive film covers the biomass when the tape is pressed onto the substrate and then removed again. In this way, the examined biomass can be assigned to a sampling location.

Limitations can arise due to the reproducibility of the biomass fixed to the film. When it is compared with the total biomass, which may also be present in deeper layers, this could lead to inaccurate conclusions. Depending on the task, a different biomass recording method may have to be used (5).

2.2 Methodology/Measuring

Adhesive tape samples (n = 37) were analysed by different test subjects under different microscopes. They used diverse image fields as well as magnifications. The samples were counted fully, partly, and minimally, and, in the end, up to 200 times. The test subjects could themselves choose to count either on the eyepiece measuring grid, camera window or the field of view. The only requirement was that they should count 3 cm length of the entire adhesive tape. The width of the measuring field was determined by the lens and measuring field. The extrapolated results per cm² with the respective microscope factor were compared. The influencing factors, especially the magnification and the counting window, can also be evaluated with these values. For reliable results, the counted part of the tape sample must cover a statistically representative area. The error related to a complete count decreases with a larger area.

The aim of evaluating an adhesive tape sample is to show the biomass status of a surface. For this reason, it is not relevant which part of the adhesive tape is counted. It is important that the heterogeneity of the surface coverage is sufficiently recorded. The result should reflect the biomass status of a wall/surface, not that of a subarea of the sample.

The samples were evaluated by two methods: the 3-LINE process and the 3-STAGE method. In the **3-LINE process**, the adhesive tape was counted in three horizontal rows, each having a length of over 3 cm. In the 3-STAGE process, the adhesive film was also counted in three 3 rows. However, with this method, the 3 cm was divided among the 3 rows (see Fig. 3).

A safe quantification of the biomass per cm² in the sample is achieved through a 3-LINE process. Depending on the microscope and magnification, it shows 30–45% of the reference surface. The statistical evaluation of the adhesive film preparations evaluated in the laboratories showed a high degree of reliability and reproducibility. This was especially so for the evaluation of surfaces, where the target values were below 500 spores per cm², for example, in the remediation control in sensitive areas or as described in the WTA leaflet on the evaluation of the cleaning of inventory.

A safe quantification regarding the classification of biomass according to UBA [6] is possible with the 3-STAGE method. Depending on the microscope and magnification, it shows 11–5% of the reference surface.

2.3 Cancellation criteria

For a fast analysis, especially in the 3-line process, cancellation criteria are defined. If the surface lines have a dense, equitable occupancy, the analysis can be cancelled after 2 lines. A third line improves the statistics but does not change the valuation of the sample.

It becomes difficult to define cancellation criteria for low and very low cell numbers. It can help to determine how much biomass can occur per measuring field, so that the target values are still achievable.

Samples with a very high cell count and/or a large number of other particles cannot be evaluated quantitatively. In this case, a semi-quantitative or qualitative analysis must be used, as described in Table 3, for instance.

3 Results

The evaluation of the analytical results has clearly shown how well the results of the individual test counters are consistent with each other and how well the values correspond with a fully counted sample. Furthermore, it was proved that even the simplified 3-stage method could achieve a high level of reproducibility. The statistics show that the methodology, as a whole, is more than 80% suitable. In the meantime, with the counting routines of the participating laboratories having been adjusted, it was possible to achieve a coefficient of determination R^2 of 0.9.

4 Discussion

It is important to have a high recovery rate when the adhesive tape samples have a low surface distribution, such as the remediation control of inventory or archaeological samples (4). The recovery rate for cell numbers below 500 spores per cm^2 is 70% in the LINE process. In the 3-STAGE method, the result is slightly lower at 65%.

The results will be comparable and highly accurate, if the same procedure is adopted for counting, regardless of the equipment used. Further, using a microscope of 100x magnitude or 60x is a matter of minor importance only. The most important factor is the reference basis of cm^2 . This is best achieved through looking at the linear extension of the sample. The measurement width depends on the device and determines, therefore, the calculation factor.

The 3-LINE method should be used for greater accuracy. Here, however, the user has the option of defining the termination criteria, if the result in the interpretation is unlikely to ensure any further accuracy. The 3-STEP method offers a fast and accurate analysis, especially for the determination of damages and the classification of the biomass according to UBA [6].

The user must decide which quality of the analysis is necessary, and this depends on the task. If the necessary quality cannot be achieved either by complete counting or by the methods presented, it might be necessary to choose a different analytical method.

The investigations have shown that the results are reliable and reproducible. The next aim would be to apply the LINE and STEP methods in practice. Therefore, a definition of the assessment criteria is required. The authors' experience of the past years had shown the significance of the difference between

case histories and removal control. Used in several research projects, the following assessment criteria were developed.

Removal control

Table 2 presents the recommended target value of mould contamination after a precision cleaning of indoor surfaces. As seen, the differentiation between porous surfaces like render or concrete and smooth surfaces like glass or metal yields two values regarding to the effect of the cleaning. There no need to differentiate between spores and hyphae, which are counting as one.

Table 2
Assessment criteria of precision cleaning according to (4)

	Smooth surface	Porous surface
	Spores (hits) pro cm ²	Spores (hits) pro cm ²
Target value	150	500

Case histories

The surface assessment in case histories differs to removal control by an accurate description of the microbial status to evaluate a damage and initiate counteractive measures. The recommended assessment standard is given in Table 3.

Table 3
Assessment criteria for indoor surfaces in case histories

Microbial Status	Spores /cm ²	Hyphae / cm ²
Normally contaminated surface (Background concentration)	< 1,000	< 150
Slightly increased contaminated (more than normally)	> 1,000–10,000	Single hyphae, no mycelia
Proliferation state Significantly contaminated, occurrence of colonization structures	> 10,000–60,000	Many, partly branched hyphae, small mycelia
Established Biomass Overgrown surface Heavily contaminated	> 60,000	Established fungal structures: mycelia, conidial heads, asci, secondary settlement

5 Decleration

Ethics approval and consent to participate

We confirm that any aspect of the work covered in this manuscript has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

No conflict of interest exists. We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Consent for publication

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Competing interests

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

We confirm that the manuscript has been read and approved by all named authors. We confirm that the order of authors listed in the manuscript has been approved by all named authors. Both authors were involved in the entire work.

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Not applicable

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Figures

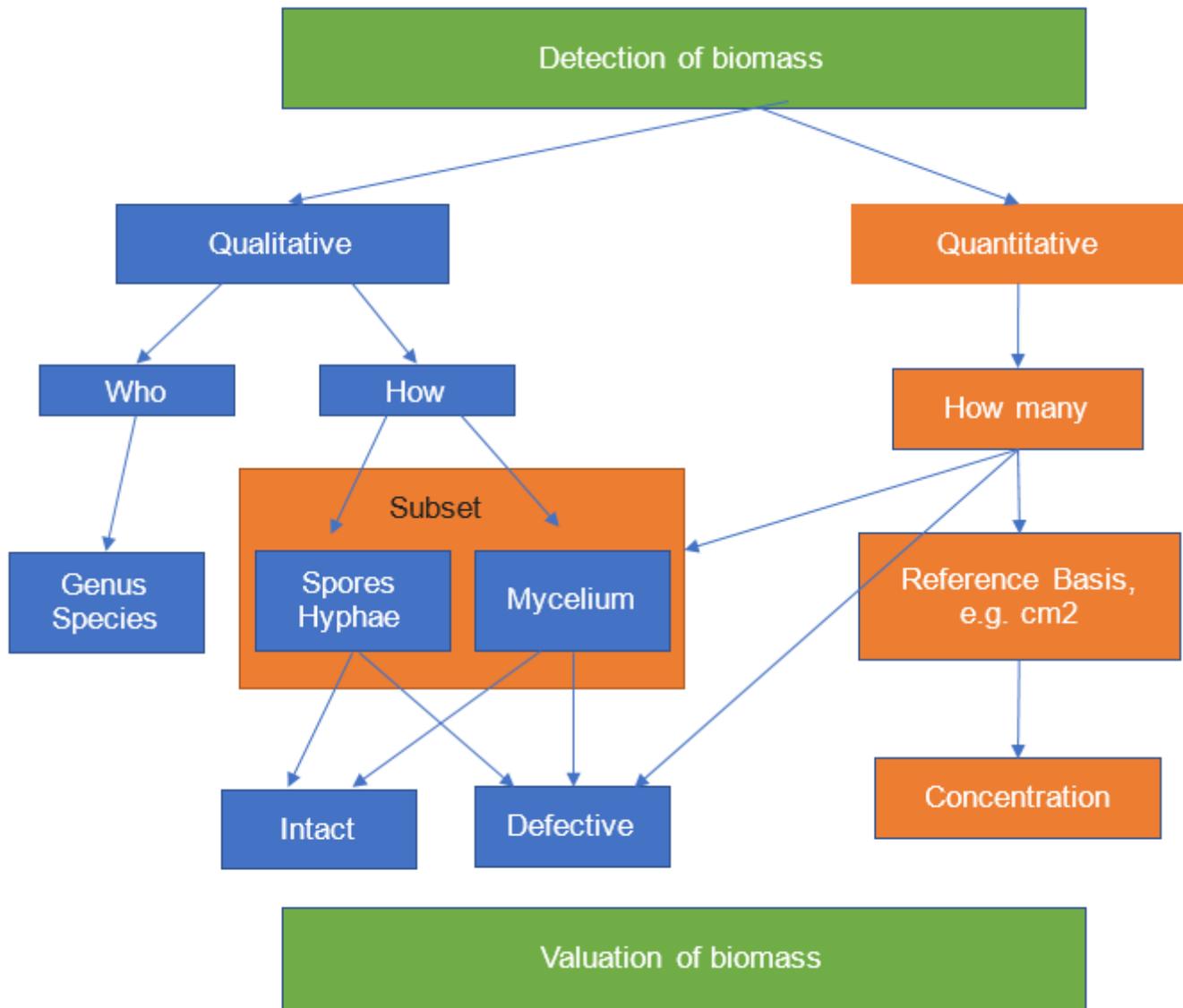


Figure 1

Definition of the task: Biomass can be recorded qualitatively and quantitatively. With an adhesive tape, the focus is more on the right side. Species and genera cannot always be determined safely.

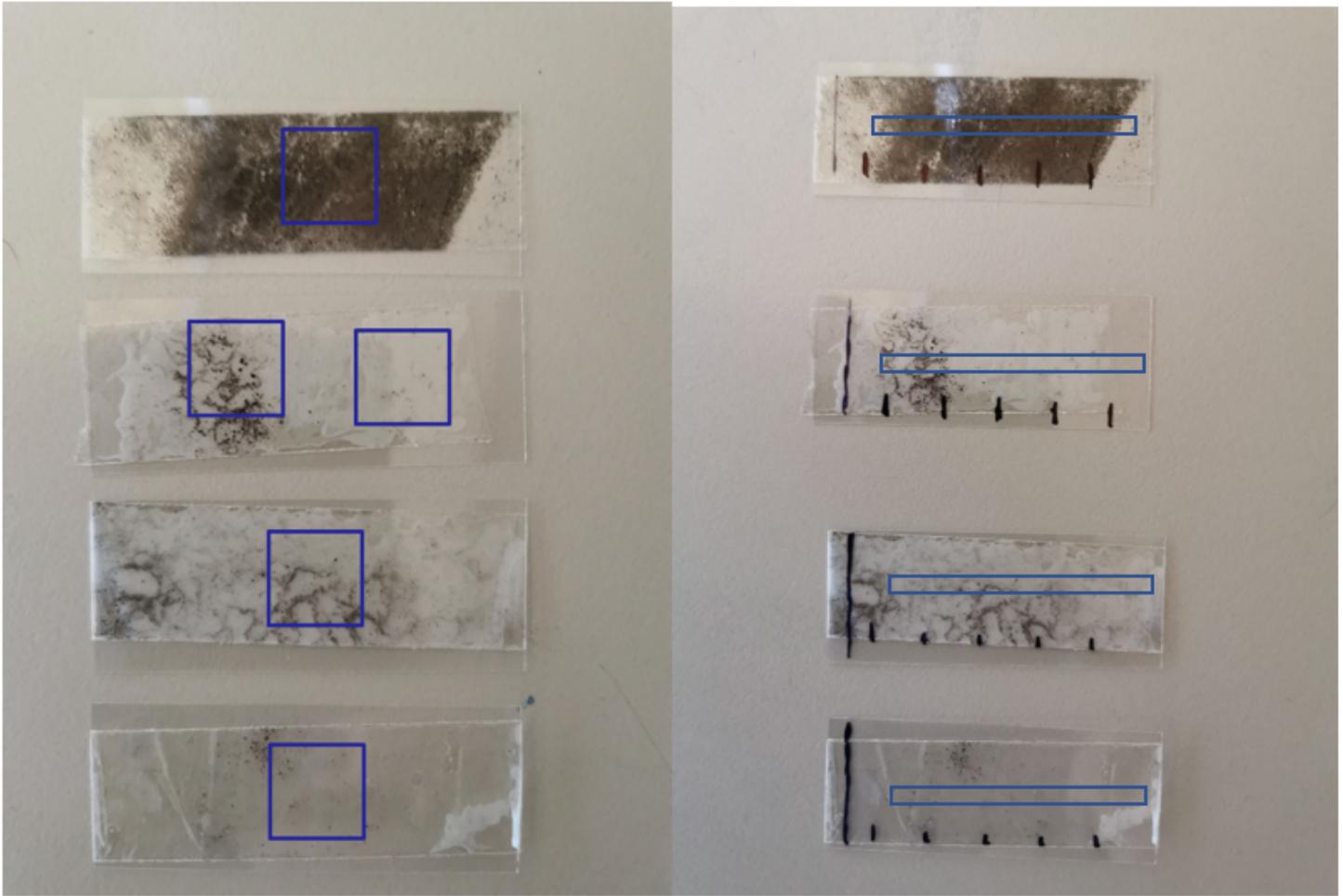


Figure 2

The measuring field determines the result. The result varies according to the position of the 'square centimetre' as a closed measuring window. However, if the surface is smeared on the entire adhesive film, the heterogeneity of the surface allocation is sufficiently recorded.

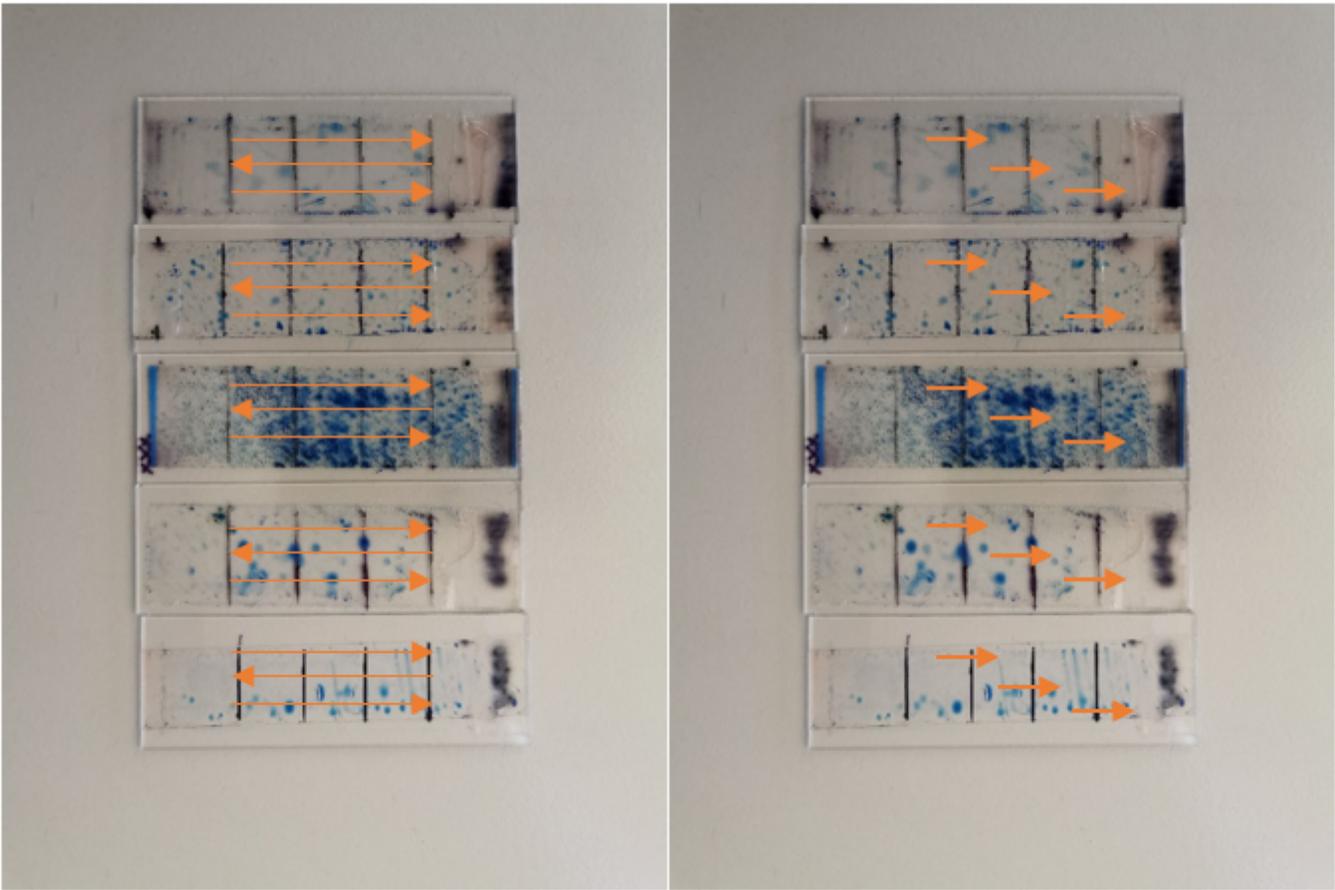


Figure 3

Counting of the adhesive films by the test counters 3 lines and in 3 stages

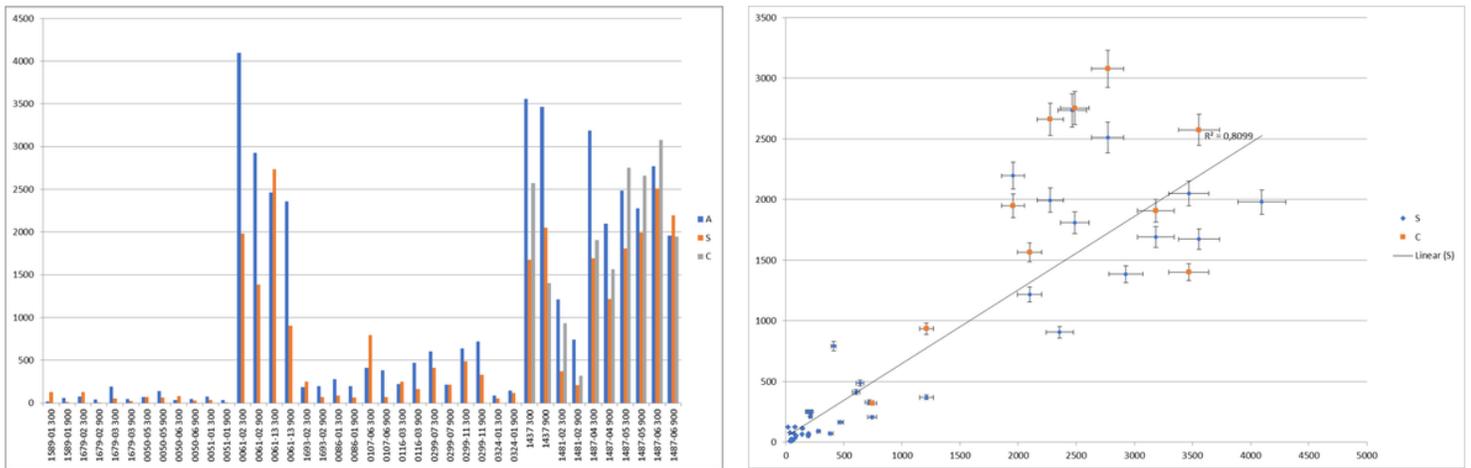


Figure 4

Comparison of the test series in 3-line process with 3 test subjects. The results show great reproducibility ($R^2 = 0.81$).

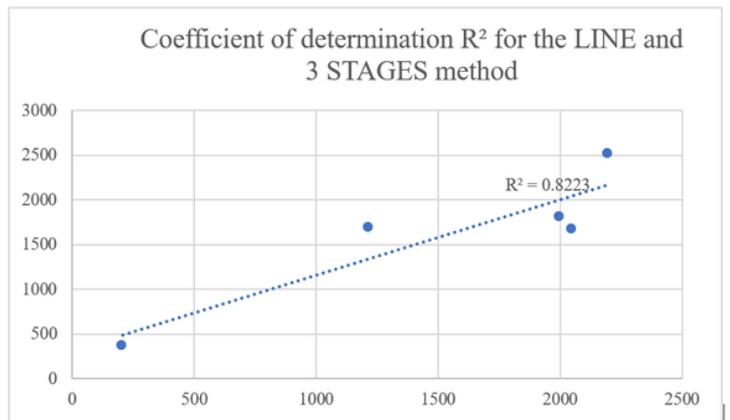
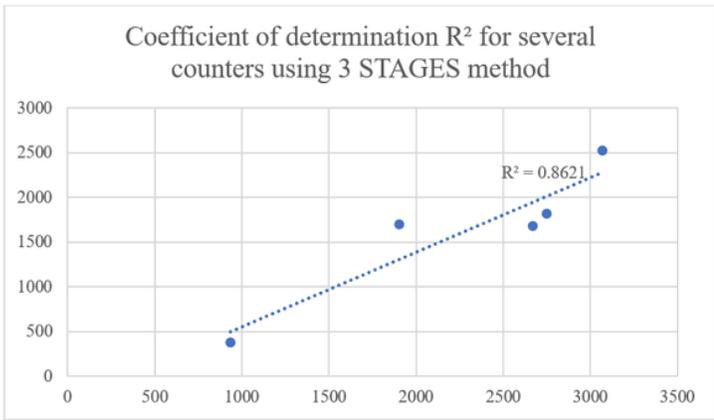


Figure 5

Comparison of the reproducibility of the results using the 3-line process compared to the 3-stage method with a grade of $R^2 = 0.82$; the quality of the counters in the 3-stage method shows a grade of $R^2 = 0.86$.