Food Hygiene

45. SAMPLING PLANS

State: 01. October 2007

• 45.1 Introduction

Modern quality assurance systems, including Hazard Analysis and Critical Control Point (HACCP), do not standardize the product but rather serve to standardize the production process. Nevertheless sampling for surveillance of microbiological criteria is not superfluous but has shifted to the level of verification instead.

Ideally the acceptance or rejection of a lot of food is decided by inspection of 100 % of the items. If the test is too slow and laborious or destroys the units, testing of representative samples has to be done instead. Only with an unrestricted random sampling plan a valid estimation of the characteristics of interest is garantueed.

Sampling plans are often drawn up "intuitively". This expression characterizes a procedure in which the sample size is negotiated chiefly from the point of view of what is economically acceptable between the parties involved; these parties then agree on plausible decision rules (e.g. acceptance numbers). Although mathematical/statistical points of view do not enter into it, one can work with plans of this kind and they may even be found worthwhile in practice. The fact, however, that pragmatic tests do not guarantee any transparent quality management, becomes evident at the latest when there are doubts about the accuracy and reliability of a decision. It often happens that the probability of false decision is not concretely established, not to mention being taken into account in the construction. The weakness of intuitive test plans is given by the fact that basic strategy questions are ignored.

- **Composing a sampling plan**

• 45.2 Sampling and sample Preparation

• 45.2.1 Sampling

Prerequisite: all units of the population or lot should be registered and available for sampling

- **Ideal random sampling:** casting dice
  pulling tickets
  random number tables
- **Practical solution:** systematic sampling with a random starting point
- **Procedures**
  - **Unitary (one-stage) procedure**
    Sometimes further division into subsamples, clusters, strata or phases
  - **Two-stage or two-step strategy**
    This strategy means that a second sampling must occur after an indifferent result in the first sampling to produce a clear acceptance or rejection result.
  - **Multiple stage strategy**
    The sample size is not established before the test begins. There are many sample units and one of three statements are made after any single analysis, that is, "accept", "reject" or "test again".
    Lots that deviate markedly from the limit, that is, particularly good and particularly bad lots, can be quickly detected with this technique, if the single analysis does not need much time.

• 45.2.2 Sample Preparations

Sample collection, identification, shipment and preparation should follow the well-known rules. For microbiological analysis the test material must be carefully homogenized to minimize sampling error.

- **Laboratory Sample** — **means a representative aliquot of one homogenized sample which is used for the analytical procedure**

- **Bulk (Gross) Sample** — **means to combine all sample units of a lot into a composite sample. Only an aliquot from the bulk composite sample is tested instead of analysing each sample separately.**
  One negative aspect of bulk sample is that all information regarding the variability of the test characteristic is lost because only one result (= realized arithmetic mean) is obtained and this has also been proven to be especially susceptible to outliers.

- **Pooled Sample** — **means to combine the sample units into one composite sample and to analyze the entire composite sample. This procedure is useful with presence/absence tests where the random sample size is n > 1 and zero tolerance exists. This sample treatment is ideal for *Salmonella* testing where samples are combined and (pre-) enriched as a composite sample in a large container. A positive pool sample leads to rejection of the entire lot just as a single (or several) positive individual samples would be done.**

• 45.3 Principles for calculating the sample size

Irrespective the user knows biometrics or not, each sampling plan has a statistical foundation that is specific to itself. This basis is made up of the following four components which affect the level of the sample size required and also the acceptance number:

- **(1) Reliability means the probability of a correct decision at a given level of stringency, that denotes rejection of 'bad' lots and acceptance of 'good' lots. If a population with fewer defects than are normally tolerated is falsely rejected on the basis of a sampling result it is called producer's risk (type 1 error, α), where as the consumer's risk (type 2 error, β) relates to a case where a 'bad' lot is wrongly accepted.**

- **(2) Stringency (discriminating power, critical difference) in quality assurance means the degree of exceeding (microbiological) limits that can be detected with a given degree of probability. Smaller differences often go unnoticed. As which reliability, the extent of testing is positively correlated with stringency requirements.**

- **(3) Variability means the homogeneity of a lot. In contrast to (1) and (2) the variance cannot be prefixed. Dispersion is inherently related to the characteristic (= distinguishing feature) itself.**

- **(3a) Qualitative (alternative, discontinuous or discrete) characteristics generally manifest in the opposites good versus bad or present versus absent. They usually follow the Poisson distribution or binomial distribution. The variance in this case is determined by correlating good and bad units. This can be calculated as a direct mathematical relationship between variance and probability of occurrence. If one estimates the number of acceptable units in a random sample, the corresponding variance can be derived.**

  Occasionally there are also contagious alternative distributions with increased variation (e.g. negative binomial distribution) for microbiological criteria. However, the sampling plans were not modified, because the clumping factor often only affects the reliability (raised consumer's risk).

- **(3b) Quantitative (continuous) characteristics may be of any possible value in a defined distance. Almost all analytic data belong to this group, including bacterial counts in food samples. Such data points usually follow a normal distribution, al least after logarithmic (or square root) transformation. One of the characteristics of normal distributions is that no relationship exists at all between the mean and variance!**

  In order to construct an appropriate testing plan, the variation must be captured and determined independently of the average.

  It is
  - ***either estimated simultaneous with the mean directly from random samples (for estimation an additional multiplicator from t-distribution is needed)***
  - ***or is known from preliminary trials - assuming sufficient information is available.***

- **(4) The importance of the relationship between sample size *n and population size N* is often overestimated. There are no interactions with reliability and accuracy in the extremely frequent cases where the relationship *n/N* < 0.1. Precision and stringency depend only on the sample numbers.**
  In the case of alternative characteristics and *n/N* > 0.1 the binomial distribution changes to the hypergeometric distribution.

- **discussion / conclusion**
- **In case of *n/N* < 0.1 constant sampling fraction means that lots of smaller size are tested with a smaller margin of safety – a philosophy which is no longer followed within modern quality control.**

- **In microbiological quality assurance infinitely large basic populations are present de facto and the required sample size (number of samples) is therefore derived from a combination of the factors power, reliability and variability (standard deviation). This interaction is expressed in the following formula:**

$$\sqrt{\text{sample size}} = \text{reliability} \times \text{stringency} \times \text{variance}$$

- **discussion / conclusion**
- **Even plans in which the level of examination is high cannot be accurate and safe at the same time, in fact the two parameters are inversely proportional. To achieve a realistic level of testing a compromise between the two requirements must be reached. There are no panoptimal plans nor is there a universal standard plan for all control situations.**
  Shifman and Kronick (1963) realized that: "Many administrators hope that they will be able to solve those problems by some formula which has universal applicability. This, of course, is a delusion ..."
- **Unless the material being examined is very homogeneous, small sample sizes lead to imprecise and uncertain decisions. The single sample is the least informative test unit . In contrast, the increase in information obtained from more than five samples ($n = 5$) is slight because of the square root function. Therefore, the addition of more random samples with complicated analysis is often not worth the effort, even for heterogeneous foods, especially when it involves a quantitative characteristic.**

- **Failed start of a statistical consultation**

- **Failed final of a statistical consultation**

• 45.4 Example for interaction of sample size, reliability and stringency

- **Even plans where there is a high level of examination cannot be both reliable and stringent. For a given sample number, the two criteria are inversely proportional as show in the table.**

Table

Minimum number of sample units required for evaluating infinitely large lots based on the number of tolerated percentage of bad units in the lot (reject quality level or RQL) as well as type 2 error* in case of a presence absence test

RQL [%]	1 – β [%]		
	95	**99**	**99.9**
25	11	17	25
10	29	44	**66**
5	**59**	90	135
1	**299**	459	688
0.5	598	919	1379
0.2	1497	2301	3451
0.1	2995	4603	6905

*** Type 2 error [1 – β (in %)] is the reliability with which a bad lot should be rejected; rejection number d ≥ 1.**

- **discussion / conclusion**
- **In the table the minimum number of sample units are shown where a bad lot can be detected under prevailing conditions such that at least one sample unit of the entire random sample yields a positive result (acceptance number *c* = 0, rejection number d ≥ 1). Attention should be paid to the sample size *n* = 60 and *n* = 300. These ensure that, for RQLs of > 5 % and > 1 % respectively, the probability of (false) acceptance does not exceed 5 %. If the prefixed probability of (false) acceptance is lowered to ≤ 0.1 % and *n* = 66 sample units are drawn, a RLQ > 10 % is given.**

• 45.5 Unitary (one-step) Attributive Two-class Sampling Plans

The typical model of acceptance sampling runs as follows:

Lot → sample → sampling plan → decision → accept/reject lot

The simplest concept to control an alternative characteristic (compare 45.3) is to determine the number of random samples required (*n*), and then to fix how many sample units at most may be defect (e.g. positive for *Salmonella*) without rejecting the lot (acceptance number *c*).

It is possible to avoid the estimation of variation for quantitative data by transferring e.g. the number of colony-forming-units/g into attributive data. Such a transformation is achieved in two steps:

first, a contamination-limit *m* is fixed;
then, as a second step, the transformation itself is carried out by assigning all test results to groups according to their being above or below the previously determined limit.

The result of this procedure is an attributive +/− structure of the characteristic being analyzed.

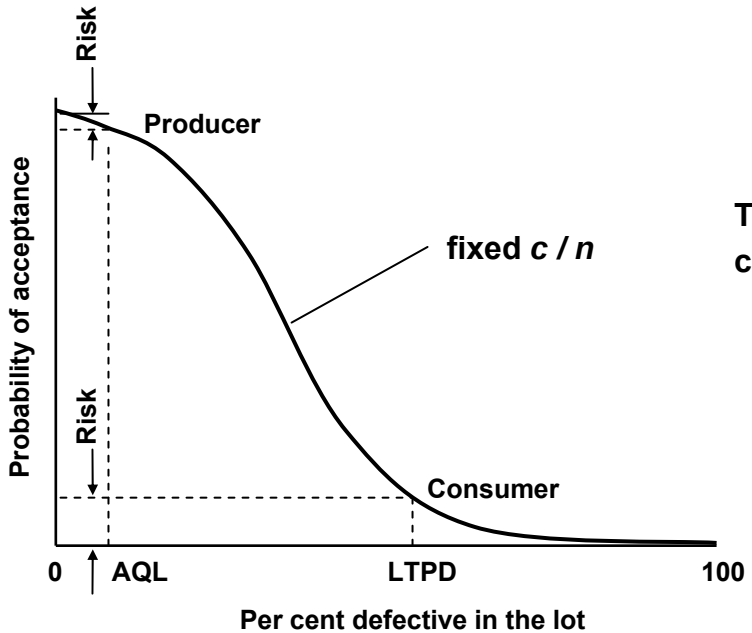
Attributive two-class plan

Number of samples examined	Acceptance number	Microbiological Limit in Case of microbiological counts
n	***C*** **≥ 0**	***m***

• 45.6 OC-Function for an attributive two-class-sampling plan

Principles

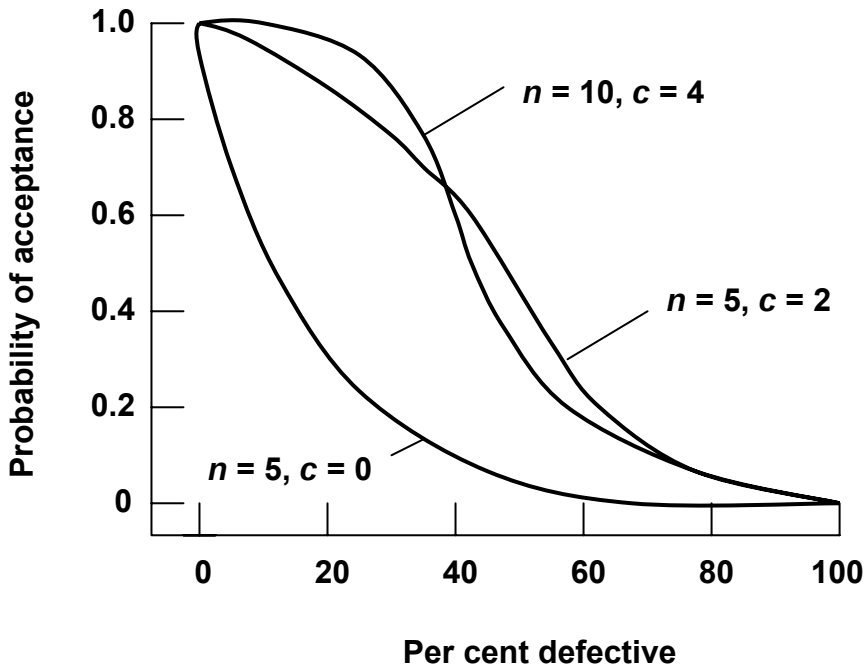
Only with the aid of a relevant OC-function (Operation characteristic) the user can imagine how a sampling plan works. The OC-function of a specified sampling plan shows the probability of acceptance as a function of per cent defective (0-100%) in the lot.



The OC-function is the identity card of a certain sampling plan

Figure: Acceptance curve (operating characteristic or OC-function) showing producer's and consumer's risk correlated with Acceptable Quality Level (AQL or H_0) and Lot Tolerance Per cent Defectives (LTPD or H_1 or RQL). The AQL is the point on the horizontal axis measured from an OC curve such that a lot with that per cent of defectives has high probability (e.g. 99%) of acceptance. This is also referred to as the producer's risk or low probability (e.g. 1%) that a good lot will be rejected. Similarly, the LTPD is referred to as the consumer's risk or the quality level at which a poor lot has a low probability (e.g. 5%) of being accepted.
In statistical terms AQL relates to H_0 and LTPD to H_1

- **Examples**



Operating Characteristic (OC) functions for three sampling plans with two different sample sizes and two different ratios of *c* to *n*.

• 45.7 Unitary Attributive Three-class Sampling Plans

• Introduction

The transformation of a quantitative into a qualitative criterion lowers the level of information considerably, because neither the actual amount of variation within the lot can be derived, nor how far away the single results are from the limit. In order not to lose the information contained in the cfu numbers or other quantitative data – as happens when applying the two-class plan – the three-class plan was developed and promoted.

• Design

Concept of the attributive two- and three-class plan

	Number of Samples Examined (*n*)	Microbiological Limit	Acceptance Number (*c*)
Two-class plan	***n* = 5**	***m***	$c_m \geq 1$
Additional limit		***M***	$c_M = 0$
Three-class plan	***n* = 5**	***m***	$c_m \geq 1$
		M	$c_M = 0$

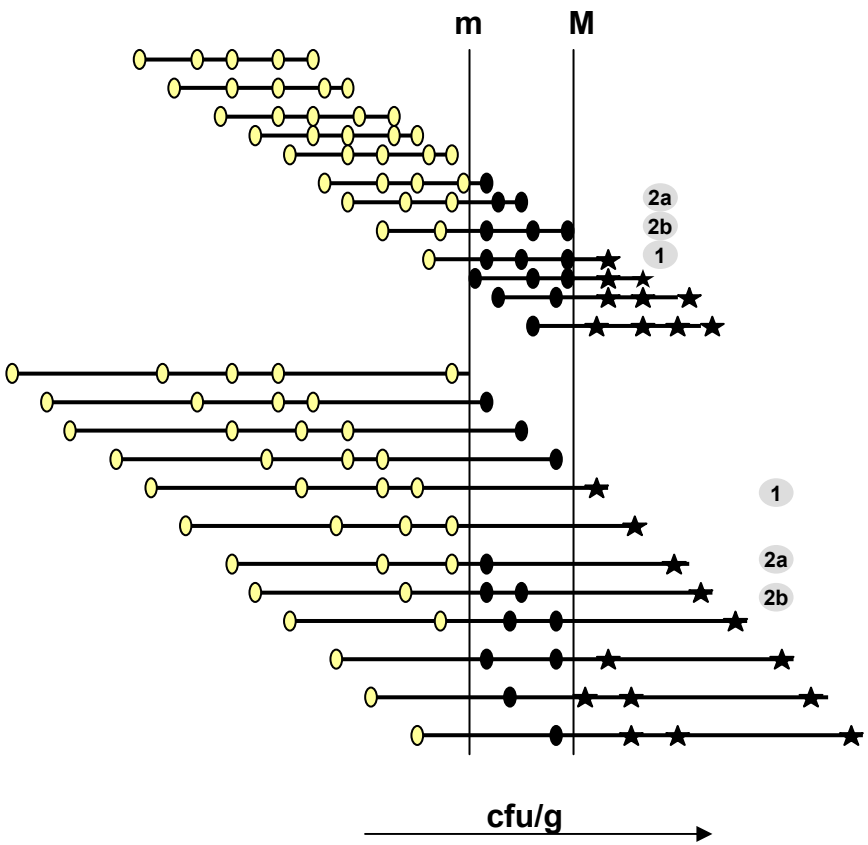
- **Remarks**
  - ***n* = 5 is common but not mandatory**
  - ***m* is equivalent to the upper limit of a good manufacturing practice (GMP).**
  - ***M* marks the borderline beyond which the quality is no longer acceptable.**
  - **There are several approaches to choosing the value of *M***
    1. **as a utility index**
    2. **as a general hygiene indicator**
    3. **as a health hazard**
  - **Because c_M for *M* is generally 0, in most three-class plans only c_m is mentioned (given as *c*).**

- **discussion / conclusion**
- **In the three-class sampling plan where *n* = 5, c_m = 2 and c_M = 0, it was demonstrated that the additional risk of rejecting a lot with an acceptable, technological unavoidable standard deviation σ (in log units) solely due to a single sample lying above *M* is reasonable, if the difference between *M* and *m* does not fall within the distance 1.84 x σ. Results of surveys indicate that the usually chosen distances (*M* – *m*) of 0.5 log units for recently homogenized foods and 1.0 log units for material with heterogeneous distributed microorganisms fulfil this condition.**

- **Example for a three-class plan: total aerobic plate count of minced meat**

Variable		Example	Definition
n	=	5	Number of units examined
m	=	5 x 10^5 CFU/g	Limit above which maximally c samples are tolerated
c_m	=	2	Maximum accepted number of results above m (acceptance number)
M	=	5 x 10^6 CFU/g	Limit that is unacceptable for any sample. If any sample result exceeds M, that lot is rejected (c_M = 0)

- **Operating mode of the attributive three-class plan in the case of 5 samples with little (above) and large (below) variance and a continuously increasing mean value**



Rejection because

1 $x_5 > M$ (c = 0)

2a $x_4 , x_5 > m$ (c = 1)

2b $x_3 , x_4 , x_5 > m$ (c = 2)

- **discussion / conclusion**
- **With increasing variance and/or closer distance between *m* and *M* lots will be more often rejected because a single sample unit exceeds *M* (c_M = 0) than c_m + 1 samples exceed m.**

• 45.10 Microbiological Criterion

Microbiological Criterion means a statement that defines acceptability of a food product or lot of food. It is applied to individual lots or consignments of food.

Components:

- **– Food of concern**
- **– Microorganism of concern and/or their toxins/metabolites**
- **– Analytical method**
- **– Analytical unit**
- **– Sampling plan (microbiological limits m/M; c/n)**
- **– Production step where the criterion is applied (Reg. (EC) 2073)**
- **– Corrective measures when failing the hygiene criteria (Reg. (EC) 2073**

- **Microbiological criteria for use in lot acceptance determinations fall into three categories:**

  - **– Microbiological Standard means a mandatory criterion that is incorporated into a law or ordinance.**
  - **– Microbiological Guideline means an advisory criterion used to inform food operators and others of the microbial content that can be expected in a food when best practices are applied.**
  - **– Microbiological Specification means a part of a purchasing agreement between a buyer and a supplier of a food; such criteria may be mandatory or advisory according to use.**

- **discussion / conclusion**
- **In contrary to the microbiological limit of a microbiological criterion a FSO is only characterized by one component, the maximum frequency or concentration of a microbiological hazard. On the basis of a FSO a microbiological criterion can be established.**

• 45.11 Choosing Sampling Plans

45.11.1 Introduction

There is no mathematical rule which leads from a distinct hazard or a certain hygiene parameter automatically to an appropriate sampling plan. For finding a suitable plan reliability and stringency must be fixed in advance. But these two parameters are mathematical values too which are not directly connected with the risk of concern. At the end every selection of a sampling plan is an arbitrary act.
Never the less the absolute size must be fixed voluntary, there should be a correlation between the seventy of food hazards and the affiliated sample sizes.
Beside of the risk the number of samples depends on other factors like economic demands or analytical uncertainties.

- **Risk based sampling plans should regard**
  - **type and extent of the hazard based on the target organism as most important aspect**
  - **probability of contamination of the raw materials**
  - **post-harvest process technology**
  - **type of treatment which the product usually receives from the consumer**
  - **consumption of the product by groups with lowered immune resistance (young, old, pregnant, immunosuppressive: YOPI).**

• 45.11.2 FOSTER-Plan

One of the most popular proposals for choosing acceptance plans for *Salmonella*-testing of food with different hazard characteristics is know as FOSTER-Plan

---

FOSTER-Plan (target: *Salmonella*; Sample unit 25g/ml)

Category I	**Non-sterile food for children, old people and the sick**
	n* = 60; *c* = 0 or *n* = 95; *c* = 1
Category II	**Food with all three hazard characteristics - sensitive ingredient, no destructive step during manufacture, likelihood of growth if abused**
	***n* = 30; *c* = 0 *or* *n* = 48; *c* = 1**
Category III	**Food with two hazard characteristics**
	***n* = 15; *c* = 0 or *n* = 24; *c* = 1**
Category IV	**Food with one hazard characteristic; does not usually need to be controlled**
	***n* = 15; *c* = 0 or *n* = 24; *c* = 1**
Category V	**Food with no hazard characteristic; does not usually need to be controlled**
	***n* = 15; *c* = 0 or *n* = 24; *c* = 1**

*** A sampling plan with an acceptance number of *c* = 1 is no longer tolerated in the case of Salmonella testing**

• 45.11.3 Sampling Plans for Combinations (called "cases") of Degrees of Health Concern and Conditions of Use

ICMSF-SAMPLING PLANS	Conditions in which food is expected to be handled and consumed after sampling in the usual course of events*		
Degree of concern relative to utility and health hazard	Conditions reduce degree of concern	Conditions cause no change in concern	Conditions may increase concern
Utility; general contamination, reduced shelf-life, incipient spoilage	Increase shelf-life Case 1 Three-class *n* = 5, *c* = 3	No change Case 2 Three-class *n* = 5, *c* = 2	Reduce shelf-life Case 3 Three-class *n* = 5, *c* = 1
Indicator; Low, indirect hazard	Reduce hazard Case 4 Three-class *n* = 5, *c* = 3	No change Case 5 Three-class *n* = 5, *c* = 2	Increase hazard Case 6 Three-class *n* = 5, *c* = 1
Moderate hazard: direct, limited spread	Case 7 Three-class *n* = 5, *c* = 2	Case 8 Three-class *n* = 5, *c* = 1	Case 9 Three-class *n* = 10, *c* = 1
Serious hazard; incapacitating but not usually life threatening, sequelae are rare, moderate duration	Case 10 Two-class *n* = 5, *c* = 0	Case 11 Two-class *n* = 10, *c* = 0	Case 12 Two-class *n* = 20, *c* = 0
Severe hazard; for (a) the general population or (b) restricted populations, causing life threatening or substantial chronic sequelae or illness of long duration	Case 13 Two-class *n* = 15, *c* = 0	Case 14 Two-class *n* = 30, *c* = 0	Case 15 Two-class *n* = 60, *c* = 0

* More stringent sampling plans would generally be used for sensitive foods destined for susceptible populations.

- **Cases and ICMSF-Sampling Plan Performance, Assuming a Standard Deviation of log 0.8. Lots having the calculated mean concentrations or greater will be rejected with at least 95%probability**

Cases, sampling plans and calculation of their performance

Case 4 (three-class, *n* = 5, *c* = 3) e.g. *m* = 1000/g, *M* = 10 000/g **Mean conc. = 5128/g**	**Case 5** (three-class, *n* = 5, *c* = 2) e.g. *m* = 1000/g, *M* = 10 000/g **Mean conc. = 3311/g**	**Case 6** (three-class, *n* = 5, *c* = 1) e.g. *m* = 1000/g, *M* = 10 000/g **Mean conc. = 1819/g**
Case 7 (three-class, *n* = 5, *c* = 2) e.g. *m* = 1000/g, *M* = 10 000/g **Mean conc. = 3311/g**	**Case 8** (three-class, *n* = 5, *c* = 1) e.g. *m* = 1000/g, *M* = 10 000/g **Mean conc. = 1819/g**	**Case 9** (three-class, *n* = 10, *c* = 1) e.g. *m* = 1000/g, *M* = 10 000/g **Mean conc. = 1575/g**
Case 10 (two-class, *n* = 5, *c* = 0) e.g. *m* = 0/25g **Mean conc. = 32/1000g (1cfu/32g)**	**Case 11** (two-class, *n* = 10, *c* = 0) e.g. *m* = 0/25g **Mean conc. = 12/1000g (1cfu/83g)**	**Case 12** (two-class, *n* = 20, *c* = 0) e.g. *m* = 0/25g **Mean conc. = 5.4/1000g (1cfu/185g)**
Case 13 (two-class, *n* = 15, *c* = 0) e.g. *m* = 0/25g **Mean conc. = 7.4/1000g (1cfu/135g)**	**Case 14** (two-class, *n* = 30, *c* = 0) e.g. *m* = 0/25g **Mean conc. = 3.6/1000g (1cfu/278g)**	**Case 15** (two-class, *n* = 60, *c* = 0) e.g. *m* = 0/25g **Mean conc. = 1.9/1000g (1cfu/526g)**

Because farm animals are not able to give informations about the statistical parameters of their products the food science expert has to do this biometric work.

• 45.12 Control Charts

• Continuous Sampling

Random sampling plans serve to evaluate uniform, discrete, defined lots. The information from a previous test does not influence the next decision. Such acceptance sampling plans for quality control of lots can also in principle be used for the continuous control of production stages. However, they represent a procedure which is more passive than active and with which the essential goals of quality control cannot be reconciled.

• The continuous control strategy – as demanded by Reg. (EC) 2073, Article 9 - should:

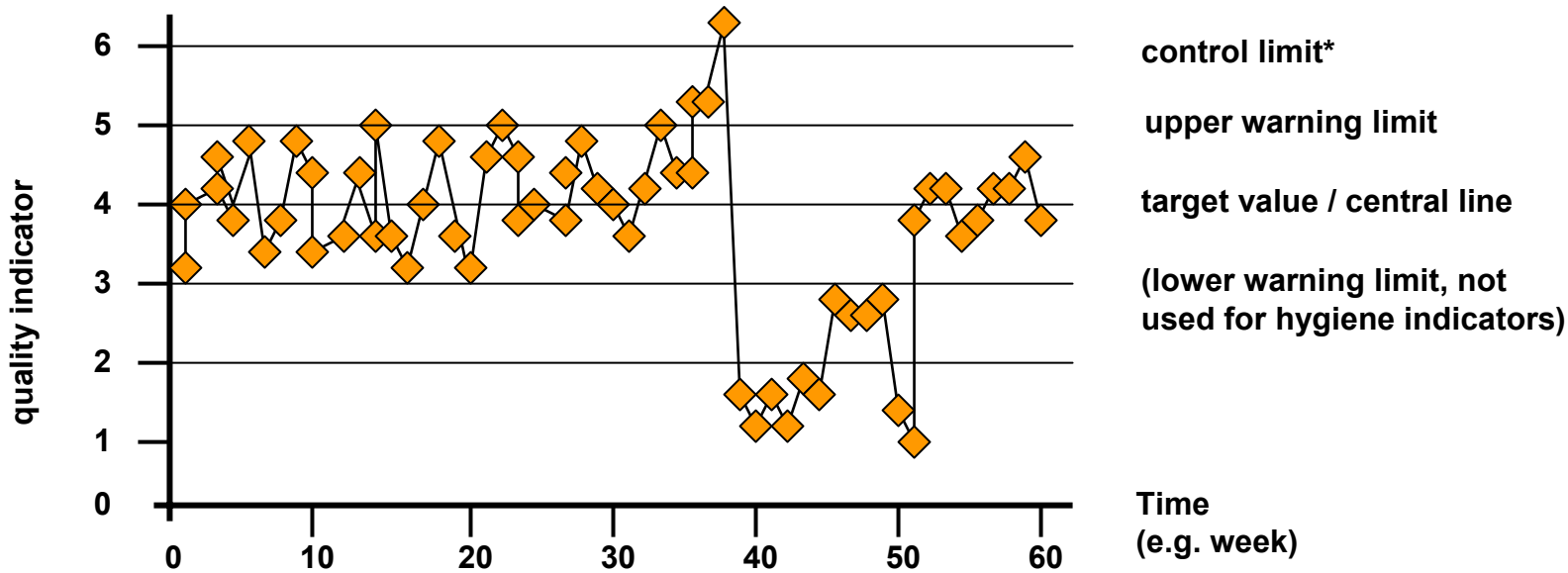
- **Yield information about the characteristics of the process, especially average quality, as well as unavoidable variations**
- **Maintain proper production as long as possible**
- **Clearly show deviations from the quality standard so that production of faulty units is recognised early.**
- **The task of lot testing, namely to accept or reject certain production units, becomes of less significance.**

A CONTROL CHART is employed

for continuous evaluation of quality

to determine the agreement between the fixed standard and the reality of practice. The essential characteristic of a control chart is that it forms a <u>continuous graphic representation</u> of the quality status of production under consideration of prescribed tolerances.

- **Example: variables control chart**



*** Results above control limit indicate that the process is out of control**

- **Types of Control Charts**

1. Control Charts: decision about corrective action after every single sample or sample unit

1.1 Attribute Control Charts (presence/absence assays) assume that a process or system under control has some fraction of defective samples which should not be exceeded ($\bar{p}$-charts)

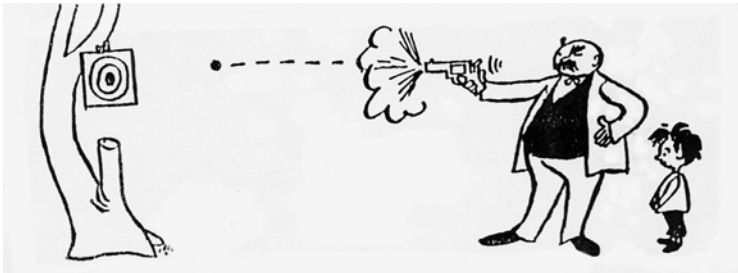
1.12 Variable Control Charts may contain

- **single individual data**
- **statistical parameters of subsamples as**
  - **median**
  - **arithmetic mean (SHEWART-chart)**
  - **standard deviation**
  - **range**
  - **range and arithmetic mean ($\bar{X}$R-chart)**

2. CUSUM Charts: Cumulative Sum Charts plot the single data or means in a cumulative fashion

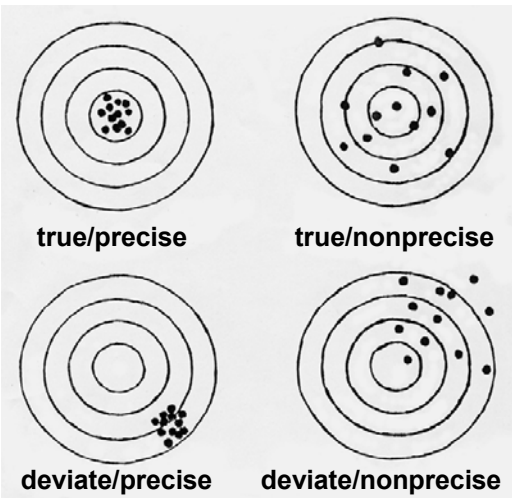
3. MOSUM Charts: Moving Sum Charts plot the single data or means of the last *n* subunits in a cumulative fashion

• 45.13 Accuracy of quantitative microbiological results



Accuracy = trueness + precision
Genauigkeit = Richtigkeit + Präzision
Justesse = justesse de la moyenne + fidélité

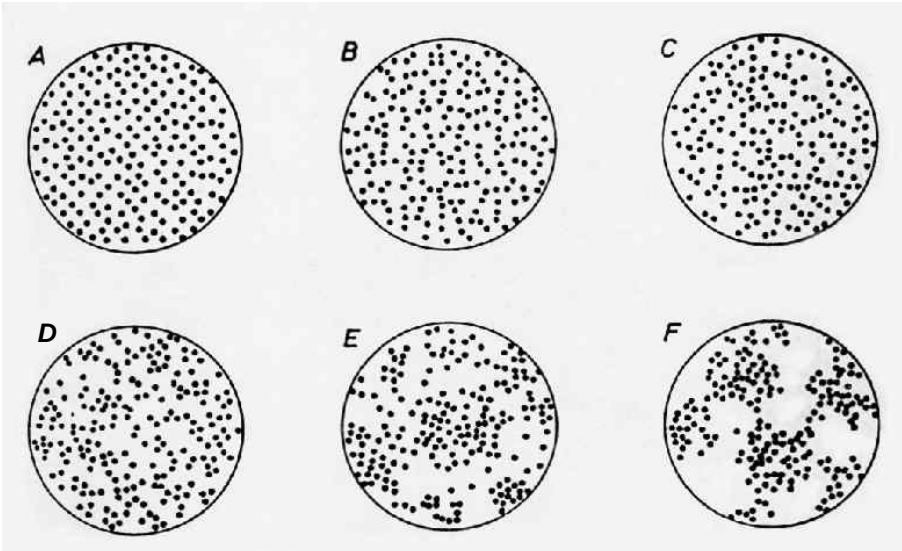
•



- **Repeatability (Wiederholbarkeit) means precision of analytical data obtained under identical conditions**
- **Reproducibility (Vergleichbarkeit) means precision of analytical data obtained under different conditions (analyst, laboratory, apparatus)**

• 45.14 Precision: Random distribution

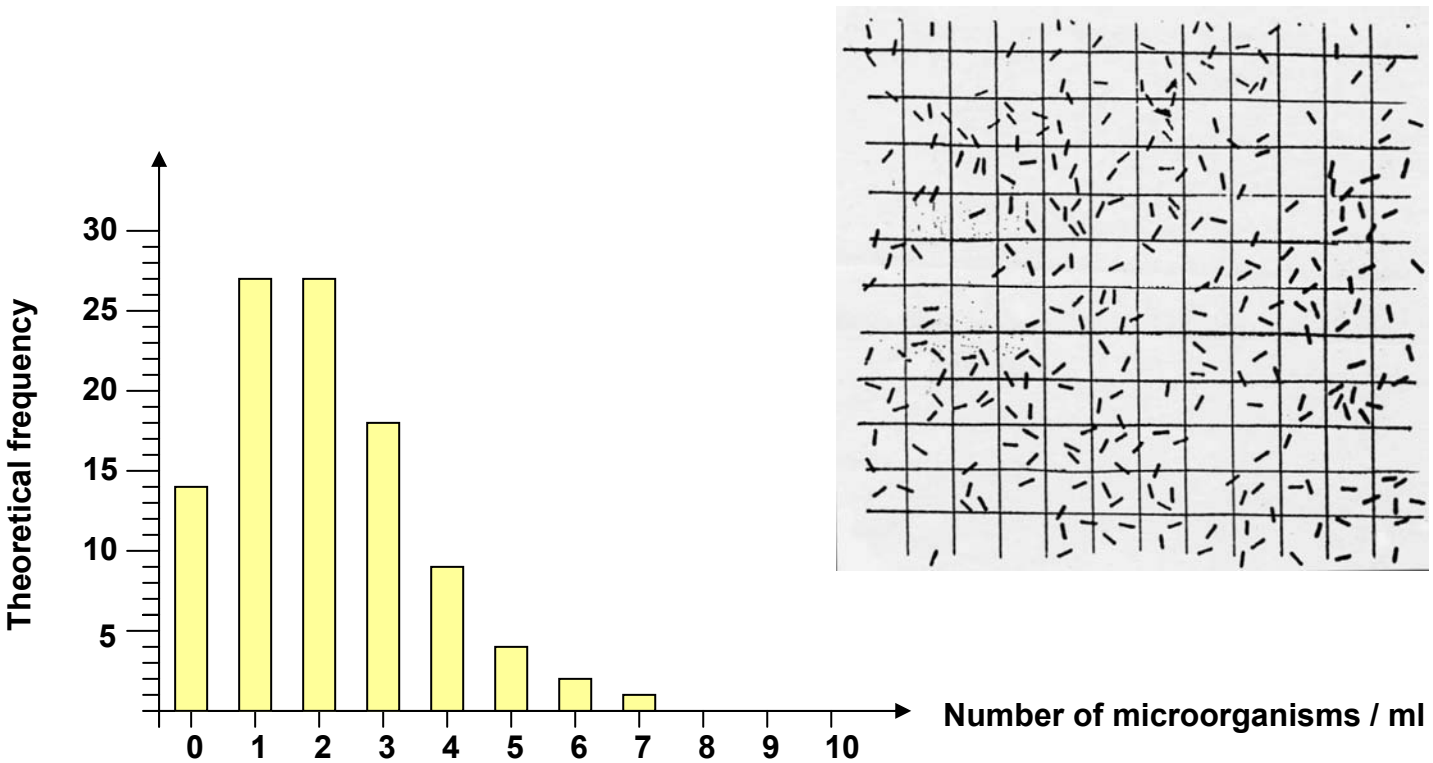
- **Which picture shows a random distribution of microorganisms in a homogenized sample?**



- **discussion / conclusion**
- **A - C show regular distributions**
  D - E show random distributions
  F shows a contagious distribution

- **Precision: <u>Poisson distribution</u>**

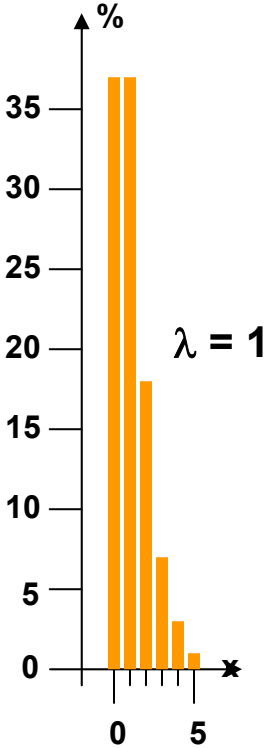
Random distribution of microbial counts for a given density of 2 microorganisms / ml

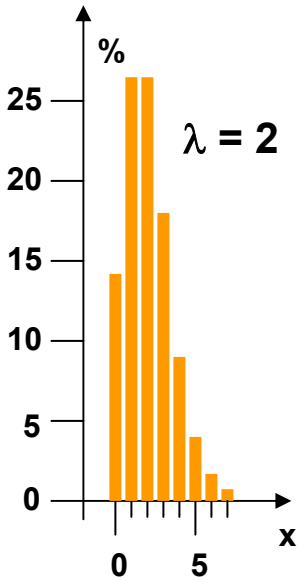


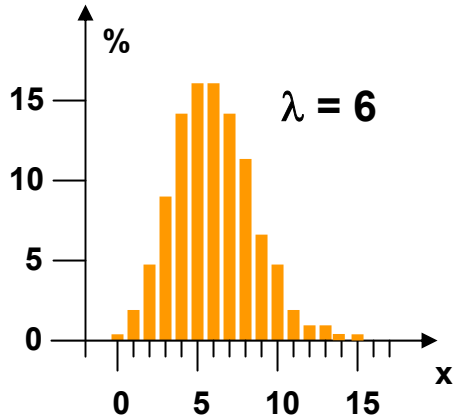
- **Poisson distributions with different λ**

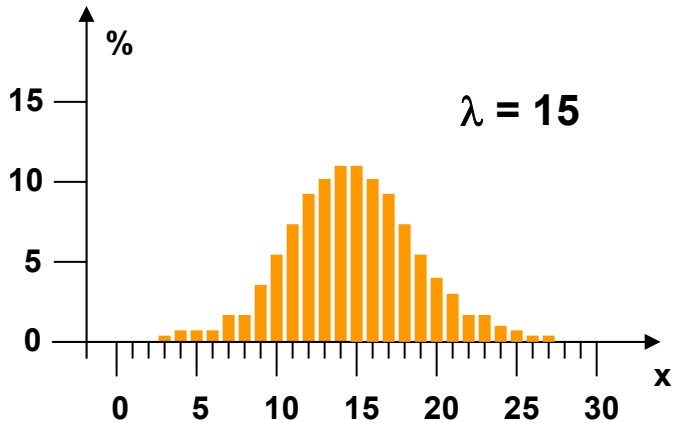
- **Poisson-distribution: $\lambda = \mu = \sigma^2$**

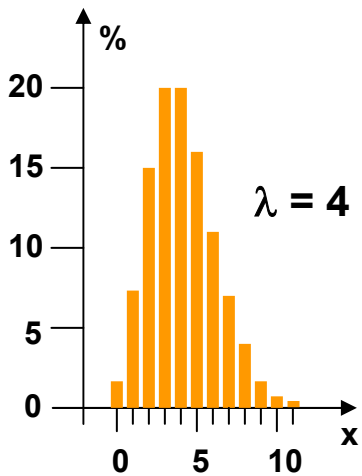
  λ = Poisson factor
  μ = real mean
  σ = real variance

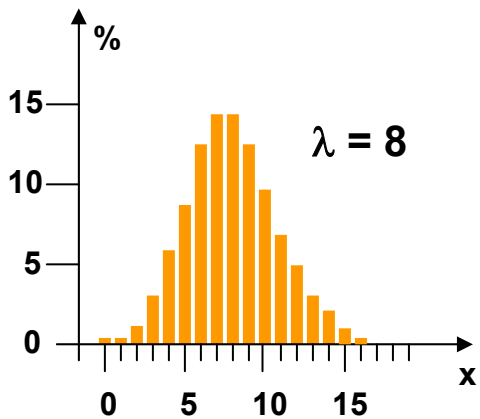


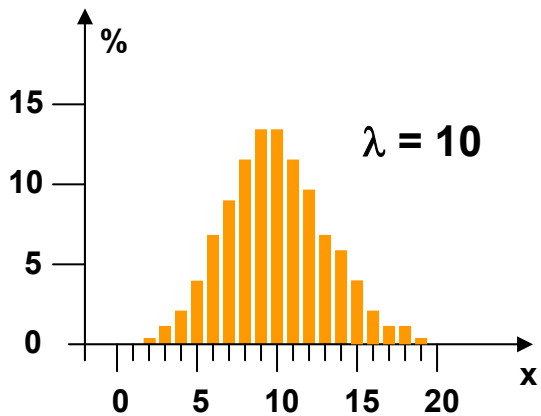












- **Monte Carlo Study for calculating the arithmetic mean $\bar{x}$ and the standard deviation s as well as repeatability r and reproducibility R of Poisson distributed data**

- **1. $\lambda = 25$**

42	21	20	26	20	24	24	19	28	23	31	21	21	25	18	22	18	27
26	20	20	28	30	31	24	30	24	31	13	20	31	26	22	25	21	21
33	23	22	15	32	26	32	26	16	24	25	27	30		n = 50			

- **2. $\lambda = 250$**

241	233	239	245	260	266	254	260	252	224	246	253	248	272
268	253	280	235	227	207	255	230	257	256	252	269	246	239
243	237	244	225	247	255	250	255	282	229	252	255	271	266
259	239	242	249	247	244						n = 50		

- **1. Monte Carlo simulation with $\lambda = 25$**

using original data: $\bar{x} = 24.6$; $S = \pm 5.4$; $s/\bar{x} = 22{,}0\ \%$

log – transformation: $\bar{x}_{(\log 10)} = 1.38$; $S_{(\log 10)} = 0.10$

$$R = r = 2.83 \times s_{(\log 10)} = 0.283$$

- **2. Monte Carlo simulation with $\lambda = 250$**

using original data: $\bar{x} = 248.9$; $S = \pm 14.6$; $s/\bar{x} = 5{,}9\ \%$

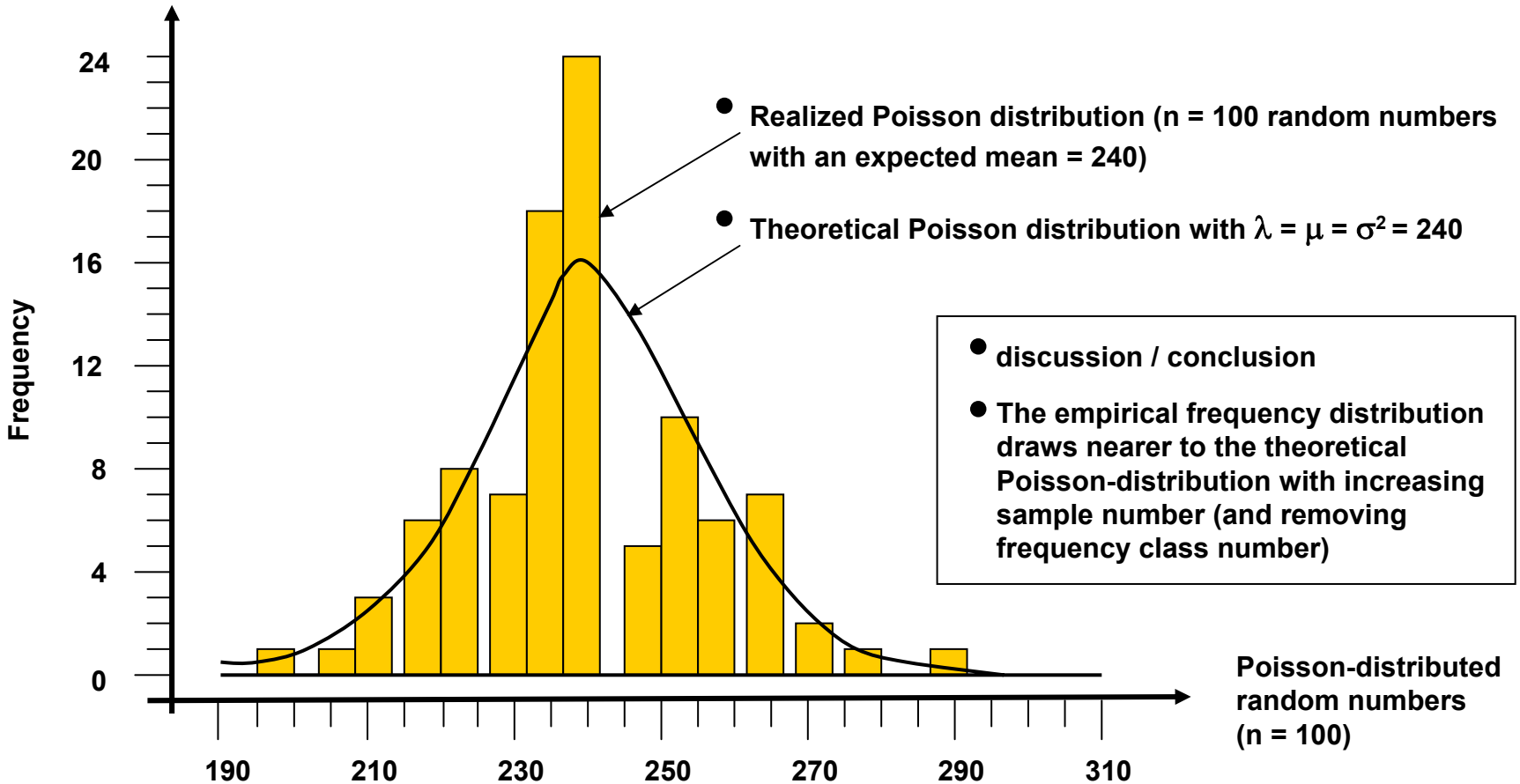
log – transformation: $\bar{x}_{(\log 10)} = 2.39$; $S_{(\log 10)} = 0.03$

$$R = r = 2.83 \times s_{(\log 10)} = 0.08$$

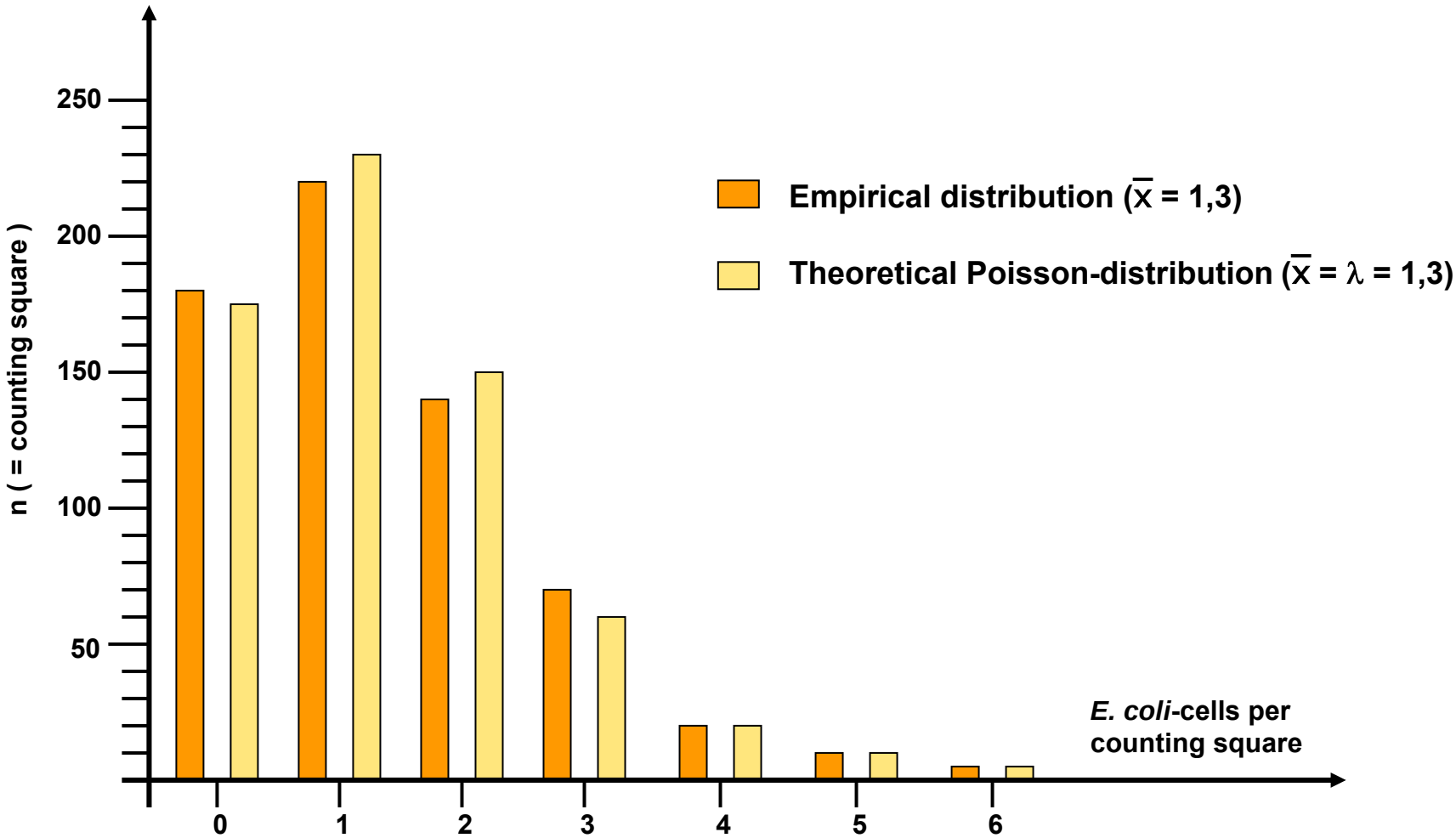
- **discussion / conclusion**

Counting e.g. $\lambda = 25$ microorganisms on the level of 1×10^{-4} ml results in a nearly fourfold higher coefficient of variation than counting $\lambda = 250$ microorganisms on the level of 1×10^{-3} ml only because of the sampling error

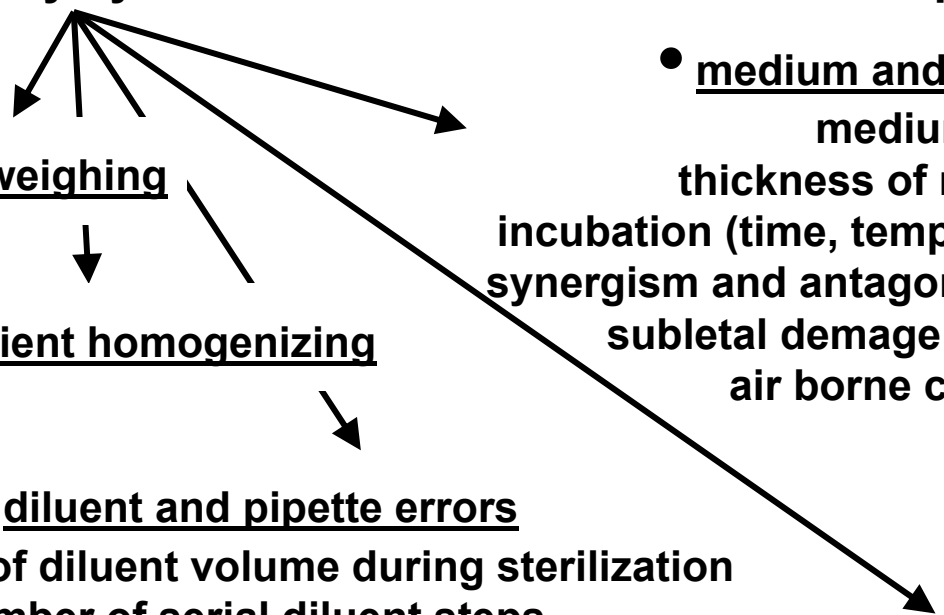
- **Poisson-distribution: theoretical and realized distributions**



- **Opposing of empirical determined and theoretically expected microbiologic counts per counting chamber squares in an experiment with an *E. coli* culture**



- **45.15 Errors of colony counting methods**
  - **1. Sampling errors (= mostly random errors)**
    - **variance between samples**
    - **variance within samples ("POISSON" – error)**
  - **2. Laboratory errors (mostly systematic errors with a random component)**



- **inaccuracies in weighing**
- **insufficient homogenizing**
- **diluent and pipette errors**
  - **decreasing of diluent volume during sterilization**
  - **number of serial diluent steps**
  - **contamination of pipettes because of manifold use**
  - **adherence of microorganisms to glass (pipette, test-tube)**
  - **pipette volume error**
  - **operator error**
- **medium and incubation error**
  - **medium recipe**
  - **thickness of medium surface**
  - **incubation (time, temperature, humidity, et. al.)**
  - **synergism and antagonisms of microorganisms**
  - **subletal demage of microorganisms**
  - **air borne contamination**
- **counting error**
  - **individual counting error**
  - **over crowding**