MAS 100 NT / MAS 100 NT EX
Flow variances and result interpretation.

Tony Ancrum PLS LB PM MHM

Roger Haas PLS LB PM MHM

Charlotte Lindhardt PLS LB PM MHM

November 2010

Low flow Problem

- It has been reported that a small percentage of customers using the 300 x 0.6mm head have seen up to a 5% drift in flow rate.
- This means instead of a flow rate of 100 litres per minute a flow rate of 95 litres per minute was achieved without an error message.
- The problem is linked to the motor control firmware and is under investigation.
- This problem could affect your results by the percentage of difference in the flow rate achieved to the flow rate expected.

Assumptions

- In Figure 1 we are trying to show that when a air sample is taken for a class “A” clean room the result is based on probability. The most appropriate model is Poisson distribution, the reasons behind this assumption are as follows.
  - You do not sample every part of the air.
  - The amount of air sampled is only a small percentage of the total volume.
  - The air in most clean rooms is changing constantly.
  - The organisms you are trying to capture are not evenly distributed.
- The guidelines for sampling are documented in the relevant GMP for your area, but these are guidelines not legal requirements.
- In most cases companies use these guidelines to set up their sampling plans and normally they set the sample volume at 1000 litres or $1M^3$ at a flow rate of 100 litres per minute.

Microbiological Monitoring

History….

The first standard written for clean room was published by the American Air force on March 1961.

It was known as Technical Manual (T.O) 00-25-203.

The concepts were:

- **Clean room design**
- **Airborne particle standards**
- **Entry procedures, clothing**
- **Restriction of certain articles**
- **Cleaning of materials**
- **Procedure for cleaning the room**

Microbiological Monitoring

History….

The first Federal standard 209 was produced in the year 1963.

- Conventional and unidirectional clean rooms were discussed.
- Measurement of particles of ≥0.5 µm by means of optical particle counters was defined.

The revisions

- **209A – 1966**
- **209B – 1973**
- **209C – 1987**
- **209D – 1988**
- **209E – 1992** (Recommendation Microbial limits)

Microbiological Monitoring

History….

ISO Standard 14644-1

TC 209: Clean rooms and associated controlled environments

The first document published in 1999 is ISO 14644-1.

“Classification of Air Cleanliness”

Standards and Regulations

- **What different Regulations for Environmental Monitoring does exist?**

  - ISO norms
  - Pharmacopeia's
  - EU-GMP / cGMP
  - cGMP

Standards and Regulations

Standards for the classification Clean rooms

Clean rooms are classified by the cleanliness of their air. This is according to ISO-14644-1. This is an international standard, having adopted by the European Union in 1999, and the USA in 2001. However the most easily understood classification of clean rooms is the obsolete Federal Standard 209 of the USA, **and it is still widely used.**

(FS 209 E gives also recommendations about microbial limits)

Standards and Regulations

What is a clean room ?

ISO 14644-1
A room in which the concentration of airborne particles is controlled, and which is constructed and used in a manner to minimize the introduction, generation, and retention of particles inside the room and in which other relevant parameters, e.g. temperature, humidity, and pressure, are controlled as necessary

Standards and Regulations

Comparison between selected equivalent classes of FS 209 and ISO 14644-1

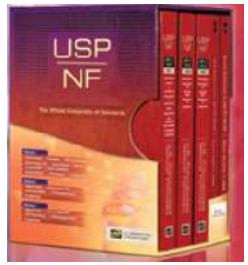
ISO 14644-1 Classes	Class 3	Class 4	Class 5	Class 6	Class 7	Class 8
FS 209 Classes	Class 1	Class 10	Class 100	Class 1000	Class 10 000	Class 6 100 000

Standards and Regulations

USP NF / Chapter <1116>

This chapter includes discussions on:

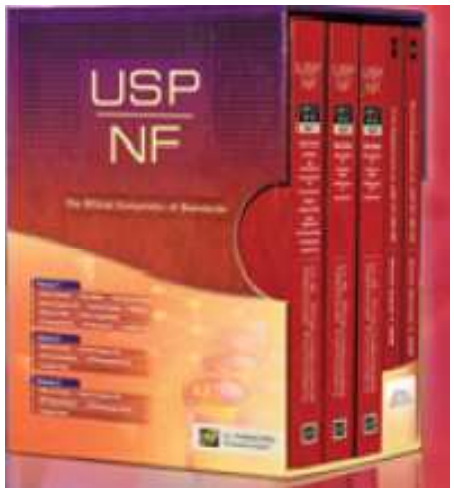
- classification of a clean room based on particulate count limits
- microbiological evaluation programs for controlled environments
- training of personnel
- critical factors in design and implementation of a microbiological evaluation program
- development of a sampling plan
- establishment of microbiological Alert and Action levels
- methodologies and instrumentation used for microbiological sampling
- media and diluents used
- identification of microbial isolates
- operational evaluation via media fills
- glossary of terms.



Standards and Regulations

USP NF / Chapter <1116>

There are alternative methods to assess and control the microbiological status of controlled environments for aseptic processing. Numerical values included in this chapter are not intended to represent absolute values or specifications, but are informational.



Sampling media and devices, and methods indicated in this chapter, are not specifications but only informational.

Standards and Regulations

USP NF / Chapter <1116>

Cleanliness Guidelines of Air, Equipment, Personal in controlled Environments

Class		cfu/m^3 of air:	cfu per Contact plate		
SI	US Customary	Air	Equipment	Gloves	Clothing
M3.5	100	<3	3 (including floor)	3	5
M5.5	10,000	<20	5 (10 floor)	10	20
M6.5	100,000	<100	-	-	-

Standards and Regulations

Pharmaceutical Clean room Classification :

Clean rooms used for pharmaceutical manufacturing have their own standards, also based on number of particles but in addition to that, the Pharmaceutical standards gives also a recommendation about the limits for microbial contamination. The two most widely used are those published in the EU and USA.

Standards and Regulations

Classification of Pharmaceutical clean rooms:

The most recent pharmaceutical standard used:

1. European Union Guide to Good Manufacturing Practice (EU GGMP)

2. **Guidance for Industry Sterile Drug Products produced by aseptic processing – Current Good Manufacturing Practice. (C GMP)**

Standards and Regulations

- **European Union Guide to Good Manufacturing Practice (EU GGMP)**

The most recent pharmaceutical standard used In Europe came into operation on January 1997. It was revised in 2003.

For the manufacture of sterile pharmaceutical products Four grades of airborne cleanliness are given.

b) Individual settle plates may be exposed for less than 4 hours

Standards and Regulations

- **European Union Guide to Good Manufacturing Practice (EU GGMP)**

For the manufacture of sterile pharmaceutical products **4** grades of airborne cleanliness are given.

Grade	Air Sample cfu/m^3	Settle Plates (diam. 90 mm), cfu/4 hours (b)	Contact Plates (diam. 55 mm), cfu/plate	Glove Print 5 fingers cfu/glove
A	< 1	< 1	< 1	< 1
B	10	5	5	5
C	100	50	25	-
D	200	100	50	-

b) Individual settle plates may be exposed for less than 4 hours

Standards and Regulations

- **Example of clean room conditions required for “Aseptic preparations” acc. to EU GGMP**

Grade A

Aseptic preparation and filling.

Grade C

Preparation of solutions to be filtered. (if not filtered; the preparation of materials and products should be done in Grade A with Grade B background)

Grade D

Handling of components after washing.

Standards and Regulations

Guidance for Industry Sterile Drug Products produced by aseptic processing – Current Good Manufacturing Practice. (C GMP)

Guideline on Sterile Drug Products Produced by Aseptic Processing.

This document was published in 1987 by USFDA.
Revised on September 2004

Critical Area
Controlled Area

Standards and Regulations

Guidance for Industry Sterile Drug Products produced by aseptic processing – Current Good Manufacturing Practice. (C GMP)

The FDA Guidance has a similar table to the EU GGMP for the required airborne particle and microbial conditions for Aseptic Processing.

Air Classifications by USFDA guideline on Sterile Drug Products

Clean Area Classification	<0.5 µm Particles/ft3	<0.5 µm Particles/mt3	Microbiological Limit	
			cfu/ft3	cfu/m3
100	100	3,500	<1	<3
1000	1000	35,000	<2	<7
10000	10000	350,000	<3	<18
100000	100000	3,500,000	<25	<88

Standards and Regulations

Guidance for Industry Sterile Drug Products produced by aseptic processing – Current Good Manufacturing Practice. (C GMP)

The FDA Guidance identifies two clean room areas of critical importance:

A) Critical AREA

B) Controlled AREA (Supporting AREA)

Standards and Regulations

Guidance for Industry Sterile Drug Products produced by aseptic processing – Current Good Manufacturing Practice. (C GMP)

Critical AREA (Class 100 / ISO class 5 / EU GGMP class A)

one in which the sterilized dosage form, containers, and closures are exposed to the environment. Activities that are conducted in this area include manipulations of these sterilized materials/product prior to and during filling/closing operations

Controlled AREA (Supporting AREA) (Class 10,000/100,000 / ISO class 7/8 / EU GGMP C/D)

one in which non-sterile components, formulated products, in-process materials, equipment and containers are prepared, held or trasferred.

Assumptions

- There is nothing in the guidelines to indicate how often you need to sample or how many samples you take in any one room. These are normally based on experience and trend analysis.
- To establish a base line test plan, a number of samples are taken over time form different positions in the room, these results are analysed against the guidelines and the results from any test batches manufactured in the room during the trial to establish if the sample plan is adequate to ensure compliance with the guidelines and product safety.

Figure 1

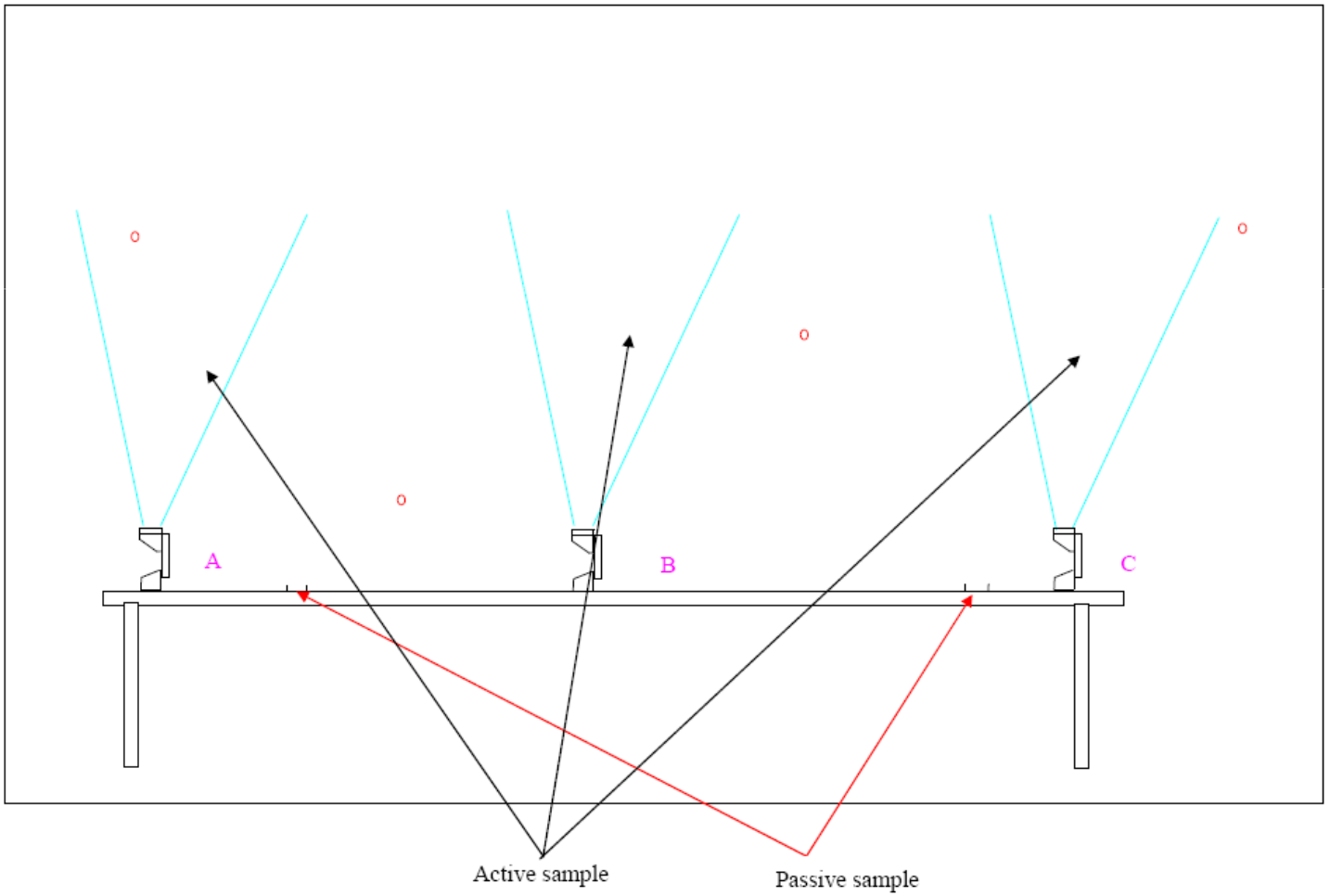
- Shows 3 air samplers in a production area all running at a flow rate of 100 litres per minute and all running for the same amount of time 10 minutes.
- Instrument “A” is the only instrument to pick up a micro-organism the other 2 would have shown a clean plate. If as is normal passive air sampling had also been used they might have picked up one more of the microbes, the only way to pick up every microbe in the room is to sample all the air in the room all the time.

Assumptions

- This is not practical so a statically technique is used to establish confidence levels.
- Using the above points as a starting point, if the normal amount of air sampled is 1000 litres or 1^2M at a flow rate of 100 litres per minute this could be classed as a 100% sample rate giving a 100% confidence level result.
- Using the same principles if the air sampled is 900 litres or $0.9M^3$ at a flow rate of 90 litres per minute this could be classed as a 90% sample rate giving a 90% confidence level result.
- This statement would hold true for 95 litres per minute with the results being 95% of the confidence level set up during the trend analysis phase.

Figure 1

o =Microbe



Results and Confidence Levels

- When both Active and Passive air samples are taken, if the result of both match, then the confidence level should be considered high that the result is good.
- The next slides show the differences to the expected results based on the main regulatory bodies with flow rates from 100 litres a minute to 90 litres a minute.

Microbiological Air Monitoring

Clean room classification

Comparison of guidelines and regulations of microbial levels for clean rooms											
Clean room grade ISO	Clean room grade (EU's GMP JP)	Clean room grade (US Fed.Std209E)	Active air sampling (cfu/m3)			Settle plates (diam. 90mm cfu/4 hours)			Contact plates (cfu/plate, surface 24-30 cm2)		
			EU's GMP JP	USP 31 <1116>	FDA 2004	EU's GMP	USP 24 <1116>	FDA 2004	EU's GMP	USP 24 <1116>	FDA 2004
5	A	100 (M 3.5)	<1	<3	1	<1	-	1	<1	3 (Including floor)	-
6	B	1000 (M 4.5)	10		7	5	-	3	5		-
7	C	10 000 (M 5.5)	100	<20	10	50	-	5	25	5 (floor: 10)	-
8	D	100 000 (M 6.5)	200	<100	50	100	-	50	50	-	-

Statistical Assumptions

- The main assumption used to calculate these results are based on the up to 10% difference in the calibrated flow rate.
- The number of tests has minimal influence on the result.
- The number of organisms found is the other main factor.
- The limits for EU GMP and the JP GMP are,
  - Less than 1 cfu per $1m^3$ for Class A / Class 5
  - Up To 10 cfu per $1m^3$ for Class B / Class 6
  - Up To 100 cfu per $1m^3$ for Class C / Class 7
  - Up To 200 cfu per $1m^3$ for Class D / Class 8

Statistical Assumptions

- The limits for USP 31 (1116) are,
  - Less than 3cfu per $1m^3$ for Class M3.5 (100)
  - Less than 3 cfu per $1m^3$ for Class M4.5 (1000)
  - Less than 20 cfu per $1m^3$ for Class M5.5 (10 000)
  - Less than 100 cfu per $1m^3$ for Class M6.5 (100 000)
- The limits for FDA 2004 are,
  - Less than 1cfu per $1m^3$ for Class 100 (M3.5)
  - Up To 7 cfu per $1m^3$ for Class 1000 (M 4.5)
  - Up To 10 cfu per $1m^3$ for Class 10 000 (M5.5)
  - Up To 50 cfu per $1m^3$ for Class 100 000 (M 6.5)

Distribution model for contamination events – Poisson:

- Based on the previous assumptions:
  - The contamination particles are randomly distributed in the air.
  - The probability of an “event” (contamination on plate) is approaching 0 for class A.
  - The volume of air sampled is small compared to the total volume of air. (This is true even in confined spaces provided the air is constantly circulated / replenished)
  - The outcome of each test (air sampling) in a series of tests is independent of the outcome of any other test.
  - Number of tests is unlimited

Distribution model for contamination events – Poisson:

- In this case, the Poisson distribution can be used as model

  $P(x) = e^{-\lambda}(\lambda^x/x!)$, where

  P(x) is the probability of x individuals occurring in a sampling unit
  λ is the Poisson parameter ($\lambda = \mu = \sigma^2$)
  λ is be estimated by m (= mean contamination per sample)

Poisson probabilities

- When sampling air the sampling can be regarded a sampling repeatedly from a continuum.
- Assuming the distribution of micro-organisms in the air is random, the population distribution can be expected to follow Poisson distribution
- One of the characteristics of the Poisson distribution is that
  $\mu = \sigma^2$ or
  $\sigma = \sqrt{\mu}$
  where μ = true mean and σ = true standard deviation
- Using the convention for 95% confidence limits $\approx$ mean $\pm$ 2 SD, this give the following examples for confidence intervals for determined plate counts

95% Confidence intervals (Poisson Distr.)

Mean	Standard Deviation	Lower 95%	Upper 95%
3	1.73	0	6.46
10	3.16	3.68	16.32
20	4.47	11.06	28.94
50	7.07	35.86	64.14
100	10	80	120
200	14.14	171.72	228.28

95% Confidence intervals (Poisson Distr.) at 90% of target air flow

Mean/0.9	Standard Deviation	Lower 95%	Upper 95%
3.33	1.73	0	6.98
11.11	3.16	4.44	17.78
22.22	4.47	12.79	31.65
55.55	7.07	40.65	70.46
111.11	10	90.03	132.19
222.22	14.14	192.41	252.04

What is the meaning of a limit?

- If a Class C clean room has a limit of 100 cfu/1000 liters, what does this mean?

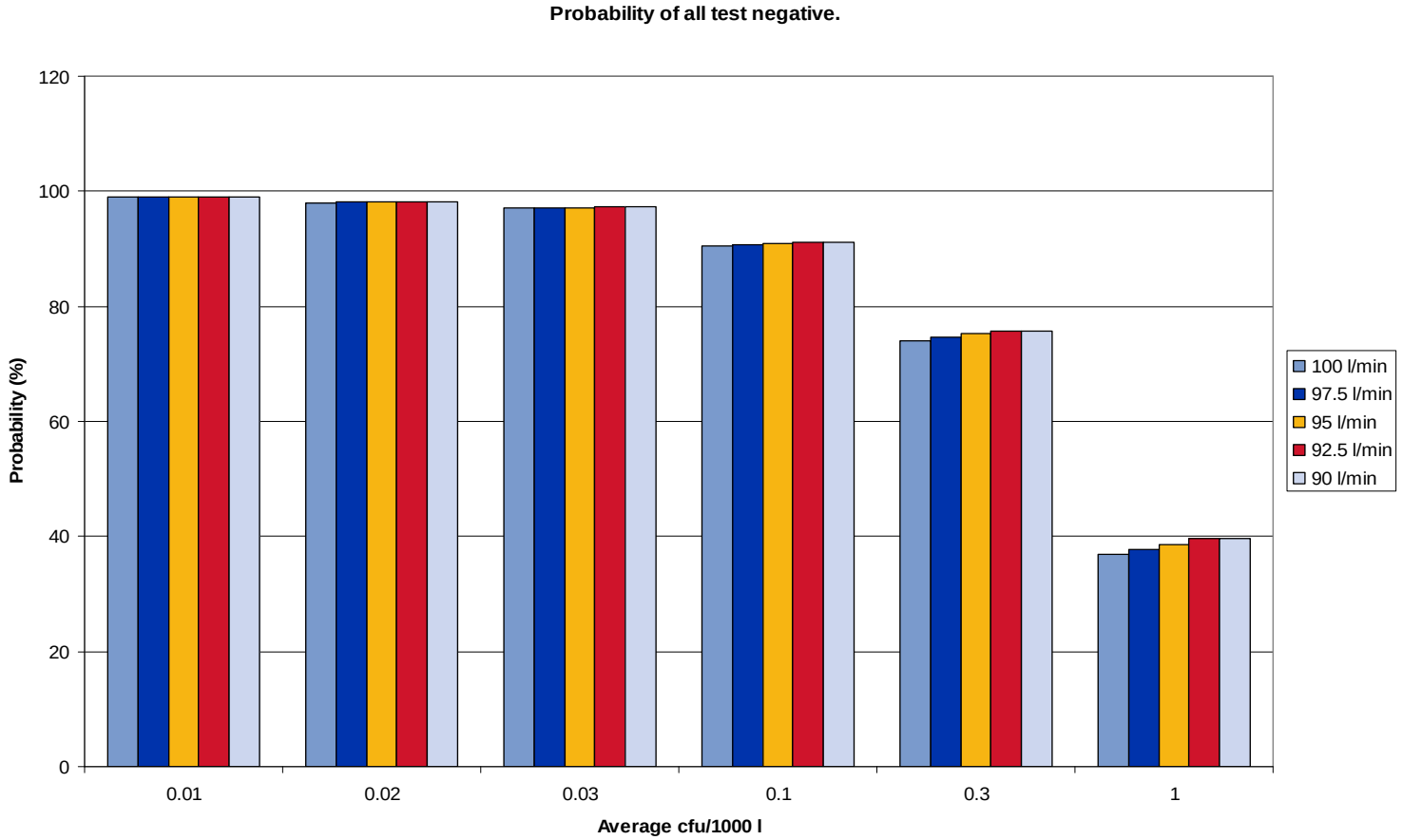
1. No single measurement can show result > 100 cfu/1000 liter
2. The average must be below 100 cfu/1000 liter with 95% confidence
3. The average must be below 100 cfu/1000 liter with >99.7% confidence

- The difference between these 3 interpretations have big implications.
- Testing only 100 liter and applying a limit of 10 cfu/100 liter drastically changes the confidence limits and thereby the probability of detection, much more than the difference between testing 900 instead of 1000 litre
  e.g. at 120 cfu in 1000 liter there is only about 5% risk of declaring the sample OK. The corresponding limit at 100 liter would be 17 cfu. At 12 cfu in 100 liter, there is a 35% risk of declaring the sample OK

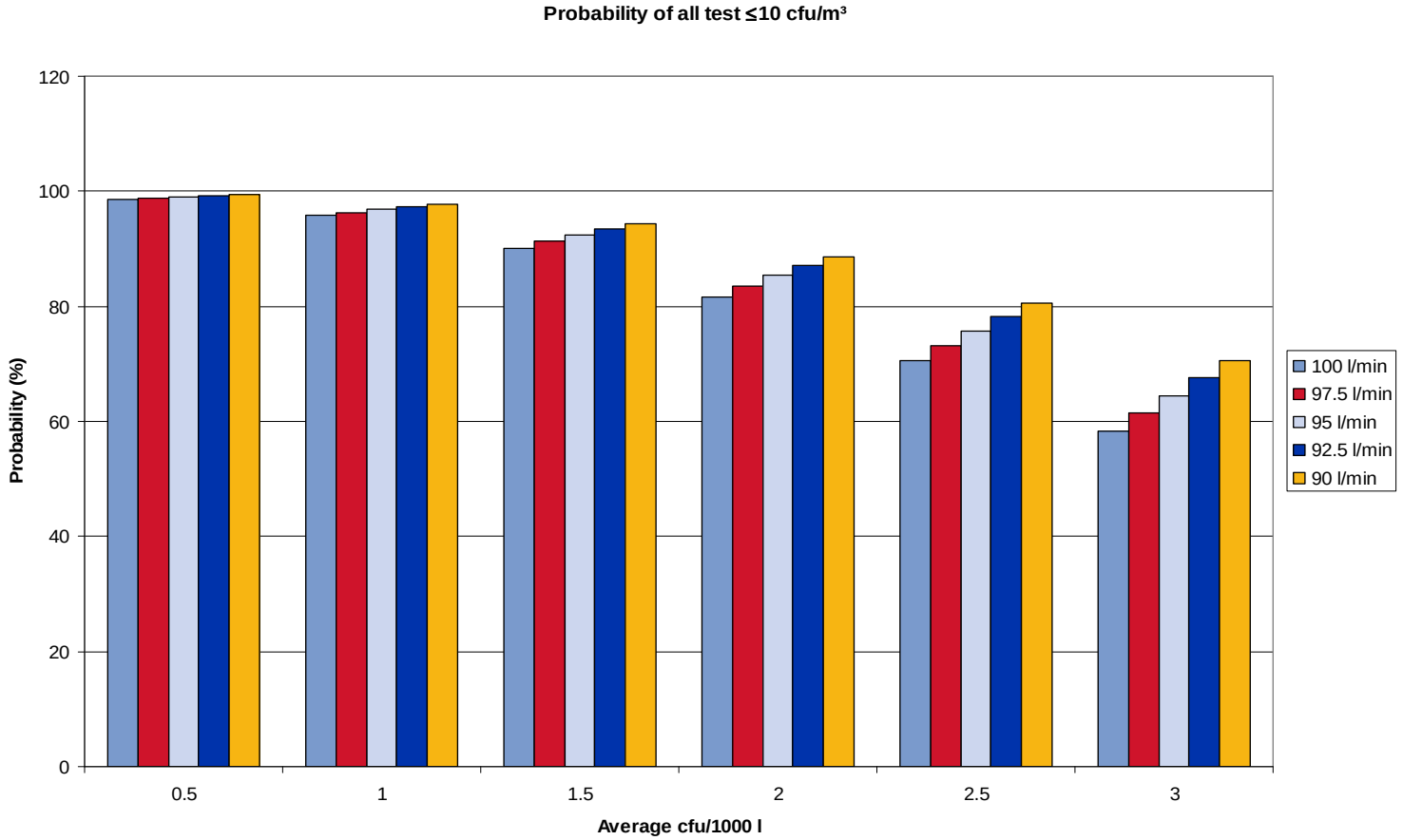
Explanation

- The charts show the estimated probability of a test being classed as “pass” for different average contamination rates and for different flow rate deviations
- The difference between the probability at a flow rate (less than 100 l/min) and that at 100 l/min for a specific contamination rate can be regarded as the probability of a contamination event going undetected (false negative)
- To select a relevant scenario, chose the contamination rate, which most closely match the expected rate of contamination events for the relevant class clean-room.

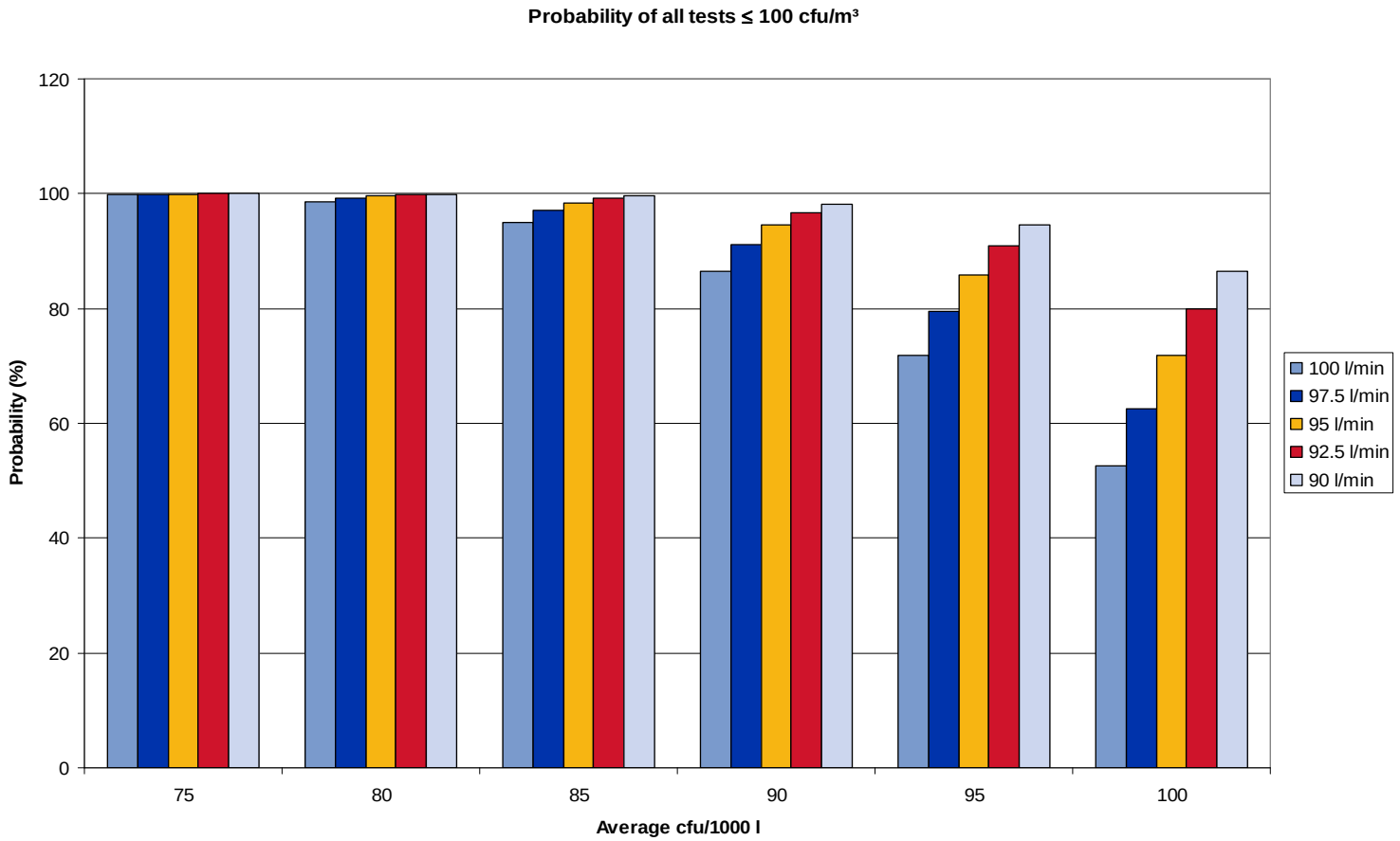
EU and JP GMP Class A <1 cfu/m³



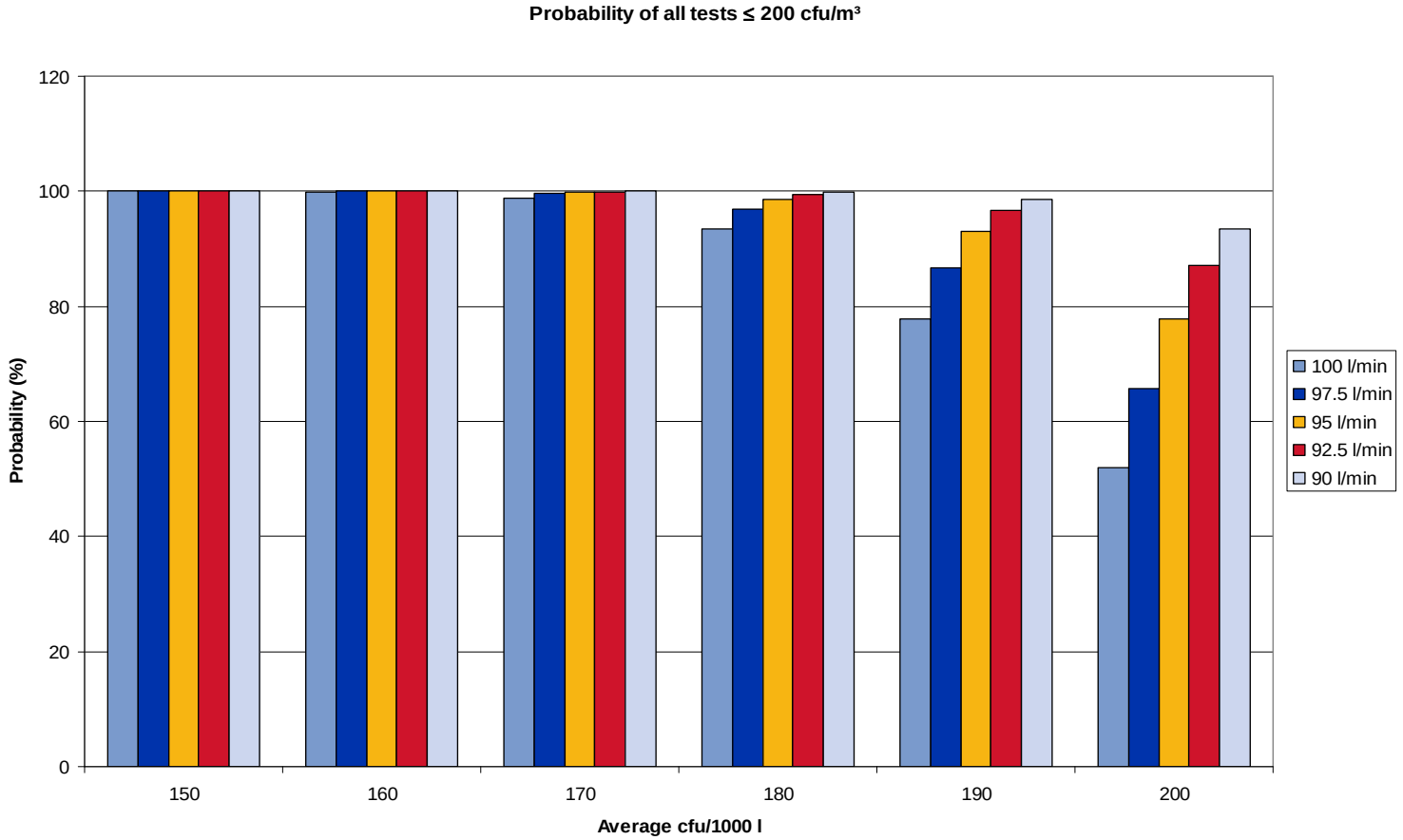
EU and JP GMP Class B 10 cfu/m³



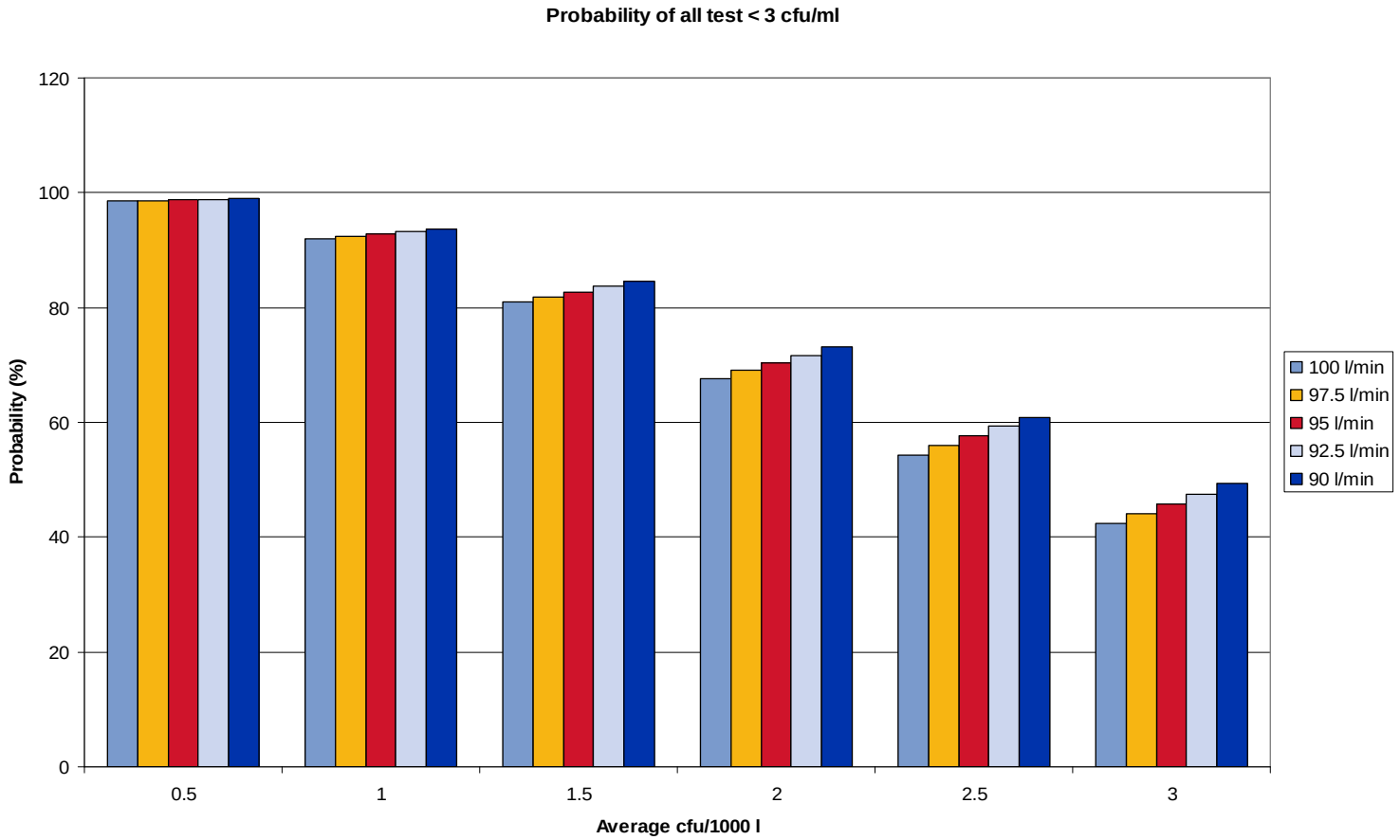
EU and JP GMP Class C 100 cfu/m³



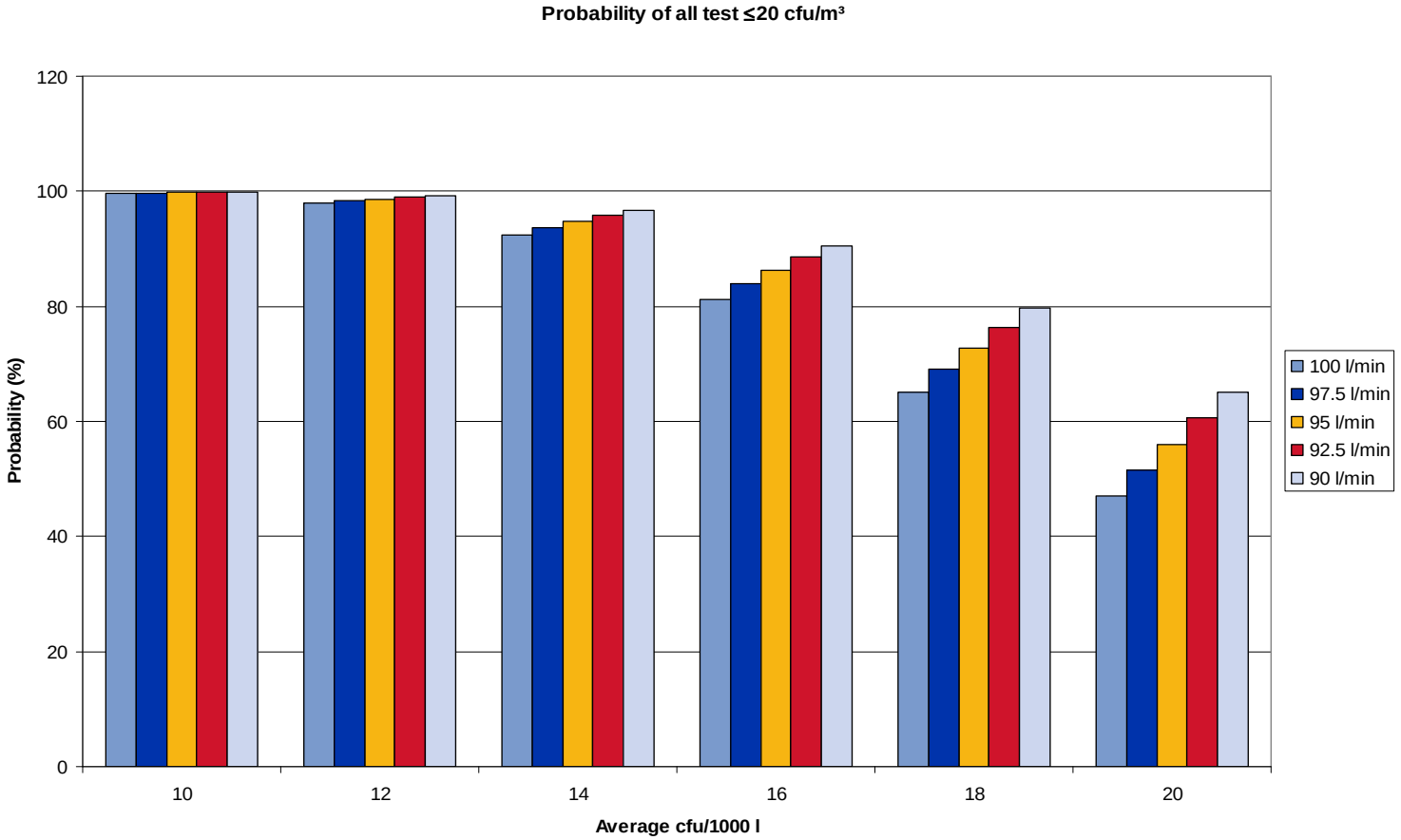
EU and JP GMP Class D 200 cfu/m³



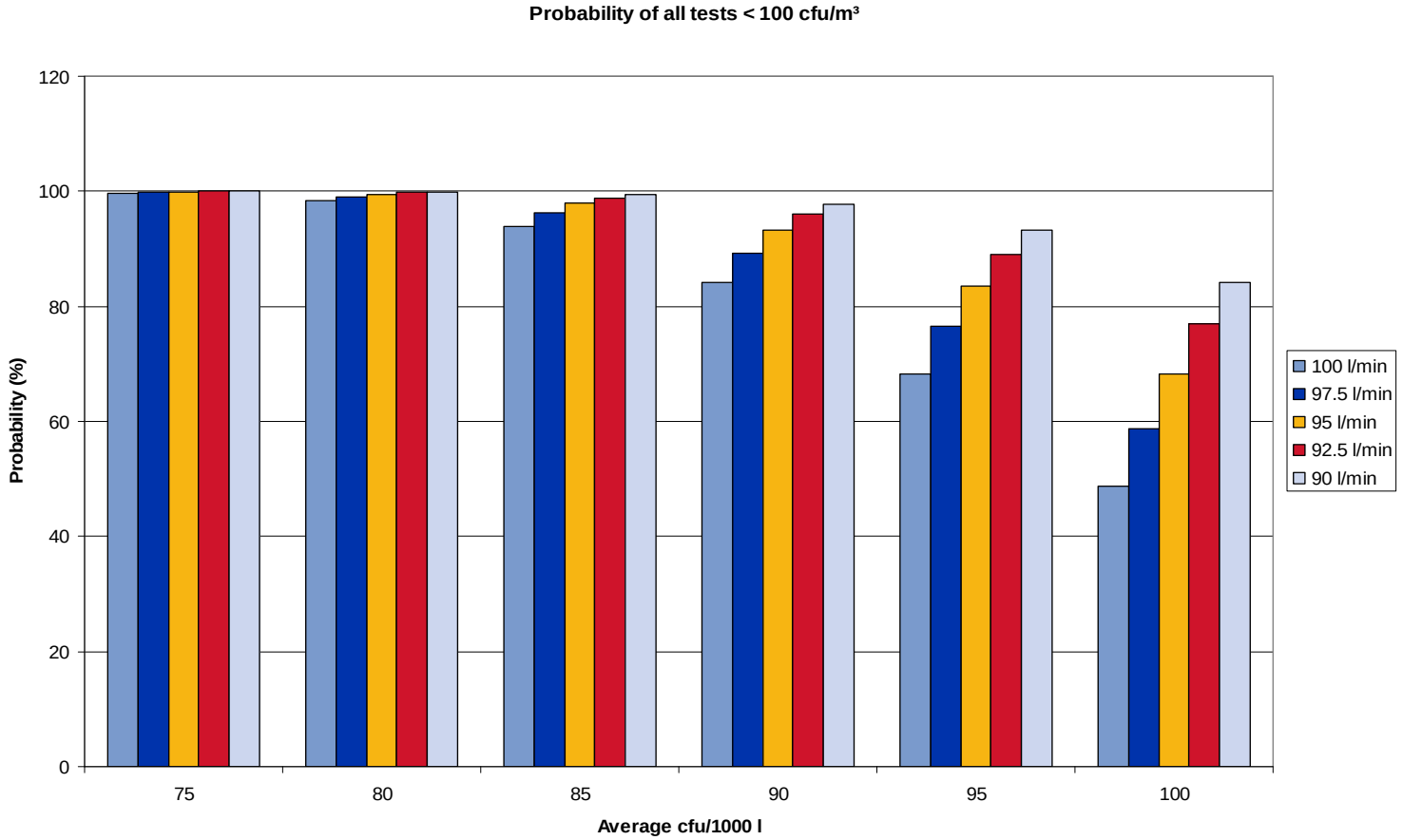
USP 31 <1116> Class M3.5 – M4.5 (100-1000) < 3 cfu/m³



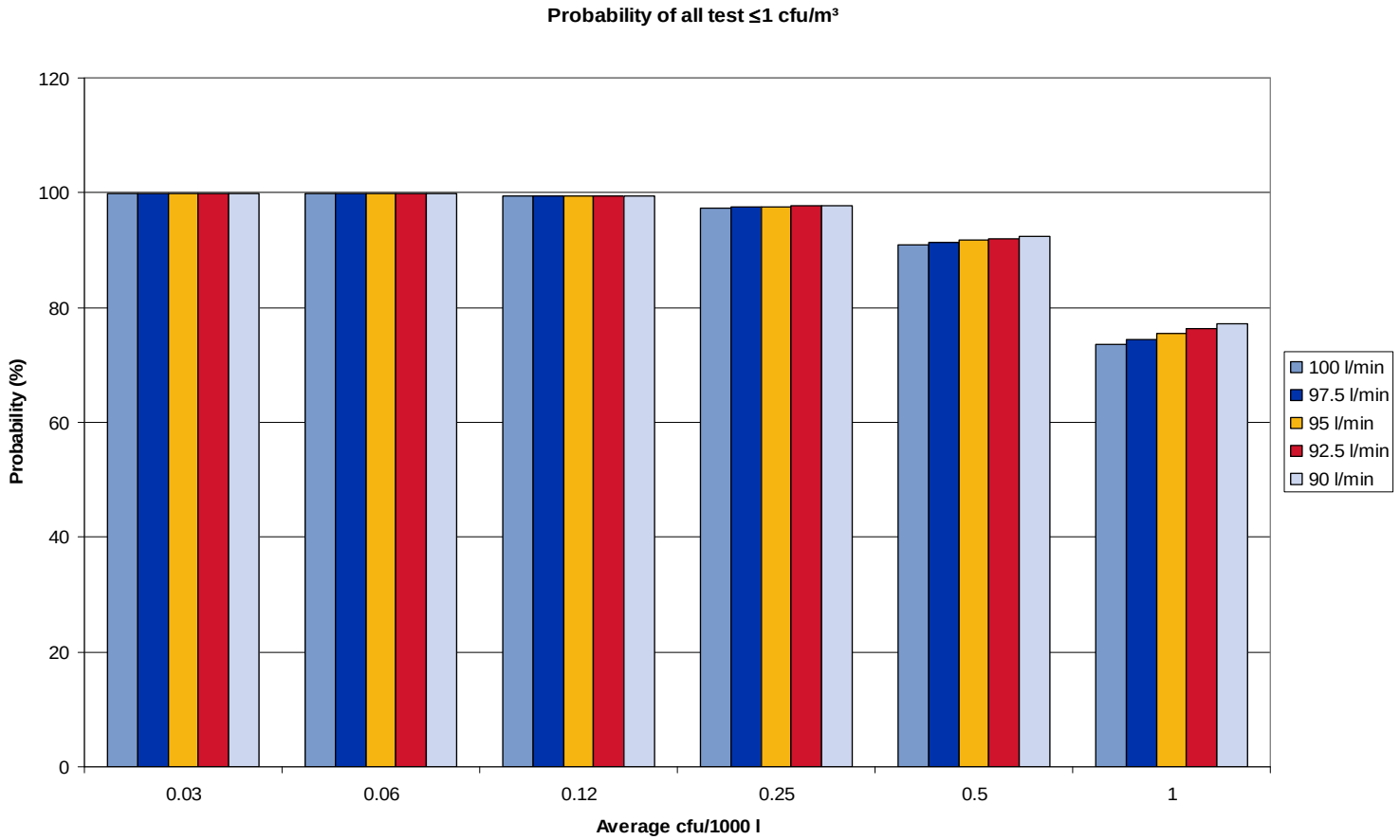
USP 31 <1116> Class M5.5 (10000) <20 cfu/m³



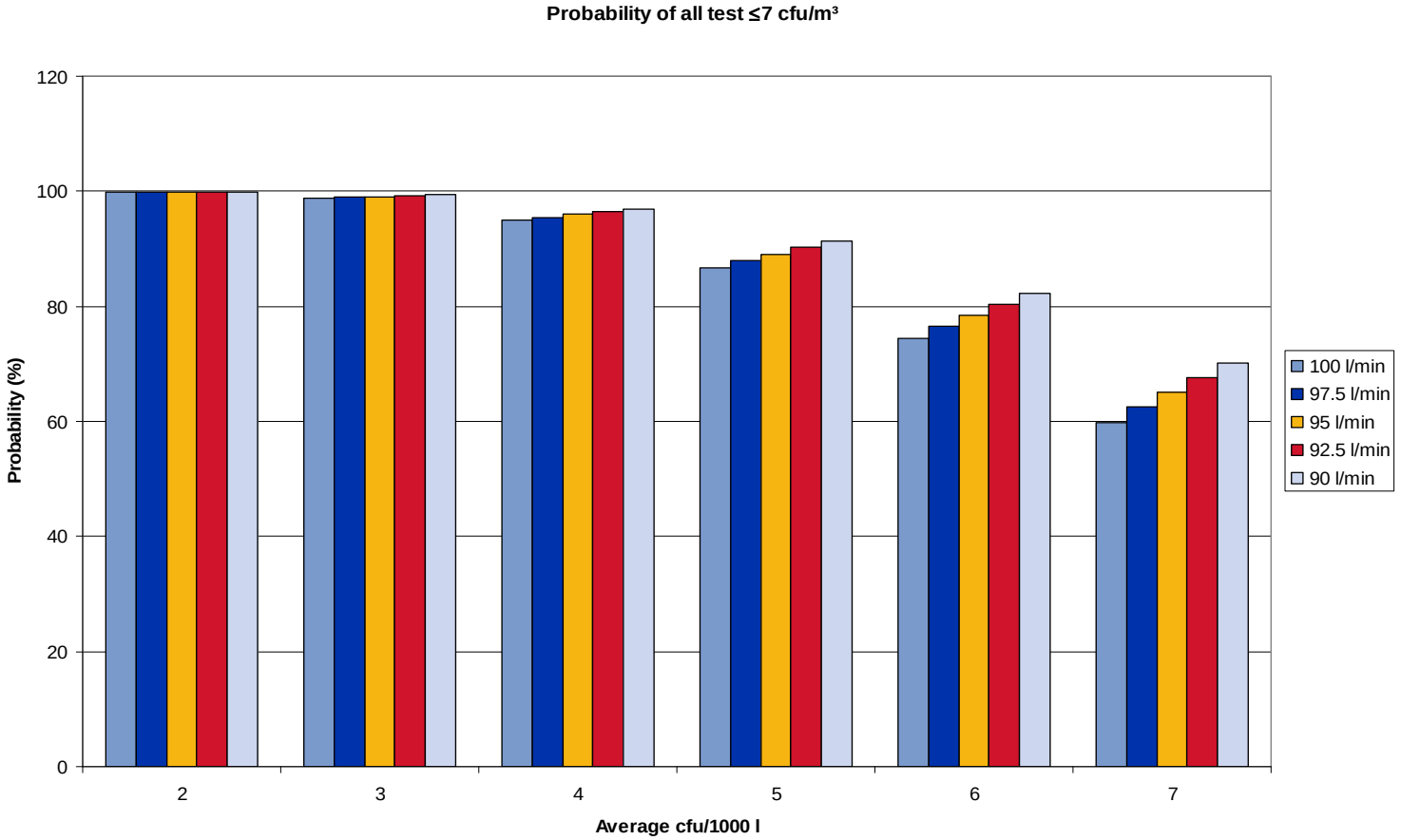
USP 31 <1116> Class M6.5 (100000)<100cfu/m³



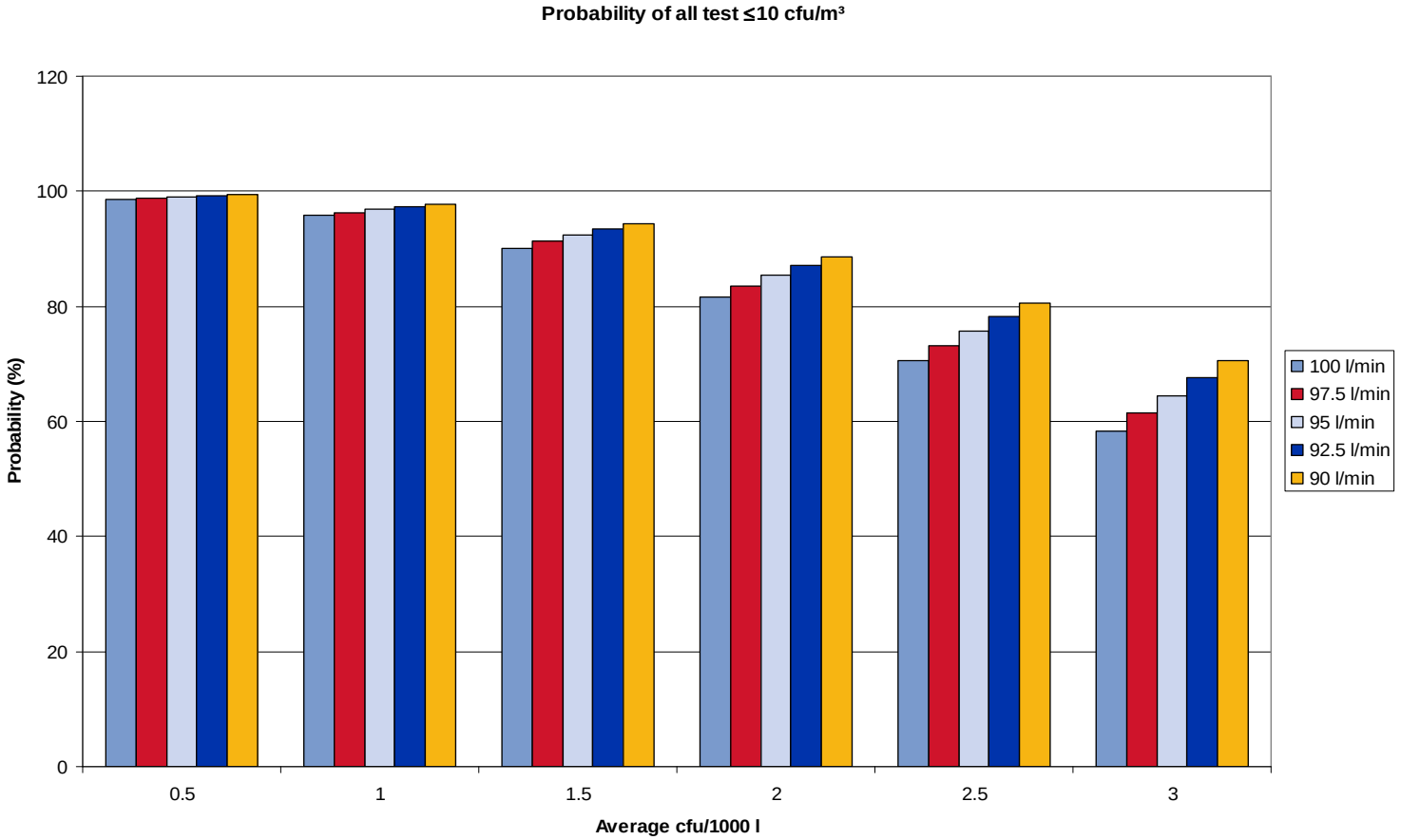
FDA 2004 Class 100 (M3.5) 1 cfu/m³



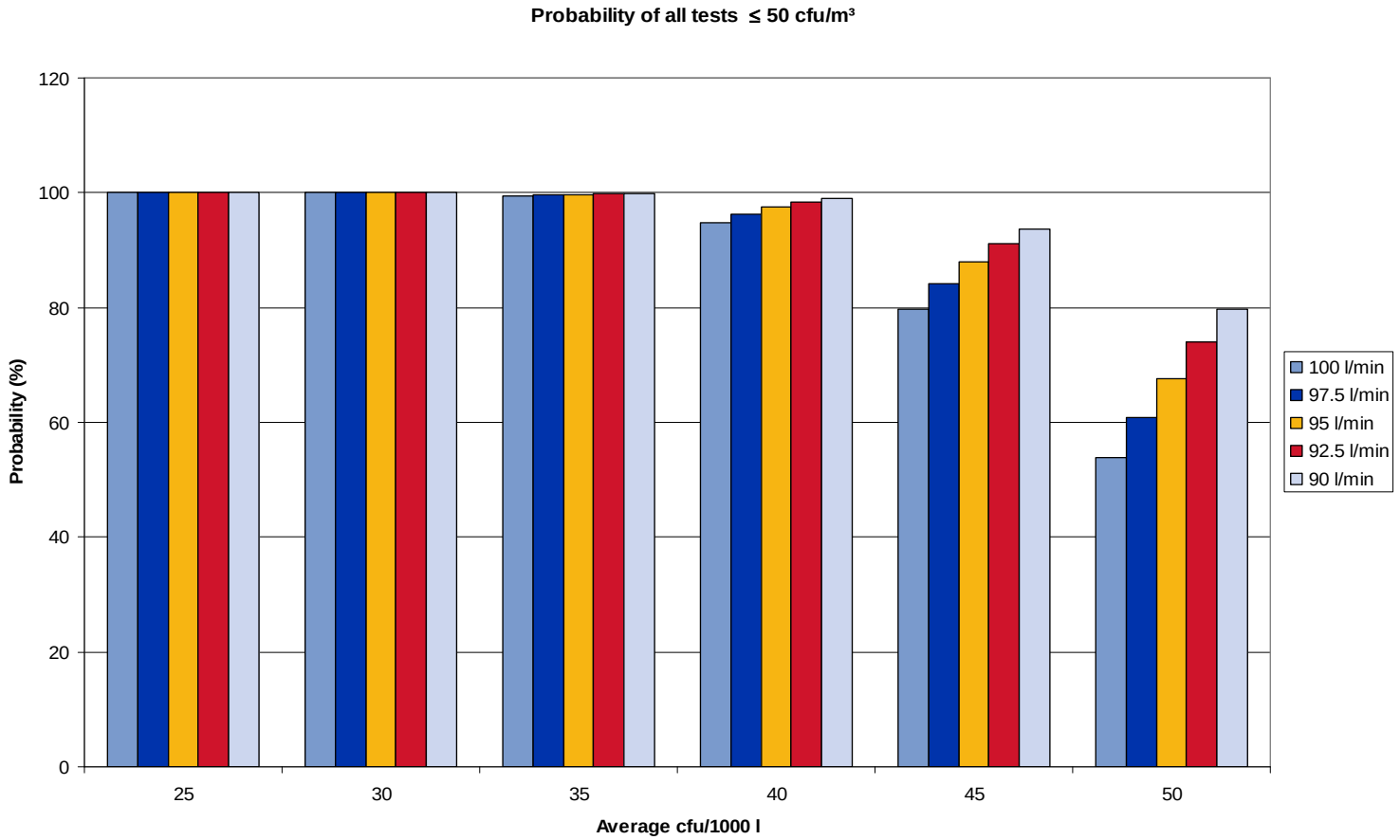
FDA 2004 Class 1000 (M4.5) 7 cfu/m³



FDA 2004 Class 10000 (M5.5) 10 cfu/m³



FDA 2004 Class 100000(M6.5) 50 cfu/m³



Conclusions:

- The most appropriate statistical model should be the Poisson distribution. The probability of obtaining false negative results at a flow rate of 90 l/min is 0.1% (of the total number of tests), at a contamination level giving an expected contamination rate of 1 in 100.
- This means that the overall risk of a false negative due to the lower flow rate is less than 0.1% at the normal industry levels.
- If your results are 10 times higher (10% of tests showing contamination), the risk of false negative results is still only 0.9%.

CAPA (Corrective & Preventive Action Plan)

- We are currently working with the manufacture to find a solution to this problem.
- A CAPA plan will be produced by the manufacture.