Millipore®
Preparation, Separation,
Filtration & Monitoring Products

Application Note

Active air sampling with Tryptic Soy Agar – ICR

ISO 14698-1 describes standard methods to measure biological contamination in cleanrooms and associated controlled environments. In areas that are used for manufacturing safe and stable pharmaceutical products, the control of biological contamination is mandatory. Part 1 of ISO 14698 specifies methods which can be used to monitor risk zones in cleanrooms or give information about sources of risk in the zones.

Annex A describes how to determine airborne biological contamination. For active air sampling, impaction, impingement or filtration samplers are recommended devices.

Annex B gives a guidance on how to validate air samplers. It is split into two parts, the physical and the biological efficiency. For the biological efficiency the use of casein-peptone soya meal-peptone containing agar is recommended. As the validation is performed with micro-organisms also the formulation and quality of the culture medium has influence on the results.

MERCK

The MAS-100® air samplers are sieve impaction systems based on the Anderson impaction principle. They use 90 – 100 mm standard petri dishes, are easy to handle and compact. They allow an appropriate suction flow rate, impact velocity and collection accuracy and efficacy.

All MAS-100® air samplers are validated according to ISO 14698-1. For validation the TSA + LTHTh – ICR+ (146683) was chosen. All members of the MAS-100® showed comparable results for biological and physical efficiency as indicated in table 1.

Table 1. Physical and biological efficiency of the MAS-100® family

Characteristics	Iso-MH (1)	Iso-MH (9)	Iso	NT	VF
Physical Efficiency for particle size of 0.8 µm (in %)	60.41	62.58	60.20		
Physical Efficiency for particle size of 1.0 µm (in %)				78.77	84.18
Physical Efficiency for particle size of 1.3 µm (in %)	71.68	82.60	76.91	84.10	85.81
Physical Efficiency for particle size of 2.2 µm (in %)	96.90	91.54	91.96	91.76	94.22
Physical Efficiency for particle size 5.4-6 µm (in %)	99.04	93.27	90.24	92.65	99.65
Biological efficiency (in %)	76.74	73.74	78.08	82.62	76.78

MilliporeSigma offers a variety of TSA – ICR formulations with and without neutralizers, which have not all been included in the validation of the air samplers according to ISO 14698-1 (listed in table 2).

Table 2. Available formulations of ICR media based on casein soya bean peptone agar (TSA)

Formulation	90 mm plate design non-lockable (order number)	90 mm plate design lockable (order number)
TSA + LTHTh	TSA + LTHTh – ICR (1460690020/1460690120)	TSA + LTHTh – ICR+ (1466830020/1466830120*)
TSA + LT	TSA + LT – ICR (1460500020/1460500120)	TSA + LT – ICR+ (1466840020/1466840120)
TSA	TSA – ICR (1460010020/1460010120)	TSA – ICR+ (1466850020/1466850120)

* pa t of ISO 14698 validation

Material and Equipment:

Table 3. Media

Product Name	Cat. No.	Format
Tryptic Soy Agar - ICR 30ml	146001	unlockable, Ready-to-Use
TSA + LT - ICR 30ml	146050	unlockable, Ready-to-Use
TSA + LTHTh - ICR 30ml	146069	unlockable, Ready-to-Use
TSA + LTHTh 90mm ICR+	146683	lockable, Ready-to-Use
Competitor A	146683	unlockable, Ready-to-Use
Competitor B	146683	lockable, Ready-to-Use

For all trials the MAS-100VF® instrument (Cat. No. 117103) was used.
The plates 146069 and 146683 contain identical agar formulation.

Method:

In a test room four MAS-100VF® instruments were placed on two tables. Each sampler had its specific position for the whole trial (see Figure 1). Positions were chosen to achieve symmetry in the room with respect to walls, ventilation inlets/outlets etc. and to ensure a good distance between the individual samplers.

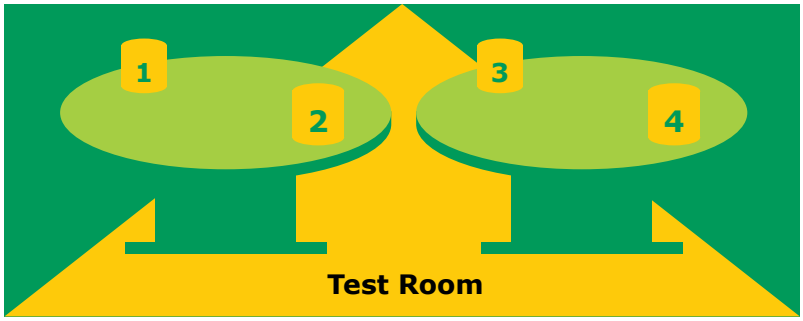


Figure 1.
Sampling concept for active air sampling. Four MAS-100VF® instruments were placed on different positions on two tables in an uncontrolled environment outside of cleanroom.

For both trials the agar plates were tested in a specific order (see table 4). Every sampling step consists of a three minutes delay and a ten minutes (equal to 1 m^3) sampling step.

After the sampling all plates were incubated for 2 days at 32.5°C (± 2.5). All lockable plates have been incubated in the lock position, MilliporeSigma plates on "closed" position. After the incubation all visible colonies were counted.

Table 4. Order of sampling.

A, B and C stands for the different agar plates that were used.

	Pos 1	Pos 2	Pos 3	Pos 4
Run 1	A	B	C	-
Run 2	B	C	- A	
Run 3	C	- A		B
Run 4	- A		B	C
Run 5	A	B	C	-
Run 6	B	C	- A	
Run 7	C	- A		B
Run 8	- A		B	C
Run 9	A	B	C	-
Run 10	B	C	- A	
Run 11	C	- A		B
Run 12	- A		B	C

Results:

The colony counts for individual plates were recorded and the results analyzed by ANOVA (general linear model, CFU versus Run, Position and Media), using Minitab 17 software.

The mean recovery per run (1 m^3) for each medium are shown in the table 5 below.

Table 5.

Product	Average recovery (cfu/m3)
TSA + LTHTh (Cat. No. 146069)	124
TSA + LT (Cat No. 146050)	127
TSA (Cat. No. 146001)	125

Interpretation:

The results indicate that there is no significant difference between the recoveries obtained with the 3 different TSA formulations from MilliporeSigma.

In comparison with the two competitor products, the MilliporeSigma formulation shows superior recovery rates, both statistically significant at 5% level.

Analysis for difference between the 3 media gave a P value of 91.9%, indicating that there is no significant difference between the recoveries on the 3 media.

For the comparison of the TSA + LTHTh agar with competitor media, the results were analyzed in the same way.

The mean recovery per run (1 m^3) for each medium are shown in the table 6 below.

Table 6.

Product	Average recovery (cfu/m3)
TSA + LTHTh 90mm ICR+ (Cat. No. 146683) 84	84
Competitor A	62
Competitor B	73

Analysis for difference between the 3 media gave a P value of 0.0%, indicating that there is a strongly significant difference between the recoveries on the 3 media.

To further investigate the recovery on TSA + LTHTh compared to each of the competitor products the results were compared pair-wise by Student's t-test (one-sided, assuming equal variances).

This recovery for Competitor A was significantly poorer than for TSA + LTHTh (t = 3.63, P = 0.07%).

The recovery for Competitor B was poorer than for TSA + LTHTh (t = 1.92, P = 3.4%).

To Place an Order or Receive Technical Assistance

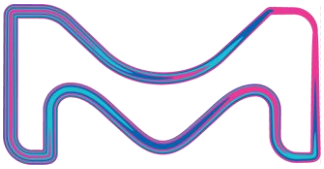
In the U.S. and Canada, call toll-free
1(800)-645-5476

For other countries across Europe, call
+44 (0) 115 943 0840

For other countries across Europe
and the world, please visit
merckmillipore.com/offices

For Technical Service, please visit
merckmillipore.com/techservice

Merck KGaA
Frankfurter Strasse 250
64293 Darmstadt
Germany



Lit. No. AN6110ENUS Ver. 1.0
BM-16-13064
06/2019