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 of safe 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 risks in the zones.[1]

Annex A describes how to determine airborne biological contamination in sterile production. 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.

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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. (Cat. No. 146683) was chosen. All members of the MAS-100showed comparable results for biological and physical efficiency as indicated in **Table 1**.

Table 1 Physical and biological efficiency of the MAS-100® family (results derived from independent validation of MAS-100® family acc. ISO 14698 with TSA + LTHTh Ref. No. 1466830120)

Characteristics	Iso-MH (1)	Iso-MH (9)	Iso	NT	VF
Physical Efficiency for particle size of 0.8 µm	60.41	62.58	60.20	n/a	n/a
Physical Efficiency for particle size of 1.0 µm	n/a	n/a	n/a	78.77	84.18
Physical Efficiency for particle size of 1.3 µm	71.68	82.60	76.91	84.10	85.81
Physical Efficiency for particle size of 2.2 µm	96.90	91.54	91.96	91.76	94.22
Physical Efficiency for particle size 5.4-6 µm	99.04	93.27	90.24	92.65	99.65
Biological efficiency	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 + LTHThio sedi	TSA + LTHThio sedi. - ICR (1467860020/1467860120)	Tryptic Soy Agar + LTHThio Sedi. – ICR+ (1467870020/1467870120)
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)

* part of ISO 14698 validation

Material

Table 3 TSA ICR test plates for air sampling

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

For both trials the agar plates were tested in a specific order (**Table 4**). Every sampling step consists of a three minutes delay and 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 (plates on "closed" position). After the incubation all visible colonies were counted.

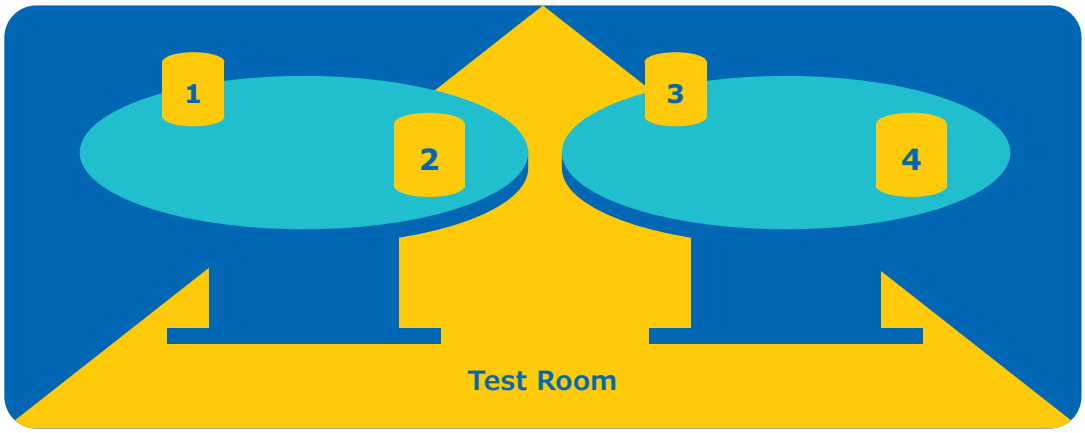

Figure 1 Sampling concept for active air sampling. Four MAS-100VF® instruments were placed on different positions on two tables in an uncontrolled environment outside of cleanroom.

Table 4 Order of sampling. A, B and C stands for the different agar plates that were used.

	Position 1	Position 2	Position 3	Position 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 Comparison of three different TSA formulations

Product	Average recovery (cfu/m^3)
Tryptic Soy Agar - ICR 30 mL (146001)	125
TSA + LT - ICR 30 mL (146050)	127
TSA + LTHTh - ICR 30 mL (146069)	124

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 Comparison different supplier

Product	Average recovery (cfu/m^3)
TSA + LTHTh 90mm ICR+ (Cat. No. 146683)	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%).

Conclusion

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

In comparison with the two competitor products, the TSA + LTHTh 90mm ICR+ formulation shows superior recovery rates, both statistically significant at 5% level.

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