Settle plates: six small mistakes that undermine your EM data

A practical guide for quality and microbiology teams running environmental monitoring in GMP cleanrooms, written for small sites without a large microbiology department.

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BEFORE YOU START

What settle plates can and can't tell you

Settle plates look simple: expose, incubate, count. The method rarely fails. The way it is applied does, and the data won't tell you when. A plate that was handled badly still gives you a number, and that number usually looks better than reality.

Passive

Plates collect particles that settle under gravity and room air movement. They do not measure a known air volume, so they complement active air sampling rather than replace it.

Semi-quantitative

A count is an estimate. Its value is in the pattern across locations and sessions over time, not in any single number.

Only as good as handling

Every step from media choice to incubation can change the result. Most of those steps lower the count, which is why the errors go unnoticed.

The limits you are working to

Grade	Settle plate action limit 90 mm, cfu / 4 hours	What that means in practice
A	No growth	Any growth should result in an investigation. Plates are exposed for the duration of operations, including set-up, and changed after a maximum of 4 hours.
B	5	Exposed for the duration of operations and changed after a maximum of 4 hours, as in grade A.
C	50	Exposure time (maximum 4 hours) and frequency based on quality risk management.
D	100	As for grade C.

Source: EU GMP Annex 1 (2022), section 9.30, Table 6 and footnotes. Exposure time should be based on validation including recovery studies, and individual plates may be exposed for less than 4 hours. Alert levels are yours to set from your own data (9.10–9.11). UK sites work to the same text through the MHRA.

How each section works

What goes wrong	The mistake as it usually happens on a busy site, and why it matters.
What good looks like	The practice an inspector expects to see, and the reason behind it.
Put it in your SOP	What your procedure should actually say, so good practice doesn't depend on who is on shift.
Ask yourself	One question to test your own programme today.

A note on judgement. This guide gives typical practice and typical figures. Exposure times, media, incubation and acceptance criteria must be justified by your own risk assessment and validation, written into your contamination control strategy (CCS), and decided with the support of your in-house quality team.

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MISTAKE

Using media that can't detect what's there

01

If the plate can't grow what lands on it, the room looks cleaner than it is.

WHAT GOES WRONG

- Plates without neutralisers are used where disinfectant or sporicide residues remain on surfaces, gloves or equipment nearby.
- In isolators and RABS decontaminated with vaporised hydrogen peroxide, residual peroxide inhibits growth on the plate.
- Media is chosen on price or habit, with no record of why.
- Growth promotion relies on the supplier's certificate alone, with no check that the media works after storage, transport and irradiation.

WHAT GOOD LOOKS LIKE

- A general-purpose medium such as tryptone soya agar, with neutralisers (commonly lecithin and polysorbate 80) chosen against the agents you actually use.
- Where VHP is used, data showing residual peroxide does not suppress recovery after decontamination and aeration.
- Irradiated, multi-wrapped plates for grade A and B, so the plates themselves don't bring contamination in.
- Growth promotion on each batch, or a documented rationale for relying on the supplier, plus a check before use for contamination, cracks and condensation.

Put it in your SOP

- Media type for each grade and the reason for it
- The neutralisers present and the disinfectants they cover
- Growth promotion organisms and acceptance criteria
- Storage conditions, shelf life and handling after receipt
- Pre-use inspection and reject criteria
- Link to the disinfectant rotation in your CCS

Why it matters. Annex 1 expects the recovery efficiency of your sampling methods to be supported by data (9.29). A medium that can't neutralise your disinfectants is the most common way to fail that expectation without knowing it.

ASK YOURSELF *Can you show, for each sampling point, why that medium was chosen and whether it neutralises the disinfectants you use there?*

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MISTAKE

Labelling the lid

02

Once a plate loses its identity, the result is unusable and traceability can't be recovered.

WHAT GOES WRONG

- Lids are labelled, then removed, swapped and placed back on the wrong base during exposure or reading.
- Labels are written across the centre of the base, hiding colonies.
- Handwritten labels smudge with condensation during incubation.
- Exposure start and stop times are written up afterwards from memory.

WHAT GOOD LOOKS LIKE

- The label goes on the base, around the edge, so colonies stay visible and the identity travels with the agar.
- Each plate carries location, date, exposure start and stop, media lot and operator, or a barcode that links to them.
- Pre-printed labels or barcodes for routine locations, so nothing is written in the cleanroom that doesn't need to be.
- Times are recorded at the moment of exposure and removal, on the plate or in the batch or session record.

Put it in your SOP

- Where the label goes and what it must contain
- Who records exposure times and when
- How plates are reconciled: number placed against number read
- What happens to an unidentifiable plate
- How results link to the batch or session record
- Label material that survives incubation

Reconcile every session. Count plates out and count them back. A missing grade A plate is a gap in continuous monitoring, and it needs to be handled as a deviation, not quietly replaced.

ASK YOURSELF *Pick any plate in today's incubator. Could you trace it back to location, time and operator without opening the lid?*

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MISTAKE

Placing plates where it's convenient

03

A plate in the wrong place gives you a clean result for a location nobody needed to know about.

WHAT GOES WRONG

- Plates sit where there is space on the bench, not where the product is exposed.
- Plates are placed in the path of first air, disrupting the protection they are meant to verify.
- Locations were set at qualification and never reviewed after changes to equipment, process or layout.
- No-one can explain why a location was chosen.

WHAT GOOD LOOKS LIKE

- Locations come from a documented risk assessment that sits within your CCS.
- Airflow visualisation (smoke studies) under dynamic conditions shows where air moves at critical points: open containers, the point of fill, stopper bowls and where interventions happen.
- Plates are as close to critical points as possible without obstructing first air or the operation.
- Every location is on a drawing with a written reason, and the map is reviewed after changes and against trend data.

Put it in your SOP

- Reference to the location drawing and its revision
- The rationale for each grade A and B location
- Rules for placement relative to first air
- When locations are reviewed and by whom
- How placement is covered in operator training
- Link to airflow visualisation studies

Annex 1 is explicit. Sampling locations, frequency, methods and incubation conditions should be defined in the monitoring programme and based on risk (section 9). An inspector will ask to see the reasoning, not just the map.

ASK YOURSELF *Is every settle plate location on a drawing, with a written reason it sits where it does?*

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MISTAKE

Over-exposing, then extrapolating

04

Contamination arrives in events, not at a steady rate. Arithmetic can't turn one into the other.

WHAT GOES WRONG

- Plates are left out beyond their validated window because a session ran long or nobody was free to change them.
- A count from a 1-hour plate is multiplied up to "cfu per 4 hours" to compare against the limit.
- Start and stop times are approximate, so the actual exposure is unknown.
- Extended exposure or larger plates are used without any validation behind them.

WHAT GOOD LOOKS LIKE

- Grade A and B plates are exposed for the whole of operations, including set-up, and changed at no more than 4 hours.
- The maximum exposure time is backed by a recovery study in your own airflow conditions.
- Exact times are recorded and actual counts are reported. A plate exposed for less than 4 hours is compared directly against the limit.
- A plate exposed beyond its validated time is a deviation, recorded and assessed, not a number to adjust.

A worked example. A grade B plate exposed for 1 hour grows 2 cfu. Multiplying to "8 cfu per 4 hours" and reporting an action limit breach invents data. So does dividing a 6-hour plate's count down to make it pass. Report 2 cfu against the limit of 5, with the exposure time, and let the trend do its job.

Put it in your SOP

- Maximum exposure time per grade and its validation reference
- How plates are changed during long sessions
- How exposure times are recorded
- That actual counts are reported, never normalised
- What to do when a plate exceeds its validated time
- Requirements for any extended-exposure method

ASK YOURSELF *Do your records show actual start and stop times for every plate, and is your exposure time backed by a recovery study?*

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MISTAKE

Accepting dried-out plates

05

A desiccated plate can read zero because particles bounce off a hard surface, not because the room was clean.

WHAT GOES WRONG

- Plates under unidirectional airflow lose water through exposure. The agar shrinks from the edge, wrinkles, cracks or turns hard and leathery.
- Dried plates are read and reported as normal results.
- An invalid plate is recorded as zero, or simply not recorded.
- Repeated desiccation at the same location is never noticed because nobody trends it.

WHAT GOOD LOOKS LIKE

- A recovery study shows how much water your plates lose at your airflow velocity and maximum exposure time, and that recovery is still acceptable.
- Written acceptance and reject criteria, with photographs, so every reader makes the same call.
- Severely desiccated plates are invalidated, recorded as invalid and assessed as a gap in monitoring.
- Invalid plates are trended by location. A pattern points to airflow, exposure time, storage or fill volume.

Put it in your SOP

- Visual grading scale with photographs
- Which grades are accepted, flagged or invalidated
- How an invalid plate is recorded and reported
- When desiccation triggers an investigation
- Trending of invalid plates by location
- Link to the exposure-time recovery study

Weight loss isn't the whole story. How much water a plate loses is a weak predictor of whether it can still recover organisms. Visual criteria backed by your own recovery data are more reliable. The decision tree on the next page shows how to apply them.

ASK YOURSELF *Is there a written rule for when a dried plate is rejected, and when did desiccation last trigger an investigation?*

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TOOL

Desiccation decision tree

Use this at plate reading. The grading scale is an example: build your own from photographs of your plates and the results of your recovery study.

1. Was the plate exposed within its validated time?

Check the recorded start and stop times against your SOP maximum.

Yes

Go to question 2.

No

Raise a deviation. Report the actual count with the actual exposure time, flag the result and assess why the plate was not changed.

▼

2. Grade the agar surface against your visual scale

Look at the edge, the surface texture and the agar thickness. Compare with an unexposed plate from the same lot if in doubt.

Grade 0

Normal. Smooth, moist surface, agar to the edge.

Accept

Grade 1

Slight shrinkage at the edge. Surface intact and smooth.

Accept and note the observation

Grade 2

Marked shrinkage or wrinkling. Surface no longer smooth.

Invalidate. Record as invalid and assess the monitoring gap

Grade 3

Cracked, split, hard or leathery.

Invalidate and raise a deviation

▼

3. Is this the second invalid plate at this location in your trending period?

Set the trigger in your SOP. Two in a quarter is a reasonable starting point for most small sites.

No

Record and continue routine trending.

Yes

Investigate the cause: airflow velocity at that point, exposure time, plate storage and age, fill volume. Consider a different plate format only with validation.

> **Never record an invalid plate as zero.** A zero says the room was clean. An invalid plate says you don't know. Those are different results, and an inspector will treat them differently.

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MISTAKE

Losing control after exposure

06

The plate is still part of the sample until it is read. What happens in transit and in the incubator changes the count.

WHAT GOES WRONG

- Plates wait for hours, sometimes overnight, before incubation.
- They travel stacked, tilted or loose, and are crammed into an incubator with a fixed fan.
- The incubation regime is whatever the lab has always done, with no data behind it.
- Plates are read once, by whoever is free, and positives are discarded before identification.

WHAT GOOD LOOKS LIKE

- A defined, validated window from end of exposure to incubation, recorded for every plate.
- Transport in closed containers, upright with lids on, without pressure on the lids.
- Incubation inverted, in an incubator mapped loaded, with a maximum load and stack height.
- A defined regime backed by recovery data. Many sites use two temperatures, such as 20–25 °C then 30–35 °C, to recover both fungi and bacteria. The order is debated: whichever you choose, justify it.
- Trained readers, defined reading times, and isolates from grade A and B kept for identification.

Put it in your SOP

- Maximum time from exposure to incubation
- Transport containers and orientation
- Incubation temperatures, durations and order
- Incubator load and mapping reference
- Reading times and reader training
- Identification requirements by grade

Identification is not optional in A and B. Annex 1 expects microorganisms found in grade A and B to be identified, and changes in flora to be part of your trending (9.11). That only works if positive plates reach the lab intact.

ASK YOURSELF *How long do plates actually wait between exposure and incubation, and has your incubator been mapped loaded?*

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TOOL

Settle plate self-audit checklist

Work through this with your SOP and last month's records in front of you. Every "no" is a finding worth closing before an inspector finds it.

01 Media

- [ ] Media and neutralisers are justified against the disinfectants in use
- [ ] VHP residues have been shown not to inhibit recovery (if VHP is used)
- [ ] Growth promotion approach is documented and followed
- [ ] Plates are inspected before use, with reject criteria

02 Identity and records

- [ ] Labels go on the base, around the edge
- [ ] Location, date, times, media lot and operator are traceable for every plate
- [ ] Exposure times are recorded at the time, not afterwards
- [ ] Plates are reconciled at the end of every session

03 Locations

- [ ] Every location is on a current drawing with a written rationale
- [ ] Grade A locations are informed by airflow visualisation
- [ ] Plates do not obstruct first air
- [ ] Locations were reviewed after the last relevant change

04 Exposure

- [ ] Grade A and B plates cover the whole of operations, changed at ≤ 4 hours
- [ ] Maximum exposure time is backed by a recovery study
- [ ] Actual counts are reported, never normalised to 4 hours
- [ ] Over-exposure is handled as a deviation

05 Desiccation

- [ ] A visual grading scale with photographs is in use
- [ ] Invalid plates are recorded as invalid, never as zero
- [ ] Invalid plates are trended by location
- [ ] Desiccation has a defined investigation trigger

06 After exposure

- [ ] Time from exposure to incubation is defined and recorded
- [ ] Transport and incubator loading are controlled
- [ ] The incubator has been mapped loaded
- [ ] Incubation regime is justified by recovery data
- [ ] Grade A and B isolates are identified and trended

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SUMMING UP

The thread running through all six

Trending only works if every plate is handled the same way, every time. Small inconsistencies don't show up as failures. They show up as a cleanroom that looks cleaner than it is, and that is the hardest kind of problem to find, because nothing ever goes red.

Each mistake in this guide has the same cure: decide what good looks like, write it into the SOP with the reason behind it, train it, and check it. None of it needs expensive equipment. All of it needs someone to own the detail.

Want a second pair of eyes on your EM programme?

A Mozeka Readiness Review looks at your quality system, your contamination control strategy and your environmental monitoring programme against EU GMP Annex 1, and gives you a prioritised list of what to fix.

Remote review from £1,950, plus VAT where applicable. **mozeka.co.uk/readiness-review**

Or email **hello@mozeka.co.uk**

References and further reading

EU GMP Annex 1 (2022)	Manufacture of Sterile Medicinal Products. EudraLex Volume 4. Section 9 covers environmental and process monitoring; Table 6 sets the settle plate limits.
USP <1116>	Microbiological Control and Monitoring of Aseptic Processing Environments.
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