20 YEARS OF INNOVATION

XENOMETRIX
Swiss Commitment for Bioassays
Ames MPF

Comparison of the Performance of the Colorimetric Ames MPF Assay with the Agar Plate Method
Ames MPF and Ames Agar Plate Test

Ames MPF is based on same principle as agar plate test but

- Liquid low-volume format
- Use of microplates and multichannel pipettes
- Colorimetric read-out
- Less test sample - up to 4 fold
- Less S9 – up to 12 fold
- Higher throughput

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Procedure Ames Microplate Assay

Bacterial stock
-80°C

Overnight culture

Assay preparation

37°C, 12-15 h
250 rpm

Exposure cultures
replicates 1 replicates 2 replicates 3

37°C, 90 min, 250 rpm
(20 min E. coli +S9)

Indicator medium

384-well plates

48 h, 37°C

8h, 37°C
Measuring Points

Agar Plate test

• 1 plate - 1 measuring point
• Individual handling:
  – 1 plate requires mixing of
  – 1 compound, agar and plating

liquid culture Ames MPF

• 1 plate - 24 measuring points
• Simultaneous handling of several replicates
Evaluation of Results

Colony counting of individual plates Automation possible

Colorimetry Counting sections of 48 Wells Automation possible

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## Throughput of compounds: Hands-on-time

1 sample, 5 concentrations, 5 strains (OECD), +/- S9, controls, triplicates manual handling, ready to use agar plates/top agar

<table>
<thead>
<tr>
<th></th>
<th>Agar Plate / 5 Conc.</th>
<th>MPF / 6 Conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample dilutions:</td>
<td>~5 min</td>
<td>~5 min</td>
</tr>
<tr>
<td>Top agar (preparation of tubes):</td>
<td>~35 min</td>
<td>-</td>
</tr>
<tr>
<td>Addition of sample, culture, S9:</td>
<td>~50 min</td>
<td>~25 min</td>
</tr>
<tr>
<td>Plating:</td>
<td>~40 min</td>
<td>-</td>
</tr>
<tr>
<td>Transfer to 384-well plates:</td>
<td>-</td>
<td>~40 min</td>
</tr>
<tr>
<td><strong>Handling time:</strong></td>
<td>~130 min</td>
<td>~70 min</td>
</tr>
<tr>
<td><strong>Counting time:</strong></td>
<td>~180 min</td>
<td>~20 min</td>
</tr>
<tr>
<td><strong>Total time:</strong></td>
<td>~5 h</td>
<td>~1½ h</td>
</tr>
</tbody>
</table>
Minimum of Test Sample Consumption

**Setup:** 5 strains (OECD 471), ½ log dilution steps, triplicates, -/+ S9

<table>
<thead>
<tr>
<th></th>
<th>Ames Agar Plate:</th>
<th>Ames MPF:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top dose:</td>
<td>5 mg/plate</td>
<td>5 mg/ml</td>
</tr>
<tr>
<td>Test sample:</td>
<td>220 mg</td>
<td>55 mg</td>
</tr>
</tbody>
</table>

- 4-fold less test sample
- Important when compound quantity is limited!
- Genotoxic impurities
S9 Consumption

Setup: 5 strains (OECD 471), ½ log dilution steps, triplicates, +/- S9

<table>
<thead>
<tr>
<th></th>
<th>Ames Agar Plate:</th>
<th>Ames MPF:</th>
</tr>
</thead>
<tbody>
<tr>
<td>S9 30%:</td>
<td>15.57 ml</td>
<td>1.35 ml</td>
</tr>
<tr>
<td>S9 10%:</td>
<td>5.25 ml</td>
<td>0.45 ml</td>
</tr>
</tbody>
</table>

- 4- up to 11-fold less S9
- Reduced number of sacrificed animals!
- Acc. to 3Rs: Replace, Reduce, Refine!
Sample Concentration - Comparison

Liquid exposure with 5 mg/ml (MPF) or 5 mg/plate (pre-incubation)*

<table>
<thead>
<tr>
<th></th>
<th>Addition</th>
<th>Stock</th>
<th>Final Volume</th>
<th>Final concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ames MPF</td>
<td>10 µl</td>
<td>125 mg/ml</td>
<td>0.25 ml</td>
<td>5.0 mg/ml</td>
</tr>
<tr>
<td>Pre-Incubation</td>
<td>100 µl</td>
<td>50 mg/ml</td>
<td>0.70 ml</td>
<td>7.1 mg/ml</td>
</tr>
</tbody>
</table>

*MPF method and Pre-incubation method: Both exposures performed in liquid media

⇒ Bacteria incubated with constant sample concentrations

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Cytotoxicity in Ames MPF

Cytotoxicity can be detected easily:

- Reduction of revertant wells and

Increased brilliance of purple medium  Lipid droplets (bubbles) without S9
Colored compounds - colorimetric read-out

Orange instead of yellow wells
Easily detectable
Best Assay for Genotoxic Impurities, ICH M7

Note ICH M7
To assess the mutagenic potential of impurities, a single bacterial mutagenicity assay can be carried out with a fully adequate protocol according to ICH S2(R1) and OECD 471 guidelines. For degradants that are not feasible to isolate or synthesize or when compound quantity is limited, bacterial mutagenicity testing could be carried out using a miniaturized assay format with proven high concordance to the ICH-compliant assay to enable testing at higher concentrations with justification...
High concordance with agar plate test

- TA98, TA1537, TAMix compared with all strains NTP
- 25 chemicals tested
- Overall agreement: 88%

- TA98, TA100, TA1535, TA1537 MPF, TAMix vs published results traditional Ames
- Ames MPF PENTAG I (strains as above plus EC Combo) vs. published traditional Ames
- Overall agreement: 89 - 100%
High concordance with agar plate test

- Overall agreement 84.2%
- trad. Ames (all strains) vs Ames II (TA98, TAMix): 84.2% (16/19)
- Inter-laboratory consistency of 89.5% (17/19).
High concordance with agar plate test

- 84% agreement agar plate and Ames MPF in identifying mutagens and non-mutagens
- Discordant results included chemicals requiring reductive metabolism using FMN, hamster liver S9
- 83% Concordance
- Ames II vs. traditional Ames using 42 company-own chemicals
- No false positive results
High concordance with agar plate test

Direct Comparison  Ames MPF - Ames Pre-incubation

• 15 equivocal to weakly positive chemicals
• Same overnight cultures, chemicals and S9 to exclude external variations (culture growth, chemical purity, weighing errors, S9 activity)
• Parallel tests with most responsive strains of the NTP database (mg/plate vs. mg/ml)
• Each test was repeated at least once
• 87% concordance (13/15)
• Excellent concordance for equivocal to weak positive chemicals
• Confirms the high concordance with the ICH-compliant assay
Conclusion

- Ames MPF – Ames agar test: same principle, same tester strains
- Well documented, excellent study data available, mean concordance of 87%
- 4 x less test sample necessary
- Liquid microplate format allows for less hands-on-time, simultaneous processing of several replicates
- Higher throughput, partly automatable
- 3Rs: Up to 12 fold less consumption of S9 rat liver fraction from sacrificed animals
- Quick, easy colorimetric read-out, less error prone
- Less plastic ware, reduced contaminated waste in environment
More information

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