

| Technical Data Sheet                 |  |                    |      |              |      |             |    |                    |      |      |    |               |      |      |     |                 |      |                  |  |  |  |
|--------------------------------------|--|--------------------|------|--------------|------|-------------|----|--------------------|------|------|----|---------------|------|------|-----|-----------------|------|------------------|--|--|--|
| <b>Use in</b>                        | <ul style="list-style-type: none"> <li>Pharmaceutical Industry in clean rooms and isolators</li> <li>For industrial, laboratory &amp; research applications only</li> </ul>  |                    |      |              |      |             |    |                    |      |      |    |               |      |      |     |                 |      |                  |  |  |  |
| <b>Use for</b>                       | <ul style="list-style-type: none"> <li>Detection of aerobic and anaerobic micro-organisms</li> <li>Contact sampling, personnel monitoring, as well as active air monitoring</li> <li>Isolation and growth of fastidious bacteria, yeasts and moulds</li> <li>Universal neutralization of residues of disinfectants</li> </ul> <p>The medium should be applied with a uniform and steady pressure to the surface for a few seconds. After sampling the surface must be cleaned to remove residues of the medium.</p>                        |                    |      |              |      |             |    |                    |      |      |    |               |      |      |     |                 |      |                  |  |  |  |
| <b>Typical composition per liter</b> | <table> <tbody> <tr> <td>Casein peptone</td> <td>15g</td> <td>Lecithin (L)</td> <td>0,7g</td> </tr> <tr> <td>Soy peptone</td> <td>5g</td> <td>Polysorbate 80 (T)</td> <td>5,0g</td> </tr> <tr> <td>NaCl</td> <td>5g</td> <td>Histidine (H)</td> <td>0,5g</td> </tr> <tr> <td>Agar</td> <td>15g</td> <td>Thiosulfate (T)</td> <td>0,1g</td> </tr> <tr> <td>Neutralizer PLUS</td> <td></td> <td></td> <td></td> </tr> </tbody> </table> <p>This medium can be adjusted / or supplemented according to the performance criteria required.</p> | Casein peptone     | 15g  | Lecithin (L) | 0,7g | Soy peptone | 5g | Polysorbate 80 (T) | 5,0g | NaCl | 5g | Histidine (H) | 0,5g | Agar | 15g | Thiosulfate (T) | 0,1g | Neutralizer PLUS |  |  |  |
| Casein peptone                       | 15g  | Lecithin (L)       | 0,7g |              |      |             |    |                    |      |      |    |               |      |      |     |                 |      |                  |  |  |  |
| Soy peptone                          | 5g   | Polysorbate 80 (T) | 5,0g |              |      |             |    |                    |      |      |    |               |      |      |     |                 |      |                  |  |  |  |
| NaCl                                 | 5g   | Histidine (H)      | 0,5g |              |      |             |    |                    |      |      |    |               |      |      |     |                 |      |                  |  |  |  |
| Agar                                 | 15g  | Thiosulfate (T)    | 0,1g |              |      |             |    |                    |      |      |    |               |      |      |     |                 |      |                  |  |  |  |
| Neutralizer PLUS                     |  |                    |      |              |      |             |    |                    |      |      |    |               |      |      |     |                 |      |                  |  |  |  |
| <b>Irradiation</b>                   | <ul style="list-style-type: none"> <li>Gamma-irradiated at 9-20 kGy</li> </ul>   |                    |      |              |      |             |    |                    |      |      |    |               |      |      |     |                 |      |                  |  |  |  |
| <b>Filling volume</b>                | <ul style="list-style-type: none"> <li>16-19ml</li> </ul>  |                    |      |              |      |             |    |                    |      |      |    |               |      |      |     |                 |      |                  |  |  |  |
| <b>Packaging</b>                     | <ul style="list-style-type: none"> <li>Triple bagged, staples of 10 plates</li> <li>Transparent</li> <li>High barrier foil for H<sub>2</sub>O<sub>2</sub> as well as for water-vapor</li> <li>10 staples of 10 plates per packaging unit</li> <li>Temperature isolated handle-bag in the cardboard-boxes</li> </ul>  |                    |      |              |      |             |    |                    |      |      |    |               |      |      |     |                 |      |                  |  |  |  |
| <b>Units per pack</b>                | <ul style="list-style-type: none"> <li>100 plates</li> </ul>   |                    |      |              |      |             |    |                    |      |      |    |               |      |      |     |                 |      |                  |  |  |  |
| <b>Shelf life</b>                    | <ul style="list-style-type: none"> <li>9 months from production date</li> </ul>  |                    |      |              |      |             |    |                    |      |      |    |               |      |      |     |                 |      |                  |  |  |  |
| <b>Storage</b>                       | <ul style="list-style-type: none"> <li>Recommended storage temperature: 15-25°C</li> <li>Can be stored at temperatures outside of the recommended storage temperature for periods up to 72 hours (e.g., down to 4°C or up to 35°C) without having an impact on growth promotion properties</li> <li>Should be stored at temperatures as stable as possible</li> </ul>  |                    |      |              |      |             |    |                    |      |      |    |               |      |      |     |                 |      |                  |  |  |  |
| <b>Label</b>                         | <ul style="list-style-type: none"> <li>On the side, at the bottom</li> </ul>   |                    |      |              |      |             |    |                    |      |      |    |               |      |      |     |                 |      |                  |  |  |  |
| <b>Label information</b>             | <ul style="list-style-type: none"> <li>Product name: TSA U+</li> <li>Expiry date: YYYYMMDD → MMM in letters (e.g.: 2023Nov04)</li> <li>Lot-number</li> <li>Individual number</li> <li>Barcode</li> </ul>   |                    |      |              |      |             |    |                    |      |      |    |               |      |      |     |                 |      |                  |  |  |  |

| <b>Technical Data Sheet</b> |   |
|-----------------------------|---|
| <b>Barcode</b>              | <ul style="list-style-type: none"> <li>• 2-dimensional (data matrix), 20 digits:</li> <li>• Digits 1-3: Art.-No.</li> <li>• Digits 4-9: Lot-Number</li> <li>• Digits 10-14: Individual-Number</li> <li>• Digits 15-20: Date (YYMMDD)</li> </ul>   |
| <b>Delivery</b>             | <ul style="list-style-type: none"> <li>• Temperature controlled delivery on request</li> <li>• For shipments of larger amounts plastic pallets in Euro-size are used</li> </ul>   |
| <b>Petri dish</b>           | <ul style="list-style-type: none"> <li>• Incubations in vent and closed position possible</li> <li>• Specific design to improve binding of agar to plate</li> <li>• Easy handling due to increased handling area</li> </ul>   |
| <b>Locking lid</b>          | <ul style="list-style-type: none"> <li>• Locking-lid plate, made from polystyrene</li> <li>• Inner diameter: ~ 56.5 mm, thus providing an area of ~25cm<sup>2</sup></li> <li>• Outer diameter: ~ 66 mm</li> <li>• Bottom part with 1cm<sup>2</sup> square grid for facilitated evaluation</li> </ul>  |
| <b>Lid positions</b>        | <ul style="list-style-type: none"> <li>• All plates are delivered in the non-locked position</li> <li>• The plate contains 2 locked positions. If turning the lid clockwise the locked positions are in the following order:               <ol style="list-style-type: none"> <li>1. Vent position</li> <li>2. Closed position</li> </ol> </li> </ul>   |
| <b>Aerobic incubation</b>   | <ul style="list-style-type: none"> <li>• Turn the lid clockwise to the right to the end into the final stop position</li> <li>• The lid locks in the closed position</li> <li>• Ideal incubation condition for aerobic micro-organisms</li> <li>• Limits the dehydration of the agar during incubation</li> </ul>   |
| <b>Anaerobic incubation</b> | <ul style="list-style-type: none"> <li>• The vent position is ideal for anaerobic incubations, as it allows an easy and effective removal of oxygen under anaerobic incubation conditions</li> <li>• Incubate in anaerobic incubator, anaerobic jar or suitable equipment</li> </ul> <ol style="list-style-type: none"> <li>1. First option:           <ul style="list-style-type: none"> <li>• Turn the lid clockwise to the right to the end into the final stop position</li> <li>• Turn the lid one click counter-clock-wise to the vent position</li> </ul> </li> <li>2. Second option:           <ul style="list-style-type: none"> <li>• Turn the lid clockwise directly into the first locked position</li> </ul> </li> </ol> |
| <b>Place of production</b>  | PharmaMedia Dr. Müller GmbH<br>Gustav-Throm-Str. 1, 69181 Leimen - Germany  |

| <b>Quality control, Certificates</b>                               |   |  |
|--|---|--|
| <b>Certificates</b>  | Each lot of product can be obtained with a certificate of analysis (CoA):   |  |
|  | <b>Physico-chemical test parameters:</b>  |  |
|  | Appearance  | Slightly turbid, yellowish                   |
|  | pH value  | 7,1 – 7,5                                    |
|  | Filling volume  | 16 – 19ml                                    |
|  | Irradiation   | 9-20 kGy                                     |
|  | <b>Growth Promotion test: 10-100 CFU</b>  |  |
|  | <i>S. aureus</i>  | ATCC 6538    30-35°C    1 day    50-200%     |
|  | <i>E. coli</i>  | ATCC 8739    30-35°C    1 day    50-200%     |
|  | <i>P. aeruginosa</i>  | ATCC 9027    30-35°C    1 day    50-200%     |
|  | <i>B. subtilis</i>  | ATCC 6633    30-35°C    1 day    50-200%     |
|  | <i>S. aureus</i>  | ATCC 6538    30-35°C    1 day    50-200%     |
|  | <i>C. albicans</i>  | ATCC 10231    20-25°C    3-5 days    50-200% |
| <i>A. brasiliensis</i>   | ATCC 16404    20-25°C    3-5 days    50-200%  |  |
| <b>Neutralizer PLUS test: 10-100 CFU, 20µl Biocide A per plate</b> |   |  |
| <i>B. subtilis</i>   | ATCC 6633    30-35°C    1 day    50-200%  |  |
| <b>Sterility control</b>   |   |  |
|  | No growth   |  |
| <b>Certificate of origin</b>                                       | <p>All media lots produced by PMM can be obtained with a Certificate of Origin (CoO). All animal derived raw materials are specified as follows:</p> <ul style="list-style-type: none"> <li>• Raw material</li> <li>• Tissue</li> <li>• Animal source</li> <li>• Country of origin</li> <li>• Infectivity category (acc. to TSE guideline: EMA/410/01 rev. 3)</li> </ul>  |  |
| <b>BSE policy</b>  | <ul style="list-style-type: none"> <li>• In compliance with the current note for guidance on minimizing the risk of transmitting animal spongiform encephalopathy via human or veterinary medicinal products, we check the CoO of raw material in respect to the specified animal source, the country of origin and the infectivity category. We neither store or process ruminant raw materials obtained from high infectivity tissues (IA) nor ruminant raw materials whose animal source originates from countries or regions with an undetermined risk (cat C/GBR IV).</li> </ul> |  |
| <b>Temperature stress</b>  | <ul style="list-style-type: none"> <li>• Art. 101.0100 has been exposed to temperature stress conditions (3 days at 2-8°C as well as 3 days at 30-35°C) and has passed shelf-life testing at least 30 days after the assigned expiry date. Shelf-life testing comprise all regular tests which are part of the normal release test of this article (see CoA).</li> </ul>  |  |

### Neutralization of residues of disinfectants

The disinfection of surfaces is crucial for maintaining an adequate environment for the production of sterile pharmaceutical drugs. To guarantee the best possible success of the disinfection process many pharmaceutical companies do perform a regular rotation of the disinfectants used. Quite often at least one of the disinfectants used contains quaternary ammonium compounds, benzalkonium compounds, biguanides or even a combination of these substances. The advantages of such disinfectants are the well proved bactericidal activity against microorganisms even if used in relatively low concentrations. However, the disadvantages are the residues which remain on treated surfaces, if not removed by a suitable cleaning step.

The removal or inactivation of residues of disinfectants is critical for the reliable detection of viable and cultivable microorganisms. If highly active residues remain on surfaces, these will be picked up with contact plates or swabs when performing environmental monitoring tests. Then these residues can interfere with the growth of potential contaminants and this could finally result in false negative results.

Whereas some residues of disinfectants can be neutralized with the standard neutralizers LTHT (Lecithin, Tween 80, Histidine and Thiosulfate – please see product description of art. 100.0100) especially the residues of quaternary ammonium compounds, benzalkonium compounds as well as biguanides are not sufficiently inactivated by these neutralizers.

To overcome this unsatisfactory inactivation of these residues, media manufacturers have tried to develop special neutralizer media. However, most of the media offered so far had different drawbacks: turbidity, precipitation, short shelf-life, low recovery rates on Gram positive strains and quite high price - and due to these disadvantages, such media have not been really accepted. PMM now offers a newly designed plate without showing these drawbacks. **TSA U+ plates** look like a regular TSA plate and are free of precipitation throughout the shelf-life of more than 9 months. However, the outstanding inactivation of all typically used disinfectants including even high concentrations of quaternary ammonium compounds, benzalkonium compounds and biguanides really is the big step forward in obtaining reliable results for the environmental monitoring.

TSA U+ plates were tested with respect to the inactivation of disinfectants using the worst-case approach by directly inoculating defined amounts of disinfectant on the agar plates. Typically, 20µl, 50µl or 100µl of disinfectant was used. 100µl of disinfectant applied to a contact plate of about 25 cm<sup>2</sup> surface correspond to about 40ml of disinfectant used to disinfect an area of one square meter, a concentration typically used in the pharmaceutical industry. After a period of 15 to 20 min the test organisms were applied to the treated plates.

Test organisms used for such neutralization tests could be for example *B. subtilis* ATCC 6633, *S. aureus* ATCC 6538 and *S. epidermidis* ATCC 14990 as well as *E. coli* ATCC 8739, *P. aeruginosa* ATCC 9027, *C. albicans* ATCC 10231 and *A. brasiliensis* ATCC 16404. However, as Gram positive microorganisms are typically more sensitive to quaternary ammonium compounds, it is recommended to perform the tests with Gram positive microorganisms.

As reference, plates not treated with disinfectant are used.

| Quality control, Certificates |  |
|-------------------------------|--|
|                               | <p>Specifications: for sufficient inactivation of disinfectants the amount of 50µl of a disinfectant applied to a contact plate must be inactivated, resulting in a recovery rate of more than 50%.</p> <p>Results: Beside the disinfectants inactivated already by our standard plate (see product description of art. 100.0100) <b>TSA U+ plates</b> are as well inactivating quite high concentrations of quaternary ammonium compounds, biguanides and benzylalkoniumchlorides. Disinfectants tested were Amphospray 41 IP, Gigasept AF (4%), Hexanios G+R, Hexaquart forte (2%), Incidin plus (2%), Biocide A, Biocide B, Lysoformin 3000 (2%), Melsept SF (2%), Microbac forte (2%) and Terralin protect (2%).</p> <p>Results obtained with the above listed disinfectants show recovery rates of more than 70% if 20 or 50µl of the disinfectant was applied directly on <b>TSA U+ plates</b>. Even when applying 100µl most recovery rates were above 70%, only few recovery rates dropped to values between 30 to 50%. In comparison to these results standard TSA plates with neutralizers did not show any or very low recovery rates even if only 20µl of these disinfectants were applied. As a conclusion <b>TSA U+ plates</b> can be considered as the universal media for performing environmental monitoring, delivering reliable results independent from the disinfectant used.</p> |

| Safety Data                       |  |
|-----------------------------------|--|
| <b>Toxic ingredients</b>          | <ul style="list-style-type: none"> <li>• None</li> </ul>                     |
| <b>Basic composition</b>          | <ul style="list-style-type: none"> <li>• See typical composition</li> </ul>  |
| <b>Solvent content</b>            | <ul style="list-style-type: none"> <li>• None</li> </ul>                     |
| <b>Safety data sheet required</b> | <ul style="list-style-type: none"> <li>• Not mandatorily required</li> </ul> |