LEAD THE WAY IN BLOOD SAFETY

THERAFLEX MB-Plasma
The safety of fresh frozen plasma (FFP) in developed countries has improved significantly over the past two decades due to stringent donor selection criteria and improved screening tests. In certain countries, safety is further enhanced by leucodepletion and pathogen inactivation. The greatest concern driving the development of pathogen reduction technologies is the prevention of blood supply contamination by new pathogens or new strains of known pathogens for which no tests currently exist. Additionally, the accumulation of separate measures such as bacterial screening + viral testing + NAT + gamma irradiation increases the overall cost of blood components. Pathogen inactivation raises the safety margin by inactivating pathogens that have gone undetected during screening due to seroconversion window periods or false results (negative or positive test). Ultimately, pathogen inactivation provides a proactive approach, inactivating emerging pathogens before they enter the blood supply chain and before screening tests have been developed and implemented.

Transfusion-transmitted infections: risk per unit transfused.

Modified from Klein et al.²

The SAFEST & USER-FRIENDLY technology for effective pathogen inactivation in SINGLE UNITS OF PLASMA
THE THERAFLEX MB-Plasma

PROCESS: ULTIMATE SAFETY FOR FFP TRANSFUSION

The THERAFLEX MB-Plasma kit incorporates a dockable set suitable for both whole blood and aphaeresis plasma. The process requires a simple dry set consisting of:
- a Plasmaflex filter for leucodepletion, removal of residual red cells, platelets and aggregates,
- a Methylene Blue pill (85μg anhydrous MB chloride),
- an illumination bag,
- a Blueflex filter for MB and photoproduct retention,
- and a storage bag.

The initial plasma volume range to be connected to the THERAFLEX MB-Plasma system is 235ml-330ml.

BLOOD SAFETY

- **FAST**
  - Short illumination cycle (~15 min.) due to optimal wavelength (630nm) of LED sources (light-emitting diodes)
- **SMALL**
  - Optimal dimensions for bench usage
  - 2 bags/cycle
  - Operated by touchscreen
- **SAFE**
  - Import/export with Local Information System (LIS) through the MacoTrace Data Management System
  - Full GMP-Procedure
  - Full IT reporting of illumination cycle

The MacoTronic B2: the newest generation of illumination device for THERAFLEX MB-Plasma

THE THERAFLEX MB-Plasma SYSTEM:
A user-friendly and effective pathogen inactivation technique against enveloped and non-enveloped viruses for single units of plasma.
MECHANISM OF ACTION

Methylene blue is a phenothiazine-based photosensitizer with particular affinity for guanosine-cytosine pairs.

It intercalates into viral nucleic acid and subsequent illumination generates singlet oxygen leading to guanosine oxidation and destruction of the viral nucleic acid preventing viral replication.\(^3,4\)

**FOCUS ON METHYLENE BLUE**

- Methylene Blue has a monograph in the European Pharmacopeia (8th edition, 2014) and the US Pharmacopeia (USP 38-NF 33, 2015)
- Methylene Blue is in clinical use for organ staining, as disinfectant drug and for reversal of methemoglobinemia in very high concentrations (1,000 to 10,000 times higher than used in the THERAFLEX MB-Plasma)

**PROCESS SPECIFICATIONS**

| Preparation before illumination (sterile connection, plasma filtration, MB dilution, purge, seal-off) | Total time 15 min 30 sec | Hands-on time 4 min |
| Illumination process (loading, cycle, labeling, unloading) | Total occupation time of the illuminator 16 min 45 sec | Hands-on time 1 min 45 sec |
| Preparation after illumination (transfer, purge, seal-off) | Total time 12 min 40 sec | Hands-on time 40 sec |

**TOTAL PROCESSING TIME** 44 min 55 sec

**TOTAL HANDS-ON TIME** 6 min 25 sec

THERAFLEX MB-Plasma

THROUGHPUT WITH THE MACOTRONIC B2

This is an example of a potential process design. Tailor made solutions corresponding to individual customer requirements are offered by Macopharma.

**PROCESS THROUGHPUT**

| Design | 1 FTE* / 3 MacoTronic B2 |
| Hourly throughput | 18.25 bags/hour |
| Daily throughput | 146 bags/day** |
| Annual throughput | 37,960 bags/year*** |

* FTE = Full Time Equivalent
** 8 hours a day, 260 worked days per year
*** 8 hours a day, 260 worked days per year

Simple and fast procedure (3 steps)

1. Preparation before illumination (sterile connection, plasma filtration, MB dilution, purge, seal-off)
2. Illumination process (loading, cycle, labeling, unloading)
3. Preparation after illumination (transfer, purge, seal-off)

Flexibility

- Time between collection & freezing ≤ 24h
- Immediate availability of the treated plasma

**BLOOD SAFETY FOCUS ON METHYLENE BLUE**

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**MECHANISM OF ACTION**

Methylene Blue molecule

- Methylene Blue is a phenothiazine-based photosensitizer with particular affinity for guanosine-cytosine pairs.
- It intercalates into viral nucleic acid and subsequent illumination generates singlet oxygen leading to guanosine oxidation and destruction of the viral nucleic acid preventing viral replication.\(^3,4\)

1. Intercalation of Methylene Blue into nucleic acid
2. Visible light
3. Formation of singlet oxygen
4. Destruction of the viral nucleic acid

**PROCESS THROUGHPUT**

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- Methylene Blue is in clinical use for organ staining, as disinfectant drug and for reversal of methemoglobinemia in very high concentrations (1,000 to 10,000 times higher than used in the THERAFLEX MB-Plasma)
Plasma quality is maintained after treatment:
- No influence on complement system, inhibitors of coagulation, fibrinolysis markers or ADAMTS13
- Coagulation factors and activation are only moderately affected (Fibrinogen, Factor V, VIII, XI) and remain within the specifications set by the Council of Europe Guidelines
- Moderate enhanced thrombin time and aPTT
- Very little effect on the strength of clot formation as assessed by thrombelastometry (Cardigan et al., Transfusion 2009)16

### Plasma Parameters of Whole Blood-Derived Plasma After MB Treatment:

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>NORMAL VALUES</th>
<th>THERAFLEX MB-Plasma (MACOPHARMA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen (F I) [Clauss] g/l</td>
<td>1.50 – 3.50</td>
<td>1.911, 2.111, 1.977, 2.000, 1.995, 2.415, 2.161, 2.231, 2.351, 2.311, 2.2921</td>
</tr>
<tr>
<td>Prothrombin (FII) U/ml</td>
<td>0.7 - 1.3</td>
<td>0.969, 0.989, 0.9999</td>
</tr>
<tr>
<td>Factor V U/ml</td>
<td>0.7 - 1.3</td>
<td>0.661, 0.799, 0.841, 0.761, 0.799, 1.011, 1.021,</td>
</tr>
<tr>
<td>Factor VIII U/ml</td>
<td>0.7 - 1.3</td>
<td>0.989, 1.021, 1.011, 1.031</td>
</tr>
<tr>
<td>Factor IX U/ml</td>
<td>0.5 - 1.5</td>
<td>0.769, 0.889, 0.839, 0.669, 0.629, 0.811, 0.901</td>
</tr>
<tr>
<td>Factor IX U/ml</td>
<td>0.5 - 1.5</td>
<td>0.749, 0.839, 0.811, 0.701, 0.769, 0.821</td>
</tr>
<tr>
<td>Factor XI U/ml</td>
<td>0.5 - 1.5</td>
<td>1.151, 0.889, 0.961, 1.001</td>
</tr>
<tr>
<td>Factor XII U/ml</td>
<td>0.7 - 1.3</td>
<td>1.029, 1.011, 1.011</td>
</tr>
<tr>
<td>Factor XII U/ml</td>
<td>0.7 - 1.3</td>
<td>0.761, 0.849, 0.521, 0.759, 0.821</td>
</tr>
<tr>
<td>Antithrombin U/ml</td>
<td>0.7 - 1.3</td>
<td>0.941, 0.969, 0.121, 0.871</td>
</tr>
<tr>
<td>Protein C U/ml</td>
<td>0.7 - 1.3</td>
<td>0.949, 0.899, 1.101, 0.801</td>
</tr>
<tr>
<td>Protein S U/ml</td>
<td>0.7 - 1.3</td>
<td>1.121, 0.991, 0.759, 0.941</td>
</tr>
<tr>
<td>vWF cleaving percollase U/ml</td>
<td>0.8 - 1.2</td>
<td>1.111, 1.311, 0.741, 0.501</td>
</tr>
<tr>
<td>PT (sec)</td>
<td>11.15</td>
<td>13.19</td>
</tr>
<tr>
<td>Fibrinogen (F I)</td>
<td>1.50 – 3.50</td>
<td>0.969, 0.989, 0.9999</td>
</tr>
<tr>
<td>aPTT (sec)</td>
<td>23 - 35</td>
<td>34.11, 40.1, 34.11</td>
</tr>
<tr>
<td>Prothrombin Fragments F1 + 2 (mM)</td>
<td>0.4 - 1.4</td>
<td>1.229, 0.859, 1.031</td>
</tr>
<tr>
<td>Total protein (g/l)</td>
<td>5.91</td>
<td></td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>K+ (mMol/l)</td>
<td>3.21</td>
<td></td>
</tr>
</tbody>
</table>
SAFETY PROFILE OF THERAFLEX MB-Plasma

When exposed to visible light, MB is highly effective in inactivating lipid-enveloped viruses such as HIV, HBV, HCV and the newly emergent West Nile Virus, non-enveloped viruses such as Parvovirus B19 and bacteria.

INACTIVATION OF NON-ENVELOPED VIRUSES

<table>
<thead>
<tr>
<th>VIRUS</th>
<th>FAMILY</th>
<th>REDUCTION RATE (log 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2A-SV40</td>
<td>Papova</td>
<td>≧ 5</td>
</tr>
<tr>
<td>Caki</td>
<td>Calici</td>
<td>≧ 3.9</td>
</tr>
<tr>
<td>SV 40</td>
<td>Papova</td>
<td>≧ 4</td>
</tr>
<tr>
<td>Parvo B19</td>
<td>Parvo</td>
<td>≧ 5</td>
</tr>
</tbody>
</table>

A final virus content below the detection limit implies a depletion at least as equivalent as the initial virus content.

INACTIVATION OF ENVELOPED VIRUSES

<table>
<thead>
<tr>
<th>VIRUS</th>
<th>FAMILY</th>
<th>REDUCTION RATE (log 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1 Retro</td>
<td></td>
<td>≧ 5.45</td>
</tr>
<tr>
<td>HIV-1 Frix</td>
<td></td>
<td>≧ 5.78</td>
</tr>
<tr>
<td>HCV Frix</td>
<td></td>
<td>≧ 5.84</td>
</tr>
<tr>
<td>DENGUE 1-4 Frix</td>
<td></td>
<td>≧ 4.66 - 5.78</td>
</tr>
<tr>
<td>H. cholera Frix</td>
<td></td>
<td>≧ 5.92</td>
</tr>
<tr>
<td>Duck HBV 437</td>
<td>Hepadna</td>
<td>≧ 6.0</td>
</tr>
<tr>
<td>PRV</td>
<td>Hepadna</td>
<td>≧ 5.48</td>
</tr>
<tr>
<td>CMV</td>
<td>Hepadna</td>
<td>≧ 4.08</td>
</tr>
<tr>
<td>Herpes Simplex</td>
<td>Hepadna</td>
<td>≧ 5.50</td>
</tr>
<tr>
<td>Bovine herpes</td>
<td>Hepadna</td>
<td>≧ 8.11</td>
</tr>
<tr>
<td>Influenza H1N1</td>
<td>Orthomyxo</td>
<td>≧ 4.40</td>
</tr>
<tr>
<td>Influenza H2N2</td>
<td>Orthomyxo</td>
<td>≧ 6.1</td>
</tr>
<tr>
<td>BIV</td>
<td>Coronaviridae</td>
<td>≧ 4.90</td>
</tr>
<tr>
<td>Semliki Forest</td>
<td>Togaviridae</td>
<td>≧ 7.00</td>
</tr>
<tr>
<td>Sindbis</td>
<td>Togaviridae</td>
<td>≧ 7.73</td>
</tr>
<tr>
<td>Chikungunya</td>
<td>Togaviridae</td>
<td>≧ 6.88</td>
</tr>
<tr>
<td>Vesicular Stomatitis</td>
<td>Rhodoviridae</td>
<td>≧ 4.89</td>
</tr>
</tbody>
</table>

Bacterial reduction is achieved after both filtration steps (Plasma filtration with the PLAS4 filter and MB-treated plasma filtration with the Blueflex filter) in the treated plasma. The overall reduction capacity of the THERAFLEX MB-Plasma system is sufficient to prevent transfusion-transmitted bacterial infections, taking into account the concentration of bacteria normally present in contaminated therapeutic plasma25.

Plasma filtration not only decreases transfusion reactions and HLA alloimmunisation but also provides the benefit of removing cell-associated pathogens such as cytomegalovirus (CMV) and human T-cell lymphotropic viruses (HTLV) I and II.

REMOVAL OF PARASITES

<table>
<thead>
<tr>
<th>PARASITE</th>
<th>LOG 10 REDUCTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trypanosoma cruzi</td>
<td>≧ 4.0 to ≧ 5.8</td>
</tr>
</tbody>
</table>

REMOVAL OF CELLS (INCLUDING INTRACELLULAR VIRUSES)

<table>
<thead>
<tr>
<th>BACTERIA SPECIES</th>
<th>CUMULATIVE LOG 10 REDUCTION FACTOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>F. cholera (PEI-8-19)</td>
<td>≧ 4.8</td>
</tr>
<tr>
<td>S. epidermidis (PEI-8-06)</td>
<td>≧ 4.9</td>
</tr>
<tr>
<td>S. aureus (PEI-8-23)</td>
<td>≧ 4.8 and ≧ 5.9</td>
</tr>
<tr>
<td>B. cereus (PEI-8-07)</td>
<td>≧ 4.9</td>
</tr>
<tr>
<td>K. pneumoniae (PEI-8-24)</td>
<td>≧ 4.8</td>
</tr>
<tr>
<td>B. subtilis spore preparation (DSM 618)</td>
<td>≧ 5.0</td>
</tr>
<tr>
<td>B. brevis (DSM 1635)</td>
<td>≧ 3.7 and ≧ 5.2</td>
</tr>
</tbody>
</table>

THERAFLEX MB-Plasma presents a HIGH SAFETY PROFILE EFFICACY ON:

- Enveloped and non-enveloped viruses
- Parasites such as Trypanosoma cruzi
- Unknown or untested pathogens
- Cells (intracellular viruses), leucocytes, red cells and platelets
- Bacteria

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CLINICAL EXPERIENCE AND WORLDWIDE PRESENCE

Since 1992, over 7 million units of MB-FFP have been transfused in various clinical settings. Currently there is clinical experience with MB-treated plasma produced with the THERAFLEX MB-Plasma system for more than 15 years in more than 19 countries.

Clinical experience of THERAFLEX MB-Plasma:
Routine use in Europe, South America and Asia Pacific.

Countries:
- Germany
- Spain
- Greece
- Italy
- United Kingdom
- Belgium
- Malaysia
- Argentina
- Russia
- Belarus
- Austria
- Brazil
- Singapore
- Armenia
- Kazakhstan
- Turkmenistan
- Hong Kong
- Saudi Arabia
- Poland
- Ukraine

www.bloodsafety.macopharma.com
BIBLIOGRAPHY


The THERAFLEX MB-Plasma is a CE marked medical device. It is not available for sale in the United States.

Worldwide regulatory approvals:
- **Germany**: PEI (Paul-Ehrlich Institute)
- **United Kingdom**: NHS (National Health Service)
- **Spain**: AEMPS (Agencia Española de Medicamentos y Productos Sanitarios)
- **Italy**: Ministry of Health
- **Switzerland**: Swissmedic
- **Canada**: Santé Canada
- **Russia**: Ministry of Health
- **Mexico**: Ministry of Health
- **Croatia**: Agency for Medicinal Products and Medical Devices
- **Ukraine**: Ministry of Health
- **Brazil**: ANVISA
- **Belarus**: Ministry of Health
- **Kazakhstan**: Ministry of Health
- **Singapore**: HSA (Health Sciences Authority)
- **Saudi Arabia**: Saudi Food & Drug Authority (SFDA)

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