Collection of Hyperimmune Plasma

Version: 001
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General Indications

• Wherever an active vaccination is possible an passive vaccination (the application of a specific immunoglobulin) could be suitable

• To proof the concept of an active vaccination is the transfer of specific immunoglobulins either in the prevention or the treatment of an disease (passive immunization)
Immunoglobulin's

Idea: polyspecific polyclonal antibodies (i.v.Ig, i.m.Ig, s.c.Ig) specific polyclonal antibodies (anti-Tetanus, anti-D, anti-...)

Mouse

Potential Repertoire: \(10^{12}\) different antibodies genetic potential

Available Repertoire: \(10^{10}\) different antibodies expressed within a life span

Actual Repertoire: \(10^8\) different antibodies expressed within a day

Known Indications: \(2 \times 10^2\) → there are much more opportunities

Main function: Humoral Immunity against bacteria (\(10^5\) different Species)

Main competitors: Antibiotics, Interleukins, Cytokines Chemokine, Growth factors (G-CSF), other fractionators

Opportunities: better description of the involvement of antibodies in the humoral immune response in combination with antibiotics, CSF’s, better description of the involvement of antibodies in the autoimmune diseases and chronic inflammations
Consequences after the binding of an Antibody

- **Agglutination**: Enhances phagocytosis and reduces number of infectious units to be dealt with.
- **Opsonization**: Coating antigen with antibody enhances phagocytosis.
- **Neutralization**: Blocks adhesion of bacteria and viruses to mucosa.
- **Activation of complement**: Cell lysis.
- **Inflammation**: Disruption of cell by complement/reactive protein attracts phagocytic and other defensive immune system cells.
- **Antibody-dependent cell-mediated cytotoxicity**: Antibodies attached to target cell cause destruction by non-specific immune system cells.
General Issues

- European self sufficiency in N, DK, SF, F
- Declaration of plasma sourcers forces European self sufficiency → but still not for Hyperimmunes!

Resulting opportunities

- Anti-RhD → limiting is the available amount of anti-D plasma (US & EU)
- i.m. IgG → sales only by weight not by volume
- s.c. IgG → sales like in i.v. Ig / Manufacturing like i.m. Ig
- i.m. Anti-Tetanus-Ig → Plasma available due to immunization programs
- i.v. anti-Tetanus → plasma available but very small market
- Anti-CMV → Plasma available due to natural infections
- Anti-HBV → Plasma available due to immunization programs
- Anti-Rabies → Plasma available due to immunization programs

New indications???
## Market Review

**Market share analysis, Define market**

Short summarizing analysis

<table>
<thead>
<tr>
<th></th>
<th>Total market 2000</th>
<th>Trends</th>
</tr>
</thead>
<tbody>
<tr>
<td>anti-Rh0</td>
<td>$33,000,000</td>
<td>increasing worldwide</td>
</tr>
<tr>
<td>anti-Tetanus</td>
<td>$30,000,000</td>
<td>decreasing in Europe, slightly increasing outside Europe</td>
</tr>
<tr>
<td>i.m. Ig</td>
<td>$9,000,000</td>
<td>decreasing worldwide</td>
</tr>
<tr>
<td>s.c. Ig</td>
<td>$10,000,000</td>
<td>increasing worldwide</td>
</tr>
<tr>
<td>Anti-RSV</td>
<td>$12,000,000</td>
<td>constant</td>
</tr>
<tr>
<td>Anti-HBV</td>
<td>$12,000,000</td>
<td>increasing in countries with incomplete vaccination</td>
</tr>
<tr>
<td>Anti-...</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## Market Developments I

**Competitive drugs and therapies**

<table>
<thead>
<tr>
<th>Indication</th>
<th>Drug</th>
<th>Implication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial infection</td>
<td>Antibiotics</td>
<td>Go for combinations</td>
</tr>
<tr>
<td>Bacterial infection</td>
<td>Cytokines</td>
<td>Go for combinations</td>
</tr>
<tr>
<td>Bacterial / viral infection</td>
<td>Cytokine neutralizing Mab’s</td>
<td>open</td>
</tr>
<tr>
<td>Bacterial infection</td>
<td>Growth factors (GCSF, GM-CSF)</td>
<td>See published literature about triple combination</td>
</tr>
<tr>
<td>Severs bacterial infection</td>
<td>sCD14, LBP*, BPIP**</td>
<td>open</td>
</tr>
<tr>
<td>Viral infection</td>
<td>Virostatics</td>
<td>Go for combination</td>
</tr>
<tr>
<td>Immune-Modulation</td>
<td>Cytokine, Chemokines</td>
<td>Go for combination</td>
</tr>
<tr>
<td>GvH, transplant rejection</td>
<td>Cytostatics</td>
<td>Go for combination</td>
</tr>
</tbody>
</table>

*LBP = LPS binding protein  
**BPIP = Bactericidal permeability increasing protein*
## Market Developments II

- **Opportunities withheld by Hyperimmunes**

<table>
<thead>
<tr>
<th>Product</th>
<th>Patient population</th>
<th>Global Market [$MM]</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. epidermidis</em></td>
<td>400,000</td>
<td>720</td>
</tr>
<tr>
<td>CMV</td>
<td>100,000</td>
<td>500</td>
</tr>
<tr>
<td><em>Pseudomonas</em></td>
<td>50,000</td>
<td>500</td>
</tr>
<tr>
<td>Gram negative</td>
<td>210,000</td>
<td>638</td>
</tr>
<tr>
<td>HBV</td>
<td>50,000</td>
<td>500</td>
</tr>
<tr>
<td>HCV</td>
<td>100,000</td>
<td>500</td>
</tr>
<tr>
<td>HAV</td>
<td>100,000</td>
<td>??? (still open)</td>
</tr>
<tr>
<td>Infection in Children (Pneumc, Meningoc, HIB)</td>
<td>500,000</td>
<td>&gt; 720</td>
</tr>
<tr>
<td></td>
<td>1,000,000</td>
<td>???</td>
</tr>
</tbody>
</table>
New product developments

**Viruses**
- CMV (Competitors only) → Vaccine available
- CMV as indication for i.v.lg
- Influenza → Vaccines available
- Ebola? → Potential vaccine available

**Bacteria**
- S. epidermidis → donor selection
- *Pseudomonas aeruginosa* → donor selection
- Gram negative bacteria *(Pseudomonas/E.coli/Klebsiella)* → donor selection
- Group A Streptococci → donor selection
- Group B Streptococci → donor selection

Main infectious agents in children:
- *H. influenzae* → vaccine available
- *Pneumococcus* → vaccine available
- *Meningococcus* → vaccine available
- *E. coli* K1O18 → donor selection
- Lipid A → Ligand apheresis / affinity chromatography

**Existing Hyperimmunes**
- HBV → Vaccine available
- HAV → Vaccine available
- Rabies → Vaccine available
- VZV → Vaccine available
Anti-Tetanus

3D structure of tetanus toxin

Tetanic mouse
Anti-Tetanus Immunoglobulin

- The tetanus prophylaxis is currently (only) present in the German-speaking countries.
- Successful!!!
- About 5 patients are killed by tetanus in Germany per annum
- About 150 in Italy (no prophylaxis in use)
**Anti-Tetanus i.m. / i.v.**

**Disease**
- **Caused by** bacterial exotoxin from *Clostridium tetani* after anaerobic infection, blocking neurotransmission to muscle cells
- **Mortality:** very high (50 %), especially in young, old patients and i.v. drug abusers
- **Morbidity:** 0,625/100.000
- **Risk:** especially in young, old patients and i.v. drug abusers
  - Incomplete immunized persons
  - Injuries like burs, surgical wounds, *post partum*, new-borns umbilicus

**Therapy**
- **Prevention of anaerobic infection**
- **Vaccination** with monovalent or polyvalent vaccines
- **Acute injuries:** passive immunization with anti-Tetanus Hyperimmunoglobulins “Tetanus prophylaxis”
  - Unknown, incomplete or uncertain immunization status: “simultaneous “Tetanus prophylaxis”
  - Immunization more than 5 year ago: passive “Tetanus prophylaxis”

**Treatment options**
- **Prophylaxis, mostly important market segment:** i.m. product
- **Therapy of the disease:** i.v. product
Hyperimmunization

- **Immunological considerations**
  - “more is better” doesn’t work in vaccinations
  - Sometimes less antigen in the vaccine give higher titters
    - Clonal selection in the specific B cells is more sufficient when antigen is limited
  - “Fast is better” doesn’t work as well
  - All immunologist were patient
    - In first time immunizations
      - Interval between 1\textsuperscript{st} and 2\textsuperscript{nd} dose $\rightarrow$ 4 weeks
      - Interval between 2\textsuperscript{nd} and 3\textsuperscript{rd} dose $\rightarrow$ 10 ... 12 weeks
    - In boosted donors
      - Interval between 1\textsuperscript{st} booster and 2\textsuperscript{nd} dose $\rightarrow$ 10 ... 12 weeks#
  - **But:** Differences between different vaccines.
    - **Pre-Trial is required!!**
Example: Tetanus-Hyperimmunization I

- Classification of pre-Titters

<table>
<thead>
<tr>
<th>Class [IE/mL]</th>
<th>Centre 1</th>
<th>Centre 2</th>
<th>Sum</th>
<th>[%]</th>
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</thead>
<tbody>
<tr>
<td>0-1</td>
<td>16</td>
<td>14</td>
<td>30</td>
<td>14.9</td>
</tr>
<tr>
<td>1-2</td>
<td>22</td>
<td>28</td>
<td>50</td>
<td>24.9</td>
</tr>
<tr>
<td>2-3</td>
<td>19</td>
<td>26</td>
<td>45</td>
<td>22.4</td>
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<tr>
<td>3-4</td>
<td>13</td>
<td>14</td>
<td>27</td>
<td>13.4</td>
</tr>
<tr>
<td>4-5</td>
<td>7</td>
<td>10</td>
<td>17</td>
<td>8.5</td>
</tr>
<tr>
<td>5-6</td>
<td>2</td>
<td>6</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>6-7</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>1.5</td>
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<tr>
<td>7-8</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>&gt;8</td>
<td>2</td>
<td>13</td>
<td>15</td>
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</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>201</td>
<td>100</td>
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</table>
Example: Tetanus-Hyperimmunization II

• Unexpected events

<table>
<thead>
<tr>
<th>Titter Class [IE/mL]</th>
<th>0-1</th>
<th>1-2</th>
<th>2-3</th>
<th>3-4</th>
<th>4-5</th>
<th>5-6</th>
<th>6-7</th>
<th>7-8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of vacc. donors</td>
<td>23</td>
<td>22</td>
<td>23</td>
<td>19</td>
<td>14</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Number of donors with UE (%)</td>
<td>6</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(26)</td>
<td>(46)</td>
<td>(22)</td>
<td>(26)</td>
<td>(7)</td>
<td>(25)</td>
<td>(0)</td>
<td>(0)</td>
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</table>

<table>
<thead>
<tr>
<th>UE / Titter Class</th>
<th>0-1</th>
<th>1-2</th>
<th>2-3</th>
<th>3-4</th>
<th>4-5</th>
<th>5-6</th>
<th>6-7</th>
<th>7-8</th>
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<tbody>
<tr>
<td>Local</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• local heat</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>• local pain</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td></td>
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<tr>
<td>• Swelling</td>
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<td>7</td>
<td>4</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• redness</td>
<td>2</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• limited movement</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• pressure pain</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• rigidification</td>
<td>3</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Systemic</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• headache</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• exhaustion</td>
<td></td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• fever</td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• dizziness</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• loss of appetite</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• growing pains</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>• sickness</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>• rash</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>• pruritus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>• neck pain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>41</td>
<td>25</td>
<td>18</td>
<td>4</td>
<td>3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
# Immune Response after Tetanus-Hyperimmunization

Proportion of donors with more than 12 IE/mL.

N.d.* = not done

<table>
<thead>
<tr>
<th>Titter class</th>
<th>1. week</th>
<th>2. week</th>
<th>3. week</th>
<th>4. week</th>
<th>5. week</th>
<th>6. week</th>
<th>7. week</th>
<th>8. week</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1</td>
<td>17%</td>
<td>48%</td>
<td><strong>57%</strong></td>
<td>52%</td>
<td>38%</td>
<td>33%</td>
<td>29%</td>
<td>19%</td>
</tr>
<tr>
<td>1-2</td>
<td>17%</td>
<td>57%</td>
<td><strong>71%</strong></td>
<td>62%</td>
<td>43%</td>
<td>32%</td>
<td>29%</td>
<td>6%</td>
</tr>
<tr>
<td>2-3</td>
<td>9%</td>
<td>52%</td>
<td>33%</td>
<td>48%</td>
<td>25%</td>
<td>25%</td>
<td>11%</td>
<td>13%</td>
</tr>
<tr>
<td>3-4</td>
<td>26%</td>
<td>50%</td>
<td><strong>56%</strong></td>
<td>50%</td>
<td>36%</td>
<td>36%</td>
<td>38%</td>
<td>25%</td>
</tr>
<tr>
<td>4-5</td>
<td>23%</td>
<td><strong>46%</strong></td>
<td>42%</td>
<td>42%</td>
<td>25%</td>
<td>8%</td>
<td>22%</td>
<td>20%</td>
</tr>
<tr>
<td>5-6</td>
<td>50%</td>
<td>46%</td>
<td>50%</td>
<td><strong>75%</strong></td>
<td>50%</td>
<td>25%</td>
<td>50%</td>
<td>25%</td>
</tr>
<tr>
<td>Total</td>
<td>19%</td>
<td><strong>51%</strong></td>
<td><strong>52%</strong></td>
<td><strong>52%</strong></td>
<td>35%</td>
<td>27%</td>
<td>25%</td>
<td>17%</td>
</tr>
</tbody>
</table>
Anti-RhO
Anti-RhO (Rh Prophylaxis)

- **Disease**
  - *Caused by alloimmunization in* Rh neg. women during pregnancy
    - This results for second und following foetus in the *Morbus hemolyticis neonatorum*
  - **Mortality:** without prophylaxis → 7%,
  - **Morbidity:** 0,625/100.000
  - **Risk:** each Rh-negative pregnant women (15% of the EU population)

- **Therapy**
  - **Prevention** of alloimmunization by *removing fetal erythrocytes from maternal circulation prior to an alloimmunization*, (recognition of the foetal erythrocytes by the maternal immune system)
  - *Rh-Prophylaxis has to take place in (every) case of possible foeto-maternal haemorrhage* in the described Rh antigen constellation
Anti-RhD (Anti-D, Anti-RhO)

Anti-D Plasma, Current Situation

- Currently is the limiting factor the availability of anti-D source plasma
- Anti-D plasma production in Germany is possible (but expansive)

<table>
<thead>
<tr>
<th>Company</th>
<th>1998</th>
<th>1999</th>
<th>2000</th>
<th>Price</th>
</tr>
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<tbody>
<tr>
<td>Serologicals</td>
<td>75000</td>
<td>85000</td>
<td>95000</td>
<td>$580</td>
</tr>
<tr>
<td>NABI US</td>
<td>40000</td>
<td>65000</td>
<td>75000</td>
<td>$580</td>
</tr>
<tr>
<td>Ortho &amp; Seractec</td>
<td>50000</td>
<td>50000</td>
<td>50000</td>
<td>$550</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>165000 L</strong></td>
<td><strong>200000 L</strong></td>
<td><strong>230000 L</strong></td>
<td></td>
</tr>
</tbody>
</table>

- Manufacturers using the high tittered for own products (anti-D i.v.)
- Increase the availability in U.S. and Europe and others countries?
Comparison of

**FDA Recommendations (1995)**
- Test potential donor for all infectious disease markers antibodies, antigen
- Collect Red Blood Cells and store frozen for **12** months
- Test donor and intended recipients for all markers antibodies, antigen
- Immunize up to 3 recipients
- Test recipients at 3, 6, 9, and 12 months for all markers antibodies, antigen
- Frozen cells qualified for routine use, Donor is "pedigreed"

**German Guidelines (1997)**
- Test potential donor and intended recipient for all infectious disease markers antibodies, antigen, PCR
- Collect Red Blood Cells and store frozen for **6** months
- Test donor at and intended recipient 3 and 6 months for all markers antibodies, antigen, PCR
- Frozen cells qualified for routine use, immunize recipients

Model: European
α-Rh0
Plasma Sourcing
Model of a European $\alpha$-RhO
Hyperimmunization

Notes
(1) Austria and Germany
(2) assumption: 17% of donors are Rh-neg., 50% are male, 25% are willing to participate =2%
(3) 70% donors qualify after phenotyping
(4) first dose 5 ml, next seven 2 ml
(5) 30% of participants reach high titters
(6) maxium no. of donations p.a. in Austria = 50, in Germany = 45
(7) average yield 800 ml
(8) average
Potential Indication

• Prophylaxis and Therapy of the CMV infection in transplanted patients
  • CMV infection is a frequent complication on transplantations
  • Either alone or
  • In combination with virostatic drugs a (e.g. Ganciclovir)
Anti-PRP

- PRP = polyribosyl-ribitol-phosphate
  - The major antigen in capsular *H. influencae*
  - Vaccine is available (HIB)
  - Natural in the donor population as well
  - Hyperimmune from selected plasma units
    - With natural immunity (and high titres)
    - After immunization programs
      - ELISA-method is available
  - Antigen for Ligand-affinity-purification is available for a high purified product
  - Preclinical models are simple
Anti-PRP

• Indication
  • Children on risk
    • After splenectomy
    • After chronic (severe) infections of the middle ear
    • After chronic (severe) infections of the respiratory tract
  • Alone or
  • In combination with antibiotics
More Specific Immunoglobulin's

• Classical
  • Anti-Tetanus
  • Anti-Diphtheria
  • Anti-CMV
  • Anti-HBV
  • Anti-PRP (*Haemophilus influenza*)
  • Anti-Pneumococcal
  • Anti-Menigococcal
  • Anti-RSV ...

• Open
  • Anti-*S. epidermidis*
  • Anti-*Pseudomonas*

• New Indications
  • Children-IgG
    • Basic variant (Combination of anti-Pneumococcal, anti-Meningococcal, anti-*Haem. Influenca* [PRP])
    • Extended variant (Combination of Pneumococci, Meningococci, *H.Influenca*, *S.epidermidis*, RSV, Anti-K1-Coli)

• Anti-inflammatory Ig’s
  • Anti-TNF
  • Anti-α-IFN
Children Ig

• Anti-PRP (HIB) alone is only indicated when the respective infection was proven!
• Often in paediatrics it’s not clear which bacteria causes the infection
• Diagnosis takes normally 24 – 48 h
• Meningitis / Encephalitis will be developed within this time
• The Ig should have specific antibodies against the most hazardous and most present bacterial in infection in new-borns and small children
  • *H. influencae*
  • Meningococcus
  • Pneumococcus
  • *E. coli K1018* (most dangerous in new-borns)
German Hyperimmunization Guideline

• Regulatory issues
  • Vaccine has to licensed (for normal immunization)
  • But will be used outside the indications (e.g. licensed immunization schedule)
    → therefore:
  • Hyperimmunization has to be handled according to GCP
    • Hyperimmunization plan
    • Ethic vote
    • Donor education (written)
    • Donor consent (written)
    • Reporting of unexpected events according to GCP (to authorities and vaccine manufacturers)
    • Donor insurance

• To ensure the donors safety at each time
Hyperimmunization, General Procedure

1. Screen the vaccination status
   - On paper
   - By measuring antigen specific titters

2. Regular immunization if possible (according to the licensing conditions of the vaccine)

3. Inclusion into the hyperimmunization plan
   - Regular booster
   - Limits for titters for a hyperimmunization, booster injections
   - Limits for titters to end the hyperimmunization
Hyperimmunization not always

Donor

Check vaccination status

Check titters

Draw plasma

Check titters

Regular vaccination possible

Immune response

Draw plasma

Check titters

Hyperimmunization program

Immune response

Draw plasma

Check titters

Booster vaccination in the Hyperimmunization plan

Immune response

Draw plasma

Check titters

Hyperimmune Plasma
Other Prerequisites for a Hyperimmunization

- Active vaccination has to be present and
- Has to be successful

Or

- Prevalence of specific antibodies due to natural infections in the population (reconvalescent plasma)
  **beware:** in epidemic situations (Influenza) an infection due to an insufficient immune response has to be excluded

and

- Available **screening assays** (closed to the mode of action: e.g. virus neutralization assays, toxin neutralization ...) → which is not that easy!!

- The same assays should be used in **clinical situation to evaluate the deficiency of neutralizing antibodies** (and give the indication for an hyperimmune)
• Thank you for your attention