The function of kidney is to remove excess salts & waste products from the human body. Nonfunctional kidneys leads to problem in maintaining the level of sodium, potassium, and minerals and which results in congenital kidney disease.

There are two types of dialysis treatment available:
1. Hemodialysis
2. Peritoneal dialysis.

Hemodialysis treatment:
• Artificial kidney (hemodialyzer) used to remove the excretory products and fluids from blood.
• Dialysis patient undergoes small surgery that creates an access point in an arm or leg, and then the blood is allowed to flow through a hemodialyzer.
• Hemodialysis treatment occurs 3 times a week, each session of 4 hr duration. Each exposure of 150 litres, one year exposure of 23400 litres of dialysis fluid
• Actual frequency and time of Hemodialysis treatment depends upon the condition of kidney, function, accumulation of toxic products in the blood etc.

Peritoneal Dialysis treatment:
• Dialysis is done without removing the blood from the body.
• Catheter is placed in the abdomen by a minor surgery and catheter point is closed with a solution called the dialysate, which then fills the inner side of the abdomen.
• Toxic substances in the blood filter through the blood vessels namely arteries and veins into the dialysate by osmotic process.

Endotoxin is the natural form of lipopolysaccharide occurring in the outer layer of the bi-layered gram negative bacterial cell wall.

The term lipopolysaccharide refers to the pure chemical form.
Why Monitor Endotoxin in HD?
- Patients receiving hemodialysis therapy are exposed to approx. 400 L of water per week which is 20-25 fold greater than drinking water. This potentially exposes them to large amounts of endotoxin.
- Endotoxin in the Dialysis Fluids may enter the blood compartment and activate monocytes to produce pro-inflammatory cytokines.
- Evidence also shows Transmembrane Stimulation of Mononuclear Cells
- Increase in cytokines, mainly IL-1, IL-6, and TNF-alpha, is thought to be related to several distinct acute and chronic problems of HD patients.
- Cytokines trigger a series of acute phase reactants and consequently, these patients exhibit a state of chronic microinflammation.
- Studies indicate a relationship between chronic microinflammation and heart disease and mortality.

The use of popular HD modalities, e.g. use of bicarbonates dialysates and high flux dialysers are predisposed to bacterial contamination of the dialysate fluid increasing the endotoxin burden of these fluids.
- Additionally, washing with water for the reuse of hemodialysers and centralized dialysate production also increases the risk of endotoxin contamination. This practice is not being used anymore in the USA, Europe, Japan, but might be used elsewhere.
- In Hemodialysis systems, bacteria escape water treatment & reach downstream sites and begin to proliferate. In case of inadequate sanitation, bacteria and endotoxin will reach dangerous levels.

Why test for Endotoxin?
- Dialysis was once only administered to patients with acute renal failure and was only administered over a very short period.
- Now, chronic hemodialysis treatment can continue for years.
- Exposure to endotoxin can result in pyrogenic reactions: Fever, chills, fall in blood pressure, Activation of the complement system, Release of cytokines, Inflammation, Resistance to recombinant human erythropoietin.

Endotoxin in Dialysate
- Endotoxin
  - Chills, fever, BP
  - Activation of Complement System
  - Release of Cytokines
  - Inflammation
  - Resistance to rHuEPO

Recombinant Human Erythropoietin (rHuEPO)
- Anemia is common in patients with renal failure.
- rHuEPO is used to treat anemia in dialysis patients.
- Inflammation induced by exposure to endotoxin reduces the effectiveness of rHuEPO resulting in higher doses.
- Studies show that reduction in endotoxin increases the effectiveness of rHuEPO.
- Low endotoxin results in lower doses of rHuEPO.
- Reduction of rHuEPO dose is a significant reason for AAMI lowering the allowable endotoxin in water and dialysate.
Hypothesis: Transmembrane Stimulation of Mononuclear Cells

Blood Compartment

Dialysate Compartment

Dialyzer Membrane

Dialyzer Membrane

Cytokines:
- TNFα
- IL1β

Gram negative bacteria and endotoxin

Pyrogenic Reactions (early 1970s)

Outbreak 1
- PR attack rate 13.5/1,000 treatments
- Attack rates were directly proportional to concentration of gram negative bacteria in the dialysate

Outbreak 2
- PR in 73% of patients
- Related to installation of DI unit


Pyrogenic reactions

- Endotoxin predominant cause of Acute Patient Reactions
  - Pyrogenic Reactions (PR): onset of objective chills (rigors) and/or fever (oral temp ≥ 37.8°C) in a patient who was afebrile and had no signs or symptoms of infection before dialysis treatment.
- In the United States, 21% of 2,808 hemodialysis centers report having ≥ 1 PR only 1.7% of these centers report PR in clusters
- From CDC investigations of outbreaks most PR clusters are associated with errors in dialyzer reprocessing

Factors Necessary for Control of Microbial Contamination

- Standards
- Surveillance Systems
- Data Systems
- Control Mechanisms

Types of Hemodialysis Fluids Generally Cultured

- Dialysis Fluids
  - Water used to prepare dialysate
  - Dialysate
  - Bicarbonate Concentrate
- Dialyzer Reuse Fluids
  - Water used to rinse/reprocess hemodialyzers
  - Water used to prepare dialyzer disinfectant
Recommended Frequency for Environmental Monitoring Hemodialysis Fluids

- After a suspected pyrogenic reaction
- After a suspected bacteremia
- After modification to the water treatment system
- At least weekly for new water treatment systems
- At least monthly for established water treatment systems

What do we do with the results of monitoring?

- Positive result:
  - Repeat test if this result is correct.
  - If repeated assay confirms original result then take appropriate action.
- Record results: Plot results over time; for a trend analysis
- DO SOMETHING!!! be pre-emptive!
- Remember manufacturer’s instructions are a guide.

Global Statistics

- Incidence of End Stage Renal Disease (ESRD) is increasing globally. The number of dialysis patients is expected to continue growing by about 4-6% annually.
- 2008, approximately 2,300,000 patients treated for ESRD
- Approximately 1,700,000 patients in 145 countries received dialysis treatment in 2008.
  - 1,580,000 received Hemodialysis
  - 190,000 received Peritoneal Dialysis
- Dialysis patients worldwide increased by 7% in 2008
- Of the 145 countries known to provide dialysis treatment, only 37 report statistics through a registry.

2008 statistics provided by Fresenius based on world population of 6.7 billion

ESRD Prevalence, Patients per Million

- Taiwan – 2,420 patients per million population
- Japan – 2,380 patients per million population
- U.S.A. – 1,780 patients per million population
- European Union – 960 patients per million population

2008 statistics provided by Fresenius Annual Report

Dialysis Patients by Region

- North America 445,000 patients +4-5%
  - U.S. (21% of global total) 370,000 patients +3-4%
  - Europe/Middle East/Africa 520,000 patients +5-6%
  - EU (17% of global total) 300,000 patients +3-4%
  - Asia-Pacific 620,000 patients +10-11%
  - Japan (16% of global total) 290,000 patients +3-4%
  - Latin America 185,000 patients +7-8%
  - Worldwide 1,770,000 patients +7%

2008 statistics provided by Fresenius Annual Report

Endotoxin: effect in Sepsis
**Severe Sepsis: A Significant Healthcare Challenge**

- Major cause of morbidity and mortality worldwide
- Leading cause of death in non-coronary ICU (US)
- 11th leading cause of death overall (US)
- More than 750,000 cases of severe sepsis in US annually
- In the US, more than 500 patients die of severe sepsis daily

**Factors in the High Incidence of sepsis**

- Aggressive use of catheters.
- Use of prosthetic devices.
- Administration of chemotherapy.
- Use of immunosuppressive drugs.
- Increased life expectancy of high-risk patients.

**Microbiological causes of sepsis & Indian scenario**

- G-ve infections

- G+ve and fungal infections

**Evidence linking LPS(endotoxin) with Sepsis**

- Endotoxin levels in Plasma can predict sepsis
- Reaction after administration of endotoxin to healthy volunteers.
- Clinical condition of sepsis may worsen despite antibiotic therapy.
- Experimental models of endotoxaemia.
LPS induces inflammatory mediators

- ICAM-1
- E-selectin
- Tissue Factor
- Nitric Oxide (NO)
- Superoxide ($O_2^-$)
- Prostaglandins
- Leukotrienes
- PAF
- TNFα
- IL-1
- IL-6
- IL-8

LPS - Pathogenesis of Sepsis (I)

- Cell-wall fragments (LPS)
- Macrophage
- LPS
- Activation of Complement & Coagulation
- Platelet activation
- Neutrophil activation
- Inflammatory Mediators (Cytokines)

LPS - Pathogenesis of Sepsis (II)

- SEPSIS
  - Capillary leak
  - Tissue damage
  - Fever
  - Disseminated Intravascular Coagulation
- SEPTIC SHOCK
  - Hypotension
  - Adult respiratory distress syndrome
  - Multiple Organ Failure
- ENDOTOXIN
  - Endothelial Damage
  - Death

Systemic Activation of Inflammation in Sepsis

Inflammation is Activated in Sepsis

- Endotoxin (ng/L)
- TNF (ng/L)
- IL-6 (U/mL)

So many levels of...
1800s
- 1860s: the word “pyrogen” was first used by a scientist named Billbroth to describe substances that caused fever.
- 1884: Danish scientist, Hans Christian Gram, discovered a method of differentiating bacteria into two groups, Gram-positive and Gram-negative, based on properties of their cell walls. This procedure is now known as the Gram stain.
- 1890s: A scientist by the name of Pfeiffer was studying cholera. While growing cholera, he discovered a toxin that was not secreted into growth medium and stayed anchored to the bacterial cell. Pfeiffer called this “endotoxin”.
- Using Gram’s staining procedure, scientists discovered that endotoxins were associated with Gram-negative bacteria.

1900s
- 1912: E.C. Hort and W.J. Penfold developed the first Rabbit Pyrogen Test and could classify bacteria as pyrogenic or non-pyrogenic.
- With Gram’s staining procedure, Hort and Penfold determined that pyrogens were associated with Gram-negative bacteria and fevers were not caused by the act of injection, but by the bacteria within the solution.
- 1920s: Florence Seibert (best known for developing a skin test for tuberculosis) proved that pyrogens were heat-stable, filterable Gram-negative bacteria. She developed a method of distillation that made solutions safer from bacteria.
- Seibert developed the Rabbit Pyrogen Test as we know it today.

1960s and 1970s
- Drs Frederick Bang and Jack Levin developed the first LAL test performed in a tube.
- Bang and Levin developed their test based on information from two other scientists, W.H. Howell and L. Loeb, from the 1800s.
- Howell observed that when exposed to the elements, the blood of a horseshoe crab would clot.
- Loeb observed that clotting only occurred in plasma containing the single blood cell or amebocyte of the horseshoe crab but not in plasma free of amebocytes.
- 1970s: The gel clot LAL test was commercially introduced.

What is Endotoxin?
Endotoxin is the natural form of lipopolysaccharide occurring in the outer layer of the bi-layered gram negative bacterial cell wall.

The term lipopolysaccharide refers to the pure chemical form
Biological Activity of LPS

- LPS initiates inflammatory responses from host systems
  - These include release of:
    - Cytokines
    - Lipids
    - Free radicals
- Activation of:
  - Complement
  - Coagulation
- LPS is a signal of infection with G-ve bacteria

Release of LPS from bacteria

- Natural shedding during bacterial growth.
- Complement-mediated lysis.
- Antibiotic-induced lysis (in vitro).
- Some organisms (N.meningitidis) shed large amounts of LPS (LOS) during infection.

LAL Reaction Cascade

- Endotoxin
- Factor C
- Activated Factor C
- Factor B
- Activated Factor B
- Proclotting Enzyme
- Clotting Enzyme
- Coagulogen
- Coagulin

Current Recognised LAL Methods

- Gel Clot: Based on LAL clotting protein and formation of visible clot in test tubes.
- Kinetic Turbidimetric: Based on LAL clotting protein. Monitors change in solution clarity as a clot forms. Most commonly performed in 96-well plates and read on an absorbance plate reader.
- Endpoint Chromogenic: A synthetic substrate replaces the clotting protein. A yellow color is generated as a response to cleaving of the substrate. Most commonly performed in 96-well plates and read on an absorbance plate reader.
- Kinetic Chromogenic: Also uses a synthetic substrate and is read in a plate on an absorbance plate reader. It is regarded as the most robust of the kinetic assays.

Gel CLOT TEST

- Can be read up to 2 minutes after incubation period
- Read with momentary inversion of tube
- Do not invert more than once

Limulus Amebocyte Lysate

Gel Clot Test

- Proenzyme
- Clotting Protein
- Electrostatic Bonding
- Clot Formation
Kinetic Assay

Limulus Amebocyte Lysate
Kinetic Turbidimetric Test

- Proenzyme -> Active Enzyme
- Coagulogen -> Clotting Protein + (Fragment)
- Clotting Protein -> Turbidity Formation

Limulus Amebocyte Lysate
Kinetic Chromogenic Test

- Proenzyme -> Active Enzyme
- Chromogenic Substrate -> Release of PNA + (Fragment)
- PNA Concentration (Yellow Colour) 
  Directly Relates to Endotoxin Concentration

History

- AAMI is the Association for the Advancement of Medical Instrumentation.
- 1982: First AAMI Standard for hemodyalisis was published.
  Maximum levels of bacteria in water and dialysate were established.
  - Pyrogenic reactions were significantly increased if bacteria in dialysate exceeded 2000 CFU/mL.
  - Dialysis machines at that time could amplify the bacteria burden by a factor of 10.
  Therefore, the limit was set at 200 CFU/mL.
- First standard did not include endotoxin.
- 2001: AAMI Standard was revised to include endotoxin.

AAMI - The Current Standards & other International standards

AAMI RD62 and RD52

- AAMI procedures require that action be taken to reaffirm, revise, or withdraw this standard no later than 5 years from the date of publication.
- AAMI RD62 - Water Treatment Equipment for Hemodialysis Applications
  a)section 3.11 talks on endotoxin effect
  b)section 4.1.1 on water quality & levels of endotoxin
  c)section 5.1.1 on methods of water collection
  d)section 4.3.2.1 on limits of endotoxin in conventional dialysate
- AAMI RD52 – Dialysate for Hemodialysis (section 4.3.2.2 Bacteriology of ultrapure dialysate)
EP Endotoxin Limits for Hemodialysis

- Water for dilution of concentrated soln. - 0.25 IU/ml
- Soln. for hemodialysis - 0.5 IU/ml
- Soln. for hemofiltration & hemodiafiltration - 0.25 IU/ml
- Soln. for peritoneal dialysis - 0.5 IU/ml
- NOTE: These are non-mandatory limits

Int’l Limits for Water for Dialysis Applications

<table>
<thead>
<tr>
<th>Organization</th>
<th>Bioburden (cfu/ml)</th>
<th>Endotoxin EU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>European Pharmacopoeia</td>
<td>&lt; 100</td>
<td>&lt; 0.25</td>
</tr>
<tr>
<td>EDTNA/ERCA* (proposed)</td>
<td>&lt; 100</td>
<td>&lt; 0.25</td>
</tr>
<tr>
<td>Japan Society for Dialysis Therapy</td>
<td>&lt; 100</td>
<td>&lt; 0.25</td>
</tr>
<tr>
<td>AAMI RD62</td>
<td>&lt; 200</td>
<td>&lt; 2</td>
</tr>
</tbody>
</table>

*European Dialysis and Transplant Nurses Association
European Renal Care Association

Additional Int’l Limits for Water for Dialysis Applications

- French Health Ministry requires a limit of 0.05 IU/ml for replacement electrolyte fluids used in hemofiltration
- BP 2010 requires a limit of 0.5 IU/ml for hemodialysis solutions and less than 0.25 IU/ml for Hemofiltration & Hemodiafiltration solutions.

EDTNA/ERCA

- The EDTNA/ERCA document endorses the EP microbial limits, but goes further by addressing frequency of testing and sampling conditions.
- It proposes that frequency be established based on historical data and maintenance procedures for the water system.
- Monthly test interval, unless history of elevated levels show a need for weekly testing.
- Recommends having in-house testing capability
- Immediate investigation of elevated results.

New AAMI Standards

- AAMI RD 62 – Current Limit – 2.0 EU/mL
- AAMI RD 62 – Current Action Level – 1.0 EU/mL
- AAMI/ISO – New Proposed Limit – 0.25 EU/mL
- AAMI/ISO – New Action Level – 0.125 EU/mL
- Water samples may be tested undilute
- No interference with any LAL tests

Water Testing
Dialysate Testing

- AAMI RD 52 – Current Limit – 2.0 EU/mL
- AAMI RD 52 – Current Action Level – 1.0 EU/mL
- AAMI RD 52 – New Proposed Limit – 0.50 EU/mL
- AAMI RD 52 – New Action Level – 0.25 EU/mL
- Ultrapure Dialysate – 0.03 EU/mL
- Dialysate may require dilution to overcome inhibition
- Can be tested with LAL tests

Dialysis system & water sampling

Monthly Monitoring Requirements

- Product water from the water system and the distribution loop.
  - This includes portable acute water units
- Conventional dialysate with sample collected from the dialysate port of the dialyzer or from a sampling port in the inlet dialysate line that can be accessed using a syringe
- Weekly Monitoring is required at start-up or if levels exceed maximum levels until a pattern of consistent compliance with limits can be demonstrated.
Sample Collecting (Water)

- Water samples should be collected directly from taps on the water distribution system.
- Do not disinfect water collection tap.
- If disinfection is necessary, only use alcohol and sterile gauze.
- Do not use bleach or other disinfectant.
- Allow alcohol to completely evaporate before collecting.
- Water tap should be opened and allowed to run for a minimum of 60 seconds before a sample is collected.

Sample Collecting (Dialysate)

- Sample at least two machines per month.
- Rotate so that all machines are tested at least once per 12 months.
- Dialysate should be collected from dialysate port of dialyzer.
- Newer machines have a dialysate sampling port that is accessed with a syringe.
- If disinfection is necessary, only use alcohol and sterile gauze and allow alcohol to evaporate.
- Do not use bleach or other disinfectant.
- A 30 mL syringe is used to aspirate and dispense dialysate out of and into the port before sample is collected.
- A new syringe should be used to collect the sample.
- A minimum of 25 mL or a volume specified by the testing laboratory should be collected in a sterile, endotoxin-free container.

Sample Collection Techniques (Water Used to Reprocess Dialyzers)

- Collect sample from water supply line.
  - Used to reprocess dialyzer.
  - Used to sample dialyzer disinfectant.
  - Used to sample the dialyzer reprocessing system.
- Allow water to flow for 30-60 seconds.
- Avoid collecting samples from:
  - tubing connected to spigots.
  - quick connect devices.
- Collect water samples in sterile endotoxin-free container. Alcohol should be used and allowed to completely dry before the sample is drawn. Bleach or other disinfectants should not be used.

Sample collection sites:

- The sample should be taken from the product water distribution piping at the following locations.
- Site 1: At the point where the water leaves the RO machine, before it enters the holding tank (Indirect System), or before it goes to the treatment room to provide water for dialysis machines (Direct System).
- Site 2: If an RO water holding tank is present, a sample should be taken at the point where the water leaves the tank.
- Site 3: At the end of the return line of the RO water distribution loop, whether it is returning to the RO or a water-holding tank. If a bacteria filter is installed anywhere in the system, a sample is to be drawn from a sample port both pre and post filter.
- Site 4: At the point where water enters into the dialyzer reprocessing system, whether it is a manual or automated system. (Note: If a sample port is not present one should be installed.)
- Site 5: At a point where water enters equipment used to prepare bicarbonate and acid concentrate. (Note: If a sample port is not present one should be installed.)
- Site 6: At the point where the dialyzer machine is hooked up to the product water loop. If a dialyzer machine is consistently attached to that location, you may culture the machine instead of the water outlet.
- Site 7: If facility uses softened, dechlorinated water as a backup water plan, it is necessary to perform cultures and a Limulus Amebocyte Lysate (LAL) test on this water, because the RO is the primary source of bacterial protection for the patients.

Action Level Exceeded

- If a sample exceeds the Action Level (currently 1 EU/mL), an investigation should be conducted:
  - the sample should be collected again and retested,
  - review compliance with disinfection and sampling procedures,
  - evaluating microbiological data for the previous 3 months to look for trends,
  - Notify Medical Director.

Thank You