

Water in the Clinical Laboratories

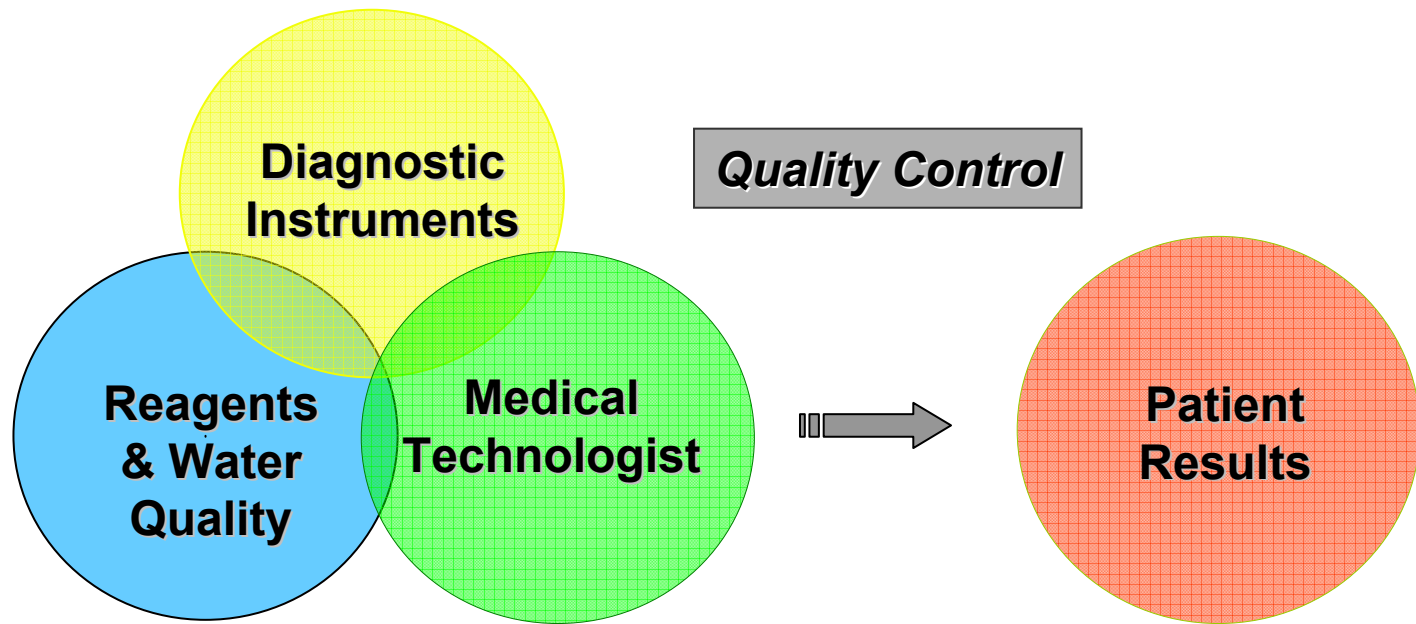


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OEM

Hong Kong, June 2008

Water in the Clinical Laboratory



Water quality as part of the quality process

Overview

- CLSI Guideline
- Water contaminants and water quality monitoring
- Bacteria and their by-products: interference with clinical assays
 - Example of Calcium
 - Alkaline phosphatase and immuno enzyme assays
- Effect of water quality on other clinical analyzer assays
- Reaching high water quality: purification technologies
 - Addressing ions and organic removal
 - Bacterial control
- Other analytical techniques in the clinical laboratory
- Summary and conclusions

CLSI Guideline



CLSI C3-A4, 2006

Revised version of the 1991 C3-A4 NCCLS document



Committee: hospital laboratory doctors and managers, hospital microbiologist, water purification systems companies

CLSI Guideline



Purpose of the guideline: to provide support to clinical laboratories to improve their quality system in regards to water. The standard defines recommendations on the selection of water purification solutions, on system maintenance, and on quality control.

- Philosophy of the CLSI guideline is that the user is responsible to check if the water used is suited for the purpose
- One grade only of water quality is defined (CLRW)
- Other water grades not specified: this needs to be decided by the user (in collaboration with the manufacturer), based on the applications and the water quality requirements

CLSI Water Grades



➤ **Clinical laboratory reagent water (CLRW)**

- Parameters defined

➤ **Special reagent water (SRW)**

- May be specified for specific applications when additional parameter are needed to insure water quality

Metal analysis, DNA/RNA assays, cell cultures, immunoassays

➤ **Instrument feed water (IFW)**

- Based on manufacturer's recommendations

➤ **Water Supplied by a Method Manufacturer for Use as a Diluent or Reagent**

- Cannot be a substitute for CLRW

➤ **Commercially Bottled, Purified Water**

- Water suitability needs to be checked

➤ **Autoclave and Wash Water Applications**

Specifications - CLRW

- Ionic purity
 - Resistivity > 10 MΩ.cm @ 25 ° C –
- Microbiology < 10 cfu/mL
 - Total heterotrophic plate count
 - plate count method, 20-28 ° C, > 5 days, R₂A media
- Organic purity
 - TOC < 500 ppb
- Particulates: particles larger than 0.22 μm should be removed using filtration (screen filter, Ultrafiltration, Reverse Osmosis)
- No recommendation for silica
- CAP is very likely to follow on

Quality: Validation & Trend Monitoring

- “Fit for purpose” water Validation
- Water Equipment Validation
- Documented parameters monitoring and trending
 - “Making sure water continues to meet specifications”
- Maintenance Procedures development
- Feed water quality requirement



Rather than a constraint, the CLSI guideline should be considered as a tool and a reference guide to select the most appropriate solution to the laboratory needs and requirements

Quality: FDA validation of Water

- FDA do show a big interest in Water for clinical analysers
- Some companies must use validated water systems
 - Dade Behring, Roche etc
- Why?
 - Instruments use water as reagents



Water Contaminants and Water Quality Monitoring

Water Contaminants

Water: H_2O and some other things

Presence of contaminants



Particles

Gases

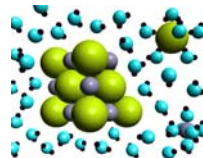
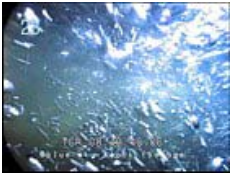
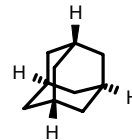
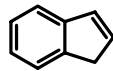
Microorganisms

Ions

Organics



Purification technologies
Monitoring



Ionic Purity

- **Conductivity** and **resistivity** used to define the overall ionic purity/contamination of water – Usually measured in-line

Conductivity

$$X = F \sum c_i z_i \mu_i$$

Conductivity (Siemens/cm) Faraday constant Concentration of each ionic species (eq/ml) Valence Mobility $\Omega^{-1} \cdot \text{cm}^{-1} \cdot \text{s}^{-1}$

$$[\text{H}^+] = [\text{OH}^-] = 10^{-7} \text{ M}$$

*Minimum theoretical conductivity is
 $0.055 \mu\text{S} \cdot \text{cm}^{-1}$ at 25°C*

Resistivity

$$R = \frac{1}{X}$$

*Maximum theoretical
 resistivity is $18.2 \text{ M}\Omega \text{cm}$ at 25°C*

Correlation Between NaCl Concentration and Resistivity

NaCl concentration (ppb = $\mu\text{g/L}$)	Conductivity ($\mu\text{S.cm}^{-1}$)	Resistivity ($\text{M}\Omega.\text{cm}$)
0	0.055	18.2
1	0.057	17.6
5	0.066	15.2
10	0.076	13.1
20.5	0.1	10
100	0.270	3.7
1,000	2.210	0.45

@ 25 °C

Organic Purity

- Total Organic Carbon (or Total Oxidizable Carbon - **TOC**) defines the overall organic purity of the water
 - TOC expressed in ppb ($\mu\text{g/L}$) of carbon
 - Measured on-line or off-line
 - Does not provide any information on specific molecules

Bacteria and Their By-Products

- Example of calcium interference
- Alkaline phosphatase
- Bacteria in water

Calcium

- *“Problem with Calcium assay”*
- Common problem on several brands of analyzers
 - Same chemistry: Arsenazo assay
- Issues with calibration, standards or patient values
- Often, decontamination solves the issues

Comments on the Calcium Assay

➤ Calcium dosed

- Normal range: ca 100 mg/L = 100 ppm
- 50% of calcium is free, 40% bound to proteins (mostly albumin in human blood)
- Test based on a colorimetric complex between calcium and the Arsenazo reagent at neutral pH

➤ Recommendations for plasma sampling

- *“Do not use citrate, oxalate or EDTA because they remove calcium by complex formation. Any substance which either chelates or contains calcium will interfere with the test.”*

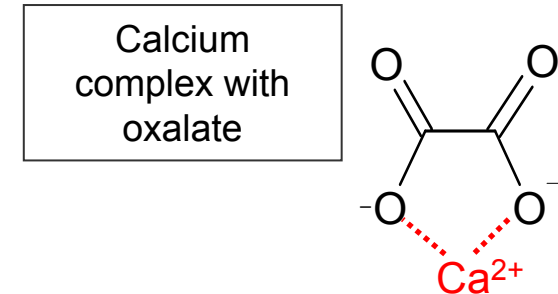
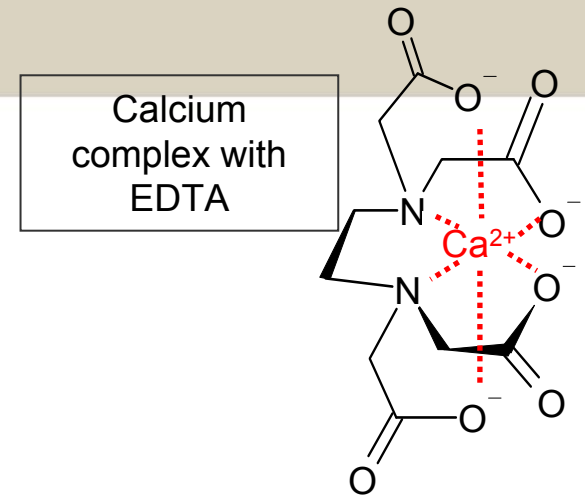
Interferences with Calcium Assay ?

- Calcium present in water
 - Water produced around 10 MΩ.cm
 - Maximum Calcium concentration: 10-15 ppb
 - Water in a reservoir
 - Water degrading in a reservoir: 1 MΩ.cm
 - Maximum Calcium concentration: 500 ppb
 - Compared to calcium dosed (100 ppm):
 - Unlikely to be the ionic purity of the water (contributes to < 1%)
- So, what else can interfere with the assay?

Interference Parameters

➤ Chelation (complexation) of Ca

- Small organic acids: e.g. Oxalate, citrate
 - From feed water
 - From bacteria
- Humic and fulvic acids
 - Present in the feed water
- Proteins
 - From bacteria
- Ions
 - Phosphate from feed water and bacteria
 - Fluoride, that might be present in the feed water



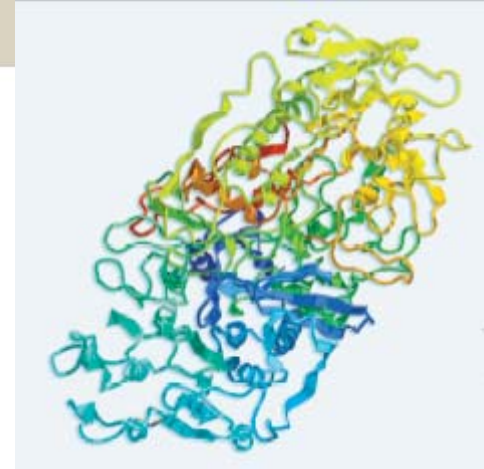
➤ Impact on pH

- Dissolution of large amounts of carbonates/bicarbonates can create a buffer. Might change the pH of the buffer used in the reaction

Calcium Assay: Conclusions

- Although calcium is an ion, it is not the ionic purity of the water that is generally the root cause of the problem in this assay
- The source of the problem stems from other contaminants present in the water
- Avoiding high organic load is important
- The decontamination removes the bacterial by-products present in the water and solves the issue

Consider the water quality in its totality
Monitoring
Maintenance



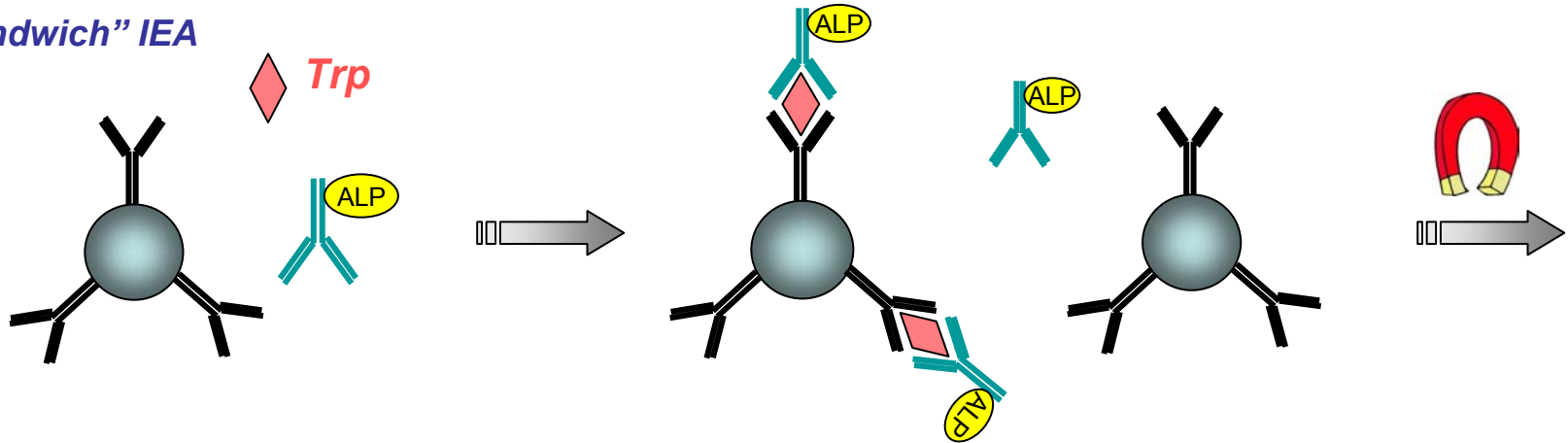
Alkaline Phosphatase

Interference with Enzyme Immunoassays

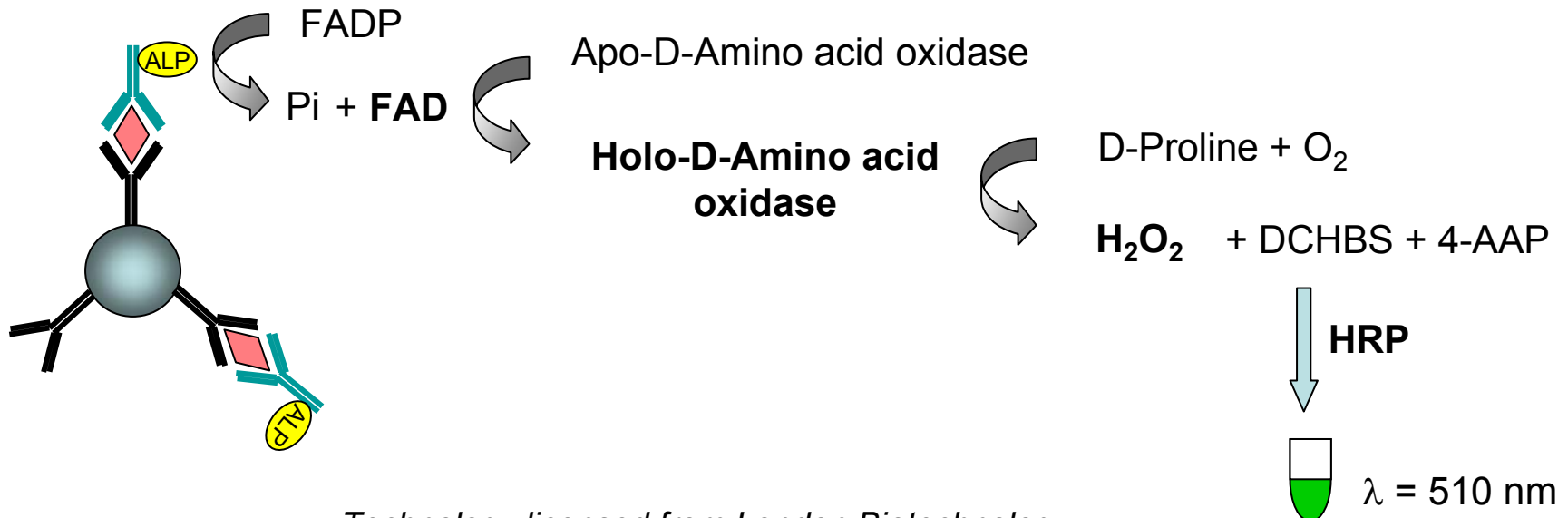
- Immuno enzyme assays used for cardiac markers
CTNI
- Stability issues reported with that assay
Need to perform calibrations more often than expected

Example of Interference: Clinical Assays for Cardiac Biomarkers

Sandwich IEA

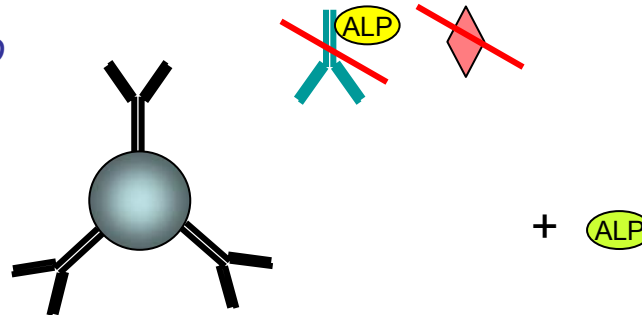


Biochemical amplification cascade



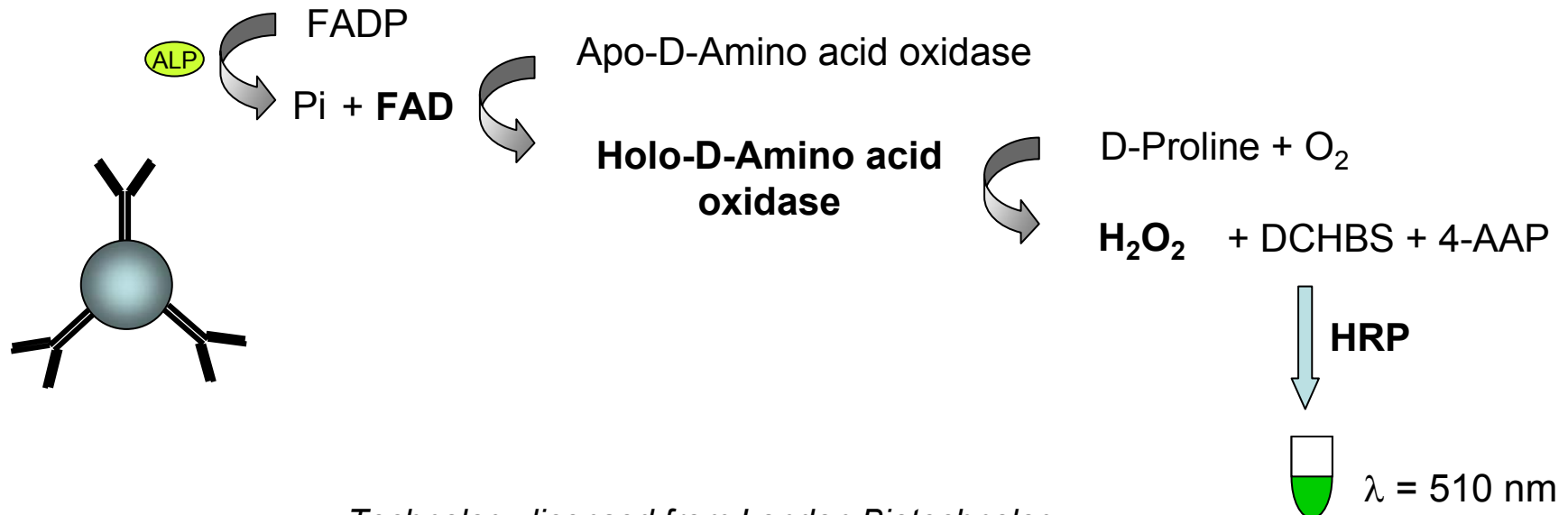
Experimental Protocol

Reaction cuvette with no Trp and no ALP-labeled antibodies



Addition of increasing concentrations of bacterial ALP

Biochemical amplification cascade

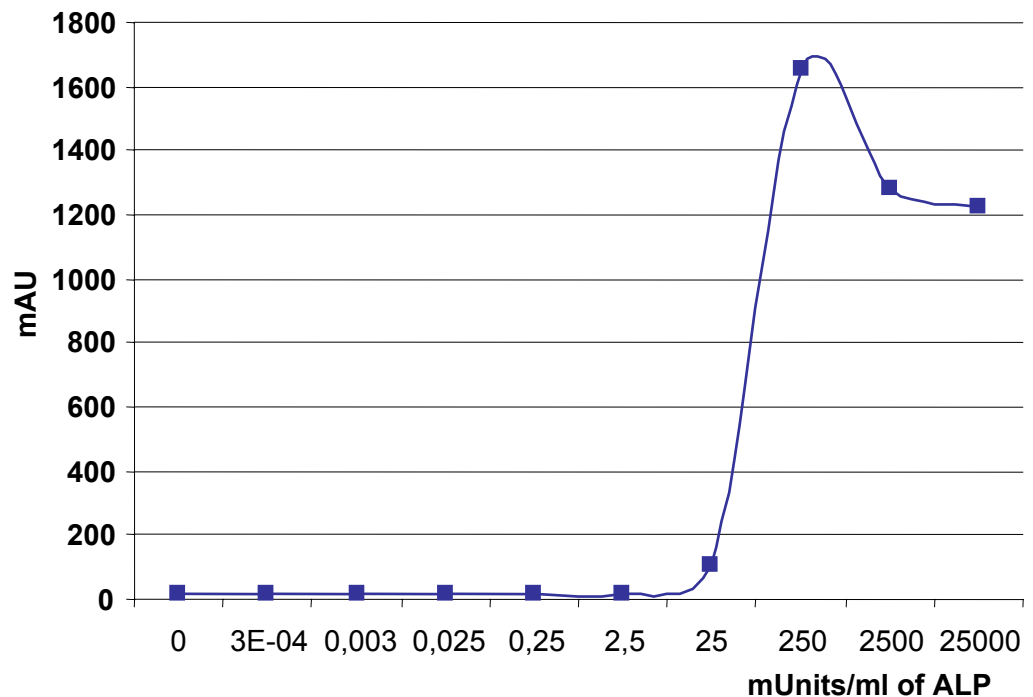


Interference of Bacterial ALP with the MILLIPORE Assay

Range of the ALP concentrations tested : 0.25 μ Units/mL to 25 Units/mL

E. Coli ALP from Sigma (ref : P-4252) 100 units. Use Ultrapure water for dilutions

[ALP]	mAU
No ALP	12,44
0,25 μ Unit/ml	12,41
2,5 μ Unit/ml	12,89
25 μ Unit/ml	12,8
0,25mUnit/ml	12,19
2,5mUnit/ml	12,7
25mUnit/ml	103,59
0,25Unit/ml	1657,7
2,5Unit/ml	1283,1
25Unit/ml	1221

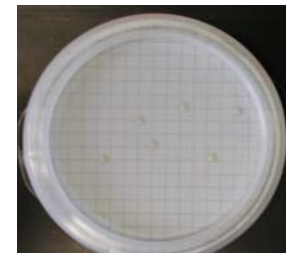


Demonstration that bacterial alkaline phosphatase in the water can cause interferences in the assay

ALP Release from Bacteria in High Purity Water

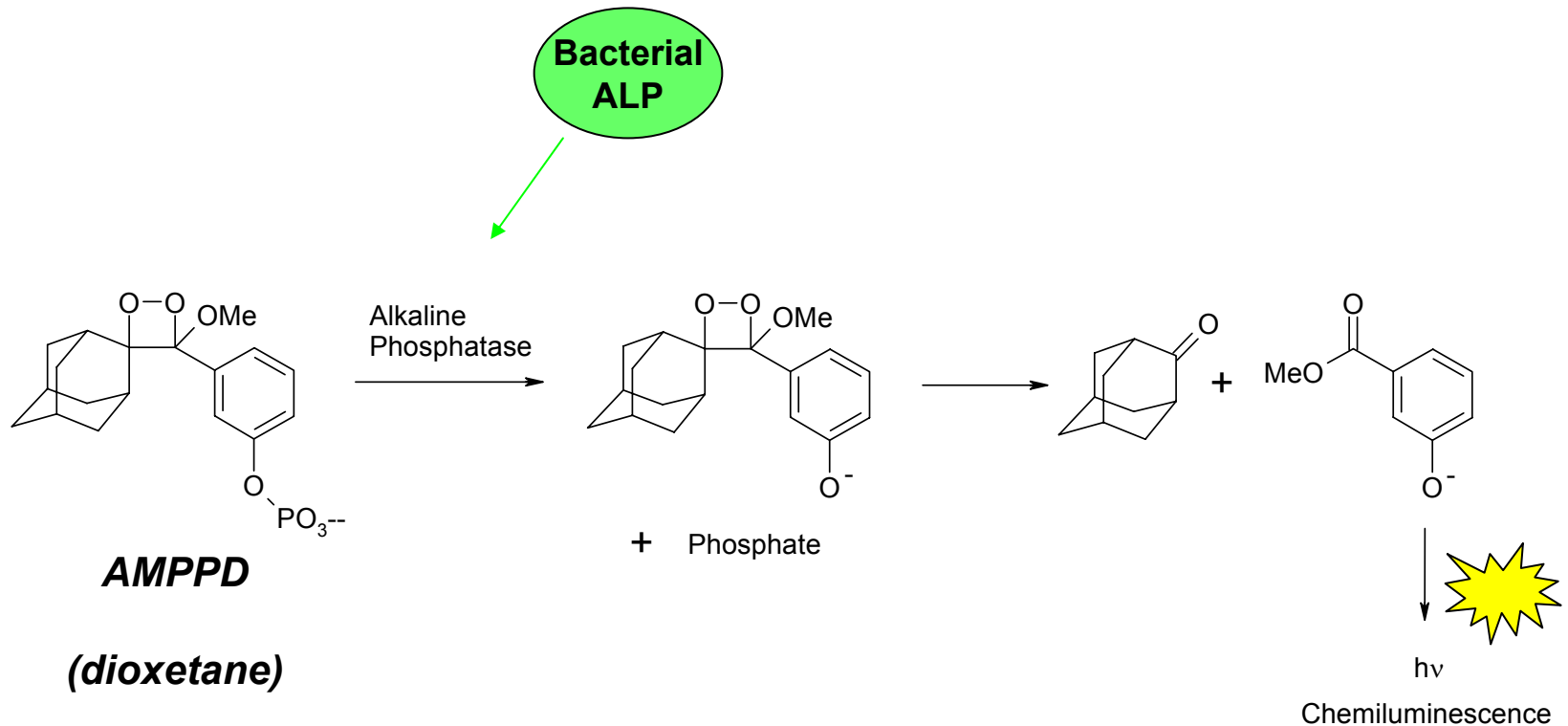
- Correlation between bacteria concentrations and levels of ALP in water

Bacteria Strain (identification by 16S rDNA sequencing)	Bacteria level (x 10 ⁶ cfu/mL)	ALP concentration (μUnit/μL)
<i>Sphingomonas paucimobilis</i>	29.2	6.22
<i>Caulobacter crescentus</i>	9.7	9.95
<i>Ralstonia pickettii</i>	29.5	8.29



Interference in Chemiluminescence Assays

Assays utilized on several brands of chemical analyzers

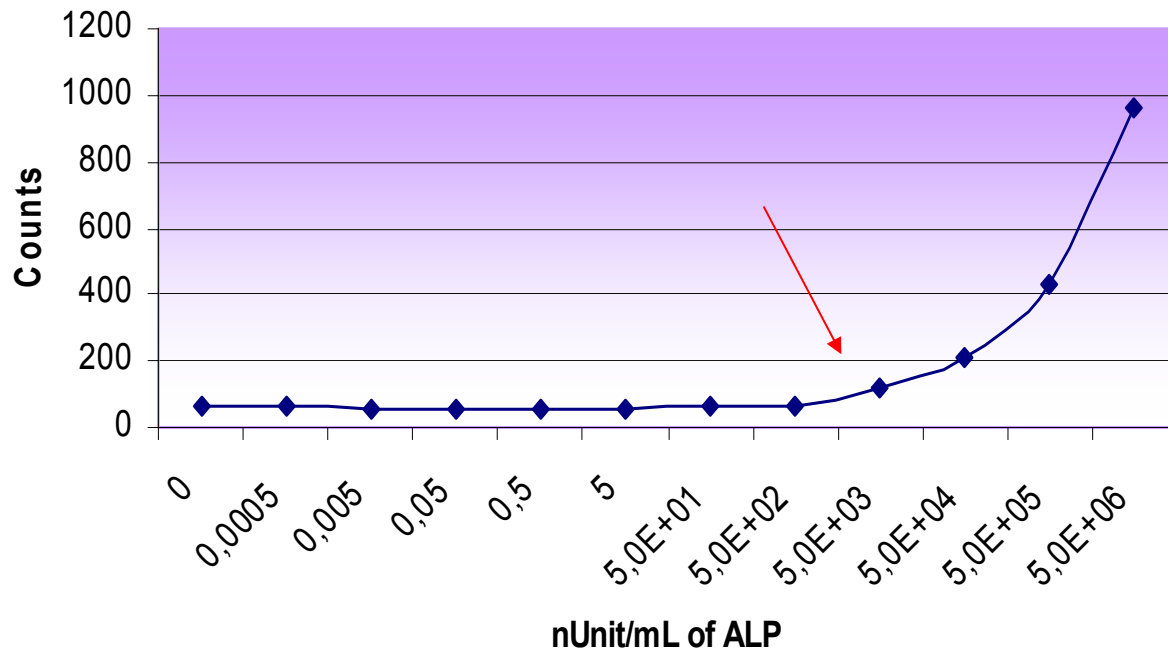


AMPPD-Based Assays: ALP Dose-Response

Addition of increasing ALP concentrations in “water test” on the Olympus AU3000i immuno analyzer

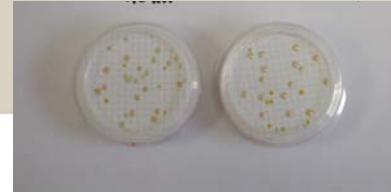
Water test is simply a test for ALP activity on the chemiluminescence substrate

E. Coli ALP from Sigma (ref : P-4252) 100 units. Use Ultrapure water for dilutions



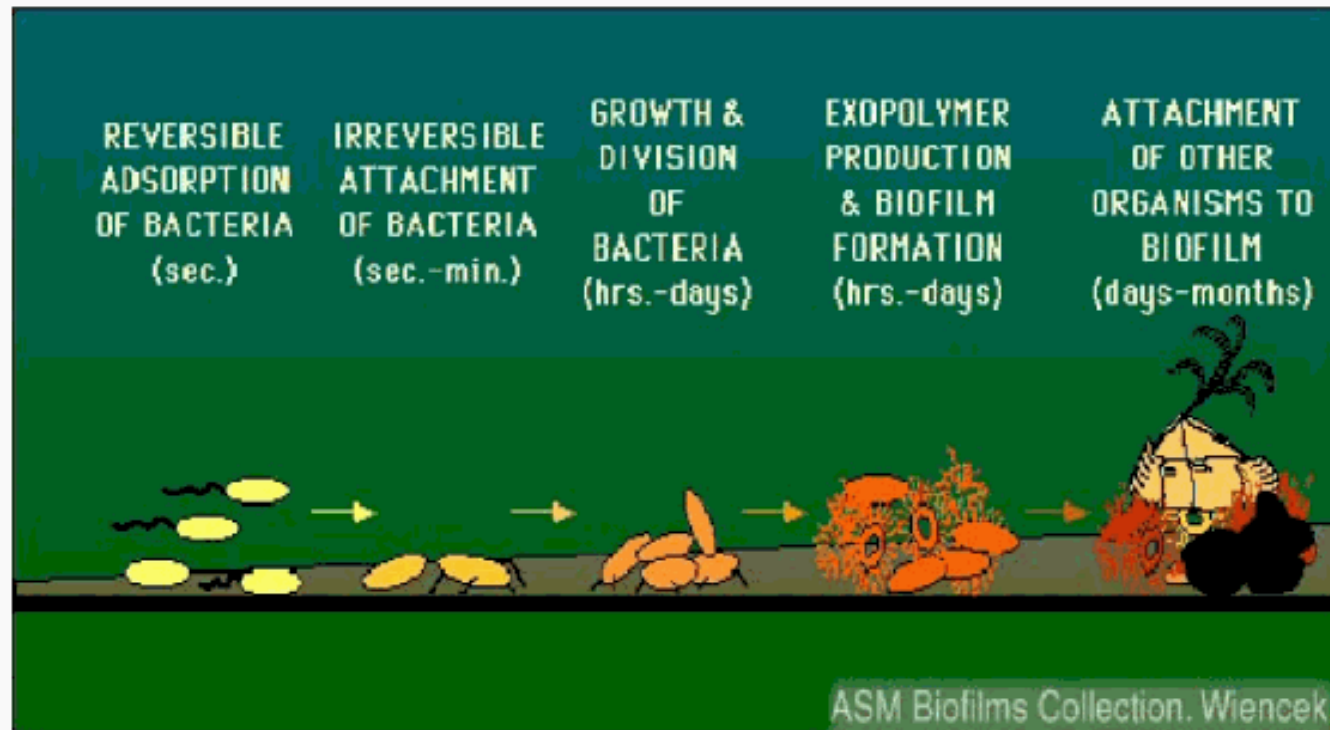
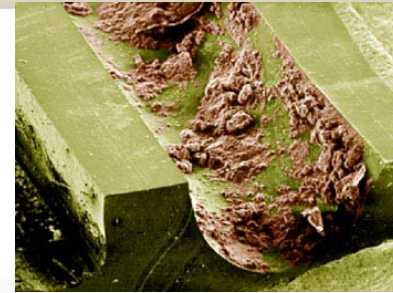
In the range of bacterial contamination $10^3 - 10^6$ CFU/mL

Bacteria in Clinical Analyzers



- Some bacteria strains are able to adapt to stringent conditions and grow in high purity water
 - Three strains are commonly identified in clinical analyzers
 - *Ralstonia pickettii*
 - *Sphingomonas paucimobilis*
 - *Caulobacter crescentus*
- Count methods
 - Total heterotrophic plate count (R₂A medium)
 - Membrane filtration or spread-plate techniques
 - Epifluorescence more recently introduced (more bacteria detected)

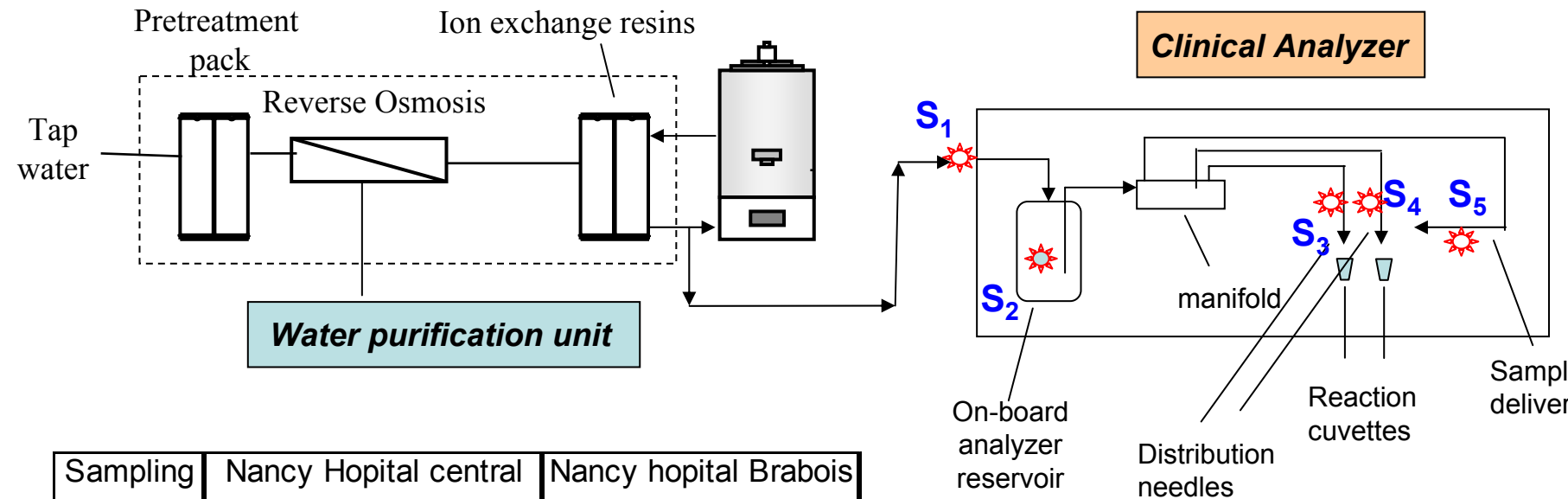
Major Issue with Bacteria: Biofilm Formation



Case Studies: The Reality in the Clinical Analyzers !

Two similar analyzers compared:

- one where assay issues (CTNi) have been reported (Hopital Central),
- one running normally (Hopital Brabois)



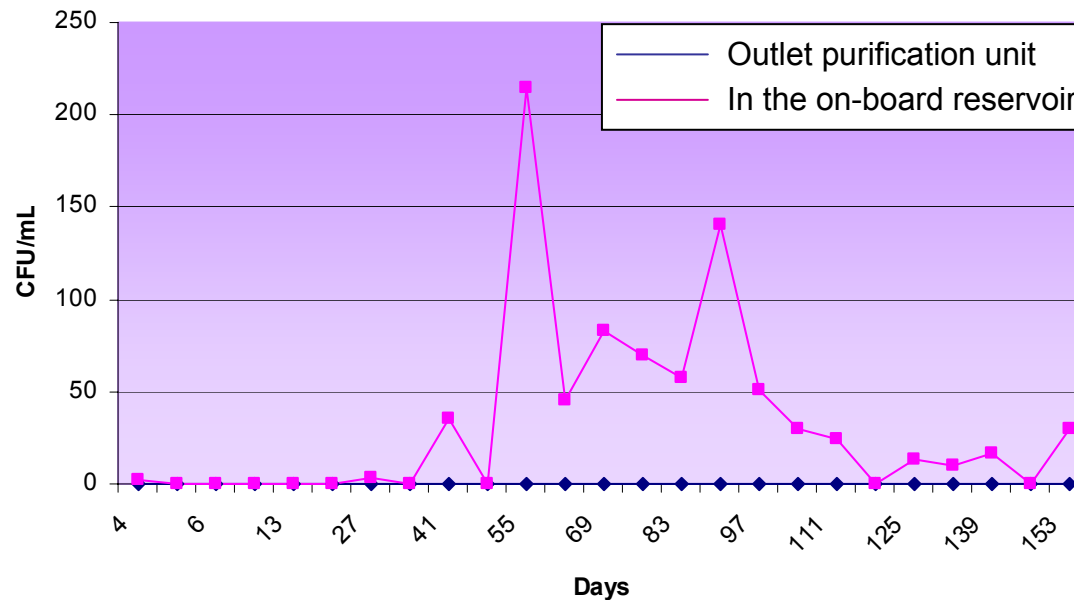
Sampling point	Nancy Hopital central	Nancy hopital Brabois
	Issue	No issue
S1	400 CFU/ml	4 CFU/ml
S2	700 CFU/ml	650 CFU/ml
S3	2350 CFU/ml	550 CFU/ml
S4	16250 CFU/ml	500 CFU/ml
S5	TNTC (100 μ L)	300 CFU/ml



CLSI recommendation at S₁:
< 10 cfu/mL

Bacteria Level in the On-Board Reservoir

- Water delivered by water purification meets CLSI specifications (< 10 CFU/mL)
- Bacteria growth in the on-board reservoir (analyzer sanitized when experiment started)



Critical to monitor and control bacterial growth on a regular basis

Frequently Reported Issues on Assays

Frequent Calibrations

High CV%

Fluctuation in quality results over the day/week/month

Interfered assays

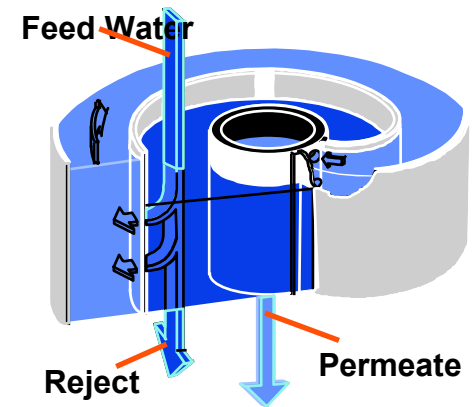
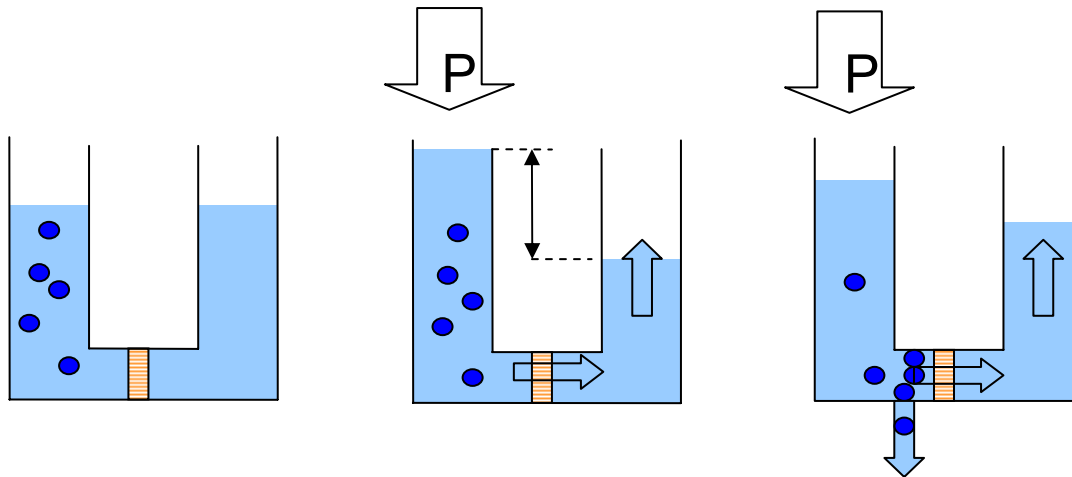
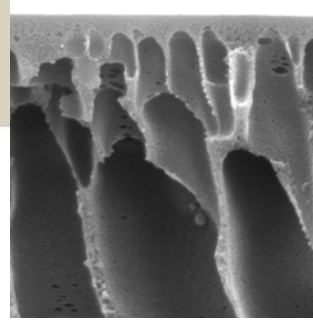
- | | |
|-----------------|--|
| – Calcium | Interfered by rocks, organics (humic and fulvic acids), biofilm |
| – ALP | Interfered by biofilm, detergent, rocks |
| – CK | Interfered by water treatment |
| – Amylase | Interfered by citrate, detergents, organics (humic and fulvic acids) |
| – LD | Interfered by effluent, organics (humic and fulvic acids), H ₂ O ₂ |
| – Phosphorus | Interfered by citrate, organics |
| – Iron | Interfered by rocks, organics (humic and fulvic acids), detergents |
| – Magnesium | Interfered by citrate, rocks |
| – Triglycerides | Interfered by plastics, chemicals |
| – Urea | Interfered by citrate, water treatment |
| – Troponin I | Interfered by biofilm |

Purification Technologies

- Addressing ionic and organic purity
 - Bacteria removal

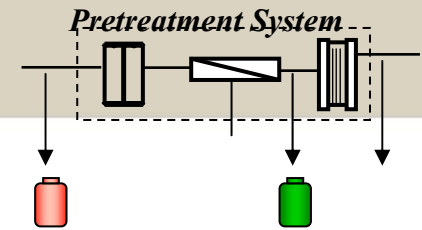
Reverse osmosis

- Removes ions ($> 95\%$), organics ($> 90\%$), particulates ($> 99\%$)



Efficiency of Reverse Osmosis

Ions	Tap water	RO permeate	% Reject
Li ⁺	2.9	0.19	93.4
Na ⁺	14 599	1 868	87.2
K ⁺	3 138	371	88.2
Mg ²⁺	6 438	20	99.7
Ca ²⁺	106 502	584	99.5
F ⁻	578	6	99.0
Cl ⁻	45 477	831	98.2
NO ₃ ⁻	13 843	1 756	87.3
SO ₄ ²⁻	117 546	232	99.8



Units: in $\mu\text{g/L}$
(ppb)

TOC (Organic content) values in ppb

	Tap	RO permeate
# 1	1410	64
# 2	1180	38

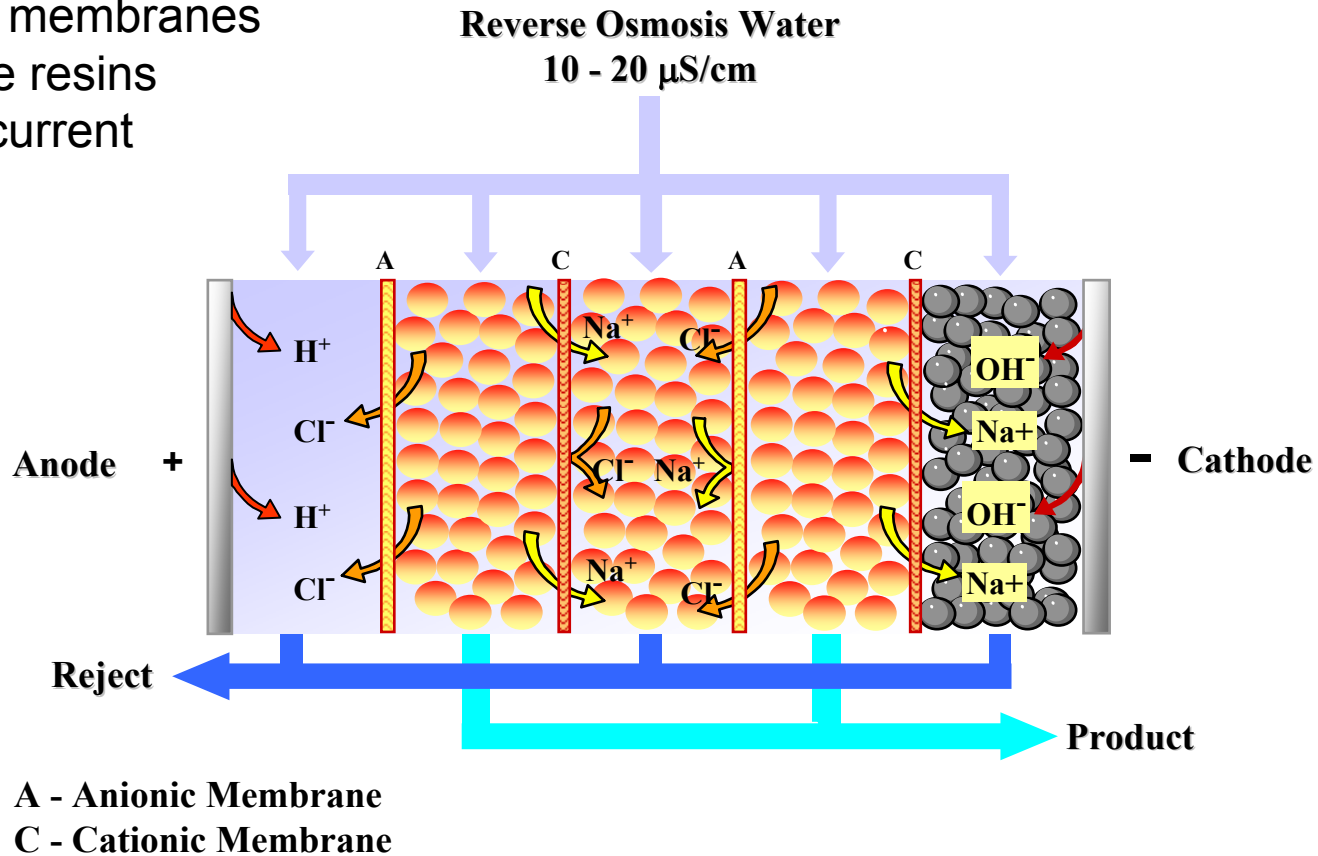
TOC measured using a Ionics-Sievers TOC analyzer
(method with acid treatment – persulfate and UV oxidation)

Overall rejection: Conductivity 569 $\mu\text{S/cm}$ to 12.3 $\mu\text{S/cm}$ – 97.8%

Technology Insight: Electro-Deionization

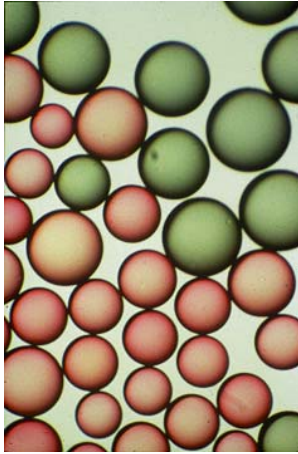
EDI module

- Ion selective membranes
- Ion exchange resins
- Continuous current



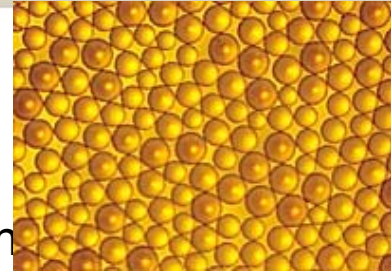
Self-regenerating system: No need for maintenance

Ion Exchange Resins



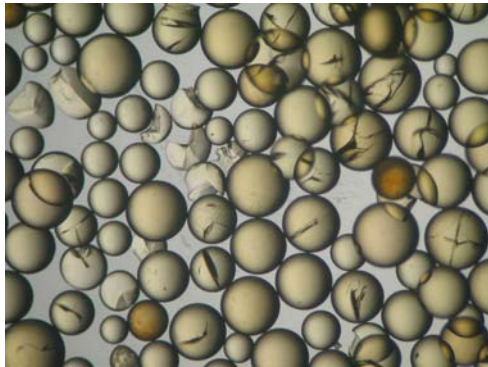
➤ Characteristics

- Removes ions (inorganic and organic)
- Mixed bed ion exchange resins
- Size and color homogeneous
- No particulates or fines
- No grain cracked (single regeneration)
- Control of each incoming resin lot



➤ As a result:

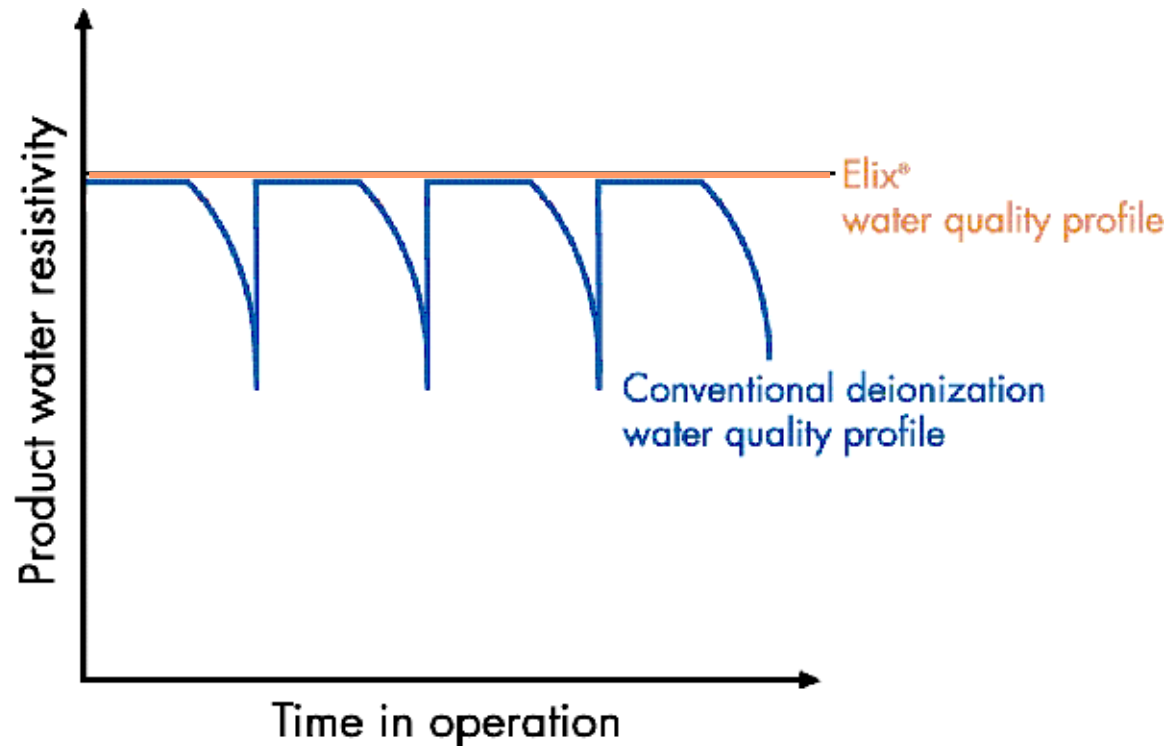
- High capacity with good binding kinetics
- Low organic release



Resin discarded:
*Standard cationic gel,
broken and cracked*

RO- DI compared with RO-EDI

➤ Consistent water quality



UV_{254 nm}: Germicidal Effect

Low pressure mercury lamps – Bacteria inactivation

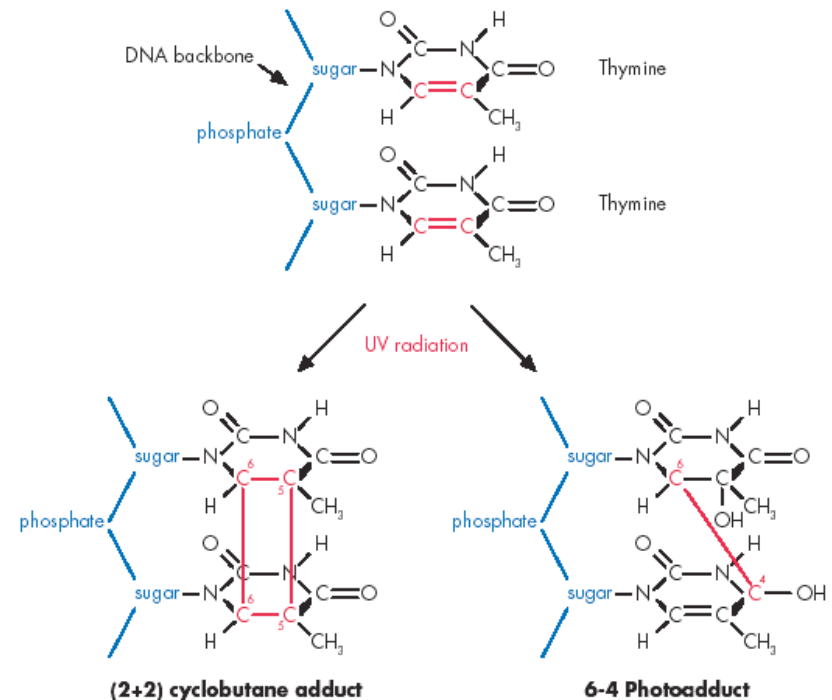
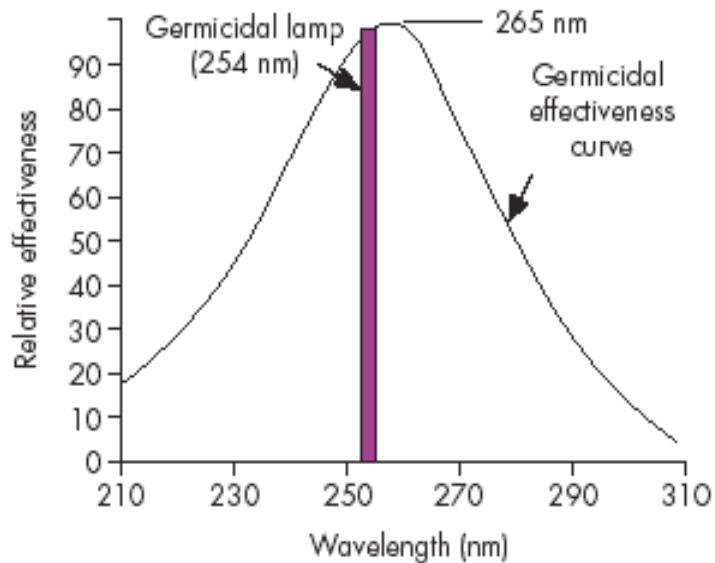


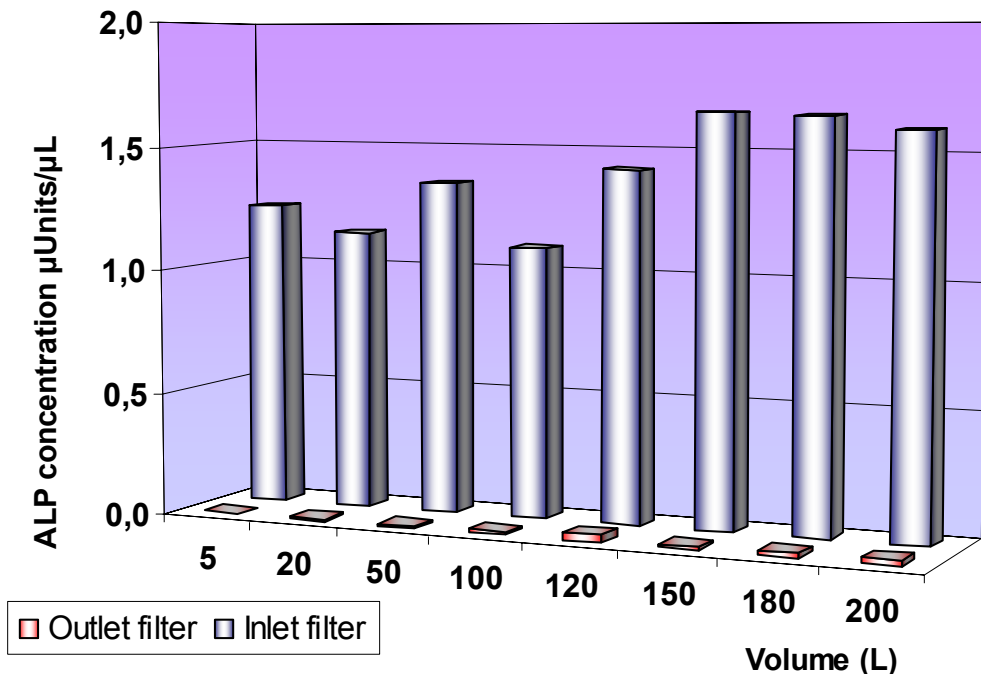
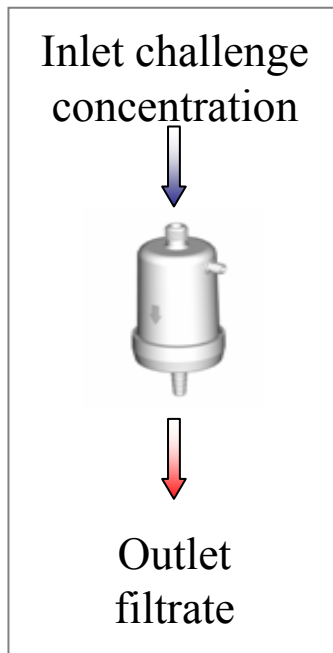
Figure 8: DNA photoadducts generated by UV 254 nm



Ultrafiltration: ALP Removal



- **Ultrafiltration:** membrane with a cut-off between 5 and 20 KDa
- Challenge solution: 200 L of high purity water + 300 Units of ALP (Sigma P-4252, 1000 Units)
- Challenge solution filtered on ultrafiltration device (delivery pressure 1 bar, flow rate 1.4 L/min)



A minimum of 300 U of ALP can be filtered out using the UF cartridge

Comparison of CTNI blanks using a 0.2 μm screen filter *versus* an ultrafilter

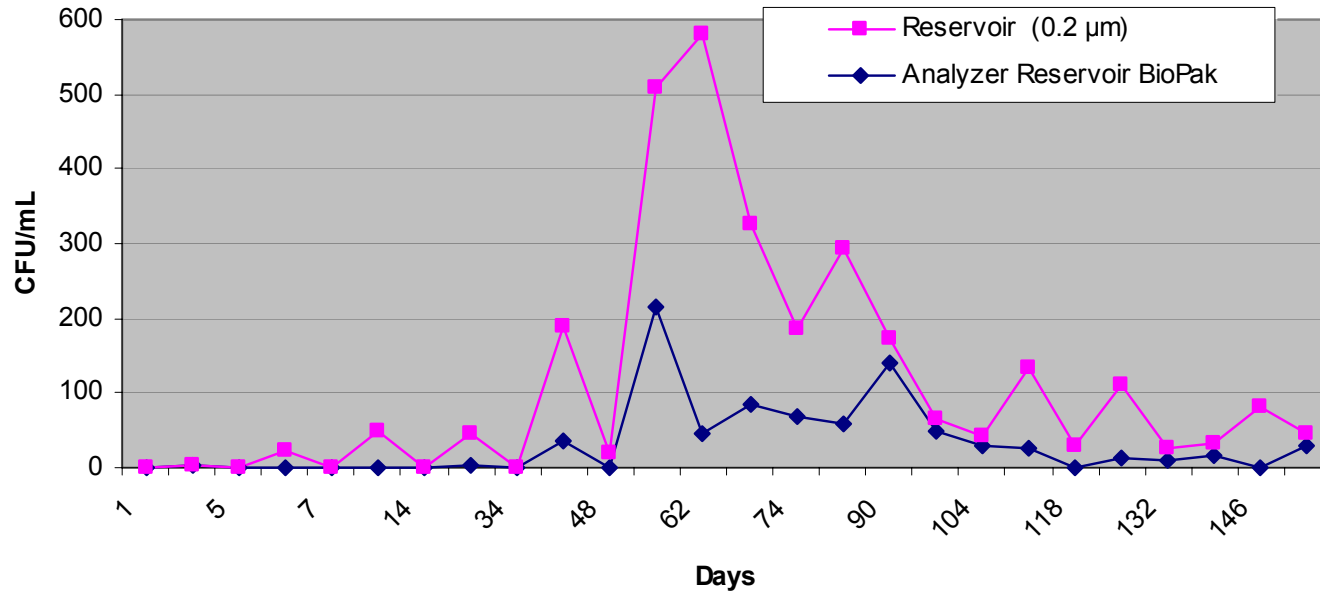
MILLIPORE

0.2 μm filter

Ultrafilter

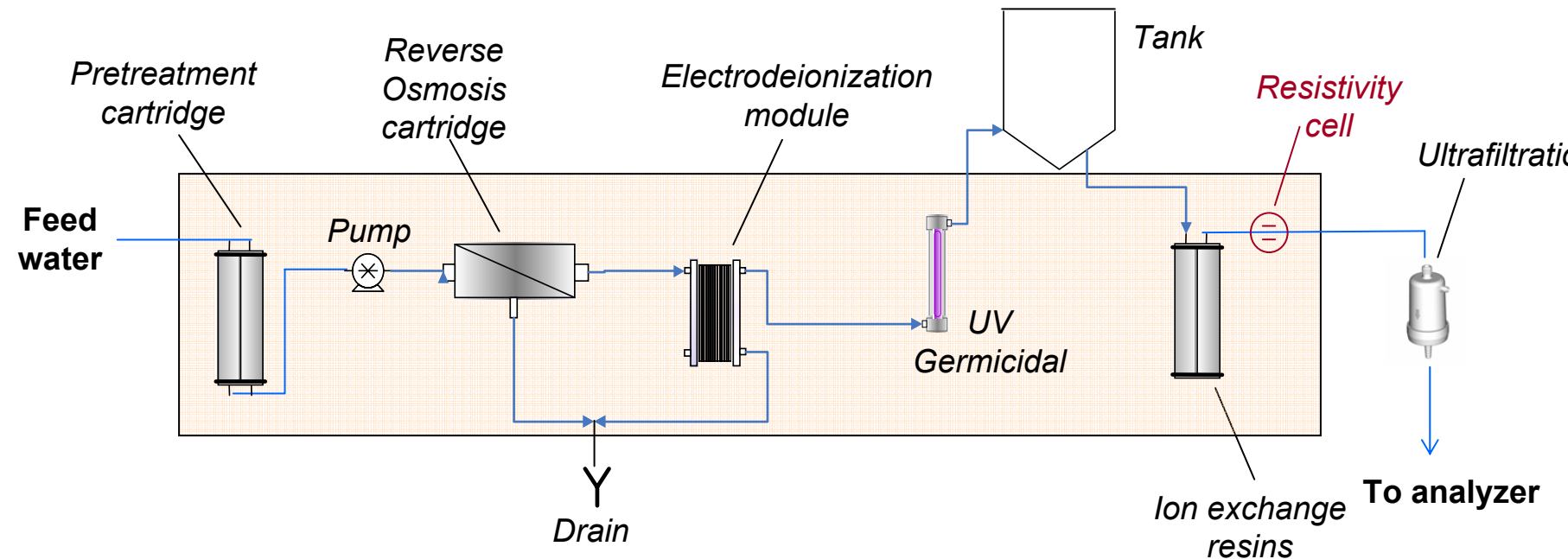
Ultrafiltration also Improves Bacterial Count

- Ultrafiltration reduces the amount of nutrients (macromolecules) in the on-board reservoir of the clinical analyzer.
- This results in a reduced bacterial growth in the reservoir and an improved bacterial control.



Example of Combination of Purification Technologies

Simplified flow schematic combining purification technologies (Elix Clinical)



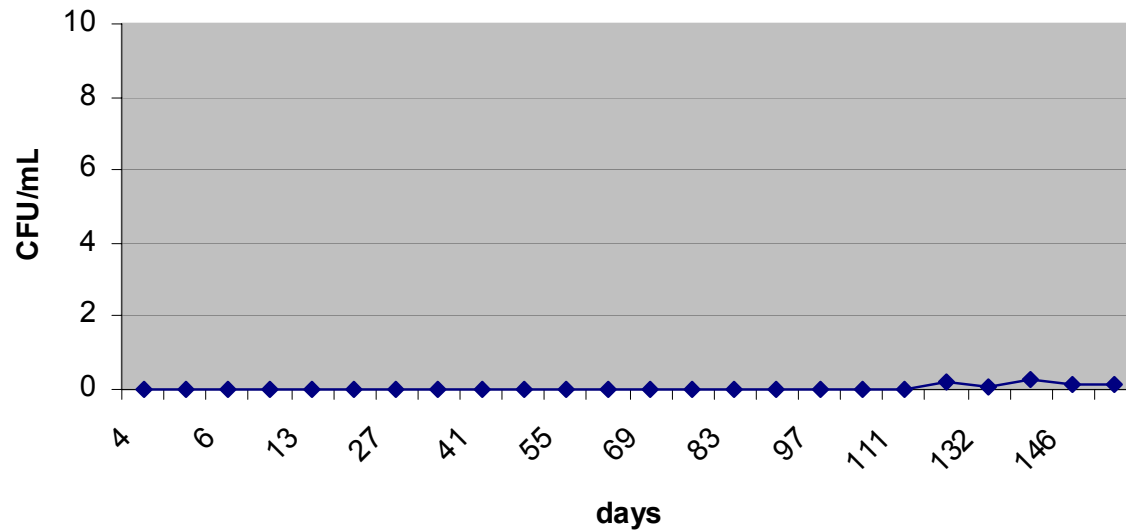
Resistivity > 10 MΩ.cm
Bacteria < 10 cfu/mL
TOC < 500 ppb

Case Study: Oregon Hospital & State University Laboratory

MILLIPORE

Field analysis: Water purification unit (Elix Clinical) equipped with ultrafiltration – Beckman Analyzer LX20

Bacterial count at the outlet of the purification unit



Summary

- Recording water quality parameters improves the risk management in the laboratory
 - Following the specifications recommended by the CLSI guideline (CLRW) reduces the risks of issues in assays run on clinical analyzers.
 - Monitoring resistivity, TOC and bacteria on a regular basis enables to detect issues before they generate downtime of the instruments
- Water purification systems need to be well designed
 - to ensure high water quality
 - To allow validation and easy monitoring of quality parameters
- Bacteria (not always coming from the water !) are a major source of issues in clinical analyzers: it is critical to sanitize and maintain the analyzers appropriately

An aerial photograph of the Great Wall of China, showing its iconic crenelated battlements and watchtowers as it snakes across a vast, densely forested mountain range. The perspective is from a high vantage point, looking down and across the terrain. The wall's path is clearly visible, with several prominent watchtowers and sections of the wall that appear to be under construction or repair, featuring modern-looking staircases and walkways. The surrounding landscape is a lush, green expanse of trees and vegetation, with the mountains rising and falling in the distance. The overall scene is one of historical grandeur set against a backdrop of nature.

谢谢