WHAT'S IN YOUR WATER?

MICROORGANISMS

- Microorganism contamination is usually bacterial and heavily impacts downstream assays involving cells or cellular material
- Directly leads to death in most cultured cells; can induce phenotypic changes in survivors
- Secrete nucleases, pyrogens, and phosphatases, affecting nucleic acid and protein integrity
- Confounds cell counts and protein/nucleic acid concentration measurements
- Interferes with downstream assays (e.g., PCR, western blot, ELISA) by destroying source material quality or impeding probing mechanisms (e.g., antibody binding specificity)

REMOVAL

- Microorganisms can be killed using UV light irradiation at 185 or 254 nm
- Dead microorganisms may leave cellular debris/internal cellular contents in solution
- Filtration can remove both microorganisms and cellular debris
- Equipment maintenance is critical as microorganisms can foul filters and form biofilms

IONS

NON-ALKALI METALS

- Metals such as Fe, Cu, Zn, and Mn are involved in many physiological processes, serving as catalysts, enzyme inhibitors, or chelators
- Heavy metals (e.g., Hg, Zn, Pb) are cytotoxic

ALKALI METALS

- Na^+ and K^+ ions are critical to electrophysiology and affect water conductivity and resistance
- Mg levels are important to PCR efficiency

REMOVAL

- Ion-exchange resins swap unwanted ions for H^+ or OH^- ions
- Resins combined with electric currents pull ions out of solution towards an anode or cathode depending on charge polarity
- Deionized water is "hungry" – highly susceptible to ionic contamination leached from storage containers
- Ion content is measured by resistivity, with 18.2 MΩ·cm at 25 °C the industry standard for pure deionized water

PARTICULATE MATTER & COLLOIDS

- Nonsoluble particulate matter can be present in the form of minerals, sediment, sand, and other similar materials
- Can clog filter pores and small channels, such as those commonly used in microfluidic or chromatography instruments
- Particulate matter can directly damage sensitive equipment used in biological assays; clogs increase pressure levels within instrument systems, indirectly damaging pumps, injectors, and sensors
- Colloids modulate water viscosity, potentially affecting system pressures and causing clogs

REMOVAL

- Microfiltration with a 0.22 µm pore size is sufficient to remove most particulate matter; this is the minimum requirement for clinical laboratory reagent-grade water under CLSI specifications

GASES

- Gases are absorbed and dissolved into water via atmospheric contact
- Excess N_2 and O_2 may confound experimental measurement accuracy, particularly in the ecological sciences
- Cl_2, CO_2, NH_3 can ionize, altering water pH
- All gases may precipitate and form bubbles, affecting spectrophotometric measurements and blocking microfluidic channels

REMOVAL

- Distillation partially removes dissolved gases, but a new equilibrium will be established upon atmospheric contact
- Specific gases can be removed by subjecting the water to chemical reactions intended to consume the target gas
- The products of ionizing gases can be removed using ion-exchange or electrodeionization (EDI); continued application will eventually exhaust gas reserves

ORGANIC CONTENT

- Organics are defined as compounds with carbon as a principle constituent
- This can include decayed plant matter, solvents (e.g., toluene, benzene), and byproducts of combustion
- Organic contamination is detectable by chromatographic assays (e.g., HPLC, LC/MS), leading to increased noise or confounding peaks; organic compounds can also generate background fluorescence
- Hydrocarbons are cytotoxic, impairing cellular function and enzyme activity

REMOVAL

- UV irradiation breaks carbon-hydrogen bonds, destroying organic molecules
- Reverse osmosis is excellent for removing organic compounds due to the low MWCO point

1 NCCLS GUIDELINES FOR CLASSIFICATION OF WATER TYPES (1998)

CONTAMINANT (PARAMETER)	TYPE III	TYPE II	TYPE I
Ions (Resistivity; MΩ·cm @ 25 °C)	0.1	1.0	1.0
Organic Materials (TOC ppb)	NS	NS	Carbon filtration
pH	NS	NS	5-8
Particulates >0.22 µm (Units/ml)	NS	NS	0.22 µm filtration
Colloids (Silica; mg/L)	1.0	0.1	0.05
Bacteria (CFU/ml)	NS	<1000	<10

2 CLSI SPECIFICATION FOR REAGENT LABORATORY WATER (2006)

WATER TYPE	SPECIFICATIONS
Clinical laboratory reagent water (CLRW)	Microbial content <10 CFU/ml >10 MΩ·cm @ 25 °C Free of particulates >0.22 µm TOC <500 ppb
Special reagent water (SRW)	Application defined
Instrument feed water (IFW)	NS

3 ASTM REAGENT GRADE WATER SPECIFICATIONS (GUIDELINE D1193-06-2011)

CONTAMINANT (PARAMETER)	TYPE IV	TYPE III	TYPE II	TYPE I
Ions (Resistivity; MΩ·cm @ 25 °C)	0.2	4.0	1.0	18
Organic Materials (TOC ppb)	NS	200	50	50
pH	5-8	NS	NS	NS
Chloride (µg/L)	<50	<10	<5	<1
Sodium (µg/L)	<50	<10	<5	<1
Colloids (Silica; mg/L)	NS	500	3	3

CONTAMINANT (PARAMETER)	TYPE A	TYPE B	TYPE C
Endotoxin (EU/ml)	<0.03	0.25	NS
Bacteria (CFU/ml)	1	10	1000

NS: not specified

4 ASTM STANDARD GUIDE FOR BIO-APPLICATIONS GRADE WATER (GUIDELINE D5196-06-2006)

PARAMETER	STANDARD
Ions (Resistivity; MΩ·cm @ 25 °C)	18
pH	NS
Organic Materials (TOC µg/L)	20
Chloride (µg/L)	NS
Sodium (µg/L)	NS
Total Silica (µg/L)	NS
Bacteria (CFU/ml)	100
Endotoxin (EU/ml)	0.01 (or as required)
Nucleases and Proteases	As required