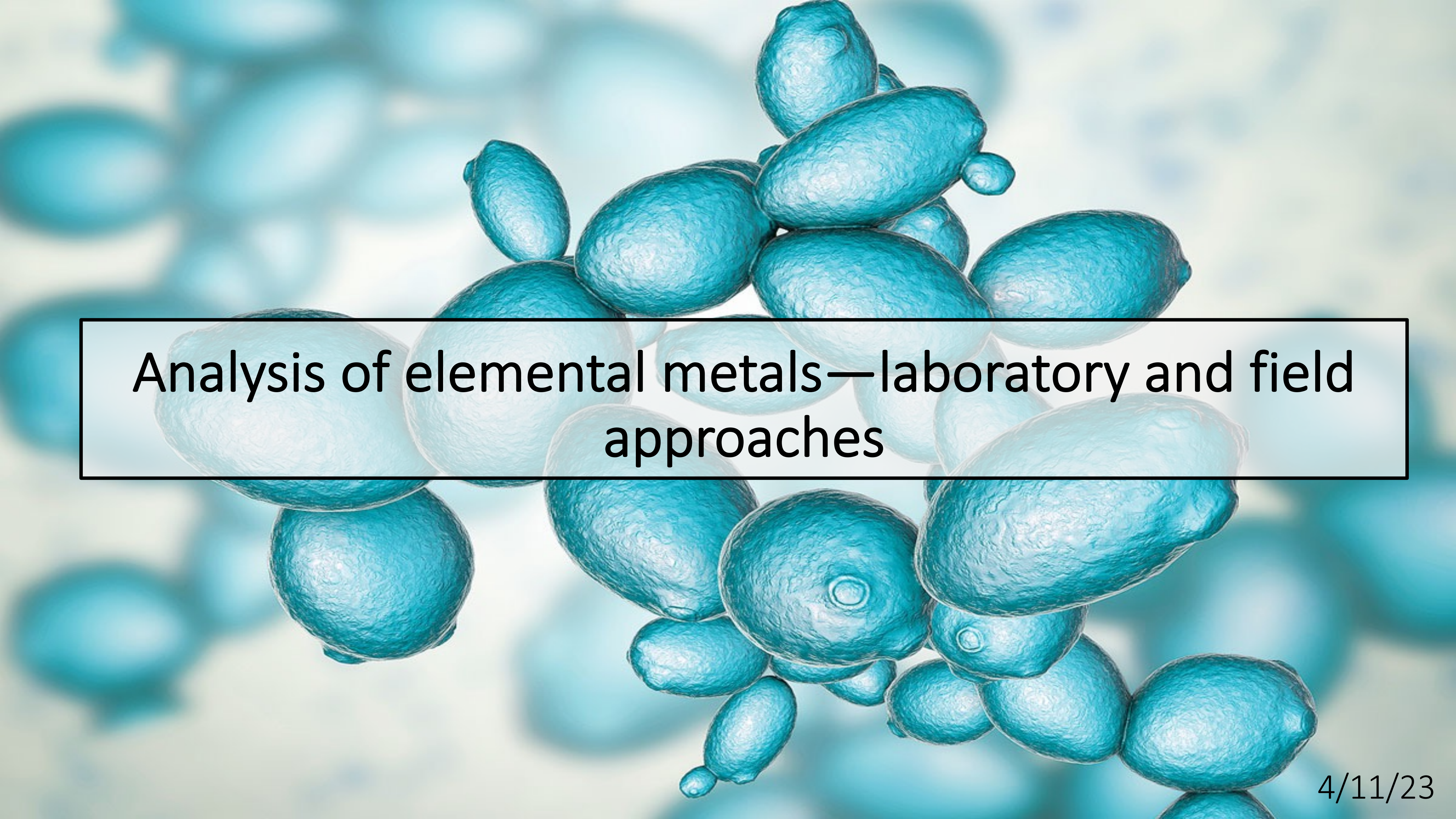


Analysis of elemental metals—laboratory and field approaches

4/11/23

Module Outline

- **M2D1**: Environmental heavy metal contamination
- **M2D2**: Model system – target selection and engineering approach
- **M2D3**: Model system – choosing a chassis host
- **M2D4**: Screening a system—assessing features of a bioremediation system
- **M2D5**: Analysis of elemental metals – laboratory and field approaches
- **M2D6**: Applying remediation strategies—advantages and pitfalls
- **M2D7**: Engineering a problem-specific bioremediation solution
- **M2D8**: Comm Lab

Intro

Design

Test

Apply

Review

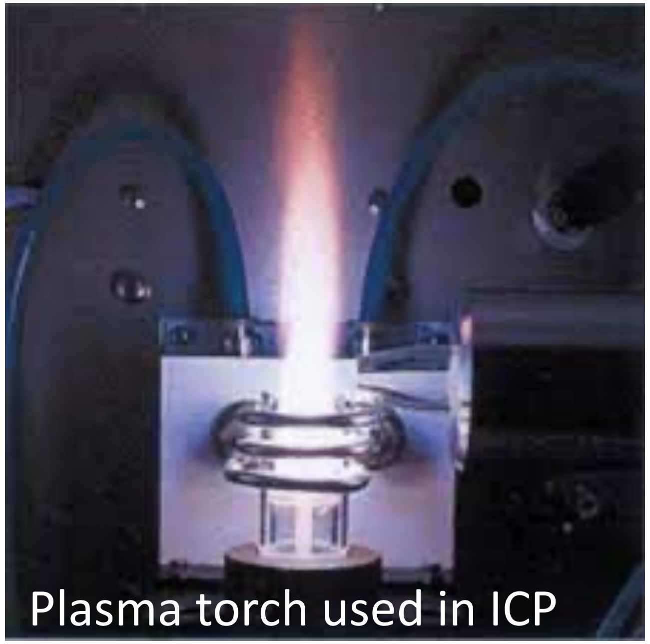
Lecture outline

- Review

- Elemental metal analysis with spectroscopy
  - ICP-OES
  - ICP-MS
  - CVAAS

- Metal analysis using chemical reactions
  - colorimetric tests

- Field tests for metal analysis
  - Color-based strips
  - Lateral flow assays



Plasma torch used in ICP

Review

Review of lecture concepts

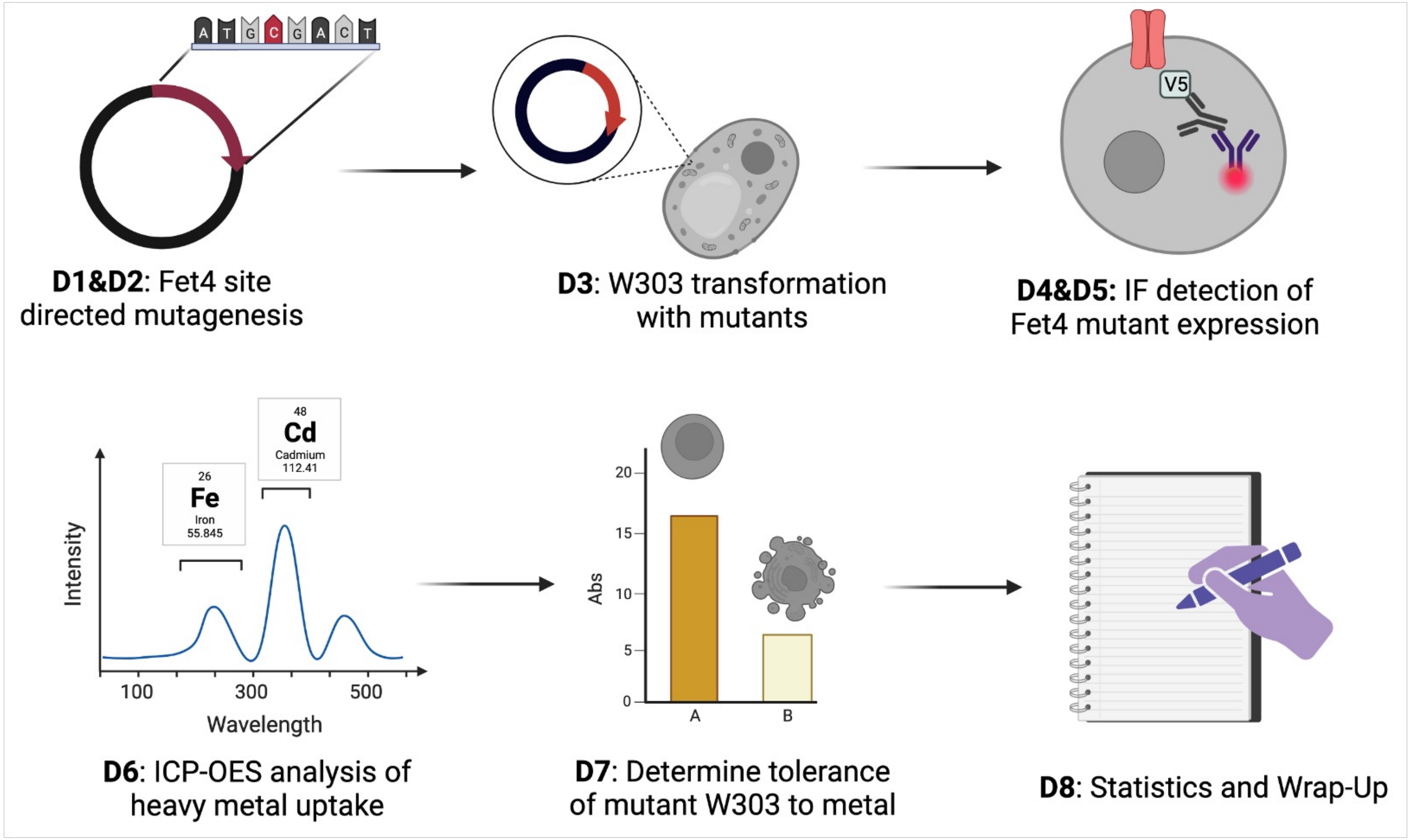
- The uses of heavy metals and how they can be released into the environment

- The effects of exposure to heavy metals on humans, plants, and microbes

- Considerations when choosing a target for modification and a a chassis to deploy genetically engineered system

- Methods for testing the created bioremediation system in the lab

Review of laboratory progress



Elemental metal analysis with spectrometry

The Environmental Protection Agency standardizes protocols for analytical metal testing from environmental sources

Cuyahoga river caught fire at least 13 times between 1868 and 1969

River fire in 1952

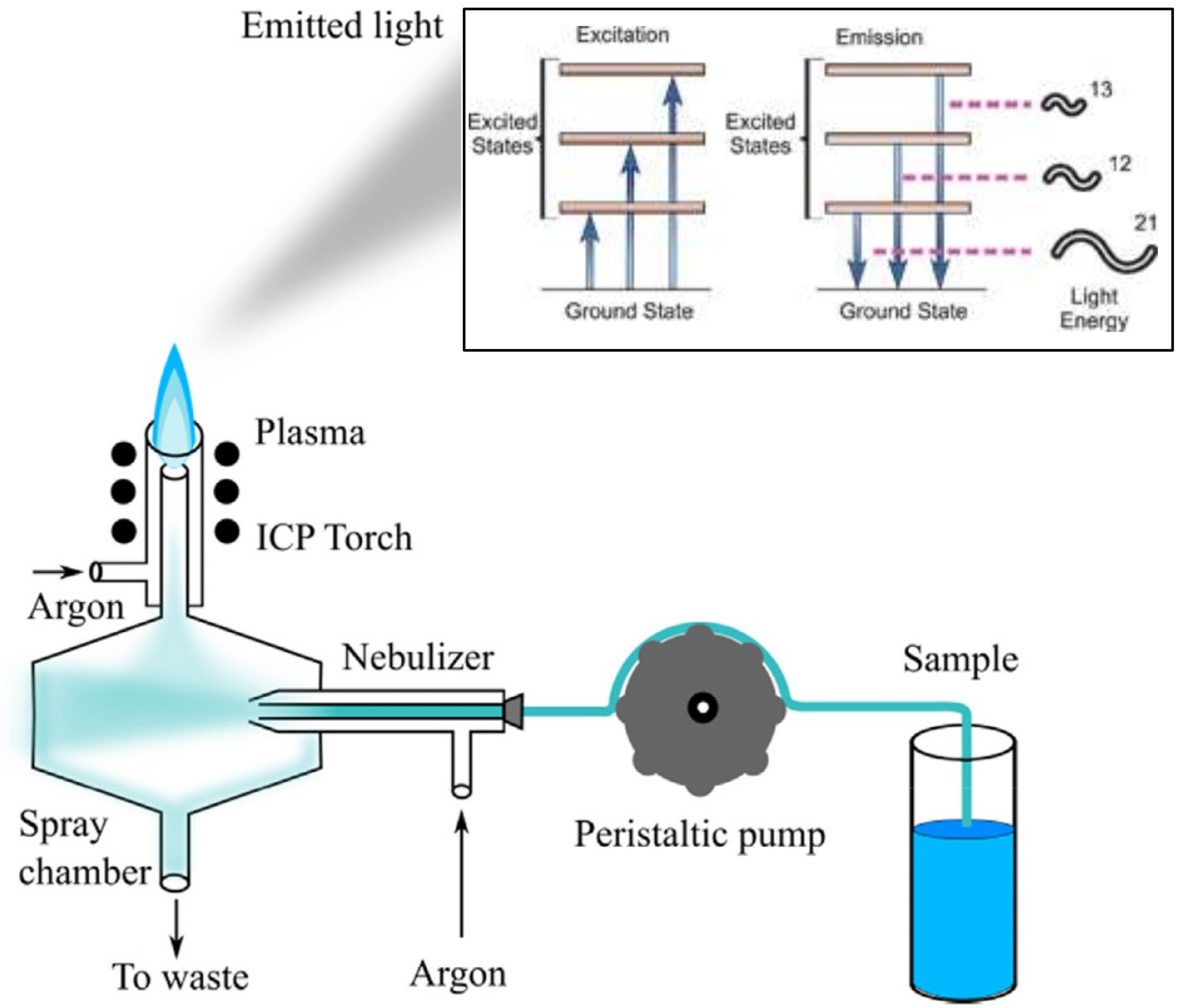
Oil residue in 1960s

EPA-approved methods for determining metal contamination

- Method 200.7 = ICP-OES
- Method 200.8 = ICP-MS
- Method 245.2 = CVAAS

Inductively coupled plasma– optical emission spectroscopy (ICP-OES)

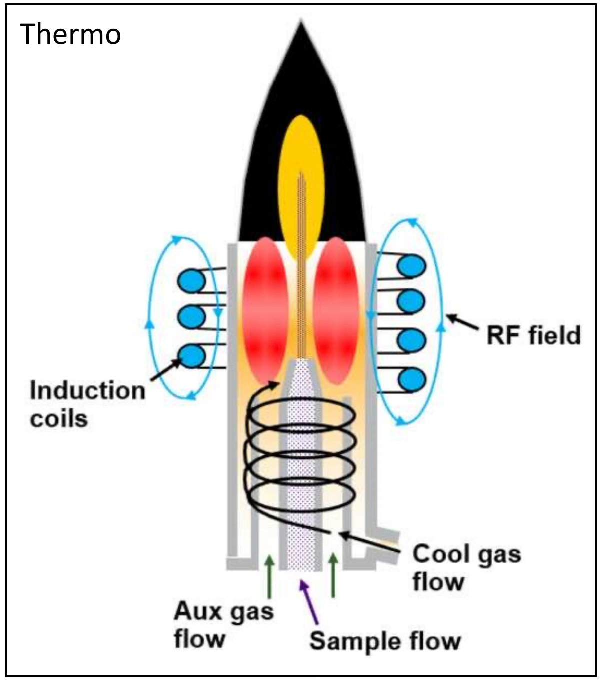
- also known as ICP-AES (atomic emission spectroscopy)
- Samples are digested with nitric acid and pumped into a nebulizer where it mixes with argon and creates an aerosol in the spray chamber
- The spray enters a central chamber with radiofrequency-induced electromagnetic field of argon plasma where the sample is vaporized, atomized, and ionized
- The plasma energy excites electrons which emit photons at specific wavelengths unique to each element as the electrons fall back to an unexcited state
- This photon emission data is collected to identify composition and relative concentration of elements in the sample

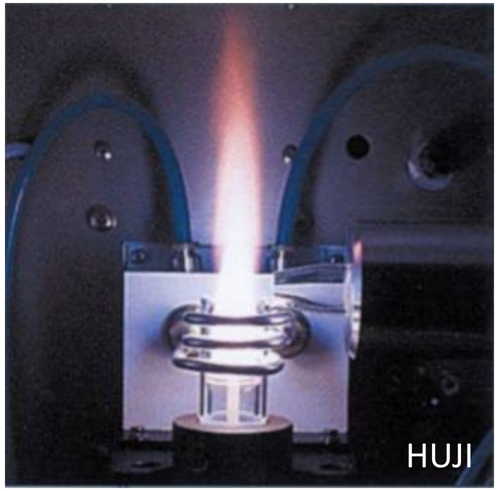


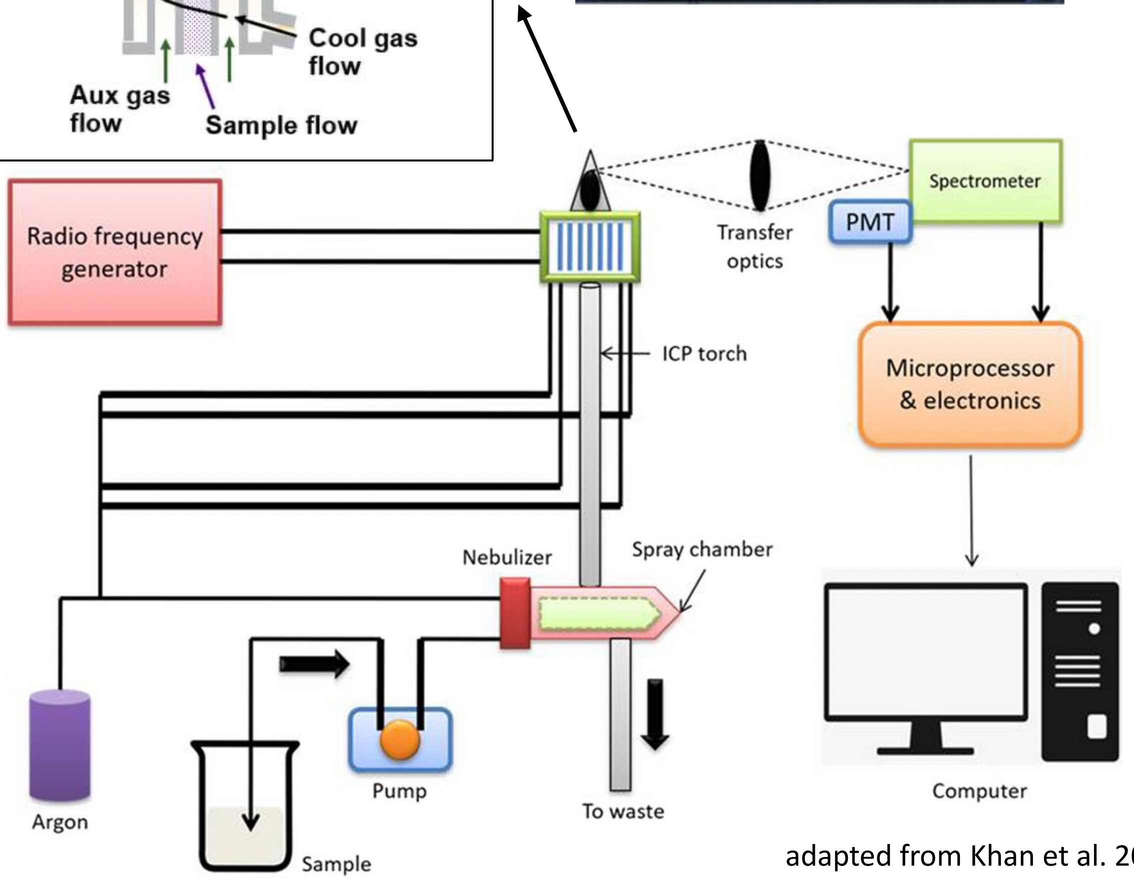
adapted from Cherevko. 2017

ICP-OES: the torch

- Inductively coupled plasma torch is created when:
  - Argon gas is pumped into a quartz tube encircled by induction coils
  - A radiofrequency generation powers these induction coils to create strong variable electromagnetic field
  - This produces a plasma torch which typically burns at ~ 6,000K
    - Same temperature as the surface of the sun



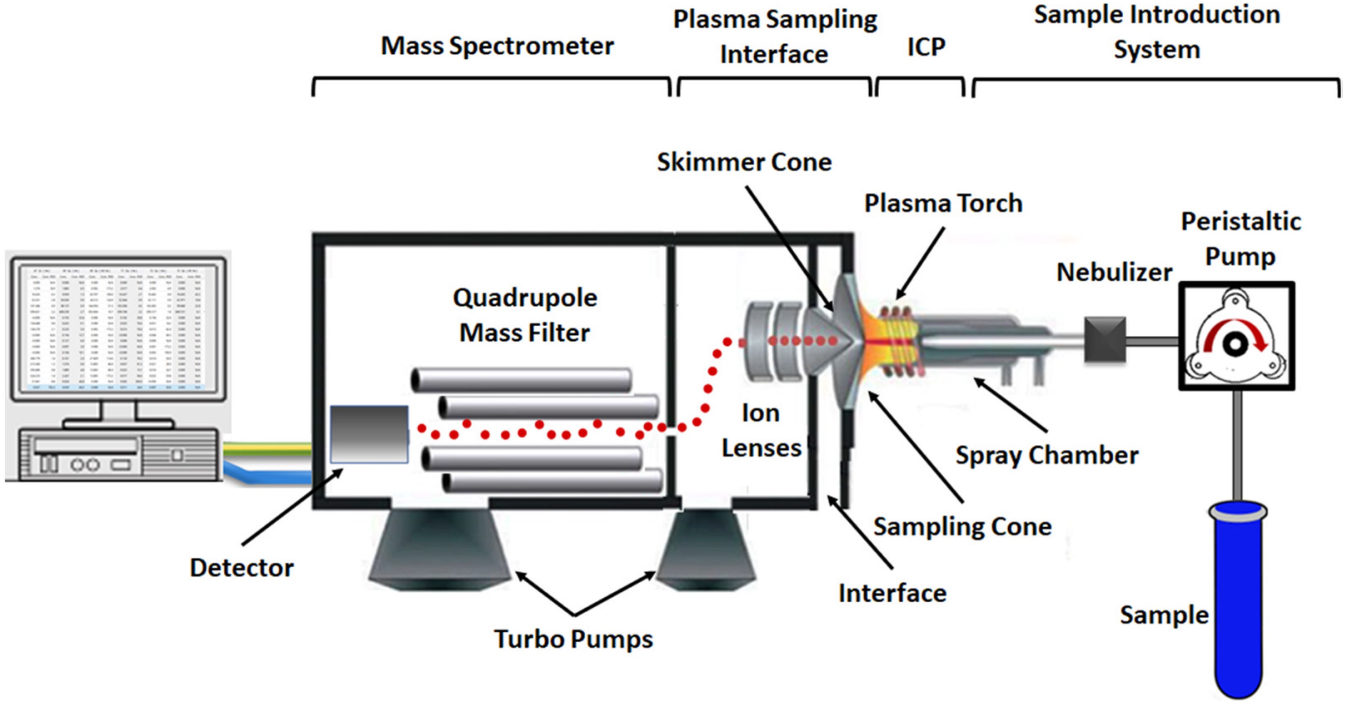




adapted from Khan et al. 2022

ICP-Mass Spectrometry (ICP-MS)

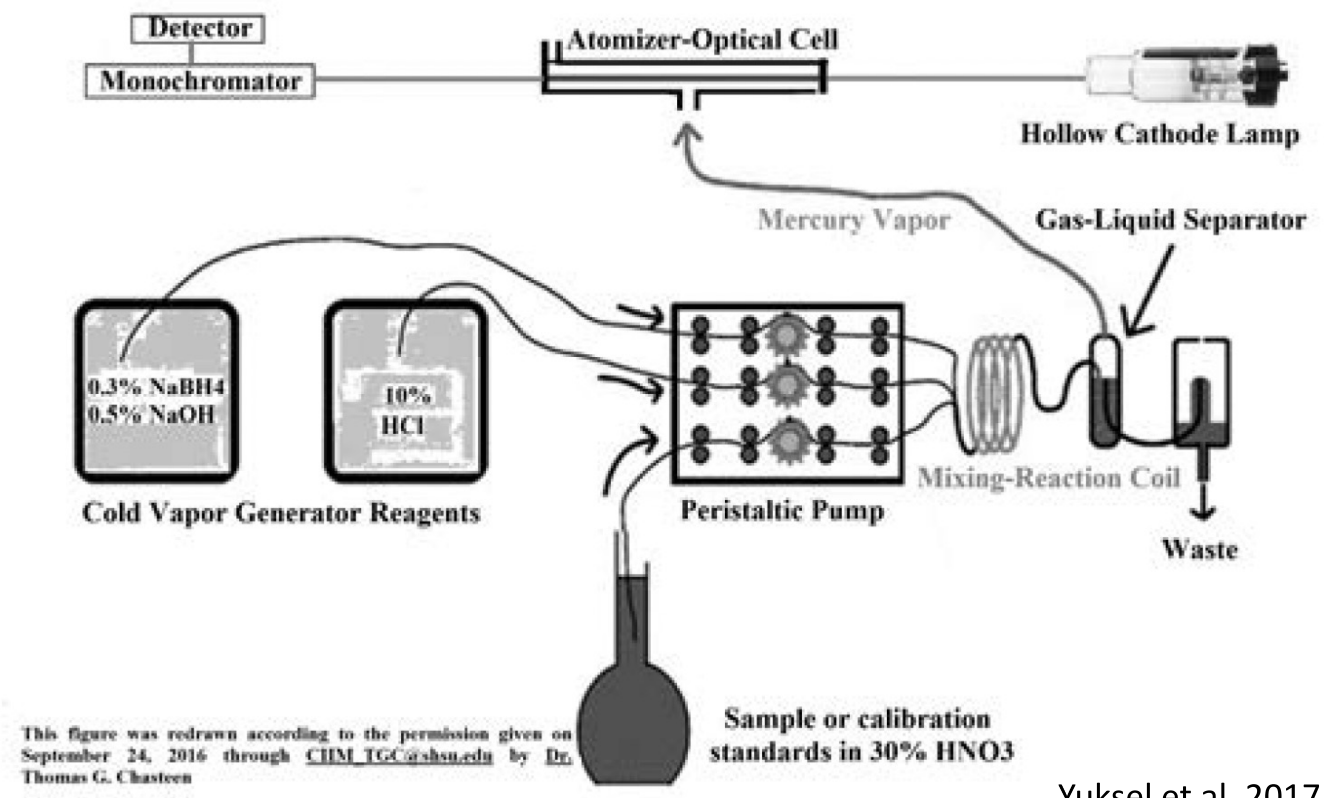
- Samples are digested with nitric acid and diluted with ultra-pure water
- Peristaltic pump moves sample into nebulizer where it mixes with argon and creates an aerosol in the spray chamber
- The spray moves into the argon plasma torch where it is ionized at the high temperature
- The ionized samples are passed through a skimmer cone to focus the ions into a beam which enters the vacuum of the quadrupole mass analyzer
- Ions are separated based on their mass/charge ratio and strike the detector resulting in a signal pulse that can be measured



Mazarakioti et al, 2022

Cold vapor atomic absorption spectroscopy (CVAAS)

- Reference method for monitoring drinking water under the Safe Drinking Water Act
- One of the primary techniques for mercury analysis
- Sample is digested in nitric acid
  - introduced into the peristaltic pump alongside reducing agent stannous chloride ($SnCl_2$) and HCl
- The $SnCl_2$ reduces Hg^{2+} to Hg^0 in the mixing vessel until equilibrium
- The Hg vapor is then pushed out of the liquid-gas separator by an inert gas (argon) and into the atomic absorption cell where the energy transfer can be recorded



Yuksel et al, 2017

Ideal spectrometry analysis approach depends on experimental parameters

ICP-OES

- Sample volume: 5ml
- LOD: ppb
- 50+ metals in single sample
  - Spectral interference between elements
- More tolerant of high concentration of dissolved solids in sample

ICP-MS

- Sample volume: 2ml
- LOD: ppt
- 50+ metals in single sample
  - Mass interference by metals with same isotopic mass or are doubly charged
- Lighter elements are more difficult to detect

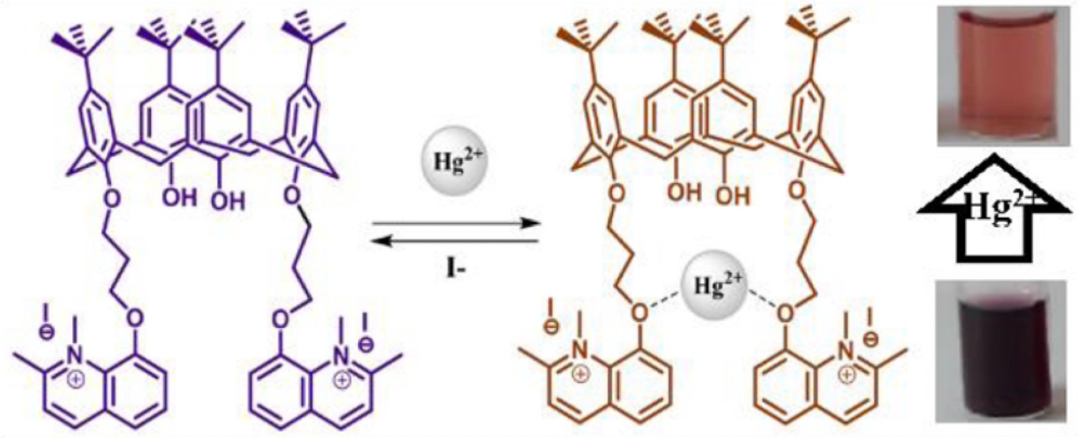
CVAAS

- Sample volume: 1-2ml
- LOD: ppt
- Best for volatile compounds like Hg
  - Better sensitivity for these compounds
- Most often used for single element analysis

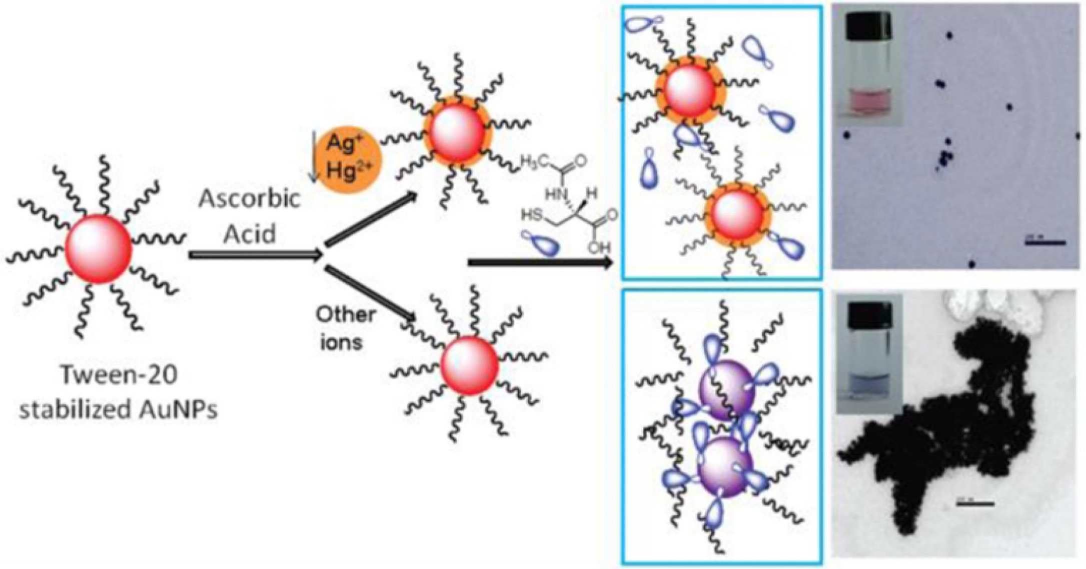
Metal analysis using colorimetric outputs

Colorimetric reactions are useful tools to determine heavy metal presence and concentration

- Organic chemicals, enzymes, etc…
  - Chelation or enzymatic reaction to generate chromogenic change



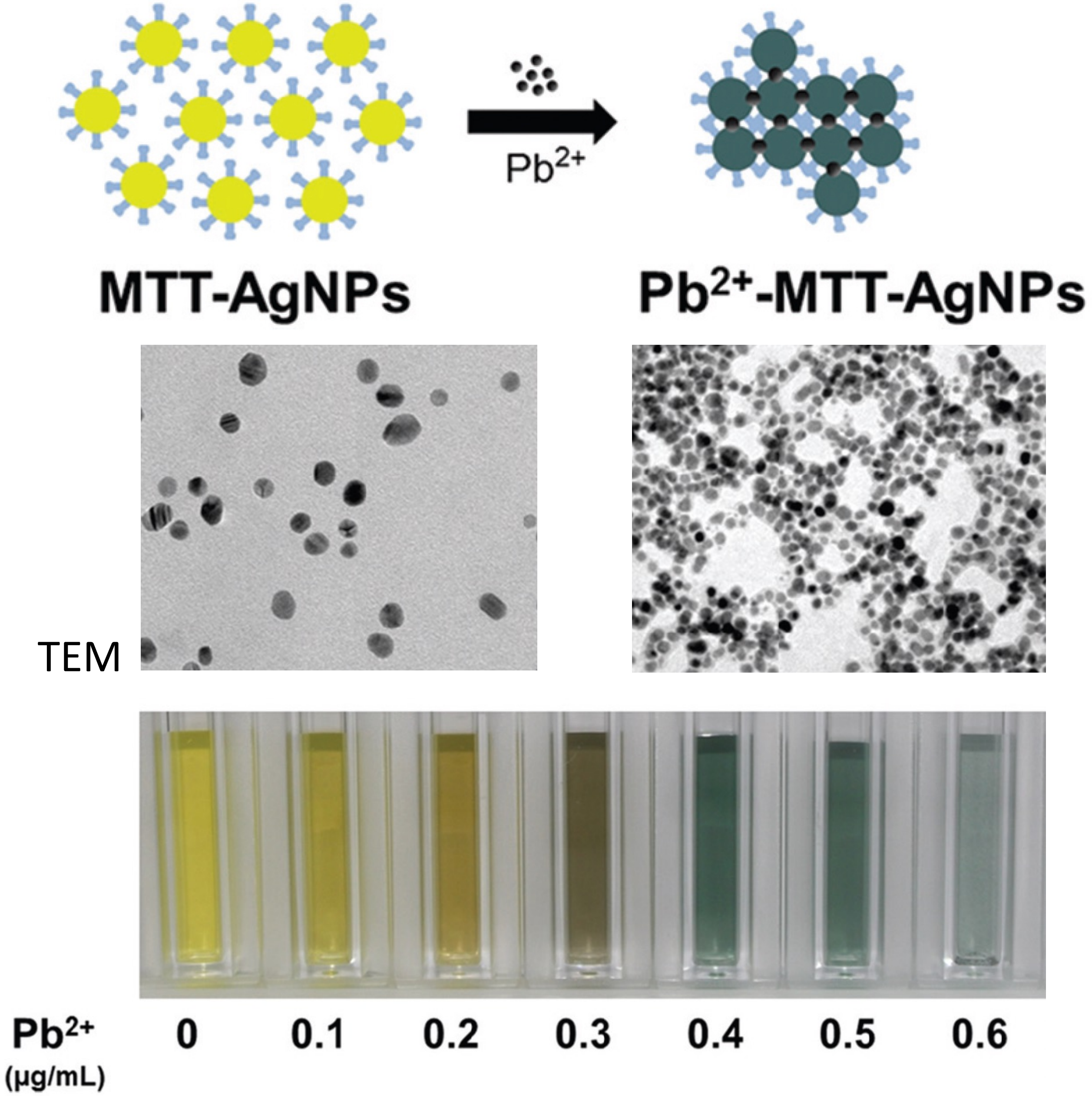
- Inorganic metal nanoparticles
  - Metal nanoparticles have different optical properties based on distance and morphology



Chen et al. 2023

Functionalized nanoparticles can indicate metal concentration through color changes

- Silver nanoparticles (AgNPs) conjugated to thiol-functionalized cyanuric acid (MTT)
- Lead interacts with the MTT groups to form aggregates of the AgNPs
- This change in distance between NPs alters their optical properties
- Type of localized surface plasma resonance (LSPR) sensor

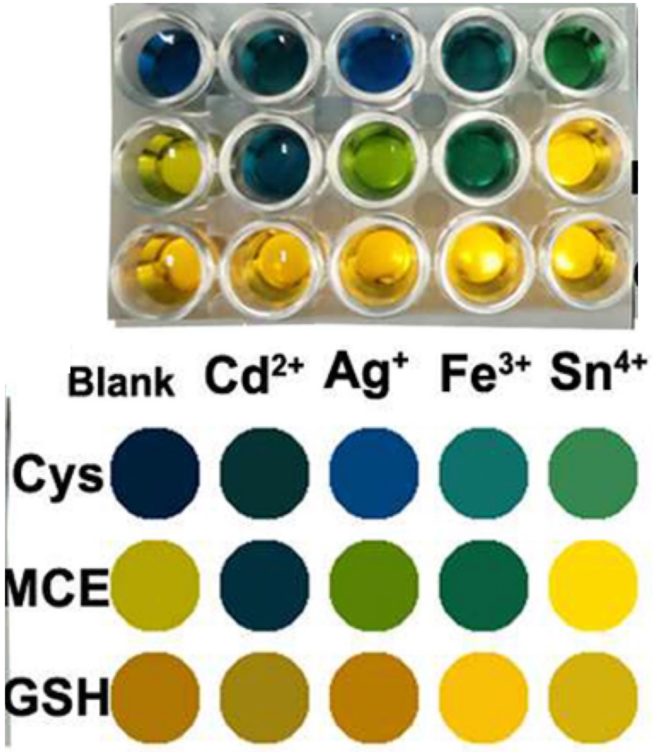


Noh et al, 2015

Organic colorimetric measures rely on characterized chemical reactions to determine metal presence

heavy metal + urease → glutathione / cysteine / β-mercaptoethanol → urea → ammonia → bromophenol blue

- metal forms a complex with urease
- GSH, Cys, MCE (3 kinds of thiols) have high binding affinity for metals
    - Affinities between thiols and metals differ
- When thiol binds metal, the urease is released
- Urease catalyzes decomposition of urea to ammonia
- Ammonia causes a pH change
- Change in pH discolors bromophenol blue dye



He et al. 2018

Metal analysis in the field

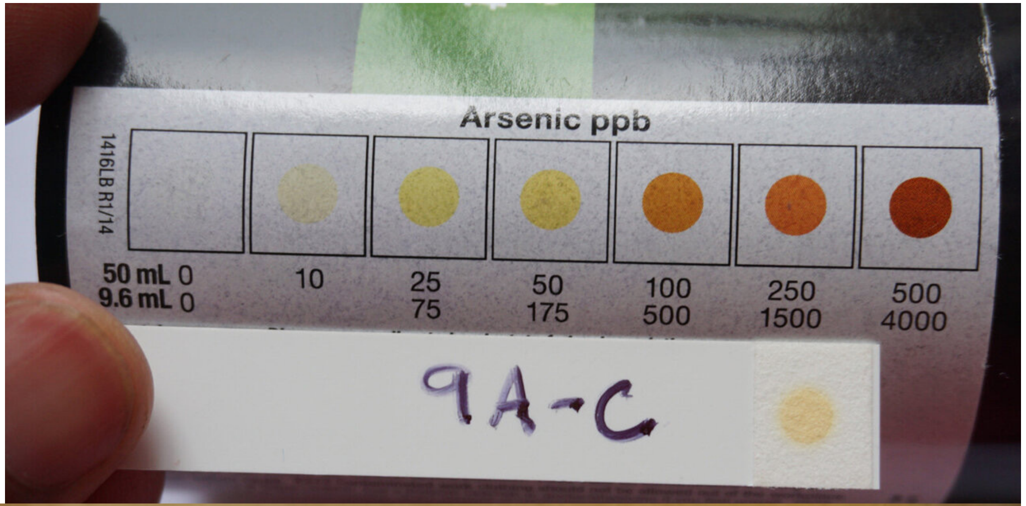
Arsenic field tests commonly utilize mercuric bromide as an indicator

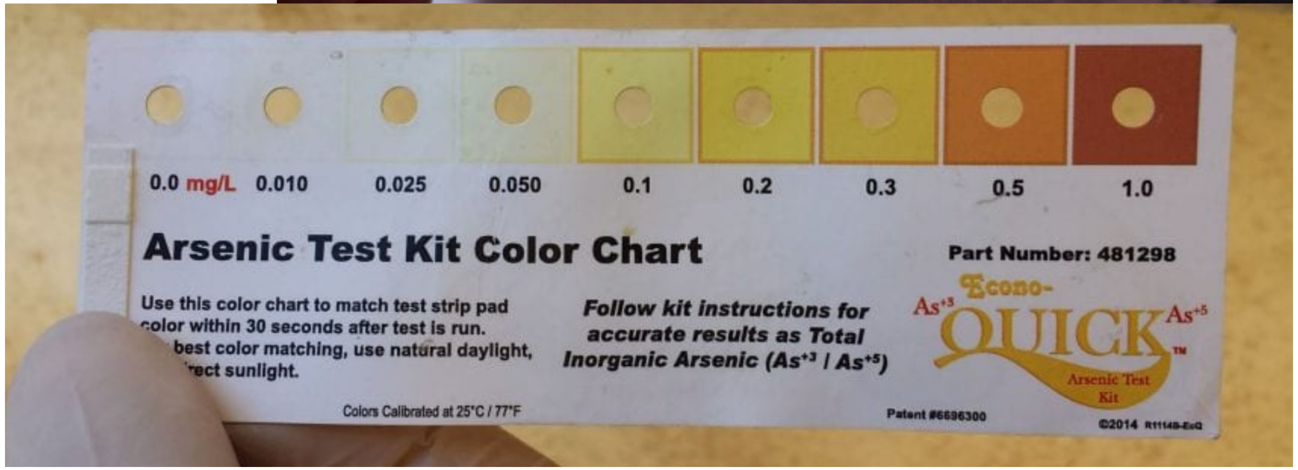
- Arsenic trioxide is reduced to arsine through the addition of zinc and hydrochloric acid

$$As_2O_3 + 6\ Zn + 12\ HCl \rightarrow 2\ AsH_3 + 6\ ZnCl_2 + 3\ H_2O$$

- Arsine gas flows through a paper test strip containing mercuric bromide

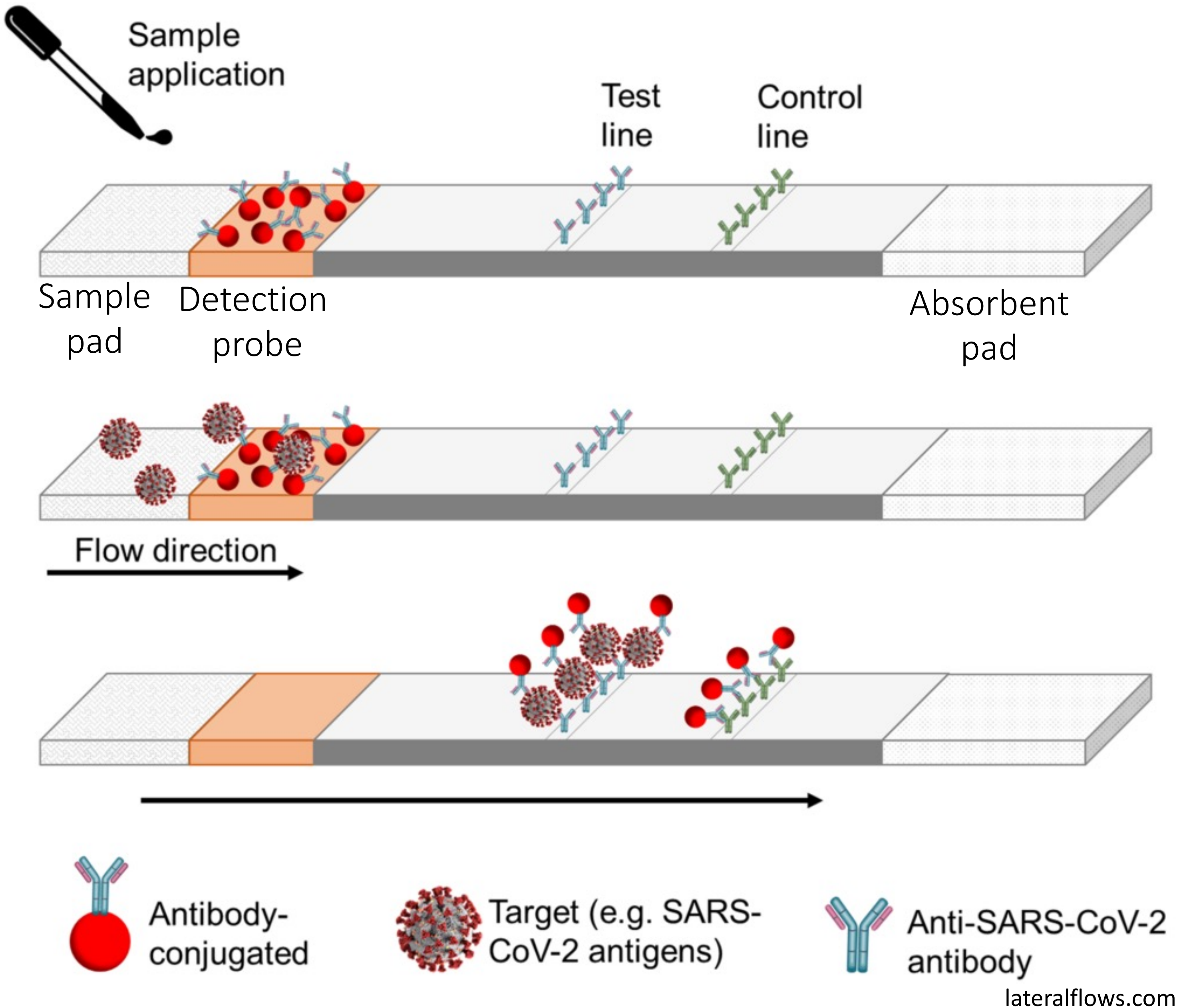
- White mercuric bromide reacts with arsine
  - light yellow -> brown
  - depends on arsine concentration





Lateral flow assay is a rapid and contained way to measure presence of antigen

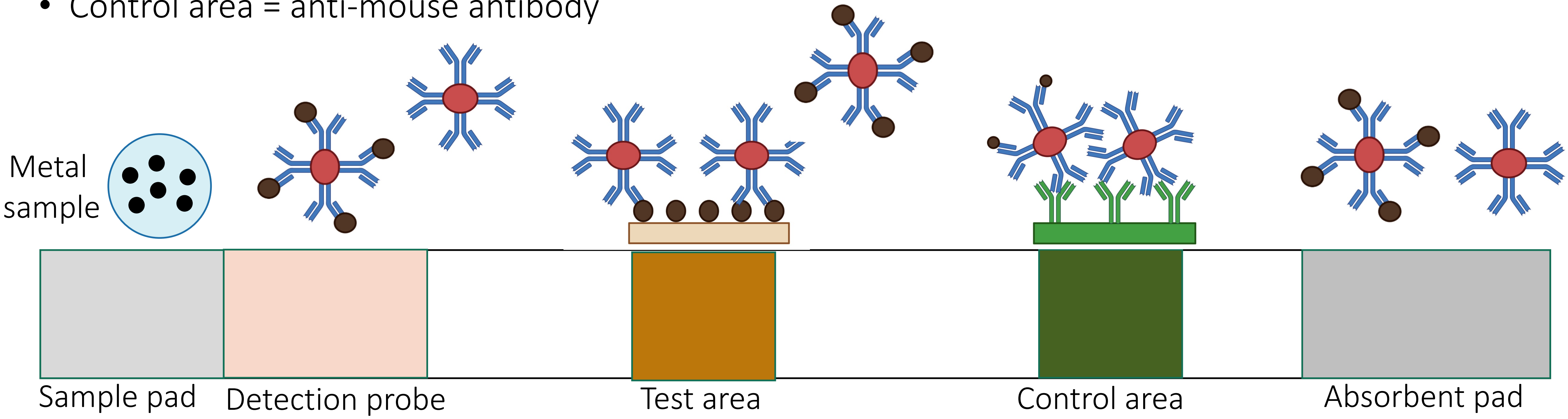
- Liquid sample with antigen added
- Antigen in samples forms complex with antibody conjugated to colloidal gold
  - temperature stable nanoparticle that appears red
- Test line = antibody against antigen
- Control line = antibody against detection probe
- modified Sandwich ELISA



Lateral flow assays can be used to measure heavy metals

- Antibodies generated against metal/chelator complex in mice
  - recognize the metal as an antigen
- anti-metal antibodies conjugated with colloidal gold
- Test area = metal conjugated to membrane
- Control area = anti-mouse antibody

- Modified Competitive ELISA
- Allows for more than -/+ output



Take home messages

- Spectrometry analysis allows for highly specific and reproducible analysis of metal composition in sample
  - EPA methods to allow standardized measurements from different sites

- Colorimetric analysis of metals allows for more accessible metrics of metal composition and concentration of a sample
  - lacks the highly specific analysis available with spectroscopy technology

- Field tests are highly accessible to the public and are easy to use
  - lack accurate quantification

What are you doing in lab?

- Begin characterization of your mutants!
- Confirm expression of Fet4_mutant in yeast system via direct immunofluorescence