



# **VIRUS RISK**

## **HEPATITIS A & NOROVIRUS**

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# COMPANY OVERVIEW



Eurofins is the **global leader in biological testing** with an unrivaled reputation for unbiased analysis



**200,000 reliable analytical methods** for characterizing the safety, identity, purity, composition, authenticity, and origin of products



Our **diverse laboratories** navigate seamlessly through a dynamic and ever-changing global marketplace



**50K+** EMPLOYEES



**800+** LABORATORIES



**50** COUNTRIES



**400M+** TESTS ANNUALLY

# AGENDA

Hepatitis A & Norovirus.

Significance in the Produce industry.

What controls the risk?

Options for testing.

# WHAT MADE YOU JOIN TODAY?

- Perhaps a customer has asked for Hep A & Norovirus testing?
  - Where is Eurofins seeing this?
    - Fruit
    - Vegetables
    - Hand-harvested products
    - Juices
    - Foodservice
- Updating your HACCP/HARPC plan?
  - An auditor noticed an absence of viruses on your plan?
  - New ingredient brought into the plant.
  - New team-member noticed it was missing.
- You've been hearing about parasites a ton and thought, "*we also need the challenge of managing viral risks!*"

# OUR JOBS IN THE FOOD INDUSTRY

Food Safety employees have the encompassing role of:

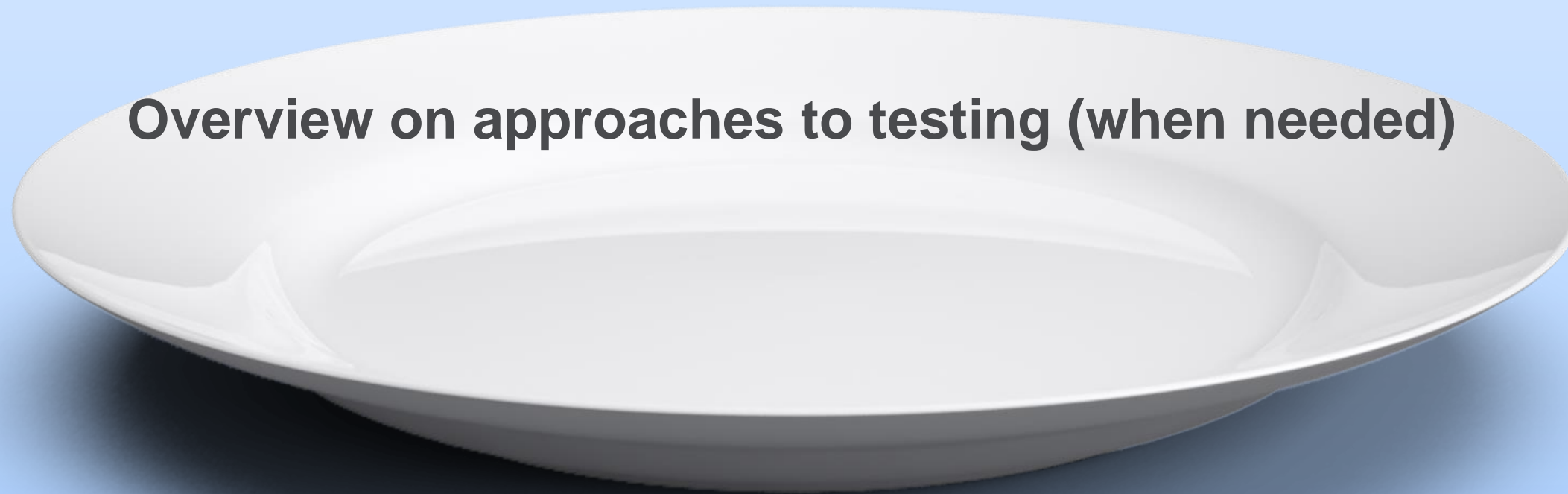


# KEY FUNCTION FOR TODAY:

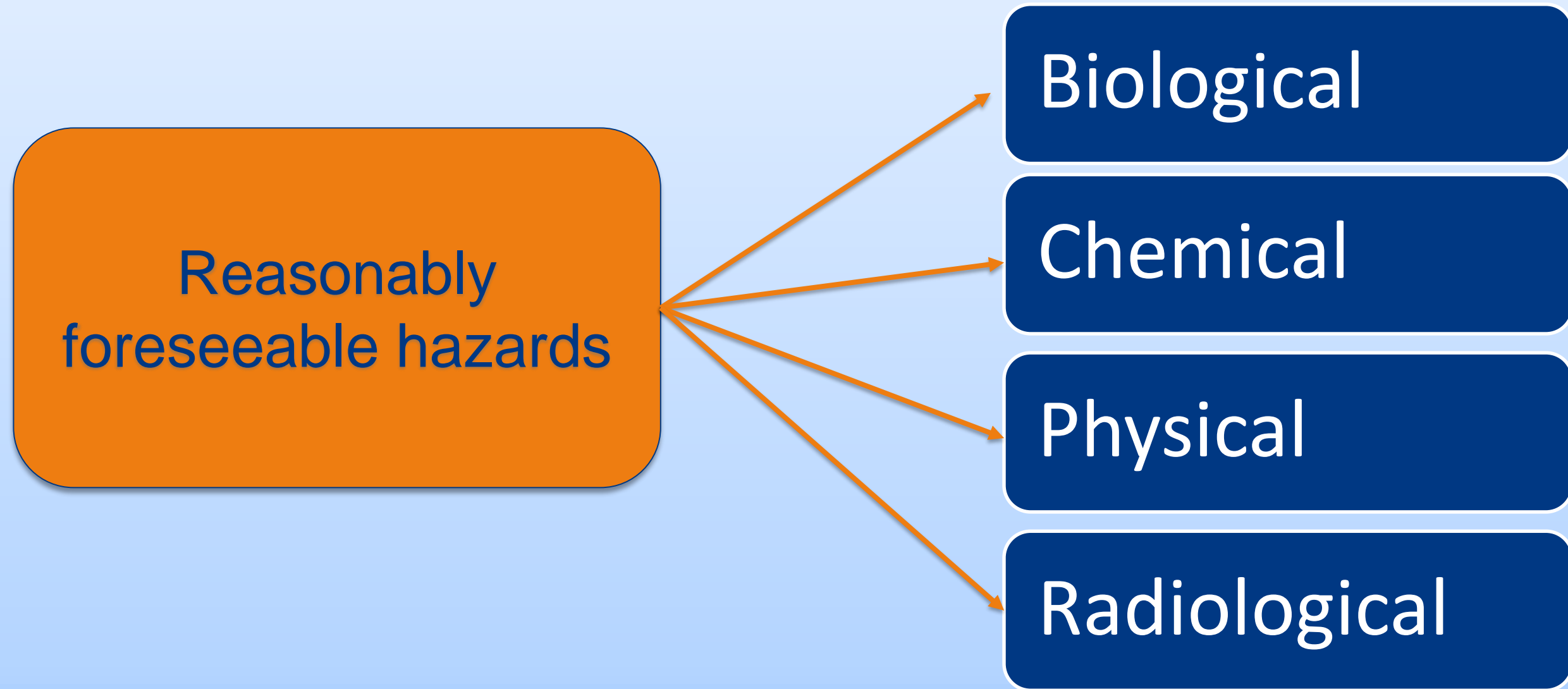
**Connect the dots on Hep A & Norovirus**

**Help provide realistic background on these risks**

**Overview on approaches to testing (when needed)**



# RISK ASSESSMENT



# BIOLOGICAL RISKS (MICROORGANISMS)

## Bacteria

- *Salmonella*
- *Listeria*
- *E.coli*
- *Shigella*
- *Campylobacter*
- *Vibrio*
- *Staphylococcus*
- Etc.

## Viruses

- Hepatitis A
- Hepatitis E
- Norovirus
- Rotavirus

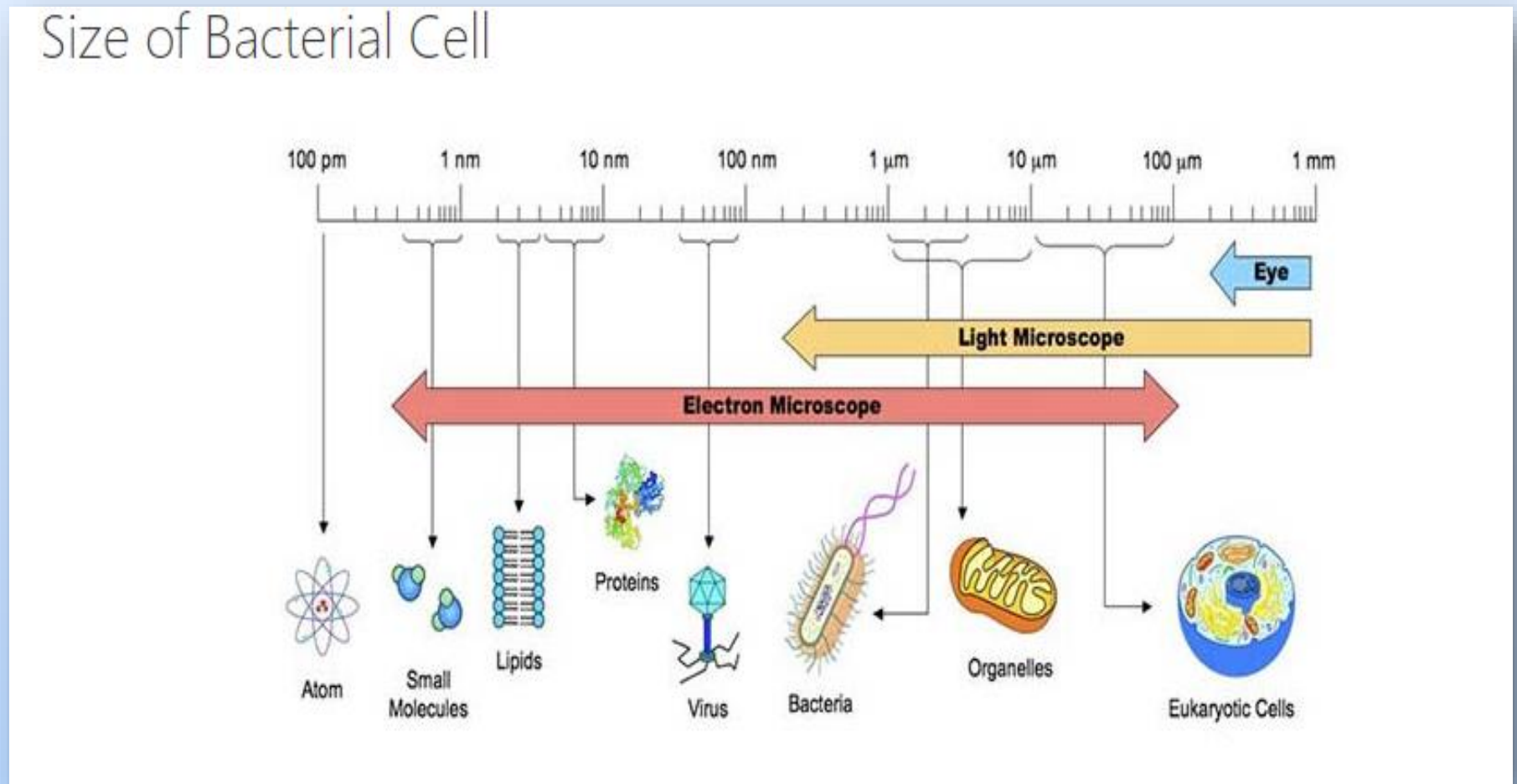
## Parasites

- *Giardia*
- *Cryptosporidium*
- *Cyclospora*
- *Toxoplasma gondii*
- *Trichinella* spp.
- Etc.



# BACTERIA VS VIRUSES

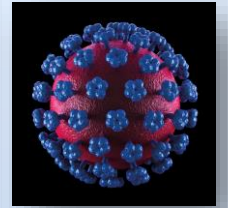
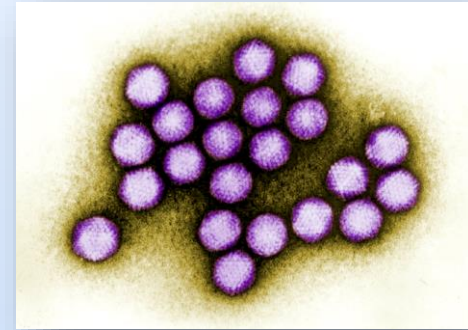
- Bacteria, 1-5 micrometers
- Viruses, 50-100 nanometers
- Bacteria are DNA based
- Viruses can be DNA, RNA
- Bacteria are living, Viruses are “waiting”



<https://microbiologyinfo.com/different-size-shape-and-arrangement-of-bacterial-cells/>

# VIRAL VS. BACTERIAL REPLICATION

- Viruses are host specific
  - Viruses attach to very specific receptor sites on a host cell
  - Receptor sites determine the specificity of the virus → basically, it defines what the virus can infect
  - In the laboratory you have to grow the host cells to research the viruses & “grow” the viruses
- Bacteria are self-sufficient
  - Replicate on their own when they have necessary nutrients
  - Many bacteria can infect many different hosts (e.g. zoonotic bacteria)



# HEPATITIS A (HAV)

## RNA virus that infects the liver

- DNA detection methods rely on Reverse Transcriptase: RNA → DNA

## Highly contagious & passed through the fecal-oral route

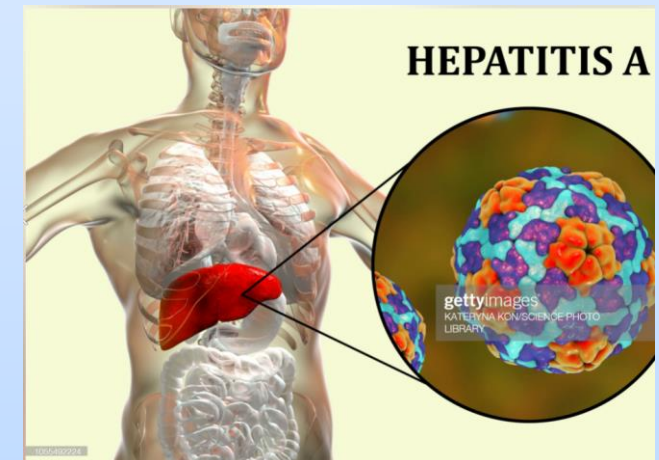
- RTE foods contaminated by food handlers
- Produce contaminated environmentally or by handlers (survives outside body 4mo)
- Shellfish contaminated by waste in water
- Incubation time ~28d

## Symptoms can last up to 2 months

- Fatigue
- Nausea
- Stomach pain
- Jaundice
- Joint pain

## Low infectious dose: 1-100 viral particles

- Infection leads to lifelong immunity
- Vaccines do exist



# NOROVIRUS

## RNA virus is a gastrointestinal virus

- DNA detection methods rely on Reverse Transcriptase: RNA → DNA

## Highly contagious & passed through the fecal-oral route

- Contamination by handlers/surfaces
- Environmental contamination
- Common stomach illness: cruise ships, day cares, etc.

## Symptoms can last 24-48hrs

- Fatigue
- Nausea
- Stomach pain
- Vomiting
- Fever

## Low infectious dose: 1-10 viral particles

- Very stable in the environment & on surfaces
- Resistant to many cleaners



Science Photo Library via Getty Images

Norovirus sufferers may experience symptoms like abdominal pain, nausea, vomiting and diarrhea.

# HEP A & NOROVIRUS RISK

- CDC estimates **35,796** cases of Hep A virus per year
- CDC estimates **20,865,958** cases of Norovirus
- Comparison of other known pathogens:
  - *Salmonella* cases (**1,229,007**)
  - *Listeria monocytogenes* (**1,662**)
  - Non-O157 STEC (**168,698**)
  - *E.coli* O157 STEC (**96,534**)

Source: <https://www.cdc.gov/foodborneburden/pdfs/scallan-estimated-illnesses-foodborne-pathogens.pdf>

# HEP A CONTROL METHODS

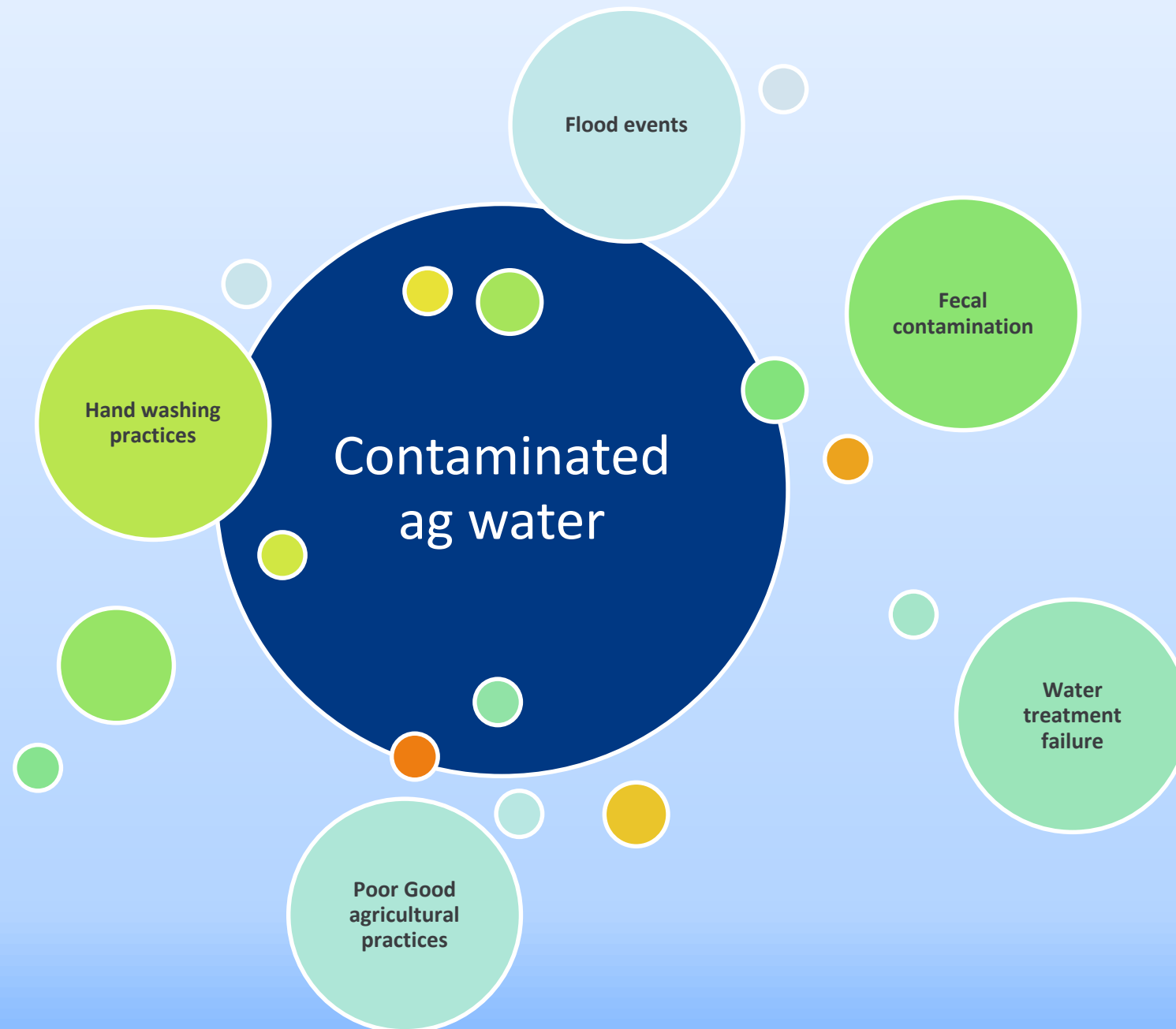
- Cooking can “kill” HAV at >185F (>85C) for 1 min
- Freezing does not inactivate the virus
- Chlorination of wastewater/municipal water should eliminate HAV

# NOROVIRUS CONTROL METHODS

- Cooking can “kill” Norovirus at  $>140\text{F}$  ( $>60\text{C}$ )
- Freezing does not inactivate the virus
- Resistant to many common disinfectants and hand sanitizers



# PRODUCE RISK FROM HAV & NOROVIRUS



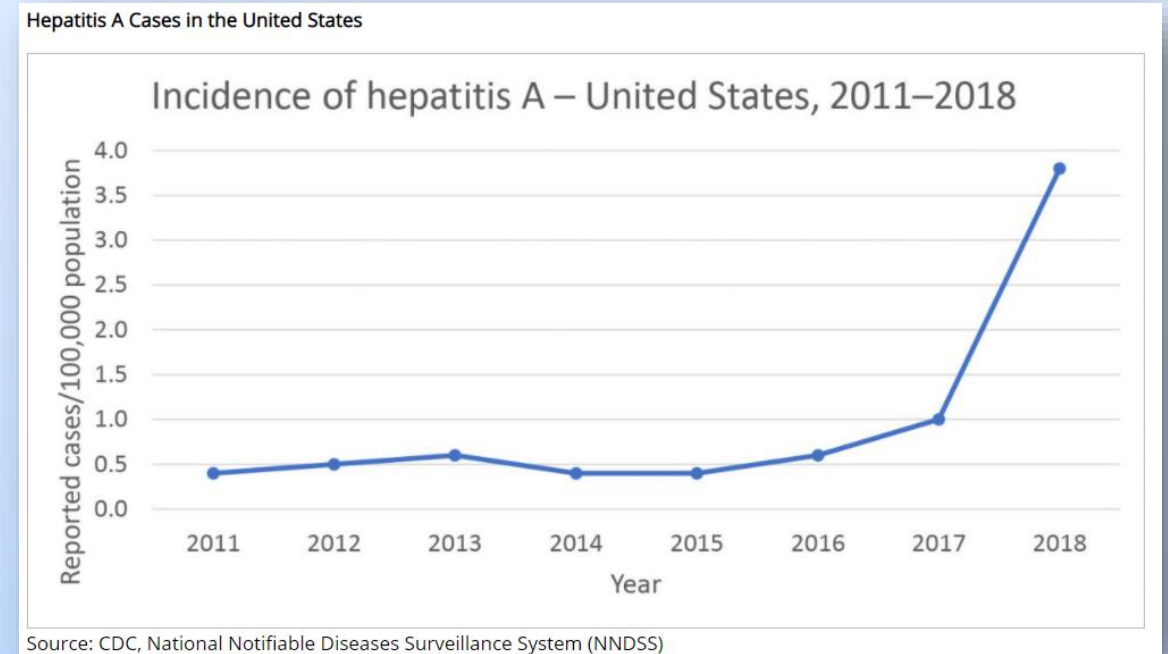


# EXAMPLES OF PRODUCE RISK



# PRODUCE HAV OUTBREAKS

- 2019 HAV multistate outbreak, blackberries
  - 20 cases, 11 hospitalizations
  - 7 States
- 2016 HAV multistate outbreak, IQF strawberries
  - 143 cases, 56 hospitalizations
  - 9 States
- 2013 HAV in Europe, frozen berries
  - 1589 cases
  - 2 deaths
- 2012 HAV multistate outbreak, pomegranate arils
  - 165 cases, 71 hospitalizations
  - 10 States



<https://www.cdc.gov/hepatitis/outbreaks/hepatitisaoutbreaks.htm>

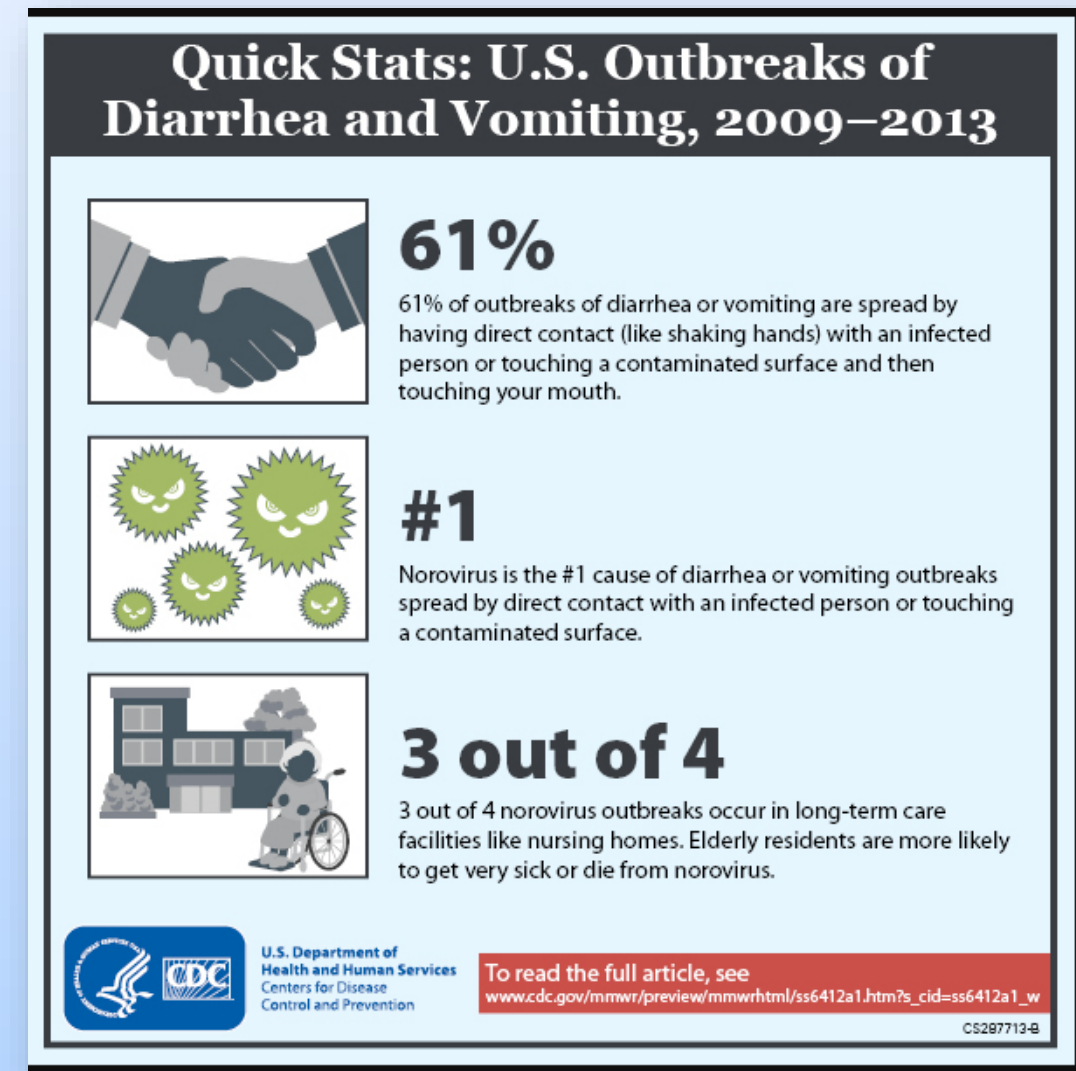
# HAV FATE IN FOOD

- Viral particles are highly stable in the environment
  - Water, soil, surfaces, hands
- Cold temperature (3-10C) does not inactivate HAV
  - Bottled water at 4C remained infective for 1 yr
  - Lettuce & carrots remained infective for 4 days, green onions for 20 days
- Freezing does not inactivate viral particles
  - Studies have shown infectivity lasting for 3 mo
  - Many large outbreaks have been associated with frozen product

Randazzo, W., Sanchez, G. (2020) Hepatitis A infections from food. *J. of Applied Microbiology*. 129, 1120-1132.

# NOROVIRUS OUTBREAKS/RECALL

- One of the leading causes of foodborne illness
- Often the culprit in cruise ship illnesses
- Foodservice risk
- Processed leafy greens, fresh/frozen fruits, oysters have all been associated with outbreaks
- 2019 frozen blackberry recall



[https://www.cdc.gov/norovirus/trends-outbreaks/outbreaks.html#:~:text=Foods%20that%20are%20commonly%20involved,shellfish%20\(such%20as%20oysters\).](https://www.cdc.gov/norovirus/trends-outbreaks/outbreaks.html#:~:text=Foods%20that%20are%20commonly%20involved,shellfish%20(such%20as%20oysters).)

# RISK *IDENTIFIED*

- Assessments of past outbreaks & recalls identify that Hep A and norovirus can lead to illness & are known risks
- Notation of a risk on an assessment? What next?
  - How do you manage the risk?
  - What can you look for?
  - Are certain regions/areas higher risk than others?
  - Does testing play a part or not?



# RISK MONITORING

## FDA Sampling Frozen Berries for Harmful Viruses



### What's New

January 22, 2020

As of September 30, 2019, the FDA has tested 339 domestic samples and 473 import samples of frozen berries.

Of the frozen berries sampled, the FDA found genetic material from hepatitis A virus in five samples and genetic material from norovirus in eight samples, using multiple-laboratory validated RT-qPCR methods for the detection of hepatitis A virus and norovirus viral sequences in soft fruit. These 13 samples were then further tested by another method called Sanger sequencing to see if the viral material could be further characterized by examining a separate, distinct section of the viral genomes. Nine of the 13 samples could be further characterized using this technique. When the FDA detected genetic material from hepatitis A virus or norovirus in a sample, the agency notified the firm of the finding(s) and worked with them to take appropriate action to protect the public health.

Testing for all pathogens is still underway and no conclusions can be drawn at this time.

View the [Microbiological Surveillance Sampling: FY 19-20 Frozen Berries \(Strawberries, Raspberries and Blackberries\) Results as of 10/1/2019](#).

## Regulatory Approach

FDA has been conducting surveillance testing on berries for Hepatitis A and Norovirus since 2019.

13 samples have been found to detect Hep A &/or norovirus

Domestic & international samples of frozen berries

# TESTING

The risk is there, now what?



# BACTERIAL DETECTION

## Detection limit (LOD)

- Too little target and the technology will miss (false negative)
- Time to result still relies on biological growth

## Increasing the target

- Enrichment to get from 1 CFU to 10,000 CFU
- LOD for most methods is 10,000 CFU, starting contamination at 1 CFU

## Presence vs. Viability

- Molecular methods detect DNA
- DNA is present even if a cell is non-living





# VIRAL DETECTION

Viral particles don't replicate on their own

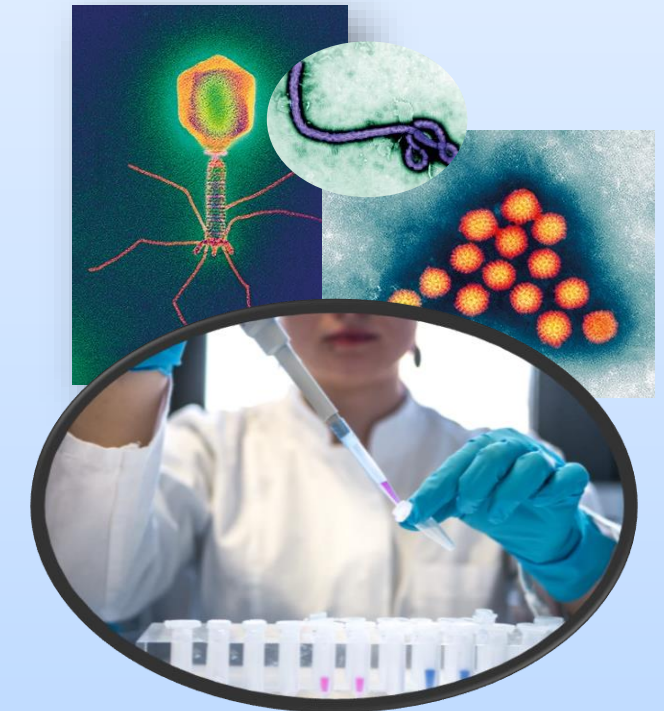
- Rely on host machinery
- Intracellular pathogens

Testing for viruses relies on concentration, not amplification

- Filtering
- Centrifugation
- Key is to find low levels of particles in original sample

Food samples are complex

- PCR inhibitors
- Large volumes
- Culture confirmations less possible due to non-living state

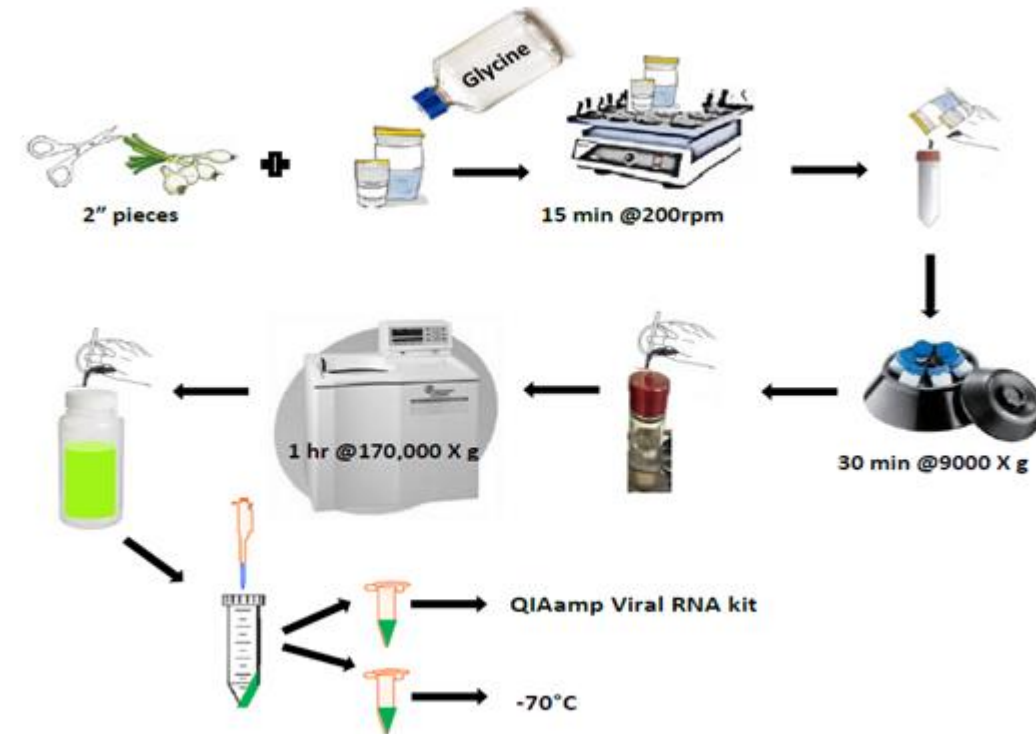


# METHOD TO DETECT HAV & NOROVIRUS

## FDA 26b BAM method

- Relies on concentration through shaking & centrifugation steps
- High speed centrifuge 170,000 x g
- DNA extraction
  - Inhibitors issue
- qPCR amplification

Figure 2. Flow diagram of HAV elution, concentration and extraction from green onion.



<https://www.fda.gov/food/laboratory-methods-food/bam-chapter-26b-detection-hepatitis-virus-foods>

# METHOD TO DETECT HAV & NOROVIRUS

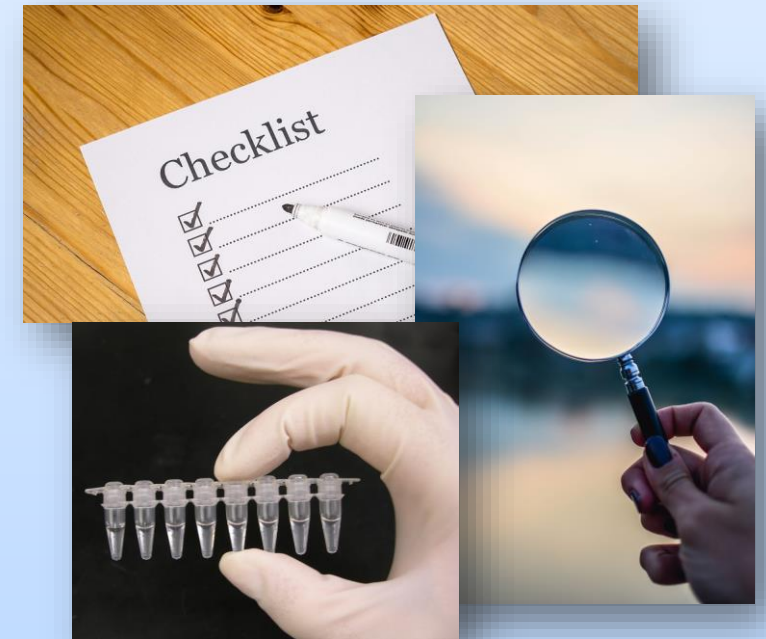
## ISO 5216-2:2019 method

- Relies on concentration through shaking & centrifugation steps
- Multiple centrifugation steps
- DNA extraction
  - Inhibitors issue
  - Process control from extraction through amplification
- qPCR amplification



# HEP A & NOROVIRUS RISK MANAGEMENT

- Risk *identified*, now what are the means to control it?
  - Supplier audits
  - Harvest audits
  - GMP review
  - Testing
- Know your higher risk items
  - Past outbreaks/recalls
  - Areas with known outbreaks/epidemics
  - Target testing where risk is elevated
- Develop surveillance methods to identify trends



# WHERE DOES TESTING FALL IN MANAGING RISK?

- **Testing identifies risk, it doesn't change it.**
- **How then do we interpret testing data on viruses?**
  - Added complexity on infective/non-infective
  - Issue with test limitations if contamination is small
  - PCR inhibitions due to concentration
- **When does testing for HAV & norovirus make sense?**

# TEST PLAN DESIGN

- Surveillance
- Lot acceptance
- Investigatory



# SURVEILLANCE TESTING

- Determines normal occurrence of target (prevalence)
- Can help identify when risk increases
- Can be used to dictate appropriate lot acceptance sampling plan



# INVESTIGATORY TESTING

- Root cause testing
- High risk suppliers/items
- Exploration





# SUMMARY

- **HAV & Norovirus risks are common in the food system**
- **Control of these viruses is through GAP, GMPs, risk assessment of supply chains**
- **Testing can be used in surveillance & investigatory situations**
- **Test method still rely on concentration & genomic identification (no infectivity)**



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**THANK YOU**