General Issues Meeting on Use of Pathogen-Agnostic Sequencing for Emergency Preparedness and Response Efforts

FDA Presentation

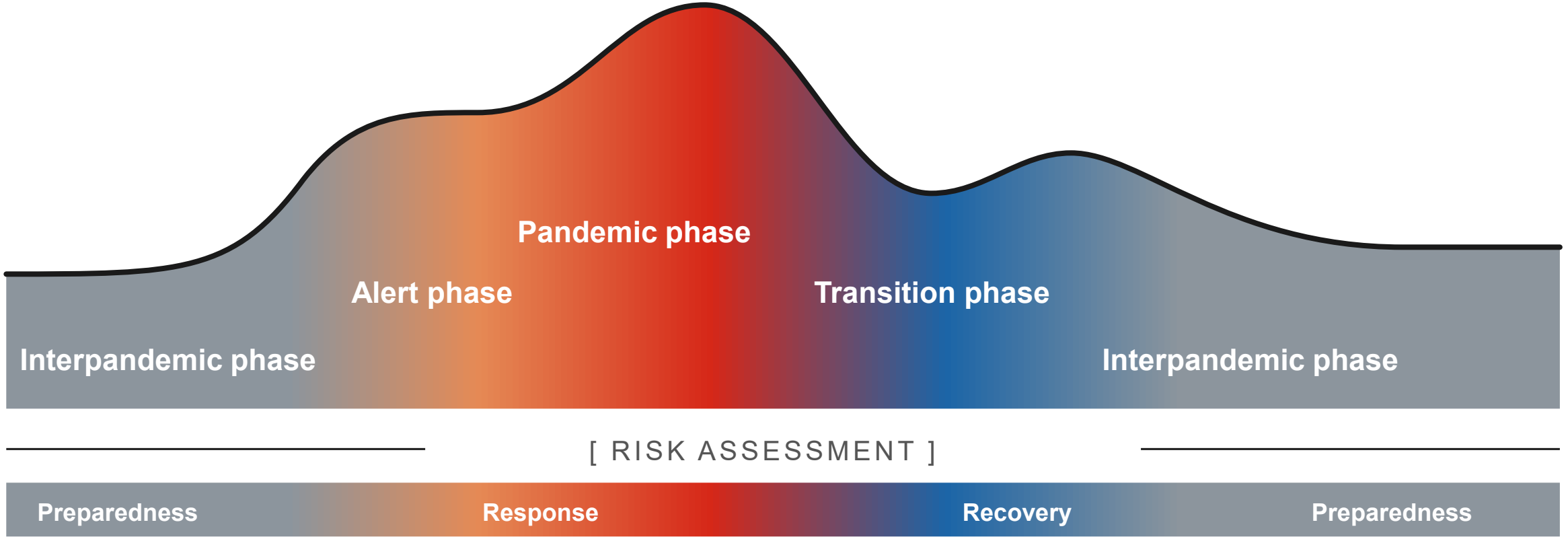
Joseph Briggs, Ph.D.
Deputy Division Director
Division of Microbiology Devices
FDA/CDRH/OPEQ/OHT7/DMD

Outline

- Background: Emergency Response and Sequencing Technology
- Pathogen Agnostic Sequencing Design Considerations
- Use of Pathogen Agnostic Sequencing in Emergency Preparedness and Response- Benefit and Risk Considerations
- Considerations for Preparedness Applications and Operational Implementation
- Summary

BACKGROUND: EMERGENCY RESPONSE AND SEQUENCING TECHNOLOGY

Overview of Use Case- Pathogen X

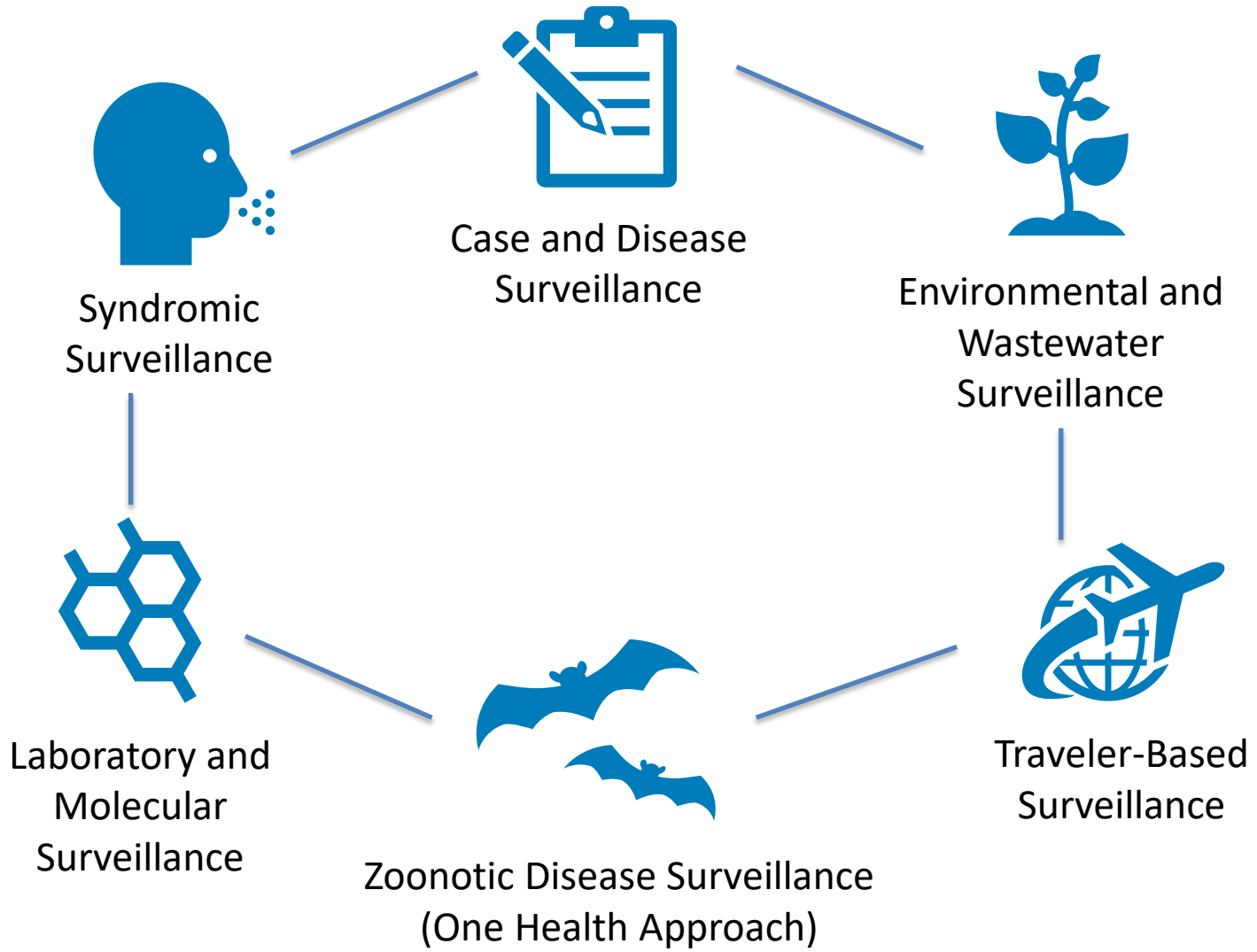


This continuum is according to a "global average" of cases, over time, based on continued risk assessment and consistent with the broader emergency risk management continuum.

Source: Centers for Disease Control and Prevention

Biosurveillance

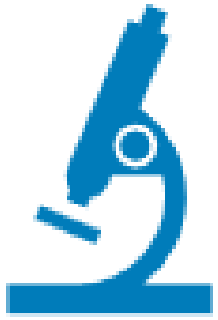
Earlier Detection → Faster Characterization → Timely Action



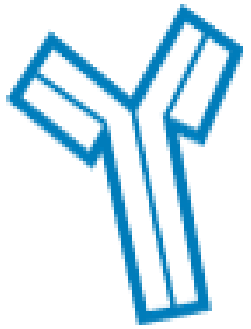
Approaches to Human Pathogen Detection and Identification



Microbial Culture



Microscopy



Immunoassays



Molecular

Each approach has strengths and limitations

Targeted vs. Untargeted Approaches

Targeted	Untargeted
Requires prior knowledge (E.g., specific primers/probes needed)	Hypothesis-free (No prior sequence data required)
Narrow scope (Detects only known, targeted organisms)	Comprehensive scope (Captures all genomic material – host & microbial)
Vulnerable to novel threats (Blind to "Disease X" or severe mutations)	Capable of detecting novel/emerging pathogens and variants

Focusing on Molecular Tests

DNA	**A** Adenine	**T** Thymine	**C** Cytosine	**G** Guanine
RNA	**A** Adenine	**U** Uracil	**C** Cytosine	**G** Guanine

The complete and specific order of the letters defines a genome sequence, a portion of which can serve as a target to identify/differentiate/discriminate between organisms or variants/strains

Primer sequence:

3'-ATATCGGTTTCATAG-5'

5'-AATGCGTTAGGGCCTATATAGCCAAAGTATCGGCAGATAGC-3' = Known organism 1

5'-AATGCGTTAGGGCCTACCGGTACAAAGTATCGGCAGATAGC-3' = Mutated organism 1

Targeted Approach: Limitations

- Critical limitation of targeted tests: the pathogen's genomic sequence has to already be known. Targeted tests may not work if:
  - The pathogen mutates
  - The pathogen is novel or unsuspected

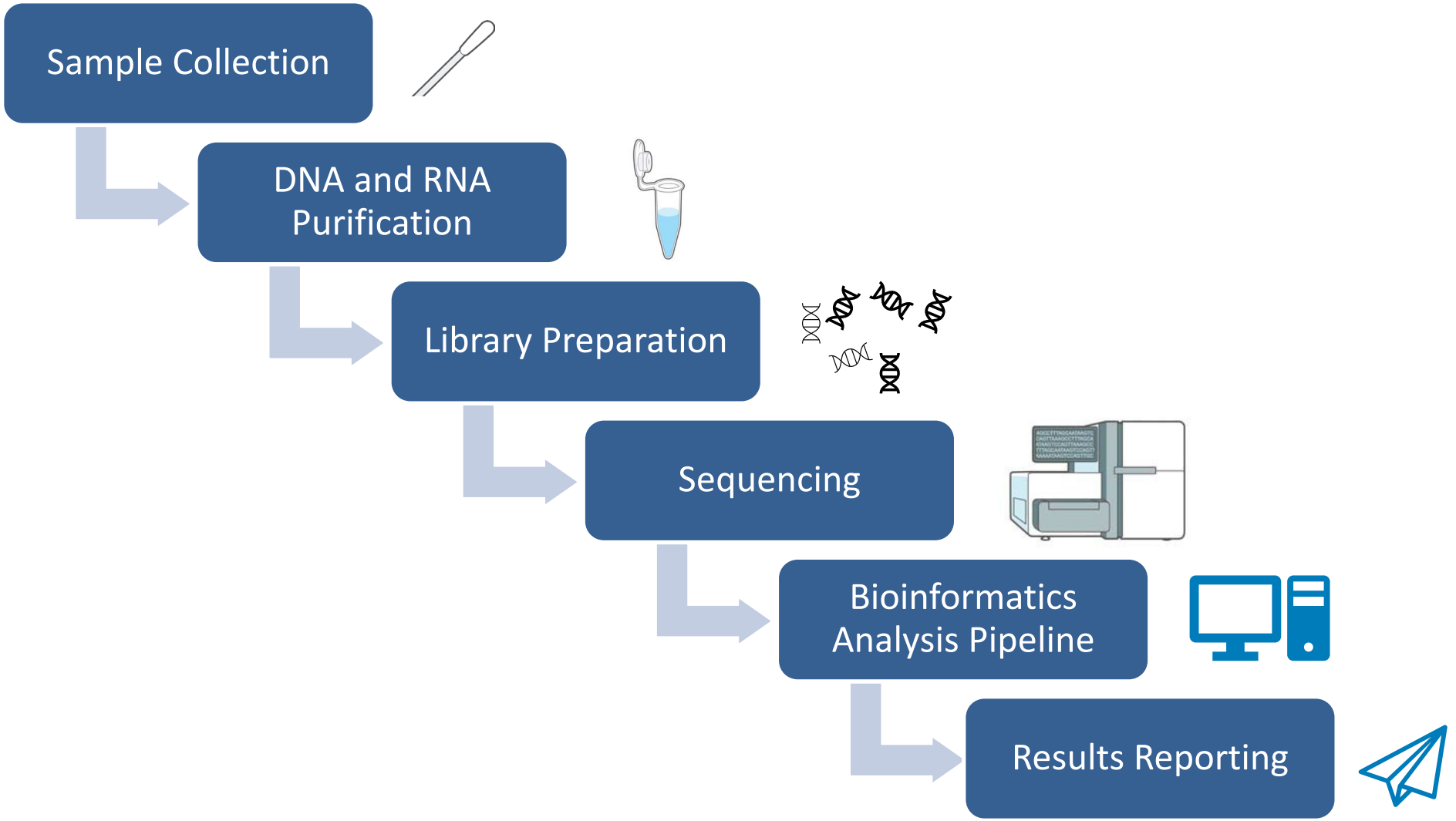


What if we don't know what we are looking for?



Pathogen agnostic sequencing (PAS) is designed to address the limitations of targeted testing

Sequencing Technologies: From Biological Sample to Actionable Information

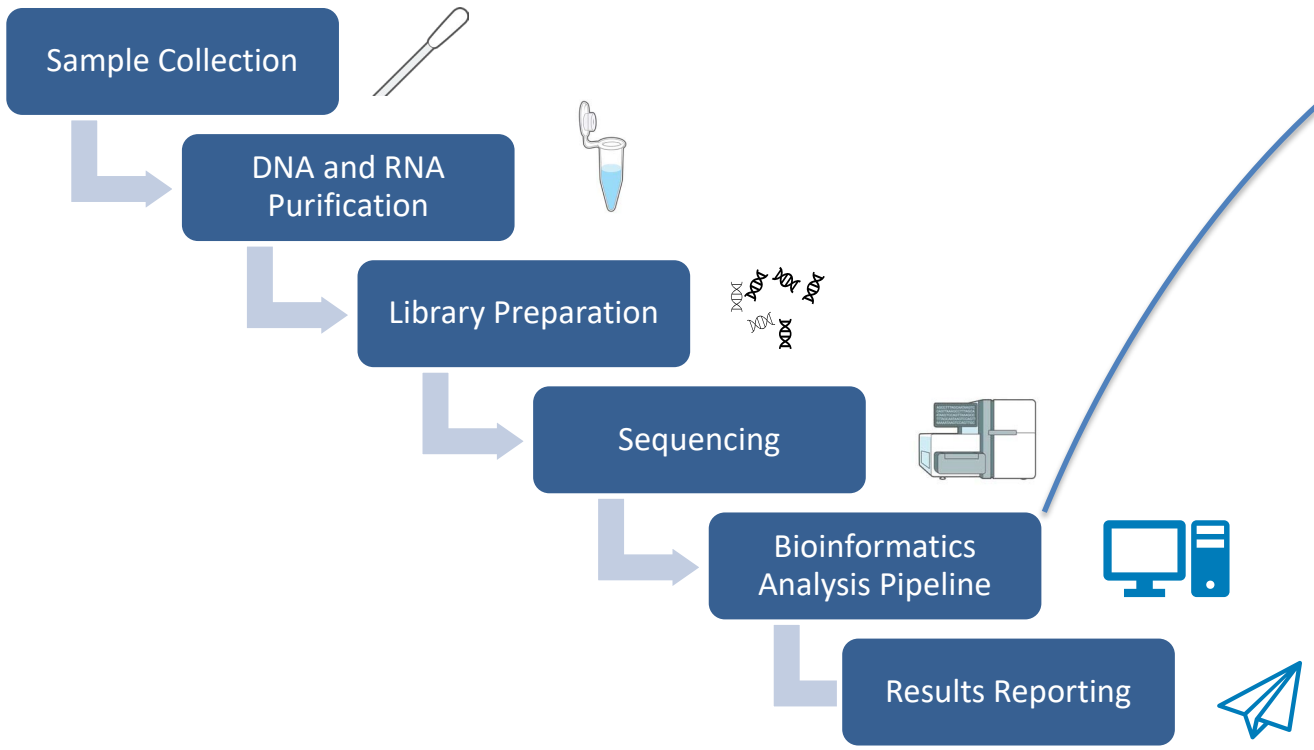


DNA/RNA

Millions of sequence reads

Interpreted Information

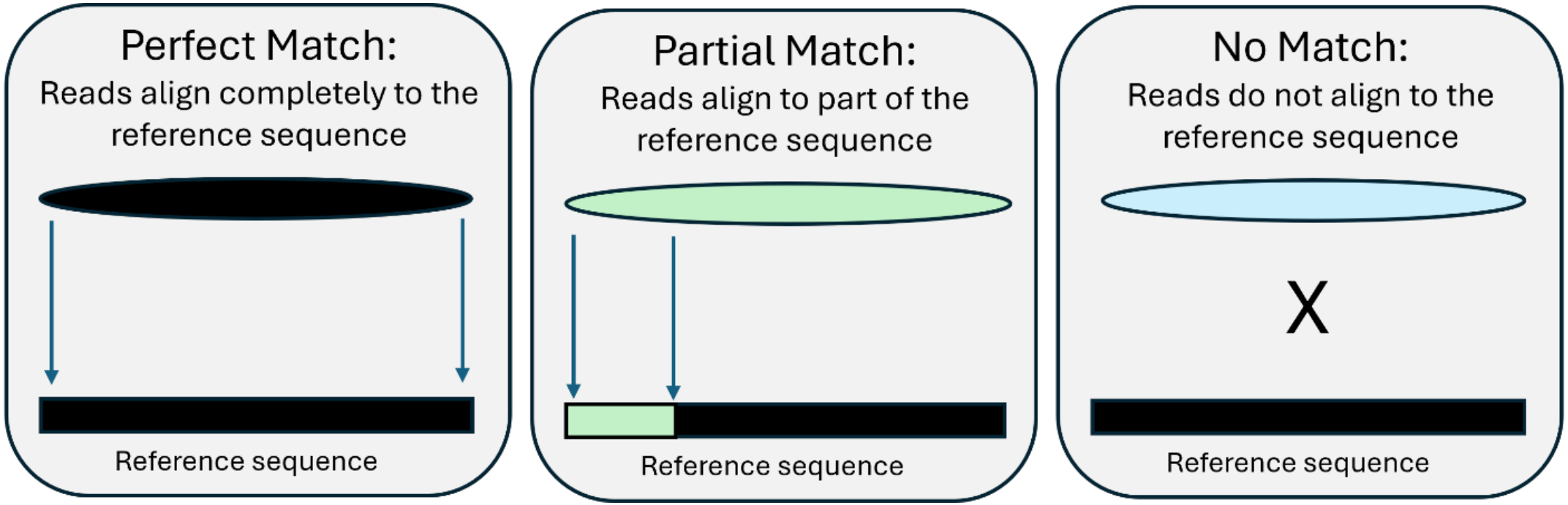
Sequencing Technologies: Bioinformatics Pipeline



Bioinformatics Pipeline:

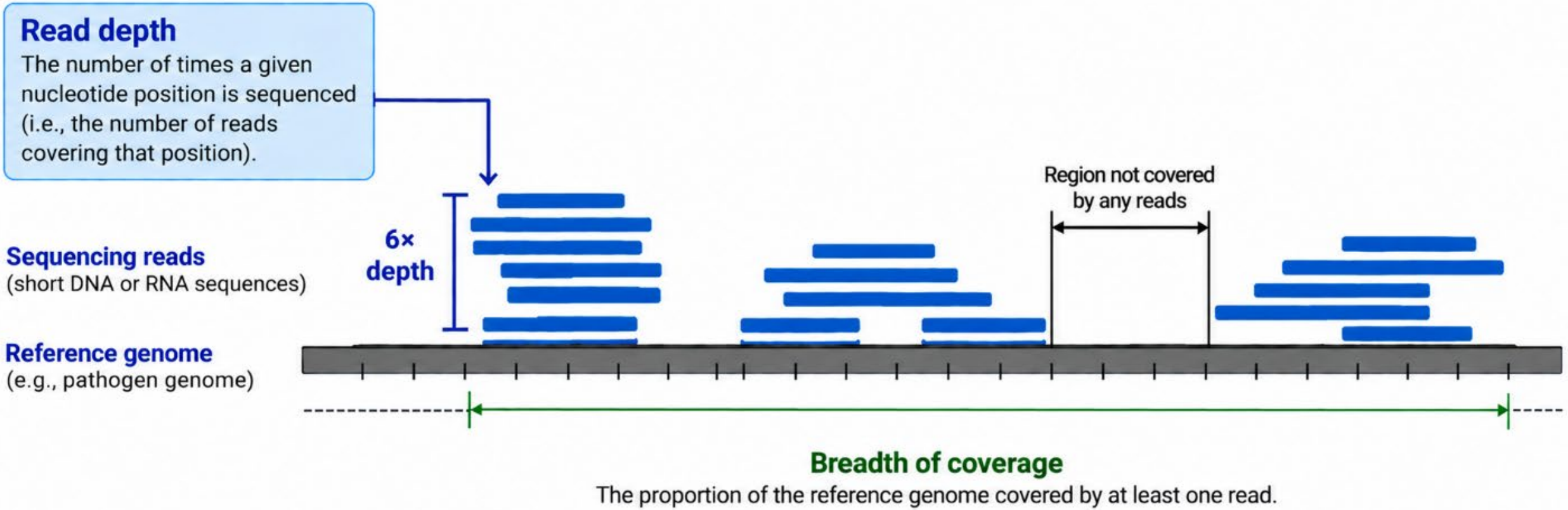
- Assess Quality of Data
- Compare sequence reads with reference database
- Identify and characterize organisms

Matching Sequencing Reads to Reference Sequences in a Database



Read Depth and Breadth of Coverage

Read depth
The number of times a given nucleotide position is sequenced (i.e., the number of reads covering that position).



De Novo Assembly

De Novo Assembly

- *De novo* assembly is the process where overlapping reads are pieced together into longer, more complete sequences called contigs.

Purpose

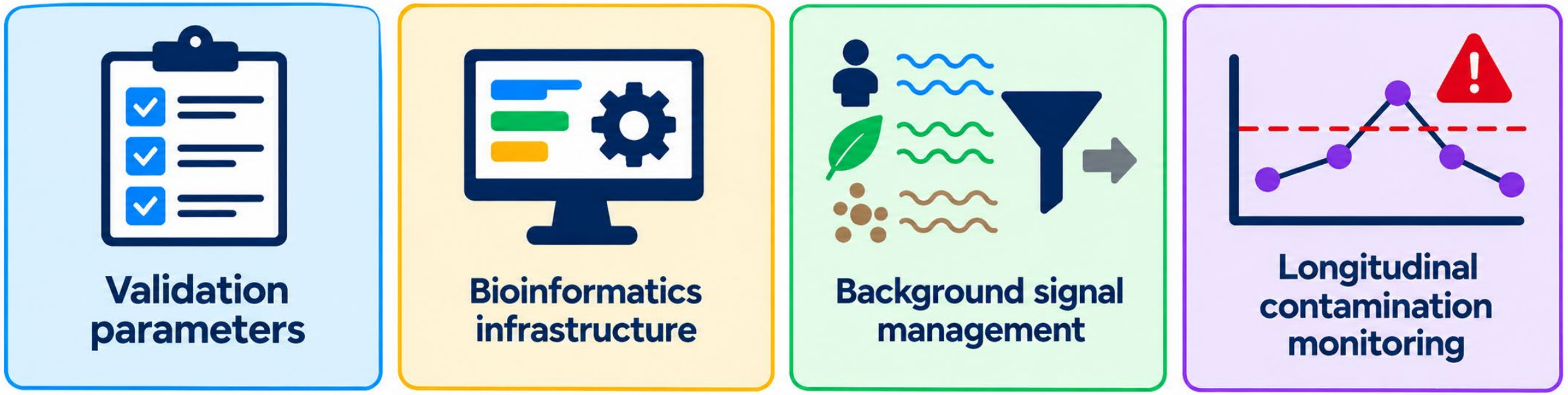
- Used when no good matches exist in a reference database for a significant portion of reads that appear to belong together.

Limitations

- Computationally intensive, requiring substantial resources.
- Short reads often cannot resolve repetitive regions, which can result in incomplete or fragmented contigs.
- Taxonomic origin can remain uncertain without a close reference sequence available for comparison.

PATHOGEN AGNOSTIC SEQUENCING DESIGN CONSIDERATIONS

Design Considerations



Design Considerations: Validation

- Designed to detect an unknown number of microbial species or sequences.
- Obtaining positive specimens for every potentially detectable microorganism is not feasible.
- Representative wet-lab testing supplemented by *in silico* analyses to evaluate performance across a broader sequence space.
- Establish evidence from representative organisms supporting generalizability across a broader range or organisms.

Design Considerations: Modifications

Updates and modifications to PAS present unique challenges for design consideration.

Changes may include updates to reference databases or bioinformatics pipelines — not just reagents or defined targets.

Modifications may have broader and less predictable implications than for targeted tests, potentially affecting performance across many organisms simultaneously.

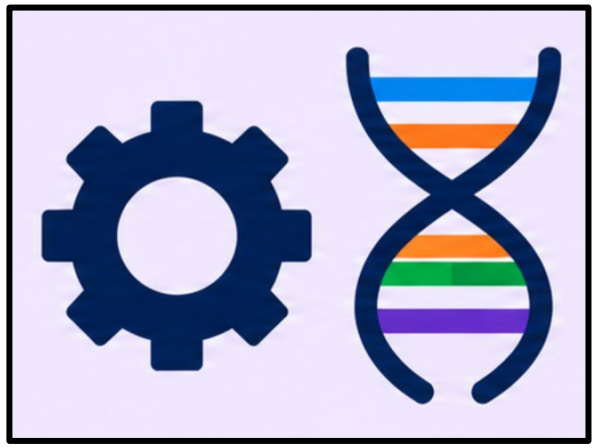
A change in computational methods could impact overall performance.

Thoughtful design anticipates future updates and maintenance needs while preserving performance.

Design Considerations: Bioinformatics



✓ Quality and completeness



✓ Technical factors



✓ Data privacy and cybersecurity

Design Considerations: Database Updates

DATABASE UPDATE LIFECYCLE

New pathogen sequences may become available rapidly during an emerging threat.



Version matters: the same sample may yield a different result after a database or pipeline update.

Design Considerations: Background Signal

COMMON BACKGROUND SOURCES

Specimen biology

host nucleic acid, commensal microbiota, colonization

Collection & environment

swabs/media, room surfaces, operators, ambient DNA/RNA

Reagents & consumables

extraction kits, enzymes, water, plastics; lot-specific "kitome"

Workflow carryover

cross-sample contamination, aerosolization, index misassignment

Informatics

reference-database contamination, near-neighbor misclassification

IF BACKGROUND IS NOT CONTROLLED

False positives

contaminants interpreted as pathogens → unnecessary alerts or interventions

False negatives

high host/microbial background consumes reads and can obscure low-level targets

Poor reproducibility

site/lot/run effects masquerade as biological differences

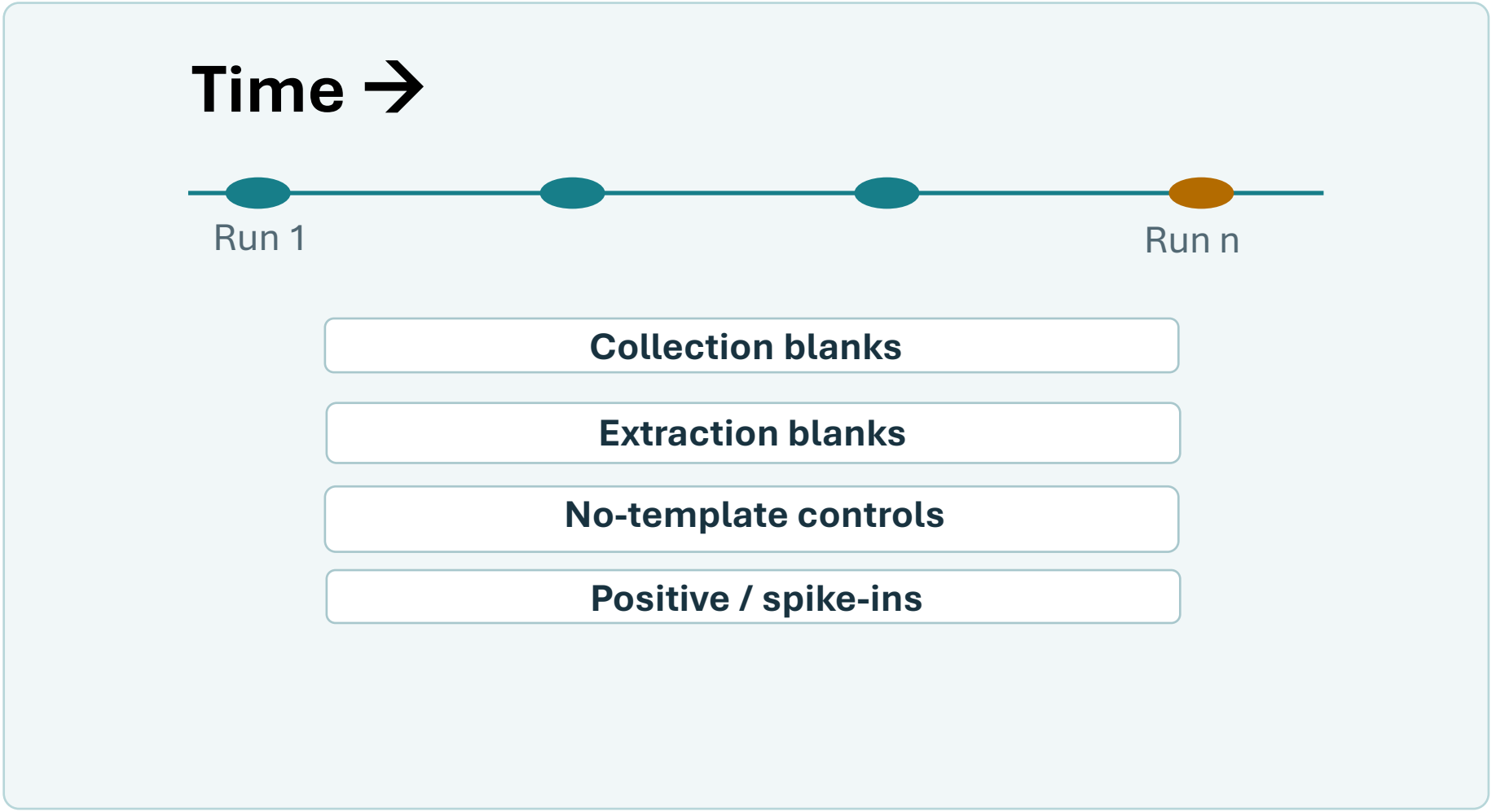
Weak surveillance signal

baseline drift can look like emergence, spread or changing prevalence

Lower confidence in results

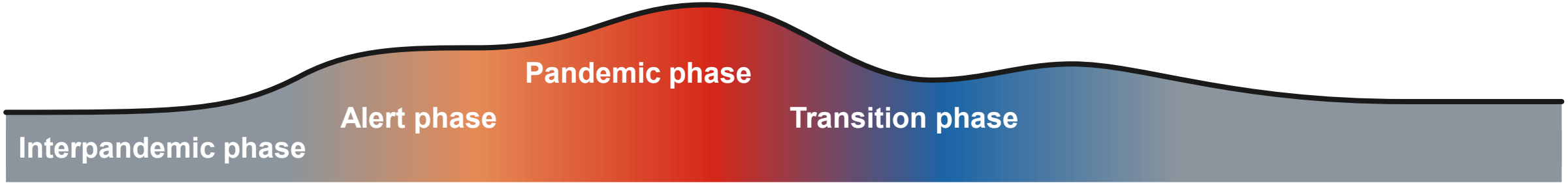
uncertain analytical specificity, thresholds and result interpretability

Design Considerations: Longitudinal Contamination Monitoring



USE OF PATHOGEN AGNOSTIC SEQUENCING IN EMERGENCY PREPAREDNESS AND RESPONSE- BENEFIT AND RISK CONSIDERATIONS

Potential Benefits of Pathogen Agnostic Sequencing

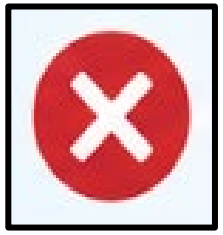


Early Alert: identification of a novel pathogen or new strain of a known pathogen

Early Response: Early sequence information can inform targeted test development leading to faster, near patient testing

Continuous Monitoring: Characterize the evolution of the pathogen genome sequence over time

Potential Risks of Pathogen Agnostic Sequencing



False Results

- Unnecessary quarantine or isolation
- Incorrect patient cohorting
- Misallocation of resources



Turnaround Time

- Results not available soon enough to inform decision-making



Result Complexity

- Detection of multiple organisms in a single specimen may cause uncertainty or delays



Taxonomic Resolution

- Identification only at the genus level may provide insufficient information when different species or strains have different implications

CONSIDERATIONS FOR PREPAREDNESS APPLICATIONS AND OPERATIONAL IMPLEMENTATION

Operational Implementation Considerations for Pathogen Agnostic Sequencing

1. **Specialized capacity**

   Sequencing instrumentation, computational infrastructure, trained personnel, and an NGS quality system.

2. **Referral & specimen pathways**

   Sites without PAS capability informed by defined criteria, logistics, and expertise to identify and route appropriate specimens to equipped laboratories.

3. **Role in the clinical workflow**

   Use may differ when no targeted test exists versus follow-on testing after targeted diagnostics are unrevealing; each pathway affects timing and interpretation.

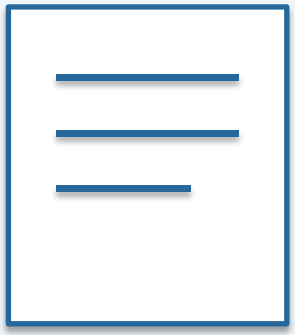
PAS Clinical Report Considerations

1 Detected organisms + metrics

Report organism(s) detected with relevant quantitative or semi-quantitative supporting metrics.

2 Unknown / novel findings

Consider how potentially novel or unclassified findings and associated uncertainty are represented.



Clinical report

Clear communication of findings, supporting evidence, uncertainty, and classification

3 Clinical context

Interpretation may depend on clinical presentation, epidemiology, specimen type, and other laboratory results.

4 Standardized terminology

There can be different terminology used to communicate findings across clinical, laboratory, and public health settings.

Summary



Pathogen-agnostic sequencing has the potential to strengthen diagnostic capacity during public health emergencies



Careful consideration of development, validation, and deployment is important to maximize its potential

Today's discussion will focus on the following topics:

Part 1: Use cases and implementation in emergency preparedness

- ❑ Benefits, risks and mitigations
- ❑ Incorporation into clinical workflow
- ❑ Results Reporting

Part 2: Pathogen-Agnostic Sequencing Design

- ❑ Cutoff considerations
- ❑ Performance generalizability
- ❑ Representative validation approach
- ❑ Background signals
- ❑ Bioinformatics systems and databases


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