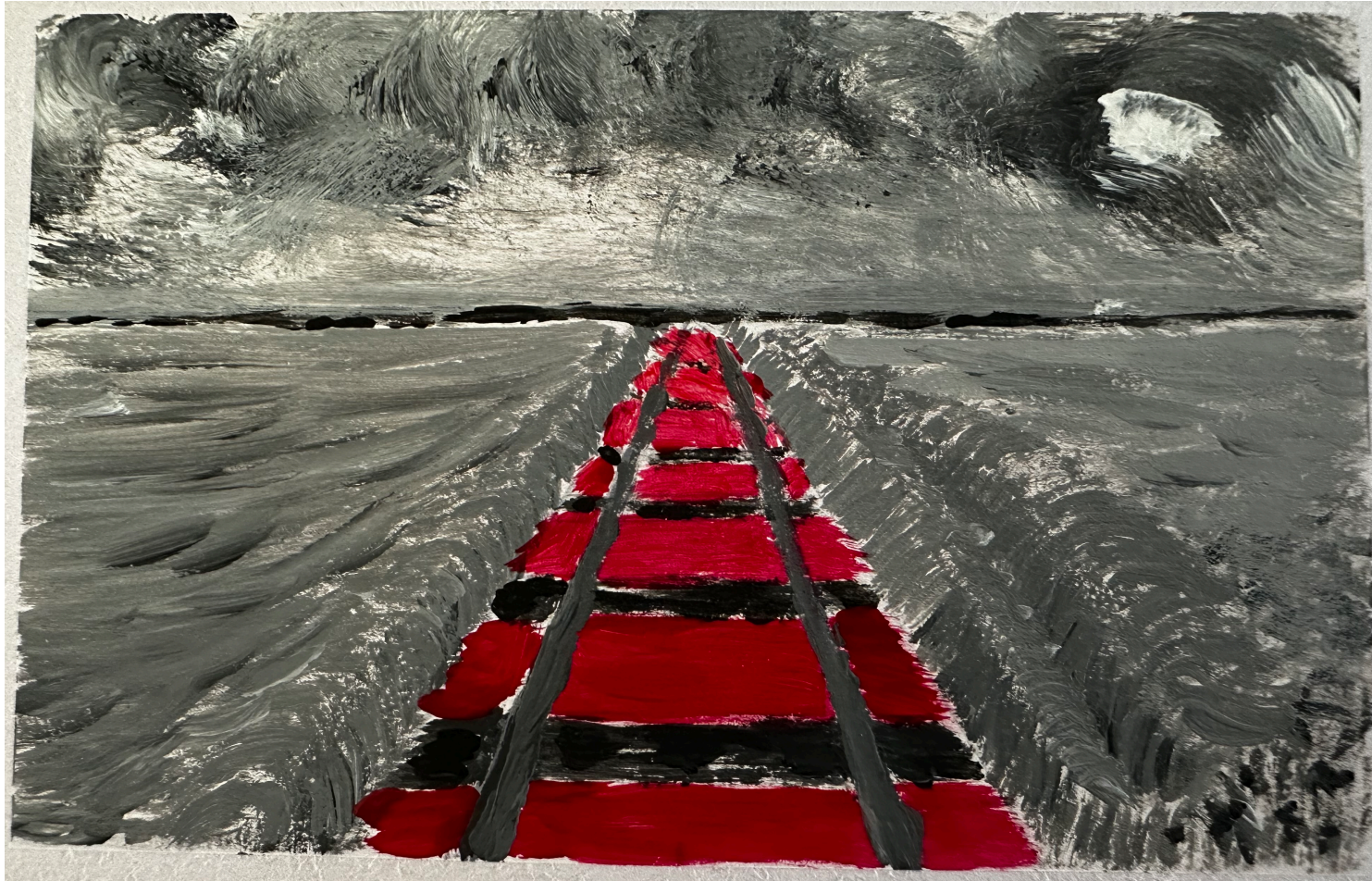


Unveiling infectious diseases: the power of next generation sequencing

Prof. dr. John W.A. Rossen

LMMI, Isala, Zwolle – MMBI, UMCG, Groningen – University of Utah, Salt Lake City



Painting by Ingrid Rossen de Vaan

Disclosure slide

Prof. dr. Rossen has received funding or honoraria for conference attendance, advisory boards, lectures and training from ALDA, ARES-genetics, IDbyDNA, Illumina, Molzym and Tecan. He has received research support from ZonMW (NL), EU-cofund (H2020), JPI-AMR (EU), Interreg (EU), the University of Groningen, EU-Horizon and ESCMID. He is the education officer of the ESCMID Study Group for Genomic and Molecular Diagnostics, and a technical assessor for the Dutch Accreditation Council.

Infectious diseases are often acute problems that require an immediate solution.

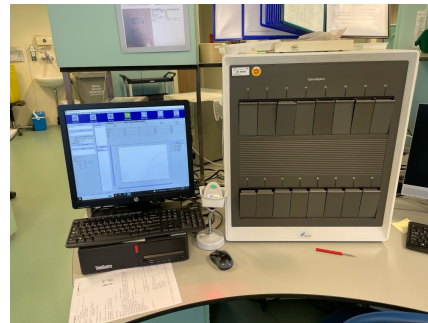
The speed of diagnosis is crucial for both treatment and prevention of spread.



DALL-E-3: Infectious patient treatment and prevention

Molecular-based testing

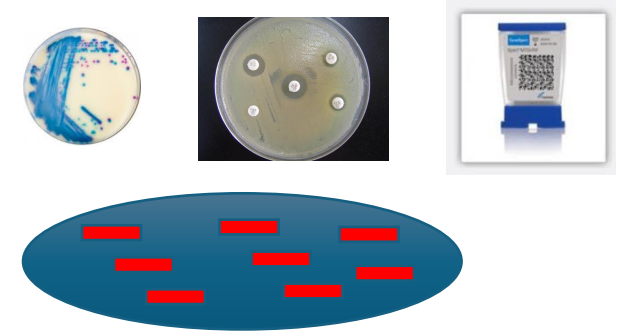
- Suitable for automation and high volumes
- Applicable to all types of patient materials
- Detection of difficult or non-cultivable microbes
- Fast (15 min – 18 hours)
- Demonstration of the presence of antimicrobial resistance and virulence genes
- Sensitive and specific



Personalised molecular microbiology

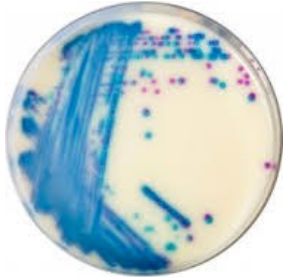
From demonstrating the presence of a microbe to understanding the infection

- Characterizing
 - Antimicrobial resistance and virulence markers
 - Microbial fingerprint



Whole-genome sequencing

Culture bacteria



Extract DNA

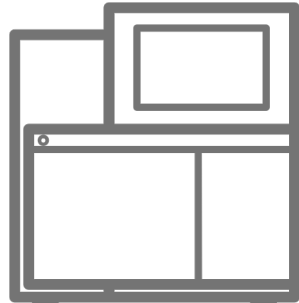


Bacterial

DNA Libraries

Fragmentation + Linkers

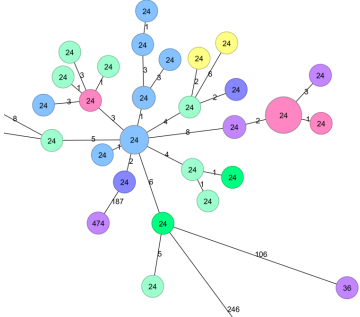
Sequencing



DNA Sequences

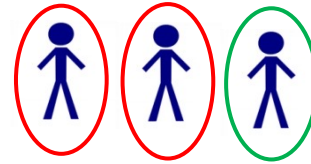
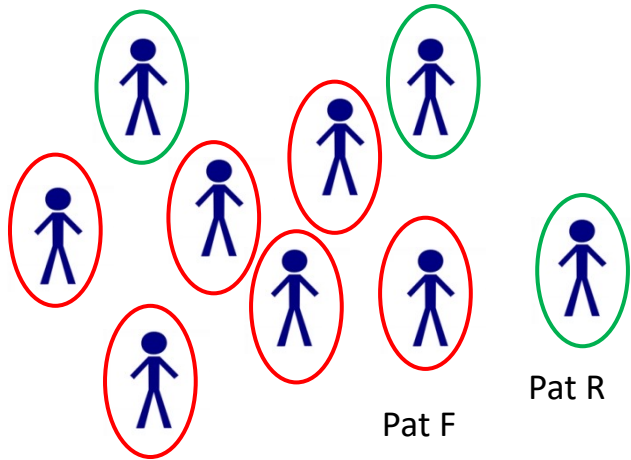
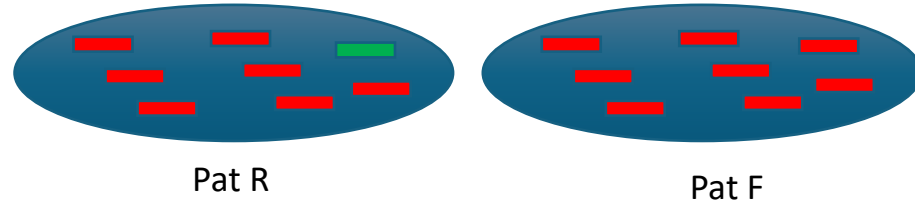
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Typing Pathogens



Typing Report

Tailor-made diagnostics



3 patients
all VRE
identical?

transmission

different variants?

no transmission

important for source tracing
infection control measures
patient management

Results analyses ≥ 5 days

Unique marker DNA



DALL-E-3: sherlock holmes en dna

Unique marker PCR (AI?)

results $\geq 8-24$ hours¹

cheaper

high throughput



Personalised molecular microbiology

From demonstrating the presence of a microbe to understanding the infection

- Characterizing
 - Antimicrobial resistance and virulence markers
 - Microbial fingerprint
- Full microbial profile
 - Microbiome/Pathogenome
 - Resistome
 - Virulome



what is present
(metagenomics)



Viral Bacterial Fungal

Metagenomics

- Characterizing all microbial DNA/RNA in clinical sample¹
- Non-biased
- Why?

No etiological diagnosis in:

80% encephalitis, meningitis²

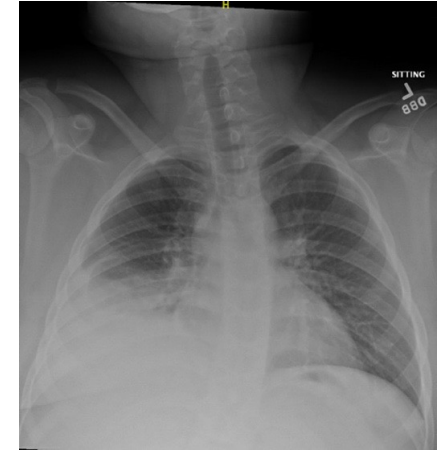
60% pneumoniae^{3,4}

40% sepsis^{5,6}



Metagenomics – an example

- 12-year-old male with a history of obesity, type II diabetes, presented to his primary care provider with pneumonia
- Admitted overnight and given ceftriaxone and azithromycin
- PCR respiratory viral panel → HCoV-OC43; Not thought to explain his lobar pneumonia
- Mild improvement and discharged the following day
- Three days later, the patient returned to the ER with worsening symptoms → again admitted
- Pleural fluid → culture negative
- Metagenomics (IDbyDNA, Salt Lake City) → HCoV-OC43 **and** *Prevotella pleuriditis*
- Patient placed on anaerobic coverage with meropenem and improved markedly



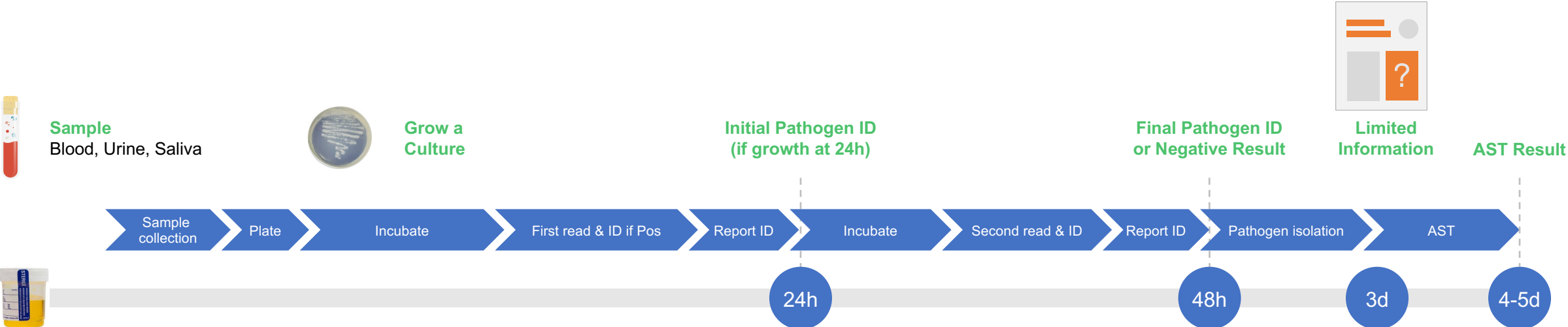
By courtesy of Schlager, Briggs, Bruursema



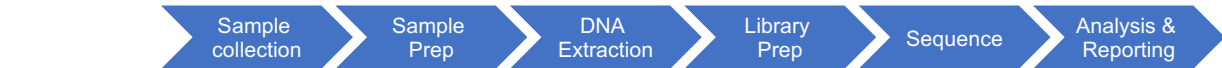
R&D team IDbyDNA

Metagenomics: Breakthrough Discoveries and Detection Through Sequencing and Analyzing Microbial DNA

Traditional lab culture – the current standard of care



Metagenomics – Much More Information and Better Understanding



Sample
Any Type of Biological Material



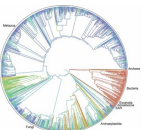
Sequence DNA & RNA
From All Pathogens Present



Digitize Sequences
Convert to Data

Final Pathogen ID or Negative Result

Antibiotic Resistance



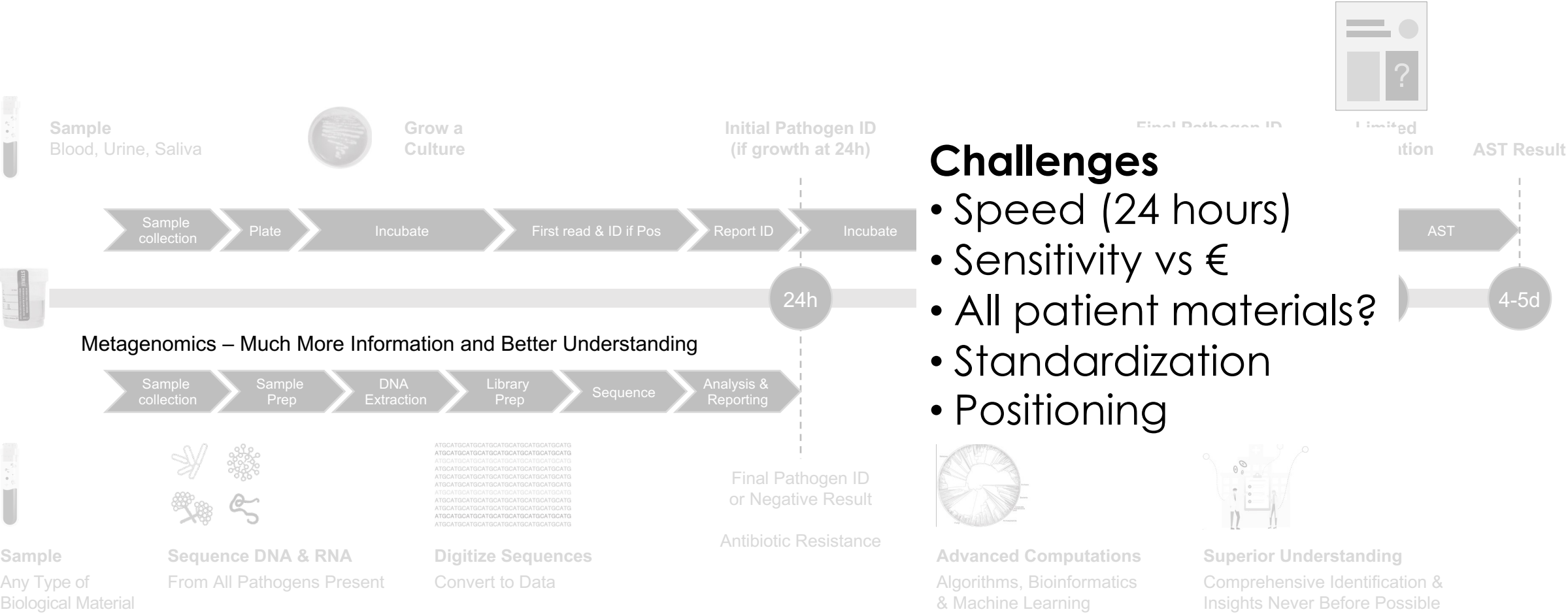
Advanced Computations
Algorithms, Bioinformatics & Machine Learning



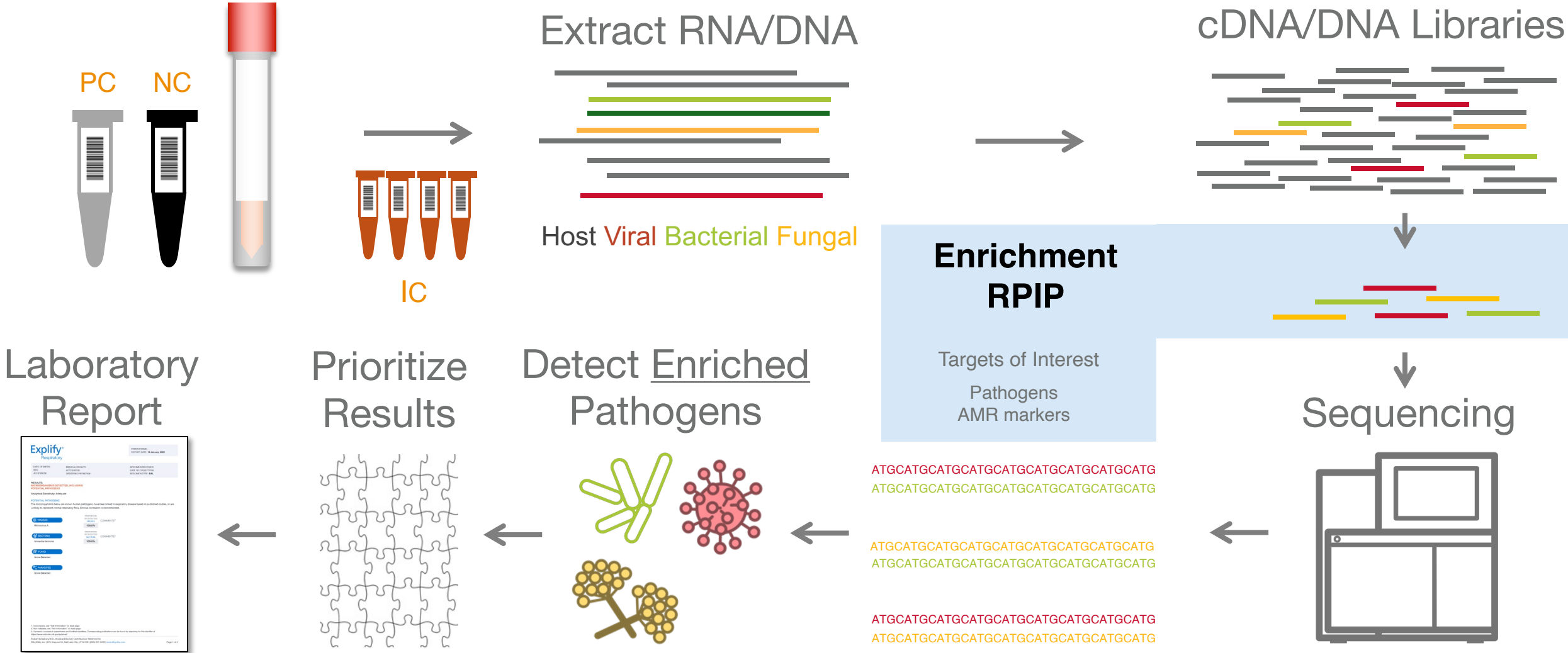
Superior Understanding
Comprehensive Identification & Insights Never Before Possible

Metagenomics: Breakthrough Discoveries and Detection Through Sequencing and Analyzing Microbial DNA

Traditional lab culture – the current standard of care



Precision metagenomics



Enrichment Approach Provides High Detection Efficiency

Table 3: Improved detection of pathogens with enrichment using the Respiratory Pathogen ID/AMR Panel

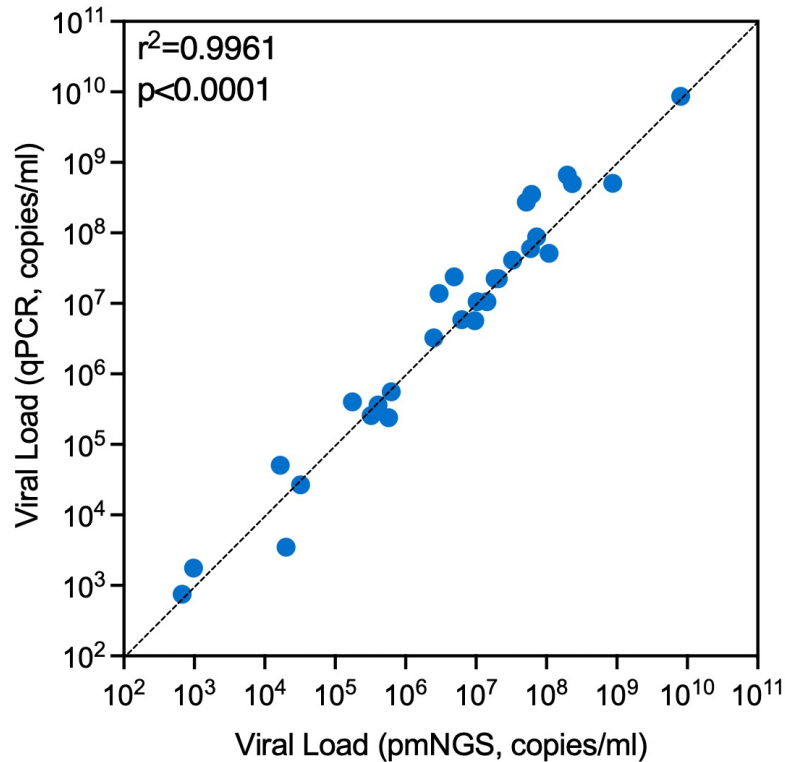
Pathogen	Detected without enrichment	Detected only with enrichment	
Viruses	Human adenovirus B ^a	1	1
	Human adenovirus C	2	2
	Influenza A virus (H1N1)	0	1
	Human metapneumovirus	1	2
	Influenza B virus	0	1
	Human parainfluenza virus 1	1	2
	Human parainfluenza virus 3	2	2
	Respiratory syncytial virus B	1	1
	SARS-CoV-2	1	2
Bacteria	<i>Enterobacter cloacae</i> complex	0	1
	<i>Escherichia coli</i>	1	2
	<i>Haemophilus influenzae</i>	2	2
	<i>Klebsiella pneumoniae</i>	1	2
	<i>Legionella pneumophila</i>	0	1
	<i>Proteus mirabilis</i>	0	1
	<i>Pseudomonas aeruginosa</i>	1	1
	<i>Serratia marcescens</i>	0	1
	<i>Staphylococcus aureus</i>	2	2
	<i>Streptococcus pneumoniae</i>	1	1
Fungi	<i>Pneumocystis jirovecii</i>	0	1
Total	17/29	29/29	

- Residual NP Swabs, BAL, Tracheal Aspirates, Sputa positive with real-time PCR
- Without enrichment **17/29** positive
- With enrichment **29/29**

a. Reported adenovirus B/E by conventional test.

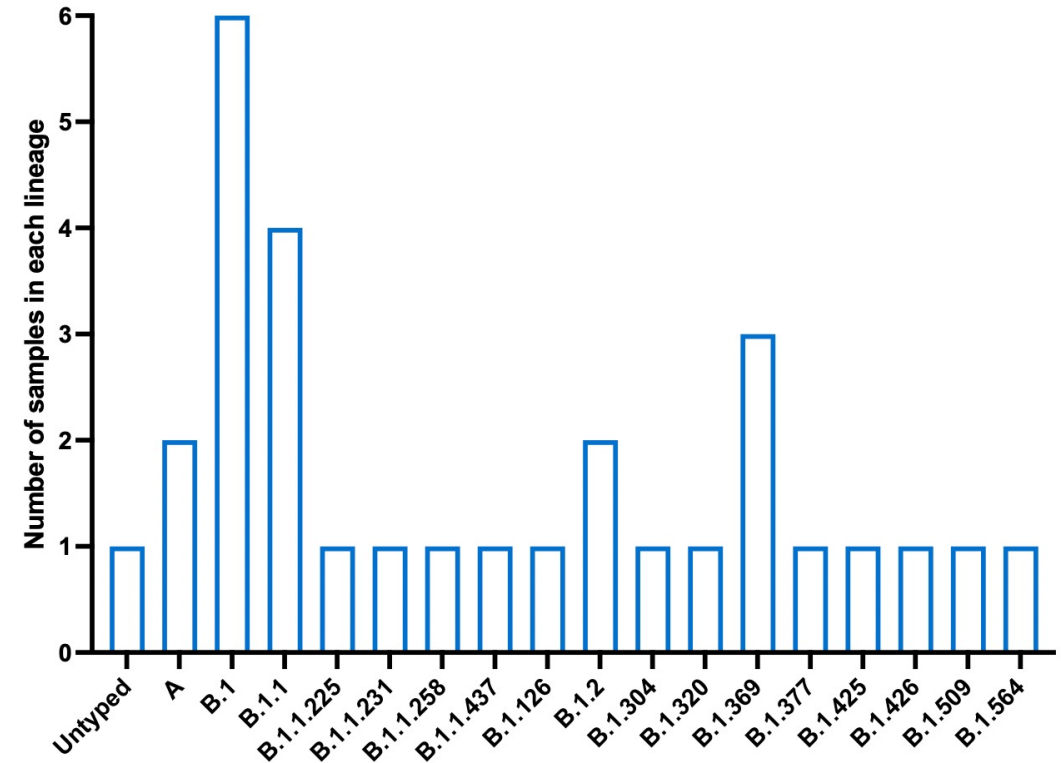
Characterization of SARS-CoV-2 by Precision Metagenomics

SARS-CoV-2 Quantification by qPCR and pmNGS



- Quantification results from qPCR and precision metagenomics showed a significant correlation ($r^2 = 0.9961$; $p < 0.0001$)
- Quantification using the the Respiratory Pathogen ID/AMR does not require the generation of a standard curve required for quantification by qPCR

SARS-CoV-2 Characterization by Precision Metagenomics



- The IDbyDNA Respiratory Pathogen ID/AMR Panel Platform for analysis provides the full SARS-CoV-2 genome and reports the Pango lineage of the virus present in the clinical samples

Other applications metagenomics

- Monitoring wastewater (population)
 - New variants existing pathogens (SARS-CoV-2)
 - Prevalence antimicrobial resistant microbes
 - Predicting outbreaks
- Identification new pathogens

"In December 2019, a cluster of patients with pneumonia of unknown cause was linked to a seafood wholesale market in Wuhan, China. A previously unknown betacoronavirus was discovered using unbiased sequencing in samples from patients with pneumonia."¹

¹Zhu et al., *N Engl J Med* 2020; 382:727-733



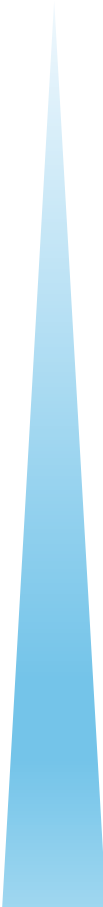
DALL-E-3: voorspellen van uitbraken metagenomics

The microbiome and infections

- A healthy microbiome can limit the growth of pathogenic bacteria
- A disturbed microbiome can lead to increased vulnerability to infections (dysbiosis)
- Personalization: microbiome 'fingerprint' can lead to personalized diagnosis, prognosis, and treatment

Personalised molecular microbiology

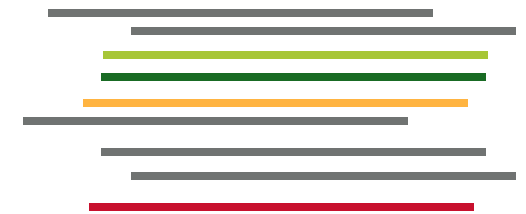
From demonstrating the presence of a microbe to understanding the infection

- 
- Characterizing
 - Antimicrobial resistance and virulence markers
 - Microbial fingerprint
 - Full microbial profile
 - Microbiome/Pathogenome
 - Resistome
 - Virulome
 - Interaction and host response
 - Active microbe?
 - Active resistance and virulence markers?
 - Interactions microbes?
 - Genetic profile host?
 - Host response?

Cost/
information



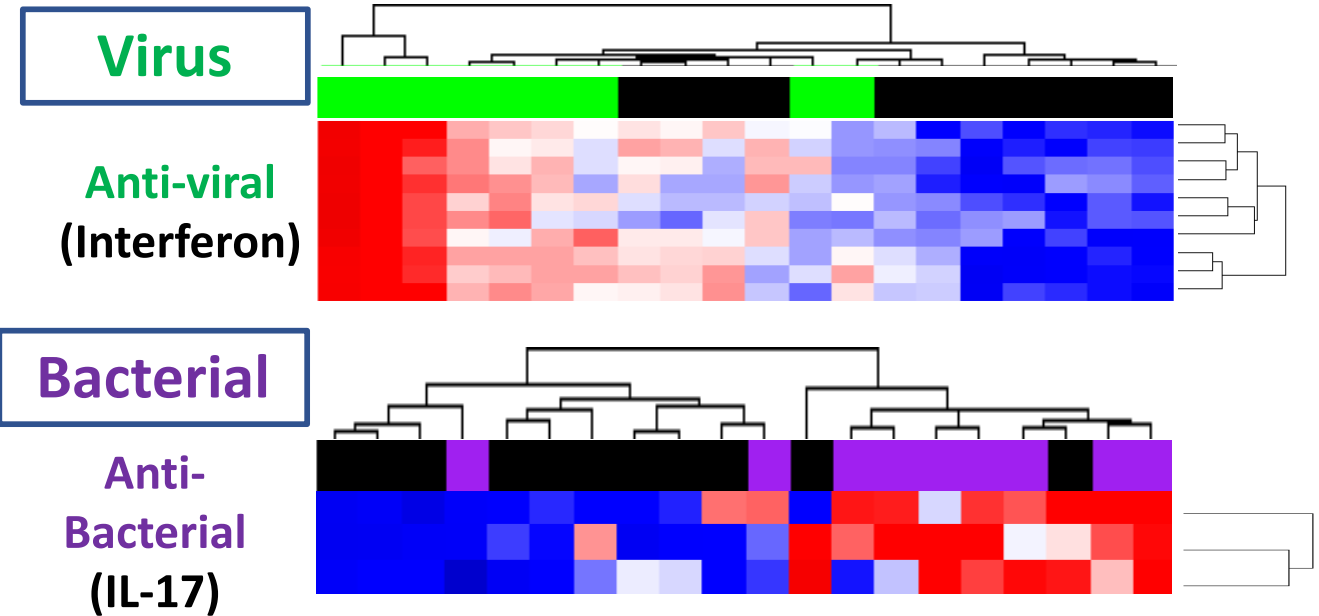
what happens
(metatranscriptomics)



Host Viral Bacterial Fungal

The host response

- Identification all genetic material in clinical sample
- Activity host (human) and microbe
- Fine-tune diagnostics with polymicrobial infections
- Personalised treatment taking host response into account



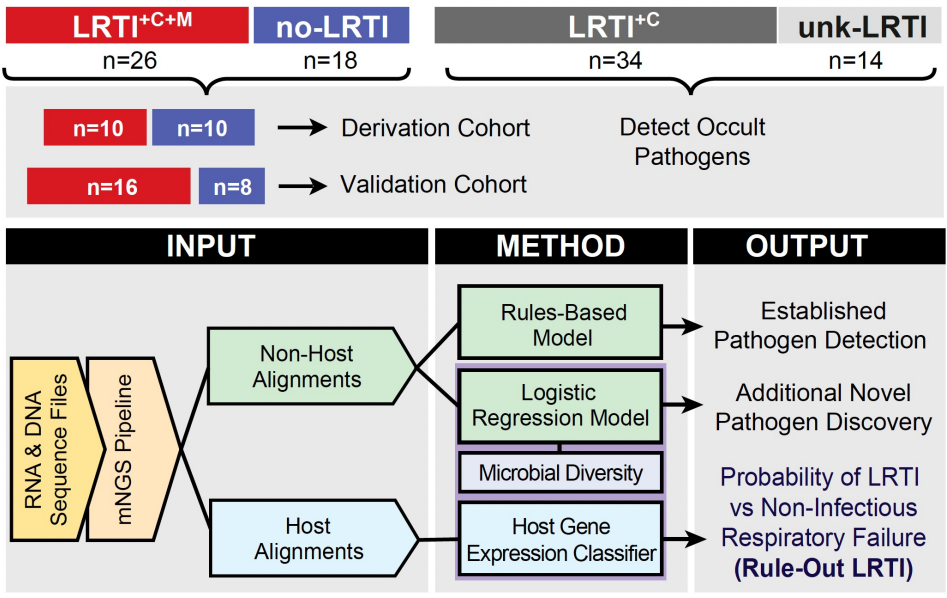
anti-virus responses → no antibiotics?

anti-bacterial response → antibiotics?

Integrating host response and unbiased microbe detection for lower respiratory tract infection diagnosis in critically ill adults

- the pathogen metric → an area under the receiver-operating curve (AUC)* of 0.96 (95% CI, 0.86–1.00) **Which pathogens are present**
- the lung microbiome diversity metric with an AUC of 0.80 (95% CI, 0.63–0.98) **Which other microbes are there**
- the host transcriptional classifier with an AUC of 0.88 (95% CI, 0.75–1.00) **How is the host response**
- Combined → a negative predictive value of 100%**

to distinguish probable pathogens from airway commensals:

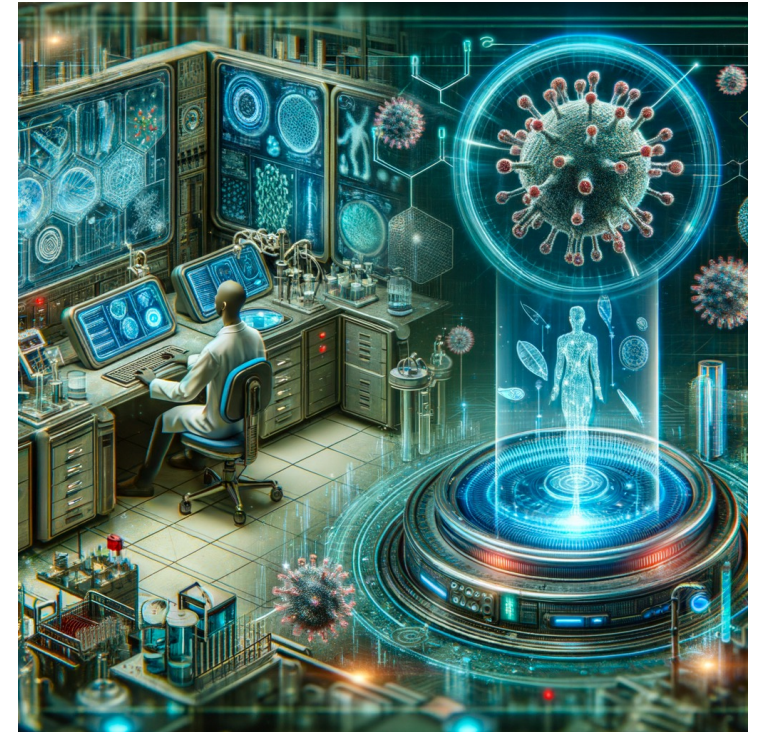


*AUC of 1.0 = 100% specificity and sensitivity
> 0.9 = highly predictive

A single streamlined protocol offering an integrated genomic portrait of pathogen, microbiome, and host transcriptome may hold promise as a tool for LRTI diagnosis

Artificial intelligence

- **Diagnostics:** Analyzing Big Data → faster and more accurate
- **Antimicrobial resistance:** monitor and predict resistance
- **Epidemiology:** modelling spread infection and predict future outbreaks
- **Personalized treatment:** role microbiome in course of infection and analyzing interactions between microbes and with their host



DALL-E-3: Medische microbiologie en kunstmatige intelligentie

Molecular microbiology: decode the unknown?

- Potential to improve the diagnosis of infectious diseases and clinical patient outcomes
- several key challenges remain to be overcome in order to harness this potential impact
 - For whom: varies - only critically ill patients? (More data needed!)
 - Which material: only primarily sterile materials? (More data needed!)
 - When: Only when all conventional tests are negative? (More data needed!)
- Need for standardized approaches
- Multidisciplinary effort and training are essential



DALL-E-3: team bestaande uit moleculair bioloog, infectioloog, arts-microbioloog, data-scientist, epidemioloog, analist, statisticus, biomedical engineer

High-throughput sequencing



reveal the path to
the unknown!