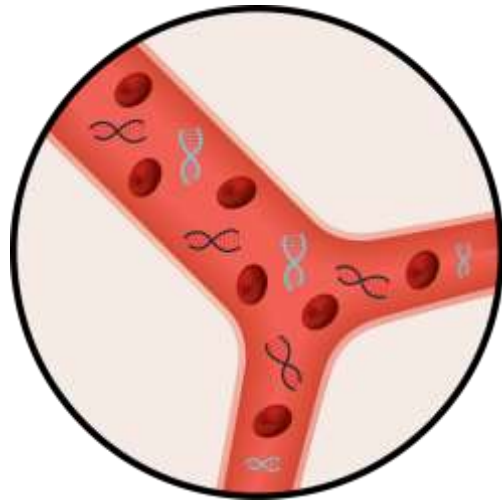


Detection of Respiratory Pathogens using a Novel Plasma-Based Next-Generation Sequencing Assay

David K. Hong, Timothy A. Blauwkamp, Mickey Kertesz,
Cynthia Truong, Niaz Banaei

Detection of pathogen DNA in cell-free plasma



Bloodstream



30 Billion
DNA fragments / mL

Non-invasive prenatal diagnostics

7-10%

Transplant rejection

3-12%

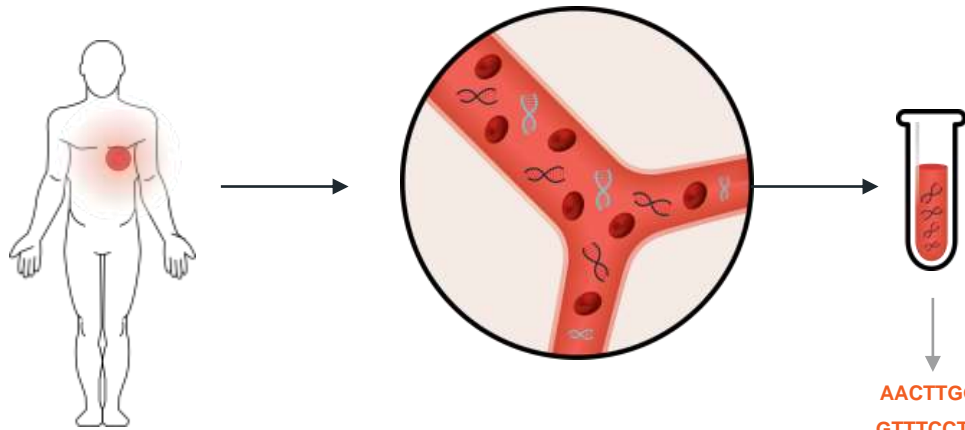
Cancer ctDNA ("Liquid biopsy")

0.1%

Pathogen detection

0.001%

Detection of pathogen DNA in cell-free plasma



Source
of
Infection

AACTGG
GTTTCCT
TAACTGT
TGAGCAA

E. coli

GAAACTCTCAACTTGGCTTAATTGTTTCCTTTGTTCTTGAGCAAT.....

S. aureus

GTGATACACCATTTTAACTGTTTGTTTCCTTAACTAATTGAGCAAAT.....

CMV

CAAATTGTCCTTAACTTAATTTGCAATTTCTGTAGTGAGGTAGTGAT.....

.....
.....



Karius Digital Culture Test™

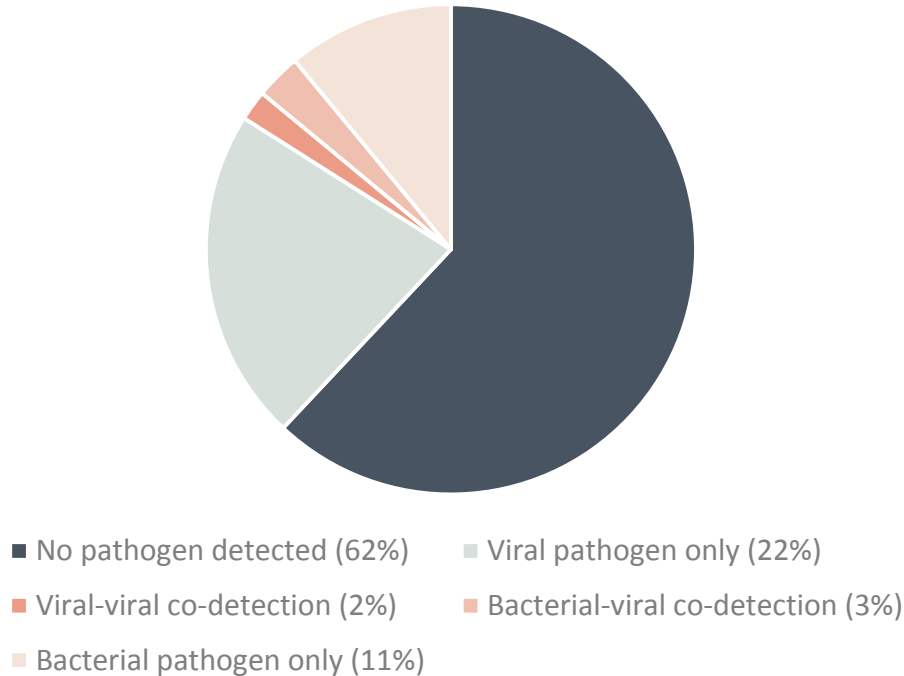


Potential Use Cases

The test may be useful in patients with:

- Culture-negative infection including sepsis, endocarditis, and osteomyelitis
- Infection with fastidious or unculturable organisms
- Susceptibility to a wide breadth of pathogens due to immunosuppression
- Deep infection that requires an invasive biopsy for diagnosis

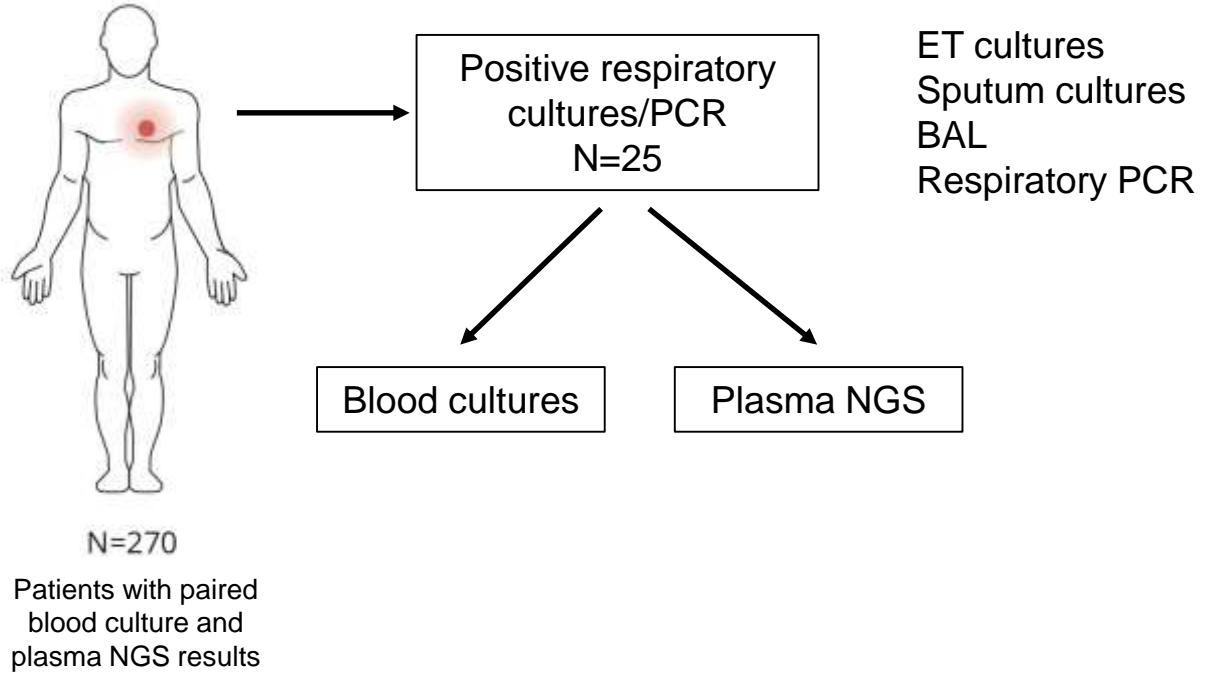
The challenge with pneumonia



- No pathogen is detected 62% of the time
- Microbiologic confirmation often requires invasive testing
- Empiric treatment leads to antibiotic overuse

Jain S et al, *NEJM* (2015); 373:415-427

Detection of Respiratory Pathogens with Plasma NGS



Respiratory pathogens detected by Blood Culture and Plasma NGS

Respiratory test	Blood Culture	Plasma NGS
1+ <i>Staphylococcus aureus</i> in sputum – same day	<i>Staphylococcus aureus</i>	<i>Staphylococcus aureus</i>
2+ <i>Klebsiella pneumoniae</i> , 4+ <i>Enterococcus species</i> in sputum – day -4	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i> , <i>E. faecium</i>
3+ <i>Enterococcus species</i> , 3+ <i>Candida species</i> in sputum – day -2	<i>Enterococcus faecium</i>	<i>Enterococcus faecium</i>
<i>Enterobacter aerogenes</i> in blood culture day -6; in sputum culture day -5	Negative	<i>Enterobacter spp.</i>
<i>Enterobacter aerogenes</i> in endotracheal culture day -2	Negative	<i>Enterobacter aerogenes</i>
2+ <i>Serratia marcescens</i> , 1+ <i>S.aureus</i> - same day in respiratory culture	Coag-neg Staph	<i>Serratia marcescens</i>
Adenovirus detected in nasopharyngeal swab PCR	Negative	Adenovirus
<i>E. aerogenes</i> and <i>Acinetobacter baumannii</i> in endotracheal culture - same day	Negative	<i>Enterobacter aerogenes</i>
4+ <i>E.coli</i> , 4+ <i>S.aureus</i> in endotracheal culture same day	Negative	<i>Escherichia coli</i>
4+ <i>Moraxella catarrhalis</i> , 1+ <i>S.aureus</i> in respiratory culture day -4	Negative	<i>Moraxella catarrhalis</i>
3+ Lactose fermenting rods in endotracheal culture on same day	Negative	<i>Escherichia coli</i>

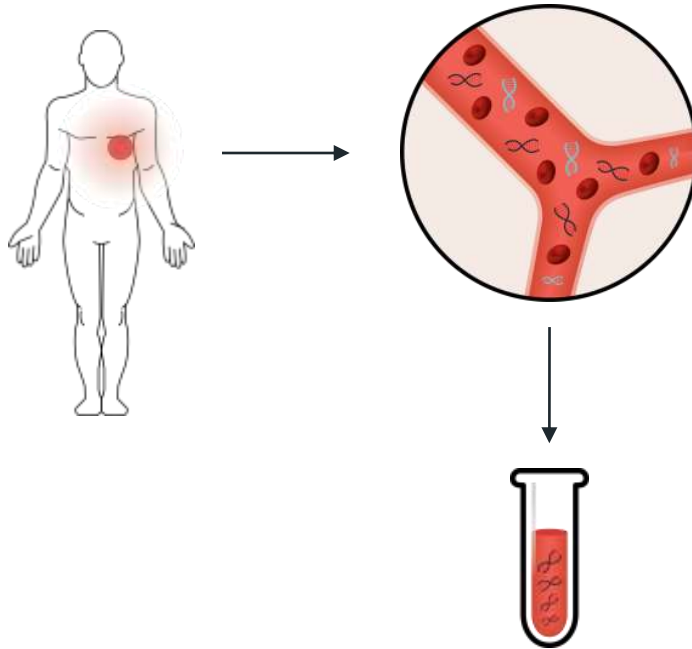
Different pathogens detected by Plasma NGS vs Respiratory Tests

Respiratory Test	Blood Culture	Plasma NGS
1+ <i>Staphylococcus aureus</i> in respiratory culture <i>Streptococcus anginosus</i> , <i>Enterococcus faecalis</i> in abdominal fluid culture	Negative	<i>Fusobacterium nucleatum</i> , <i>Bacteroides thetaiotamicron</i> , <i>Bacteroides fragilis</i> , <i>Streptococcus constellatus</i>
Parainfluenza 4; Adenovirus detected in Respiratory Virus PCR Panel	Negative	<i>Moraxella catarrhalis</i>
10-50 colonies of yeast – induced sputum	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i>
4 colonies of <i>Aspergillus fumigatus</i> – induced sputum	<i>Pseudomonas aeruginosa</i>	<i>Pseudomonas aeruginosa</i>
4+ <i>Stenotrophomonas maltophilia</i> - sputum	<i>Escherichia coli</i>	<i>Escherichia coli</i>

Both Blood Culture and Plasma NGS are negative

Respiratory Test	Blood Culture	Plasma NGS
1+ <i>K. pneumoniae</i> , rare number <i>Bacillus</i> sp. - BAL	Negative	Negative
2+ <i>E. cloacae</i> complex, 2+ <i>S. maltophilia</i> - Sputum	Negative	Negative
20-50 colonies yeast, 3 colonies <i>A. fumigatus</i> complex - sputum	Negative	Negative
BAL - 2+ <i>S. aureus</i>	Negative	Negative
Endotracheal (ET) - 1 colony Yeast, 1+ <i>E. cloacae</i>	Negative	Negative
ET culture - 1+ <i>S. aureus</i>	Negative	Negative
BAL – 50-100 colonies <i>C. glabrata</i>	Negative	Negative
ET – 3+ <i>E. cloacae</i> , 3+ <i>Acinetobacter</i> sp, 3+ <i>S. aureus</i>	Negative	Negative
Sputum – 1+ <i>S. aureus</i> Sputum – 2+ <i>S. aureus</i>	Negative	Negative

Detection of respiratory pathogens by cell-free plasma NGS

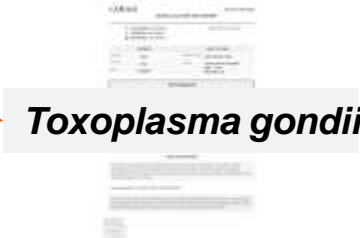
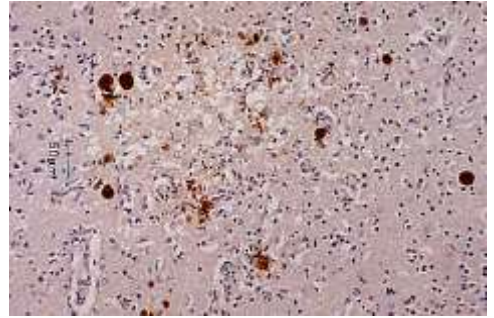
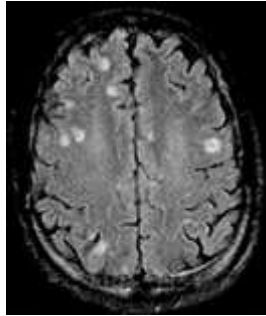
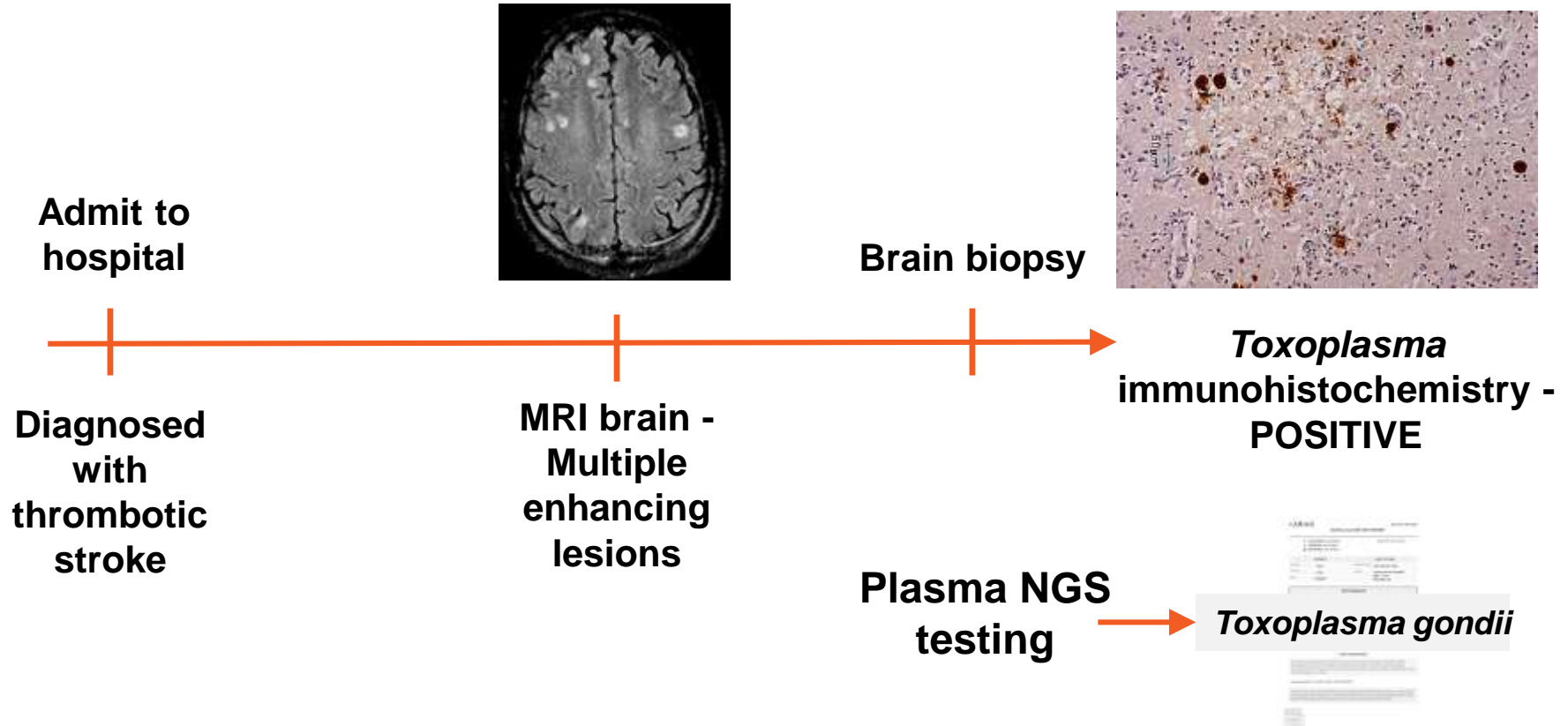


- The Karius Digital Culture test is a validated cell-free plasma NGS test for pathogen detection
- Pathogen DNA from respiratory infections can be detected directly from cell-free plasma
- Non-invasive test can rapidly identify a broad array of pathogens in 48 hours

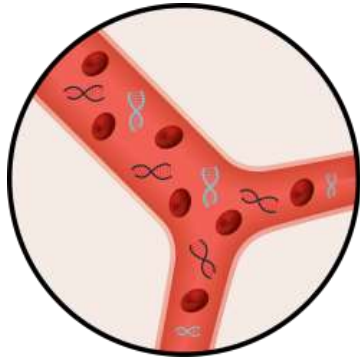
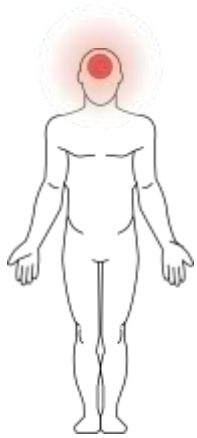
Liquid biopsy for Infectious Diseases: Performance of a Next-generation Sequencing Cell-free Plasma Assay to Detect Pathogen DNA from Deep Infections

David K. Hong, Timothy A. Blauwkamp, Mickey Kertesz,
Cynthia Truong, Niaz Banaei

29yo male with right-sided paralysis



Detection of pathogen DNA in cell-free plasma



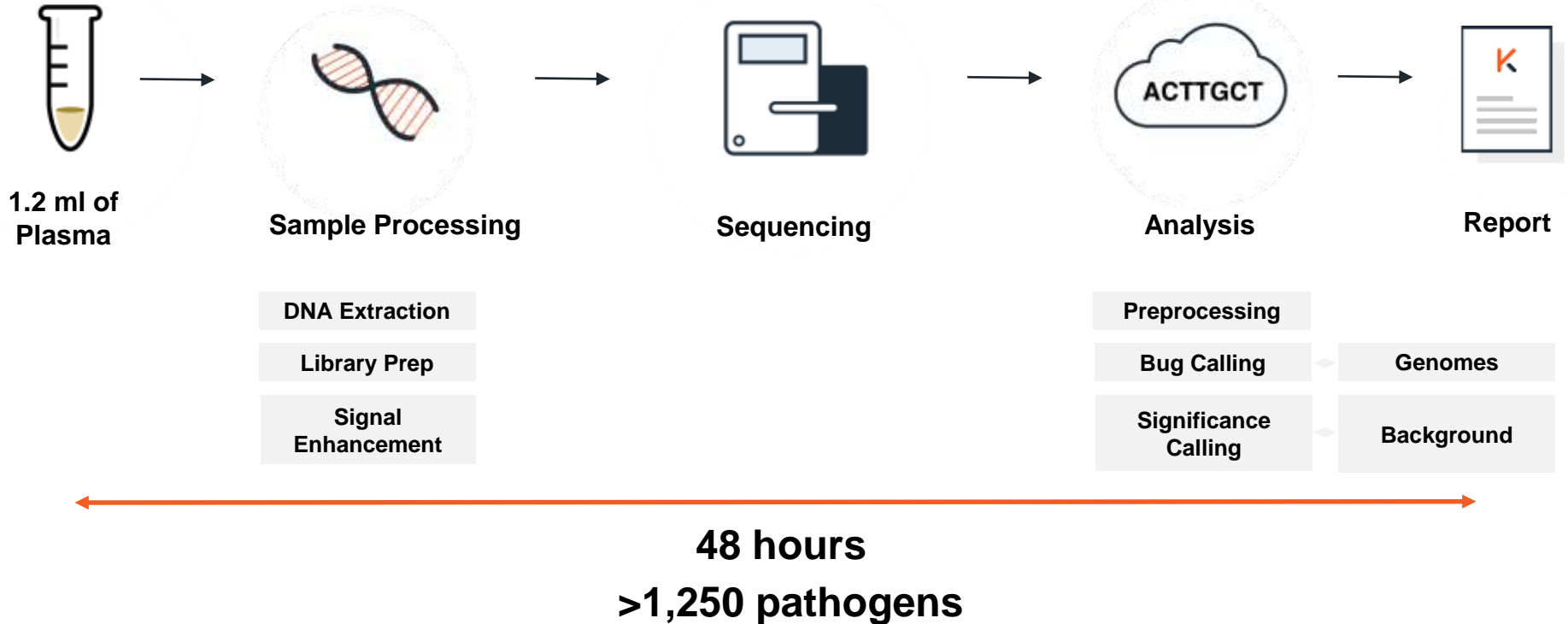
E. coli **AACTTGG**
GAAACTCTCAACTTGGCTTAATTGTTTCCTTTGTTCTTGAGCAAT.....

S. aureus **TTAACTGT** **GTTTCCTT** **TGAGCAA**
GTGATACACCATTTTAACTGTTTGTTTCCTTA ACTAATTGAGCAAAT.....

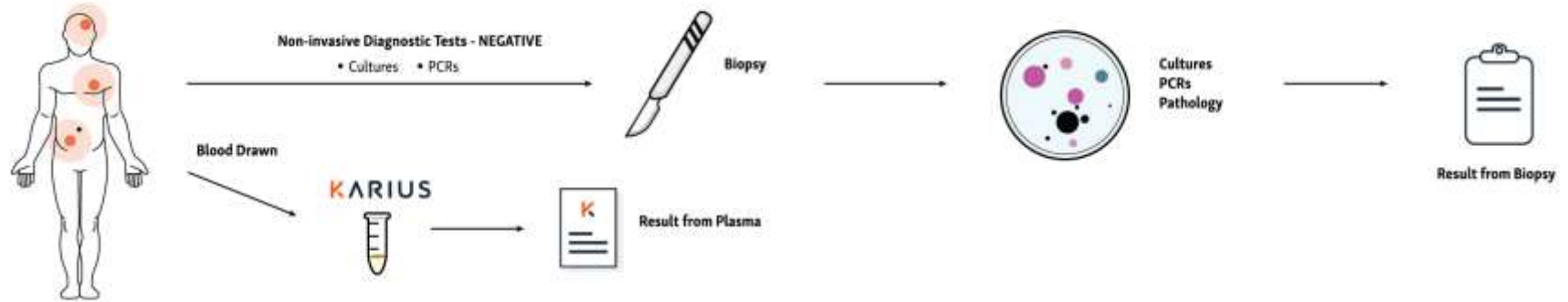
CMV
CAAATTGTCCTTAACCTTAATTTGCAATTTCTGTAGTGAGGTAGTGAT.....
.....
.....



Karius Digital Culture Test™



Liquid biopsy for infection



Patients with infection diagnosed via invasive sampling of various body sites including:

- Broncho-alveolar lavage fluid
- Lung tissue
- Heart tissue
- Intra-abdominal abscess
- Bone biopsy tissue
- Joint fluid
- Cerebrospinal fluid
- Brain biopsy tissue
- Sinus biopsy tissue

Non-invasive pathogen detection

Pathogen from deep infection site	Number	Plasma NGS result matched biopsy result
Bacteria	29	
<i>Mycobacterium</i> (tuberculosis and NTM)	9	
Fungi	20	
Total	58	

Non-invasive pathogen detection

Pathogen from deep infection site	Number	Plasma NGS result matched biopsy result
Bacteria	29	14
<i>Mycobacterium</i> (tuberculosis and NTM)	9	4
Fungi	20	8
Total	58	26

Bacterial DNA detected in plasma from patients with invasive disease

Clinical Diagnosis	Diagnostic Method	Plasma NGS
<i>Pseudomonas aeruginosa</i>	Abdominal wall tissue culture	<i>Pseudomonas aeruginosa</i>
<i>Escherichia coli</i>	Synovial fluid culture	<i>Escherichia coli</i>
<i>Staphylococcus aureus</i>	LVAD fluid culture	<i>Staphylococcus aureus</i>
<i>Staphylococcus aureus</i>	Knee synovial fluid culture	<i>Staphylococcus aureus</i>
<i>Enterococcus faecalis</i>	Pleural fluid	<i>Enterococcus faecalis</i>
<i>Streptococcus pyogenes</i>	Genital wound	EBV, <i>Streptococcus pyogenes</i>
<i>Yersinia enterocolitica</i>	Hepatic abscess	<i>Yersinia enterocolitica</i>, <i>H.pylori</i>

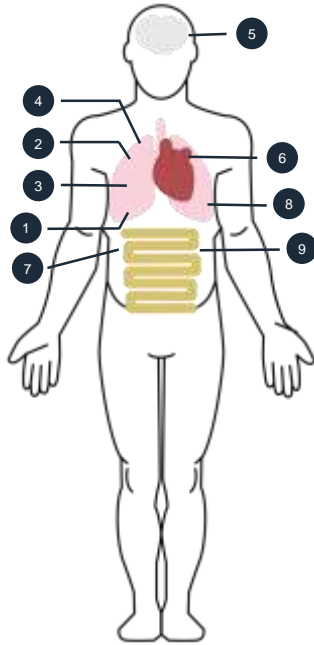
Mycobacterium DNA detected in plasma from patients with invasive disease

Clinical Diagnosis	Diagnostic Method	Plasma NGS
<i>Mycobacterium avium complex</i>	Lymph node, liver, blood, bone marrow AFB cultures	<i>Mycobacterium intracellulare, Enterobacter cloacae</i>
<i>Mycobacterium tuberculosis, CMV</i>	Mtb PCR in CSF CMV PCR (blood) – 35,376	<i>Mycobacterium tuberculosis, Cytomegalovirus</i>
<i>Mycobacterium tuberculosis</i>	BAL – AFB culture	<i>Mycobacterium tuberculosis</i>
<i>Mycobacterium tuberculosis</i>	Tissue adrenal gland – AFB culture	<i>Mycobacterium tuberculosis</i>

Fungal DNA detected in plasma from patients with invasive disease

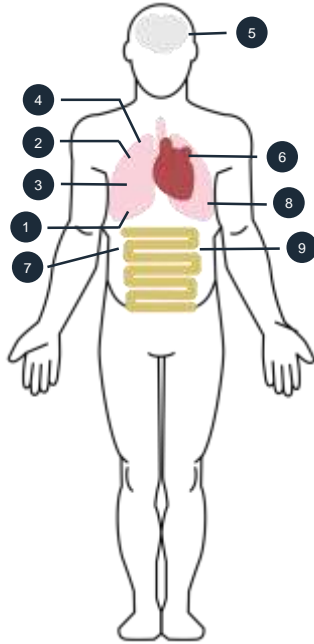
Clinical Diagnosis	Diagnostic Method	Plasma NGS
<i>Cunninghamella sp.</i>	Tissue right lung culture - positive	<i>Cunninghamella bertholletiae</i>
<i>Aspergillus fumigatus</i>	Sputum culture positive; Galactomannan positive	<i>Aspergillus fumigatus</i>
<i>Rhizopus sp.</i>	Sternal tissue culture positive	<i>Rhizopus microsporus</i>
<i>Scedosporium boydii</i>	Brain tissue culture - >100 colonies of <i>Scedosporium</i>	<i>Scedosporium apiospermum</i>
<i>Rhizopus oryzae</i>	R sinus tissue – Fungal Sequencing	<i>Rhizopus oryzae var delemar</i>
<i>Rhizopus sp.</i>	Heart tissue fungal culture positive	<i>Rhizopus microsporus</i>
Presumed fungal disease	Fungal elements seen in bone marrow – negative by fungal culture/fungal sequencing	<i>Candida dubliniensis</i>
<i>Candida kefyr</i>	Fungal culture	<i>Kluyveromyces marxianus</i> (<i>Candida kefyr</i>)

Non-invasive pathogen detection



	Signs and symptoms	Plasma NGS results	Microbiology confirmation
1	Ventilator-associated pneumonia	<i>Enterobacter cloacae/aerogenes</i>	Blood cultures and sputum cultures: <i>Enterobacter cloacae/aerogenes</i>
2	Invasive fungal disease of the lungs	<i>Cunninghamella bertholletiae</i>	Tissue right lung culture - positive
3	Late-stage ALS on ventilator presents with fever	<i>Serratia marcescens</i>	Endotracheal cultures: <i>Serratia marcescens</i>
4	5 days of fever, cough and blood-streaked sputum	<i>Adenovirus</i>	Nasopharyngeal swab PCR: Adenovirus
5	Fungal infection of the brain	<i>Scedosporium apiospermum</i>	Brain tissue - >100 colonies <i>Scedosporidium boydii</i>
6	Fungal endocarditis	<i>Rhizopus delemar</i>	Heart tissue, culture positive <i>Rhizopus</i>
7	Hepatic abscess	<i>Yersinia enterocolitica</i>	Liver biopsy culture – <i>Yersinia enterocolitica</i>
8	Pleural effusion	<i>Enterococcus faecalis</i>	Pleural fluid culture – 4+ <i>Enterococcus</i>
9	Adrenal gland biopsy	<i>Mycobacterium tuberculosis complex</i>	AFB culture of tissue from adrenal gland biopsy

Conclusions



The Karius Digital Culture test is a validated cell-free plasma NGS test for pathogen detection

- **Rapid** – 48 hours
- **Non-invasive** – can detect pathogens from a variety of deep infection sites directly from cell-free plasma
- **Broad** – can detect DNA viruses, bacteria including Mycobacterium, fungi

Acknowledgements

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