



Walter+Eliza Hall

Institute of Medical Research

DISCOVERIES FOR HUMANITY

# c-FIND: Using CRISPR Frontier Infection Diagnostics to Detect, Prevent and Respond to Infectious Threats

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CANCER

|

IMMUNE

DISORDERS

|

INFECTIOUS

DISEASE

# Diagnostic results inform optimal patient care

## **Types of clinical encounters**

- Patient feels unwell and visits clinician
- Screening programs (asymptomatic)
- Hospital testing (patients admitted for other conditions)

## **Patient sample**

*Patient samples are taken for testing and diagnosis*

- Blood / plasma / serum
- Stool / rectal swab
- Tissue swab / biopsy
- Fluid - urine, aspirate, sputum, central nervous system (CNS)
- Throat swab



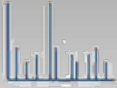

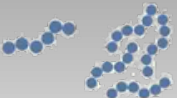

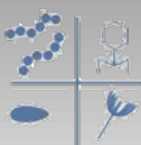





## **Diagnostic test**

*Patient samples are tested in pathology laboratories*

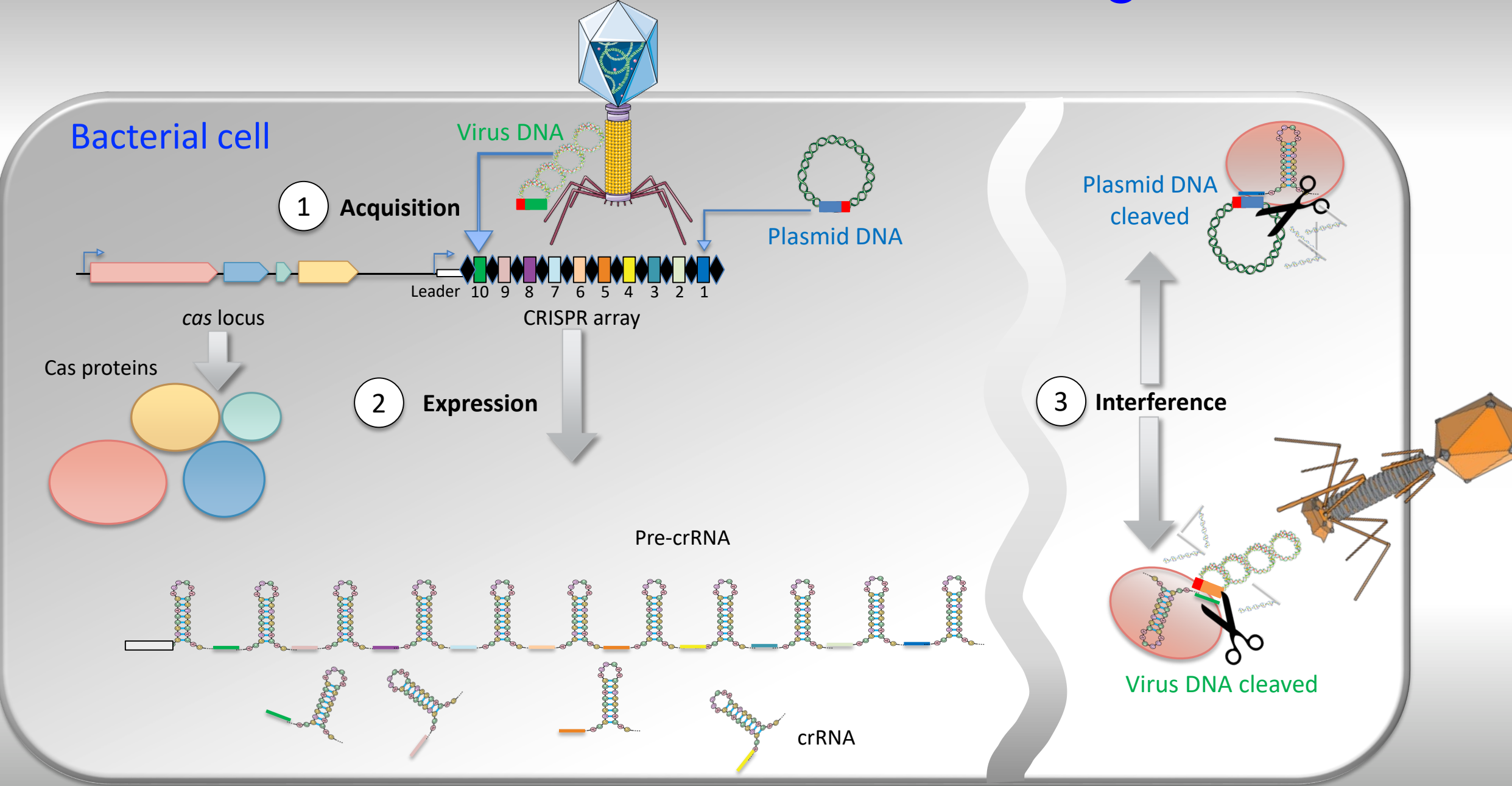
- Bacterial / fungal /viral culture
- Serology
- Microscopy + histology
- PCR
- DNA sequencing
- Antibody panel
- Imaging
- Other laboratory tests

***Patient sample + reagents  
+ diagnostics instruments***

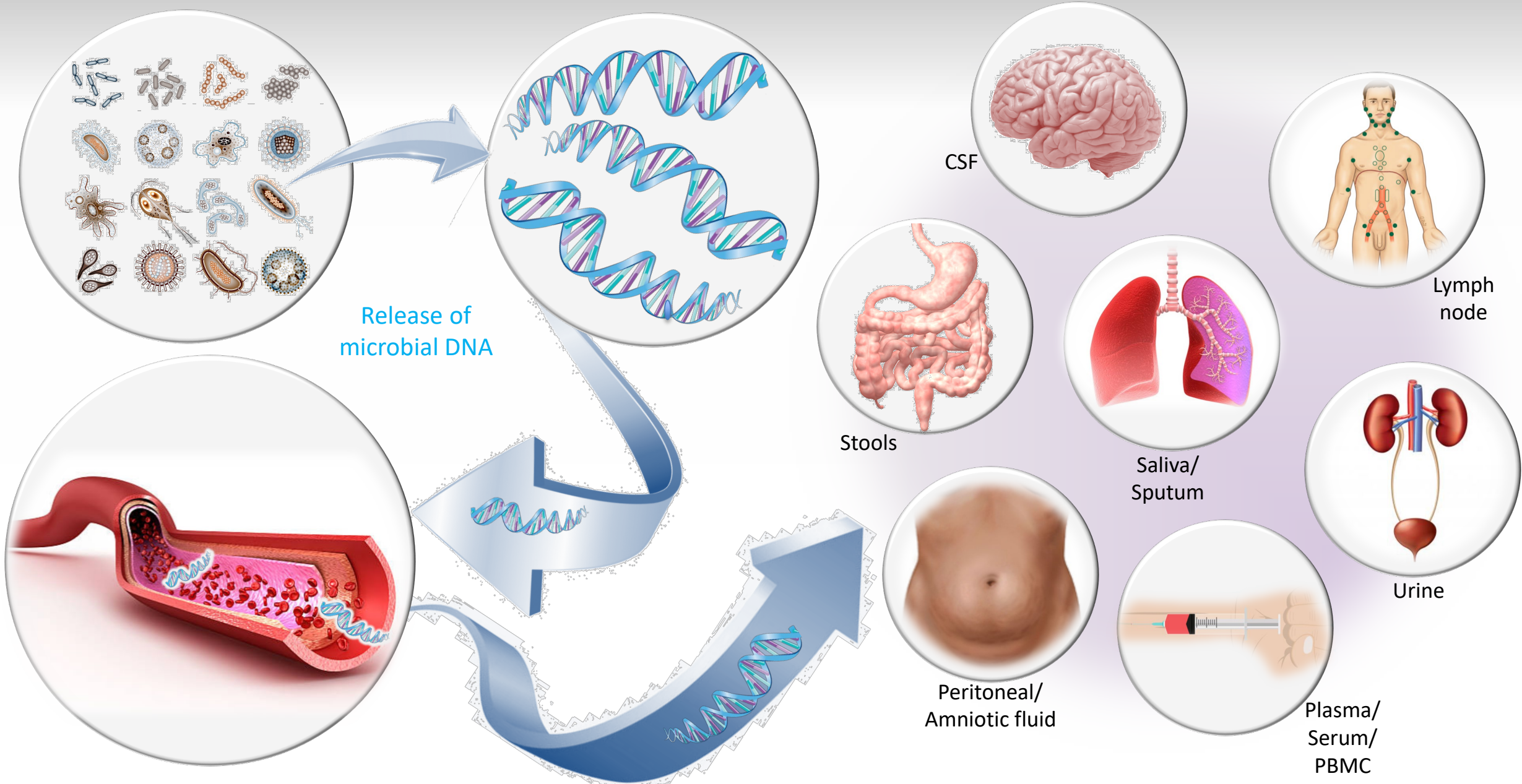
***Diagnostic results inform optimal patient care***

|   | <br>Point-of-care | <br>Time to diagnosis | <br>Sensitivity | <br>Specificity | <br>Antimicrobial resistance | <br>Rapid development | <br>Application | <br>Throughput | <br>Infrastructure | <br>Training | <br>Specimen / stability | <br>Multiplexing |
|---|---|---|---|--|--|---|---|--|--|--|--|--|
| <b>CRISPR</b><br>Identification of DNA signatures of disease  | ✓✓  | MINUTES   | ↑↑↑↑  | ↑↑↑↑   | ✓✓   | ✓✓  | BROAD   | ↑↑   | \$   | MINIMAL  | ANY/<br>STABLE   | ✓✓   |
| <b>MASS SPECTROMETRY</b><br>Ionizes chemical species and sorts the ions based on their mass-to-charge ratio | ~   | HOURS to DAYS   | ↑↓  | ↓↓   | ×  | ×   | PATCHY  | ↑  | \$\$   | MODERATE   | LIMITED/<br>LABILE   | ~  |
| <b>PCR</b><br>DNA amplification and detection   | ~   | HOURS to DAYS   | ↑↓  | ↓  | ~  | ✓   | BROAD   | ↑  | \$\$   | MINIMAL to SIGNIFICANT   | LIMITED  | ✓✓   |
| <b>IMMUNOASSAY</b><br>Antibody or antigen measurements to ID the concentration of chemical molecules        | ~   | MINUTES to DAYS   | ~   | ~  | ×  | ×   | NARROW  | ↓  | \$ - \$\$\$  | MINIMAL to SIGNIFICANT   | LIMITED  | ×  |
| <b>BACTERIAL CULTURE</b><br>Multiplication of bacteria under specific laboratory conditions                 | ×   | DAYS to WEEKS   | ↓↓  | ↑  | ~  | ×   | NARROW  | ↓↓   | \$\$\$   | SIGNIFICANT  | ANY/<br>UNSTABLE   | ×  |

# CRISPR-Cas is a bacteria's defence against viruses



# CRISPR-Cas can be adapted as a diagnostic

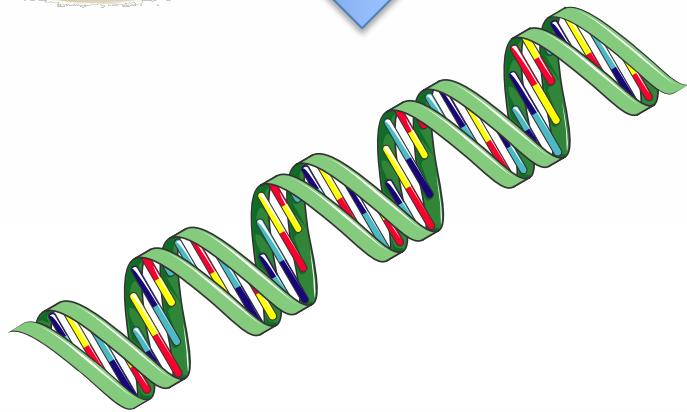


# CRISPR-Cas can be adapted as a diagnostic

Collect specimen at clinical encounter



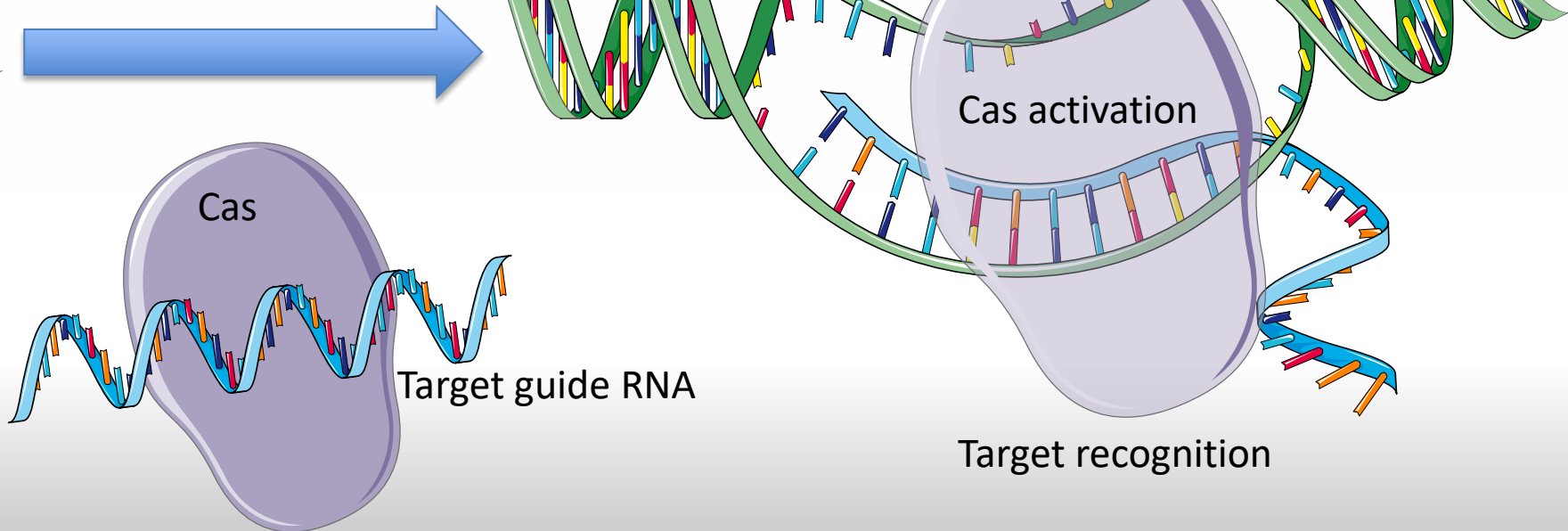
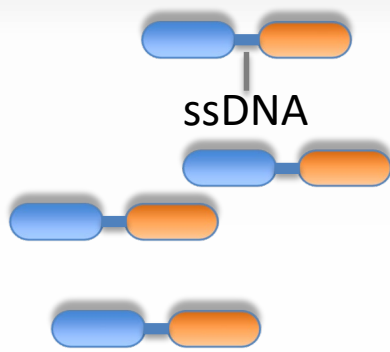
Extract DNA



Microbial nucleic acid

- Pathogen specific sequences
- Genes conferring AMR

Quencher Fluorophore



Fluorescence

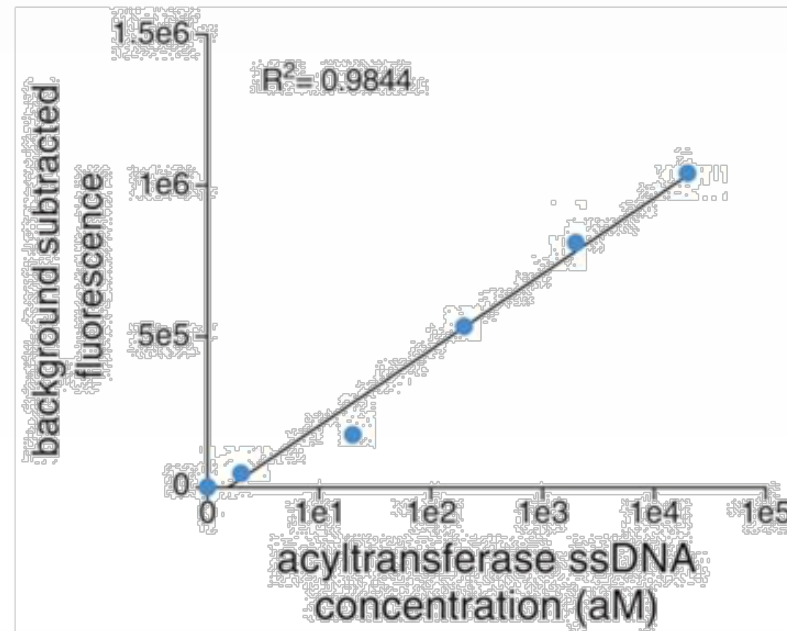
Cas activation

Target guide RNA

Target recognition

# CRISPR-Cas combined with conventional PCR has exquisite sensitivity and specificity

Correlation of *P. aeruginosa* synthetic DNA concentration with detected fluorescence



- Single base pair mismatch detected @  $10^{-18}$  moles /litre
- Can be pushed to 8 zM ( $10^{-21}$ ) with larger sample input (1ml)

# We created a c-FIND team to develop infection diagnostics

Lead organisation:



**Walter+Eliza Hall**  
Institute of Medical Research  
**DISCOVERIES FOR HUMANITY**

Commercialisation partner:



**axxin**  
INNOVATION TO IMPACT.

Partner organisations:



**THE UNIVERSITY OF SYDNEY**



**THE UNIVERSITY OF MELBOURNE**



**THE UNIVERSITY OF WESTERN AUSTRALIA**



**Doherty Institute**



**MELBOURNE HEALTH**



**Peter Mac**  
Peter MacCallum Cancer Centre  
Victoria Australia



**Burnet Institute**  
Medical Research. Practical Action.



**mater** research

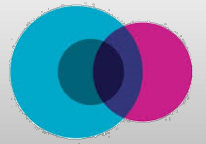


**icpmr**



**The Royal Children's Hospital Melbourne**

Affiliated organisations:



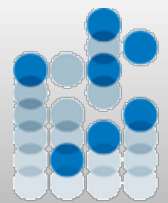
**NCIC**  
NATIONAL CENTRE FOR  
CANCER  
RESEARCH



**WHO Collaborating Centre  
for Reference and  
Research on Influenza  
VIDRL**



**TELETHON KIDS INSTITUTE**  
Discover. Prevent. Cure.



**VIDRL**



**Microbiological Diagnostic  
Unit Public Health Laboratory**



**NSW GOVERNMENT Health Pathology**



**Problem**

**Consequence**

**Gap**

**Inability to diagnose (no rapid / point-of-care diagnostic tests are available)**

- Increased patient morbidity and mortality
- Inconsistent, inadequate and inappropriate treatment
- Spread of disease

- Paediatric patients with fever
- Emerging threats imported by travellers with fever – Pandemic Flu / MERS / Ebola / Zika / SARS / Dengue
- Recognition of complex antimicrobial resistance in infections caused by fastidious/difficult to culture pathogens

**Failure to detect infection in high-risk hospital populations**

- Increased patient morbidity and mortality
- Inconsistent, inadequate and inappropriate treatment
- Spread of disease

- Paediatric patients
- Cancer patients
- Immunosuppressed patients

**Lengthy delays in diagnosis due to limitations of current diagnostic tools**

- Spread of disease
- Economic impact – reduced productivity, school closures, hospital closures, impact on tourism and travel
- Increased patient morbidity and mortality

- Biosecurity risk
- Emerging infectious threats
- Epidemics / pandemics
- Delayed effective antimicrobial therapy in the case of serious bacterial, fungal or viral infections where routine diagnostic testing is unavailable or indeterminate.

**Unknown antimicrobial resistance profiles**

- Spread of antimicrobial resistance
- Inappropriate antimicrobial therapy

- Microbes that cannot be grown / tested adequately
- Latent tuberculosis, fungal infections

**Access to diagnostics limited to highly resourced pathology laboratories**

- Biosecurity risks
- Logistics of centralised testing sites delay diagnosis
- Lack of diagnostic access in point-of-care settings

- Biosecurity risk
- Returned travellers
- Emerging infectious threats
- Epidemics / pandemics
- Latent tuberculosis
- Failure to eliminate malaria reservoir in endemic areas

# c-FIND can inform clinical management

## Detection and Diagnosis

- Diagnostic tests
- Screening

## Response

- Action plans
- Isolation
- Early intervention
- Risk management

## Correct treatment

- Antibiotics
- Antivirals
- Antifungals
- Antiparasitics

## Patient Outcome

- Decreased mortality
- Decreased morbidity
- Reduced transmission
- Improved quality of life

***Match the right patient with the right treatment at the right time***

# CRISPR has capacity to offer rapid POC test that can be updated in real time

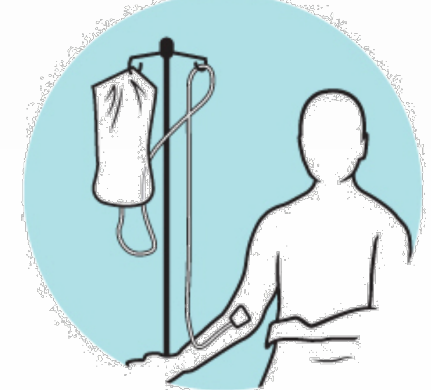
**Clinical encounter**



**Sample collection**

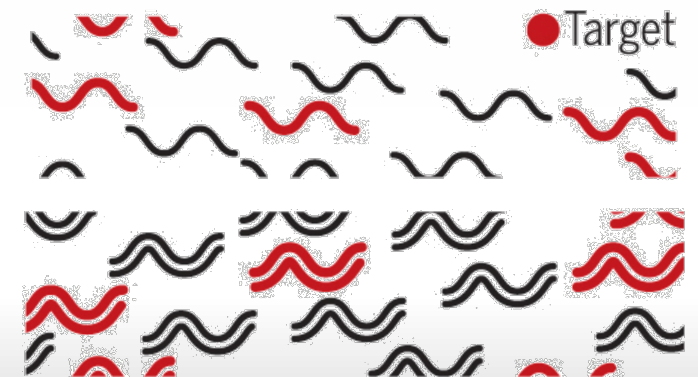


**Infection control**  
Optimal clinical care

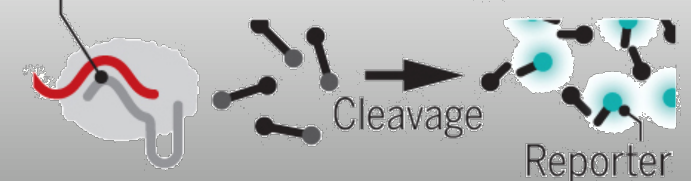


## DIAGNOSTIC TEST

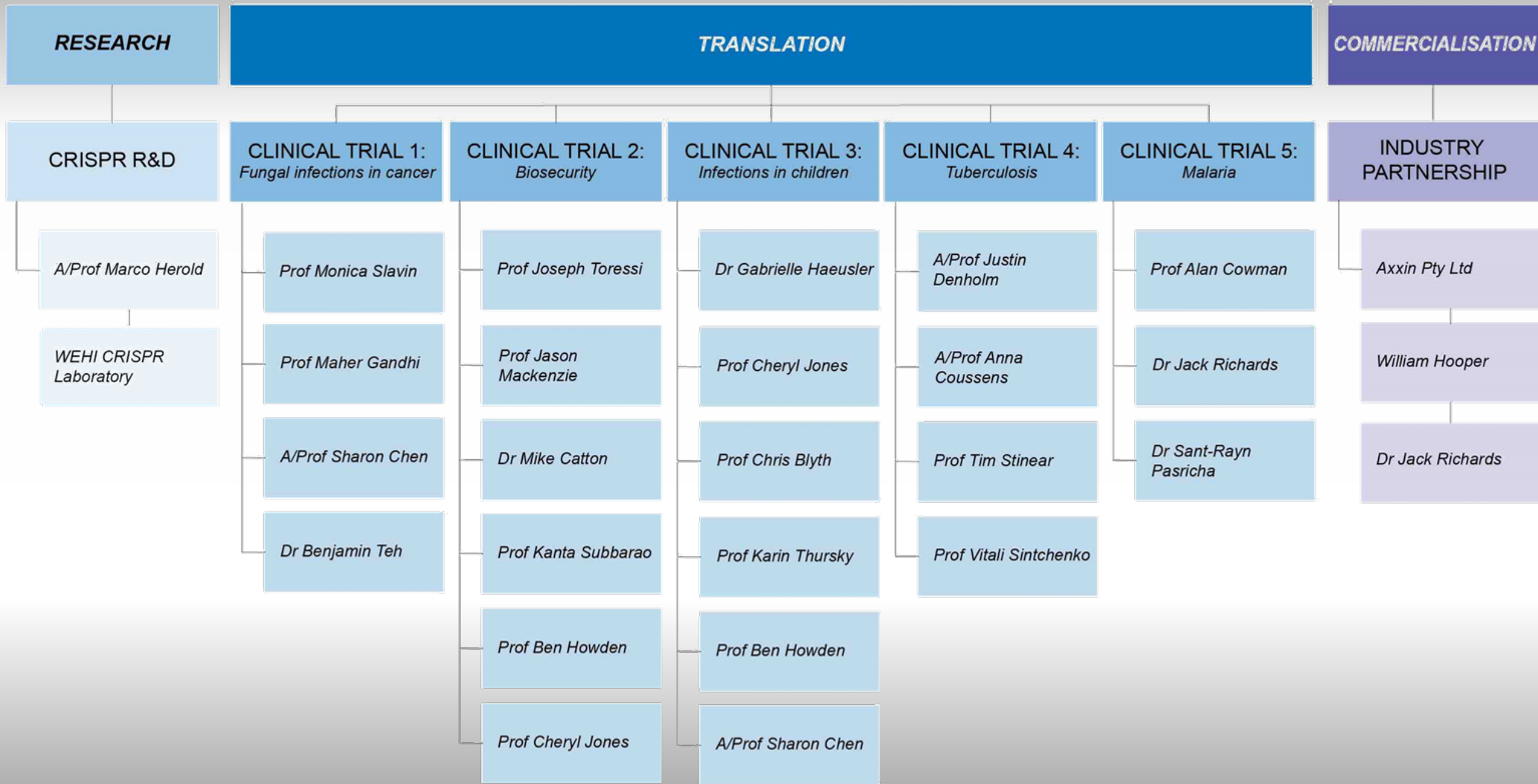
1. Prepare sample, release and protect nucleic acid (HUDSON)
2. Amplify DNA and RNA
3. Use gRNA to detect specific sequence and amplify signal



Cas-crRNA detection of target



c-FIND Frontier Program  
Program Lead: Professor Marc Pellegrini



# The research involves 5 clinical trials

*c-FIND guide design and targets for clinical trials*

| <b>Clinical trial</b>   | <b>Validation samples</b> | <b>gRNA guide design</b>  | <b>Pathogen target</b>  | <b>Antimicrobial resistance target</b>  |
|---|---------------------------|---|---|---|
| <b>Clinical trial 1:<br/>Life threatening fungal infections in cancer patients, immunocompromised patients and children</b> | CIDMLS (NSW)              | Sharon Chen<br>Christopher Blyth  | <i>Candida spp.</i><br><i>Aspergillus spp.</i><br><i>Mucorales</i><br><i>Lomentospora prolificans</i>   | Azole<br>Echinocandin<br>Amphotericin B   |
| <b>Clinical trial 2:<br/>National Biosecurity, emerging epidemic and pandemic infectious threats</b>                        | VIDRL (VIC)<br>SCHN (NSW) | Jason Mackenzie<br>Joseph Torresi<br>Mike Catton<br>Kanta Subbarao<br>Cheryl Jones<br>Christopher Blyth | MERS-CoV<br>SARS-CoV<br>Ebola<br>Influenza A viruses<br>Flaviviruses<br>Norovirus<br>ESBL producing enterobacteriaceae (PE)<br>Real time response to infectious threats | Zanamivir<br>Oseltamivir<br>Baloxavir<br>Antibiotics including extended spectrum penicillins, third generation cephalosporins, quinolones and monobactams |

# The research involves 5 clinical trials

| <i>Clinical trial</i>   | <i>Validation samples</i>  | <i>gRNA guide design</i>  | <i>Pathogen target</i>   | <i>Antimicrobial resistance target</i>  |
|---|--|---|--|---|
| <b>Clinical trial 3:<br/>Infections in infants<br/>and children</b> | RCH – Lab services (VIC)<br>RWH – Lab services (VIC)<br>VIDRL (VIC)<br>MDU (VIC)<br>SCHN (NSW) | Sharon Chen<br>Ben Howden<br>Mike Catton<br>Cheryl Jones<br>Christopher Blyth | <i>Streptococcus spp.</i><br><i>Staphylococcus spp.</i><br><i>Escherichia coli</i><br><i>Klebsiella spp.</i><br><i>Pseudomonas spp.</i><br><i>Neisseria spp.</i><br><i>Haemophilus spp.</i><br>Enteric pathogens (non-typhoidal <i>Salmonella</i> , <i>Shigella</i> )<br><i>Listeria spp.</i> (neonates)<br><i>E. faecium</i> (VRE)<br><i>Actinobacter</i> / | $\beta$ -lactam / carbapenems<br>Methicillin<br>Cephalosporins<br>Fluoroquinolones<br>Aminoglycosides<br>Macrolides<br>Trimethoprim-sulphamethoxazole |

# The research involves 5 clinical trials

| <i>Clinical trial</i>   | <i>Validation samples</i>                          | <i>gRNA guide design</i>                       | <i>Pathogen target</i>                                  | <i>Antimicrobial resistance target</i>  |
|---|--|--|---|---|
| <b>Clinical trial 4:<br/>Spread of imported<br/>undiagnosed<br/>Tuberculosis Disease<br/>in adults and children</b> | VIDRL (VIC)<br>South Africa                        | Vitali <u>Sintchenko</u><br>Tim <u>Stinear</u> | <i>Mycobacterium tuberculosis</i>                       | Isoniazid<br>Rifampicin<br>Pyrazinamide<br>Ethambutol<br>Ethionamide<br>Streptomycin<br>Para- <u>aminosalicylic acid</u><br>Fluroquinolones |
| <b>Clinical trial 5:<br/>Malaria detection to<br/>guide elimination</b>   | Papua New Guinea<br>Indonesia<br>Vietnam<br>Africa | Alan Cowman<br>Sant- <u>Rayn Pasricha</u>      | <i>Plasmodium falciparum</i><br><i>Plasmodium vivax</i> | Chloroquine<br>Artemisinin<br><b>Other targets:</b><br>G6PD   |