

PCR - past, present, future

Lyn Gilbert,
CIDM-Public Health,
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Polymerase chain reaction
“PCR” (\equiv zipper; biro etc.)
or nucleic acid (amplification)
test - “NA(A)T”

Stanley Falkow's prediction

- ASM, Melbourne c1976

- DNA probes

- for diagnosis of diarrhoea

Faecal extract spots on membrane

- DNA probes for known pathogens

- Store and retest for new pathogens

- Numerous potential applications

- Promise never fulfilled



Why we needed PCR in 1980s

- Culture –ve meningoencephalitis
 - TBM – CSF ZN –ve (usually)
 - HSVE - Viral culture (biopsy) slow +/or –ve
- Intrauterine infections
 - *Toxoplasma gondii* – mouse inoculation
 - CMV; rubella – culture slow
- Chlamydial infections
 - Culture unavailable; antigen tests unreliable
 - Diagnosis rarely confirmed in males

Background to the PCR

- 1953 double helix structure of DNA
 - Watson, Crick, Wilkins (Franklin)
- 1960 DNA-RNA hybridization - Rich
- 1971 replication of short DNA template with primers - Kleppe
- 1976 – Taq polymerase – Chien
- 1983 – PCR – Mullis (+LSD)

Invention of the PCR



**Kary Mullis,
Nobel Prize - 1993**

"Beginning with a **single molecule** of .. DNA, the PCR can generate **100 billion** similar molecules in an afternoon.

The reaction is **easy** to execute. It requires ... a test tube, a few simple reagents and a source of heat.

The DNA sample ... can be **pure**, or .. a minute part of ... **complex** biological mixture ... from a hospital **tissue specimen**, a single **human hair**, a drop of **dried blood**, .. tissues of a **mummified brain** or .. a 40,000-year-old **woolly mammoth** frozen in a glacier."

Scientific American 1983

What we do with “PCRs” in 1990-2000s

Diagnose:

- Infections due to slowly growing, fastidious, nonculturable organisms
- Individual/multiple (syndrome) pathogens +/- rapidly growing pathogens
- **Many viruses:**
 - e.g. parvo; HCV; HBV, HIV; polyoma; EBV; VZV; noro; metapneumo. etc
- **CNS:** *M. tuberculosis*; HSV; enterovirus
- **Respiratory:** *B. pertussis*, *M. pneumoniae*; *C. pneumoniae*; *L. pneumophila* + viruses
- **Genital/STD:** *C. trachomatis*; HPV; *T. pallidum*; *M.genitalium*; *T. vaginalis*

What we do with “PCRs” in 1990-2000s

Discover:

- new pathogens: broad range primers
PCR/sequencing
 - e.g. numerous mycobacteria,
Tropheryma whippelii, *B. henselae*, etc.

Identify:

- virulent strains of common
commensals/pathogens
 - e.g. *E. coli*; HPV

What we do with “PCRs” in 1990-2000s

- **Viral load** – HIV; CMV
- **Antimicrobial resistance** –
 - *S. aureus* (*mec*); enterococci (*van*); HIV
- **Rapid screening for carriage**
 - GBS; MRSA

The ideal diagnostic NAT - “horses for courses”

- Appropriate for clinical indication
- Sensitive and specific (quantified)
 - DNA quality;
 - target – multiple vs single copies;
 - primer design; PCR protocol;
 - nesting; probes; signal amplification
 - controls (inhibition & contamination);
- Clinically evaluated – predictive values

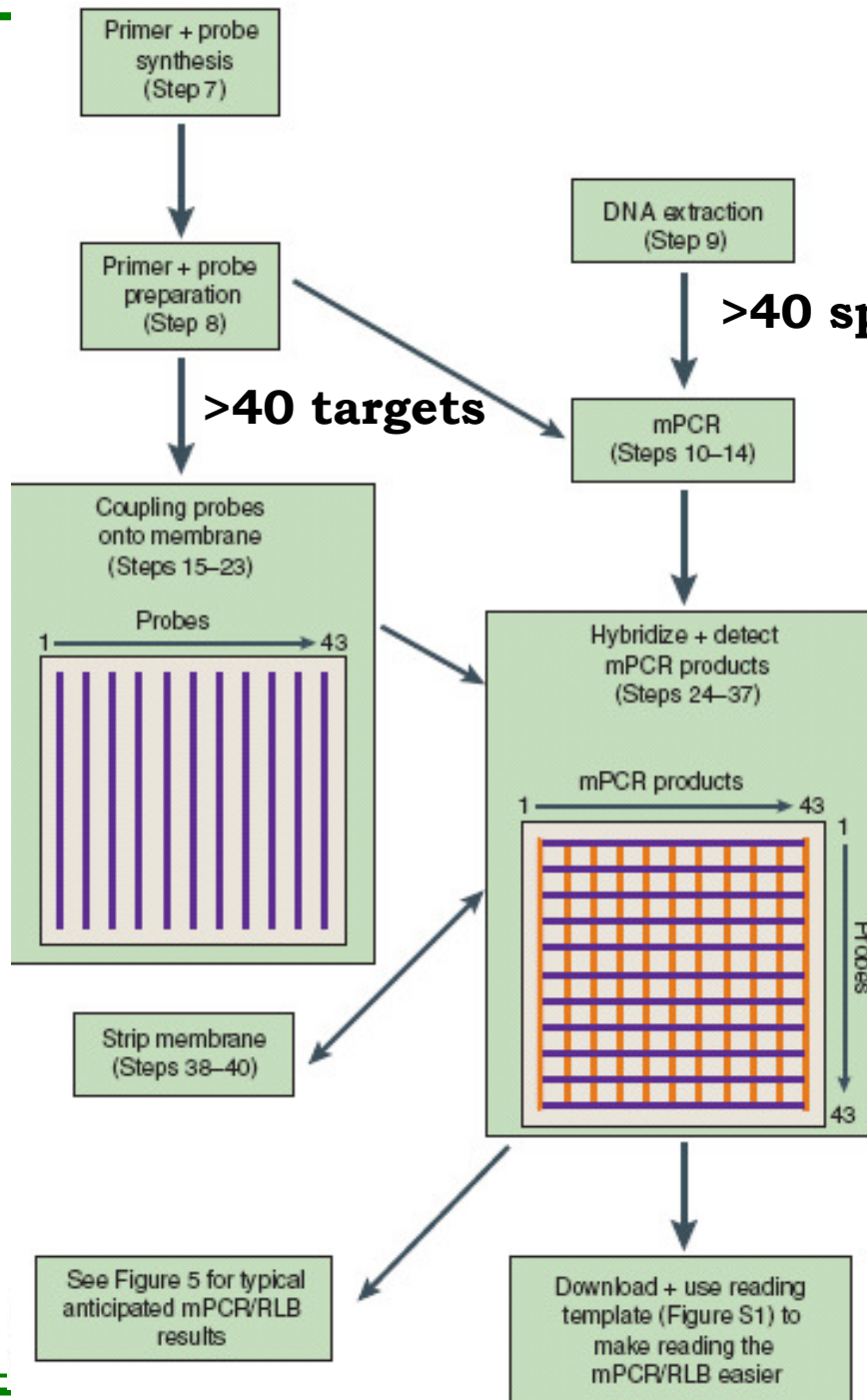
The ideal diagnostic NAT

- Convenient specimen types
 - e.g. urine; throat swab etc.
- Rapid – “real-time”
- Random access or automated
- Fool-proof/robust
- (Semi-)quantitative
 - Prognosis; monitor treatment
 - Latency from infection

Multi-multiplex assays

- Multiplex tandem (MT)-PCR - later
- mPCR/reverse line blot (RLB) assay
 - Up to ~40 sets of primers
 - Careful primer design for sens., spec.
 - Amplicons detected by probes on membrane
 - 40 specimens per run
 - Suitable for
 - non-urgent diagnosis; surveys; batch testing
 - microbial typing; fingerprinting

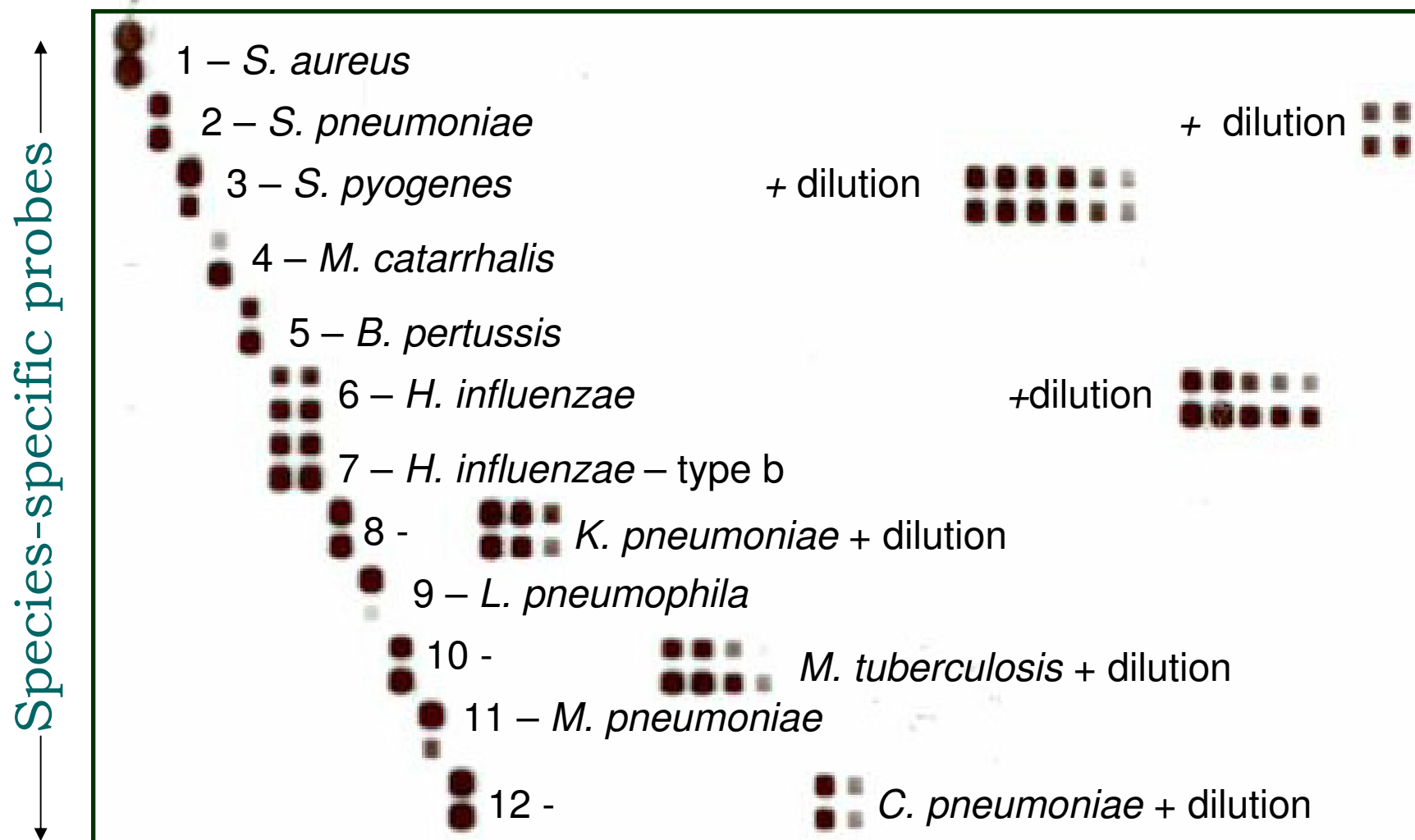
mPCR/RLB



- Simple; versatile
- High volume
- Reproducible
- Inexpensive
 - Equipment cheap
 - Reusable membranes
- Relatively rapid
 - 6-8 hours
- Objective

Kong & Gilbert, Nature Protocols, 2007

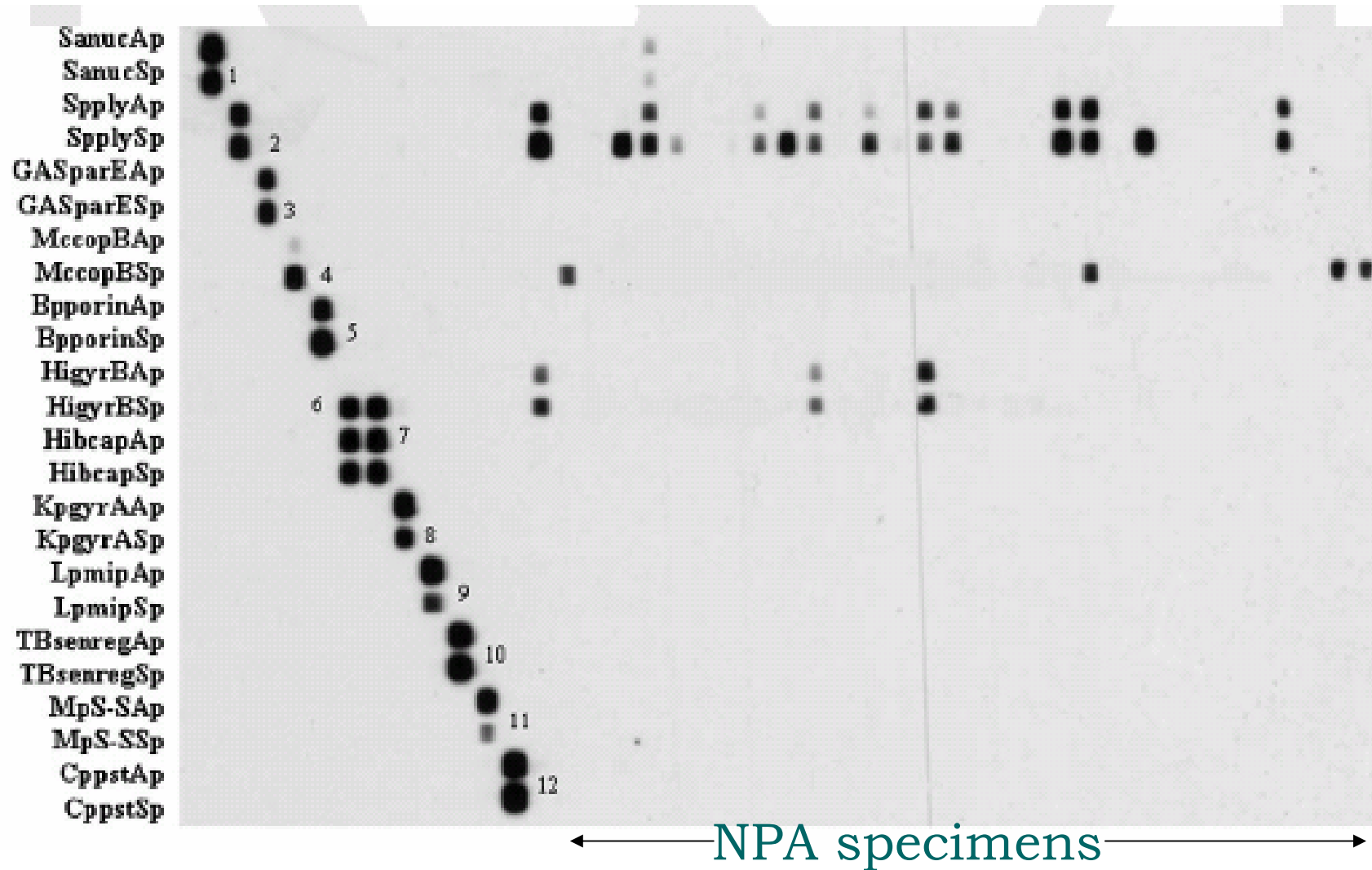
Respiratory pathogen mPCR/RLB



Culture extracts or DNA of species +/- dilutions

Y Wang et al, *Pediatr Pulmonol* 2008;43:150-9

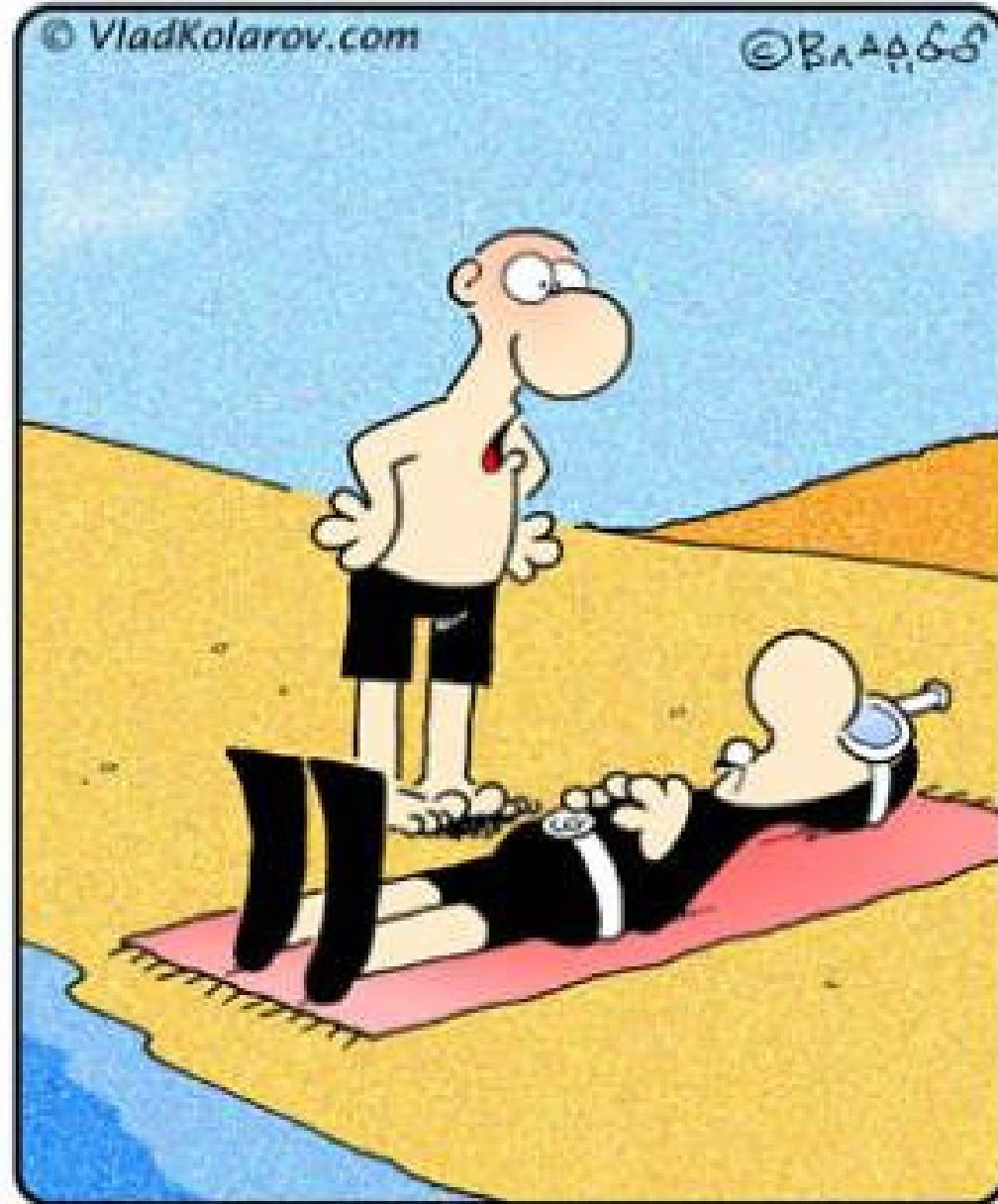
Respiratory pathogen mPCR/RLB



Future

- Closer to ideal NAT
- Fast, automatable +/- POC
- Multiple targets
 - Species; subtype; epidemiological markers
 - Virulence & antibiotic resistance profiles
- Automated reporting & notification
 - Medical record, infection control unit, health dept.
 - Spatio-temporal cluster identification
 - Transmission events; infection/disease control
 - Risk assessment; decision support





*"This time you've prepared yourself
for the tide, eh?"*

thankyou

Developing an in-house NAT

- Define clinical indications
- Literature review
 - use/adapt validated methods if possible
 - or design/order primers
- Choose platform; optimise assay
 - incl. specimen prep.; controls
- Measure sensitivity/limits of detection
 - cultures; spike/actual specimens
 - compare vs “gold standard” (if applicable)
- Measure specificity – test similar species
- Clinical evaluation – PPV, NPV
 - vs clinical other lab diagnosis