

What I think should be the standard in diagnosing
human *Mycoplasma (pneumoniae)* infections

Meet-the-Expert Session

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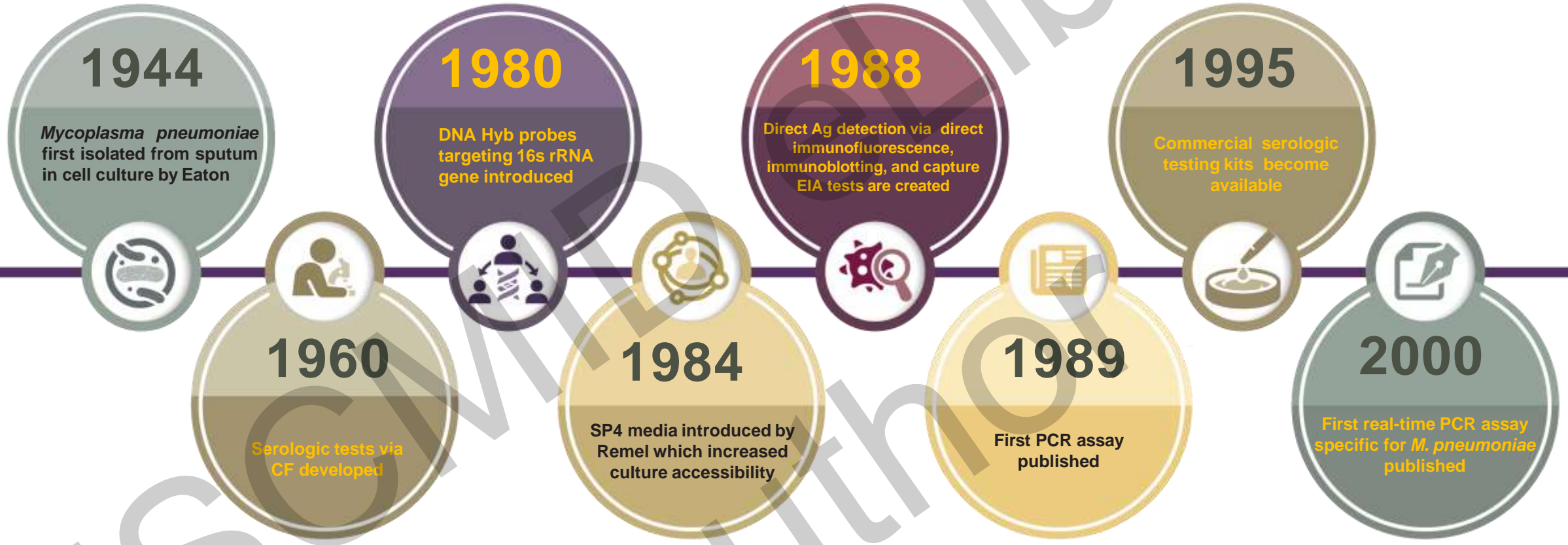
zdx2@cdc.gov (404) 395-3389



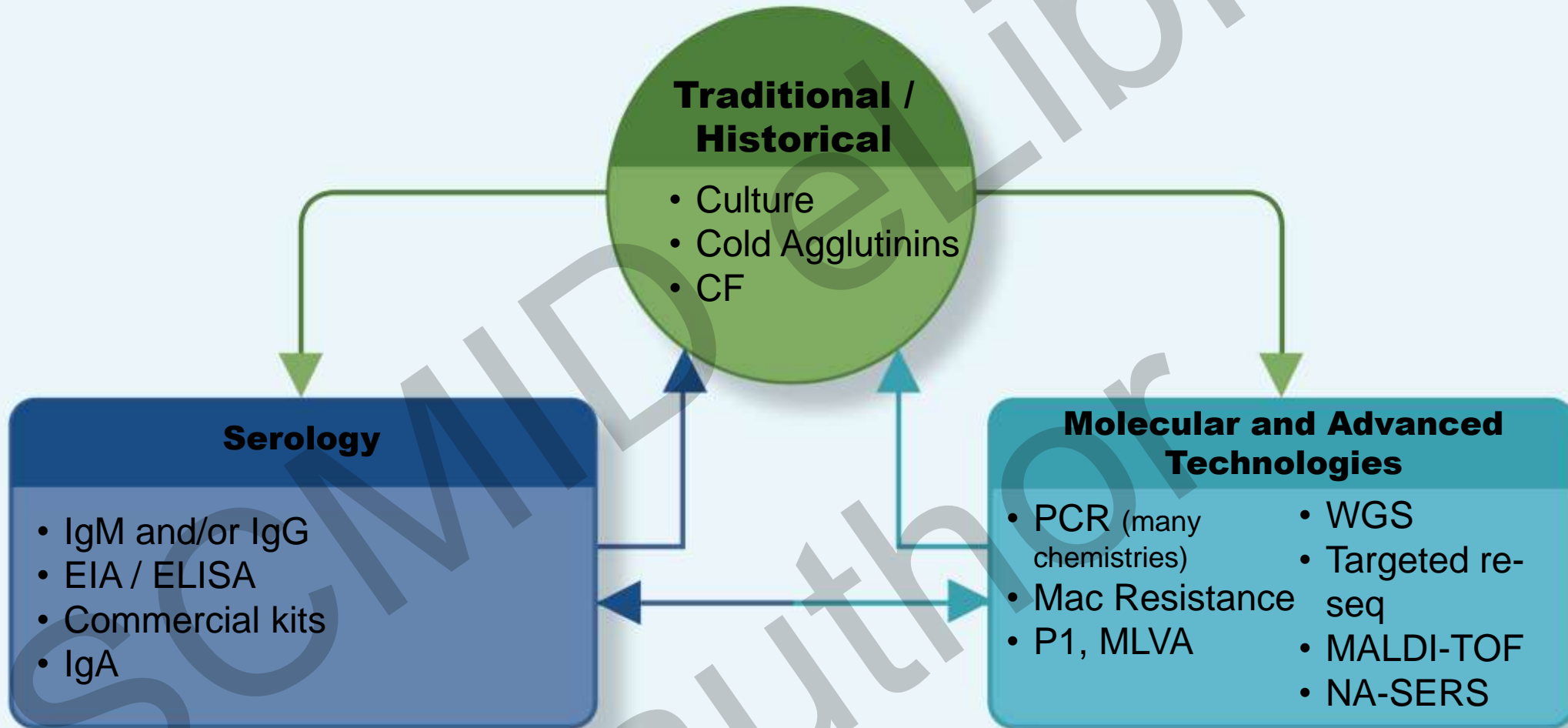
Before we begin, a quick poll:

1. How many of you regularly test for *M. pneumoniae* (Mp)?
2. How many solely use some sort of serological test for Mp diagnostics?
3. How many solely use some sort of molecular-based (e.g. PCR) tests for Mp diagnostics?
4. How many use a combination of the above?
5. How many use something other than the above?

MYCOPLASMA PNEUMONIAE DIAGNOSTICS HISTORY TIMELINE



M. pneumoniae Testing Categories



The choice of which diagnostic(s) will depend on your objectives / responsibilities

What is the situation?

1. Is time a factor? (e.g. outbreaks, PH emergency) or is time not critical (e.g. surveillance study)
2. Single case, cluster of patients, or population-based (do you need through-put)
3. Age of patient(s), stage of disease
4. Capacity and sophistication of the laboratory
 - i. Which is linked to funding...



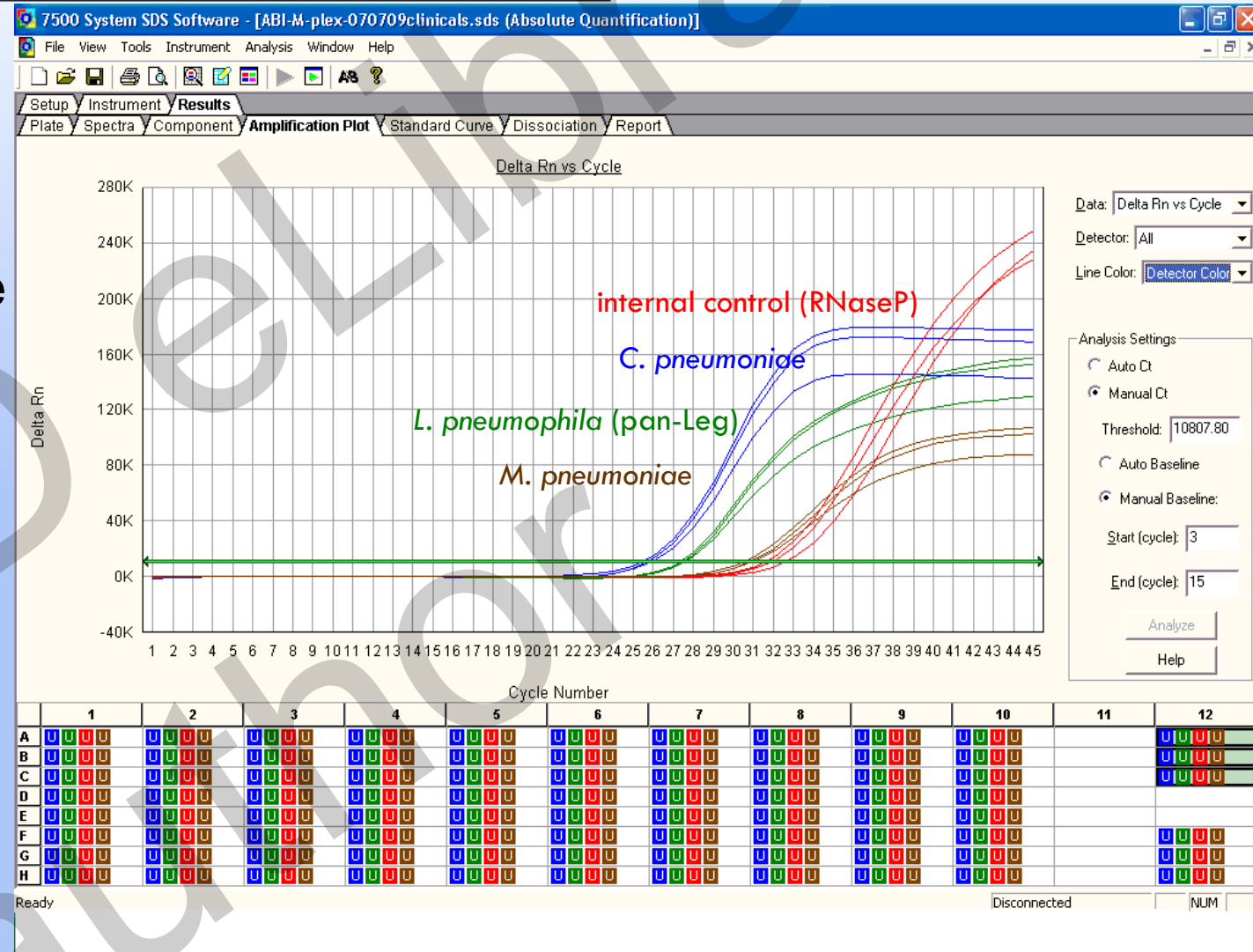
In our lab at CDC, if we *only* suspected Mp (based on previous testing data or strong Epi), we would run:

- A validated real-time PCR in triplicate
- If positive, we would immediately run a molecular test to determine the macrolide genetic profile based on SNPs
- Would subsequently type it (P1 / MLVA)
- If the Ct values are low enough (typically under 32) we would initiate culture
- May or may not do more advanced studies (WGS)



CDC Multiplex real-time PCR

- Detection of 4 targets in a single reaction
- Reduces reagent and specimen usage
- Test simultaneously for multiple targets
- Most real-time PCR instruments capable of multi-channel detection
- CLIA approved



Detection of Macrolide Resistance

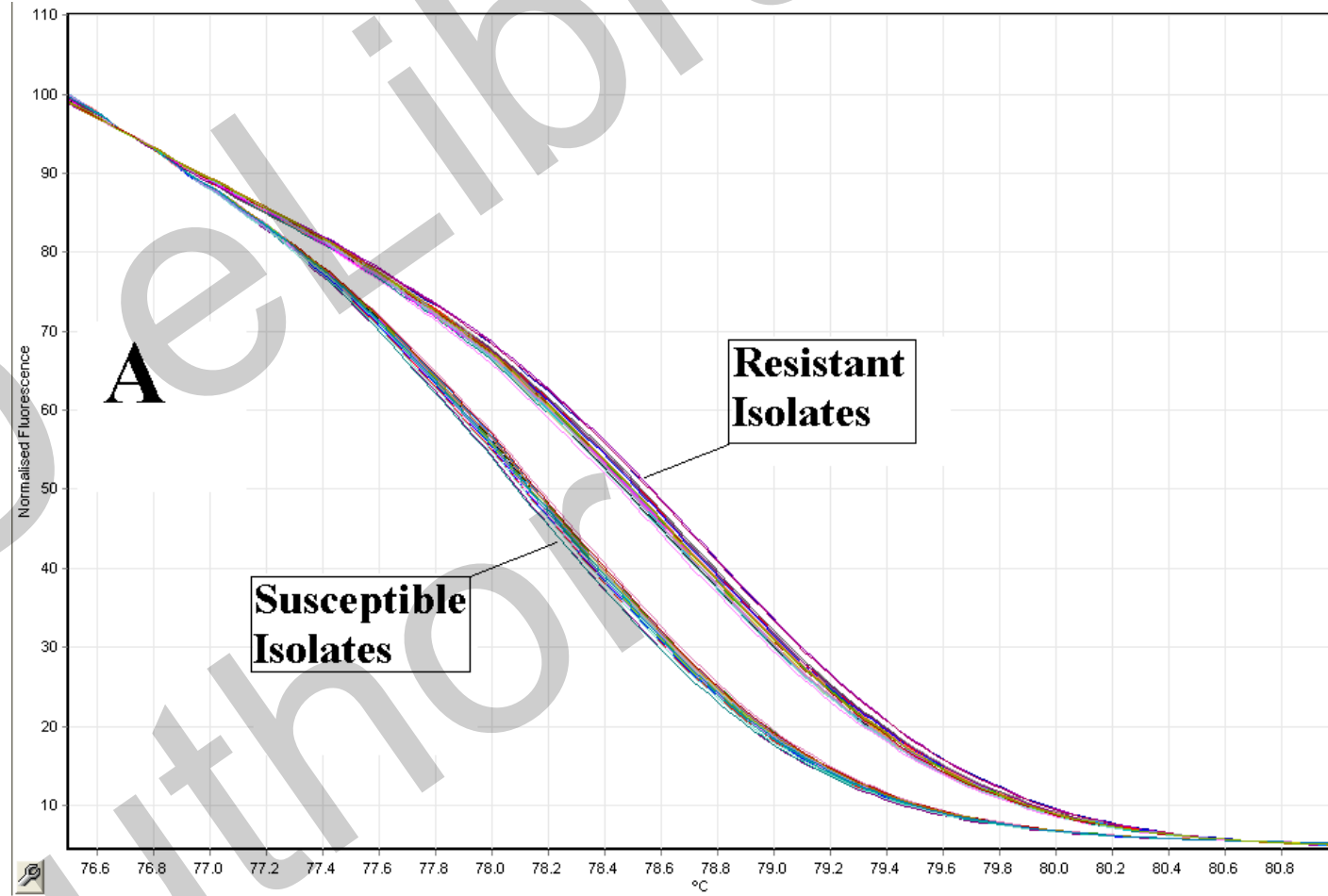
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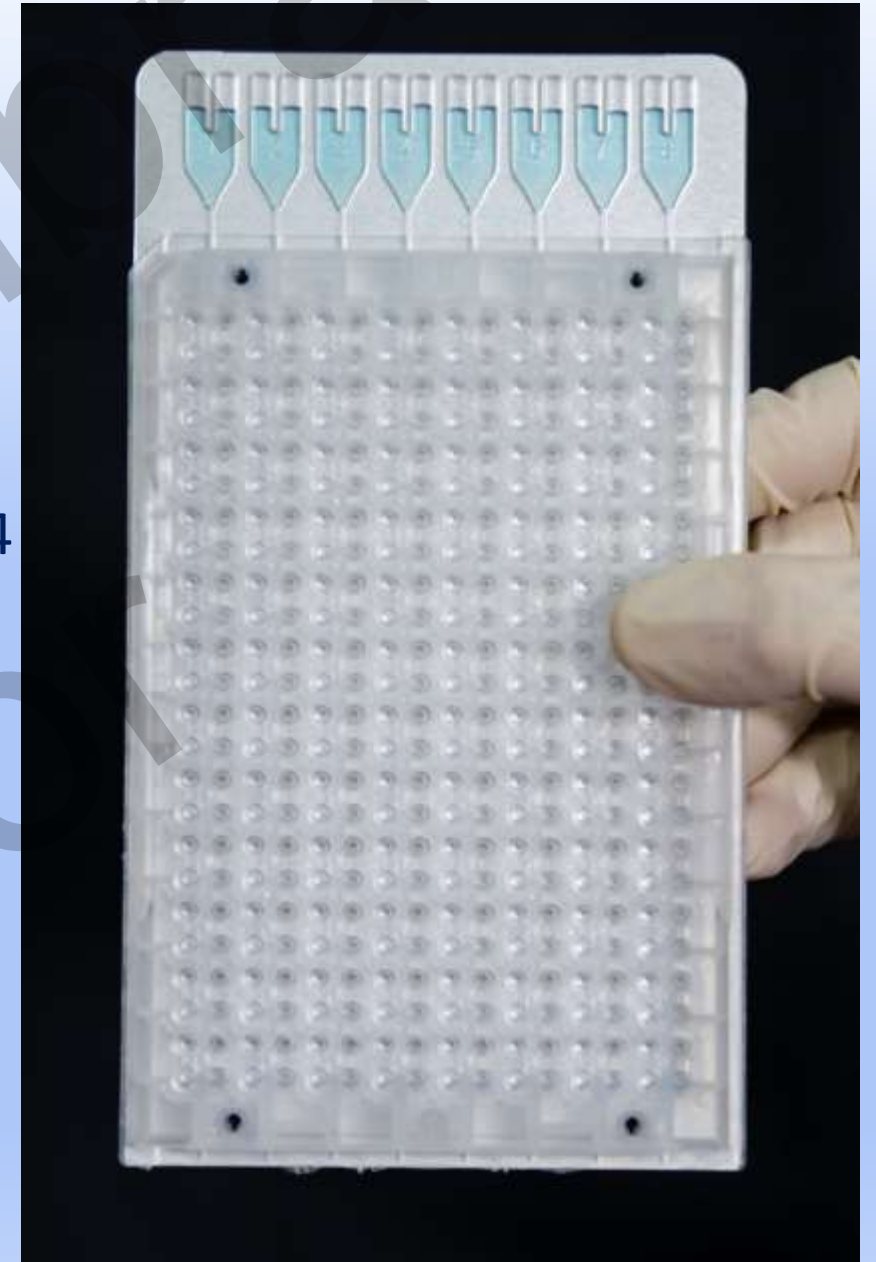
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Multi-pathogen testing

(URDOs)

TaqMan[®] Array Cards (TAC)

- Microfluidic card with 8 loading ports
- TaqMan[®] (5'-hydrolysis) real-time PCR assays
 - Primers and probes are pre-loaded and dried onto 384 1 μ L-wells
 - 48 wells available per specimen x 3 assays (multiplexing) = 144 results per specimen
- Easy-to-use (24 pipetting steps)
- Requires minimal TNA extract (20-50 μ l)
- Custom designed panels
- Many syndromes



Serology:

Not used at CDC since ~2008

Mainly two reasons why:

1. The molecular tests we have (NAATs) are so reliable, robust, & almost invariably unequivocal
2. Serology testing is fraught with shortcomings



Limitations of Serology

- Lack of standardization between procedures, studies, products, antigen variability, scoring criteria
- Cross comparison between studies can be very difficult or impossible
- Acute vs. Convalescent (timing of draws can be uncertain)
- Specimen quality / integrity
- Cross RxN (Specificity)
- Highly variable sensitivity
- Some with subjective interpretation
- Patient population – varied response potential (no response to persistent Abs)



Potential Consequences of using serology-based assays

- Over-diagnosis potential due to wide variations among serological tests
 - Many studies document healthy donors as high as ~20% IgM, 69% IgA;
 - non-Mp + patients: 42% IgG, 100% IgA → → False Positives → → misuse of Abx
- False Negative potential if reliant on early IgM results (7-10 days before response detected)
- Misled on a “contrived” outbreak when solely dependent on serology



So, are there *any* instances when serology testing would provide value?

1. If serological assays became better standardized and QC'd
2. Perhaps in settings where molecular testing is simply not possible
 - Still requires skilled interpretation and context (does the epi fit?)
3. Some suggest that serology testing can be supportive data in the context of PCR results...still questionable



Discussions Points

- So, what did I miss?
 - What is your relative confidence in molecular tests versus serological tests?
 - Are there other diagnostics that would be reasonable, practical, and timely?
 - Suggestions on best testing algorithms
 - Open: your ideas / comments / thoughts overall
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- A big thank you to ECCMID, ESGMAC, Dr. Ran Nir-Paz, Dr. Cécile Bébéar and you, the audience

