Pooled PCR Testing of SARS-COV-2: Practical Considerations

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05 November 2020

Presentation Outline

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- Consideration affecting the efficiency of pooling
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- Consideration when implementing pooled testing strategies
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- Challenges and considerations in Pooled Testing
- Resources
- Acknowledgements

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Background

- The COVID-19 cases are still rising in Africa: over 1.8 Million cases and ~44 000 deaths
- SARS-COV-2 diagnosis is mostly by RT-qPCR which is:
  - Expensive (price per test)
  - Not easily scalable when testing volumes are high (long turn around time)
- Pooling: testing several specimen in one tube is an attractive option to reduce the price per test

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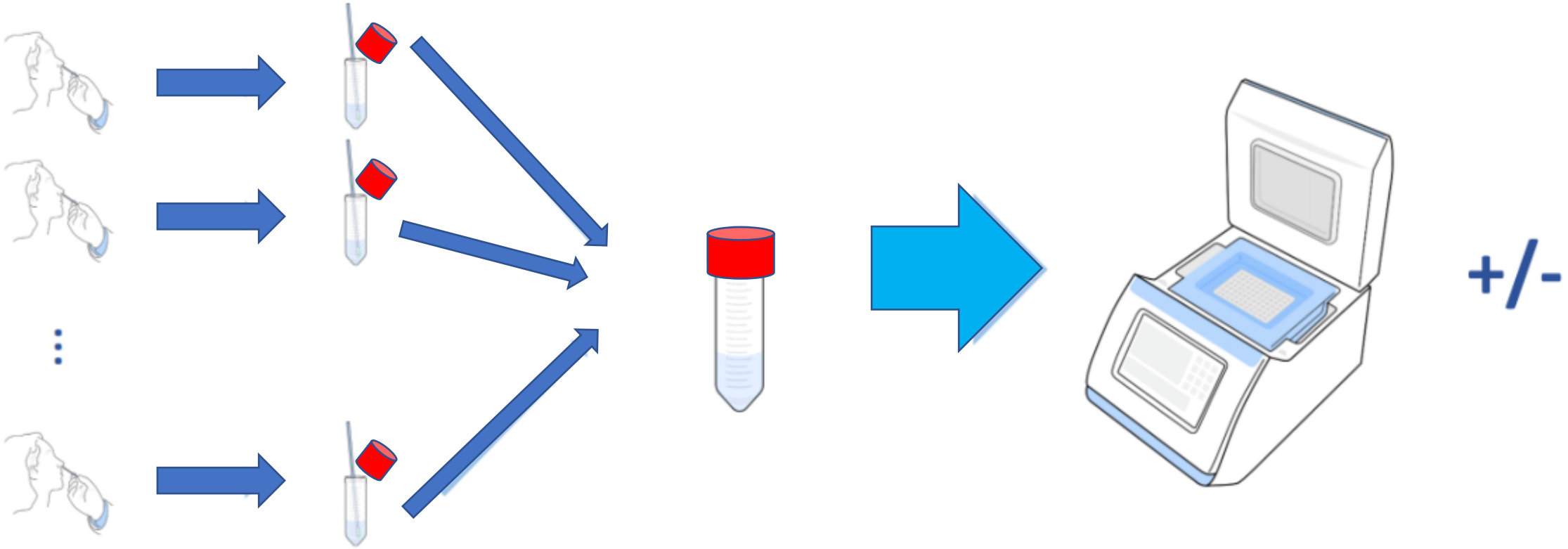
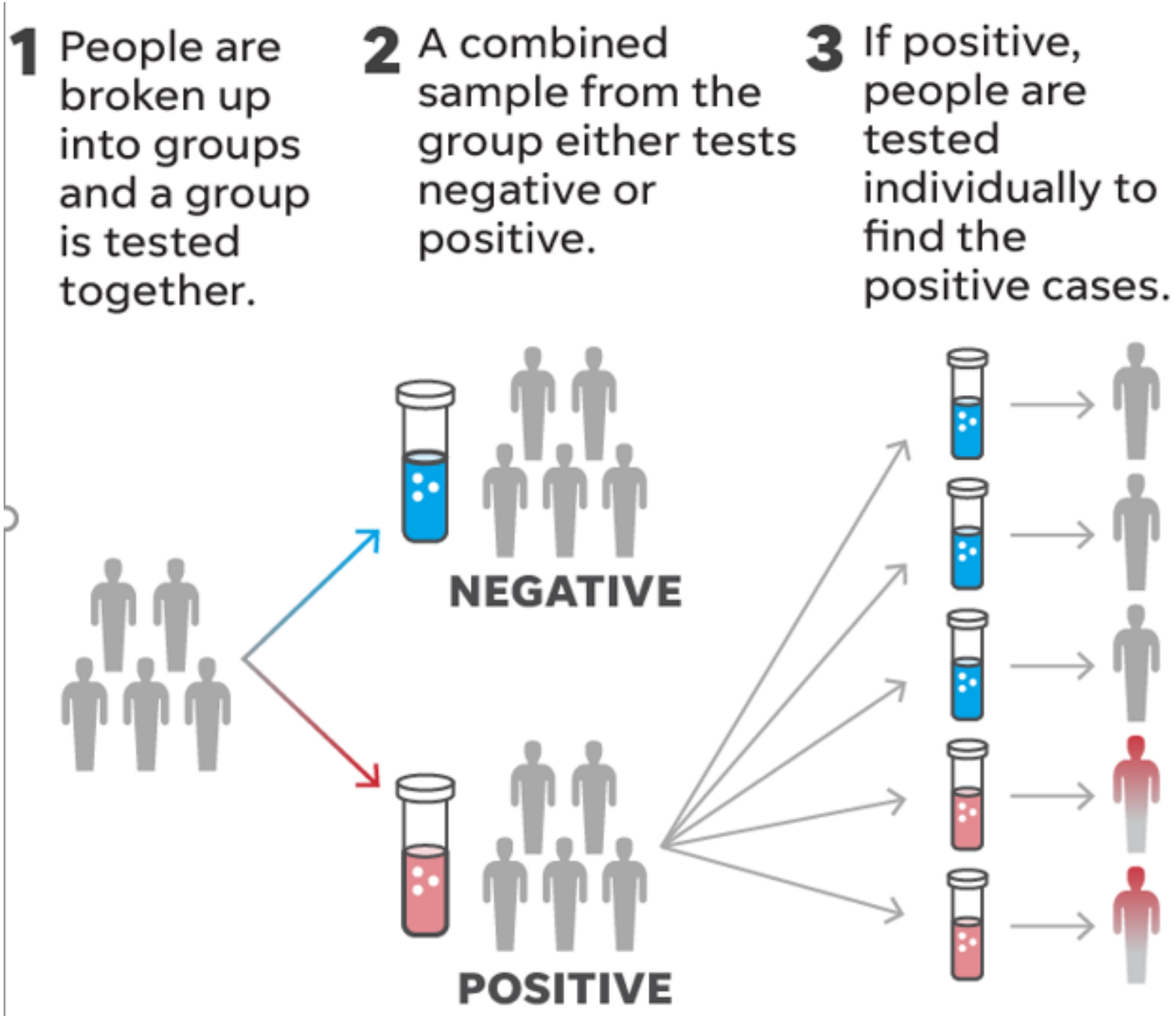
What is Pooling for COVID-19 testing?



It is combining respiratory samples from several people and conducting one laboratory test on the combined pool of samples to detect SARS-COV-2

How does pooled testing work?

Less PCR reactions are necessary to identify the positive cases



Why pooling?

To save on resources:

- less qPCR reactions
- less test kits
- faster turnaround times
- Possibility to test more people

Example 1 (*Garg et al., J Med Virol. 2020;1–6)*

- 100 samples (5% prevalence)
- 10 samples pool strategy
- test sensitivity of 95%
- **40 - 60% saving in reagents.**

Example 2

(Cherif et al., *AMA Network Open.* 2020;3(6):e2013075.)

- 94 samples (1% prevalence)
- 3 sample pools strategy
- test sensitivity of 70%
- **84% saving in reagents**

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Africa CDC Recommendations

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Africa Centres for Disease Control and Prevention (Africa CDC)

Guidance on pooled testing for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

August 2020

If SARS-CoV-2 prevalence	Sensitivity of molecular diagnostic test used	Optimal pool size can be
<1%	≥96%	10
≥1% and ≤5%	≥96%	5

- The interpretation of test results for pooled RT-PCR tests is similar to that with testing of individual samples (negative results).
- If the pool test result is **positive or indeterminate**, all samples in the pool must be **re-tested individually**.

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Considerations affecting the efficiency of pooling

- **Prevalence of SARS-COV-2 in the population**
  - The higher the prevalence, the less effective is the pooling (too many positive batches)
- **Sensitivity of the RT-PCR assay**
  - The lower the sensitivity, the higher the yield of false negative
- **Pool size**
  - Balances out the dilution effect and the reduction in test number

How many tests are required on average to test 1,000 people

Incidences	Pool Size 1	2	3	4	5	6	7	8	9	10	12	15
1%	1000	520	363	289	249	225	211	202	198	196	197	207
2%	1000	540	392	328	296	281	275	274	277	283	299	328
5%	1000	598	476	435	426	432	445	462	481	501	543	603
8%	1000	654	555	534	541	560	585	612	639	666	716	780
9%	1000	672	580	564	576	599	626	655	683	711	761	824
10%	1000	690	604	594	610	635	665	695	724	751	801	861
11%	1000	708	628	623	642	670	701	731	761	788	836	893
12%	1000	726	652	650	672	702	734	765	795	821	868	920
15%	1000	778	719	728	756	790	822	853	879	903	941	979
20%	1000	860	821	840	872	905	933	957	977	993	1015	1031
30%	1000	1010	990	1010	1032	1049	1061	1067	1071	1072	1069	1062
40%	1000	1140	1117	1120	1122	1120	1115	1108	1101	1094	1081	1066
50%	1000	1250	1208	1188	1169	1151	1135	1121	1109	1099	1083	1067

From leonardo Baso et al Universidad de Chile: https://isci.cl/wp-content/uploads/2020/06/MULTIPLYING-TESTING-CAPACITY-RT-PCR-POOL-TESTING.pdf

This is an example: **You need to consider the sensitivity of the kit being used**

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Pooled Testing Strategies

Hierarchical Algorithms: testing specimens in non-overlapping pools over a set number of stages.

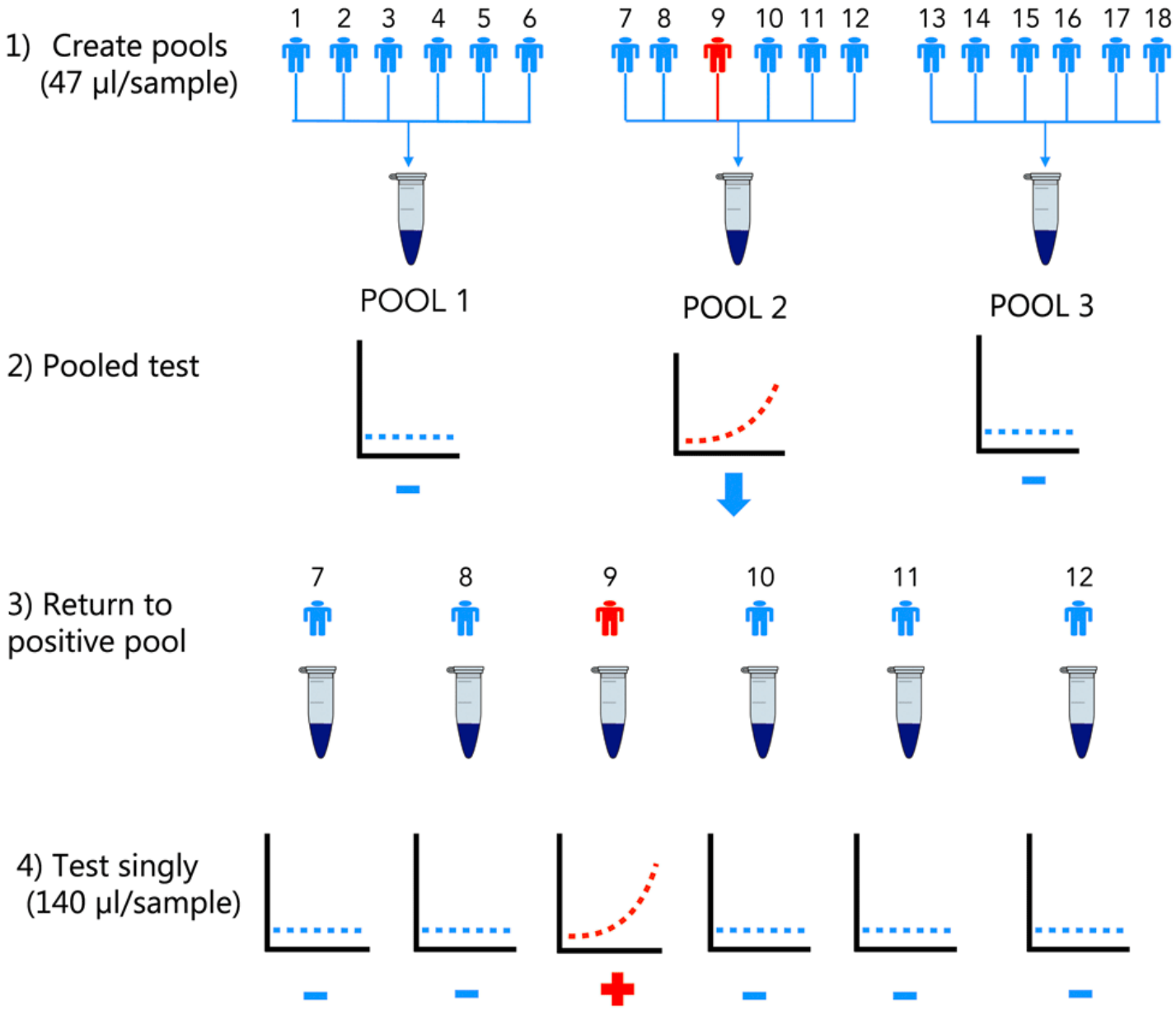
e.g. Hierarchical 2 or 3-Stages

Non-Hierarchical algorithms: testing specimens over stages, but individuals may be tested more than once per stage via overlapping pools

e.g. 10 x 10 Array or 8 x 12 Array

Hierarchical algorithms 2 stage

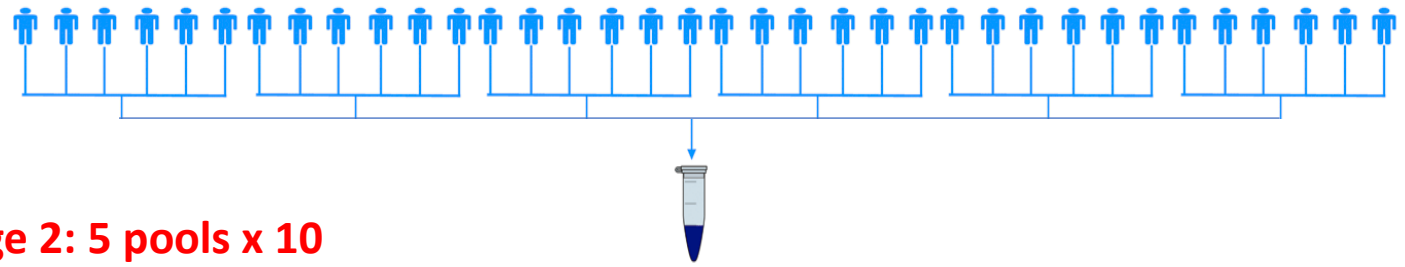
The 2-step pooling is suitable for most African laboratories because of its simplicity and avoids carryover or cross-contamination



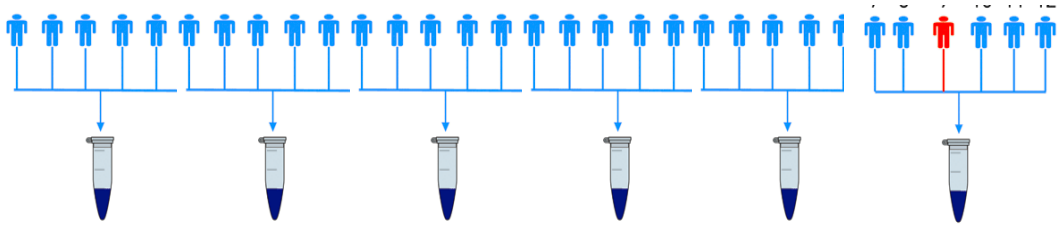
Hierarchical algorithms 3 stage

Can be efficient for large screening if the prevalence is very low

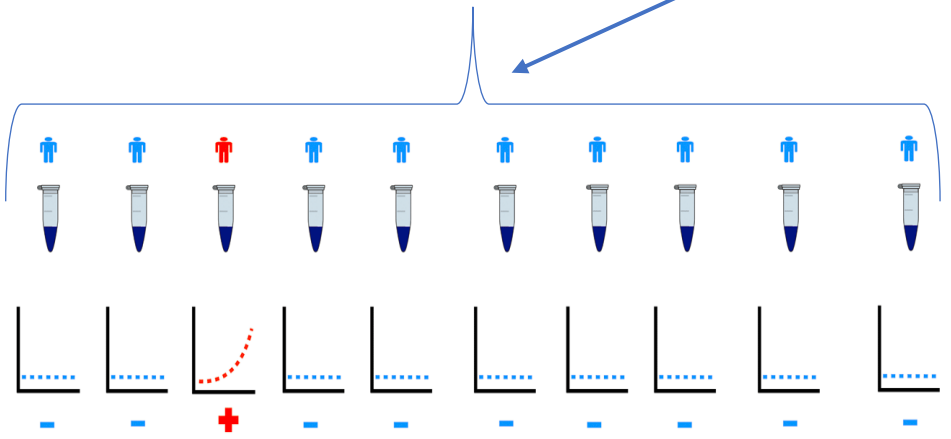
Stage 1: 1 pool x 50



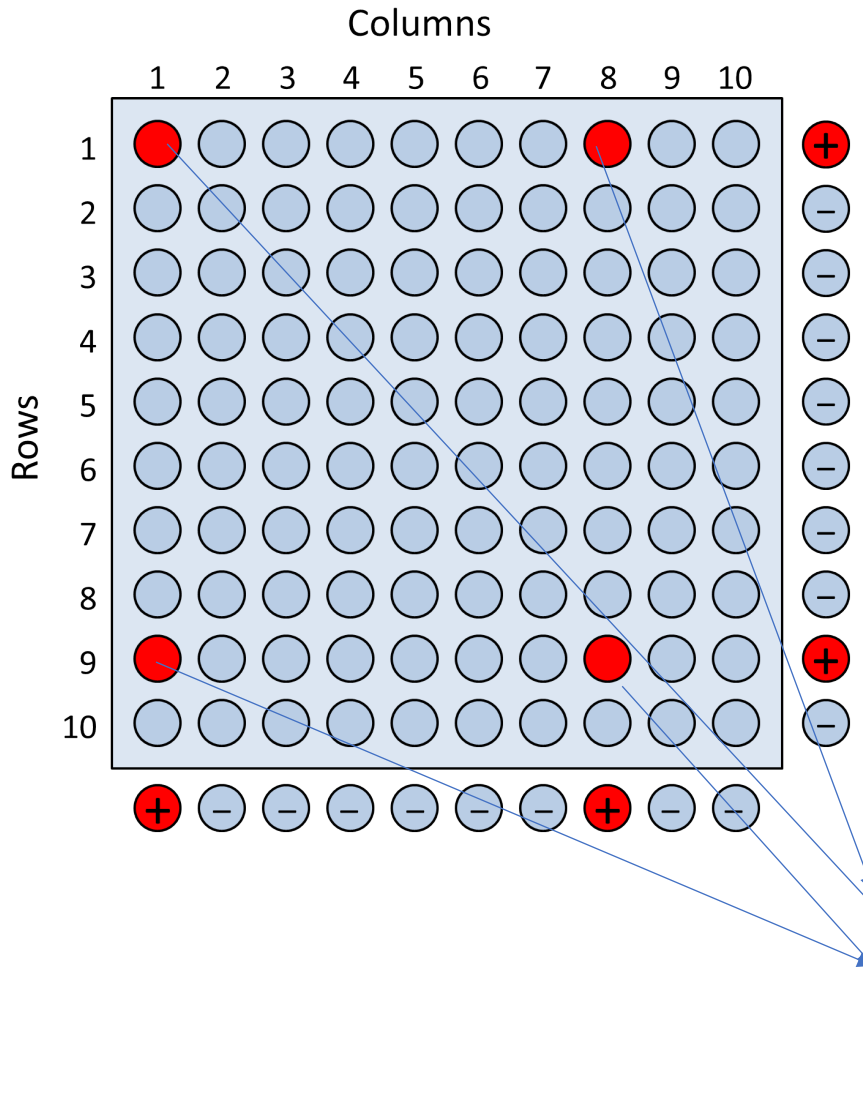
Stage 2: 5 pools x 10



Stage 3: Single testing of positive pool



Non-Hierarchical algorithms



- Testing over stages
- Individuals may be tested more than once per stage *via overlapping pools*.
- The most common of these algorithms is array testing (*row and column pools*)
- These pools are tested in the ***first stage*** of the algorithm.
- The ***second stage*** involves separately retesting those specimens that lie at intersections of positive rows and columns
- Advantage – Each sample tested in 2 pools (in stage 1) which INCREASES chances of correct classification

Considerations when implementing Pooled Testing strategies

- **Standardization of sample collections and other QA procedures**
  - Sample transport,
  - Quality of swabs,
  - Transport media,
  - Efficiency of extraction

- **Test performance and validation**
  - Of all test that are to be used for pooled testing before implementation
  - Examine the Dilution effects and increased Ct values in pools (False Negatives)
  - test performance (pooled) should **yield ≥85% percent positive agreement** (PPA) as compared to tests (individual testing (FDA).

A Shiny App for Pooled Testing – Optimal Testing Configuration

https://www.chrisbilder.com/shiny/

A Shiny App for Pooled Testing

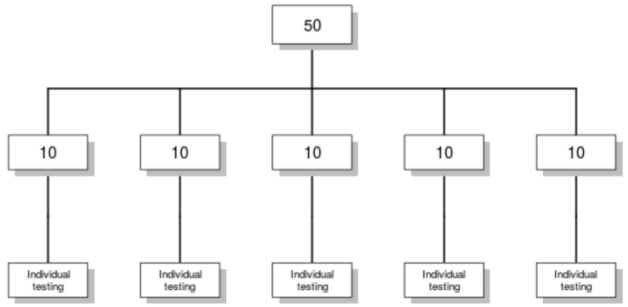
Pooled testing is the process of testing amalgamations of specimens in a "pool" (or "group") rather than testing specimens separately. This process is used in a wide variety of applications and is an indispensable tool for laboratories when testing high volumes of clinical specimens for infectious diseases. Choosing pool sizes is an important decision that needs to be made prior to any implementation of pooled testing. The purpose of our app is to help laboratories make this decision. We provide tools to calculate the expected number of tests and to choose the "best" set of pool sizes, known as the optimal testing configuration.

This Shiny application allows the user to choose either hierarchical or array testing algorithms for pooled testing. For more information on these algorithms, click here. Operating characteristics such as the expected number of tests and accuracy measures are calculated and the optimal testing configuration can be found given the overall probability of disease, the sensitivity and specificity of the assay, and other specifications.

Use the tabs to the left or the hyperlinks below to get started.

Hierarchical testing	Array testing
Calculate for one testing configuration	Calculate for one testing configuration
Find the optimal testing configuration	Find the optimal testing configuration

Operating Characteristics | Algorithm Diagram | Results



Hierarchical testing

Calculate the operating characteristics for a given configuration

Specifications

How many diseases for the assay?
(•) 1 ( ) 2

What is the overall disease prevalence?
0.03

What is the sensitivity of the assay?
Note: Sensitivity is treated as equal for each stage; if unequal, provide values separated by commas and ordered by stage.
0.95

What is the specificity of the assay?
Note: Specificity is treated as equal for each stage; if unequal, provide values separated by commas and ordered by stage.
0.99

How many stages for the pooling algorithm?
( ) 2 (•) 3

What is the initial pool size?
Note: The minimum size allowed is 3.
50

What are the pool sizes for the second stage of testing?
Note: Pool sizes should be specified as a list of numbers, separated by commas, and should sum to the initial pool size.
10,10,10,10,10

Calculate | Example

Operating Characteristics | Algorithm Diagram | Results

16.82
Expected number of tests

0.34
Expected number of tests per individual

The configuration has an initial pool size of 50 and subsequent pool sizes of 10, 10, 10, 10, and 10.

The expected number of tests for the configuration is 16.82. This leads to an expected number of tests per individual of 16.82 / 50 = 0.34. Thus, three-stage hierarchical testing reduces the expected number of tests by 66% when compared to individual testing.

For the *overall* implementation of the algorithm, the sensitivity is 0.8574, the specificity is 0.9978, the positive predictive value is 0.9229, and the negative predictive value is 0.9956.

The inputs used for these calculations were

- Diseases: 1
- Overall disease probability: 0.03
- Sensitivity: 0.95 for stage 1, 0.95 for stage 2, 0.95 for stage 3
- Specificity: 0.99 for stage 1, 0.99 for stage 2, 0.99 for stage 3
- Stages: 3
- Initial pool size: 50
- Second stage pool sizes: 10,10,10,10,10

Resources

- **A Shiny App for Pooled Testing – Optimal Testing Configuration**

  https://www.chrisbilder.com/shiny/

- Online tools: http://www.bios.unc.edu/~mhudgens/SARS-CoV-2.pooling.2.htm

- Africa Centres for Disease Control and Prevention (Africa CDC) Guidance on pooled testing for severe acute respiratory syndrome coronavirus 2

  https://africacdc.org/download/guidance-on-pooled-testing-for-severe-acute-respiratory-syndrome-coronavirus-2-sars-cov-2/

- US Centres for Disease Control and Prevention (Africa CDC) - Interim Guidance for Use of Pooling Procedures in SARS-CoV-2 Diagnostic, Screening, and Surveillance Testing

  https://www.cdc.gov/coronavirus/2019-ncov/lab/pooling-procedures.html

Acknowledgements

ASLM team

- Pascale Ondoa
- Marguerite Massinga Loembe
- Luc-Christian Ngwom
- Blessing Marondera
- Anafi Mataka
- Samba Diallo
- Collins Otieno

Africa CDC

- Abebaw Kebede

RESOLVE project Subject matter experts

- Maria Bonitz
- Joel Fleury DJOBA SIAWAYA
- Joseph FOKAM
- Almoustapha Maiga

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