

# REVIEW OF DAY 1

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Victorian Infectious Disease Laboratory (VIDRL)

# Schedule Day 1

MONDAY	ACTIVITY	PRESENTER
8:45 – 9:00	Registration	Lisa
9:00 – 9:15	Overview of the Doherty Institute/CPG/VIDRL/MDU	Lisa
9:15 – 9:45	Welcome and Introductions	
9:45 – 10:00	Training Overview	Jean
10:00 – 10:30	<b>LAB:</b> Pipetting exercise	Louise
10:30 – 11:00	Morning tea	
11:00 – 11:30	<b>LECTURE:</b> Introduction to Mpox and MPXV genomics at VIDRL	Jean
11:30 – 12:30	<b>LECTURE:</b> Tiled amplicon for Mpox	Jean
12:30 – 13:30	Lunch	
13:30 – 15:30	<b>LAB:</b> Tiled amplicon PCR	Louise
15:30 – 16:00	Afternoon tea	
16:00 – 16:30	<b>LECTURE:</b> Introduction to ONT sequencing viruses	Louise
16:30 – 17:00	Group discussion: Opportunity for Q&A and further discussion	Nicole

# Mpox surveillance workflow.

The core stages of this workflow are:

- a) Specimen collection  - Mpox panel.
- b) Sample preparation  - Mpox PCR - AMPLICON GENERATION
- c) Genome sequencing – DAY 2
- d) Processing of sequencing results – DAY 3 + 4
- e) Sequence data interpretation and data sharing.- DAY 5

# Wet Lab Training Goals

## DAY 1

### Sample preparation

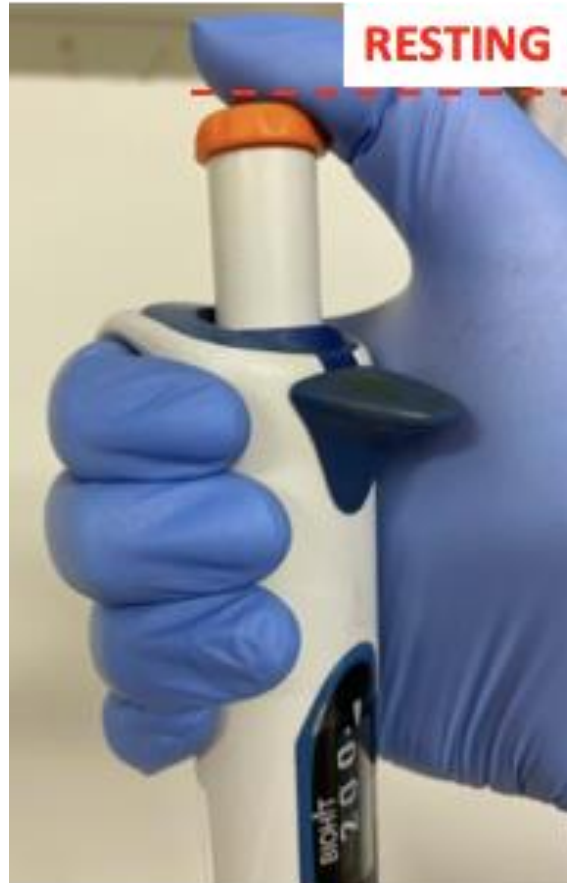
- ✓ Pipetting skills refresher
- ✓ MPXV Panel (7 samples)
- ✓ Amplicon PCR Scheme: Artic-inrb-mpox/2500/V1.0.1  
2500 bp with Pool 1 and 2

## DAY 2

### Genome sequencing

- Quantify amplicons
- Create a ONT library using Rapid Barcoding
- Quantify library
- Load and run libraries on a MinION R10.4.1 for <24 hours

# Pipette plunger positions



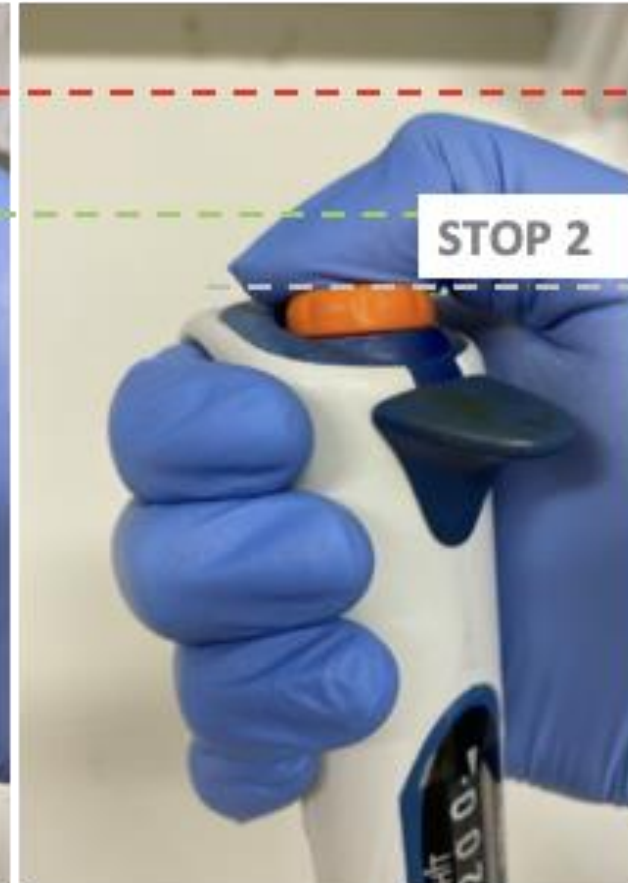
## Rest

No pressure on the plunger



## Desired measurement Stop 1

Feel for a light  
stopping pressure



## Full discharge Stop 2

Press down until liquid  
is discharged

# Accurate pipetting examples



**bubbles in liquid**

Plunger lifted too quickly,  
inconsistent liquid uptake.



**incorrect volume**

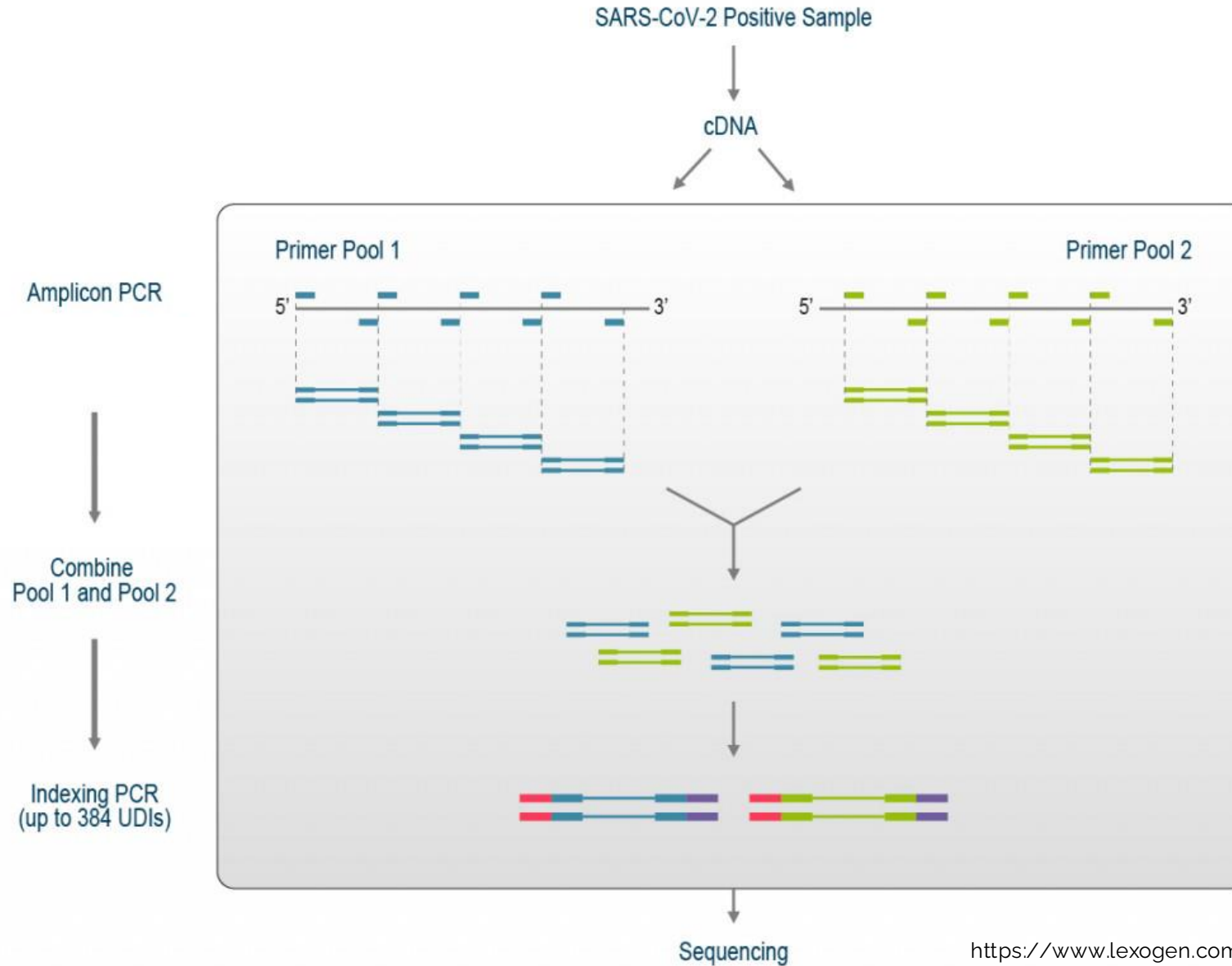
Pipette lifted out of tube  
before full amount of liquid  
has been measured



**correct volume**

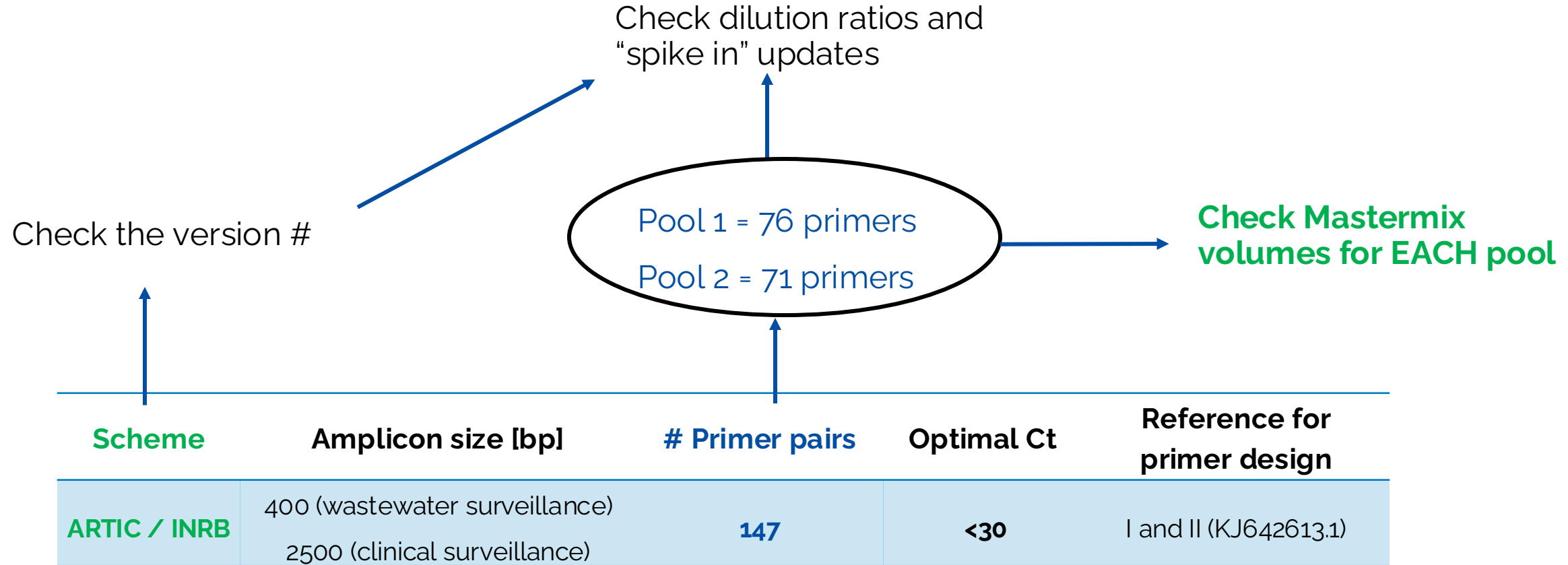
No bubbles

# Tiled Amplicon and Pools





# Amplicon Schemes Updates

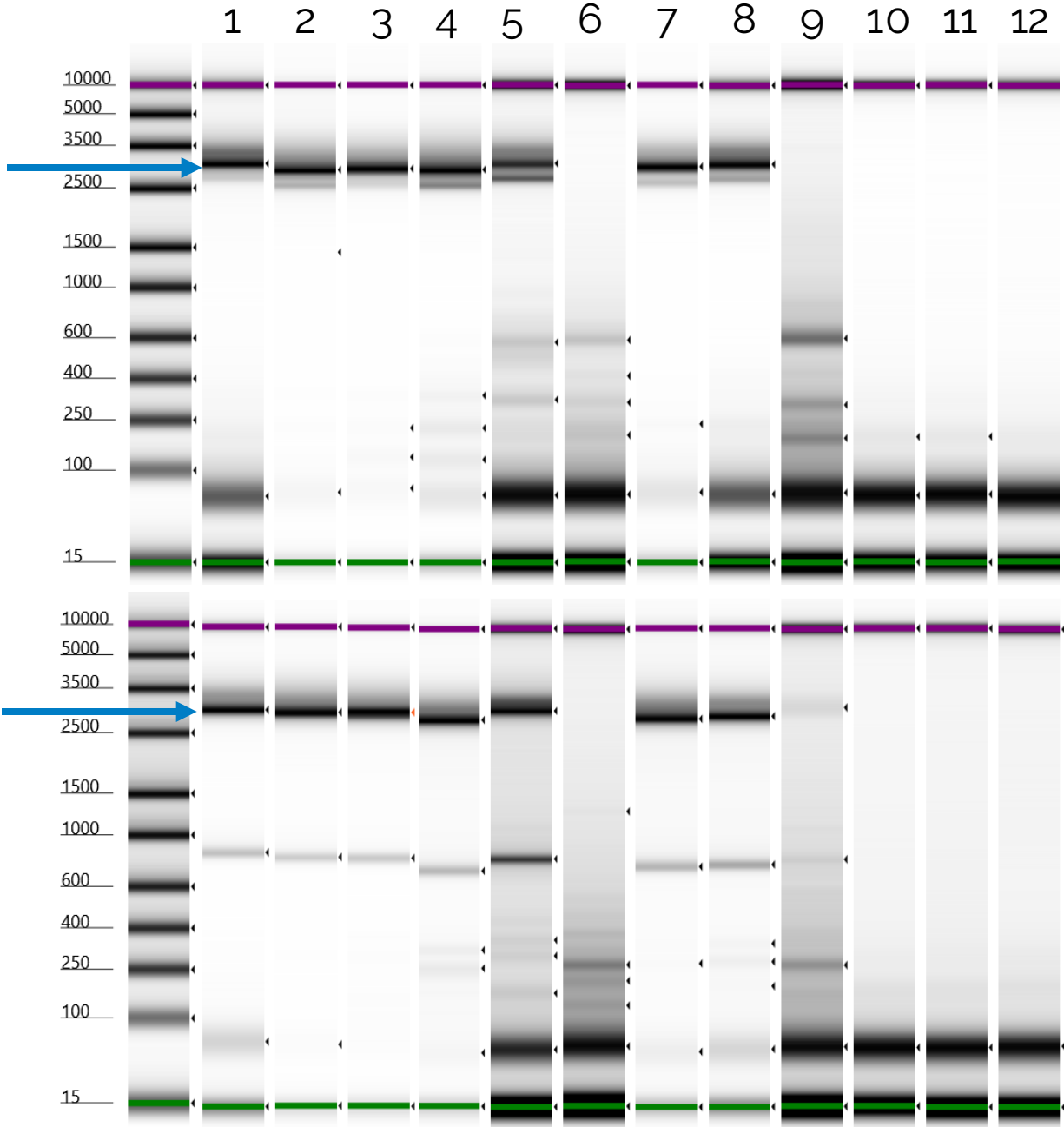




# Amplicon Concentration

POOL 1

POOL 2



## QuBit Assay

Sample	Mpox Ct	POOL (ng/ul)	
		Pool 1	Pool 2
1	25.6	22.5	34.6
2	21.9	73.4	57.7
3	19	82.8	76.2
4	21	42.9	61.2
5	29.1	14.5	13.8
6	Not Det	13.8	10
7	24	42.2	49
8	26	25.3	31.2
9	35.3	13.8	14.3
10	Not Det	6.98	6.21
11	Not Det	9.08	50.6
12	Not Det	7.48	6.47

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