WET LAB DAY 1: TRAINING OVERVIEW

Jean Moselen
Senior Medical Scientist
Victorian Infectious Disease Laboratory (VIDRL)

CENTRE FOR PATHOGEN GENOMICS

World Health Organization
Western Pacific Region

IPSN International Pathogen Surveillance Network
WHO hosted network

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	**LAB:** Pipetting exercise	Louise
10.30 – 11.00	Morning tea	
11.00 – 11.30	**LECTURE:** Introduction to Mpox and MPXV genomics at VIDRL	Jean
11.30 – 12.30	**LECTURE:** Tiled amplicon for Mpox	Jean
12.30 – 13.30	Lunch	
13.30 – 15.30	**LAB:** Tiled amplicon PCR	Louise
15.30 – 16:00	Afternoon tea	
16:00 – 16.30	**LECTURE:** Introduction to ONT sequencing viruses	Louise
16.30 – 17:00	Group discussion: Opportunity for Q&A and further discussion	Nicole

Schedule DAY 2

TUESDAY	ACTIVITY	PRESENTER
9.00 – 9.10	Review of Day 1	Jean
9.10 – 10.00	**Lab:** Amplicon QC	Louise
10.00 – 10.30	Morning tea	
10.30 – 12.30	**Lab:** Library Preparation & Loading	Jean
12.30 – 13.30	Lunch	
13.30 – 14.15	**Lab:** Flow cell loading practice and Run Reviewing	Louise
14.15 – 14.30	**Lecture:** Review of laboratory workflow	Jean
14.45 – 15.30	**Lecture:** Genomics pathogen topic to be chosen from participants. Questions from the day before	Jean + Louise
15.30 – 16.00	Afternoon Tea	
16.00 – 16.30	**Lecture:** MPXV Illumina Sequencing Options	Jean
16.30 – 17.00	Group discussion: Opportunity for Q&A and further discussion	Nicole

Trainers

A joint venture between The University of Melbourne and The Royal Melbourne Hospital

Jean

Louise

Nicole

Dave

Jess

Taylor

Day 1 and 2 wet lab training will be in the Doherty Teaching Lab

Training Groups

Jean - A

Louise - B

Nicole - C

Dave - D

Jess - E

Taylor - F

GROUPS OF 4-5 PEOPLE

TRAINER	TEAM		COUNTRY
Jean	A	Dr Janin Nouhin	Cambodia
		Mrs Ushananthiny Ravi	Malaysia
		Mr Francis Lelngei	PNG
		Ms Minh Hang Duong	Viet Nam
Louise	B	Ms Om Sovandar	Cambodia
		Dr Vilayouth Phimolsannousith	Lao PDR
		Dr Naranzul Tsedenba	Mongolia
		Mr Paul Raymund J. Yap	Philippines
		Le Thanh Dat	Viet Nam
Nicole	C	Mrs Phan Phally	Cambodia
		Mr Sinakhone Xayadeth	Lao PDR
		Ms Sarnai Yadam-Erdene	Mongolia
		Mr Neil Tristan M. Yabut	Philippines
		Narcisse Joseph	Malaysia
Dave	D	Ms Farheen Hussein	Fiji
		Dr Khayri Azizi Kamel	Malaysia
		Ms Rebecca Narokobi	PNG
		Dr Văn Đình Tráng	Viet Nam
Jessica	E	Ms Shanon Prasad	Fiji
		Dr Muhammad Hasyim Chew Abdullah	Malaysia
		Dr Barne Willie	PNG
		Dr Le Thi Thanh	Viet Nam
Taylor	F	Mr Sreng Panha	Cambodia
		Mr Chittaphone Vanhnollat	Lao PDR
		Dr Tsogjargal Burentogtokh	Mongolia
		Mr John Leonard R. Chan	Philippines

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Viral detection & sequencing methods

Real time PCR

Basics of using PCR as a **detection method** are very similar for different viruses

Sanger sequencing

Sequencing only the **gene/s of interest** for identification of pathogen & it's strain/lineage

Whole-genome sequencing (WGS)

WGS is a comprehensive method for **analysing entire genomes**.

Next Generation Sequencing (NGS)

Technology for sequencing any human, animal, plants, or pathogen microbes.

MPXV genomic surveillance workflow.

The core stages of this workflow are:

a) Specimen collection
b) Sample preparation
c) Genome sequencing
d) Processing of sequencing results
e) Sequence data interpretation and data sharing.

Mpox surveillance workflow.

The core stages of this workflow are:

a) **Specimen collection ✅ - Mpox panel for trainees.**
b) Sample preparation – DAY 1
c) Genome sequencing – DAY 2
d) Processing of sequencing results – DAY 3 + 4
e) Sequence data interpretation and data sharing.- DAY 5

Mpox Panel for trainees

A joint venture between The University of Melbourne and The Royal Melbourne Hospital

a) Specimen collection ✅ - MPXV Panel for trainees.

PANEL OF CLINICAL SWABS RECEIVED AT VIDRL FOR MPXV TESTING

DNA EXTRACTED FROM SWAB

REAL TIME PCR SCREEN

Extracting Mpox DNA

Kits we have tested

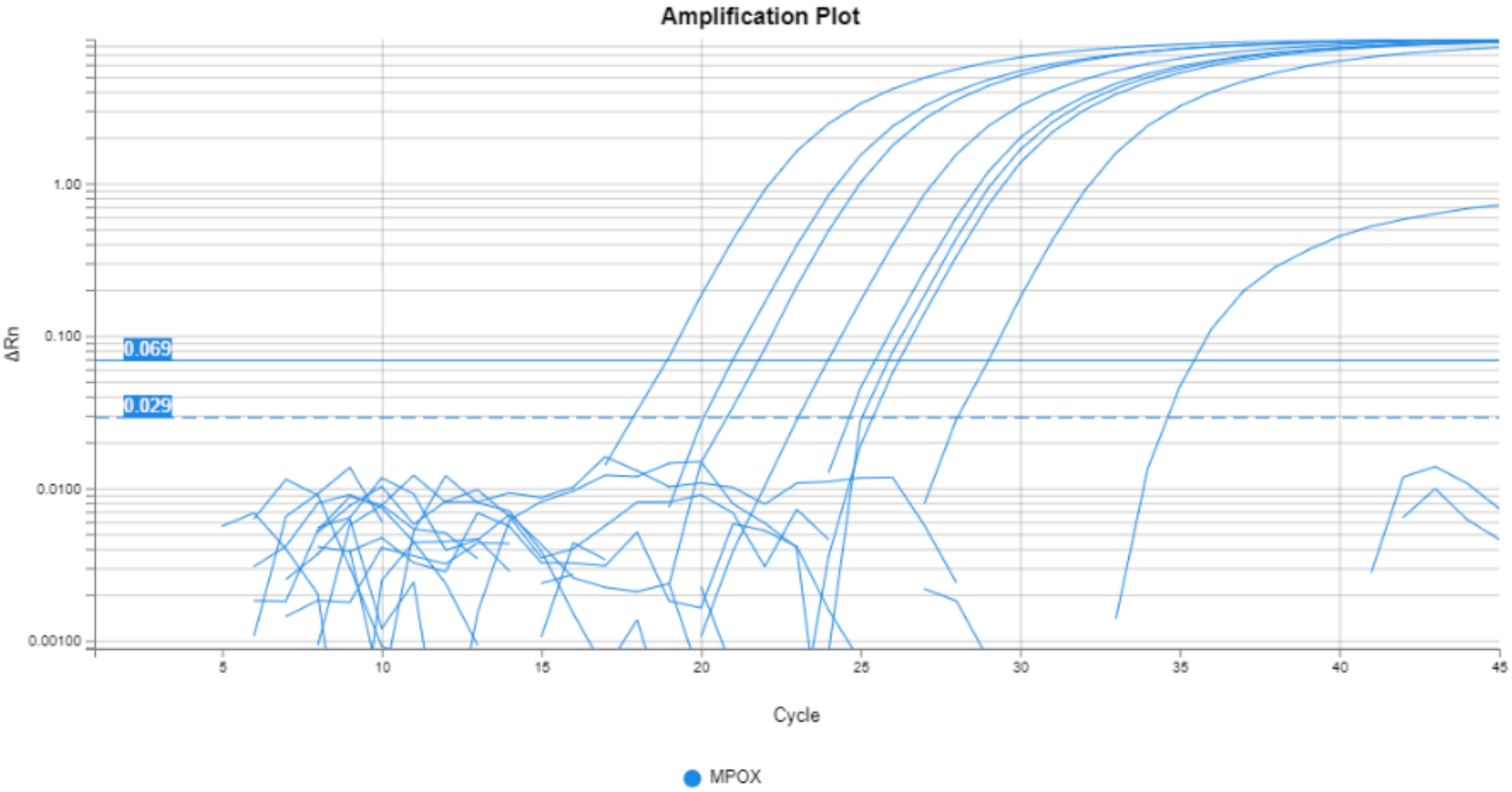
Automated:

- Qiagen EZ2
- **MagMAX Viral/Pathogen Nucleic Acid Isolation Kit (MVP II)**
- QIAamp DNA QIAcube Kit
- TANbead OptiPure Viral Kit

Manual:

- Qiagen QIAamp MinElute Virus Spin Kit

Mpox panel for trainees



Sample	Real Time
1	25.6
2	21.9
3	Not Det
4	21
5	29.1
6	19
7	Not Det

IN-HOUSE VIDRL MPOX REAL TIME– HAEMAGGLUTININ TARGET

Wet Lab Training Goals

CENTRE FOR PATHOGEN GENOMICS
Doherty Institute
THE UNIVERSITY OF MELBOURNE
The Royal Melbourne Hospital
A joint venture between The University of Melbourne and The Royal Melbourne Hospital

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

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