How to Choose the Right Equipment/Platforms for your Laboratory

(A Public Hospital Laboratory Perspective)

Michelle J. Francis
Overview

• Public Hospital Pressures / Challenges
• Commercial vs In-house assays
• Platform considerations - “Can’t Afford That”
• Laboratory space requirements - “No Room”
• Batching considerations - “Today Please”
• Assay cost - “Too Expensive”
• Assay Verification
• Assay Validation
Public Hospital Pressures

• Monash Health covers 2312 km²

• Comprises of:
  • 6 Public Hospitals : >1700 beds
  • 1 Private Hospital
  • 5 Aged Residential Care Facilities : 300 beds
Public Hospital Challenges

- 3 Emergency Departments
- 3 ICU’s
- 2 NICU’s

- Clinicians require a wide range of assays be run
- Demand for frequent batches
  - Smaller batches
  - Random access assays

“Need the result, now”
Monash Pathology

• Microbiology Laboratory at Central Hospital only
  • Runs 24/7

• Molecular Microbiology
  • Mon – Fri
  • 8.00am – 16.30pm
Most frequently requested NAT results

• Influenza A / B result

• *M. tuberculosis* complex result

• CSFs : Enterovirus / HSV result
New Molecular Assay

• What do you want to detect?
  • Bacteria / Virus / Parasite / Fungi
  • RNA
  • DNA
TYPES OF MOLECULAR ASSAYS
Commercial or In-house

• The single biggest decision to make

• Affects the equipment you will use

• Many commercial systems are “locked”
  • Cannot be programmed for an in-house assay
Commercial Assays

• Validated
• TGA approved
• Can search literature for meaningful evaluations
• Require **laboratory verification**
• Assessed during NATA accreditation
• More expensive option

[Roche AmpliPrep / TaqMan system]
In-House Assays

- Choose assay designs from trustworthy literature
- Design your own assay
  - Primer 3
  - Primer-BLAST
  - Primer Express
- **Must validate** to NPAAC requirements
- **Must** register with TGA
  - Assay / QC material
- Assessed during NATA accreditation
Class 4 IVDs – Don’t Go There!!

An IVD medical device intended to be used for any of the following purposes is classified as a Class 4 IVD medical device or a Class 4 in-house IVD:

“To detect the presence of, or exposure to, a transmissible agent that causes a serious disease with a high risk of propagation in Australia.”

“All assays for the clinical diagnosis of infection by HIV 1&2, Hepatitis C virus, Hepatitis B virus and HTLV I/II are Class 4 IVDs.”

TGA Website:  www.tga.gov.au/ivd-classification.html
Class 4 IVD – what’s involved

• More difficult to register Class 4 IVDs
  • Includes a TGA Conformity Assessment

• Laboratories manufacturing Class 4 in-house IVDs must: fulfil the same regulatory requirements as other commercially supplied Class 4 IVDs

• Pay the fees applicable to commercial manufacturers

• Class 4 in-house IVDs are treated as commercial IVDs
COST CONSIDERATIONS
Platforms

• Buy equipment outright?

• Reagent rental?
  • Flexibility

• Commercial assays
  • Not such a bad idea to reagent rent because of the speed of which assays / platforms are evolving
  • What other assays may be potentially run?
Associated Equipment Costs

- Service Contract
- LIS Interface
- Printers
- UPS
Cost of the Assay

• Need to calculate “Cost per reportable test”

• Includes costs of:
  • Equipment
  • Reagents
  • Consumables
  • Controls
  • Quality Assurance

With this much money, the only experiment we can do is “flip a coin”
SPACE REQUIREMENTS
How many separate Molecular “Areas” does the assay need:

- Some platforms “are all in one”
- Some require a separate extraction / amplification area
- Some require more (many in-house assays)
  - Mastermix Area
  - Post-Amplification Area
Space - Size

- What equipment will fit into the available laboratory space?

Biomerieux EasyMag

Qiagen Symphony
ASSAY
CONSIDERATIONS
Assay Targets

• Research common target choices for chosen assay
  • Read literature
  • Compare two assays

• Choose high copy number targets for sensitivity
• Confirm with more specific targets if necessary
  • N.gonorrhoea / B.pertussis

• Add appropriate comment if target is not specific
Sample Batching

- Number of tests per year

- What is the requirement for the turn-around-time?

- Expect to run assay:
  - daily / weekly / on demand

= How large do my batch sizes need to be??
= What are my platform options
Assay Complexity

• Platforms with assays requiring very little technical skill:
  • Cepheid GeneXpert
  • Small labs
  • Place in Bacteriology Laboratory

• Complex assays requiring accurate pipetting:
  • BD ProbeTec
  • All in-house assays
  • Place in Molecular Laboratory
Staff

Suitable for Molecular Microbiology

Not suitable for Molecular Microbiology
Extraction Options

• **Organic Extraction**
  • Rapid denaturation nucleases & RNA stabilisation
  • Laborious
  • Organic waste produced

• **Filter Based Spin Column Formats**
  • Convenient & easy
  • Can automate
  • Filter clogging
Extraction

• **Magnetic Particle Technology**
  - Excellent efficiency of target capture
  - Magnetic format = rapid collection/concentration sample
  - Can automate
  - Magnetic particles can be carried though to eluate

• **Direct Lysis**
  - Extremely easy & fast
  - Potential for residual RNAse activity if lysates not handled properly
ASSAY EVALUATION
Assay Evaluation: Verification

- Independent Laboratory Verification for Commercial assays:

  “Confirm that the performance claims of the assay have been met”

- Laboratory must show it can obtain the performance specifications
Assay Verification

- **Accuracy** – ability to detect the correct result
- **Precision** – reproducibility
- **Analytical Sensitivity / Specificity**
- **Linearity** – if a quantitative assay
- **Limit of Detection**
Verification Report

- Summarise performance

- Did it meet your acceptance criteria?
  - As defined in your verification protocol

- Is it fit for use in your laboratory?

- Attach raw data

- Sign off
Staff - Pipette Tip Removal

THERE ARE 2 KINDS OF PEOPLE IN THE WORLD
Make that THREE..................
Assay Evaluation: Validation

• Requirement for In-house assays:

“Validation shall be as extensive as is necessary to confirm that the specific requirements for the intended use of the assay have been met”

• Need to establish performance characteristics of your in-house assay
Particular requirements

• Accuracy & Precision of in-house assay:
  • Certified reference material
  • Comparison with reference method
  • Use of validated in-house reference material
  • Performance in external proficiency testing
  • Laboratory sample exchange
Assay Validation

- Analysis of Primers +/- Probes
- Result Acceptance Criteria

**ESTABLISH**

- Accuracy & Precision
- Analytical Sensitivity & Specificity
- PPV / NPV
- Limit of Detection / Quantitation
- Linearity
More Validation Criteria

- Measurement Uncertainty
- Measurement of Robustness
- Interfering Substances
- Reagent Stability
- Control Material Validation
Validation Report

- Developmental validation

- Did it meet your acceptance criteria?
  - As defined in your validation protocol

- Attach raw data

- Sign off
EVALUATION PLAN
Overview

• While verification / validation of an assay is a labour intensive process, it is the responsibility of a laboratory to ensure this is done as required before the assay is introduced into the laboratory
Evaluation Plan

• What will be the “Gold Standard”
  • Another NAT
  • Culture

• How many samples?
  • The more the better
  • Range of patient samples covering all expected specimen types (strains / genotypes)
  • External well-characterised control material
  • Pooled samples for repeatability testing
## Guidelines

<table>
<thead>
<tr>
<th></th>
<th>Verify</th>
<th>Validate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Accuracy</strong></td>
<td>20 – 40</td>
<td>At least 40 samples</td>
</tr>
<tr>
<td><strong>Precision</strong></td>
<td>5 – 7 concentrations</td>
<td>2-3 samples in triplicate</td>
</tr>
<tr>
<td></td>
<td>Across reportable range</td>
<td>Across reportable range</td>
</tr>
<tr>
<td></td>
<td>Run at least X5</td>
<td>Run at least X20</td>
</tr>
<tr>
<td><strong>Reportable Range (Quantitative)</strong></td>
<td>5-7 concentrations across stated linear range</td>
<td>7-9 concentrations across stated linear range</td>
</tr>
<tr>
<td></td>
<td>2 Replicates</td>
<td>3 Replicates</td>
</tr>
</tbody>
</table>

Controls

- **Controls**
  - Positive
  - Negative
  - Internal

- **Checkerboard analysis**
  - High concentrations next to negatives
  - Check for cross-contamination for automated platforms
Discordant Results

- Check sample
  - Integrity
  - Labelling

- Repeat testing
  - Different staff member
  - Different laboratory

- Test using another assay
- Sequence products
Summary

• Which assay / platform fulfils requirements of:
  • Cost
  • Space
  • Staff
  • Batching

• AND performs well in the evaluation?
GOOD LUCK!!
OOPS!

NEVER SAY THAT IN A DNA LAB!