Multi-analyte testing
- for HIV and other infections

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World Health Organization
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Definitions

- **Multiplex testing**
  - Simultaneous detection of different analytes in a single specimen using one test procedure/test run
  - May/may not be discriminatory detection

- **Multi-analyte platforms**
  - A platform that tests multiple analytes using same assay principle e.g. serology

- **Multi-platforms**
  - A platform comprised of multiple modules to give the capability to test for multiple analytes using different assay principles
Why multi-analyte testing?

- Fewer fingerpricks!
  - Less specimen volume required than for a series of tests

- Service delivery efficiencies
  - Less time required than a series of tests
    - E.g. ANC clinics where HIV & syphilis screening are desirable
  - Streamlined procurement

- Cost efficiencies
  - Linked to better service delivery
  - Cost per multiplexed test lower than the total for a series of tests
Why not multi-analyte testing?

- Does one size fit all?
  - Endless combinations & permutations
  - Different specimen types for different analytes

- Epidemiology driven or programmatically driven?

- Risks associated with de facto testing for other analytes
  - Particularly important that pre/post test counselling is performed for HIV testing
  - Ensuring treatment availability for all analytes
    - Same day treatment and/or link to treatment programmes
Laboratory Networks

- Tiered laboratory network with integrated testing provision
  - HIV, malaria, TB, hepatitis B, hepatitis C, syphilis, etc
## Diagnosis – multiplex diagnostics

<table>
<thead>
<tr>
<th>Format (qualitative)</th>
<th>Examples* (non-exhaustive list)</th>
<th>Menu</th>
<th>Complexity/infrastructure</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAT</td>
<td>Novartis Procleix ULTRIO (for blood screening)</td>
<td>HIV-1/HCV/HBV</td>
<td>High, constant electricity, maintenance</td>
<td>Level IV</td>
</tr>
<tr>
<td>Serology (RDT)</td>
<td>Combiquic HIV/HCV</td>
<td>HIV/HCV</td>
<td>Low, minimal</td>
<td>Level I</td>
</tr>
<tr>
<td></td>
<td>Multiplo HIV/HCV/HBV Antibody Test</td>
<td>HIV/HCV/HBV</td>
<td>Low, minimal</td>
<td>Level I</td>
</tr>
<tr>
<td></td>
<td>SD Bioline HIV/syphilis Duo</td>
<td>HIV/syphilis</td>
<td>Low, minimal</td>
<td>Level I</td>
</tr>
<tr>
<td></td>
<td>DPP Dual Platform HIV/syphilis</td>
<td>HIV/syphilis</td>
<td>Low, minimal</td>
<td>Level I</td>
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Multiplex RDTs

- Different types of detection
  - Combined detection of two or more analytes simultaneously on the test device
  - Discriminatory detection of two or more analytes, simultaneously on the test device

- Multiplex RDTs are mostly still in development
  - Proof of concept, demonstration studies, clinical evaluations

- Assay developers are uncertain of market needs
## Qualpro Diagnostics
### Combiquic HIV/HCV

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HIV-1&amp;2 Ab</th>
<th>HCV Ab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity*</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Specificity*</td>
<td>100%</td>
<td>99.6%</td>
</tr>
</tbody>
</table>

*Data supplied by manufacturer

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## MedMira Multiplo Rapid HIV/HCV Antibody Test

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HIV-1/2 Ab</th>
<th>HCV Ab</th>
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</thead>
<tbody>
<tr>
<td>Sensitivity*</td>
<td>99.8%</td>
<td>99.1%</td>
</tr>
<tr>
<td>Specificity*</td>
<td>99.7%</td>
<td>99.7%</td>
</tr>
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</table>

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Standard Diagnostics
SD Bioline HIV/Syphilis Duo

Parameter | HIV-1&2 Ab | TP (Syphilis)
---|---|---
Sensitivity* | 100% | 99%
Specificity* | 99.6 | 99.5%

*Data supplied by manufacturer

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Chembio
DPP (Dual Path Platform) HIV/syphilis

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## Diagnosis - multi-analyte platforms

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<tr>
<td>Serology (manual EIA)</td>
<td>bioMerieux</td>
<td>Vironostika HIV Ag/Ab Hepanostika HBsAg</td>
<td>Medium, electricity, maintenance</td>
<td>Level II, III</td>
</tr>
<tr>
<td></td>
<td>BioRad</td>
<td>Genscren HIV Monolisa HCV</td>
<td>Medium, electricity, maintenance</td>
<td>Level II, III</td>
</tr>
<tr>
<td></td>
<td>DiaSorin Murex</td>
<td>HCV, HBV, HIV, HIV Combo</td>
<td>Medium, electricity, maintenance</td>
<td>Level II, III</td>
</tr>
</tbody>
</table>

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Manual EIA for Serology

- **Specimen requirements**
  - 50 – 100µl serum or plasma, therefore one blood draw can be used to perform many tests

- **Equipment requirements**
  - Plate incubator
  - Plate washer
  - Plate reader
### Diagnosis - multi-analyte platforms contd.

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</thead>
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<tr>
<td>Serology (immunoanalyser)</td>
<td>bioMerieux VIDAS/miniVIDAS</td>
<td>Measles, Rubella, CMV, Toxo, Chlamydia, HAV, HBV, HCV, HIV</td>
<td>Medium, electricity, maintenance</td>
<td>Level II, III</td>
</tr>
<tr>
<td></td>
<td>Abbott AxSYM</td>
<td>CMV, Rubella, Toxo, HAV, HBV, HCV, HIV</td>
<td>Medium, electricity, maintenance</td>
<td>Level II, III</td>
</tr>
<tr>
<td>Serology (immunoanalyser with increased automation)</td>
<td>Abbott ARCHITECT range</td>
<td>CMV, Rubella, Toxo, HAV, HTLV, Chagas, syphilis, HBV, HCV, HIV, HIV Combo</td>
<td>High, constant electricity, maintenance</td>
<td>Level IV, III</td>
</tr>
<tr>
<td></td>
<td>Ortho Vitros range</td>
<td>CMV, Rubella, Toxo, HAV, HBV, HCV, HIV, HIV Combo</td>
<td>High, constant electricity, maintenance</td>
<td>Level IV, III</td>
</tr>
<tr>
<td></td>
<td>Siemens ADVIA Centaur</td>
<td>CMV, Rubella, Toxo, syphilis, HAV, HBV, HCV, HIV, HIV Combo</td>
<td>High, constant electricity, maintenance</td>
<td>Level IV, III</td>
</tr>
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## Diagnosis - multi-analyte platforms contd.

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<tr>
<td>NAT (quantitative)</td>
<td>GenXpert</td>
<td>MTB/RIF (tuberculosis) MRSA VRE influenza (H1N1) <em>C. difficile</em> Strep B Meningitis BCR-ABL HIV (expected 2013-14?) STI (expected ?)</td>
<td>Medium, electricity, maintenance</td>
<td>Level III, II</td>
</tr>
</tbody>
</table>

*Format Examples*
Multi-analyte platforms
## Diagnosis/toxicities – multi-platforms

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<tr>
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</thead>
<tbody>
<tr>
<td>Serology + chemistry + haematology</td>
<td>Roche cobas 4000</td>
<td>Includes cobas c 311 for clinical chemistry and cobas e 411 for serology</td>
<td>High/constant electricity</td>
<td>Level IV, III</td>
</tr>
<tr>
<td></td>
<td>Vitros 5600</td>
<td>120 analytes including serology, clinical chemistry, lipids, renal, anaemia, oncology, etc.</td>
<td>High/constant electricity</td>
<td>Level IV, III</td>
</tr>
</tbody>
</table>

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## Monitoring - multi-analyte platforms

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</tr>
</thead>
<tbody>
<tr>
<td>NAT (quantitative)</td>
<td>Roche COBAS® AmpliPrep/COBAS® TaqMan® (CAP/CTM)</td>
<td>CMV, HCV, HBV, HIV</td>
<td>High, constant electricity, maintenance</td>
<td>Level IV, III?</td>
</tr>
<tr>
<td></td>
<td>Abbott RealTime</td>
<td>Chlamydia, Gonorrhea, HCV, HBV, HIV-1</td>
<td>High, constant electricity, maintenance</td>
<td>Level IV, III?</td>
</tr>
</tbody>
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Specimen logistics

- Specimen collection
  - RDTs utilize serum/plasma, and fingerstick whole blood
  - EIA and NAT formats utilize serum and/or plasma
  - Most preferable option will depend on the testing setting
    - Single blood draw vs. multiple fingerpricks

- Specimen transportation
  - For diagnosis, same visit results are desirable
  - For treatment initiation, same visit results may be desirable
  - For treatment monitoring, next visit results might be acceptable
    - Decentralized specimen collection with centralized testing
Quality Control – did the test work?

- QC is still poorly executed
  - For most RDTs, the in-built control line is reagent addition control rather than specimen additional control
    - the latter is preferred
  - Few RDT test kits include test kit control specimens i.e. HIV positive and HIV negative controls
  - Little or no national programmes to provide QC specimens to fill these gaps
  - Dried tube specimen (DTS) approach could used for local production of QC specimens
    - Suitable for both serology-based & NAT-based testing
External Quality Assessment

- EQA assesses the likelihood that the correct testing result was given & is complementary to QC

- EQAS is still poor diffused to all levels of the health system
  - Level IV (National Reference Laboratories) may participate in international EQAS
    - Such as WHO, US/CDC, NRL Australia, UKNEQAS, etc.
  - Level III (provincial laboratories) to Level I (health centres) facilities are generally not covered by any national EQAS
  - DTS approach may also be used for local production of EQAS specimens
Pipeline for multiplex diagnostics

- Adapting existing instruments
  - E.g. GenXpert

- Microfluidics for lab-on-a-chip
  - Miniaturizing existing techniques
  - Paper-based techniques
  - Capillary action
  - Current prototypes still require instrumentation to read result
  - Cost?
Yager Group (DxBox)

- Portable instrument (US $1000)
  - Immunoassay using disposable plastic cards containing reagents
  - Cards could be stored at ambient temperature for 12 months

- Applied to diagnosis
  - Fever differentiation between 6 pathogens

- Barriers
  - Patent issues
  - No cheaper than RDTs & still requires an instrument

- Any added value?
Sia group (mChip)

- HIV and syphilis detection
- Miniaturized ELISA
- Requires a reader
- Similar to RDTs
  - Comparable cost (10-50¢)
  - Comparable time (20 mins)
  - 1µl whole blood
- Any added value?
Whitesides group

- Microfluidic paper-based analytical devices
- Colourmetric
- Requires reader
- Currently applied to
  - liver function tests
- Any added value?
Yager Group (microfluidics origami)

- Instrument-free DNA extraction only
- Heat-stable reagents
- Time to extract
  - 90 mins without power
- Then DNA extract can be applied
  NAT-based system for amplification/detection
- Currently applied to
  - Sputum for TB
Ideal multiplex diagnostics

- Multiplexing for HIV & concomitant infections of interest

- Depends on
  - Epidemiology or disease burden and/or
  - Testing purpose/setting e.g. ANC, STI, blood screening, etc.

- Potential combinations
  - HIV + syphilis, HIV + syphilis + other STIs
  - HIV + syphilis + malaria
  - HIV + HCV, HIV + HBV, HIV + HCV + HBV
Ideal multiplex diagnostics

- Multiplex diagnostics for HIV care

- HIV diagnosis & treatment eligibility
  - HIV diagnosis + CD4 count
  - HIV diagnosis + marker of HIV recent infection
  - HIV diagnosis + HIV quantitative (viral load)

- HIV treatment monitoring including toxicity
  - HIV quantitative (viral load) + chemistry/haemoglobin
Conclusions

- In comparison to existing diagnostics, pipeline multiplex Dx must at least be
  - as cheap & easy to use with similar specimen requirements
  - with equivalent or better performance

- Ideally, pipeline multiplex Dx should diagnose & monitor infection status
  - Including treatment initiation, treatment monitoring, treatment failure