Feedback from the G6PD Workshop

Eijkman Institute for Molecular Biology
Jakarta, Indonesia (September 3-4, 2018)
Point-of-Care (POC) G6PD Diagnostics

What do we know and what do we need to know?

Objectives:

- Update on G6PD diagnostics
- Identify issues to be addressed in order to standardize evaluation studies
- Population surveillance for populations at risk
### Session 1: Recent Advances in G6PD Tests

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| **Product Pipeline for G6PD Testing** | Overview of:  
- Challenges of PQ versus TQ radical cure  
- Technical versus commercial risks for manufacturers  
- New “kids on the block” in R&D (qualitative RDTs):  
  1) MoloCare®  
  2) IVDS  
  3) Axxin®/Burnet  
  4) BD™  
- Importance of QC/QA material  
- Considerations for manufacturers include:  
  a) Regulatory processes  
  b) Teaching & training  
  c) Need beyond malaria → Leprosy, neonatal jaundice and antibiotics |
<p>| <strong>Carestart® RDT vs FST Trinity Biotech</strong> | - Both tests very sensitive at 30% threshold value; huge cost implications: US$ 6 Trinity® FST versus US$ 4.5 Carestart® RDT in some countries, and high repetition rates for RDT |</p>
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| Trinity® FST in Newborns (Thailand)            | **Beyond malaria: Neonatal bilirubinaemia** – 25% caused by G6PDd  
- 8-10% G6PDd missed by Day0 measurement in Cord Blood (CB)  
The study highlights the need to test & validate **quantitative** G6PD tests in CB                                                                                       |
| Trinity® versus Pointe Scientific®             | Detailed report available online: **Pointe Scientific® comparable to Trinity®**  
However, high user-to-user and inter-lab variability: Call for **Proficiency Panel & Testing** and standardized procedure to be implemented across labs.                                                 |
| New Instruments on the way                     | Several with plus points:  
- Membrane blood separator cassette  
- Hb measurement  
- Parasite detector                                                                                                                                              |
| AccessBio® versus SD Biosensor® in Bangladesh  | - Both devices showed good correlation with spectrophotometry (=current reference method). Issues with Carestart Biosensor in terms of blood volumes.  
However, results highlight the need for reporting **Bland-Altman plots in different activity ranges**                                                                 |
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<td>Spectrophotometry: Venous versus Capillary</td>
<td>- Overall, cap. blood (non-coagulated) consistently higher G6PD activity than venous blood&lt;br&gt;- Issues related to blood volumes and type of sample with Accessbio® Biosensor&lt;br&gt;Results highlight the need for further research into:&lt;br&gt;1. Other technologies/approaches&lt;br&gt;2. More rigorous x-checking and validation procedures, using a variety of sample types and/or a proficiency panel</td>
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<td>Sampling: Does it Matter?</td>
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<td>Delay of Testing by Spectrophotometry:</td>
<td>- RT: Significant drop in activity after 24 hours – Recommendation: Test ASAP&lt;br&gt;- 4-8°C: Significant drop in activity after 9 days – Recommendation: Test after max. 7 days</td>
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<td>What is Acceptable?</td>
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<td>Essential Performance Indicators for</td>
<td>1) Analytical - robustness, linearity and bias (accuracy and repeatability)&lt;br&gt;2) Clinical level – ability to discriminated those at risk&lt;br&gt;3) Establish threshold on the point of care devices</td>
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<tr>
<td>Quantitative Diagnostics</td>
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<td>others</td>
<td>Both malaria and primaquine can induce hemolysis. Higher baseline Hb usually drops more than lower baseline Hb. G6PD activity in malaria tend to shift higher than in normals.</td>
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Conclusions (Sessions 1 & 2)

- For PQ: A **qualitative** POC test is sufficient
- For TQ: We need a **quantitative** POC test
- Careful considerations have to be given to the **complexity of a test and the costs**
- Previous APMEN guidelines for what to use, how to use, and how to interpret have to be revised/updated
- Standardized reporting of test results (e.g., units per dl Hb for quantitative tests) is required
- Recognized need for an **EQA scheme**
- Further ‘downstream policies’ in regards to patient management according to test results need to be translated into specific and clear practical job aids/guidelines for health care providers
### Session 3: Standardizing Definitions and Cut offs.

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| Current G6PD classification by the WHO and its limitations | - First WHO recommendations in the late 60s; minor, but various changes over the years, with reasons/evidence unclear  
The presentation highlighted the impact of varying thresholds on the balance between  
a) The mitigation of PQ-induced risk of hemolysis, versus  
b) Prevention of radical cure (i.e., increased risk of morbidity/mortality plus sustained transmissibility)                                                                                                                                                                                                                                                                                                                                                                                                  |
| G6PD pooled analyses – Understanding intra- and inter-assay variability | Pooled analyses that seek to explain variabilities on various levels:  
- Individual: Repeatability of test, impact of (other) patient factors  
- Labs/Studies: Intra- and inter-assay variability  
- Population: Variability between sites; meta-analysis  
The outcome of these analyses will lead to a better understanding of the observed variabilities in general, and the drug-attributable risk of hemolysis in particular                                                                                                                                                                                                                                                                                                             |

**DISCUSSION**

WHAT DO WE NEED TO STANDARDIZE? WHAT DATA DO WE NEED TO DO THIS?
Conclusions (Session 3)

- A better understanding of the variabilities is a prerequisite to inform on what level of standardization is required.
- A better understanding of the variabilities is important to inform on more specific guidelines for testing and case management.
  - Pooled analyses will dissect the sources of variabilities (e.g. various host factors, drug regimen/dosage, etc.):
    - Do we have to consider gender-specific guidelines?
    - Different test results may require different drug dosing regimens?
- Global standards and guidelines may not be applicable. The level of standardization may have to be applied on various levels (i.e., geography, malaria endemicity, ethnicity, etc.)
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| **What’s the problem?** | - Importance of differentiating gametocytocidal (=safe) versus hypnozoiticidal (=risk) dosage/regimen  
- Re-emphasis: PQ with qualitative test is OK – TQ requires quantitative test  
- For malaria elimination, we need to tackle the hypnozoites (i.e., radical cure) |
| **Hemolytic risk in heterozygous females** | - Presentation of various clinical scenarios and its associated challenges  
- The complexity of the risk may require complex guidelines (i.e., different dosing regimens for people with different G6PD activity levels, etc.) - Additional challenge to healthcare providers  
- Additional consideration on population level WRT implementation of guidelines include:  
  a) Access to health care  
  b) How to explain dosing regimens to HC workers and patients (adherence)  
  c) How to explain community versus individual benefit (i.e., clinical versus public health benefit) |
| **Hemolytic risk after CQ and PQ – Pooled analyses** | Clinical considerations because of combinations of:  
  • Anemia  
  • Phenotype (symptomatic vs asymptomatic)  
  • Concomitant infections  
  • G6PD status  
  • Hematocrit drop |
| **DISCUSSION** | **WHAT METRICS DO WE NEED TO DEFINE SEVERE HEMOLYSIS? ROLE OF PHARMACOVIGILANCE** |
Conclusions (Session 4)

- Need for better practical guidelines and tools for early detection and monitoring of hemolytic events
- Need for further studies investigating the association between G6PD genotypes and phenotypes
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| **Mapping G6PD deficiency and variants**   | - Part of Global Burden of Disease Project – Annual updates  
- Presentation of:  
a) Global longitudinal *P. vivax* burden  
b) G6PDd prevalence and variant distribution                                                                                                                                                                                                                                                                                                                                                                           |
| **Validation of novel variants**           | - Genotype-phenotype associations are important for G6PD severity classification and risk prediction maps  
- Discovery and validation of novel genotypes on various levels:  
a) Biochemical  
b) *In vitro*  
c) Animal models  
d) Computational tools (with x-validation)                                                                                                                                                                                                                                                                                                                                                     |
| **High throughput genotyping and interpretation** | Comprehensive overview of:  
- G6PD genetic properties and associated challenges with sequencing/genotyping  
- The application and cost-effectiveness of various typing tools and platforms in the context of research versus surveillance (i.e., amplicon sequencing versus typing of single known polymorphisms, level of throughput, etc.)                                                                                                                                                                                                                     |

**DISCUSSION**

**DISCOVERING AND MAPPING NOVEL VARIANTS**
Conclusions (Session 5)

- Need for more in-depth studies on the association between G6PD genotyping variants and phenotypes in order to assess the relevance of genotypes on severity of G6PDd and risk of hemolysis
- Current advances in technologies allow simpler sampling methods and strategies that are amenable to real-time surveillance
### Challenges for deploying G6PD testing in Indonesia

- Current policy: PQ low dose regimen without G6PD testing, but patient monitoring for hemolytic events
- Data on G6PD prevalence and variant distribution are limited
- Challenges to introduce testing in Indonesia include:
  a) Lack of knowledge
  b) Uncertainties about guidelines
  c) Logistical challenges
  d) Cost

### Barriers for G6PD deployment

Presentation of published data from Bangladesh, Cambodia, China, and Malaysia
- Main barriers in Bangladesh, Cambodia, China included:
  a) Perceived low risk of PQ-induced hemolysis,
  b) Perception of vivax malaria being benign, and
  c) Additional costs incurred by testing
In contrast, the current test-and-treat algorithm in the close-to-elimination context of Malaysia was deemed suitable
Conclusions (Session 6)

- Strong evidence is required to make a case for policy change
  - Need for further studies (national and local, if applicable) to provide evidence
- Improved communication strategies are needed to translate appropriate messages from research into policies and corresponding clinical guidelines/practical job aids
Concluding remarks upon closure

- For malaria elimination, we need effective radical cure (i.e., short-course high-dose PQ, or single high-dose TQ)
- For short-course high-dose PQ, qualitative G6PD POC tests are appropriate to use
- For single high-dose TQ, we need to use quantitative G6PD POC tests → need universal cut off for “go/no go” treatment
- There is an urgent need for better/clearer clinical practical guidelines for the prescription of radical cure against *P. vivax* malaria
Round Table Discussion:

1. For countries that have already adopted or decided to adopt G6PD testing:
   a) What has been your experience?
   b) What difficulties did you encounter in the process of adoption or decision-making, and what challenges are you still facing?

2. For countries that have not yet adopted G6PD testing:
   a) What is your level of interest in testing G6PD for radical cure?
   b) What concerns do you have about adopting G6PD testing?
   c) What challenges are you facing in making decisions to implement it?