Diagnosis of G6PD Deficiency
G6PDd Diagnostics

• Discovered 1956 in clinical trials of primaquine
• Heinz body test 1960
• Glutathione stability test 1965
• Quantitative spectrophotometric assay
• NADPH spot test 1979
• Dye reduction tests 1980s & 1990s
• PCR mutation detection 1990s
Researchers Ohno & Beutler
To sons, not daughters.
G6PD Standard Testing Approach

- **Quantitative** – provides precise measure of G6PD activity/gHb in any given RBC population
- **Cytology** – provides precise measure of mosaicism among female heterozygotes
- **Qualitative** – provides normal vs. deficient classification
- **Genetic** – provides unambiguous diagnosis of a specific gene mutant
G6PD Standard Testing

Analytical Downsides

• **Quantitative** – averaging in heterozygotes, and possible confounding by disease states

• **Cytology** – no measure of degree of enzyme activity

• **Qualitative** – averaging in heterozygotes, and possible confounding by disease states, variable & subjective cut-offs for normal vs. deficient

• **Genetic** – blind to mutants not specifically evaluated
G6PD Standard Testing

Practical Downsides

- **Quantitative** – Expensive, sophisticated laboratory expertise & special equipment
- **Cytology** – Expensive, laborious, technically very difficult & subjective end point
- **Qualitative** – Cold chain, special equipment, expensive, subjective end point
- **Genetic** – Expensive, laborious, sophisticated laboratory expertise & equipment, likely very often insensitive
Basis of Assays

G-6-P → G-6-PD → 6-PG

NADP ↔ NADPH

Reduced dye → Oxidized dye

Orange or Blue

340nm
Available Qualitative Tests

• Fluorescent Spot Test (NADP+ reduction), formerly sold by Sigma Chem. Co., now Trinity (Ireland).

• Dye Reduction Test (several kits available including Trinity & Dojindo)

• Binax Now G6PD Test (Alere Inc. USA)
Typical results are shown in Figure 1 below:

**Fluorescent Spot Test (FST)**
- Trinity

**Dye Reduction Test (DRT)**
- Dojindo

**Equipment and Costs:**
- Cold chain Pipettor
- Water bath
- UV lamp
- $4.35/test (300)
- Cold chain Pipettor
- $2/test (200)
5. The test must be performed at temperatures between 18-25°C (64°F to 77°F); failure to perform testing in the specified temperature range could lead to erroneous results. If the temperature is outside this range, DO NOT PERFORM THE TEST.

$400 for box of 25 tests; $16/test
Available Qualitative Tests

**Pro**
- “Go” versus “No go” on PQ therapy at or near point of care

**Con**
- “Maybe”? Common
- Blind to some heterozygotes
- Variable & poorly defined cut-offs
- Not validated with malaria & other disease states
- Cold chain
- Specialized equipment
- Laboratory skills
- Expensive
- Suited to mass screening rather than patient management
Experimental Qualitative Tests

Truly Point-of-Care (POC)

- Black: Individuals classified as G6PD normal by using CareStart G6PD deficiency RDT
- Red: Individuals classified as G6PD deficient by using CareStart G6PD deficiency RDT

G6PD activity (U/g Hg)
Key Gaps in POC Qualitative Tests

- Appropriate & well-defined residual enzyme activity level for PQ “go” versus “no go” readouts
- Primaquine sensitivity phenotype quantitatively linked to residual enzyme activity
- Risk to heterozygous females at “appropriate” cut-off for male hemizygotes
- Impact of demographic, nutritional, infections, and chronic disease on test read out (e.g., anemia, pregnancy, parasitemia, thrombocytopenia, paroxysm, hemolysis, dehydration, shock, etc.)
Testing Technology for Addressing the Gaps in POC Tests: Flow Cytometry

• Quantitative, unambiguous measures of G6PD activity within RBC populations
• Develop as “Gold Standard” against which qualitative kits are assessed, optimized, and validated
A novel cytofluorometric assay for the detection and quantification of glucose-6-phosphate dehydrogenase deficiency

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Ideal Experimental Setting/Design

• Prospective analysis of G6PDd by standard quantitative, standard qualitative, experimental qualitative, and flow cytometry
• Patients admitted to hospital with a primary diagnosis of malaria confirmed by certified expert microscopy (also by flow cytometry and perhaps PCR)
• Patients systematically evaluated clinically and laboratory (syndromes, CBC, chemistries, etc.)
• Both species, mixed infections, and illness ranging from mild to fatal
• Multivariate logistic regression analysis of variables that impact each of the G6PD methodologies. What can mislead G6PD diagnostics in patients with malaria?