Anti-Malarial Drug Testing: Tests, Reference Standards and Assays

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Part funded by;
Topics

- Reference Standards
- Validated Assays for Artemisinin
- Laboratory Tests
- In-Field Tests
- Metabolic profiling, plant characterisation and traceability for A2S2
Reference Standards

• Current supply of anti-malarial standards is limited and sparse
• Comprehensive portfolio of standards, fully characterised
• Contains 40 standards and 20 plant extracts
  o artemisinin and artemisinin derived pharmaceuticals
  o other anti-malarial pharmaceuticals
  o artemisinin derivatives
  o Useful plant derived metabolites
  o A. annua variety Artemis extracts and leaf samples
Reference Standards - Examples

ART & ART derived pharmaceuticals
- Artemisinin
- Arteether
- Artemether
- Artesunate

Artemisinin derivatives
- Dihydroartemisinin
- Deoxyartemisinin

Other anti-malarial pharmaceuticals
- Lumefantrine
- Piperaquine phosphate
- Sulphamethoxypyrazine
- Trimethoprim

A. *annua* variety Artemis extracts
- Hexane- EtOAc
- R134A
- Acetonitrile
- Toluene

Useful plant derived metabolites
- Quinine, Quercetin, Coumarin
- Artemisinic acid, DHAA
- Arteannuin B, Artemisitene

Artemisia leaf samples
- *Artemisia abrotanum*
- *Artemisia absinthium*
- *Artemisia vulgaris*
Validated Assay for Artemisinin using LCMSMS
The need for an accredited assay for the detection and measurement of ART

- Current methodologies are not best suited to the detection and measurement of Artemisinin.
- Current methods for ‘pure’ Artemisinin analysis could be improved.
- We need an assay that is suited for quantitative analysis of both ‘pure’ Artemisinin and Artemisinin present in leaf extracts with options for its derivatives and impurities.
Validated Assay

- We have now launched a validated LCMSMS assay service for artemisinin and related compounds
- BS 17025 accreditation assessment in November and is based upon ICH Q2A guidelines
- Available for inter laboratory calibration...not intended to replace existing assays, but to help provide consistency of analysis in the industry
- ...send us 1 in every 10 samples for calibration
Antibody based detection systems for Artemisinin

ELISA and Lateral Flow Devices
Overview

Conjugate and antibody production

Antibody purification and testing

ELISA development, optimisation and testing

LF strip production, wet assay tests

Working dry assay with reader

Test batch manufacture, validation and calibration

Sampling kit development

Testing on wet and dry leaf

Software rewrite and test

Manual production

ELISA kit production **Now available**

LFD β test, re assessment, **field testing est. Jan 2011**
ELISA for Artemisinin;

- Limits of detection = 1mg/ml-50ng/ml
- Limits of quantification = 2-64µg/ml

- ELISA give excellent correlation with other analytical techniques
- Supplement to TLC, more reproducible
- Use in conjunction with validated LCMSMS
In Field Quantitation Kit

- For use with Lateral Flow Device and Device reader
- Configured for field use with all equipment an instructions included
- Looking for beta testing partners
Principle Component Analysis using Multivariate Data Analysis (PCA) for metabolic profiling in A. annua
The advantage of PCA:

- A mathematical technique based upon real data which allows identification of molecules responsible for key characteristics.

- It is the only way to clearly identify the molecules (or groups of molecules) which are likely to relate to industry issues such as; agronomy (region of growth, pesticides, fertilizers, irrigation, sunlight and daylight effects, high yielding plants, extraction and crystallization efficiency, API impurities*, API stability, future processing.

- This technique will give us the tools required for rational design of industry protocols.

- * Important implications for supply chain traceability from plant to API to final product.
PCA breaks down a large table of data into two smaller ones

Plots of scores and loadings turn data into pictures

Correlations among observations and variables are easily seen

Information resides in the correlation structure of the data

Interpretation of PCA scores shows patterns and trends
Data acquisition for PCA:

- We initially chose to use HPLC at 216 and 280 nm to establish the model.

- Q-TOF MS under way...to identify all components in a plant and to analyze against relevant variables.

- Studies in progress with FTIR as rapid analysis technique.
Example analysis....this is for a particular localized region of growth, but we have also examined the compounds extracted by different solvents, from internationally sourced leaf samples and from different cultivars.

Yellow- Mediplant std.

Others- Same variety grown in different areas....they can be distinguished by molecular analysis
Example of PCA model Observations

• In proof of principle studies we have shown that the components of *A. annua* leaf from different geographical origins, and extracted using different solvents can be separated into distinct groups.

• The differences in metabolic profiles which are impossible to interpret with the naked eye, can be identified using multivariate analysis e.g. to identify compounds related to crystallisation and yield.

• The models developed may also be used for monitoring the batch-to-batch consistency of raw materials and for traceability in the supply chain (A2S2)
Summary

- Our aim is to promote market normalisation and stability by providing a helpful portfolio of relevant products and services. These include:
  - A new portfolio of anti-malarial reference standards
  - New in-field and laboratory based tests
  - Validated assay services for artemisinin, several other related compounds and other anti-malarials
  - Metabolic profiling and supply chain traceability using multivariate data analysis
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