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

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Medicines for Malaria Venture

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Topics

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

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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
    - artemisinin and artemisinin derived pharmaceuticals
    - other anti-malarial pharmaceuticals
    - artemisinin derivatives
    - Useful plant derived metabolites
    - *A. annua* variety Artemis extracts and leaf samples

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Reference Standards - Examples

ART & ART derived pharmaceuticals

- Artemisinin
- Arteether
- Artemether
- Artesunate

Artemisinin derivatives

- Dihydroartemisinin
- Deoxyartemisinin

Other anti-malarial pharmaceuticals

- Lumefantrine
- Piperaquine phosphate
- Sulphamethoxypyrazine
- Trimethoprim

Useful plant derived metabolites

- Quinine, Quercetin, Coumarin
- Artemisinic acid, DHAA
- Arteannuin B, Artemisitene

***A. annua* variety Artemis extracts**

- Hexane- EtOAc
- R134A
- Acetonitrile
- Toluene

***Artemisia* leaf samples**

- *Artemisia abrotanum*
- *Artemisia absinthium*
- *Artemisia vulgaris*

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Validated Assay for Artemisinin using LCMSMS

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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.

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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

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Antibody based detection systems for Artemisinin

ELISA and Lateral Flow Devices

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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**

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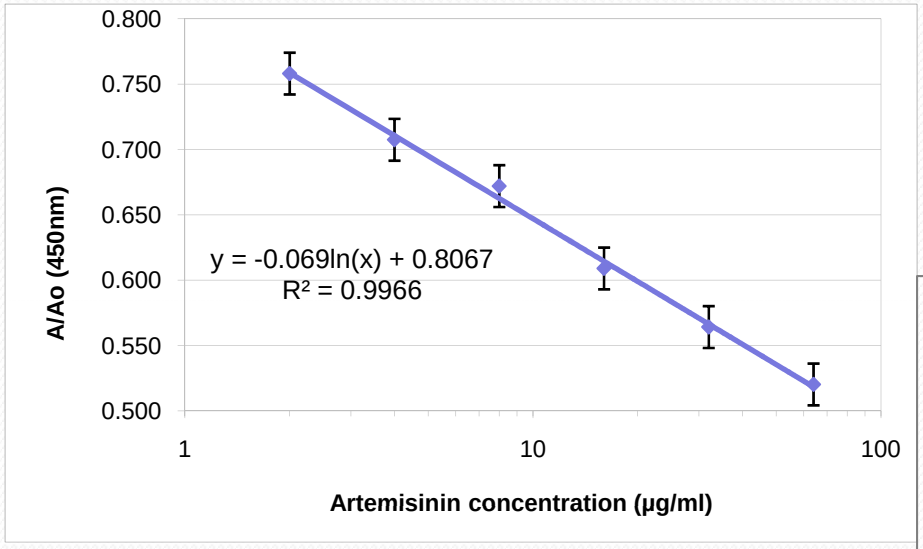
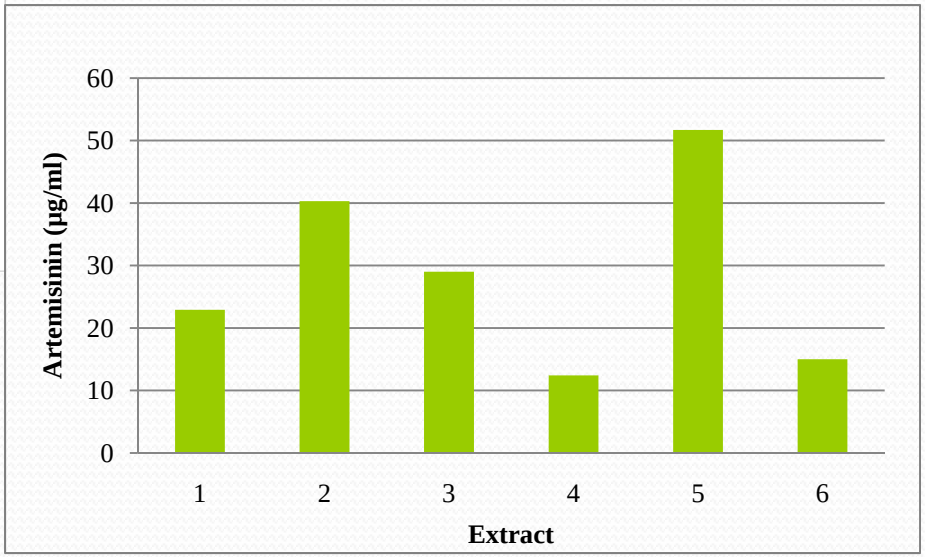
ELISA for Artemisinin;



- 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

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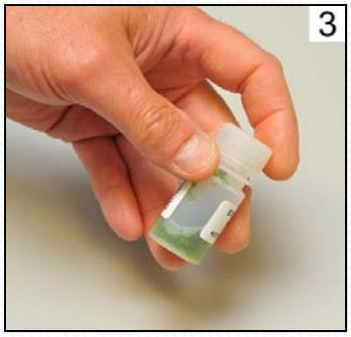
In Field Quantitation Kit

- For use with Lateral Flow Device and Device reader
- Configured for field use with all equipment an instructions included
- Looking for beta testing partners



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Principle Component Analysis using Multivariate Data Analysis (PCA) for metabolic profiling in A. annua

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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

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Data for PCA:

OBSERVATIONS ↓ **VARIABLES** →

Primary Obs	ClassID	Maturity	2,564"_DA	2,659"_DA	2,892"_DA	3,048"_DA	3,219"_DA	3,40"_DAC	3,50"_DAC	3,653"_DA	3,842"_DA	4,0"_DAD	14,1"_DAD	14,303"_DA	4.475"_DA	4,739"_DA	4,8"_DAD
SO82-37	Iboaka 1045 Mat-4,2	4,2	26.86961	44.9733	52.41531	54.34677	51.66421	123.5335	0	58.88955	41.57663	0	0	631.0388	0	174.1241	741.97
SO82-38	Iboaka 1046 Mat-4,4	4,4	25.25257	43.72847	51.26069	0	142.8474	155.5588	0	0	246.9885	557.2141	0	0	76.8434	0	846.5731
SO82-39	Ambalaseva 3194 Mat-	Ambalaseva	26.86961	50.38239	82.44022	0	218.0575	100.0604	0	0	878.6672	0	0	78.72085	89.85306	0	805.6885
SO82-41	Antisrabe 1225 Mat-4	4	1093.804	0	611.4351	0	0	59.09922	518.197	335.384	0	0	78.61356	65.32234	80.53956	0	646.5154
SO82-42	Antisrabe 1226 Mat-4,9	4,9	1060.033	0	479.2135	0	111.7254	0	965.2594	0	0	88.69537	81.01097	0	226.5649	0	902.8701
SO82-43	Antisrabe 1021 Mat-3,6	3,6	953.4064	0	308.4754	195.9851	0	0	571.5104	500.549	0	0	50.18599	0	102.5747	0	518.682
SO82-44	Antisrabe 1023 Mat-3,1	3,1	857.1718	0	294.5936	176.8587	0	0	428.0541	536.7195	0	0	43.52091	0	78.47832	0	416.9307
SO82-45	Iboaka 1238 Mat-5,1	5,1	123.6765	69.77823	334.3663	0	136.9739	0	1093.075	0	0	70.13799	59.47911	0	176.0726	0	942.0013
SO82-46	Iboaka 1201 Mat-4,7	4,7	145.984	68.87493	379.0592	0	135.1142	0	842.2739	0	0	46.51926	58.01641	63.22187	75.76952	0	741.4986
SO82-47	Iboaka 1204 Mat-4,9	4,9	0	0	124.6906	161.2865	76.73167	0	316.0786	613.0707	0	0	0	129.1609	93.39915	0	731.0713
Med ACN1	Mediplant	Med ACN1	177.3451	180.6178	105.9363	320.3188	86.90177	601.4019	0	199.4794	143.51	0	0	144.8257	0	0	0
Med ACN2	Mediplant	Med ACN2	168.3885	181.7006	105.922	332.5286	94.85378	602.0241	0	282.0712	0	0	53.20174	159.8341	0	0	0
Med ACN3	Mediplant	Med ACN3	163.7602	294.3558	0	338.8649	98.37358	597.8733	0	284.6949	50.27254	0	0	163.7376	0	0	0

- 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

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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

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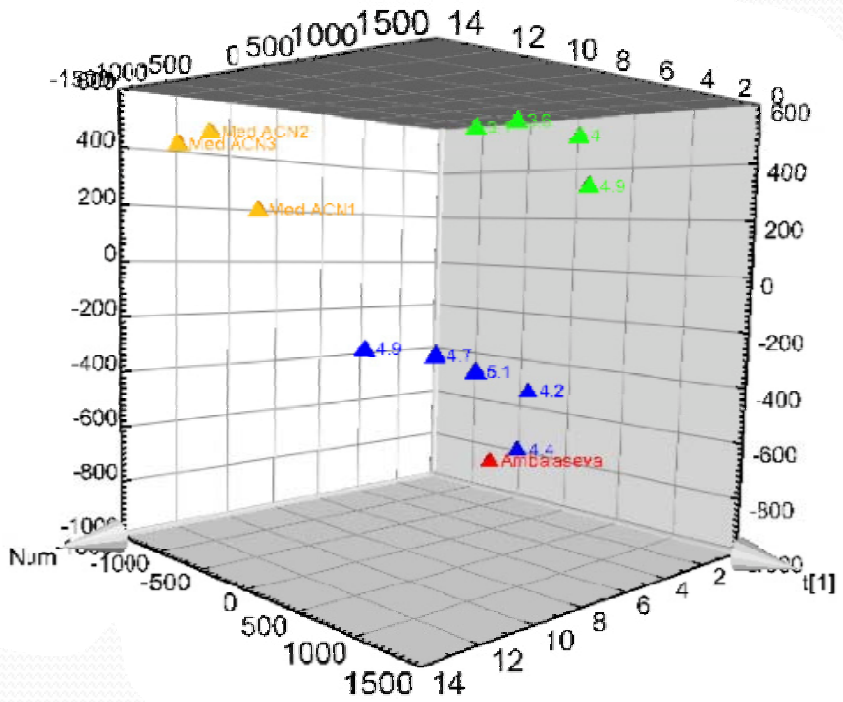
Example analysis….this is for a particular localized region of growth, but we have also examined the compounds extracted by different solvents, from internationally sourced leaf samples and from different cultivars.



Yellow- Mediplant std.

Others- Same variety grown in different areas….they can be distinguished by molecular analysis

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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)

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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
- **Please contact us at: neil@sensapharm.eu**

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