

CLINICAL UTILITY OF AUTOMATED MALARIAL DISCRIMINANT FACTORS FOR DETECTING MALARIA ON THE BECKMAN COULTER LH750

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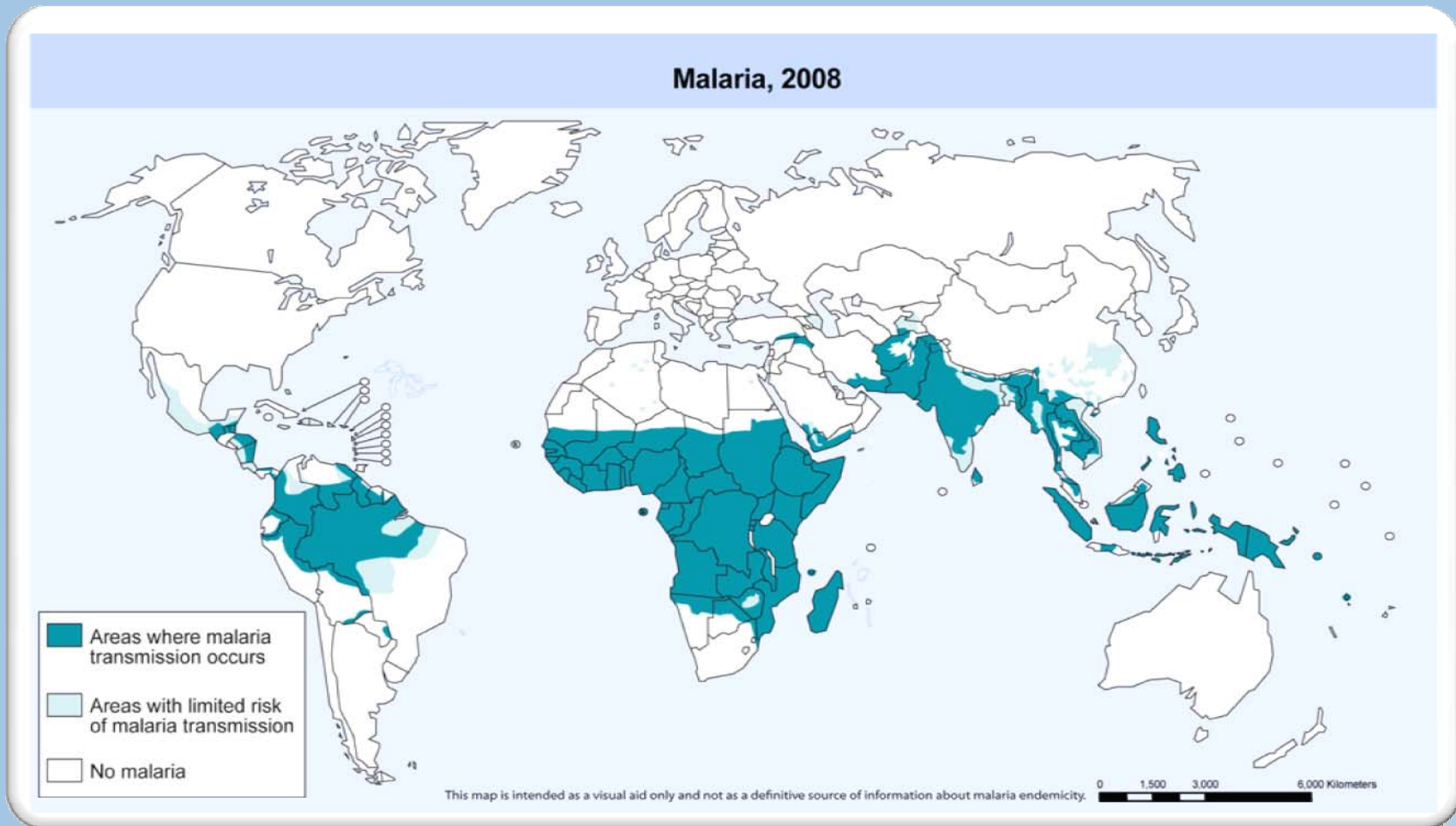
Southern Health

Better Health for Our Community

WHO-Malaria statistics today

- Half the world's population is at risk of malaria.
- In 2006 there were 247 million cases of malaria, causing an estimated 881,000 deaths.
- 80% of the cases were in 13 African countries.
In Africa 85% were children under 5 years of age.
- Every 30 seconds a child dies from malaria.
- Outside the African region, 80% of the cases were in India, Myanmar, Bangladesh, Indonesia, Papua New Guinea and Pakistan.

WHO-Malaria statistics today



http://gamapserver.who.int/mapLibrary/Files/Maps/Global_Malaria_ITHRiskMap.JPG

Malaria parasites

- Plasmodium falciparum
- Plasmodium vivax
- Plasmodium ovale
- Plasmodium malariae
- Plasmodium knowlesi

Transmission of malaria

- Principal mode of spread of malaria is by the bites of female anopheles mosquito.



Malaria diagnosis

- Microscopic diagnosis
Gold standard: thick and thin films
- Rapid immunochromatography diagnostic tests (ICT): antigen detection-screening test.
- PCR-mainly used for research confirmation

Alternative screening methods

- Fluorescent microscopy:
fluorescent dyes - acridine orange (AO)
Studies showed sensitivities around 96% and
specificity 95%.

Disadvantages:

Microscopy expertise still required.

Requires special equipment-fluorescent
microscope.

Trans.Royal Society of Tropical Medicine and Hygiene, Vol 90,1996,516-518.

Alternative screening methods

- Fluorescent microscopy –QBC method.
Acridine Orange capillary with blood, centrifuged, examined under fluorescent microscope.

Disadvantages:

- expensive
- difficulty in species identification and quantification
- technical problems

Alternative screening methods

Flow cytometry:

- offers automated counts for parasitaemias
- Sensitivity 91%, specificity 86%
- False positives in high reticulocyte levels.

Southeast Asian J Trop Med Public Health, 2004 Sep; 35(3): 552-559

Haematology analysers-new parameters

Automated blood cell analysers.

New parameters continue to be introduced:

- NRBC
- immature granulocytes
- immature reticulocyte fraction
- immature platelet fraction
- red cell fragments.
- % hypochromic red cells, retic Hb content

Malaria diagnosis using automation

Several studies conducted to evaluate feasibility of automated malaria detection.

- Abbott
 - Cell Dyn 4000 and 3500
- Beckman Coulter:
 - Gen S
 - LH 750

Malaria diagnosis using automation.

Abbott Cell-Dyn 4000

- Multi-angle light scattering cytometry to differentiate white cells.
- Detection of atypical light depolarisation of leucocytes which had ingested the malaria pigment haemozoin.
- Sensitivity 91% and specificity 100%

Malaria diagnosis using automation

Beckman Coulter

- VCS technology used to detect changes to volume, conductivity and scatter of monocytes and lymphocytes that have been activated by malarial parasites

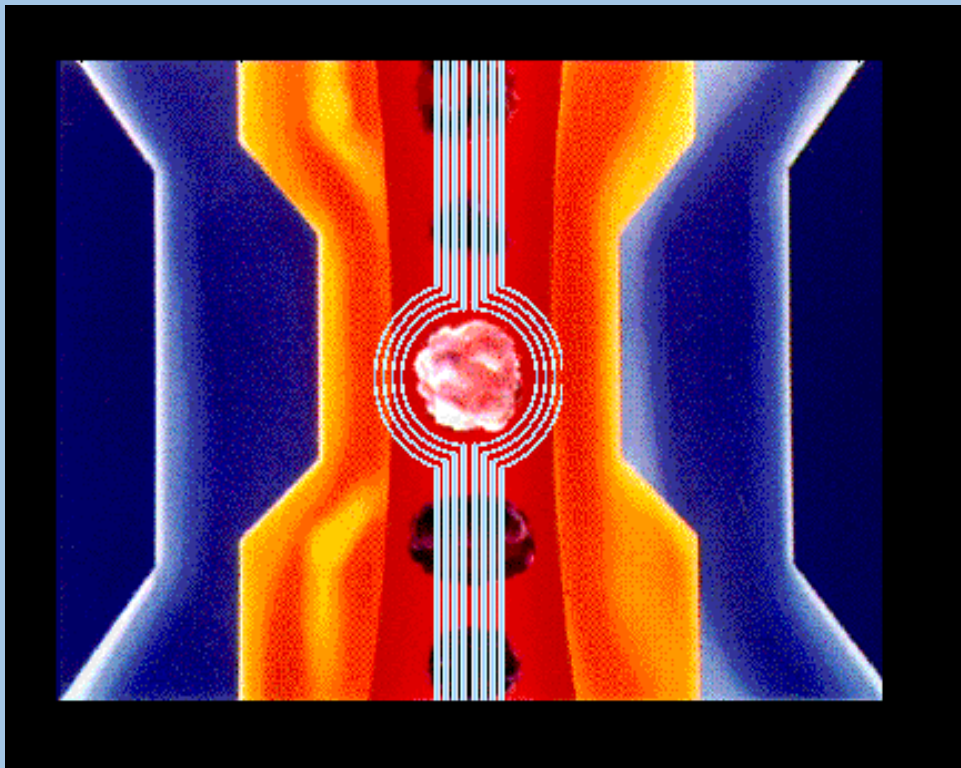
Beckman Coulter VCS technology

- V-volume
- C-conductivity
- S-scatter

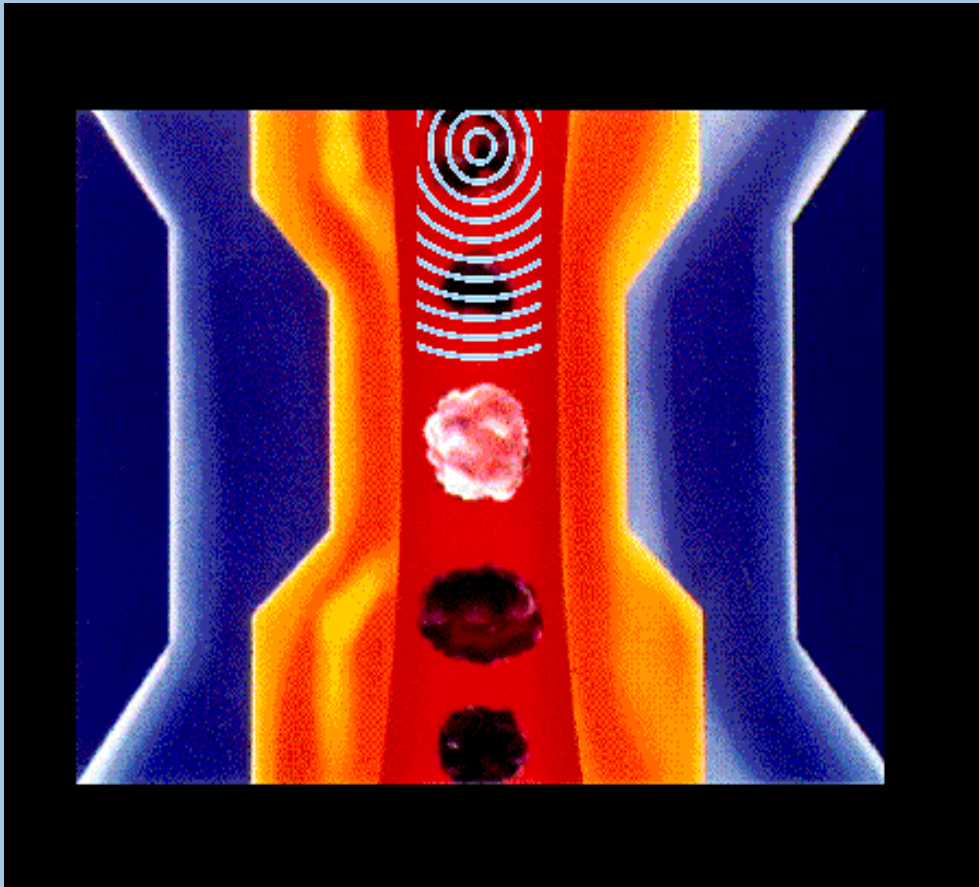
VCS-technology

Volume:

Coulter principal of direct current (DC) impedance to measure the cell volume.



VCS-technology



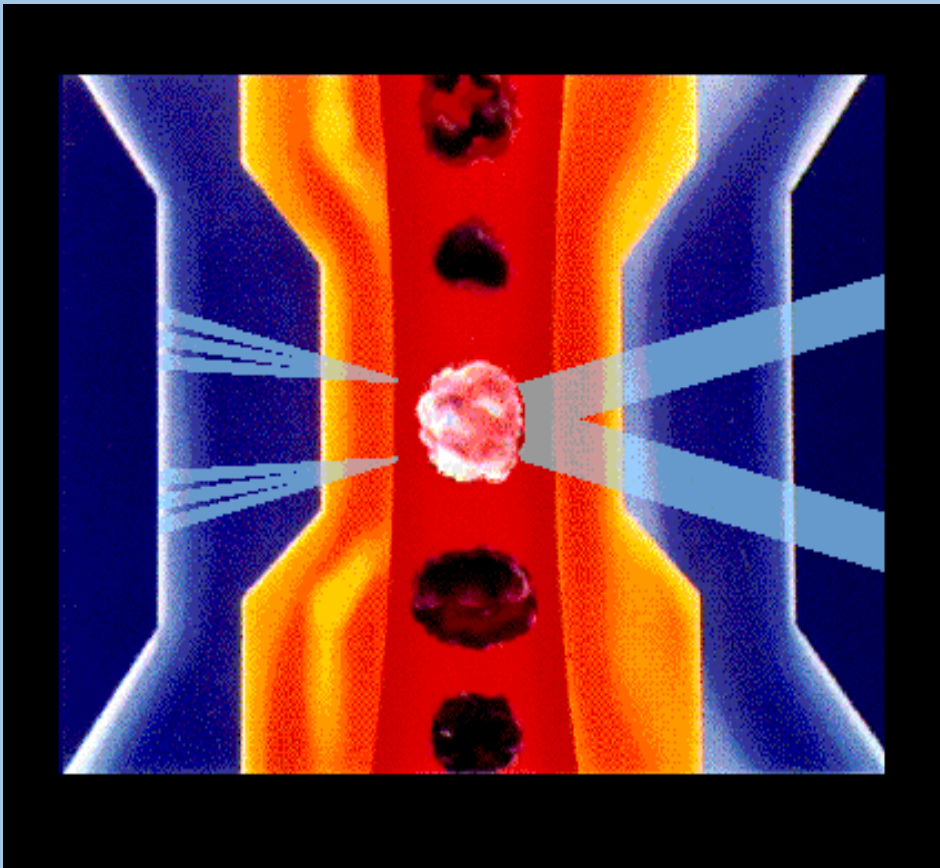
Conductivity:

High radio frequency (RF) current at 23 MHz is used that penetrates the cell membrane and measure the internal structure of the cell.

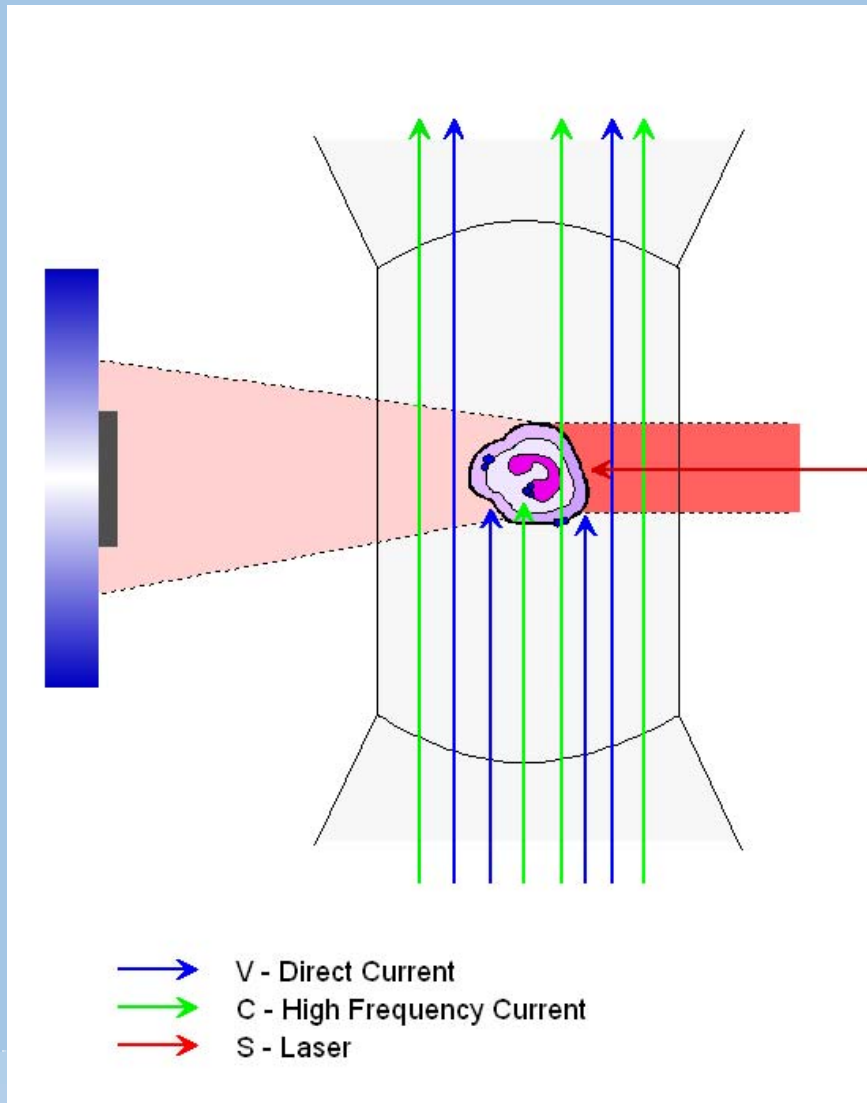
VCS technology

Scatter:

a laser light is directed onto the cell scattering light in different directions. It measures cell surface and subsurface granularity using a broad range of laser light angles.



VCS technology



VCS:

all 3 measurements (DC, RF and scatter) in a single channel analysis, interrogate 8192 cells simultaneously.

Malaria diagnosis using VCS

Fourcade et al used VCS technology on Gen S and LH 750 Beckman Coulter analysers to detect malaria positive patients.

	NE		LY		MO		EO	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
V	169	49.9	82	25.4	184	26.6	161	28.8
C	147	17.8	130	31.8	130	9.7	143	9.5
S	128	17.8	69	22.5	92	11.4	193	7.9

Malaria diagnosis using VCS

- Changes in monocytes and lymphocytes caused by the presence of activated cells were analysed.
- A discriminant value for malaria detection was established by this formula.

$$D = \frac{V_{SD} (\text{lymphocytes}) \times V_{SD} (\text{monocytes})}{100}$$

Malaria diagnosis using VCS

- Using ROC (receiver operator curve) analysis they established two discriminant factors:
 - Discriminant Factor of 5.06 had 97% sensitivity and 83% specificity
 - Discriminant Factor of 4.81 had 100% sensitivity and 77% specificity.

Malaria diagnosis using VCS

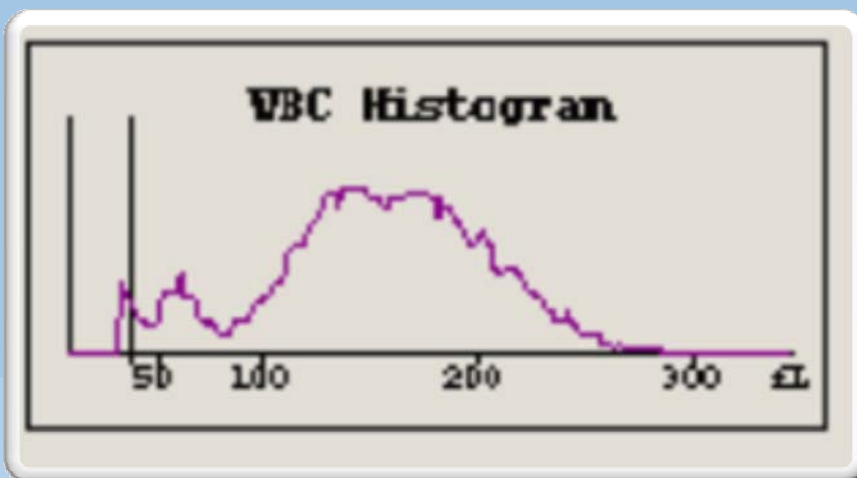
Briggs et al conducted similar study. They used four LH750 and one Gen S. They performed statistical analysis and they obtained the following results:

- Malaria Discriminant Factor of 3.7
- 98% sensitivity
- 94% specificity

Malaria diagnosis using VCS

Additional parameters:

- eosinophil percentage $<0.15\%$
- platelet count $<150 \times 10^9/L$
- presence of a peak on the WBC histogram



Sensitivity still 98%,
specificity improved to
95%. Malarial factor 3.8

At Dandenong Hospital

We were excited!



At Dandenong Hospital

Dandenong is home to a large African refugee population as well as locals who travel to malaria endemic areas such as India, Pakistan, Papua New Guinea.

We have a refugee clinic in place where blood testing for different types of diseases is performed including malaria.

At Dandenong hospital

- We conducted our own study to see if we can apply the same concept to our population.
- We applied the published malaria factors of Briggs and Fourcade to a population of 68 samples from 56 patients with suspected malaria of whom 15 had detectable parasites.

At Dandenong hospital

Sensitivities and specificities on published malarial factors

	Fourcade factor 4.8	Briggs Factor 3.7	Briggs Factor 3.8
Sensitivity	27%	40%	7%
Specificity	93%	76%	98%

At Dandenong Hospital

We were disappointed!



At Dandenong Hospital

- Three instruments used
 - LH750 – Dandenong
 - LH755 – Monash Medical Centre
 - LH755 – Monash Medical Centre
- Latron, 5C
- RCPA QAP - Southern Health Subgroup
- Weekly Inter Laboratory QC

VCS comparisons

- Samples processed through three LHs.
First at DDH then 2 MMC analysers.
- The VCS parameters were compared to check if the analysers gave the same result.
- P value <0.0001 indicates that the values obtained for the same parameter from the 3 analysers are statistically significantly different.

At Dandenong hospital

Comparison of the VCS parameters using ANOVA: P values

	Neutrophils		Lymphocytes		Monocytes		Eosinophils	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
V	<0.001	<0.001	<0.001	0.007	<0.001	0.009	0.37	0.92
C	<0.001	<0.001	<0.001	0.43	<0.001	<0.001	0.52	0.26
S	<0.001	<0.001	<0.001	0.002	<0.001	0.004	0.08	0.77

VCS Reference Range

- Establishing a Reference Interval
- Collected 50 samples with normal Haematology parameters and unremarkable clinical notes (pre operative outpatients)
- Standard Deviation Volume (Lymphocytes)
 - 13.3 +/- 1.5 (11.8 – 14.8)
- Standard Deviation Volume (Monocytes)
 - 16.3 +/- 4.1 (12.3 – 20.4)

Lymphocyte SD-V Reference Range

- Review the 68 ?Malaria patients by applying the established Lymphocyte Reference Range
- 11 of the patients with malaria had an abnormal SD Volume Lymphocytes
4 were within normal range
- 37 of the patients with no observed parasites had an abnormal SD Volume Lymphocytes
Only 16 were normal

Monocyte SD-V Reference Range

- Review the 68 ?Malaria patients by applying the established Monocyte Reference Range
- 10 of the patients with malaria had an abnormal SD Volume Monocytes
5 were within range
- 24 of the patients with no observed parasites had an abnormal SD Volume Monocytes
29 were normal

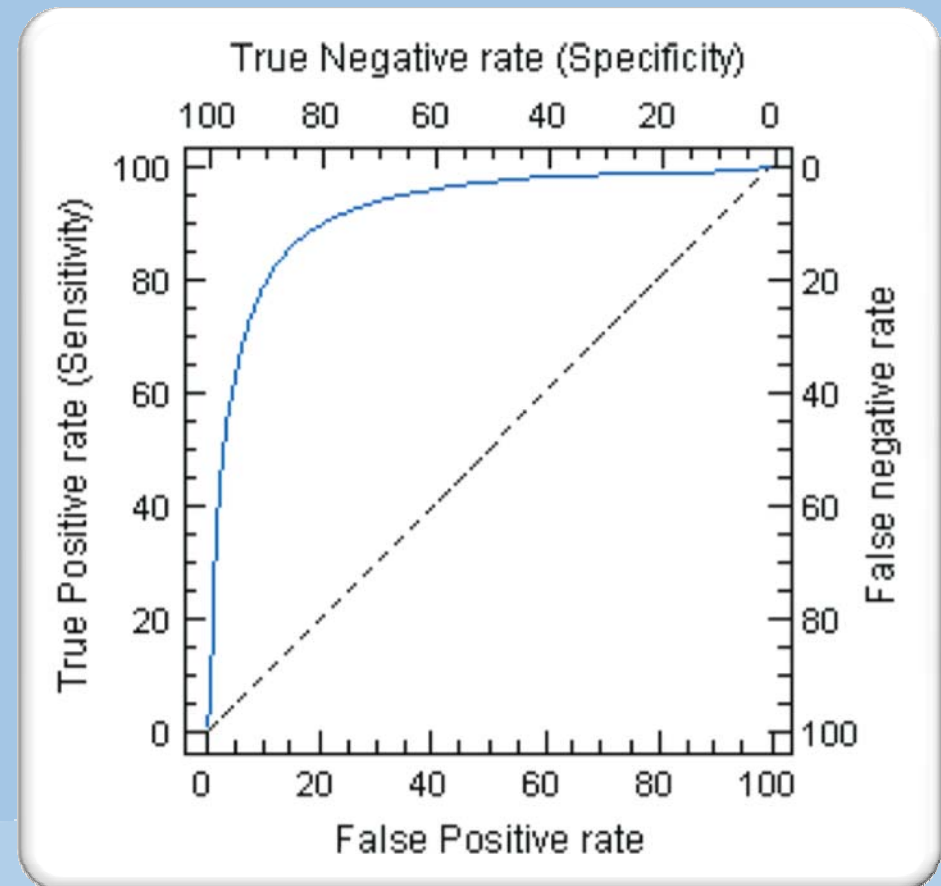
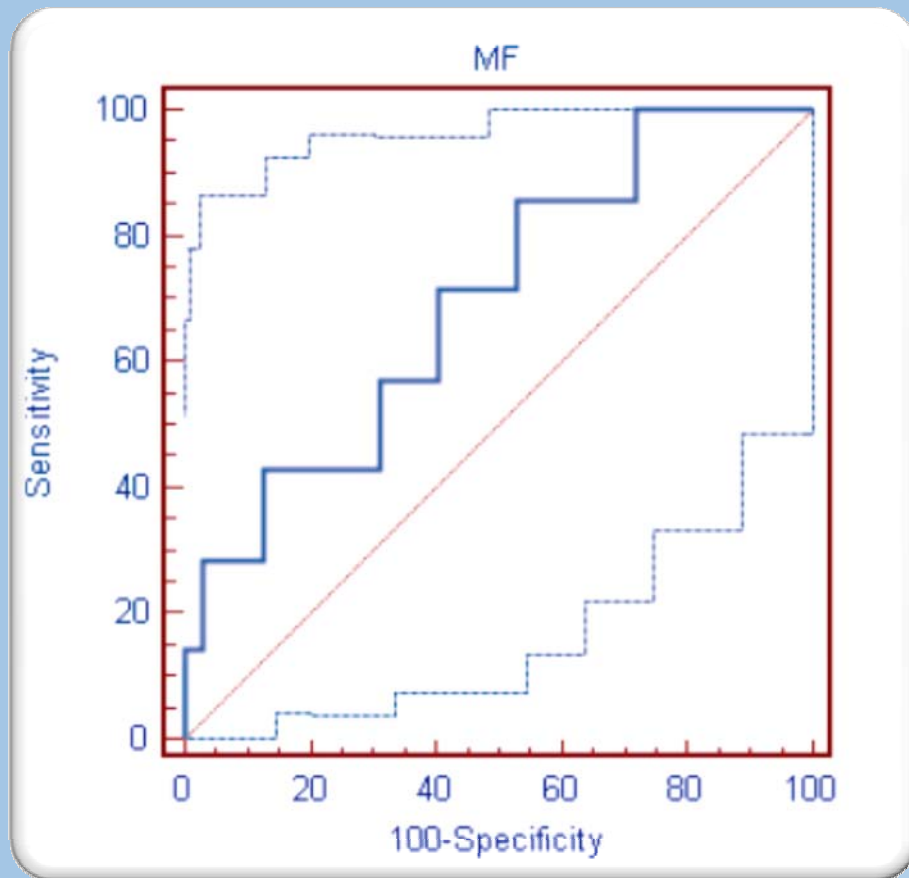
VCS Reference Range

- Reviewed 200 routine hospital patients by applying the established Reference Range
- 35% of the hospital patients had an abnormal SD Volume Lymphocytes
- 15% of the hospital patients had an abnormal SD Volume of Monocytes

ROC analysis

- Applied Receiver Operator Curves analysis to our ? Malaria database
- Established a local Southern Health Discriminant Factor

At Dandenong hospital-ROC curve



At Dandenong Hospital

	Southern Health Factor >2.5	Southern Health Factor >3.5
Sensitivity	80%	53%
Specificity	43%	72%

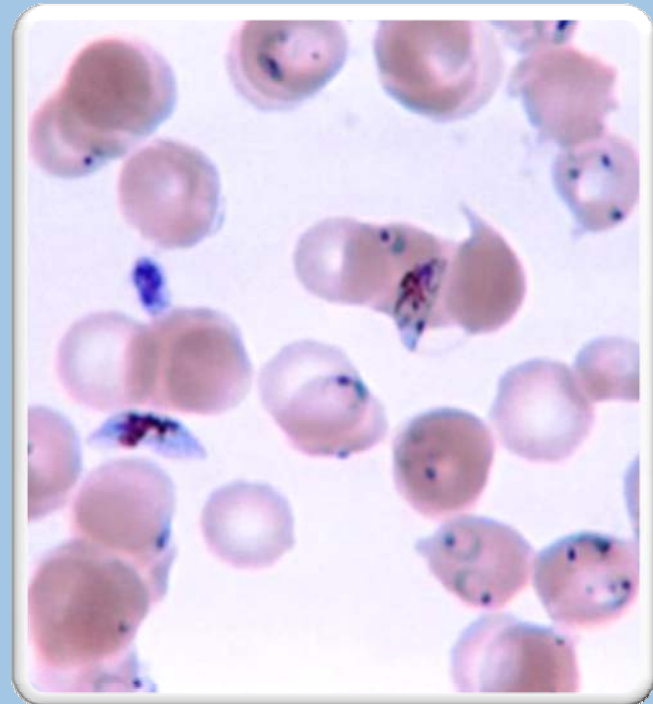
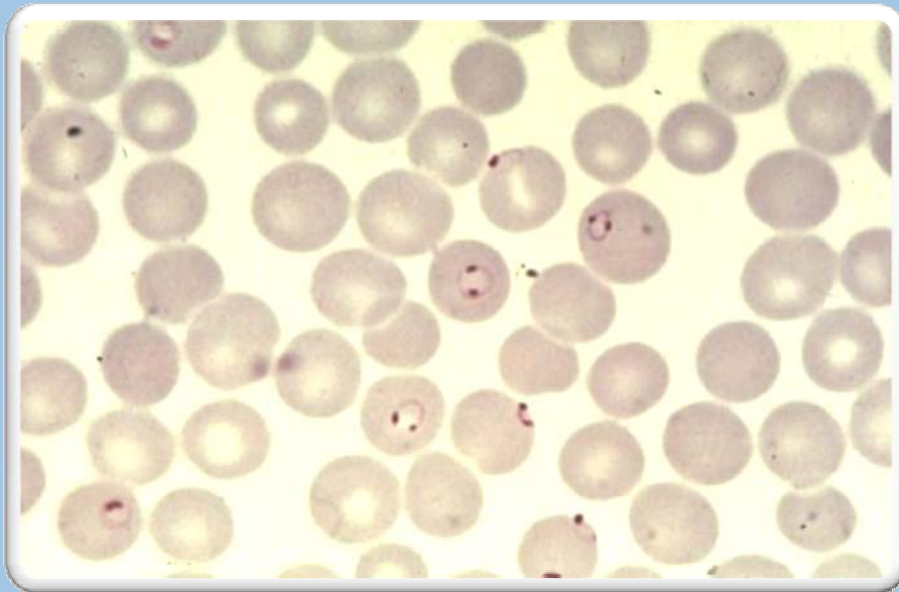
Aim Of Study

- The aim was to add a 'flag' in the analyser.
- Other flags and lab actions already in place.
- Emergency Department/refugee clinic patients.
- Study not successful. 22% of routine hospital patients with no malaria flagged positive.

In Summary

- Malarial parasites cause alterations in the VCS positional parameters
- Inter instrument variability requires local establishment of discriminant factors
- Difficulty in collecting sufficient data to establish and then validate local discriminant factors
- ? What happens when we change a laser – no external QAP comparative program for VCS positional parameters.

Stick To Microscopy!



1. <http://www.rph.wa.gov.au/malaria/teach.html>
2. <http://fuschmu.wordpress.com/2009/09/03/malaria-vaccine/>



Southern Health

Acknowledgments

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