**Diplomarbeit**

# Synergism of Pyronaridine and Retinol against P.falciparum In vitro


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unter der Anleitung von
**Prof. Dr. Walther H. Wernsdorfer**

Ort, Datum……. (Unterschrift)

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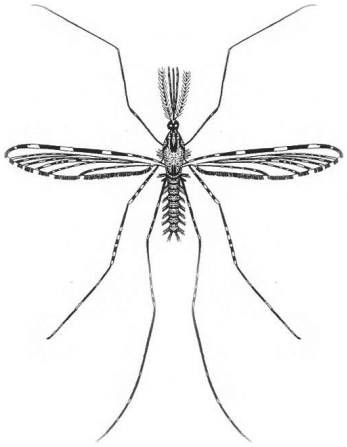

**This day relenting God**
**Hath placed within my hand**
**A wondrous thing; and God**
**Be praised. At his command**

**Seeking his secret deeds**
**With tears and toiling breath**
**I find thy cunning seeds,**
**O million-murdering Death.**

**I know this little thing**
**A myriad men will save**
**O Death, where is thy sting?**
**Thy victory, O Grave**

Ronald Ross
Poem, written august 22, 1897, following his discovery of malaria parasites in anopheline mosquitoes fed on malaria-infected patients[1].

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| | |
|---|---|
| Aa.: | Anopheline |
| ACT: | Artemisinin-based combination therapy |
| ACPR: | Adequate clinical and parasitological response |
| ARDS: | Adult respiratory distress syndrome |
| BMM: | Blood-medium-mixture |
| CDC: | Department of Communicable Disease Control |
| CI: | Confidence interval |
| DIC: | Disseminate intravascular coagulation |
| DBB: | Desbutylbenflumetol |
| DHFR : | Dihydrofolate reductase |
| EC: | Effective drug concentration |
| ETF: | Early treatment failure |
| FIC: | Fractional inhibitory concentration |
| $f_{PR}$: | Factor of the potency ratio |
| LPF: | Late parasitological failure |
| LCF: | Late clinical failure |
| LUM: | Lumefantrine |
| MDR: | Multi-drug-resistance |
| MCOC: | Arithmetic mean cut off concentration |
| MCP: | Malaria Control Programme |
| MOPH: | Ministry of Public Health |
| ml: | Mililiters |
| µl: | Micro liter |
| n: | Number |
| nmol: | Nanomol |
| nM: | Nanomolar |
| PCR: | Polymerase Chain Reaction |
| PR: | Potency ratio |
| PYR: | Pyronaridine |
| RBC: | Red Blood Cells |
| RET: | Retinol |
| SMI%: | Schizont maturation inhibition |
| SP: | Sulfadoxine-pyrimethamine |

# Abstract

*Keywords*: P.falciparum, multi-drug-resistant strains, in vitro test, drug sensitivity, correlation, drug interaction Pyronaridine, Retinol, Lumefantrine, DBB

The study was carried out in Mae Sot, north western Thailand between June and July 2008. This area is affected by multi-drug-resistant strains of P.falciparum.

Aim of the study was to analyze the synergistic interaction between Pyronaridine, Retinol and combinations of both in Plasmodium falciparum in vitro. The applied Retinol concentrations corresponded to the $50^{th}$, $65^{th}$ and $80^{th}$ percentile of the physiological serum retinol level. Furthermore the blood schizontocidal activities of Lumefantrine and Desbutylbenflumetol were tested and compared with earlier studies made in this area.

The investigation was based on the WHO standard in vitro micro-test Mark II for determining the inhibition of schizont maturation. Included in the study were 38 fresh blood isolates of P.falciparum. Besides the analyzation of the drug sensitivity of P.falciparum for the drugs mentioned above a correlation analysis was performed for all four substances as well as an interaction analysis for the interaction of Pyronaridine and Retinol.

The $EC_{50}$ values for Pyronaridine, Retinol, and Pyr-Ret low, medium and high were 12,65nM, 36365,61nM, 1,21nM, 0,63nM, 0,93nM, the $EC_{90}$ values were 261,35nM, 23046357,36nM, 13,99nM, 7,42nM, 8,15nM and the $EC_{99}$ were 3084,23nM for Pyronaridine, 102,60nM, 54,75nM and 47,80nM for Pyr-Ret low, medium and high. The $EC_{99}$ values for Retinol were not calculated, because the necessary Retinol concentration would have been too high and well above physiological levels in humans. The arithmetic mean cut off concentrations (MCOC) of schizont maturation of Pyronaridine, and for Pyronaridine in the combinations low, medium and high were 4878,95nM, 226,32nM, 114,05nM and 122,43nM.

In the correlation analysis of Lumefantrine and Pyronaridine, the $EC_{50}$ and $EC_{90}$ had a positive correlation while the $EC_{99}$ showed to be negative. All three EC values were none significant. Also the correlation analysis of DBB and Pyronaridine showed no significance for all three EC values, the $EC_{50}$ had a positive correlation while the $EC_{90}$ and the $EC_{99}$ showed to be negative. Only the correlation analysis of Pyronaridine and Pyr-Ret-l showed to be significant for the $EC_{50}$ and $EC_{90}$, but none significant for the $EC_{99}$. The correlation with between Pyronaridine and Pyr-Ret-m/h was none-significant for all EC values.

The interaction analysis showed for all combinations (low, medium and high) a similar percentage of synergistic behaviour of around 83,9% for the $EC_{50}$. Also for the $EC_{90}$ the synergistic behaviour of the combination was similar with around 98%. These results indicate strong synergism between Pyronaridine and Retinol and it can be suggested that the combination of both substances is augmenting the antimalarial effectiveness of Pyronaridine.

The sensitivity to Lumefantrine has declined between 2005 and 2008 but the $EC_{99}$ is still within the therapeutic range without a sign of resistance. Also DBB showed a slight reduction in its effectiveness compared to 2005. The correlation analysis of Lumefantrine and DBB showed a positive correlation for the all three EC values, $EC_{50}$ ($r=0,444842$; $p=0,01$), $EC_{90}$ ($r=0,558751$; $p=0,001$) and $EC_{99}$ ($r=0,358325$; $p=0,05$). All results are significant, the one of the $EC_{50}$ and $EC_{90}$ even highly significant. This outcome represents the similar mode of action of both substances, which was expected since they are chemically related drugs.

# Zusammenfassung

*Schlüsselwörter*: P.falciparum, mehrfach resistente Stämme, In vitro Test, Medikamentensensibilität, Korrelationen, Medikamenteninteraktion, Pyronaridine, Retinol, Lumefantrine, DBB

Die vorliegende Studie wurde im Zeitraum zwischen Juni und Juli 2008 in Mae Sot, Nordwestthailand, durchgeführt. Dieses Gebiet ist bekannt für seine Multiresistenzen von P.falciparum gegen viele gängige Antimalariamedikamente.

Ziel der Studie war es, den Synergismus zwischen Pyronaridin und Retinol gegenüber P.falciparum in vitro zu untersuchen. Die verwendeten Retinol Konzentrationen entsprachen der 50., 65. und 80. Perzentile der physiologischen Retinol-Serumkonzentration im gesunden Menschen. Des Weiteren wurde die blutschizontozide Wirkung von Lumefantrin und DBB getestet und mit Daten früherer Studien aus Mae Sot verglichen.

Die Untersuchung wurde mittels des WHO Standard In vitro micro-test Mark II zur Messung der Hemmung der Schizontenreifung durchgeführt. In die Studie wurden 38 frische Parasitenisolate von P.falciparum aufgenommen. Neben der Untersuchung der Sensibilität von P.falciparum gegenüber den schon erwähnten Medikamenten wurden auch die Korrelationen zwischen den einzelnen Medikamenten und eine Interaktionsanalyse gemacht. Die $EC_{50}$ Werte für Pyronaridin, Retinol und Pyr-Ret low, medium und high waren 12,65nM, 36365,61nM, 1,21nM, 0,63nM, 0,93nM, die jenigen der $EC_{90}$ waren 261,35nM, 23046357,36nM, 13,99nM, 7,42nM, 8,15nM und jene der $EC_{99}$ 3084,23nM für Pyronaridin, 102,60nM, 54,75nM und 47,80nM für Pyr-Ret low, medium and high. Die $EC_{99}$ Werte für Retinol konnten nicht berechnet werden, da die dafür notwenige Retinol-Konzentration weit über die physiologische Serumkonzentration hinausgegangen wäre.

Die arithmetischen Mittelwerte für die vollständige Hemmung der Schizontenreifung betrugen für Pyronaridin alleine und für Pyr-Ret low, medium, high 4878,95nM, 226,32nM, 114,05nM and 122,43nM.

Die Korrelationsanalyse zwischen Lumefantrin und Pyronaridin ergab für die $EC_{50}$ und $EC_{90}$ eine positive Korrelation, für die $EC_{99}$ war sie jedoch negativ. Es zeigte sich keine Signifikanz für alle drei EC Werte. Auch die Korrelationsanalyse zwischen DBB und Pyronaridin zeigte keine Signifikanz für alle drei EC Werte. Die $EC_{50}$ war positiv, die beiden anderen EC Werte jedoch negativ.

Nur die Korrelationsanalyse zwischen Pyronaridin und Pyr-Ret low zeigte eine Signifikaz und zwar für die $EC_{50}$ und die $EC_{90}$, für die $EC_{99}$ war diese jedoch nicht mehr gegeben.
Zwischen Pyronaridin und Pyr-Ret medium und high zeigte die Korrelationsanalyse wiederum keine Signifikanz.
Für die $EC_{50}$ zeigte die Interaktionsanalyse bei allen Kombinationen einen ähnlichen Prozentsatz an synergistischem Verhalten von 83,9%. Auch für die $EC_{90}$ zeigte eine ähnlichere Anzahl an Isolaten von rund 98% ein synergistisches Verhalten.
Die Ergebnisse weisen auf einen starken Synergismus zwischen Pyronaridin und Retinol hin. Dies lässt vermuten, dass die Zugabe von Retinol zu Pyronaridin die therapeutische Wirksamkeit von Pyronaridin noch verstärkt.
Zwischen 2005 und 2008 hat die Wirkung von Lumefantrin abgenommen, die $EC_{99}$ befindet sich aber immer noch im therapeutischen Bereich, weswegen man nicht von Resistenz sprechen kann. Auch DBB zeigte einen leichten Wirkungsverlust verglichen mit 2005. Die Korrelationsanalyse zwischen Lumefantrin und DBB ergab für alle EC Werte eine positive Korrelation, $EC_{50}$ ($r=0,444842$; $p=0,01$), $EC_{90}$ ($r=0,558751$; $p=0,001$) und $EC_{99}$ ($r=0,358325$; $p=0,05$). Diese zeigte sich auch für alle Werte signifikant, für die $EC_{50}$ und $EC_{90}$ sogar hochsignifikant. Das Ergebnis zeigt ein ähnliches Verhalten der beiden strukturell verwandten Substanzen.

# 1 Introduction

## 1.1 A short history of Malaria

Malaria parasites and humans have a long evolutionary association. It is presumed that host-parasite relations had already existed when humans where still living as nomads, but life in small itinerant groups limited a vast malaria transmission. With the first settlements along the river valleys of the Nile, the Euphrat and Tigris, the Indus, Ganges, the Hoangho and the Jangtsekiang[2], resulting from agricultural and pastoral activities, the population began to grow, and this was the beginning of substantial malaria endemicity. This development was favoured by the warm climate in these regions and periodical inundations. Later the plasmodial species were taken along during the immigration of humans to Europe. Here the climate was much colder, so malaria transmission was limited to the warm seasons.

In ancient texts from Egypt and China one can already find reports about periodical fevers which lead one to assume that these were of malarial origin[3].The first one to give a clear description of the clinic of malaria was Hippocrates of Kos (5$^{th}$ century B.C.). At this time he had already distinguished different patterns of fever. In his writings he describes these patterns as daily-fever (qoutidiana), tertian fever (tritaios) and four-day fever (tetartaios). In ancient roman times this classification changed to Malaria tropica for qoutidiana, Malaria tertiana for tritaios and instead of tetartaios they used the term Malaria quartana. In the following centuries many people died of Malaria and there was no knowledge of how to cure this disease[2]. Not until 1630 when Fathers of the Society of Jesus introduced a bark of a tree called Cinchona to Europe. This tree originated from Peru and was used there to cure the tertian fever. It was widely promoted in Europe and was called “Jesuit's bark”[4]. Although this newly introduced medicine promised to be a successful remedy against Malaria, two hundred years ago malaria was still widely distributed throughout the world, even in areas where today nobody would suspect it, like Canada, Finland or some parts in the north of Russia. Almost 90% of the world population was living in malaria endemic areas[3].The word “Malaria” comes from Italian and means “bad air”. This name was used by the Italian scientist F. Torti and referred to the fact that in Europe these periodic fevers were mostly common in marshy areas in the south of Europe, such as the Po plane in central Italy, and malaria transmission there was intense. According to Torti, Malaria was caused by the “bad air” of the swamps[2].

The first big breakthrough in malaria research was in the nineteenth century. Scientists like Virchow, Meckel and Frerichs had discovered that the pigment found in the organs of people who had died from malaria, and the pigment they had observed in the blood of malaria patients, resulted from the destruction of erythrocytes.

In the 1870s many scientists presumed that the cause of malaria was a bacteria. It was a French surgeon, Charles Louise Laveran, who disproved this theory and proclaimed that malaria was evoked by a parasite. But it was not until several years later that Ronald Ross, a Scottish physician found the rout of infection. He demonstrated that mosquitoes were the carrier of the parasite and identified the Anopheles mosquito as the vector of human malaria. Both discoveries led to the award of Nobel prizes for Laveran and Ross[4].

These new revelations provided a basis for reliable diagnosis and an establishment of malaria control.

The Second World War stimulated the search for new antimalarial methods, and DDT was developed as an insecticide to kill the mosquitoes, while the chloroquine group of drugs were synthesised as an effective treatment[5]. As a result of these discoveries, the malaria transmission in Europe and North America was more and more eradicated.

Shortly after, malaria was eliminated from Europe, USA, Australia and from some parts of northern Asia, and in other parts of the world malaria incidence was reduced. This success led to the euphoric possibility that malaria could be eradicated from the whole planet using these methods, and major campaigns were carried out to kill the mosquitoes and distribute drugs[5]. And indeed, in many countries the malaria transmission was reduced and in some countries like Sri Lanka malaria was almost eliminated. By 1975 around 50% of the world population lived in malaria-free areas[3]. This was a big achievement in the battle against malaria. Unfortunately this success was not long-lasting. Overconfidence led to a reduction in the research and control efforts.

As a result, in some areas malaria transmission began increasing again, and the situation deteriorated due to a widespread resistance of P.falciparum and P.vivax against the formerly used cheap and effective antimalarial drugs.

With the emergence of resistant parasites, the search for more effective drugs was necessary. Various drugs and drug combinations were assessed, and methods were developed to control and monitor degrees of resistance.

## 1.2 Malaria today and its distribution

### 1.2.1 Global

Still today, Malaria is one of the three major communicable diseases worldwide among AIDS and Tuberculosis. The world malaria report released by the WHO in 2008 gives an overview of the Malaria situation and its global distribution today. It's reported that the world wide malaria incidences in 2006 were estimated at up to 247 million clinical cases among 3.3 billion people at risk, causing nearly a million deaths, mostly children under the age of 5 in sub-Saharan Africa. But it has to be noted that these estimations are only based on numbers of national malaria control programmes (NMCPs).These case reports are far from complete in most countries, and the real malaria incidences are probably much higher[6].

**Illustration 1:** Approximate distribution of malaria

The malaria burden is not evenly distributed. The global pattern of malaria transmission suggests a disease centred in the tropics but with a slight distribution in the subtropics as well. 109 countries were endemic for malaria in 2008, 45 within the African region. The most affected among African countries were Nigeria, the Democratic Republic of Congo, Ethiopia, Tanzania and Kenya[6], and 90% of the clinically manifest malaria cases there

were due to P.falciparum[3], the only fatal human malaria parasite. The countries with the highest incidences outside Africa were India, Sudan, Myanmar, Bangladesh, Indonesia, Papua New Guinea and Pakistan[6].

Although big efforts had been made in the 60s and 70s of the last century to eliminate or at least to minimize malaria cases by evolving malaria programmes and major campaigns to kill the mosquitoes and distribute drugs[5], they could not solve the malaria problem. The failure of the global malaria eradication programme and the fact that malaria was eradicated in Europe and most parts of North America led to a loss of interest in the disease. The development of new insecticides and new effective and cheap drugs was not an interest for the industry anymore. Thus, the world today is facing an increasing disease burden[7].

Some of the reasons for this critical development are population movements into malarious regions, agricultural practices including the building of dams and irrigation schemes, deforestation, the weakening of public health systems in some poor countries afflicted by civil wars and unrest, and long-term climate changes. Another very important cause for the increasing malaria incidences is the development of resistance to drugs and insecticides worldwide[7]. Resistance mainly affects P.falciparum and on a lower percentage P.vivax. Even though a resistance to quinine was already documented in 1910, the dissemination of resistance stayed small. Today the situation has deteriorated due to a widespread resistance of P.falciparum and P.vivax against a big range of cheap and effective antimalarial drugs such as Chloroquine and Sulphadoxine/Pyrimethamine[8].

The ecological and social problems which result out of this situation are massive. Most of the countries where Malaria is endemic are suffering from poverty, and the consequences of the disease are multiple, including effects on fertility, population growth, saving and investment, worker productivity, absenteeism, premature mortality, severe anaemia in children and pregnant women, low birth weight and medical costs[7].

### 1.2.2 Thailand

Like in several other countries of Southeast Asia, Malaria used to be endemic all over Thailand[9]. While in 1949 malaria was the leading cause of death with 201.5 deaths/100.000 population, today the central part of Thailand is practically malaria-free. Only along the international borders, mainly the Thai-Cambodian and the Thai-Myanmar border, malaria is still prevalent. The reason why Thailand managed to become almost a malaria-free country was the establishment of sufficient malaria eradication programmes in the 50s of the last century. The Thai government developed a country-wide malaria control programme, first with the help of the WHO and later with the support of the United States[10]. Soon the malaria death rate decreased. By 1970 the attack phase was almost completed and a country-wide surveillance was started. Since then malaria incidences have dropped intermitted only by two increases in the 80s of the last century caused by drug resistant malaria[9]. The main goals today are vector control and monitoring the health status in the population as well as intensive information campaigns and putting insecticide treated bed nets at the population's disposal.

The structure of the current Malaria Control Programme (MCP) reflects the continuous downward trend of malaria during the last years. The Department of Communicable Disease Control (CDC) of the Ministry of Public Health (MOPH) decided to merge the MCP with the Filariasis and Dengue Haemorrhagic Fever Control Programmes. The idea behind the restructuring was to make the best utilization of human resources, budget and equipment for control of all mosquito-born diseases and to minimize relatively high cost of the MCP.

The current programme consists of 12 regional Disease Control and Prevention offices, each one directed by a medical officer, 39 Vector Borne Disease Control centres and 302 Vector Borne Disease Units at a provincial level[10]. Today, one of the biggest challenges for the MCP is the situation at the Cambodian and Myanmar border and the problem of resistance to the most commonly used drugs.

The reasons for the still-intense transmission in the border areas are the highly efficient vectors, the enhanced vector longevity, and the immense border trafficking.

The main vectors are Anopheles dirius, mostly found in the forest areas, and Anopheles minimus, which is widely distributed in the forest fringe areas and the rice paddies[10].

Recently P.vivax became the leading species, closely followed by P.falciparum. Other species, such as P.malariae and P.ovale are very rare[9].

## 1.3 Human-pathogenic Plasmodia

### 1.3.1 Plasmodium falciparum

Almost all deaths and severe malaria are caused by P.falciparum or Malaria Tropica, as it is also called. Compared with the other species, in evolutionary terms P.falciparum is a relatively young parasite of man (about 10.000 years old). Since most parasites need the host to survive, they endeavour not to kill it but to keep it alive. With its potential to kill, P.falciparum has been cited as an example of evolutionary immaturity. Severe or deadly diseases are very rare in the other human Plasmodia.

P.falciparum is mainly distributed in Africa south of the Sahara, Papua New Guinea, Haiti, South East Asia, Oceania and in some parts of Latin America[4].

Its perilousness results from its potential to invade all ages of Red Blood Cells (RBC), and its incubation period of 8-11 days. Compared to the other plasmodial species, this is relatively short. Its fever pattern is continuous remittent, and in early stages of the disease periodicity of the cycle cannot be observed. This often leads to an incorrect diagnosis which can be a fatal mistake. Therefore, its fast and reliable diagnosis in the peripheral blood is very important. The morphology of P.falciparum differs from the other human plasmodia.

It is possible that one RBC is invaded by more then one parasite, and often it shows double chromatin. Its pigment is brown-black. A mature schizont contains 24-36 merozoites. The duration of the erythrocytic schizogony is around 48h and the gametozytogony 12 days. An untreated primary attack usually last around 2-3 weeks. True relapses from the liver do not occur, and after a year, recurrences are rare. The maximum time duration of P.falciparum in the host is about 4 years[11].

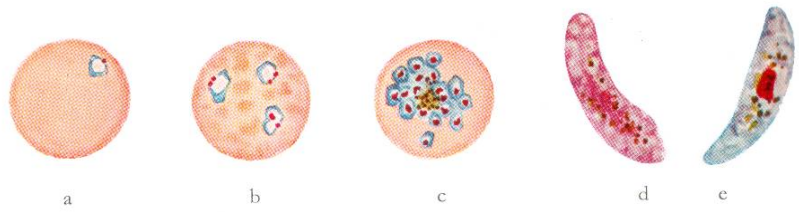


**Illustration 2:** *Plasmodium falciparum*, thin film, giemsa-stained.
a, b) Ringforms, c) Mature schizont, d) Microgametocyt, e) Macrogametocyt

### 1.3.2 Plasmodium vivax

P.vivax is one of the three “benign” malaria species along with P.ovale and P.malariae. Severe complications are rare, but coma and sudden death of cerebral involvement have been reported[11]. It is predominant in Central and South America, India, North Africa, and the Middle East, and in other parts of the world it coexists with P.falciparum, as in South East Asia and Oceania[4]. Its incubation period is 8-17 days, and it invades only the reticulocytes, the young RBC. Therefore the parasitaemia is usually limited to around 2-5% of the available RBC.

Together with P.ovale, P.vivax is called Malaria Tertiana because of its periodic fever pattern of 48h. Because of its limited parasitaemia, it can sometimes be hard to diagnose in the peripheral blood. The morphology of the trophozoit is mostly represented as the typical amoeboid form. The invaded young RBC is enlarged, deformed and covered with light pink dots, so called Schüffner-dots[12]. Its pigment is a delicate yellow-brown. A mature schizont contains 16-24 merozoites. The duration of the erythrocytic schizogony is around 48h and the one of the gametozytogony 48-96h. The parasite forms hypnozoits which can survive in hepatocytes in the human liver, and this can lead to relapses. An untreated primary attack usually last around 3-8 weeks, but true relapses can occur after weeks, months, or up to 5 years or more[11]. Because of its ability to develop these latent forms in the liver, the course of treatment has to be different from the one for P.falciparum infections.

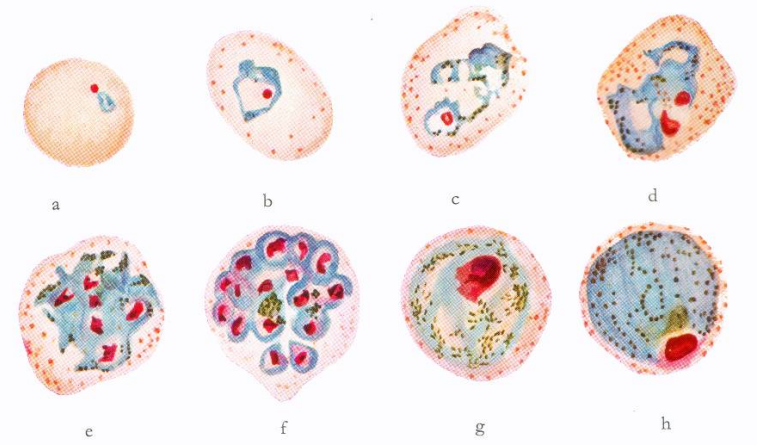


**Illustration 3:** *Plasmodium vivax,* thin film, giemsa-stained.
a,b) Ringforms, c) Amoeboid form,d, e) Young schizont,
f) Mature schizont, g) Microgametocyt,h) Macrogametocyt

### 1.3.3 Plasmodium ovale

The distribution of P.ovale is rare outside of tropical Africa but can also be found in the Middle East, Papua New Guinea, and Irian Jaya in Indonesia[11]. Reported cases of P.ovale infections from South East Asia are probably caused by different types of P.ovale, namely P.fieldi or P.simiovale, which can be regarded as zoonosis, originating from primates[13].
The morphology and the clinical symptoms of P.ovale are very similar to the ones of P.vivax, but it's not as severe as an infection caused by P.vivax. The incubation period is 10-17 days[11]. The object of invasion are reticulocytes, therefore the parasitaemia is also limited to 2-5%. Unlike P.falciparum, the invaded reticulocytes are not, or only minimally, enlarged and are oval shaped. Schüffner dots are also found[12]. Its pigment is black. A mature schizont contains 8-12 merozoites. The duration of the erythrocytic schizogony is around 48h and the one of the gametozytogony 48-96h. Like P.vivax the parasite forms hypnozoits, and this can lead to relapses but they are not as common as in a P.vivax infection[11]. An untreated primary attack can last around 2-3 weeks, and the parasite has a life span of around 5-7 years. However spontaneous recovery is often found[11].

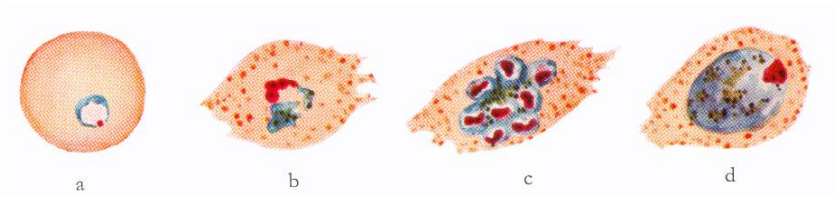


**Illustration 4:** *Plasmodium ovale*, thin film, giemsa-stained.
a,b) Ringforms, c) Schizont, d) Macrogametocyt

### 1.3.4 Plasmodium malariae

On a phylogenetic basis, this parasite is the oldest of all human pathogenic plasmodial species[13]. It's the most benign malaria infections of all plasmodial infections. It's found in most areas but is not very common outside Africa[4]. Coinfections with other plasmodial species, like P.falciparum, are not uncommon.

It is also called Malaria Quartana, because of its typical fever pattern - a fever attack every fourth day. The incubation period ranges from 27-40 days. The clinical symptoms are similar to the ones found in P.vivax but asymptomatic infections also exist.
The parasite invasion is restricted to old RBC, therefore parasitaemia is relatively low[11]. The morphology of young Trophozoits is similar to the one of P.falciparum, but they only have one nucleolus. Very characteristic is the ribbon-shaped amoeboid trophozoit inside the RBC. The invaded old RBCs are neither enlarged nor deformed and no Schüffner dots can be found[12]. Its pigment is black. A mature schizont contains 6-8 merozoites. The duration of the erythrocytic schizogony is around 72h and the one of the gametozytogony 72-120h. In some patients a latent infection can persist more then 20 years or even a lifetime. Proteinuria is commonly found; in children it may be associated with clinical signs of a nephrotic syndrome. The membranoproliferative type of glomerulonephritis is a dreaded complication of a chronic infection and is usually not reversible with therapy[11].

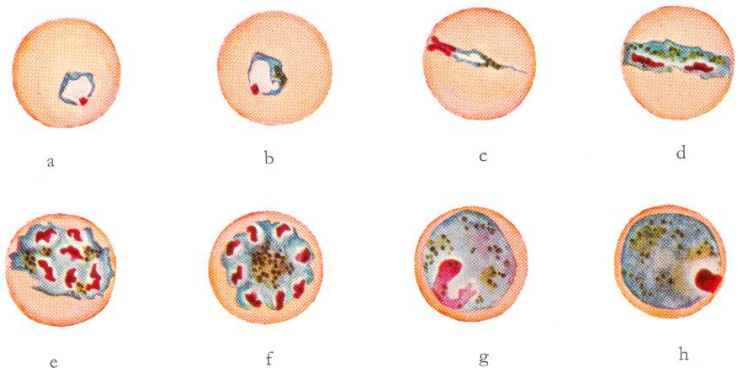


**Illustration 5:** *Plasmodium malariae*. thin film, giemsa-stained
a,b) Ringforms, c) Amoeboid form,d, e) Young schizont,
f) Mature schizont, g) Microgametocyt,h) Macrogametocyt

### 1.3.5 Plasmodium cynomolgi

This species is normaly observed only in apes, Maccaca fascicularis, and is transmitted by A. balabcensis introlatus, but infections of human can occur when habitats of men and animal overlap. The morphology of P.cynomolgi is very similar to the one of P.vivax, and it also shows an expression of hypnozoits.
The dissemination of this parasite is restricted to some areas in South East Asia[13].

### 1.3.6 Plasmodium knowlesi

This parasite occurs also in Maccaca fascicularis and is transmitted by A.hackeri. So far it has been only found in Malaysia, and since it is very similar to the morphology of P.malariae, it is sometimes confused with this plasmodial species[13].

## 1.4 Life Cycle

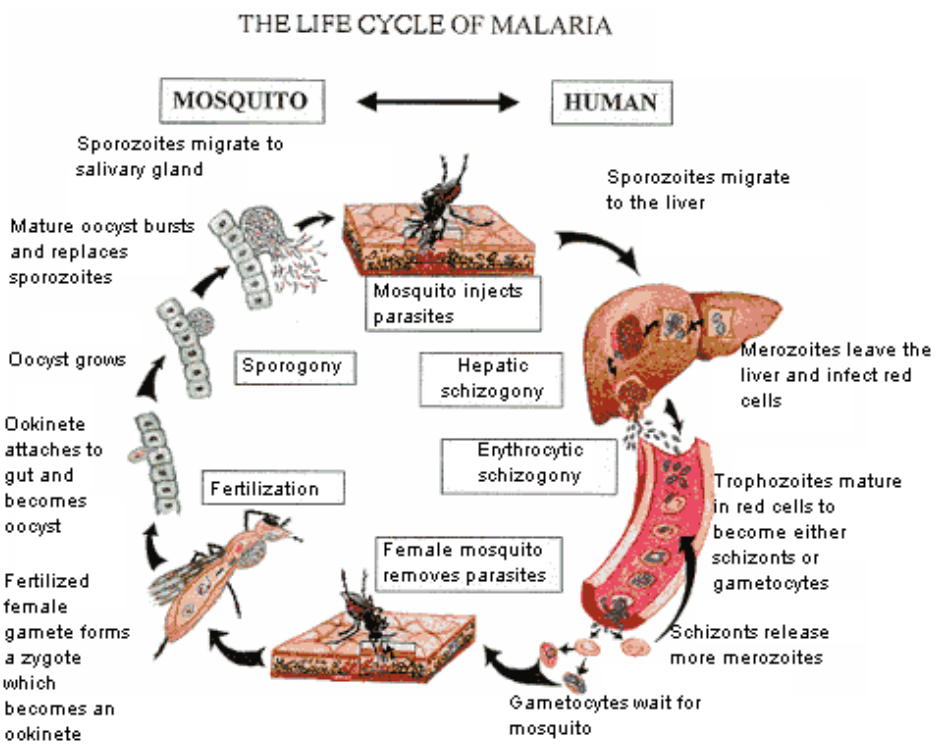


**Illustration 6**: Life Cycle

### 1.4.1 The Vector

The carrier for the human pathogen plasmodium is the female anopheline mosquito. Out of 400 different anopheline species, only 80 can transmit malaria. Its distribution is not restricted to the tropics and subtropics; anophelines are found worldwide, even in the arctic territories.

But to transmit malaria, a special temperature is needed otherwise development in the mosquito (sporogony) can not take place. Below 16C° and above 33C° malaria transmission does not occur, therefore a temperature range in between 20C° and 30C° and humidity form the optimum conditions. In many parts of the world malaria transmission is limited only to the rainy seasons, which provides water for mosquito breeding.

The lifespan of one mosquito is several weeks. But there is a big variety in the behaviour of the anophelines mosquitos in different parts of the world and between the different species[4]. For example, A. dirus which is, among A. minimus, the main vector for malaria transmission in Thailand lives in the forest while A. minimus plays a major role due to its wide distribution in the forest fringe areas[10]. Even the potential to transmit malaria differs between the species. The most potent malaria vector of all is the A. gambiae, which mainly lives in sub-Saharan Africa. Its effectiveness results from its ability to bite humans frequently, long lifespan and its high density[4].

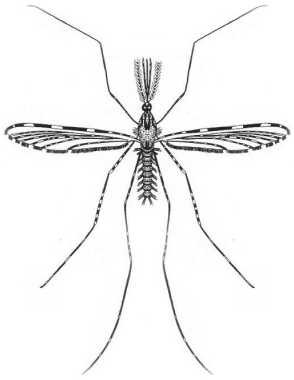

**Illustration 7**: Anopheline mosquito

### 1.4.2 The human host

In the distribution of malaria, the behaviour of men plays an important role. Appointed men- made actions, like changing the pattern of land use and water management, led to an eradication of malaria in Europe and parts of North America[3]. But in other parts of the world, like Africa or Asia, deforestation, building of dams and irrigation schemes, and population movement into malarious regions contributes to an increase of malaria incidences[7]. Besides this, on a smaller level, vector contact depends also on occupation, settlement patterns, age, sex, housing conditions[3] and the access to effective malaria treatment. In hyperendemic or holoendemic areas, infants and children less than five years old are the most vulnerable members of society. They represent the main reservoir for gametocytes, because parasitaemia in this age group is very high.

Protective immunity which is sufficient to prevent mortality usually develops within the first five years after birth. Older age groups also show infections, many of which are accompanied by mild symptoms or are asymptomatic[4], a sign of acquired semiimmunity. This suppresses parasite development and therefore, parasitaemia is very low, sometimes below microscopical detection[3].

In other parts of the world where malaria is hypoendemic or mesoendemic, all age groups are equally affected by malaria infections, and semiimmunity is not acquired.

P.falciparum malaria is more severe in pregnant women, especially in primigravidae[4].

### 1.4.3 Pre- erythrocytic development

When the anopheline mosquito takes its blood meal it simultaneously discharges sporozoits out of its salivary glands into the puncture wound. Via the blood stream, these stages of the parasite are transported to the liver where they penetrate the hepatocytes. Here they begin to grow until each infected hepatocyt contains several merozoites. This stadium is called pre-erythrocytic cycle. After the hepatocytes are ruptured the merozoits leave the liver and invade the RBCs in the peripheral blood, thus initiating the so called erythrocytic cycle.

In two malaria species, P.vivax and P.ovale, some of the parasites remain in the liver as hypnozoits, which can lead later on to a relapse of the infection[11].

### 1.4.4 Asexual blood stage development

Inside of the RBC the parasite grows and feeds on haemoglobin. The merozoit, or young trophozoit, is vacuolated, ring shaped and in most species has only one nucleolus.
The malarial pigment, produced by the parasite, consists of protein, hematin and iron porphyrin. When the trophozoit grows and the nucleolus begins to divide, it becomes a pre-schizont and later a mature schizont, containing several merozoiets. With the release of merozoiets into the blood stream, the first fever attack occurs, because they stimulate the immunity by releasing malaria toxins and haemozoin. Many of these merozoites get destroyed by the immune system but some escape and invade new RBCs and a new cycle begins[11].

### 1.4.5 Sexual stages and development in the mosquito

After several of these erythrocytic cycles some of the merozoites do not develop into schizonts, but form female (macrogametocytes) or male (microgametocytes) gametocytes, the sexual forms of the parasite.
These gametocytes are ingested while a mosquito feeds. In the mosquito gut they mature into gametes. The male microgamete undergoes a process which is called exflagellation and then penetrates the female macrogamete. Together they form a zygote, and this develops into an ookinete. This form of the parasite then migrates from the gut to the midgut and becomes an oocyst. The oocyst grows into the mature form within two days to two weeks and contains hundreds of sporozoits. When the oocyst ruptures, these sporozoits are set free and some of them make their way into the salivary glands of the mosquito. With the next blood meal of the anopheline mosquito these sporozoites are discharged into the puncture wound, and the life cycle begins all over again[11].

## 1.5 Pathophysiology and clinical symptoms of P. falciparum

Since P.falciparum tends to infect any RBC regardless of age, very heavy infections may result from this. The mass of parasitized RBCs can cause a massive internal ischemia by plugging vessels in different organs. This can lead to several different symptoms depending on the organ which is affected. The cause of this plugging of vessels by infected RBCs is not yet fully known, but it is suggested that the RBCs lose their ability to change shape while passing through the small capillaries. They become inflexible and tend to stick to each other and to the endothelial lining of the capillaries of the internal organs. Therefore, in the peripheral blood only the ring forms and the sexual forms can be observed[11]. P.falciparum infections can be roughly divided into uncomplicated and severe malaria, depending on the clinical symptoms.

### 1.5.1 Uncomplicated malaria

The first symptoms of an infection caused by malaria tropica occur 8-12 days after the mosquito bite and the inoculation of the sporozoites. In the beginning these symptoms often can't be distinguished from a normal influenza. Headache, pains, fatigue, anorexia, nausea, chills, or vomiting are very common non-specific symptoms. Its fever pattern is continuous remittent, and in early stages of the disease periodicity of the cycle cannot be observed. This often leads to a misdiagnosis which can be a fatal mistake. If the patient lives in a holo–or hyperendemic area, he might have already acquired semiimmunity, and an untreated primary attack usually ends within 2-3 weeks. In a patient with nonacquired semiimmunity the primary attack can be fatal[11].

### 1.5.2 Severe malaria

Although only a minority of cases progress to severe life threatening illness, the absolute number is enormous, with around one million deaths each year in sub-Saharan Africa only. Severe malaria is an extremely complex multi-process and multi-system disorder[14].
The progression to severe disease can be rapid and is related to the above described plugging of vessels in the internal organs[11].

In young children, the history of symptomatic malaria can be as short as one day before they develop severe malaria. In adults the development of severe malaria usually is not as fast as in children[4].

**Definition of severe falciparum malaria proposed by the WHO[15]:**

- Prostration
- Severe anaemia
- Renal impairment
- Pulmonary oedema or ARDS
- Hypoglycaemia
- Circulatory collapse or shock
- Spontaneous bleeding, or DIC
- Impaired consciousness
- Multiple convulsions
- Jaundice
- Haemoglobinuria
- Hyperparasitaemia
- Hyperlactataemia
- Acidosis

One or more of the symptoms described above and an asexual parasitaemia diagnosed in the patient's blood defines severe falciparum malaria.

In many cases, multiple vital organ dysfunctions occurs[4]. When severe falciparum malaria is diagnosed the patient should be immediately delivered into a clinic where specific antimalarial-treatment, adjunctive therapy and supportive care are assured.

The mortality of untreated severe P.falciparum infection is, depending on the acquired semiimmunity of the patient, up to 100%. With correct antimalarial-treatment the mortality falls down to 15-20%[15].

## 1.6 Diagnosis

Although the case history is very important for the diagnosis of malaria, it is not a clinical diagnosis. The diagnosis should be laboratory-based by a microscope. This is a relatively simple method to ascertain a plasmodial infection[12].

Both the thin as well as the thick film are prepared by taking blood from the patients' fingertips. A drop of the blood should be placed at each of the slides. The thick film has to be dried while the thin film gets smeared until a very fine layer of blood is visible. This thin film than has to be fixed in anhydrous methanol. Both slides are then Giemsa stained, and after drying in a slide rack they can be examined under oil immersion at a magnification of x1000[4]. Since one single negative example will not rule out a plasmodial infection, it is very important to examine not only one set of films, but additional blood specimens should be examined over a 36-h time frame[11].

There are alternative ways to diagnose malaria, like antigen detection tests (also known as rapid or “dipstick” tests), molecular tests and serology[16], but the gold standard is still the microscopic examination of a thin and a thick blood smear because it’s a cheap, simple, fast and reliable method[11].

## 1.7 The Antimalarial drugs

### 1.7.1 Classification and mode of action

One can classify antimalarial drugs according to their activity or to their structure.
Activity of antimalarial drugs:

Different antimalarial drugs interfere selective in different development stages of the malaria parasite. They can be classified in tissue schizonticides for causal prophylaxis, tissue schizonticides for preventing relapses, blood schizonticides and gametocytocides[17].

| | |
|---|---|
| **Exoerythrocytic cycle:** | |
| tissue schizonticides for causal prophylaxis | These drugs block the development of primary tissue forms of the plasmodia. By inhibiting these early stages, further development of the infection can be theoretically prevented.<br>Agents: Pyrimethamine and Primaquine[17]. |
| tissue schizonticides for preventing relapses | Drugs of these category act on the hypnozoites of P.vivax/P.ovale in the liver and therefore prevent relapses.<br>Agents: Primaquine and Pyrimethamine[17]. |
| **Erythrocytic cycle:** | |
| blood schizonticides | By oppressing the asexual reproduction of the parasite in the erythrocytes, these drugs can terminate the clinical attacks. They act as prophylaxis as well as treatment.<br>Agents: Chloroquine, Quinine, Mefloquine, Halofantrine, Sulfadoxine, Sulfones, Pyronaridine, Pyrimethamine, Tetracyclines, Proguanil, Amodiaquine, Artemisinins, Atovaquone, Lumefantrin[18,11,17]. |
| gametocytocides | These drugs are acting on the sexual forms of the plasmodia therefore prevent transmission from men to mosquito.<br>Agents: Chloroquine and Quinine only against P. vivax/P.malariae, Primaquine also against P.falciparum. |

**Table 1:** Classification of drugs

Structure of antimalarial drugs:

- Aryl-amino-alcohols:
  Quinine, Quinidine (cinchona alkaloids), Mefloquine, Halofantrine.

- 4-aminoquinolines:
  Chloroquine, Amodiaquine.

- Folate-synthesis inhibitors:
  Type 1: competitive inhibitors of dihydropteroate synthase - Sulfones and Sulfonamides.
  Type 2: inhibit dihydrofolate reductase – biguanides like Proguanil and Chloroproguanil, Diaminopyrimidine like Pyrimethamine.

- 8-aminoquinolines:
  Primaquine.

- Antimicrobials:
  Tetracycline, Doxycycline, Clindamycin, Azithromycin, Fluorochinolones.

- Peroxides:
  Artemisinin (Qinghaosu) derivatives and analogues like Artemether, Arteether, Artesunate, Arteinic acid.

- Naphtoquinones:
  Atovaquone.

- Iron chelating agents:
  Desferrioxamine.

- Aryl alcohols: Lumefantrine (Benflumetol).

- 1,5 Naphthyridine/Mannich-base: Pyronaridine.

- Bisquinoline: Piperaquine.

In their mode of action antimalarial drugs can be classified as inhibitors of hem polymerase, inhibitors of the synthesis of folic acids and respiratory chain and agents which act as free radicals.

Inhibitors of hem polymerase:

By inhibiting the parasites hem polymerase, drugs like Quinine, Chloroquine, Mefloquine Halofantrine are effective against blood schizonts[19]. For its reproduction the parasite is dependent on essential amino acids which it gains from degradation of haemoglobin of the host to haem (haematin).

Haem oxidizes to the toxic ferric form, ferriprotoporphyrine IX, a membrane damaging substance which can kill the plasmodia. By polymerization of ferriprotoporphyrine IX to haemozoin, the parasite is detoxifying this substance. The haemozoin (malaria pigment) is then stored in the parasites food vacuole. For this process, the enzyme hem polymerase is essential. If this enzyme gets inhibited by drugs, the transformation of haemoglobin to haemozoin is impossible and the parasite is killed[18].

Inhibitors of the synthesis of folic acid and respiratory chain:
Another way to block the asexual reproduction of the parasite is interference in the synthesis of folic acid or inhibiting the parasites respiratory chain. Agents which interfere with folic acid synthesis by blocking the bifunctional enzyme dihydrofolate reductase-thymidilate synthase (DHFR) are Pyrimethamine and Proguanil. Sulfonamides and Sulfones are interfering in the synthesis of folic acid as well, only in a previous step in the synthetic pathway[4].
Atovaquone acts on the parasites mitochondria. It inhibits the respiratory chain of the parasite by interfering with mitochondrial electron transport[20].

Oxidative damaging agents:
The mechanism of action of Artemisinin and its derivatives is not yet fully known but it appears to involve oxidative damage. His endoperoxid structure can be catalysed by hem-bounded iron to free radicals. These are interfering with the parasites membrane proteins, thereby causing oxidative damage of the plasmodia[4,19]. These drugs are fast acting and have a very short half life[20].

### 1.7.2 Antimalarial Treatment

Because of the widespread patterns of drug resistance in the world today to many antimalarial drugs, it is not recommended to treat malaria without knowing the local resistance patterns. For example, in the treatment of a Plasmodium falciparum infection Chloroquine can no longer be used in most parts of the world, therefore alternatives must be considered[21]. There are special drug regimes for the different plasmodial infections.

Treatment of benign malarias (P.vivax, P. ovale, P.malariae):

The recommended standard therapy is still Chloroquine unless the infection is acquired in geographical regions where Chloroquine resistance is reported. Since the hypnozoite stages in P.vivax and P ovale are not affected by Chloroquine, Primaquine must be given to prevent relapses and achieve a radical cure. In case of Chloroquine resistance Amodiaquine in combination with Primaquine should be given as an alternative[24].

Treatment of uncomplicated P.falciparum malaria:

The current recommended treatment of choice for uncomplicated malaria is an Artemisinin-based combination therapy (ACT), monotherapy should be abandoned.

ACT means the simultaneous use of two or more blood schizonticidal agents with independent modes of action. One of these agents is an Artemisinin derivative, one of the most rapidly acting antimalarial drugs. Examples are Artemether and Lumefantrine or Artesunate and Mefloquine. The idea behind these combinations is to keep effectiveness and to prevent resistance[24].

Treatment of severe malaria:

Severe malaria is always an emergency and should be treated in a hospital because of its increasing morbidity and mortality by delays in proper medical care and the need for parenteral antimalarial therapy[21]. There are different treatment options depending on the transmission likelihood:

The WHO recommended therapy in non-malaria endemic areas with low transmission and in high transmission areas is Artesunate.

Other WHO recommended drugs for use in high transmission areas are Quinine and Artemether.

Quinidine should only be used if the other, recommended, parenteral antimalarial drugs are not available[24].

### 1.7.3 Drug resistance

Resistance to antimalarial drugs is proving to be a challenging problem in malaria control in most parts of the world.

Over the last generation, P.falciparum has become widely resistant to all known antimalarial drugs, except of the artemisinin derivatives. This resistance has developed to various degrees in many countries. More recently, resistance of P.vivax to Chloroquine and/or Primaquine[16]has also been observed but is much more limited.

The chloroquine resistant P.falciparum strains are distributed in almost all areas where P.falciparum is transmitted except for malarious areas in Central America north of the Panama Canal, Haiti, The Dominican Republic, and the Middle East[22].

Resistance against Sulfadoxine-Pyrimethamine (SP) affects South East Asia and South America. It is becoming more prevalent in Africa south of the Sahara as SP has been used there as a replacement for Chloroquine. Resistance against Mefloquine occurs in the Amazonian region and in some parts of South-East Asia as well as in some areas in Africa[16]. Drug resistance against antimalarial drugs is defined by the WHO as:

"Ability of a parasite strain to survive and/or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within tolerance of the subject"[16].

If a parasite is not only resistant to an antimalarial drug of one class but to antimalarial drugs of two or more classes this is called Multi-drug-resistance (MDR).

There is evidence of MDR in some parts of South-East-Asia. Mainly affected is the border region of Thailand- with Cambodia in the east and Myanmar in the west.[23]

There are several molecular mechanisms for the evolution of drug resistance.

In some cases, like in Chloroquine, the parasite finds a way to increase its capacity to excrete the applied drug out of its food vacuole. In this way, the drug can't reach the necessary level which is needed to inhibit the haem-polymerization.

In other cases, resistance is conferred to single point mutations, like resistance to Atovaquone.

These point mutations lead to an alteration of the target protein of the drug. The result is a loss of function of the drug, because it has lost its specific target[16].

Drug resistance is genetically determined and is independent of drug pressure. Wild plasmodial strains are more or less sensitive to antimalarial drugs. But naturally resistant parasites have a survival advantage when confronted with antimalarial drugs[4].

Under certain circumstances these mutants can spread rapidly and form a new resistant plasmodial strain. For example, widespread use of drugs in sub-therapeutic levels can provoke drug resistance. The administered drug concentration is not enough to kill all parasites. While most of the parasite population gets eradicated by the drug, some parasites can survive because they can cope with the administered drug level, thus resulting in a survival advantage and reproduction of these parasites. A similar problem is observed when drugs with a long half-life (like Mefloquine or Chloroquine) are used in areas with massive malaria transmission. A long half-life is related to longer periods of sub-therapeutic drug levels.

Every new infection, received in the long post-therapeutic elimination phase, can lead to resistance with the same mechanism as it is described above[3]. But not all treatment failures are caused by drug resistance. There are cases where treatment failure has other reasons than drug resistance. For example, non compliance of the patient in the duration of dosing, incorrect dosing, poor ingestion of antimalarial drugs by very sick patients, expired drugs or poor quality of the drugs[16]. It is important to discriminate these two causes of treatment failure. While a treatment failure caused by the reasons described above can be solved, treatment failure caused by drug resistance is yet an unsolved problem. Therefore big efforts have to be made to counter this problem. The surveillance of therapeutic efficacy and development of resistance plays a major roll in malaria control. It can be made by several methods: in vivo tests, in vitro tests, molecular methods and animal models.

In the In vivo test a group of infected patients gets treated with known dosages of an antimalarial drug while their clinical and parasitological response is constantly monitored[16].

Their response to treatment is classified in: Early treatment failure (ETF), Late clinical failure (LCF), Late parasitological failure (LPF) and Adequate clinical and parasitological response (ACPR)[24].

| ETF | LCF | LPF | ACPR |
|---|---|---|---|
| Development of danger signs or severe malaria on day 1 to 3 in the presence of parasitaemia.<br><br>Parasitaemia on day 2 higher than the day 0 count irrespective of the axillary temperature.<br><br>Parasitaemia on day 3 with axillary temperature ≥37,5°C.<br><br>Parasitaemia on day 3 that is ≥25% of count on day 0. | Development of danger signs or severe malaria After day 3 in the presence of parasitaemia, without previously meeting any ETF criteria.<br><br>Presence of parasitaemia and axillary temperature ≥37,5°C on any day from day 4 to day 28, without previously meeting any ETF criteria. | Presence of parasitaemia on any day from day 7 to day 28 and axillary temperature <37,5°C, without previously meeting any of the criteria of ETF or LCF. | Absence of parasitaemia on day 28 irrespective of axillary temperature without previously meeting any of the criteria of ETF, LCF or LPF. |

**Table 2:** Classification of response of treatment

The advantage of this test is its reflection of actual clinical and epidemiological situations.

The disadvantage compared to the In vitro test is the influence of external factors, and therefore an exact prediction of the atimalarial drug resistance can not be made.

The in vitro test measures the inhibition of schizont maturation in fresh blood isolates of P.falciparum by increasing drug concentrations in a controlled experimental environment, thus avoiding the confounding factors described above. This test is useful to get information about the pure drug resistance, but it measures only the interaction between the parasite and the drugs and leaves out the host response. While the in vivo test is easy and cheap, the in vitro test is relatively expensive and complicated for routine work in the field[16].

The molecular methods may be the future tests to study and survey antimalarial drug resistance because they have many advantages compared to the methods described above. Polymerase Chain Reaction (PCR) is used to determine mutations which lead to drug resistance and to detect the mechanisms of MDR[25].

Another important method for identifying and monitoring antimalarial drug resistance is case reports of treatment failure. It is relatively easy and cheap and can be carried out by health care clinics on a regional basis[16].

While these monitoring methods described above are an essential component of malaria control, there are further strategies to combat the problem of drug resistance and to prevent the development of new resistance against still-efficient drug regimes.

These strategies include:

- Replacing none efficient antimalarial-drugs with effective ones such as artemisinin-based combination therapy (ACT) worldwide (use of monotherapy in the treatment of P.falciparum should be banned).
- Better access to public sector health care for everybody and a health care personal which is adequately trained, sufficient supplied and efficient supervised.
- Provision with prompt access to antimalarial drugs for those who need to be treated while at the same time reducing the inappropriate use of those same drugs.
- Treatment should be based on a laboratory-confirmed diagnosis.
- Intensified research for new drugs and drug combinations[6].

However, besides the need for the development of new antimalarial drugs it is important to use the available and still effective drugs in a way to retain its effectiveness as long as possible[3]. This can be achieved by combining them with one another and with substances like retinol.

## 1.7.4 Drugs used in the study

### 1.7.4.1 Lumefantrine

Lumefantrine, also known as Benflumetol, is a racemic fluorine derivative. Its chemical name is 2-dibutylamino-[2,7-dichloro-9-(4-chlorobenzylidene)-9H-fluren-4yl]-ethanol.

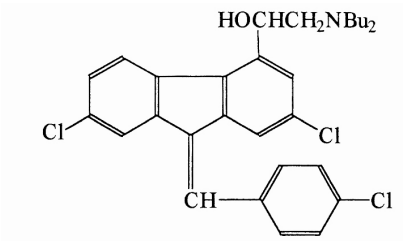


**Illustration 8:** Lumefantrine

It has a chiral centre and appears therefore in a laevorotatory and dextrorotatory form. Both have similar activity against P.falciparum[26].

Structurally, physicochemically, and in the mode of action it has similarities to the aryl-amino-alcohol-group of antimalarial substances like Quinine, Mefloquine, and Halofantrine[27].

It shows blood schizontocidal action against different plasmodial species, also to Chloroquine resistant strains[28]. It interferes with haem, a degradation product of haemoglobin but its specific mechanism of action is still not exactly known[29].
Compared with the antimalarial activity of artemisinine derivatives it is slower acting but the recrudescence rate is lower when used at the recommended dose regime[28].
Pharmacokinetically, Lumefantrine has a variable oral bioavailability, a large volume of distribution, and an elimination half life between 4 to 5 days[27]. It gets metabolized in the liver by the enzyme CYP3A4[30]. It is highly lipophil, therefore if given after meals rich in fat oral bioavailability can be augmented. Compared to the similar aryl-amino- alcohol group of antimalarial substances like Quinine and Halofantrine, Lumefantrine has little or no effect on ventricular repolarisation and can therefore be used in patients with long QTc syndrome or any other cardiac abnormalities[27].
It was originally synthesized in the 1970s by the Academy of Military Medical Sience in Beijing, China. In using Lumefantrine as monotherapy of P.falciparum infections in China, it showed a cure of more than 90%[27]. Today it is mostly used in combination with other antimalarial drugs such as Artemether. Well known drug combinations are Co-artem® or Riamet® (Artemether and Lumefantrine)[30].

### 1.7.4.2 Desbutylbenflumetol (DBB)

DBB is a 2,3 benzindene and is closely related to Lumefantrine (Benflumetol) of which it is assumed to be a metabolite. Like Lumefantrine it has a blood schizontocidal effect on malaria parasites, including multidrug-resistant strains of P.falciparum.

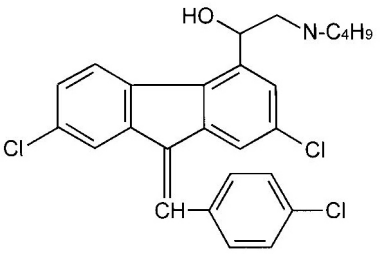


**Illustration 9:** DBB

While Lumefantrin has a dibutylamino-ethanol as a side chain in the 4-position, DBB carries a monobutylamino-ethanol at this position. Although it is assumed to be a metabolite of Lumefantrine, so far DBB has not been detected in patients treated with Lumefantrine. Since the structures of the amino-aryl-alcohol antimalarials and DBB are related, the mechanism of action could be similar. But until now, there exists not much data about the pharmacodynamic and pharmacokinetic action of DBB[31].

In two earlier in-vitro studies made in Mae Sot and Mae Hong Son/ Thailand[31, 32], DBB showed a high efficacy in fresh isolates of multi-drug-resistant P.falciparum strains.
Its in-vitro activity was more than four times higher than the one of the closely related Lumefantrien[31].

### 1.7.4.3 Pyronaridine

Pyronaridine was originally developed at the National Institute of Parasitic diseases in Shanghai, China.
This drug, a 2-methoxy-7-chloro-10[3',5'-bis(pyrrolidinyl-1-methyl-) 4'hydroxyphenyl]aminobenzyl-(b)-1,5-naphthyridine, is a highly active blood schizontocidal mannich base antimalarial drug.

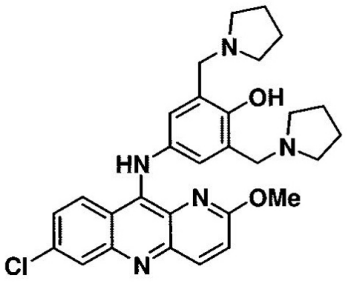


**Illustration 10:** Pyronaridine

It is structurally related to the aminoacridine drug Quinacrine. About twenty years ago it was registered and since then used in China and neighboring countries as an antimalarial drug. It is highly effective against P.falciparum and P.vivax, mainly in treating malaria infected patients in regions of Chloroquine resistance[33, 34].
Its mechanism of action is not yet fully known, but it probably acts similar to the 4-aminoquinoline Chloroquine. In vitro studies showed a ß-hematin formation inhibition (a process which is similar to the hemozoin formation within the parasite food vacuole). Moreover it forms a drug-hematin-complex, inhibits glutathione-dependent degradation of hematin and enhances hematin induced lysis of erythrocytes[34].
Pyronaridine given orally, intramuscularly or by intravenous drip is not only effective against malaria but is showing low toxicity and few side effects[33]. The disadvantage of using Pyronaridine as an antimalarial drug is its fast introduction of resistance by high drug pressure. This led to a decrease of its effectiveness against P.falciparum on the border region between Thailand and Myanmar[35] where it has been used as monotherapy.
Recently, a study in Gabon has analyzed the effectiveness of a Pyronaridine-Artesunate combination therapy for the treatment of uncomplicated falciparum malaria in children. The results showed a good tolerability and safety profile, at all dose levels of the drug[36].
Another study, made by the London school of Hygiene and Tropical Medicine, also reports that the combination of Pyronaridine and Artesunate presumably has a potential in areas of multidrug resistant malaria[37]

1.7.4.4 Retinol

Vitamin A or Retinol, a fat soluble retinoid, and its metabolites play diverse roles in physiology of humans.

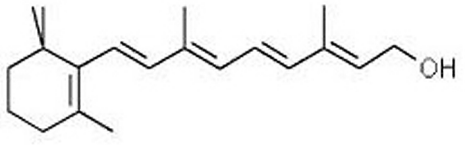


**Illustration 11:** Retinol

The two important active metabolits of Retinol are Retinoic acid, which acts like an intracellular messenger that affects gene expression and thereby influence numerous physiological processes, and Retinal. This metabolite is a necessary structural component of rhodopsin[38]. The main dietary source of Retinol is from animal sources, of which liver contains the most Retinol of all. Vegetables or any other plants do not contain Retinol but they contain provitamin A carotenoids, which are converted with the intestinal mucosa to Retinol during absorption[39]. Besides the visual function, Retinol and its metabolites have profound physiological effects. They are essential in reproduction, bone remodelling, epithelial cell "integrity" and in the immunological function[38].

The important role which Retinol is playing in the human body is evidenced by the severe disorders that accompany deficiency or excess states. Pre-existing Retinol deficiency is associated with a range of infectious diseases which can lead to increasing numbers of morbidity and mortality[40].

Additionally, there exists data that infections per se can reduce synthesis of retinol-binding protein which leads to reduced circulating levels of Retinol[38, 40]. A reduction of Retinol concentration in human serum was also observed in patients infected with falciparum malaria[41], in inverse proportion to disease severity and parasite burden[42]. In healthy persons, the average Retinol serum concentration is 1-3µmol/l[40], while in patients with malaria this level decreases by 50%[43].

Several studies were made to investigate if additional given Retinol has an impact on malaria severity. Randomized controlled trials of Vitamin A supplementation showed a decreased risk of clinical malaria in children in Papua New Guinea[44], and decreased likelihood of developing active placental malaria in Ghanaian primigravidae[45], while in another investigation made in Ghana no effect was reported[46].

In vitro studies have shown that Retinol inhibits the growth of cultured P.falciparum[42] and in combination with antimalarial drugs can enhance the activity of these substances[32, 42, 47].

These outcomes show that P.falciparum is a Retinol-sensitive parasite and that supplementation of specific antimalarial drugs by Retinol may accelerate parasite dysfunction and death in patients with malaria falciparum infection[40].

The exact mechanisms of these findings yet remain unclear. It is proposed that Retinol might inhibit DNA, protein synthesis and membrane effects of the parasite and may act as a pro-oxidant in certain situations[40]. Furthermore, it was shown that 9-cis-Retinoid acid is able to enhance phagocytic activity of macrophages and monocytes, which is leading to an increasing parasite clearance[48].

Besides an antimalarial activity, Retinol might have other benefits since host factors such as immunfunction and the integrity of epithelial barriers may also get better[40].

# 2 Material and methods

## 2.1 Study area and study period

**Mae Sot** is a town in the Tak province with a population of about 106,413. Its located 550 km northwest from Bangkok on the border to Myanmar, and it is the most important border city on the Thai-Myanmar border. The Moei River is a natural boundary between these two countries. Its population is divided into many different ethnic group such as Burmese, Chinese, Karen, Shan and Thai. It's surrounded by rice and cotton paddies, natural forests and mountains.

The weather in Tak province is hot in summer and very cold in winter. Summer begins in February and last until May. The rainy season starts from May to October and winter starts from October to February[49].

Tak is one of the provinces in Thailand which suffers most of malaria. Despite massive government actions, it still has the highest rate of infection in the country, and one of the major problems to solve is the Multi-drug resistant (MDR) malaria.


**Illustration 12:** Map of Thailand

The nearby border to Myanmar, and the heavy border trafficking in between these two countries, is one of the main reasons for this situation, because most of the malaria cases in Mae Sot are imported from Myanmar.

The most important vectors in this region are A. minimus and A. dirus, and the leading species which cause malaria are P.vivax and P.falciparum.

The main transmission period is between May and September, and there is a secondary peak in November/December[9].

The study took place between June and August 2008. The blood samples were taken from patients of the local Malaria Clinic in Mae Sot.

Most of the patients which attended the Clinic were either from Myanmar or worked there. Since the health system in Myanmar underdeveloped and malaria is very frequent, due to the lack of governmental antimalaria monitoring programs, patients come to Thailand, where diagnosis and treatment of malaria is free of charge. The idea behind the free governmental treatment in clinics like the one in Mae Sot is to prevent self treatment which often implements low compliance or incorrect use of antimalarial drugs. This is one of the reasons why resistance is increasing. In the clinic malaria was diagnosed by microscopic examination of a Giemsa-stained thick blood film by a medical assistant. The standard treatment of P.falciparum infections is a combination therapy of Mefloquine and Artesunat. Usually patients are treated as outpatients, and only a severe P.falciparum infection has to be treated in a hospital.

**Illustration 13, 14:** Malaria clinic Mae Sot

## 2.2 Patients

### 2.2.1 Inclusion criteria/Exclusion criteria

Blood samples were taken from 48 patients, of which only 38 were finally included in the study because 10 of them did not comply with the inclusions criteria.
Inclusion criteria were monoinfection with P.falciparum and an asexual pre-incubation parasitemia in the range between 1000 and 280.000 parasites/µl blood in consideration of a haematocrit of 2% in the blood-medium-mixture.
Exclusion criteria were pregnancy, severe or complicated malaria, age < 6 years and recent treatment with antimalarial drugs and antibiotics (mefloquine within the last 9 weeks, all other drugs within the last 4 weeks).
After confirmation of the diagnosis and blood sampling for the tests the patient was treated with artsunate and mefloquine, the standard therapy in Thailand.

### 2.2.2 Epidemiology

Before taking blood samples, written or oral informed consent was obtained from all patients.
From all patients relevant data such as sex, age, nationality, occupation, place of residence, origin of infection, and the registration number of the Clinic in Mae Sot were taken.

#### 2.2.2.1 Parasitaemia and sex / age

Out of all patients which were included in the study (n=38), only 6 were female (15,8%), the remaining 32 patients were male (84,2%).
The calculated arithmetic mean age in women was 33,8, the one for men 22, 34 years.

| | **Number (n)** | **Age*** | **Parasitaemia/µl*** |
|---|---|---|---|
| **Female** | 6 ( 15,8% ) | 33,8 | 89596,833 |
| **Male** | 32 ( 84,2% ) | 22,34 | 62668,2 |

*arithmetic mean

**Table 3**

2.2.2.2 Parasitaemia and place of residence / origin of infection

15 patients (39,4%) out of the 38 included in the study, lived in Thailand, the other 23 (60,6%) were residents of Myanmar. While 34 of the patients got infected in Myanmar (87,5%), a minority of only 4 patients had been infected in Thailand (10,5%).
Noticeable is a broad difference in the origin of infection. This is probably due to a poor health system in Myanmar and an underdeveloped malaria control program in this country.

| | **Residence in Thailand** | **Residence in Myanmar** | **Infection in Thailand** | **Infection in Myanmar** | **Parasitaemia/µl*** |
|---|---|---|---|---|---|
| **Female** | 4 ( 10,5% ) | 2 ( 5,3% ) | 1 ( 2,6% ) | 5 ( 13,2% ) | 89596,833 |
| **Male** | 11 ( 28, 9%) | 21 (55,3% ) | 3 ( 7,9% ) | 29 ( 76,3% ) | 62668,2 |

*arithmetic mean

**Table 4**

2.2.2.3 Parasitaemia and profession

With 22 patients (57,9%) the most commonly occupation group found were labour (mainly woodcutters), followed by 9 students (23,7%) and 7 farmers (18,4%). These numbers are not unexpected since the exposition to mosquitoes is much higher in outdoor activities.

| | **Labour** | **Students** | **Farmer** | **Parasitaemia/µl*** |
|---|---|---|---|---|
| **Female** | 1 ( 2,6% ) | 1 ( 2,6% ) | 4 ( 10,5% ) | 89596,833 |
| **Male** | 21 ( 55,3% ) | 8 ( 21% ) | 3 ( 7,9% ) | 62668,2 |

*arithmetic mean

**Table 5**

## 2.3 Invitro test system

The investigation was based on the WHO standard in vitro micro-test Mark II for the determination of drug sensitivity in Plasmodium falciparum (WHO-MAP/87.2rev.1,1990). This test measures the inhibition of schizont maturation in fresh blood isolates of P.falciparum by increasing drug concentrations.

The correct performance of the micro-test Mark II was as follows:

With a heparinized capillary, 100-200µl blood (depending on the density of parasites) was taken from the fingertips of the patient. The collected blood was then diluted into a plastic tube, containing 6,8 ml RPMI 1640 LPLF-Medium. The tube with the blood-medium-mixture (BMM) was gently agitated and marked with the serial number of the sample. It then was placed in an incubator at 37.5°C until it was inoculated into the micro titre test plates later on. At the same time a thick blood film was prepared to determine the initial parasitaemia of the patient (pre-incubation slide). This was necessary to check if the blood sample was filling in the criteria of the study. The counting procedure for the pre-incubation slide was done by a microscopic examination of the slide. The number of asexual parasites was simultaneously counted against 200 leucocytes and filled in the following formula:

**parasites/µ = parasites x 8000 / leucocytes**

If the parasitaemia was in between 1000 and 280.000 parasites/µl blood the sample was included in the study.

The micro titre plates (Falcon 3070 Becton Dickinson) had already been prepared in advance by Professor Wernsdorfer in Vienna at the Institut für spezifische Prophylaxe und Tropenmedizin, Zentrum für Physiologie und Pathophysiologie der Medizinischen Universität Wien. The plates each contained 8x12 wells, which had been filled with increasing concentrations (well B to well H) of the substance to be tested, leaving out well A, which served as a drug free control (an illustration of a micro titre plate is shown below).

| | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| **LUM** | **0** | **3** | **10** | **30** | **100** | **300** | **1000** | **3000** |
| **DBB** | **0** | **1** | **3** | **10** | **30** | **100** | **300** | **1000** |
| **PYR** | **0** | **3** | **10** | **30** | **100** | **300** | **1000** | **3000** |
| **RET** | **0** | **3** | **10** | **30** | **100** | **300** | **1000** | **3000** |
| **PYR-RET-LOW** | **0** | **3** | **10** | **30** | **100** | **300** | **1000** | **3000** |
| **PYR-RET-MED:** | **0** | **3** | **10** | **30** | **100** | **300** | **1000** | **3000** |
| **PYR-RET-HIGH** | **0** | **3** | **10** | **30** | **100** | **300** | **1000** | **3000** |

**Table 6:** Micro–Titre–Plate and concentrations of the substances used in the study

The plates were then sealed to protect them from contamination. Furthermore, the ones containing retinol were protected from light being wrapped with aluminium foil.

After 3 to 4 hours the BMM was taken from the incubator and 50µl aliquots were added to well A-H of the scheduled test plates. Then the plates where closed with a Falcon 3071 lid and carefully agitated before being placed in a so called candle box, containing a lid candle and a small container of water. The candle was used to get rid of the oxygen and to elevate the $CO_2$. The box was sealed airtight and returned to the incubator at 37,5°C and was held there for 23,5 h. After this period the box was taken out from the incubator and the plates were harvested by decanting the supernatant while the thick film was prepared from the sediment.

The sediment of one test was placed in a set order of eight thick films on one microscopical slide. The slides were then dried for several hours ( 18-24h) and on the next day stained with a 3% Giemsa stock solution at pH 6,85 for about 90 minutes[50].

**Illustration 15,16**: Inoculation/Incubation

## 2.4 Evaluation and statistical analysis

The reading of the prepared slides was performed microscopically at 1000x amplification with oil immersion. Beginning with well A (control) the preschizonts (3-8 nuclei) and schizonts (> 8 nuclei) were counted against asexuall forms to a total of 200 parasites. The control was necessary to see if the test is valid. Criteria for validity were either ≥ 20 schizonts, or ≥ 10% schizonts of all asexual parasites in the count. If these criteria were fulfilled the number of preschizonts and schizonts / 200 parasites were counted in the remaining wells B-H. Usually the number of preschizonts and schizonts dropped with the increase of the concentration of the substance until it reached the point where no more preschizonts and schizonts could be found in the well (cut-off-point or cut-off-concentration).

The situation of the cut-off-points gives a rough indication of the isolates' sensitivity.

The statistical analysis of the tested isolates was done with the log-probit method of Litchfield and Wilcoxon[51], because of the assumed log-normal distribution of drug resistance in basically all parasite populations with no recent drug contact.

With this method the concentration of the drug is transformed in logarithms and the inhibition in % in probite. Furthermore the calculation of parameters such as the individual effective drug concentration (EC) for $EC_{50}$, $EC_{90}$ and $EC_{99}$ and their 95% confidence intervals (CI), heterogeneity and the slope of regression was made by a computer model of Wernsdorfer & Wernsdorfer[50].

The line of regression of each substance was then represented graphically as well as the arithmetic mean cut off concentration (MCOC) of the monocompounts Lumefantrin, DBB, Pyronaridine and the combinations of Pyronaridine/Retinol low, medium and high.
Moreover, the correlation coefficient according to Pearson and Spearman and the interaction following a method of Berenbaum[52] were analysed.
The correlation coefficient is the calculated outcome of the correlations in the mode of activity between two drugs comparing the individual $EC_{50}$, $EC_{90}$ and $EC_{99}$.
The result was then also displayed graphically.
In the interaction analysis the interaction between Pyronaridin and Retinol was shown, based on the calculation and comparison of the fractional inhibitory concentrations (FIC) of one single substance (Pyronaridin or Retinol) with the combination of the two drugs and the calculation of the sum of these FICs (∑FIC). This sum of the FICs gives information about the mode of interaction of the two drugs:
Strong synergism = ∑FIC< 0,5, moderate synergism = ∑FIC 0,5< 1, addidive effect = ∑FIC 1< 2, antagonism = ∑FIC >2.

# 3 Results

## 3.1 Activity of tested drugs

Activity of a drug means its potential to inhibit the maturation of schizonts in the tested isolates. In some of the drugs which were tested in this study or combinations of these drugs a good potential of inhibition was observed. Other drugs used in the study did show only a very weak inhibition of the schizont maturation in the range of tested concentration.

### 3.1.1 Lumefantrin

Out of originally 48 extracted isolates, only 38 (79,2%) were presenting an adequate maturation of schizonts, that is >10% schizonts in well A, and were therefore included in the study.

The arithmetic mean schizont concentration in well A per 200 asexual parasites was 63.

The cut-off-point or cut-off-concentration signifies a point where no more preschizonts and schizonts per 200 trophzoites can be found in the well. 29 isolates were sensitive to one of the tested drug concentrations which means, a cut-off-point was reached as seen in an illustration below. In 9 isolates this cut-off-concentration could not be demonstrated.

The calculated arithmetic mean cut off concentration (**MCOC**) was 4200,00 nmol/l BMM, that is 4200,00 nM ( 95% confidence interval 3100,59-5299,41).

Cut off points

| **Conc. (nmol/l)** | **300** | **1000** | **3000** |
|---|---|---|---|
| **Number of Isolates ( n=38)** | **2 (5,3%)** | **6 (15,8%)** | **21 (55,3%)** |

**Table 7:** Cut off points

The relative schizont maturation inhibition (**SMI%**) demonstrates the maturation inhibition of schizonts with increasing drug concentration in comparison to the number of schizonts in the control well A.

| Drug conc. | SMI% |
|---|---|
| 3,0 | 11,28 |
| 10,0 | 23,91 |
| 30,0 | 53,70 |
| 100,0 | 77,43 |
| 300,0 | 90,68 |
| 1000,0 | 96,28 |
| 3000,0 | 99,51 |

**Table 8:** Drug concentration (LUM) and SMI%

At a concentration of 30 nmol Lumefantrin in all tested isolates the SMI% already showed a 53% inhibition but it never reached 100%, to a total maturation inhibition even with the highest tested concentration of 3000 nmol Lumefantrin. The steepest increase of the SMI% could be found at drug concentrations between 10 and 30 nmol/l.

| | | | | | |
|---|---|---|---|---|---|
| n = | 38 | S = | 6,0413 | $f_s$ = | 1,5122 |
| a = | 3,1413 | A = | 1,6739 | $f_{EC-50}$ = | 1,5946 |
| b = | 0,5529 | K = | 7 | $f_{EC-90}$ = | 2,0378 |
| r = | 0,9962 | N' = | 114 | $f_{EC-95}$ = | 2,2857 |
| $\chi^2$ = | 0,8112 | R = | 1000 | $f_{EC-99}$ = | 2,9390 |

**Table 9:** Parameters of regression

According to Lichfield and Wilcoxon (1949): n=number of isolates; a=intercept of regression; b=slope of regression; r=correlation of regression; X²=heterogeneity; S=slope function; A=intermediate term for fs,; K=number of drug concentration; N'=number of data points EC16 to EC84; R=highest/lowest drug concentration tested; fs= factor of S; fec=multiplication/division factor for obtaining 95% confidence intervals of EC.

| EC | Mean | 95% Confidence Intervals Lower | Higher |
|---|---|---|---|
| $EC_1$ | 0,4293 | 0,1461 | 1,2617 |
| $EC_{16}$ | 4,7732 | 2,9933 | 7,6113 |
| $EC_{50}$ | 28,8361 | 18,0836 | 45,9820 |
| $EC_{84}$ | 174,2068 | 109,2480 | 277,7901 |
| $EC_{90}$ | 292,7976 | 143,6818 | 596,6689 |
| $EC_{95}$ | 564,8345 | 247,1207 | 1291,0212 |
| $EC_{99}$ | 1936,9847 | 659,0610 | 5692,8109 |

**Table 10:** EC Parameters (schizont maturation inhibition in%

The line of regression was also represented graphically by transforming the concentrations of Lumefantrin in logarithms and the SMI % in probite as one can see below.

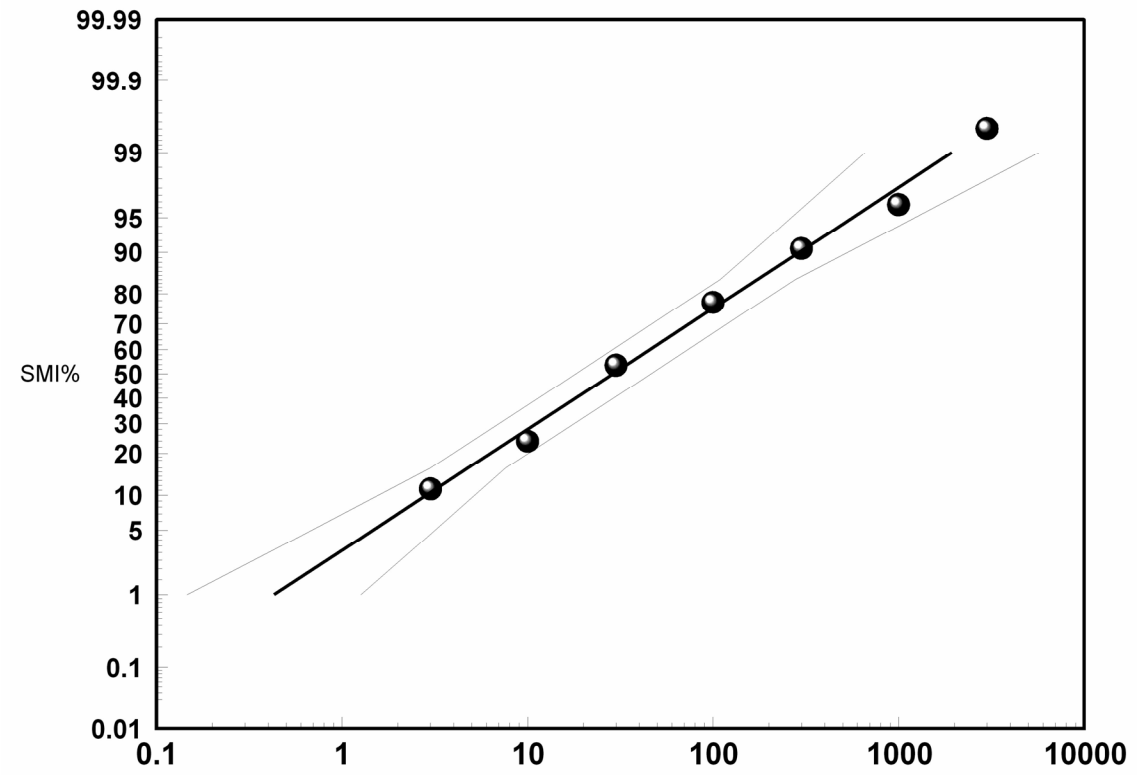


**Diagram 1**: Mae Sot 2008 Lumefantrin

### 3.1.2 Desbutylbenflumentol (DBB)

Out of 48 originally extracted isolates, only 38 (79,2%) were presenting an adequate maturation of schizonts, that is >10% schizonts in well A, and were therefore included in the study.

The arithmetic mean schizont concentration in well A per 200 asexual parasites was 63.

The cut-off-point or cut-off-concentration signifies a point where no more preschizonts and schizonts per 200 trophzoites can be found in the well. All tested isolates were sensitive to one of the tested drug concentrations which means a cut-off-point was reached, as seen in an illustration below, and none of the samples was insensitive to any of the drug concentrations.

The calculated arithmetic mean cut off concentration (**MCOC**) was 994,74 nmol/l BMM, that is 994,74 nM (95% confidence interval 686,30-1303,17).

| **Conc. (nmol/l)** | **100** | **300** | **1000** | **3000** |
|---|---|---|---|---|
| **Number of isolates (n=38)** | **5 (13,2%)** | **11 (28,9%)** | **16 (42,1%)** | **6 (15,8%)** |

**Table 11**: Cut off points

The relative schizont maturation inhibition (**SMI%**) demonstrates the maturation inhibition of schizonts with increasing drug concentration in comparison to the number of schizonts in the control well A.

| Drug conc. | SMI% |
|---|---|
| 3,0 | 29,80 |
| 10,0 | 57,74 |
| 30,0 | 83,29 |
| 100,0 | 92,95 |
| 300,0 | 97,69 |
| 1000,0 | 99,53 |
| 3000,0 | 100,00 |

**Table 12:** Drug concentration (LUM) and SMI%

At a concentration of 10 nmol DBB in all tested isolates the SMI% already showed a 57,7% inhibition and at the highest concentration tested, at 3000 nmol DBB, a 100% SMI% was observed. The steepest increase of the SMI% could be found at drug concentrations between 3 and 10 nmol/l.

| | | | | | |
|---|---|---|---|---|---|
| n = | 38 | S = | 5,9573 | $f_s$ = | 1,5025 |
| a = | 3,9251 | A = | 1,6606 | $f_{EC-50}$ = | 1,5888 |
| b = | 0,5573 | K = | 7 | $f_{EC-90}$ = | 2,0202 |
| r = | 0,9943 | N' = | 114 | $f_{EC-95}$ = | 2,2612 |
| $\chi^2$ = | 0,6055 | R = | 1000 | $f_{EC-99}$ = | 2,8948 |

**Table 13:** Parameters of regression

According to Lichfield and Wilcoxon (1949): n=number of isolates; a=intercept of regression; b=slope of regression; r=correlation of regression; X²=heterogeneity; S=slope function; A=intermediate term for fs,; K=number of drug concentration; N'=number of data points EC16 to EC84; R=highest/lowest drug concentration tested; fs= factor of S; fec=multiplication/division factor for obtaining 95% confidence intervals of EC.

| EC | Mean | 95% Confidence Intervals | |
|---|---|---|---|
| | | Lower | Higher |
| $EC_1$ | 0,1059 | 0,0366 | 0,3064 |
| $EC_{16}$ | 1,1552 | 0,7271 | 1,8354 |
| $EC_{50}$ | 6,8818 | 4,3314 | 10,9339 |
| $EC_{84}$ | 40,9966 | 25,8032 | 65,1361 |
| $EC_{90}$ | 68,6269 | 33,9700 | 138,6415 |
| $EC_{95}$ | 131,7123 | 58,2501 | 297,8216 |
| $EC_{99}$ | 447,3677 | 154,5419 | 1295,0396 |

**Table 14:** EC Parameters (schizont maturation inhibition in %)

The line of regression was also represented graphically by transforming the concentrations of DBB in logarithms and the SMI % in probite as one can see below.

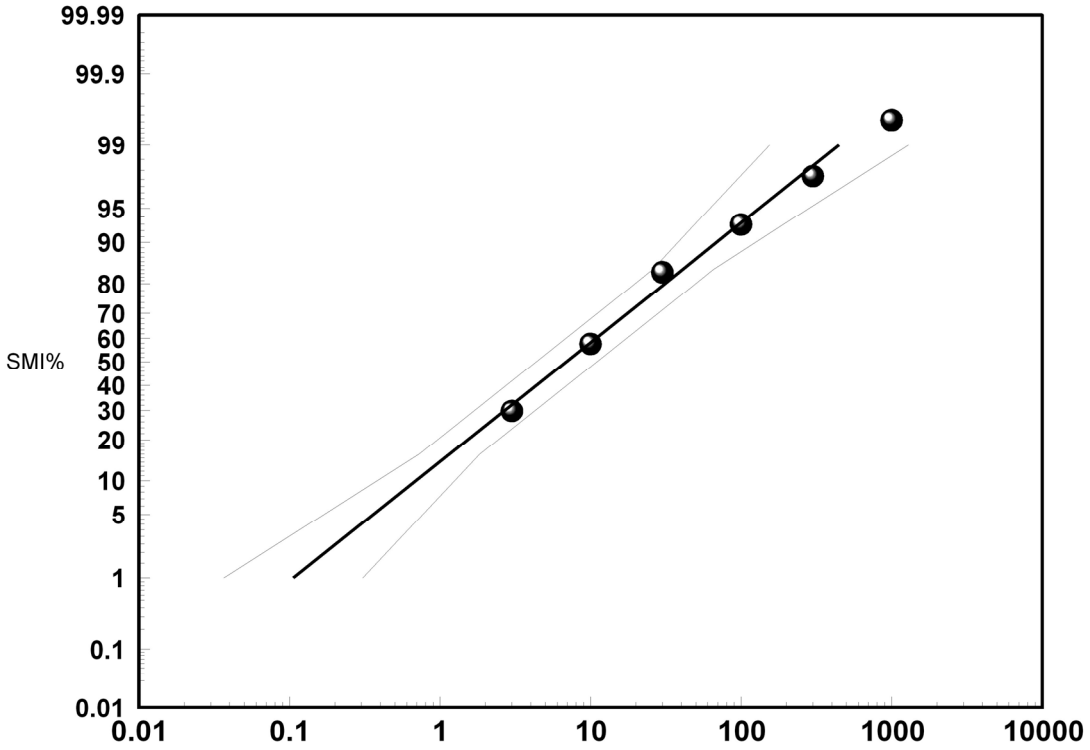


**Diagram 2**: Mae Sot 2008 DBB

### 3.1.3 Pyronaridine

Out of 48 originally extracted isolates, only 38 (79,2%) were presenting an adequate maturation of schizonts, that is >10% schizonts in well A, and were therefore included in the study.

The arithmetic mean schizont concentration in well A per 200 asexual parasites was 67.

The cut-off-point or cut-off-concentration signifies a point where no more preschizonts and schizonts per 200 trophzoites can be found in the well. 25 isolates were sensitive to one of the tested drug concentration which means a cut-off-point was reached as seen in an illustration below. In 13 isolates this cut-off-concentration could not be demonstrated.

The calculated arithmetic mean cut off concentration (**MCOC**) was 4878,95 nmol/l BMM, that is 4878,95 nM (95% confidence interval 3632,00-6125,90).

| Conc. (nmol/l) | 100 | 300 | 1000 | 3000 |
|---|---|---|---|---|
| Number of Isolates (n=38) | 1 (2,6%) | 1 (2,6%) | 7 (18,4%) | 16 (42,1%) |

**Table 15:** Cut off points

The relative schizont maturation inhibition (**SMI%**) demonstrates the maturation inhibition of schizonts with increasing drug concentration in comparison to the number of schizonts in the control well A.

| Drug conc. | SMI% |
|---|---|
| 3,0 | 25,73 |
| 10,0 | 46,73 |
| 30,0 | 66,02 |
| 100,0 | 80,77 |
| 300,0 | 91,24 |
| 1000,0 | 96,22 |
| 3000,0 | 98,95 |

**Table 16:** Drug concentration (Pyronaridine) and SMI%

At a concentration of 10 nmol Pyronaridine in all tested isolates the SMI% almost showed a 50% inhibition but it never reached 100%, to a total maturation inhibition even with the highest tested concentration of 3000 nmolPyronaridine. The steepest increase of the SMI% could be found at drug concentrations between 3 and 10 nmol/l.

| | | | | | |
|---|---|---|---|---|---|
| n = | 38 | S = | 10,4810 | $f_S$ = | 1,8426 |
| a = | 3,9257 | A = | 2,4087 | $f_{EC-50}$ = | 1,6954 |
| b = | 0,4233 | K = | 7 | $f_{EC-90}$ = | 2,5958 |
| r = | 0,9990 | N' = | 152 | $f_{EC-95}$ = | 3,1213 |
| $\chi^2$ = | 0,1477 | R = | 1000 | $f_{EC-99}$ = | 4,6190 |

**Table 17:** Parameters of regression

According to Lichfield and Wilcoxon (1949): n=number of isolates; a=intercept of regression; b=slope of regression; r=correlation of regression; X²=heterogeneity; S=slope function; A=intermediate term for fs,; K=number of drug concentration; N'=number of data points EC16 to EC84; R=highest/lowest drug concentration tested; fs= factor of S; fec=multiplication/division factor for obtaining 95% confidence intervals of EC.

| EC | Mean | 95% Confidence Intervals | |
|---|---|---|---|
| | | Lower | Higher |
| $EC_1$ | 0,0519 | 0,0112 | 0,2398 |
| $EC_{16}$ | 1,2074 | 0,7122 | 2,0470 |
| $EC_{50}$ | 12,6549 | 7,4645 | 21,4546 |
| $EC_{84}$ | 132,6360 | 78,2349 | 224,8649 |
| $EC_{90}$ | 261,3598 | 100,6849 | 678,4432 |
| $EC_{95}$ | 616,5964 | 197,5461 | 1924,5697 |
| $EC_{99}$ | 3084,2398 | 667,7230 | 14246,2306 |

**Table 18:** EC Parameters (schizont maturation inhibition in %)

The line of regression was also represented graphically by transforming the concentrations of Pyronaridine in logarithms and the SMI % in probite as one can see below.

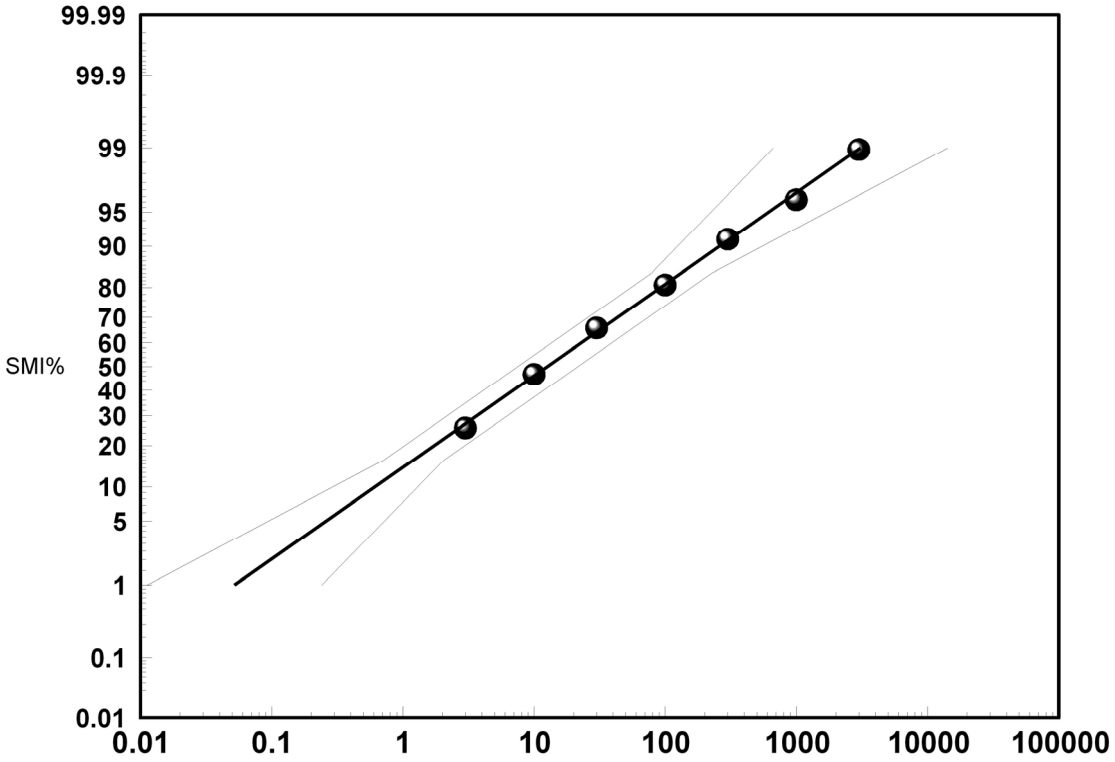


**Diagram 3**: Mae Sot 2008 Pyronaridine

### 3.1.4 Retinol

Out of 48 originally extracted isolates, only 36 (75%) were presenting an adequate maturation of schizonts, that is >10% schizonts in well A, and were therefore included in the study.

The arithmetic mean schizont concentration in well A per 200 asexual parasites was 65.

The cut-off-point or cut-off-concentration signifies a point where no more preschizonts and schizonts per 200 trophzoites can be found in the well. Of the 36 successfully tested isolates none of them was sensitive to one of the tested concentrations of retinol. Since there was no cut of point observed in any of the isolates it was not possible to calculate a arithmetic mean cut off concentration (**MCOC**).

The relative schizont maturation inhibition (**SMI%**) demonstrates the maturation inhibition of schizonts with increasing drug concentration in comparison to the number of schizonts in the control well A.

| Drug conc. | SMI% |
|---|---|
| 30,0 | 5,78 |
| 100,0 | 12,52 |
| 300,0 | 17,78 |
| 1000,0 | 24,89 |
| 3000,0 | 32,76 |
| 10000,0 | 39,82 |
| 30000,0 | 46,04 |

**Table 19:** Drug concentration (Retinol) and SMI%

At a concentration of 30 nmol Retinol in all tested isolates the SMI% only showed a 5,8% Inhibition, and even with the highest concentration tested, 30000 nmol Retinol, the SMI% was only 46 %. The steepest increase of the SMI% could be found at drug concentrations between 1000 and 3000 nmol/l.

| | | | | | |
|---|---|---|---|---|---|
| n = | 36 | S = | 149,3606 | $f_s$ = | 12,8054 |
| a = | 2,9139 | A = | 54,1183 | $f_{EC-50}$ = | 2,8113 |
| b = | 0,1986 | K = | 7 | $f_{EC-90}$ = | 32,2089 |
| r = | 0,9913 | N' = | 180 | $f_{EC-95}$ = | 76,1260 |
| $\chi^2$ = | 0,4167 | R = | 1000 | $f_{EC-99}$ = | 437,3273 |

**Table 20:** Parameters of regression

According to Lichfield and Wilcoxon (1949): n=number of isolates; a=intercept of regression; b=slope of regression; r=correlation of regression; X²=heterogeneity; S=slope function; A=intermediate term for fs,; K=number of drug concentration; N'=number of data points EC16 to EC84; R=highest/lowest drug concentration tested; fs= factor of S; fec=multiplication/division factor for obtaining 95% confidence intervals of EC.

| EC | Mean | 95% Confidence Intervals | |
|---|---|---|---|
| | | Lower | Higher |
| $EC_1$ | 0,2984 | 0,0007 | 130,4968 |
| $EC_{16}$ | 243,4753 | 86,6073 | 684,4717 |
| $EC_{50}$ | 36365,6113 | 12935,7101 | 102233,0953 |
| $EC_{84}$ | 5431589,1870 | 1932085,3030 | 15269595,5251 |
| $EC_{90}$ | 23046357,3623 | 715527,6677 | 742297763,8523 |
| $EC_{95}$ | 143506216,7830 | 1885115,1937 | ######## |
| $EC_{99}$ | ######## | 10134025,4708 | ######## |

**Table 21**: EC Parameters (schizont maturation inhibitionin %)

The line of regression was also represented graphically by transforming the concentrations of Retinol in logarithms and the SMI % in probite as one can see below.

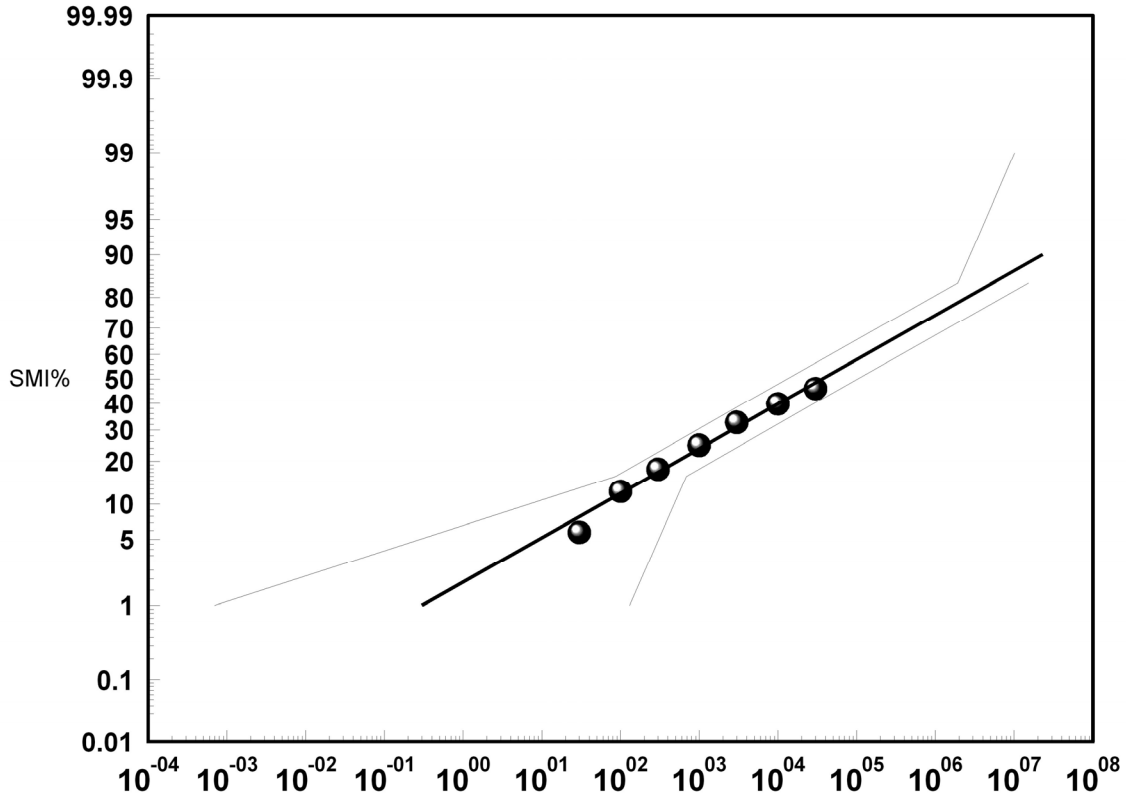


**Diagram 4:** Mae Sot 2008 Retinol

## 3.1.5 Combination of drugs

### 3.1.5.1 Pyronaridine-Retinol low (25,8ng/l)

Out of 48 originally extracted isolates, only 38 (79,2%) were presenting an adequate maturation of schizonts, that is >10% schizonts in well A, and were therefore included in the study.

The arithmetic mean schizont concentration in well A per 200 asexual parasites was 61.

The cut-off-point or cut-off-concentration is signifies a point where no more preschizonts and schizonts per 200 trophzoites can be found in the well. All tested isolates were sensitive to one of the tested drug concentration which means a cut-off-point was reached as seen in an illustration below and none of the samples was insensitive to any of the drug concentrations.

The calculated arithmetic mean cut off concentration (**MCOC**) was 226,32 nmol/l BMM, that is 226,32 nM (95% confidence interval 65,02-387,61).

| **Conc.(nmol/l)** | **10** | **30** | **100** | **300** | **1000** |
|---|---|---|---|---|---|
| **Number of isolates (n=38)** | **4 (10,5%)** | **12 (31,6%)** | **9 (23,7%)** | **11 (28,9%)** | **1 (2,6%)** |

**Table 22**: Cut off points

The relative schizont maturation inhibition (**SMI%**) demonstrates the maturation inhibition of schizonts with increasing drug concentration in comparison to the number of schizonts in the control well A.

| Drug conc. | SMI% |
|---|---|
| 3,0 | 65,97 |
| 10,0 | 89,32 |
| 30,0 | 95,77 |
| 100,0 | 98,27 |
| 300,0 | 99,83 |
| 1000,0 | 99,98 |
| 3000,0 | 100,00 |

**Table 23:** Drug concentration (Pyr.Ret.low) and SMI%

At a concentration of 3 nmol Pyronaridine-Retinol low in all tested isolates the SMI% already showed a 66% inhibition and at the highest concentration tested, at 3000 nmol Pyronaridine-Retinol low, a 100% SMI% was observed. The steepest increase of the SMI% could be found at drug concentrations between 3 and 10 nmol/l.

| | | | | | |
|---|---|---|---|---|---|
| n = | 38 | S = | 6,6612 | $f_s$ = | 2,2171 |
| a = | 4,8977 | A = | 1,7729 | $f_{EC-50}$ = | 2,3446 |
| b = | 0,5244 | K = | 7 | $f_{EC-90}$ = | 3,8217 |
| r = | 0,9922 | N' = | 38 | $f_{EC-95}$ = | 4,7870 |
| $\chi^2$ = | 0,5703 | R = | 1000 | $f_{EC-99}$ = | 7,8146 |

**Table 24:** Parameters of regression

According to Lichfield and Wilcoxon (1949): n=number of isolates; a=intercept of regression; b=slope of regression; r=correlation of regression; X²=heterogeneity; S=slope function; A=intermediate term for fs,; K=number of drug concentration; N'=number of data points EC16 to EC84; R=highest/lowest drug concentration tested; fs= factor of S; fec=multiplication/division factor for obtaining 95% confidence intervals of EC.

| EC | Mean | 95% Confidence Intervals Lower | Higher |
|---|---|---|---|
| $EC_1$ | 0,0144 | 0,0018 | 0,1125 |
| $EC_{16}$ | 0,1825 | 0,0778 | 0,4278 |
| $EC_{50}$ | 1,2155 | 0,5184 | 2,8498 |
| $EC_{84}$ | 8,0965 | 3,4533 | 18,9828 |
| $EC_{90}$ | 13,9973 | 3,6626 | 53,4935 |
| $EC_{95}$ | 27,9830 | 5,8457 | 133,9532 |
| $EC_{99}$ | 102,6041 | 13,1299 | 801,8065 |

**Table 25:** EC Parameters (Schizont maturation inhibition in %)

The line of regression was also represented graphically by transforming the concentrations of Pyronaridine-Retinol low in logarithms and the SMI % in probite as one can see below.

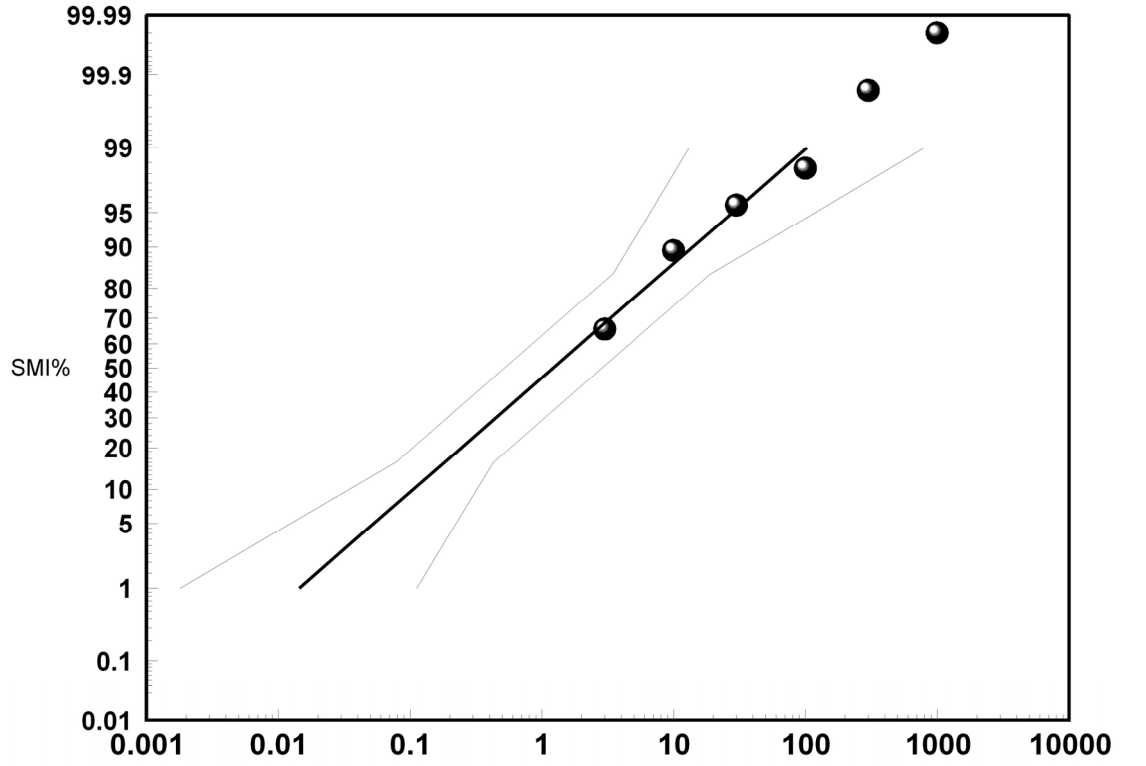


**Diagram 5**: Mae Sot 2008 Pyronaridine-Retinol low

3.1.5.2 Pyronaridine-Retinol medium (31,6ng/l)

Out of 48 originally extracted isolates, only 37 (77,1%) were presenting an adequate maturation of schizonts, that is >10% schizonts in well A, and were therefore included in the study.

The arithmetic mean schizont concentration in well A per 200 asexual parasites was 60.

The cut-off-point or cut-off-concentration is signifies a point where no more preschizonts and schizonts per 200 trophzoites can be found in the well. All tested isolates were sensitive to one of the tested drug concentration which means a cut-off-point was reached as seen in an illustration below and none of the samples was insensitive to any of the drug concentrations.

The calculated arithmetic mean cut off concentration (**MCOC**) was 114,05 nmol/l BMM, that is 114,05 nM (95% confidence interval 38,87-189,24).

| **Conc.(nmol/l)** | **10** | **30** | **100** | **300** | **1000** |
|---|---|---|---|---|---|
| **Number of Isolates (n=37)** | **10 (27)** | **13 (35,1%)** | **9 (24,3%)** | **3 (8,1%)** | **2 (5,4%)** |

**Table 26**: Cut off points

The relative schizont maturation inhibition (**SMI%**) demonstrates the maturation inhibition of schizonts with increasing drug concentration in comparison to the number of schizonts in the control well A.

| Drug conc. | SMI% |
|---|---|
| 3,0 | 78,46 |
| 10,0 | 93,14 |
| 30,0 | 98,00 |
| 100,0 | 99,56 |
| 300,0 | 99,92 |
| 1000,0 | 100,00 |
| 3000,0 | 100,00 |

**Table 27:** Drug concentration (Pyr.Ret.med.) and SMI%

At a concentration of 3 nmol Pyronaridine-Retinol medium in all tested isolates the SMI% already showed a 78,5% inhibition and at the highest concentration tested, at 3000 nmol Pyronaridine-Retinol medium, a 100% SMI% was observed. The steepest increase of the SMI% could be found at drug concentrations between 3 and 10 nmol/l.

| | | | | | |
|---|---|---|---|---|---|
| n = | 37 | S = | 6,7030 | $f_s$ = | 2,5575 |
| a = | 5,2339 | A = | 1,9846 | $f_{EC-50}$ = | 2,3783 |
| b = | 0,5227 | K = | 6 | $f_{EC-90}$ = | 4,4679 |
| r = | 0,9988 | N' = | 37 | $f_{EC-95}$ = | 5,9012 |
| $\chi^2$ = | 0,0420 | R = | 333,3333333 | $f_{EC-99}$ = | 10,7045 |

**Table 28**: Parameters of regression

According to Lichfield and Wilcoxon (1949): n=number of isolates; a=intercept of regression; b=slope of regression; r=correlation of regression; X²=heterogeneity; S=slope function; A=intermediate term for fs,; K=number of drug concentration; N'=number of data points EC16 to EC84; R=highest/lowest drug concentration tested; fs= factor of S; fec=multiplication/division factor for obtaining 95% confidence intervals of EC.

| EC | Mean | 95% Confidence Intervals Lower | Higher |
|---|---|---|---|
| $EC_1$ | 0,0075 | 0,0007 | 0,0799 |
| $EC_{16}$ | 0,0954 | 0,0401 | 0,2268 |
| $EC_{50}$ | 0,6392 | 0,2688 | 1,5202 |
| $EC_{84}$ | 4,2844 | 1,8015 | 10,1898 |
| $EC_{90}$ | 7,4204 | 1,6608 | 33,1538 |
| $EC_{95}$ | 14,8686 | 2,5196 | 87,7426 |
| $EC_{99}$ | 54,7523 | 5,1149 | 586,0965 |

**Table 29**: EC Parameters (Schizont maturing inhibition in %)

The line of regression was also represented graphically by transforming the concentrations of Pyronaridine-Retinol medium in logarithms and the SMI % in probite as one can see below.

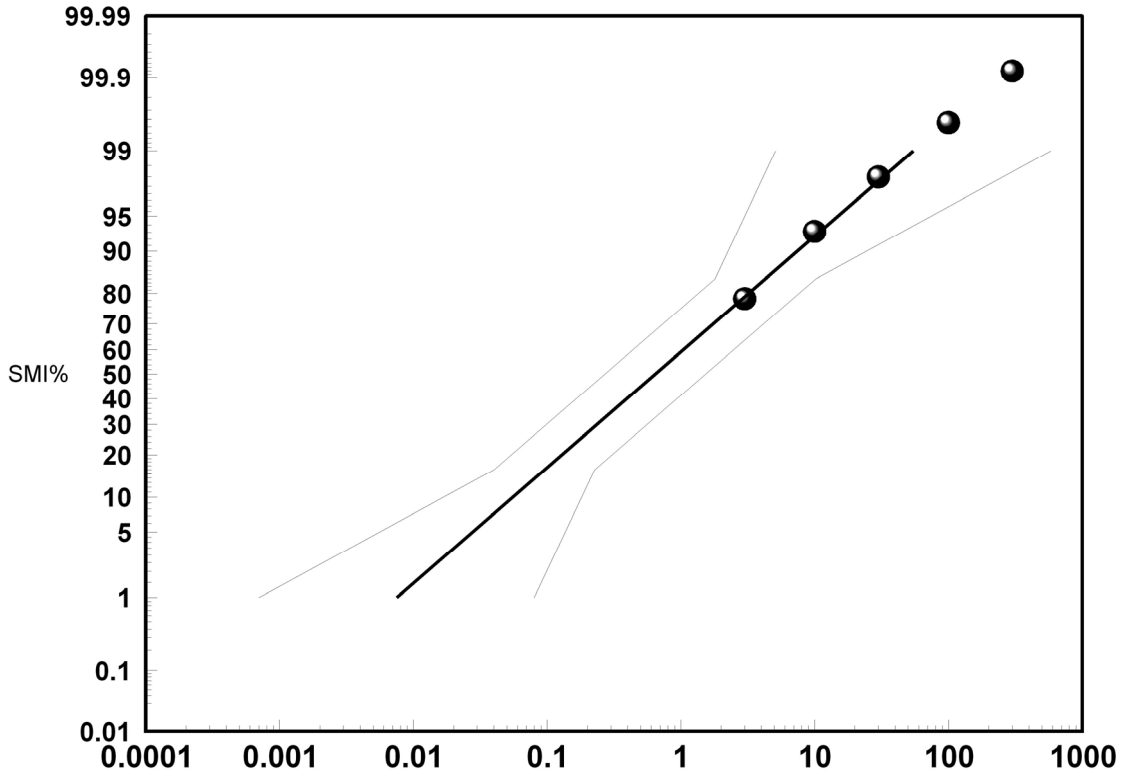


**Diagram 6**: Mae Sot 2008 Pyronaridine-Retinol medium

3.1.5.3 Pyronaridine-Retinol high (37,3ng/l)

Out of 48 originally extracted isolates, only 37 (77,1%) were presenting an adequate maturation of schizonts, that is >10% schizonts in well A, and were therefore included in the study.

The arithmetic mean schizont concentration in well A per 200 asexual parasites was 64.

The cut-off-point or cut-off-concentration signifies a point where no more preschizonts and schizonts per 200 trophzoites can be found in the well. All tested isolates were sensitive to one of the tested drug concentration which means a cut-off-point was reached as seen in an illustration below and none of the samples was insensitive to any of the drug concentrations.

The calculated mean cut off concentration (**MCOC**) was 122,43 nmol/l BMM, that is 122,43 nM (95% confidence interval 46,65-198,21).

| **Conc.(nmol/l)** | **10** | **30** | **100** | **300** | **1000** |
|---|---|---|---|---|---|
| **Number of isolates (n=37)** | **15 (40,5%)** | **6 (16,2%)** | **10 (27%)** | **4 (10,8%)** | **2 (5,4%)** |

**Table 30**: Cut off points

The relative schizont maturation inhibition (**SMI%**) demonstrates the maturation inhibition of schizonts with increasing drug concentration in comparison to the number of schizonts in the control well A.

| Drug conc. | SMI% |
|---|---|
| 3,0 | 73,86 |
| 10,0 | 94,06 |
| 30,0 | 98,27 |
| 100,0 | 99,63 |
| 300,0 | 99,94 |
| 1000,0 | 100,00 |
| 3000,0 | 100,00 |

**Table 31**: Drug concentration (Pyr.Ret.high) and SMI%

At a concentration of 3 nmol Pyronaridine-Retinol high in all tested isolates the SMI% already showed a 73,9% inhibition and at the highest concentration tested, at 3000 nmol Pyronaridine-Retinol high, a 100% SMI% was observed. The steepest increase of the SMI% could be found at drug concentrations between 3 and 10 nmol/l.

| | | | | | |
|---|---|---|---|---|---|
| n = | 37 | S = | 5,3832 | $f_s$ = | 2,0856 |
| a = | 5,0416 | A = | 1,7101 | $f_{EC-50}$ = | 2,1523 |
| b = | 0,5908 | K = | 6 | $f_{EC-90}$ = | 3,4043 |
| r = | 0,9933 | N' = | 37 | $f_{EC-95}$ = | 4,1987 |
| $\chi^2$ = | 0,3170 | R = | 333,3333333 | $f_{EC-99}$ = | 6,6181 |

**Table 32**: Parameters of regression

According to Lichfield and Wilcoxon (1949): n=number of isolates; a=intercept of regression; b=slope of regression; r=correlation of regression; X²=heterogeneity; S=slope function; A=intermediate term for fs,; K=number of drug concentration; N'=number of data points EC16 to EC84; R=highest/lowest drug concentration tested; fs= factor of S; fec=multiplication/division factor for obtaining 95% confidence intervals of EC.

| EC | Mean | 95% Confidence Intervals Lower | Higher |
|---|---|---|---|
| $EC_1$ | 0,0182 | 0,0027 | 0,1203 |
| $EC_{16}$ | 0,1731 | 0,0804 | 0,3726 |
| $EC_{50}$ | 0,9320 | 0,4330 | 2,0060 |
| $EC_{84}$ | 5,0172 | 2,3311 | 10,7986 |
| $EC_{90}$ | 8,1565 | 2,3960 | 27,7668 |
| $EC_{95}$ | 15,0855 | 3,5929 | 63,3387 |
| $EC_{99}$ | 47,8021 | 7,2229 | 316,3592 |

**Table 33**: EC Parameters (Schizont maturation inhibition in %)

The line of regression was also represented graphically by transforming the concentrations of Pyronaridine-Retinol high in logarithms and the SMI % in probite as one can see below.

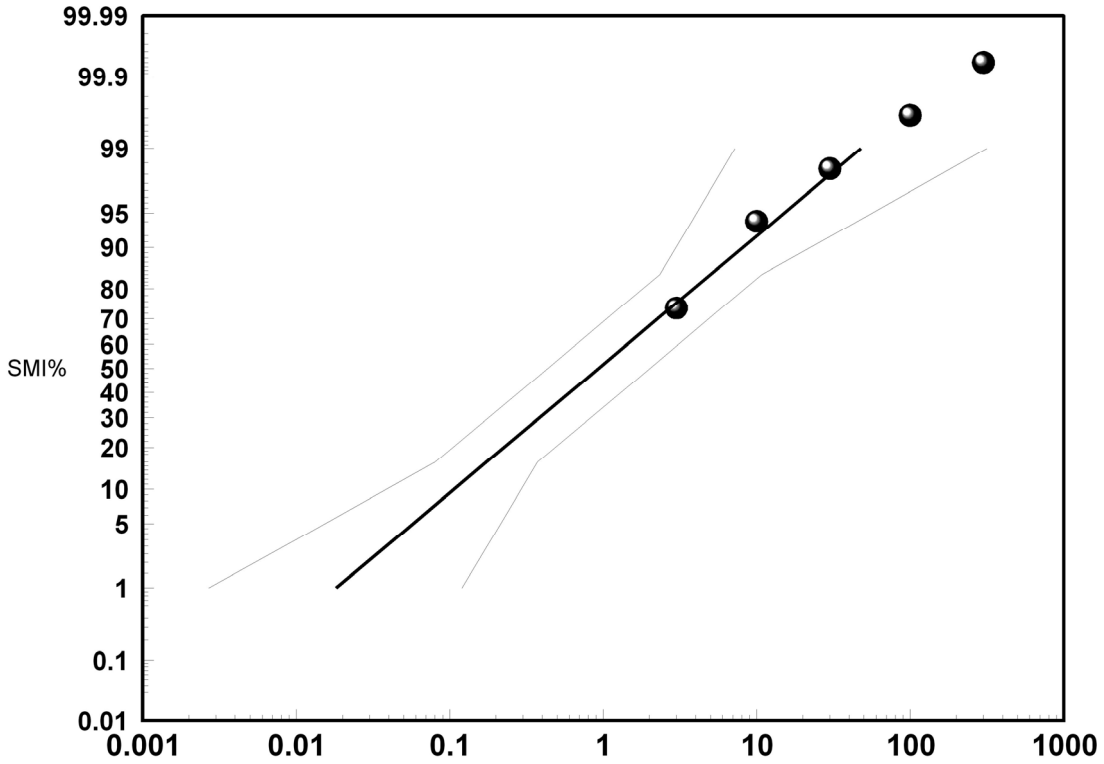


**Diagram 7**: Mae Sot 2008 Pyronaridine-Retinol high

## 3.2 Correlation analysis

The correlation coefficient is the calculated outcome of the correlations in the mode of activity between two drugs comparing the individual $EC_{50}$, $EC_{90}$ and $EC_{99}$ of all 38 isolates.

If there is a high correlation between two substances high affinity in structure or mode of action can be presumed. If $p \leq 0,05$, the correlation is significant, values with $p \leq 0,01$ are highly significant (highlighted results are significant).

$EC_{50}$

| | LUM | RET | PYR | PYR-RETL | PYR-RETM | PYR-RETH |
|---|---|---|---|---|---|---|
| DBB | **0,444842** | 0,257122 | 0,145887 | **0,458064** | 0,095793 | 0,238620 |
| LUM | | 0,137618 | 0,149872 | 0,296067 | -0,032865 | -0,060678 |
| RET | | | 0,1469817 | 0,1680135 | 0,000436 | 0,192263 |
| PYR | | | | **0,444847** | 0,151288 | 0,303536 |
| PYR-RETL | | | | | 0,224350 | **0,354222** |
| PYR-RETM | | | | | | 0,268438 |
| | r | r | r | r | r | r |

**Table 34**

$EC_{90}$

| | LUM | RET | PYR | PYR-RETL | PYR-RETM | PYR-RETH |
|---|---|---|---|---|---|---|
| DBB | **0,558751** | -0,09819 | -0,11708 | 0.0464948 | -0,052285 | 0,033887 |
| LUM | | -0,11708 | 0,048732 | 0,017327 | -0,108839 | -0,159243 |
| RET | | | 0,063287 | -0,050617 | -0,076790 | -0,093175 |
| PYR | | | | **0,429588** | 0,247703 | 0,074144 |
| PYR-RETL | | | | | 0,029641 | 0,096388 |
| PYR-RETM | | | | | | **0,415104** |
| | r | r | r | r | r | r |

**Table 35**

$EC_{99}$

| | LUM | RET | PYR | PYR-RETL | PYR-RETM | PYR-RETH |
|---|---|---|---|---|---|---|
| DBB | **0,358325** | | -0,030966 | 0,014387 | -0,028173 | -0,011332 |
| LUM | | | -0,059449 | -0,044912 | -0,190651 | -0,118609 |
| RET | | | | | | |
| PYR | | | | -0,023724 | -0,096333 | 0,011973 |
| PYR-RETL | | | | | 0,092345 | 0,003498 |
| PYR-RETM | | | | | | 0,225805 |
| | r | r | r | r | r | r |

**Table 36**

## 3.3 Interaction analysis

In the interaction analysis, the interaction between Pyronaridine and Retinol was performed based on the calculation and comparison of the FICs of one single substance (Pyronaridin or Retinol) with the combination of the two drugs and the calculation of the sum of these FICs (∑FIC). The sum of the FICs gives information about the mode of interaction of the two drugs:

Strong synergism = ∑FIC< 0,5, moderate synergism = ∑FIC 0,5< 1, additive effect = ∑FIC 1< 2, antagonism = ∑FIC >2.

The retinol concentration used in the combination with Pyronaridine for $EC_{50}$ values was 25,8ng/l, for $EC_{90}$ values, the concentration of retinol was 31,6ng/l.

### 3.3.1 Interactions for $EC_{50}$ values

The observed geometric mean sum of the $FIC_{50}$ of PYR-Ret-L was 0,21, the one of PYR-Ret-M was 0,22910479, and the geometric mean sum of the $FIC_{50}$ of PYR-Ret-H was 0,31839464.

The vast majority of interactions between the combinations of Pyronaridine-Retinol display a strong synergism or moderate synergism for the $EC_{50}$ values. Only a minority of the interactions present an additive or antagonistic effect.

A real difference in the outcome of the tests with lower concentrations of Retinol added to Pyronaridine compared to the ones with higher concentrations could not be observed.

| **$EC_{50}$** | **Strong Synergism ∑FIC< 0,5** | **Moderate Synergism ∑FIC 0,5< 1** | **Additive Effect ∑FIC 1< 2** | **Antagonism ∑FIC >2** |
|---|---|---|---|---|
| **PYR-RET-L**<br><br>**Number of isolates (n=38)** | **28 (73,7%)** | **4 (10,5%)** | **4 (10,5%)** | **2 (5,3%)** |
| **PYR-RET-M**<br><br>**Number of isolates (n=37)** | **27 (73%)** | **5 (13,5%)** | **2 (5,4%)** | **3 (8,1%)** |
| **PYR-RET-H**<br><br>**Number of isolates (n=37)** | **22 (59,5%)** | **8 (21,6%)** | **2 (5,4%)** | **5 (13,5%)** |

**Table 37**

The calculated outcome was also displayed graphically as one can see in the illustration below.

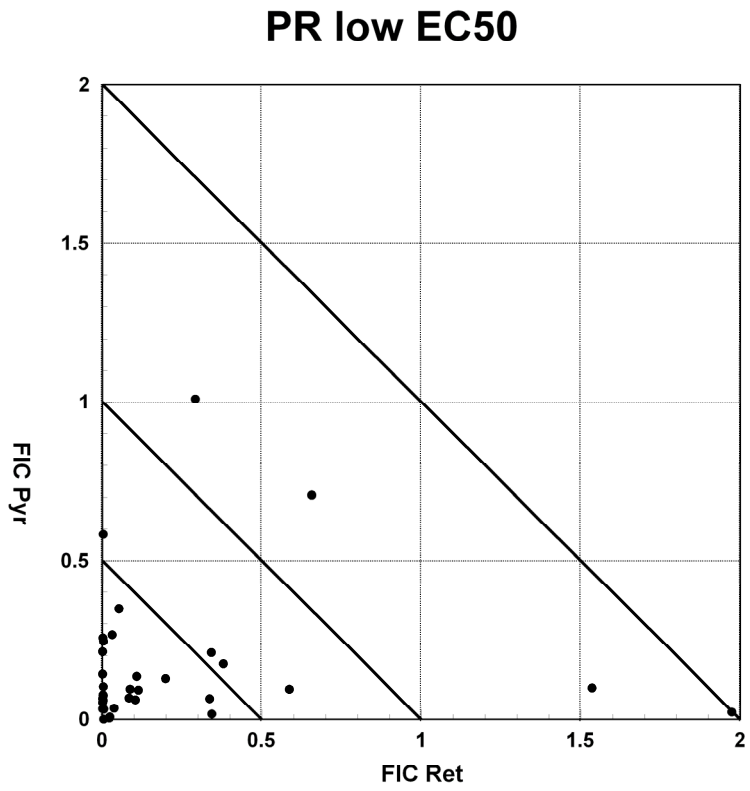


**Diagram 8**

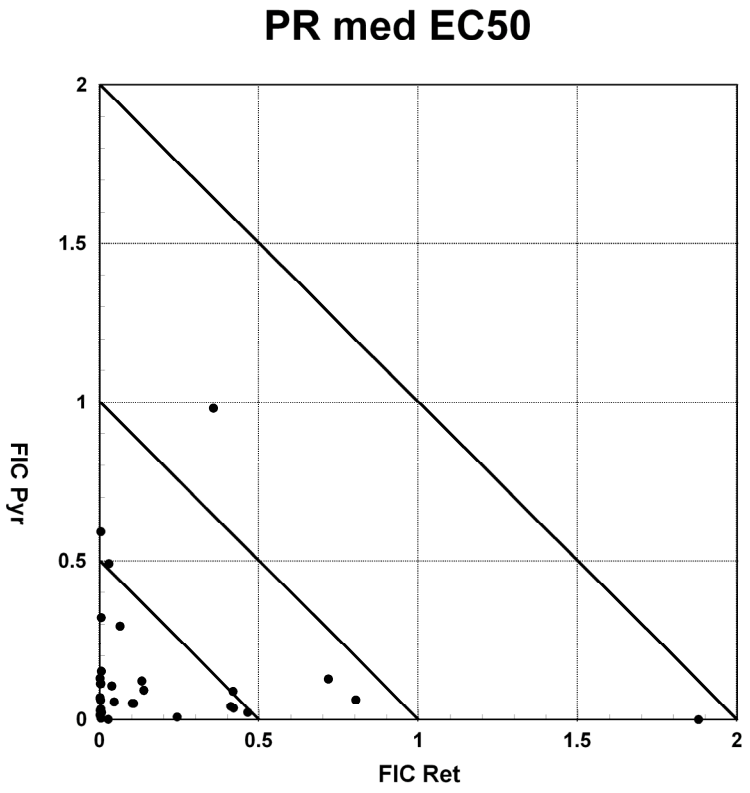


**Diagram 9**

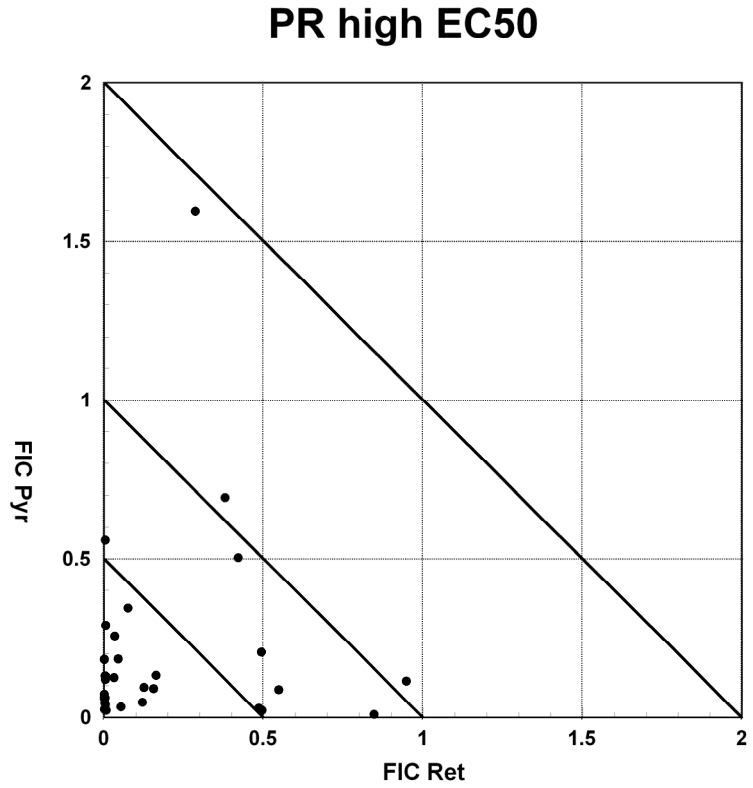


**Diagram 10**

### 3.3.2 Interactions for $EC_{90}$ values

The observed geometric mean sum of the $FIC_{90}$ of PYR-Ret-L was 0,04784586, the one of PYR-Ret-M was 0,01927674 and the geometric mean sum of the $FIC_{90}$ of PYR-Ret-H was 0,03076946.

The vast majority of interactions between the combinations of Pyronaridine-Retinol display a strong synergism. Only a minority of PYR-Ret-L and PYR-Ret-H show a moderate synergism for the $EC_{90}$ values, but this was not observed for PYR-Ret-M. And again, only a minority of the interactions present an additive (PYR-Ret-L) or antagonistic effect (PYR-Ret-M).

A real difference in the outcome of the tests with lower concentrations of Retinol added to Pyronaridine compared to the ones with higher concentrations could not be observed.

| $EC_{90}$ | Strong Synergism ∑FIC< 0,5 | Moderate Synergism ∑FIC 0,5< 1 | Additive Effect ∑FIC 1< 2 | Antagonism ∑FIC >2 |
|---|---|---|---|---|
| **PYR-RET-L**<br><br>**Number of isolates (n=37)** | **35 (94,6%)** | **1 (2,7%)** | **1 (2,7%)** | **0** |
| **PYR-RET-M**<br><br>**Number of isolates (n=37)** | **36 (97,3%)** | **0** | **0** | **1 ( 2,7%)** |
| **PYR-RET-H**<br><br>**Number of isolates (n=37)** | **36 (97,3%)** | **1 (2,7%)** | **0** | **0** |

**Table 38**

The calculated outcome was also displayed graphically as one can see in the illustration below.

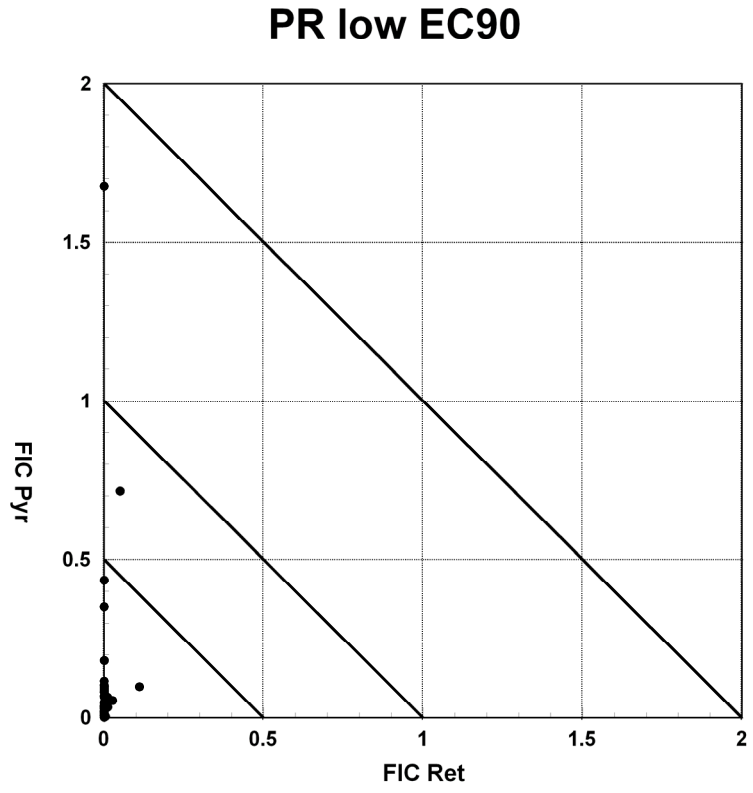


**Diagram 11**

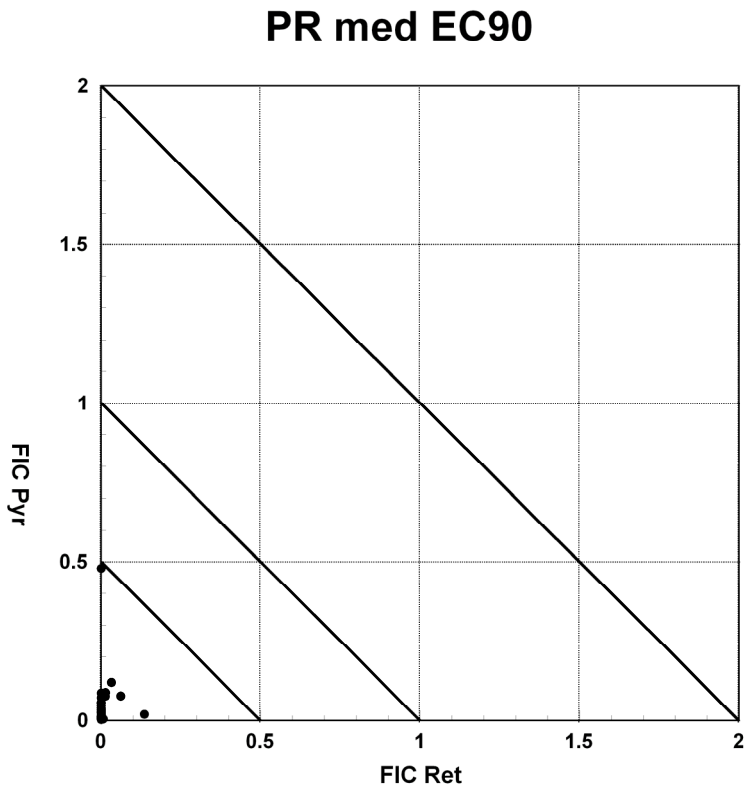


**Diagram 12**

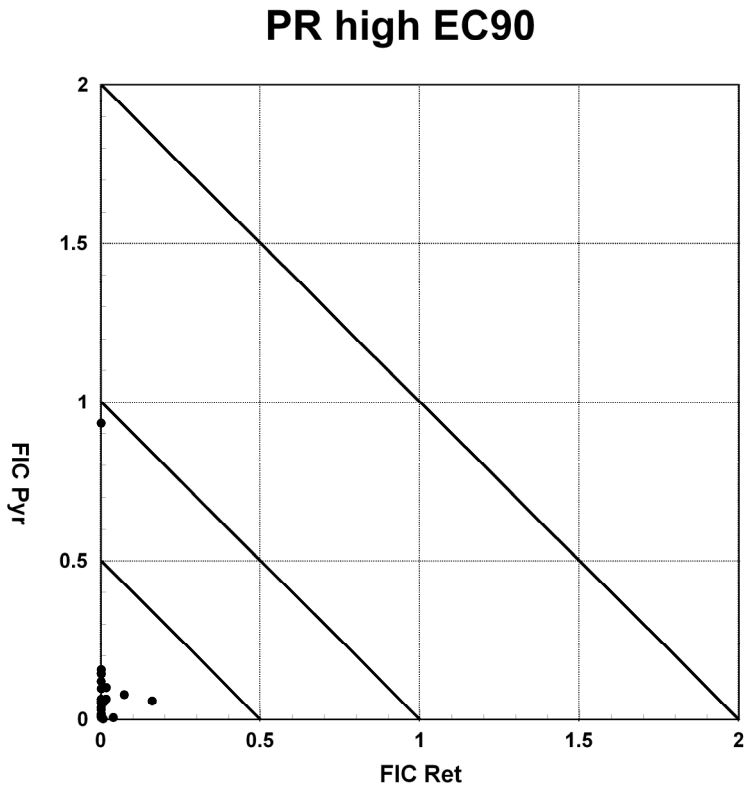


**Diagram 13**

# 4 Discussion

To determinate differences in drug sensitivity of P.falciparum to the substances used in the study, the regression lines were compared among each other. In this way, the effects of the different substances can be compared and at the same time, it is possible to contrast the results of the individual substance with earlier studies[50].

## 4.1 Lumefantrine

At a Lumefantrien concentration of 30 nmol/l a relative schizont maturation inhibition (SMI%) of 53,70% was observed, while the SMI% of the highest tested drug concentration of 3000 nmol/l was 99,51%.

Lumefantrine resistance is defined as schizont maturation in the presence of approximately 10000nmol drug/l blood. There were no signs of resistance observed in this study but compared to a study made 2005 in Mae Sot[29] there has been a decrease in the effectiveness of Lumefantrine. By comparing the regression lines of Lumefantrine, provided 2005 and 2008, for parallelism and activity differences, the slope ratio of the regression lines showed to be parallel. Therefore the two could be compared for an efficacy difference.

| Lum | S | $f_s$ | $EC_{50}$ | $EC_{90}$ | $EC_{99}$ |
|---|---|---|---|---|---|
| 2005 | 4,1834 | 1,3189 | 22,6319 | 143,1124 | 643,5456 |
| 2008 | 6,0413 | 1,5122 | 28,8361 | 292,7976 | 1936,9847 |

S=slope function, fs= factor of S

**Table 39**: Comparison of regression parameters LUM. 2005/2008

| LUM 2005/2008 | SR | $f_{SR}$ | Slope of regression | PR | $f_{PR}$ | |
|---|---|---|---|---|---|---|
| $EC_{50}$ | 1,44411 | 1,64484 | parallel | 1,27413 | 1,84000 | n. significant |
| $EC_{90}$ | | | | 2,04592 | 2,43270 | n. significant |
| $EC_{99}$ | | | | 3,00986 | 3,73920 | n. significant |

SR=Slope Ratio, $f_{SR}$=Factor of slope ratio, PR= Potency ratio, $f_{PR}$=Factor of the potency ratio

**Table 40**: Comparison of regression parameters LUM. 2005/2008

In the comparison of regression parameters of Lumefantrine from 2005 and 2008 there appeared to be no significant efficacy difference for all three EC's (50/90/99).
Until now, it was thought that Lumefantrine is not used as an antimalarial drug in the area of the investigation therefore reduction in the effectiveness of Lumefantrine was thought to be due to its similarity to the class-two-blood schizonticides. These are commonly used drugs at the area around the Thai-Myanmar border to treat complicated and uncomplicated malarial infections. But recently there have been presumptions that in Myanmar, Lumefantrine is used in a combination with Artemether. This could be also an explanation for the reduction in the effectiveness of Lumefantrine.

Correlation analysis

The correlation analysis of Lumefantrine and DBB showed a positive correlation for the all three EC values, $EC_{50}$ (r=0,444842; p=0,01), $EC_{90}$ (r=0,558751; p=0,001) and $EC_{99}$ (r=0,358325; p=0,05). All result are significant, the one of the $EC_{50}$ and $EC_{90}$ even highly significant. This outcome represents the similar mode of action of both substances, since they are chemically related drugs and it is presumed that DBB is a metabolite of Lumefantrine.
Although the correlation analysis of Lumefantrine and Retinol showed a positive correlation for the $EC_{50}$ values, the result is not significant. For the $EC_{90}$ the correlation was negative and none significant. The analysation of the $EC_{99}$ was not possible, because there was no data for the $EC_{99}$ of Retinol. Since this two substances are not chemically related these results are not unexpected.
Also the correlation analysis of Lumefantrine and Pyronaridine showed no significance for all three EC values, the $EC_{50}$ and $EC_{90}$ had a positive correlation while the $EC_{99}$ showed to be negative. This can be also explained by the difference in the mode of action of the two drugs and its unrelated structures.
By comparing Lumefantrine with the combinations Pyr-Ret L/M/H no significans was observed for all EC values.

## 4.2 Desbutylbenflumetol (DBB)

The schizont maturation inhibition at a DBB concentration of 10nmol/l BMM showed to be already 57, 74%, at a DBB concentration of 300nmol/l it was 97, 69%. At the highest used drug concentration of 3000nmol/l the SMI% was 100%.

The slope ratio of the regression lines of DBB provided 2005 and 2008 showed to be parallel. Therefore the two regressions could be compared for an efficacy difference.
There was no significant efficacy difference for all three EC's (50/90/99) although compared to the EC values of 2005, DBB showed, like Lumefantrine, a slight reduction in its effectiveness. This could be due to the same reason like the reduction in the effectiveness of Lumefantrine since both substances are closely related and DBB is assumed to be a metabolite of Lumefantrine.

| DBB | S | $f_s$ | $EC_{50}$ | $EC_{90}$ | $EC_{99}$ |
|---|---|---|---|---|---|
| 2005 | 5, 1552 | 1,338 | 5,0162 | 41,5148 | 232,509 |
| 2008 | 5,9573 | 1,5025 | 6,8818 | 68,6269 | 447,3677 |

S=slope function, fs= factor of S

**Table 41**: Comparison of regression parameters of DBB 2005/2008

| DBB 2005/2008 | SR | $f_{SR}$ | Slope of regression | PR | $f_{PR}$ | |
|---|---|---|---|---|---|---|
| $EC_{50}$ | 1,155590 | 1,6496090 | parallel | 1,371914 | 1,797920 | n. significant |
| $EC_{90}$ | | | | 1,6530707 | 2,4014864 | n. significant |
| $EC_{99}$ | | | | 1,9240876 | 3,722603 | n. significant |

SR=Slope Ratio, $f_{SR}$=Factor of slope ratio, PR= Potency ratio, $f_{PR}$=Factor of the potency ratio

**Table 42**: Comparison of regression parameters of DBB 2005/2008

By comparing the regression parameters of both substances, DBB and Lumefantrine, the regression lines showed to be parallel, therefore DBB and Lumefantrine could be compared for an efficacy difference.

| 2008 | S | $f_s$ | $EC_{50}$ | $EC_{90}$ | $EC_{99}$ |
|---|---|---|---|---|---|
| DBB | 5,9573 | 1,5025 | 6,8818 | 68,6269 | 447,3677 |
| LUM | 6,0413 | 1,5122 | 28,8361 | 292,7976 | 1936,9847 |

S=slope function, fs= factor of S

**Table 43**: Comparison of regression parameters DBB/LUM 2008

| DBB/LUM 2008 | SR | $f_{SR}$ | Slope of regression | PR | $f_{PR}$ | |
|---|---|---|---|---|---|---|
| $EC_{50}$ | 1,0141003 | 1,786641 | parallel | 4,1901973 | 1,929638 | significant |
| $EC_{90}$ | | | | 4,2665135 | 2,7199744 | significant |
| $EC_{99}$ | | | | 4,3297374 | 4,544615 | n. significant |

SR=Slope Ratio, $f_{SR}$=Factor of slope ratio, PR= Potency ratio, $f_{PR}$=Factor of the potency ratio

**Table 44**: Comparison of regression parameters DBB/LUM 2008

The $EC_{50}$ and $EC_{90}$ values appeared to have significant differences in the effectiveness, while for the $EC_{99}$ no significant difference could be observed.

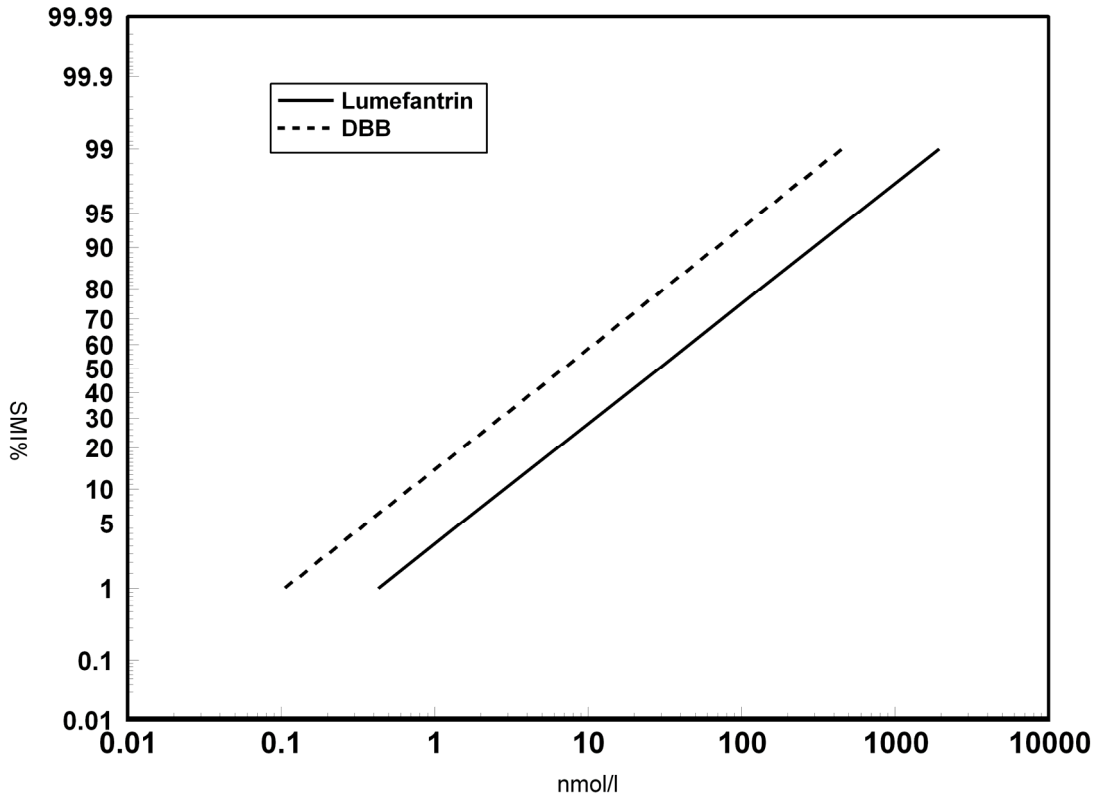


**Diagram 14**: Comparison Lumefantrine/DBB

Although the regression lines of DBB and Lumefantrine (see diagram above) are parallel, by comparing them, they show a significant difference. DBB already shows a much higher schizont maturation inhibition in a lower concentration than Lumefantrine (at 100nmol/l DBB, the SMI% was 92,95%, whereas Lumefantrine in the same concentration has only a SMI% of 77,43%).

By comparing the MCOCs of both substances (see diagram below), with an arithmetic mean cut off concentration of Lumefantrine of 4200,00nmol/l BMM, and a MCOC of DBB of 994,74nmol/l BMM, a significant difference can be also observed (t=5,54102; p=0,0001). This results display a much higher antimalarial drug activity of DBB compared with the one of Lumefantrine.

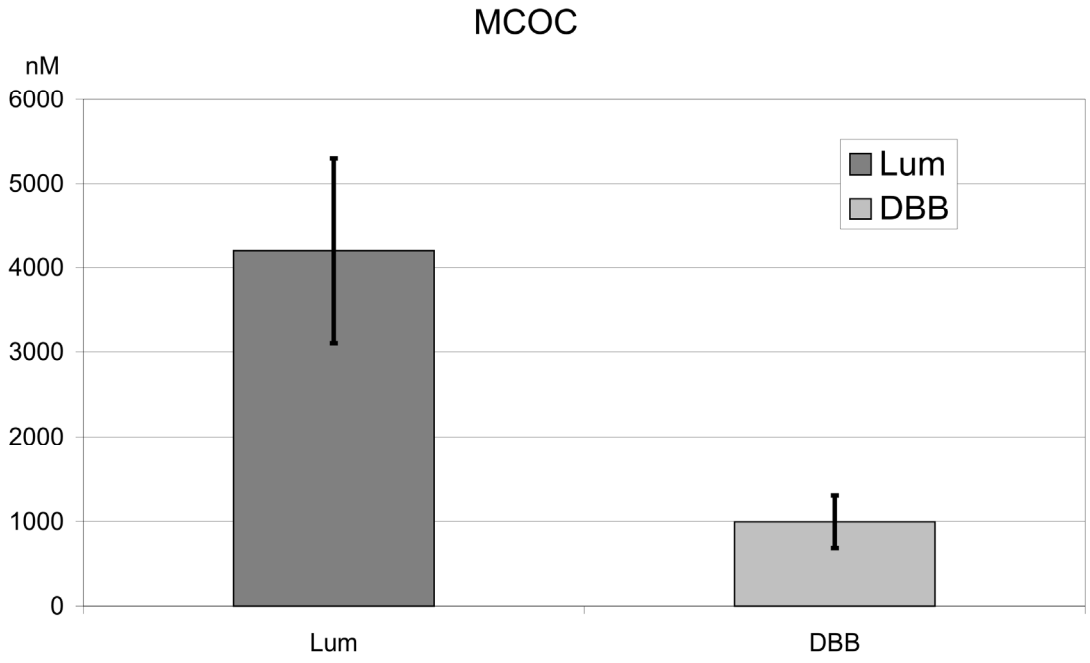


**Diagram 15**: MCOC DBB-LUM 2008

So far there has never been a clinical testing with DBB. The antimalarial effect of this drug has only been ascertained in In vitro tests. But it is presumed that it may be used in combination with Artesunate instead of Lumefantrine if the reduction in its effectiveness will progress and resistance towards this substance will increase.

Correlation analysis:

In the correlation analysis between DBB and Retinol a positive correlation was shown for the $EC_{50}$, for the $EC_{90}$ it was negative. No significance was observed for the $EC_{50}$ and $EC_{90}$ values, the $EC_{99}$ was not calculated. Also the correlation analysis of DBB and Pyronaridine showed no significance for all three EC values, the $EC_{50}$ had a positive correlation while the $EC_{90}$ and the $EC_{99}$ showed to be negative. This can be explained by the difference in the mode of action of the two drugs and its unrelated structures. By comparing DBB with the combinations Pyr-Ret L/M/H no significans was observed for all EC values.

## 4.3 Retinol

At a concentration of 3000 nmol/l Retinol the SMI% observed was only 32, 76%, and even with the highest concentration tested, 30000 nmol Retinol, the SMI% reached only 46,04 %. In comparison with studies from 2005, in which the activity of Retinol against P.falciparum as a single substance was already tested, the results 2008 showed a reduction in its effectiveness.

| Retinol | S | $f_s$ | $EC_{50}$ | $EC_{90}$ | $EC_{99}$ |
|---|---|---|---|---|---|
| 2005 | 7,3479 | 1,7141 | 622,3141 | 8261,1423 | 67995,7349 |
| 2008 | 149,3606 | 12,8054 | 36365,6113 | 23046357,3623 | //////////////////// |

S=slope function, fs= factor of S

**Table 45**: Comparison of regression parameters Retinol 2005/2008

| Retinol 2005/2005 | SR | $f_{SR}$ | Slope of regression | PR | $f_{PR}$ | |
|---|---|---|---|---|---|---|
| $EC_{50}$ | 20,3269777 | 13,547325 | none parallel | | | |
| $EC_{90}$ | | | | | | |
| $EC_{99}$ | | | | | | |

SR=Slope Ratio, $f_{SR}$=Factor of slope ratio, PR= Potency ratio, $f_{PR}$=Factor of the potency ratio

**Table 46**: Comparison of regression parameters Retinol 2005/2008

The factor of slope ratio $f_{SR}$ was smaller than the slope ratio SR, the regression lines of 2005 and 2008 turned out to be not parallel. Therefore it was not possible to calculate data of PR and $f_{PR}$ according to the method of Litchfield and Wilcoxon[51].

The EC values of Retinol from 2008 have increased compared to 2005 and it was not possible to calculate the $EC_{99}$ values, because the necessary Retinol concentration would have been too high and well above physiological levels in humans.

The high EC values probably demonstrate the fact that Retinol alone has only little effect on the maturation of schizonts, that is, the activity of Retinol against P.falciparum is low.

## 4.4 Pyronaridine

In this study no evidence of resistance against Pyronaridine was observed.

At a Pyronaridine concentration of 30nmol/l a SMI% of 66,02% was found. At the highest used drug concentration of 3000nmol/l the SMI% was 98,95%.

Altough the effectiveness of Pyronaridine 2008 has slightly increased again compared to a study made 2007 in Mae Sot, the sensitivity to pyronaridine of *P. falciparum* has decreased significantly in the last nine years.

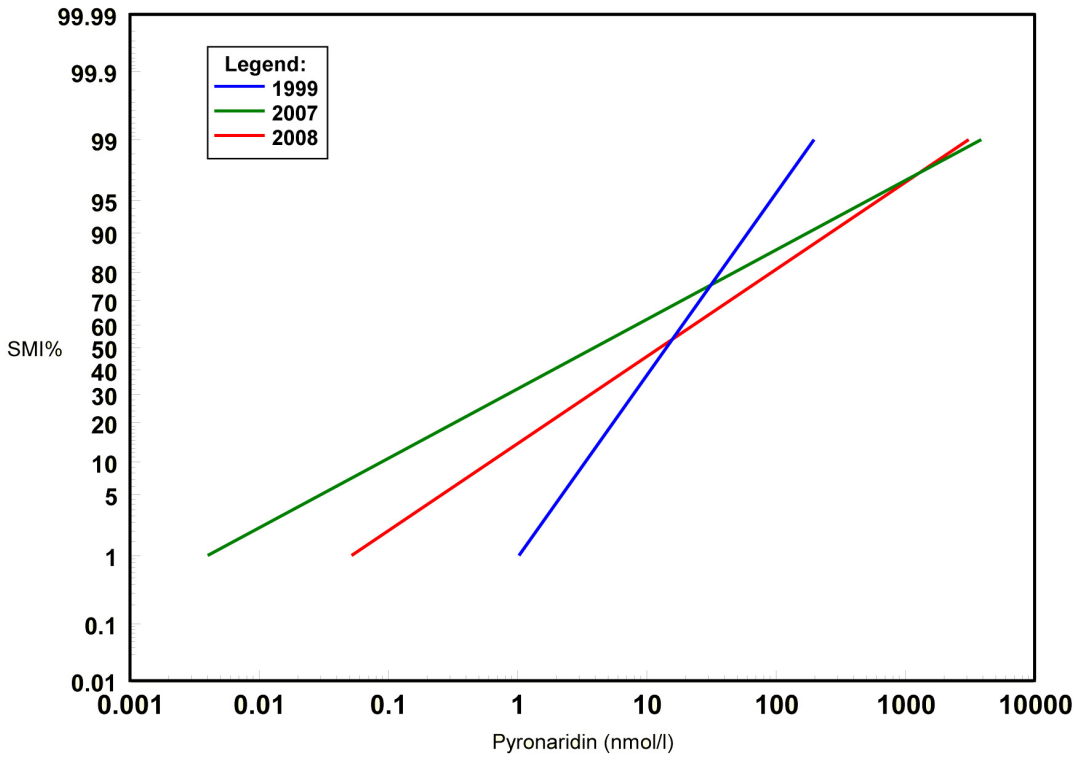


**Diagram 16**: Pyronaridine, Mae Sot 1999/2007/2008

Between 1999 and 2007 a flattening of the regression lines was observed- evidence of decreasing activity of Pyronaridine against P.falciparum. This was probably due to frequent and uncontrolled use of Pyronaridine during this period in the area of investigation.
The regression line of 2008 shows again a slight inclination, a sign of increasing drug sensibility. Apparently as a consequence of a decreasing effect of the substance in the years before, thus resulting in lesser popularity and therefore falling drug pressure.

Pyronaridine and Retinol showed a positive correlation for the $EC_{50}$ and $EC_{90}$ values but none of them was significant.
The correlation analysis of Pyronaridine and Pyr-Ret-L showed to be positive and significant for the $EC_{50}$ (r=0,444847; p=0,01) and $EC_{90}$ (r=0,429588; p=0,01) but negative and none significant for the $EC_{99}$.
By comparing Pyronaridine with the combinations Pyr-Ret-M a positive correlation between the $EC_{50}$ and $EC_{90}$ was observed, while for the $EC_{99}$ this could not be demonstrated. None of the correlations were significant.
A positive correlation was shown for all three EC values of Pyr-Ret-H, but again, none of them were significant.

## 4.5 Combinations Pyronaridine / Retinol

### 4.5.1 Pyronaridine-Retinol low (25,8ng Retinol)

The schizont maturation inhibition at a Pyronaridine-Retinol low concentration of 3nmol/l showed to be already 65,97%, at 300nmol/l it was 99,83%. At the highest used drug concentration of 3000nmol/l the SMI% was 100%.
While the activity of Retinol alone against P.falciparum is low, in combination with Pyronaridine it already showed a significant effect, even at the lowest concentration of 25,8ng Retinol. This combination has also more antimalarial activity than Pyronaridine used as a monocompount as one can see by comparing the EC values and the regression parameters of Pyronaridine alone and of the combination.

| 2008 | S | $f_s$ | $EC_{50}$ | $EC_{90}$ | $EC_{99}$ |
|---|---|---|---|---|---|
| PYR-Retinol low | 6,6612 | 2,2171 | 1,2155 | 13,9973 | 102,6041 |
| PYR | 10,4810 | 1,8426 | 12,6549 | 261,3598 | 3084,2398 |

S=slope function, fs= factor of S

**Table 47**: Comparison of regression parameters PYR-Ret low/PYR 2008

| PYR-Retinol low /PYR 2008 | SR | $f_{SR}$ | Slope of regression | PR | $f_{PR}$ | |
|---|---|---|---|---|---|---|
| $EC_{50}$ | 1,5734 | 2,7284 | parallel | 10,4112 | 2,7248 | significant |
| $EC_{90}$ | | | | 18,67215 | 5,183142 | significant |
| $EC_{99}$ | | | | 30,0596 | 12,9736 | significant |

SR=Slope Ratio, $f_{SR}$=Factor of slope ratio, PR= Potency ratio, $f_{PR}$=Factor of the potency ratio

**Table 48**: Comparison of regression parameters PYR-Ret low/PYR 2008

For all EC values of Pyronaridine and Pyronaridine-Retinol low a significant difference in the effectiveness could be demonstrated.

### 4.5.2 Pyronaridine-Retinol medium (31,6ng Retinol)

The schizont maturation inhibition at a Pyronaridine-Retinol medium concentration of 3nmol/l was already 78,46%, and at the next higher concentration of 10nmol/l the SMI% showed to be 93,14% . A complete schizont maturation inhibition of a 100% was reached at a concentration of 1000nmol/l.

This outcome reflects the fact that with rising Retinol concentration, the antimalarial activity of Pyronaridine is increasing significantly.

| 2008 | S | $f_s$ | $EC_{50}$ | $EC_{90}$ | $EC_{99}$ |
|---|---|---|---|---|---|
| PYR-Retinol med. | 6,7030 | 2,5575 | 0,6392 | 7,4204 | 54,7523 |
| PYR | 10,4810 | 1,8426 | 12,6549 | 261,3598 | 3084,2398 |

S=slope function, fs= factor of S

**Table 49**: Comparison of regression parameters PYR-Ret med/PYR 2008

| PYR-Retinol med. /PYR 2008 | SR | $f_{SR}$ | Slope of regression | PR | $f_{PR}$ | |
|---|---|---|---|---|---|---|
| $EC_{50}$ | 1,5636 | 3,0661 | parallel | 19,7980 | 2,7581 | significant |
| $EC_{90}$ | | | | 35,2217 | 5,9003 | significant |
| $EC_{99}$ | | | | 56,3307 | 16,8039 | significant |

SR=Slope Ratio, $f_{SR}$=Factor of slope ratio, PR= Potency ratio, $f_{PR}$=Factor of the potency ratio

**Table 50**: Comparison of regression parameters PYR-Ret med/PYR 2008

By comparing the regression parameters of Pyronaridine-Retinol medium with Pyronaridine, a significant difference in the effectiveness is demonstrated for all EC values.

### 4.5.3 Pyronaridine-Retinol high (37,3%)

The SMI% of this combination was 73,86% at the lowest concentration of Pyronaridine-Retinol high of 3nmol/l and reached a complete inhibition of a 100% at a drug concentration of 1000nmol/l.

In comparison with the schizont maturation inhibiting activity of Pyronaridine-Retinol medium the activity of Pyronaridine-Retinol high was still slightly higher but the largest increase of effectiveness was in between an added Retinol concentration of 25,8ng (low) and 31,6ng (medium).

| 2008 | S | $f_s$ | $EC_{50}$ | $EC_{90}$ | $EC_{99}$ |
|---|---|---|---|---|---|
| PYR-Retinol high | 5,3832 | 2,0856 | 0,9320 | 8,1565 | 47,8021 |
| PYR | 10,4810 | 1,8426 | 12,6549 | 261,3598 | 3084,2398 |

S=slope function, fs= factor of S

**Table 51**: Comparison of regression parameters PYR-Ret high/PYR 2008

| PYR-Retinol high/PYR 2008 | SR | $f_{SR}$ | Slope of regression | PR | $f_{PR}$ | |
|---|---|---|---|---|---|---|
| $EC_{50}$ | 1,9469 | 2,601148 | parallel | 13,5782 | 2,5363 | significant |
| $EC_{90}$ | | | | 32,0431 | 4,7238 | significant |
| $EC_{99}$ | | | | 64,5210 | 11,3774 | significant |

SR=Slope Ratio, $f_{SR}$=Factor of slope ratio, PR= Potency ratio, $f_{PR}$=Factor of the potency ratio

**Table 52**: Comparison of regression parameters PYR-Ret high/PYR 2008

The regression lines of both substances, Pyronaridine and the combination Pyronaridine-Retinol high, were parallel.

All calculated EC values ($EC_{50}$, $EC_{90}$, $EC_{99}$) showed a significant difference in the effectiveness.

### 4.5.4 Relation between all combinations, MCOC and interaction analysis

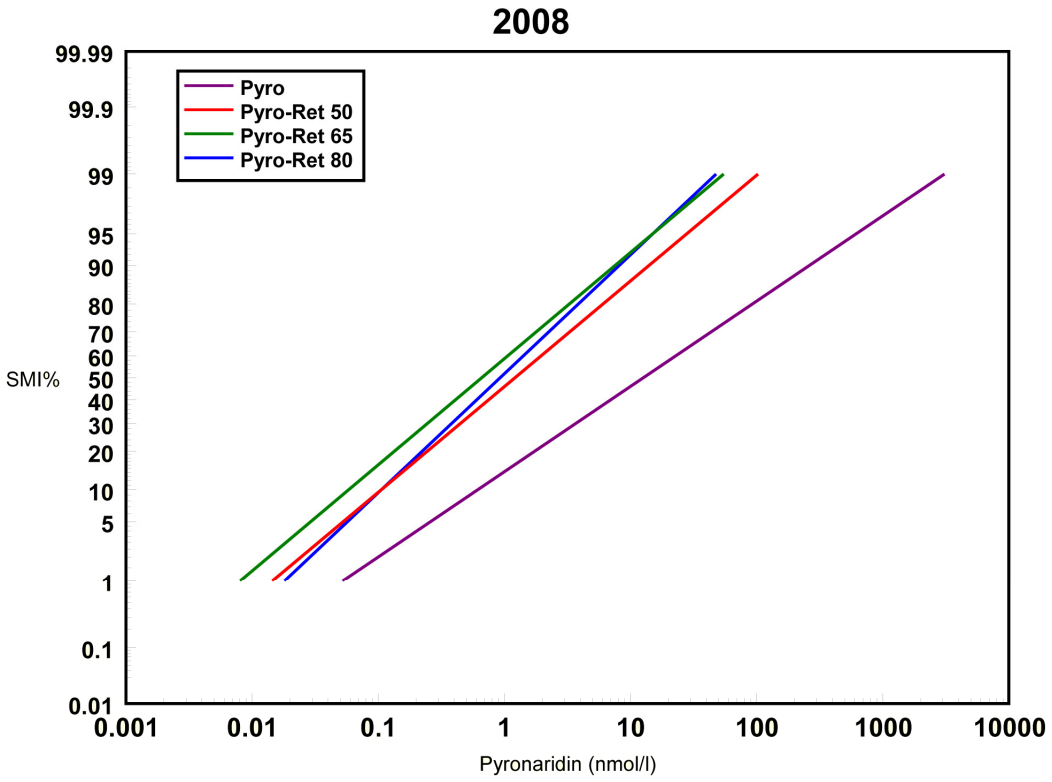


**Diagram 17**: Regression lines Pyronaridine/ Combinations

In the diagram above the sensitivity of P. falciparum to Pyronaridin and to the combinations with Retinol are graphically displayed. As compared to pyronaridine alone, the slopes of the regressions observed with the pyronaridine + retinol combinations were steeper. The steepest increase of the slope of the regression was between the regression lines of Pyronaridie alone (violet) and Pyronaridine-Retinol low (red). Between Pyronaridine-Retinol low and Pyronaridine-Retinol medium (green) this increase still improved, with little further increment between Pyronaridine-Retinol medium and Pyronaridine-Retinol high (blue).

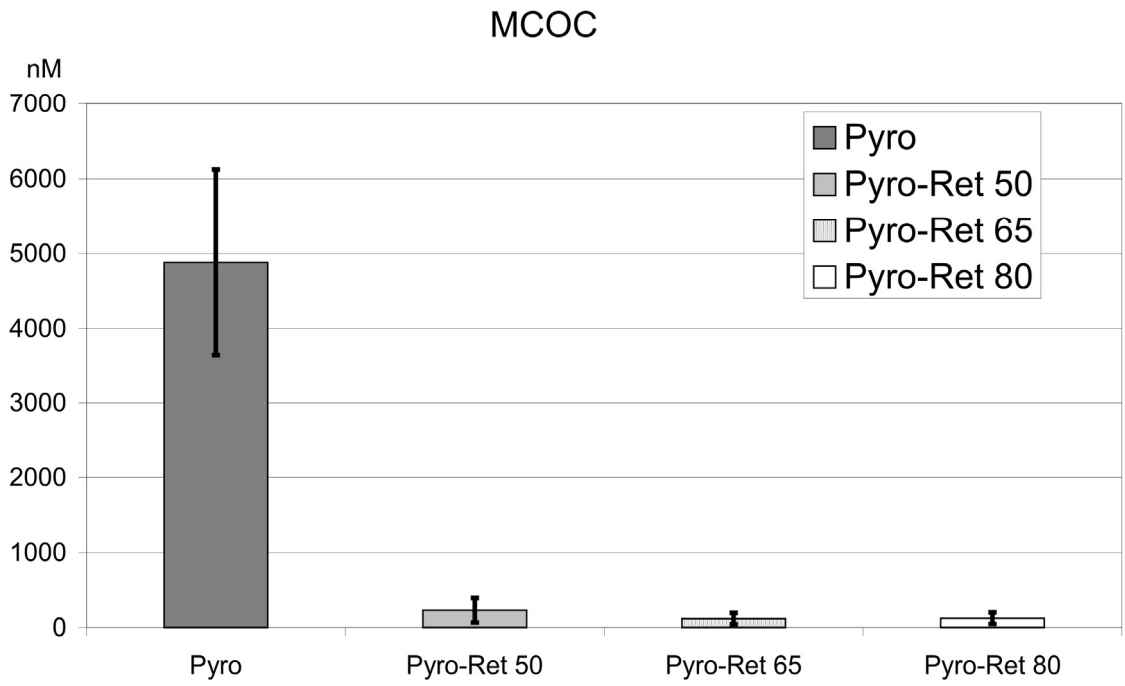


**Diagram 18**: MCOC Pyronaridine and combinations 2008

The MCOC was calculated and then graphically displayed first for the monosubstance Pyronaridine (MCOC of 4878,95 nmol/l BMM) and then for the combinations Pyronaridine / Retinol low, medium and high (MCOC of 226,32 nmol/l BMM, 114,05 nmol/l BMM and 122,43nmol/l BMM).

The MCOC of all combinations was decreasing compared to the one of Pyronaridine alone. A significant difference in the MCOC can be observed when Pyronaridine alone is compared with Pyronaridine + Retinol low ($t = 7.4009$ and $p = 5.3266 \times 10^{-10}$), Pyronaridine + Retinol medium ($t = 1.8337$ and $p = 1.8337 \times 10^{-10}$), and Pyronaridine + Retinol high ($t = 7.6156$ and $p = 1.9290 \times 10^{-10}$).

This outcome shows a much higher potential of schizont maturation inhibition by the drug combinations then by the monocompount. Therefore, the increasing concentrations of Retinol added to Pyronaridine are displaying the rising efficiency of the combination against P. falciparum.

Interaction analysis

The $EC_{50}$ for the combination of Pyronaridine-Retinol-low (25,8ng Retinol) showed synergism in 84,2% while 10,5% had an additive effect and another 5,3% were antagonistic. A difference in the interaction between a lower concentration of Retinol added to Pyronaridine compared with higher concentrations (31,6ng and 37,3ng Retinol) could not be observed.

But synergism increases with the ECs. The $EC_{90}$ showed a synergistic pattern in 94,6% for the combination Pyr-Ret-L, while 2,7% of the isolates showed additive behaviour. None of the isolates had an antagonistic effect. The $EC_{90}$ for higher concentrations (31,6ng and 37,3ng Retinol) showed similar outcomes, 97,3% of both had synergistic effects, none of them had a additive effect while only Pyr-Ret-medium showed an antergonistic behaviour in 2,7%. Overall, this outcome indicates strong synergism between both substances, Pyronaridine and Retinol.

# 5 Conclusion

Although the in vitro sensitivity to Lumefantrine has declined between 2005 and 2008 in the study area of Mae Sot, the $EC_{99}$ is still within the therapeutic range without a sign of resistance. The effectiveness of DBB as well has slightly decreased in the last three years, but still, it shows more activity against P.falciparum in vitro than Lumefantrine.
In both drugs a nearly parallel regressionline was observed and the MCOC values were 4200nmol/l BMM for Lumefantrine, and 994,74nmol/l BMM for DBB, representing a significant difference between them (t=5,54102; p=0,0001).
The results suggest that DBB may replace Lumefantrine in association with Arthemeter in the treatment of uncomplicated malaria in the future, in case the decrease of the sensitivity to Lumefantrine will continue.
Also the in vitro sensitivity of Pyronaridine against P.falciparum has decreased compared with earlier made investigations, due to its uncontrolled and frequent use as a monocompound. But in combination with Retinol, a considerable increase of the effectiveness of Pyronaridine was observed.
The suggestion that Retinol has an antimalarial effect by inhibiting the growth of the parasite has been already confirmed in several studies[40, 44, 46].
Also earlier made investigations with Retinol in combination with other antimalarial drugs demonstrated synergistic interaction[47,32, 42]. The exact underlying mechanism of how Retinol acts against the parasite is not yet clear, nor is the nature of the pharmacodynamic interaction between Retinol and Pyronaridine. This should be an object of further investigations. But the observed synergism between Retinol and Pyronaridine in our study could indicate the possibility to maintain or even enhance the effectiveness of Pyronaridine in the future.

# 6 Annex

**Patient data**

| DATE | PAT. NO. | PAT. REG. MC. | SEX | AGE | OCCUPATION | RESIDENCE COUNTRY | ORIGIN OF INFECTION |
|---|---|---|---|---|---|---|---|
| 02/06/08 | 1 | 68B | m | 16 | Labour | Myanmar | Myanmar |
| 02/06/08 | 2 | 21B | m | 24 | Labour | Thailand | Myanmar |
| 02/06/08 | 3 | 69B | m | 26 | Labour | Myanmar | Myanmar |
| 02/06/08 | 4 | 84B | m | 22 | Labour | Myanmar | Myanmar |
| 02/06/08 | 5 | 489/51 | m | 39 | Labour | Myanmar | Myanmar |
| 02/06/08 | 6 | 93B | f | 30 | Labour | Thailand | Myanmar |
| 02/06/08 | 7 | 23/108 | m | 41 | Labour | Thailand | Thailand |
| 03/06/08 | 8 | 99B | m | 20 | Labour | Myanmar | Myanmar |
| 03/06/08 | 9 | 102B | m | 23 | Labour | Myanmar | Myanmar |
| 04/06/08 | 10 | 110B | m | 15 | Student | Myanmar | Myanmar |
| 05/06/08 | 11 | 120B | m | 50 | Farmer | Myanmar | Myanmar |
| 05/06/08 | 12 | 123B | m | 9 | Student | Myanmar | Myanmar |
| 05/06/08 | 13 | 30B | m | 30 | Labour | Thailand | Myanmar |
| 06/06/08 | 14 | 133B | m | 15 | Student | Myanmar | Myanmar |
| 09/06/08 | 15 | 530B | m | 28 | Labour | Myanmar | Myanmar |
| 09/06/08 | 16 | 538B | m | 44 | Labour | Myanmar | Myanmar |
| 10/06/08 | 17 | 32B | m | 37 | Farmer | Thailand | Myanmar |
| 10/06/08 | 18 | 165B | m | 27 | Labour | Myanmar | Myanmar |
| 11/06/08 | 19 | 171B | f | 13 | Student | Myanmar | Myanmar |
| 11/06/08 | 20 | 169B | m | 22 | Labour | Thailand | Thailand |
| 11/06/08 | 21 | 177B | f | 22 | Farmer | Myanmar | Myanmar |
| 12/06/08 | 22 | 110A | m | 42 | Farmer | Thailand | Myanmar |
| 12/06/08 | 23 | 112 | m | 40 | Farmer | Thailand | Thailand |
| 12/06/08 | 24 | 113 | f | 42 | Farmer | Thailand | Myanmar |
| 12/06/08 | 25 | 186B | m | 30 | Labour | Myanmar | Myanmar |
| 12/06/08 | 26 | 187B | m | 27 | Labour | Myanmar | Myanmar |
| 12/06/08 | 27 | 188B | m | 31 | Labour | Myanmar | Myanmar |
| 13/06/08 | 28 | 197B | m | 17 | Student | Myanmar | Myanmar |
| 16/06/08 | 29 | 48B | m | 15 | Student | Thailand | Myanmar |
| 16/06/08 | 30 | 49B | m | 19 | Labour | Thailand | Myanmar |
| 16/06/08 | 31 | 205B | m | 40 | Labour | Myanmar | Myanmar |
| 16/06/08 | 32 | 541/51 | m | 11 | Student | Myanmar | Myanmar |
| 16/06/08 | 33 | 540/51 | m | 30 | Labour | Myanmar | Myanmar |
| 16/06/08 | 34 | 206B | m | 27 | Labour | Myanmar | Myanmar |
| 17/06/08 | 35 | 212B | m | 32 | Labour | Myanmar | Myanmar |
| 18/06/08 | 36 | 223B | f | 35 | Farmer | Thailand | Myanmar |
| 18/06/08 | 37 | 226B | m | 32 | Labour | Myanmar | Myanmar |
| 18/06/08 | 38 | 61B | m | 26 | Labour | Thailand | Myanmar |
| 19/06/08 | 39 | 230B | f | 40 | Farmer | Myanmar | Myanmar |
| 19/06/08 | 40 | 228B | m | 9 | Student | Myanmar | Myanmar |
| 20/06/08 | 41 | 73B | m | 15 | Student | Thailand | Myanmar |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 25/06/08 | 42 | 25B | m | 9 | Student | Myanmar | Myanmar |
| 27/06/08 | 43 | 58B | m | 23 | Labour | Myanmar | Myanmar |
| 27/06/08 | 44 | 35A | m | 56 | Labour | Thailand | Myanmar |
| 30/06/08 | 45 | 37A | m | 15 | Student | Thailand | Thailand |
| 30/06/08 | 46 | 67B | m | 19 | Student | Myanmar | Myanmar |
| 30/06/08 | 47 | 21B | m | 35 | Farmer | Thailand | Myanmar |
| 30/06/08 | 48 | 24B | f | 43 | Farmer | Thailand | Thailand |

**Pre-incubation slides**

| **ISOLATE NO:** | PRE-INCUB. PARASITAEMIA/µl | % MED&LARGE TROPHOZOITES | WELL A SCHIZONTS/ 200 TROPHOZOITES | RESULT |
|---|---|---|---|---|
| **1** | 62400 | 40 | 20 | OK |
| **2** | 2480 | 51 | 44 | OK |
| **3** | 135900 | 68 | 70 | OK |
| **4** | 270000 | 69 | 59 | OK |
| **5** | 3990 | 61 | 77 | OK |
| **6** | 42824 | 25 | 20 | OK |
| **7** | 50037 | 44 | 20 | OK |
| **8** | 23652 | 69 | 104 | OK |
| **9** | 233393 | 23 | 24 | OK |
| **10** | 29393 | 61 | 57 | OK |
| **11** | 16444 | 47 | 56 | OUT |
| **12** | 220000 | 83 | 23 | OK |
| **13** | 8160 | 55 | 163 | OK |
| **14** | 41882 | 47 | 159 | OK |
| **15** | 3200 | 23 | 18 | OUT |
| **16** | 40471 | 84 | 168 | OK |
| **17** | 28800 | 5 | <20 | OUT |
| **18** | 1000 | 60 | 74 | OUT |
| **19** | 20480 | 69 | 138 | OK |
| **20** | 4471 | 80 | 118 | OK |
| **21** | 108667 | 65 | <20 | OUT |
| **22** | 12903 | 74 | 22 | OK |
| **23** | 26933 | 33 | 28 | OK |
| **24** | 270545 | 15 | 52 | OK |
| **25** | 32523 | 37 | 42 | OK |
| **26** | 35733 | 6 | <20 | OUT |
| **27** | 22054 | 58 | <20 | OUT |
| **28** | 106667 | 40 | 52 | OK |
| **29** | 133818 | 84 | 152 | OK |
| **30** | 6316 | 40 | 34 | OK |
| **31** | 62545 | 25 | 26 | OK |
| **32** | 15273 | 67 | 63 | OK |
| **33** | 1524 | 89 | 61 | OK |

| | | | | |
|---|---|---|---|---|
| **34** | 18667 | 17 | 21 | OK |
| **35** | 76571 | 57 | 21 | OK |
| **36** | 48696 | 15 | 21 | OK |
| **37** | 2256 | 27 | 39 | OK |
| **38** | 61895 | 97 | 37 | OK |
| **39** | 12267 | 59 | 123 | OK |
| **40** | 34667 | 28 | 20 | OUT |
| **41** | 7273 | 70 | 20 | OUT |
| **42** | 9524 | 30 | 27 | OK |
| **43** | 7226 | 52 | 79 | OK |
| **44** | 9481 | 63 | 40 | OK |
| **45** | 2479 | 31 | <20 | OUT |
| **46** | 282667 | 37 | 49 | OK |
| **47** | 12364 | 39 | 130 | OK |
| **48** | 142769 | 34 | 80 | OK |

**Detailed test record**

**LUMEFANTRINE Mae Sot 2008**

| | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| **Pat. No.:** | Control SCHIZ | Conc. SCHIZ | Conc. SCHIZ | Conc. SCHIZ | Conc. SCHIZ | Conc. SCHIZ | Conc. SCHIZ | Conc. SCHIZ |
| | 0 | 3 | 10 | 30 | 100 | 300 | 1000 | 3000 |
| **1** | 49 | 44 | 39 | 23 | 14 | 5 | 2 | 0 |
| **2** | 36 | 28 | 25 | 17 | 12 | 7 | 4 | 1 |
| **3** | 83 | 83 | 83 | 58 | 41 | 19 | 7 | 3 |
| **4** | 75 | 54 | 39 | 22 | 12 | 3 | 1 | 0 |
| **5** | 55 | 55 | 19 | 13 | 7 | 5 | 2 | 1 |
| **6** | 29 | 21 | 15 | 6 | 2 | 1 | 0 | 0 |
| **7** | 29 | 23 | 19 | 12 | 8 | 4 | 2 | 0 |
| **8** | 113 | 86 | 73 | 24 | 1 | 1 | 0 | 0 |
| **9** | 20 | 20 | 20 | 9 | 2 | 1 | 1 | 0 |
| **10** | 54 | 46 | 44 | 25 | 6 | 4 | 2 | 0 |
| **12** | 48 | 40 | 33 | 14 | 6 | 2 | 0 | 0 |
| **13** | 163 | 152 | 129 | 63 | 12 | 10 | 3 | 0 |
| **14** | 159 | 159 | 80 | 43 | 23 | 7 | 2 | 0 |
| **16** | 81 | 80 | 77 | 74 | 17 | 5 | 2 | 1 |
| **19** | 121 | 121 | 121 | 111 | 24 | 3 | 2 | 0 |
| **20** | 87 | 76 | 76 | 50 | 10 | 6 | 2 | 0 |
| **22** | 33 | 28 | 23 | 19 | 15 | 9 | 5 | 1 |
| **23** | 34 | 28 | 23 | 16 | 11 | 3 | 0 | 0 |
| **24** | 32 | 30 | 29 | 12 | 5 | 2 | 0 | 0 |
| **25** | 90 | 76 | 65 | 21 | 7 | 4 | 2 | 0 |
| **28** | 49 | 49 | 42 | 12 | 6 | 3 | 1 | 0 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **29** | 177 | 165 | 160 | 84 | 42 | 21 | 9 | 2 |
| **30** | 27 | 26 | 25 | 14 | 8 | 5 | 2 | 0 |
| **31** | 26 | 24 | 21 | 9 | 4 | 2 | 1 | 0 |
| **32** | 45 | 45 | 45 | 27 | 11 | 5 | 2 | 0 |
| **33** | 33 | 30 | 27 | 14 | 7 | 4 | 2 | 0 |
| **34** | 34 | 31 | 29 | 22 | 16 | 6 | 2 | 0 |
| **35** | 32 | 32 | 32 | 20 | 13 | 6 | 3 | 1 |
| **36** | 44 | 27 | 17 | 5 | 1 | 0 | 0 | 0 |
| **37** | 20 | 15 | 5 | 2 | 2 | 1 | 0 | 0 |
| **38** | 54 | 49 | 44 | 7 | 1 | 0 | 0 | 0 |
| **39** | 101 | 101 | 97 | 82 | 13 | 2 | 1 | 0 |
| **42** | 23 | 16 | 11 | 9 | 8 | 4 | 2 | 0 |
| **43** | 29 | 26 | 23 | 21 | 19 | 6 | 2 | 0 |
| **44** | 34 | 25 | 19 | 16 | 13 | 4 | 1 | 0 |
| **46** | 44 | 40 | 37 | 32 | 27 | 5 | 1 | 0 |
| **47** | 133 | 129 | 126 | 78 | 23 | 8 | 3 | 1 |
| **49** | 101 | 100 | 93 | 74 | 15 | 3 | 2 | 1 |

**DBB Mae Sot 2008**

| | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| **Pat. No.:** | Control SCHIZ | Conc. SCHIZ | Conc. SCHIZ | Conc. SCHIZ | Conc. SCHIZ | Conc. SCHIZ | Conc. SCHIZ | Conc. SCHIZ |
| | 0 | 3 | 10 | 30 | 100 | 300 | 1000 | 3000 |
| **1** | 48 | 29 | 18 | 10 | 6 | 2 | 0 | 0 |
| **2** | 40 | 22 | 20 | 11 | 8 | 6 | 4 | 0 |
| **3** | 74 | 66 | 51 | 22 | 11 | 4 | 1 | 0 |
| **4** | 59 | 25 | 11 | 5 | 2 | 0 | 0 | 0 |
| **5** | 85 | 40 | 15 | 5 | 2 | 1 | 0 | 0 |
| **6** | 45 | 16 | 6 | 3 | 0 | 0 | 0 | 0 |
| **7** | 27 | 19 | 13 | 5 | 2 | 0 | 0 | 0 |
| **8** | 148 | 107 | 32 | 6 | 2 | 0 | 0 | 0 |
| **9** | 26 | 14 | 8 | 1 | 1 | 0 | 0 | 0 |
| **10** | 52 | 27 | 15 | 2 | 2 | 1 | 0 | 0 |
| **12** | 41 | 20 | 10 | 3 | 0 | 0 | 0 | 0 |
| **13** | 156 | 129 | 35 | 10 | 4 | 2 | 0 | 0 |
| **14** | 168 | 167 | 91 | 20 | 5 | 0 | 0 | 0 |
| **16** | 75 | 70 | 65 | 31 | 1 | 1 | 0 | 0 |
| **19** | 120 | 113 | 52 | 8 | 3 | 2 | 0 | 0 |
| **20** | 76 | 62 | 6 | 4 | 2 | 2 | 1 | 0 |
| **22** | 25 | 22 | 20 | 11 | 6 | 1 | 0 | 0 |
| **23** | 26 | 19 | 14 | 7 | 4 | 1 | 0 | 0 |
| **24** | 32 | 16 | 8 | 3 | 1 | 0 | 0 | 0 |
| **25** | 71 | 40 | 23 | 5 | 1 | 0 | 0 | 0 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **28** | 33 | 21 | 7 | 4 | 2 | 2 | 0 | 0 |
| **29** | 169 | 159 | 93 | 53 | 11 | 8 | 2 | 0 |
| **30** | 22 | 17 | 13 | 5 | 1 | 0 | 0 | 0 |
| **31** | 25 | 25 | 24 | 5 | 1 | 0 | 0 | 0 |
| **32** | 46 | 45 | 18 | 3 | 1 | 0 | 0 | 0 |
| **33** | 48 | 34 | 5 | 1 | 0 | 0 | 0 | 0 |
| **34** | 30 | 16 | 20 | 9 | 4 | 1 | 0 | 0 |
| **35** | 33 | 28 | 23 | 14 | 8 | 2 | 0 | 0 |
| **36** | 24 | 10 | 4 | 1 | 0 | 0 | 0 | 0 |
| **37** | 20 | 16 | 12 | 4 | 2 | 1 | 0 | 0 |
| **38** | 57 | 17 | 5 | 2 | 0 | 0 | 0 | 0 |
| **39** | 100 | 100 | 81 | 18 | 1 | 1 | 0 | 0 |
| **42** | 32 | 18 | 10 | 8 | 6 | 2 | 1 | 0 |
| **43** | 36 | 25 | 18 | 8 | 4 | 1 | 0 | 0 |
| **44** | 32 | 22 | 15 | 11 | 8 | 2 | 0 | 0 |
| **46** | 51 | 36 | 25 | 5 | 1 | 0 | 0 | 0 |
| **47** | 136 | 113 | 56 | 40 | 16 | 4 | 1 | 0 |
| **48** | 104 | 84 | 40 | 6 | 2 | 1 | 0 | 0 |

**RETINOL Mae Sot 2008**

| | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| **Pat. No.:** | Control SCHIZ | Conc. SCHIZ | Conc. SCHIZ | Conc. SCHIZ | Conc. SCHIZ | Conc. SCHIZ | Conc. SCHIZ | Conc. SCHIZ |
| | 0 | 30 | 100 | 300 | 1000 | 3000 | 10000 | 30000 |
| **1** | 29 | 27 | 25 | 23 | 21 | 20 | 18 | 16 |
| **2** | 41 | 37 | 33 | 29 | 26 | 23 | 21 | 18 |
| **3** | 92 | 86 | 83 | 80 | 74 | 69 | 64 | 59 |
| **4** | 36 | 32 | 28 | 25 | 22 | 19 | 17 | 15 |
| **5** | 85 | 75 | 66 | 58 | 51 | 45 | 39 | 34 |
| **6** | 25 | 25 | 24 | 21 | 18 | 16 | 14 | 12 |
| **7** | 26 | 26 | 25 | 25 | 24 | 23 | 21 | 19 |
| **8** | 108 | 106 | 104 | 103 | 90 | 84 | 76 | 69 |
| **9** | 30 | 24 | 20 | 20 | 15 | 11 | 8 | 6 |
| **10** | 51 | 46 | 42 | 38 | 34 | 31 | 28 | 25 |
| **12** | 64 | 57 | 51 | 50 | 48 | 47 | 45 | 43 |
| **13** | 169 | 157 | 147 | 137 | 127 | 118 | 108 | 99 |
| **14** | 166 | 161 | 155 | 150 | 145 | 139 | 134 | 130 |
| **16** | 109 | 96 | 84 | 74 | 65 | 57 | 50 | 44 |
| **19** | 133 | 112 | 87 | 68 | 49 | 35 | 18 | 9 |
| **20** | 75 | 72 | 68 | 65 | 61 | 58 | 55 | 52 |
| **22** | 26 | 26 | 25 | 25 | 24 | 23 | 22 | 21 |
| **23** | 21 | 21 | 21 | 20 | 19 | 18 | 17 | 16 |
| **24** | 29 | 28 | 26 | 25 | 24 | 23 | 22 | 20 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **25** | 85 | 82 | 79 | 77 | 74 | 72 | 69 | 66 |
| **28** | 50 | 42 | 35 | 29 | 24 | 20 | 16 | 13 |
| **29** | 182 | 173 | 164 | 158 | 148 | 141 | 134 | 127 |
| **30** | 34 | 33 | 31 | 30 | 29 | 28 | 27 | 26 |
| **31** | 31 | 29 | 26 | 23 | 21 | 19 | 17 | 15 |
| **32** | 53 | 51 | 49 | 48 | 46 | 22 | 11 | 6 |
| **33** | | | | | | | | |
| **34** | 27 | 27 | 26 | 26 | 25 | 24 | 23 | 22 |
| **35** | 28 | 28 | 27 | 26 | 25 | 24 | 23 | 21 |
| **36** | 23 | 22 | 20 | 18 | 17 | 16 | 14 | 12 |
| **37** | | | | | | | | |
| **38** | 67 | 64 | 60 | 57 | 54 | 52 | 49 | 46 |
| **39** | 115 | 110 | 103 | 98 | 92 | 62 | 42 | 28 |
| **42** | 22 | 21 | 19 | 19 | 18 | 17 | 16 | 15 |
| **43** | 26 | 25 | 23 | 21 | 19 | 17 | 15 | 13 |
| **44** | 22 | 21 | 20 | 19 | 18 | 17 | 16 | 15 |
| **46** | 36 | 36 | 35 | 31 | 27 | 24 | 21 | 18 |
| **47** | 112 | 104 | 96 | 89 | 83 | 65 | 46 | 32 |
| **48** | 95 | 92 | 88 | 86 | 60 | 41 | 29 | 20 |

**PYRONARIDINE Mae Sot 2008**

| | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| **Pat. No.:** | Control SCHIZ | Conc. SCHIZ | Conc. SCHIZ | Conc. SCHIZ | Conc. SCHIZ | Conc. SCHIZ | Conc. SCHIZ | Conc. SCHIZ |
| | 0 | 3 | 10 | 30 | 100 | 300 | 1000 | 3000 |
| **1** | 34 | 29 | 25 | 21 | 17 | 5 | 2 | 0 |
| **2** | 40 | 22 | 7 | 7 | 7 | 5 | 1 | 1 |
| **3** | 150 | 38 | 21 | 9 | 3 | 0 | 0 | 0 |
| **4** | 81 | 64 | 50 | 28 | 15 | 4 | 1 | 0 |
| **5** | 57 | 36 | 21 | 10 | 2 | 1 | 1 | 0 |
| **6** | 39 | 28 | 20 | 14 | 10 | 3 | 0 | 0 |
| **7** | 36 | 22 | 13 | 6 | 3 | 1 | 0 | 0 |
| **8** | 108 | 107 | 90 | 82 | 15 | 6 | 2 | 1 |
| **9** | 20 | 18 | 9 | 4 | 3 | 1 | 1 | 0 |
| **10** | 56 | 15 | 12 | 11 | 10 | 10 | 3 | 1 |
| **12** | 56 | 51 | 47 | 35 | 26 | 5 | 1 | 0 |
| **13** | 172 | 69 | 28 | 15 | 9 | 6 | 3 | 1 |
| **14** | 179 | 160 | 143 | 100 | 28 | 9 | 4 | 2 |
| **16** | 116 | 115 | 65 | 47 | 8 | 5 | 3 | 1 |
| **19** | 137 | 127 | 109 | 66 | 50 | 15 | 9 | 3 |
| **20** | 82 | 68 | 16 | 4 | 3 | 2 | 1 | 0 |
| **22** | 40 | 31 | 24 | 18 | 13 | 5 | 2 | 0 |
| **23** | 27 | 19 | 13 | 10 | 8 | 4 | 2 | 0 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **24** | 32 | 31 | 31 | 10 | 3 | 1 | 0 | 0 |
| **25** | 74 | 56 | 43 | 16 | 6 | 3 | 1 | 0 |
| **28** | 38 | 17 | 9 | 6 | 4 | 1 | 0 | 0 |
| **29** | 170 | 160 | 140 | 106 | 54 | 36 | 24 | 16 |
| **30** | 23 | 16 | 11 | 5 | 2 | 1 | 0 | 0 |
| **31** | 21 | 15 | 11 | 7 | 5 | 3 | 1 | 0 |
| **32** | 78 | 49 | 31 | 11 | 4 | 2 | 1 | 0 |
| **33** | 48 | 34 | 25 | 20 | 15 | 11 | 7 | 3 |
| **34** | 29 | 23 | 19 | 12 | 8 | 4 | 2 | 0 |
| **35** | 38 | 33 | 29 | 10 | 9 | 5 | 3 | 1 |
| **36** | 22 | 13 | 8 | 6 | 4 | 2 | 0 | 0 |
| **37** | 20 | 13 | 9 | 8 | 5 | 3 | 1 | 0 |
| **38** | 61 | 41 | 27 | 15 | 8 | 2 | 0 | 0 |
| **39** | 121 | 109 | 32 | 5 | 0 | 0 | 0 | 0 |
| **42** | 27 | 15 | 8 | 7 | 5 | 3 | 2 | 1 |
| **43** | 29 | 23 | 19 | 12 | 7 | 3 | 1 | 0 |
| **44** | 34 | 31 | 29 | 18 | 11 | 5 | 2 | 0 |
| **46** | 38 | 32 | 27 | 12 | 5 | 2 | 1 | 0 |
| **47** | 136 | 123 | 74 | 54 | 45 | 27 | 16 | 9 |
| **48** | 86 | 76 | 75 | 74 | 21 | 6 | 4 | 1 |

**PYR-RET low Mae Sot 2008**

| | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| **Pat. No.:** | Control SCHIZ | Conc. SCHIZ | Conc. SCHIZ | Conc. SCHIZ | Conc. SCHIZ | Conc. SCHIZ | Conc. SCHIZ | Conc. SCHIZ |
| | 0 | 3 | 10 | 30 | 100 | 300 | 1000 | 3000 |
| **1** | 60 | 23 | 9 | 4 | 0 | 0 | 0 | 0 |
| **2** | 50 | 11 | 6 | 4 | 2 | 0 | 0 | 0 |
| **3** | 83 | 60 | 18 | 6 | 2 | 0 | 0 | 0 |
| **4** | 55 | 24 | 10 | 3 | 0 | 0 | 0 | 0 |
| **5** | 51 | 11 | 6 | 3 | 1 | 0 | 0 | 0 |
| **6** | 43 | 16 | 6 | 2 | 0 | 0 | 0 | 0 |
| **7** | 26 | 7 | 2 | 0 | 0 | 0 | 0 | 0 |
| **8** | 89 | 5 | 1 | 0 | 0 | 0 | 0 | 0 |
| **9** | 20 | 4 | 2 | 1 | 0 | 0 | 0 | 0 |
| **10** | 49 | 3 | 1 | 1 | 0 | 0 | 0 | 0 |
| **12** | 32 | 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| **13** | 156 | 8 | 4 | 0 | 0 | 0 | 0 | 0 |
| **14** | 176 | 164 | 3 | 2 | 1 | 0 | 0 | 0 |
| **16** | 154 | 101 | 7 | 4 | 1 | 0 | 0 | 0 |
| **19** | 116 | 113 | 17 | 13 | 7 | 3 | 1 | 0 |
| **20** | 73 | 6 | 5 | 4 | 1 | 0 | 0 | 0 |
| **22** | 28 | 8 | 2 | 0 | 0 | 0 | 0 | 0 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **23** | 43 | 9 | 2 | 0 | 0 | 0 | 0 | 0 |
| **24** | 30 | 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| **25** | 60 | 8 | 1 | 0 | 0 | 0 | 0 | 0 |
| **28** | 31 | 3 | 1 | 0 | 0 | 0 | 0 | 0 |
| **29** | 173 | 106 | 67 | 59 | 52 | 7 | 0 | 0 |
| **30** | 27 | 7 | 2 | 0 | 0 | 0 | 0 | 0 |
| **31** | 22 | 5 | 1 | 0 | 0 | 0 | 0 | 0 |
| **32** | 44 | 25 | 12 | 6 | 3 | 0 | 0 | 0 |
| **33** | 38 | 15 | 6 | 3 | 1 | 0 | 0 | 0 |
| **34** | 31 | 8 | 2 | 0 | 0 | 0 | 0 | 0 |
| **35** | 30 | 20 | 13 | 4 | 1 | 0 | 0 | 0 |
| **36** | 32 | 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| **37** | 20 | 7 | 4 | 1 | 0 | 0 | 0 | 0 |
| **38** | 42 | 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| **39** | 108 | 30 | 6 | 2 | 0 | 0 | 0 | 0 |
| **42** | 23 | 8 | 3 | 0 | 0 | 0 | 0 | 0 |
| **43** | 28 | 11 | 4 | 2 | 1 | 0 | 0 | 0 |
| **44** | 32 | 10 | 3 | 0 | 0 | 0 | 0 | 0 |
| **46** | 40 | 15 | 6 | 2 | 0 | 0 | 0 | 0 |
| **47** | 123 | 17 | 4 | 1 | 0 | 0 | 0 | 0 |
| **48** | 89 | 63 | 19 | 6 | 2 | 0 | 0 | 0 |

**PYR-RET medium Mae Sot 2008**

| | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| **Pat. No.:** | Control SCHIZ | Conc. SCHIZ | Conc. SCHIZ | Conc. SCHIZ | Conc. SCHIZ | Conc. SCHIZ | Conc. SCHIZ | Conc. SCHIZ |
| | 0 | 3 | 10 | 30 | 100 | 300 | 1000 | 3000 |
| **1** | 48 | 22 | 10 | 3 | 0 | 0 | 0 | 0 |
| **2** | 32 | 2 | 1 | 0 | 0 | 0 | 0 | 0 |
| **3** | | | | | | | | |
| **4** | 55 | 7 | 0 | 0 | 0 | 0 | 0 | 0 |
| **5** | 51 | 4 | 1 | 0 | 0 | 0 | 0 | 0 |
| **6** | 37 | 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| **7** | 29 | 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| **8** | 88 | 3 | 1 | 0 | 0 | 0 | 0 | 0 |
| **9** | 20 | 6 | 2 | 1 | 0 | 0 | 0 | 0 |
| **10** | 41 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| **12** | 41 | 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| **13** | 160 | 9 | 1 | 0 | 0 | 0 | 0 | 0 |
| **14** | 165 | 19 | 2 | 1 | 0 | 0 | 0 | 0 |
| **16** | 77 | 30 | 6 | 2 | 1 | 0 | 0 | 0 |
| **19** | 125 | 35 | 10 | 3 | 1 | 0 | 0 | 0 |
| **20** | 88 | 28 | 15 | 8 | 3 | 1 | 0 | 0 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **22** | 39 | 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| **23** | 30 | 5 | 1 | 0 | 0 | 0 | 0 | 0 |
| **24** | 46 | 7 | 1 | 0 | 0 | 0 | 0 | 0 |
| **25** | 77 | 8 | 0 | 0 | 0 | 0 | 0 | 0 |
| **28** | 35 | 2 | 1 | 1 | 0 | 0 | 0 | 0 |
| **29** | 162 | 29 | 11 | 6 | 0 | 0 | 0 | 0 |
| **30** | 24 | 5 | 1 | 0 | 0 | 0 | 0 | 0 |
| **31** | 22 | 5 | 1 | 0 | 0 | 0 | 0 | 0 |
| **32** | 52 | 8 | 3 | 1 | 0 | 0 | 0 | 0 |
| **33** | 43 | 18 | 9 | 2 | 0 | 0 | 0 | 0 |
| **34** | 24 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| **35** | 27 | 13 | 6 | 2 | 0 | 0 | 0 | 0 |
| **36** | 42 | 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| **37** | 20 | 15 | 10 | 3 | 1 | 0 | 0 | 0 |
| **38** | 49 | 7 | 1 | 0 | 0 | 0 | 0 | 0 |
| **39** | 101 | 5 | 1 | 0 | 0 | 0 | 0 | 0 |
| **42** | 22 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| **43** | 31 | 6 | 1 | 0 | 0 | 0 | 0 | 0 |
| **44** | 32 | 6 | 1 | 0 | 0 | 0 | 0 | 0 |
| **46** | 43 | 7 | 1 | 0 | 0 | 0 | 0 | 0 |
| **47** | 135 | 32 | 8 | 2 | 0 | 0 | 0 | 0 |
| **48** | 101 | 71 | 42 | 11 | 6 | 2 | 0 | 0 |

**PYR-RET high Mae Sot 2008**

| | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| **Pat. No.:** | Control SCHIZ | Conc. SCHIZ | Conc. SCHIZ | Conc. SCHIZ | Conc. SCHIZ | Conc. SCHIZ | Conc. SCHIZ | Conc. SCHIZ |
| | 0 | 3 | 10 | 30 | 100 | 300 | 1000 | 3000 |
| **1** | 58 | 20 | 7 | 2 | 0 | 0 | 0 | 0 |
| **2** | 35 | 14 | 5 | 1 | 0 | 0 | 0 | 0 |
| **3** | 91 | 43 | 14 | 3 | 0 | 0 | 0 | 0 |
| **4** | 57 | 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| **5** | 39 | 7 | 4 | 2 | 0 | 0 | 0 | 0 |
| **6** | 47 | 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| **7** | 34 | 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| **8** | 161 | 117 | 47 | 15 | 5 | 2 | 0 | 0 |
| **9** | 20 | 4 | 2 | 1 | 0 | 0 | 0 | 0 |
| **10** | 45 | 8 | 3 | 1 | 0 | 0 | 0 | 0 |
| **12** | 48 | 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| **13** | 160 | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| **14** | 169 | 139 | 4 | 2 | 1 | 0 | 0 | 0 |
| **16** | 152 | 97 | 19 | 4 | 2 | 0 | 0 | 0 |
| **19** | 118 | 99 | 25 | 6 | 2 | 1 | 0 | 0 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **20** | 86 | 17 | 5 | 1 | 0 | 0 | 0 | 0 |
| **22** | 29 | 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| **23** | 27 | 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| **24** | 28 | 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| **25** | 60 | 7 | 0 | 0 | 0 | 0 | 0 | 0 |
| **28** | 42 | 5 | 2 | 1 | 0 | 0 | 0 | 0 |
| **29** | 156 | 81 | 11 | 3 | 1 | 0 | 0 | 0 |
| **30** | 33 | 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| **31** | 21 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| **32** | 61 | 11 | 2 | 1 | 0 | 0 | 0 | 0 |
| **33** | 24 | 5 | 1 | 0 | 0 | 0 | 0 | 0 |
| **34** | 26 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| **35** | 32 | 8 | 2 | 0 | 0 | 0 | 0 | 0 |
| **36** | 21 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| **37** | 31 | 21 | 14 | 5 | 2 | 0 | 0 | 0 |
| **38** | 43 | 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| **39** | 110 | 10 | 1 | 0 | 0 | 0 | 0 | 0 |
| **42** | 24 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| **43** | 29 | 5 | 1 | 0 | 0 | 0 | 0 | 0 |
| **44** | 31 | 6 | 1 | 0 | 0 | 0 | 0 | 0 |
| **46** | | | | | | | | |
| **47** | 122 | 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| **48** | 93 | 8 | 1 | 0 | 0 | 0 | 0 | 0 |

**CURRICULUM VITAE**

Date of birth: 16.11.1979
Place of birth: Innsbruck / Austria
Family status: unmarried

| | |
|---|---|
| School education: | 1986-1998 Rudolf-Steiner school Linz / Austria |
| | January- July 1997 Arlington High school / Arlington, Massachusetts (USA) |
| 1998-1999 | General qualification for university entrance |

University education:

| | |
|---|---|
| 2001-2003 | Slavic studies/ Russian, University of Vienna |
| since 2003 | Medical studies, Medical University of Graz |

Clinical electives / practical work in Austria:

| | |
|---|---|
| July/ August 2000 | Practical work, orthopedy, Barmherzige Schwestern / Linz |
| 2001-2003 | Summer job as receptionist in a youth hostel / Vienna |
| January 2004 | Practical work, urology, LKH / Graz |
| February 2005 | Clinical elective general surgery SMZOst / Danubehospital Vienna |
| July/ August 2005 | Nurse assistant gastroenterology SMZOst / Danubehospital Vienna |
| February 2006 | Clinical elective pneumology, LKH / Graz |
| July 2007 | Clinical elective gynecology/obstetrics, Landes- Kinder – und Frauenklinik / Linz |
| September 2007 | Clinical elective general internal medicine / LKH Freistadt |

International Clinical electives / practical work:

| | |
|---|---|
| August/ September | 2002 Practical work in an orphanage, Kostroma/ Russia |
| August 2004 | Practical work in an orphanage, Slujdjanka/ Siberia |
| August 2006 | Clinical elective internal medicine, hospital of Jacaltenango / Guatemala |
| February 2007 | Clinical elective gynecology/obstetrics, hospital San Carlos / Chiapas / Mexico |
| May/June 2008 | Field work for my thesis, Mae Sot/Thailand |
| April-July 2009 | ERASMUS Universidad Complutense de Madrid/Spain |

Language abilities:

English fluently,
Spanish and Russian working knowledge

# 7 References

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