QUALITY CONTROL PROTOCOL.

GENERAL RULES:

1. Keep **ALL** the OPD slides read by the lab in the previous month (see annex III: how to keep the slides).

2. Store the slides daily and **separate the positive slides from the negative**. The sample size calculation are done separately for negative and positive slides as the main measure of performance are:
   - Sensitivity, based on the total number of positive slides.
   - Specificity, based on the total number of negative slides.

3. If you have to send a sample of slides **always take the sample randomly**: DO NOT CHOOSE THE SLIDES.

4. Always send slides of **the previous** month to SMRU as soon as possible: Old slides of 2 or 3 months old should never be send as the number of slides unreadable is expected to increase with time.

5. **Take good care when recording the QC form** (**date**, **code**, **result** and lab tech name): If you record wrongly a code or a result then the recording mistake will be interpreted as a reading mistake.

6. The result must show the specie(s) found, the stage of development (T, S and/or G), and the parasitaemia with a percentage of RBC parasitised for all PFT +++ (3 +) and ++++ (4 +). Note also the presence of pigment in WBC when found.

7. Avoid as much as possible to send slides through post. If you have to, prepare a good secure packing.

THE NUMBER OF POSITIVE SLIDES AND NEGATIVE SLIDES YOU HAVE TO SEND IS RELATED TO DIFFERENT FACTORS:

- The precision you want for your sensitivity and specificity value.
  The smaller the precision the bigger the sample taken will be. **We should aim for a precision of at most p ± 0.03 with a 95 % confidence interval (level of significance α = 0.05).**

  Example: if your sensitivity is p = 0.94 (94 %) with a precision p ± 0.03, it's means that the real sensitivity value is comprised between 0.91 and 0.97 with a 95 % confidence interval.

- The **expected values** for the sensitivity and specificity: the expectation taken into account will be your last QC results.
  Use the following table to calculate the size of the sample needed.

- The number of slides invalid (unreadable), which cannot be cross-checked because of deterioration through time. This number varies especially with the season. **All sample sizes in the table have been increased by 20 % to take this into account.**
  If more than 20 % of slides are expected to be invalid the sample size should be increased accordingly.

FREQUENCY OF THE CONTROL

- It is related to the quality of the laboratory (previous QC) and the turnover of the staff: a good laboratory (sensitivity & specificity $\geq$ 95 %) with stable staff and continuous supervision of the smears and stains quality, needs a QC once or twice a year.

ANALYSIS

The following indicators should be assessed.

- Smears quality: thick smears, thin smear and stain.
- The global percentage of errors.
- Sensitivity, Specificity, Predictive positive value (PPV), Predictive negative value (PNV). All these values should be given with a precision (see annex I).
- Kappa value. It gives a range of agreement (from poor to very good) in the species differentiation (positive slides) between the lab and the control. This value should be given with a precision (see annex II).
- The detection accuracy of the different development stages (T, S & G). This is important especially for *Plasmodium falciparum*.
- The parasitaemia accuracy.

Important:

For the analysis of your QC **we need the total number of slides positive** and **the total number of slides negative** read by your lab during the dates matching the sample of slides sent for QC. Please send these 2 numbers altogether with the slides.

TABLE: Sample of **POSITIVE & NEGATIVE** slides for QC

N $_{-ve}$ = total number of **Negative** slides read during the month
N $_{+ve}$ = total number of **Positive** slides read during the month

p_1= **Specificity or Sensitivity** expected $p_1 \geq 93$ %
p_2= **Specificity or Sensitivity** expected $87\ \% \leq p_2 < 93\ \%$.
p_3= **Specificity or Sensitivity** expected $82\ \% \leq p_3 < 87\ \%$.

N $_{-ve}$ or N $_{+ve}$	***< 110***	***150***	***200***	***250***	***300***	***350***	***400***
p_1	All slides	110	130	145	155	155	170
p_2	All slides	130	170	190	220	230	250
p_3	All slides	All slides	180	220	240	265	290

N $_{-ve}$ or N $_{+ve}$	***500***	***800***	***1000***	***1500***	***2000***	***2500***	***3000***
p_1	170	205	205	215	230	230	230
p_2	275	325	350	380	410	420	430
p_3	320	400	430	480	530	550	580

Count N $_{-ve}$ and N $_{+ve}$ separately for the past month.

Take into account your **sensitivity** expected (last QC) for the number of **positive** slides to be taken.
Take into account your **specificity** expected (last QC) for the number of **negative** slides to be taken.

Example 1:

Last month your Total number of ***positive*** slides was 220. Your last QC showed a ***sensitivity*** of 92 %.

How many positive slides do you need to send for QC?

Look into the second row: p_2 (Sensitivity expected 87 % $\leq p_2 <$ 93 %). N_{+ve} (220) is roughly between 200 and 250, so the number of positive slides to be sent is between 170 and 190: **180**.

Important remark: this number is an **estimation** for a significant sample size. It does not need to be respected exactly. If you are 5 less or 5 more, that does not matter.

How to select them randomly?

If you have 220 positive slides and you want to send 180, you need to take off 40 slides randomly.
40 slides out of 220 (40 / 220 = 0.182) is close to 1 out of 6 (1 / 6 = 0.167).
Therefore, **without choosing the slides**, you will take off 1 slide every 6 slides: 183 will remain to be sent for QC.

Example 2:

Last month your Total number of ***negative*** slides was 613. Your last QC showed a ***specificity*** of 98 %.

How many slides do you need to send for QC?

Look into the first row: p_1 (Specificity expected $p_1 \geq$ 93 %). N_{-ve} (613) is between 500 and 800, so the number of negative slides to be sent is between 170 and 205: about 190.

How to select them randomly?

If you have 613 negative slides and you want to send around 200, you need to keep 1 slide out of 3 randomly (200 / 600 = 2 / 6 = 1 / 3).
Therefore, **without choosing the slides**, you will keep 1 slide every 3 slides for QC.

ANNEX I: SENSITIVITY, SPECIFICITY, P.P.V, P.N.V CALCULATION & PRECISION.

Example: Total number of slide positive read by the lab in that month: 102
Total number of slide negative read by the lab in that month: 383

Positive / Negative cross-tabulation.

		SMRU control		
		+	Neg	Total
L	+	99	3	102
A	Neg	6	174	180
B	Total	105	177	282

Sensitivity: 94,3 % (99 / 105)
Specificity: 98,3 % (174 / 177)

P.P.V: 97,1 % (99 / 102)
P.N.V: 96,7 % (174 / 180)

Precision: $p \pm 1.96 \times se$

p is the proportion: sensitivity, specificity, P.P.V or P.N.V

$$se = \sqrt{\frac{p(1-p)}{N} \times \frac{N_{pop} - N}{N_{pop}}}$$

1. **Sensitivity:** p = 99 / 105 = 0.943

The sensitivity is related to the number of slides positive. All the positive slides were crosschecked (no sample taken randomly) therefore the sensitivity value is the reel one and there is no precision value.

Sensitivity = 94.3 %

2. **Specificity:** p = 174 / 177 = 0.983

N_{pop} = N $-_{ve}$ = Total number of negative slides read by the lab in the month =383.
N = Denominator for the specificity =177.

se = 0.007
Specificity = 0.983 $\pm$ 0.0139
Specificity = 98.3 % precision 95 % CI (96.9 %, 99.7 %)

3. **P.P.V:** p = 99 / 102 = 0.971

The P.P.V is related to the number of slides positive. All the positive slides were crosschecked (no sample taken randomly) therefore the P.P.V is the reel one and there is no precision value.

P.P.V = 0.971 = 97.1 %

4. **P.N.V:** p = 174 / 180 = 0.967

N_{pop} = N $-_{ve}$ = Total number of negative slides read by the lab in the month =383.
N = Denominator of the P.N.V = 180.

se = 0.0097
P.N.V = 0.967 $\pm$ 0.019
P.N.V= 96.7 % precision 95 % CI (94.8 %, 98.6 %)

***Particular case*:**

When sensitivity, specificity, P.P.V or P.N.V = 100 %, Then p = 1 and the above formula is inapplicable.

Then the precision is: $(\alpha^{1/n}, 1)$

α is the level of significance, α = 0.05
n is the sample size

Example: Specificity: p = 143 / 143 = 1

$$\alpha^{1/n} = 0.05^{1/143} = 0.979$$

Specificity = 100 % precision (97.9 %, 100 %)

ANNEX II: KAPPA CALCULATION & CONFIDENCE INTERVAL.

Kappa (K) is a statistical value used to quantify the agreement between results.
Kappa ratings are as follow:

- $0 \leq K \leq 0.2$: poor agreement
- $0.2 < K \leq 0.4$: fair agreement
- $0.4 < K \leq 0.6$: moderate agreement
- $0.6 < K \leq 0.8$: good agreement
- $0.8 < K \leq 1$: very good agreement

Example:

Species (positive / positive) cross-tabulation.

		SMRU control				
		Pf	Pf + Pv	Pv	Pm	***Total***
LAB	Pf	74	2	0	0	*76*
	Pf + Pv	0	5	0	0	*5*
	Pv	2	1	72	0	*75*
	Pm	0	0	1	4	*5*
	Total	*76*	*8*	*73*	*4*	***161***

p_0 = proportion observed agreement.

$$K = \frac{p_0 - p_e}{1 - p_e}$$

p_e = proportion expected agreement.

$$p_0 = \frac{74 + 5 + 72 + 4}{161} = 0.963$$

$$p_e = \frac{\frac{(76 \times 76)}{161} + \frac{(5 \times 8)}{161} + \frac{(75 \times 73)}{161} + \frac{(5 \times 4)}{161}}{161} = \frac{70.25}{161} = 0.436$$

$$K = \frac{0.963 - 0.436}{1 - 0.436} = \frac{0.527}{0.564} = 0.934$$

Precision: $K \pm 1.96 \times se$

$$se = \sqrt{\frac{p_0 (1 - p_0)}{N (1 - p_e)^2}}$$ With N = total of positive slides cross-checked

$$se = \sqrt{\frac{0.963 (1 - 0.963)}{161 (1 - 0.436)^2}} = 0.0264$$

$K = 0.934 \pm 1.96 \times se = 0.934 \pm 0.052$

Kappa = 0.934 Precision (0.882, 0.986) **Very good agreement.**

ANNEX III: HOW TO KEEP AND STORE THE SLIDES IN GOOD CONDITION.

Store and keep slides in good condition is mainly a fight against humidity (fungus growth) and dust.
The fight against humidity is a difficult challenge in the peripheral laboratories, e specially during the rainy season.

The following points might help:

- To keep a slide in good condition, it has to be in good condition in the first place: smearing, drying, fixing and staining techniques must be correctly performed.
- Before starting to read the slides place beside the microscope pre-cut bands of newspaper (about 8-cm width).
- Put the MINIMUM quantity of immersion oil necessary for the reading.
- When the reading is finished, remove the slide from the stage and put it **FACE DOWN** (protection from dust) on the news paper band.
  Start to separate positive slides from negative slides at this point: one newspaper band for the positive and another one for the negative.
  The newspaper will absorb the oil from the slides.
- When the work of the day is finished, take a NEW band of newspaper and roll the slides into it (separate the positive from the negative).
- Then, pack the slides in a plastic bag with zip and record the date and "positive" or "negative" slides on the bag.

Note: this method is one among others and does not pretend to be the best. However it is one of the cheapest.
It is also possible to store slides in hermetic boxes with silicate gel or to send the slides regularly to a suitable place with air-conditioned.