International Consortium for Trials of Chemotherapeutic Agents in Tuberculosis (INTERTB)

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1. INTRODUCTION:

An international consortium has been created to evaluate the clinical and bacteriological outcomes of chemotherapeutic agents for the treatment of tuberculosis.

The members of the consortium are:

1. **Department of Cellular and Molecular Medicine, St. George's Hospital Medical School, London**
2. **MRC Clinical Trials Unit, London**
3. **Centro Internacional de Entrenamiento e Investigaciones Médicas (CIDEIM), Colombia**
4. **Dr. Bernard Fourie, South African Medical Research Council, Pretoria, South Africa**
5. **Harvard Medical School, Boston, Massachusetts, USA**

The participants in the Centre for Infection, Division of Cellular and Molecular Medicine, St George's Hospital Medical School, are **Professors Anthony Coates, Philip Butcher, Denis Mitchison** and **Dr Tom Harrison** (Consultant), **Mr. David Coleman** (Senior Research Technologist) and **Dr. Amina Jindani,** (Honorary Senior Lecturer).

Professor Andrew Nunn, MRC Clinical Trials Unit (CTU), Data management and analyses of trials will be carried out at the CTU under the supervision of Professor Andrew Nunn.

The Centro Internacional de Entrenamiento e Investigaciones Médicas CIDEIM based in Cali, Colombia, is a non-profit, non-governmental organization dedicated to biomedical research in infectious diseases and the development of research capability. The Director of Scientific Activities is **Dr. Nancy Saravia.**

Dr. Bernard Fourie is Associate Researcher with the South African Medical Research Council.

Drs Jennifer Furin, Carole Mitnick and Mercedes Becarra of the Harvard Medical School, will be involved with aspects of the Programme which concern multidrug resistant tuberculosis (MDRTB).

The consortium is a not-for-profit charitable organisation responsible for designing, conducting and analyses of randomised controlled clinical trials, to be carried out in countries with a high burden of tuberculosis, through a substantial network of treatment services and laboratories with which relationships already have been established. The primary objective of these trials will be to define regimens of treatment that will have high cure rates and will be simple to administer by the National Tuberculosis Programmes of these countries.

Because of their importance in assessing the activity of new antituberculosis drugs, basic studies of factors affecting response to chemotherapy and the study of surrogate markers of relapse will form a major part of the work of the consortium.

In addition, capacity strengthening, to increase the ability of centres to participate in trials, will be an integral part of the consortium's functions. Expertise is available in trial design and analysis, and in laboratory techniques.

The Coordinating Centre is based at St. George's Hospital Medical School, London. The International Coordinator, Dr. Amina Jindani, will coordinate all the aspects of the multi-centre

trials, including recruitment of participating centres, training local staff, through regular site visits and close monitoring of the general conduct of the trials. Members of the consortium will collaborate in whatever form of supervision is seen to be the most necessary and effective.

2. SCIENTIFIC PROGRAMME

The scientific programme is aimed partly at improvement of current regimens, either by shortening durations of treatment or through the development of intermittent regimens, and partly at assessing new anti-tuberculosis drugs. For the moment, at least, the work will be confined to tuberculosis infections and not to infections with other mycobacteria.

Some of the methods used in assessing chemotherapeutic agents have been described by Mitchison[1,2].

It is planned to study, through basic research and clinical trials, the speed of bactericidal activity of drugs during chemotherapy and the phenomenon of bacterial persistence.

2.1. Basic research on chemotherapy

2.1.1 The phenomenon of bacterial persistence

Clinically, persistent bacteria, related to those in the latent state, are mainly responsible for the current lengthy duration of chemotherapy. It has been demonstrated [3] that these persistent bacilli are metabolically active. There are probably several distinct bacillary populations in lesions with different characteristics such as ability to grow in liquid or solid medium and tolerance to rifampicin. This implies that it may be possible to find new drugs that specifically target some or all of these persistent bacilli [4]. It also implies that it is possible to design *in vitro* tests that will distinguish between the power of different drugs to kill the different populations of bacilli [5,6].

The following relevant research topics and techniques are extant at St George's:

1. Studies on the use of Hu/Coates models of persistence[3,4,5] to measure the sterilising activities of anti-tuberculosis drugs, new or old (Coates).
2. Studies on the action of individual drugs during current chemotherapy and their effects on the different bacterial populations (Mitchison)
3. Studies on gene expression changes during chemotherapy using a new amplified microarray technique and RT-qPCR (Butcher)
4. Facilities for large scale studies of drug action in experimental murine tuberculosis

All of these topics lead to an understanding of bacterial persistence during chemotherapy, the main reason for the current lengthy duration of treatment.

Initial *in vitro* screening against dormant tuberculosis of drugs and drug combinations is now possible and validation of the models in clinical trials is important.

2.2. Surrogate markers of relapse

Studies of surrogate markers of relapse are required to obtain a preliminary indication of efficacy and of the sterilising activity of a drug. The advantage of such studies is that they require small groups of patients and results are available within a short space of time. These studies are also

necessary because of the great difficulty of assessing a new drug or change of drug dosage in ethical phase III trials.

Surrogate markers would be one of the major areas of investigation by the consortium. The only marker that has been well validated so far is the culture result at two months following chemotherapy. These studies will include estimations of early bactericidal activity (EBA) and the several markers of sterilising activity.

2.2.1 Early Bactericidal Activity.

Studies of the early bactericidal activity (EBA) of drugs have been carried out on most of the chemotherapeutic agents for tuberculosis [7-11]. The first study [7], estimated the effect of single and multiple drugs on the fall in colony counts during the first 14 days of treatment.

The fall in counts during the first 2 days, with the drug given in progressively smaller dose sizes, can be used to estimate the therapeutic margin, the ratio of dose sizes between the usual dose and the dose that just produces a measurable EBA The therapeutic margin measures the ability to penetrate into all lesions. These measurements allow an estimation of the activity of the actively growing bacterial population but not the persisters. If the duration of the study is prolonged for 5-7 days, suggestive evidence may be obtained on the activity against persisters and therefore on the ability of the drug to shorten treatment.

Estimations are needed on drugs for which EBA studies have not been done including third line drugs, such as ethionamide, kanamycin and capreomycin, for the treatment of multi drug resistant cases of tuberculosis (MDRTB).

2.2.2 Markers of sterilising activity.

This can be aimed either at assessing the sterilising activity of a drug or at the identification of patients who are not likely to relapse and whose treatment can therefore be shortened. Although these two aims use the same procedures, they are clearly different in aim. If a sufficiently good association between the markers and relapse could be found, then it might be possible to try out treatments in which patients at risk of relapse would have longer treatment than the remaining patients, thus shortening overall the average duration of treatment.

Markers can measure the response of either the bacteria or the host immune system. The summation of both effects can be measured by the Wallis whole blood assay [12,13], but separate measures of both factors are really necessary. These should include studies of bacterial tolerance and also measures of immunity. Currently it is of interest to examine the use of the early expression patterns in the 4 key human genes identified by a recent study as prognostic for relapse in the Western Cape.

Current markers under study are:

1. The proportion of patients with positive 2-month sputum bacteriology – a well validated procedure.
2. Serial sputum cfu counts (SSCC) studies. Likely to be a very useful assessment. Probably more sensitive than 2-month bacteriology.
3. Time to sputum conversion, using appropriate statistical methods. This has never been done in a study aimed at measuring the effects of introducing a new drug.

4. Tolerance of the pre-treatment strains to drugs, such as rifampicin and gatifloxacin
5. Wallis whole blood assay. This measures tolerance and host immunity simultaneously.
6. Measures of host immunological activity.

2.2.2.1 Culture result at 2 months after treatment

Comparisons of the effects of adding rifampicin or pyrazinamide to the initial phase or to the entire regimen were made in 8 clinical trials [15]. In all of these trials, the addition resulted in a lower proportion of patients with positive cultures at 8 weeks. The difference was statistically highly significant in comparing regimens with 150-200 patients in each arm. The associations were less good at 4 weeks and at 12 weeks. Since the method is so well validated, it should be a standard surrogate marker method to be used until other methods are shown to be more efficient.

2.2.2.2 Serial Sputum Colony Counts (SSCCs)

While the EBA is a measure of the bactericidal activity at the start of treatment, it does not indicate the sterilising activity of single drugs or combinations of drugs. Serial counts of colony forming units (SSCCs) during the first two months of treatment may give an indication of the sterilising activity of treatment regimens. Regression coefficients are fitted to the exponential fall in the counts for each patient, and these regression coefficients can then be analysed and their means for each of the regimens compared. SSCC estimates have been done after decontamination of the sputum with NaOH [14], which kills a proportion of bacilli, or on selective medium that does not kill bacilli[16]. We do not know as yet how many patients are required or how to get the most efficient spacing of the viable counts in such studies

2.2.2.3 Bacterial tolerance to drugs

Evidence that pre-treatment strains of tubercle bacilli differ in the speed with which they are killed in vitro by anti-tuberculosis drugs (particularly rifampicin) was first obtained by Wallis.[14] He showed that these differences were not related to the MIC of the drug or to the speed with which the culture grew. No genetic changes occurred so that the phenomenon is phenotypic. In a small series of patients, the strains that survived longest (that is the most tolerant) tended to come from patients whose treatment failed or relapsed. Clearly this attribute could be of considerable importance in determining the speed with which sputum conversion is obtained and therefore the chance of ultimate relapse.

2.2.2.4 Measures of host immunity

As judged by the effect of HIV infection, low host immunity has a only weak effect on the sterilizing activity of drugs, and perhaps surprisingly immunity antagonises sterilizing activity. However, low immunity may predispose to relapse after chemotherapy.
A large study in the Western Cape district of South Africa has explored gene expression patterns in patients classified after chemotherapy as having (i) latent infections; (ii) active disease; (iii) recurrent relapse; and (iv) cure. The expression of 9 genes, including 4 with a major effect, was found to characterise these four clinical states. Currently a study is planned (EDCTP) to see whether the expression patterns from RNA obtained much earlier during treatment has a similar prognostic value. In this study blood as a source of mRNA will be obtained pre-treatment, at 8 weeks and at 6 months (end of treatment) from a sample of patients in a current chemotherapy study (Oflotub).

2.2.2.5 Bacillary gene expression patterns

Studies to develop a new linear amplification system for the study of bacillary gene expression by microarray and to apply this method to human and animal tissues. We hope to try out this new system on the bacilli in sputum. Sputum will be obtained pre-treatment, when it probably contains a mixture of actively growing bacilli and persistent bacilli, and after 4-5 days of treatment of the patient with isoniazid alone, when the actively growing bacilli will have been killed leaving the persistent population. Thus we hope to obtain the expression pattern for persistent bacilli that can be compared with published patterns obtained from cultures under various environmental conditions

2.2.2.6 Pharmacokinetic studies during chemotherapy.

Current pharmacological studies on chemotherapeutic drugs concentrate on two issues: (i) do environmental factors, such as HIV infection, malnutrition and unusual diet, alter the levels of drug in patients under treatment, and (ii) are there consistent differences from patient to patient in blood levels of the important drugs, and do these difference influence the results of chemotherapy or cause toxicity. In addition, there may be a need for specialised studies, for instance to categorize the state of the human NAT2 gene so as to be able to identify the speed with which isoniazid is inactivated by acetylation.

2.3 Clinical trials

The major end-points in randomised clinical trials are failures during treatment and relapses after treatment while additional information is available from the proportion of patients with positive sputum cultures at 8 weeks.

2.3.1 Proposed trials.

(i) The effect of substituting a fluoroquinolone (either moxifloxacin or gatifloxacin) for isoniazid during the initial 8-week intensive phase. This was suggested by a long-term mouse experiment, in which this substitution increased sterilizing activity during treatment[18].

(ii) The possible antagonistic action of ethambutol on the sterilizing activity of other drugs as suggested by an analysis of an EBA study[11] and by the results of another clinical trial[17].

(iii) The effects of increasing the dose size of rifapentine from 600 mg given in earlier studies to 900 mg and 1200 mg given once weekly. The aims will be to avoid the creation of rifamycin mono-resistance in relapse cultures in HIV positive patients, as occurred in HIV-positive patients in CDC study 22 [19], and to improve the overall sterilizing activity of the regimens containing rifapentine. An increase in dose size will increase the length of time that bacilli are inhibited from growth by the presence of inhibitory rifapentine concentrations and the subsequent lag due to the post antibiotic effect (PAE)[20]. This increase will leave less time for bacterial multiplication, necessary to create resistance, towards the end of the 7 days between doses.

(iv) The effects of giving a long-life fluoroquinolone (either moxifloxacin or gatifloxacin) once weekly, instead of isoniazid in the continuation phase of treatment. This substitution is suggested because a deficiency of isoniazid action was suggested by an association between low isoniazid blood levels and relapse in CDC study 22[21] (though

no such deficiency was found in the Hong Kong rifapentine study [22]). The replacement with a quinolone was suggested by the excellent results obtained in a long term mouse experiment [23].

2.3.2 Other clinical trials.

2.3.2.1 Rifampicin

There is a need to explore the possibility of giving rifampicin in doses higher than 600 mg daily. This is required because of the possible development of more active rifamycins and the possibility that rifampicin itself might be more active if given at a higher dose.

Studies with high doses of rifampicin[24,25] show that doses of up to 1800 mg may be given and are well tolerated. It would be better to give the drugs daily rather than intermittently to avoid inducing 'flu syndrome' when high doses are used intermittently.

Furthermore, there is evidence that EBA increase, during the first two days of monotherapy, increases with an increase in dose size[7].

As a first step, it would be essential to carry out further EBA studies with the higher doses, to ensure that there is a progressive increase in the EBA and that there is no tailing off of the response as the dose sizes are increased above 600 mg.

2.3.2.2.Pyrazinamide

A review of pyrazinamide activity [26] indicates that one would expect greater bactericidal activity as the metabolism of the bacilli wound down. From this, it could be expected that pyrazinamide would be increasingly bactericidal during the course of chemotherapy as the remaining persisting organisms had increasingly low metabolism.

There is some evidence [16] that the action of pyrazinamide starts after day 14. EBA data[7] show that pyrazinamide is moderately bactericidal during the first 14 days at a rate similar to that of the other drugs. There is also evidence that pyrazinamide is not active after the first two months of treatment [28]. What is not known is its activity between 14 and 60 days. This information could be obtained from a clinical trial comparing treatment with and without pyrazinamide during the IIP. The control group would receive the standard 2EHRZ/4HR and the test group, 2EHR/6HR where the continuation phase would be prolonged for an additional two months. Overnight sputum collections would be made on days 2, 14, 21, 28, 35 and 42.

It would not be easy to get ethical approval for such a trial but a case could be made because of the toxic effects of pyrazinamide, particularly joint pains and hepatic dysfunction.

Third line drugs

There is some discussion about how best to treat cases of tuberculosis that are resistant to both isoniazid and rifampicin. These cases are defined as being multi-drug resistant (MDR) and their management poses problems in centres where susceptibility tests are not readily available.

A major problem is that the activity of these drugs is not known and they have never been graded according to their activity.

For instance, the third line drugs for which the EBA activity is known are PAS, thiacetazone, amikacin and paromomycin (aminosidine). Despite the fact that the latter has greater EBA activity, it is the former that is that is recommended in third line treatment of MDRTB.

Studies of EBA need to be done on ALL the drugs used in third line treatment, particularly ethionamide so that effective regimens can be recommended for the treatment for MDRTB.

The partners from the Harvard Medical School have considerable experience of delivery of third line drugs to patients with MDRTB with a cure rate of 80%. This experience will be used to carry out EBA studies of third line drugs as well as randomised clinical trials to establish the most efficacious regimens of treatment.

3. CAPACITY STRENGTHENING IN OPERATIONS RESEARCH

Participating centres would be selected according to certain criteria. These are :

That they are in a country with an established National Tuberculosis Programme (NTP); they have access to a laboratory capable of microscopy, culture and susceptibility testing with a system of quality control; there are adequately trained personnel capable of supervising treatment and monitoring progress. This should include a home visiting service for defaulter tracing; and an agreement from the Ministry of Health for participation in clinical trials.

This capacity would have to be strengthened so that they are better able to participate in trials of chemotherapeutic agents.

The objective of capacity strengthening not only entails training in the conduct of randomised clinical trials and of data management. It requires also quality assurance of laboratories and expanding the ethical principles in carrying out trials using human subjects. This capacity will be developed and sustained in several ways.

3.1 Conduct of clinical trials

We believe that participation in the trials itself will strengthen the capacity of the participating centres to perform internationally significant operations research. By following the protocol, in every detail, collaborating institutions in low-income countries will develop their capacity, in terms of designing and carrying out studies relevant to their own situations. They will acquire knowledge as to how to conduct randomised clinical trials.

3.2 Good clinical practice (GCP)

The International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use has defined Good Clinical Practice (GCP) as an international ethical and scientific quality standard for designing, recording and reporting trials that involve the participation of human subjects. Compliance with this standard provides public assurance that the rights, safety and well being of trial subjects are protected, consistent with the principles that have their origin in the Declaration of Helsinki, and that the clinical data are credible.

In order to meet international standards on experiments in human subjects and Good Clinical Practice, there is a need to develop and enlarge the local ethical guidelines and strengthen the local institutional review boards to evaluate and monitor such trials.

The system of local ethics review clearly needs to be standardised, as far as it is possible to do so, in order to meet international guidelines for experiments conducted on human subjects. But this would require visiting individual centres in order to examine their existing systems and help them to develop their own ethics committees.

The International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use has also published guidelines for the obtaining and documenting of the informed consent procedure.

Episodic training through workshops will be given to all those involved in conducting clinical trials.

3.3 Good laboratory practice (GLP)

In order to have consistently reliable results a system of quality assurance, carried out by microbiologists, will be set up. This will consist of, in the first place, the completion of a questionnaire by each laboratory so that their actual situation may be evaluated. Regular visits to the participating laboratories by microbiologists, who would evaluate the laboratory methods dealing with equipment and recording in order to ensure that the results are reliable, would be envisaged as well as methods dealing with health and safety aspects.

'Good Laboratory Practise' (GLP) is a quality management system for the organisational process and the conditions under which laboratory studies during clinical trials are planned, performed, recorded, monitored and archived. GLP compliance is a necessary requirement of laboratories undertaking clinical trials, but this is a dynamic process requiring reviewing and updating of systems, surveillance and auditing.

A sample of laboratories in the developing world taking part in clinical trials were evaluated for GLP compliance and 'Health and Safety' by questionnaire and inspection. It was concluded that these laboratories, on the whole, produced moderate to good levels of technical standards and of training, especially so on their limited budgets and resources, but they had a very limited concept of GLP and lacked information and guidance on 'Health and Safety' issues; additionally, there were too many variations on standard methods used, without the validating evidence to support their use.

Furthermore, it was observed that the laboratories with the highest technical standards, were supported by laboratories in the developed world, but the majority of methods currently used in the developed world were inappropriate or too expensive and there were a very limited number of people currently with a knowledge and understanding of these earlier methods.

Training needs include:

- GLP and document control
- 'Health and Safety'
- Equipment calibration, monitoring and maintenance
- Quality Control, Quality Assurance

And support needs include:

- Technical
- Problem solving
- Strategic planning for GLP
- Strategic planned replacement of equipment
- IT and the internet
- Purchasing of equipment and consumables
- National Reference Laboratories, to provide leadership for the laboratory network in their country

- Validation of methods and procedures
- Development of new methods and procedures that are appropriate for the conditions that prevail in these countries.

The flow charts in APPENDIX I show the outline of evaluation, training and follow up that is necessary so that all laboratories are GLP compliant.

3.4 Workshops and Seminars

Investigators from the participating centres as well as other personnel of National Tuberculosis Programmes would be invited to attend workshops and seminars. Since we consider that, one of the most important things a programme can do is evaluate the efficacy of its interventions or of proposed new interventions, the participants will consist of staff already taking part in the clinical trials network and thus in settings that require the application of such skills.

A typical workshop would address the following topics:

a. Framing the research question
b. Preparing a research protocol
c. Statistical matters: confidence, significance, power, sample size, sampling methods
d. Study procedures: randomisation, enrolment, treatment phase, follow-up phase
e. Drafting forms and questionnaires
f. Laboratory procedures relevant to the TB programme's studies
g. Human subjects protection and trial oversight
h. Data management
i. Analysis of the data: basic approaches, impact of confounding and losses, etc.
j. Critical issues in clinical trials:
    - when to stop?
    - outcome events?
    - critical data or specimens?
    - serious adverse events
k. Reporting the results
l. Intellectual property
m. Resources available

These topics would be summarised in a publication on doing randomised clinical trials.

The process of training in operations research, and of capacity strengthening to do such research, will require a sustained effort over a period of, at least, five years. Activities will consist of repeated workshops as research issues, especially statistical procedures and protection of human subjects. The training will, therefore, have to be a continuous process. Staff changes at participating centres will also mandate a continuous training scheme in order to strengthen the performance of new and old participants as well as the NTPs.

Although this episodic training is extremely important, it should also be noted that participating centres receive continuous on site training through regular site visits by experts and through continuous monitoring and evaluation of performance by the Trial Monitors. This helps the staff to deal with local situations and strengthens the performance of the NTPs. There is a need to extend this kind of training to centres not yet participating in a clinical trial.

Training of local staff also requires their participation in international conferences so that they are made aware of developments in other countries and that they are able to meet their colleagues from other countries for discussions.

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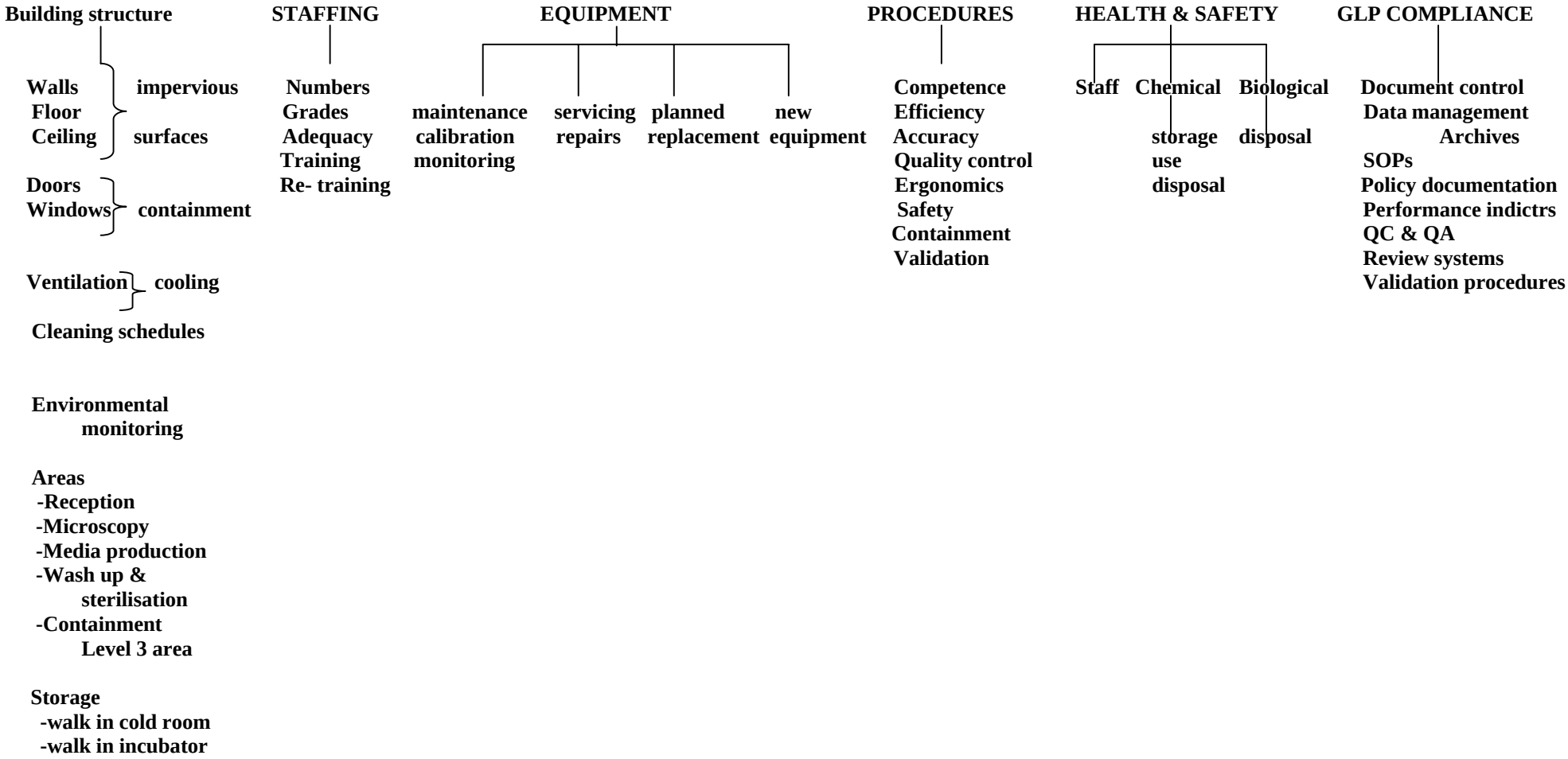
APPENDIX I

A STRATEGY FOR STRENGTHENING THE CAPACITY OF MYCOBACTERIAL LABORATORIES TO PARTICIPATE IN CLINICAL TRIALS OF TUBERCULOSIS

STEP 1 – EVALUATION OF PRESENT POSITION



STEP II – WHAT TO DO NEXT

Staff training

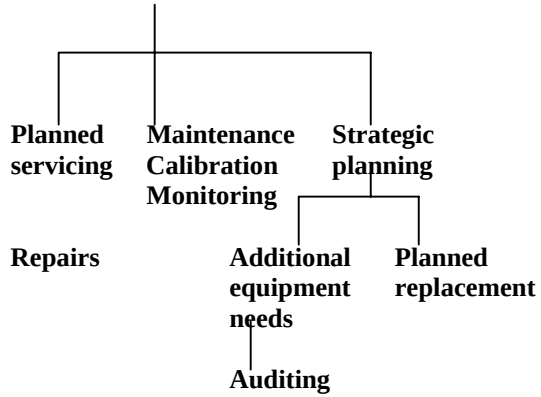
INF TECH
- **Basic concepts**
- **Managing files**
- **Word processing**
- **Spreadsheets**
- **Data bases**
- **Presentations**
- **Information & Communication**

Distance learning
- **CD ROM**
- **Reference manual**

Web site
- **Troubleshooting**
- **Problems**

Workshops
- **Regional**
- **GLP & Doc control**
- **Health & Safety**
- **Equipment calibration, monitoring & maintenance**

Equipment



QC & QA situations

Internal
External
Interlaboratory exchange
Performance indication - laboratory - individual

Review systems

Regular evaluations

Pre-trial
Every two years

R&D new procedures

New procedures
Improved methodology
Rapid methods
Responsive