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## **Dedication 2005**

This report is once again dedicated to all patients who are served by the laboratory tests provided by the National Health Laboratory Services. It is hoped that this report and the information gained and distributed by the NICD staff involved in producing the EQA Programmes will contribute to improving the services delivered by the NHLS.

## **Acknowledgements**

To the staff who produce the EQA Services  
Emma Goetsch, Vivian Fensham, Rebecca Landsberg, Helen Haritos, Martin Masango, John Frean, Adrian Puren,  
Kerrigan McCarthy, Ann Higgs, Lauren Buckton, Hollis Miles, Leigh Dini, Rita van Deventer, Ntabo Ndou,  
Susan Gould, Jay Patel, Gloria Zulu.

# Introduction

Since the compilation of the previous report (2004), the South African health fraternity has become increasingly aware of the pressure upon South Africa as a signatory to the World Health Association (WHA) to conform to the International Health Regulations; these ordinances were signed into effect at the National General Assembly of the WHA in May 2005; member states were given 2 years (i.e. until 2007) to conform. Quoting from Article 2 of the IHR; “The purpose and scope of these Regulations is to prevent, protect against, control and provide a public health response to the international spread of disease”. In essence the IHR detail the obligations of signatory nations to undertake surveillance activities within the borders of their nations so that diseases with potential for international spread can be detected, reported and acted upon, for the benefit of all communities. Global health security is recognized to be achievable when national health is secured. To this end, the international community has become increasingly aware of the role of public health laboratories in the verification of disease outbreaks. Public health authorities need to have confidence in results of laboratory testing. This is precisely the information that EQA programmes are able to provide.

This annual report presents an objective summary of NHLS and other (international, and South African non-NHLS) laboratory’s performance in EQA programmes provided by the NICD. It represents our health infrastructure’s capacity to detect and verify cases of disease for which appropriate EQA services tests are available. As such, this report is of interest to South African health authorities and all those who use the diagnostic services of the NHLS both for individual patient management and public health decision making. Although EQA results represent the best possible result a laboratory can produce and tend to overestimate laboratory diagnostic capacity in South Africa, they are one source of information from which we may derive our trust in statistics produced by laboratory services.

This annual report concerning EQA activities undertaken by the NICD during 2005 is the second year of production of such a report. During this year there has been an increasing awareness of the need for diagnostic medical laboratories to become accredited facilities according to ISO15189 and to participate in External Quality Assessment Programmes. Consequently local and international laboratories have approached the NICD requesting to enlist; laboratories that currently participate in EQA programmes are regarding their results more seriously; requests for EQA programmes for other diagnostic services have been made to the NICD EQA Unit and the NHLS QA division; where we have been unable to accommodate these requests, laboratories have commenced their own inter-laboratory comparison programmes. In 2006, state diagnostic medical laboratories in Kwa-Zulu Natal will join the NICD EQA programmes from January.

In conclusion, EQA is a growing area in the NICD, and one which in parallel with ongoing in-service training requires increasing managerial commitment and emphasis. The programmes reported on in this document would not have been possible without NHLS financial provision and NICD management environment. Finally, the staff who tirelessly produce these services that have monotonous administrative components deserve the most thanks!!

## **A note regarding EQA Survey Logistics**

NHLS laboratories are enrolled in surveys by their business manager who liaises with the QA division of the NHLS. Private and international laboratories enrol with the QA division of the NHLS. Surveys are generally distributed three times per annum. The closing date for survey results is four weeks after distribution of survey material (Tuberculosis culture surveys close after 8 weeks as results take longer to generate). Survey material is distributed to participating laboratories by the existing NHLS transport infrastructure. Results are faxed, posted or emailed to NICD co-ordinators by the closing date. The NICD co-ordinators or EQA Unit staff enter data onto Access databases. The method of evaluation of participant responses is detailed in the individual reports that follow. Participating laboratories and NHLS Business and Quality managers are issued with reports concerning laboratory performance. Corrective action following discrepant results is the responsibility of the participating laboratory or Business manager.

# Programme Co-ordinators and contact details.

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# Bacteriology EQA Programme

## Overview of Scheme

Three Bacteriology EQA surveys were sent out in 2005 to 108 laboratories, of which 87 are NHLS laboratories (11 academic, 76 regional) and 21 are private, mining or laboratories from neighbouring countries. The format of the surveys was identical to 2004; each survey contained three specimens for processing and one paper challenge. In each survey, the first, second and third specimens were respectively chosen to challenge the following important aspects of bacteriology:

- Specimen A: An identification challenge
- Specimen B: An antimicrobial susceptibility challenge
- Specimen C: A clinical relevancy challenge

'Specimen D' was a 'paper challenge' that details a clinical scenario in which laboratory input is required. It challenges pre- or post-analytical variables that cannot be addressed by simulated specimens. Laboratories are provided with multiple choices, and are requested to choose an appropriate course of action.

## Evaluation of laboratory responses

A marking template is drawn up by the Programme Co-ordinator, and evaluated by appropriate experts from the NICD Reference Units. The marking template takes into account the status or level of participating laboratories by having less stringent and more appropriate acceptable responses for regional laboratories.

All specimens are marked using the following mark scheme: The laboratory's final answer is graded as 'Acceptable' or 'Not acceptable'. Then aspects of the processing are evaluated using the criteria below, and the laboratory is awarded a 'mark' or percentage. Occasionally some of these criteria are not relevant; in that case marks are not allocated for that grading area and the total is reduced accordingly. This 'mark' and the individualised comments which are given to each laboratory is intended to assist laboratories that obtain a 'Not acceptable' grade to improve their performance in grading areas where they are weak.

Table 1. Grading categories and mark allocations for evaluation of laboratory performance in the NHLS Bacteriology EQA programme.

Grading Category	Mark allocation
Microscopy (from semi-solid, simulated smear or culture)	10
Processing	10
Culture conditions	10
Culture and ID	
Basic biochemical ID	15
Specific biochemical ID	10
Serotyping	10
Antimicrobial testing	
Choice of antibiotics	10
Susceptibility result	15
Clinical significance/ interpretation	10
<b>TOTAL</b>	<b>100</b>
<b>PERCENTAGE</b>	<b>100</b>

## Survey Contents

After evaluation of laboratory performance in 2004 it was decided that the theme for 2005 would be antimicrobial susceptibility testing. Survey contents were decided upon after consultation with NICD Epidemiology and Microbiology Reference Units and are tabulated below. All shipments were quality controlled to ensure that the organism of interest was viable one week after closing date of survey. After completion of each survey, laboratory results in the form of a report, a commentary (including explanations of marking scheme and highlighting areas of weakness) and a teaching exercise are sent to participating laboratories. The subject of the teaching exercise following each survey is listed below:

- Survey 0105: Part 1: Introduction and principles of Antimicrobial susceptibility testing  
Part 2: Susceptibility testing of *Staphylococcus* species
- Survey 0205: Processing of urethral swabs and susceptibility testing of *Neisseria gonorrhoeae*
- Survey 0305: Bacterial agents of pneumonia and meningitis, p5-63 from the 'Manual for the Laboratory Identification and Antimicrobial Susceptibility Testing of Bacterial Pathogens of Public Health Importance in the Developing World', written by the CDC and WHO, 2003.

The three paper challenges covered respectively the interpretation of a staphylococcal susceptibility test, interpretation of quality control results for amoxicillin and amoxicillin-clavulanate discs, and the appropriate use of the bacterial latex antigen detection in the diagnosis of bacterial meningitis.

**Survey Results**

Figures 1,2, 3 and 4 detail overall performance by participating laboratories in each challenge. Table 2,3 and 4 describe ‘Acceptable’ and ‘Not acceptable’ responses for each challenge contained in the Survey. Figures 5 onward demonstrate specific bacteriological issues raised by survey results, and indicate specific strengths and weaknesses of bacteriology diagnostics within the NHLS laboratories that were revealed by the Programme over 2005. Non-NHLS laboratories are a diverse group consisting of South African private, and international (Namibia, Zimbabwe and Ghana) public health laboratories. Analysis of their performance in Figures 4 onward is not included as it is not meaningful.

Figure 1. Percentage of non returns by survey for 2005 categorised by Academic, Regional and non-NHLS laboratory status

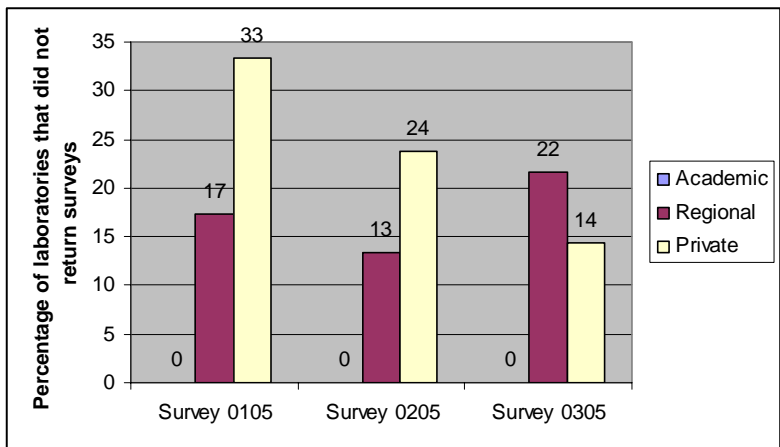


Figure 2. The percentage of academic, regional NHLS and non-NHLS laboratories that obtained acceptable responses for specimens A-D of Survey 0105.

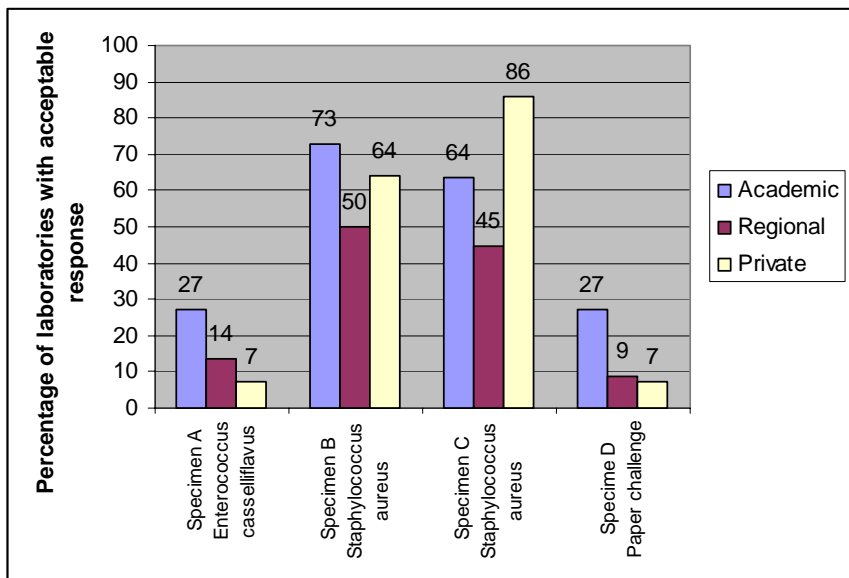


Table 2. Acceptable and Unacceptable responses for academic and regional/private laboratories for Survey 0105 challenges\*

<b>Specimen A</b>	<b>Acceptable responses</b>	<b>Unacceptable responses</b>
All laboratories	<i>Enterococcus casseliflavus</i> with low level vancomycin resistance <i>Enterococcus</i> species, referred for ID, with low level vancomycin resistance	<i>Enterococcus casseliflavus</i> with incorrect susceptibility <i>Enterococcus</i> species with incorrect susceptibility
Regional laboratories only	<i>Enterococcus</i> speciation incorrect, with low level vancomycin resistance	
<b>Specimen B</b>		
All laboratories	<i>Staphylococcus aureus</i> ; penicillin, erythromycin and clindamycin resistant; oxacillin, trimethoprim-sulphamethoxazole, vancomycin sensitive, +/- comment that "D" test is positive for inducible clindamycin resistance <i>Staphylococcus aureus</i> ; penicillin and erythromycin resistant; oxacillin, trimethoprim-sulphamethoxazole and vancomycin sensitive, clindamycin not tested.	<i>Staphylococcus aureus</i> ; major incorrect susceptibility result in one or more of oxacillin/ penicillin/ vancomycin /erythromycin results)  Incorrect organism identification
Academic laboratories only		<i>Staphylococcus aureus</i> ; penicillin and erythromycin resistant; oxacillin, chloramphenicol, rifampicin, trimethoprim-sulphamethoxazole, vancomycin and clindamycin sensitive, with NO comment that "D" test is positive for inducible clindamycin resistance.
Regional laboratories only	<i>Staphylococcus aureus</i> , penicillin, erythromycin resistant, oxacillin, chloramphenicol, rifampicin, cotrimoxazole, vancomycin, clindamycin sensitive, and NO comment that "D" test is positive for inducible clindamycin resistance.	
<b>Specimen C</b>		
All laboratories	<i>Staphylococcus aureus</i> , penicillin and oxacillin resistant; erythromycin, clindamycin, trimethoprim-sulphamethoxazole and vancomycin sensitive.	<i>Staphylococcus aureus</i> , incorrect susceptibility result for penicillin, oxacillin, erythromycin, clindamycin, trimethoprim-sulphamethoxazole or vancomycin Incorrect organism identification

\*Academic and regional laboratories are evaluated using criteria listed in rows called "All laboratories"; however only regional laboratories (or academic laboratories) were evaluated using criteria listed in rows called "Regional laboratories only" (or "Academic laboratories only")

Figure 3. The percentage of academic, regional NHLS and non-NHLS laboratories that obtained acceptable responses for specimens A-D of Survey 0205

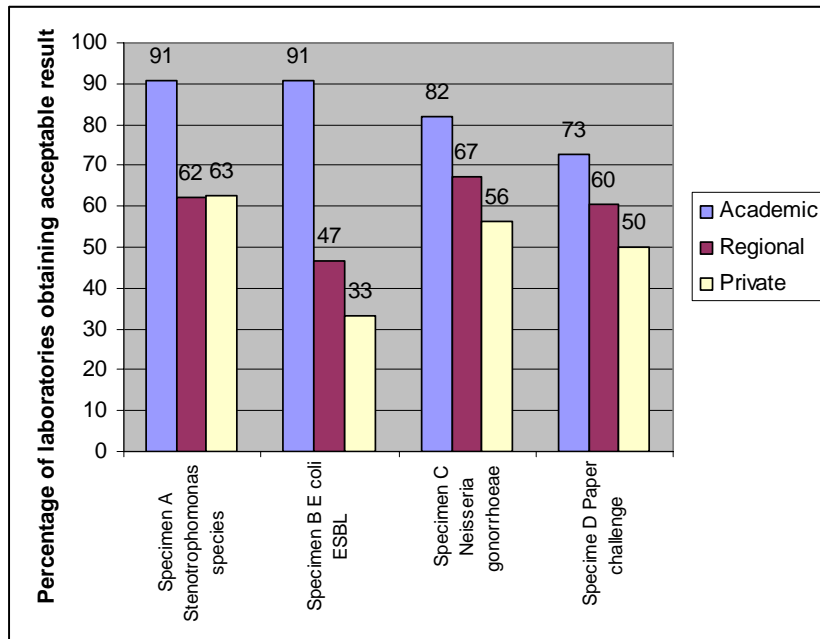


Table 3. Acceptable and Unacceptable responses for academic and regional/private laboratories for Survey 0205 challenges\*

Specimen A	Acceptable responses	Unacceptable responses
All laboratories	<i>Stenotrophomonas maltophilia</i> with correct susceptibility	<i>Stenotrophomonas maltophilia</i> /other old nomenclature, with incorrect susceptibility Any other response
Regional laboratories only	<i>Pseudomonas</i> species/ <i>Xanonthomas</i> species/ <i>Stenotrophomonas</i> species referred for ID and susceptibility testing <i>Pseudomonas</i> species/ <i>Xanonthomas</i> species/ <i>Stenotrophomonas</i> species referred for ID, with correct susceptibility	
Specimen B		
All laboratories	<i>Escherichia coli</i> with correct susceptibility (ESBL producer)	<i>Escherichia coli</i> with incorrect susceptibility (ESBL not detected)
Specimen C		
All laboratories	<i>Neisseria gonorrhoeae</i> , correct susceptibility	<i>Neisseria gonorrhoeae</i> presumed from microscopy only, culture negative. Any other response
Non-STI reference laboratories only	<i>Neisseria gonorrhoeae</i> , susceptibility not performed <i>Neisseria gonorrhoeae</i> presumptive ID only, susceptibility not performed	

\*Academic and regional laboratories are evaluated using criteria listed in rows called "All laboratories"; however only regional laboratories (or academic laboratories) were evaluated using criteria listed in rows called "Regional laboratories only" (or "Academic laboratories only")

Figure 4. The percentage of academic, regional NHLS and non-NHLS laboratories that obtained acceptable responses for specimens A-D of Survey 0305.

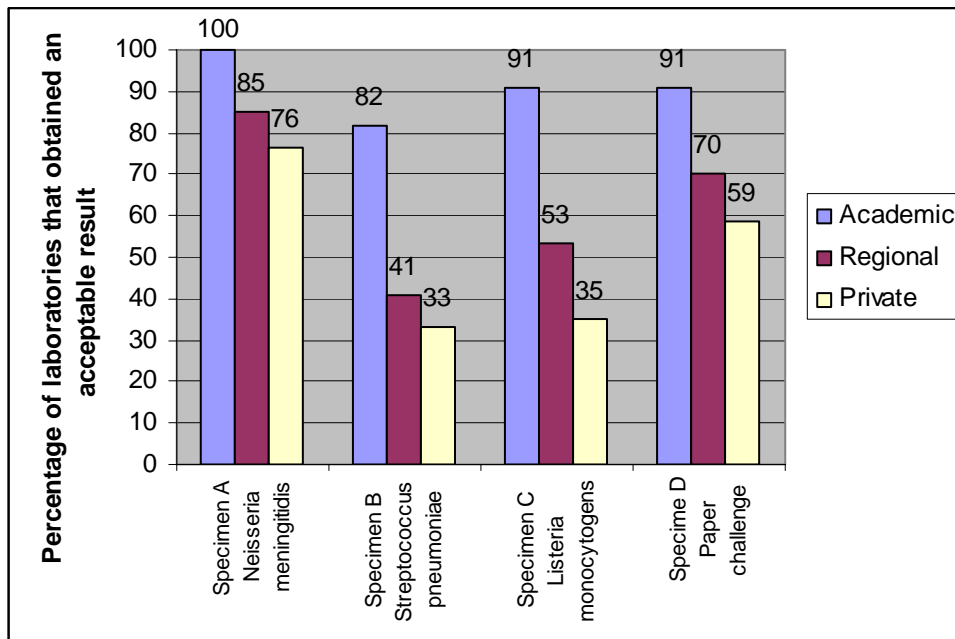


Table 4. Acceptable and Unacceptable responses for academic and regional/private laboratories for Survey 0305 challenges\*

Specimen A	Acceptable responses	Unacceptable responses
All laboratories	<i>Neisseria meningitidis</i>	Any other response
Specimen B		
All laboratories	<i>Streptococcus pneumoniae</i> with correct susceptibility	<i>Streptococcus pneumoniae</i> with incorrect susceptibility Incorrect ID
Specimen C		
All laboratories	<i>Listeria monocytogenes</i>	Any other response

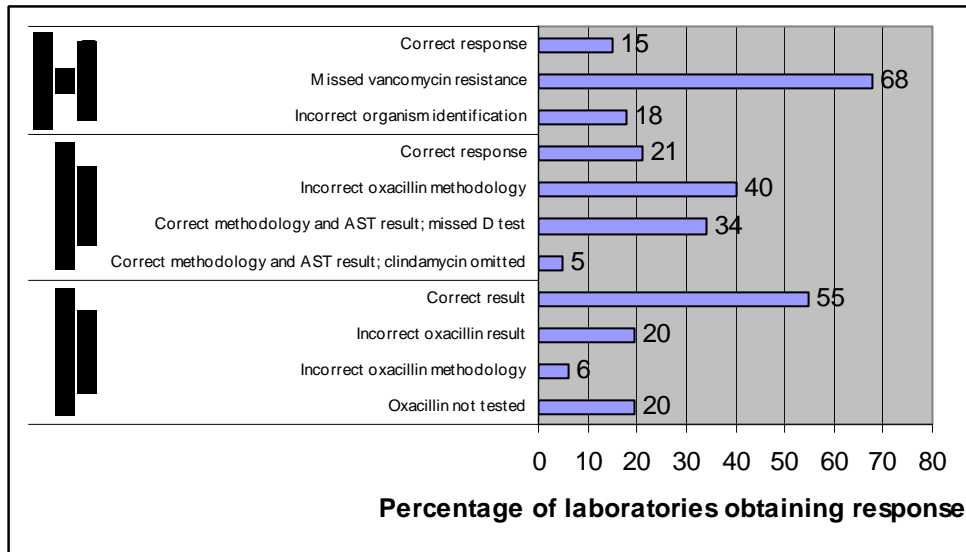
\*Academic and regional laboratories are evaluated using criteria listed in rows called "All laboratories"; however only regional laboratories (or academic laboratories) were evaluated using criteria listed in rows called "Regional laboratories only" (or "Academic laboratories only")

### Analysis and general comments

#### Survey 0105

This survey highlighted a pervasive problem (in both academic and regional NHLS laboratories) with the detection of vancomycin resistance and susceptibility testing of *Staphylococci*. Specific errors and omissions are tabulated in Table 5. Organism A, (*Enterococcus casseliflavus*) was obtained from the CDC in Atlanta (through the participation of the NICD in the WHO/CDC EQAS programme on susceptibility testing) and contained a *VanC* genetic element conferring intermediate (and intrinsic) vancomycin resistance. This was almost universally missed by those laboratories that did not do MIC testing or the vancomycin agar screen test. During the preparation of the commentary it was realised that the NHLS SOP on susceptibility testing of oxacillin for *Staphylococci* did not conform to the CLSI methodology (which the NHLS has decided in principle to follow). Consequently the SOP was revised and the use of 30µg cefoxitin discs incubated on Mueller Hinton agar for 16-18 hours to predict oxacillin susceptibility was introduced into the SOP. A teaching exercise detailing the correct CLSI procedure for determining susceptibility to oxacillin was sent out with the commentary. This issue will be visited again in 2006.

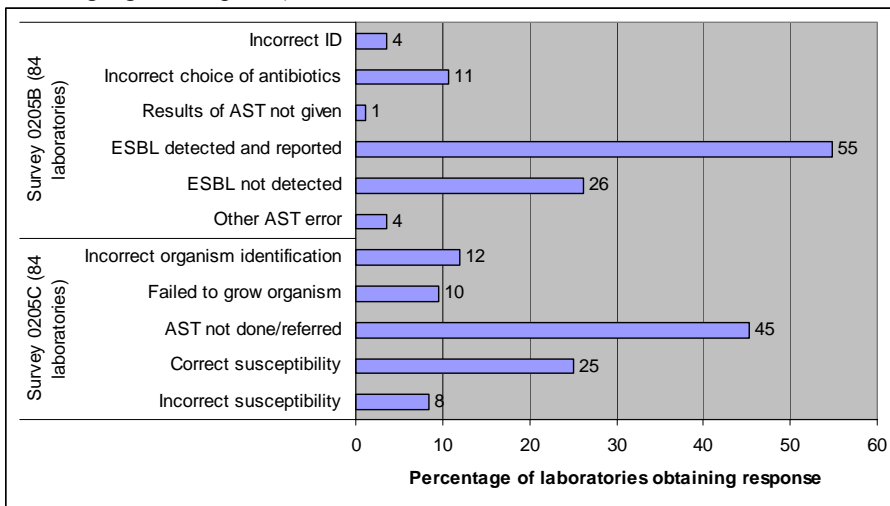
Figure 5. Specific errors in susceptibility testing of Gram-positive bacteria included in Survey 0105 (listed as percentage of laboratories obtaining a given response)



Survey 0205

Organisms in survey 0205 proved difficult to culture and identify for some laboratories, and this is of concern particularly for *Neisseria gonorrhoeae*. Extended spectrum  $\beta$ -lactamase production in specimen B missed by many regional laboratories; although these are usually nosocomial isolates, the increasing prevalence and clinical relevance of this enzyme means that even smaller laboratories should report its presence.

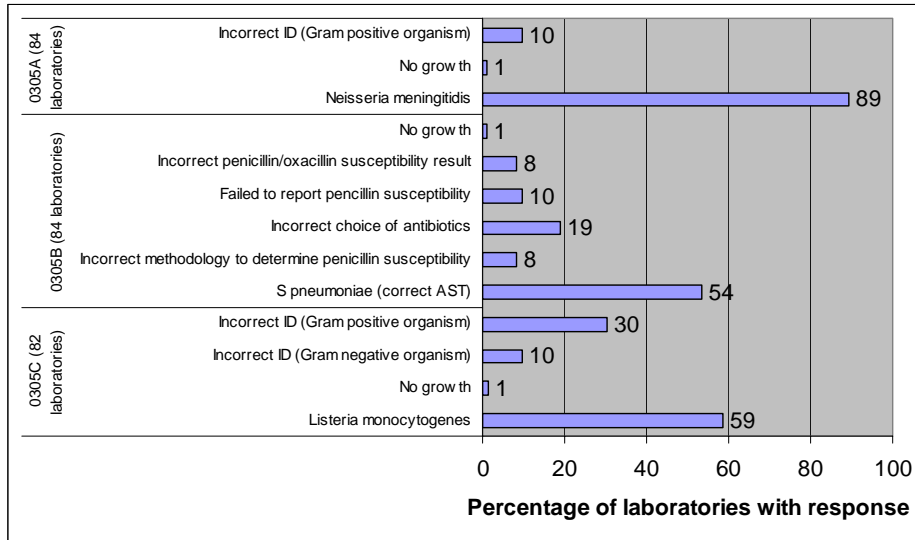
Figure 6. Specific errors made by participating laboratories in Survey 0205 (listed as percentage of laboratories obtaining a given response)



### Survey 0305

Pathogens in survey 0305 were those commonly isolated from CSF in cases of bacterial meningitis. The results of this survey revealed problems in identification of *Neisseria meningitidis* (10% of laboratories, all of which were regional or private, failed to identify the organism correctly, calling it a Gram-positive organism), and *Listeria monocytogenes*, which 40% misidentified. Susceptibility testing of pneumococci is still problematic even for some academic laboratories; Figure 7 below categorises the nature of errors made in this challenge (incorrect choice of antibiotic, incorrect methodology to determine penicillin resistance, failure to report penicillin susceptibility result and incorrect penicillin susceptibility result despite use of correct methodology)

Figure 7. Specific errors made by participating laboratories in Survey 0305 (listed as percentage of laboratories obtaining a given response)



### Conclusion

The bacteriology EQA programme continues to be useful to participants and the NHLS management; the programme provides objective evidence of excellent performance by participants and conversely also of deficiencies in laboratory performance that require training interventions. Antimicrobial susceptibility testing of pathogens is an area that continues to require attention and this theme will be continued in 2006.

## HIV Serology Programme

### Overview of Scheme and Survey Contents

The EQA Unit produces a HIV serology EQA programme that is sent out three times a year. Currently, there are 221 laboratories which participate in the scheme of which 168 are NHLS laboratories and 53 non-NHLS laboratories (including Partners in Prevention (PIP), HIV Vaccine Trial Network (HVTN), International AIDS Vaccine Initiative (IAVI) vaccine sites). Six serum samples are sent out at ambient temperature together with instructions and a report form. Sites have one month to complete the testing and return report forms.

The reference plasma is obtained from the South African National Blood Service. The plasma is converted to serum. Samples that are reactive for HIV antibodies are heat-inactivated at 56°C for 60 minutes. In the NICD laboratories, the samples are characterised using three different anti-HIV-1/2 EIA tests and three different anti-HIV-1/2 rapid tests. The NHLS QA division aliquots and distributes the panels. Participants are required to test the samples for HIV antibodies using their routine methods and submit their results on the result form provided. Survey contents are listed in Table 1 below.

Table 1. Survey contents and number of returns for HIV serology EQA Survey, 2005.

Survey Number	0105	0205	0305
Number of participants	179	221	221
<b>No. Samples</b>	6	6	6
Survey Contents	H01: <i>Positive</i> H02: <i>Negative</i> H03: <i>Negative</i> H04: <i>Positive</i> H05: <i>Negative</i> H06: <i>Negative</i>	H01: <i>Positive</i> H02: <i>Positive</i> H03: <i>Negative</i> H04: <i>Positive</i> H05: <i>Negative</i> H06: <i>Positive</i>	H01: <i>Positive</i> H02: <i>Negative</i> H03: <i>Positive</i> H04: <i>Negative</i> H05: <i>Negative</i> H06: <i>Negative</i>
Number of responses			
<b>Total</b>	143	194	200
NHLS Labs	169	150	157
Non-NHLS Labs	10	44	43
Non>Returns	36	27	21

### Evaluation of laboratory responses

Laboratory responses are scored according to the overall result which they report on the report form using the evaluation scheme in the table below:

Table 2. Scheme for evaluation and scoring of participant's results.

Specimen Category	Participants' Response	Score
<b>Anti-HIV positive</b>	Positive	2
	Equivocal	1
	Negative	0
<b>Anti-HIV negative</b>	Positive	0
	Equivocal	1
	Negative	2

Survey number, NHLS HIV Serology EQA programme																		
Response category*	0105						0205						0305					
	Specimen (Numbers of laboratories)						Specimen (Numbers of laboratories)						Specimen (Numbers of laboratories)					
	<u>H01</u>	H02	H03	H04	H05	H06	<u>H01</u>	H02	H03	H04	H05	H06	<u>H01</u>	H02	H03	H04	H05	H06
E	+	-	-	+	-	-	+	+	-	+	-	+	+	-	+	-	-	-
1	141	143	142	142	141	141	194	194	192	193	192	193	199	199	200	198	200	198
2	2	1	1	1	2	2	0	0	2	1	2	1	1	1	0	2	0	2
3	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0

### Survey Results

Table 3. Results of HIV serology EQA Surveys, 2005

\*E=Expected response, 1=Correct Results, 2=Incorrect Results, 3=No result submitted or Not tested

### Analysis

The following analysis has been drawn from the table above; a total of 21 reported incorrect results were obtained; These are described below and summarised in Table 5.

#### Survey 0105: eight incorrect results

Sample H01 (positive) was reported by two laboratories as negative and by a third laboratory as equivocal; these results were obtained using the AxSYM Combo test kit.

Sample H03 (negative) was reported as equivocal by one laboratory using the Determine rapid kit.

Sample H04 (positive) was reported as negative by one laboratory using Double-check Gold rapid kit.

Sample H05 (negative) was reported as positive and as equivocal by one laboratory each in which Double-check Gold and Determine HIV rapid kits were used, respectively.

Sample H06 (negative) was reported as positive (test method not stated) and as equivocal (Determine HIV rapid kit) by two laboratories respectively.

#### Survey 0205: six incorrect results:

Sample H03 (negative) was reported as positive by two laboratories, one in which AxSYM method was used; the laboratory producing the second false positive result did not state their test method.

Sample H04 (positive) was reported as negative by one laboratory using Determine rapid kit.

Sample H05 (negative) was reported as positive by two laboratories in which two test methods namely IMX and Kat Rapid kits were used in one and the other test method was not stated by second lab.

Sample H06 (positive) was reported as negative (test method not stated) by one laboratory.

#### Survey 0305: seven incorrect results

Sample H01 (positive) was reported as negative by one laboratory, test method not stated.

Sample H02 (negative) was reported as positive by two laboratories; where one lab was using both AxSYM and Vitros as methods of testing.

Sample H04 (negative) was reported as positive by two laboratories in which one laboratory used Determine HIV rapid kit and the other test method is not stated.

Sample H06 (negative) was reported as positive and as equivocal by two laboratories (test methods not stated).

Table 4. Test kit used and summary of incorrect results obtained with each kit

KIT NAME	Number of laboratories using each kit				Incorrect results obtained with each kit		
	0105	0205	0305	All surveys combined	False positive	False negative	Equivocal
<b>ELISA test formats</b>							
AxSYM/AxSYM HIV Ag/Ab Combo	19	11	8	38	2	2	1
Enzygnost Integral	1	1	1	3			
Core	1	1	0	2			
Vironostika HIV Uniform II Plus	6	8	5	19			
Biorad Access	3	3	0	6			
Vidas Duo	3	2	2	7			
IMX	7	7	5	19			
Vitros	1	1	1	3			
<b>Rapid test formats</b>							
Capillus	3	2	2	7			
Determine	99	89	91	279	2	1	4
Double-Check Gold	1	2	1	4	2	1	-
First Response	1	2	1	4			
Kat Rapid	1	0	0	1			
<b>Test method not stated</b>	0	65	83	148	4	2	1
<b>Total responses</b>							
	146	194	200	540			

### **Conclusion and comments**

The panel for 2005 was sent at ambient temperature and no problems were experienced using this format.

There is an excellent response rate and in particular for surveys two and three. The reason (s) for the non-response in the case of the remaining labs and sites requires investigation. EQA forms part of the activities for a lab to qualify to perform testing. It is critical that every effort is made to assist laboratories to participate in EQA as part of the qualification process.

Regarding completion of response forms, over 50% of sites did not fill in the report forms completely or complete the details regarding test kit or technology used. It appears that the number of laboratories using AxSYM declined markedly by survey three; however this could be an artifact of incomplete reporting. Seventy-three to 85 NHLS laboratories currently use HIV-1 rapid kits that are produced by five different manufacturers. The ‘Determine’ rapid kit is the major kit used. We were not able to establish the test algorithm used by participating laboratories, or whether these rapid tests are used as the first line of testing.

The scores achieved by the majority of the labs and sites were excellent. Nevertheless, both false positive and false negative results were reported. Reasons for this may be clerical error (mixed up samples), poor machine maintenance, and incorrect interpretation of test results. In addition, certain test kits have been shown in field trials to be linked to false positive and negative results. We would recommend that laboratories/sites review their results and take appropriate corrective action. The correct use of tests and a recognized algorithm of testing regarding rapid and ELISA formats are areas where ongoing training is required. Results are still reported as “equivocal” using rapid HIV tests despite information in previous reports that highlighted this problem.

# Mycobacteriology EQA Programmes

## Overview and survey contents

External Quality Assessment programmes for 2005 included “TB Microscopy”, which aims to assess technologist and laboratory proficiency in staining and microscopy of sputum smears for tuberculosis, and “TB Culture”, which aims to assess laboratory proficiency at isolation and susceptibility testing of Mycobacterium species. The other components of EQA for tuberculosis diagnostic services that are advised by the WHO (slide rechecking and laboratory supervisory visits) are not covered by the NICD EQA Unit, and are the responsibility of the NHLS managerial structure. The number of laboratories currently participating in Mycobacteriology EQA programmes are as follows:

TB Microscopy : 197 laboratories (152 NHLS and 45 non-NHLS)  
 TB Culture : 20 laboratories (10 NHLS and 10 non-NHLS)

## **TB Microscopy**

The NICD Tuberculosis (TB) Microscopy EQA Programme was upgraded in 2004 to conform to recommendations endorsed by the World Health Organisation (WHO) and the International Union against Tuberculosis and Lung Disease (IUATLD) that were published in 2002, 2005 being the second year of operation according to these principles, thus allowing comparisons to be made. The TB Microscopy EQA programme is sent out three times per year. Each laboratory receives 8 slides that are prepared in the EQA Unit. Positive slides are prepared by using clinical material and diluted to appropriate concentrations of bacilli according to procedures adapted from the above recommendations. Negative slides are made using simulated sputum (No clinical material is used). Four slides are stained using Ziehl Neelsen method by the EQA unit prior to distribution. To ensure accuracy and consistency of slides, random samples are distributed among four internal referee laboratories before and after each survey. On receipt of specimens, laboratories stain the unstained slides, perform microscopy and complete the response forms using the IUATLD Quantification Scheme<sup>1</sup>. Stained slides are sent out to evaluate the participants’ microscopy technique, whilst unstained slides are sent out as a check on both the quality of staining procedures and microscopy technique in the laboratories.

## **TB Culture**

This programme is sent out twice a year. Cultures are prepared and quality controlled for the EQA Unit by the Braamfontein NHLS TB Reference Laboratory. Each survey consists of four specimens: one of which is lyophilized sputum; three are mycobacterial cultures on Lowenstein-Jensen slopes. Laboratories are required to decontaminate the first specimen and culture for tuberculosis, perform identification only on the second specimen and perform susceptibility testing on the remaining two specimens.

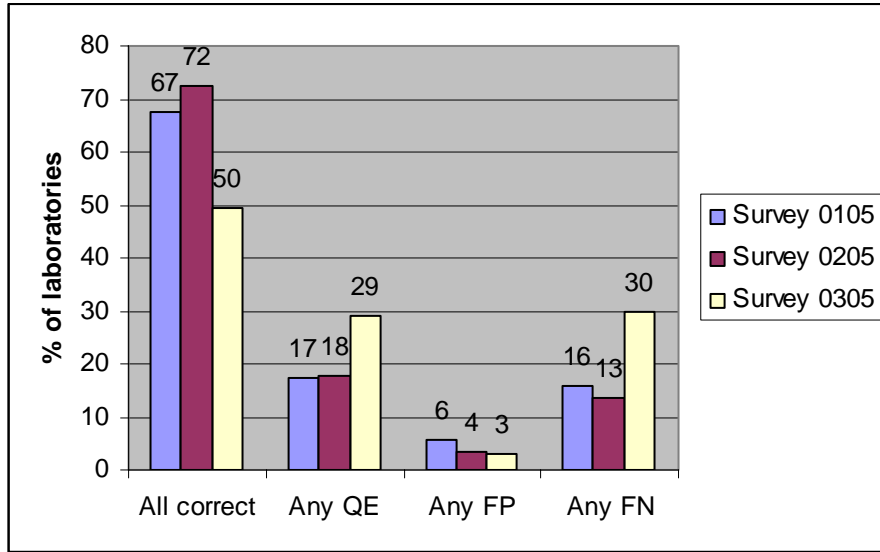
Table 1. Summary of TB Microscopy surveys for 2005

Survey number	0105	0205	0305
<b>Overall participants</b>	197	197	197
NHLS laboratories	155	152	153
Non NHLS laboratories	42	45	44
Number of samples	8	8	8
Survey contents	2 Negative slides 2 ‘3+’ 2 ‘2+’ 2 ‘1+’	2 Negative slides 2 ‘3+’ 2 ‘2+’ 2 ‘1+’	2 Negative slides 4 ‘2+’ 2 ‘1+’
<b>Returns (%)</b>			
Total	181 (92)	178 (90)	166 (84)
NHLS laboratories	144 (93)	139 (91)	130 (85)
Non NHLS laboratories	37 (88)	39 (87)	36 (82)
<b>Non-returns (%)</b>			
Total	16 (8)	19 (10)	31 (16)
NHLS laboratories	11 (7)	13 (9)	9 (6)
Non NHLS laboratories	5 (12)	6 (13)	8 (18)
NHLS labs not evaluated	-	-	*14 (9)

\*Exceptional case: Survey 0305 QC Samples for Limpopo East went missing (Transport department)



Figure 1. Percentage of NHLS laboratories that obtained correct responses for all eight slides of each survey, any quantitation error (QE) in a given survey, any false positive result in a given survey (FP) and any false negative (FN) result in a given survey in Surveys 0105, Survey 0205, and Survey 0305 respectively.

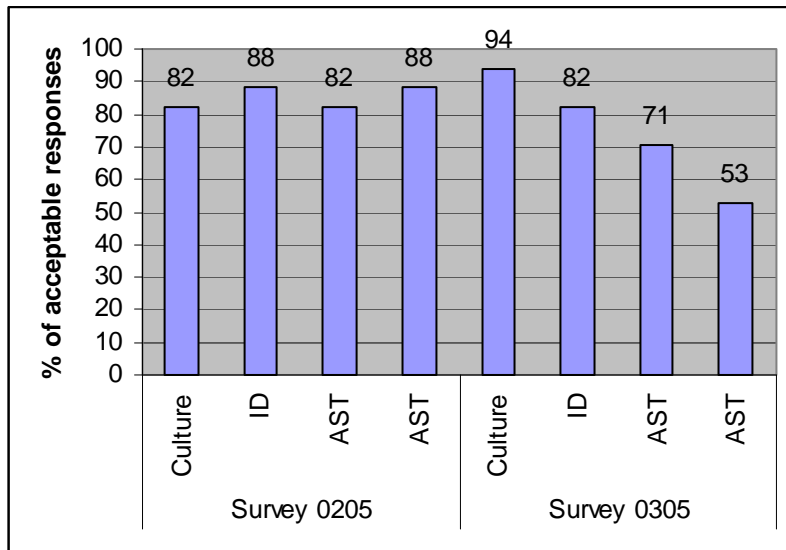


**Survey Results: TB Culture surveys**

Table 5. Numbers of laboratories producing acceptable and non-acceptable results in TB Culture Survey 2005

	0205 17 laboratories returned results 3 Non returns				0305 18 laboratories returned results 3 Non returns			
	Specimen number				Specimen number			
	1	2	3	4	1	2	3	4
Acceptable	14	15	14	15	16	14	12	9
Not acceptable	3	2	3	2	1	3	5	8
Not evaluated	0	0	0	0	1	1	1	1

Figure 1. Results of TB culture surveys 2005 (ID=identification, AST=Antimicrobial Susceptibility Testing)



## **Analysis and General comments**

### **TB Microscopy**

Laboratory performance was less good in Survey 0305 compared with the first two surveys of the year; Survey 0305 was 'more difficult' in that it did not contain any '3+' slides and 30% of laboratories obtained a false negative result for at least one slide in the survey. False negative results are a disastrous clinical error that has bad implications both for the patient and the National TB Control Programme; laboratories recording these results should undergo extensive review of their procedures, and participate in a slide rechecking programme. The percentage of laboratories obtaining false positive results is reassuringly small. Quantitation errors have no clinical significance.

### **TB Culture**

The TB Culture results highlighted a problem with susceptibility testing of rifampicin by participating laboratories, in that Specimen 4 of Survey 0305 was deemed by the NICD Braamfontein TB laboratory to be rifampicin resistant; however several laboratories reported that it was rifampicin sensitive. The MRC Tuberculosis laboratory will be requested to participate as a referee in 2006 programmes.

## **References**

1. IUATLD Quantification Scheme for Acid Fast Bacilli (AFB) Microscopy. See <http://www.sahealthinfo.org/tb/tbmicroscopy.htm>
2. Ridderhof J, Humes R, Boulahbal F, K Weyer et al. External Quality Assessment for AFB Smear Microscopy. Association of Public Health Laboratories September 2002. Endorsed by the IUATLD, CDC, WHO, APHL, RIT and KNCV.

# Mycology EQA Programme

## Overview of Scheme

The Mycology EQA programme consists of a basic and an advanced programme. In 2005 three surveys for both the basic and advanced program were sent out. CPD points are available to participants who complete each program.

### Basic Mycology

The basic program tests laboratory proficiency in detection of fungal elements on microscopy and basic identification of yeast isolates. All laboratories that perform bacteriology should have proficiency in these aspects of mycology. The basic survey consists of two specimens. One is a slide for staining and microscopy and the second is a lyophilised yeast isolate for culture and identification. Eighty-nine laboratories participate in the basic mycology programme; this includes all NHLS laboratories that participate in the NHLS Bacteriology EQA programme.

### Advanced Mycology

The advanced program is intended for those laboratories that process specimens for mycology culture and identification. Four specimens are sent in each survey. Three of the four specimens are evaluated. The fourth specimen is a bonus isolate, and this allows for the less common organisms to be included in the program. This serves as a teaching exercise and as a challenge for those laboratories that are proficient in mycology. Moulds are selected for inclusion based on availability of an isolate and clinical considerations. Isolates are identified morphologically in our laboratory, and these results are correlated with participating laboratory identifications prior to evaluation of results. No external laboratory referees each survey. Tutorials on identification of these isolates are sent to the participating laboratories. Twenty-one laboratories participate in the Advanced Mycology EQA program.

## Evaluation of laboratory responses

### Basic Mycology

Laboratory performance is evaluated and graded as “Acceptable” or “Not-acceptable”. In each challenge only a single laboratory process is evaluated. Specimen 1 evaluates the laboratories ability to perform and interpret a smear that may or may not contain yeasts. Specimen 2 tests the ability of the laboratory to identify a *Candida* yeast as an *albicans* or non-*albicans* species, or to identify a *Cryptococcus neoformans*. Every laboratory that performs microbiology should be able to perform and interpret these tests correctly.

### Advanced Mycology

Laboratories are graded by allocation of points for their performance in six grading areas: Culture medium used, culture conditions, biochemical findings or microscopic morphology, final identification of the isolate and clinical relevance. The total number of points per specimen is 25. Laboratories that obtain more than 18 points have an ‘Acceptable performance’. Laboratory reports reflect this grading scheme.

## Survey Contents

Table 1. Basic mycology contents for 2005

	First survey	Second survey	Third survey
Specimen 1	Slide with bacteria only for Gram’s stain	Slide with yeast and bacteria for Gram stain	Slide with hyphae and conidia( <i>Curvularia</i> )
Specimen 2	<i>Candida species (C. tropicalis)</i> for identification using Germ tube test	<i>Cryptococcus neoformans</i> identification using Niger seed agar	<i>Candida species (C. krusei)</i> ) for identification using Germ tube test

Table 2. Advanced mycology challenges for 2004

	First Survey F01-05	Second Survey F02-05	Third Survey F03-05
Specimen 1	<i>Candida tropicalis</i>	<i>Cryptococcus neoformans</i>	<i>Candida guilliermondii</i>
Specimen 2	<i>Microsporium audouinii</i>	<i>Microsporium canis</i>	<i>Curvularia</i> species
Specimen 3	<i>Fusarium</i> species	<i>Alternaria</i> species	<i>Candida krusei</i>
Specimen 4	<i>Microsporium nanum</i>	<i>Chaetomium</i> species	<i>Microsporium gypseum</i>

**Survey Results**

Table 3. Number of laboratories that did not return surveys for Basic and Advanced mycology programme

	Survey 1	Survey 2	Survey 3
Basic mycology	25/89	24/89	28/89
Advanced mycology	4/21	6/21	7/21

Figure 1. Results for Basic Mycology surveys 2005

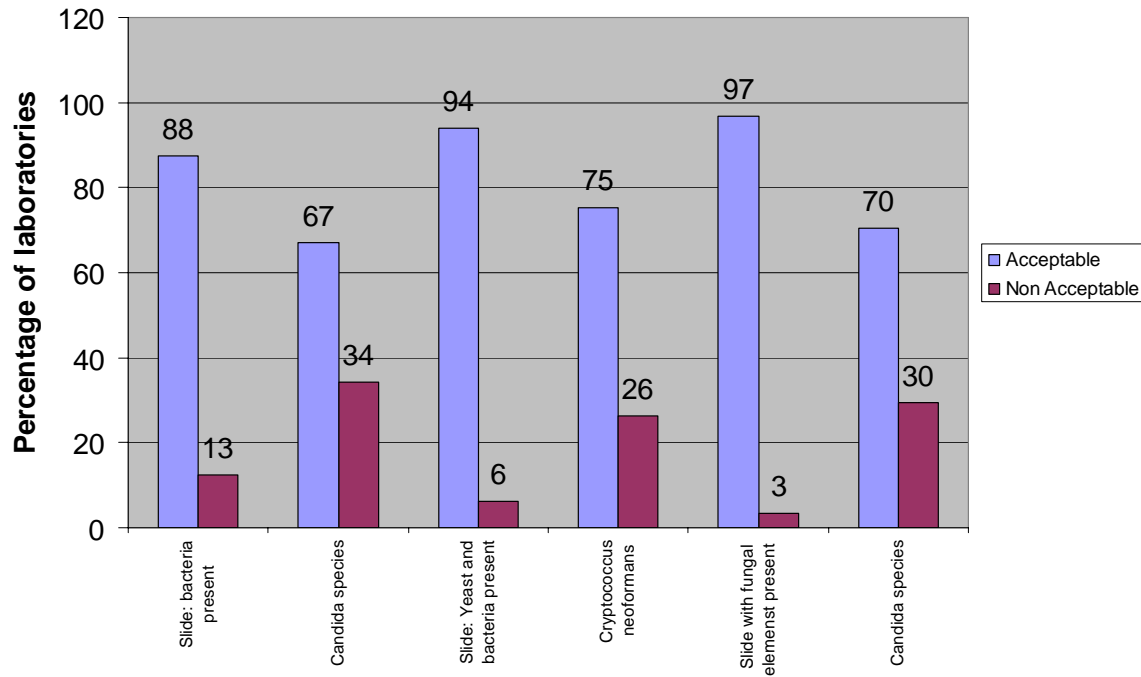


Table 4. Summarised results for laboratories participating in the Advanced Mycology surveys 2005

	Survey 1			Survey 2			Survey 3		
	<i>Candida tropicalis</i>	<i>Microsporium audouinii</i>	<i>Fusarium</i> species	<i>Cryptococcus neoformans</i>	<i>Microsporium canis</i>	<i>Alternaria</i> species	<i>Candida guilliermondii</i>	<i>Curvularia</i> species	<i>Candida krusei</i>
Number of laboratories	21	21	21	21	21	21	21	21	21
Average percentage obtained by participating laboratories (%)	75.2	33.6	72	95.1	77.4	82	91.2	86.4	53.6
Number of laboratories with an acceptable response	10	2	11	14	10	12	12	10	6
Number of laboratories with a non-acceptable response	7	15	6	1	5	3	2	4	8

Table 5. Mould identifications obtained by participating laboratories for specific challenges in 2005

Survey 1 (Number of laboratories)	Survey 2 (Number of laboratories)	Survey 3 (Number of laboratories)
<b>Specimen 1</b> <i>Candida tropicalis</i>	<b>Specimen 1</b> <i>Cryptococcus neoformans</i>	<b>Specimen 1</b> <i>Candida guilliermondii</i>
<i>Candida tropicalis</i> 10 <i>Candida</i> species 3 <i>Torulopsis candida</i> 1 No result given 2	<i>Cryptococcus neoformans</i> 14 No result given 1	<i>Candida guilliermondii</i> 12 <i>Candida</i> sp. not <i>C.albicans</i> 1 <i>Candida famata</i> 1
<b>Specimen 2</b> <i>Microsporium audouinii</i>	<b>Specimen 2</b> <i>Microsporium canis</i>	<b>Specimen 2</b> <i>Curvularia</i> species
<i>Microsporium audouinii</i> 2 <i>Trichophyton</i> species 5 <i>Epidermophyton</i> species 1 <i>Penicillium</i> species 2 <i>Aspergillus flavus</i> 1 <i>Basidiobolus</i> species 1 No result given 5	<i>Microsporium canis</i> 10 <i>Malessezia furfur</i> 1 <i>Microsporium</i> species 1 <i>Microsporium vanbreuseghemii</i> 1 <i>Microsporium audouinii</i> 1 No result given 1	<i>Curvularia</i> species 10 <i>Curvularia lunata</i> 2 <i>Microsporium nanum</i> 1 <i>Drechslera</i> species 1
<b>Specimen 3</b> <i>Fusarium</i> species	<b>Specimen 3</b> <i>Alternaria</i> species	<b>Specimen 3</b> <i>Candida krusei</i>
<i>Fusarium</i> species 11 <i>Microsporium</i> species 3 <i>Trichophyton</i> species 1 No result given 2	<i>Alternaria</i> species 10 <i>Alternaria chlamydospora</i> 1 <i>Alternaria infectoria</i> 1 <i>Microsporium cookei</i> 1 <i>Epidermophyton</i> species 1	<i>Candida krusei</i> 6 <i>Candida lipolytica</i> 5 <i>Candida inconspicua</i> 1 <i>Rhodotorula glutinis</i> 1 No result on Vitek given 1

## **Analysis and general comments**

### Basic programme

The most pressing problem with the basic programme is the exceedingly large number of non-returned responses. Laboratories that do not return results have as a primary problem a failure of management processes leading to failure to submit a response. Basic mycology is an integral part of bacteriology diagnostic services as the presence of yeast or moulds in specimens may have significant clinical implications. Regarding results of participating laboratories, the microscopy challenges are handled acceptably well; however laboratories have difficulty in identifying *Cryptococcus neoformans*. In South Africa where this AIDS-defining illness is very prevalent, this deficiency needs to be addressed. A large proportion of laboratories are not able to identify non-albicans *Candida* species; this simple germ-tube test is easy to perform and requires no special equipment or controls. NHLS Business managers should follow up on these poor results.

### Advanced programme

As with the Basic programme, a significant number of participants failed to return surveys. Several interesting isolates were sent out during the year which highlighted some controversial findings:

1. Specimen 2 of survey 1 (*Microsporum audouinii*). This mould is particularly difficult to identify and requires specialised media in order to induce it to sporulate.
2. Specimen 4 of survey 3 (*Candida krusei*). This organism was identified in our laboratory using an API 20C; however, laboratories that used the VITEK system (all laboratories in the Western Cape, and several private laboratories) identified the organism as a *Candida lipolytica*. The Molecular laboratory of the Mycology Reference Laboratory under Dr Jenny Rossouw is in the process of sequencing the isolate in order to establish unequivocally the organisms identity.

### Future developments

In 2006 the Basic program will be renamed the Yeast program but the structure will remain unchanged. The evaluation of laboratory performance for the yeast isolate will depend on the participating laboratory's resources; i.e. those laboratories which have access to commercial yeast identification systems will be marked out of 25 as it was for the Advanced program in 2005. The Advanced program will be renamed the Mould program and will consist of 3 or 4 mould isolates for culture and identification. The marking procedure for this program will remain as it is for 2005. Regarding the Advanced programme and as a consequence of the controversy arising from Specimen 3 of Survey 3, the Division of Mycotic Diseases of the Centers for Disease Control and Prevention in Atlanta will be approached to act as referee; in addition we shall investigate the possibility of sequencing all moulds prior to inclusion in the EQA programme.

# Parasitology EQA Programme

## Overview of schemes

The objectives of the parasitology EQA schemes are to build capacity in the field of human diagnostic parasitology in Southern Africa, to obtain an objective measure of the diagnostic ability of participating laboratories and to improve knowledge about human diagnostic parasitology in general. Two Parasitology EQA surveys are offered: 'Stool and urine parasites' and 'Blood and tissue parasites'. Surveys are issued 3 times per year. Survey challenges encompass parasite identification and laboratory techniques. A teaching series is included in each survey to encourage participants to learn more about parasites. Both Parasitology EQA programmes are CPD accredited.

## Evaluation of participant responses

To ensure the quality of material sent out for each survey and the standard of assessment, the model (expected) results are compared against those returned by a subset of historically good participants who are designated 'referee laboratories'. Referee laboratories are selected on an annual basis for each EQA programme and their selection is based on consistent excellent EQA performance over the past 2 to 3 years. There needs to be at least 80% consensus among the referee laboratory results for a challenge to be scored.

Participant results are graded using a four-tiered grading system (see table 1), and participants are assigned an overall percentage based on their performance in the entire survey. An individual report is issued to each participant detailing their performance and year-to-date percentage, expected results, statistics for the current survey and an analysis of the performance of all other unnamed participants. It is accompanied by a detailed commentary, which includes marking schedules, illustrations of the parasites found in that particular survey, comments on pitfalls and errors, additional educational material and a corrective action form. Regional summaries and corrective actions for NHLS participants are sent to the regional managers. Executive summaries are sent to the CEO, NICD director, Microbiology EQA Unit and QA Division head.

Table 1: Grading system for NHLS Parasitology EQA Programmes

Score:	Result:	Definition:	Performance assessment:
4	Completely correct result	A result accepted as the most correct and clinically relevant result.	Acceptable
3	Almost completely correct result	A result not technically correct, but the error or omission has little or no clinical impact; a deviation from what is considered the most clinically relevant result.	Acceptable
	<i>Separator</i>	<i>To divide the acceptable from unacceptable responses.</i>	N/A
1	A significantly incorrect result	A clinically relevant result that could lead to a <b>minor</b> diagnostic or treatment error.	Unacceptable
0	Completely incorrect result	A clinically relevant result that could lead to a <b>major</b> diagnostic or treatment error.	Unacceptable
0	No result	No result submitted by participant.	Unacceptable

## Survey contents and results: Stool and Urine Parasites

Table 2: Stool & Urine Parasite EQA Programme survey challenges

Survey PS1/05		
Code	Challenge	Description
PS1/05	A=stool concentrate for microscopy and ID B=stool smear for mod ZN stain, microscopy and ID	Stool concentrate with <i>Strongyloides stercoralis</i> larvae & <i>Isospora belli</i> oocysts
PS2/05	Stool smear for mod ZN stain, microscopy and ID	Stool smear with <i>Cryptosporidium</i> species oocysts
PS1/05	Teaching series	Flowchart for stool parasite processing
Survey PS2/05		
PS3/05	Microscopy and ID	Stool concentrate with <i>Taenia</i> species ova
PS4/05	Microscopy and ID	Urine with <i>Schistosoma haematobium</i> ova
PS5/05	Stool smear for mod ZN stain, microscopy and ID	Stool smear: <i>Cryptosporidium</i> species oocysts and artifacts
PS2/05	Teaching series	Formalin-ethyl acetate SOP
Survey PS3/05		
PS6a/05	Microscopy and ID	NPS with yeasts (Candida)
PS6b/05	Smear from PS6a/05 for mod ZN stain, microscopy and ID	NPS with yeasts (Candida)
PS7/05	Stool smear for mod ZN stain, microscopy and ID	Stool smear with <i>Cyclospora cayetanensis</i> oocysts
PS8/05	Stool smear for mod ZN stain, microscopy and ID	Stool smear with <i>Cryptosporidium</i> species oocysts
PS3/05	Teaching series	Modified ZN stain method

Table 3: Results of Stool and Urine Parasite EQA Programme

Survey number	PS1/05	PS2/05	PS3/05	Overall
Number of participants	159	159	158	159
Number of responses	135	140	117	148
Average score	76%	79.2%	77.1%	64.3%
Number of participants with score of 100%	50	57	64	18
Number of participants with score of $\geq 50\%$	112	126	100	116
Number of participants with score of $< 50\%$ *	23	14	17	32

\* Excludes non-responders who automatically receive 0

## Survey contents and results: Blood and Tissue Parasites

Table 4: Blood and Tissue Parasite EQA Programme survey challenges:

Survey PB1/05		
Code	Challenge	Description
PB1/05	Giemsa stain, microscopy & ID	Unstained thin smear with <i>Trypanosoma brucei</i> species
PB2/05	Giemsa stain, microscopy & ID	Unstained thick smear with NPS
PB3/05	Malaria antigen test	<i>P. falciparum</i> antigen positive blood
PB1/05	Teaching series	Flowchart for malaria specimen processing
Survey PB2/05		
PB4a/05	Microscopy & ID	Stained thin smear with <i>P. falciparum</i>
PB4b/05	Parasite count	Use slide PB4a/05
PB5a/05	Giemsa stain, microscopy & ID	Unstained thin smear with <i>P. falciparum</i>
PB5b/05	Parasite count	Use slide PB5a/05
PB2/05	Teaching series	Giemsa stain SOP
Survey PB3/05		
PB6a/05	Giemsa stain, microscopy of thick smear	Unstained NPS thick smear
PB6b/05	Giemsa stain, microscopy of thin smear	Unstained NPS thin smear
PB6c/05	Malaria antigen test	<i>P. falciparum</i> antigen negative blood
PB7/05	Microscopy and ID of thick smear	Stained thick smear with <i>Trypanosoma brucei</i> species
PB3/05	Teaching series	Malaria microscopic SOP

Table 5: Results of Blood and Tissue Parasite EQA Programme

Survey number	PB1/05	PB2/05	PB3/05	Overall
Number of participants	132	131	132	132
Number of responses	109	110	98	122
Average score	71%	61.3%	85.2%	62%
Number of participants with score of 100%	54	9	55	4
Number of participants with score of $\geq 50\%$	103	74	90	93
Number of participants with score of $< 50\%$ *	6	36	8	29

### Analysis and general comments

As with last year, two issues are of concern: the high rate of non-returns for each survey and the number of laboratories failing to meet an acceptable standard. The standard of these two Parasitology EQA Programmes is set at a minimum level for a routine diagnostic laboratory, so participants should easily achieve over 50%. However 21.6% of participants that submitted responses for the Stool and Urine Parasite EQA Programme failed and similarly 23.7% failed the Blood and Tissue Parasite EQA Programme. Participants that fail the parasitology EQA programmes should realistically not offer parasitic diagnosis until they are able to sustain a pass grade.

# Non-Treponemal Syphilis Serology EQA Programme

## Overview and survey contents

The EQA Unit produces a non-treponemal (RPR) syphilis serology EQA programme that is sent out three times a year. There are currently 173 participants (162 NHLS and 11 non-NHLS). Three lyophilized serum samples per survey are provided together with reconstitution instructions and request forms. Reference material (serum) is purchased from the South African Blood Services and lyophilized by the NHLS QA division. A representative portion of the lyophilized samples are submitted to two referee laboratories for quality control purposes. Participants are required to perform qualitative and quantitative non-treponemal tests on reconstituted serum.

## Evaluation of laboratory responses

This year laboratories were evaluated on performance at two levels; a qualitative level and a quantitative level. The qualitative level required the participants' results (positive or negative result of a non-treponemal syphilis test) to agree with the predetermined result obtained by the EQA Unit (after quality control by external referee laboratories). The quantitative result was evaluated using consensus results of participating laboratories in that laboratory responses are deemed acceptable if their titre agreed with majority responses obtained by participants. These results were stratified by the type of test kit used by participants (Immutrep Carbon Antigen (Omega diagnostics), Macro-vue RPR card test (Beckton Dickinson)).

## Survey results

Numbers of participants and returned surveys are tabulated in Table 1 below. Qualitative results are presented by the kit used in Table 2. Analysis of quantitative results is not provided, as with consensus reporting, all laboratories with a titre result that falls within two standard deviations of the mean distribution of titres will have an acceptable result.

Table 1. RPR Serology for 2005

Survey number	0105	0205	0305
Overall participants	174	177	177
No. of samples	3	3	3
Sample contents (expected responses after EQA Unit QC)	S01 (1:4) S02 (Negative) S03 (1:16)	S01 (1:4) S02 (1:2)* S03 (Negative)	S01 (1:16) S02 (Negative) S03 (1:16)
<b>Returns</b>			
Total	161	166	152
NHLS Labs	151	157	144
Non-NHLS Labs	10	9	8
Not Evaluated	0	0	14**
Non-returns	13	11	11

\* Survey 0205 S02 (1:2) results were not evaluated as most of the labs reported it as negative.

\*\* Limpopo East survey 0305 samples were lost in transit; the NHLS managed to find them on the 15 February 2006, reports were sent out as not evaluated.

Table 2. Percentage of NHLS laboratories that produced acceptable non-treponemal serology results

	Survey 0105	Survey 0205	Survey 0305
Immutrep Carbon Antigen (Omega diagnostics)	93	96	100
Macro-vue RPR card test (Beckton Dickinson)	94	99	100
Other kit	93	96	100
All results	94	98	100

## Analysis and general comments

Procurement of reference material with sufficiently high titres from the blood bank is difficult as the incidence of active syphilis amongst voluntary blood donors is small. Consequently our challenges are limited in number and titre. It was reassuring that in all surveys at least 90% of laboratories obtained qualitatively correct results. In the third survey 100% of laboratories obtained qualitatively correct results for all three challenges with no false positive or false negative results being recorded.

# WHO Bacteriology EQA Programme

## Overview of Scheme

The primary objective of this scheme is to provide external quality assessment (EQA) specimens for the laboratory confirmation of agents of bacterial meningitis, diarrhoea and plague in the WHO AFRO region. The Scheme is produced by the NICD EQA Unit, together with the NICD Reference Units (Respiratory and Meningeal Pathogens, Enteric Diseases, Special Bacterial Pathogens). The WHO Communicable Diseases Surveillance and Response Unit and the WHO African Regional Office (WHO-AFRO) provides finance through an APW with the WHO head office that is subject to annual renewal, as well as managerial input assistance.

In 2005 the programme was sent to 72 laboratories in 47 countries within the WHO AFRO region. There are 3 surveys per year and 12 surveys have been sent since the programme's inception in May 2002. A complete report for the 4 years of the survey is available on request. All laboratories receive 2 enteric and 2 meningeal samples per survey. There are 16 laboratories that also receive an additional 2 plague samples. Survey contents is decided upon by NICD Reference Units (Enteric Diseases Reference Unit, Respiratory and Meningeal Pathogens Unit, Special Bacterial Pathogens Unit) and the Microbiology EQA Unit. All samples undergo rigorous quality control in our laboratory prior to and after distribution. Several national and international referee laboratories validate survey contents, and assist with moderation of laboratory responses.

In the last survey of 2005 two microscopy programmes were added under a separate APW: these include tuberculosis (acid-fast bacilli) and malaria microscopy. This survey was commenced in October 2005, and will continue in 2006. All laboratories receive 8 slides (4 stained with Ziehl-Neelsen stain and 4 unstained) for tuberculosis microscopy and 16-20 stained blood slides for Malaria microscopy. The Malaria slides are prepared by the Parasitology Reference Unit and the TB slides by the Microbiology EQA Unit.

All EQA surveys are packed according to International Air Transport Association PI-650 (IATA) and are classed as diagnostic specimens. Shipments are sent by air courier (DHL) from Johannesburg to the various African, European and North American destinations. The communication with participating laboratories is in English, French and Portuguese. Documents are prepared in English by NICD coordinators and translated by Dr Antoine Pierson and Sebastien Cognat into French, and by Mrs Linda de Gouveia into Portuguese.

## Evaluation of laboratory responses

Laboratory responses are assessed in four or five grading areas; Microscopy, Culture and Identification, Serotyping, Choice of antimicrobial agents, Antimicrobial susceptibility testing result. Each specimen is evaluated independently. The response in each grading area is evaluated as being acceptable or unacceptable. All specimens are sent to international and South African national referee laboratories, whose comments and results are used to moderate participants responses. Referee laboratories are listed in Table 1.

Table 1. Referee laboratories for the NICD/WHO AFRO EQA programme December 2005

<p><u>Bacterial meningitis specimens:</u></p> <p>WHO Collaborating Center for Meningitis, CDC, Ms S Schmink, Atlanta, GA, USA,            Service de Sante des Armees, IMTSSA, Dr P. Nicolas, Marseille, France            Microbiology Department, Dr A Whitelaw, Groote Schuur Hospital, Cape Town, SA            Microbiology Department, Dr O Perovic, Johannesburg General Hospital, SA</p>
<p><u>Plague specimens:</u></p> <p>Institut Pasteur, Dr Lila Rahalison, Antananarivo, Madagascar            CDC, Fort Collins, Jeannine Petersen</p>
<p><u>Enteric specimens:</u></p> <p>WHO Collaborating Center for Enteric disease, CDC, Ms. Cheryl Bopp, Atlanta, GA, USA            Hopital d'Instruction des Armees, Service Des Biologie Clinique, Dr Pierre Hance, Marseille, France            Microbiology Department, Dr A Whitelaw, Groote Schuur Hospital, Cape Town, SA            Microbiology Department, Dr O Perovic, Johannesburg General Hospital, SA</p>

Table 1. continued

<u>Malaria Microscopy:</u> LSDI/Malaria, DPS Maputo, Dr Elizabeth Streat, Mozambique Hospital for Tropical Diseases, Department of Clinical Parasitology, Prof Peter Chiodini, London
<u>Mycobacteria Microscopy:</u> Microbiology Department, Dr A Whitelaw, Grootte Schuur Hospital, Cape Town, SA

**Survey contents**

The organisms in the enteric and meningitis disciplines were selected on the basis of their role as major public health pathogens on the African continent. Plague challenges were selected on basis of diagnostic tests thought to be available in AFRO-region laboratories. The plague F1 antigen dipstix tests are currently manufactured in Madagascar by Institut Pasteur and were distributed to participating laboratories in the NICD EQA packages.

Table 2. Survey contents in NICD/WHO AFRO bacteriology EQA Programme 2005

Survey No	Survey 1		Survey 2		Survey 3	
	1A	1B	2A	2B	3A	3B
<b>Enteric challenges</b>	Normal flora	<i>Vibrio cholerae</i> serogroup O1 serotype Inaba	<i>Salmonella enterica</i> serovar <i>enterica</i> serotype Paratyphi C	<i>Shigella boydii</i>	<i>Shigella dysenteriae</i> type 2	<i>Vibrio cholerae</i> serogroup O1 serotype Ogawa
	<b>1C</b>	<b>1D</b>	<b>2C</b>	<b>2D</b>	<b>3C</b>	<b>3D</b>
<b>Meningitis challenges</b>	<i>Cryptococcus neoformans</i>	<i>Neisseria meningitidis</i> serogroup B	Viridans Streptococcus species	<i>Streptococcus pneumoniae</i>	<i>Haemophilus influenzae</i> serotype c	<i>Neisseria meningitidis</i> serogroup C
	<b>1E</b>	<b>1F</b>	<b>2E</b>	<b>2F</b>	<b>3E</b>	<b>3F</b>
<b>Plague challenges</b>	<i>Yersinia enterocolitica</i>	<i>Pasteurella multocida</i>	<i>Pasteurella multocida</i>	<i>Klebsiella pneumoniae</i>	F1 antigen positive	F1 antigen positive
<b>Mycobacteria microscopy*</b>	-	-	-	-	4 ZN stained slides (2x Neg; 2x 2+ ) 3 unstained slides (2 x Neg; 1x 1+)	
<b>Malaria microscopy*</b>	-	-	-	-	6 specimens with both thick and thin smears (2x Neg; 3x P.falcip; 1x NE)	

\*Malaria and TB microscopy was only included from survey 3 of 2005.

**Survey results**

The response rate for the three surveys over 2005 was 87%, 69% and 87% respectively. The average turnaround time over three surveys for all participating laboratories was 23 days. The percentage of laboratories obtaining acceptable results in each survey for the enteric, meningitis and plague challenges are shown in Figures 1, 2 and 3 respectively. Enteric challenges are generally performed well by participating laboratories, however in the meningitis discipline, serotyping and susceptibility testing still pose significant challenges to participants. Processing of plague samples by the laboratories yielded varied results.

Figure 1. Survey results for enteric challenges indicating percentage of laboratories that obtained an acceptable result.

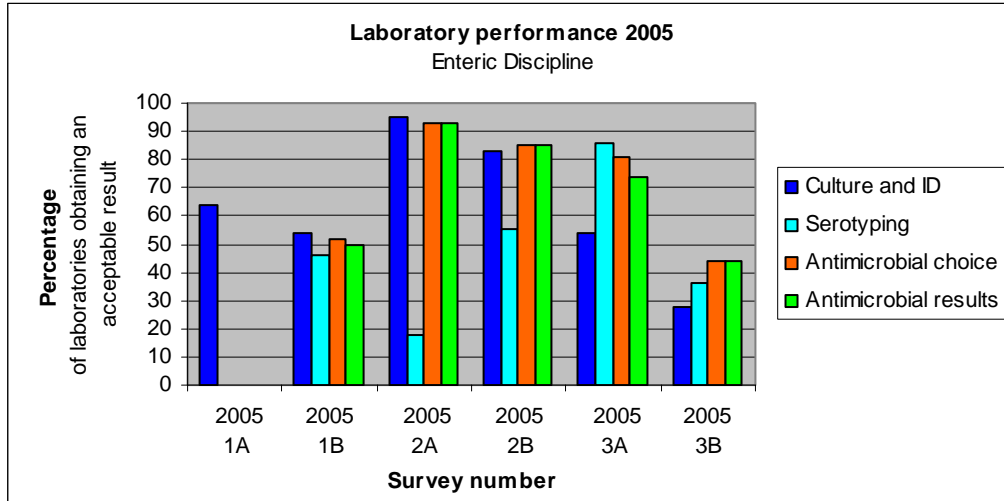


Figure 2. Survey results for meningitis challenges indicating percentage of laboratories that obtained an acceptable result.

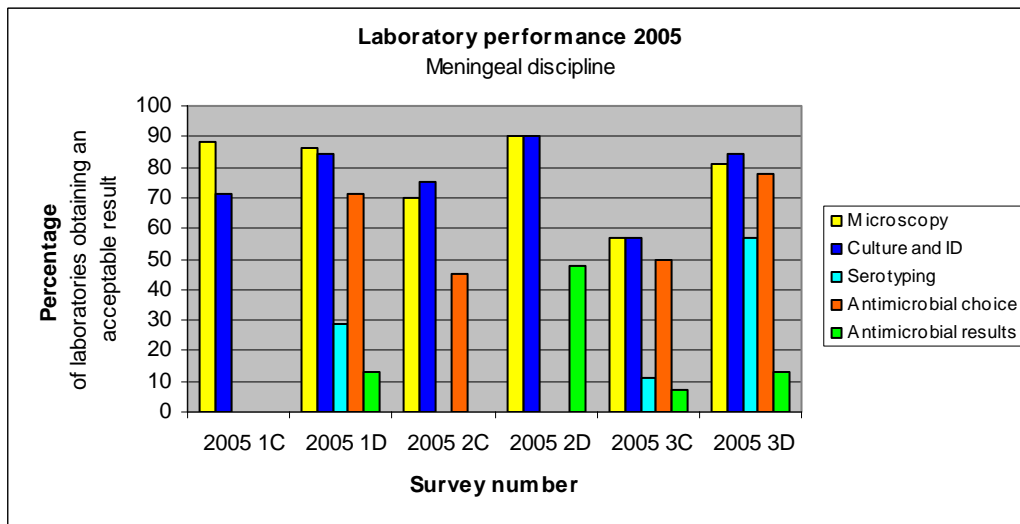
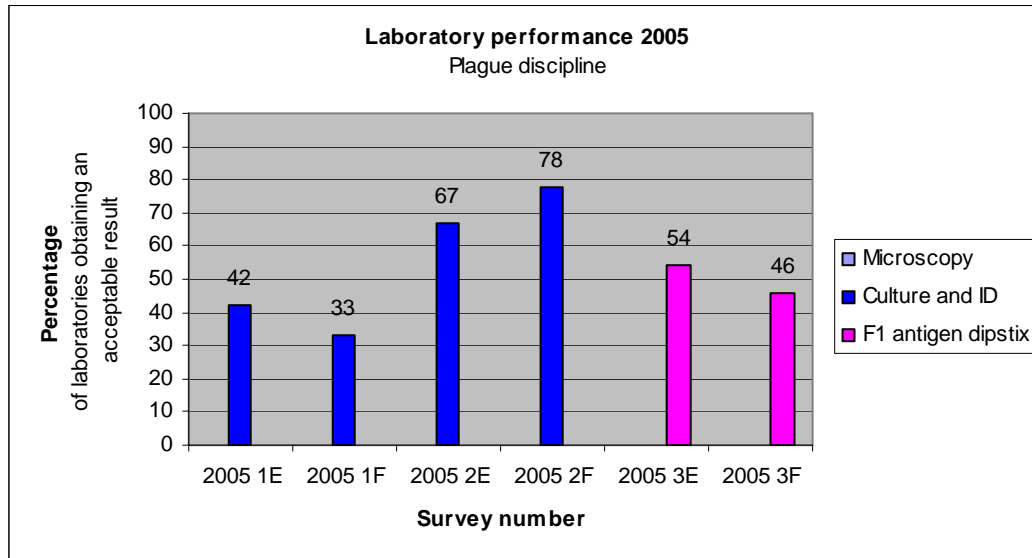


Figure 3. Survey results for plague challenges indicating percentage of laboratories that obtained a correct result. (No microscopy challenges were included in the plague discipline in 2005)



### Analysis and General comments

The EQA programme has provided objective data on laboratory capacity for epidemic prone bacterial disease in African public health laboratories for the funders, the WHO-Communicable Diseases Surveillance and Response Division. Communication co-operation with participating laboratories and WHO-headquarters, WHO-AFRO and the NICD has been promoted. Additionally, through our involvement in the EQA scheme, the NICD has participated in interventions to improve laboratory capacity in the AFRO region; these have included training workshops (WHO EMRO delegates, April 2005), site visits with on-site training (Ethiopian Health and Nutrition Research Institute, Addis Ababa, Ethiopia, October 2005) and distribution of teaching material in survey packages including significant publications such as the CLSI guidelines on antimicrobial susceptibility testing by disc methodology (M2-A8), and the “Manual for the Laboratory Identification and Antimicrobial Susceptibility Testing of Bacterial Pathogens of Public Health Importance in the Developing World” published in 2003 by the CDC and WHO.

## Participating laboratories 2005

	Microbiology	Parasitology - Stool	Parasitology - Blood	Mycology - Basic	Mycology - Advanced	TB Microscopy	TB Culture	Serology RPR	Serology HIV	Bacteriology WHO
<b>South African Laboratories</b>										
Chris Hani Main Lab	X	X	X	X	X	X		X	X	
Chris Hani Stat Lab										
Central	X					X	X	X	X	
Johannesburg Hospital	X	X	X	X	X	X		X	X	X
Infection Control	X									
Special Pathogens	X									
Central STI								X	X	
Helen Joseph	X	X	X	X	X	X		X	X	
Helen Joseph (Chemistry)									X	
Coronation										
Leratong	X	X	X	X	X	X		X	X	
Yusuf Dadoo			X					X	X	
Carletonville	X	X	X	X		X		X	X	
South Rand	X	X	X	X		X		X	X	
Tambo Memorial	X	X	X	X		X		X	X	
Far East Rand	X	X	X	X		X		X	X	
Pholosong			X			X		X	X	
Germiston	X	X	X	X		X		X	X	
Natalspruit	X	X	X	X		X		X	X	
Kopanong	X	X	X	X		X		X	X	
Sebokeng	X	X	X	X		X		X	X	
Tembisa	X	X	X	X		X		X	X	
Edenvale	X	X	X	X		X		X	X	
Tshepong	X	X	X	X		X	X	X	X	
Klerksdorp			X						X	
Potchefstroom	X	X	X	X		X		X	X	
Wolmaransstad	X	X	X	X		X		X	X	
Rustenburg	X	X	X	X		X		X	X	
George Stegman		X	X			X		X	X	
Mafikeng/Bophelong	X	X	X	X		X		X	X	
Lehurutshe	X	X	X	X		X		X	X	
Thusong	X	X	X	X		X		X	X	
Gelukspan	X	X	X	X		X		X	X	
Pelonomi			X					X	X	
Botshabelo	X	X	X	X				X	X	
Bloemfontein	X	X		X		X	X			
Welkom	X	X	X	X		X		X	X	
Kroonstad	X	X	X	X		X		X	X	
Bethlehem	X	X	X	X		X		X	X	
Manapo	X	X	X	X		X		X	X	
Kimberley	X	X	X	X		X	X	X	X	
De Aar	X	X	X	X		X		X	X	
Upington	X	X	X	X		X		X	X	
Springbok	X	X	X	X		X		X	X	
Ganyesa		X	X			X		X	X	
Tswaragano	X	X	X	X		X		X	X	
Taung	X	X	X	X		X		X	X	
Huhudi		X	X			X		X	X	
Universitas	X	X		X		X	X	X	X	
Groote Schuur Hospital	X	X	X	X	X	X		X		X
Tygerberg	X	X		X	X	X		X	X	
Green Point	X	X	X	X		X	X	X	X	
Paarl	X	X	X	X		X		X	X	
Vredenburg						X		X	X	
Karl Bremer						X				
G F Jooste										
Worcester	X	X	X	X		X		X	X	
George	X	X	X	X		X		X	X	
Oudtshoorn						X				
Beaufort West						X		X	X	
Somerset West						X		X	X	

	Microbiology	Parasitology - Stool	Parasitology - Blood	Mycology - Basic	Mycology - Advanced	TB Microscopy	TB Culture	Serology RPR	Serology HIV	Bacteriology WHO
Umtata	X	X	X	X		X	X	X	X	
St. Elizabeth						X		X	X	
St. Margaret's						X		X	X	
St. Patrick's						X		X	X	
Sipetu						X		X	X	
Maluti						X		X	X	
Mt. Ayliff						X		X	X	
Holy Cross		X				X		X	X	
Bambizana						X		X	X	
Mary Theresa						X		X	X	
Taylor Bequest						X		X	X	
Rietvlei						X		X	X	
Greenville						X		X	X	
Tabankulu						X		X	X	
Nessy Knight						X		X	X	
Qumbu						X		X	X	
St. Lucy	X			X		X		X	X	
All Saints						X		X	X	
Canzibe	X			X		X				
Zitulele	X			X		X		X	X	
Port St. Johns						X		X	X	
St. Barnabas						X		X	X	
Isilimela						X		X	X	
Madwaleni		X				X		X	X	
Cala						X		X	X	
Mqamakwe						X		X	X	
Butterworth	X	X		X		X		X	X	
Njanyana						X		X	X	
Tafalofefe	X	X		X		X		X	X	
Willowvale	X			X		X		X	X	
Cofimvaba	X			X		X		X	X	
East London Stat Lab										
East London Main Lab	X	X	X	X	X	X		X	X	
Aliwal North			X			X		X	X	
Queenstown	X	X	X	X		X		X	X	
Bisho	X	X	X	X		X		X	X	
Glen Grey			X			X		X	X	
Empilisweni						X		X	X	
SS Gida						X		X	X	
Victoria Hospital			X			X		X	X	
Cecilia Makiwana			X			X		X	X	
Port Elizabeth	X	X		X	X	X	X	X	X	
Port Elizabeth M2										
Livingstone			X					X	X	
Uitenhage	X	X	X	X		X		X	X	
Dora N'ginza			X					X	X	
Somerset East			X			X		X	X	
Cradock								X	X	
Graaff Reinet			X			X		X	X	
Grahamstown			X			X		X	X	
Port Alfred			X			X		X	X	
Humansdorp			X					X	X	
Tshwane Academic	X	X	X	X	X	X	X	X	X	
Tshwane Academic										
Dr George Mukhari Tertiary Lab	X	X	X	X	X	X	X	X	X	
Dr George Mukhari Virology									X	
Witbank	X	X	X	X		X		X	X	
Middelburg		X	X			X		X	X	
Philadelphia		X	X			X		X	X	
Kwa-Mhlanga			X			X		X	X	
Ermelo	X	X	X	X		X		X	X	
Embhleni		X	X			X		X	X	

	Microbiology	Parasitology - Stool	Parasitology - Blood	Mycology - Basic	Mycology - Advanced	TB Microscopy	TB Culture	Serology RPR	Serology HIV	Bacteriology WHO
Standerton		X	X			X		X	X	
Piet Retief		X	X			X		X	X	
Evander			X					X	X	
Nelspruit	X	X	X	X		X		X	X	
Barberton	X	X	X	X		X		X	X	
Themba	X	X	X	X		X		X	X	
Shongwe	X	X	X	X		X		X	X	
Mapulaneng	X	X	X	X		X		X	X	
Tintswalo		X	X			X		X	X	
Matikwana		X	X					X	X	
Tonga		X	X			X		X	X	
Tzaneen	X	X	X	X		X		X	X	
CN Phathudi		X	X			X		X	X	
Letaba		X	X			X		X	X	
Kgapane		X	X			X		X	X	
Sekororo		X	X			X		X	X	
Phalaborwa	X	X	X	X		X		X	X	
Namakgale		X	X			X		X	X	
Elim	X	X	X	X		X		X	X	
Donald Frazer		X	X			X		X	X	
Giyani		X	X			X		X	X	
Malamulele		X	X			X		X	X	
Messina		X	X			X		X	X	
Siloam		X	X			X		X	X	
Tshilidzini	X	X	X	X		X		X	X	
Louis Trichardt		X	X			X		X	X	
Kalafong	X	X	X	X	X	X		X	X	
Pretoria West	X	X	X	X		X		X	X	
Folang						X		X	X	
Jubilee	X	X	X	X	X	X		X	X	
Warmbaths						X		X	X	
Nylstroom	X	X	X	X	X	X		X	X	
Mmametlhake		X	X			X		X	X	
Odi	X	X	X	X		X		X	X	
Brits						X		X	X	
Thabazimbi	X	X	X	X				X	X	
Polokwane	X	X	X	X	X	X		X	X	
Mankweng	X	X	X	X	X	X		X	X	
Helen Franz						X		X	X	
Knobel						X		X	X	
Zebediela (Grootshoek)						X		X	X	
Ellisras	X	X	X	X	X	X		X	X	
George Masebe						X		X	X	
Mokopane						X		X	X	
Dilokong (Mandagshoek)	X	X	X	X		X		X	X	
Mecklenberg	X	X	X	X		X		X	X	
Botlokwa						X		X	X	
Seshego						X		X	X	
Jane Furse	X	X	X	X	X	X		X	X	
Matlala						X		X	X	
St. Ritas	X	X	X	X		X		X	X	
Voortrekker (Potgietersrus)						X		X	X	
Lebowakgoma	X	X	X	X		X		X	X	
NICD Serology								X		
Bedford										
Ngangelizwe Health Centre								X	X	
Mbekweni Health Centre								X	X	
Mhlakulo Health Centre								X	X	
Addington		X	X							
King Edward V111		X								
Prince Mshiyeni		X	X							
R K Khan		X	X							
Albert Luthuli		X								

	Microbiology	Parasitology - Stool	Parasitology - Blood	Mycology - Basic	Mycology - Advanced	TB Microscopy	TB Culture	Serology RPR	Serology HIV	Bacteriology WHO
Edendale		X	X							
Greys		X	X							
Northdale		X	X							
Bethesda		X	X							
Benedictine		X	X							
Ladysmith		X	X							
Madadeni		X	X							
Ngwelezane		X	X							
Port Shepstone		X	X							
Public Health		X	X							
Leslie Williams	X	X	X			X		X	X	
Western Deep	X	X	X			X		X	X	
KDM	X	X	X		X			X	X	
Westvaal	X	X	X			X	X	X		
1 Military		X		X		X				
Niehaus Dyson & Lancet	X	X			X	X				
SANBS Natal										
Jacobs & v Blerk (Modderfontein)										
Orapa	X	X						X		
van Drimmelen		X	X	X		X				
Ampath Little com Mary										
Lancet TB Richmond						X	X			
Lancet Nelspruit		X								
Duff Scott	X					X				
Pathcare Dietrich & ptnrs CT		X			X	X	X			
Pathcare Mulligan & ptnrs EL	X	X				X				
Pathcare PE	X	X				X				
Pathcare Bloemfontein		X			X	X				
Pathcare Welkom						X	X			
Pathcare Kimberley		X				X				
Pathcare George		X				X				
Pathcare Namibia		X		X		X	X			
Ampath Bower & ptnrs Durban		X		X		X	X			
Vermaak & ptnrs Wilgers										
Vermaak & ptnrs Eugene Marais										
Vermaak & ptnrs Unitas										
Contract Research								X	X	
CLS Donald Gordon									X	
Contract Research Cape Town									X	
MDP RHRU Chris Hani									X	
MDP Africa Center Ref Lab									X	
MDP Durban MRC Lab Tongaat									X	
Malaria Institute Tzaneen			X							
Toga Lab										
PHRU Clinical site Chris Hani Bara PIP									X	
<b>INTERNATIONAL LABORATORIES</b>										
Windhoek	X	X		X		X			X	
Gobabis		X				X				
Keetmanshoop	X	X				X			X	
Mariental		X				X				
Rehoboth	"					X			X	
Otjiwarongo	X	X				X			X	
Swakopmund	X	X				X			X	
Walvis Bay		X				X			X	
Oshakati	X	X				X			X	
Engela	X	X				X				
Eenhana		X				X				
Outapi		X				X			X	
Onandjokwe	X	X				X			X	
Oshikuku		X				X			X	

	Microbiology	Parasitology - Stool	Parasitology - Blood	Mycology - Basic	Mycology - Advanced	TB Microscopy	TB Culture	Serology RPR	Serology HIV	Bacteriology WHO
Rundu	X	X				X			X	
Katimo Mulilo	X	X				X			X	
Grootfontein		X				X				
Nankudu		X				X				
Tsumeb						X				
Mananga Swaziland						X			X	
W.P.B.T.S.										
ZIMPACT Zimbabwe						X	X		X	
Project San Francisco (IAVI)		X						X	X	
Vermaak & ptrns Microbiology				X		X	X			
Holy Trinity Ghana	X	X	X			X		X	X	
Ndlovu Medical Centre										
Gugutainer Lab (Toga)										
Montana (Ampath)										
KEMRI Kilifi (IAVI)									X	
MDP Zambia CIDRZ Ref Lab									X	
MDP Zambia Mazabuka Lab									X	
MDP Uganda MRC Entebe Ref									X	
MDP Uganda MRC Masaka Lab									X	
MDP Tanzania Ref Lab (NIMR)									X	
MDP Tanzania Lab									X	
MDP Mtubatuba Africa Centre Lab									X	
Nairobi PIP Lab									X	
Nairobi PIP Clinic									X	
Kisumu Kenya PIP Lab									X	
Kisumu Kenya PIP Clinic									X	
Moi Hosp Eldoret Kenya Lab PIP									X	
Moi Hosp Eldoret Kenya Clinic PIP									X	
Botswana Harvard HIV Ref Lab PIP									X	
Botswana Harvard HIV Clinic PIP									X	
TDRC Ref Lab Ndola Zambia PIP									X	
Ndola clinical site Zambia PIP									X	
Kitwe clinical site Zambia PIP									X	
Kampala Ref Lab Uganda PIP									X	
Kampala Uganda Clinic PIP									X	
Moshi Ref Lab Tanzania PIP									X	
Moshi Tanzania Clinic PIP									X	
Kongo (Namibia)		X				X				
Andara (Namibia)		X				X			X	
Nyangana (Namibia)		X				X				
ZEHRP Lusaka Zambia (IAVI)								X	X	
UVRI Uganda (IAVI)								X	X	
Bodene Lab	X									
KEMRI Nairobi							X			
UTH Lusaka		X	X							
Karonga Malawi		X				X	X		X	
JCRC Uganda (IAVI)	X	X	X			X		X	X	
MRC Masaka (IAVI)								X		
ANGOLA - NPHL Nacional do Salude										X
ALGERIA - NPHL Centre Hospitalier d'Oran										X
BENIN - NPHL Laboratoire National de Sante Publique										X
BENIN-PBM Centre National Hospitalier et Universitaire de COTONOU										X
BOTSWANA - NPHL+PBM National Public Health Refeerence laboratory										X
BURUNDI - NPHL+PBM Laboratoire National de Reference										X
BURKINA FASO - NPHL+PBM Centre Hospitalier National										X
BURKINA FASO - NPHL Hospital pediatric Charles de Gaulles										X
BURKINA FASO - NPHL Laboratoire national de Sante Publique										X
CAMEROON / CAMEROUN - NPHL Laboratoire de bact, Centre Pasteur, Garoua										X
CAPE VERDE - NPHL Laboratorio Nacional de Referencia/										X
CENTRAL AFRICAN REPUBLIC - NPHL Laboratoire National de Sante Publique										X
CHAD - NPHL Hopital general de Reference nationale										X
COMORES- NPHL Laboratoire de Biologie EL-Maarouf,Hopital El Maarouf										X
CONGO BRAZZAVILLE - NPHL Laboratoire National de Sante Publique										X
CÔTE D'IVOIRE - NPHL+PBM CHU Yopougon										X

DEMOCRATIC REPUBLIC CONGO - NPHL Institut National de Recherche Bio-Medicales	X
EQUATORIAL GUINEA - NPHL INSESO Laboratory ( Instituto de Seguridad Social )	X
ERITREA - NPHL National Health Laboratory	X
ETHIOPIA - NPHL Ethiopian Health and Nutrition Research Institute	X
ETHIOPIA - PBM Tikuer Anbessa Hospital	X
GABON - NPHL National Reference laboratory	X
GAMBIA - NPHL+PBM National Health Laboratories, Royal Victoria Hospital	X
GHANA - NPHL Public Health and Reference Laboratory	X
GHANA - PBM U.G.M.S , Department of Microbiology	X
GHANA- Kumasi - PBM Komfo Anokye Teaching hospital (KATH)	X
GUINEE CONAKRY - NPHL Laboratoire National de Sante Publique	X
GUINEE CONAKRY - PBM Laboratoire de Bacteriologie, Chu Donka	X
GUINEE BISSAU - NPHL Laboratoire National de Santé Publique	X
KENYA NPHL- National Public Health Laboratory Service	X
KENYA - PBM Kenyatta National Hospital	X
LESOTHO - NPHL Ministry of Health and Social Welfare	X
MADAGASCAR - NPHL Laboratoire de Bactériologie du Centre Hospitalier d'Antanarivo-HRJA	X
MALAWI - NPHL Public Health Laboratory	X
MALAWI - PBM Wellcome Trust Clinical Research Programme Laboratory	X
MALI - NPHL+PBM Institut National de Recherche en Sante Publique	X
MAURITANIA - NPHL Centre National d'Hygiene	X
MAURITANIA- Laboratoire de Biologie Medicale	X
MOZAMBIQUE - NPHL Faculty of Medicine, Eduardo Mondlane University	X
MOZAMBIQUE - PBM Hospital Centre de Maputo	X
NAMIBIA - NPHL Namibia Institute of Pathology	X
NAMIBIA-OSHAKATI - NPHL Namibia Institute of Pathology, Oshakati State Hospital	X
NIGERIA - NPHL Central Public Health Laboratory	X
NIGER - NPHL+PBM Reseau National de Laboratoire	X
RWANDA - NPHL-Kigali Laboratoire National de Reference et de Sante Publique	X
RWANDA - NPHL- Butare Bacteriologie Laboratoire, CHU de Butare	X
RWANDA - PBM Laboratoire de Biologie Medicale	X
SAO TOME ET PRINCIPE Centre de Hospitalier de Tome	X
SENEGAL - NPHL+PBM Centre Hospitalier National de FANN	X
SENEGAL - PBM Hopital d'Enfants	X
SENEGAL new Laboratoire de Bacteriologie, CHU Aristide Le Dantec, Dakar	X
SEYCHELLES - NPHL National Public Health Laboratory	X
SIERRA LEONE - NPHL+PBM Central Referral Laboratory	X
SWAZILAND - NPHL+PBM National ReferenceLaboratoryl	X
TANZANIA - NPHL Muhimbili Medical Centre	X
TOGO - NPHL+PBM Ministere de la Sante	X
UGANDA - NPHL Central Public Health Laboratories	X
UGANDA - PBM Microbiology Department, Mulago Hospital	X
UGANDA - PBM St Mary's Hospital, Lacor	X
ZAMBIA - NPHL Tropical Diseases Research Centre	X
ZAMBIA - PBM University Teaching Hospital	X
ZIMBABWE - NPHL+PBM Natinal Microbiology Reference Laboratory	X
ZANZIBAR - NPHL Mnazi Mmoja Hospital	X
MALAWI (NEW) QUEEN ELIZABETH CENTRAL HOSPITAL	X
BURKINA FASO WHO Multi Disease Surveillance Centre	X
CAMEROON Centre Pasteur, Yaoundé	X
DJIBOUTI Hopital Général Peltier	X
DJIBOUTI Office de Protection Sociale, Ministère du travail	X
DRC Laboratoire Provincial de Lubumbashi	X
NIGER Cermes, Niamey	X
SOMALIA C/o WR / SOMALIA, Hargeiza Office	X
SUDAN National Health Lab, Khartoum	X

## National Stock Culture Collection

The National Stock Culture Collection (NSCC) was established in April 2004 under the NICD EQA Unit, with the mandate to provide a quality controlled and reliable source of reference bacterial, fungal and mycobacterial strains for NHLS laboratories. Microbiology laboratories that are accredited according to ISO15189 as diagnostic medical laboratories are required to control all procedures and tests. Certain procedures such as antimicrobial susceptibility testing, and biochemical tests on bacteria require reference bacterial strains on which control tests are performed. The NSCC has an invaluable role to play in facilitating the NHLS laboratories' compliance with accreditation authorities. This report summarizes the activities of the NSCC over 2005.

### Management issues and Information Technology

The NSCC policies and procedures are documented in SOP NSCC#001. This SOP details characterization procedures, storage procedures (including master, seed, distribution and reserve stock of cultures), distribution procedures and record keeping of isolates. Information pertaining to cultures is recorded on paper and in an Access database format. Costing of the production and maintenance of these reference cultures was performed; single isolates are sold to laboratories at a nominal fee of R150.00.

### Characterisation and storage of reference cultures

During 2005 over 201 strains have been characterized into the NSCC database (including 171 bacteria, 20 yeasts and moulds, 10 enterovirulent *Escherichia coli*) These comprise ATCC (American Type Culture Collection) strains for quality control of antimicrobial susceptibility testing using the disc method according to the Clinical Laboratory Standards Institute (CLSI) recommendations M2-A8, *Mycobacterium* species, yeasts and moulds, diarrhoeagenic *E. coli* and strains for quality control of media, biochemical testing and teaching purposes. Sources of these isolates include the former SAIMR culture collection; isolates obtained from participation in international EQA programmes, or clinical isolates from the NICD Reference Units. A full catalogue is available on request. Information pertaining to each strain is recorded on an access database including the following information (where appropriate):

- Biochemical analysis
- Antimicrobial susceptibility profiles (including results of disc susceptibility testing and minimum inhibitory concentrations (MICs))
- Serotyping results where applicable
- PCR results with accompanying agarose gel pictures (where applicable, to confirm the presence of toxigenic genes)
- Full colour images of colonial morphology and spore formation (yeasts and moulds)

When the EQA Unit lacks expertise to characterize organisms, this is outsourced to the NICD Reference Units, or to appropriate NHLS laboratories. Over the past year, and in order to refresh existing cultures that have indeterminate passage number, 34 strains listed in Table 1 below have been purchased from international culture collections (ATCC and National Culture Type Collection of the United Kingdom).

Table 1. Reference cultures purchased from international culture collections during 2005.

Organism name	Reference culture number	Organism name	Reference Culture number
<i>Bacillus subtilis</i>	ATCC6051	<i>Clostridium botulinum</i>	NCTC 7272
<i>Bordetella pertussis</i>	ATCC 9797	<i>Clostridium botulinum</i>	NCTC 7273
<i>Candida albicans</i>	ATCC 90028	<i>Clostridium botulinum</i>	NCTC 8266
<i>Candida krusei</i>	ATCC 6258	<i>Clostridium botulinum</i>	NCTC 10281
<i>Corynebacterium diphtheriae</i>	ATCC 13812	<i>Vibrio mimicus</i>	ATCC 33653
<i>Enterococcus faecalis</i>	ATCC 29212	<i>Vibrio parahaemolyticus</i>	ATCC 17802
<i>Escherichia coli</i>	ATCC 25922	<i>Yersinia pseudotuberculosis</i>	ATCC 11960
<i>Escherichia coli</i>	ATCC 35218	<i>Trypanosoma lewisi</i>	ATCC 30085
<i>Haemophilus influenzae</i>	ATCC 49247	<i>Enterococcus faecium</i>	ATCC 19434
<i>Haemophilus influenzae</i>	ATCC 49766	<i>Morganella morganii</i>	ATCC 25830
<i>Haemophilus parainfluenzae</i>	ATCC 7901	<i>Proteus mirabilis</i>	ATCC 43071

<i>Neisseria gonorrhoeae</i>	ATCC 49226	<i>Proteus vulgaris</i>	ATCC 13315
<i>Neisseria lactamica</i>	ATCC 23971	<i>Klebsiella pneumoniae</i>	ATCC 700603
<i>Pseudomonas aeruginosa</i>	ATCC 27853	<i>Staphylococcus aureus</i>	ATCC 43300
<i>Shigella sonnei</i>	ATCC 25931	<i>Aeromonas hydrophila</i>	ATCC 7966
<i>Staphylococcus aureus</i>	ATCC 29213	<i>Alcaligenes faecalis</i>	ATCC 19018
<i>Stenotrophomonas maltophilia</i>	ATCC 13637	<i>Streptococcus pneumoniae</i>	ATCC 49619

### Distribution of reference cultures

During 2005, approximately 249 strains were distributed on request to NHLS, non-NHLS South African laboratories and international laboratories (excluding beaded vials which were a mass distribution).

Regarding antimicrobial susceptibility testing, NHLS laboratories have elected to comply with the CLSI guidelines; this necessitates that each laboratory have a supply of fresh cultures of several ATCC strains with passage number not more than six. In order to facilitate this, the NSCC developed a method whereby a given strain is lyophilized in a glass vial containing sterilized traditional African glass beads. When stored in the fridge (<4°C), these organisms remain viable for extended periods of time; a single bead can be placed in broth and reconstituted, allowing for a fresh strain of organism to be available for up to a year. This method minimizes the need for repeated subculture of strains and hence the possible contamination, mutation and loss of viability. These sets of organisms are shown in Figures 1 and 2. Eight-six sets were distributed to NHLS laboratories only in May 2005, along with the following documentation: an NSCC validation certificate for each strain, diagrammatic and textual instructions for reconstitution; storage instructions for beaded vials and viable QC strains, log sheets for recording QC of antibiotic discs (on the back of each validation certificate), a table listing the QC procedures for which each strain could be used, and a Department of Health Permit application form. Internal quality control was performed on these organisms and results are tabulated in Table 2 below. In summary, extended QC of strains sent out showed viability for 12 months for *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E. coli*, *Candida albicans*, *Candida tropicalis* and *Streptococcus pneumoniae*. Shortened viability for *Haemophilus influenzae* and *Cryptococcus neoformans* was observed. Reasons for loss of viability of the more fastidious strains are being investigated.



Figure 1. Complete set of quality control strains distributed to NHLS laboratories

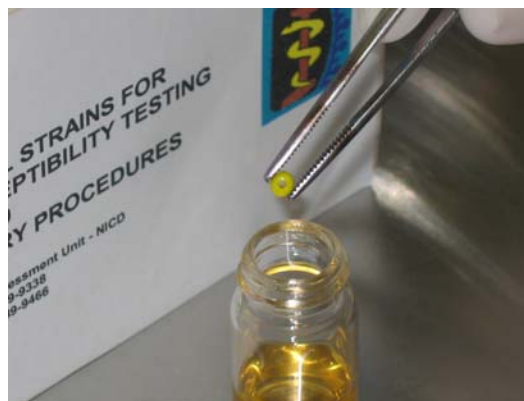


Figure 2. Procedure for reconstitution of a single bead in broth.

Table 2. Internal quality control results of beaded culture sets distributed in May 2005 to NHLS laboratories for quality control of antimicrobial susceptibility testing.

	Date of production of batch	Date of last viability of sample of batch	Duration of viability (months)	Comments
<i>E coli</i> ATCC 35218	22/02/2005	17/03/2006	12	Excellent survival
<i>E coli</i> ATCC 25922	9/03/2005	02/02/2006 – 17/03/2006	11-12	Variable growth; dependent upon how much lyophilized material is present on the bead
<i>S aureus</i> ATCC 25923	4/03/2005	17/03/2006	12	Excellent survival
<i>Pseudomonas aeruginosa</i> ATCC 27853	1/02/2005	17/03/2006	12	Excellent survival
<i>Streptococcus pneumoniae</i> ATCC 49619	9/03/2005	17/03/2006	12	Excellent survival
<i>Haemophilus influenzae</i> ATCC 49766	22/03/2005	17/03/2006	12	Variable growth; dependent upon how much lyophilized material is present on the bead
<i>Haemophilus influenzae</i> ATCC 49247	30/03/2005	18/11/2005-17/03/2006	8-12	Variable growth; dependent upon how much lyophilized material is present on the bead
<i>Candida albicans</i> ATCC 90028	30/03/2005	17/03/2005	12	Excellent survival
<i>Candida tropicalis</i> ATCC 750	30/03/2005	18/11/2005-17/03/2006	8-12	Variable growth; dependent upon how much lyophilized material is present on the bead
<i>Cryptococcus neoformans</i> ATCC 66031	30/03/2005	22/06/2005	3	Very poor survival.

NHLS laboratories were requested to evaluate the sets by answering a questionnaire distributed in November 2005. Comments from laboratories that used the strains and replied to the questionnaire (20 laboratories only) revealed the following:

- General comments that the strains were useful and a welcome innovation that facilitated provision of quality controlled reference cultures
- Excellent viability of *S. aureus*, *E coli*, *Pseudomonas aeruginosa*.
- Poor or no viability of *C. neoformans*
- Some laboratories reported decreased quantities of colonies after 24 hour incubation over the months of usage of the beaded vials for the fastidious organisms *Haemophilus influenzae* and *Streptococcus pneumoniae*.
- Occasional reports that organisms were contaminated. This must have occurred in the laboratory where strains were used, as no vials were found to be contaminated on QC in the EQA Unit.
- Evidence that strains were not correctly stored, and that certain laboratories were not aware of proper storage and reconstitution procedures.

Following distribution of the sets, certain laboratories requested that specific organisms (which they required as regular isolates for quality control or other purposes) be placed onto beaded vials. The following organisms were distributed in this manner; *Clostridium perfringens*, *Serratia Marcescens*, *Salmonella typhimurium*, *Shigella flexneri*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Lactobacillus fermentum*, *Lactobacillus planterium*, *Lactobacillus casei*, *Vibrio cholerae*, *Vibrio parahaemolyticus*.

#### **Compliance with Department of Health, and Department of Trade and Industry regulations.**

The NSCC organisms were distributed with strict adherence to the following regulations that govern the transmission of live cultures or microorganisms.

- Department of Health: Regulation 2 and 3 of Government Notice 2306 of December 1920: Authorisation to keep, transmit, or use cultures and microorganisms. This regulation covers all bacterial organisms that are used for quality control purposes or are kept in the laboratory (excluding clinical isolates). All laboratories are expected to comply with this regulation; the Department of Health issues permits that are valid for one year, after which the laboratory needs to re-apply. The NSCC cultures are distributed on receipt of a relevant permit.

- Department of Trade and Industry ‘Council for Non-proliferation of Weapons of Mass Destruction’ Government Gazette Volume 468, Number 26444 8 June 2004, Government Notice 712; Non-proliferation of weapons of mass destruction Act 87/1993. Notice under section 13: Declaration of certain biological goods and technologies to be controlled and control measures applicable to such goods. This regulation requires that all facilities using or maintaining organisms with potential for biological warfare be registered with the Council for Non-proliferation of Weapons of Mass Destruction. The NICD facilitated registration of the NHLS divisions with the Council, and distributes relevant cultures only on receipt of proof of registration with the Council

#### **Provision of reference strains for EQA activities**

The NSCC provides quality controlled, fully characterized strains for inclusion in EQA programmes, for use in training workshops (both local and international) that are undertaken by the EQA Unit; the Group for Enteric, Respiratory and Meningitis surveillance of South Africa (GERMS-SA i.e. the surveillance network co-ordinated by the NICD) and the School of Laboratory Medicine of the NHLS.

#### **Future activities**

The NSCC is working towards accreditation, along with the EQA Unit. Beaded vials will be distributed to NHLS bacteriology laboratories in 2006.