NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES

Division of the National Health Laboratory Service

EQuAFRICA

External Quality Assessment for AMR Testing

National Institute for Communicable Diseases (NICD) team in the Fleming fund EQuAfrica external quality assurance program

11th March 2022

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ASLM

AFRICAN SOCIETY FOR LABORATORY MEDICINE

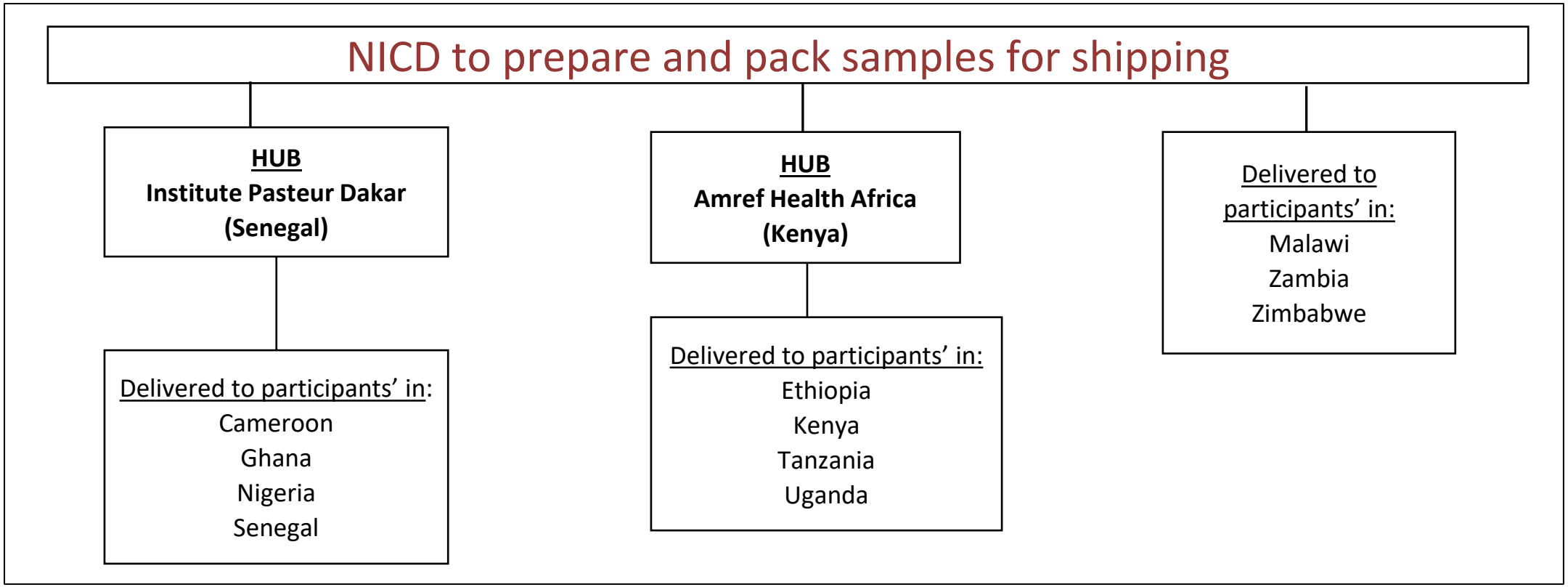
The Fleming Fund

Background

- NICD is a consortium partner and a member of the steering committee working in coordination with other partners for the strengthening of in-country capacity for the coordination of national EQA and the selection and strengthening of centers of excellence for additional capacity for international/regional EQA.
- Personnel involved:
  - Professor Olga Perovic: Team leader role – Organize the EQA programme at NICD
  - Mrs. Marshagne Smith: Technical leader role – Involved in management of the programme, design the programme, oversee all segments and contribute to the final annual report.
  - Mrs. Rubeina Badat: Technical support in all segments of the programme – Preparation of samples, quality control of samples, managing the EQA scheme.
- Activities :
  - Involved in assessment of informatics system and the programme design.
  - Part of stakeholders meetings to provide detailed outline of the programme implementation stages, activities, roles and responsibilities for establishment of regional EQA providers.
  - Involved in assessments and procurements for IPD and Amref laboratories.
  - Involved in training of IPD and Amref and face to face training of IPD team.
  - Responsible for the pilot cycle for all and for cycle one provision of panels for Amref and NICD laboratories and QC strains for all participants.

Preparation for Pilot survey/s

- Primary objective of the Pilot was for testing logistics of shipping and informatics system.
- NICD contributed to the program and informatics system design.
- Provided information for informatics system drop down fields.
- Weekly meetings to discuss and iron out challenges encountered.
- Agreed proposed plan for distribution of panels:



NICD responsibilities for the pilot survey

- Planning for the pilot survey
  - NICD shared a draft programme of work (POW) with additional steps required for planning. NICD provided design of survey with the list of organism. Pilot cycle – 05/03/2021
- Activities performed:
  - Number and list of participating laboratories
  - Confirmation of referee laboratories
  - Confirmation of couriers to be used for distribution of shipments
  - Quality control strains provided to participants. PHE supplied NCTC QC strains to NICD. NICD prepared beaded lyophilised vials for distribution.
  - The French translation were provided by ASLM team.
  - The Quality control procedures were performed for the duration of the shipment.
- POW finalised grading areas.

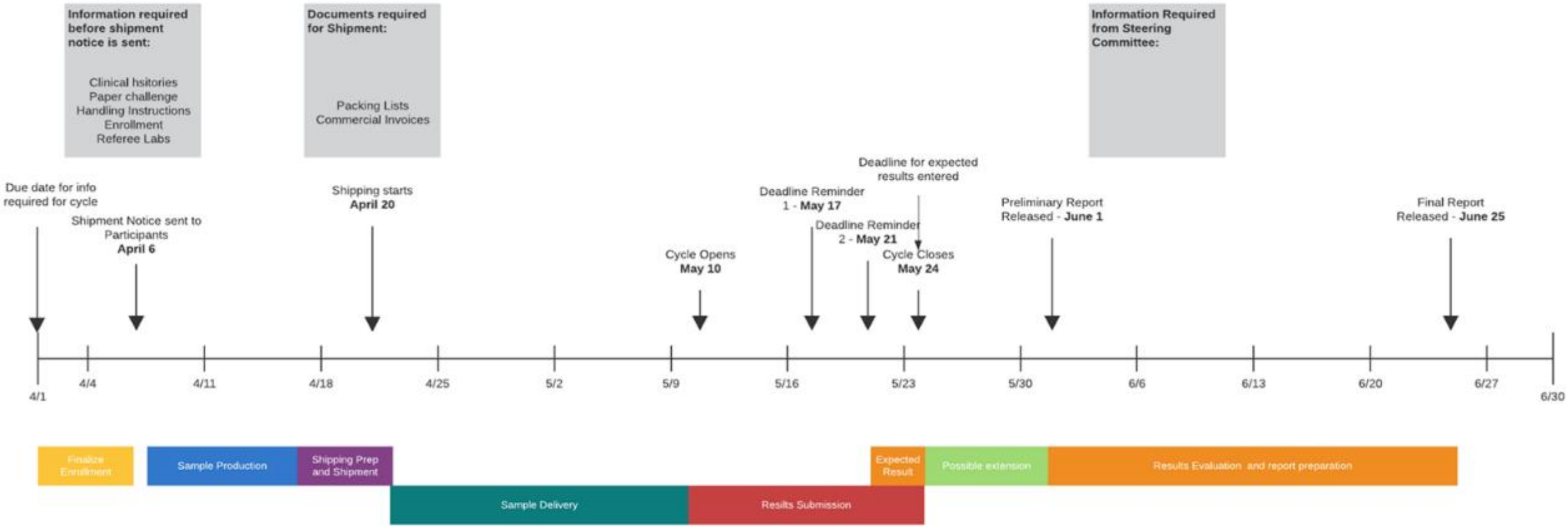
Pilot cycle details

- Five samples (in duplicate) were to be included in the cycle – lyophilised.

Sample ID	Organism identification	Microscopy - Evaluated	Final identification - Evaluated	AST - Evaluated
Sample A	*Escherichia coli*	√	√	√ - Susceptible
Sample B	*Staphylococcus aureus*	√	√	√ - MRSA
Sample C	*Escherichia coli*	√	√	√ - ESBL
Sample D	*Salmonella* species (non-Typhi)	√	√+serotyping	√ - Susceptible
Sample E	*Vibrio parahaemolyticus*	√	√	

- Four NCTC quality control (QC) strains were provided to participants as beaded lyophilised cultures.
- Referee consensus were used to determine acceptability of results for grading.
- NICD was doing weekly QC of prepared samples. It was suggested that one of the other Hub sites perform weekly QC for the samples as well. IPD agreed to perform.
- Cycle open date 10 May 2021, final Cycle close date 24 May 2021.
- Endorsing the Technical Advisory Group (TAG) for the pilot programme with representatives from DTU, PHE and NICD.
- Sixty–seven participating laboratories across the three sites were enrolled for the pilot programme.

Final Programme of work - pilot



NICD implementation plan

Programme of work finalised –
Organism to be included are agreed upon. Dates for survey activities agreed upon i.e. shipping date, testing start date etc.

Week of 23 March 2021:
Start with organism work ups -
Identify organism to be used. Pull out from-70, perform full organism work-up (all ID and AST) – as per our (NICD) SOPs in place

Numbers to be prepared were confirmed. Included participant as well as referee laboratories.
Samples were sent in duplicate. Additional samples for weekly QC and homogeneity and stability testing were prepared

Start lyophilisation of panel organisms and QC strains according to our (NICD) SOPs in place. Label etc.

Samples packed according to IATA specifications – packed samples to "hub" PT provider sites for distribution to participants

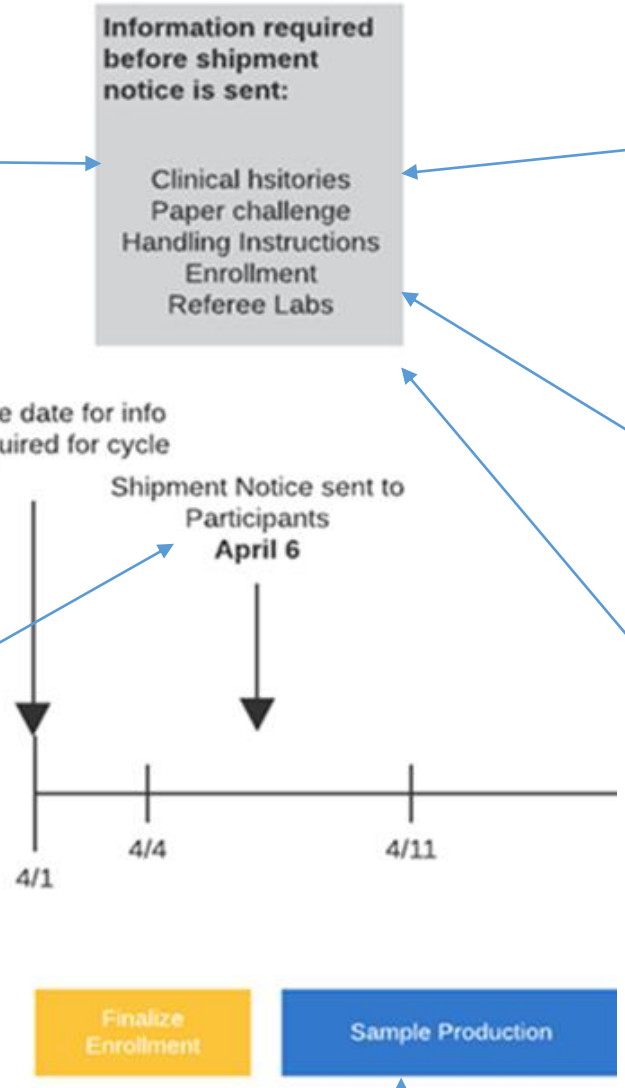
Ship to other PT providers (Hubs) week of 18 April 2021

Enrolment in Informatics system

The final number of participants required in order to start preparation of sample and QC strains.

A communication sent to participants informing them of shipping date of Pilot cycle samples

Selection and confirmatory testing of isolates to be included in the cycle being completed at NICD – week of 23 March



Five Referee labs confirmed:

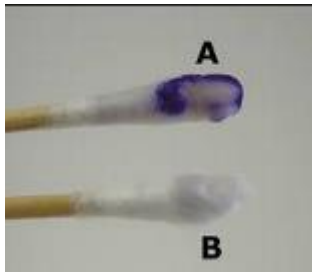
- DTU
- Ampath
- NHLS-CMJAH – replaced by NHLS infection control for pilot.
- NHLS-Groote Schuur
- Vermaak and Partners

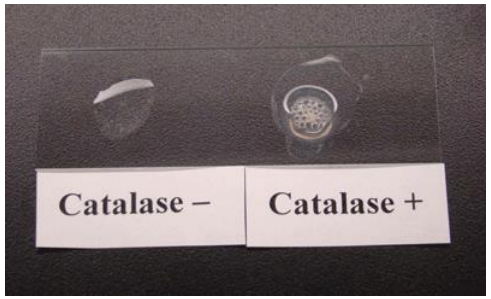
Clinical data for human health compiled by NICD, circulated to advisory group for input and approval. DTU supplied the clinical data for animal health laboratories. Translated to French by ASLM where required.

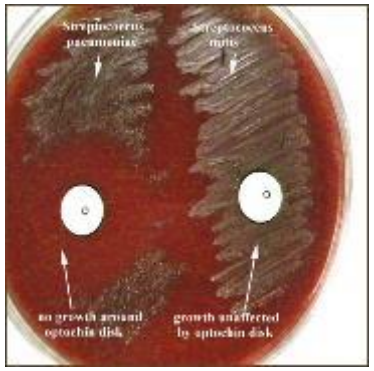
Handling instructions for sample processing and use of beaded QC strains provided by NICD. Fleming Fund provided French translation.

Preparation of lyophilised samples and beaded QC strains at NICD

Complete organism work-up before inclusion in the cycle

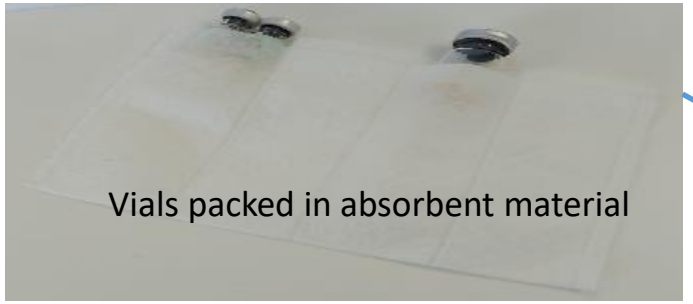






Shipping conditions

Prepared samples – Capped and labelled



Vials packed in absorbent material

Samples in absorbent material placed into Pathopouch
Separate sections for documents e.g. clinical data

All participant samples received completely packed according to IATA specifications – Distribution site to provide NICD with their information that appeared on the senders label address. Name of laboratory, address, contact information.
Distributors to confirm the courier that were used for the delivery of shipments, we made sure the courier was aware that these panels would be distributed as **UN3373 Biological substances category B,** arrange for door to door delivery.

Place pathopouch into shipping box

Box will be sealed using a security label
And the relevant sender and recipient labels Added.

Instructions and documents included

EQuAFRICA AMR Pilot programme – Animal health laboratories

NB: All samples are lyophilised and must be reconstituted before culture. Store samples between 2-8°C on receipt

Sample details

Sample A	A farm encountered an outbreak with peracute death in healthy, well-conditioned, recently weaned pigs. The pigs suffered from loss of coordination, periocular oedema and extensive oedema of the stomach and mesocolon. Diarrhoea preceded the signs of oedema disease. Specimen type: Small intestine tissue from a pig Report the following on the pathogen isolated: > Microscopy > Identification > Antimicrobial susceptibility testing. Report the following antimicrobial agents:		
	Ampicillin	Ceftriaxone	Imipenem
	Amoxicillin/clavulanate	Amikacin	Meropenem
	Cefepime	Gentamicin	Piperacillin/Tazobactam
	Cefotaxime	Tobramycin	Trimethoprim/sulfamethoxazole
	Cefoxitin	Ciprofloxacin	
	Ceftazidime	Ertapenem	
Sample B	A routine visit by the veterinarian due to occasional cases of abscesses in a very few pigs. In general, no clinical signs were observed in the pig farm. Specimen type: Nasal swab from a pig Report the following on the pathogen isolated: > Microscopy > Identification > Antimicrobial susceptibility testing. Report the following antimicrobial agents:		
	Ampicillin	Clindamycin	Rifampicin
	Cefoxitin	Gentamicin	Quinupristin/Dalfopristin
	Chloramphenicol	Linezolid	Tetracycline
	Ciprofloxacin	Oxacillin	Trimethoprim/sulfamethoxazole
	Erythromycin	Penicillin	Vancomycin
Sample C	A veterinarian observed in a poultry breeding farm birds with systemic infection, which manifested in diverse ways, including acute fatal septicaemia. Thus, high doses of ceftiofur was administered. Specimen type: Enlarged, hyperaemic liver of a chicken Report the following on the pathogen isolated: > Microscopy > Identification > Antimicrobial susceptibility testing. Report the following antimicrobial agents:		
	Ampicillin	Ceftriaxone	Meropenem
	Amoxicillin/clavulanate	Amikacin	Nitrofurantoin
	Cefepime	Gentamicin	Trimethoprim/sulfamethoxazole
	Cefotaxime	Tobramycin	
	Cefoxitin	Ertapenem	
	Ceftazidime	Imipenem	

Instructions for use and maintenance of beaded lyophilised quality control strains

As participants in the EQuAFRICA pilot program laboratories, have received a set of quality control (QC) strains. Additional information for strain usage is outlined in Table 1 below. Strains provided are:

- NCTC 12241 – equivalent to *Escherichia coli* ATCC 25922
- NCTC 12934 – equivalent to *Pseudomonas aeruginosa* ATCC 27853
- NCTC 12973 – equivalent to *Staphylococcus aureus* ATCC 29213
- NCTC 12981 – equivalent to *Staphylococcus aureus* ATCC 25923

Provided in this document are instructions on how to proceed with use and maintenance of beaded lyophilised QC strains. Each lyophilised culture is in a vial containing a minimum of 12 glass beads.

The vial you receive in your laboratory contains a pure culture of organism that has been authenticated and fully characterised. Good laboratory practice requires that organisms used in standardised test methods (e.g. antimicrobial susceptibility testing according to CLSI recommendations) should not be used beyond 6 or 7 passages or sub-cultures before being discarded. This practice will minimise accidental loss of the organism, contamination, genetic drift and human error in transfer and labelling.

Storage of vials

All vials must be stored at 2-8°C. Ensure that once opened the vials are properly re-sealed.

Continual refrigeration at 2-8°C:

- Ensure that your refrigerator temperature is constant
- These organism will also do well in the freezer.
- Replace vials in the refrigerator immediately after removal of a bead.

Ensure that vials are re-sealed:

- Prevents lyophilised material from absorbing moisture.
- Moisture absorption allows the organism to commence metabolism; if this occurs when the organisms are in the beaded vial no nutritive media is available and the organism will die.

Method for retrieving cultures from lyophilised vials with beads

NB: Carry out all procedures aseptically, preferably in a class II Biosafety cabinet

1. Label a sterile test tube with the organism name.
2. Aseptically add 2-3 drops of broth (BHI, TSB, serum) to the test tube.
3. Carefully remove the metal cap from the culture vial using forceps.
4. Remove the rubber stopper and place upside-down on a clean, disinfected work surface.
5. Immerse the ends of one or two pairs of stainless steel forceps in alcohol or rectified spirits.
6. Remove a pair of forceps from the alcohol and flame until dry and allow to cool down.
7. Carefully loosen the beads at the bottom of the vial.
8. Remove one bead from the vial, and drop into the labelled tube containing the broth.
9. Reseal the vial with the rubber stopper.
10. Incubate the broth for 15-30 minutes at 37°C.
11. Transfer a loop full or a few drops of broth onto appropriate solid media (organism dependant) and plate out for single colonies.

Handling instructions

Store samples between 2-8°C on receipt.

Your shipment includes two sets of isolates, labelled accordingly:

- EQA Samples for testing

Samples for testing

Process samples according to clinical data provided, submit results once complete.

- Quality control strains for your use

Quality control strains

Instructions for use and maintenance of beaded lyophilised quality control strains will be provided.
List of QC strains provided:

- NCTC 12241 (*Escherichia coli*)
- NCTC 12934 (*Pseudomonas aeruginosa*)
- NCTC12973 (*Staphylococcus aureus*)
- NCTC12981 (*Staphylococcus aureus*)

Des Instructions pour récupérer une souche lyophilisée:

- Faites basculer la partie ronde du bouchon métallique en utilisant des forceps. (Fig. 1)
- À ce stade, le capuchon métallique et le bouchon en caoutchouc peuvent être retirés, et avec l'aide d'une pipette Pasteur ou une autre pipette, peut être utilisé pour prélever et transférer 0.5 ml de bouillon** dans le lyophilisat afin de le remettre en suspension, tout en respectant les techniques d'asepsie adéquates. (Fig. 2)
- Placez le flacon en incubation à 37 °C pendant 10-15 minutes
- Inoculer la gélose compatible avec la souche et pratiquer un étalement de la souche pour obtenir des colonies isolées. (Fig. 6)

OR

- Désinfecter la partie exposée du bouchon en caoutchouc avec 70% d'alcool. (Fig. 3)
- En utilisant une seringue et une aiguille, prélever 0.5 ml de bouillon en respectant les techniques d'asepsie adéquates
- Insérez l'aiguille dans le flacon à travers le bouchon et injecter le contenu de la seringue dans le flacon pour remettre le lyophilisat en suspension dans le bouillon prélevé. (Fig. 4)*
- Placez le flacon en incubation à 37 °C pendant 10-15 minutes.
- Prélevez quatre gouttes du contenu/lyophilisat du flacon et les déposer sur une gélose appropriée (Fig. 4) puis étalez la souche pour obtenir des colonies isolées (Fig.5)
- Jetez l'aiguille dans un conteneur pour objets tranchants
- Le flacon contenant le reste de bouillon peut aussi être incubé avec les plaques.
- En cas d'absence de croissance sur le milieu gélosé après 24 heures, il est possible d'étaler une goutte du bouillon sur un nouveau milieu gélosé.
- Incuber le milieu gélosé et bouillon à la température optimale de croissance pour la souche Incubate media at

Figure 1

Figure 2

Figure 3

Figure 4 *

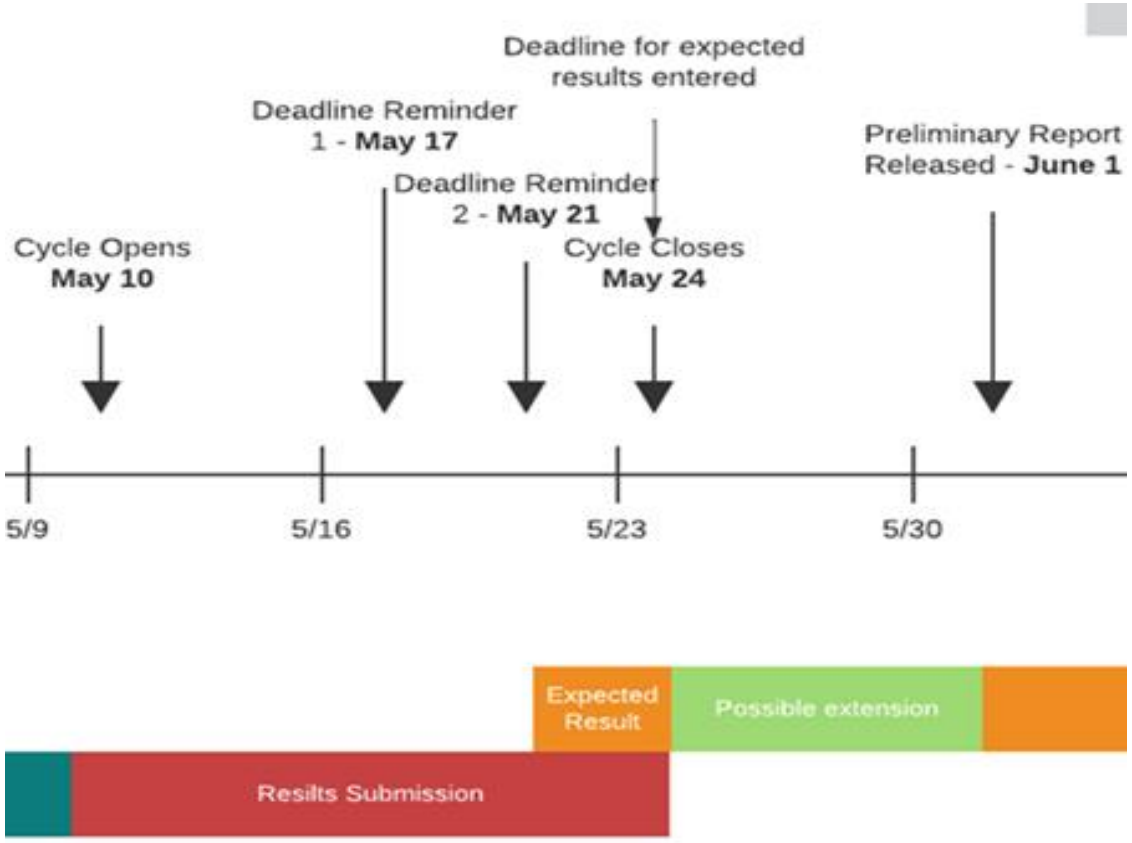
Figure 5

Figure 6

* Si vous utilisez cette méthode, conformez-vous aux mesures de sécurité universelles.

** BHI is the media of choice to reconstitute most organisms. Other alternatives would be serum broth or any suitable nutrient broth.

Program of work continue



- Each site was responsible for monitoring the delivery of shipment and noting challenges.
- The official cycle open date was 10 May 2021 and closing date for result submission was 24 May 2021.
- Due to delays in the delivery of some of the shipments, participants informing they had received panels late were granted additional time for processing and submitting results. For these participants the closing date for submission of results was extended to 01 June 2021.
- Expected results report was made available after the cycle had officially closed.

Results evaluation and report preparation

- Review of referee participants results for drawing up of mark scheme. The ≥80% referee consensus per graded area was needed.
- TAG approved final mark scheme and was consulted for queries arising.
- Participant results received in an excel spreadsheet.
- The grading system outlined in the program design was used initially, this was revised and the CMPT grading system was used, and was done twice.
- Individual participant report layout was designed and circulated for approval.
- Grading in the excel spreadsheet was used by IT support team for individual participant report generation.
- Commentary report generated, circulated to TAG for comments and approval.
- A scoring guide was also made available to participants to understand how they were graded.
- A TA feedback session was held with all participants to review the Pilot cycle.

Breakdown of participant numbers for EQuAfrica Pilot programme

IPD

Countries	Pilot
Cameroon	11
Gabon	0
Senegal	4
Ghana	6
Nigeria	4
Sierra Leone	0
Total	25

Amref

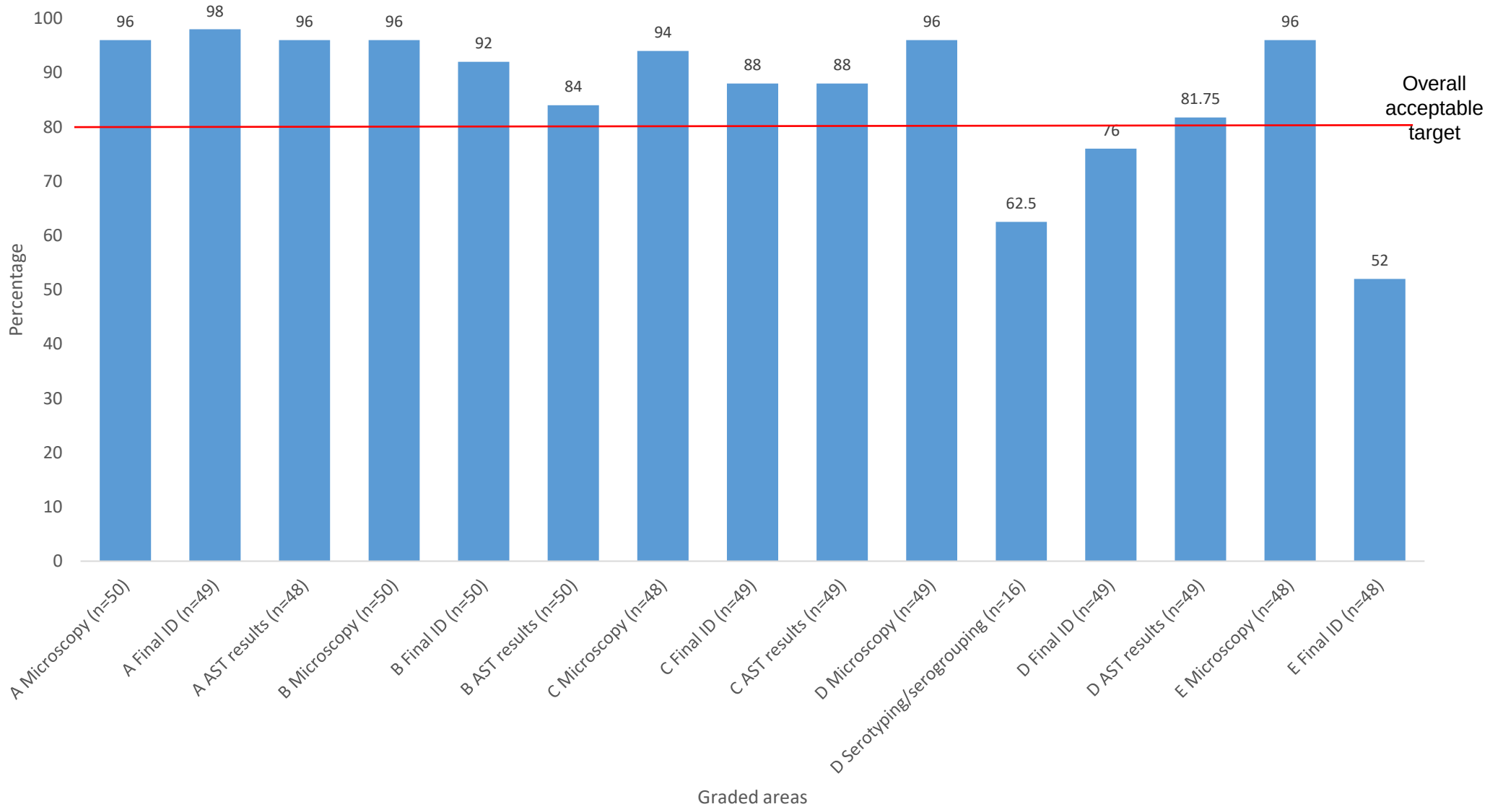
Countries	Pilot
Ethiopia	2
Kenya	14
Uganda	8
Tanzania	2
Total	26

NICD

Countries	Pilot
Eswatini	0
Malawi	4
Zambia	3
Zimbabwe	9
Total	16

	Number of participants	%
Received EQA samples	67	100%
Declined participation - reason provided	3	4.5%
Results received	51	76%
No results submitted	13	19.5%

Overall participant performance for Pilot Programme



Activities following pilot cycle

- IPD team visited NICD for training on PT scheme management and preparation.
- Covered design and management of a PT scheme, preparation of lyophilised and swab PT samples, QC and stability testing, mark scheme, grading and procedure for report generation.
- On completion of training, IPD was requested to perform a competency exercise by preparing a mock shipment to send to NICD.

Cycle 1 planning activities

- Additional laboratories were invited to participate in Cycle 1 – final numbers were needed before samples and QC strains could be prepared.
- Program of work was drawn up by NICD and circulated for approval. Suggested organism with areas to be graded and timeline for activities for the cycle.
- Five samples sent in duplicate and six NCTC control strains to be supplied by PHE

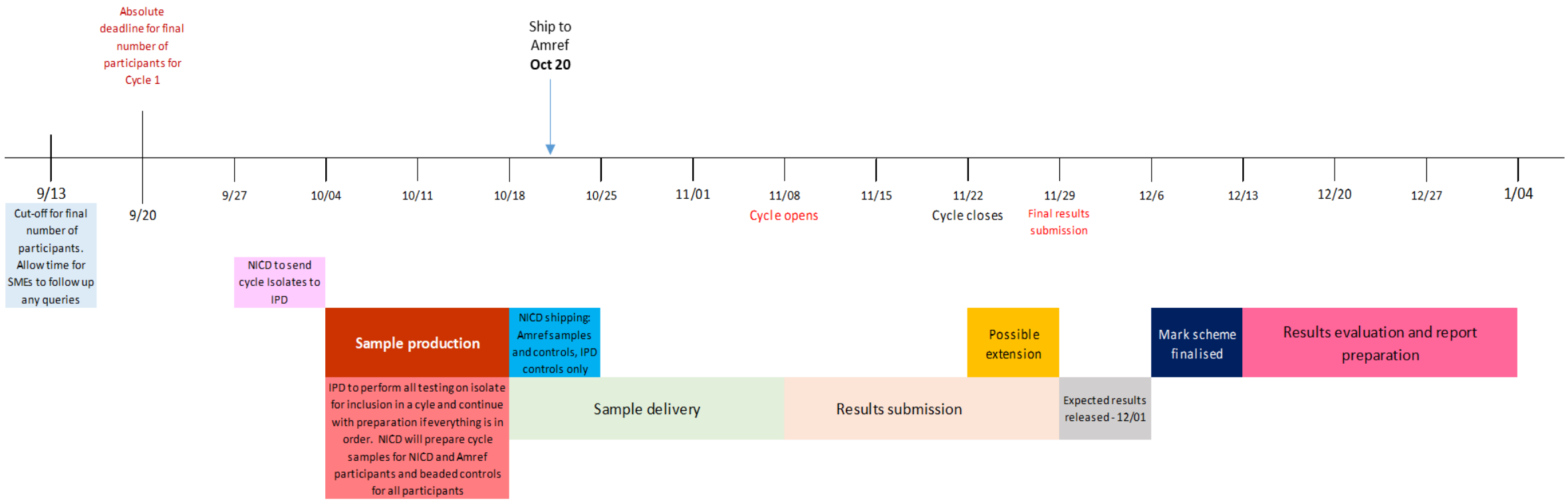
Sample ID	Organism identification	Microscopy - Evaluated	Final ID - Evaluated	AST - Evaluated
Sample A	*CPE – Klebsiella pneumoniae*		√	√ - CRE+CPE
Sample B	*Enterococcus faecium*	√	√	√ - Vancomycin R
Sample C	*Pseudomonas aeruginosa*		√	√ - MDR
Sample D	*Staphylococcus aureus*		√	√ - Inducible clindamycin R
Sample E	*Listeria monocytogenes*	√	√	

- Other sites had more involvement in packing and preparing of samples.
- Due to time constraints, the new set of QC strains were sent as swabs. Lyophilised beaded cultures will be provided in Cycle 2. New enrolled participants also received a set of the previous control strains sent in the Pilot.

Cycle 1 further planning

- Representatives from Amref and IPD were included in the TAG.
- Documents used for the Pilot were used and content changed according to Cycle 1 requirements.
- NICD provided human health clinical scenarios and DTU for animal health.
- Progress regarding the informatics system. Hub sites were provided with orientation and training for “Admin” activities using the informatics system.
- Amref continued with the same allocation.
- Results for Amref and NICD were combined, both were doing grading of results. Training exercise and a feedback session on completion was completed.

Program of work – Cycle 1



IPD

- To prepare samples for their participants. NICD shipped the organisms to be included in the Cycle to IPD.
- IPD performed full workup on organisms before preparing samples for participants – confirmed that results matched that of NICD.
- NICD prepared NCTC QC strains for IPD participants'. Packed according to IATA specifications for shipping to IPD.
- Supplied IPD with shipping boxes, security seal labels and overpacks.
- Content for clinical data was supplied to IPD. To be formatted and provided to participants.

IPD

Countries	Cycle 1
Cameroon	12
Gabon	0
Senegal	9
Ghana	9
Nigeria	20
Sierra Leone	3
Total	53

Amref

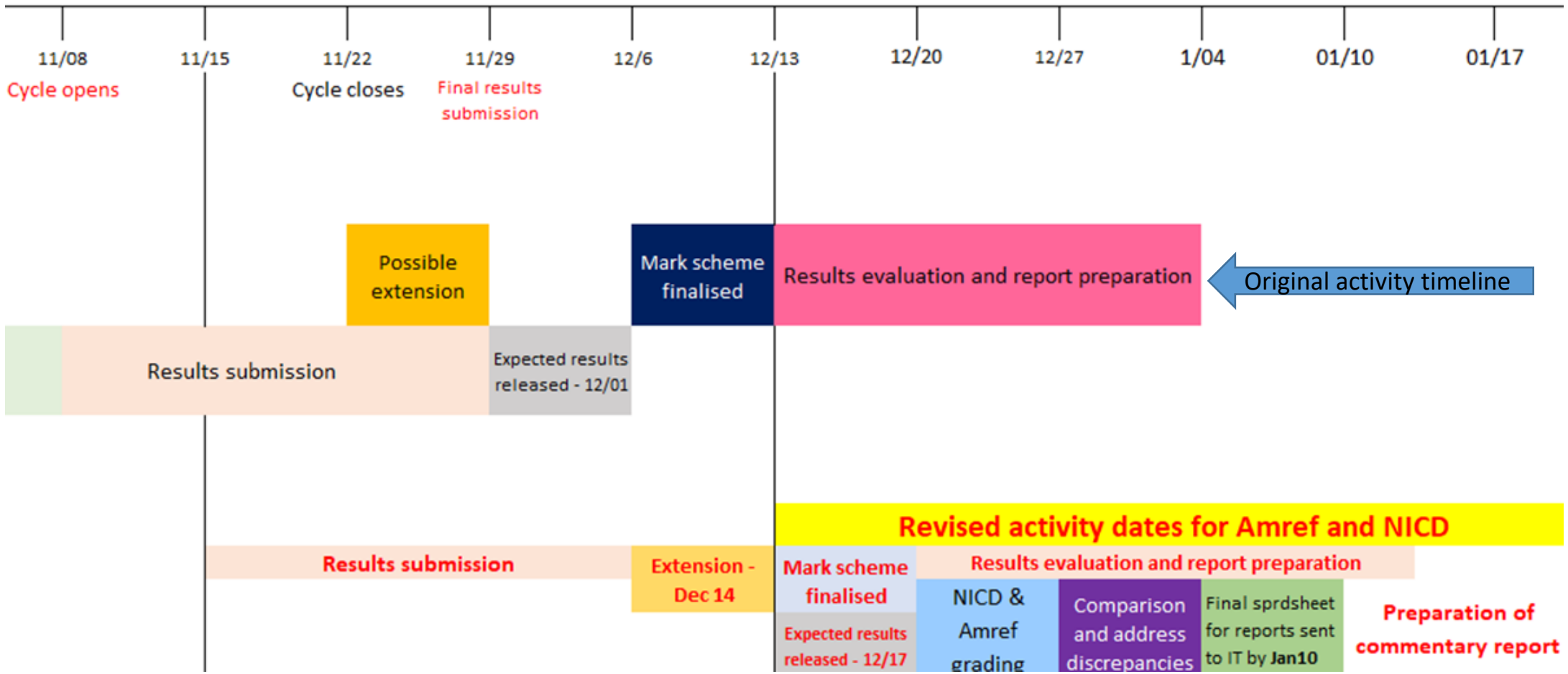
Countries	Cycle 1
Ethiopia	8
Kenya	26
Uganda	10
Tanzania	10
Total	54

NICD

Countries	Cycle 1
Eswatini	8
Malawi	17
Zambia	5
Zimbabwe	16
Total	46

- Each site was responsible for monitoring the delivery of shipment and noting challenges.
- The official cycle open date was 08 November 2021 and closing date for result submission was 22 November 2021.
- Participants were granted an extension to 07 December 2021 due to late delivery of shipments.
- Extended delays in delivery of shipments were experienced by some participants. For these participants the closing date for submission of results was extended to 14 December 2021.
- Expected results report was made available after the cycle had officially closed.

Revised POW due to shipping delays



Challenges experienced by NICD

- Logistics:

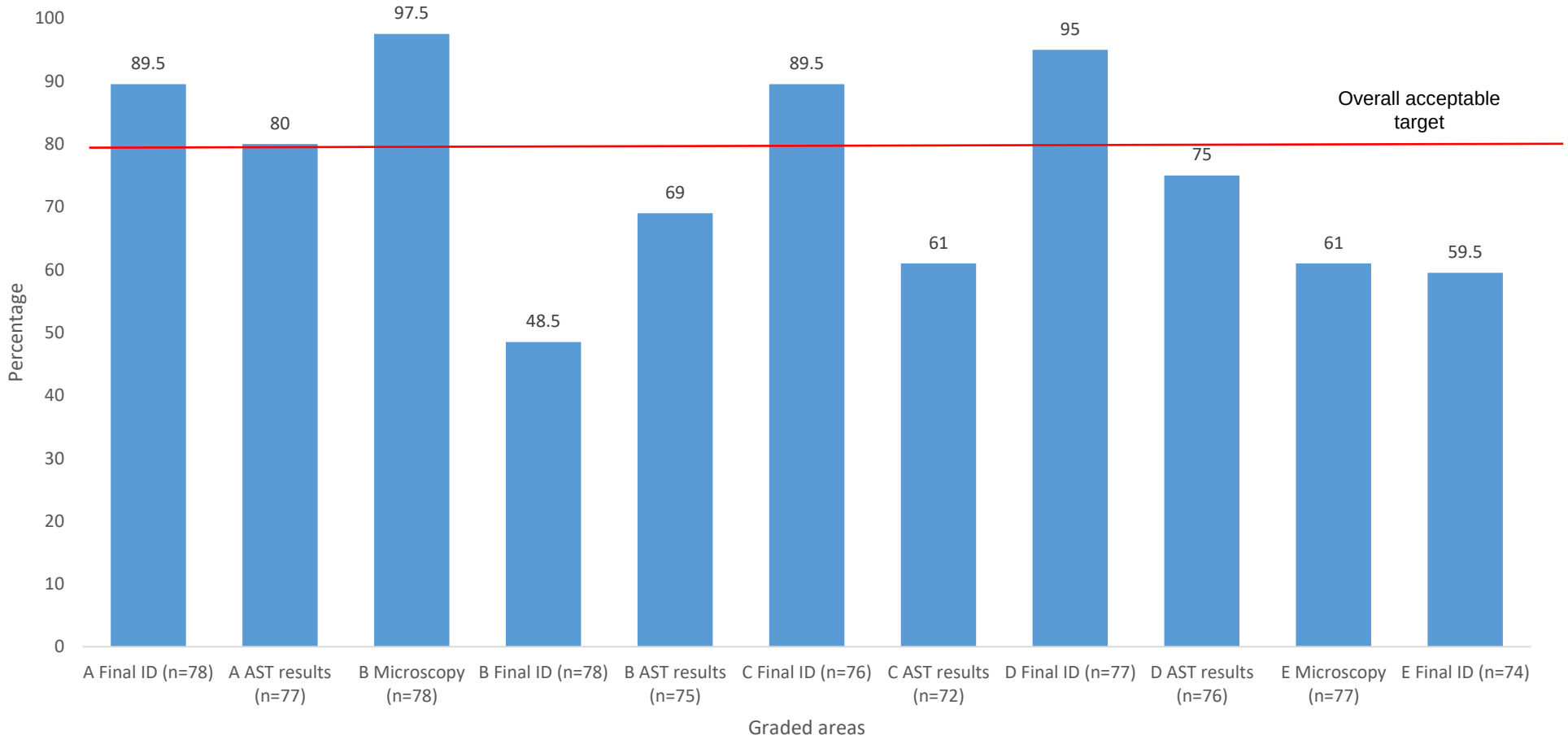
  - Delays due to availability of shipping permits.

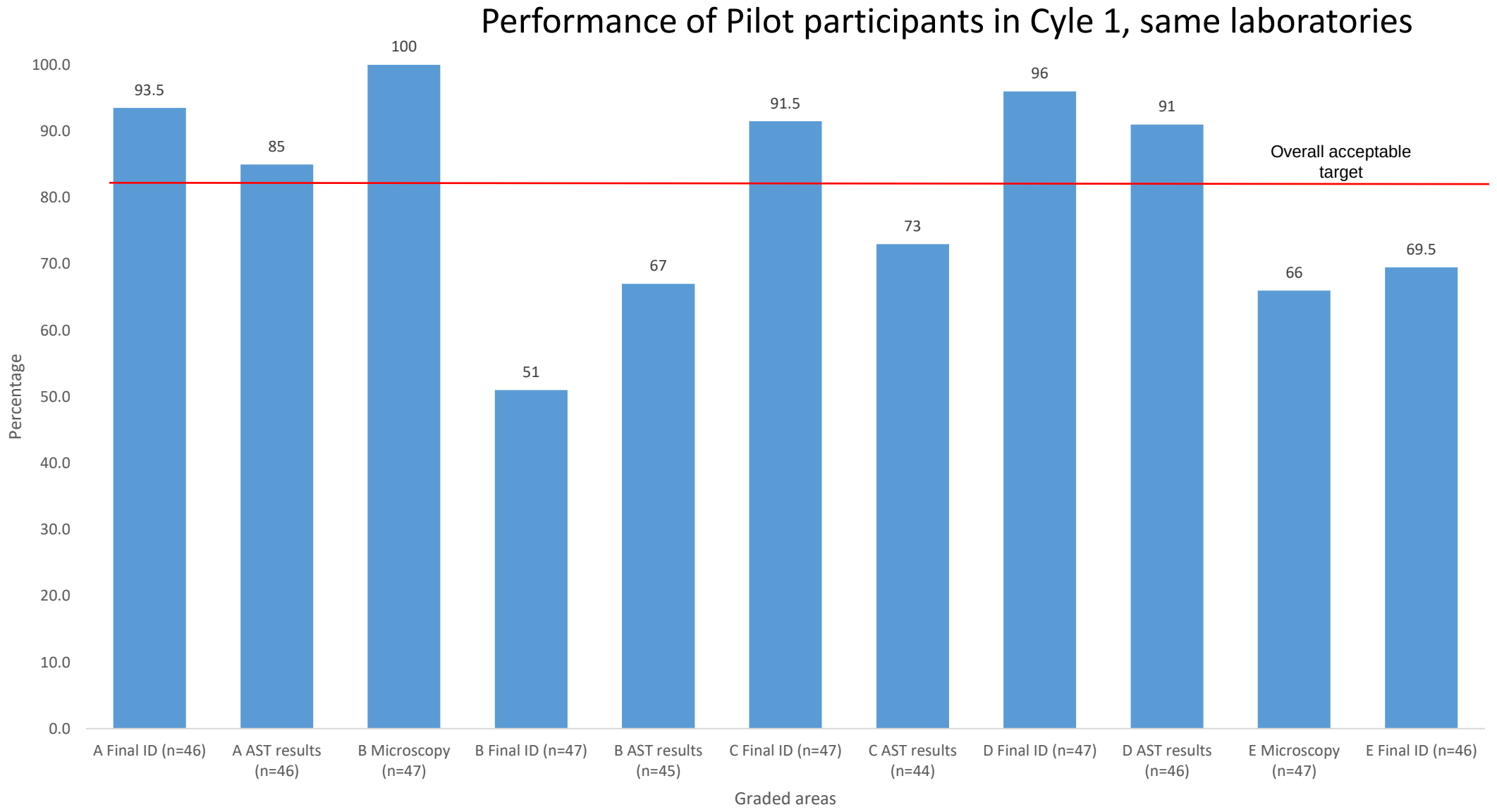
  - Multiple couriers were used for shipping to different countries. Delays in delivery of shipment cannot be controlled by providers. This is dependant on regulations of the each countries.

- Grading and reports generation:

  - Delays in closing of cycle causes a in a delay in grading of (including grading and report generation and circulation).

Overall participants performance in cycle 1




Performance of Pilot participants in Cyle 1, same laboratories
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Percentage
93.5
85
100
51
67
91.5
73
96
91
66
69.5
Overall acceptable target
A Final ID (n=46)
A AST results (n=46)
B Microscopy (n=47)
B Final ID (n=47)
B AST results (n=45)
C Final ID (n=47)
C AST results (n=44)
D Final ID (n=47)
D AST results (n=46)
E Microscopy (n=47)
E Final ID (n=46)
Graded areas

Take home messages

- Overall delays should be addressed.
- To standardise the approach to mark scheme generation, grading and report generation across all hub sites.
- To avoid festive season time for sending shipments.

Thank you for your attention!