SADC SECRETARIAT

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Promotion of Regional Integration in the SADC Livestock Sector
(PRINT Livestock Project)

9 ACP SAD 002

Report of a Mission to the Republic of Tanzania
Reinforcement of the capacity on Contagious Bovine Pleuropneumonia (CBPP)
Diagnostics for SADC Veterinary Laboratories Network

PRINT Report N°: CBPP-TANZ-09-2007

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Dates: 17th sept to 21st sept 2007

Reinforcement of the capacity on contagious bovine pleuropneumonia (CBPP)
Diagnostics for the SADC Veterinary Laboratories Network

PRINT project
Local intervention in SADC
Tanzania, Dar es Salam Sept17th to 21st 2007

Organizer Institution
CIRAD-UPR15 World reference laboratory for CBPP for the FAO, OIE reference laboratory

Experts
Dr F. Thiaucourt (Cirad)

Local organisation
Dr Gabriel Mkilema Shirima (ADRI)

Overall objective of the workshop
To improve the capacity of each SADC veterinary laboratory that is involved in CBPP surveillance and diagnostic, especially those in CBPP-free countries which are "at risk".

Program

Monday
Introduction, medium preparation, culture (liquid, solid media)
Tuesday
Serology, cELISA, pipetting, preparing CFT reagents
Wednesday
Complement fixation Test, Observation of MmmSC cultures, sample preparation for PCR
Thursday
PCR, agarose gel electrophoresis
Friday
General discussion on critical points, harmonized SOPs, round-robin organization

Specific objective of the workshop
Each CBPP diagnostic technique will be implemented in a demonstration with a focus on critical points that affect the quality of the results. These critical points will be discussed by the various participants of the workshop to ensure a list of recommendations to be implemented in the SADC participating veterinary laboratories before interlaboratory testing is organized. Recommendations for the definition of harmonized Standard Operating Procedures will also be issued.

The two workshops organized in Zambia in June and in Tanzania in September will be followed by an interlaboratory testing that is to take place in the following months. This testing should allow each laboratory to evaluate its own capabilities in terms of CBPP diagnosis and take action for improvement.

THE TRAINING COURSE ON REINFORCEMENT OF THE CAPACITY ON CONTAGIOUS BOVINE PLEUROPNEUMONIA (CBPP) DIAGNOSTICS FOR THE SADC VETERINARY LABORATORIES NETWORK.

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ACTIVITIES

Monday Sept 17th

General principles of quality management
External and internal auditing
Ways to write and control SOPs
Training on quality management
Equipment servicing and validation

Recommendations for
ADRI
- Perform training on quality management
- Start by writing some SOPs under QM principles
- Quality policy to be signed and implemented by the direction

Malawi
- Director to sign a QM policy

Botswana
- Verify that the written SOPs are effectively put in action

DRC
- Director to sign a QM policy
- Training is needed
- Servicing equipment

Namibia
- More training on QM
- Equipment servicing

Zambia
- Improve the commitment of direction to implement QM
- Servicing of equipment

Mozambique
- Equipment servicing
- Review the SOPs

Zimbabwe
- Write SOPs for CBPP testing

Angola
- - Director to sign a QM policy

BVI
- ISO 9001
- Start implementing ISO17025 for internal testing

Tuesday 18th

Critical point of serological tests
Selection of suppliers for critical reagents (and how to assess them)
Calibration of micropipettes (theory)
Practical's on micropipette testing according to SOP
- Visualization of errors in pipetting (precision and accuracy)
- Regularity of pipette calibration

Practicals on cELISA
Practicals on Mycoplasma culture (4 different M. species, liquid and solid media)

Recommendations
ADRI
- Perform qualibration
- Improve their knowledge on ELISA reader and ways to send OD values to computer without using EDI: use of "Hyperterminal" using correct BAUD rate (9600)

Malawi
- Select an ELISA reader to work with

Botswana
- Switch from EDI to another software for ELISA data transmission

DRC
- Perform locally some calibration of pipettes

Namibia
- Perform locally some calibration of pipettes
- Implement ways to manage who is using the QM assured equipment

Zambia
- Buy an extra bulb for the ELISA reader
- Calibration of pipettes

Mozambique
- Check the ELISA reader through interlaboratory trial
- Buy a software for transmission of data

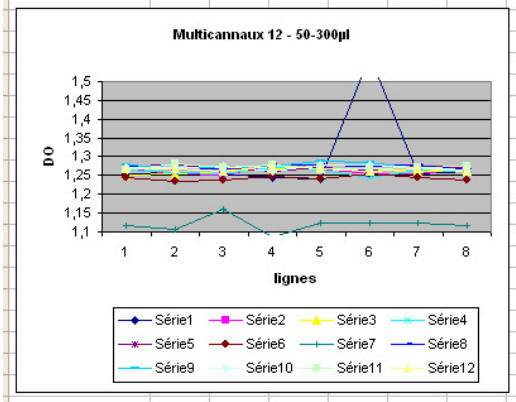
Zimbabwe
- Intensify pipette calibration
- Buy a software for transmission of data

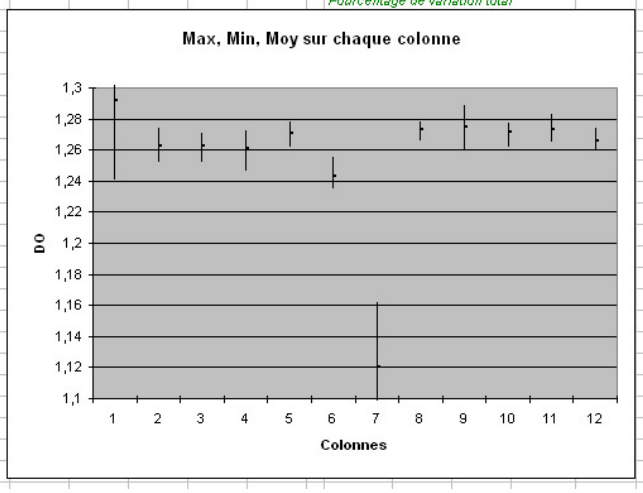
Angola
- Testing ELISA reader
- Implement the connection between ELISA reader and computer

BVI
- Intensify technician and pipette calibration
- Buy a software for transmission of data

Results of Pipette check at
ADRI.

Date:	#######			**Pipette multicannaux testée:**			06Y7978			ADRI									
Opérateur:	x,x			**cones / tips:**		Labor-1-100													
Mode opératoire:	Pipette			**colorant : n°**		2007-115													
				Lecteur ELISA		ADRI	x							Acceptability					re
				Filtre:		n°2 : 450								*Global variation*			<5%		W
				Plaque:										*Between mean variation*			<2,5%		W
	07082701-multicanaux12-100µl																		
	1	2	3	4	5	6	7	8	9	10	11	12							
	1,255	1,268	1,252	1,266	1,276	1,243	1,118	1,275	1,278	1,271	1,265	1,267							
	1,258	1,256	1,256	1,257	1,278	1,235	1,107	1,271	1,271	1,272	1,283	1,268							
	1,254	1,252	1,261	1,253	1,272	1,237	1,162	1,269	1,272	1,275	1,273	1,262		CV					
	1,244	1,274	1,268	1,272	1,262	1,247	1,085	1,278	1,276	1,262	1,277	1,274		4,202					
	1,241	1,268	1,271	1,265	1,27	1,241	1,124	1,277	1,289	1,277	1,268	1,268							
	1,566	1,256	1,265	1,246	1,266	1,255	1,125	1,274	1,284	1,277	1,269	1,26							
	1,253	1,256	1,26	1,266	1,274	1,246	1,125	1,278	1,267	1,273	1,273	1,268							
	1,263	1,268	1,265	1,261	1,27	1,238	1,116	1,266	1,26	1,264	1,276	1,262							
																	% variation entre		
Max	1,566	1,274	1,271	1,272	1,278	1,255	1,162	1,278	1,289	1,277	1,283	1,274	1,566				*moyennes*		
Min	1,241	1,252	1,252	1,246	1,262	1,235	1,085	1,266	1,26	1,262	1,265	1,26	1,085				*de colonnes*		
moynne	1,292	1,262	1,262	1,261	1,271	1,243	1,120	1,274	1,275	1,271	1,273	1,266	1,256	1,292	1,120	13,657			
ecartype	0,1110	0,0081	0,0063	0,0084	0,0052	0,0065	0,0215	0,0044	0,0093	0,0056	0,0057	0,0045	0,0528						
(Max-Min)/moy	0,252	0,017	0,015	0,021	0,013	0,016	0,069	0,009	0,023	0,012	0,014	0,011	38,302						
													Pourcentage de variation total						





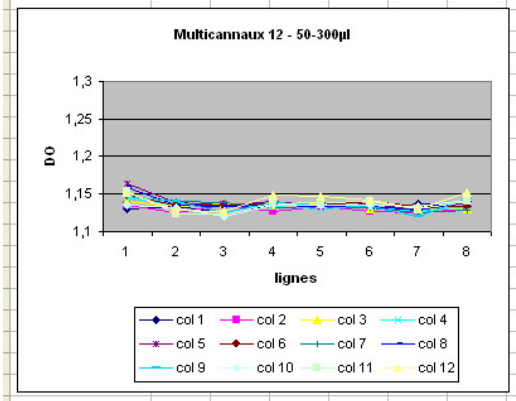
This first trial of multichannel 06Y7978 yielded unsatisfactory results:
- There is one aberrant value in column 1 6th well (OD 1.566)
- All values from column 7 are bellow the values of other columns.

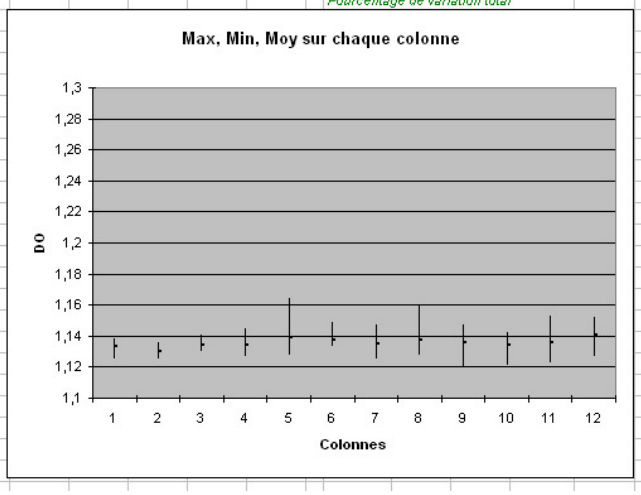
These non-conformancies could be attributed to the multichannel pipette itself or the incorrect fitting of the tips.

A second check was performed two days later with the same pipette

Date:	70920			**Pipette multicannaux testée:**			06Y7978			ADRI										
Opérateur:	F. Thiaucourt			**cones / tips:**		Labor-1-100														
Mode opératoire:				**colorant : n°**		2007-115														
				Lecteur ELISA		ADRI	x													
				Filtre:		n°2 : 450														
				Plaque:																
	07082701-multicanaux12-100µl																			
	1	2	3	4	5	6	7	8	9	10	11	12								
	1,131	1,136	1,141	1,145	1,164	1,149	1,147	1,159	1,147	1,136	1,153	1,152								
	1,133	1,125	1,134	1,136	1,138	1,135	1,141	1,136	1,14	1,132	1,123	1,128								
	1,125	1,135	1,137	1,135	1,135	1,136	1,139	1,133	1,125	1,121	1,123	1,127		CV						
	1,136	1,127	1,135	1,133	1,141	1,137	1,132	1,135	1,139	1,136	1,137	1,148		0,677						
	1,138	1,133	1,137	1,131	1,134	1,135	1,133	1,134	1,135	1,136	1,139	1,147								
	1,131	1,127	1,13	1,133	1,137	1,14	1,132	1,133	1,133	1,136	1,14	1,142								
	1,137	1,125	1,13	1,127	1,128	1,134	1,125	1,128	1,12	1,133	1,131	1,13								
	1,134	1,131	1,131	1,134	1,138	1,133	1,129	1,141	1,144	1,142	1,14	1,152								
																		% variation entre		
Max	1,138	1,136	1,141	1,145	1,164	1,149	1,147	1,159	1,147	1,142	1,153	1,152		1,164				moyennes		
Min	1,125	1,125	1,13	1,127	1,128	1,133	1,125	1,128	1,12	1,121	1,123	1,127		1,12				de colonnes		
moynne	1,133	1,130	1,134	1,134	1,139	1,137	1,135	1,137	1,135	1,134	1,136	1,141		1,136	1,141	1,130	0,958			
ecartype	0,0042	0,0045	0,0039	0,0051	0,0107	0,0052	0,0071	0,0095	0,0092	0,0060	0,0100	0,0108		0,0077						
(Max-Min)/moy	0,011	0,010	0,010	0,016	0,032	0,014	0,019	0,027	0,024	0,019	0,026	0,022		3,875						
														Pourcentage de variation total						

Acceptability			res
Global variation		<5%	Ok
Between mean variation		<2,5%	Ok





Results were then passing the threshold values.

These results show that:

1) Pipette 06Y7978 passes this quality check
2) The previous individual aberrant value may have occurred due to incorrect pipetting
3) The previous aberrant values for channel N°7 was due to an incorrect fitting of the tip

As a consequence, the SOP for the multichannel pipette 06Y7978 should mention that the tips should be well adjusted before starting pipetting.

cELISA demonstration

Results
The initial values given by EDI software were outside limits (MAb OD too low)
Analysis of the problem showed that the EDI software was unable to set the correct filter of the ELISA reader.
After entering the correct wavelength directly on the ELISA reader, cELISA values were all returning to acceptable values.

As a conclusion
1) The cELISA kit proved very robust as it gave correct values although it was kept at the custom without cooling for more than 7 days.
2) Laboratories performing ELISAs should ensure that they are using correct settings, hence the importance of operating manuals for each equipement and software.

Wednesday 19th

PCR sample preparation
Comparison of advantages and drawbacks of PCR and isolation
Q principles of Plan Do Check and Act
Continuous training for technicians is needed and evaluation of training is also needed (qualification of trainer and trainees)

Practicals on PCR for CBPP (it worked!)

ADRI
- Training on PCR is needed as very good equipment and facilities exists

Malawi
- Find facilities and agreement to perform PCR

Botswana
- Continuous training on PCR needed

DRC
- Purchase the necessary reagents for CBPP PCR

Namibia
- Training on QM of PCR technique

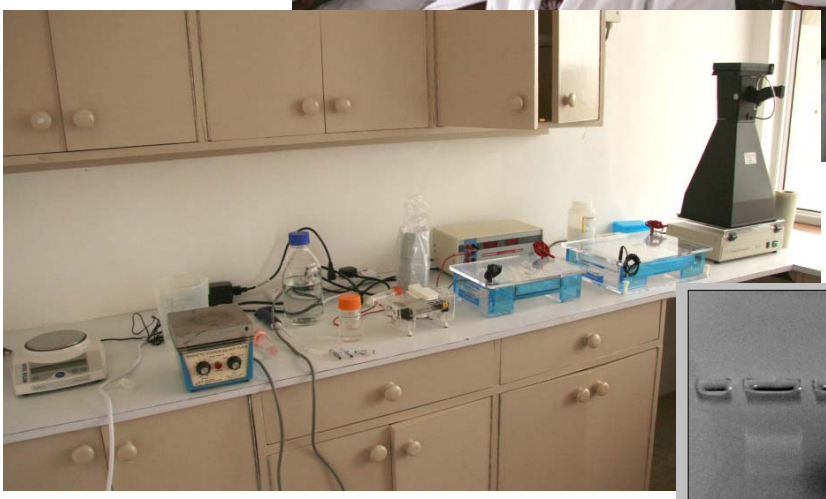
Zambia
- Reinforcement of the links with other institutions performing PCR

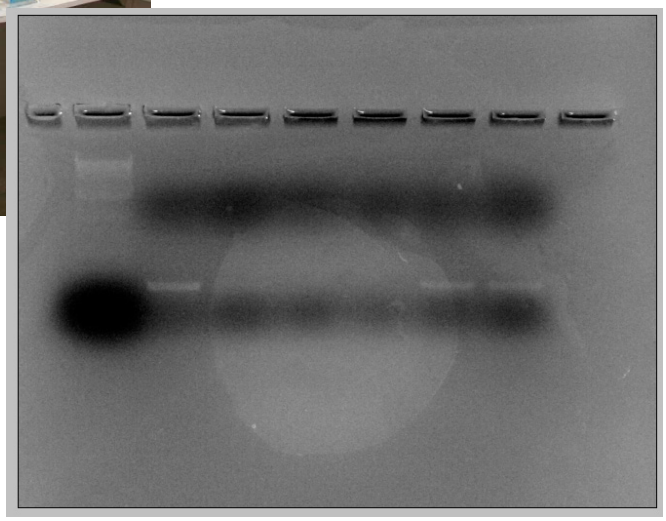
Mozambique
- PCR technique has to be established

Zimbabwe
- Write SOPs for CBPP PCR

Angola
- PCR technique has to be established

BVI
- Use of PCR for checking cell culture contaminations
- Use of PCR for checking T1 vaccine identity





PCR exercise pointed out the need to manipulate with the correct safety precautions Etidium Bromide (use of gloves, SOPs to eliminate gels…)

In spite of incorrect storage at custom for more than 7days, the PCR results were correct. This shows the robustness of the technique and its usefulness in case of emergency situations.

The main drawback of PCR is the risk of contaminations which was discussed at length during the exercise

Thursday 20th

Agarose gel electrophoresis (Various technical points)
Safety precautions for EtBr manipulations
Review of the advantages and drawbacks of PCR

CFT, review of the various critical points for the CFT
(temperature for decomplementation, Sheep red blood cells, complement activity…)
Practicals on complement activity testing
Re-testing pipettes from ADRI using hyperterminal as a way to transmit data

Controls of Mycoplasma cultures (solid: aspect of colonies, liquid: pH indication)

ADRI
- Improve stereo microscope
- Improve the SOPs for medium preparation and control of quality of medium
- Seed larger plates to be able to perform isolation
- Modify SOP for Complement titration
- Records of laboratory work have to be improved

Malawi
- Procure reagents for isolation of mycoplasmas
- Training for CFT

Botswana
- Obtain ready made medium and compare with locally made medium
- Monitor better the critical points of the CFT

DRC
- Training on CFT
- Purchase of medium to implement isolation

Namibia
- Reagents needed for isolation, training
- setting SOPs for CFT

Zambia
- Stereomicroscope needed

Mozambique
- Buy medium for isolation
- Need of reagents

Zimbabwe
- Training on culture and identification (and CFT)
- Procurement of reagents (CFT and isolation)
- Stereomicroscope needed
- Control cultures needed

Angola
- Reagent procurement (CFT and isolation)

BVI
- Purchase of reagents for testing vaccines

The complement titration performed on Thursday 20th failed to give proper values as no well showed complete haemolysis.

A first hypothesis was that the haemolytic system had not been prepared correctly or that the agitation was not sufficient.

A second trial failed to give proper results again.

The most likely conclusion was that the complement activity had been lost during the improper storage of the kit at the custom.

Recommendations could be as follow:
1) Ensure that procedures to retrieve parcels at the custom are fast enough
2) Keep Guinea pigs as alternative source of complement

Interlaboratory trials

- Emails sent to Heads of labs on
- Official answers still awaited for many labs
- Official letters to be sent by fax or DHL

Interlaboratory trial to be organized between October November 2007
Report of interlaboratory trial to be sent before the end of December 2007

Need to repeat interlaboratory trials within SADC vet lab network