Sterility control

Intended use: mAECT columns are produced in batches. Each batch is identified by its production date. Quality control of each batch is performed on a sample of each batch produced. SOP M/10 describes the sterility control procedures for aerobic and anaerobic bacteria as well as for fungi.

Material

Safety cabinet with gas source
Incubator at 36°C with CO_2
Incubator at 28°C

Consumables

Aerobic culture medium:
- Brain Heart Infusion, in tubes
- *Optional: Chocolate Agar/HgB + isovitalex, in Petri dishes*

Anaerobic culture medium:
- Thioglycollate medium, in tubes
- *Optional: Anaerobic Blood Agar, in Petri dishes*

Culture medium for fungi:
- Sabouraud + chloramphenicol, in inclined tubes
- Sabouraud + chloroform + actidione, in inclined tubes

Procedure

1. Control that all materials and reagents are available and clean.
2. Prepare three columns for testing (N° 2, 9 and 16 of the series tested in SOP M/9).
3. Under sterile conditions, inoculate each liquid culture medium with 0.5 ml of supernatant buffer from each column (4 media).
4. Put the aerobic and anaerobic cultures in the incubator with CO_2 at 36±2°C.
5. Put the fungi cultures at 28°C.
6. Check the cultures daily for 7 days for the presence of bacteria. If bacterial growth is visible, subinoculate the aerobic or anaerobic cultures on agar in order to identify the nature of the contaminant.
7. If agar cultures are positive for coagulase-negative staphylococci, the result is considered as a non-specific contamination during culture and does not lead to batch refusal.
8. Check the cultures daily for 3 weeks for the presence of fungi. If growth is visible, indicate the nature of the contaminant, if possible.

PRODUCTION SHEET
Sterility control
Anaerobic bacteria

INOCULATION DATE		PERSON WHO PREPARED	

DATE ON COLUMN LABEL	

LIQUID CULTURE MEDIUM	

DAYS AFTER INCOCULATION	COLUMN 2 N= negative P = positive	COLUMN 9 N= negative P = positive	COLUMN 16 N= negative P = positive	IDENTIFIED BACTERIA
1				
2				
3				
4				
5				
6				
7				

AGAR CULTURE MEDIUM	

DAYS AFTER INCOCULATION	COLUMN 2 N= negative P = positive	COLUMN 9 N= negative P = positive	COLUMN 16 N= negative P = positive	IDENTIFIED BACTERIA
1				
2				
3				
4				
5				
6				
7				

COMMENTS

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PRODUCTION SHEET
Sterility control
Aerobic bacteria

INOCULATION DATE	

PERSON WHO PREPARED	

DATE ON COLUMN LABEL	

LIQUID CULTURE MEDIUM	

DAYS AFTER INCOCULATION	COLUMN 2 N= negative P = positive	COLUMN 9 N= negative P = positive	COLUMN 16 N= negative P = positive	IDENTIFIED BACTERIUM
1				
2				
3				
4				
5				
6				
7				

AGAR CULTURE MEDIUM	

DAYS AFTER INCOCULATION	COLUMN 2 N= negative P = positive	COLUMN 9 N= negative P = positive	COLUMN 16 N= negative P = positive	IDENTIFIED BACTERIUM
1				
2				
3				
4				
5				
6				
7				

COMMENTS

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PRODUCTION SHEET
Sterility control
Fungi

INOCULATION DATE		PERSON WHO PREPARED	

DATE ON COLUMN LABEL	

LIQUID CULTURE MEDIUM	

DAYS AFTER INCOCULATION	COLUMN 2 N= negative P = positive	COLUMN 9 N= negative P = positive	COLUMN 16 N= negative P = positive	IDENTIFIED FUNGUS
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				

COMMENTS

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Functionality control

Intended use: mAECT columns are produced in batches. Each batch is identified by its production date. Quality control of each batch is performed on a sample of each batch produced. SOP M/11 describes the functionality control procedures.

Storage: Columns are stored at 4 °C.

Safety: **Animal and human bloods are potentially infectious. *Trypanosoma brucei gambiense*, which is used to determine the functionality of columns, is infectious. Usual cautions for infectious agents should be taken (wear gloves and lab coats, properly dispose of infectious and contaminated material).**

Material

Microscope
Rack for mAECT
Viewing chamber for mAECT
Automatic 5-40 µL pipette
Automatic 100-1000 µl pipette
Grip
Centrifuge
Chronometer
Indelible marker

Consumables

Heparin
Disposable gloves
Absorbing paper
Liquid disinfectant
Microscope slides
Microscope slide coverslips 24 x 24 mm
200-µl pipette tips
1000-µl pipette tips
URIGLASS counting chamber (slide with 10 1-µl chambers)
Polypropylene pastettes (3.5 ml)
Polypropylene tubes (3 – 13 ml)
Monovette for heparinized blood with needle
Collector tubes
14-ml centrifuge tubes
Ice
PSG buffer (see SOP M/3). *Note: PSG buffer may be frozen in small volumes.*

Organisms

Mouse previously infected with *T.b. gambiense*.

Blood

Human blood drawn with heparin. May be used for 2 days. The required volume depends on the number of columns to be tested. 0.350 ml of blood is required for each column.

Procedure

3-4 days before the experiment

1. Infect a mouse with *T.b. gambiense*. If required, sub-inoculate for future tests.

On the day of the experiment

2. Control that all materials and reagents are available and clean.
3. Put the PSG buffer on ice or at 4°C.
4. Put the freshly drawn heparinized blood on ice or at 4°C.
5. Control the mouse parasitemia.
6. Prepare a trypanosomes suspension in blood (100 tryps/ml). This step should be done as quickly as possible and must be done with cold blood and buffer.
    - Draw blood with heparin from the infected mouse (from the tail or by cardiac punction) and, depending on the initial parasitemia, immediately dilute 1:10 to 1:100 in cold PSG.
    - Estimate the number of trypanosomes using a Uriglass counting chamber (one chamber contains 1 µl)
    - Dilute further in PSG to obtain a suspension with 10 trypanosomes/µl.
    - Fill 3 Uriglass counting chambers and let stand for 5 minutes. Count the number of trypanosomes per chamber and calculate the average of the 3 chambers.
    - Add X µl of this trypanosomes suspension to Y ml of cold human blood to obtain a suspension with 100 trypanosomes/ml.
    - Keep the 10 trypanosomes/µl suspension in PSG on ice to check trypanosomes viability at the end of the experiment.
7. Perform the mAECT test with 5 columns and 350 µl of blood per column following the mAECT kit instructions. The 5 columns are numbers 3, 4, 10, 11 and 17 from the series tested in SOP M/9.
8. For each column, note the time of the beginning and of the end of flow-through.
9. Count the number of trypanosomes in each collector tube.
10. Check the absence of foreign particles on the bottom of each collector tube and indicate the nature of the observed particles if any (gel, fibers, filter debris....).
11. Check trypanosomes viability in the original 10 tryps/µl in PSG suspension.
12. Calculate the gel percentage that is occupied by blood
    - Measure the gel height that is occupied by blood in mm = h
    - Measure the gel height between the lower and upper filters in mm = H
    - Gel percentage occupied by blood=h/Hx100=%
13. Note under the comments section any other observations made during quality control.

PRODUCTION SHEET 1

Functionality control

DATE	
PERSON WHO PREPARED	
DATE ON COLUMN LABEL	

MOUSE PARASITEMIA CONTROL

TRYPANOSOME STRAIN	
COLLECTION TIME	
PARASITEMIA IN DILUTION 1/10-1/100 IN PSG	

PREPARATION OF A 10 TRYPS/µL SUSPENSION IN PSG

FIRST DILUTION: X µL IN Y ML OF PSG	X = ... µL
	Y = ... ML
NUMBER OF TRYPANOSOMES IN COUNTING CHAMBER	
IF REQUIRED, SECOND DILUTION: X µL IN Y ML OF PSG	X = ... µL
	Y = ...ML
NUMBER OF TRYPANOSOMES IN COUNTING CHAMBER 1	
NUMBER OF TRYPANOSOMES IN COUNTING CHAMBER 2	
NUMBER OF TRYPANOSOMES IN COUNTING CHAMBER 3	
AVERAGE NUMBER OF TRYPANOSOMES / µL	

PREPARATION OF A 100 TRYPS/ML SUSPENSION IN HUMAN BLOOD

ADD X µL OF 10 TRYPS/µL SUSPENSION IN Y ML OF BLOOD TO OBTAIN 100 TRYPS/ML IN BLOOD	X = ... µL Y = ...ML

END OF PREPARATION TIME	

PRODUCTION SHEET 2

Functionality control

DATE	
PERSON WHO PREPARED	
DATE ON COLUMN LABEL	

COLUMN NUMBER	START OF FLOW-THROUGH TIME *HH:MM*	END OF FLOW-THROUGH TIME *HH:MM*	TEST DURATION: *HH:MM*	NUMBER OF TRYPANOSOMES	BLOOD HEIGHT IN GEL	GEL HEIGHT BETWEEN FILTERS	GEL PERCENTAGE OCCUPIED BY BLOOD	ABSENCE (A) OR PRESENCE OF PARTICLES OR DEBRIS	NATURE OF THE OBSERVED PARTICLES OR DEBRIS
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									

CONTROL OF TRYPANOSOMES VIABILITY IN THE ORIGINAL SUSPENSION IN PSG

TIME		NUMBER OF LIVE TRYPANOSOMES	

OTHER OBSERVATIONS OR COMMENTS

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Stock of 10 columns for future quality control

Intended use: For each batch, a few mAECT minicolumns are kept in case negative comments are received from users and a new quality control is needed.

Storage: The 10 mAECT minicolumns put aside for future QC are kept on their plastic tray (blister) and stored in the refrigerator.

Material

Cardboard box
Plastic tray with 10 spaces for columns

Procedure

1. Put 10 columns (the remaining columns from the series tested in SOP M/9) in a plastic tray.
2. Store these columns in the refrigerator dedicated to long-term storage.
3. Write in the Excel file "production and stock monitor.xls" the location, date and number of columns stored.

PRODUCTION SHEET

Stock of 10 columns for future quality control

DATE	
BATCH NUMBER	
PERSON WHO PREPARED	

	NUMBER
NUMBER OF MINICOLUMNS	
LOCATION (REFRIGERATOR NUMBER)	

Independent-site Quality Release Control (ITM)

Procedure

At INRB, Kinshasa

Per batch of 700-800 columns produced at INRB:

1. Select 20 columns from the batch for Independent-site QRC at ITM. Take 7 from the start, 6 from the middle and 7 from the end of the assembling chain and number them immediately from 1 to 20.
2. Prepare with these columns 2 complete mAECT kits containing each 10 columns and accessories.
3. Sent the mAECT kits to IMT by DHL or other carrier (no cold chain is required). Address below.
4. Communicate Air Way Bill or flight number if carried by traveller, to ITM by email.

Address: P. Büscher
Department of Parasitology
Institute of Tropical Medicine
Nationalestraat 155, B-2000 Antwerpen, Belgique
Tél: +32 3 247 63 71
email: pbuscher@itg.be

At ITM, Antwerp

1. Record duration of transport
2. Check contents of shipment
3. Visual inspection on integrity of mAECT kit package and contents if package damaged
4. If critical parts (columns, collector tubes) are damaged or lacking, communicate with INRB for sending other mAECT kits.
5. Check contents of each mAECT kit (columns, collector tubes, instruction leaflet, accessory materials)
6. Visual inspection of columns (label, gel volume, position of filters, leakage, air bubbles, visible contamination etc.)
7. Check pH and conductivity of pooled supernatant buffer from 4 columns
8. Check sterility for bacteria and fungi on 3 columns
9. Check functionality with live trypanosomes cultured in mice on 13 columns
10. Prepare Batch Release document (**last page of this SOP**)
11. Communicate decision on Batch Release or Batch Rejection to INRB
12. If applicable, propose corrective measures to INRB

Batch number:

Independent-site Quality Control (ITM-ATP)

Condition of received materials at arrival

DATE	

AWB NUMBER OR FLIGHT NUMBER (IF CARRIED BY TRAVELLER)	
DATE DISPATCHING BY INRB	
DATE ARRIVAL AT ITM	
DURATION OF TRANSPORT	
NUMBER OF KITS RECEIVED (EXPECTED #2)	
CONDITION OF KITS AT ARRIVAL GOOD/DAMAGED/PARTS LACKING	
SPECIFY DAMAGE AND/OR PARTS LACKING	

DECISION 1

1. If condition of goods at arrival is good
   => **proceed with QC**
2. If condition of goods at arrival is damaged but not the columns neither the collector tubes
   => **proceed with QC but propose corrective measures**
3. If columns and/or collector tubes are damaged or lacking
   => **communicate with INRB and have new kits sent.**

Batch number:

Contents of each mAECT kit

DATE	

ITEM		EXPECTED	OBSERVED		DEVIATION	
			Kit #1	Kit #2	Kit #1	Kit #2
LABEL ON UPPER LID OF BOX						
	Present and readable	Y				
	Batch number	Y				
	Expiry date	Y				
	Storage instructions	Y				
	For *in vitro* use only	Y				
INSTRUCIONS ENGLISH						
	Number	1				
	Readable	Y				
	Number	1				
	Readable	Y				
INSTRUCIONS PORTUGUESE						
	Number	1				
	Readable	Y				
COLUMNS		10				
COLLECTOR TUBES						
	Number	10				
	Clean	Y				
14 ML CENTRIFUGATION TUBES						
	Number	10				
	Clean	Y				
PLASTIC TRANSFER PIPETTES						
	Number	10				
	Clean	Y				

DECISION 2

1. If no deviation observed
   => **proceed with QC**
2. If deviation observed is not critical (all 10 colomns and collector tubes are present)
   => **proceed with QC but propose corrective measures**
3. If deviation observed is critical (at least 1 column or collector tube is missing)
   => **communicate with INRB to have new kits sent**

PRODUCTION mAECT
SOP M/26

Operator: Peter Ilegems...................

Batch number:

Physical and chemical inspection of columns

DATE	

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
DATE ON LABEL (Y/N)																				
POSITION OF LABEL (P/N)																				
POSITION OF GREEN UPPER STOPPER (P/N)																				
POSITION OF WHITE LOWER STOPPER (P/N)																				
POSITION OF UPPER FILTER (P/N)																				
POSITION OF LOWER FILTER (P/N)																				
AIR BUBBLES (Y/N)																				
GEL PACKING (P/N)																				
GEL COLOR NORMAL (Y/N)																				
BUFFER COLORLES (Y/N)																				
VISIBLE CONTAMINATION (Y/N)																				
HEIGHT OF GEL (in mm)																				
HEIGHT OF BUFFER ON TOP (in mm)																				

	EXPECTED	MEASURED
MEAN HEIGHT OF GEL (in mm)	14 (12-18)	0
MEAN HEIGHT OF BUFFER (in mm)	28 (24-32)	0
Temperature	-------	
pH	8,0 - 8,1	0
Conductivity	9 mmho/cm or mS/cm	0

Physical and chemical inspection of columns

DATE	

OTHER OBSERVATIONS

DECISION 3

1. If no deviation observed
   => **proceed with QC and send 3 columns to CLKB**
2. If deviation observed is not critical
   no visual contamination
   gel and buffer volume within expected limits
   upper filter in correct position
   => **proceed with QC but propose corrective measures and send 3 columns to CLKB**
3. If deviation observed is critical
   visual contamination
   gel and buffer volume beyond expected limits
   upper filter absent or not retaining the gel
   => **note deviation on Batch Release Document and Reject Batch, propose corrective measures**

Functionality with live trypanosomes cultured in mice
(see also SOP M/11)

This is done with the 13 remaining columns.

DATE	

PARASITEMIA IN THE MOUSE

TRYPANOSOME STRAIN	LITAT 1/3
TIME OF BLOOD SAMPLING	
PARASITEMIA OF THE DILUTION 1/10-1/100 IN PSG	

PREPARATION OF THE SUSPENSION WITH 10 TRYPS/µL IN PSG

FIRST DILUTION: X µL IN Y ML OF PSG	X = ... µL
	Y = ... ML
NUMBER OF TRYPANOSOMES IN COUNTING CHAMBER (ONE COUNTING CHAMBER CONTAINS 1 µL)	
IF NECESSARY, SECOND DILUTION: X µL IN Y ML OF PSG	X = ... µL Y = ... ML
	VERDUNNING
NUMBER OF TRYPANOSOMES IN COUNTING CHAMBER 1	
NUMBER OF TRYPANOSOMES IN COUNTING CHAMBER 2	
NUMBER OF TRYPANOSOMES IN COUNTING CHAMBER 3	
MEAN NUMBER OF TRYPANOMES / µL	

PREPARATION OF THE SUSPENSION OF 100 TRYPS/ML IN HUMAN BLOOD

ADD X µL OF THE SUSPENSION 10 TRYPS/µL IN Y ML OF BLOOD TO OBTAIN 100 TRYPS/ML IN BLOOD	X = ... µL Y = ... ML

TIME OF END OF PREPATION	

Operator: Peter Ilegems ....

Batch number:

Functionality with live trypanosomes cultured in mice

DATE	

COLUMN NUMBER	START TIME OF ELUTION: *hh:mm*	END TIME OF ELUTION: *hh:mm*	TEST TIME: *hh:mm*	NUMBER OF TRYPANOSOMES	HEIGHT IN MM OF BLOOD IN GEL	HEIGHT OF GEL IN MM	% OF GEL OCCUPIED BY BLOOD	ABSENCE (A) OR PRESENCE (P) OF PARTICLES OR ARTIFACTS IN SEDIMENT	NATURE OF PARTICLES OR ARTIFACTS
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
MEAN			0,0	0,0			0,00		
MIN			0,0	0,0			0,00		
MAX			0,0	0,0			0,00		

Functionality with live trypanosomes cultured in mice

CHECK VIABILITY OF TRYPANOSOMES IN THE ORIGINAL SUSPENSION IN PSG

TIME	

NUMBER OF LIVE TRYPANOSOMES	

CHECK MEAN FUNCTIONALITY PARAMETERS FOR COLUMN BATCH

	TEST TIME: *HH:MM*		**NUMBER OF TRYPANOSOMES**		**PERCENTAGE OF GEL OCCUPIED BY BLOOD**		**PARTICLES**	
	EXP	**OBSD**	**EXP**	**OBS**	**EXP**	**OBS**	**EXP**	**OBS**
MEAN	**29**		**2.5**		**80%**		**Absent**	p
MIN	**20**		**0**		**60%**		**na**	na
MAX	**60**		**na**		**100%**		**na**	na
ACCEPTED	**Y (only when all values between the limits)**		**Y (only when mean and min values between the limits)**		**Y (only when all values between the limits)**		**Y (only when particles do not hamper reading and no blood is running through)**	

DECISION 5

1. If all parameters acceptable
   => **prepare Batch Release Document**
2. If one of the parameters unacceptable
   => **note on Batch Release Document and Reject Batch, propose corrective measures**

Independent-site Quality Control (ITM-CLKB)

Batch number:

Bacteriological and fungal sterility

Culture media

Aerobe culture media
Brain Heart Infusion, liquid in tubes
Facultative: Agar Chocolat/HgB + isovitalex, in Petri dishes

Anaerobe culture media
Thioglycollate medium, liquid in tubes
Facultative: Anaerobe Blood Agar, in Petri dishes
Culture medium for fungi
Sabouraud + chloramphenicol, in Petri dishes

Procedure

1. Verify whether all reagents and materials are available
2. Prepare 3 columns to test
3. Under sterile conditions, inoculate liquid medium for anaerobic and aerobic cultures, each with 0.5 ml of each column
4. Monitor the bacteria cultures for 7 days
   If cultures become positive, subinoculate agar media for identification of the contaminating organism.
   If positive for coagulation negative *Staphylococcus*, this is considered as non specific contamination during culture inoculation and will not lead to rejecting of the batch.
5. Check the fungi cultures daily for 3 weeks. If growth is visible, indicate the nature of the contaminant, if possible.

Aerobe bacteria

BATCH NUMBER		**OPERATOR**	
INOCULATION DATE		**SIGNATURE**	

AEROBE LIQUID CULTURE MEDIUM	

INOCULATION DAY	COLUMN 1 N= negative P = positive	COLUMN 2 N= negative P = positive	COLUMN 3 N= negative P = positive	BACTERIA SPECIES
1				
2				
3				
4				
5				
6				
7				

AEROBE AGAR CULTURE MEDIUM	

INOCULATION DAY	COLUMN 1 N= negative P = positive	COLUMN 2 N= negative P = positive	COLUMN 3 N= negative P = positive	BACTERIA SPECIES
1				
2				
3				
4				
5				
6				
7				

COMMENTS

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Anaerobe bacteria

BATCH NUMBER		OPERATOR	
INOCULATION DATE		SIGNATURE	

ANAEROBE LIQUID CULTURE MEDIUM	

INOCULATION DAY	COLUMN 1 N= negative P = positive	COLUMN 2 N= negative P = positive	COLUMN 3 N= negative P = positive	BACTERIA SPECIES
1				
2				
3				
4				
5				
6				
7				

ANAEROBE AGAR CULTURE MEDIUM	

INOCULATION DAY	COLUMN 1 N= negative P = positive	COLUMN 2 N= negative P = positive	COLUMN 3 N= negative P = positive	BACTERIA SPECIES
1				
2				
3				
4				
5				
6				
7				

COMMENTS

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Fungi

BATCH NUMBER		**OPERATOR**	
INOCULATION DATE		**SIGNATURE**	

DAYS AFTER INCOCULATION	COLUMN 2 N= negative P = positive	COLUMN 9 N= negative P = positive	COLUMN 16 N= negative P = positive	IDENTIFIED FUNGUS
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				

COMMENTS

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Decision by:

Batch number:

BATCH RELEASE DOCUMENT

	OK? YES / NO	NEW KITS TO BE SENT	CORRECTIVE MEASURES NEEDED
DECISION 1 (damage)		WHEN "NO"	WHEN "YES" BUT NON-CRITICAL DAMAGE OBSERVED (= COLUMNS OR COLLECTOR TUBES NOT DAMAGED)
DECISION 2 (kit contents)		WHEN "NO"	WHEN "YES" BUT NON-CRITICAL DEVIATIONS OBSERVED (=ALL 10 COLUMNS AND COLLECTOR TUBES PRESENT)

IF NEW KITS TO BE SENT RESTART SOP "LOT RELEASE QC" FROM THE BEGINNING

OTHERWISE CONTINUE BELOW

	OK? YES / NO	REJECT	CORRECTIVE MEASURES NEEDED
DECISION 3 (physical and chemical shortcomings)		WHEN "NO"	WHEN "YES" BUT NON-CRITICAL SHORTCOMINGS ARE OBSERVED OR WHEN REJECTED
DECISION 4 (sterility)		WHEN "NO"	WHEN REJECTED
DECISION 5 (functionality)		WHEN "NO"	WHEN REJECTED

CONCLUSION □ **PASSES** □ **FAILS**

APPROVED BY ....

DATE ....

Revision history	
Changes made with respect to the previous published version dated feb 08:	P10-11-12: Name “Frank Anthonissen” removed P10-11-12: Signature added

In-site Quality Release Control (INRB)

Use A batch release document is the final proof of the quality of the produced mAECT kits.
This batch release document is delivered when the mAECT kits of the batch correspond to the different physical and chemical requirements and when all production sheets are available and complete.
The batch release document established after On-site Quality Release Control is needed to allow dispatching of a sample kits to ITM for Independent-site Quality Release Control. This QRC will generate a batch release document that is needed for delivery of the batch to the end-user.

General procedure

Per batch of 700-800 columns produced:

1. Select 2 complete mAECT kits containing each 10 columns and accessories.
2. Check contents of each mAECT kit (columns, collector tubes, instruction leaflet, accessory materials)
3. Visual inspection of columns (label, gel volume, position of filters, leakage, air bubbles, visible contamination etc.)
4. Check critical production sheets for presence, completeness and compliance with requested values
5. Prepare Batch Release document (**last page of this SOP**)
6. Communicate decision on Head of Production Unit
7. If applicable, propose preventive and corrective measures to Head of Production Unit

Contents of each mAECT kit

BATCH NUMBER	

ITEM	**EXPECTED**	**OBSERVED**	**DEVIATION Y/N**
LABEL ON UPPER LID OF BOX: PRESENT *AND READABLE* BATCH NUMBER EXPIRY DATE STORAGE INSTRUCTIONS FOR IN VITRO USE ONLY	 Y Y Y Y Y		
INSTRUCTIONS ENGLISH *NUMBER*	1		
INSTRUCTIONS ENGLISH *READABLE*	Y		
INSTRUCTIONS FRENCH *NUMBER*	1		
INSTRUCTIONS FRENCH *READABLE*	Y		
INSTRUCTIONS PORTUGUESE *NUMBER*	1		
INSTRUCTIONS PORTUGUESE *READABLE*	Y		
COLUMNS *NUMBER*	10		
COLLECTOR TUBES *NUMBER*	10		
COLLECTOR TUBES *CLEAN*	Y		
14 ML CENTRIFUGATION TUBES *NUMBER*	10		
14 ML CENTRIGUATION TUBES *CLEAN*	Y		
PLASTIC TRANSFER PIPETTES *NUMBER*	10		
PLASTIC TRANSFER PIPETTES *CLEAN*	Y		

DECISION 1

1. If no deviation observed
   => **proceed with QC**
2. If deviation observed is not critical (all 10 colomns and collector tubes are present)
   => **proceed with QC but propose corrective measures**
3. If deviation observed is critical (at least 1 column or collector tube is missing)
   => **communicate with Head of Production Unit for corrective measures**

Visual inspection of columns

DATE	
BATCH NUMBER	

OPERATOR	

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
DATE ON LABEL																				
POSITION OF LABEL																				
POSITION OF GREEN UPPER STOPPER																				
POSITION OF WHITE LOWER STOPPER																				
POSITION OF UPPER FILTER																				
POSITION OF LOWER FILTER																				
AIR BUBBLES ABSENT																				
GEL COLOR NORMAL																				
BUFFER COLORLESS																				
NO VISIBLE CONTAMINATION																				
HEIGHT OF GEL (IN MM)																				
HEIGHT OF BUFFER ON TOP (IN MM)																				

	EXPECTED	MEASURED
MEAN HEIGHT OF GEL IN MM	14 (12 – 16)	
MEAN HEIGHT OF BUFFER IN MM	28 (26-29)	

Visual inspection of columns (continued)

DATE	
BATCH NUMBER	

OPERATOR	

OTHER OBSERVATIONS

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DECISION 2

1. If no deviation observed
   => **proceed with QC**
2. If deviation observed is not critical
   no visual contamination
   gel and buffer volume within expected limits
   upper filter in correct position
   no air bubbles
   => **proceed with QC but propose corrective measures**
3. If deviation observed is critical
   visual contamination
   gel and buffer volume beyond expected limits
   upper filter absent or in wrong position
   air bubbles
   => **note deviation on Batch Release Document and Reject Batch, propose corrective measures**

Inspection of production sheets

The production sheets corresponding to the following SOPs should be checked for:
- Presence in the batch production file (Y/N)
- Completeness (batch number, date, operator, data) (Y/N)
- Values within the expected limits (Y/N)

DATE	
BATCH NUMBER	

OPERATOR	

SOP	PRODUCTION SHEET		
Number	Present	Complete	Values within limits
1 PBS 10x			
2 PBS			
3 PSG			
4 Phosphoric acid dilution			
5 Gel equilibration			
6 Assembling			
7 Tyndallisation			
9 Physical and chemical check			
10 Sterility check			
11 Functionality check			

DECISION 3

1. If no deviation observed
   => **proceed with QC**
2. If deviation observed is not critical
   all production sheets present
   all values noted and within limits
   => **proceed with QC but propose corrective measures**
3. If deviation observed is critical
   one or more production sheet is missing
   one or more values are missing
   one or more values are not within limits
   => **note deviation on Batch Release Document and Reject Batch, propose corrective measures**

BATCH RELEASE DOCUMENT

BATCH NUMBER	

	OK? YES / NO	NEW KITS TO BE CHOSEN	CORRECTIVE MEASURES NEEDED
DECISION 1 (kit contents)		WHEN "NO"	WHEN "YES" BUT NON-CRITICAL DAMAGE OBSERVED (=ALL 10 COLUMNS AND COLLECTOR TUBES PRESENT)
DECISION 2 (visual inspection of columns)		WHEN "NO"	WHEN "YES" BUT NON-CRITICAL DEVIATIONS OBSERVED (=NO VISUAL CONTAMINATION, GEL AND BUFFER VOLUME WITHIN EXPECTED LIMITS, UPPER FILTER IN CORRECT POSITION, NO AIR BUBBLES)

IF NEW KITS TO BE CHOSEN, RESTART SOP "IN-SITE QUALITY RELEASE CONTROL" FROM THE BEGINNING

OTHERWISE CONTINUE BELOW

	OK? YES / NO	REJECT	CORRECTIVE MEASURES NEEDED
DECISION 3 (production sheets)		WHEN "NO"	WHEN "YES" NON-CRITICAL SHORTCOMINGS ARE OBSERVED OR WHEN REJECTED

CONCLUSION □ **PASSES** □ **FAILS**

APPROVED BY ....

DATE ....

Stability test

Intended use: mAECT minicolumns are sterilized to allow storage for some time without any loss of functionality. This SOP describes a procedure to evaluate the storage conditions and estimate the maximum storage period of the columns.

Material and storage equipment

Incubator at 45°C
Incubator at 37°C
Refrigerator at 4°C
Cupboard at room temperature (variable)
Electronic thermometers with memory (min/max)
Record sheet for temperatures
100 mAECT columns, marked (# 25) with a code depending on storage conditions (# 4).

Procedure

1. Control that all materials are available
2. Number and weigh each column
3. Put **25** columns, packed in a box (like for normal kit packaging), in the following conditions:
   - Incubator at 45°C
   - Incubator at 37°C
   - Refrigerator at 4°C
   - Cupboard at room temperature (variable)
4. Note every day the temperatures of the incubators, refrigerator and cupboard.
5. After 1 month, weigh all columns.
6. After 3 months, remove 5 columns from each condition, check them visually for contamination, weigh them and control the sterility of 3 columns and the functionality of 2 columns (see specific protocols below)
7. Immediately report the test results to FIND for corrective actions if needed.
8. Repeat the tests with 5 columns after 6, 12, 18 and 24 months.

VISUAL INSPECTION OF COLUMNS

CONTROLLER	

INDICATE VISIBLE CONTAMINATION (1 or 0)

		MONTHS								
		3		**6**		**12**		**18**		**24**
CONDITION	**NR**		**NR**		**NR**		**NR**		**NR**	
37°C	1		6		11		16		21	
	2		7		12		17		22	
	3		8		13		18		23	
	4		9		14		19		24	
	5		10		15		20		25	
ROOM TEMP.	26		31		36		41		46	
	27		32		37		42		47	
	28		33		38		43		48	
	29		34		39		44		49	
	30		35		40		45		50	
4°C	51		56		61		66		71	
	52		57		62		67		72	
	53		58		63		68		73	
	54		59		64		69		74	
	55		60		65		70		75	
45°C	76		81		86		91		96	
	77		82		87		92		97	
	78		83		88		93		98	
	79		84		89		94		99	
	80		85		90		95		100	

WEIGHTS OF COLUMNS

STORAGE AT 37°C

CONTROLLER	

	MONTHS						
	0	1	3	6	12	18	24
NUMBER							
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							
16							
17							
18							
19							
20							
21							
22							
23							
24							
25							

WEIGHTS OF COLUMNS

STORAGE AT ROOM TEMPERATURE

CONTROLLER	

	MONTHS						
	0	1	3	6	12	18	24
NUMBER							
26							
27							
28							
29							
30							
31							
32							
33							
34							
35							
36							
37							
38							
39							
40							
41							
42							
43							
44							
45							
46							
47							
48							
49							
50							

WEIGHTS OF COLUMNS

STORAGE ÀT 4°C

CONTROLLER	

	MONTHS						
	0	1	3	6	12	18	24
NUMBER							
51							
52							
53							
54							
55							
56							
57							
58							
59							
60							
61							
62							
63							
64							
65							
66							
67							
68							
69							
70							
71							
72							
73							
74							
75							

WEIGHTS OF COLUMNS

STORAGE ÀT 45°C

CONTROLLER	

	MONTHS						
	0	1	3	6	12	18	24
NUMBER							
76							
77							
78							
79							
80							
81							
82							
83							
84							
85							
86							
87							
88							
89							
90							
91							
92							
93							
94							
95							
96							
97							
98							
99							
100							

BACTERIOLOGIC STERILITY

CONTROLLER	

Culture media

Aerobic culture medium:

Brain Heart Infusion, in tubes

Optional: Chocolate Agar/HgB + isovitalex, in Petri dishes

Anaerobic culture medium:

Thioglycollate medium, in tubes

Optional: Anaerobic Blood Agar, in Petri dishes

Procedure

1. Control that all materials and reagents are available and clean.
2. Prepare three columns for testing.
3. Under sterile conditions, inoculate each liquid culture medium with 0.5 ml of supernatant buffer from each column (4 media).
4. Put the aerobic and anaerobic cultures in the incubator with CO_2 at 36±2°C.
5. Check the cultures daily for 7 days for the presence of bacteria. If bacterial growth is visible, subinoculate the aerobic or anaerobic cultures on agar in order to identify the nature of the contaminant.

Aerobic bacteria

CONTROLLER	

INDICATE IF POSITIVE WITHIN 7 DAYS

		MONTHS								
		3		**6**		**12**		**18**		**24**
CONDITION	**NR**		**NR**		**NR**		**NR**		**NR**	
37°C	1		6		11		16		21	
	2		7		12		17		22	
	3		8		13		18		23	
Room temp.	26		31		36		41		46	
	27		32		37		42		47	
	28		33		38		43		48	
4°C	51		56		61		66		71	
	52		57		62		67		72	
	53		58		63		68		73	
45°C	76		81		86		91		96	
	77		82		87		92		97	
	78		83		88		93		98	

IF POSITIVE, INDICATE THE NATURE OF THE CONTAMINANT, IF POSSIBLE

....

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....

....

Anaerobic bacteria

CONTROLLER	

INDICATE IF POSITIVE WITHIN 7 DAYS

		MONTHS								
		3		6		12		18		24
CONDITION	**NR**		**NR**		**NR**		**NR**		**NR**	
37°C	1		6		11		16		21	
	2		7		12		17		22	
	3		8		13		18		23	
Room temp.	26		31		36		41		46	
	27		32		37		42		47	
	28		33		38		43		48	
4°C	51		56		61		66		71	
	52		57		62		67		72	
	53		58		63		68		73	
45°C	76		81		86		91		96	
	77		82		87		92		97	
	78		83		88		93		98	

IF POSITIVE, INDICATE THE NATURE OF THE CONTAMINANT, IF POSSIBLE

....

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Functionality control

Safety: **Animal and human bloods are potentially infectious. *Trypanosoma brucei gambiense*, which is used to determine the functionality of columns, is infectious. Usual cautions for infectious agents should be taken (wear gloves and lab coats, properly dispose of infectious and contaminated material).**

Material

Microscope
Rack for mAECT
Viewing chamber for mAECT
Automatic 5-40 µL pipette
Automatic 100-1000 µl pipette
Grip
Centrifuge
Chronometer
Indelible marker

Consumables

Heparin
Disposable gloves
Absorbing paper
Liquid disinfectant
Microscope slides
Microscope slide coverslips 24 x 24 mm
200-µl pipette tips
1000-µl pipette tips
URIGLASS counting chamber (slide with 10 1-µl chambers)
Polypropylene pastettes (3.5 ml)
Polypropylene tubes (3 – 13 ml)
Monovette for heparinized blood with needle
Collector tubes
14-ml centrifuge tubes
Ice
PSG buffer (see SOP M/3). *Note: PSG buffer may be frozen in small volumes.*

Organisms

Mouse previously infected with *T.b. gambiense*.

Blood

Human blood drawn with heparin. May be used for 2 days. The required volume depends on the number of columns to be tested. 0.350 ml of blood is required for each column.

Procedure

3-4 days before the experiment

1. Infect a mouse with *T.b. gambiense*. If required, sub-inoculate for future tests.

On the day of the experiment

2. Control that all materials and reagents are available and clean.
3. Put the PSG buffer on ice or at 4°C.
4. Put the freshly drawn heparinized blood on ice or at 4°C.
5. Control the mouse parasitemia.
6. Prepare a trypanosomes suspension in blood (100 tryps/ml). This step should be done as quickly as possible and must be done with cold blood and buffer.
   - Draw blood with heparin from the infected mouse (from the tail or by cardiac punction) and, depending on the initial parasitemia, immediately dilute 1:10 to 1:100 in cold PSG.
   - Estimate the number of trypanosomes using a Uriglass counting chamber (one chamber contains 1 µl)
   - Dilute further in PSG to obtain a suspension with 10 trypanosomes/µl.
   - Fill 3 Uriglass counting chambers and let stand for 5 minutes. Count the number of trypanosomes per chamber and calculate the average of the 3 chambers.
   - Add X µl of this trypanosomes suspension to Y ml of cold human blood to obtain a suspension with 100 trypanosomes/ml.
   - Keep the 10 trypanosomes/µl suspension in PSG on ice to check trypanosomes viability at the end of the experiment.
7. Perform the mAECT test with 5 columns and 350 µl of blood per column following the mAECT kit instructions.
8. For each column, note the time of the beginning and of the end of flow-through.
9. Count the number of trypanosomes in each collector tube.
10. Check the absence of foreign particles on the bottom of each collector tube and indicate the nature of the observed particles if any (gel, fibers, filter debris....).
11. Check trypanosomes viability in the original 10 tryps/µl in PSG suspension.
12. Calculate the gel percentage that is occupied by blood
    - Measure the gel height that is occupied by blood in mm = h
    - Measure the gel height between the lower and upper filters in mm = H
    - Gel percentage occupied by blood=h/Hx100=%
13. Note under the comments section any other observations made during quality control.

Functionality results: 3 months

DATE	
PERSON WHO PREPARED	

MOUSE PARASITEMIA CONTROL

TRYPANOSOME STRAIN	
COLLECTION TIME	
PARASITEMIA IN DILUTION 1/10-1/100 IN PSG	

PREPARATION OF A 10 TRYPS/µL SUSPENSION IN PSG

FIRST DILUTION: X µL IN Y ML OF PSG	X = ... µL
	Y = ... ML
NUMBER OF TRYPANOSOMES IN COUNTING CHAMBER	
IF REQUIRED, SECOND DILUTION: X µL IN Y ML OF PSG	X = ... µL
	Y = ...ML
NUMBER OF TRYPANOSOMES IN COUNTING CHAMBER 1	
NUMBER OF TRYPANOSOMES IN COUNTING CHAMBER 2	
NUMBER OF TRYPANOSOMES IN COUNTING CHAMBER 3	
AVERAGE NUMBER OF TRYPANOSOMES / µL	

PREPARATION OF A 100 TRYPS/ML SUSPENSION IN HUMAN BLOOD

ADD X µL OF 10 TRYPS/µL SUSPENSION IN Y ML OF BLOOD TO OBTAIN 100 TRYPS/ML IN BLOOD	X = ... µL Y = ...ML

END OF PREPARATION TIME	

Functionality results: 3 months

DATE	
PERSON WHO PREPARED	

STORAGE CONDITION	COLUMN NUMBER	START OF FLOW-THROUGH TIME *HH:MM*	END OF FLOW-THROUGH TIME *HH:MM*	TEST DURATION: *HH:MM*	NUMBER OF TRYPANOSOMES	BLOOD HEIGHT IN GEL	GEL HEIGHT BETWEEN FILTERS	GEL PERCENTAGE OCCUPIED BY BLOOD	ABSENCE (A) OR PRESENCE OF PARTICLES OR DEBRIS	NATURE OF PARTICLES OR DEBRIS
37°C	4									
	5									
Room temp.	29									
	30									
4°C	54									
	55									
45°C	79									
	80									

CONTROL OF TRYPANOSOMES VIABILITY IN THE ORIGINAL SUSPENSION IN PSG

TIME		NUMBER OF LIVE TRYPANOSOMES	

OTHER OBSERVATIONS OR COMMENTS

....

....

Functionality results: 6 months

DATE	
PERSON WHO PREPARED	

MOUSE PARASITEMIA CONTROL

TRYPANOSOME STRAIN	
COLLECTION TIME	
PARASITEMIA IN DILUTION 1/10-1/100 IN PSG	

PRÉPARATION D'UNE SUSPENSION DE 10 TRYPS/µL DANS LE PSG

FIRST DILUTION: X µL IN Y ML OF PSG	X = ... µL
	Y = ... ML
NUMBER OF TRYPANOSOMES IN COUNTING CHAMBER	
IF REQUIRED, SECOND DILUTION: X µL IN Y ML OF PSG	X = ... µL
	Y = ...ML
NUMBER OF TRYPANOSOMES IN COUNTING CHAMBER 1	
NUMBER OF TRYPANOSOMES IN COUNTING CHAMBER 2	
NUMBER OF TRYPANOSOMES IN COUNTING CHAMBER 3	
AVERAGE NUMBER OF TRYPANOSOMES / µL	

PREPARATION OF A 100 TRYPS/ML SUSPENSION IN HUMAN BLOOD

ADD X µL OF 10 TRYPS/µL SUSPENSION IN Y ML OF BLOOD TO OBTAIN 100 TRYPS/ML IN BLOOD	X = ... µL Y = ...ML

END OF PREPARATION TIME	

Functionality results: 6 months

DATE	
PERSON WHO PREPARED	

STORAGE CONDITION	COLUMN NUMBER	START OF FLOW-THROUGH TIME *HH:MM*	END OF FLOW-THROUGH TIME *HH:MM*	TEST DURATION: *HH:MM*	NUMBER OF TRYPANOSOMES	BLOOD HEIGHT IN GEL	GEL HEIGHT BETWEEN FILTERS	GEL PERCENTAGE OCCUPIED BY BLOOD	ABSENCE (A) OR PRESENCE OF PARTICLES OR DEBRIS	NATURE OF PARTICLES OR DEBRIS
37°C	4									
	5									
Room temp.	29									
	30									
4°C	54									
	55									
45°C	79									
	80									

CONTROL OF TRYPANOSOMES VIABILITY IN THE ORIGINAL SUSPENSION IN PSG

TIME		NUMBER OF LIVE TRYPANOSOMES	

OTHER OBSERVATIONS OR COMMENTS

....

....

Functionality results: 12 months

DATE	
PERSON WHO PREPARED	

MOUSE PARASITEMIA CONTROL

TRYPANOSOME STRAIN	
COLLECTION TIME	
PARASITEMIA IN DILUTION 1/10-1/100 IN PSG	

PREPARATION OF A 10 TRYPS/µL SUSPENSION IN PSG

FIRST DILUTION: X µL IN Y ML OF PSG	X = ... µL
	Y = ... ML
NUMBER OF TRYPANOSOMES IN COUNTING CHAMBER	
IF REQUIRED, SECOND DILUTION: X µL IN Y ML OF PSG	X = ... µL
	Y = ...ML
NUMBER OF TRYPANOSOMES IN COUNTING CHAMBER 1	
NUMBER OF TRYPANOSOMES IN COUNTING CHAMBER 2	
NUMBER OF TRYPANOSOMES IN COUNTING CHAMBER 3	
AVERAGE NUMBER OF TRYPANOSOMES / µL	

PREPARATION OF A 100 TRYPS/ML SUSPENSION IN HUMAN BLOOD

ADD X µL OF 10 TRYPS/µL SUSPENSION IN Y ML OF BLOOD TO OBTAIN 100 TRYPS/ML IN BLOOD	X = ... µL Y = ...ML

END OF PREPARATION TIME	

Functionality results: 12 months

DATE	
PERSON WHO PREPARED	

STORAGE CONDITION	COLUMN NUMBER	START OF FLOW-THROUGH TIME *HH:MM*	END OF FLOW-THROUGH TIME *HH:MM*	TEST DURATION: *HH:MM*	NUMBER OF TRYPANOSOMES	BLOOD HEIGHT IN GEL	GEL HEIGHT BETWEEN FILTERS	GEL PERCENTAGE OCCUPIED BY BLOOD	ABSENCE (A) OR PRESENCE OF PARTICLES OR DEBRIS	NATURE OF PARTICLES OR DEBRIS
37°C	4									
	5									
Room temp.	29									
	30									
4°C	54									
	55									
45°C	79									
	80									

CONTROL OF TRYPANOSOMES VIABILITY IN THE ORIGINAL SUSPENSION IN PSG

TIME		NUMBER OF LIVE TRYPANOSOMES	

OTHER OBSERVATIONS OR COMMENTS

....

....

Functionality results: 18 months

DATE	
PERSON WHO PREPARED	

MOUSE PARASITEMIA CONTROL

TRYPANOSOME STRAIN	
COLLECTION TIME	
PARASITEMIA IN DILUTION 1/10-1/100 IN PSG	

PREPARATION OF A 10 TRYPS/µL SUSPENSION IN PSG

FIRST DILUTION: X µL IN Y ML OF PSG	X = ... µL
	Y = ... ML
NUMBER OF TRYPANOSOMES IN COUNTING CHAMBER	
IF REQUIRED, SECOND DILUTION: X µL IN Y ML OF PSG	X = ... µL
	Y = ...ML
NUMBER OF TRYPANOSOMES IN COUNTING CHAMBER 1	
NUMBER OF TRYPANOSOMES IN COUNTING CHAMBER 2	
NUMBER OF TRYPANOSOMES IN COUNTING CHAMBER 3	
AVERAGE NUMBER OF TRYPANOSOMES / µL	

PREPARATION OF A 100 TRYPS/ML SUSPENSION IN HUMAN BLOOD

ADD X µL OF 10 TRYPS/µL SUSPENSION IN Y ML OF BLOOD TO OBTAIN 100 TRYPS/ML IN BLOOD	X = ... µL Y = ...ML

END OF PREPARATION TIME	

Functionality results: 18 months

DATE	
PERSON WHO PREPARED	

STORAGE CONDITION	COLUMN NUMBER	START OF FLOW-THROUGH TIME *HH:MM*	END OF FLOW-THROUGH TIME *HH:MM*	TEST DURATION: *HH:MM*	NUMBER OF TRYPANOSOMES	BLOOD HEIGHT IN GEL	GEL HEIGHT BETWEEN FILTERS	GEL PERCENTAGE OCCUPIED BY BLOOD	ABSENCE (A) OR PRESENCE OF PARTICLES OR DEBRIS	NATURE OF PARTICLES OR DEBRIS
37°C	4									
	5									
Room temp.	29									
	30									
4°C	54									
	55									
45°C	79									
	80									

CONTROL OF TRYPANOSOMES VIABILITY IN THE ORIGINAL SUSPENSION IN PSG

TIME		NUMBER OF LIVE TRYPANOSOMES	

OTHER OBSERVATIONS OR COMMENTS

....

....

Functionality results: 24 months

DATE	
PERSON WHO PREPARED	

MOUSE PARASITEMIA CONTROL

TRYPANOSOME STRAIN	
COLLECTION TIME	
PARASITEMIA IN DILUTION 1/10-1/100 IN PSG	

PREPARATION OF A 10 TRYPS/µL SUSPENSION IN PSG

FIRST DILUTION: X µL IN Y ML OF PSG	X = ... µL
	Y = ... ML
NUMBER OF TRYPANOSOMES IN COUNTING CHAMBER	
IF REQUIRED, SECOND DILUTION: X µL IN Y ML OF PSG	X = ... µL
	Y = ...ML
NUMBER OF TRYPANOSOMES IN COUNTING CHAMBER 1	
NUMBER OF TRYPANOSOMES IN COUNTING CHAMBER 2	
NUMBER OF TRYPANOSOMES IN COUNTING CHAMBER 3	
AVERAGE NUMBER OF TRYPANOSOMES / µL	

PREPARATION OF A 100 TRYPS/ML SUSPENSION IN HUMAN BLOOD

ADD X µL OF 10 TRYPS/µL SUSPENSION IN Y ML OF BLOOD TO OBTAIN 100 TRYPS/ML IN BLOOD	X = ... µL Y = ...ML

END OF PREPARATION TIME	

Functionality results: 24 months

DATE	
PERSON WHO PREPARED	

STORAGE CONDITION	COLUMN NUMBER	START OF FLOW-THROUGH TIME *HH:MM*	END OF FLOW-THROUGH TIME *HH:MM*	TEST DURATION: *HH:MM*	NUMBER OF TRYPANOSOMES	BLOOD HEIGHT IN GEL	GEL HEIGHT BETWEEN FILTERS	GEL PERCENTAGE OCCUPIED BY BLOOD	ABSENCE (A) OR PRESENCE OF PARTICLES OR DEBRIS	NATURE OF PARTICLES OR DEBRIS
37°C	4									
	5									
Room temp.	29									
	30									
4°C	54									
	55									
45°C	79									
	80									

CONTROL OF TRYPANOSOMES VIABILITY IN THE ORIGINAL SUSPENSION IN PSG

TIME		NUMBER OF LIVE TRYPANOSOMES	

OTHER OBSERVATIONS OR COMMENTS

....

....

Physical and chemical quality control

Intended use: mAECT columns are produced in batches. Each batch is identified by its production date. Quality control of each batch is performed on a sample of each batch produced. SOP M/9 describes the physical and chemical quality control procedures. Physical control is done on the whole sample (#21), chemical control is done on 3 columns. The rest of the columns is reserved for sterility control (#3, SOP M/10), functionality control (#5, SOP/M11) and long term storage for eventual delayed quality control (#10, SOP 23).

Storage: Columns are stored at 4 °C.

Safety: Reagents are not toxic, explosive or corrosive.

Material pH meter, Conductimeter
Slide caliper, 15-ml Falcon tube

Reagents

Standard buffers for pH meter

Procedure

1. Check that all materials and reagents are available and clean.

Physical parameters control

2. Select 21 columns to be tested from the mounting racks: one third (#7) from the beginning of the batch, one third (#7) from the middle, one third(#7) from the end.
3. Number each column following the batch sequence order (1, 2, 3 ....)
4. Check the label with the production date.
5. Check the position of the label.
6. Check that the green cap is positioned on the top of the column and the white cap on its bottom.
7. Check the positions of the upper and lower filters.
8. Check the absence of air bubbles in the gel.
9. Check the colors of the gel and buffer.
10. Check the absence of any visible precipitation or contamination.
11. Gel height: measure the distance in mm between the bottom of the tube and the gel surface.
12. Buffer height: measure the distance in mm between the bottom of the tube and the buffer surface.

Chemical parameters control

13. Calibrate the conductimeter (see the conductimeter manual).
14. Calibrate the pH meter (see the pH meter manual).
15. Open **three** columns (*beginning/middle/end*) and pool the buffer supernatants in a 15-ml Falcon tube.
16. Note the temperature and measure the conductivity of the buffer.
17. Measure the pH of the buffer.

Other comments

18. Note on the production sheet.

PRODUCTION SHEET
Physical and chemical quality control

DATE	
NUMBER OF COLUMNS TO BE TESTED	

PERSON WHO PREPARED	
DATE ON LABEL	

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
DATE ON LABEL																					
LABEL POSITION																					
UPPER CAP POSITION																					
LOWER CAP POSITION																					
UPPER FILTER POSITION																					
LOWER FILTER POSITION																					
ABSENCE OF AIR BUBBLES																					
GEL COLOR																					
BUFFER COLOR																					
ABSENCE OF CONTAMINATION																					
GEL HEIGHT (IN MM)																					
BUFFER HEIGHT (IN MM)																					

	EXPECTED	MEASURED
AVERAGE GEL HEIGHT IN MM	14 (12-16)	
AVERAGE BUFFER HEIGHT IN MM	28 (26-29)	
TEMPERATURE	---------------	
CONDUCTIVITY	9 mmho/cm or mS/cm	
pH	8,0 – 8,1	

PRODUCTION SHEET

Physical and chemical quality control

DATE	

PERSON WHO PREPARED	

DATE ON LABEL	

OTHER COMMENTS

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