Australian Government
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Title: Bacterial Endotoxin - Appendix 2K - Kinetic LAL Routine Assay Worksheet	

Appendix 2K - ROUTINE ASSAY WORKSHEET – KQCL or rFC

Assay ID: [redacted] Operator: 19July2021

Limulus Amoebocyte Lysate (LAL)

Lysate batch and expiry recorded on software for each assay

Ensure sensitivity of LAL batch has been confirmed. Confirmation date: 1 June 2021

Recombinant Factor C (rFC)

rFC Enzyme, Fluorogenic Substrate & rFC Assay Buffer batches and expiry dates recorded on software for each assay-

Ensure sensitivity of the rFC batch has been confirmed. Confirmation date: -

Control Standard Endotoxin (CSE)

CSE batch and expiry recorded on software for each assay

Reconstitution details for either LAL or rFC

Date Reconstituted: 29 June 2021 to 50 EU/ml with LRW: 0000830072

Or Date Reconstituted: - to 20 EU/ml with LRW: -

Use by Date: 27 July 2021 *Use by date is 4 weeks (ie. 28 days) from reconstitution when stored at 2-8°C*

LAL Reagent Water (LRW)

How many samples were linked to this assay?2

This Appendix is used for recording the assay details and results and only gives the method in point form. See the full SOP (Appendix 8K) for the detailed method.

To avoid endotoxin contamination, use careful technique and **pyrogen free** equipment.

Preparation of Software

- Print and fill out the appropriate paperwork from the Quality Management System.

- Retrieve the required kit reagents from cold room to equilibrate to room temperature before use.
- Turn on plate reader. Wait until "ready"
- Turn on computer and log on using your current Windows password
- Open and log in to WinKQCL
- Click on the Templates tab and click "New"
- Under "Assay Type" select either "Kinetic QCL" for a LAL or "PyroGene" for an rFC
- Under "Test Type" select "Routine Assay"
- Click the "Lot/Exp" tab and enter lot number and expiry of the reagents to be used in the assay
- Confirm the concentrations of the points on the standard curve (5 points at 50 – 0.005 EU/ml for a LAL or 4 points at 5 – 0.005 EU/ml for an rFC)
- Ensure that the pipettes, tips, tubes and other accessories are correctly recorded in the accessories section of the assay template in WinKQCL
- Add the details of the samples to be tested from the master list
- When the PPC box is ticked a default value will enter. Change this to the correct value
    - Most assays will use a final PPC concentration of 0.5 EU/ml
- Name the template: Date (DDMmmYY) Operator initials Test information, and then Save
- Print Plate Layout in "Landscape" to help set up the dilutions if required

Preparation of Control Standard Endotoxin

- CSE dilutions can be dispensed to the plate as they are prepared to save mixing time.
- Open reaction plate from packet
- If required, reconstitute the endotoxin with the volume of LRW specified on the Lonza C of A.
- This will make up either 50 EU/ml for a LAL kit or 20 EU/ml for an rFC kit
- Vortex vigorously for at least 15 minutes.
- Rack and label dilution tubes/bottles as in the tables below and dispense LRW into them

For a LAL (KQCL) assay

Concentration	Volume LRW	Volume Endotoxin	Plate wells	% CV
50 EU/ml	Direct from vial	-	F1 – F2	1.12
5 EU/ml	900 µl LRW	100 µl of 50 EU/ml	E1 – E2	1.11
0.5 EU/ml	900 µl LRW	100 µl of 5 EU/ml	D1 – D2	1.87
0.05 EU/ml	900 µl LRW	100 µl of 0.5 EU/ml	C1 – C2	1.99
0.005 EU/ml	900 µl LRW	100 µl of 0.05 EU/ml	B1 – B2	2.07
Blank	Direct from vial	-	A1 – A2	

For an rFC assay

Concentration	Volume LRW	Volume Endotoxin	Plate wells	% CV
5 EU/ml	750 µl LRW	250 µl of 20 EU/ml	E1 – E2	-
0.5 EU/ml	900 µl LRW	100 µl of 5 EU/ml	D1 – D2	-
0.05 EU/ml	900 µl LRW	100 µl of 0.5 EU/ml	C1 – C2	-
0.005 EU/ml	900 µl LRW	100 µl of 0.05 EU/ml	B1 – B2	-
Blank	Direct from vial	-	A1 – A2	

- Immediately after removing each CSE dilution from the vortex, dispense 100 µl of the appropriate dilution of CSE into the appropriate wells of the plate
- Pipette 100 µl into the next tube/bottle as instructed above to make the next dilution.
- Vortex the tube/bottle for 1 minute pipette to the appropriate wells of the plate
- Continue to prepare dilutions and dispense into the reaction plate as instructed above, vortexing for 1 minute between dilutions
- Dispense 100 µl LRW into the appropriate wells of the plate (A1 & A2) – can alternatively be added at the beginning
- If preferred, PPC additions of 10 µl (as below) can be placed into the appropriate wells during this process to save mixing time

Preparation of Samples

- Retrieve the required samples from the designated storage to equilibrate to room temperature before use
- Rack and label the appropriate tubes for predilution and testing of samples
- For routine assays a PPC with a final concentration approximating 0.5 EU/ml is used. The 100 µl sample in the PPC wells is "spiked" with 10 µl of the 5 EU/ml standard to obtain a final concentration of 0.45 EU/ml.
- Vortex the 5 EU/ml standard for 1 minute
- Pipette 10 µl of the 5 EU/ml standard to the appropriate PPC wells as per the plate layout
- If required remove the label from the first sample and place it on the corresponding Sample Results Sheet
- Vortex the first sample on full speed for 10-20 seconds
- Transfer sample to the appropriate tube/bottle labelled "Neat"
- Prepare sample dilutions as in table in Appendix 1K, vortexing for 5 seconds before transferring to the next dilution tube. Repeat until all dilutions of the first sample are prepared.
- Dispense 100 µl of the final dilution into the 4 appropriate wells as per the plate layout
- Repeat with remaining samples

Starting the Assay

- The plate is then ready for the reaction. Prepare the software for the reaction as follows:
- Return to "Templates" screen
- Select the correct Available Template, Add it to the Merged Template field and click "Run"
- Follow the prompts on the screen
- Pre-warming. Leave the lid of the plate on, place the plate into the reader and click OK, this starts the 10 minute warm-up

If performing a LAL assay

- Reconstitute the required lysate vial/s with the specified volume of LRW stated on the vial
- Gently swirl /roll the vial to reconstitute lysate. Avoid frothing.
- Immediately before use, pour the lysate from the vial/s into the reagent reservoir, removing the final drops with a pipette

OR

If performing an rFC assay

- Make up the required volume of rFC reagent directly into the reservoir (ensuring thorough mixing) using with the volumes specified for each reagent from the table in the kit insert. This is dependent on the number of wells needed
  - Fluorogenic Substrate -
  - rFC Assay Buffer -
  - rFC Enzyme Solution -
- Open cover – if using the Spectramax use the software to open and close the draw
- Take plate out and as quickly as possible carefully add 100 µl lysate to each of the assay wells
- Return the plate to the reader and click OK to start the run. **Do not open cover.**

Acceptance Criteria – for LAL

The CSE standard curve must meet the following parameters for the assay to be valid	
Correlation coefficient (r) absolute value ≥ 0.980	-0.997
Slope between -0.400 and -0.100	-0.226
Y intercept between 2.500 and 3.500	3.146
Mean reaction times of blank ≥ mean reaction times of lowest standard	Yes
Coefficient of variation (CV) values for all standards are < 10%	Yes
Were all acceptance criteria for the standard curve met?	Yes

Acceptance Criteria – for rFC

The CSE standard curve must meet the following parameters for the assay to be valid	
Correlation coefficient (r) absolute value ≥ 0.980	-
Slope between 0.760 and 1.110	-
Y intercept between 2.500 and 5.000	-
Mean RFU of blank ≤ mean RFU of lowest standard	-
Coefficient of variation (CV) values for all standards are < 25%	-
Were all acceptance criteria for the standard curve met?	-

Conclusions

Prior to printing, the Operator should electronically sign the report and record any deviations from the method during the e-signature procedure

Transcribe results to Assay Worksheet and Sample Result Sheet/s, i.e. % CV's, acceptance criteria parameters, EU/ml, and % PPC Recovery

Have another operator, preferably the person responsible for endotoxin testing, record any explanation of unexpected outcomes and sign electronically as a "Reviewer"

The results are stored as part of the assay in the WinKQCL software. Exported or printed versions are stored in Endotoxin Testing Results folders.

Notes and Comments

Click or tap here to enter text.