Bid Submission Form
Participation in Assignment of Infectious Titer
RFP 9.0

Please complete the following fields:

Contact Information – RFP 9.0

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***If laboratories are submitting a proposal as a group, a main contact should be provided along with contact information for each participating laboratory (attach additional copies of this form).**

Please indicate if your institution is also submitting proposals for the other activities:

- [X] Determination of Particle Concentration
- [ ] Short-term/Field Stability Studies
- [ ] Long-term Stability Study
- [ ] Other Characterization
- [ ] Donation of Supplies/Other Services for Characterization Phase

Each laboratory submitting a proposal should provide a statement describing their experience and capacity to perform the spectrophotometric particle assay described in the standard operating procedure. The statement should specifically address:

- the qualifications of the personnel involved in performing the procedure and reviewing the data,
  - All staff are qualified to graduate, or above, academic level and all have a minimum of 2yr experience in support of a current Cobra Therapeutics clinical programme using an Adenovirus vector generated in PER.C6.

- the equipment that will be used and its calibration status,

  Class II biological safety cabinets (Envair)

  These are serviced every 6 months by a qualified service engineer.

  Variable volume pipettes (Gilson)

  All pipettes are cleaned and calibrated every 6 months to a specification set by the manufacturer.

- how long it will take the laboratory to perform the procedure, and review and report results back once the sample is received,
  - 1 month from receipt to report

- the laboratory's readiness to begin testing in mid to late September 2001.
  - Lab ready and available for September 2001

For laboratories wishing to also submit a proposal to perform additional methods of determining the particle concentration, the proposal should include:

- the amount of Ad5 WT Reference Material that will be required to perform the proposed analysis,
  - The assay for infectious titre by "Plaque Assay" will require one sample, equivalent to that supplied for the CPE assay.
- a complete description of the method, preferably in the form of an operating procedure
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PRINCIPLE

After a virus particle has infected a cell, that cell is then made to produce more virus particles, which will eventually lyse from the cells and infect other cells.
By adding a layer of agar over the cells, this restricts the movement of the virus to the neighbouring cells. Over a period of several replication cycles, the lysed cells form a plaque of dead cells that has arisen from one initial infectious particle.
By diluting the virus sufficiently so that there are between 5 – 20 plaques per well, an estimate of infectious particles can be gained, plaque forming units/ml (pfu/ml).

EQUIPMENT

All virus work must be carried out in a Class II Biological Safety Cabinet

REAGENTS

Dulbeccos Minimum Essential Media with 10% Foetal Bovine Serum and 1% Penicillin/Streptomycin (DF10)
911 cells
Merck agar (Cat number: 101615) prepared at 2% and autoclaved on a liquid cycle for 15 minutes. Stored at 4^{o}C and used with 3 months
2 x Modified Eagle Media (Gibco, cat number: 21935) with 3.76% Foetal Bovine Serum and 2.5% 1M $MgCl_2$ – Overlay Media
Neutral Red Solution (Sigma cat number: N-2889). Stock at 0.33%

PROCEDURE

1. Cell Seeding
   Seed 911 cells into 6-well plates, 5x10^{5} cells/well in 3ml DF10. Incubate at 37^{o}C 5% CO_2 for 2/3 days until even monolayers have formed.

2. Viral Dilutions
   Prepare appropriate viral dilutions, starting with the addition of 4µl stock virus to 2ml DF10 (gives 2 x 10^{-3} dilution). Mix and transfer 4µl to 4ml DF10 to obtain 2 x 10^{-6} dilution. Further dilute by transferring 400µl to 3.6ml medium to obtain 2 x 10^{-6}, 2 x 10^{-7}, 2 x 10^{-8}, 2 x 10^{-9} and 2 x 10^{-10} dilutions.

3. Transfection
   Replace growth media on cells with 0.5ml/well of virus at each dilution in triplicate. Include Mock wells (media alone). Incubate plates in 37^{o}C, CO_2 incubator for 90 minutes.

4. Overlay
   Melt pre-autoclave agar in the microwave, leaving the bottle cap loose and ensuring that the agar is fully molten. Aliquot into 20ml amounts and keep at 55^{o}C in a waterbath for at least 30 minutes prior to use.
   Aliquot overlay media into 20ml amounts and keep at 37^{o}C in a waterbath for at least 30 minutes prior to use.
   When ready, add one tube of agar to one tube of media and mix. Take 2 plates and remove the transfection medium. Add 3ml/well of overlay. Allow to set in hood.
   Cover with plate sealer and incubate at 37^{o}C / 5% CO_2.

5. Feeding

Feed cells every 3-4 days as necessary. Make up overlay as described above and feed the cells by adding 2mls/well.

6. Staining

Stain on days 12-14 depending on the size of the plaques and state of cells. Dilute stock neutral red to 0.02% in dH2O and add 2ml/well. Incubate plates at 37oC for 2 hours, then remove the stain and incubate overnight. Count plaques the following day.

CALCULATION

At a given dilution, multiply the number of plaques by 2 (to allow for volume/well) and divide by the virus dilution. This gives the pfu/ml.
For example, Average number of plaques = 50 at 2 x 10^{-9} dilution,
So, (50 x 2)/(2 x 10^{-9}) = 5 x 10^{10} pfu/ml

To get the P:I ratio, divide the number of particles/ml of virus stock by the pfu/ml.

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- the laboratory's experience in performing the proposed procedure,
  - The procedure has been used to support entry into a UK clinical trial and to provide data as part of the ongoing stability study (currently at 2yr) that is reviewed by UK MCA.
- the qualifications of the personnel involved in performing the procedure and reviewing the data,
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