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| **Standard Operating Procedure** | |
| **Document Title** | **28-Day *In Vitro* Assay for the Presence of Viral Contaminants** |
| **Reference Number** | **BPBT3107** |
| **Client-specific** | Yes  No |

1. PURPOSE
   1. The purpose of this assay is to determine if viral contaminants are present in the test article material.
2. SCOPE

**Location:**  US  UK  Singapore

**Division:**  Biologics  Toxicology

**Compliance:**  GLP  GMP  N/A

1. REFERENCES
   1. PROC-BREL\_GLOB-OP-036137 Use of a Hemocytometer to Determine Viable Cell Counts by Trypan Blue Exclusion Method (BPTD0011)
   2. PROC-BREL\_GLOB-OP-034732 Mammalian Cell Cultures (BPBT0600)
   3. PROC-BREL\_GLOB-OP-035069 Hemagglutination And Hemadsorption Testing For The *In Vitro* Assay (BPBT3103)
   4. PROC-BREL\_GLOB-OP-035071 Observation Of Cell Cultures For Viral CPE in *In Vitro* Virus Assays (BPBT3104)
   5. PROC-BREL\_GLOB-OP-034740 Cell Culture Medium Preparation (BPBT0609)
   6. PROC-BREL\_GLOB-OP-034757 Maintenance of Insect Cell Cultures (BPBT0630)
   7. PROC-BREL\_GLOB-OP036051 Events and Associated Records (BPQP8101)
   8. PROC-BREL\_GLOB-POL- 034539 (QMS-23) Control of Non-Conforming Products and Services: Investigation of Laboratory Events Including OOS, Anomalous and Positive Results
   9. PROC-BREL\_GLOB-OP-052639 Positive Control Concentrations Required for Routine *in Vitro* Assays (BPBT6376) (UK and Singapore only)
   10. PROC-BREL\_GLOB-OP-052637 Testing LIMS (BPBT5000)
   11. PROC-BREL\_US-OP-038220 US Testing LIMS – *In Vitro*
   12. PROC-BREL\_US-OP-050243 Vi-CELL XR CELL ANALYZER (OPTD9149) (US only)
2. MATERIALS/equipmEnt and/or critical reagents
   1. Materials
      1. Sterile six-well tissue culture plates, tissue culture flasks, and other tissue culture-ware as appropriate (SKU #A0000075) used for mammalian cell cultures)
      2. Pipettes, various sizes, sterile.
      3. Hemocytometer.
      4. Centrifuge tubes, sterile.
      5. Media and media components as detailed in SOP PROC-BREL\_GLOB-OP-034740 (BPBT0609) Cell Culture Medium Preparation.
   2. Equipment
      1. Low speed refrigerated centrifuge (Series 118 in the UK) or equivalent
      2. Waterbath set at 36 ± 2°C (Series 59, 787, 128 or 015 in the UK) or equivalent
      3. Inverted microscope (Series 100, 148, 668, 64 or 305 in the UK) or equivalent
      4. Incubator, 36 ± 2°C, 5 ± 2% CO2 (5 ± 1.5% CO2 for UK facility)(humidified as appropriate) (Series 571, 018, 765, 437 in the UK) or equivalent
      5. Incubator at other temperature and CO2 ranges as appropriate (as required)
      6. Incubator, 27 ± 2°C, to be used for insect cell cultures (as required)
      7. Incubator, 28 ± 2°C, 5 ± 2% CO2, to be used for insect cell cultures (as required)
   3. Critical reagents
      1. Dulbecco’s Phosphate Buffered Saline (DPBS). (UK SKU 100004079, Singapore Cat # 14190-086, Supplier Invitrogen (suppliers, catalogue numbers and SKU numbers are site specific).
      2. Trypsin-EDTA. (UK SKU A25300062) or TrypLE, as appropriate (suppliers, catalogue numbers and SKU numbers are site specific).
      3. Hanks’ Balanced Salt Solution (HBSS), or other appropriate balance salt solution (UK SKU 100000794) (suppliers, catalogue numbers and SKU numbers are site specific).
      4. The maintenance media for the indicator cell lines contains anti-fungal agents such as fungizone (UK SKU 100000670) or amphotericin B (0.1%), and/or antibiotics such as gentamicin (UK SKU 100001267) (at 10-50 μg/ml), penicillin (at 100units/ml), streptomycin (pen/strep UK SKU 100000777) (at 100 μg/ml), or Ciprofloxacin (at 5 μg/ml). Other antibiotics or anti-fungal agents may be used, or substituted, as required. (suppliers, catalogue numbers and SKU numbers are site specific).
      5. As per regulatory guidance, MRC-5 and Vero indicator cell lines are required when testing for adventitious agents. At least one additional indicator cell line must be tested and should be the same species of origin in which the test material is produced (i.e. if a CHO-K1 working cell bank or unprocessed bulk material, then CHO-K1 would be selected as the third indicator cell line).
      6. The qualified indicator cell lines and corresponding positive control are listed in Appendix 1. Other variations or combinations will be specified in the protocol, technical specification, LIMS Study Plan, Study Specific Protocol Supplement (SSPS), Study Specific Technical Specification Supplement (SSTS) or indicated in test article submission paperwork.
      7. Erythrocytes as appropriate (specified in study plan, study protocol technical specification sheet, SSPS or SSTS).
         1. Chicken erythrocytes (UK SKU 100002723, SKU 100010009 or equivalent approved vendor)
         2. Chicken erythrocytes (SG SKU:500000325, 500000327 or equivalent approved vendor)
         3. Guinea Pig erythrocytes (UK SKU A0000991, SKU 100010008 or equivalent approved vendor)
         4. Guinea Pig erythrocytes (SG SKU:500000324, 500000326 or equivalent approved vendor)
         5. Rhesus monkey erythrocytes (UK SKU 100002722)
         6. Cynomolgus monkey erythrocytes (SG SKU:500000323 or equivalent approved vendor)
         7. Human Type O erythrocytes (UK SKU A0000096)
3. PROCEDURE
   1. Seeding Six-Well Tissue Culture Plates (Monolayers) in Biological Safety Cabinet
      1. Label each six-well culture plate with study number, date to be inoculated (or date of seeding, if desired), type of cell and type of inoculum (i.e. test article, negative or positive control). For LIMS automated studies, labels will be printed from LIMS containing this information. Refer to SOP PROC-BREL\_GLOB-OP-052637 (BPBT5000) for the UK and Singapore or SOP PROC\_BREL\_US\_OP 038220 for the US for details related to LIMS automation.
      2. Label one six well plate per inoculum type, or 8 wells if four blood types are required.
      3. Prepare the cell suspension according to PROC-BREL\_GLOB-OP-034732 (BPBT0600) and perform a cell count according to PROC-BREL\_GLOB-OP-036137 (BPTD0011) or PROC-BREL\_US-OP-050243 (OPTD9149) (US only). Seed wells at 1.0 - 8.0 x 105cells/well by adding 2 ml of cell suspension/well.

**Note**: Seed plates the day prior to inoculation.

* 1. Seeding Six-Well Tissue Culture Plate (Suspension) in Biological Safety Cabinet.
     1. Seeding of suspension cultures is done during the inoculation step. See 5.5.2 inoculation of suspension cultures.
     2. If four blood types are required in the assay, all sample types should be inoculated with 8 replicate wells independently of the positive control expected results for CPE, HA and HAD.
  2. If the assay is to be performed using tissue culture flasks, details will be provided in study specific protocol, protocol amendments, technical specification, SSTS or SSPS.
  3. Preparation of Test Article Inoculum
     1. Cell Lysates
        1. If the test article is frozen, thaw in waterbath at 36 ± 2°C and then place the material on ice, or maintain at 2 - 8°C until inoculated.
        2. Clarify the lysate by centrifugation at 800 x *g* for 10 minutes at 2 - 8°C. Keep lysate on ice, or maintain at 2 - 8°C, until ready to inoculate. Inoculation should occur as soon as possible after thawing of frozen test articles.

In cases where the supernatant is not clarified after centrifugation step, supernatant will be transferred to a new conical tube and an additional centrifugation will be performed at 800 x *g* for 10 minutes at 2 - 8C. Study manager approval is required to perform this additional step. The supernatant will be used to inoculate the indicator cell lines.

* + - 1. If test article is not frozen, use as provided or clarify as in section 5.4.1.2.
  1. Inoculation of Indicator Cells
     1. Adherent Cultures
        1. Prior to inoculation, examine the six-well plates to be inoculated for the presence of a uniform, subconfluent monolayer.

**NOTE: If the monolayers are not uniform or appear unhealthy, notify the Study Director/Responsible Scientist and/or Laboratory Management immediately.**

* + - 1. Positive control viruses are diluted to a level pre-determined to cause appropriate end-point responses within a 14-day time period. [Refer to PROC-BREL\_GLOB-OP-052639 (BPBT6376), UK and Singapore only]. The diluted virus is then inoculated onto the appropriate indicator cell line as described below.
      2. Aspirate the growth medium and wash the monolayer with 1.0 ml/well of DPBS. Aspirate the wash buffer and add 0.5 ml of the test article or control to each well of the appropriate six-well plate.
      3. Inoculate 6 wells per inoculum type, or 8 wells if four blood types are required.
      4. Inoculate the negative control first, then the test article, and then the positive control. Inoculate a separate plate for each test article and control.
      5. To ensure appropriate segregation, test articles are brought into the hood one at a time and inoculated onto their specified indicator cell types. Any remaining test article material is removed from the hood prior to inoculation of subsequent inoculations.
      6. Allow the inoculum to adsorb at 36 ± 2°C for 70 ± 10 minutes (Optional: plates may be rocked gently during the adsorption period).
      7. Aspirate inoculum and add 2 ml/well of maintenance medium (appropriate to cell type) and return to incubator.
    1. Suspension Culture
       1. Positive control viruses are diluted to a level pre-determined to cause appropriate end-point responses within a 14-day time period. [Refer to Positive Control Concentrations Required for Routine *in Vitro* Assays (BPBT6376), UK and Singapore only]. The diluted virus is then inoculated onto the appropriate indicator cell line as described below.
       2. Tap each tissue culture flask to evenly suspend the cells and mix cells by aspirating with a pipette.
       3. Determine cell concentration by performing a viable cell count in accordance with PROC-BREL\_GLOB-OP-036137 (BPTD0011) for the use of a haemocytometer to determine cell counts.
       4. Transfer 1.2 x 106 cells, as determined in section 5.5.2.3, to an appropriately labeled centrifuge tube. Prepare enough tubes to account for each sample type to be inoculated.
       5. Pellet the cells by centrifugation at 200 x *g* for 10 minutes at 2-8°C and aspirate the resulting supernatant.
       6. To inoculate the cells, resuspend the pellet in 3.0 ml of the appropriate test article or control. Inoculate the negative control first, then the test article, and finally the positive control.
       7. Incubate at 36 ± 2°C for 70 ± 10 minutes.
       8. Pellet the cells by centrifugation at 200 x *g* for 10 minutes at 2 - 8°C and aspirate the resulting supernatant.
       9. Resuspend the cell pellet in 12 ml of the appropriate maintenance media.
       10. Dispense 2.0 ml of the cell suspension into each well of a six-well plate and return to the incubator at 36 ± 2°C.
  1. Cytopathic Effect Observations and Refeeds
     1. The cells should be observed at least 3 times/week, which must include an observation at day 14. Record their appearance as normal or abnormal (if abnormal, document the appearance). Evidence of cytopathic effect (CPE) in the test article or negative control plates, determined according to PROC-BREL\_GLOB-OP-035071 (BPBT3104), must be verified by the Study Director/Responsible Scientist or a designated alternate and assessed at the conclusion of the study by the Study Director/Responsible Scientist or Laboratory Management.
     2. Refeed the plates at least once per week of incubation, using the appropriate maintenance medium for each cell line.
     3. For suspension cultures, remove between 50-70% of the culture media from all wells and refeed to a total volume of 2 ml of maintenance media per well. (Cultures exhibiting low level confluence should be centrifuged at 200 x g for 10 minutes at 2 – 8°C and the cells then resuspended in 2ml of maintenance medium). At this time, a viability count on the culture fluid removed from the wells may be performed if required, according to PROC-BREL\_GLOB-OP-036137 (BPTD0011).
     4. There are certain occasions where sub-culture of the assay cultures may be performed to maintain the integrity of the cell monolayers:

1) For certain cell lines which grow to high densities (e.g. MDBK, NIH/3T3, 324K, 293 and PPK).

2) In certain circumstances cell lines may detach in patches due to static electricity; sometimes referred to as “plate effect”. The resultant assay plates are observed to exhibit variable cell densities between the six wells of a given condition.

* + 1. The decision to sub-culture must be made by the Study Director/Responsible Scientist or study management and be documented in LIMS, the assay workbook or batch record. Sub-culture should not be performed less than seven days prior to the scheduled testing for haemadsorption (with the exception of PPK cultures which may require a second subculture) to allow sufficient time for cell monolayer integrity to return and for cells to express potential adventitious viruses. When sub-culturing any given condition, all available associated conditions inoculated onto the same cell line, not exhibiting CPE, should be sub-cultured on the same day (e.g. should you perform sub-culture on an MRC-5 negative control condition, you must sub-culture the MRC-5 positive control, if CPE is not present, as well as all MRC-5 test article cultures which share these controls).

Performance of sub-culture as well as the reason for deciding to take this approach must be described in the relevant Final Report/Certificate of Analysis.

* + 1. To perform subculture, harvest the cells by removing the medium, rinsing the monolayer once with 1ml/well of DPBS, and then adding 0.5ml of Trypsin-EDTA to each well. After allowing sufficient time for cell detachment, collect the cells from each well in a volume of 1.5ml of fresh maintenance medium giving a total volume of 2ml per well. Pool the cells from all 6 wells by adding to a 15-ml tube and mixing well. Add 0.5ml of cell suspension to each well of a new 6-well plate followed by 1.5ml of maintenance media, giving a total of 2ml per well. Then return the plates to the incubator.
    2. If CPE is observed in the indicator cells inoculated with test article, a sample of the conditioned medium will, at the Study Director's/Responsible Scientist’s discretion, be collected and stored at -60°C or below. The sample may, as authorized by Sponsor, be passaged onto fresh indicator cells to confirm cytopathology or a cytotoxic effect, or may be used in additional procedures as requested by the Sponsor.
  1. Haemagglutination and Haemadsorption (HA/HAD)
     1. Adherent Cultures

5.7.1.1 On day 14 post-inoculation, or earlier at the appearance of CPE in all wells, if required (For UK testing only refer to appendix 2), test the conditioned medium and the remaining cell monolayers for hemagglutination and hemadsorption according to the procedures described in PROC-BREL\_GLOB-OP-035069 (BPBT3103).

* + 1. Suspension Cultures
       1. On day 14 post-inoculation, or earlier at the appearance of CPE, centrifuge the culture media from each pair of wells at 200 x *g* for 10 minutes at 2 - 8°C. If required, retain some of the media in each well to prevent desiccation.
       2. Pool the supernatants and test for haemagglutination using appropriate erythrocytes at 2 - 8°C and 36 ± 2°C according to PROC-BREL\_GLOB-OP-035069 (BPBT3103), noting any exceptions in the SSPS, SSTS, protocol or LIMS Study Plan.
       3. Resuspend the pellet in 1.0 ml of the appropriate erythrocyte suspension. Remove any remaining culture fluid from the plate wells and transfer 0.5ml of each suspension back to its original pair of wells.
       4. Incubate the plates at 2 - 8°C for approximately 30 minutes and read according to PROC-BREL\_GLOB-OP-035069 (BPBT3103).
       5. Transfer the plates to an incubator at 36 ± 2°C and incubate for approximately 30 minutes and read according to PROC-BREL\_GLOB-OP-035069 (BPBT3103).
  1. Blind Passage Inoculation Day 14:
     1. Seed detector cells for passage according to section 5.1 – 5.3.
     2. On day 14 post-inoculation, test article and negative control cultures not showing CPE in all wells will be blind passaged. Pool 7 - 12 ml of culture medium from all available wells (5 or 6 minimum). If necessary, clarify the culture medium by centrifugation at 800 x *g* for approximately 10 minutes.
* **Note:** Blind passage positive controls are only inoculated if applicable, on Study Director's/Responsible Scientist’s instructions. Inoculate a new set of positive controls for the indicator cells as performed in section 6.5.
  + - 1. If the culture is a suspension, clarify the culture medium by centrifugation at 200 x *g* for 10 minutes prior to blind passage inoculation.
    1. Inoculation
       1. For adherent cultures, repeat steps under 5.5, using 3 ml of clarified (if applicable) supernatant from 5.8.1 as the inoculum.
       2. For suspension cultures, place two volumes, each containing cells into two centrifuge tubes, one labeled “negative control blind passage” and one with the test article designation. Then, repeat steps under 5.5, using 3 ml of clarified supernatant from 5.8.1 as the inoculum.
       3. The cells should be observed for CPE at least 3 times a week, which must include an observation on day 28 of the assay. Repeat steps   
          5.6 – 5.7.
       4. Optional: Store the remaining sample (4 - 6 ml) at -60°C or below as a back-up sample. Label with: study number, inoculum type, date collected, and storage conditions.

After a minimum of 28 days, or earlier if necessary, an additional HA/HAD procedure will be performed as described in section 5.7.

1. Criteria for a VALId test
   1. Positive Controls
      1. Each positive control virus must cause cytopathic effects in all wells of the indicator cell plates into which it is inoculated and which can be evaluated. A minimum of 5 of 6 wells inoculated with each positive control must be able to be evaluated. See section 6.3.
      2. Each positive control virus must produce haemadsorption and/or haemagglutination with at least one type of erythrocyte at 2 - 8°C and/or 36 ± 2°C, both prior to and following blind passage, for the indicator cell line into which it is inoculated.
   2. Negative Controls
      1. The indicator cell lines inoculated with the negative control must not exhibit any cytopathic effects.
      2. The indicator cell lines inoculated with the negative control must not exhibit any hemadsorption and hemagglutination.
   3. Loss of Replicates

No more than one well of the six wells inoculated for any given condition (test article, negative control, or positive control) may be lost due to non-specific reasons (i.e., mold, bacterial contamination, desiccation, etc.).

Should between two and six wells of the six wells inoculated for a given control condition (negative control or positive control) be lost due to non-specific reasons (i.e., mould, bacterial contamination, desiccation, etc.), this may be replaced by an alternative control condition. The alternative control condition from another batch of assays must have six wells inoculated for a given control condition and be able to meet **all** of the following criteria:

* Alternative control was prepared on the same day as original control condition.
* Alternative control was inoculated onto cells at the same passage number as original control condition.
* Alternative control was inoculated using the same media recipe as original control condition.
* Alternative control (for positive control condition) was inoculated using the same virus lot and titer.

A copy of the raw data for the preparation of the alternative control must be added to the data packet. In the case of LIMS studies, a cross-reference should be made to the alternative control in the comments section for the appropriate reads. The use of any alternative control(s) must be described in the relevant Final Report(s) or Certificate(s) of Analysis. Any loss of replicates would be considered an unexpected occurrence and as such a BRIQS record should be opened to investigate appropriately.

* 1. Should between two and six wells of any control condition (negative control or positive control) be lost due to non-specific reasons (i.e., mold, bacterial contamination, desiccation, etc.) and there is no alternative control available, the assay does not meet the criteria for a valid test.

1. evaluation of test results
   1. In the absence of viral cytopathic effects and hemagglutination or hemadsorption activity, the test article will be concluded to be negative in the 28-day *in vitro* assay. If viral cytopathic effects are observed during the course of the study, or if a positive haemagglutination or haemadsorption result is obtained with any erythrocytes at 2 - 8°C or 36 ± 2°C, the conclusion of the study may be that the test article is possibly contaminated with an adventitious virus. In this case, samples of conditioned medium obtained during the course of the study may be used, upon authorization by the sponsor, for confirmatory studies or for the purpose of identifying the adventitious viral contaminant.

7.2 For UK and Singapore Testing only, if spiked Test Article is included, each Test Article culture spiked with virus must cause cytopathic effects in all wells of the indicator cell plates into which it is inoculated and which can be evaluated. A minimum of 5 of 6 wells inoculated with each spiked Test Article must be able to be evaluated. See section 7.3. In addition, if the virus is an HAD/HA control, each spiked Test Article culture spiked with virus must produce haemadsorption and/or haemagglutination with at least one type of erythrocyte at 2 -8°C and/or 36 ± 2°C.

If the acceptance criteria are not met for the spiked Test Article cultures, an event will be raised. Following conclusive investigation, the Test Article will be reported as inhibitory if the above acceptance criteria are not met.

7.3 Any of the following findings may trigger an Event to be entered into BRIQS [according to PROC-BREL\_GLOB-OP-036051 (BPQP8101)] which may result in the performance of an investigation:

a) a positive finding for a test article, according to QMS 23

b) a positive finding for a negative control,

c) failure of a positive control and

d) bacterial or mould contamination in more than one well of a given plate.

1. Summary of revisions

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| ***SUMMARIZE CHANGES FROM LAST REVISION*** | |
|  | 1. *Updated to new EDMS template; some section numbers altered as a result.* 2. *Change of author.* 3. *Section 4.3.7 – SKUs updated to reflect additional blood supplier.* |

**Appendix 1 – Cell/Virus Combinations**

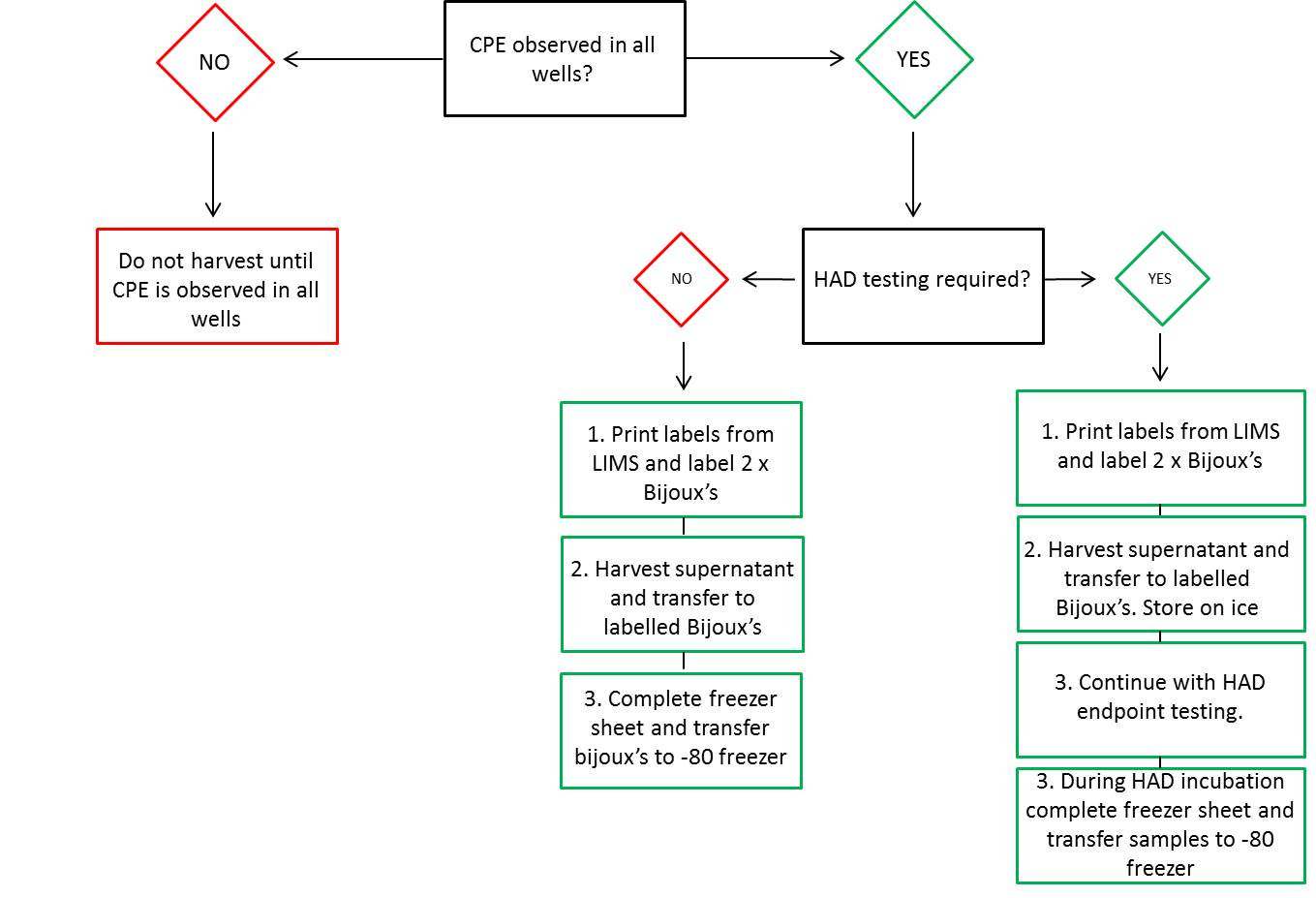
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| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Species of Origin | Cell Line | Source | UK SKU # | Singapore SKU | Positive Controls | Source | UK SKU # | Singapore SKU |
| Required | MRC-5 (Human diploid lung) | ATCC CCL 171 | 100000232 | | Measles | ATCC VR-24 | 100000281 | |
| Vero (African Green Monkey Kidney) | ATCC CCL 81 | 100000224 | | Parainfluenza virus type 3 | ATCC VR-281 | 100000276 | |
| Hamster | CHO-K1 (Chinese Hamster Ovary) | ATCC CCL 61 | 100000233 | | Simian virus type 5 | ATCC VR-288 | 100000513 | |
| BHK-21 (Baby Hamster Kidney) | ATCC CCL 10 | 100000229 | Not Applicable | Parainfluenza virus type 3 | ATCC VR-281 | 100000276 | Not Applicable |
| Human | HeLa (Human) | ATCC CCL 2 | 100000230 | Parainfluenza virus type 3 | ATCC VR-281 | 100000276 |
| PER.C6 (Human) | Crucell | Not Applicable | Parainfluenza virus type 3 | ATCC VR-281 | 100000276 |
| 324K (Human Newborn Kidney) | Yale University | 100000252 | Murine minute virus (MMV) | ATCC VR-1346 | 100000541 |
| ATCC VR-663 | 100000502 |
| 293 (Human Embryonic Kidney) | ATCC CRL 1573 | 100000226 | Herpes Simplex Virus Type 1 | ATCC VR-539 | 100000551 |
| Parainfluenza virus type 3 | ATCC VR-281 | 100000276 |
| Rabbit | RK13 (Rabbit Kidney) | ECACC 00021715 | 100007052 | Parainfluenza virus type 3 | ATCC VR-281 | 100000276 |
| Canine | MDCK (Canine Kidney Epithelial) | ATCC CCL 34 | 100000261 | Canine Parainfluenza virus | ATCC VR-399 | 100000138 |

(Continued) **Appendix 1 – Cell/Virus Combinations**

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| Species of Origin | Cell Line | Source | UK SKU # | Singapore SKU | Positive Controls | Source | UK SKU # | Singapore SKU |
| Required | MRC-5 (Human diploid lung) | ATCC CCL 171 | 100000232 | | Measles | ATCC VR-24 | 100000281 | |
| Vero (African Green Monkey Kidney) | ATCC CCL 81 | 100000224 | | Parainfluenza virus type 3 | ATCC VR-281 | 100000276 | |
| Hamster | CHO-K1 (Chinese Hamster Ovary) | ATCC CCL 61 | 100000233 | | Simian virus type 5 | ATCC VR-288 | 100000513 | |
| BHK-21 (Baby Hamster Kidney) | ATCC CCL 10 | 100000229 | Not Applicable | Parainfluenza virus type 3 | ATCC VR-281 | 100000276 | Not Applicable |
| Human | HeLa (Human) | ATCC CCL 2 | 100000230 | Parainfluenza virus type 3 | ATCC VR-281 | 100000276 |
| PER.C6 (Human) | Crucell | Not Applicable | Parainfluenza virus type 3 | ATCC VR-281 | 100000276 |
| 324K (Human Newborn Kidney) | Yale University | 100000252 | Murine minute virus (MMV) | ATCC VR-1346 | 100000541 |
| ATCC VR-663 | 100000502 |
| 293 (Human Embryonic Kidney) | ATCC CRL 1573 | 100000226 | Herpes Simplex Virus Type 1 | ATCC VR-539 | 100000551 |
| Parainfluenza virus type 3 | ATCC VR-281 | 100000276 |
| Rabbit | RK13 (Rabbit Kidney) | ECACC 00021715 | 100007052 | Parainfluenza virus type 3 | ATCC VR-281 | 100000276 |
| Canine | MDCK (Canine Kidney Epithelial) | ATCC CCL 34 | 100000261 | Canine Parainfluenza virus | ATCC VR-399 | 100000138 |

(Continued) **Appendix 1 – Cell/Virus Combinations**

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| Species of Origin | Cell Line | Source | UK SKU # | Positive Controls | Source | UK SKU # |
| Chicken | CEF (Chick Embryo Fibrobalst) | Veterinary Laboratory Agency (VLA), UK | A0001025 | Canine Parainfluenza virus | ATCC VR-399 | 100000138 |
| Herpes Simplex Virus Type 1 | ATCC VR-733 | 100000955 |
| or ATCC VR-539 | 100000551 |
| Charles River Labs, SPAFAS | Not Applicable | Canine Parainfluenza virus | ATCC VR-399 | 100000138 |
| Herpes Simplex Virus Type 1 | ATCC VR-733 | 100000955 |
| or ATCC VR-539 | 100000551 |
| Duck | DEF (Duck Embryo Fibroblast) | ATCC CCL 141 | 100001828 | Herpes Simplex Virus Type 1 | ATCC VR-733 | 100000955 |
| Insect | D.Mel-2 (Drosophila) | Invitrogen | Not Applicable | Baculovirus | Invitrogen | 100002010 |
| Sf9 (Spodoptera frugiperda) | ATCC CRL 1711 | 100000838 | Baculovirus | Invitrogen | 100002010 |
| 100000837 | Baculovirus | Invitrogen | 100007994 |
| C6/36  (Ades albopictus) | ATCC CRL 1660 | 100000203 | Sindbis | ATCC VR 68 | 100001173 |
| Sf21  (Spodoptera frugiperda) | Life Technologies  (11497) | 100008339 | Baculovirus | Invitrogen | 100000837 |
| Porcine | PPK (Primary porcine kidney) | Veterinary Laboratories Agency (VLA), Surrey, UK | Not Applicable | Porcine Parvovirus (PPV) | ATCC VR-742 | 100000959 |
| Porcine Parvovirus (PPV) | Lonza | 100000959 |
| PPK (Primary porcine kidney) | Lonza | Not Applicable | Porcine Parvovirus (PPV) | ATCC VR-742 | 100000959 |
| Porcine Parvovirus (PPV) | Lonza | 100000959 |
| PT-1  (Porcine Testicle) | American Bioresearch Inc.  Seymour, Tennessee | 100007100 | PPV | ATCC VR-742 | 100000959 |
| PK-15  (Porcine kidney) | ATCC CCL 33 |  | Parainfluenza virus type 3 | ATCC VR-281 |  |
| Sponsor supplied | Not applicable | Not applicable | To be determined | To be determined | Not applicable | Sponsor supplied |

**Appendix 2: Procedure for early harvest of supernatant from positive control cultures**