Standard Operating Procedure N°1

HEALTH MONITORING ACROSS PDX CENTRES

Purpose of this SOP

The microbiological status of patient-derived cancer xenograft (PDX) models directly influences experimental variability, scientific projects and animal welfare, but also imposes a risk to the personnel in charge of the experiments. In addition, reproducibility of data will be improved by standardization of procedures and tools, including the health status and biological characteristics of the applied preclinical model.

Although the transfer of models across PDX centres should be minimized as much as possible to avoid time consuming logistical aspects and associated costs, transfers are needed within EU funded projects such as the EurOPDX Research Infrastructure (RI), and for any other collaborative project among different institutes. Therefore, we need to ensure the transfer is facilitated by standard procedures across animal facilities for screening the health status of the models at several levels during the transfer of models, their biobanking and use in *in vivo* studies.

Scope of this SOP

As an integrated part of the quality assurance system set-up in the frame of the EDIReX project work package 2 (EU Grant #731105), the present SOP represents a health monitoring program to be established to meet scientific, legal and welfare requirements.

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The health monitoring program will be applied at **four different levels** as detailed below:

❖ *Level 1:* Health monitoring of the animal facility of origin

- According to the FELASA[1] recommendations (Mähler M. *et al.*, Laboratory animals, 2014), a **general health monitoring program should be set up in each animal facility involved**, and tailored to PDX-specific and project needs, including microbiological unit definition (cage-level containment housing: IVC), the animal species and immune status (immunocompromised animals), the number of animals, the health history of the facility, the sampling and detection methods (sentinels, filters etc.) and sample analysis.
- We will keep the ***Health Status Questionnaire*** of animal facility(ies) at each node of the EurOPDX RI (and for other project partners providing PDXs – optional) and 1-year health screening history at all times available in a Dropbox folder accessible to project partners, and available to EurOPDX RI users.

Health Status Questionnaire template: 

Click here!

- **Pathogens to be screened** are listed in the Excel document included below and have been agreed as relevant for PDX intrinsic features. Accordingly, those pathogens have been **classified in different levels**: **E (Excluded)** for pathogens that no facility will accept, **P (Problematic)** for pathogens which might have an impact on the research such as *Corynebacterium bovis*, **M (Minor)** for pathogens with no expected impact on the research. It is up to each facility to accept or not pathogens P and M.

Detailed list of pathogens to be screened (1st tab "L1 screening facility"): 

Click here!

- **Frequency of screening** is determined by the biological characteristics and prevalence of the infectious agent, mainly monitored **3-monthly or annually**.

❖ *Level 2:* Health monitoring of pre-transfer PDX tissue

- **Upon transfer of a PDX model to a node of the EurOPDX RI for biobanking purpose, cryopreserved or fresh tissue will be sent to a service company which will perform a pathogen screening according to the PDX standards agreed.**
- Pathogens to be screened in this case are listed in the attached Excel file (see above, 2nd tab "screening tumour pieces").

[1] Federation of European Laboratory Animal Science Associations (www.felasa.eu)

- The human origin of the samples, PDX models and humanized mice models, implies an additional risk and need to be addressed accordingly. Although high-risk patients are mainly avoided for sampling, the majority of the current PDXs have an unknown human pathogen status and a lack of patient consent to screen for those specific microbiological components, a limitation which should be taken into account. Primary human cell lines in a passage 6 (P6) or higher are normally considered as low-risk biological material and can be treated at biosafety level 1. A similar approach can be used with PDX models. In general, irrespective of passage, PDX models and derivatives should be treated as high-risk biological material and manipulated accordingly in a laminar flow, at biosafety level 2.

❖ ***Level 3:*** **Health monitoring of *in vivo* PDX tissue for biobanking procedures**

- **To compensate for tumour sample heterogeneity in Level 2, an additional level of screening will be included during the effective biobanking of the models**. **Two options** are possible as detailed below, which will be considered equivalent:

  1. PDX tissue will be transplanted for biobanking purposes to immune deficient mice (NSG in the case of the EurOPDX RI), and in parallel in immunocompetent mice (e.g. FVB strain) for outgrowth in a quarantine unit and treated as one biological unit. After 5 weeks of implantation and possibly development of an immune response against (a) pathogen(s) in the immunocompetent mice, a blood-based screening by serology will be performed on the immunocompetent mice. Pathogens to be screened are listed in the attached Excel file (see above, 3rd tab "screening serology").

  2. PDX tissue will be transplanted for biobanking purposes to immune deficient mice (NSG in the case of the EurOPDX RI), and tumour pieces from a number of animals (at least n=3) will be monitored by PCR. In that case, to diagnose Corynebacterium bovis, skin swabs and/or feces pellets will also be sampled of immune deficient mice during outgrowth. All harvested outgrowth material will be tested by PCR.

- For each model, an **overview of all monitoring at different levels will be summarized in the "PDX Passport"**.

❖ ***Level 4:*** **Health monitoring of PDX tissue during an *in vivo* study**

- To define the microbiological status of an experimental unit, **a health screening should be performed at the end of an *in vivo* study, to monitor possible infections occurring during the study**. Tumour pieces from a number of animals (at least n=3) will be monitored by PCR.
- Pathogens to be screened are listed in the same document as mentioned above.
- Infections, apparent or inapparent, that may confound the scientific results could be diagnosed and included in the interpretation of the results.

❖ Procedure in case of positive result

- A positive result means finding a PDX sample positive for a pathogen E, P or M, independently of the animal facility health status.
- The owner of the model and originating animal facility should be notified, as well as the main EDIReX project contacts as listed below (e.g. for modification of the corresponding PDX Passport, …).
- Decision with regard to the transfer of a positive model (e.g. inclusion or not in the EurOPDX Data Portal, transfer between facilities or not for a particular project) will be taken according to the levels E/P/M defined above and discussions between representatives of the animal facilities concerned. **For the avoidance of doubt, no samples should be shipped without the explicate approval of the relevant contact at the animal facility of the receiving PDX centre.**
- In case of emerging pathogens, please notify the contact persons below for them to update the present SOP.
- On a longer term, procedures to cope with contaminated models should be discussed.

C. bovis: A procedure has been described in Manuel C.A. et al., JAALAS 2017. Procedure not validated yet across the EurOPDX Consortium members.

❖ Contact

For any question or comment about this SOP, please contact us by email at **contact@europdx.eu**.