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ITF Briefing Meeting report

PathoQuest

ITF Briefing meeting held virtually with the European Medicines Agency (EMA) on 23/02/2024.

The objective of the ITF Briefing Meetings is to provide for a preparatory discussion on scientific and regulatory topics relevant to the development of new medicinal products and technologies complementing and reinforcing existing formal procedures.

Name/identifier:	NGS Agnostic Transcriptome Assay - **iDTECT®** **Transcriptome**
Product / technology / method / methodology description:	Next Generation Sequencing. Agnostic methods of virus testing based on Next Generation Sequencing (NGS) have been developed and suggested as an alternative technology to *in vivo* testing methods not only in the different European Pharmacopeia chapters but has been strongly recommended in the revision to the ICH Q5A (R2) adopted in Nov 2023. This is a GMP validated new approach methodology (NAM) to replace the use of animals in the testing of medicines, in line with the 3Rs principles (replacement, reduction, refinement).
Intended use:	Viral Safety Testing of Medical Product

Participants**Applicant:**

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Disclaimer presented at beginning of meeting

The views expressed in this document are the opinion of the participating members of the Innovation Task Force and the experts, and may not reflect the opinion of the EMA scientific committees. Therefore, the answers provided should not be interpreted as regulatory guidance or review recommendations for an application, but as a preliminary set of scientific considerations of the information presented.

Should aspects of the subject matter discussed herein become part of a formal data submission, application, or supplement, it is at the full discretion of the appropriate working party, evaluation team or scientific committee to completely and independently assess the product(s)/technology(ies) in question.

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1. Background

<Brief description of the product/strategy/technology/proposed indication>.

iDTECT® Transcriptome is PathoQuest's Next Generation Sequencing Agnostic Transcriptome Assay (ATA) - RNA-only sequencing (RNA-Seq)

The recently adopted ICHQ5A(R2) guideline 'encourages' the replacement of *in vivo* virus testing by next generation sequencing based methods. It also states that NGS can be used to replace or supplement *in vitro* virus testing.

The **iDTECT® Transcriptome** method for virus testing has been developed and proposed as an alternative technology to traditional *in vivo* and *in vitro* testing methods.

PathoQuest's **iDTECT®** platform is a GMP validated approach designed to replace the use of animals in the testing of cells used in the production of medicines or biotechnological products, in line with the 3Rs principles of replacement, reduction, and refinement (**1**).

iDTECT® Transcriptome differs from other approaches as it detects viral RNA transcripts expressed in virus-infected cells instead of targeting viral genomes present in viral particles. This viral transcription phase is common to all families of viruses, regardless of type, and therefore viral transcripts can be used as markers of viral infection. This specific methodology enables the detection of viral infection in cells, which can be readily differentiated from viral nucleic acid carry-over with no infectivity risk.

As per ICH Q2, the validation strategy for this assay consisted of two (2) steps to meet pharmaceutical requirements; the method was initially validated by PathoQuest as a "generic platform assay" (step 1) with subsequent method adoption (step 2) to verify that assay performance is not negatively impacted by any potential matrix effect from the test sample itself. These steps are detailed below.

Step 1: the materials used for this initial validation were MRC-5 cells, a virus-free cell line (negative control) and a mix consisting of a cell line chronically infected with EBV (B95-8 cells) combined with cells infected by MuLV (Ramos cells) (positive control). The infected cell model was developed to reflect host cells naturally infected with respective viruses rather than directly spiking virus particles into a test matrix (artificial spike). The infected cell model better represents the patterns of nucleic acids synthesized during viral infections, including viral RNA transcripts that are detected using PathoQuest's transcriptomic approach.

Step 2: method adoption is performed via a reference standard approach. The assay is performed using samples artificially spiked with exogenous synthetic RNAs (controls available from the External RNA Control Consortium (ERCC)) to evaluate the interference of the matrix on the detection of viral RNAs. Detection of the ERCC spiked control sequences in the sample is compared to their detection in the companion MRC-5 and B95-8/RAMOS controls used to insure bridging with step 1.

PathoQuest's Agnostic Transcriptomic Assay "ATA" is known as **iDTECT® Transcriptome** and PathoQuest's Agnostic Viromic Assay "AVA" is known as **iDTECT® Virome.** Both assays have different method validation approaches and different applications based on their respective actions. The objective session was to discuss the application strategy of **iDTECT® Transcriptome** (for the detection of replicating viruses in cells) based on the publication data (1)**.**

2. Topics discussed

<Topic 1>

In the experts' opinion, can PathoQuest's presented iDTECT® Transcriptome replace *in vivo* and *in vitro* tests for cell banks for gene therapy products, for Active Product Ingredients and Drug Products for cell therapy and *ex vivo* gene therapies?

<Topic 2>

Can PathoQuest's presented iDTECT® Transcriptome be used to qualify cell banks (*e.g.,* master (MCB), working (WCB)) and uninfected cells (corresponding controls) at the end of production (EOPC or Limit of In Vitro Cell Age (LIVCA)) of vaccines and vectors to replace *in vivo* and *in vitro* assays?

<**Applicant's position** (e.g. experience, acceptance in the scientific community, status of development or validation efforts etc)> **for topics 1 and 2:**

In the light of the assay validation approach per ICHQ5A(R2) and ICHQ2(R2) supported by the presented head-to-head comparison study data, the applicant's position is that iDTECT® Transcriptome can replace *in vivo* and *in vitro* testing.

<ITF: Key points on topic 1 et 2>

While ITF experts shared that replacement of *in vivo* testing should be possible with iDTECT® Transcriptome, regarding *in vitro* assay a discussion was further engaged with a focus on 2 main topics:

1/ Validation strategy:

- The sustainability of the ERCC use should be followed by a periodic assessment as instability of synthesis sequences was reported in the literature. The supplier/product should be assessed for the quality of the sequences of control sequence-containing plasmids which have been certified by NIST as a reference material.
- A discussion on different Illumina NextSeq models was raised regarding their use for IVD application or for GMP application in the perspective of multiple Medical Product Testing. Indeed, GMP provides for flexibility in the selection of equipment model as input & output parameters are setup by the owner with the Computerized System Validation process according to annex 11 of the GMP.
- the iDTECT® Transcriptome validation approach was discussed and compared with the validation approach used classically by PathoQuest for **iDTECT® Virome** according to WHO https://www.who.int/publications/m/item/WHOBS2020-2394.
  The following points were resulting from this discussion:
  - iDTECT® Transcriptome detects only transcripts produced by infected cells in a cellular Test Article,
  - i.e for iDTECT® Transcriptome, the matrix interference is resulting from the capacity of the test to overcome the background of RNAs issued from infected cells and RNAs from other origins (like the viruses' carry-over or ribosomal RNA for example),

Subsequently, if virus particles are spiked in cell lysates (like in the context of **iDTECT® Virome)** without enabling cell infection, no signal will be detected.

- Considering these elements, the ERCC approach was proposed as validation approach supported by the head-to-head comparison study where different infection phases were simulated during the sample preparation as described in the presented study: Evaluation of a viral transcriptome Next Generation Sequencing assay as an alternative to animal assays for viral safety testing of cell substrates - ScienceDirect

  9 virus infected cell lines were inoculated to assess the transcripts subsequently produced by the cells:

Viruses and cell lines used in the current and Gombold *et al.* studies [17].

Virus	Study	Strain	Virus family	Genome	Source	Propagation/Detection cell line	Cell source
Influenza A	J.Gombold et al.	A/PR/8/34	Orthomyxoviridae	ssRNA (-)	ATCC VR-1469	MDCK	ATCC CCL-34
	H:H Comparison study	A/PR/8/35			ATCC VR-1470	MDCK	ATCC CCL-34
VSV	J.Gombold et al.	Indiana	Rhabdoviridae	ssRNA (-)	CRL 4–705-111	Vero / Vero 76	ATCC CCL-81 / ATCC CRL-1587
	H:H Comparison study	Indiana			ATCC VR-1238	Vero 76	ATCC CRL-1587
HSV	J.Gombold et al.	McIntyre	Herpesviridae	dsDNA	CRL 1–575-94	Vero / Vero 76	ATCC CCL-81 / ATCC CRL-1587
	H:H Comparison study	McIntyre			ATCC VR-539	Vero 76	ATCC CRL-1587
Cox B3	J.Gombold et al.	–	Picornaviridae	ssRNA (+)	CRL 1–1240-177	LLC-MK2	ATCC CCL-7
	H:H Comparison study	Nancy			ATCC VR-30	Vero 76	ATCC CRL-1587
Cox A16	J.Gombold et al.	–	Picornaviridae	ssRNA (+)	Zeptometrix 0810107CF	Vero	ATCC CCL-81
	H:H Comparison study	–			Zeptometrix 0810107CF	Vero 76	ATCC CRL-1587
Mumps	J.Gombold et al.	Enders	Paramyxoviridae	ssRNA (-)	CRL 1–1240-150	Vero	ATCC CCL-81
	H:H Comparison study	Enders			ATCC VR-106	Vero 76	ATCC CRL-1587
Measles	J.Gombold et al.	Edmonston	Paramyxoviriadae	ssRNA (-)	CRL 1–647-74	Vero	ATCC CCL-81
	H:H Comparison study	Edmonston			ATCC VR-24	Vero 76	ATCC CRL-1587
Echo 11	J.Gombold et al.	Gregory	Picornaviridae	ssRNA (+)	CRL 1–240-181	LLC-MK2	ATCC CCL-7
	H:H Comparison study	Gregory			ATCC VR-41	Vero 76	ATCC CRL-1587
BVDV 1	J.Gombold et al.	New York 1	Flaviviriadae	ssRNA (+)	CRL 647–59-111502	BT	ATCC CRL-1390
	H:H Comparison study	New York 1			ATCC VR-1561	BT	ATCC CRL-1390

H:H is Head to Head comparison.

Based on this supportive data combined with

- the iDTECT® Transcriptome validation strategy,
- and ERCC use as referent standard to assess the matrix interference for each Test Article,

the applicant's position is that iDTECT® Transcriptome can facilitate the replacement for *in vivo and in vitro* testing.

The relevance of the spiking of virus particles in cell lysates (used for iDTECT® Virome validation) versus the use of infected cells (as for iDTECT® Transcriptome validation) and their respective application were discussed.

A last option was raised to make available additional supporting data regarding the range of detection by spiking different viral synthetic RNAs encompassing viral variability within each species at different concentrations to assess the variability of the LOD.

2/ Place of iDTECT® Transcriptome in the NGS strategy:

It was highlighted that iDTECT® Transcriptome is designed to detect viral transcriptomic pattern if the cell tested are infected. This limitation can be a limit of iDTECT® Transcriptome application if live DNA viruses unable to infect production cells but able to infect humans are introduced through raw materials.

The applicant answered that:

1/ any process can be contaminated by live viruses, even those that are unable to amplify viruses (like bacterial products that use peptones and either proteins as raw material in medium). Nevertheless, viral testing is only mandatory for cell-based product because they are able to replicate and amplify viral contaminants. Such risk of replication/amplification is targeted by iDTECT® Transcriptome.

2/testing of raw materials like FBS can be done either directly with the viromic assay iDTECT® Virome on FBS (at the risk of detecting inactivated viruses) or indirectly with the transcriptomic assay on cells (bovine and/or human cells) cultivated in presence of FBS.

ICHQ5A(R2) states that the *in vitro* cell culture infectivity assays have general limitations e.g. susceptibility of cell lines to infection and specific limitations of the production system.

The classical 3-cell line *in vitro* virus assay is not designed to detect a full range of (bovine) viruses. This is why it is often supplemented by the 9CFR *in vitro* test for cell bank characterisation. Also, manufacturers typically use FBS that has been tested for viruses (as per 9CFR, CPMP and EP documents) and irradiated according to a validated process.

The *in vitro* assay is also often supplemented with PCR testing to detect MVM and other species-specific viruses.

Taking all together and based on case-by-case, the iDTECT® Transcriptome is an appropriate approach to test cellular sample as cells used in the production of biological products or ATMPs based on human eukaryotes cell therapy or *ex vivo* gene therapy while the lack of cell infection is important for the safety of the product. Furthermore, the alternative propositions are limited.

<Topic 3>

In the experts' opinion, can iDTECT® Transcriptome be used to replace *in vivo* and *in vitro* testing of cell banks (MCB, WCB, EOPC, LIVCA) used in the manufacturing of recombinant proteins without testing isolated medium?

<**Applicant's position** (e.g. experience, acceptance in the scientific community, status of development or validation efforts etc)>

For *in vitro* testing of non-purified bulk harvests, the ICH guidelines R1 require testing of both cells and culture medium, as infectious particles can be released into the medium or remain associated with the cells depending on the virus, in the event of an infection or viral contamination. The iDTECT® Transcriptome approach, which did not exist when the ICH Q5A(R1) guidance was first published, makes it possible to identify all viruses infecting the production cells, by specifically detecting the RNA phase of a replicating virus, as detailed above, therefore mitigating the need to assess the culture medium itself, and streamlining the assay. As mentioned by ICHQ5A guideline R2 (extract below), this is considered as part of the rational for selecting sample.

Extract from ICHQ5A **R2** 3.2.5.2 next generation sequencing:

When analysing cell culture-derived materials, nucleic acids prepared from cells are used for genomics and transcriptomics while cell culture supernatants or cell free virus preparations are used for the viromics. **The rationale for selecting these different strategies should be provided.**

<ITF: Key points on topic 3>

In case of recombinant product testing, the importance of the sampling strategy in continuous fermentation processes was discussed and the opportunity to test cells at different time of the fermentation as a monitoring tool was addressed.

It was said by ITF experts that as soon as live cells are available, iDTECT® Transcriptome can be relevant to test Bulk Harvest.

(1) Beurdeley-Fehlbaum P. *et al*. Evaluation of a viral transcriptome Next Generation Sequencing assay as an alternative to animal assays for viral safety testing of cell substrates, *Vaccine* 41(37) 5383-5391 (2023). https://doi.org/10.1016/j.vaccine.2023.07.019.
Link to publication: Evaluation of a viral transcriptome Next Generation Sequencing assay as an alternative to animal assays for viral safety testing of cell substrates - ScienceDirect