
Evidence Based Toxicology Collaboration (EBTC) In Vitro Critical Appraisal Tool (IV-CAT): Tool Development Protocol

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Acknowledging the input of EBTC staff and EBTC In Vitro WG

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IV-CAT Protocol

Draft 1.0 | 8 October 2018

Procedural Notes

The reporting of this systematic review protocol (part 1 of the project) adheres to items 1 through 18 of the *Environment International* modified PRISMA-P statement for environmental health research, insofar as they can be interpreted as applying to systematic reviews of critical appraisal tools for evaluating the quality of *in vitro* research and any standards for reporting thereof.

The EBTC Scientific Advisory Council were consulted for comment and approval prior to publication of this protocol. Any disputes were arbitrated by the Working Group chairs RdV and PW. The protocol was reviewed by the EBTC Board of Trustees and signed off by EBTC's Executive Director, Dr Katya Tsaion. After sign-off, this protocol was published in the Zonodo.org repository, prior to commencement of screening the search results for the systematic review.

Part 4 of the project (developing the release version of the tool) will be articulated in more detail, if necessary, at a later date when more information about the structure of the tool and its forecast usability is available.

Overall project structure and plan

Context

In vitro studies are becoming an increasingly important source of data in chemical risk assessment. There are concerns, however, about the methodological quality of these studies and multiple interventions being undertaken to improve them. These interventions to improve study quality can focus on three target groups: (1) researchers designing, conducting and reporting primary *in vitro* exposure studies, (2) peer reviewers of journals advising on whether to publish a submitted manuscript and (3) authors of systematic reviews aiming to assess the risk of bias/study quality of the primary *in vitro* studies included in their review. Given that there are already interventions available or being developed for target groups (1) and (3), we here focus on target group (2).

Objective

The objective is to develop a tool, IV-CAT (In-Vitro Critical Appraisal Tool) which will help ensure comprehensive and exacting peer-review of *in vitro* toxicology studies, to increase the quality (understood as “fitness-for-purpose”) of published *in vitro* research.

We define “fit for purpose” as meeting a minimum acceptable standard for publication as determined by consensus of a group of expert practitioners. The standard for publication includes the potential value of the study to scientific progress and/or decision-making, whether the study can be considered to have produced credible results, whether the results have been appropriately presented and interpreted, and whether the study has been reported in sufficient detail to permit critical appraisal and potential replication by third parties.

We define “*in vitro* research” as a study in which living parts of animals (including humans) up to and including the level of organisation of tissues, but not whole organs, are exposed to chemicals in order to detect adverse effects. Our definition is intended to exclude: (1) efficacy studies, (2) whole embryo and *ex vivo* studies (in which whole organs are removed and studied outside the animal’s body) and (3) viral toxicity studies. Our definition includes: studies in cell cultures (including stem cell and organo-typic cultures), and tissue slices. Studies into the safety of drugs (pharmaceutical toxicology) as well as biological effects of exposure to chemical substance (environmental toxicology) are both included for potential appraisal by IV-CAT.

Overview of methods

The development of IV-CAT will proceed in three steps: (1) the development of an initial draft version of the tool for testing and feature development; (2) comprehensive testing and iterated improvement of the draft tool to develop a release version of the tool; (3) and publication of the release version of the tool and accompanying manuscript.

The draft version will be developed by:

1. Systematically reviewing existing critical appraisal tools and reporting standards for *in vitro* research for potential criteria which could be included in IV-CAT;
2. Consulting with field experts as to the “ideal” structure of an *in vitro* study, to determine a conceptual model for organising the potential criteria identified from the SR into a provisional appraisal structure;
3. Employing a Delphi process to select from the existing criteria which are most important for use in a tool, and identify any potential missing criteria;
4. Creating a draft of the tool based on the results of Step 4 and an iterated testing and development process.

If budget permits, an online version of the tool will be created, adapting software being developed by PW for a critical appraisal tool evaluating publishability of environmental health systematic reviews.

Part 1: Systematic review of existing *in vitro* CATS

Title

Quality Constructs Applied by Critical Appraisal Tools for In Vitro Toxicology Studies: A Systematic Review Protocol. This is a protocol for a systematic review of the tools and criteria used or proposed for appraising the quality of *in vitro* toxicological research. It is not an update of an existing systematic review.

Registration

Publish protocol in Zenodo.org. No pre-registration of protocol.

Authors

Primary authors: RdV and PW. Guarantor: Katya Tsaïoun, EBTC

Support

The project is funded as a project of the Evidence Based Toxicology Collaboration at Johns Hopkins University Bloomberg School of Public Health. PW was a consultant to EBTC from July 1 2017 to June 30 2018 and an employee of Lancaster University, with salary funded by EBTC, from 2 July 2018 to 1 July 2019. RdV is an employee of Radboud UMC seconded to JHU BSPH. RdV and PW developed the project plan in consultation with EBTC staff, the EBTC Risk of Bias and Publishing Standards Working Group, and the EBTC Scientific Advisory Council. Final approval of the project was from the EBTC Board.

Objectives

Survey the criteria which have been proposed in critical appraisal tools as characteristics of good quality *in vitro* research and study reports. “Good quality” is understood in its broadest sense, with reviewers extracting whatever quality constructs (desirable characteristics of an *in vitro* research project or study report) are offered in any of the included literature, according to the operating or implied definitions in the included tools.

“Good quality” for the purpose of identifying quality criteria in the SR is distinguished from the concept of being “fit for purpose”, the latter being the final set of characteristics most relevant to appraising the publishability of a study as determined by the consensus process of this project.

Eligibility criteria

Inclusion criteria

- Documents which either present or utilise a set of formally-specified criteria for appraising post-conduct the quality of planning, conduct and/or reporting of an in vitro study, via critical appraisal of study reports (“critical appraisal tools”).
- Documents which present a formally-specified set of characteristics which should be included in a report of an in vitro study (“reporting standards”)
- English language
- Any date
- Any publication status

Exclusion criteria

- Exclude documents which give guidance or define standards for the conduct of in vitro studies without presenting a critical appraisal tool or reporting standard.
- Exclude but hold for hand-searching documents which review or survey critical appraisal tools and/or reporting standards for in vitro research (“reviews of critical appraisal tools and/or reporting standards”)

Explanatory Notes for Eligibility Criteria

“Formally specified” means criteria which are explicitly listed rather than implicit in a narrative critique or appraisal of a piece of research, e.g. a framework or checklist.

Documents which describe standards or give guidance for the conduct of in vitro studies are to be excluded because they do not directly present quality criteria for distinguishing higher quality studies from lower quality ones. While the standards are based on an implicit understanding of what constitutes high-quality research, these criteria must be inferred from the standard. This requires the review authors to derive criteria from the standard, i.e. to create an appraisal tool based on the standard. The tool would then be the work of the review authors and not a tool which would necessarily be endorsed by or represent the intent of the authors of the original standard or guidance. We therefore do not think it appropriate to include such standards in the systematic review.

We consider it unlikely that critical appraisal tools will be published only in a non-English language, so the data set we generate from our search and selection strategy should be

sufficiently comprehensive to provide sound basis for a critical appraisal tool. Any criteria missing from our data set should be identified via our Delphi process; given this process will include participants from different countries, we believe our final set of potential criteria should be sufficiently comprehensive without having to resource a comprehensive non-English search strategy.

Information Sources

We will search PubMed, Embase, and Web of Science for reporting standards and critical appraisal tools. We will not conduct a specific grey literature search, as we believe that searching the reference lists of reviews of critical appraisal tools we find in our literature search will provide sufficient coverage of appraisal tools to populate a comprehensive dataset of proposed quality criteria. We acknowledge that our approach will not locate any uncited tools which have not been indexed in the three literature databases we are searching.

We will create a concept saturation curve, which keeps track of the number of new concepts each time a tool is added, and see if it is tailing off. If so, then it is highly unlikely that the tools not found by our search strategy would add to the concepts we already have.

Search Strategy

Search Strategy Notes

Each search consists of two strategies: one to identify systematic reviews of in vitro studies (as a source of critical appraisal tools for primary studies); and a second search to identify guidelines for conduct or appraisal of in vitro studies (while guidelines will be excluded at point of screening, for the search to be sufficiently sensitive they are included as a search concept). The two strategies are combined with an OR operator.

The PubMed SR search strategy uses the systematic review filter from Shojania KG, Bero LA. "Taking advantage of the explosion of systematic reviews: an efficient MEDLINE search strategy." *Eff Clin Pract.* 2001 Jul-Aug;4(4):157-62. [[PMID: 11525102](#)]. The Embase SR search strategy adapts a search strategy from Wilczynski NL, Haynes RB, Hedges Team, exchanging a small drop in specificity for a large increase in sensitivity. "[EMBASE search strategies achieved high sensitivity and specificity for retrieving methodologically sound systematic reviews.](#)" *J Clin Epidemiol.* 2007;60(1):29-33). The Web of Science SR search strategy is a translation of the Embase strategy.

PubMed Search Strategy

PubMed Search Part 1 – Systematic Reviews of in vitro Studies.

((meta-analysis[pt] OR meta-analysis[tw] OR metanalysis[tw]) OR ((review[pt] OR guideline[pt] OR consensus[ti] OR guideline*[ti] OR literature[ti] OR overview[ti] OR review[ti]) AND ((Cochrane[tw] OR Medline[tw] OR CINAHL[tw] OR (National[tw] AND Library[tw])) OR (handsearch*[tw] OR search*[tw] OR searching[tw]) AND (hand[tw] OR manual[tw] OR electronic[tw] OR bibliographi*[tw] OR database* OR (Cochrane[tw] OR Medline[tw] OR CINAHL[tw] OR (National[tw] AND Library[tw]))))) OR ((synthesis[ti] OR overview[ti] OR review[ti] OR survey[ti]) AND (systematic[ti] OR critical[ti] OR methodologic[ti] OR quantitative[ti] OR qualitative[ti] OR literature[ti] OR evidence[ti] OR evidence-based[ti])) NOT (case*[ti] OR report[ti] OR editorial[pt] OR comment[pt] OR letter[pt])

AND

"In Vitro Techniques"[Mesh] OR "Organoids"[Mesh] OR "Diffusion Chambers, Culture"[Mesh] OR "Animal Testing Alternatives"[Mesh] OR "High-Throughput Screening Assays"[Mesh] OR animal testing alternative*[tw] OR alternative to animal testing[tw] OR alternatives to animal testing[tw] OR animal use alternative*[tw] OR "animal testing reduction"[tw] OR "animal testing reductions"[tw] OR "animal testing refinement"[tw] OR "animal testing refinements"[tw] OR "animal testing replacement"[tw] OR "animal testing replacements"[tw] OR in vitro[tw] OR invitro[tw] OR ex vivo[tw] OR culture technique*[tw] OR axenic culture*[tw] OR aseptic culture*[tw] OR sterile culture*[tw] OR cell culture*[tw] OR batch culture*[tw] OR shake-flask culture*[tw] OR cell engineer*[tw] OR cellular engineer*[tw] OR tissue engineer*[tw] OR organoid*[tw] OR culture diffusion chamber*[tw] OR semi-permeable chamber*[tw] OR semipermeable chamber*[tw] OR tissue cage*[tw] OR cocultur*[tw] OR co-cultur*[tw] OR cocultivation*[tw] OR co-cultivation*[tw] OR continuous culture*[tw] OR dissociated culture*[tw] OR "culture of dissociated cells"[tw] OR "cultures of dissociated cells"[tw] OR microcarrier culture*[tw] OR monolayer culture*[tw] OR primary culture*[tw] OR primary cell colon*[tw] OR primary cell system*[tw] OR suspension culture*[tw] OR synchronous culture*[tw] OR xeno-free culture*[tw] OR "xenofree culture"[tw] OR "xenofree cultures"[tw] OR "xenogeneic-free culture"[tw] OR "xenogeneic-free cultures"[tw] OR erythrocyte culture*[tw] OR cultured erythrocytes[tw] OR cultured erythroid cells[tw] OR cultured red blood cells[tw] OR RBC culture*[tw] OR fibroblast culture*[tw] OR yeast culture*[tw] OR astrocyte culture*[tw] OR microglial culture*[tw] OR oligodendrocyte culture*[tw] OR heart culture*[tw] OR "heart myoblast culture"[tw] OR "heart myoblast cultures"[tw] OR kidney culture*[tw] OR leukocyte culture*[tw] OR cultured leucocytes[tw] OR cultured leukocytes[tw] OR cultured white blood cells[tw] OR leucocyte culture*[tw] OR lymphocyte culture*[tw] OR cultured lymphocytes[tw] OR NK culture*[tw] OR macrophage culture*[tw] OR monocyte culture*[tw] OR hepatocyte culture*[tw] OR liver culture*[tw] OR mixed culture*[tw] OR muscle fiber culture*[tw] OR muscle culture*[tw] OR myocyte culture*[tw] OR cultured nerve cells[tw] OR cultured neurons[tw] OR nerve culture*[tw] OR neuron culture*[tw] OR neuronal culture*[tw] OR cortical culture*[tw] OR cultured brain cells[tw] OR cultured brain neurons[tw] OR cultured cerebral cells[tw] OR cultured cerebral neurons[tw] OR cultured cortical cells[tw] OR "cultured cortical nerve cells"[tw] OR

cultured cortical neurons[tw] OR cultured hippocampal neurons[tw] OR cultured hippocampus neurons[tw] OR "cultured spinal cells"[tw] OR cultured spinal cord cells[tw] OR "cultured spinal cord nerve cells"[tw] OR cultured spinal cord neurons[tw] OR cultured spinal neurons[tw] OR spinal cord culture*[tw] OR spinal culture*[tw] OR protoplast culture*[tw] OR feeder cell*[tw] OR feeder layer*[tw] OR primary cell*[tw] OR cultured tumor cells[tw] OR cultured tumour cells[tw] OR tumor culture*[tw] OR tumour culture*[tw] OR cancer culture*[tw] OR tumor spheroid*[tw] OR tumour spheroid*[tw] OR embryo culture*[tw] OR blastocyst culture*[tw] OR organ culture*[tw] OR tissue culture*[tw] OR bone marrow culture*[tw] OR skin culture*[tw] OR slice culture*[tw] OR high-throughput screen*[tw] OR high-throughput drug screen*[tw] OR high-throughput biological assay*[tw] OR high-throughput chemical assay*[tw]

5,477 results, 7/30/19

PubMed Search Part 2 – Guidelines/Research Design/in vitro Studies

"Guidelines as Topic"[Mesh:NoExp] OR "Validation Studies as Topic"[Mesh] OR "Validation Studies" [Publication Type] OR guideline*[tw] OR validation*[tw] OR tool*[tw] OR checklist*[tw] OR check list*[tw] OR guidance[tw] OR best practice*[tw] OR recommendation*[tw] OR requirement*[tw] OR criterion[tw] OR criteria[tw]

AND

"Research Design"[Mesh:NoExp] OR "Control Groups"[Mesh] OR "Random Allocation"[Mesh] OR "Sample Size"[Mesh:NoExp] OR "Data Accuracy"[Mesh] OR "Dimensional Measurement Accuracy"[Mesh] OR "Quality Control"[Mesh] OR "Bias"[Mesh] OR "Peer Review"[Mesh:NoExp] OR "Peer Review, Research"[Mesh] OR study design[tw] OR study designing[tw] OR study designs[tw] OR research design[tw] OR research designing[tw] OR research designs[tw] OR research strateg*[tw] OR error source*[tw] OR scoring method*[tw] OR matched group*[tw] OR research technique*[tw] OR research technic[tw] OR research technics[tw] OR research method*[tw] OR problem formulation*[tw] OR experimental design*[tw] OR methodological research[tw] OR research instrument[tw] OR research instruments[tw] OR research protocol*[tw] OR control group*[tw] OR blind[tw] OR blinded[tw] OR blindedness[tw] OR blinding[tw] OR blindly[tw] OR masked[tw] OR random allocation*[tw] OR randomization*[tw] OR randomisation*[tw] OR sample size*[tw] OR minimal data set*[tw] OR minimum data set*[tw] OR experimental error*[tw] OR scientific error*[tw] OR experimental mistake*[tw] OR scientific mistake*[tw] OR research error*[tw] OR measurement accurac*[tw] OR quality control*[tw] OR good laboratory practice*[tw] OR proficiency test*[tw] OR bias[tw] OR biases[tw] OR analytic error*[tw] OR analytical error*[tw] OR analysis error*[tw] OR "research mistake"[tw] OR "research mistakes"[tw] OR measurement error*[tw] OR random error*[tw] OR sampling error*[tw] OR sample error*[tw] OR ecological fallac*[tw] OR observer variation*[tw] OR interobserver variation*[tw] OR inter-observer variation*[tw] OR interobserver variabilit*[tw] OR inter-observer variabilit*[tw] OR intraobserver

variation*[tw] OR intra-observer variation*[tw] OR intraobserver variabilit*[tw] OR intra-observer variabilit*[tw] OR systematic error*[tw] OR peer review*[tw] OR study quality[tw] OR study qualities[tw] OR data accuracy[tw] OR data quality[tw] OR quality appraisal*[tw] OR study appraisal*[tw] OR research quality[tw] OR evidence quality[tw] OR quality of evidence[tw] OR validity[tw] OR multitrait multimethod*[tw] OR multi-trait multi-method*[tw] OR MTMM[tw] OR reliability[tw] OR quality assessment*[tw] OR quality assurance*[tw] OR quality management[tw] OR reporting[tw]

AND

"In Vitro Techniques"[Mesh] OR "Organoids"[Mesh] OR "Diffusion Chambers, Culture"[Mesh] OR "Animal Testing Alternatives"[Mesh] OR "High-Throughput Screening Assays"[Mesh] OR animal testing alternative*[tw] OR alternative to animal testing[tw] OR alternatives to animal testing[tw] OR animal use alternative*[tw] OR "animal testing reduction"[tw] OR "animal testing reductions"[tw] OR "animal testing refinement"[tw] OR "animal testing refinements"[tw] OR "animal testing replacement"[tw] OR "animal testing replacements"[tw] OR in vitro[tw] OR invitro[tw] OR ex vivo[tw] OR culture technique*[tw] OR axenic culture*[tw] OR aseptic culture*[tw] OR sterile culture*[tw] OR cell culture*[tw] OR batch culture*[tw] OR shake-flask culture*[tw] OR cell engineer*[tw] OR cellular engineer*[tw] OR tissue engineer*[tw] OR organoid*[tw] OR culture diffusion chamber*[tw] OR semi-permeable chamber*[tw] OR semipermeable chamber*[tw] OR tissue cage*[tw] OR cocultur*[tw] OR co-cultur*[tw] OR cocultivation*[tw] OR co-cultivation*[tw] OR continuous culture*[tw] OR dissociated culture*[tw] OR "culture of dissociated cells"[tw] OR "cultures of dissociated cells"[tw] OR microcarrier culture*[tw] OR monolayer culture*[tw] OR primary culture*[tw] OR primary cell colon*[tw] OR primary cell system*[tw] OR suspension culture*[tw] OR synchronous culture*[tw] OR xeno-free culture*[tw] OR "xenofree culture"[tw] OR "xenofree cultures"[tw] OR "xenogeneic-free culture"[tw] OR "xenogeneic-free cultures"[tw] OR erythrocyte culture*[tw] OR cultured erythrocytes[tw] OR cultured erythroid cells[tw] OR cultured red blood cells[tw] OR RBC culture*[tw] OR fibroblast culture*[tw] OR yeast culture*[tw] OR astrocyte culture*[tw] OR microglial culture*[tw] OR oligodendrocyte culture*[tw] OR heart culture*[tw] OR "heart myoblast culture"[tw] OR "heart myoblast cultures"[tw] OR kidney culture*[tw] OR leukocyte culture*[tw] OR cultured leucocytes[tw] OR cultured leukocytes[tw] OR cultured white blood cells[tw] OR leucocyte culture*[tw] OR lymphocyte culture*[tw] OR cultured lymphocytes[tw] OR NK culture*[tw] OR macrophage culture*[tw] OR monocyte culture*[tw] OR hepatocyte culture*[tw] OR liver culture*[tw] OR mixed culture*[tw] OR muscle fiber culture*[tw] OR muscle culture*[tw] OR myocyte culture*[tw] OR cultured nerve cells[tw] OR cultured neurons[tw] OR nerve culture*[tw] OR neuron culture*[tw] OR neuronal culture*[tw] OR cortical culture*[tw] OR cultured brain cells[tw] OR cultured brain neurons[tw] OR cultured cerebral cells[tw] OR cultured cerebral neurons[tw] OR cultured cortical cells[tw] OR "cultured cortical nerve cells"[tw] OR cultured cortical neurons[tw] OR cultured hippocampal neurons[tw] OR cultured hippocampus

neurons[tw] OR "cultured spinal cells"[tw] OR cultured spinal cord cells[tw] OR "cultured spinal cord nerve cells"[tw] OR cultured spinal cord neurons[tw] OR cultured spinal neurons[tw] OR spinal cord culture*[tw] OR spinal culture*[tw] OR protoplast culture*[tw] OR feeder cell*[tw] OR feeder layer*[tw] OR primary cell*[tw] OR cultured tumor cells[tw] OR cultured tumour cells[tw] OR tumor culture*[tw] OR tumour culture*[tw] OR cancer culture*[tw] OR tumor spheroid*[tw] OR tumour spheroid*[tw] OR embryo culture*[tw] OR blastocyst culture*[tw] OR organ culture*[tw] OR tissue culture*[tw] OR bone marrow culture*[tw] OR skin culture*[tw] OR slice culture*[tw] OR high-throughput screen*[tw] OR high-throughput drug screen*[tw] OR high-throughput biological assay*[tw] OR high-throughput chemical assay*[tw]

8,832 results, 7/29/18

PubMed Search, Part 1 OR Part 2

13,715 results, 7/30/18

Embase Search Strategy

Embase Search Part 1 – Systematic Reviews of in vitro Studies

'meta-analysis':ab,ti OR 'systematic review':ab,ti OR MEDLINE:ab,ti

AND

'in vitro study'/de OR 'cell, tissue or organ culture'/exp OR 'shake flask culture'/exp OR 'ex vivo study'/exp OR 'cell engineering'/exp OR 'tissue engineering'/exp OR 'organoid'/exp OR 'diffusion chamber'/exp OR 'tissue cage'/exp OR 'embryo culture'/exp OR 'animal testing alternative'/exp OR 'high throughput screening'/exp OR ('animal testing alternative*' OR 'alternative* to animal testing' OR 'animal use alternative*' OR 'animal testing reduction*' OR 'animal testing refinement*' OR 'animal testing replacement*' OR 'in vitro' OR 'invitro' OR 'ex vivo' OR 'culture technique*' OR 'axenic culture*' OR 'aseptic culture*' OR 'sterile culture*' OR 'cell culture*' OR 'batch culture*' OR 'shake-flask culture*' OR 'cell engineer*' OR 'cellular engineer*' OR 'tissue engineer*' OR organoid* OR 'diffusion chamber*' OR 'semi-permeable chamber*' OR 'semipermeable chamber*' OR 'tissue cage*' OR cocultur* OR 'co-cultur*' OR cocultivation* OR 'co-cultivation*' OR 'continuous culture*' OR 'dissociated culture*' OR 'culture of dissociated cells' OR 'cultures of dissociated cells' OR 'enrichment culture*' OR 'microcarrier culture*' OR 'monolayer culture*' OR 'primary culture*' OR 'primary cell colon*' OR 'primary cell system*' OR 'suspension culture*' OR 'synchronous culture*' OR 'xeno-free culture*' OR 'xenofree culture*' OR 'xenogeneic-free culture*' OR 'erythrocyte culture*' OR 'cultured erythrocytes' OR 'cultured erythroid cells' OR 'cultured red blood cells' OR 'RBC culture*' OR 'fibroblast culture*' OR 'yeast culture*' OR 'astrocyte culture*' OR 'microglial culture*' OR 'oligodendrocyte culture*' OR 'heart culture*' OR 'heart myoblast culture*' OR 'kidney culture*' OR 'leukocyte culture*' OR 'cultured leucocytes' OR

'cultured leukocytes' OR 'cultured white blood cells' OR 'leucocyte culture*' OR 'lymphocyte culture*' OR 'cultured lymphocytes' OR 'NK culture*' OR 'macrophage culture*' OR 'monocyte culture*' OR 'hepatocyte culture*' OR 'liver culture*' OR 'mixed culture*' OR 'muscle fiber culture*' OR 'muscle culture*' OR 'myocyte culture*' OR 'cultured nerve cells' OR 'cultured neurons' OR 'nerve culture*' OR 'neuron culture*' OR 'neuronal culture*' OR 'cortical culture*' OR 'cultured brain cells' OR 'cultured brain neurons' OR 'cultured cerebral cells' OR 'cultured cerebral neurons' OR 'cultured cortical cells' OR 'cultured cortical nerve cells' OR 'cultured cortical neurons' OR 'cultured hippocampal neurons' OR 'cultured hippocampus neurons' OR 'cultured spinal cells' OR 'cultured spinal cord cells' OR 'cultured spinal cord nerve cells' OR 'cultured spinal cord neurons' OR 'cultured spinal neurons' OR 'spinal cord culture*' OR 'spinal culture*' OR 'protoplast culture*' OR 'feeder cell*' OR 'feeder layer*' OR 'primary cell*' OR 'cultured tumor cells' OR 'cultured tumour cells' OR 'tumor culture*' OR 'tumour culture*' OR 'cancer culture*' OR 'tumor spheroid*' OR 'tumour spheroid*' OR 'embryo culture*' OR 'blastocyst culture*' OR 'organ culture*' OR 'tissue culture*' OR 'bone marrow culture*' OR 'skin culture*' OR 'slice culture*' OR 'high-throughput screen*' OR 'high-throughput drug screen*' OR 'high-throughput biological assay*' OR 'high-throughput chemical assay*'):ab,de,kw,lnk,ti

5,207 results, 7/30/19

Part 2 – Guidelines/Research Design/in vitro Studies

'validation study'/exp OR (guideline* OR validation* OR tool* OR checklist* OR 'check list*' OR guidance OR 'best practice*' OR recommendation* OR requirement* OR criterion OR criteria):ab,de,kw,lnk,ti

AND

'study design'/exp OR 'matched groups'/exp OR 'problem formulation'/exp OR 'methodology'/de OR 'control group'/exp OR 'experimental design'/exp OR 'randomization'/de OR 'sample size'/exp OR 'measurement accuracy'/exp OR 'data quality'/exp OR 'quality control'/de OR 'good laboratory practice'/exp OR 'statistical bias'/exp OR 'confirmation bias'/exp OR 'analytical error'/exp OR 'experimental error'/exp OR 'measurement error'/exp OR 'random error'/exp OR 'sampling error'/exp OR 'systematic error'/de OR 'error sources'/exp OR 'peer review'/exp OR 'validity'/exp OR 'reliability'/de OR 'interrater reliability'/exp OR 'intrarater reliability'/exp OR 'test retest reliability'/exp OR 'reporting'/exp OR ('study design' OR 'study designing' OR 'study designs' OR 'research design' OR 'research designing' OR 'research designs' OR 'research strateg*' OR 'error source*' OR 'scoring method*' OR 'matched group*' OR 'research technique*' OR 'research technic' OR 'research technics' OR 'research method*' OR 'problem formulation*' OR 'experimental design*' OR 'methodological research' OR 'research instrument' OR 'research instruments' OR 'research protocol*' OR 'control group*' OR blind OR blinded OR blindedness OR blinding OR blindly OR masked OR 'random allocation*' OR randomization* OR randomisation*

OR 'sample size*' OR 'minimal data set*' OR 'minimum data set*' OR 'experimental error*' OR 'scientific error*' OR 'experimental mistake*' OR 'scientific mistake*' OR 'research mistake*' OR 'research error*' OR 'measurement accurac*' OR 'quality control*' OR 'good laboratory practice*' OR 'proficiency test*' OR bias OR biases OR 'analytic* error*' OR 'analysis error*' OR 'measurement error*' OR 'random error*' OR 'sampling error*' OR 'sample error*' OR 'ecological fallac*' OR 'observer variation*' OR 'interobserver variation*' OR 'inter-observer variation*' OR 'interobserver variabilit*' OR 'inter-observer variabilit*' OR 'intraobserver variation*' OR 'intra-observer variation*' OR 'intraobserver variabilit*' OR 'intra-observer variabilit*' OR 'systematic error*' OR 'peer review*' OR 'study quality' OR 'study qualities' OR 'data accuracy' OR 'data quality' OR 'quality appraisal*' OR 'study appraisal*' OR 'research quality' OR 'evidence quality' OR 'quality of evidence' OR validity OR 'multitrait multimethod*' OR 'multi-trait multi-method*' OR MTMM OR reliability OR 'quality assessment*' OR 'quality assurance*' OR 'quality management' OR reporting);ab,de,kw,lnk,ti

AND

'in vitro study'/de OR 'cell, tissue or organ culture'/exp OR 'shake flask culture'/exp OR 'ex vivo study'/exp OR 'cell engineering'/exp OR 'tissue engineering'/exp OR 'organoid'/exp OR 'diffusion chamber'/exp OR 'tissue cage'/exp OR 'embryo culture'/exp OR 'animal testing alternative'/exp OR 'high throughput screening'/exp OR ('animal testing alternative*' OR 'alternative* to animal testing' OR 'animal use alternative*' OR 'animal testing reduction*' OR 'animal testing refinement*' OR 'animal testing replacement*' OR 'in vitro' OR 'invitro' OR 'ex vivo' OR 'culture technique*' OR 'axenic culture*' OR 'aseptic culture*' OR 'sterile culture*' OR 'cell culture*' OR 'batch culture*' OR 'shake-flask culture*' OR 'cell engineer*' OR 'cellular engineer*' OR 'tissue engineer*' OR organoid* OR 'diffusion chamber*' OR 'semi-permeable chamber*' OR 'semipermeable chamber*' OR 'tissue cage*' OR cocultur* OR 'co-cultur*' OR cocultivation* OR 'co-cultivation*' OR 'continuous culture*' OR 'dissociated culture*' OR 'culture of dissociated cells' OR 'cultures of dissociated cells' OR 'enrichment culture*' OR 'microcarrier culture*' OR 'monolayer culture*' OR 'primary culture*' OR 'primary cell colon*' OR 'primary cell system*' OR 'suspension culture*' OR 'synchronous culture*' OR 'xeno-free culture*' OR 'xenofree culture*' OR 'xenogeneic-free culture*' OR 'erythrocyte culture*' OR 'cultured erythrocytes' OR 'cultured erythroid cells' OR 'cultured red blood cells' OR 'RBC culture*' OR 'fibroblast culture*' OR 'yeast culture*' OR 'astrocyte culture*' OR 'microglial culture*' OR 'oligodendrocyte culture*' OR 'heart culture*' OR 'heart myoblast culture*' OR 'kidney culture*' OR 'leukocyte culture*' OR 'cultured leucocytes' OR 'cultured leukocytes' OR 'cultured white blood cells' OR 'leucocyte culture*' OR 'lymphocyte culture*' OR 'cultured lymphocytes' OR 'NK culture*' OR 'macrophage culture*' OR 'monocyte culture*' OR 'hepatocyte culture*' OR 'liver culture*' OR 'mixed culture*' OR 'muscle fiber culture*' OR 'muscle culture*' OR 'myocyte culture*' OR 'cultured nerve cells' OR 'cultured neurons' OR 'nerve culture*' OR 'neuron culture*' OR 'neuronal culture*' OR 'cortical culture*' OR 'cultured brain

cells' OR 'cultured brain neurons' OR 'cultured cerebral cells' OR 'cultured cerebral neurons' OR 'cultured cortical cells' OR 'cultured cortical nerve cells' OR 'cultured cortical neurons' OR 'cultured hippocampal neurons' OR 'cultured hippocampus neurons' OR 'cultured spinal cells' OR 'cultured spinal cord cells' OR 'cultured spinal cord nerve cells' OR 'cultured spinal cord neurons' OR 'cultured spinal neurons' OR 'spinal cord culture*' OR 'spinal culture*' OR 'protoplast culture*' OR 'feeder cell*' OR 'feeder layer*' OR 'primary cell*' OR 'cultured tumor cells' OR 'cultured tumour cells' OR 'tumor culture*' OR 'tumour culture*' OR 'cancer culture*' OR 'tumor spheroid*' OR 'tumour spheroid*' OR 'embryo culture*' OR 'blastocyst culture*' OR 'organ culture*' OR 'tissue culture*' OR 'bone marrow culture*' OR 'skin culture*' OR 'slice culture*' OR 'high-throughput screen*' OR 'high-throughput drug screen*' OR 'high-throughput biological assay*' OR 'high-throughput chemical assay*'):ab,de,kw,lnk,ti

24,363 results, 7/30/18

Part 1 OR Part 2

28,791 results, 7/30/18

Web of Science Search Strategy

Web of Science Search Strategy Part 1 – Systematic Reviews of in vitro Studies

TS=("meta-analysis" OR "systematic review" OR MEDLINE)

AND

TS=("animal testing alternative*" OR "alternative* to animal testing" OR "animal use alternative*" OR "animal testing reduction*" OR "animal testing refinement*" OR "animal testing replacement*" OR "in vitro" OR "invitro" OR "ex vivo" OR "culture technique*" OR "axenic culture*" OR "aseptic culture*" OR "sterile culture*" OR "cell culture*" OR "batch culture*" OR "shake-flask culture*" OR "cell engineer*" OR "cellular engineer*" OR "tissue engineer*" OR organoid* OR "diffusion chamber*" OR "semi-permeable chamber*" OR "semipermeable chamber*" OR "tissue cage*" OR cocultur* OR "co-cultur*" OR cocultivation* OR "co-cultivation*" OR "continuous culture*" OR "dissociated culture*" OR "culture of dissociated cells" OR "cultures of dissociated cells" OR "enrichment culture*" OR "microcarrier culture*" OR "monolayer culture*" OR "primary culture*" OR "primary cell colon*" OR "primary cell system*" OR "suspension culture*" OR "synchronous culture*" OR "xeno-free culture*" OR "xenofree culture*" OR "xenogeneic-free culture*" OR "erythrocyte culture*" OR "cultured erythrocytes" OR "cultured erythroid cells" OR "cultured red blood cells" OR "RBC culture*" OR "fibroblast culture*" OR "yeast culture*" OR "astrocyte culture*" OR "microglial culture*" OR "oligodendrocyte culture*" OR "heart culture*" OR "heart myoblast culture*" OR "kidney culture*" OR "leukocyte culture*" OR "cultured leucocytes" OR "cultured leukocytes" OR "cultured white blood cells" OR "leucocyte culture*" OR "lymphocyte culture*" OR

"cultured lymphocytes" OR "NK culture*" OR "macrophage culture*" OR "monocyte culture*" OR "hepatocyte culture*" OR "liver culture*" OR "mixed culture*" OR "muscle fiber culture*" OR "muscle culture*" OR "myocyte culture*" OR "cultured nerve cells" OR "cultured neurons" OR "nerve culture*" OR "neuron culture*" OR "neuronal culture*" OR "cortical culture*" OR "cultured brain cells" OR "cultured brain neurons" OR "cultured cerebral cells" OR "cultured cerebral neurons" OR "cultured cortical cells" OR "cultured cortical nerve cells" OR "cultured cortical neurons" OR "cultured hippocampal neurons" OR "cultured hippocampus neurons" OR "cultured spinal cells" OR "cultured spinal cord cells" OR "cultured spinal cord nerve cells" OR "cultured spinal cord neurons" OR "cultured spinal neurons" OR "spinal cord culture*" OR "spinal culture*" OR "protoplast culture*" OR "feeder cell*" OR "feeder layer*" OR "primary cell*" OR "cultured tumor cells" OR "cultured tumour cells" OR "tumor culture*" OR "tumour culture*" OR "cancer culture*" OR "tumor spheroid*" OR "tumour spheroid*" OR "embryo culture*" OR "blastocyst culture*" OR "organ culture*" OR "tissue culture*" OR "bone marrow culture*" OR "skin culture*" OR "slice culture*" OR "high-throughput screen*" OR "high-throughput drug screen*" OR "high-throughput biological assay*" OR "high-throughput chemical assay*")

5,079 results, 7/30/18

Web of Science Search Strategy Part 2 – Guidelines/Research Design/in vitro Studies

TS=(guideline* OR validation* OR tool* OR checklist* OR "check list*" OR guidance OR "best practice*" OR recommendation* OR requirement* OR criterion OR criteria)

AND

TS=("study design" OR "study designing" OR "study designs" OR "research design" OR "research designing" OR "research designs" OR "research strateg*" OR "error source*" OR "scoring method*" OR "matched group*" OR "research technique*" OR "research technic" OR "research technics" OR "research method*" OR "problem formulation*" OR "experimental design*" OR "methodological research" OR "research instrument" OR "research instruments" OR "research protocol*" OR "control group*" OR blind OR blinded OR blindedness OR blinding OR blindly OR masked OR "random allocation*" OR randomization* OR randomisation* OR "sample size*" OR "minimal data set*" OR "minimum data set*" OR "experimental error*" OR "scientific error*" OR "experimental mistake*" OR "scientific mistake*" OR "research mistake*" OR "research error*" OR "measurement accurac*" OR "quality control*" OR "good laboratory practice*" OR "proficiency test*" OR bias OR biases OR "analytic* error*" OR "analysis error*" OR "measurement error*" OR "random error*" OR "sampling error*" OR "sample error*" OR "ecological fallac*" OR "observer variation*" OR "interobserver variation*" OR "inter-observer variation*" OR "interobserver variabilit*" OR "inter-observer variabilit*" OR "intraobserver variation*" OR "intra-observer variation*" OR "intraobserver variabilit*" OR "intra-observer variabilit*" OR "systematic error*" OR "peer review*" OR "study quality" OR "study qualities" OR "data accuracy" OR "data quality" OR

"quality appraisal*" OR "study appraisal*" OR "research quality" OR "evidence quality" OR "quality of evidence" OR validity OR "multitrait multimethod*" OR "multi-trait multi-method*" OR MTMM OR reliability OR "quality assessment*" OR "quality assurance*" OR "quality management" OR reporting)

AND

TS=("animal testing alternative*" OR "alternative* to animal testing" OR "animal use alternative*" OR "animal testing reduction*" OR "animal testing refinement*" OR "animal testing replacement*" OR "in vitro" OR "invitro" OR "ex vivo" OR "culture technique*" OR "axenic culture*" OR "aseptic culture*" OR "sterile culture*" OR "cell culture*" OR "batch culture*" OR "shake-flask culture*" OR "cell engineer*" OR "cellular engineer*" OR "tissue engineer*" OR organoid* OR "diffusion chamber*" OR "semi-permeable chamber*" OR "semipermeable chamber*" OR "tissue cage*" OR cocultur* OR "co-cultur*" OR cocultivation* OR "co-cultivation*" OR "continuous culture*" OR "dissociated culture*" OR "culture of dissociated cells" OR "cultures of dissociated cells" OR "enrichment culture*" OR "microcarrier culture*" OR "monolayer culture*" OR "primary culture*" OR "primary cell colon*" OR "primary cell system*" OR "suspension culture*" OR "synchronous culture*" OR "xeno-free culture*" OR "xenofree culture*" OR "xenogeneic-free culture*" OR "erythrocyte culture*" OR "cultured erythrocytes" OR "cultured erythroid cells" OR "cultured red blood cells" OR "RBC culture*" OR "fibroblast culture*" OR "yeast culture*" OR "astrocyte culture*" OR "microglial culture*" OR "oligodendrocyte culture*" OR "heart culture*" OR "heart myoblast culture*" OR "kidney culture*" OR "leukocyte culture*" OR "cultured leucocytes" OR "cultured leukocytes" OR "cultured white blood cells" OR "leucocyte culture*" OR "lymphocyte culture*" OR "cultured lymphocytes" OR "NK culture*" OR "macrophage culture*" OR "monocyte culture*" OR "hepatocyte culture*" OR "liver culture*" OR "mixed culture*" OR "muscle fiber culture*" OR "muscle culture*" OR "myocyte culture*" OR "cultured nerve cells" OR "cultured neurons" OR "nerve culture*" OR "neuron culture*" OR "neuronal culture*" OR "cortical culture*" OR "cultured brain cells" OR "cultured brain neurons" OR "cultured cerebral cells" OR "cultured cerebral neurons" OR "cultured cortical cells" OR "cultured cortical nerve cells" OR "cultured cortical neurons" OR "cultured hippocampal neurons" OR "cultured hippocampus neurons" OR "cultured spinal cells" OR "cultured spinal cord cells" OR "cultured spinal cord nerve cells" OR "cultured spinal cord neurons" OR "cultured spinal neurons" OR "spinal cord culture*" OR "spinal culture*" OR "protoplast culture*" OR "feeder cell*" OR "feeder layer*" OR "primary cell*" OR "cultured tumor cells" OR "cultured tumour cells" OR "tumor culture*" OR "tumour culture*" OR "cancer culture*" OR "tumor spheroid*" OR "tumour spheroid*" OR "embryo culture*" OR "blastocyst culture*" OR "organ culture*" OR "tissue culture*" OR "bone marrow culture*" OR "skin culture*" OR "slice culture*" OR "high-throughput screen*" OR "high-throughput drug screen*" OR "high-throughput biological assay*" OR "high-throughput chemical assay*")

25,255 results, 7/30/18

Part 1 OR Part 2

29,215 results, 7/30/18

Data Management

We referred to the [Kohl et al. \(2018\)](#) review of existing tools to identify appropriate screening, data extraction and management software.

- Literature search: DistillerSR
- Filtering for duplicates: EndNote and manual filtering
- Screening and selection: DistillerSR
- Extraction and coding:
 - Bibliographic information, data storage: DistillerSR
 - Coding extracted data: DistillerSR

Selection Process

Screening will be conducted by US National Toxicology Program contractors.

Screening by title and abstract: Titles and abstracts are to be screened by two reviewers. All titles and abstracts will be screened using the method of liberal acceleration (also known as “safety first”) via DistillerSR. In this method, only one author needs to classify a record as “include” or “unsure” for it to be marked for a full-text screening, whereas two authors need to independently classify a record as “exclude” for it to be excluded. There is an assumption of relevance unless there is direct evidence of irrelevance from title and abstract.

Screening by full text: Full texts are to be screened by two reviewers, with discussion to resolve conflict. Reviewers will follow the pre-specified decision-tree (see Figure 1), selecting pre-specified reasons for exclusion. All reviews will be tagged as such for later hand-searching for relevant citations. Pilot phase of initially 5% of full texts screened, conflicts resolved, and any additional instructions and clarity in decision-tree and list of exclusion criteria to be articulated before continuing screening. Additional check-point at 15% of full texts.

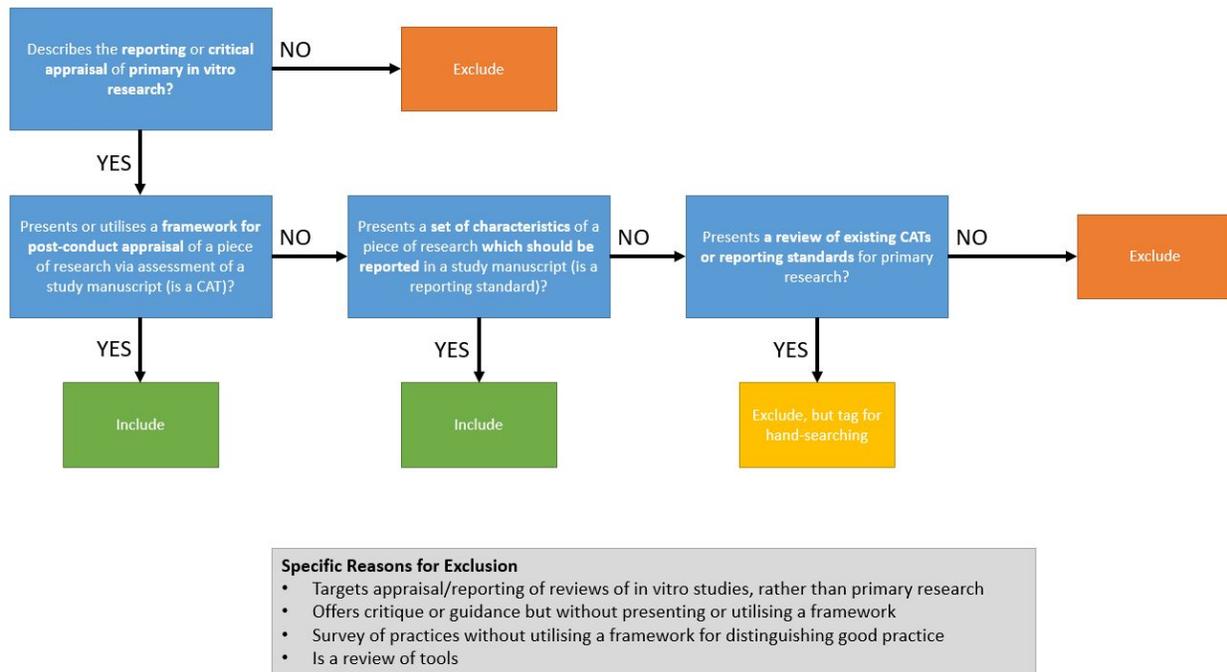


Figure 1: Literature screening decision-tree

Data collection process

Data extraction forms: Pilot forms drafted in DistillerSR and tested by each reviewer (PW and RdV) on 2 critical appraisal tools and 1 reporting standard. Forms modified and tested on an additional 2 appraisal tools and 1 reporting standard. If determined satisfactory, forms approved and used for extraction. The sub-headings into which quality criteria will be extracted are determined in Part 2 of this protocol (see below).

Data extraction process: Data will be extracted in duplicate. DistillerSR's agreement function will be used to identify disagreements between reviewers which will be resolved by discussion.

Data items

Data collection items will be finalised after a pilot data extraction process for 20 included critical appraisal tools. The initial data collection sheet will be based on that developed by PW as part of a systematic review of critical appraisal tools of systematic reviews. Data collection will be the minimum amount required for the purpose of aggregating quality criteria and readily associating them with their source documents. The search strategy, inclusion criteria and full collection of extracted data will be published as a comprehensive data set for other researchers interested in critical appraisal of in vitro studies to investigate for their own purposes. The data to be collected for IV-CAT includes:

- Bibliographic details (title, journal, lead author, year)

-
- Name of tool (full name and abbreviation)
 - Year of creation
 - DOI of the tool, if available
 - URL of the tool, if available
 - PubMed ID of the tool, if available
 - Quality or reporting criteria presented in the tool

Reviews of critical appraisal tools and reporting standards are included primarily as resources for ensuring full coverage of the literature. Data will only be extracted from critical appraisal tools and reporting standards.

Part 2: Development of In Vitro Study Model

Context

Systematic reviews are often presented as a flow diagram or framework which breaks down the conduct of an evidence synthesis into a series of discrete steps (see figure 2 for an example). The steps can be used as an organising principle or set of headings for a list of best practices in conduct of a systematic review, to provide structure to an otherwise unwieldy list and help users recognise when they have completed each phase of a review project. As each item in each stage of the SR can be treated as a reporting item or evaluated in terms of how well it has been done, such frameworks can provide the structure of a critical appraisal tool.

Objective

To facilitate coding of the quality criteria proposed in existing CATs, and provide structure for a straw-man EBTC IV-CAT tool, we will define a flow diagram / framework which captures each stage in the planning and conduct of an in vitro study.

Methods

Experts in the EBTC In Vitro Methods Working Group will be presented with a SR flow chart and asked to develop a similar scheme for planning and conduct of an in vitro study. We will start with a one hour conference call with the experts to begin defining the stages and then refine via email exchanges and additional calls as necessary until we have consensus on a flow diagram.



Figure 2. Example of flowchart summarisation of a SR, from Whaley et al. (in preparation) “Conduct of Systematic Reviews in Toxicology and Environmental Health Research (COSTER): A Code of Practice”

Part 3: Development of draft version of tool

Objective

- Develop a list of criteria for assessing fitness-for-purpose of an in vitro study report
- Formulate those criteria into a draft tool for testing (see part 4)

Methods

Step One: Identifying relevant quality criteria

Aim: to reach consensus among a group of experts representing key stakeholder groups about the criteria to be included in the draft version of the tool, through a process of selecting, prioritizing and adding to the potential criteria collected by the systematic review.

Groups of stakeholders:

- Editors of journals frequently publishing in vitro exposure studies
- Reviewers of journals frequently publishing in vitro exposure studies
- Researchers frequently conducting in vitro exposure studies
- Systematic review experts

-
- Regulators/risk assessors
 - Tool developers

Starting point: A list of potential criteria categorized according to the elements of the “ideal” model for an in vitro exposure study. The Delphi participants will be asked to imagine themselves in the position of a peer-reviewer advising an editor about the strengths and weaknesses of an in vitro study, in order to come to a decision as to whether a manuscript is fit to be published.

Expected rounds:

- Round 1: Participants score the extracted criteria on 9-point Likert scale of importance in determining fitness for purpose (“publishability”) of a tool (1=hardly relevant, 9=extremely relevant), using an anonymised on-line survey tool
- Round 2: Conduct telephone interviews with individual participants to elicit additional criteria which were not mentioned in the included CATs and reporting standards
- Round 3: Participants score the additional criteria from the interviews
- Round 4: Consensus meeting based on results of rounds 1 through 3 to finalise list of criteria for inclusion in, and structure of, draft tool to be put forward to testing phase

The criteria scored in round 1 will be divided into 3 categories:

- Category 1 “Likely to be important”: At least 70% of respondents rated item 7, 8 or 9
- Category 2 “Likely to be unimportant”: 100% of respondents rated item 1, 2 or 3
- Category 3 “Controversial”: Any other distribution of responses

The primary purpose of the scoring process is to triage criteria for which there is readily-identifiable consensus as to their being important or unimportant, to allow focus in the face-to-face meeting on controversial cases and the structuring of the criteria into a useful tool.

We considered two other knowledge elicitation methods as an alternative to the Delphi-based approach, the Sheffield method and Cooke’s method. We determined that these methods are intended to have experts estimate quantities (including a measure of uncertainty of that estimate) in the absence of empirical data about that quantity. Our goal is to reach consensus among relevant experts on which ‘quality’ criteria, according to their judgement, should be part of the in vitro appraisal tool. For these reasons, we believe the Delphi approach to be most suitable for our objectives.

Step 2: Create the draft tool

PW and RdV will take the structured list of criteria from the Delphi-style process and develop from these a set of elicitation questions and prompts which will be the beta version of IV-CAT, which will be advanced to iterated testing and development.

Part 4: Development of release version of tool

Objective

Develop the beta version of the tool into a release version suitable for use by the toxicology community.

Methods

A test pool of users will be selected, who will apply the beta version of the tool to a set of in vitro study manuscripts. Feedback on the structure, criteria, utility and format of the tool will be collected via written comments and interview. Feedback will be incorporated in revision of the tool into an updated beta version. Testing will be halted when development plateaus. The beta version will then be published as a release version and accompanied by a manuscript.