Chapter 4

Antibody screening

Hoff FW1, Lu Y2, Kornblau SM3

1. Department of Pediatric Oncology/Hematology, Beatrix Children’s Hospital, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands;
2. Department of Systems Biology, The University of Texas M.D. Anderson Cancer Center, Houston, TX, USA;
3. Department of Leukemia, The University of Texas M.D. Anderson Cancer Center, Houston, TX, USA.

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Abstract

Antibodies are among the most frequently used tools in research and have had a profound impact on the discovery of diagnostic and therapeutic targets and the understanding of the molecular background of diseases. In particular in reverse phase protein arrays (RPPA), where there is no separation of the proteins according to molecular weight, it is crucial that antibodies are proven to be highly specific, selective, and reproducible. However, numerous studies have shown that many antibodies frequently do not recognize the protein that they are supposed to detect, that multiple antibodies do often function in one application but not in another, and that antibodies are not stable over time or between different batches. So far, no universally accepted guidelines or standardized methods for determining the validity of antibodies have been established. This chapter discusses the urgent need for antibody validation, current strategies that are used for RPPA antibody validation, as well as proposes a new strategy about how to report, score, and integrate antibody validation from multiple users.
Introduction

Reverse phase protein array (RPPA) is an antibody-based proteomic approach in which the antigens (proteins) are immobilized on the solid phase of the array which are subsequently probed with a primary and a secondary antibody to measure the target of interest. This makes the reliability of the RPPA methodology highly dependent on the quality of the antibodies that are used. Unlike in a Western blot, with RPPA there is no separation of the proteins according to molecular weight; therefore, antibody validation is crucial for the outcome of the assay, as signals from potential cross-reactivities cannot be distinguished from the intended signals. Although they would have great utility, universally accepted guidelines or standardized methods for validating antibodies for use on RPPA have not yet been established. In this chapter, we will discuss the current need for better antibody validation in general, as well as provide information about how RPPA antibodies are currently validated. Additionally, we discuss which standards should be used to evaluate the quality of antibodies used on RPPA in the future and propose how to report and integrate validation from multiple users.

The need for antibody validation

Antibodies are among the most frequent tools used in research and have proven to be invaluable for the discovery of novel potential targets for diagnostics, therapeutics, and disease monitoring. According to the Antibodypedia, there are currently more than three million commercial antibodies available targeting over 19,000 human protein targets which are most commonly used for Western blot (WB), followed by immunohistochemistry (IHC) and immunocytochemistry (ICC). To successfully perform antibody binding-based identification experiments, it is crucial that an antibody must be shown to be specific, selective, and reproducible in the context for which it is to be used, that it must perform robustly across different sample types, and that it acts consistently over time. However, numerous reports have shown that this is frequently not the case as antibodies often do not recognize the protein that they are supposed to detect, instead recognizing a different antigen (possibly in addition to the target of interest), and frequently function in some applications but not others, or are not reproducible over different batches.
Non-specific antibodies ("cross-reactivity")

The production of commercial antibodies starts with the generation and selection of a specific antigen of interest selected from a host species (e.g., human, mouse, rabbit) which is then injected into a host animal. Most of the antibodies that are used in research setting to detect a protein (or antigen) of interest are either monoclonal or polyclonal. Polyclonal antibodies are produced by a number of different B cells each targeting a different epitope found on that specific antigen, resulting in a heterogeneous mixture of antibodies. Once a sufficient immune response has been achieved, the antiserum (containing the antibodies) is harvested, and purification steps are performed to increase the sensitivity and specificity of the antibodies. For monoclonal antibodies, the antibody-generating plasma cells are isolated and fused with hybridoma cells to generate different clones of cells that generate a single antibody which can then be cultured in perpetuity. Because each antibody only detects a specific epitope of a protein, “cross-reactivity” can occur if that specific epitope is shared with other proteins (e.g., isomers or common motifs). Consequently, significant caution is needed when performing antibody-based experiments; strict validation of an antibody specificity is required as non-specificity can potentially invalidate the results of an experiment.

To test the antibody quality, Algenäs and colleagues systematically analyzed 13,000 antibodies on WB with lysates from human cell lines, tissues, and plasma. In that study under half of the antibodies yielded the expected results with the other antibodies either detecting no protein bands or bands of the wrong size, potentially due to cross-reactivity.1 Furthermore, the influence of protein abundance on the apparent specificity of the antibody was assessed by performing new WB analyses for the 1369 genes that initially gave unsupportive WB results, using cell lysates with overexpressed full-length proteins. This time, the re-analysis showed specific bands corresponding to the full-length protein in more than 82% of the antibodies. Since antibodies are often raised against large amounts of the target and validation tested against purified protein, it is not surprising that results are better when the protein is abundant compared to when it is rare. A study by Jensen et al. tested the ten most cited commercially available antibodies targeting the alpha 1-adrenergic receptor subtypes using WB from tissues from wild-type mice and knockout mice with the subtypes genetically deleted (negative control).2 They reported that none of the antibodies detected the appropriately sized band, or a band somewhat larger or smaller, that was present in the wild-type samples but absent in the knock out mice, concluding that all ten antibodies were non-specific for their target protein. Another important study investigated the cross-reactivity of 246 antibodies targeting 3
unmodified histones and 57 distinct histone modifications, reporting that more than 73% of the tested antibodies performed well in terms of specificity or utility but that the other 27% failed WB or dot plot specific blots.³

Berglund et al. screened 5436 antibodies from a large number of commercial vendors and showed that less than 50% of the antibodies could be validated using WB and IHC, based on a staining pattern that was consistent with existing literature or bioinformatics data.⁴ Edwards et al. developed a mass spectrometry (MS)-based approach to quantitatively compare different antibodies from immunoprecipitation experiments. All samples were corrected with a background subtraction, and non-specific binders were eliminated using computational approaches. Proteins were sorted based on their abundance, and for each sample, the placement of the protein of interest was noted. Overall, 41% (452 out of 1124 tested antibodies) recognized their intended antigen, of which 33% (n = 354) were defined a “golden standard,” meaning that the protein of interest had the highest abundance.

Application-specific antibodies

Even though the majority of the antibodies have only been tested for the intended purpose, most antibodies are used in more than one research application, including WB, IHC, ICC, flow cytometry, and enzyme-linked immunosorbent assay. For different applications, proteins are analyzed in different conformations which present the target differently, and relatively minor changes in the antigen structure can markedly affect the strength of the interaction. A folded protein in its natural conformation may have target amino acids that are separated from each other in linear amino acid sequence, but which can be in physical proximity to form an epitope, while the target sequence on a denatured protein may be a linear sequence of amino acids. The impact of conformational changes is smaller for polyclonal antibodies than for monoclonal antibodies, as they recognize multiple epitopes, of which some are likely to be linear.⁵ Various techniques also use different experimental conditions, including sample preparation, fixation time, antibody incubation, and the use of buffer solutions. Therefore, unpredictable cross-reactivity to non-related proteins, as well as binding to other materials in the samples, could occur, which could associate with antibody molecules in a manner distinct from an antibody-antigen interaction. This inaccuracy has been demonstrated by the Human Protein Atlas, which has examined over 60,000 antibodies. Their screening showed that from the pool of antibodies validated in WB, only 50% worked satisfactorily in applications as IHC and ICC.⁶ Another study by Algienäs et al. also showed the application-specific dependence of many antibodies,
when they analyzed 13,000 antibodies in both IHC and WB, and demonstrated a weak correlation between WB and IHC.\(^1\) Egelhofer et al. performed ChIP-chip or ChIP-seq experiments with antibodies against histone modification proteins and generated reproducible results between the experiments for 78% (115 of 147) antibodies.\(^3\) Gilda et al. investigated the effect of variations in WB procedures, such as the amount of protein loaded, blocking solution, and antibody concentration, and showed that different experimental conditions can have drastic effects on the results,\(^7\) indicating that different techniques indeed make their own distinct demands of antibody reagents.

**Inconsistent production of antibodies**

A third important criterion for antibody usage is the stability and reproducibility of results obtained with an antibody across different aliquots, lots, or batches over time. Although it is often assumed that new lots or aliquots of the same antibody, especially for monoclonal antibodies, would yield results similar to those previously obtained with that antibody, a couple of studies have shown that probing with the same antibody over time or from different lots or batches can lead to different results.\(^8,9\) The variability between different batches will be greater for polyclonal antibodies, where the supply of antibodies is limited to immunized animals, than for monoclonal antibodies, which are available in a theoretically unlimited supply. Furthermore, even with the most stringent of production quality control, loss of integrity of an antibody can occur during shipping or by handling in the lab. In 2007, Pozner-Moulis et al. assessed the antibody reproducibility of different lots of the Met antibody in 640 breast cancers using different tissue microarray blocks. They identified that for four of their five tested antibodies, results were not reproducible at a quantitative level.\(^10\) Most strikingly, two different lots of the monoclonal 3D4 Met antibody showed opposite staining patterns. In addition, they found that lots of the MAB3729 monoclonal Met antibody lost binding specificity after 4–5 months despite proper storage. Another study by Gilda et al. showed that antibodies from different vendors targeting the same ubiquitinated proteins gave different banding patterns depending on the antibody utilized, independently of the blotting conditions.\(^7\) Consequently, all of these issues create the potential for significant variation in results and can adversely affect the reproducibility of antibody-based research, highlighting that extreme care in the selection, vetting, storage, and use of antibodies are required to mitigate this problem.
Antibody validation

The Food and Drug Administration defines validation as “the process of demonstrating that an analytical procedure is suitable for its intended purpose.” Inconsistently, for most of the antibody-based techniques, no scientific standardized guidelines exist to validate antibodies, and antibodies are often used in a context for which they were not validated. Given the complexity of the antibody, it is not possible to define guidelines to validate antibodies for the full range of applications.

Methods for antibody validation

The potential for problems with antibody-based research has led to collaborative efforts to develop standards to deal with these issues. In 2016, the Global Biological Standards Institute (GBSI) established several working groups to create series of application-specific antibody validation guidelines, and within each working group, they suggested five conceptual pillars for validation:

Genetic strategy

Genetic strategies seek to show specificity of a target by manipulating a genetic target and obtaining the anticipated change in the signal from the antibody, thereby providing a direct link between the gene, the protein expression, and the antibody. The principle is to look for change in signal between control cells and cells in which the target gene or epitope has been knocked out or knocked in (e.g., CRISPR-CAS9 or RNA interference).

Orthogonal approach

The second approach “orthogonal validation” seeks to confirm antibody sensitivity by demonstrating highly correlated results with another non-antibody-based method, for example, correlating WB results with MS results.

Independent antibody strategies

Different independent (e.g., different epitopes) antibodies targeting the same protein target may be used to assess specificity. This allows validation in a specific application, if the correlation between the two antibodies is sufficient. In this way, possible cross-reactivity of an antibody can also be identified.
Expression of tagged proteins
Expression of an affinity tag or a fluorescent protein on the target of interest or on the antibody that then binds to the antigen can confirm the specificity of an antibody, when the detection pattern of the tagged protein that binds to a regular non-tagged antibody and of the tagged antibody that binds to the protein are similar. For instance, a fusion of a protein with a peptide tag resulting in a detection pattern that is similar to that of the tag-specific antibody indicates specificity, whereas discordance between the patterns suggest cross-reactivity.

Immunocapture followed by MS
The identity of a protein pulled down by an antibody can be confirmed by then performing MS, and if only peptides from the expected protein are found, then the antibody is validated. The presence of unexpected peptides by MS would demonstrate non-specificity. This could arise if the antibody reacts with completely different proteins through off-target interactions, but if the proteins are probed in a conformed state, the antibody could bind to the intended target, which in turn is bound to another protein that it normally interacts with. The first would be non-specificity, and the second would be acceptable. In the setting of RPPA where the proteins are denatured, and hence they are not bound to any partners they normally interact with, this issue does not exist. Therefore, for the purpose of validating antibodies for use in RPPA by MS, this should be performed with denatured proteins.

Unfortunately, although the GBSI did discuss the RPPA standards during their policy meeting in 2016, they did not produce validation guidelines for RPPA.

Human protein atlas
With more than three million antibodies to be evaluated and a significant proportion of them not working sufficiently, an important question is whether there are techniques available that could be used to screen antibodies, thereby reducing the workload. The Human Protein Atlas approached this by constructing high-throughput high-density protein fragment microarrays with up to 21,120 different antigens from 12,412 genes on them. They used these arrays to look for off-target interactions of an antibody and screened 54,000 antibodies, of which 47,000 were approved and more the 21,000 were fully validated and published. Validation was defined based on two different criteria: (1) no more than three “weak” off-target proteins with a signal strength up to 15% of the intended target and (2) no “strong” off-target proteins with an intensity of 40% of the desired target. However, this approach would likely be inadequate for
the use of RPPA, as two to three different off-target proteins with a signal of 15% of the intended signal (meaning a cumulative of target percentage of 30–45%) are too much error for RPPA. Therefore, for this approach to be utile for RPPA, significantly more rigorous standards that do not allow for any off-target interactions would be required. However, with a rigorous standard, this approach, used in a high-throughput setting, could enable the rapid disqualification of many antibodies and the selection of a select group with monospecificity of target.

**DigiWest for antibody validation**

The DigiWest approach represents a high-throughput combination of an adapted WB to a bead-based microarray platform for signal generation.\(^\text{12}\) It uses a gel-based separation of proteins based on their molecular weight and subsequently transfers proteins within a defined molecular weight to a bead set microarray platform for hundreds of antibodies. DigiWest exhibits comparable sensitivity, linearity, and reproducibility with WB.

**Requirements for quantitative analysis on RPPA**

**Conventional RPPA antibody validation**

Antibodies must be certified for use in RPPA; up till now, the validation procedure for RPPA typically includes three different steps:

During the first step of antibody validation, WB is performed using samples from a panel of cell lines along with a molecular weight calibration marker. Ideally, cell lines with a known knockout or knockdown or cell lines that are known expressors/ non-expressors of the protein in question should be included. From here, only those antibodies that demonstrate a single band at the correct molecular weight in the known positive controls (or other cell lines), and which do not show a signal in the knockout lines, are considered suitable for further validation. In this case, at least a part of the potential cross-reactivity of the antibody could be detected by WB before the antibody is used for RPPA. Quantification of signal strength is determined for later correlation with RPPA cell line control arrays. The WB-screened antibody can then be tested on a RPPA, typically with many cell lines, preferably including the ones that were tested on the WB to allow for correlation and to determine optimal primary and secondary antibody concentrations. Once a quality cell line RPPA is obtained, the Pearson correlation coefficient between the cell lines present on both the RPPA and the WB will be determined. Based on the
strength of the correlation, antibodies are classified as $>0.7$ (valid), 0.5 - 0.7 (use with caution), and $<0.5$ (not reliable). Antibodies are validated singly since they are used singly. Each new lot of antibody should be revalidated at the WB level (Figure 1).

Simultaneously, an additional important aspect of the validation procedure includes quality control of the slide, which may be decreased due to several reasons, including the functional quality of the antibody (degradation) or a suboptimal primary dilution. A good-quality slide would be expected to have a large dynamic range of spot intensities from the lightest spots to the darkest spots, especially if the samples are printed in a five-step dilution series (resulting in a 16-fold change in concentration from the most concentrated to the most diluted spot). Also, as over a thousand samples are printed on a single slide, this would likely contain large variations in protein concentration. If a large dynamic range of spot intensities is not observed, it may be due to factors such as a lack of variation in protein concentration (due to under- or overloading) in the spots or due to poor antibody affinity in the RPPA setting of the antibody to its target protein (for instance, use of an antibody that recognizes a conformed protein against the denatured protein found in RPPA).

Another criterion for quality control is the signal-to-noise ratio, in this case between surrounding areas where nothing was printed and “negative controls” where non-protein-containing spots from lysate controls were printed, in comparison to that of the actual samples. High background signal can degrade the dynamic range of an otherwise adequate antibody. Slides with high background ratios may be due to non-specificity of the antibody or due to technical issues with the secondary antibody or detection methodology. The former is a problem with the antibody being tested, possibly correctable with greater antibody dilution, and the latter a problem with the staining procedure that can be corrected.

Finally, when a positive control is repeatedly printed across the slide (we use a mixture of 11 cell lines that are printed in a 5-step dilution along with a negative control in 192 repetitions across the slide), variation among the positive controls and their dilution series can provide another quality check. Under perfect conditions, the 192 positive controls should all yield identical values, and their dilution series should sequentially increase proportional to the twofold difference in concentration from one spot to the next. However, in reality the values can deviate from ideal conditions, and the amount of deviation can be measured and quantified. Greater deviations can result from background noise, and low spot intensities can be measured
if the amount of protein in the positive control is too little, the antibody affinity is too weak, or the antibody dilution is too low.

**Figure 1. Conventional RPPA antibody validation.** (A) Schematic workflow of RPPA antibody validation method. (B) Western blot (WB) is performed on a panel of cell lines. Only antibodies that demonstrate to be specific by showing a single band on WB are suitable for further validation. (C) RPPA on a panel of cell lines showing a large dynamic range in spot intensity within the diluted samples and between samples containing different protein concentrations. (D) and (E) Correlation between WB and RPPA results. This antibody shows a good correlation between the WB and RPPA (Pearson’s correlation of 0.85), meeting the criteria to be validated as RPPA antibody.

**New orthogonal approach to validate antibodies for RPPA**

Over the last years, the RPPA Core Facility of the Department of Systems Biology at the University of Texas MD Anderson Cancer Center has developed a new approach to improve validation of antibodies for the use of RPPA. This approach incorporates data derived from different resources, including MS and messenger RNA (mRNA) sequencing data. Their validation process first utilizes RPPA against 330 cell line samples, and each slide probed with a single antibody has to pass the strict quality control criteria, similar to the conventional method.

RPPA information is then correlated (calculation of Pearson’s correlation coefficient from linear data) with publicly available MS information which is available for most of the cell lines.
that are printed on the RPPA slide. The data used for this analysis was collected by the National Cancer Institute 60 proteome resource and facilitates a comprehensive proteome analysis of a panel of human cell lines from nine different tissues. Data comprises a high proteome coverage and contains details about protein and peptide identification together with the corresponding fragment spectra and quantitative information. Antibodies with a correlation between RPPA and MS data greater than 0.5 are defined as “valid.” For those antibodies, no further WB analysis is needed. Antibodies with a correlation coefficient of less than 0.5 but greater than 0.3 are defined as “use with caution,” and those antibodies should be evaluated further. For any antibody that has a correlation of less than 0.3 with MS data, a new search for a better antibody is needed, and those antibodies should not be used on RPPA. However, this assumes that protein expression is consistent over time between cell lines, so that the cells grown for the MS analysis and those grown later for the cell line RPPA would give consistent results across the orthogonal platforms. Ideally, a collection of cell lines should be cultured and have samples collected for RPPA and MS at the same time so that a truly comparable set of orthogonally comparable standards would exist.

Step three is comparable to step two but includes correlation between the RPPA and mRNA sequencing data; however, this is limited to non-PTM forms of the protein. mRNA data used for this step is derived from the Cancer Cell Line Encyclopedia that has data available for 1934 different cell lines and contains expression for 54,675 probes corresponding to 20,067 unique genes. Similar to the MS correlation, any antibody with a correlation coefficient above 0.5 is defined as “valid,” and for those antibodies, no further WB or analysis is needed. Antibodies with a correlation less than 0.5 but greater than 0.3 are defined as “use with caution,” and those need additional validation. However, the same concerns exist about non-concurrently sampling of the cell lines for mRNA GEP and RPPA as mentioned above.

According to this schema, antibodies with RPPA/mRNA correlation below \( r < 0.5 \) do not meet the full validation criteria. However, this is problematic as several studies have demonstrated limited correlation (typically less than 40–45% between mRNA and protein expression correlation). This discordance can be biologically accurate, arising from variability in the kinetics of mRNA translation and protein stability (long lived vs. short lived). Therefore, the RPPA/mRNA concordance is valid for those proteins demonstrated to have correlation with some antibodies, but lack of correlation does not necessarily invalidate an antibody. Consequently, mRNA validation must be used cautiously for a limited percentage of proteins.
Antibodies that showed a good correlation between RPPA and MS and those where there is demonstrated RPPA and mRNA correlation are defined as doubly “valid”. Antibodies that positively correlated with MS data, but negatively correlate with mRNA, or that were positively correlated with mRNA but, not with MS were defined as “use with caution” but may actually be fully valid if there is known mRNA/protein discordance. An antibody was designated to have “failed” when RPPA negatively correlated with MS, but MS positively correlates with mRNA. Special attention should be paid to antibodies targeting PTM (e.g., phosphorylation, cleavage, etc.) and antibodies recognizing isoforms. For those antibodies, WB should be performed as we are doing currently.

As this emphasizes that different laboratories use different approaches and criteria to validate their antibodies, this potentially means that antibodies that are validated in one lab by one set of methods and criteria could be scored as “invalid” in another. Therefore, a more consistent set of “rules” should be defined that aligns the validations process across different laboratories.

**Database integration from multiple users**

**Proposal of integrative database**

To validate each individual antibody for each separate proteomic application requires a lot of time and money. Therefore, it would be highly beneficial to the RPPA community to accurately document the results once the validation process of an antibody has been done and to make this data available to other researchers as a reference. There are already several existing repositories (e.g., Antibodpedia, Antibodies-online, The Antibody Registry) of antibodies with varying degrees of validation information; however, most of them lack proper validation data, require registration and log-in to access, and are thus not accessible to everyone, are limited in the scope of the antibodies they contain information on, may not be user-friendly, or are dependent on antibody manufacturers for source information, with a potential conflict of interest. Therefore, a possible approach would be to set up a new database or to codify and complete an existing resource that will include all the basic facts, usage facts, and validation process of an antibody. We propose the following standards to be included in such an antibody validation database.
Basic facts of an antibody

In order to document all the required information of an antibody, one approach would be to collect and store the basic facts of an antibody. This information should include:

- Antibody and protein target name, including HUGO, HUPO, and MiMI naming system and the PTM;
- Manufacturer, catalog number, batch number, and lot number;
- Animal source, clonality, isotype, recognized epitope (preferably with the amino acid components delineated), conformation status (e.g., native, denatured), and any known cross-reactivity;
- The DNA sequence of the antibody coding gene or amino acid sequence of the antibody should be listed. Antibodies are often sold by one manufacturer to another and then sold under a different name. This increases the number of antibodies available by catalog number, but not in actual number. Therefore, having this data would enable a firmer ID for the antibody;
- The weight in kilodaltons (kDa);
- Modifications, such as enzyme or fluorescence labelling.

PTM convention

Since neither the HUGO, HUPO, nor MiMI naming systems account for PTM, a nomenclature is required that allows for notation of the PTM detected. We have adopted a convention in which the HUGO name is followed by a period and then the type of PTM, “p” for phosphorylated, “cl” for cleaved, or “Me” for methylation, followed by the letter code for the affected amino acid and its sequence position, e.g., (S)erine, (Y)rosine, and (T)reonine. For example, AKT1_2_3-pThr308 is AKT phosphorylated on threonine at position 308. Placing the PTM after the protein name enables alphabetical sorting by the primary target (whereas putting a “p” in front of the protein name would sort all the phosphorylated targets together) and inclusion of the affected site allows for multiple different PTMs to be described for the same protein.

Usage facts of an antibody

Documentation of the performed validation process should provide exact information about the optimal usage conditions. As it is clear that antibodies may work well in one setting, but not another, orthogonal confirmation may not always be helpful as it may lead to the exclusion
of some antibodies that actually see their target in RPPA or in other applications. Therefore, documentation of antibody validation should include exact information about the validation of antibodies for each application separately:

- How was the antibody validated and for which application, including information about the experimental setup (antibody dilutions, exposure time, buffer composition, etc.), quantification, and dilution curves?
- Are there known negative controls, inducible controls, and knockdown controls available? Were these used in validation of this antibody?
- For which sample type was the antibody validated? Does the antibody also work on other species, diseases, or cell types? Were the cells used for the validation purpose enriched for a particular cell population, how were samples preserved, and was there any time delay between collection and protein isolation that may have led to alterations in levels?
- What results can we expect? What is the target size on WB, and where is the protein predominantly localized (e.g., nucleus, cytoplasm)?

**Antibody scoring system**

To consistently score antibody validation in a way that is interpretable for everyone, we propose to include a “scoring” of the validation, resulting in a final “confidence” score. Antibodies with no testing would be unscored. For example, for WB, antibodies that are “not usable” are those with multiple non-specific bands. “Use with caution” is intended for antibodies with one to two non-specific bands on WB or where the non-specific band is weak relative to the expected band. “Valid” or “usable” is reserved for those without non-specific bands that have a band at expected size relative to size control. The scoring could be extended by adding information about the negative control (NC), the inducible control (IC), or the knockdown (KD) control.

From here, it would be possible to define an interpretable single line of text that tells everything about the antibody and its validation:

- (B)ands = 1,2, many, including % of extra band relative to expected;
- (S)ize expected = (@) kDa, (W)rong;
- Type = (T)otal, (P)hospho, (C)leaved, (M)ethylated;
Validation: Yes/No and result (P)ass/(C)aution/(F)ail;
- (NC) negative control: Yes/No and result (P)ass/(F)ail;
- (IC) inducible control: Yes/No and result (P)ass/(F)ail;
- (KD) knockdown: Yes/No and result (P)ass/(F)ail.

A great total antibody would be B1, S@, T, NC-YP, IC-YP, KD-YP, and a weak antibody would be BM, SW, T, NC-YF, IC-YF, KD-YF.

The development of a database that collected, collated, and housed all of this information obtained from multiple laboratories would have significant utility to all of those doing RPPA work. However, this would require a significant effort in terms of workload and would require significant financial support to establish and maintain.

Conclusions

As an antibody-based methodology, the results obtained with RPPA live and die based on the quality of the antibodies that are utilized. Many available antibodies are non-specific and therefore not suitable for use in RPPA. Rigorous antibody validation is required, and the methodology used to validate them needs to be included in all RPPA publications to establish a degree of confidence in the results. A codified system for antibody validation would enable greater confidence in RPPA results, and the development of a repository of all of this information would benefit all performing RPPA.
Reference list

Part II

Protein expression patterns in acute leukemia