

# WHITE PAPER

## INFECTIOUS DISEASE ASSAY DEVELOPMENT: ESTABLISHING INCLUSIVITY/EXCLUSIVITY

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**Optimizing experimental conditions during assay development can be challenging, particularly with respect to establishing analytical sensitivity and specificity, as well as identifying and employing the appropriate external controls. Here, we will discuss the importance of inclusivity/exclusivity testing, and will provide information on how to establish these parameters when evaluating your experimental design.**

Analytical reactivity and analytical specificity are often described using the terms inclusivity and exclusivity, but what do these terms mean? Depending on whether your assay is culture- or molecular-based, inclusivity can be defined as the percentage of target microbial strains or DNA samples that give the correct positive result. In contrast, exclusivity can be defined as the percentage of non-target microbial strains or DNA samples that give the correct negative result.<sup>1-2</sup> For example, if you are developing an assay for the detection of *Staphylococcus aureus* in clinical samples, you would want to ensure that your assay is inclusive for each of the different *S. aureus* subspecies while being exclusive for other related species or non-related genera such as *Staphylococcus epidermidis* or *Escherichia coli*, respectively.

Establishing ideal inclusivity/exclusivity parameters is an essential part of assay validation, particularly when evaluating diagnostic and epidemiological assays whose results can affect public health. In many cases, the rapid and accurate identification of an infectious pathogen is critical for the timely administration of appropriate therapeutic agents as well as the prevention of transmission. Thus, to ensure the precision of your diagnostic assay, choosing a suitable sample size of the appropriate representative strains or nucleic acids is imperative.

Determining which strains to choose for inclusivity/exclusivity testing can be a daunting task. Prior to selecting your test strains, it is important to know basic information about your target organism so that it can be applied in the development of your inclusivity and exclusivity testing panels. For inclusivity testing, the use of microbial or nucleic acid panels that encompass common strain variants as well as those representing all known subspecies of the target organism is recommended. In contrast, exclusivity can be established and evaluated through the use of cross-reactivity panels that include genetically related species that are in the same genus or family, genera that share an environmental or clinical niche with the target organism, and microbial species commonly observed in the test sample.

For instance, let's say you developed a molecular-based diagnostic assay for the detection of *Klebsiella pneumoniae* in respiratory infections and you wanted to evaluate its analytical reactivity and analytical specificity. First, you would want to gather a bit of background on this microbial species. Based on previous studies, this particular bacterium has been commonly found in the normal flora of the mouth, skin, and intestines, and can cause respiratory and urinary tract infections in immunologically compromised individuals. Moreover, *K. pneumoniae* is a significant member of the *Enterobacteriaceae* family, is related to at least three other species in the *Klebsiella* genus, and comprises three known subspecies.<sup>3-4</sup> With this in mind, you would want to ensure that your inclusivity testing panel included nucleic

acids isolated from strains representing the three known *K. pneumoniae* subspecies as well as strain variants frequently isolated from clinical samples. For your exclusivity panel, you would want to include nucleic acids isolated from strains representing other known *Klebsiella* species (eg, *K. granulomatis*, *K. oxytoca*, *K. terrigena*), isolates that share the same clinical and natural niches as *K. pneumoniae* (eg, *E. coli*, *Citrobacter* spp., *Proteus* spp.), and other organisms commonly found in clinical respiratory samples from both healthy and immunologically compromised patients (eg, *Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Streptococcus pneumoniae*). In addition to choosing the appropriate strains, both panels should comprise a large enough sample set to aid in determining the significance of your experimental results.

When obtaining strains for inclusivity and exclusivity testing, it is important to go to a reliable source that provides authenticated reference standards. This will ensure that your strains are accurately identified down to the species or strain level, as well as functionally characterized for any important traits such as serotype, toxin production, drug-resistance, or clinical relevance, including newly emerging subtypes. Currently, biological reference standards are developed and produced by a number of entities, including government agencies, commercial companies, and non-profit institutions. ATCC, for example, maintains a portfolio that encompasses a vast variety of relevant strains, variants, and nucleic acids. Moreover, ATCC Genuine Cultures are fully characterized using genotypic, phenotypic, and functional analyses to establish identity as well as confirm characteristic traits, making them ideal for inclusivity/exclusivity validation studies.

Overall, evaluating inclusivity and exclusivity is critical in assay development and validation. Through the use of a diverse array of authenticated, highly characterized strains that represent your target organism or non-target species, these parameters can be established.

## REFERENCES

- 1 Saah AJ, Hoover DR. "Sensitivity" and "Specificity" Reconsidered: The Meaning of These Terms in Analytical and Diagnostic Settings. *Ann Intern Med* 126(1): 91-94, 1997.
- 2 OIE. *Terrestrial Manual*. Chapter 1.1.5. Principles and Methods of Validation of Diagnostic Assays for Infectious Diseases, 2013.
- 3 Struve C, Angeliki Krogfelt K. Pathogenic Potential of Environmental *Klebsiella pneumoniae*. *Environ Microbiol* 6(6): 584-590, 2004.
- 4 Qureshi S. *Klebsiella* Infections. <http://emedicine.medscape.com/article/219907-overview>, October 2016.

### ADDITIONAL RESOURCES

- Resources for Vector-borne Research
- Sexually Transmitted Infection Research Materials
- Human Respiratory Strains
- Human Anaerobes for Microbiome Research
- ATCC Enteric Disease Research Materials
- Multidrug Resistant & Antimicrobial Reference Strains

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