A Method Development Case Study – Successes and Learnings from a CRO-Pharma Alliance

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AstraZeneca – Covance Partnership
Scientist-to-Scientist Communication

Established in 2011
Joint teams and governance
Dedicated Scientific Advisory Group
- Encourage direct scientist to scientist communication
- Support joint publications and presentations
- Proactively evaluate portfolio technology needs and changing regulatory landscape
- Provide scientific guidance and awareness to operational teams

Executive Steering Team
Operational Steering Team
Delivery Team
Scientific Advisory Group

Method Development Process
Planning and Preparation

Scientific Advisory Group
- Proactive portfolio review
- Early engagement
- Elevate and educate scientific workforce
- Issue guidance for operational teams

Joint Bioanalytical Leads
- Requirements intake
- Timing
- Intended use of the data
- Regulatory considerations
- Bioanalytical plan

Project Execution
- Leverage previous knowledge
- Direct scientist-scientist communication
- Issue escalation
Spotlight on Antisense Oligonucleotides

Bioanalytical Plan

Overview

- Antisense Oligonucleotide (ASO)
  - Short, Single stranded nucleic acids
  - Target a single genetic pathway
  - Bind RNA by Watson-Crick base pairing
  - Highly polar

Figure 1. ASO binding to the targeted RNA*

*C. Frank Bennett, Therapeutic Antisense Oligonucleotides Are Coming of Age. Annual Review of Medicine (2019)0:307-21
Spotlight on Antisense Oligonucleotides

Bioanalytical Plan

Platform Considerations

► Common Technical Challenges

- Stability
- Cross-reactivity
- Non-specific binding
- Sensitivity
- Chromatography

Figure 8. Dependence of assay sensitivity on the length/size of analyte oligonucleotide

Knowledge Transfer
CASE STUDY 1

CHALLENGE
Transfer of complex method details

SITUATION
► Upcoming project:
  • Human plasma PK analysis
  • Antisense oligonucleotide
  • Low LLOQ required
► Team held knowledge transfer meeting months in advance of lab work

KEY TAKEAWAY
► Restructure timing of communication to better align with project activities
  • Initial discussion: align materials, understand expertise needed, general schedule
  • Kick-off meeting: Techniques and Details

Optimize Knowledge Transfer Processes
Complex Troubleshooting

CASE STUDY 2

CHALLENGE
Scientific issues during method development/method transfer

SITUATION
- Human plasma PK assay
- Method issues when the assay changed hands – determined to be due to assay performance issues with freshly prepared, never frozen standards
- High pressure, high visibility
- Multiple stakeholders involved

KEY TAKEAWAY
- Operations team drove troubleshooting
- Work as team focused on the solution
- Balance communication
  - Provide clear summary of plans and results
  - Seek feedback
  - Don’t delay to await approval

Earn Trust and Empower Teams
CASE STUDY 3

CHALLENGE
Unsurmountable obstacle, requires pivot

SITUATION
► Human plasma and urine assays
► Initial bioanalytical plan called for hybridization based assay
► Scientific issues were escalated to the joint bioanalytical leads
► Team decided to commence LC-MS/MS method development in parallel

KEY TAKEAWAY
► Methods were successfully developed and validated
► Scientists came together to assess
► When faced with unsurmountable obstacle, team quickly pivoted to alternative solution to deliver results

Operate with Transparency and Flexibility
CASE STUDY 3

Clinical Method Development

Human Plasma LC-MS/MS Method

- Leveraged lessons learned from previous experience
- Stable isotope labelled internal standard
- 0.500 ng/mL LLOQ
- RP-IPC utilizing TEA and HFIP
- Confirmed selectivity against 5 metabolites
- BSA included in intermediate solutions to avoid non-specific binding
- MD and VAL progressed quickly and smoothly

Human plasma 0.5 ng/mL
CASE STUDY 3

Clinical Method Development

Human Urine LC-MS/MS Method

- Leveraged previous experience
- Stable isotope labelled internal standard
- 1 ng/mL LLOQ
- Non-specific binding to tubes
  - 53% loss after 5 transfers
  - Added Tween to address
- Further testing discovered non-specific binding to urine precipitate as well
  - Recovery with CHAPS
CASE STUDY 3

Clinical Method Development

Conclusions

► Suite of methods validated
  ► Human Plasma via LC-MS/MS and ECL
  ► Human Urine via LC-MS/MS
  ► Anti-drug antibody

► Issues were escalated with transparency leading to decision to commence parallel method development on alternate platform to commence

► Enables head-to-head comparison of platforms to better inform strategy

► Approach evolved from hELISA/ECL to LC-MS/MS as primary technology
Method Development Process
Overview & Highlights

Scientific Advisory Group
- Proactive portfolio review
- Early Engagement
- Sharing previous methods & lessons learned
- Issue guidance for operational teams

Joint Bioanalytical Leads
- Requirements intake
- Timing
- Intended use of the data
- Regulatory considerations
- Bioanalytical plan

Project Execution
- Leverage previous knowledge
- Direct scientist-scientist communication
- Issue escalation

Communicate openly and honestly

Optimize processes for efficient knowledge transfer

Leverage each contributor's unique expertise

Learn and progress
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