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PEARLS OF LABORATORY MEDICINE

Pearl Title: Immunoglobulin and T Cell Receptor Genetics

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DOI: 10.15428/CCTC.2020.318055

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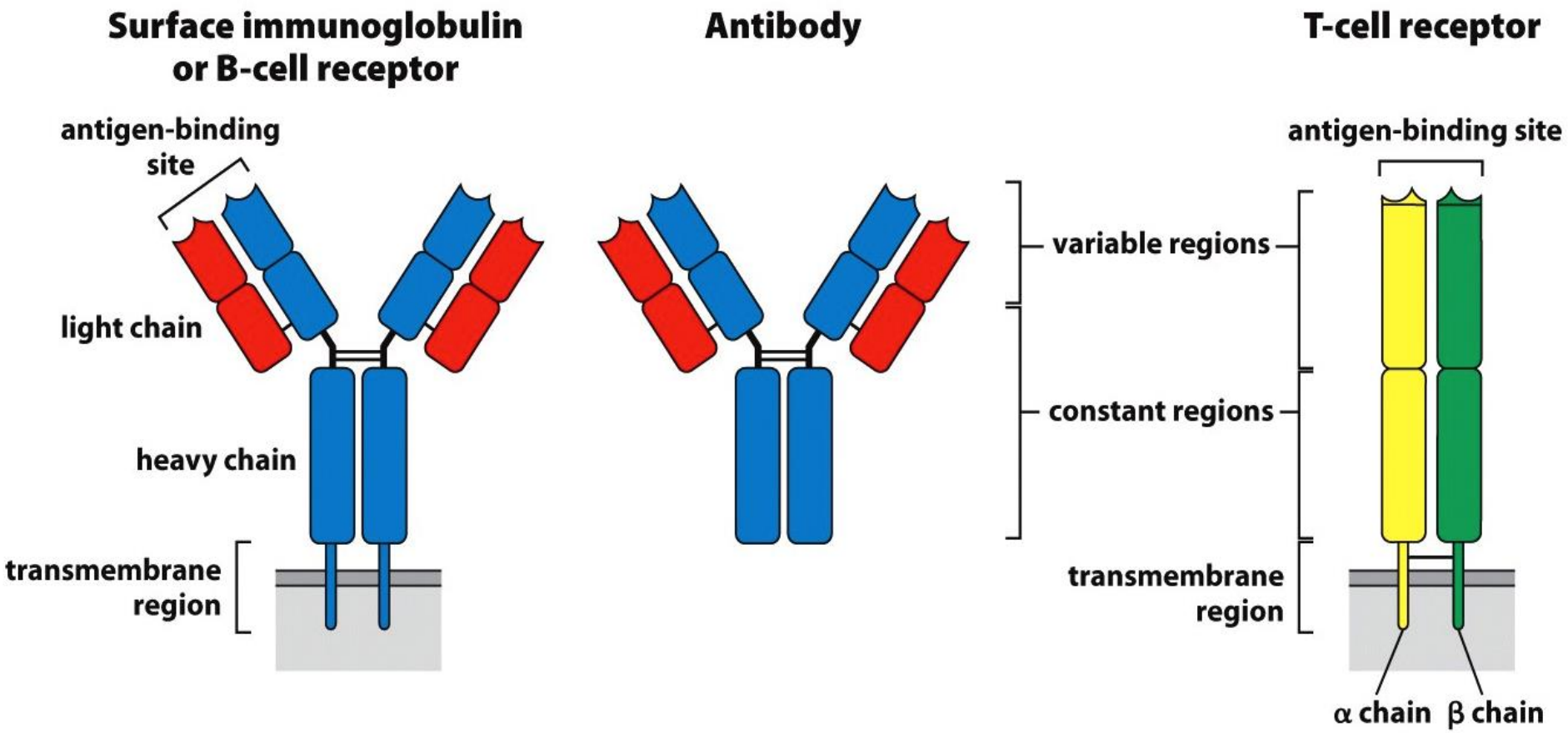
Outline

- Structure of Immunoglobulin (Ig) and TCR (T cell receptor)
- TCR/Ig genetics
- T/B cell development and TCR/Ig gene rearrangement
- Molecular testing of TCR/Ig rearrangement
- Clinical utility of molecular TCR/Ig rearrangement analysis in lymphoid malignancies

Immunoglobulin (Ig) and T Cell Receptor (TCR)

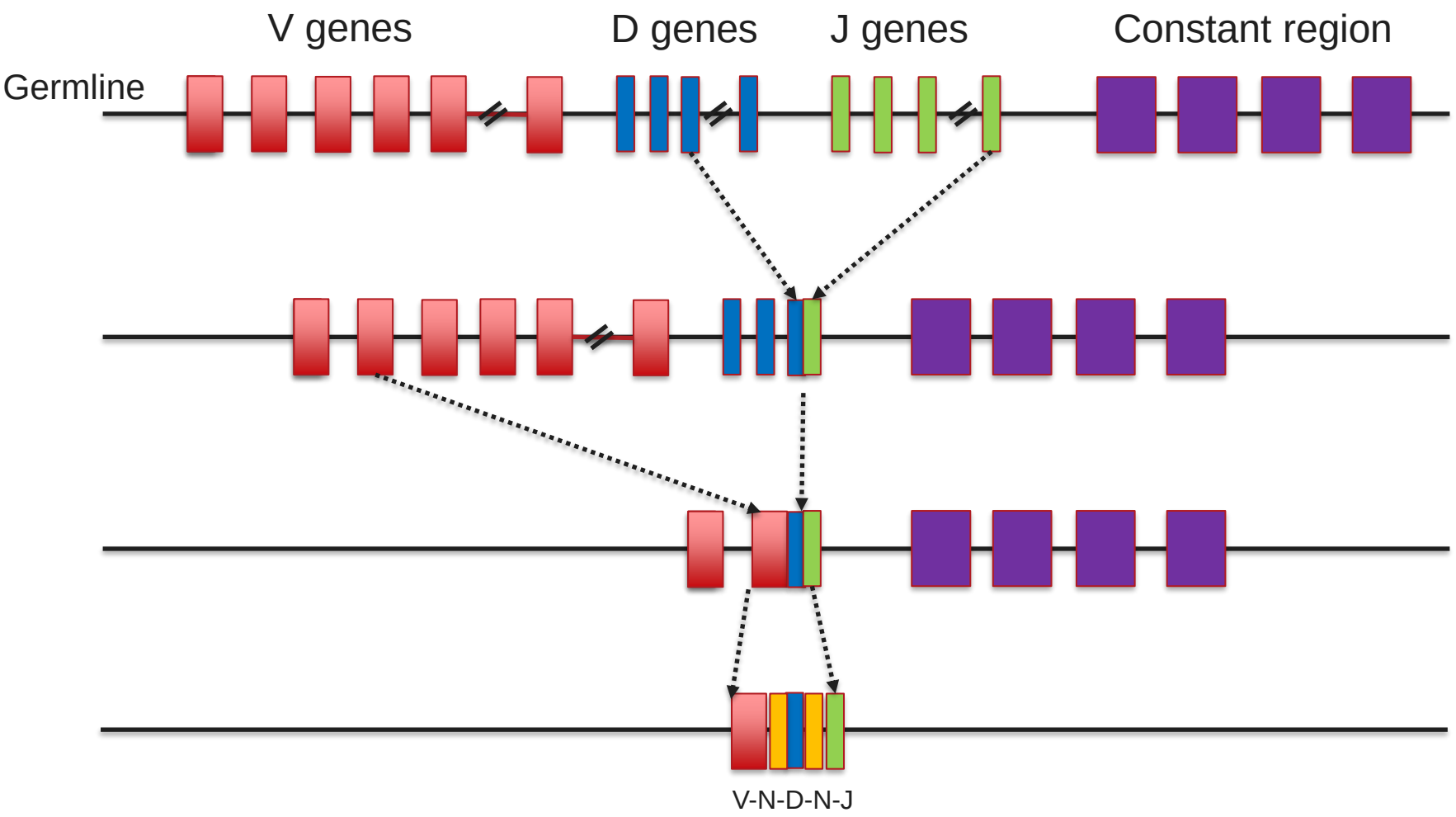


The Immune System, 3ed. (© Garland Science 2009)

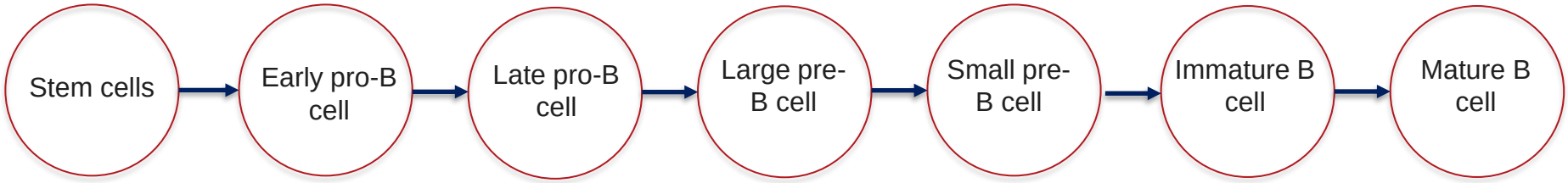
TCR/Ig Genetics

- Each receptor chain is encoded in the germline genome as different gene segments (V, D, J).
- During T/B lymphocyte development, the gene segments undergo random somatic DNA recombination (VDJ or VJ) to form a complete variable region sequence, known as gene rearrangement.
- Non-templated insertion and deletion of random nucleotides at the junctional regions.
- Somatic hypermutation: high rate of point mutations in V region of Ig genes (mature B cells).

IGH Gene Rearrangement



B cell development and BCR/Ig gene rearrangement



IgH: germline	D-J joining	V-DJ joining	VDJ rearranged	VDJ rearranged	VDJ rearranged	VDJ rearranged
IgK/L: germline	germline	germline	germline	V-J joining	VJ rearranged	VJ rearranged

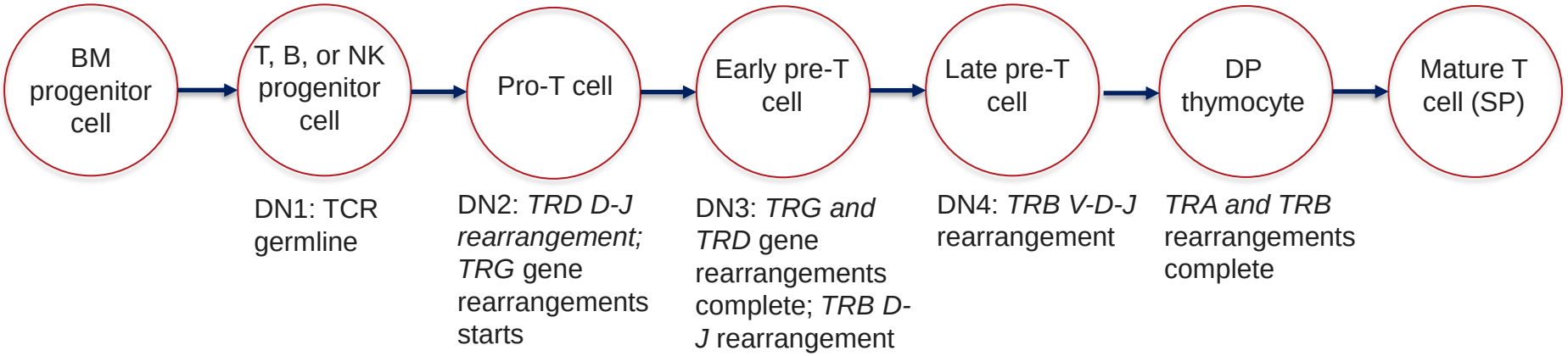
Order of gene rearrangements:

IGH → *IGK*

If *IGK* rearrangement fails → *IGL* gene rearrangement

The Immune System, 3ed. (Garland Science 2009)

T cell development and TCR gene rearrangement



Hierarchical order of TCR gene rearrangements:

TRD gene rearranges, followed by *TRG* → TCRγδ expression

Or

Followed by *TRB* rearrangements and *TRD* deletion, then *TRA* rearranges → TCRαβ expression

Staal et al. 2001

Estimated Number of Nonpolymorphic Human V, D, J Gene Segments Potentially Involved in TCR/Ig Gene Rearrangements

Gene segment		Ig genes			TCR genes			
		IGH	*IGK*	*IGL*	*TRA*	*TRB*	*TRG*	*TRD*
V	Functional	44	43	38	46	47	6	8
	Rearrangeable	66	76	56	54	67	9	8
D	Rearrangeable	27	-	-	-	2	-	3
J	Functional	6	5	4	53	13	5	4
	Rearrangeable	6	5	5	61	13	5	4

Adapted from van Dongen et al. Leukemia 2003

Clonal TCR/Ig Rearrangements in Lymphoid Neoplasms

- Lymphoid neoplastic cells derived from the single common precursor cell would be expected to harbor identical TCR/Ig DNA sequences (tumor markers).

- Although vast majority of lymphoid malignancies contain identical TCR/Ig gene sequences, TCR/Ig gene rearrangements are not necessarily lineage-specific.

Molecular Testing of TCR/Ig Rearrangements

- Methods/Techniques:
  - Southern blotting
  - PCR-capillary electrophoresis
  - Next generation sequencing

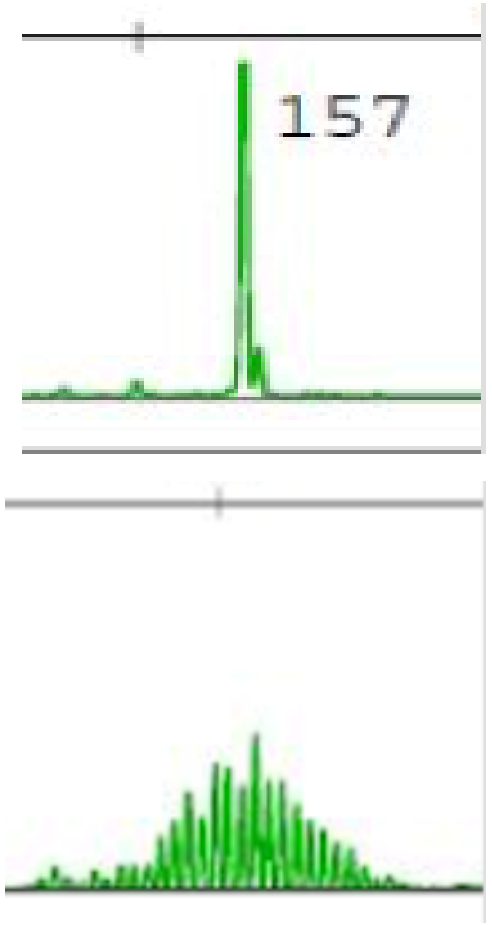
- Types of applications:
  - Clonality analysis
  - MRD

Southern Blotting-Based TCR/Ig Rearrangement Analysis

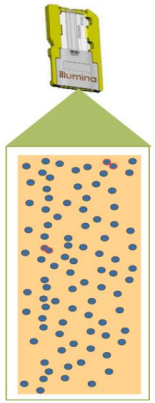
- Used to be considered as gold standard for clonality testing
- Require high quantity of high molecular weight DNA
- Lower false positive rate
- 5-10% analytical sensitivity
- Require restriction enzyme digestion; labor intensive
- Turn around time: 1-2 weeks
- Not suitable for tracking clones

PCR/CE-Based TCR/Ig Clonality Analysis

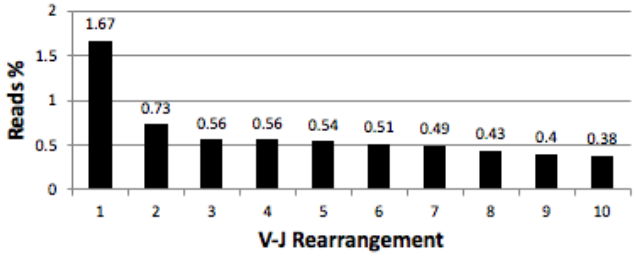
- Require small quantity of DNA
- Degraded DNA acceptable (FFPE)
- Fragment size-based interpretation; No need of restriction enzyme digestion
- Sensitivity: 2-5%
- Fast: 1-2 day TAT
- Not suitable for MRD analysis



NGS-Based TCR/Ig Rearrangement Analysis



Name	Total count	Rank	Sequence	Length	Merge count	V-gene	J-gene	% total reads
18-CLONE29-A008-	1109327	1	CAAAGCTGCTGTTCCACTACTATGACAAAGATTTTAACAATGAAGCAGACACCCCTGATAACTTCCAATCCAGGAC	254	18498	Vb15	Jb2-5	1.6674975
18-CLONE29-A008-	1109327	2	CCTTCCCATTTTAATTCACTGCCTTTGTCTTTTCCAAGCCCCACACAGTCAGACTAACCTCTGCCACCTGCGCTTCCTG	193	8146	Db2	Jb2-7	0.7343191
18-CLONE29-A008-	1109327	3	TTCAGTTCCTCTTTGAATACTTCAGTGAGACACAGAGAAACAAAGGAAACTTCCCTGGTCGATTCTCAGGGCGCCA	197	6240	Vb5-1	Jb1-2	0.5625032
18-CLONE29-A008-	1109327	4	ATGGGCTGAGGCTGATCCATTACTCATATGGTGTTCAAGACACTAACAAAGGAGAAGTCTCAGATGGCTACAGTG	197	6188	Vb10-1	Jb2-1	0.5578157
18-CLONE29-A008-	1109327	5	GGAGGTGAGAAGGAAGCCCCCGGCCTGGTCCATACCCCACCACCAACTTGCATAATGGGGGGTGATGTCACCCAC	215	5990	Db1	Jb1-2	0.5399670
18-CLONE29-A008-	1109327	6	TTCAGTTCCTCTTTGAATACTTCAGTGAGACACAGAGAAACAAAGGAAACTTCCCTGGTCGATTCTCAGGGCGCCA	191	5630	Vb5-1	Jb1-1	0.5075149
18-CLONE29-A008-	1109327	7	GGAGGTGAGAAGGAAGCCCCCGGCCTGGTCCATACCCCACCACCAACTTGCATAATGGGGGGTGATGTCACCCAC	206	5486	Db1	Jb1-1	0.4945341
18-CLONE29-A008-	1109327	8	AACAATCGATTCTTAGCTGAAAGGACTGGAGGGACGTATTCTACTCTGAAGGTGCAGCCTGCAGAACTGGAGGAT	138	4720	Vb14	Jb2-7	0.4254832
18-CLONE29-A008-	1109327	9	CCTTCCCATTTTAATTCACTGCCTTTGTCTTTTCCAAGCCCCACACAGTCAGACTAACCTCTGCCACCTGCGCTTCCTG	200	4426	Db2	Jb2-2	0.3989806
18-CLONE29-A008-	1109327	10	CATGACAAAATGTACTGGTATCAACAAGATCCAGGAATGGAACTACACCTCATCCACTATTCCTATGGAGTTAATT	252	4265	Vb25-1	Jb2-7	0.3844673



Clinical Utility of Molecular TCR/Ig Rearrangement Analysis in Lymphoid Malignancies

- Aid diagnosis of challenging or inconclusive cases of lymphoproliferative disorders
- Evaluation of clonal relationship between two or more lesions in the same patient
- Discrimination between a relapse and a second malignancy
- Lymphoma staging (dissemination/localization)
- Monitoring/evaluation of treatment effectiveness

Summary

- TCR/Ig genes are encoded in the germline genome as different gene segments.
- During T/B cell development, the V, (D), and J gene segments undergo random recombination to form complete V-region sequence, known as TCR/Ig gene rearrangement.
- Additional non-templated nucleotide insertions at the junctional regions during gene rearrangements and somatic hypermutations in Ig genes further increase TCR/Ig repertoire diversity.
- Molecular analysis of TCR/Ig gene rearrangements has important clinical utility in diagnosis and monitoring of lymphoid malignancies.

Figure/Table Titles

- Immunoglobulin (Ig) and T Cell Receptor (TCR)
- B cell development and BCR/Ig gene rearrangement
- T cell development and TCR gene rearrangement
- *IGH* Gene Rearrangement
- Estimated Number of Human V, D, J Gene Segments Involved in TCR/Ig Gene Rearrangements

Text/Figure/Table/Image Citation
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Disclosures/Potential Conflicts of Interest

Upon Pearl submission, the presenter completed the Clinical Chemistry disclosure form. Disclosures and/or potential conflicts of interest:

- **Employment or Leadership**: No disclosures
- **Consultant or Advisory Role:** No disclosures
- **Stock Ownership:** No disclosures
- **Honoraria:** Invivoscribe
- **Research Funding:** No disclosures
- **Expert Testimony:** No disclosures
- **Patents:** No disclosures

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