Fluorescence Polarization Immunoassay

FPIA

FPIA

- Fluorescein-labelled drug competes with unlabelled drug for antibody
- Sample excited with plane polarized light (490 nm)
- Fluorescein emits plane polarized light (520 nm)
- Small, free drug-fluorescein, rotates faster leading to less emission
- Larger, antibody-drug-fluorescein, rotates slower and produces more emission

FPIA

- Drug in sample competes for antibody with fluorescein bound drug
- More drug in the sample; less fluorescein labelled drug bound to antibody; lower emission of plane polarized light
- Higher drug concentration results in lower light emission values

FPIA

- Available for a variety of drugs
- Advantages
  - Rapid turnaround times, sensitivity, ease of operation
- Disadvantages
  - Background interference in serum sample (requires blank measurement)

FPIA Equipment

Redrawn from: Pieper and Rutledge, Laboratory Techniques for Pharmacists, Upjohn 1989, page43, figure 14

Comparing Analytical Methods

- Sample (Type and size)
- Analysis Time
- Sensitivity, Specificity
- Accuracy, Precision
- Ease of Use, Versatility
- Cost of Equipment
- Cost of Supplies

Comparing Analytical Methods

	HPLC	GLC	RIA	EMIT	FPIA
Size	100-500	100-500	100	50	50
Type	P,S,U,Sa	P,S,U,Sa	P,S,U,Sa	P,S,U	P,S
Time	0.5	0.5	0.25	0.1-0.3	0.1-0.2
Sensitivity	1	1	2	3	2
Specificity	1	1	4	4	3
Accuracy	2	2	2	2	2

with 1 best

Comparing Analytical Methods

	HPLC	GLC	RIA	EMIT	FPIA
Precision	3	3	2	1	1
Training	5	5	3	3	2
Versatile	1	3	3	3	2
Equipment Cost	4	4	5	3	4
Reagent	3	2	2	3	2
Clinical	4	5	4	2	1
Research	1	2	3	3	3

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