

Diamedix
A Subsidiary of IVAX Diagnostics, Inc.

Diamedix Test Kits and Disease States

A. Schrandt (updated 01.27.12)

Diamedix ELISA Test Kits

Diamedix ELISA Test Kits Provide:

- Extended stability (most tests = 18 months)
- High affinity-binding plastic wells (retain coating well)
- Effective Post-coat treatment (blocks background, affords greater stability)
- Sensitivity and Specificity
 - Antigen coating of highest purity
 - Recombinant, plasmid, peptide, immunoaffinity purified
 - Wash Buffer
 - Optimized for each analyte's individual requirements
 - Sample Diluents
 - Specialized to reduce interference

Diamedix ELISA Test Kits Provide:

- Positive-Negative Discrimination
 - Well-characterized clinical sample panels maintain consistent calibration, lot-to-lot, year-to-year
- Well-to-Well, Plate-to-Plate Repeatability
 - Plates coated/tested to ensure consistent responses throughout the lot, according to known military standards
- Lot-to-Lot Consistency
 - Secondary standards maintained for long-term use
 - Process validation enforced
 - Each lot matched, and traceable to original (released) assay

Diamedix ELISA Test Kits Provide:



- Ready-to-Use Reagents
 - Liquids in final use form AND in automation-ready containers
 - No pour off required
- Common Component Reagents
 - Within group, optimized components
- Breakapart Wells
 - Eliminates waste/inconvenience of batch testing

Diamedix ELISA Test Kits Provide:

- Extensive Menu
 - >50 Autoimmune/Infectious tests and growing
 - Additional tests/methodologies from Strategic Partnerships
- Overall recognized high quality results
 - Based on more than 25 years of ELISA knowledge
 - Extensive qualification of raw materials
 - Adequate inventory of raw materials maintained
 - Rigorous control of manufacturing steps
 - Quality testing of individual reagents throughout each lot preparation

Diamedix Kits Feature

- Color coded Antigen wells
 - Prevents accidental mixup
- Indicator desiccant
 - Ensures correct well storage/stability
- Color coded reagents and caps
 - Prevents cross-contamination
- Standardized Calibrators/Control Values
 - Easier to use
 - No correction factors necessary
 - No change in ranges from lot-to-lot
 - No possibility to use incorrect values

DETAIL OF ELISA REAGENT KIT COMPONENTS

- Antigen Wells:
 - 96 microwells/plate
 - 12 strips of 8 wells/plate
 - Strips are break-apart
 - Use Blank Wells (analyte appropriate) to complete a strip of 8
 - Strips are color-coded...See ALL PARAMETERS CHART
 - **Unused strips should be put back into kit and refrigerated**
 - **Stable until Expiration date on label of plate package**

DETAIL OF ELISA REAGENT KIT COMPONENTS

- WASH Buffer:
 - 2 bottles Wash Concentrate per kit
 - Add contents of 1 Wash Concentrate bottle to 1000 ml H₂O
 - Intended Instrument WASH bottle is designated by screen commands
 - Wash solutions are shared by different kits...See Parameters Chart
- **Stable at room temperature**
- **Inspect/Discard if buffer appears cloudy**

DETAIL OF ELISA REAGENT KIT COMPONENTS

- Sample Diluent:

- Ready to use
- Volume of 60 ml
- Mix by 'gentle' inversion
- Color of solution , and cap, in most kits is **BLUE**
- Some kits have **GREEN** sample diluent (contain additives to reduce interference) and cap
- Sample Diluents are shared by different kits
- **Stable until Expiration date on label, when kept refrigerated after use.**

DETAIL OF ELISA REAGENT KIT COMPONENTS



- Conjugate:
 - Ready to use
 - (Capture IgM kits require conjugate 'tracer' to be prepared)
 - Volume of 25 ml
 - Mix by swirling
 - Color of solution, and cap, **RED**
 - Conjugates are UNIQUE to most kits
 - **Stable until Expiration date on label, when kept refrigerated after use.**

DETAIL OF ELISA REAGENT KIT COMPONENTS



■ HRP Substrate:

- Ready to use
- Volume of 25 ml
- Mix by swirling
- Color of bottle, **BROWN** and opaque
- Substrates are shared by different kits
- **Stable until Expiration date on label, when kept refrigerated**
- **after use.**

DETAIL OF ELISA REAGENT KIT COMPONENTS



■ Stop Solution:

- Ready to use
 - Volume of 30 ml
 - Mix by swirling
 - Color of bottle, WHITE
 - Stop solutions are shared by different kits
- **Stable until Expiration date on label, when kept refrigerated after use.**

DETAIL OF ELISA REAGENT KIT COMPONENTS



- Controls/Calibrators:

- Pre-diluted:

- Ready to use
 - Not to be diluted with sample diluent
 - Added to microwells directly from tube
 - Volumes stated on the label
 - Mix by gentle inversion
 - Color of solution, **BLUE** liquid, cap colors vary
 - **Stable until Expiration date on label, when kept refrigerated after use.**

DETAIL OF ELISA REAGENT KIT COMPONENTS



■ Controls/Calibrators:

Undiluted:

- Are to be diluted with sample diluent
- Volumes stated on label
- Mix by 3 quick bursts on a vortex
- Color is NEG = **BLACK**
 - POS = WHITE
 - CAL = **BLUE**
- **Stable until Expiration date on label, when kept refrigerated after use.**

REVERSE CAPTURE IgM KITS

- Tracer Reagent:
 - 3 components used to make Tracer
 - **Lyophilized Antigen:**
 - 6 vials of powder/kit
 - **Tracer Diluent:**
 - 1 bottle/kit
 - 30 ml
 - Color of solution, **Red**
 - **30X Tracer:**
 - 1 tube/kit
 - 1 ml
 - Color of cap, **Red**

REVERSE CAPTURE IgM KITS

■ PREPARATION:

- Tap powder down in antigen vial
- Add 2.9 ml Tracer Diluent
- Dissolve by gentle inverting and swirling
- Add 100ul (0.1ml) 30X Tracer
- Invert gently to mix
- Transfer to tube for assay
- Discard prepared Tracer after testing
- Tracer reagents (**diluent and 30x concentrate**) are stable until Expiration date on label, when kept refrigerated after use.

DO's and DON'T's

- **DO** Check volume of Controls/Calibrators before starting a run.

Undiluted Controls/Calibrators should be > 100ul

Pre-diluted Controls/Calibrators should be > 600ul

- **DO** Replace all caps to the same vials and bottles
- **DO** Replace unused strips into **tightly closed bags** from kits and refrigerate once run has started

More DO's and DONT's

- **DO** Try to put Controls/Calibrators back into refrigerated kit once sample incubation has started
- **DO** Always check kit label on end of box to make sure components are being placed in the correct kit
- **DON'T** Combine strips from different kit lot numbers
- **DON'T** Pour conjugates from tubes back into bottle-
Save remaining in tube. Add conjugate to it for the next run.

REAGENT BY ANALYTE

AUTOIMMUNE

TEST	WASH SOLUTION	SAMPLE DILUENT	CONJUGATE	SUBSTRATE	STOP
Is-SSA	ENA Wash	ENA Diluent	ENA Conjugate	ENA Substrate	ENA Stop
Is-SSB	ENA Wash	ENA Diluent	ENA Conjugate	ENA Substrate	ENA Stop
Is-Sm	ENA Wash	ENA Diluent	ENA Conjugate	ENA Substrate	ENA Stop
Is-Sm/RNP	ENA Wash	ENA Diluent	ENA Conjugate	ENA Substrate	ENA Stop
Is-Scl-70	ENA Wash	ENA Diluent	ENA Conjugate	ENA Substrate	ENA Stop
Is-Jo-1	ENA Wash	ENA Diluent	ENA Conjugate	ENA Substrate	ENA Stop
Is-ENA-6 Screen	ENA Wash	ENA Diluent	ENA-6 Screen Conjugate	ENA Substrate	ENA Stop
Is-ANA ELISA Screen	U Wash	E Diluent	ANA ELISA Screen Conjugate	HRP Substrate	O Stop
Is-dsDNA	U Wash	E Diluent	dsDNA Conjugate	HRP Substrate	O Stop
Is-Rheumatoid Factor	U Wash	E Diluent	RF Conjugate	HRP Substrate	O Stop
Is-Cardiolipin IgG or IgM	X Wash	F Diluent	(various) ACA IgG or IgM Conjugate	H Substrate	P Stop
Is-Cardiolipin IgA	X Wash	F Diluent	ACA IgA Conjugate	H Substrate	P Stop
Is-Cardiolipin Screen	X Wash	F Diluent	(combined) ACA IgG,IgM,IgA Conjugate	H Substrate	P Stop
Is-b2 Glycoprotein IgG or IgM	X Wash	F Diluent	(various) b2-Glycoprotein IgG or IgM Conjugate	H Substrate	P Stop
Is-Gliadin IgG	Gliadin Wash	Gliadin Diluent	Gliadin IgG Conjugate	Gliadin Substrate	N Stop
Is-Gliadin IgA	Gliadin Wash	Gliadin Diluent	Gliadin IgA Conjugate	Gliadin Substrate	N Stop
Is-MPO	T Wash	A Diluent	MPO Conjugate	HRP Substrate	N Stop
Is-PR-3	T Wash	A Diluent	PR-3 Conjugate	HRP Substrate	N Stop
Is-TPO	T Wash	A Diluent	TPO Conjugate	HRP Substrate	N Stop
Is-TG	T Wash	A Diluent	TG Conjugate	HRP Substrate	N Stop

REAGENT BY ANALYTE

INFECTIOUS DISEASE

TEST	WASH SOLUTION	SAMPLE DILUENT	CONJUGATE	SUBSTRATE	STOP
Is-EBV-VCA IgG	T Wash	B Diluent	VCA IgG Conjugate	HRP Substrate	N Stop
Is-EBV-VCA IgM	T Wash	C Diluent	VCA IgM Conjugate	HRP Substrate	N Stop
Is-EBV EA-D IgG	T Wash	B Diluent	EA IgG Conjugate	HRP Substrate	N Stop
Is-EBV EA-D IgM	T Wash	C Diluent	EA IgM Conjugate	HRP Substrate	N Stop
Is-EBNA-1 IgG	T Wash	B Diluent	EBNA IgG Conjugate	HRP Substrate	N Stop
Is-EBNA-1 IgM	T Wash	C Diluent	EBNA IgM Conjugate	HRP Substrate	N Stop
Is-H. pylori IgG	T Wash	A Diluent	Hpylori IgG Conjugate	HRP Substrate	N Stop
Is-Mycoplasma pneumoniae IgG	T Wash	A Diluent	Mycopneu IgG Conjugate	HRP Substrate	N Stop
Is-Mumps IgG	T Wash	B Diluent	Mumps IgG Conjugate	HRP Substrate	N Stop
Is-Toxoplasma IgG	S Wash	A Diluent	Toxo IgG Conjugate	HRP Substrate	M Stop
Is-Toxoplasma IgM Capture	S Wash	A Diluent	Toxo IgM Conjugate	G Substrate	M Stop
Is-Rubella IgG	S Wash	A Diluent	Rubella IgG Conjugate	HRP Substrate	M Stop
Is-Rubella IgM Capture	S Wash	A Diluent	Rubella IgM Conjugate	G Substrate	M Stop
Is-CMV IgG	S Wash	A Diluent	CMV IgG Conjugate	HRP Substrate	M Stop
Is-CMV IgM Capture	S Wash	A Diluent	CMV IgM Conjugate	G Substrate	M Stop
Is-HSV 1 & 2 IgG	S Wash	A Diluent	HSV IgG Conjugate	HRP Substrate	M Stop
Is-HSV 1 & 2 IgM	S Wash	D Diluent	HSV IgM Conjugate	G Substrate	M Stop
Is-Measles IgG	S Wash	A Diluent	Measles IgG Conjugate	HRP Substrate	M Stop

COLOR SCHEME FOR COATED WELLS



WELL COLOR	INFECTIOUS PRODUCTS
Yellow	Is-EBV-VCA IgG
Purple	Is-EBV-VCA IgM
Light Green	Is-EBV EA-D IgG
Pink	Is-EBV EA-D IgM
Orange	Is-EBNA-1 IgG
Red	Is-EBNA-1 IgM
Dark Green	Is-H. pylori IgG
Dark Green	Is-Mycoplasma pneumoniae IgG
Yellow	Is-Mumps IgG
Silver	Is-Toxoplasma IgG
Yellow	Is-Toxoplasma IgM Capture
Purple	Is-Rubella IgG
Orange	Is-Rubella IgM Capture
Red	Is-CMV IgG
Pink	Is-CMV IgM Capture
Light Green	Is-HSV 1 & 2 IgG
Gold	Is-HSV 1 & 2 IgM
Dark Blue	Is-Measles IgG
Gold	Is-VZV IgG

ASSAY PARAMETERS BY ANALYTE

AUTOIMMUNE

AUTOIMMUNE DISEASE TEST	CAT. NO	CAL/CONTROLS	DILUTION	INCUBATION TIMES	WAVELENGTH*	TEMPERATURE	CALIBRATION**	UNITS
Is-SSA	720-250	Undiluted	1:101	30:30:30	405	18 - 30°C	SP or C/O	EU/ml or Index
Is-SSB	720-260	Undiluted	1:101	30:30:30	405	18 - 30°C	SP or C/O	EU/ml or Index
Is-Sm	720-240	Undiluted	1:101	30:30:30	405	18 - 30°C	SP or C/O	EU/ml or Index
Is-Sm/RNP	720-270	Undiluted	1:101	30:30:30	405	18 - 30°C	SP or C/O	EU/ml or Index
Is-Scl-70	720-280	Undiluted	1:101	30:30:30	405	18 - 30°C	SP or C/O	EU/ml or Index
Is-Jo-1	720-290	Undiluted	1:101	30:30:30	405	18 - 30°C	SP or C/O	EU/ml or Index
Is-ENA-6 Screen	720-230	Undiluted	1:101	30:30:30	405	18 - 30°C	C/O	Index
Is-ANA ELISA Screen	720-790	Undiluted	1:101	30:30:30	450	18 - 30°C	C/O	Index
Is-dsDNA	720-700	Undiluted	1:101	30:30:30	450	18 - 30°C	SP, 6P	IU/ml
Is-Rheumatoid Factor	720-710	Undiluted	1:101	30:30:30	450	18 - 30°C	SP, C/O	IU/ml or Index
Is-Cardiolipin IgG or IgM	720-400	Prediluted	1:101	30:30:30	450	18 - 30°C	3P, 6P	GPL or MPL
Is-Cardiolipin IgA	720-420	Prediluted	1:101	30:30:30	450	18 - 30°C	3P, 6P	APL
Is-Cardiolipin Screen	720-480	Prediluted	1:101	30:30:30	450	18 - 30°C	4P	U/ml
Is-b2 Glycoprotein IgG or IgM	720-830	Prediluted	1:101	30:30:30	450	18 - 30°C	3P, 6P	U/ml
Is-Gliadin IgG	720-800	Prediluted	1:51	30:30:30	450	18 - 30°C	3P, 6P	U/ml
Is-Gliadin IgA	720-810	Prediluted	1:51	30:30:30	450	18 - 30°C	3P, 6P	U/ml
Is-MPO (6-point or Qual)	720-430	Prediluted	1:21	30:30:30	450	18 - 30°C	6P or C/O	EU/ml or Index
Is-PR-3 (6-point or Qual)	720-440	Prediluted	1:21	30:30:30	450	18 - 30°C	6P or C/O	EU/ml or Index
Is-TPO (6-point or Qual)	720-450	Prediluted	1:101	30:30:30	450	18 - 30°C	6P or C/O	IU/ml or Index
Is-TG (6-point or Qual)	720-460	Prediluted	1:101	30:30:30	450	18 - 30°C	6P or C/O	IU/ml or Index

* Primary wave length indicated, differential filter for all tests (600-630nm)

** SP = Single Point, C/O = Cut-Off, 6P = 6 Point, 5P = 5 Point, 4P = 4 Point, 3P = 3 Point

ASSAY PARAMETERS BY ANALYTE

INFECTIOUS DISEASE

INFECTIOUS DISEASE TEST	CAT. NO	CAL/CONTROLS	DILUTION	INCUBATION TIMES	WAVELENGTH*	TEMPERATURE	CALIBRATION**	UNITS
Is-EBV-VCA IgG	720-600	Undiluted	1:21	30:30:30	450	18 - 30°C	C/O	Index
Is-EBV-VCA IgM	720-610	Undiluted	1:101	30:30:30	450	18 - 30°C	C/O	Index
Is-EBV EA-D IgG	720-640	Undiluted	1:21	30:30:30	450	18 - 30°C	C/O	Index
Is-EBV EA-D IgM	720-650	Undiluted	1:101	30:30:30	450	18 - 30°C	C/O	Index
Is-EBNA-1 IgG	720-620	Undiluted	1:21	30:30:30	450	18 - 30°C	C/O	Index
Is-EBNA-1 IgM	720-630	Undiluted	1:101	30:30:30	450	18 - 30°C	C/O	Index
Is-H. pylori IgG	720-130	Undiluted	1:21	30:30:30	450	18 - 30°C	C/O	Index
Is-Mycoplasma pneumoniae IgG	720-850	Undiluted	1:101	30:30:30	450	18 - 30°C	C/O	Index
Is-Mumps IgG	720-540	Undiluted	1:21	30:30:30	450	18 - 30°C	C/O	Index
Is-Toxoplasma IgG (3 pt or Qual)	720-300	Prediluted	1:101	60:60:20	450	37 ± 3°C	3P or C/O	IU/ml or Index
Is-Toxoplasma IgM Capture	720-310	Undiluted	1:101	60:60:20	450	37 ± 3°C	C/O	Index
Is-Rubella IgG (3 pt or Qual)	720-360	Prediluted	1:101	60:60:20	450	37 ± 3°C	3P or C/O	IU/ml or Index
Is-Rubella IgM Capture	720-370	Undiluted	1:101	60:60:20	450	37 ± 3°C	C/O	Index
Is-CMV IgG (3 pt or Qual)	720-320	Prediluted	1:101	60:60:20	450	37 ± 3°C	3P or C/O	EU/ml or Index
Is-CMV IgM Capture	720-330	Undiluted	1:101	60:60:20	450	37 ± 3°C	C/O	Index
Is-HSV 1 & 2 IgG	720-340	Prediluted	1:101	60:60:20	450	37 ± 3°C	SP or C/O	EU/ml or Index
Is-HSV 1 & 2 IgM	720-350	Undiluted	1:101	60:60:20	450	37 ± 3°C	C/O	Index
Is-Measles IgG	720-520	Prediluted	1:101	1.271180556	450	18 - 30°C	SP or C/O	EU/ml or Index
Is-VZV IgG	720-380	Prediluted	1:101	30:30:30	450	18 - 30°C	SP or C/O	EU/ml or Index

* Primary wave length indicated, differential filter for all tests (600-630nm)

** SP = Single Point, C/O = Cut-Off, 6P = 6 Point, 5P = 5 Point, 4P = 4 Point, 3P = 3 Point

ASSAY PARAMETERS BY ANALYTE

OTHER

OTHER		CAL/CONTROLS	DILUTION	INCUBATION TIMES	WAVELENGTH*	TEMPERATURE	CALIBRATION**	UNITS	Source
EZ Complement Sheep RBC Hemolysis)	789-001	Undiluted	none	60	415	18 - 30°C	SP	CH50 or %Ref	Diamedix

Anti-C6 Lyme Test	DK-E352-096AA	Undiluted	1:21	30:30:04	450/600-630	18 - 30°C	C/O	Index	Immunetics
HerpeSelect-1 &-2 IgG	EL0910-G & -20-G	Undiluted	1:101	60:30:10	450/(600-630)	18 - 30°C	C/O	Index	Focus
West Nile Virus IgG&IgM Select	EL0300G & M	Undiluted	1:101	60:30:10	450/(600-630)	18 - 30°C	C/O	Index	Focus
Anti-CCP	7975-Z	Prediluted	1:101	60:30:30	550	18 - 30°C	5P	U/ml	Axis-Shield
Anti-tTransglutaminase IgA & IgG	30-7503US &-04US	Prediluted	1:101	30:30:30	450/(600-630)	18 - 30°C	C/O	Index	AESKU
TREP-SURE	TS-96 & TS-960	Prediluted	none	60:30:15	450/(600-630)	18 - 30°C	C/O	Index	Phoenix

* Primary wave length indicated, differential filter for all tests (600-630nm)

** SP = Single Point, C/O = Cut-Off, 6P = 6 Point, 5P = 5 Point, 4P = 4 Point, 3P = 3 Point

ANALYTES GROUPED ACCORDING TO WASH SOLUTIONS USED - AUTOIMMUNE

Autoimmune Test	Wash Solution
Is-anti SSA	Is-ENA Wash
Is-anti SSB	Is-ENA Wash
Is-anti Sm	Is-ENA Wash
Is-anti Sm/RNP	Is-ENA Wash
Is-anti Scl-70	Is-ENA Wash
Is-anti Jo-1	Is-ENA Wash
Is-ENA-6 Screen	Is-ENA Wash
ANA ELISA Screen	U Wash
dsDNA	U Wash
Rheumatoid Factor	U Wash

Autoimmune Test	Wash Solution
Is-anti MPO	T Wash
Is-anti TPO	T Wash
Is-anti PR-3	T Wash
Is-anti TG	T Wash
Is-anti Cardiolipin Screen	X Wash
Is-anti Cardiolipin IgA	X Wash
Is-anti Cardiolipin IgG or IgM	X Wash
Is-anti B2-Glycoprotein IgG or IgM	X Wash
Is-anti Gliadin IgA	Is-Gliadin Wash
Is-anti Gliadin IgG	Is-Gliadin Wash

ANALYTES GROUPED ACCORDING TO WASH SOLUTIONS USED - INFECTIOUS

Infectious Test	Wash Solution
Is-Toxoplasma IgG	S Wash
Is-Toxoplasma IgM Capture	S Wash
Is-Rubella IgG	S Wash
Is-Rubella IgM Capture	S Wash
Is-CMV IgG	S Wash
Is-CMV IgM Capture	S Wash
Is-HSV 1 & 2 IgG	S Wash
Is-HSV 1 & 2 IgM	S Wash
Is-Measles IgG	S Wash
Is-VZV IgG	S Wash

Infectious Test	Wash Solution
Is-anti B. burgdoreferi IgG/IgM	T Wash
Is-EBNA-1 IgG	T Wash
Is-EBNA-1 IgM	T Wash
Is-EBV-VCA IgG	T Wash
Is-EBV-VCA IgM	T Wash
Is-EBV EA-D IgG	T Wash
Is-EBV EA-D IgM	T Wash
Is-H. pylori IgG	T Wash
Is-Mycoplasma IgG	T Wash
Is-Mumps IgG	T Wash

Diamedix and Third Party Kits



- Diamedix manufactures 39 ELISA Test Kits
- 300+ other test kits and components from 3rd party manufacturers
- ALL Diamedix test kits have been validated on all instrumentation we supply
- Some 3rd party test kits have been verified on Diamedix instrumentation
 - Refer to the Master Distributed Product For Sales excel sheet to see what kits have been verified

Autoimmune Disease

Autoimmune Disease



- Body develops antibodies against its own tissue
- Most have generic, non-descript symptoms that can mimic many other conditions
 - Joint pain
 - Muscle pain
 - Fatigue
 - Rash

Diseases Associated with ANA



- ANA = Antinuclear Antibodies
 - Antibody directed against proteins in the nucleus, nucleic acids or nucleoprotein
- ANA test is a sensitive SCREENING test
- ANA alone is not diagnostic for autoimmune diseases
 - Also found in patients with other non-immune conditions such as infections, cancer, or certain medications
- ANA's not always associated with illness
 - Found in approximately 5% of the normal population
 - Found in approximately 75% of normal elderly

Diseases Associated with ANA: System Lupus Erythematosus (SLE)



- Most common disease associated with positive ANA
- More prevalent than leukemia or MS
- Recognized in early stages in only 35% of patients
 - Can take an average of 5 years to diagnose SLE
- May have involvement of many organs
- Symptoms
 - 80% have cutaneous manifestation
 - Renal failure
 - Seizures

Diseases Associated with ANA: Sjogren's Syndrome



- Primary
 - Dry eyes, dry mouth, and arthritis
 - Enlargement of salivary glands, difficulty swallowing
- Secondary
 - Symptoms occur together with another autoimmune disease
- 90% are female

Diseases Associated with ANA: Scleroderma



- Women to men 2:1
- Appears in age 20-30's
- Replacement of smooth muscle throughout body by fibrous tissue
 - Stiffness of fingers due to tightening of the skin
 - Raynaud's phenomenon (pain in digits on exposure to cold)
 - Difficulty swallowing
 - Deep organ involvement of heart, lungs, kidneys can be severe
 - Joint manifestations similar to rheumatoid arthritis

Diseases Associated with ANA: CREST

- Calcinosis
 - Calcium deposits in soft tissue
- Raynaud's Phenomenon
 - Pain in digits on exposure to cold
- Esophageal dysmotility (difficulty swallowing)
- Sclerodactyl (stiffness of fingers)
- Telangiectasia (red lesions on skin or mucous membranes)
- Prognosis
 - 11.7 years if centromere negative, 1.1 years if centromere positive

Autoimmune Disease of Muscle



- Extremely difficult to diagnosis
 - Nonspecific symptoms
 - Numerous spontaneous remissions
- Polymyositis
 - Symmetrical weakness of larger joints
 - Difficulty swallowing
 - Weakness of neck muscles
- Dermatomyositis
 - In addition to polymyositis, also have erythematous rash

Mixed Connective Tissue Disease (MCTD)



- “Overlap disease with clinical features of SLE, scleroderma, and polymyositis
- Clinically milder form of autoimmune disease than SLE

Diagnosis of Autoimmune Disease Associated with ANA

Who should be tested?



- Patients complaining of any of the symptoms already described
- Most common = joint pain
- May initially see Primary Care Physician, General Practice MD, Internist
 - May be referred to a Rheumatologist

Testing Methods



- Screen assays
 - IFA
 - EIA – screen used primarily to rule out systemic autoimmune disease
- Confirmatory assays
 - IFA titer and pattern
- Antigen specific marker assays
 - EIA

ANA Associated Reagents

- ANA ELISA Screen
- dsDNA
- ENA-6 Screen
- SSA
- SSB
- Sm
- Sm/RNP
- Scl-70
- Jo-1

ANA ELISA Screen



- Each well contains dsDNA, histones, SSA/Ro, SSB/La, Sm, Sm/RNP, Scl-70, Jo-1, centromere, and other Ag extracted from the Hep-2 nucleus
- Recommend EIA to screen out the negatives
- Objective, not subjective test

dsDNA



- IgG against double stranded DNA
- Found in 50-70% of patients with SLE
- Not found in other systemic diseases
 - Scleroderma, Sjogren's, MCTD, CREST
 - Sometimes found in low levels in Rheumatoid arthritis
- Wells contain purified plasmid dsDNA Ag
 - No ss region – important because anti-ssDNA is found in the normal population

ENA (Extractable Nuclear Antigen)



- speckled pattern on fluorescent IFA
 - Not all speckled will be ENA positive
- Most common are:
 - Sm, Sm/RNP, SSA, SSB, Scl-70, Jo-1
 - Each associated with particular disease
- May order as screen or individual ENA's
 - Screen contains all 6 in one well

ENA and Disease

Disease Association	Antigen	Frequency of Ag
SLE	Sm	25-40%
MCTD	RNP	>95%
Sjogren's Syndrome	SSA	35-70%
Sjogren's Syndrome	SSB	50-60%
Scleroderma	Scl-70	15-30%
Polymyositis	Jo-1	20-30%
SLE	dsDNA	60%
CREST	Centromere	60-90%

Cascade



- Positive ANA
 - Run dsDNA and ENA Screen or titer and pattern by IFA
- Positive ENA Screen
 - Run individual ENAs

Non-ANA Related Systemic Autoimmune Diseases and Kits

Non-ANA Related Systemic Autoimmune Disease Kits



- Rheumatoid Factor (RF)
- MPO (P-ANCA)
- PR3 (C-ANCA)
- TPO
- TG
- Cardiolipin tests
- B2 Glycoprotein
- Gliadin

Rheumatoid Arthritis



- Systemic autoimmune disease
- Women to men 2:1, usually after 30 years of age
- Pain and swelling in small joints of hands
 - Symmetrical = both hands
 - May also involve skin, heart, lungs, skeletal muscle and other organs
- Preceded by low grade fever and weakness
- RF is not specific to Rheumatoid Arthritis
 - May be increased during acute infectious diseases

Rheumatoid Factor



- RF is the Ab associated with Rheumatoid Arthritis
 - Also associated with some bacterial and viral infections, parasitic disease, and cancer
- Elevated levels may be seen in 15% of the normal population greater than 65 years of age
- 75% of patients with RA have IgM
- Detects IgM to RF

Wegener's Granulomatosis

- Rare condition, no known cause
 - Small arteries throughout the body (kidney, lungs, and upper respiratory tract) damaged by inflammation of blood vessels resulting in glomerulonephritis (kidney disease where the part of the kidney that filters waste is damaged)
- Middle aged men
- Symptoms
 - Pneumonia, chronic sinusitis, joint and muscle pain, skin rash, night sweats, hematuria (red blood cells in the urine)
- Diagnosis can take up to 15 months
 - Includes chest X-ray, kidney biopsy, CT scans
 - ANCA tests and “confirm” assays for diagnosis and follow-up

ANCA

- Anti-Neutrophil Cytoplasmic Antibodies
 - Ab directed against cytoplasmic components of neutrophil granulocytes and monocytes
- Diagnostic marker for Wegener's Granulomatosis
- Also detected in RA, SLE, and inflammatory bowel disease but are not useful as a diagnostic test for these diseases
- Two types of cytoplasmic ANCA based on IFA pattern
 - C-ANCA – Cytoplasmic, Target Ag = PR-2 (Proteinase 3)
 - P-ANCA – perinuclear, Target Ag = MPO (myeloperoxidase)
- MPO and PR-3 are the predominant Ag in ANCA. They are not the only antigen.

MPO and PR3



- MPO
 - IgG to purified Myeloperoxidase
 - Gives p-ANCA pattern on IFA
- PR3
 - IgG to purified proteinase 3
 - Gives c-ANCA pattern on IFA

Hashimoto's Thyroiditis



- Enlargement of thyroid gland
- Female:male ratio = 10:1
- Almost all have Ab to Thyroglobulin

Grave's Disease



- Also called Thyrotoxicosis
- Hyperthyroid symptoms
 - Bulging eyes
- 1/3 of patients will have anti-TG
- Most will have antimicrosomal Ab

Anti-Thyroglobulin



- Ab to thyroid gland
- Detects IgG
- Seen with Hashimoto's and Grave's disease

Anti-TPO



- Ab to Thyroid Peroxidase
- Associated with Hashimoto's and Grave's diseases
- Not the same as anti-microsomal
- IgG Only

Celiac Disease



- Sensitivity of bowel to gliadin, fraction of gluten
- Some consider it a hypersensitivity disease, others an autoimmune disease
- Symptoms
 - Abdominal pain, diarrhea, weight loss

Gliadin



- Kits for IgG or IgA
- IgG Abs are more sensitive but less specific than IgA
- IgA Abs are more specific but less sensitive than IgG
- Normal population may have Gliadin IgG
- Used in conjunction with tissue transglutaminase and Endomysial Antibody tests

Testing for Celiac

- Aesku kit has tTg cross-linked with a deaminated Gliadin peptide
 - Will pick up patients with positive tTg OR positive Gliadin
 - This can be problematic since normal population may be Gliadin IgG positive
- IMMCO sells a Celiac kit for tTg and separate kits for Gliadin which contain deaminated gliadin peptides
- If only positive for anti-Gliadin IgG, not highly suggestive of Celiac disease
- Endomysial is excellent screening for Celiac and considered gold standard, should correlate well with tTg if the tTg kit is ONLY tTg

Anti-Cardiolipin



- Found in patients with SLE (10%) and other connective tissue diseases
- Disease state is called the Anti-phospholipid syndrome
 - Patient makes Ab to phospholipid in coagulation cascade (leads to blood clots in veins and arteries)
- Patients may produce IgG, IgM or IgA Ab to phospholipids
 - Cardiolipin is one of the phospholipids
- Separate IgG, IgM or IgA kits or Cardiolipin Screen

Anti-B2 Glycoprotein IgG and IgM



- Co-factor required for binding of Ab to cardiolipin

Infectious Diseases and Kits

Infectious Disease



- Contagious
- Ab to a micro-organism (bacteria, virus, parasite, or other)
- Organism is difficult or impossible to culture
- Evidence of Ab indicates exposure/infection
- Some can be transmitted post transplantation
 - Transplant donors and recipients must be monitored
 - CMV, Toxo, EBV, Syphilis, HIV, Hepatitis

Infectious Disease Assays



- Rubella IgG
- Rubella IgM Capture
- CMV IgG
- CMV IgM Capture
- Toxoplasma IgG
- Toxo IgM Capture
- Mycoplasma IgG
- VZV IgG
- Measles IgG
- Mumps IgG
- EBV VCA IgG or IgM
- EBNA IgG or IgM
- EBV-EA-D IgG or IgM
- HSV 1 and 2 IgG
- HSV 1 and 2 IgM
- H. pylori

Helicobacter pylori



- Gastritis, ulcers affect approx. 10% of US adults
- Treatable with antibiotics
- IgM not present at time of clinical symptoms
 - IgG and IgA produced in second week of infection
- Diamedix test kit is IgG Only

Epstein Barr Virus



- Infectious mononucleosis
- Symptoms
 - Sore throat, fatigue, aches
- Virus may be reactivated years later
 - Important in transplant cases
- Most common method of testing is a heterophile tests (card test), sheep RBC's agglutinate; not specific for EBV

Epstein Barr Virus



- Diamedix test kits – 6 kits can be run as a panel to identify stage of disease
 - EBV VCA IgG and IgM
 - VCA's appear within 1 week of infection
 - IgM precedes IgG
 - EBNA IgG and IgM
 - Develops 3-4 weeks later
 - EBV EA-D IgG and IgM
 - Detects EA-D, IgM appears early in infection

ToRCH Assays



- Toxoplasma IgG and IgM, Rubella IgG and IgM, CMV IgG and IgM, Herpes 1 and 2 IgG and IgM
- Prenatal test
 - Can cause life threatening infection for fetus or cause birth defects
- For pregnant women it is critical to determine if she is immune (has IgG), is infected but not immune (has IgM), or is not infected/susceptible.

Toxoplasma



- Host animal is cat
- *Toxoplasma gondii* – intracellular parasite
- Infection in humans
 - Ingested in raw or undercooked meat (lamb, pork, beef)
- Can cross placenta and infect fetus
 - Leads to spontaneous abortion, stillbirth or neurologic disorders
- Important to know if pregnant woman has IgG (immune) or IgM (current infection)

Rubella



- Virus, causes German Measles
- Can cross placenta
 - Infection in first trimester may cause spontaneous abortion, stillbirth or birth defects
 - Important to know if pregnant woman is immune or susceptible
- Part of pre-natal screen
- Part of MMR vaccination

CMV



- Cytomegalovirus
 - Asymptomatic in normal individuals
 - Can be sexually-transmitted
- Can cross placenta
 - Active infection during pregnancy can cause hearing loss, mental retardation, or other CNS disorders in fetus

HSV



- Herpes Simplex Virus
 - Type 1 – oropharyngeal area and eyes
 - Type 2 – genital and neonatal
- Rarely crosses placenta
- Baby infected when passing through birth canal
- 1/2 untreated neonatal cases are fatal

VZV



- *Varicella zoster virus*
 - Highly contagious
 - Primary infection – chicken pox
 - Can be very serious infection in adults
 - Re-occurs as shingles
- Test kit is IgG Only

Measles



- Part of MMR vaccine
- If vaccinated from 1963-1967
 - May be re-infected with wild type causing atypical disease clinically confused with Rocky Mountain Spotted Fever
- Test kit is IgG only

Mumps



- Contagious
 - Secondary involvement of testes, ovaries, CNS and other organs
- Test kit is IgG Only
- Part of MMR

Mycoplasma



- *Mycoplasma pneumoniae*
 - 20% community acquired pneumonias
 - Up to 50% in military setting
 - Causes walking pneumonia
- Test kit is IgG



QUESTIONS???