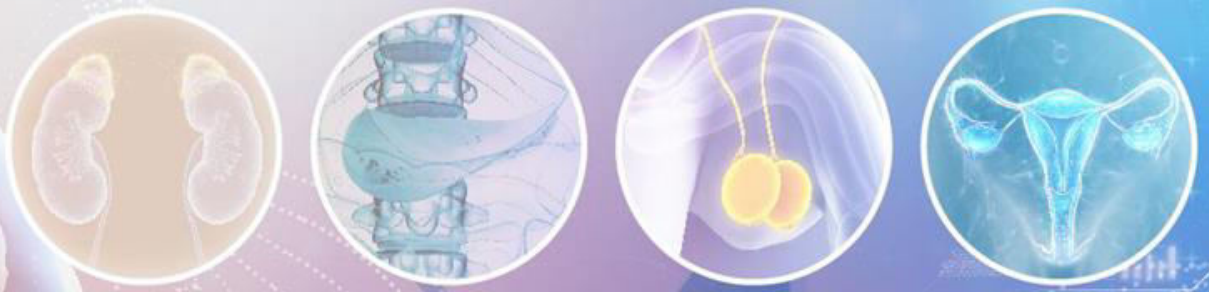
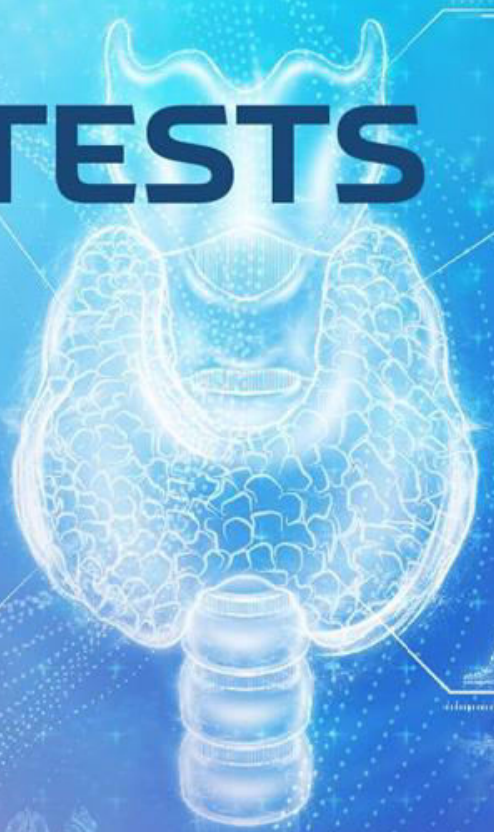
First Edition 2024

PRACTICAL GUIDE TO

ENDOCRINE DYNAMIC TESTS

KKM Endocrine Subspecialty Service





MEMS
Established 1981

Malaysia Endocrine
& Metabolic Society

Ministry of Health
Malaysia

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Specimen Handling in Complex Dynamic Tests

5.12.2024

Content

- Overview of Dynamic Tests
- The importance of proper sample handling in complex dynamic tests
- Challenges in handling complex dynamic tests
- Adrenal venous sampling
  - Pre-analytical Factors
  - Specimen collection in AVS
  - Handling and Transport in AVS

OVERVIEW OF DYNAMIC TESTS

WHAT ARE DYNAMIC TESTS?

- Assess hormone secretion in response to
  - stimulation
  - suppression

- Purpose:
  - diagnosing, confirming, or ruling out endocrine disorders by evaluating how hormones or other biomarkers respond to controlled challenges over time.

KEY FEATURES OF DYNAMIC TESTS

01	PURPOSE	• To differentiate between normal and pathological hormonal responses • To confirm and localize the source of endocrine dysfunction
02	TYPES	• STIMULATION TESTS • SUPPRESSION TESTS
03	COMPONENTS	• Baseline measurement • Administration of stimulant or suppressor • Time sequential sampling to monitor response
04	CLINICAL RELEVANCE	• Provide insight into hormonal regulation and feedback mechanism • Essential for targeted therapeutic interventions

CLINICAL EXAMPLES

TEST NAME	PURPOSE	COMPLEXITY LEVEL
Serum Insulin Test (Glucose Challenge)	To assess insulin secretion and diagnose conditions like insulin resistance or hypoglycemia by stimulating insulin release.	Simple
ACTH Stimulation Test (Cosyntropin Test)	To evaluate adrenal insufficiency, including Addison's disease or secondary adrenal insufficiency, by stimulating cortisol production.	Moderate
Oral Glucose Tolerance Test (OGTT)	To assess glucose metabolism and diagnose diabetes, impaired glucose tolerance, or gestational diabetes by measuring glucose and insulin responses.	Moderate
Growth Hormone (GH) Stimulation Test	To evaluate growth hormone deficiency or excess (e.g., acromegaly or dwarfism) by stimulating GH release with substances like arginine or clonidine.	Moderate
Fludrocortisone Suppression Test (FST	To assess aldosterone production and to help diagnose conditions such as primary aldosteronism or hyperaldosteronism.	Moderate

TEST NAME	PURPOSE	COMPLEXITY LEVEL
LHRH (Luteinizing Hormone-Releasing Hormone) Stimulation Test	To assess gonadal function, particularly in suspected cases of hypogonadism, by measuring LH and FSH levels after LHRH administration.	Complex
Insulin Tolerance Test (ITT)	To evaluate pituitary and hypothalamic function by inducing hypoglycemia to stimulate growth hormone and ACTH release.	Complex
Dexamethasone Suppression Test	To diagnose Cushing's syndrome or identify cortisol production abnormalities by assessing cortisol suppression following dexamethasone administration.	Complex
Metyrapone Test	To evaluate adrenal cortisol production and the HPA axis, especially in cases of suspected adrenal insufficiency or Cushing’s syndrome.	Complex
AVS	Identifies the source of aldosterone overproduction	Complex
IPSS	Differentiates between pituitary and ectopic ACTH production	Complex

CRITERIA OF A COMPLEX DYNAMIC TEST

1. Multiple Phases of Testing

- Involves multiple stages or measurements taken at different time intervals.
- Requires precise timing of sample collection and intervention.
- Example: **ACTH Stimulation Test**, **Growth Hormone Stimulation Test**.

2. Invasive Procedures

- Requires intravenous injections or infusions (e.g., hormones, glucose).
- Blood or other fluid samples are taken at multiple time points.
- Example: **AVS, IPSS**.

3. Specialized Equipment

- Involves the use of specialized equipment for administration or monitoring.
- May include controlled hormone delivery devices, monitoring for adverse effects.
- Example: **AVS, IPSS**

4. Tight Monitoring and Timing

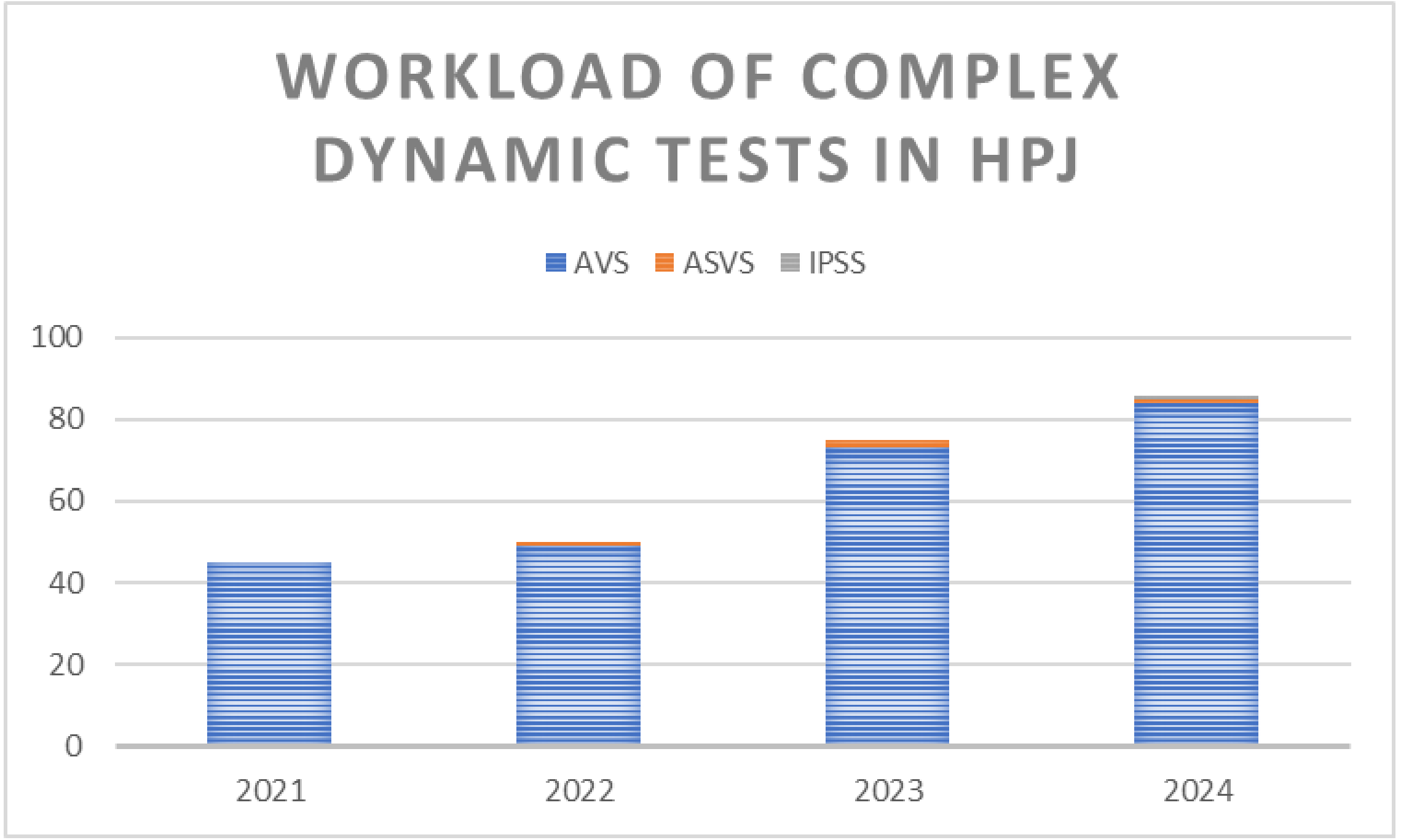
- Blood samples or physiological responses are measured at exact intervals.
- Careful monitoring is needed throughout the test to prevent complications.
- Example: **Insulin Tolerance Test**, **Dexamethasone Suppression Test**.

5. Potential for Serious Side Effects

- May induce reactions like hypoglycemia, cardiovascular effects, or hormonal imbalances.
- Requires immediate medical supervision to manage side effects.
- Example: **Insulin Tolerance Test (ITT)**, **ACTH Stimulation Test**.

6. Complex Interpretation of Results

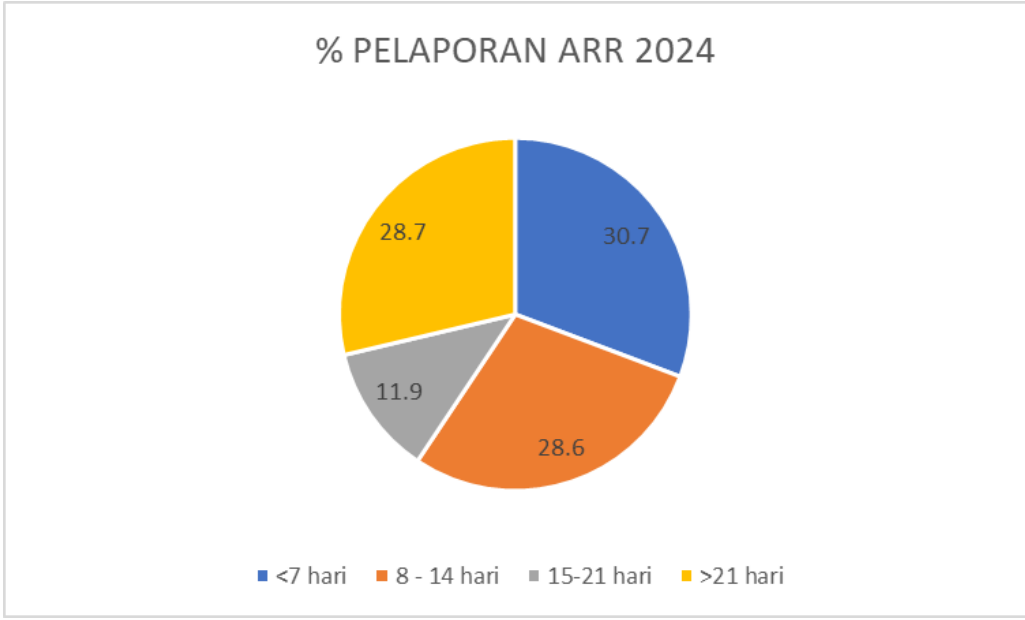
- Involves analysis of multiple hormone or metabolic responses.
- Requires specialized knowledge to differentiate normal and abnormal findings.
- Example: **ACTH Stimulation Test**, **CRH Stimulation Test**

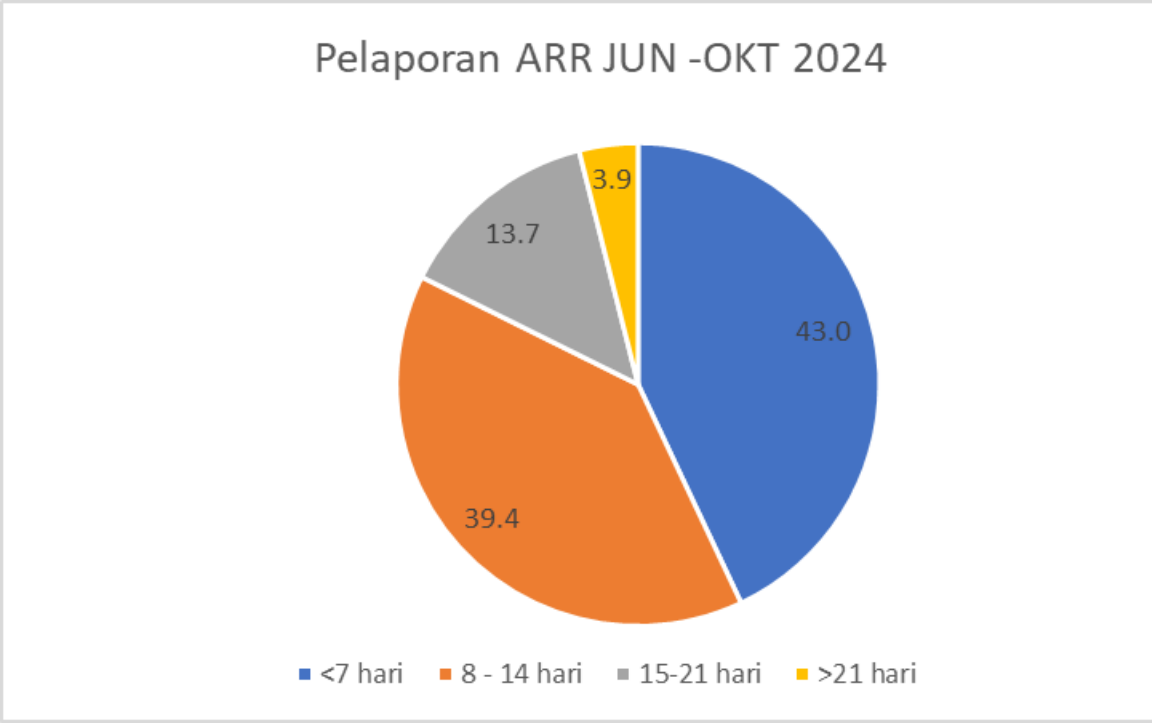

WORKLOAD OF COMPLEX DYNAMIC TESTS IN HPJ
AVS
ASVS
IPSS
100
80
60
40
20
0
2021
2022
2023
2024

ALDOSTERONE RENIN RATIO
Turn Around Time 2024

2024	Bebankerja ARR	LTAT				% Pencapaian			
		<7 hari	8 - 14 hari	15-21 hari	>21 hari	<7 hari	8 - 14 hari	15-21 hari	>21 hari
Jan	277	49	164	62	2	17.7	59.2	22.4	0.7
Feb	271	173	63	9	26	63.8	23.2	3.3	9.6
Mar	313	2	2	18	291	0.6	0.6	5.8	93.0
Apr	252	1	0	20	231	0.4	0.0	7.9	91.7
May	299	3	7	46	243	1.0	2.3	15.4	81.3
Jun	272	39	57	130	46	14.3	21.0	47.8	16.9
Jul	314	182	130	2	0	58.0	41.4	0.6	0.0
Aug	343	195	146	2	0	56.9	42.6	0.6	0.0
Sep	289	115	170	4	0	39.8	58.8	1.4	0.0
Oct	324	149	107	59	9	46.0	33.0	18.2	2.8
Jumlah	**2954**	**908**	**846**	**352**	**848**	**30.7**	**28.6**	**11.9**	**28.7**

*disclaimer: ARR is NOT a DYNAMIC TEST





IMPORTANCE OF PROPER SAMPLING

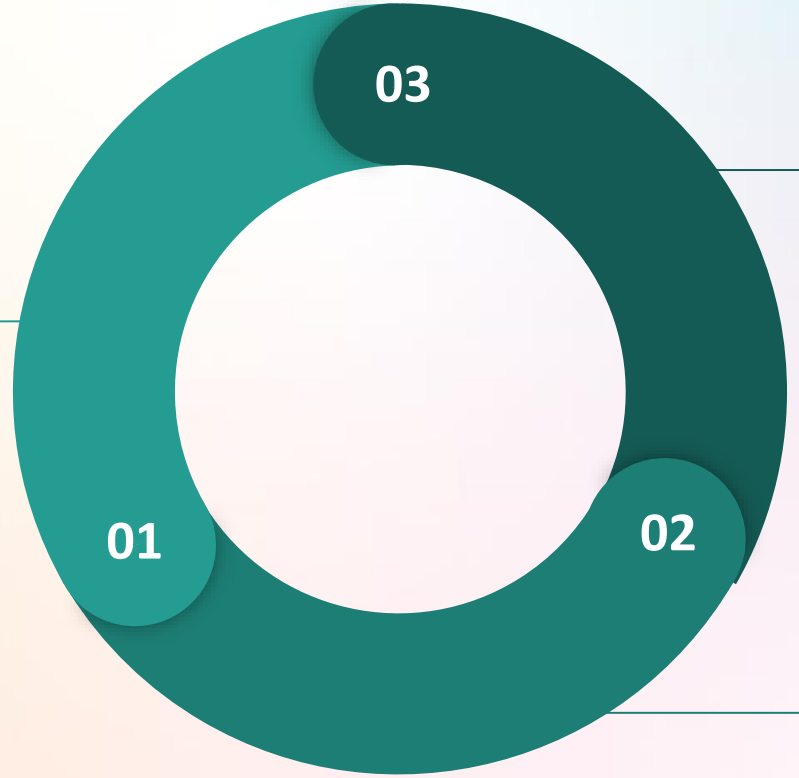
IMPORTANCE OF PROPER SAMPLING

PROPER SAMPLING ENSURES RELIABLE AND ACCURATE RESULTS

- ★ Dynamic tests are time-sensitive and analyte-specific.

- ★ Improper handling can lead to:
  - False-positive or false-negative results.
  - Delayed or incorrect diagnoses.

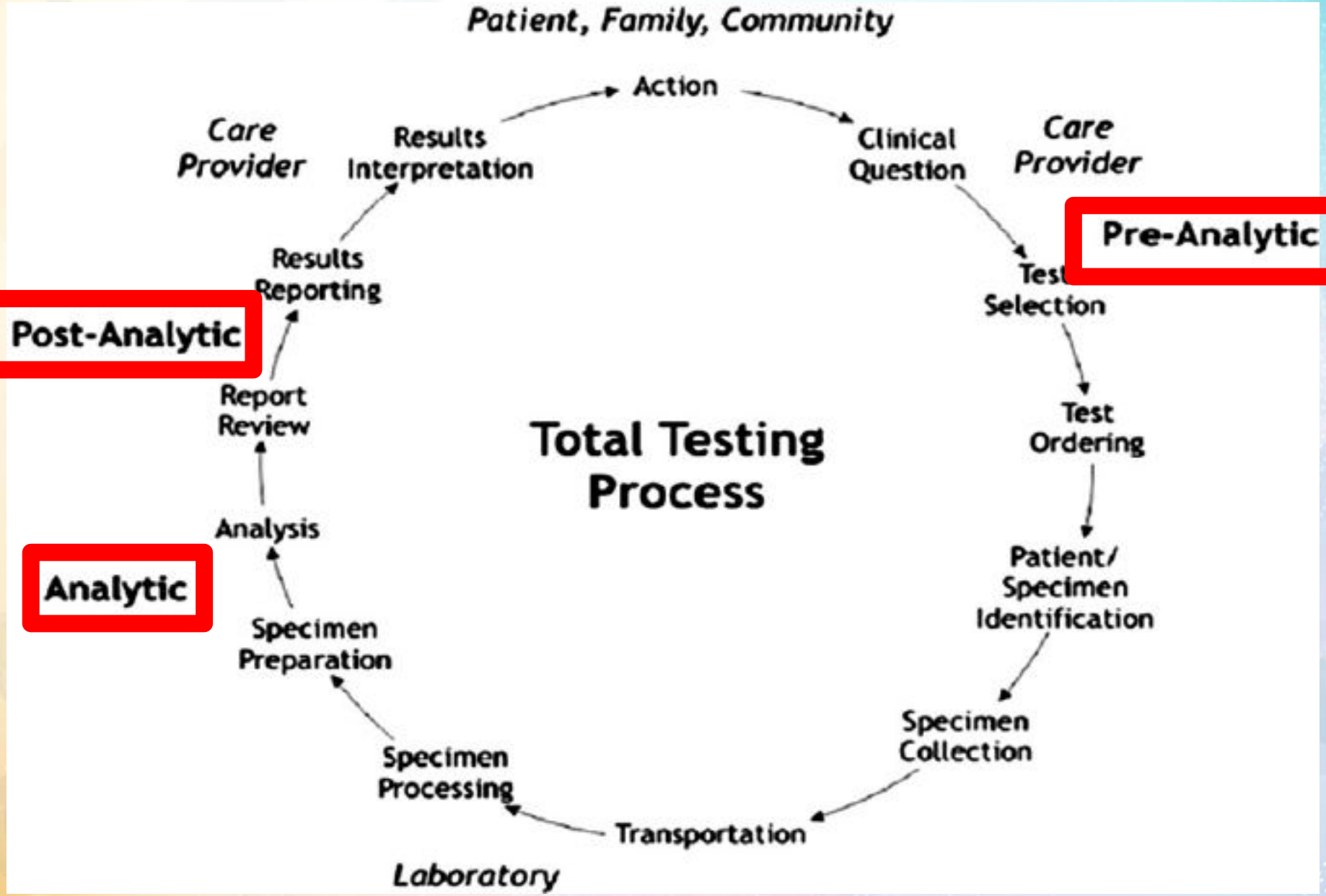
STAGES OF TESTING PROCESS



PRE-ANALYTICAL

POST-ANALYTICAL

ANALYTICAL


Patient, Family, Community
Action
Clinical Question
Care Provider
Test Selection
Test Ordering
Patient/ Specimen Identification
Specimen Collection
Transportation
Specimen Processing
Specimen Preparation
Analysis
Report Review
Results Reporting
Care Provider
Results Interpretation
Pre-Analytic
Post-Analytic
Analytic
Total Testing Process
Laboratory

IMPORTANT OF SPECIMEN HANDLING IN COMPLEX DYNAMIC TESTS

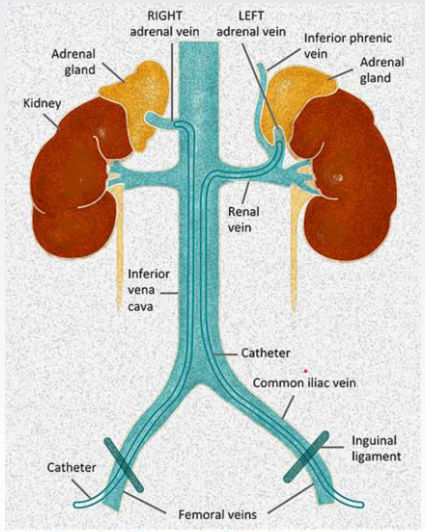
- Ensures Accurate Results
- Prevents Misdiagnosis
- Preserves patient safety
- Maintains workflow efficiency
- Complies with Standards and Guidelines
- Build Trust in Laboratory Testing

IMPORTANCE OF PROPER SAMPLING

- Minimizes pre-analytical errors such as:
  - Hemolysis or clotting of samples.
  - Incorrect timing or sequence of collection.
  - Inappropriate storage and transportation.

Challenges in Sampling

1. **Pre-Analytical Errors:**
   - Delays in transport affecting analyte stability (e.g., aldosterone degradation).
   - Incorrect anticoagulants leading to invalid results.
2. **Technical Challenges:**
   - Specialized techniques (e.g., catheter placement in AVS).
   - Patient factors (e.g., obesity or vascular anatomy).
3. **Logistical Issues:**
   - Coordinating between clinical and laboratory teams.



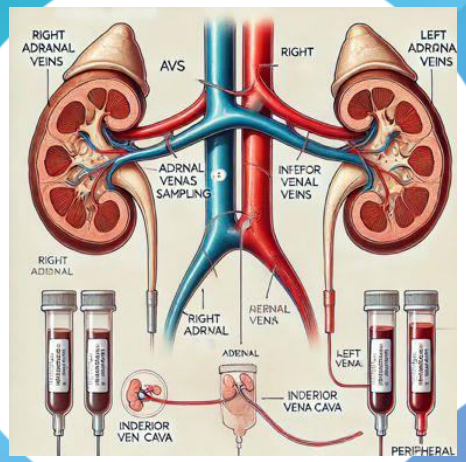
Source: Diagnosis and Management of Primary Aldosteronism: the Endocrine Society Guideline 2016 Revisited ©Bioscientifica. Used with permission.

• Key Steps for Proper Sampling

- **Pre-Test Preparation:**
  - Patient fasting or medication withdrawal as appropriate.
  - Clear communication between clinicians and lab personnel.
- **Sample Collection:**
  - Use appropriate collection tubes (e.g., EDTA for ACTH).
  - Precise timing for sequential samples.
    - Label samples clearly with time and date
    - Collect at exact prescribed intervals.
- **Post-Collection Handling:**
  - Immediate centrifugation if required.
  - Appropriate storage (e.g., refrigeration or freezing).

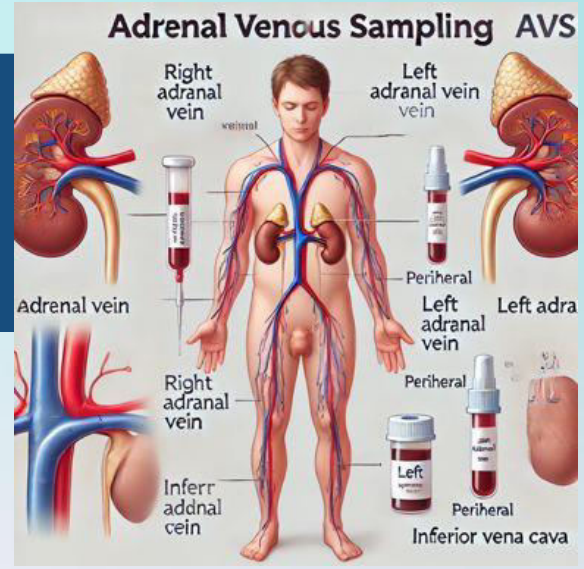
COMMON PRE-ANALYTICAL ERRORS

- Improper specimen collection techniques
- Inadequate specimen volume
- Incorrect labeling
- Inappropriate handling during transportation, e.g. temperature fluctuations or delay in transportation can cause degradation of the analytes being tested.



ADRENAL VENOUS SAMPLING

AVS



- Invasive and expensive test
  - making appropriate patient selection essential.
- It is a highly specialised and technically challenging procedure that requires multidisciplinary involvement
- It is a complex test whose success rate depends on the interventional radiologist
- The aim of AVS is to subtype primary aldosteronism (PA) into either bilateral or unilateral idiopathic hyperaldosteronism (IHA), often caused by an aldosterone-producing adenoma (APA).

ADRENAL VENOUS SAMPLING (AVS)

Purpose: Differentiate unilateral from bilateral causes of primary aldosteronism (e.g., aldosterone-producing adenoma vs. bilateral adrenal hyperplasia).

Key Handling Steps:

1. Catheterization:
   a. Blood drawn from both adrenal veins and a peripheral vein.
   b. Ensure adrenal vein catheterization success using cortisol ratios.
2. Timing and Transport:
   a. Samples should be processed immediately for cortisol and aldosterone levels.

Challenges:

- Ensuring adequate venous sampling.
- Maintaining analyte stability during transport

AVS: PRE-ANALYTICAL FACTORS

Patient Preparation:

- Fasting required for tests like OGTT and AVS.
- Discontinue interfering medication
  - For AVS: Stop diuretics (e.g., spironolactone) 2–4 weeks before.

Environmental Conditions:

- Reduce patient stress, especially for tests involving venous sampling.

Team Preparation:

- Cross-check protocols, labels, and equipment.

Preparation

- The appointment for an AVS is made by contacting the Interventional Radiologist in the respective hospital, and should be arranged as the 1st case in the morning, i.e., by 9 a.m.
- 6 weeks before the test: Stop spironolactone, amiloride and diuretics. Hypertension may be controlled with α-blockers (prazosin or terazosin) or CCBs (verapamil is preferred) or oral hydralazine.
- Within 1 week of the test: Check serum potassium to ensure normokalaemia. The serum potassium should preferably be >4.0 mmol/l before the test. Patients may require oral potassium supplementation with Slow K or potassium chloride mixture.
- One day before the test:
  - Admit the patient and ensure normokalaemia, optimised BP and obtain blood samples for full blood count, prothrombin time and partial thrombin time.
  - Insert a branula and keep the patient fasted overnight.
  - Obtain the consent for the AVS procedure.
  - Prepare and correctly label the specimen bottles.
  - Order for Synacthen® (Adrenocorticotropic hormone [ACTH] – Cosyntropin® 250 µg) from the pharmacy.
  - Inform the endocrine laboratory once the patient is admitted and arrange for laboratory staff to be present on site after sampling to transport the blood samples.
  - Make the most recent contrast-enhanced computed tomography (CT) adrenal available for the Interventional Radiologist to review.

SPECIMEN BOTTLES FOR AVS

Specimen bottles	**Investigation Specimen bottle type**
Serum aldosterone	EDTA tube Purple top with gel vacuum tube
Serum cortisol	Plain tube Yellow top with gel vacuum tube

SPECIMEN COLLECTION IN AVS

Key Steps in AVS:

- Insert catheters under fluoroscopic guidance into:
  - Right and left adrenal veins.
  - Peripheral vein (for comparison).
- Validate successful adrenal vein catheterization using cortisol ratios:
  - Adrenal-to-peripheral cortisol ratio >5 confirms correct sampling.
- Collect samples and transport to the lab promptly.

Common Errors:

- Mislabeling of adrenal vein samples.
- Incorrect interpretation of lateralization index.

Appendix 10 –
Adrenal Vein Sampling (AVS)

Adrenal Vein Sampling (AVS)

Name: **Patient ID:**
Date:

Time start:

Site	Aldosterone (pmol/l)	Cortisol (nmol/l)	Cortisol AV/P (SI)	A/C ratio
Left AV1				
Peripheral 1				
Left AV2				
Peripheral 2				
Left AV3				
Peripheral 3				
Right AV1				
Peripheral 1				
Right AV2				
Peripheral 2				
Right AV3				
Peripheral 3				

A/C, aldosterone to cortisol ratio; AV, adrenal vein; AV/P, adrenal vein to peripheral vein ratio; P, peripheral; SI, selectivity index.

Sequential AVS is the method usually used where a SINGLE femoral vein access will be made by the Interventional Radiologist.

SITE	ALDOSTERONE	CORTISOL	CORTISOL AV/P	A/C RATIO	Lateralization Index (LI)
LEFT AV1					
RIGHT AV1					
PERIPHERAL					
LEFT AV2					
RIGHT AV2					
PERIPHERAL					

Simultaneous AVS involves sampling of the right adrenal vein and left adrenal vein and peripheral sample from the antecubital vein (PV) for PAC and serum cortisol by individually designated staff for each sampling site, using pre-labeled bottles.

HANDLING & TRANSPORT IN AVS

Best Practices:

- Maintain temperature stability for aldosterone and cortisol
- Samples should be sent to the lab and processed immediately.
- Label samples clearly with vein origin (e.g., “Right Adrenal” or “Peripheral”).

Case Study:

- Error: Delayed transport led to aldosterone degradation.
- Solution: Implementing strict transport protocols improved stability

CHALLENGES IN COMPLEX TESTS

- Timing: Delayed sample collection
- Volume: Insufficient blood volumes for analysis.
- Labeling Errors: Mix-ups between adrenal veins and peripheral veins in AVS.
- Temperature Control: Improper storage

SOLUTIONS TO SAMPLING ERRORS

Preventive Strategies:

- Standardized protocols for dynamic tests.
- Staff training on timing, techniques, and labeling
- Use of detail checklists to ensure compliance for sample labeling, timing and storage

BEST PRACTICES

- Standardized protocols for each test.
- Training for staff on dynamic test procedures.
- Use of automation where possible for consistency.
- Close collaboration between clinicians and laboratory personnel.

INNOVATIONS IN SPECIMEN HANDLING

- Automated Sample Labeling Systems: Minimize errors in AVS and IPSS.
- Point-of-Care Processing: Reduces analyte degradation risks.
- Advanced Tube Technology: Preserves hormone stability over time.

SUMMARY AND TAKEAWAYS

- ★ Specimen handling in dynamic tests like AVS requires precision and vigilance.
- ★ Adherence to protocols minimizes errors and ensures diagnostic accuracy.
- ★ Continuous training and system improvements are critical for optimal outcomes.

- ★ Takeaway Message: Reliable results depend on meticulous handling at every stage.

THANK YOU

Reference

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