A new WHO manual – what must be done by the laboratories now?

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Learning Objectives

- What is the manual about?
- What is new to the laboratory?
- How is this related to the ESHRE-SIGA recommendations?
- What about reference ranges?
- What should the clinician ask the lab about?

Editorial Committéen 2005-7

Dr Trevor G. Cooper*
Münster, Germany

Do we need a new edition?

- Contradictions and ambiguities
  - Choice of technique
  - Details in procedures

- Scientific basis
  - Evidence wherever possible
  - Less ”expert”

- Standardization

What is the manual about?

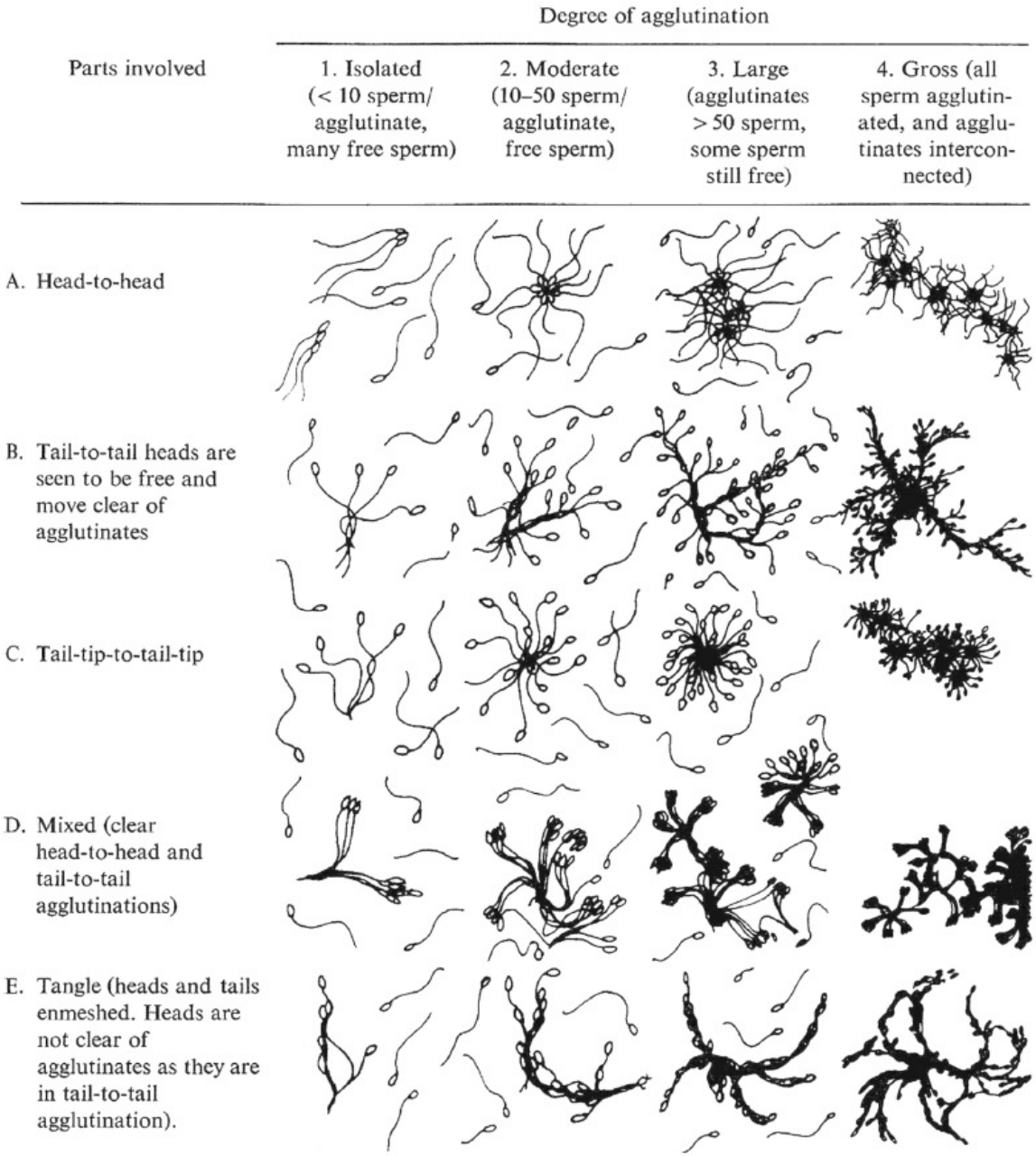
- How to do the lab work
  - Best available methods
    - Robust
    - Easiest to control
    - Easiest to learn and maintain
- Awareness of sources of errors
- Basic methods for laboratory quality control

News in the new edition

- New or expanded chapters:
  - sperm *preparation*
  - *cryopreservation* of spermatozoa
  - *cervical mucus* - optional procedures + Appendix

- New Format
  - separate chapters on basic semen analysis (routine, optional, research)
  - details of all working solutions, procedures, calculations and interpretation
  - minimal cross reference to other parts

News in the new edition



Parts involved	Degree of agglutination			
	1. Isolated (< 10 sperm/ agglutinate, many free sperm)	2. Moderate (10–50 sperm/ agglutinate, free sperm)	3. Large (agglutinates > 50 sperm, some sperm still free)	4. Gross (all sperm agglutinated, and agglutinates interconnected)
A. Head-to-head				
B. Tail-to-tail heads are seen to be free and move clear of agglutinates				
C. Tail-tip-to-tail-tip				
D. Mixed (clear head-to-head and tail-to-tail agglutinations)				
E. Tangle (heads and tails enmeshed. Heads are not clear of agglutinates as they are in tail-to-tail agglutination).				

News in the new edition

– Sources of errors and remedies:

Procedure	*Prevention*	*Control*
Microscope – cleanliness, proper alignment, proper magnification	Training, SOP, Equipment	IQC, EQC
Inadequate training before performing analysis	Training	IQC, EQC
Subjective techniques without clear guidelines	Training, SOP	IQC, EQC
Hearing about, reading about or being trained on classification systems (may cause inconsistency or changes during analysis)	Training	IQC (control charts)
Semen inadequately mixed when smear was prepared	Training, SOP	IQC
Poor smear preparation (i.e. too thick or too thin)	Training, SOP	IQC
Poor staining technique (i.e. light, dark or too much background staining)	Training, SOP	IQC
Assessing spermatozoa on edge of slide	Training, SOP	IQC
Attempting to score spermatozoa that are not flat, or are overlapping other spermatozoa	Training, SOP	IQC
Not scoring all spermatozoa in area but selecting spermatozoa for assessment	Training, SOP	IQC
Fading of stain over time (i.e. for stored IQC samples)	Training, SOP	IQC (control chart)
Errors in calculating percentages if not counted in multiples of one hundred	Training, SOP	IQC, EQC
Malfunction of multi-key counter	Equipment maintenance	IQC, EQC

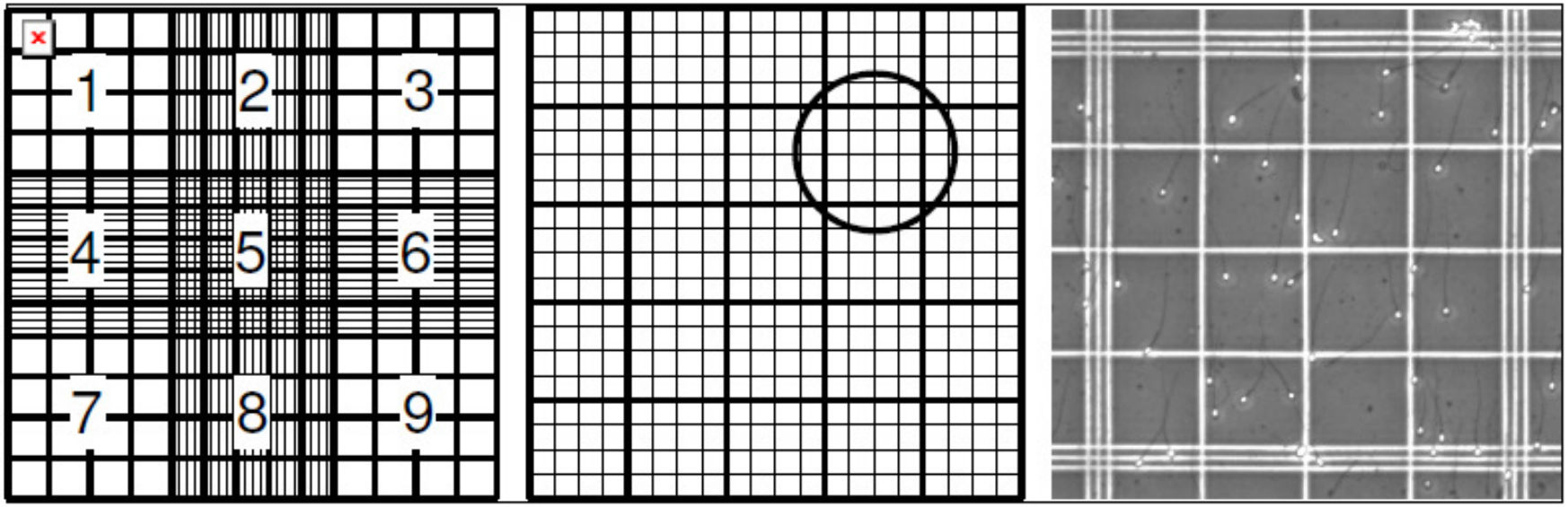
How to do the lab work

- Patient instructions
- Sample handling from collection to investigation
- Suitable equipment and lab materials
- Techniques including controls
- Calculations and presentation of results

What is new to the laboratory?

- Not much, for the state-of-the-art laboratory…
  - Number of spermatozoa assessed
  - Duplicate counts with comparisons

  - Concentration – count peripheral fields of numbers too low



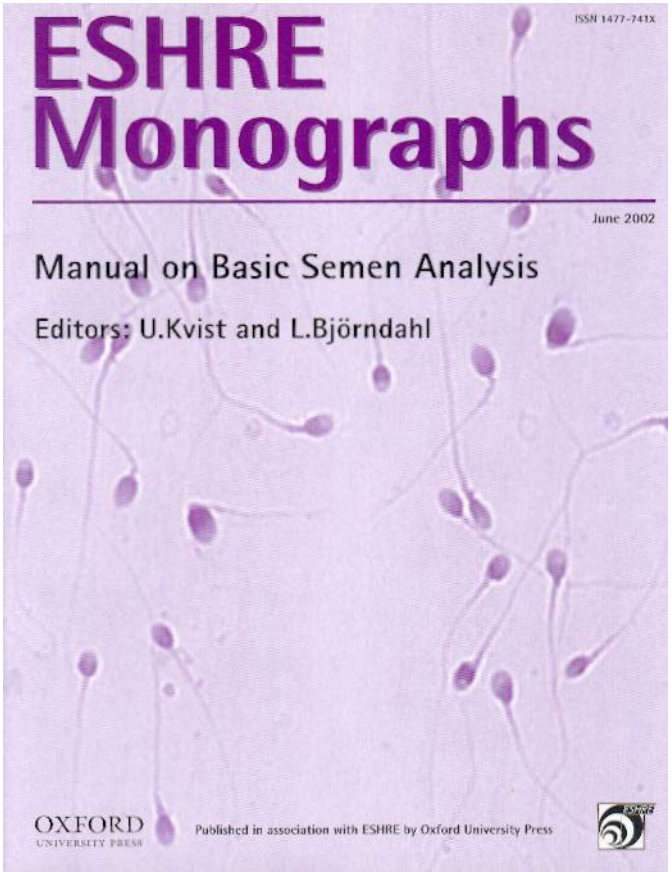
What is new to the laboratory?

- Not much, for the state-of-the-art laboratory…
  - Number of spermatozoa assessed
  - Duplicate counts with comparisons

  - Concentration – count peripheral fields of numbers too low
  - Sperm vitality – one-step Eosin-Nigrosin test
  - Motility: assessing rapid progressive ”optional”
  - Morphology: ”Tygerberg strict criteria”
    - Teratozoospermia index ”optional” but as in WHO ’92

How is it related to the ESHRE-SIGA recommendations?

– Will be discussed in SIGA



How is it related to the ESHRE-SIGA recommendations?

- Will be discussed in SIGA – my forecast:
  - *Step-by-step procedure* descripton adopted from the ESHRE
  - Sperm counting at *low concentrations* could be simpler…
  - The proportion *rapid progressively* can be assessed
    - Absence of this group is a very negative indicator for IVF
  - Vitality testing is adopted from SIGA
  - Morphology – maintain Teratozoospermia index (WHO 92)

What about reference ranges?

- All queries have referred to reference ranges!!
- All data from recent fathers
  - Useful for men in infertile couples?
- When can you use WHO reference ranges?
  - Methods
  - Populations
  - EQA

What should the clinician ask the lab about?

- **Do you comply with** methods recommended by ESHRE/SIGA or by WHO (*edition?*) ?
  - (does your vitality test unintentionally kill sperm?)
- How is your staff **trained** for semen analysis?
- **How many sperm** do you assess for each investigation?
- Do you make **duplicate counts** and **compare**?
- Do you run an **internal quality control programme**?
- Do you participate in **External Quality Assessment** programme **based on recommended methods**?

Some final thoughts

- **To comply with the WHO Manual is not to…**
  - *use the reference limits without bothering about which lab methods that are used*

- **The major factor for variation in sperm morphology between laboratories is…**
  - *which criteria are used*

- **The overall most important factor for quality in semen analysis is**
  - *the awarness of sources of error*

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Thanks for your attention!

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