

Diagnostic methods for intestinal protozoan parasites

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Intestinal Protozoal infection

Diarrhoeal complaints

Diarrhoea is a frequent, worldwide complaint. On any given day, 200 million people suffering from gastroenteritis will pass a volume of diarrhoeal water comparable with the flow of water over the Victoria Falls in one minute.

In most attacks, routine laboratory techniques will fail to demonstrate the cause.

Diagnostic workup of medical doctors

- The advantage of time
- In most cases a stool culture / virology is ordered
- Parasites are underestimated

Diagnostic work-up

- The not uncommon habit of medical doctors to order only a stoolculture in diarrheal patients appeared to be suboptimal
- In those cases 34% of protozoal diarrhoea would have been missed

Laboratory

- Sensitivity problems!
 - *Giardia lamblia*
sensitivity single fresh stool sample: 60 - 80 %

Danciger M et.al. *Am.J.Trop.Med.Hyg* 1975;24:237-42

Burke JA et.al. *Rev.Clin.Lab.Science* 1977;7:372-91

Conventionele procedure

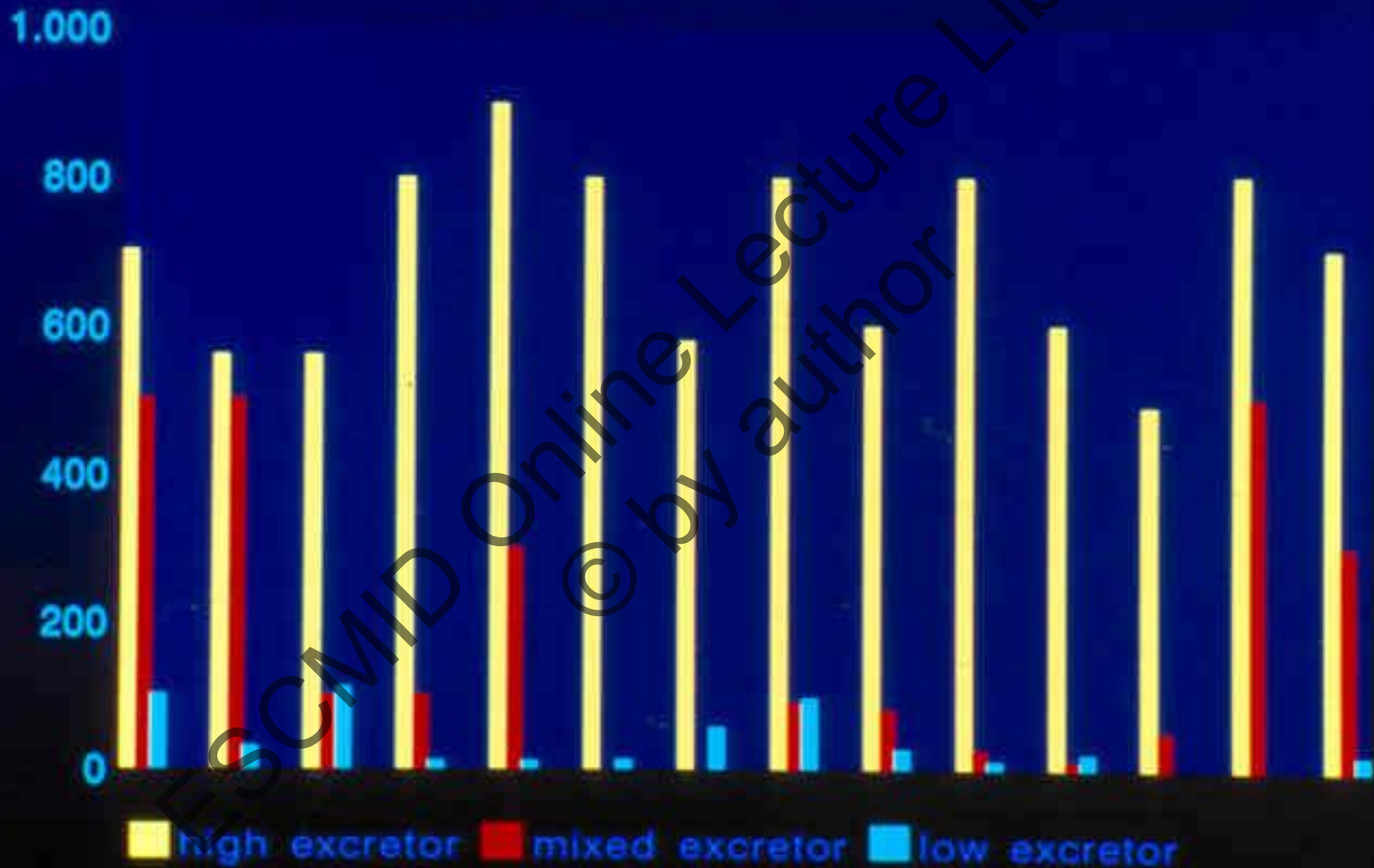
- Fresh stool sample
- Direct smear (eosin, JKJ, Saline)
- Concentration acc Ridley (JKJ, saline)
- Specific
 - Ziehl Neelsen - *Cryptosporidium* spp)

conventional procedure

critical notes

- fresh is not fresh (cave: trophozoites!)
- direct smears: suboptimal sensitivity
- concentration according Ridley

Course of cyst excretion



How to improve the sensitivity?

- multiple sampling
- fixation techniques / permanent staining
- non morphological techniques
 - copro-ELISA
 - PCR

Fixatives and Permanent stains in the laboratory diagnosis of intestinal protozoal infections

A review of the possibilities

Formalin 4%

Preparation:	easy (1:10 dil)
Fixation Quality:	doubtful (trofozoites)
Ridley concentration:	possible
Permanent staining:	not possible

P.V.A.

PolyVinylAlcohol-Schaudinn

contains:	Polyvinylalcohol, Acetic acid, Glycerin, Hg
preparation:	difficult
fixation quality:	excellent
ridley concentration:	not possible
permanent staining:	Trichroom only
CAVE:	poison, environment!

S.A.F.

Sodiumacetate-Acetic Acid-Formalin

contains:	Sodium acetate, Acetic acid formalin
preparation:	simple
Fixation quality:	very good
Ridley concentration:	good
Permanent staining:	Trichrome: bad results CB/ IHK: good Ziehl Neelsen: good

M.I.F.

Merthiolate-Iodine-Formalin

contains:	Methiolate, Formalin, glycerin, Iodine
preparation:	difficult
Fixation quality:	good
Ridley concentration:	good
Permanent staining:	not possible (Iodine)
CAVE:	shelf life limited

Permanent Stains

Trichrome

Experience:	standard procedure USA
Preparation :	easy
External influences:	none; very robust
Fixatives:	PVA fixed feces (SAF -)
Glue:	PVA network
Staining results:	colorfull; batch variation lots of kontrast, not too sharp

Permanent stains

Chlorazol Black

- Preparation: easy; batch control !
- External influences: depends on experience
- Fixatives: SAF fixed feces (PVA -)
- Glue: Mayers Albumin
- Staining results: not colourfull, no contrast
zwart in blue grey/ green
very sharp

Permanent Stains

Ironhaematoxylin-Kinyoun

Preparation:	easy
External influences:	none, robust (automated)
fixatives:	SAF-fixed feces (PVA -)
Glue:	Mayers Albumin
Staining results:	not colorfull, no contrast, black in grey; acid fast: rood scharp

De fixatives pro's and con's

	Formalin	PVA	SAF	MIF
general		Hg		shelf life
preparation	+	-	+	-
fixation	+/-	++	++	+
ridley	+	-	++	+
Kleuringen	-	only Trichr ++	CB/IHK ZN Trichr. -	

L' Histoire se répète

- Fixative: S.A.F.
- Staining: Chlorazol Black
IronHaematoxyline-Kinyoun

The “profit” of a parasitological examination using fixed stoolsamples

Comparative study fresh *versus* fixed

- 247 patients included in the study
 - 170 patients with persistent diarrhoeal complaints (<1week) consulting their GP
 - 77 temporary Asylum seekers in Haarlem
- Fresh and SAF-preserved stoolsamples

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Laboratory workup

👉 Fresh stoolsample

- 👉 direct smears (JKJ- en Eosine)

- 👉 Formalin ether concentration acc Ridley

👉 SAF-fixed stoolsample

- 👉 direct smear (JKJ)

- 👉 permanent staining (IHK)

- 👉 Formalin ether concentration acc Ridley

Yield of (pot) pathogenic protozoa according conventional
versus new method using fixed stoolsamples
(N=247 patients)

new
(SAF)

conventional
(fresh)

positive

negative

positive

40

30

70

negative

0

177

177

40

207

247

p<0.0001

In those 30; what do we see?

- 2 patients: *Giardia lamblia* trophozoites
 - in 38 out of the 247 patients, giardiasis was diagnosed
- 2 patients: *E. histolytica/dispar* trophozoites
 - in 11 out of the 247 patients, amoebiasis was diagnosed
- 3 patients: *Cryptosporidium* spp
- 24 patients: *Dientamoeba fragilis*

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Yield of protozoal stages (cysts / trophozoites)
in 247 paired stoolsamples
(fresh and SAF-fixed)

Species	Cysten	Trofo's	C+T	Totaal
<i>D.fragilis</i>	-	24	-	24
<i>E.nana</i>	12	15	33	60
<i>E.coli</i>	10	1	8	19
<i>E.histo/dispar</i>	4	2	5	11
<i>G.lambliia</i>	19	2	17	38

Persistent diarrhoea in a General Practice population in the Netherlands

Prevalence of protozoal and other
intestinal infections

Methods

study population

Inclusion:

- February 1994 - February 1996
- 80 General Practitioners
- Patients: age between 0 - 65 years
- Diarrhoeal complaints of more than 1 week's duration *or* intermittent diarrhea

Exclusion:

- Known etiology (e.g. M. Crohn, diabetes)

Methods

Laboratory techniques

Fresh stool samples

- stoolculture (S,S,Y,C)
- Rota/Adeno latex agglutination tests + 4 cell cultures

SAF-preserved stool samples

- IronHaematoxylin-Kinyoun stain
- Formalin-Ether concentration (Ridley and Hawgood)
- G.lambliia specific ELISA

Results

Prevalence (in percentages) of bacterial-, viral-, and parasitic species in stool samples from patients with persistent diarrhea in General Practice (N=892 patients)

Species	Prevalence	Most Prevalent
Bacteria	7.7	<i>Campylobacter jejuni</i>
Viruses	5.8	Rota
Pathogenic Protozoa	25.3	<i>Giardia lamblia</i>
Nonpathogenic Protozoa	36.2	<i>Blastocystis hominis</i>
Helminths	0.6	<i>Trichuris trichiura</i>

Results

Prevalence (in percentages) of protozoal species in stool samples from patients with persistent diarrhea and asymptomatic subjects in General Practice

Protozoal species	Persistent Diarrhea (n=892)	Asymptomatic subjects (n=205)
<i>Cryptosporidium spp</i>	3.3	0.5
<i>Dientamoeba fragilis</i>	8.2	4.4
<i>Entamoeba histo/dispar</i>	1.4	--
<i>Giardia lamblia</i>	14.6	2.0
<i>Blastocystis hominis</i>	25.7	37.1
<i>Endolimax nana</i>	15.2	12.2
<i>Entamoeba coli</i>	3.5	6.3

How to improve the sensitivity?

- multiple sampling
- fixation techniques / permanent staining
- non morphological techniques
 - copro-ELISA
 - PCR

Diagnosis of intestinal parasites

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Different stages of intestinal parasites present in stool samples

- Intestinal protozoa (i.e. *G. lamblia*)
 - vegetative stages / trophozoites
 - cyst stages
- Intestinal helminths (i.e. *Ascaris*, *Taenia*)
 - ova
 - larvae
 - adult worm (segments)

Solutions

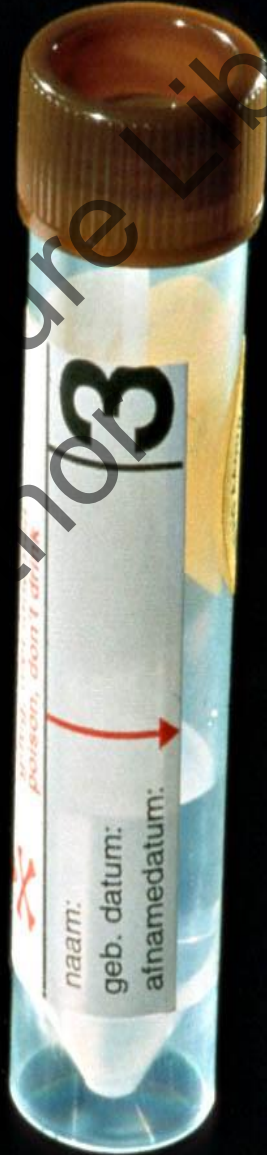
- ➔ use of preservatives (fixatives) and permanent stains
- ➔ practical device for multiple sampling

Improved diagnosis of intestinal parasites

T.F.T. = Triple - Faeces - Test

Characteristics of the TFT

- Use of fixative:
 - SAF
- Use of multiple sampling:
 - 3 consecutive days
- Use of permanent stain:
 - Chlorazol black or Iron Haematoxylin Kinyoun



Bağırsak parazitleri için dışkı tahlili T.F.T. © testi İçeriği

Test 3 kutudan oluşmaktadır: 2 kutunun içinde sıvı olup, 1 kutu boştur.

Testin kullanımı

* Peşpeşe 3 gün dışkı, aptest edildikten hemen sonra, kutulara konmalıdır:

1. gün: içinde sıvı bulunan bir kutuya;
2. gün: boş kutuya;
3. gün: içinde sıvı bulunan bir kutuya.

* **Boş kutuya** üç büyük kaşık dışkı koyun.

* **İçinde sıvı bulunan** kutuya sıvı seviyesini kırmızı oka gelmesini sağlayacak kadar dışkı koyun.



* **Doldurduktan** sonra kapağı sıkıca kapatıp, içinde sıvı bulunan kutuları **yavaşça** çalkalayın (ortalama 20 saniye).

* Her kutunun üstüne ad ve soyadınızı, doğum tarihinizi ve dışkının alındığı günü yazın.

* Üçüncü kutu dolduktan sonra, bütün paketin en kısa zamanda laboratuvara verilmesi gereklidir.

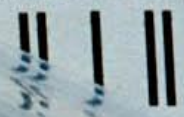
NOT:

1. **Dışkısında görülür şekilde kan bulunan** hastaların aynı gün, aptesten hemen sonra, bir sıvı bulunan ve bir boş kutuyu doldurması gereklidir. Kutulara dışkıyla birarada **kan ve salgı** konması da gereklidir. Parazitler burada bulunabilir. Her 2 kutunun hemen laboratuvara getirilmesi gereklidir.
2. **Eğer dışkı toplamanın son günü hafta sonuna rastlıyorsa, kutuları pazartesi günü verebilirsiniz.** Eğer bir günde **bir kaç kez** büyük aptest yapıyorsanız, bir günde, bir kez, bir kutuyu doldurun. Eğer her gün büyük apteste çıkmıyorsanız, 3 kutuyu doldurana kadar bekleyin.
3. Bazı tuvaletlerde (**doorspoeltoiletten**) dışkı doğrudan suyun içine düştüğünden, dışkı toplamak zordur ve bu parazitoloji araştırmasının bulgularını azaltabilir. Aptest etmeden önce bir kaç, ikiye katlanmış tuvalet kağıdını suyun üstüne koyarak, suyu aşağı yukarı kapatabilirsiniz. Böylece dışkıyı daha basit bir şekilde kağıdın üstünden alabilirsiniz.
4. Kutular oda ısısında saklanabilir.

Biologisch materiaal



postzegel niet nodig



havenziekenhuis

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Evaluation of the TFT in routine clinical practice

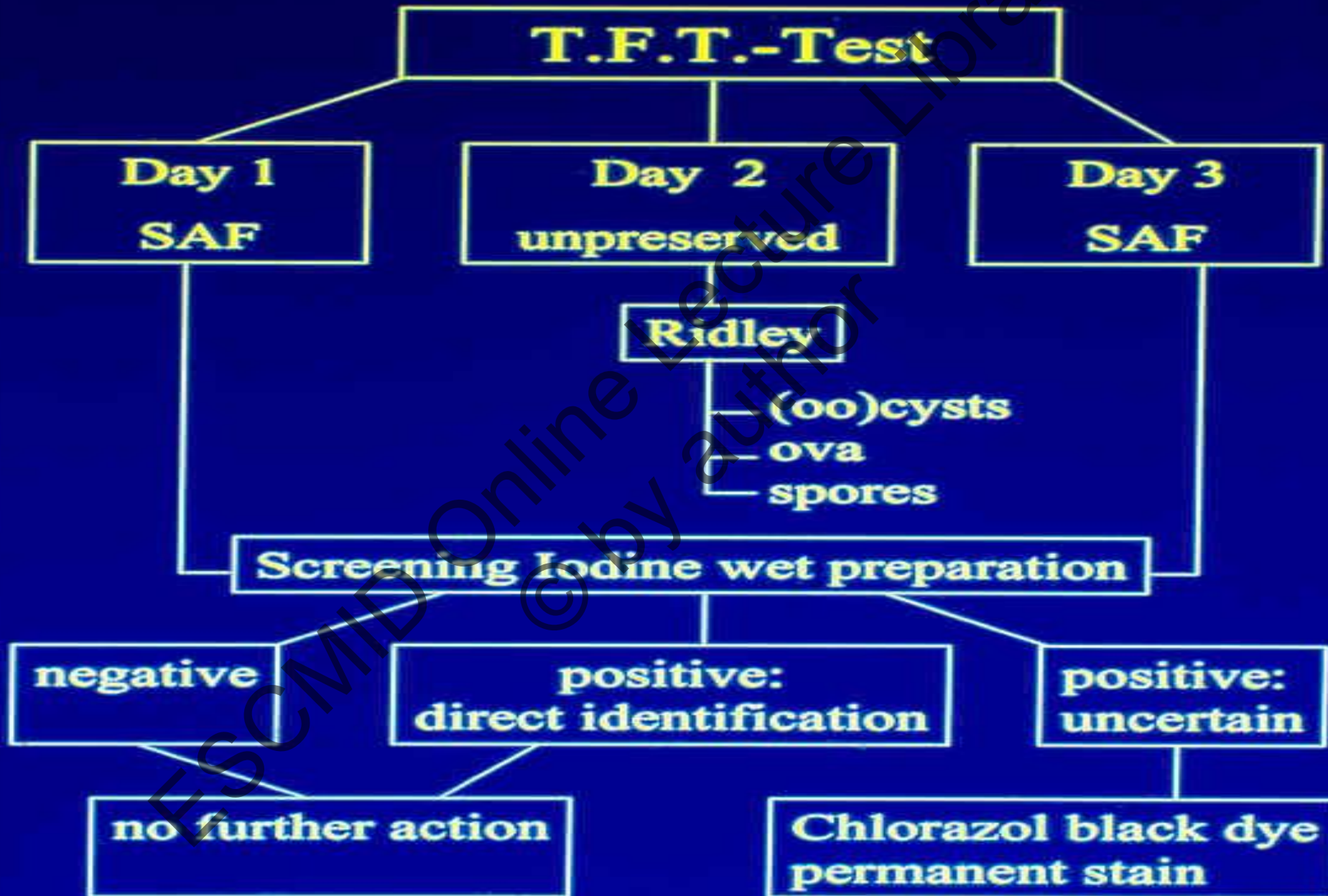
462 patients,
AMC, Amsterdam
Public Health laboratory, Haarlem

Compliance of the T.F.T.

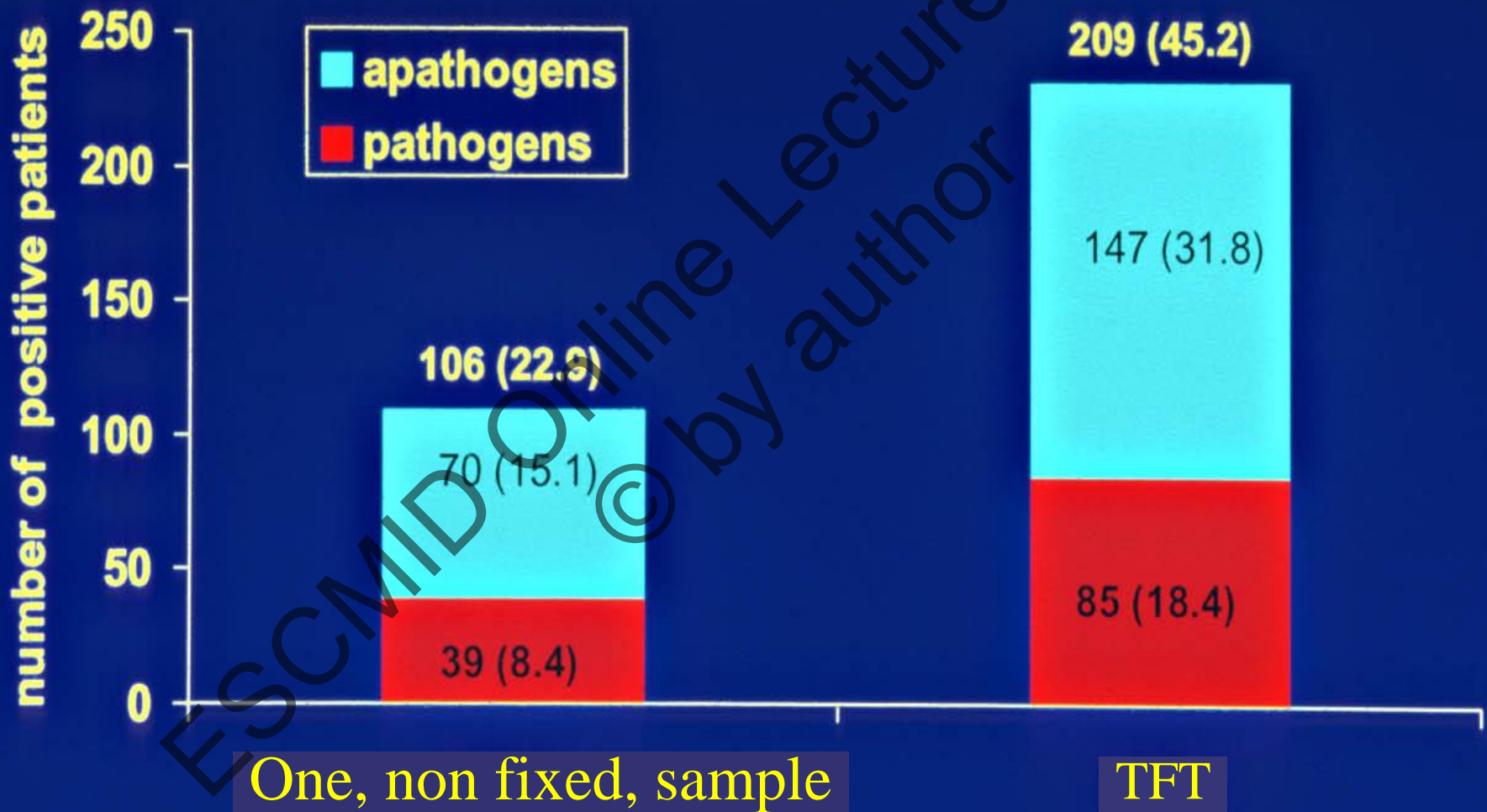
all 3 tubes delivered and filled
according to instructions

 87 %

Laboratory handling of the T.F.T.-test.



Results with one, non fixed, sample compared to TFT (no. patients = 462)



Increased recovery of intestinal protozoa in TFT (462 patients)

Organism	One stool sample(NF)	TFT	Increase with TFT
pathogen			
<i>G. lamblia</i>	18	24	6
<i>E. histolytica</i>	14	18	4
<i>D. fragilis</i>	0	45	45
apathogen			
<i>E. coli</i>	52	65	13
<i>E. hartmanni</i>	10	23	13
<i>E. nana</i>	42	47	5
<i>C. mesnili</i>	7	10	3
<i>I. bütschlii</i>	6	12	6
<i>B. hominis</i>	0	124	124

Intermittent excretion of *D. fragilis*

45 cases with
D. fragilis

Parasite present in

T.F.T 1

T.F.T 2

T.F.T 3

30

+

-

+

7

+

-

-

8

-

-

+

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Conclusions TFT in practice:

- High sensitivity and specificity
- Multiple sampling with high compliance
- Low cost sampling and laboratory material
- Additional labour-time acceptable