



# TUBEX<sup>®</sup> TF

## HOW TO PERFORM THE ASSAY TO ENSURE CORRECT RESULTS



IDL Biotech AB (publ) 2007

# TUBEX<sup>®</sup> TF

- Introduction
- Assay procedure
- Assay principle
- Interpretation of results
- Summary

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# INTRODUCTION

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*rapid typhoid detection*

# Typhoid Fever (TF) Facts

- Annual occurrence ~ 22 million cases
  - ~ 500.000 deaths worldwide
- Endemic disease, particularly in Asia, Africa and South America
- Humans are the only natural host & reservoir
  - Causative organism *Salmonella typhi*
- Disease transmission by contaminated food and water

# Issues in TF Management

- Un- & under diagnosed disease
- Targeting of correct management
- Rural disease – lack of diagnostic prerequisites
- Unnecessary high mortality rates
- Minimizing disease spread and development of multidrug resistance

# TUBEX<sup>®</sup> TF Introduction

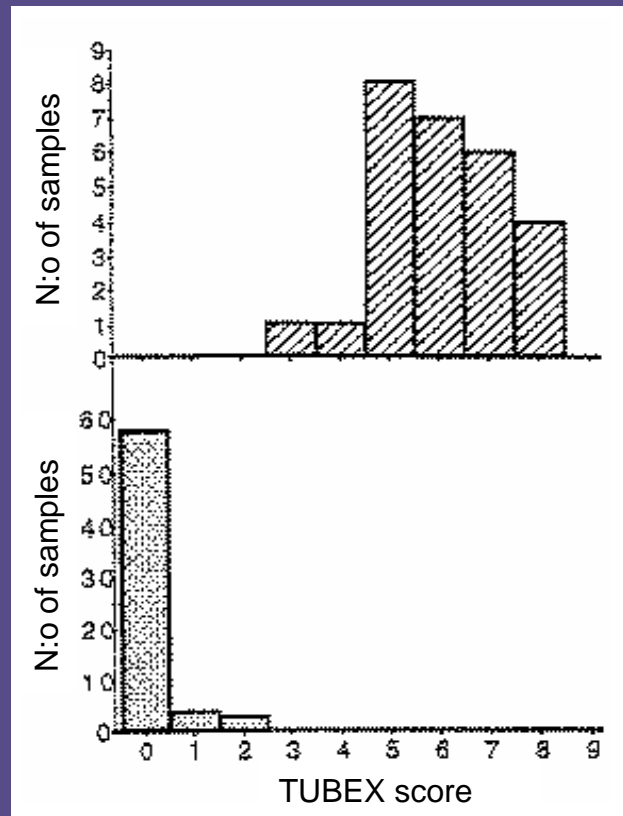
- Rapid and simple serum test
  - Less than 10 minutes
- Specific IgM detection (O9 antigen)
  - Only current infection
- No need for heavy instrumentation
  - Analysis performed in specific test strips
  - Results obtained by magnetic separation

# TUBEX<sup>®</sup> TF Clinical Benefits

TUBEX<sup>®</sup> TF specifically detects IgM, thus:

- Immediate indication of acute TF
  - One test gives direct result
  - No need for additional antibody class separation from the patient's serum
- Enhanced diagnostic specificity
- No false results due to previous infections and existing IgG

# TUBEX<sup>®</sup> TF Endemic scores



- TUBEX<sup>®</sup> TF score frequency distribution in an endemic area
- typhoid patients (*upper*)
  - control individuals (*lower*)

# TUBEX<sup>®</sup> TF 1<sup>st</sup> comparison

Assay	Sensitivity (%)	Specificity (%)
TUBEX <sup>®</sup> TF	100	100
ELISA IgM	100	96.9
Widal (TO+TH)	81.3	43.3
Direct slide agglutination	18.2	91.3
Inhibition slide agglutination	38.5	100

# TUBEX<sup>®</sup> TF Clinical data

- TUBEX TF Overall figures

Sensitivity 78 – 94%

Specificity 80 – 94%

PPV 95-98% NPV 60%

- TUBEX TF Culture positive

Sensitivity 87 – 90%

Specificity 76 – 85%

PPV 77-86% NPV 84-88%



# TUBEX<sup>®</sup> TF

Unique assay format

Easy to perform

Based on an advanced technology



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# TUBEX<sup>®</sup> technology

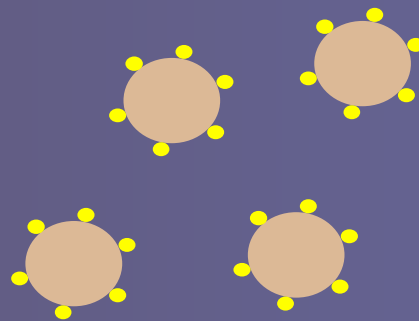
What is unique for TUBEX<sup>®</sup> ?

- Special designed reaction wells
- Special designed magnetic stands
- Visual reading compared to a color scale for interpretation of results
- Inhibition binding in solution
- A semi quantitative rapid assay

# TUBEX<sup>®</sup> TF - components

- Brown Reagent

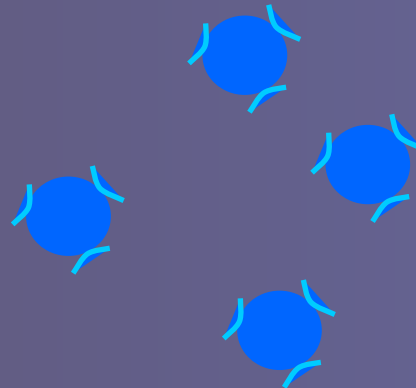
- SuperParamagnetic Particles coated with LPS O9 antigen



# TUBEX<sup>®</sup> TF - components

- Blue Reagent

- Blue Dyed Latex Microspheres coated with monoclonal IgM specific for LPS O9 antigen



# TUBEX<sup>®</sup> TF - components

- Kit controls
  - Negative control – score 0-2
  - Positive control – score 8-10

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## ASSAY PROCEDURE

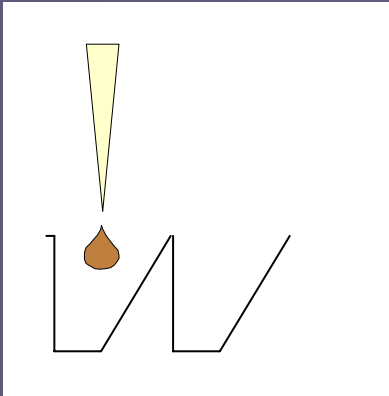
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# Assay procedure, preparations

- Room-temperature reagents and samples
- Place the Reaction Wells Strip upright on the bench

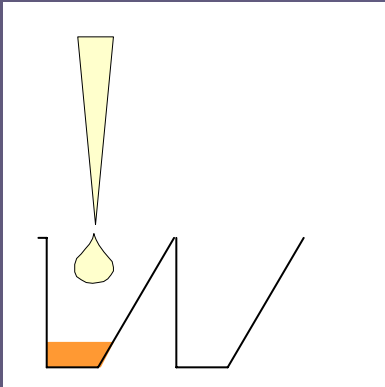
# Assay procedure, Brown Reagent

- Vortex the Brown Reagent.
- Add 45µl Brown Reagent to each well to be used.



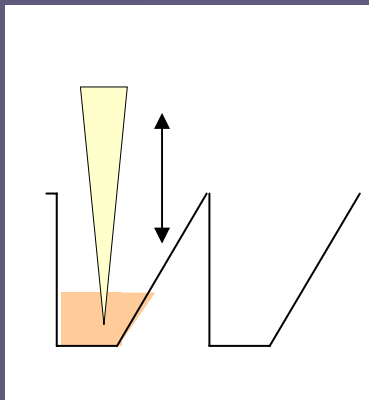
# Assay procedure, sample

- Add 45µl of patient sample, positive control and negative control to appropriate wells



# Assay procedure, incubation

- Mix thoroughly by pipetting up & down 10 times.
- Incubate Reaction Wells Strip on the bench for 2 minutes.

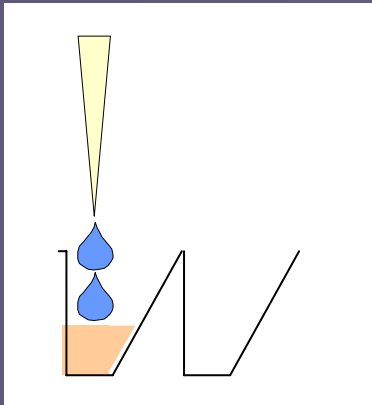


WAIT  
⌚ 2min

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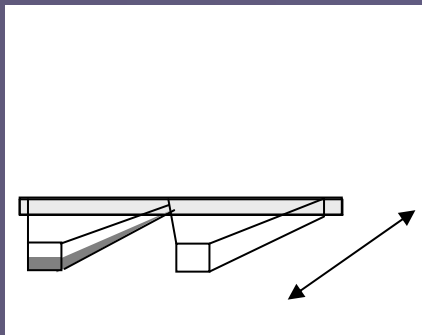
# Assay procedure, Blue Reagent

- Vortex the Blue Reagent.
- Add 90µl Blue Reagent to each well.



# Assay procedure, incubation

- Cover Reaction Wells Strip with Sealing Tape
- Hold across and tilt Reaction Strip 90°
- Shake rapidly for 2 minutes

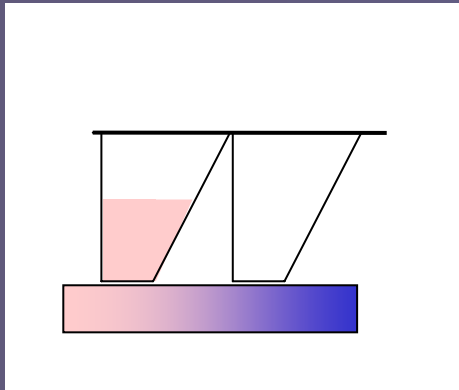


SHAKE

 2min

# Assay procedure, separation

- Place the Reaction Strip on the magnetic stand (Color Scale)
- Wait 5 minutes
- Read the results

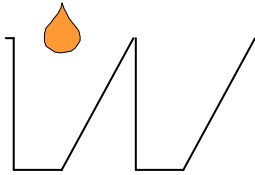


WAIT  
🕒 5min

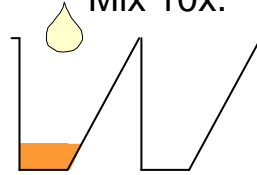
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# TUBEX<sup>®</sup> TF Assay procedure

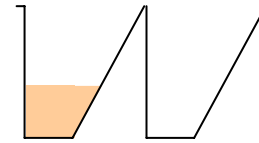
1 TUBEX<sup>®</sup> TF  
Brown Reagent  
(45 $\mu$ l).



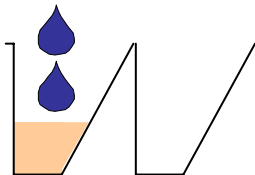
2 Sample or  
TUBEX<sup>®</sup> TF  
Control (45 $\mu$ l).  
Mix 10x.



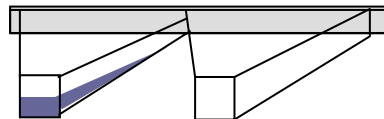
3 Incubate 2 min.



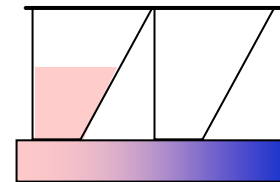
4 TUBEX<sup>®</sup> TF  
Blue Reagent  
(90 $\mu$ l).



5 Cover strip. Hold  
across and tilt 90°.  
Shake 2 min.



6 Separate 5 min on  
TUBEX<sup>®</sup> Color Scale.  
Read results.



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## ASSAY PRINCIPLE

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# Assay principle

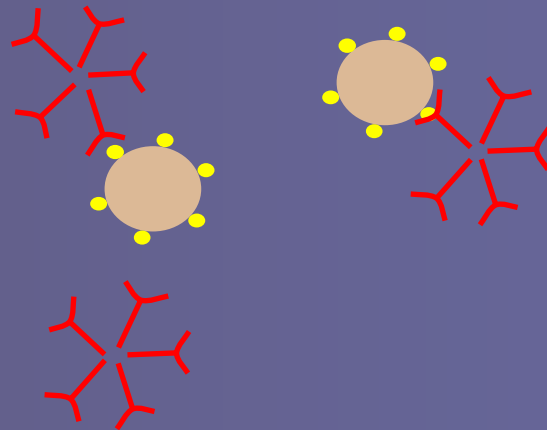
Understanding the assay principle is vital to avoid errors due to performance

# Assay principle

- immunoreaction between the LPS O9 antigen of *S. Typhi* and specific IgM antibodies
- patient IgM inhibit binding between Blue Reagent and Brown Reagent
- inhibition is proportional to the concentration of specific O9 IgM antibodies in the sample

# Positive patient sample

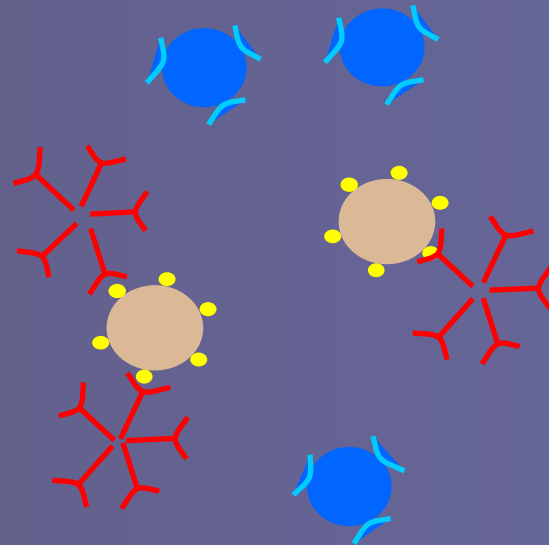
IgM antibodies in the sample bind to antigen on the paramagnetic microspheres.



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# Positive patient sample

Bound patient antibodies inhibit the binding of Blue Reagent

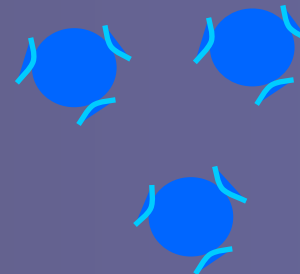


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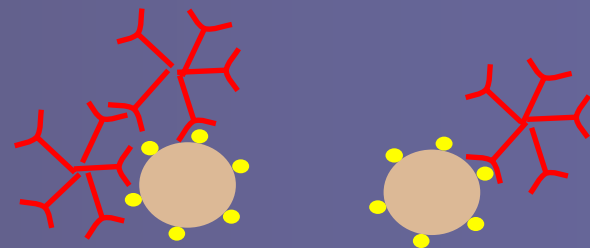
# Positive patient sample

## Result

Color is from  
bluish to intense  
blue



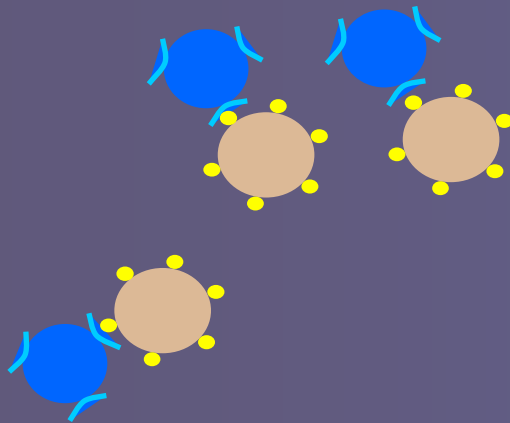
Score is read  
4 to 10



# Negative patient sample

In a negative sample there is no inhibition –

the coated particles bind to each other



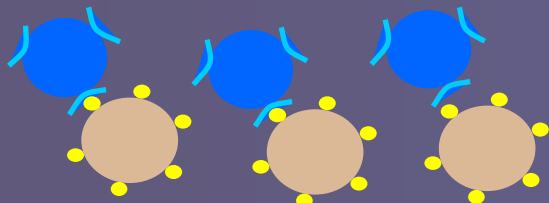
# Negative patient sample

Result

Color is pink

Score is read

0 - 2



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# Assay outcome

- Negative test

absence of IgM O9 specific antibodies in patients serum

- Positive test

presence of IgM O9 specific antibodies in patients serum

- The specificity is dependent on the right proportions of reagents and sample

# What can go wrong? / Error sources

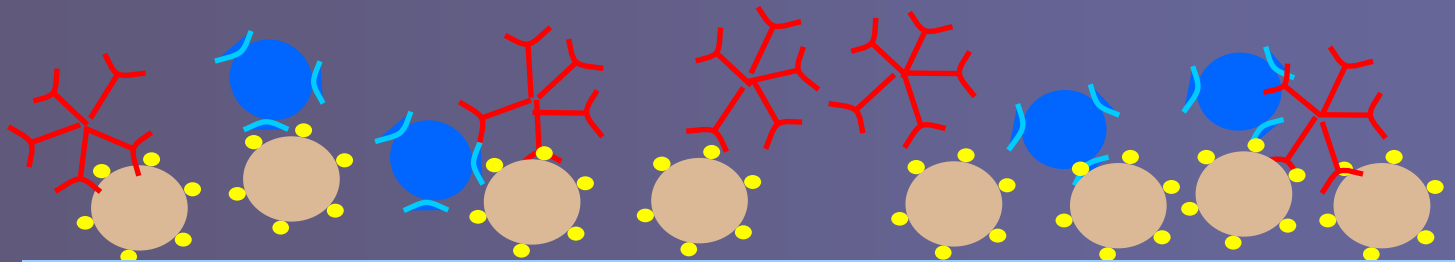
- Wrong proportions of reagents and sample
- Non-homogeneous particle suspension
- Insufficient mixing during incubation
- Pipetting error
- Insufficient mixing of reagents
- Too slow shaking during incubation

## What can go wrong? / Error sources

- Wrong samples
  - EDTA or Citrate plasmas
  - Icteric or hemolysed samples
- Disturbed reaction mixture after separation
  - Re-separation of a disturbed reaction mixture

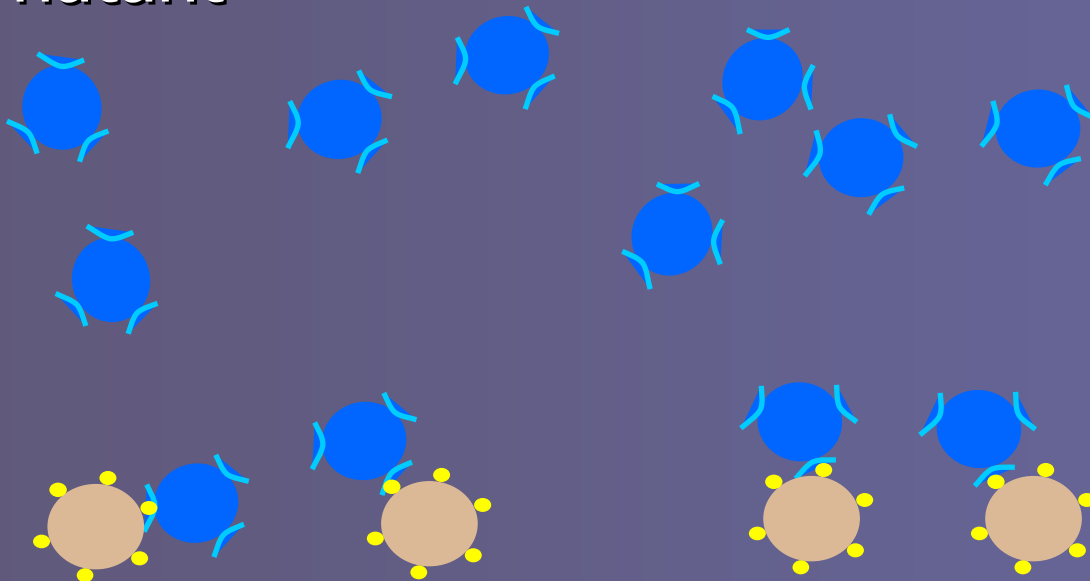
# Error sources, false negative

Too much Brown Reagent - results in binding of both patient IgM and assay IgM



# Error sources, false positive

Too much Blue Reagent or too little Brown Reagent – will cause saturated Brown Reagent – Leaving unbound Blue Reagent in the supernatant



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## INTERPRETATION of RESULTS

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# TUBEX<sup>®</sup> TF Assay result



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# TUBEX® TF Assay result

- Compare the color in each reaction well to the color scale on the magnetic stand
- The bluer the color in the supernatant the higher the concentration of antibodies in the sample
- Interfering colors in patient samples unables interpretation of results

# Interpretation

Score	Interpretation Guide
≤ 2	<b>NEGATIVE</b> – Does NOT indicate current typhoid fever infection. TUBEX® TF Negative Control
3	<b>Borderline</b> , inconclusive score. Repeat analysis. If still inconclusive, repeat sampling at a later date.
4	<b>WEAK POSITIVE</b> – Indication of current typhoid fever infection.
6 - 10	<b>POSITIVE</b> – Strong indication of current typhoid fever infection. TUBEX® TF Positive Control

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## SUMMARY

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# For correct results!

- Use only serum or heparinized plasma
- Avoid icteric or hemolysed samples
- Make sure that particles are resuspended
- Make sure that the volumes are correct
- Mix Brown reagent and sample properly
- Mix thoroughly after addition of Blue reagent
- Do not re-separate a disturbed reaction mixture

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