Implementation of IGRA: Identifying Sources of Variability

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Stanford University Medical Center Clinical Laboratories

CAP inspected, CLIA certified

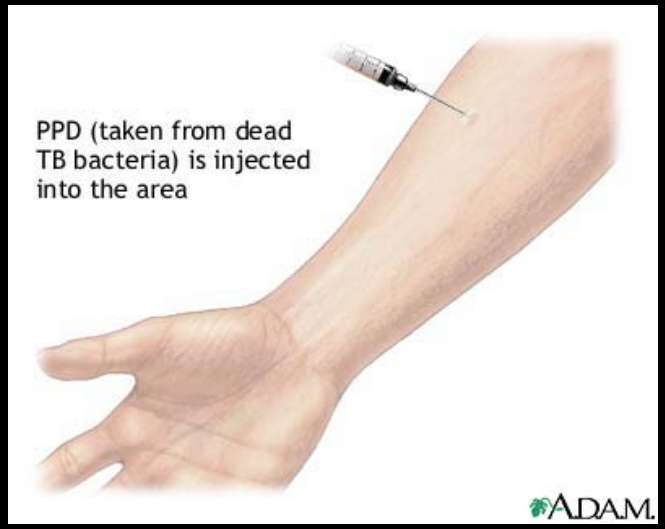
- Institutional Policy

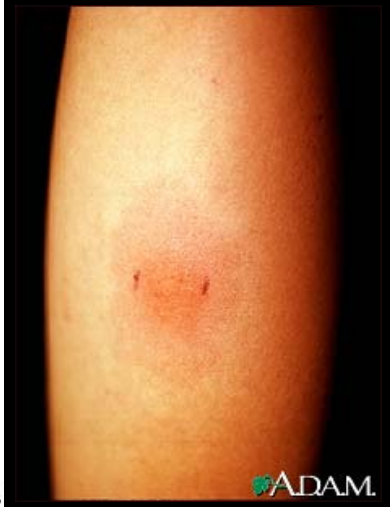
  - Screen new HCW and LTBI negative HCW annually*

  - >10,000 screenings per year

Diagnosis of LTBI Until Dec 2006

- Tuberculin Skin Test

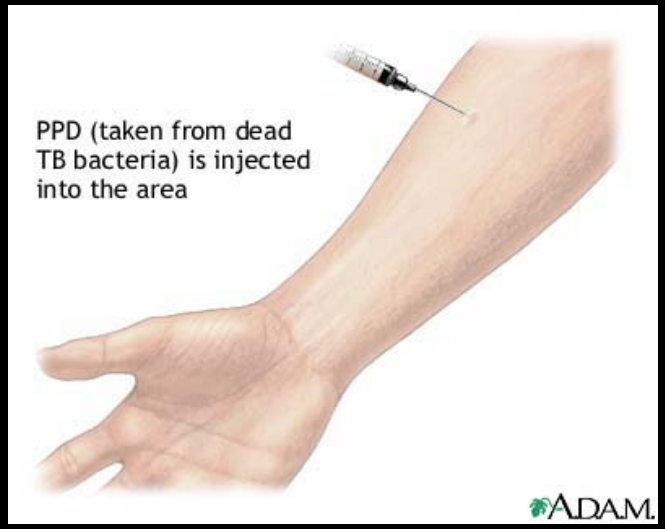


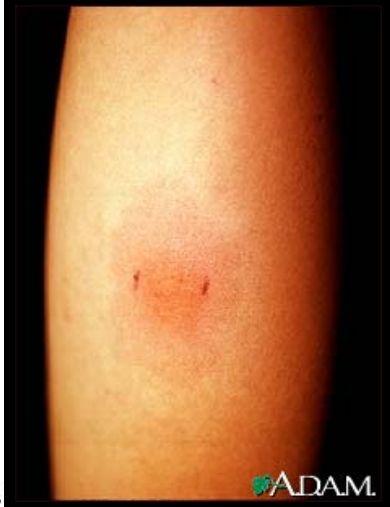


Diagnosis of LTBI Until Dec 2006

- Tuberculin Skin Test

- Disadvantages
  - Subjective
  - In vivo
    - Adverse effects
    - Boosting
  - Affected by BCG vaccination
  - Requires two visits





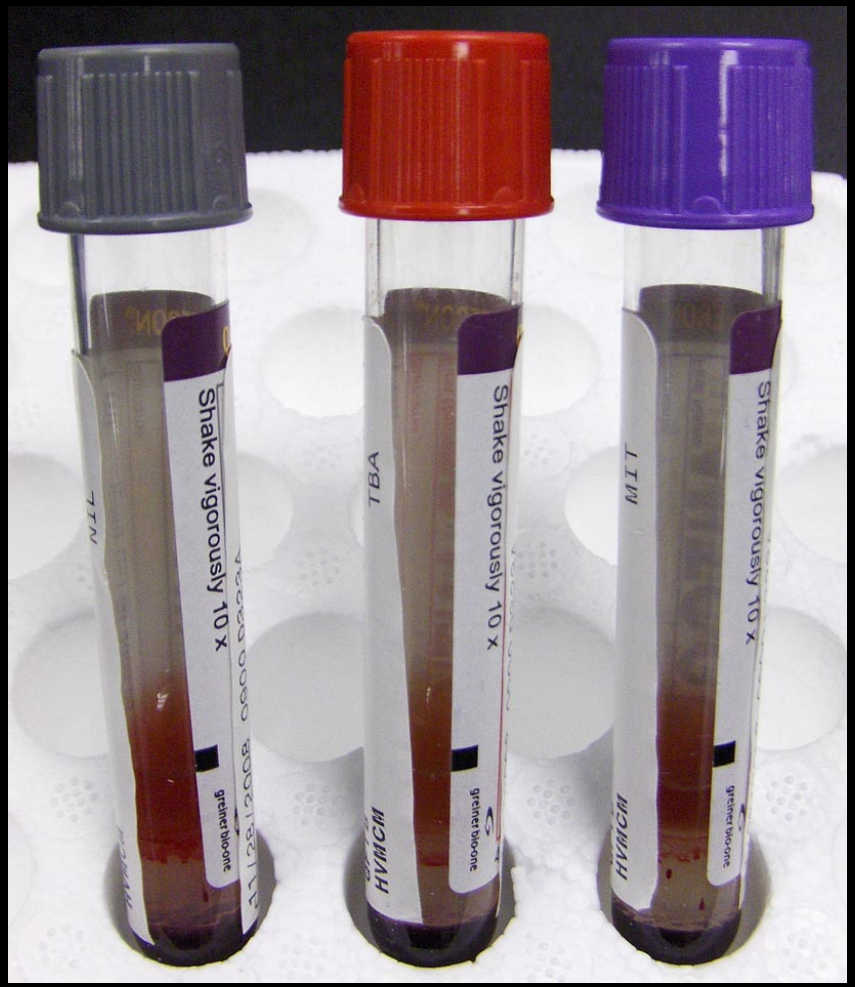
Diagnosis of Latent Tuberculosis

- Interferon-γ Release Assays

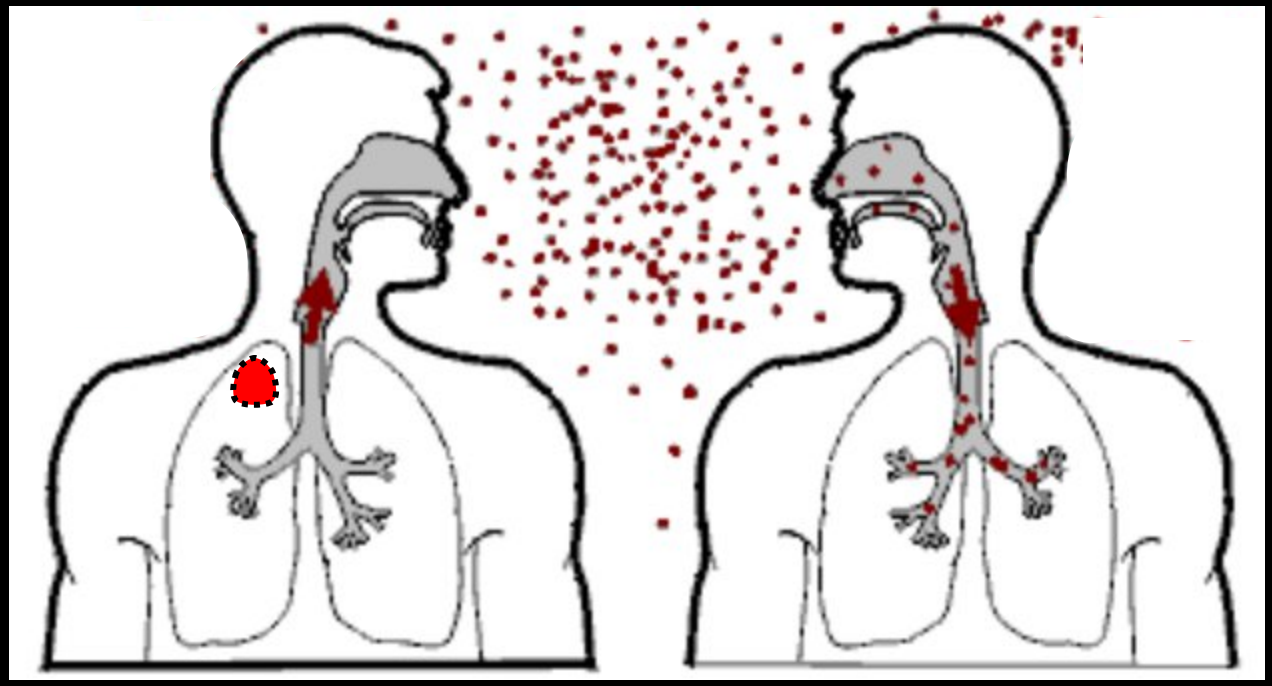
  - Quantiferon assay (Cellestis Inc.)
    - FDA approved 2001, 2005, 2007

  - T-SPOT.TB (Oxford Immunotec Inc.)
    - FDA approved 2008

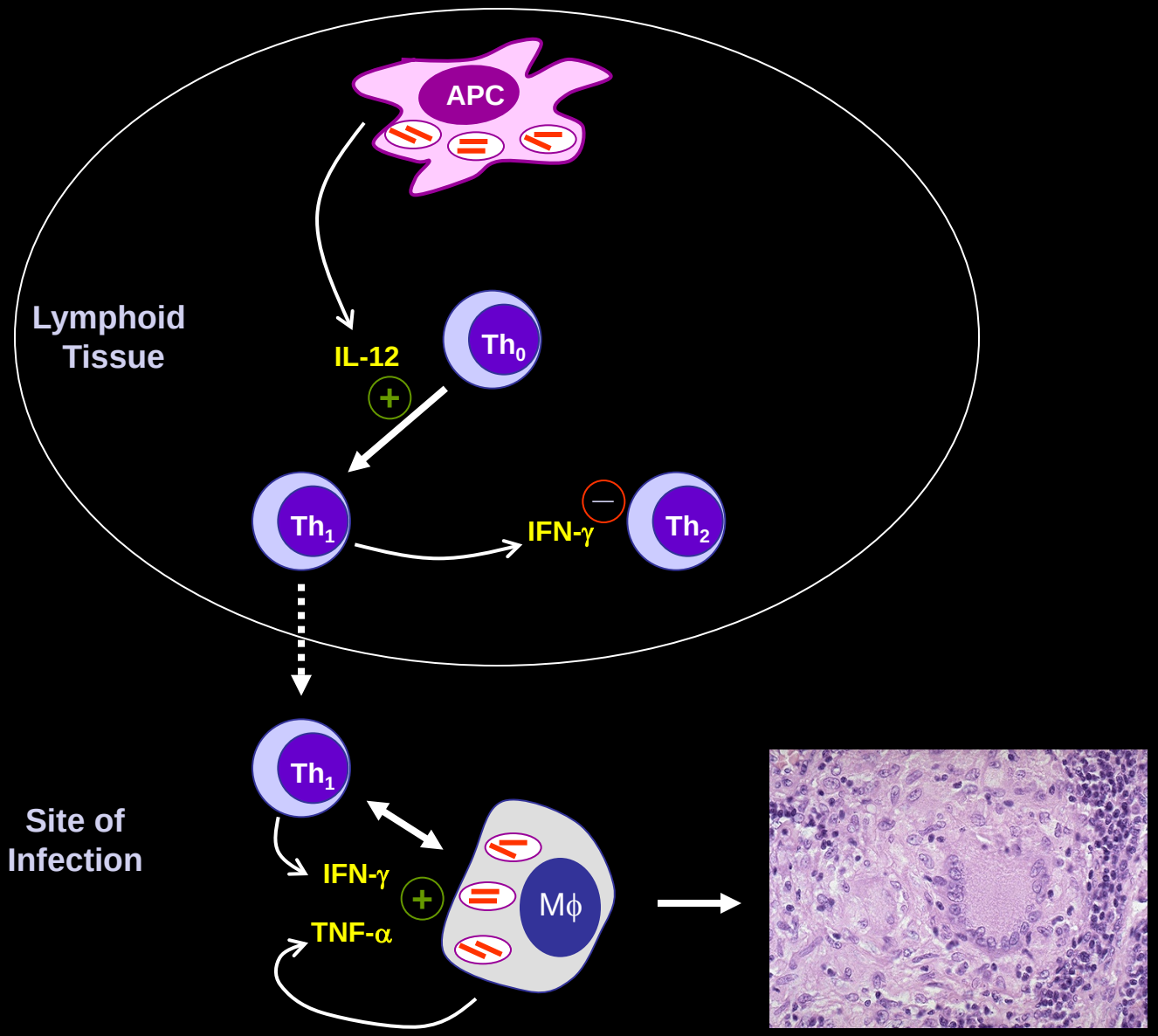
Quantiferon Gold Intube Assay



Pathogenesis of Tuberculosis



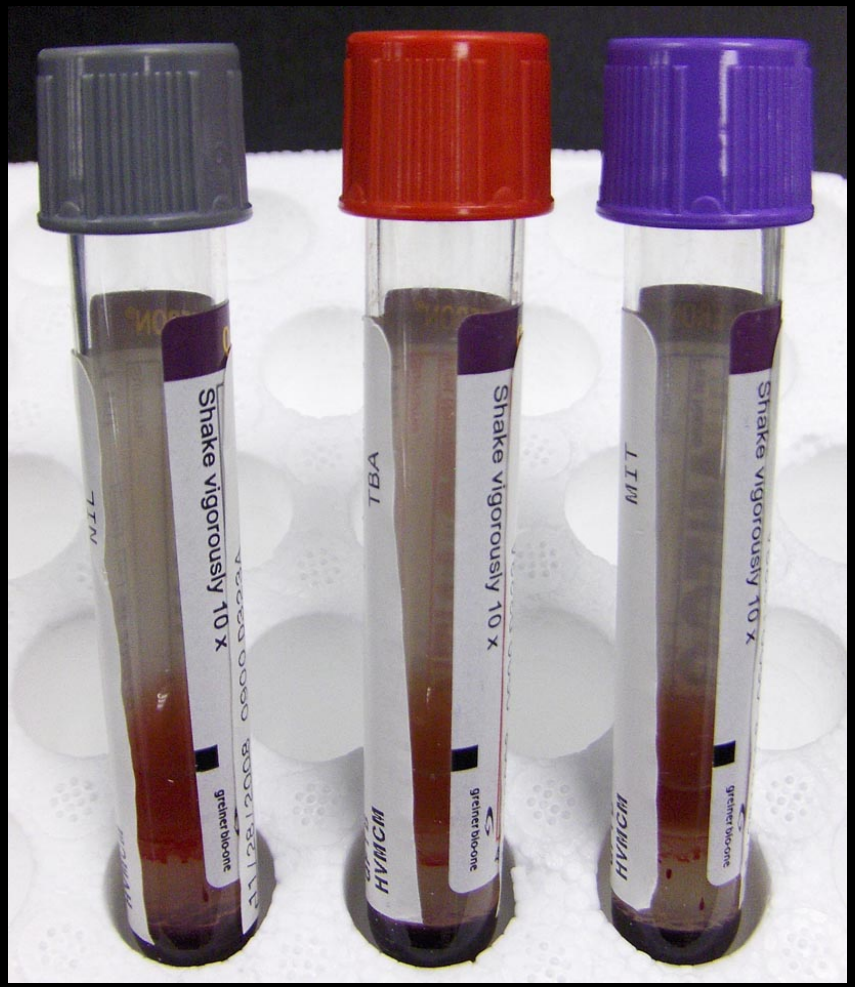
Th1 Cell-Mediated Immunity



Quantiferon Gold Intube Assay
Sample Collection

- Draw 1 ml of blood
- Shake until …

Quantiferon Gold Intube Assay
Sample Processing



- Transport to incubator (≤16h)
- Incubate 37°C 16-24 h
- Store rm temp x3 days
- Perform ELISA for IFNγ

Quantiferon Gold Intube Assay
DSX: Automated ELISA Instrument

Interpretation Criteria for QFT Gold-Intube

Nil [IU/mL]	TB Antigen minus Nil [IU/mL]	Mitogen minus Nil [IU/mL][1]	QuantiFERON®-TB [IU/mL]	Report/Interpretation
≤ 8.0	< 0.35	≥ 0.5	**Negative**	*M. tuberculosis* infection NOT likely
	≥ 0.35 and < 25% of Nil value	≥ 0.5		
	≥ 0.35 and ≥ 25% of Nil value	Any	**Positive**[2]	*M. tuberculosis* infection likely
	< 0.35	< 0.5	**Indeterminate**[3]	Results are indeterminate for TB Antigen responsiveness
	≥ 0.35 and < 25% of Nil value	< 0.5		
> 8.0[4]	Any	Any		

Local Validation of FDA Approved Tests

- How many samples does CAP requires labs to test in their validation?
- CAP requires that labs report performance for “molecular tests”

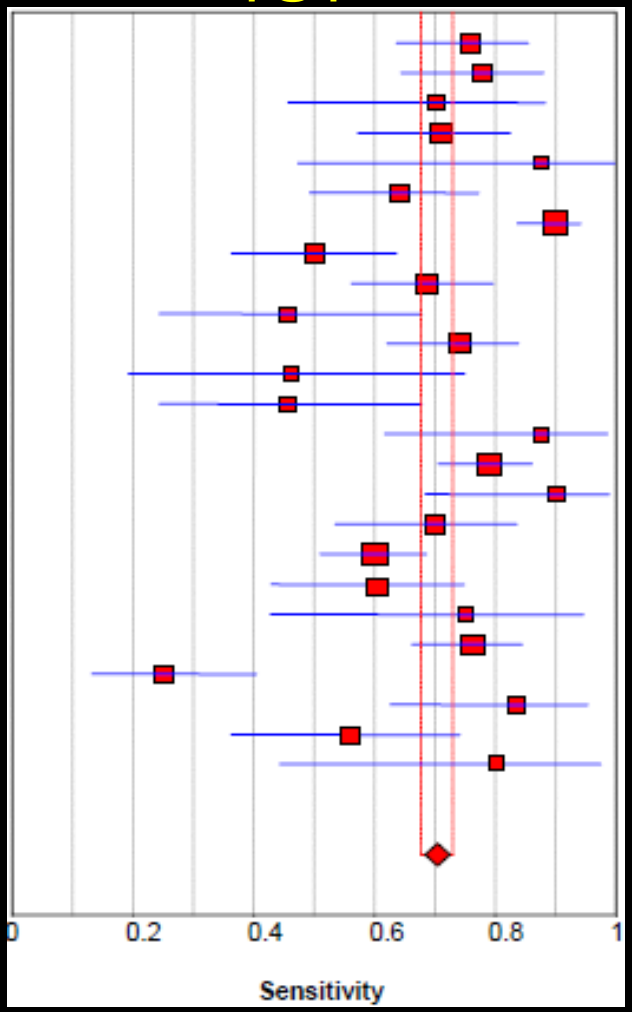
Performance of IGRA for Detection of Latent TB Given Lack of Gold Standard

- Estimated sensitivity
  - In active TB patients
  - In “high-risk” individuals
  - Concordance with TST
- Estimated specificity
  - In “low-risk” individuals
- Reproducibility
  - Repeat testing

IGRAs for Screening of HCWs

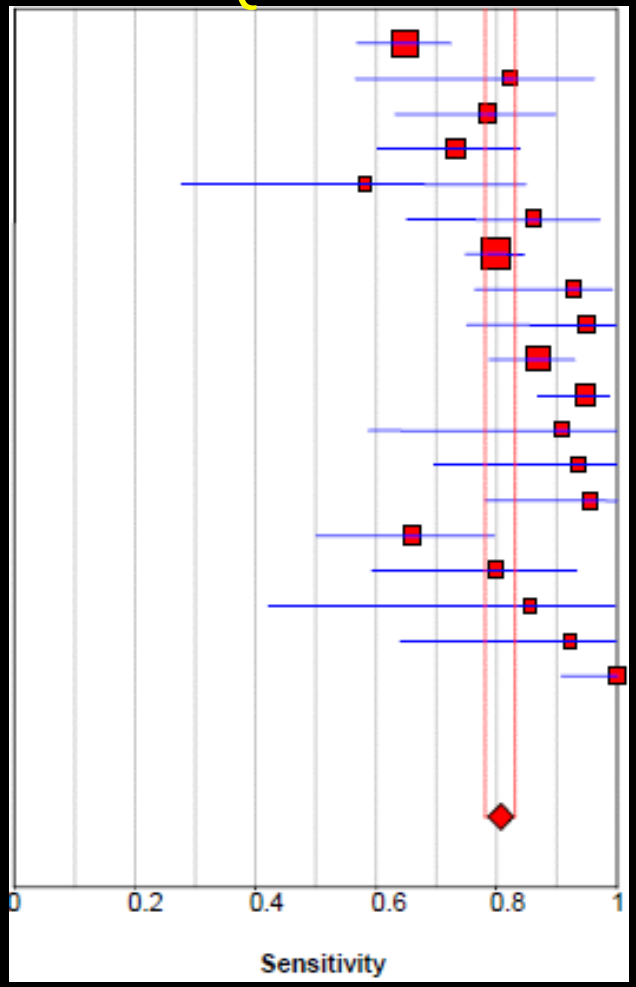
Sensitivity of TST & IGRAs in patients with active TB

TST



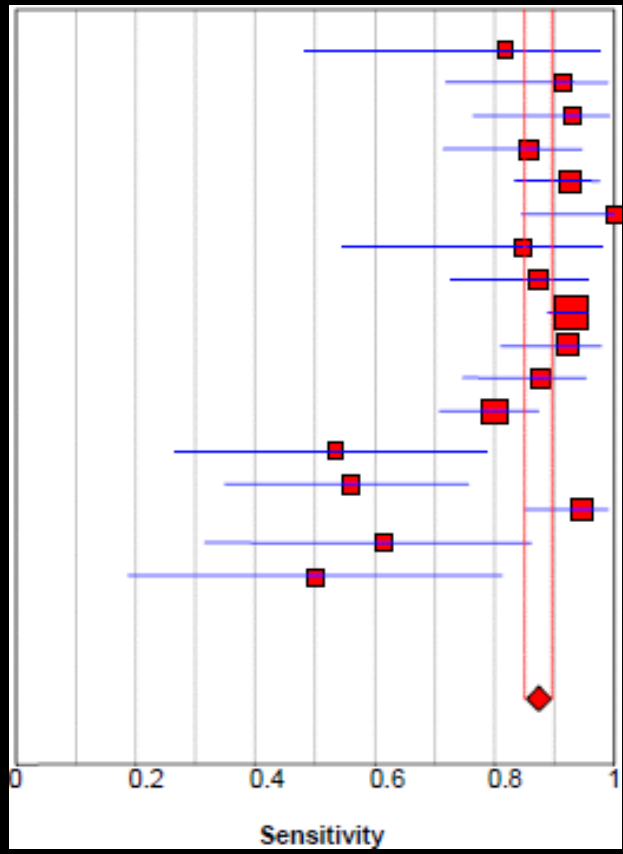
Pooled Sensitivity = 0.70 (0.67 to 0.72)
Chi-square = 128.00; d.f. = 24 (p = 0.0000)
Inconsistency (I-square) = 81.3 %

QFT-IT



Pooled Sensitivity = 0.81 (0.78 to 0.83)
Chi-square = 79.90; d.f. = 18 (p = 0.0000)
Inconsistency (I-square) = 77.5 %

T-SPOT.*TB*

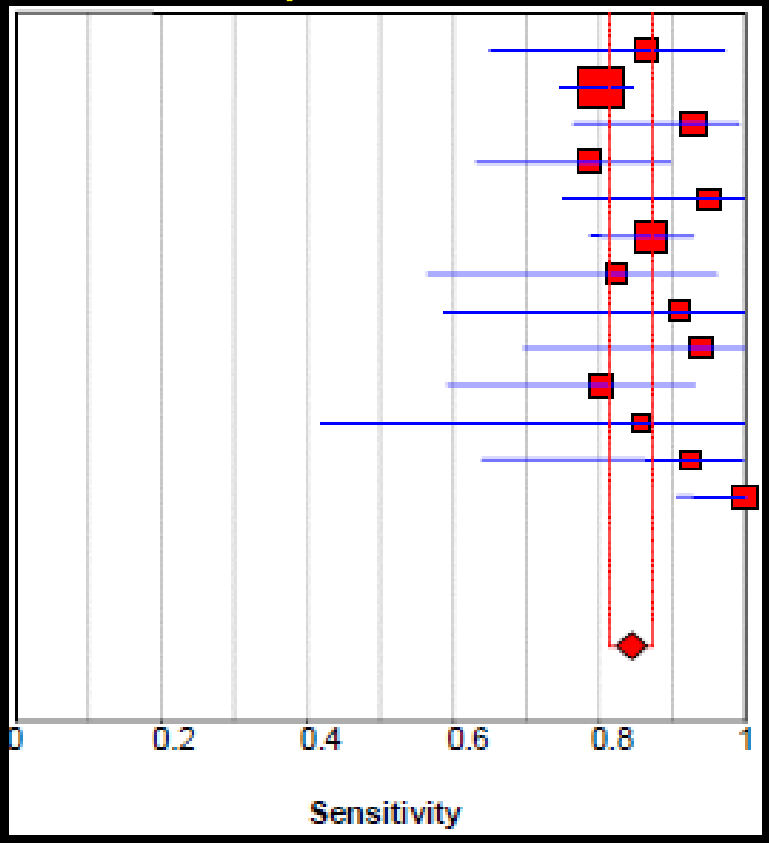


Pooled Sensitivity = 0.875 (0.85 to 0.90)
Chi-square = 65.59; d.f. = 16 (p = 0.0000)
Inconsistency (I-square) = 75.6 %

Diel et al Chest 2010

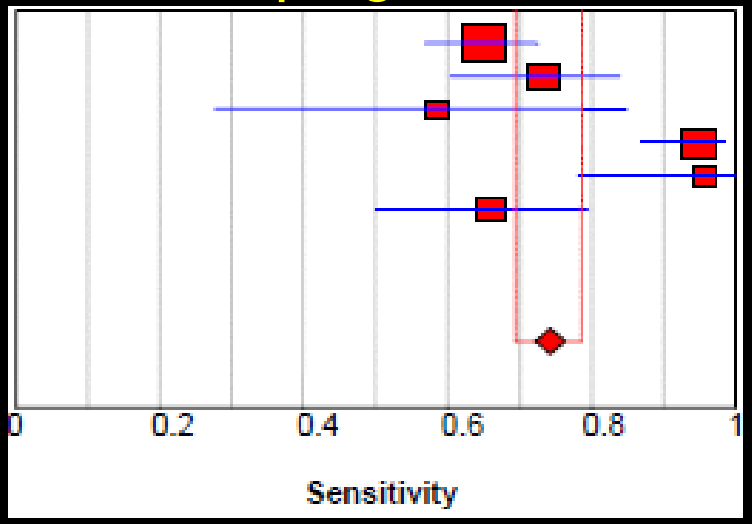
Sensitivity of QFT-IT in patients with active TB

Developed Countries



Pooled Sensitivity = 0.84 (0.81 to 0.87)
Chi-square = 25.25; d.f. = 12 (p = 0.0137)
Inconsistency (I-square) = 52.5 %

Developing Countries



Pooled Sensitivity = 0.74 (0.69 to 0.79)
Chi-square = 39.48; d.f. = 5 (p = 0.0000)
Inconsistency (I-square) = 87.3 %

Diel et al Chest 2010

Sensitivity in patients with latent TB using progression to active TB as gold standard

Country [study[a]]	No. of subjects	Follow-up period (yrs)	Active TB cases	Case isolates available for RFLP typing([b])	% sensitivity([d])		
					TST[c]	QFT-IT	T-SPOT
Netherlands [20]	339	2	9	6(6)	100(100)	63(40)	75(60)
Germany [21]	954	4	19	11(11)	52(36)	100(100)	ND
Japan [26]	3012	2	39	ND	ND	51	ND
Colombia [27]	2060	3	26	ND	ND	ND	78
Gambia [28]	2348	2	26	9(6)	56(67)	ND	52(50)[e]

Herrera et al CID 2011

Reproducibility of QFT-IT in HCW

TABLE 1
Serial testing studies of interferon-gamma release assays in health care workers (HCWs) in low and intermediate incidence countries

Author (reference), year, country	Duration between testing	Conversion, n/N (%)		IGRA reversions*, n/N (%)
		Tuberculin skin test	IGRA*	
Joshi et al (15), 2012, USA	2 to 30 days	N/A	N/A	18/45 (40)
Rafiza et al (16), 2012, Malaysia	1 year	N/A	69/703 (9.8)	14/59 (23.7)
Fong et al (17), 2012, USA	1 year or 1 to 6 months for repeat of positive IGRA	N/A	52/1857 (2.8)	8/10 (80)†
Torres Costa et al (18), 2011, Portugal	1 year	61/199 (30.7)	51 /462 (11)	
Schablon et al (19), 2010, Germany	High-risk HCWs tested annually, all others evaluated every other year	Reversion rates: 4/188 (2.1)	15/245 (6.1)	46/208 (22.1)
Ringshausen et al (20), 2010, Germany	18 weeks	N/A	3/162 (1.9)	13/42 (32.6)
Park et al (21), 2010, South Korea	1 year	N/A	14/244 (5.7)	6/18 (33.3)
Lee et al (22), 2009, South Korea	1 year	N/A	21/146 (14.4)	N/A
Chee et al (23), 2009, Singapore	1 year	16/75 (21.3)	9/182 (4.9)	N/A
Yoshiyama et al (24), 2009, Japan	2 and 4 years	0/18 (Note: denominator includes only baseline concordant positives)	5/277 (1.8)	N/A
Pollock et al (25), 2008, USA	1 to 7 months	N/A	2/43 (4.6). Selected HCWs at 'increased risk' and negative at baseline	13/32 (41)

**All conversions/reversions using simple negative/positive; †Testing was performed among individuals with positive QuantiFERON-TB (Cellestis Ltd, Australia) results close to the cut-off point. IGRA Interferon-gamma release assay; N/A Not available*

Coversions 2% to 15%
Reversions 20 to 40%

Pai and Elwood Can Respir J. 2012

QFT-GIT Assay Standardization

Standardized

- Pre-analytical
  - Blood collection*
  - 37 ºC incubation
  - Plasma separation

- Analytical
  - ELISA
  - Interpretation

Not Standardized

QFT-GIT Assay Standardization

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Not Standardized

- Pre-analytical
  - Skin preparation
  - Incubation delay*
  - Incubation duration*
  - Time of day
  - Day of month
  - Season
  - Diet
  - Infection
  - Antibiotics

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Promise versus Reality: Optimism Bias in Package Inserts for Tuberculosis Diagnostics

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Laboratorians and clinicians often rely on package inserts of diagnostic tests to assess their accuracy. We compared test accuracy for tuberculosis diagnostics reported in 19 package inserts against estimates in published meta-analyses and found that package inserts generally report overoptimistic accuracy estimates. However, package inserts of most tests approved by the U.S. Food and Drug Administration (FDA) or endorsed by the World Health Organization provide more realistic estimates that agree with meta-analyses.

QFT-GIT Assay Standardization

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Not Standardized

- Pre-analytical
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  - Season
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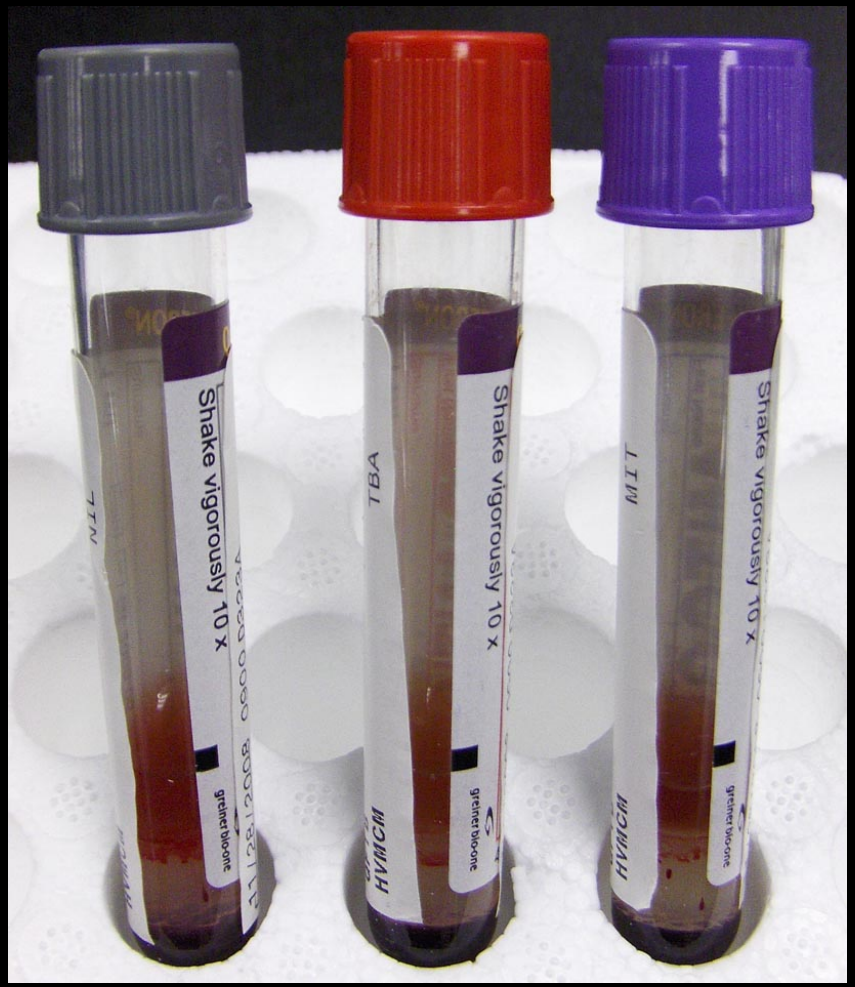
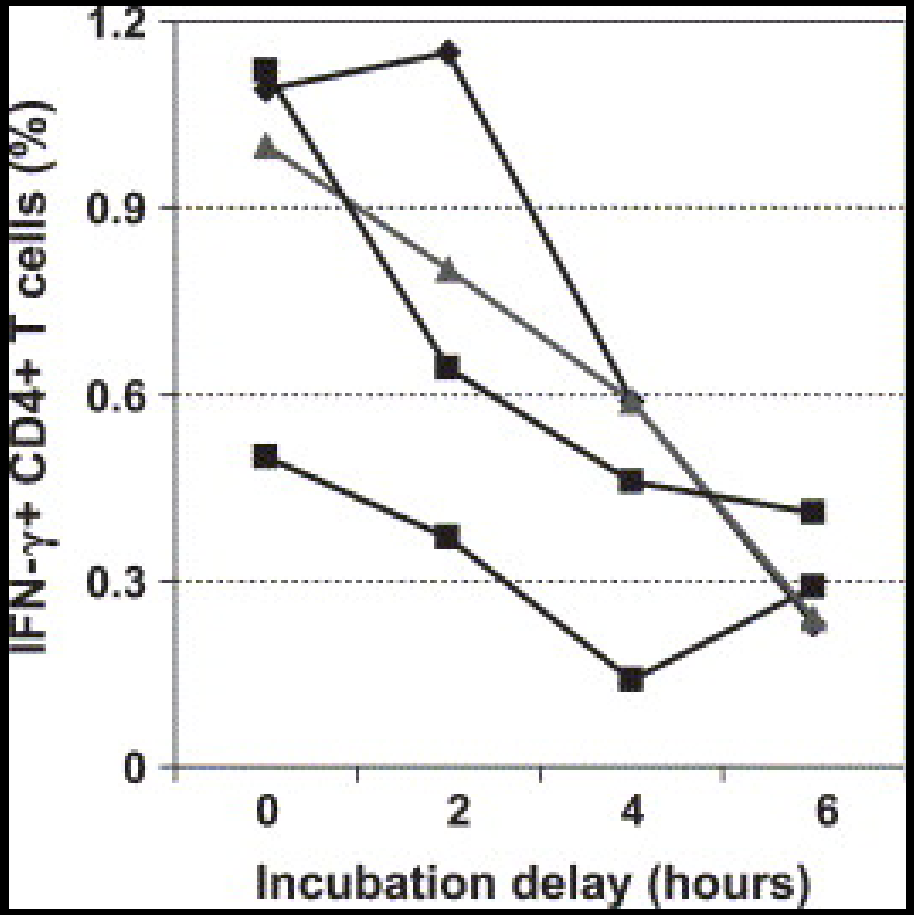
  - Infection
  - Antibiotics

Quantiferon Gold Intube Assay
Sample Processing



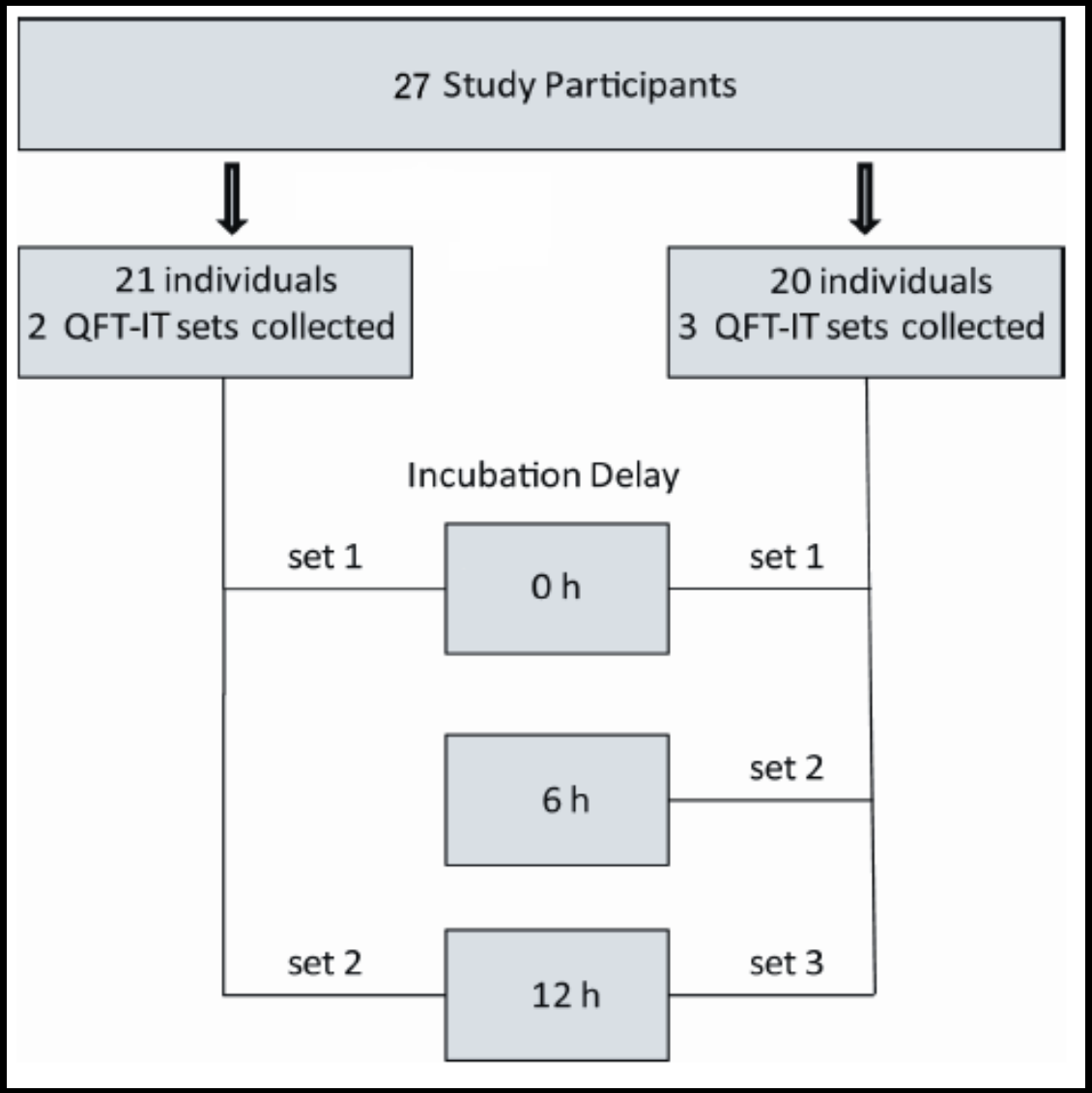
- Transport to incubator (≤16h)

Effect of delay in blood processing, following collection, on assay performance



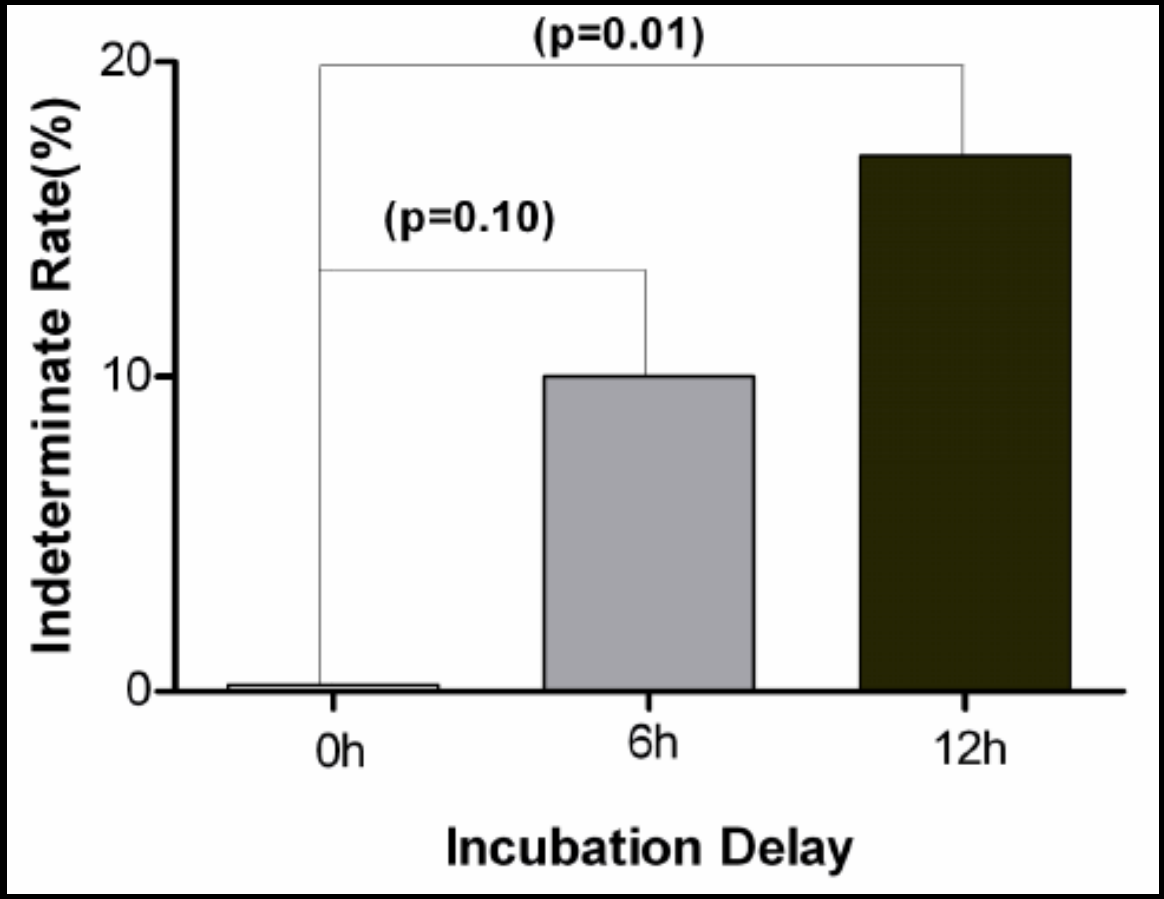
Hanekom et al *2004J. Immunol. Methods 291:185–195*

Effect of incubation delay on QFT-IT results



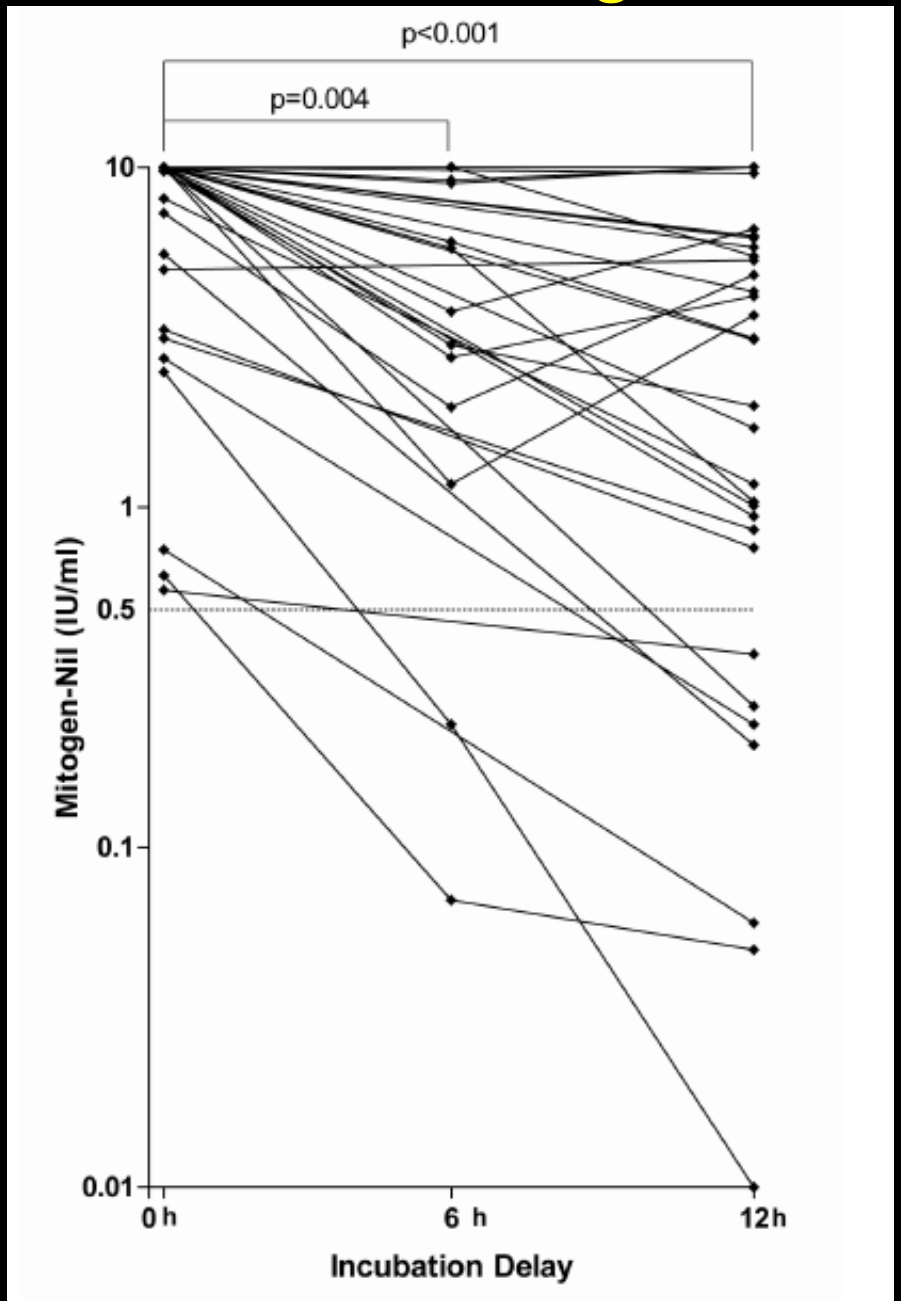
Herrera et al JCM 2010

Incubation delay increases indeterminate results



Herrera et al JCM 2010

Mitogen Results Following Incubation Delay



Herrera et al JCM 2010

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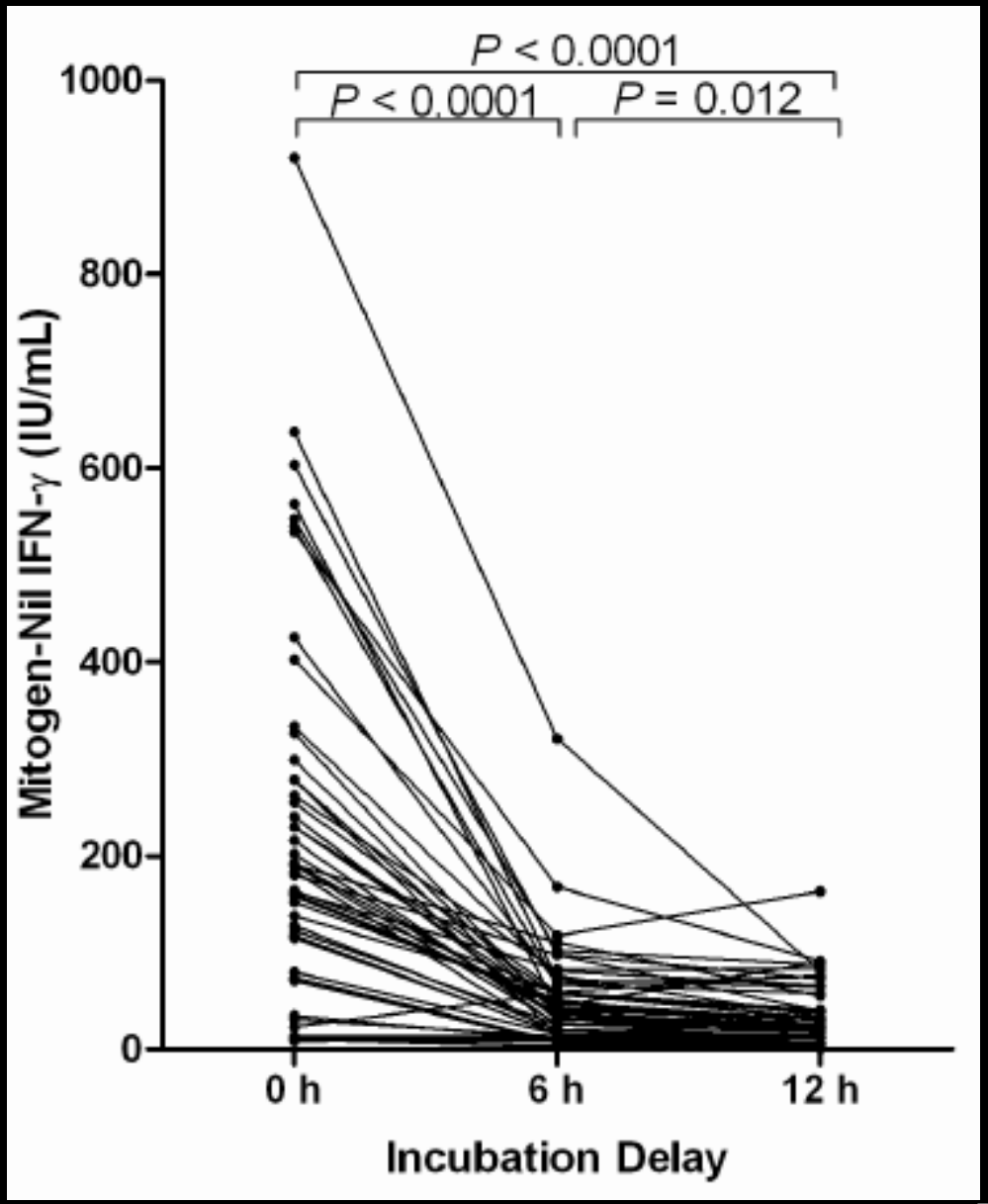


Doberne et al JCM 2011

Indeterminate Results in Studies Using QFT-IT

TABLE 4—Indeterminate results in studies using the QFT-IT

Study, Year [reference]	No. Subjects	Type of subjects	Country	No. Indeterminates	% Indeterminates
Totals	**21,922**			**469**	**2.14%**

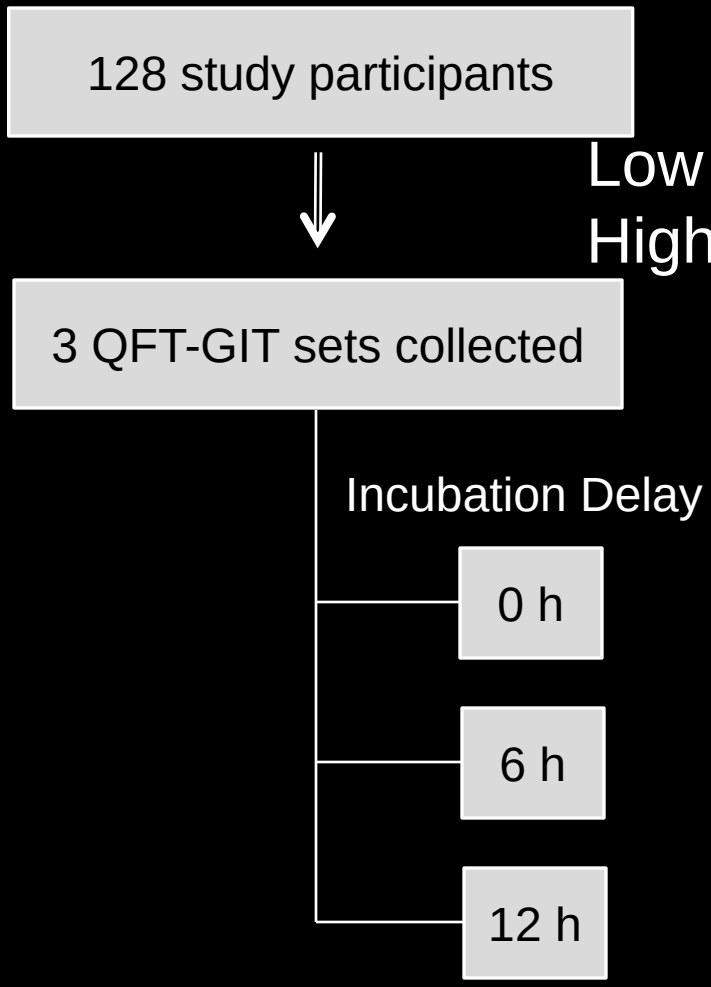
Pooled percentage of indeterminate results = 0.0214 [95% CI 0.02-0.023]
Heterogeneity χ^2 = 965.11 (d.f.= 71) P = .000
Inconsistency (I-square) = 92.6%
No. studies = 71.

Range: 0 - 41%

Implementation of the immediate incubation at Stanford yielded an indeterminate result rate of 0.36% in 14,830 HCW tested during the first 12 months

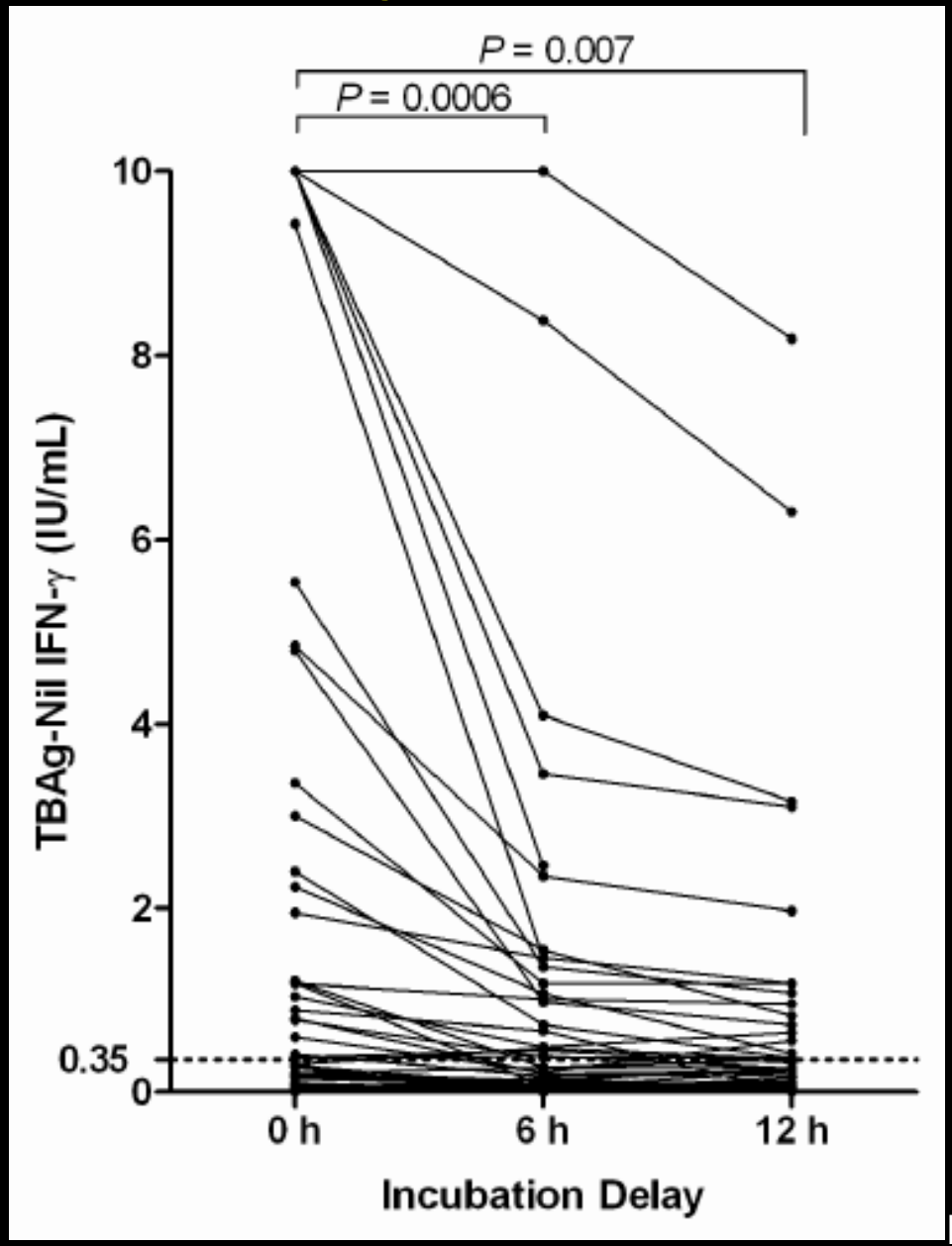
Diel et al Chest 2010

Effect of Incubation Delay on the Accuracy of QFT-IT Results



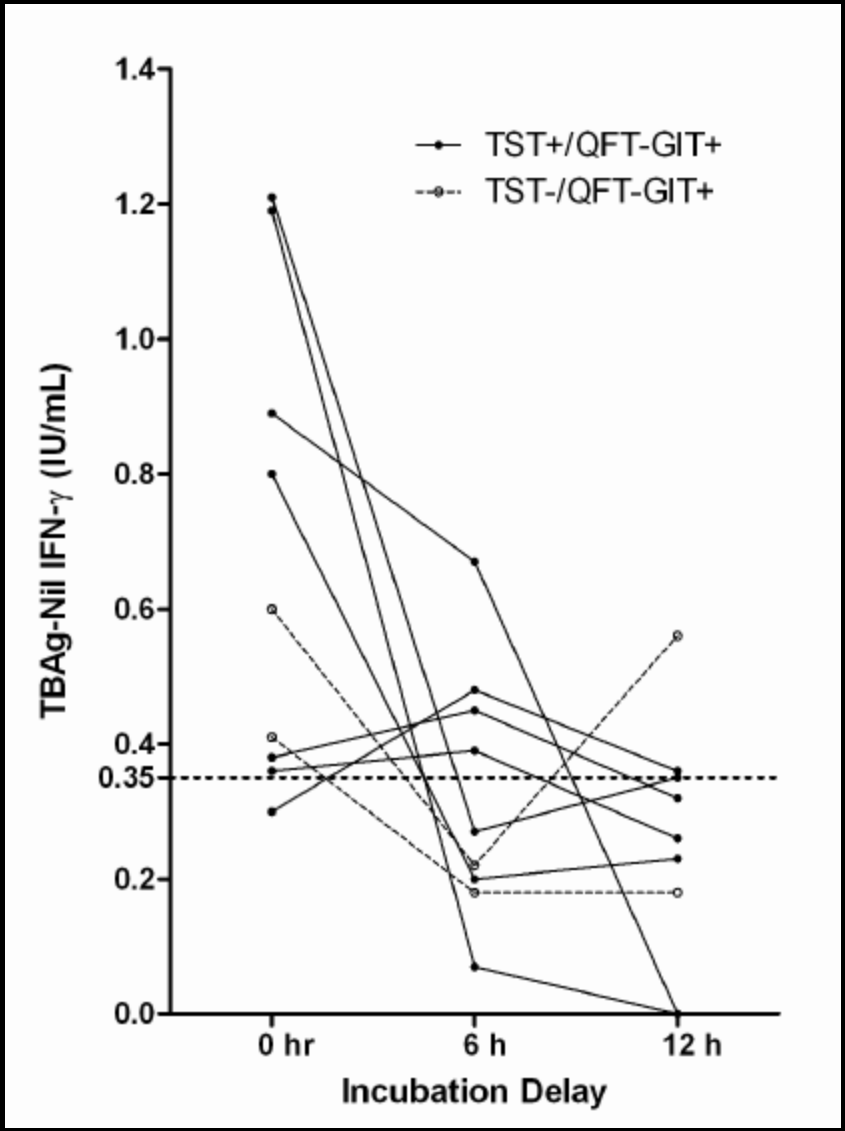
Doberne et al JCM 2011

TB Ag results following immediate and delayed incubation



Doberne et al JCM 2011

TB-Ag Results for Subjects with Discordant Results



Reversion rate:
19% (5/26) with 6 h delay
22% (5/23) with 12 h delay

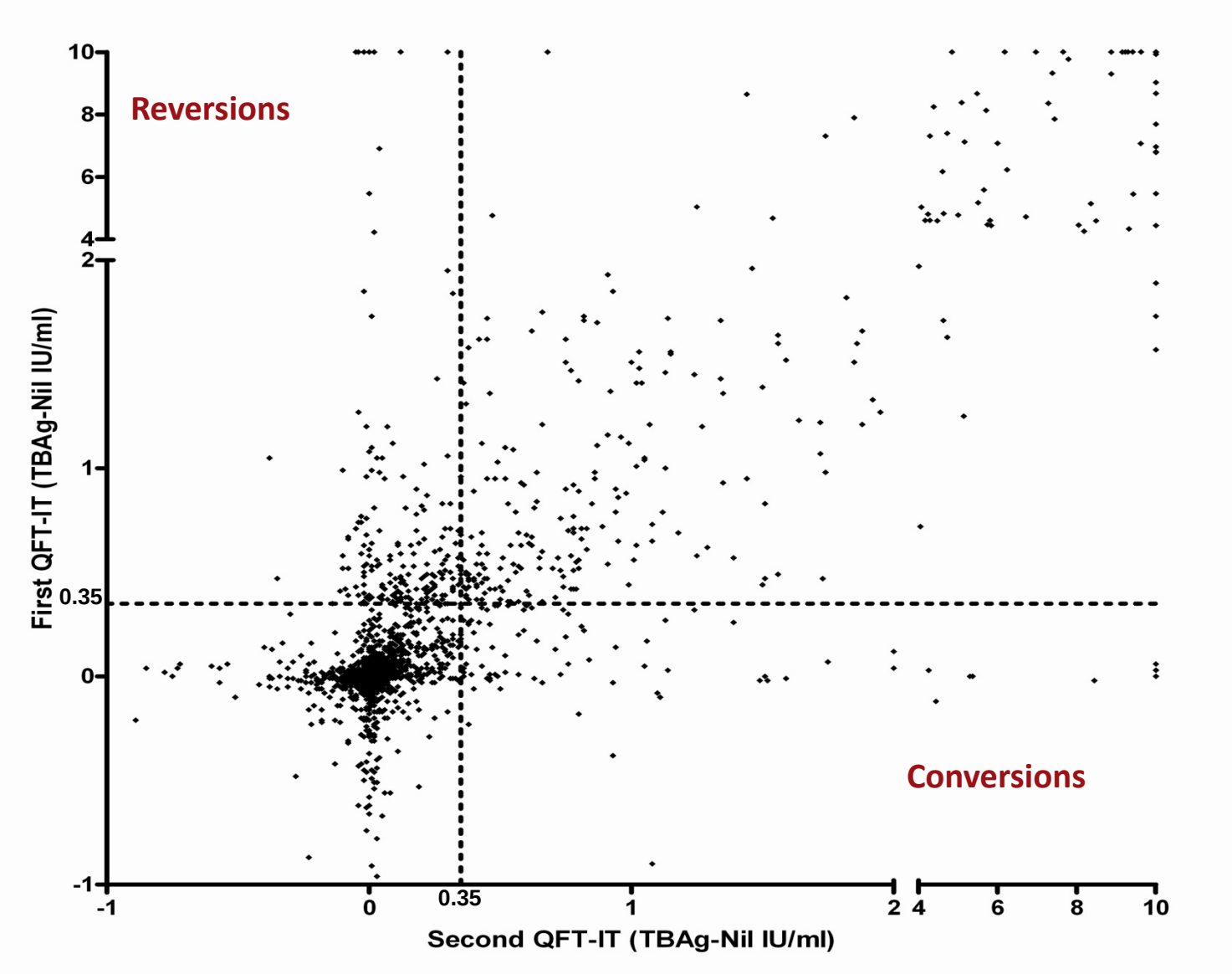
Doberne et al JCM 2011

Risk factors for latent TB infection in volunteers with discrepant results

Volunteer # (0, 6, 12 h result)	Country of Birth	TB Contact	Prior TST[a]	Prior QFT-GIT	CXR
003 (Pos/Neg/Neg)	USA	Pos	Neg	Pos	Neg
008 (Pos/Pos/Neg)	Philippine	Neg	Pos	Pos	Neg
090 (Pos/Neg/Pos)	Mexico	Pos	Pos	Pos	Neg
093 (Pos/Pos/Neg)	Philippine	Pos	Pos	Pos	Pos
103 (Pos/Neg/Pos)	USA	Neg	Neg	Pos	Neg
106 (Neg/Pos/Pos)	Philippine	Pos	Pos	Pos	Neg
122 (Pos/Pos/Neg)	Philippine	Neg	Pos	Pos	Neg
132 (Pos/Neg/IDT[b])	China	Neg	Pos	Pos	Pos
134 (Pos/Neg/Neg)	Hungary	Pos	Pos	Pos	Pos

Doberne et al JCM 2011

Reproducibility of QFT-IT in Stanford HCW



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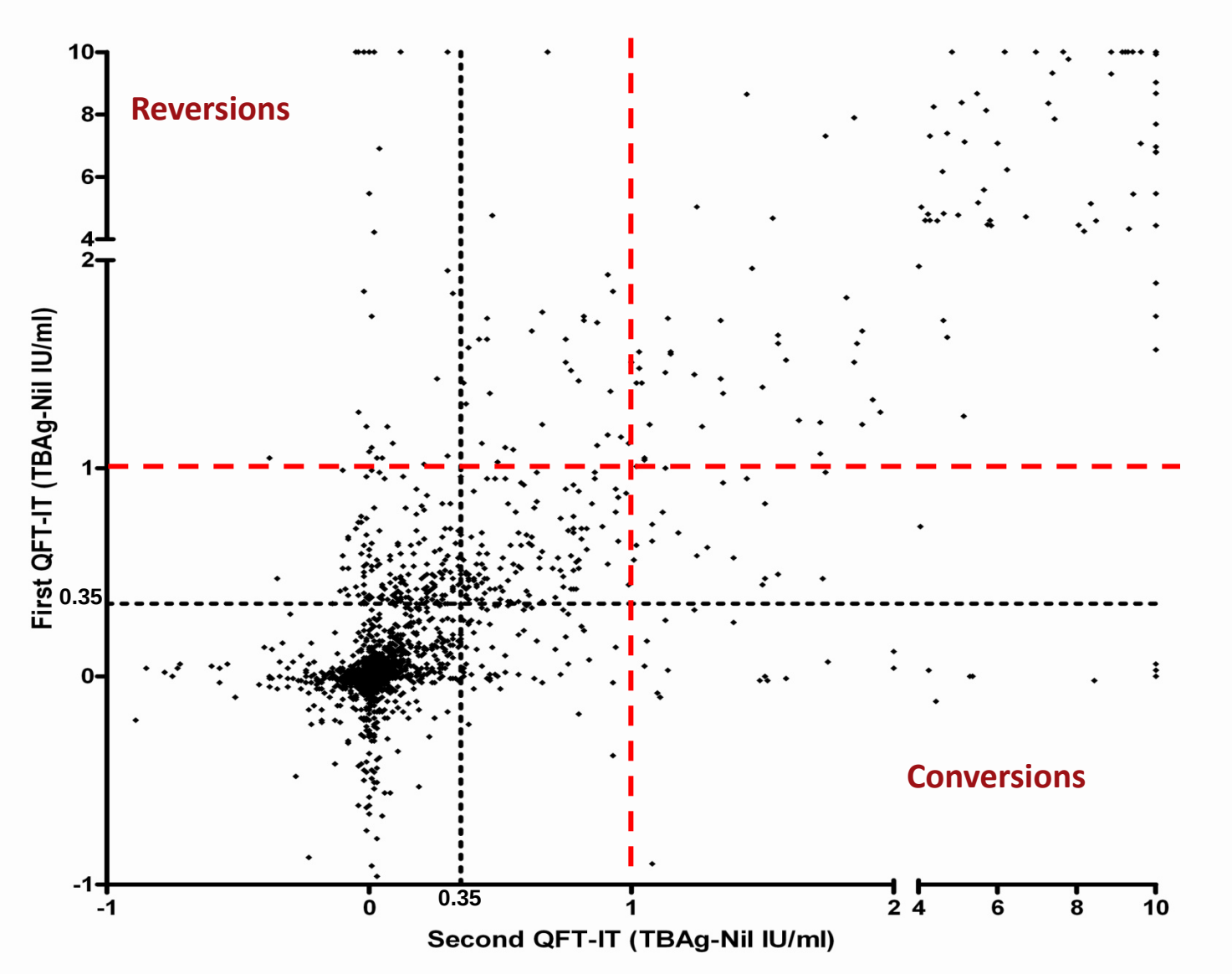

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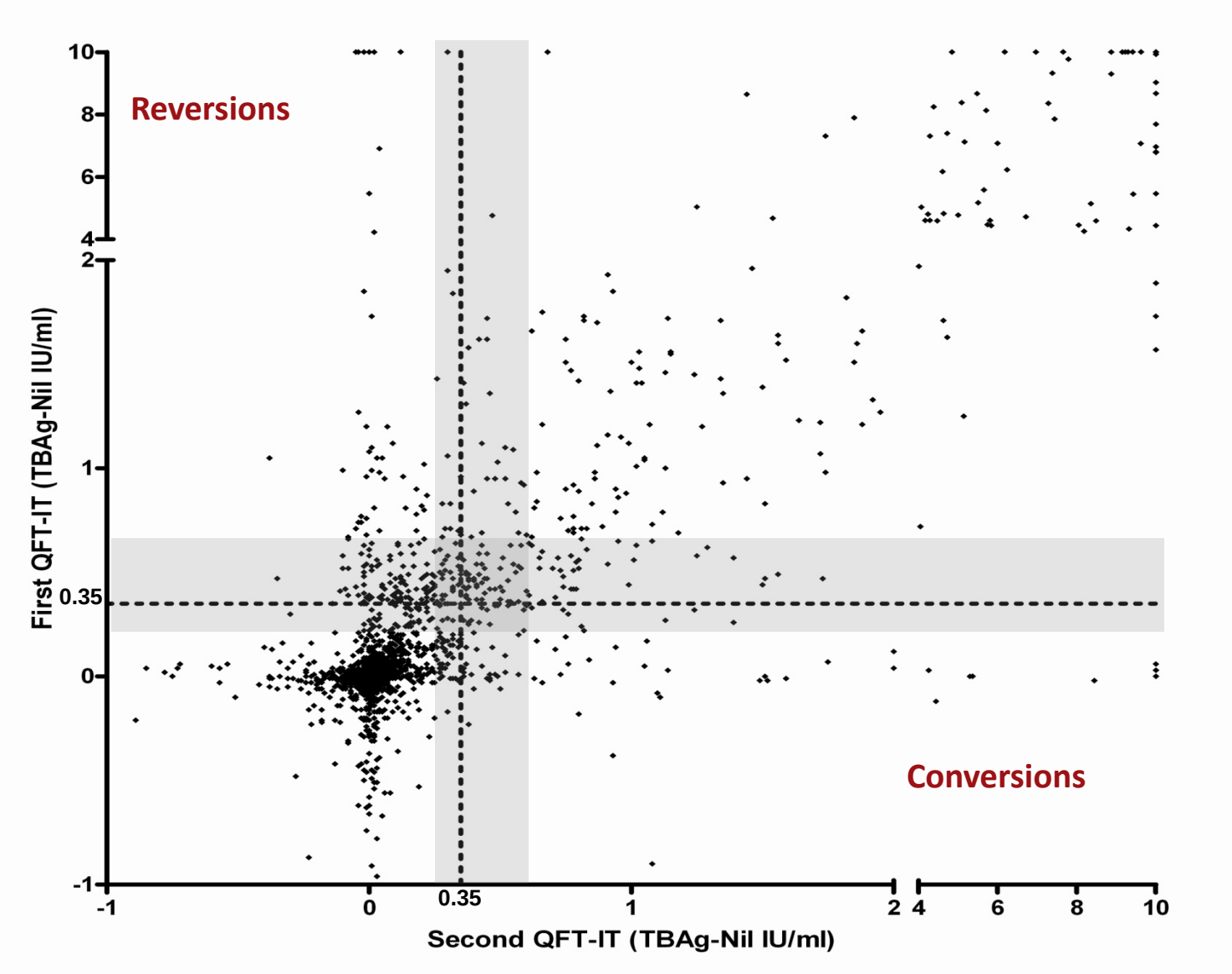
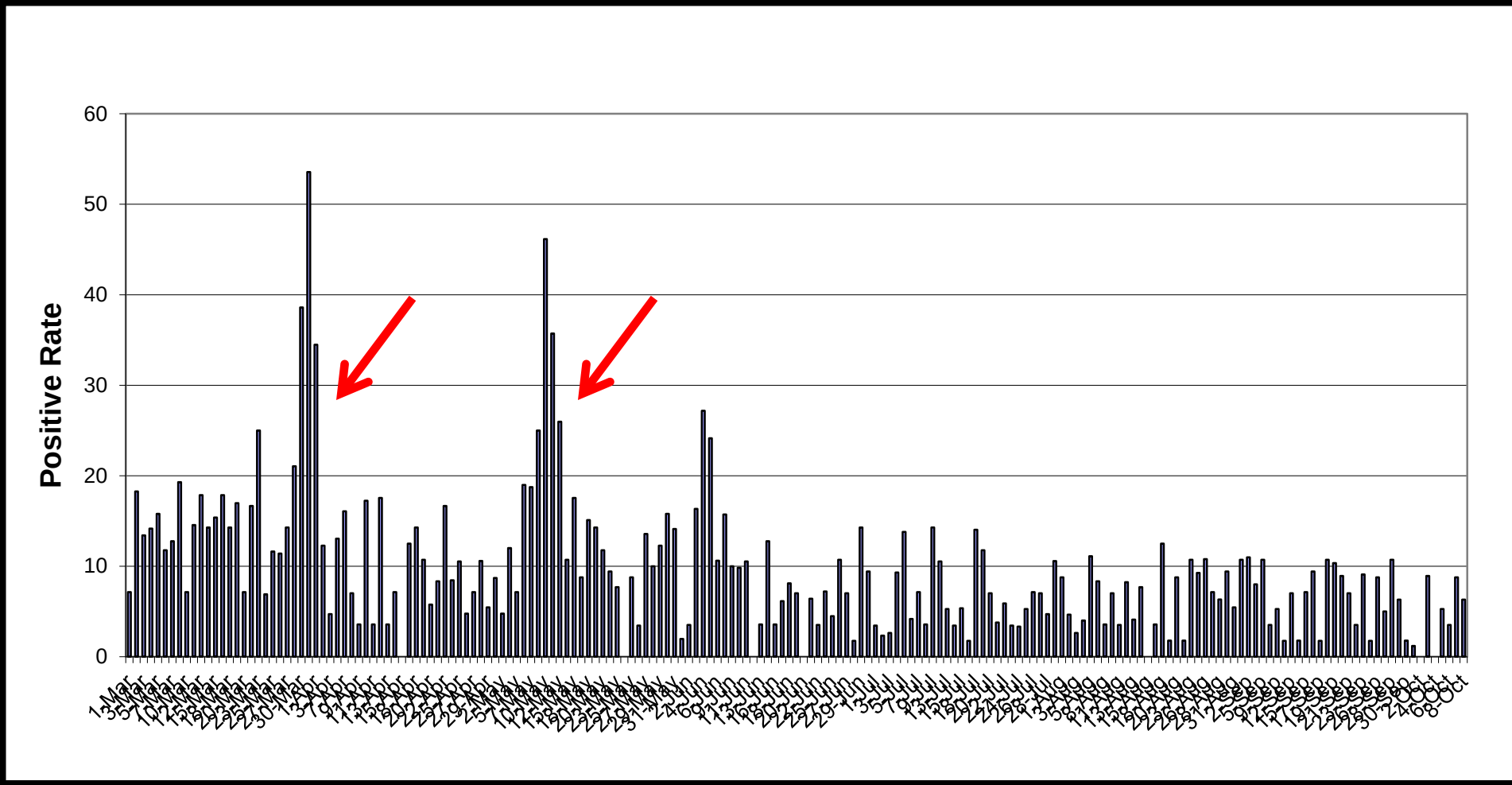
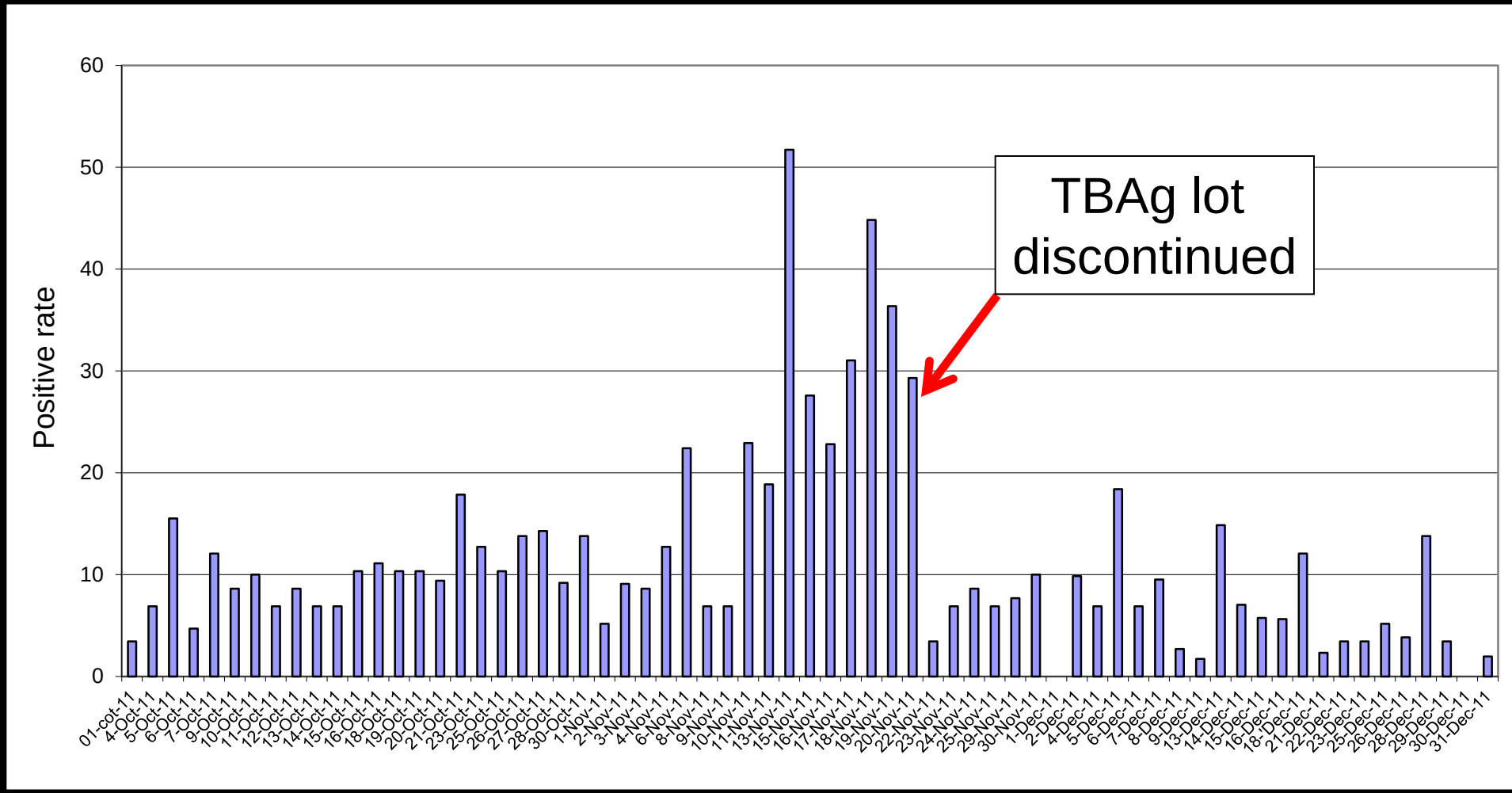


IGRAs Screening of HCWs: Need for Surveillance Program

The QFT-GIT Surveillance Graph: Daily Positive Rate Mar-Oct 2010



The QFT-GIT Surveillance Graph Showing Daily Positive Rate at Stanford



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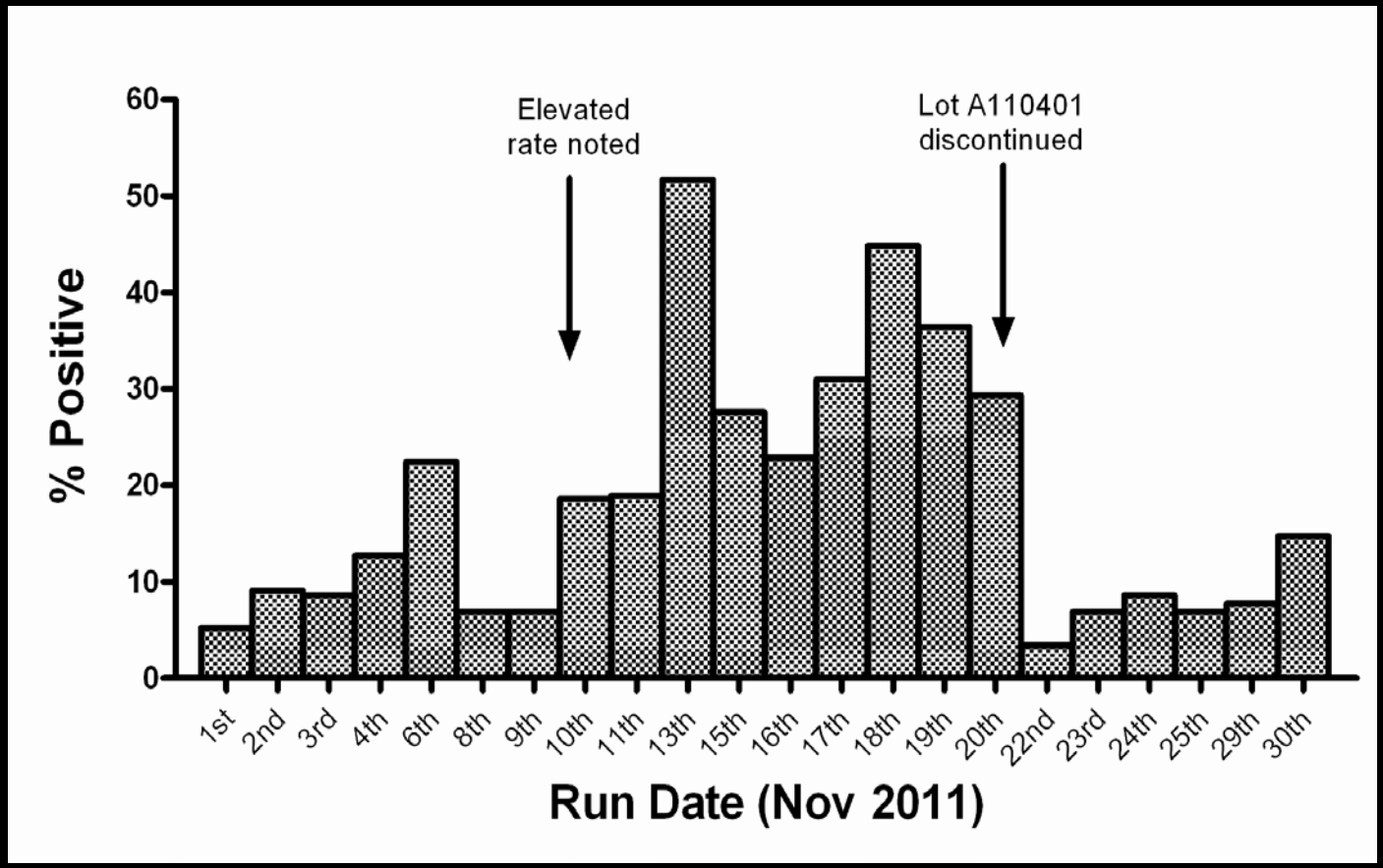


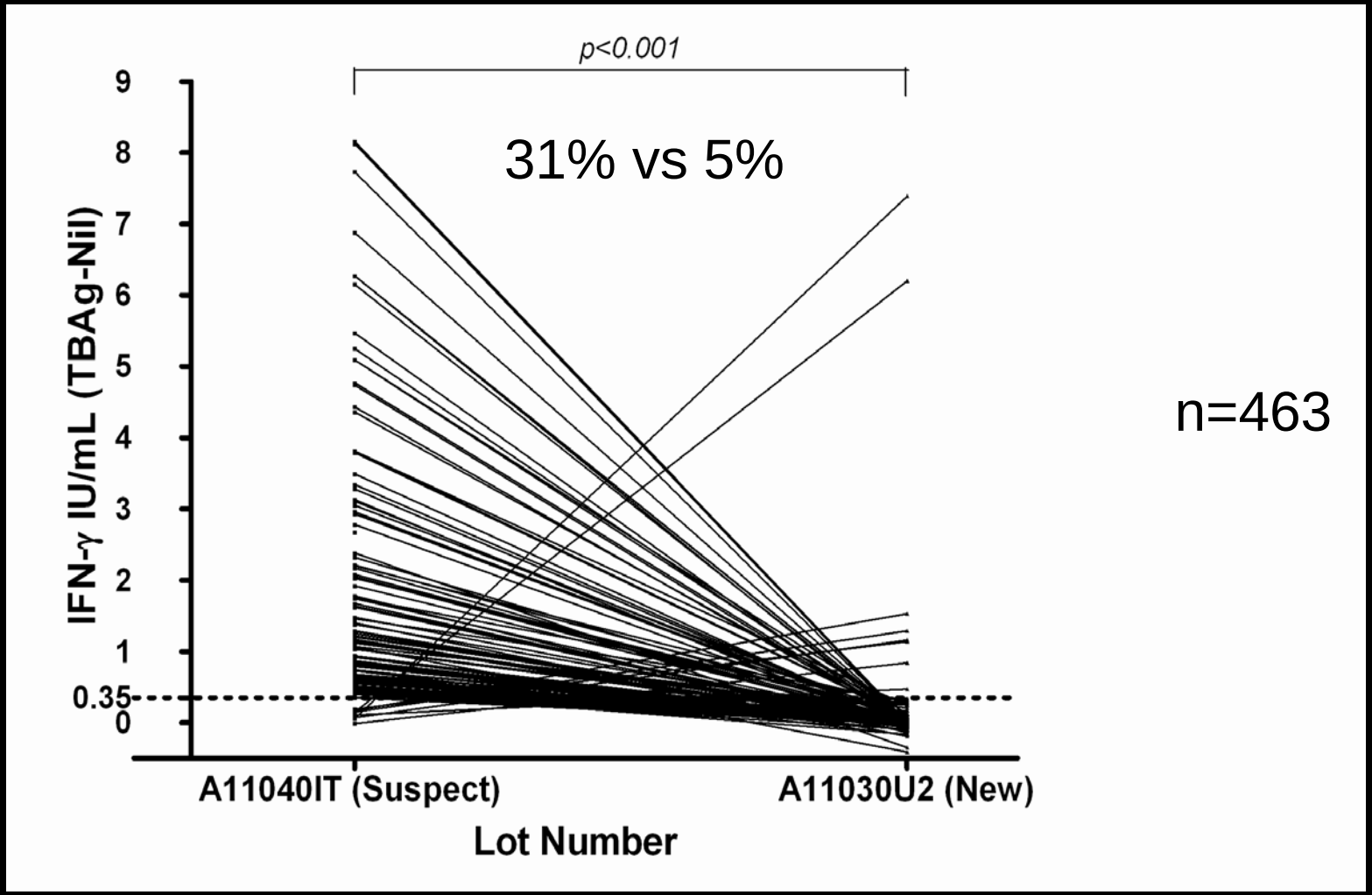
Slater et al JCM 2012

QFT-GIT Responses Pre, During, and Post

	Pre	Suspect	Post	P Value* (Pre, Post)
No. of tests	435	370	705	
Nil Mean, IU/mL	0.1	0.12	0.16	0.99, 0.23
TB Ag† Mean	0.33	0.87	0.26	<0.001, <0.001
Mitogen Mean	9.2	8.69	7.9	<0.001, <0.001
TB Ag-Nil Mean	0.23	0.77	0.1	<0.001, <0.001
% Positive Results	11%	31%	6.8%	<0.001, <0.001

Slater et al JCM 2012

Within Subject Comparison of QFT-GIT Results



n=463

Slater et al JCM 2012

Investigation Outcomes

- FDA informed via CDC.
- Cellestis conducted an internal investigation and “could not reproduce” our findings.
- We could not culture viable organisms.

Investigation Findings

- Manufacturing defects of a faulty TB Antigen tube lot could contribute to QFT-GIT variability.
- Need for implementation of surveillance programs by end users in the clinical laboratories.

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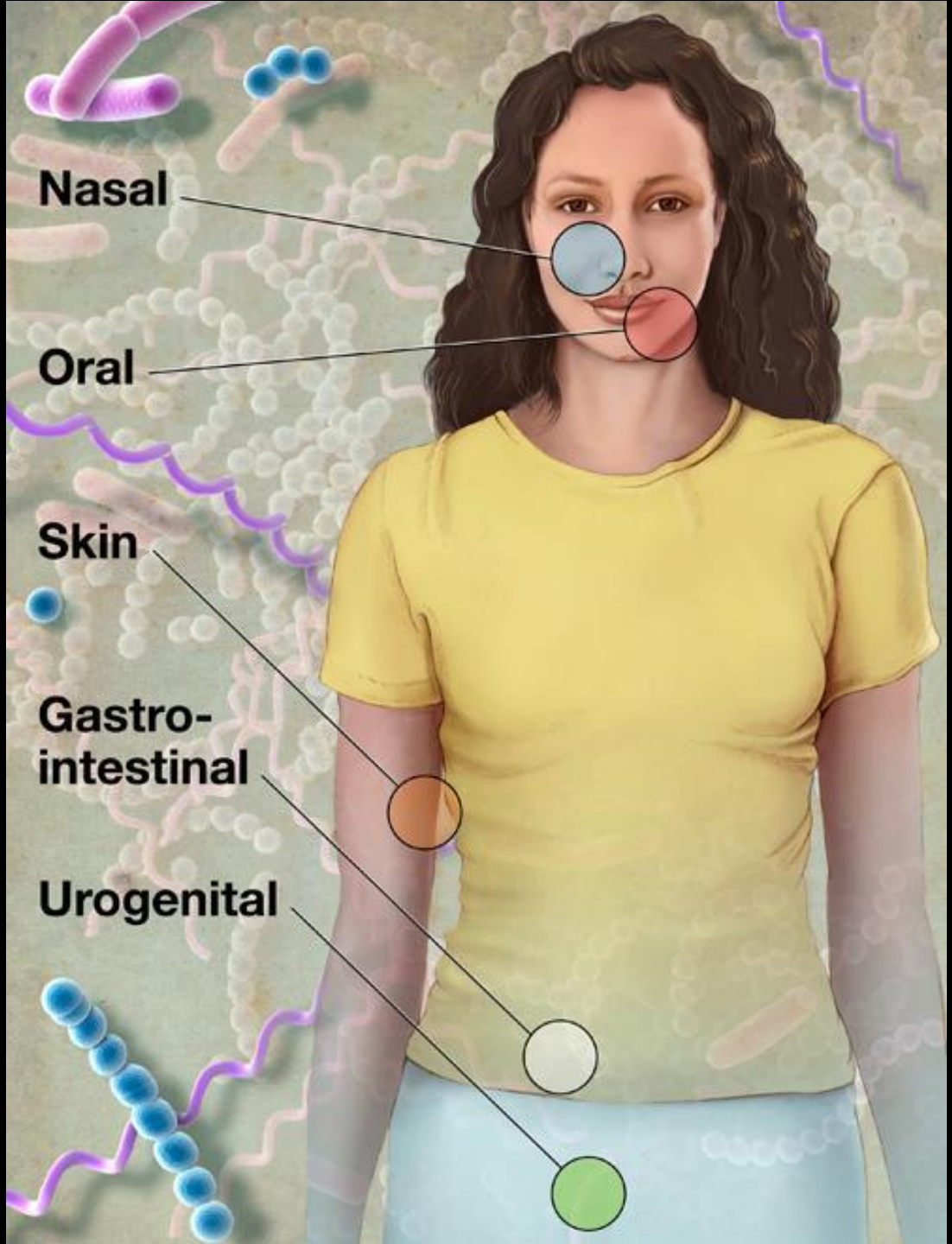
Nasal
Oral
Skin
Gastro-
intestinal
Urogenital

Microbiota regulates immune defense against respiratory tract influenza A virus infection

Takeshi Ichinohe[a,b,1], Iris K. Pang[a,1], Yosuke Kumamoto[a], David R. Peaper[c], John H. Ho[a], Thomas S. Murray[c,d], and Akiko Iwasaki[a,2]

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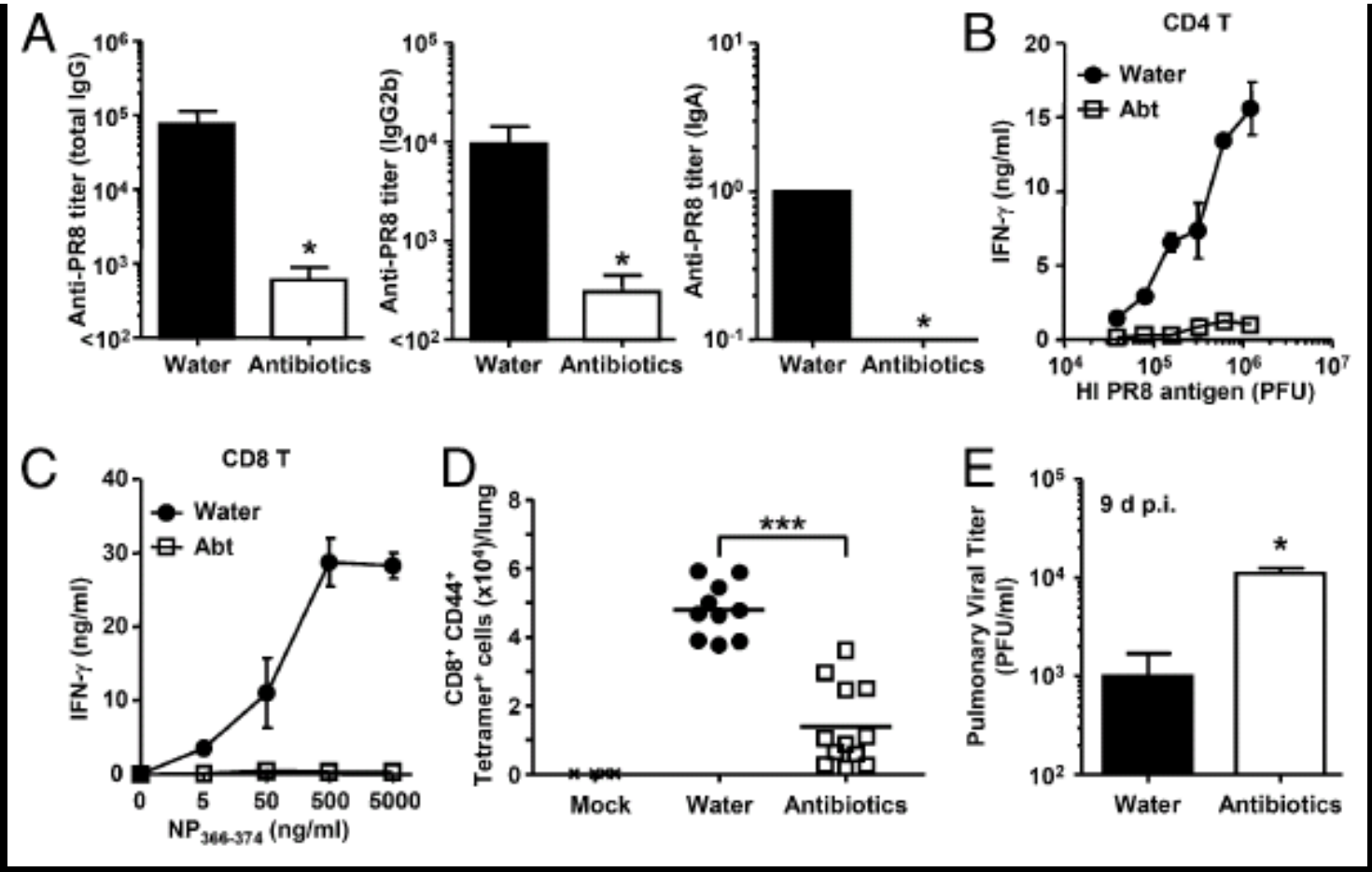
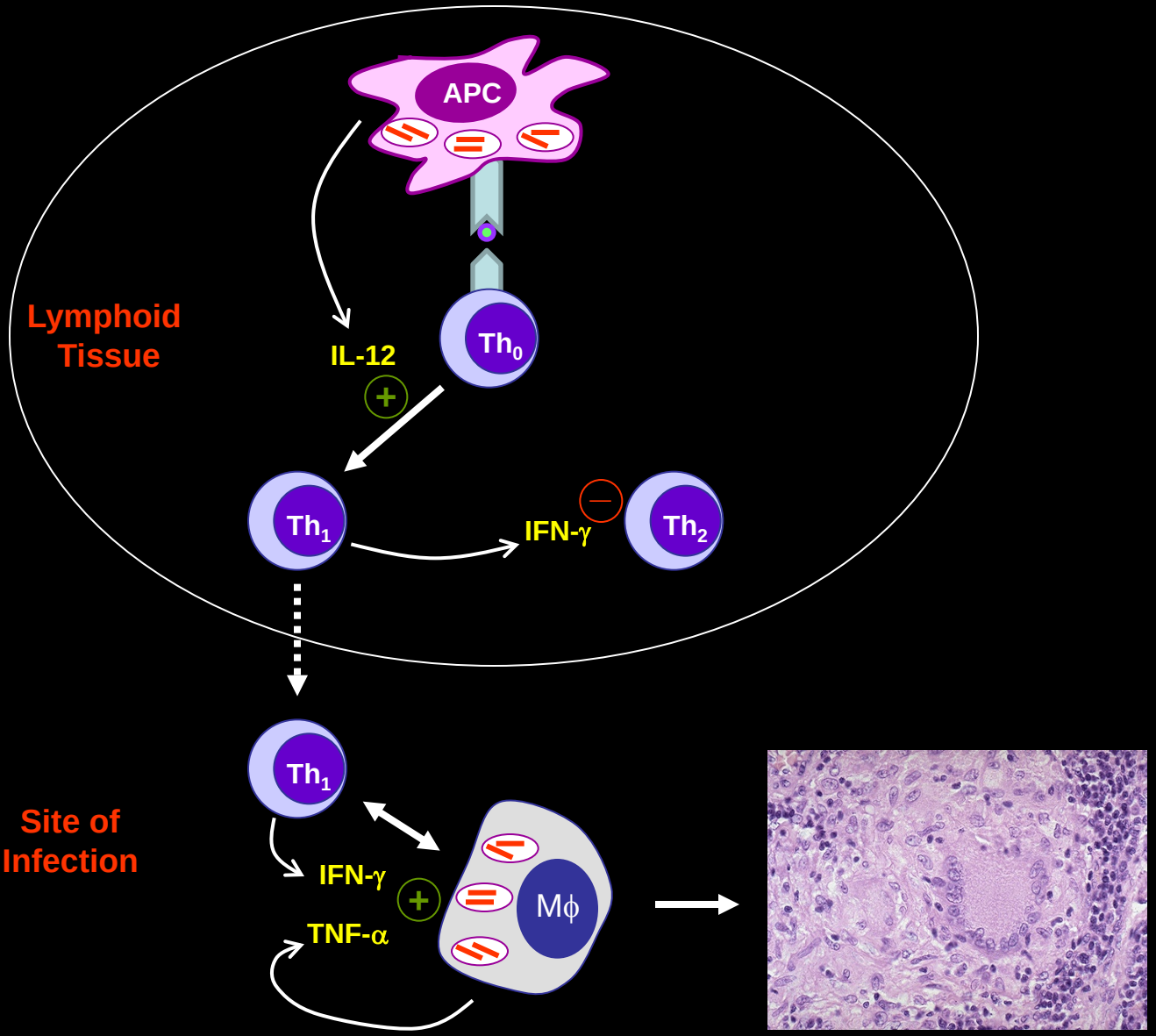
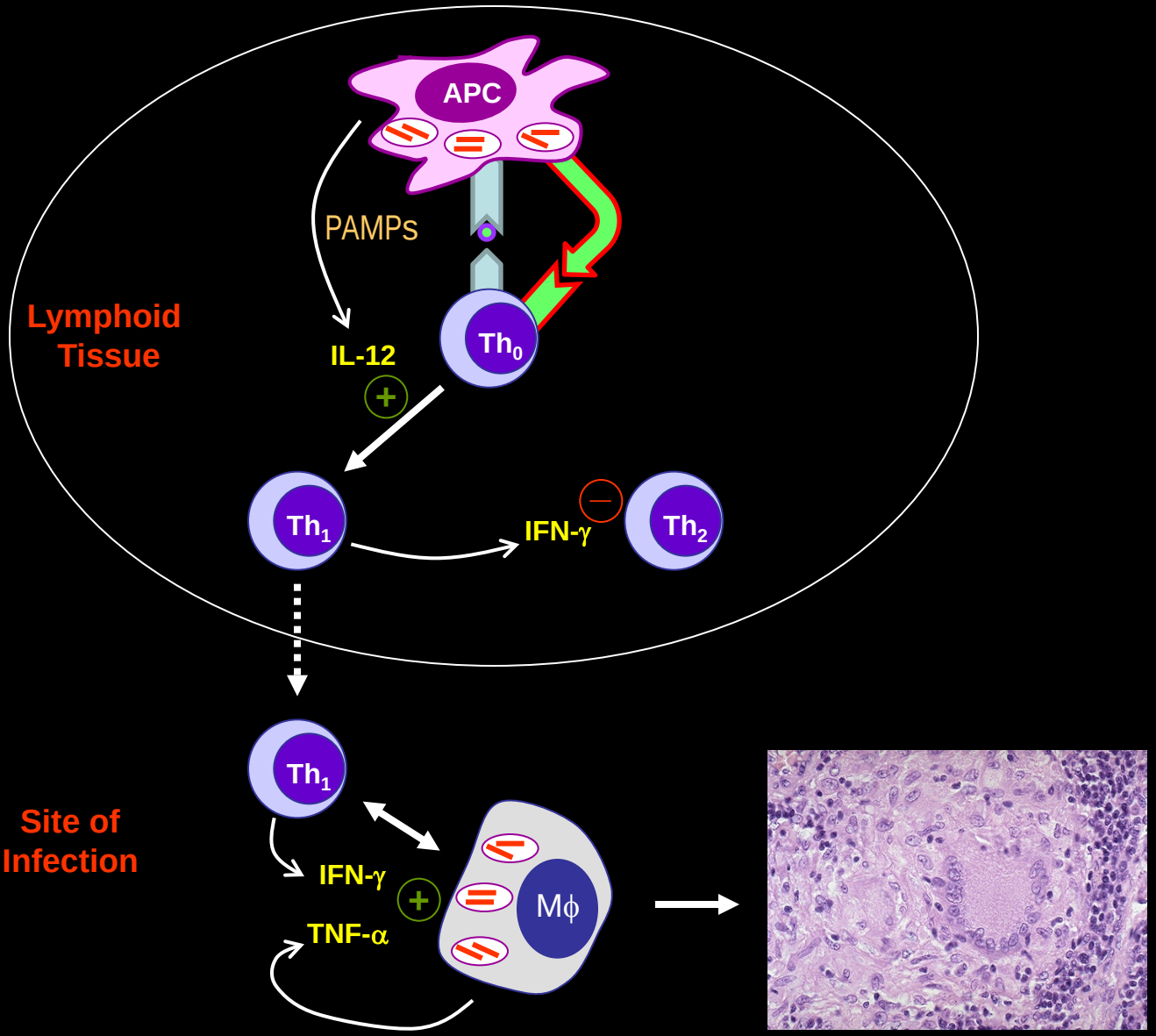


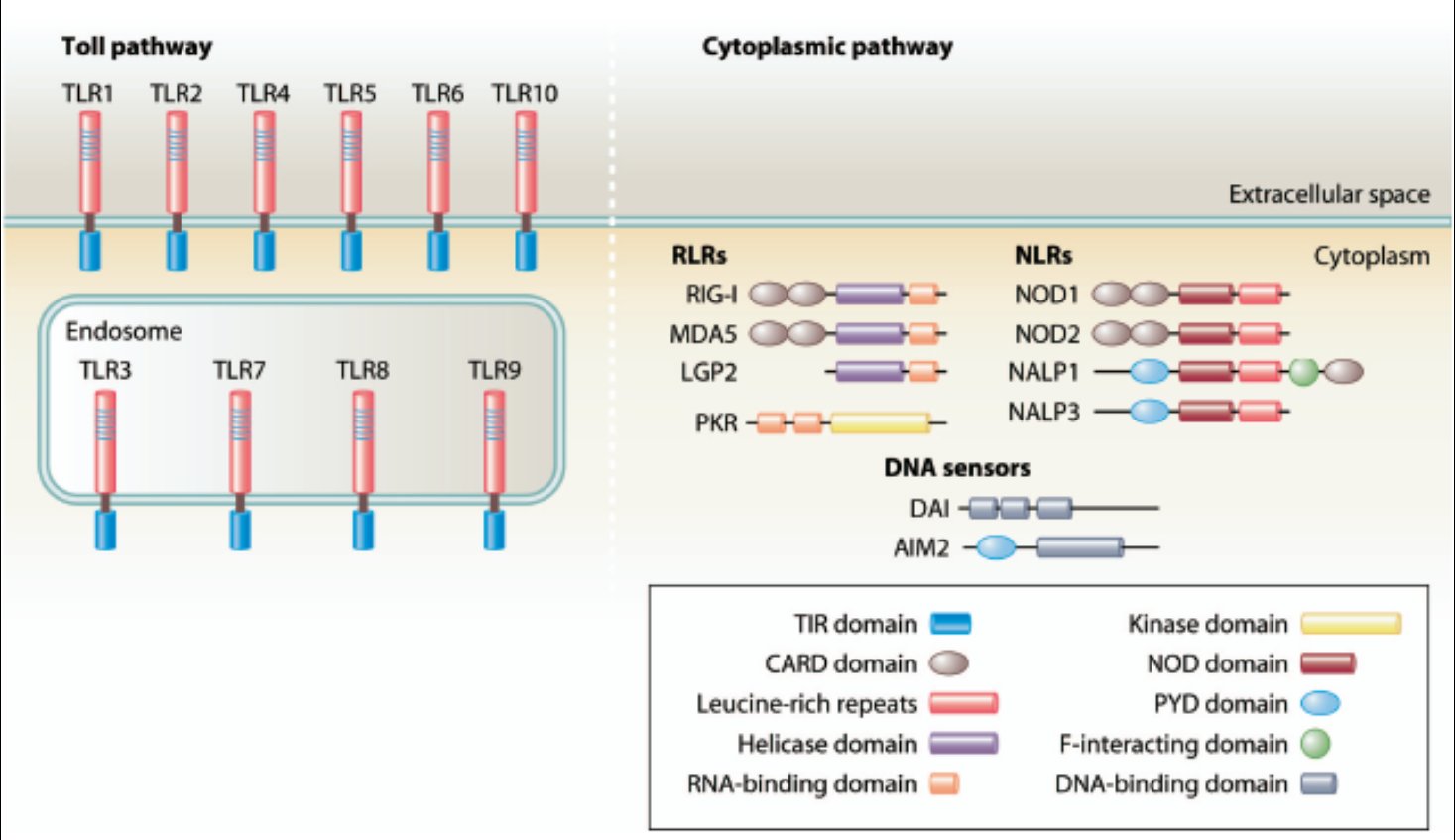
Th1 Cell-Mediated Immunity



Th1 Cell-Mediated Immunity



Pathogen Recognition Receptors



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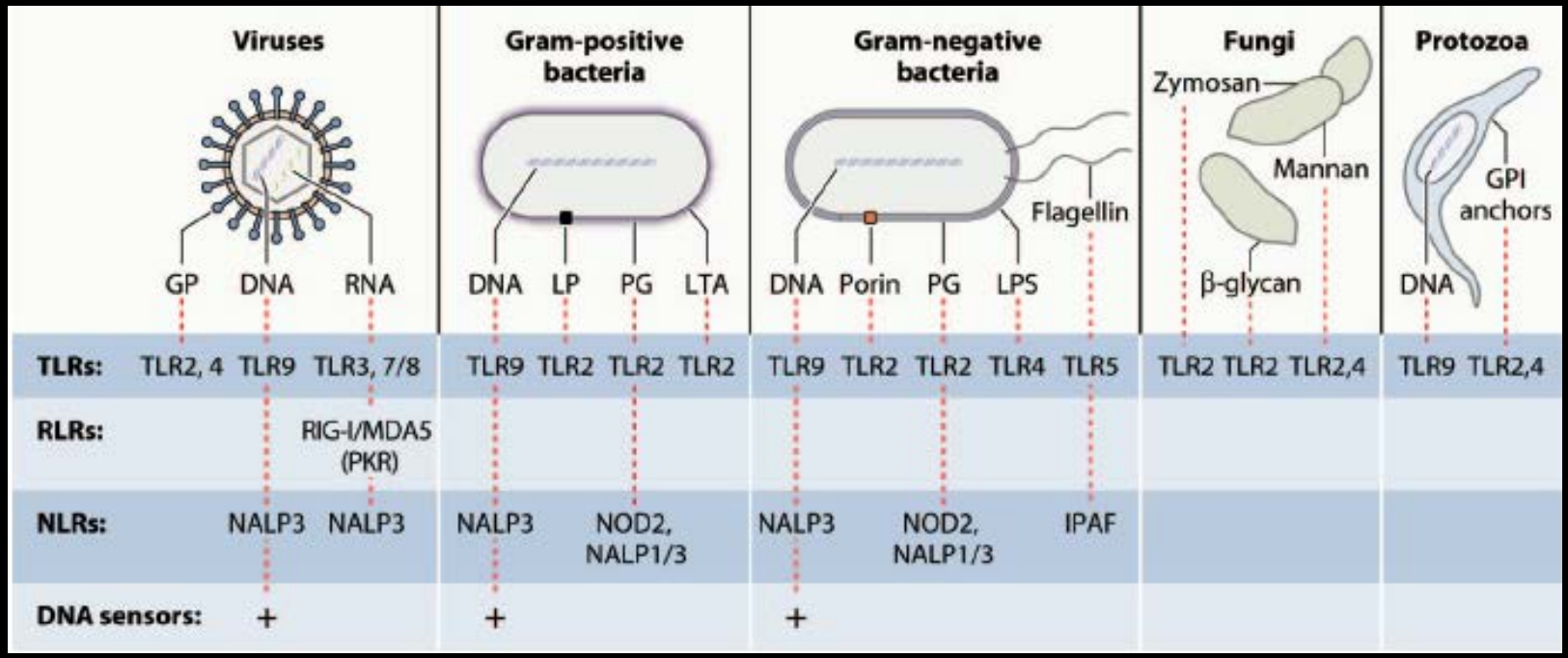
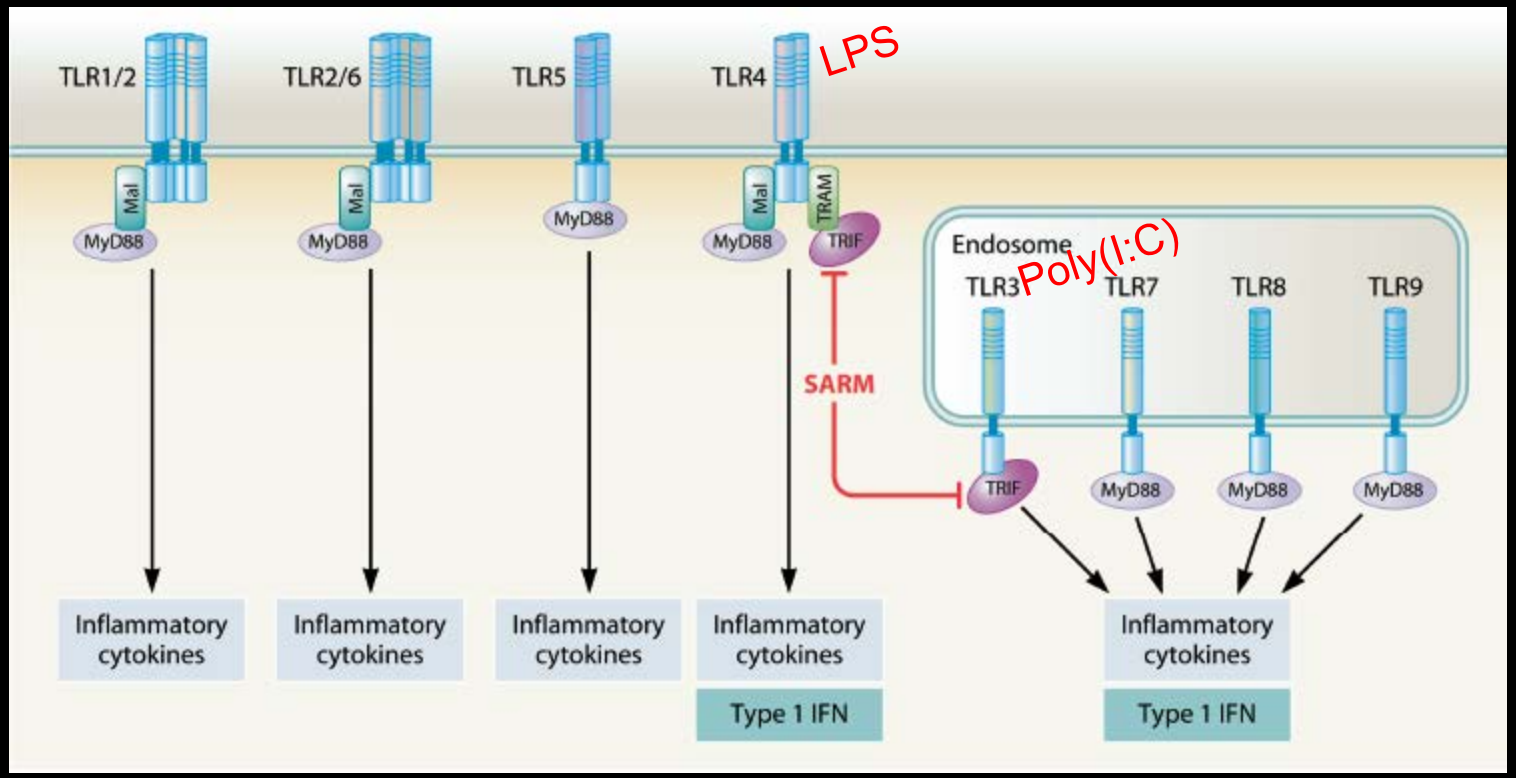
Recognition of Different PAMPs by PRRs



TLR Agonists Activate Adaptive Immune Responses



Naïve T-cell activation ← Antigen presentation Corss-presentation ← Cell recruitment DC maturation: ↑Costimulatory molecules, ↑MHC

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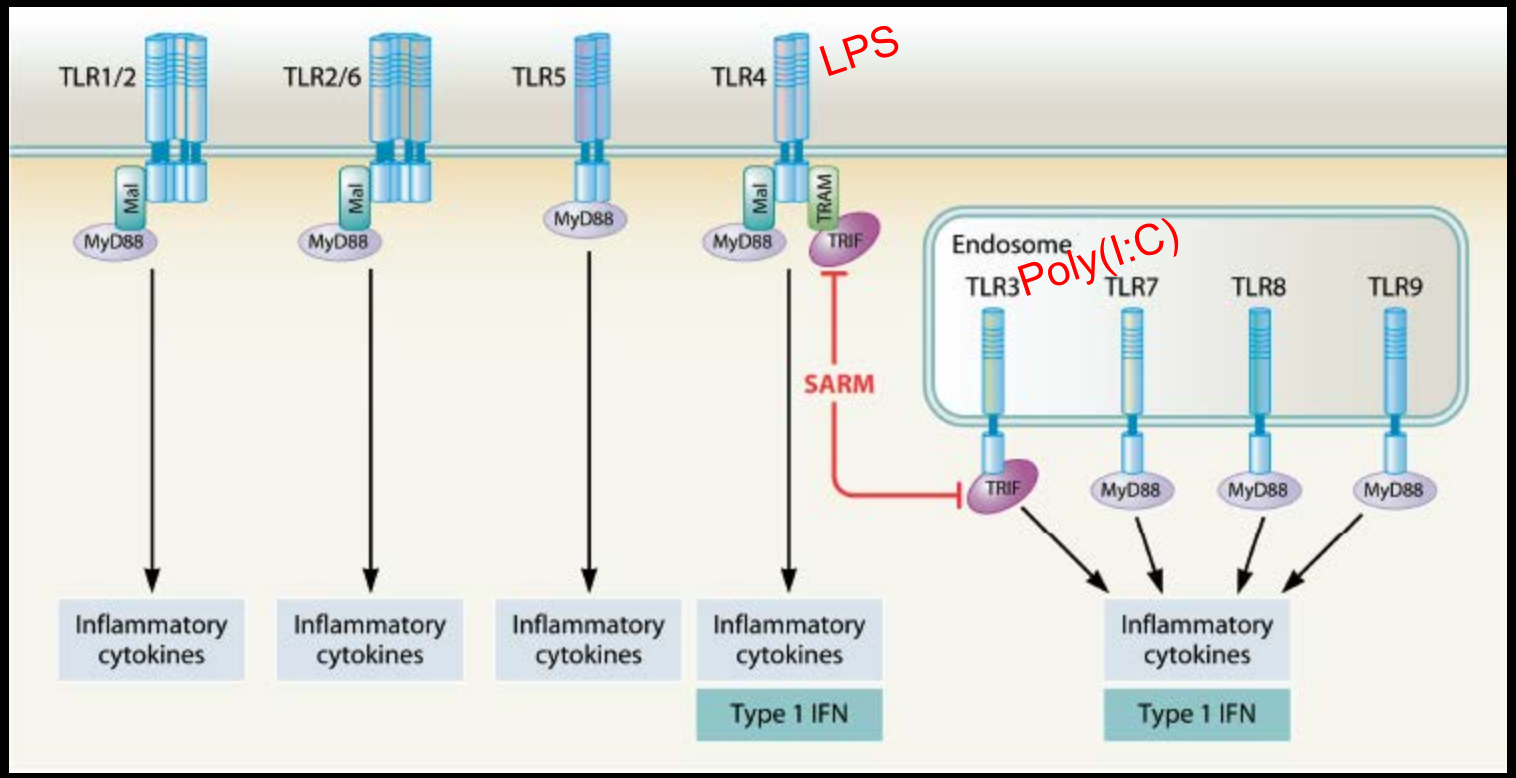
TLR Agonists Activate Adaptive Immune Responses



Naïve T-cell activation ← Antigen presentation Corss-presentation ← Cell recruitment DC maturation: ↑Costimulatory molecules, ↑MHC

Effector T-cell Activation

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ORIGINAL ARTICLE

Imiquimod Enhances IFN-γ Production and Effector Function of T Cells Infiltrating Human Squamous Cell Carcinomas of the Skin

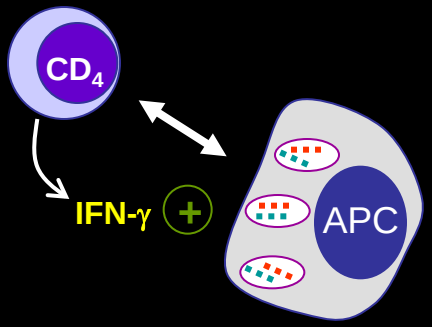
Susan J. Huang[1], Dirkjan Hijnen[2], George F. Murphy[3], Thomas S. Kupper[1], Adam W. Calarese[1], Ilse G. Mollet[4], Carl F. Schanbacher[1], Danielle M. Miller[1], Chrysalyne D. Schmults[1] and Rachael A. Clark[1]

Squamous cell carcinomas (SCCs) are sun-induced skin cancers that are particularly numerous and aggressive in patients taking T-cell immunosuppressant medications. Imiquimod is a topical immune response modifier and Toll-like receptor 7 (TLR7) agonist that induces the immunological destruction of SCC and other skin cancers. TLR7 activation by imiquimod has pleiotropic effects on innate immune cells, but its effects on T cells remain largely uncharacterized. Because tumor destruction and formation of immunological memory are ultimately T-cell-mediated effects, we studied the effects of imiquimod therapy on effector T cells infiltrating human SCC. SCC treated with imiquimod before excision contained dense T-cell infiltrates associated with tumor cell apoptosis and histological evidence of tumor regression. Effector T cells from treated SCC produced more IFN-γ, granzyme, and perforin and less IL-10 and transforming growth factor-β (TGF-β) than T cells from untreated tumors. Treatment of normal human skin with imiquimod induced activation of resident T cells and reduced IL-10 production but had no effect on IFN-γ, perforin, or granzyme, suggesting that these latter effects arise from the recruitment of distinct populations of T cells into tumors. Thus, imiquimod stimulates tumor destruction by recruiting cutaneous effector T cells from blood and by inhibiting tonic anti-inflammatory signals within the tumor.

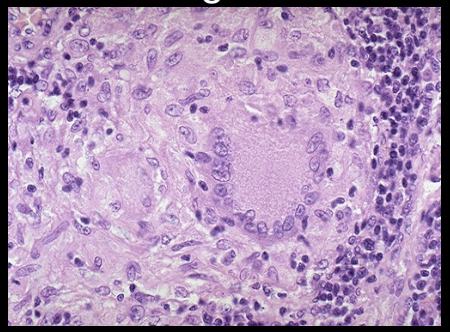
Journal of Investigative Dermatology (2009) **129,** 2676–2685; doi:10.1038/jid.2009.151; published online 11 June 2009

Interferon-γ Release Assays

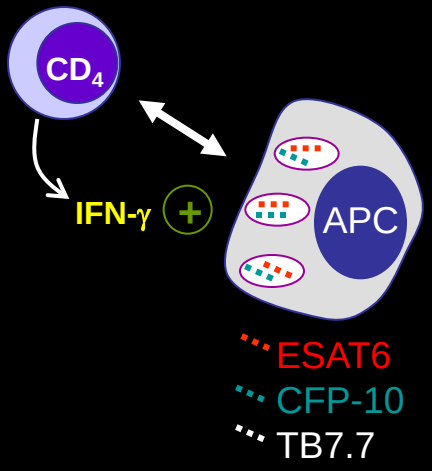
in vivo



Human granuloma



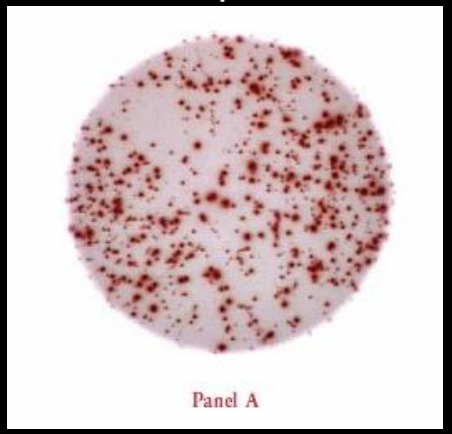
in vitro



ELISA

Quantiferon

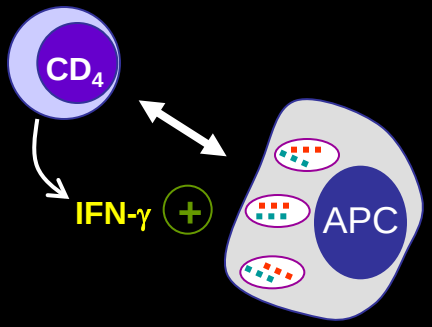
Elispot



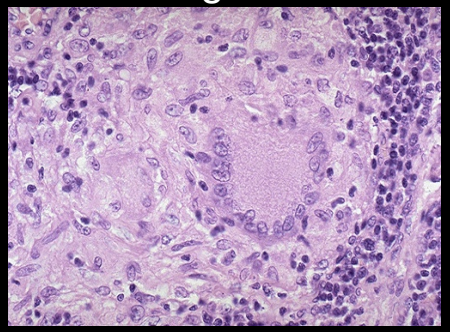
T-SPOT.TB

Interferon-γ Release Assays

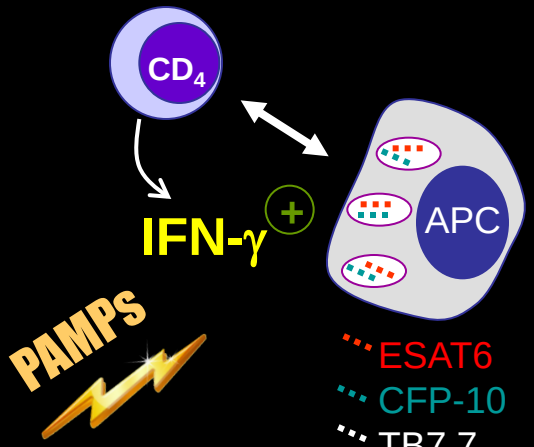
in vivo



Human granuloma



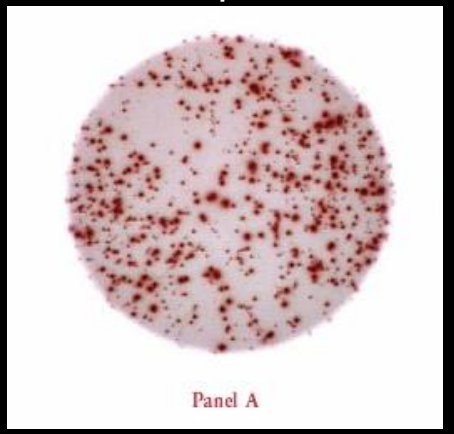
in vitro



ELISA

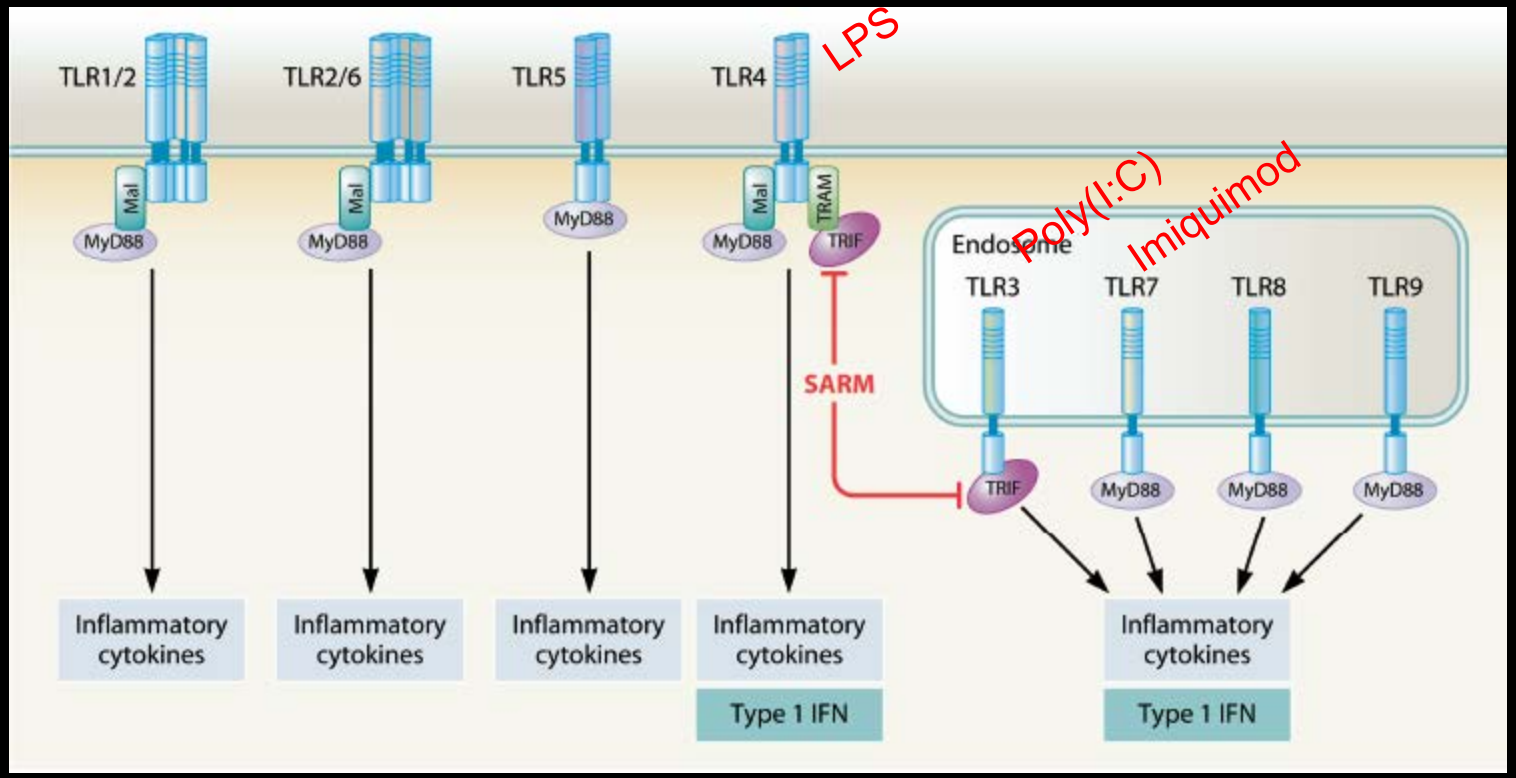
Quantiferon

Elispot



T-SPOT.TB

TLR Agonists Activate Adaptive Immune Responses



Naïve T-cell activation ← Antigen presentation Corss-presentation ← Cell recruitment DC maturation: ↑Costimulatory molecules, ↑MHC

Enhance IGRA response ?

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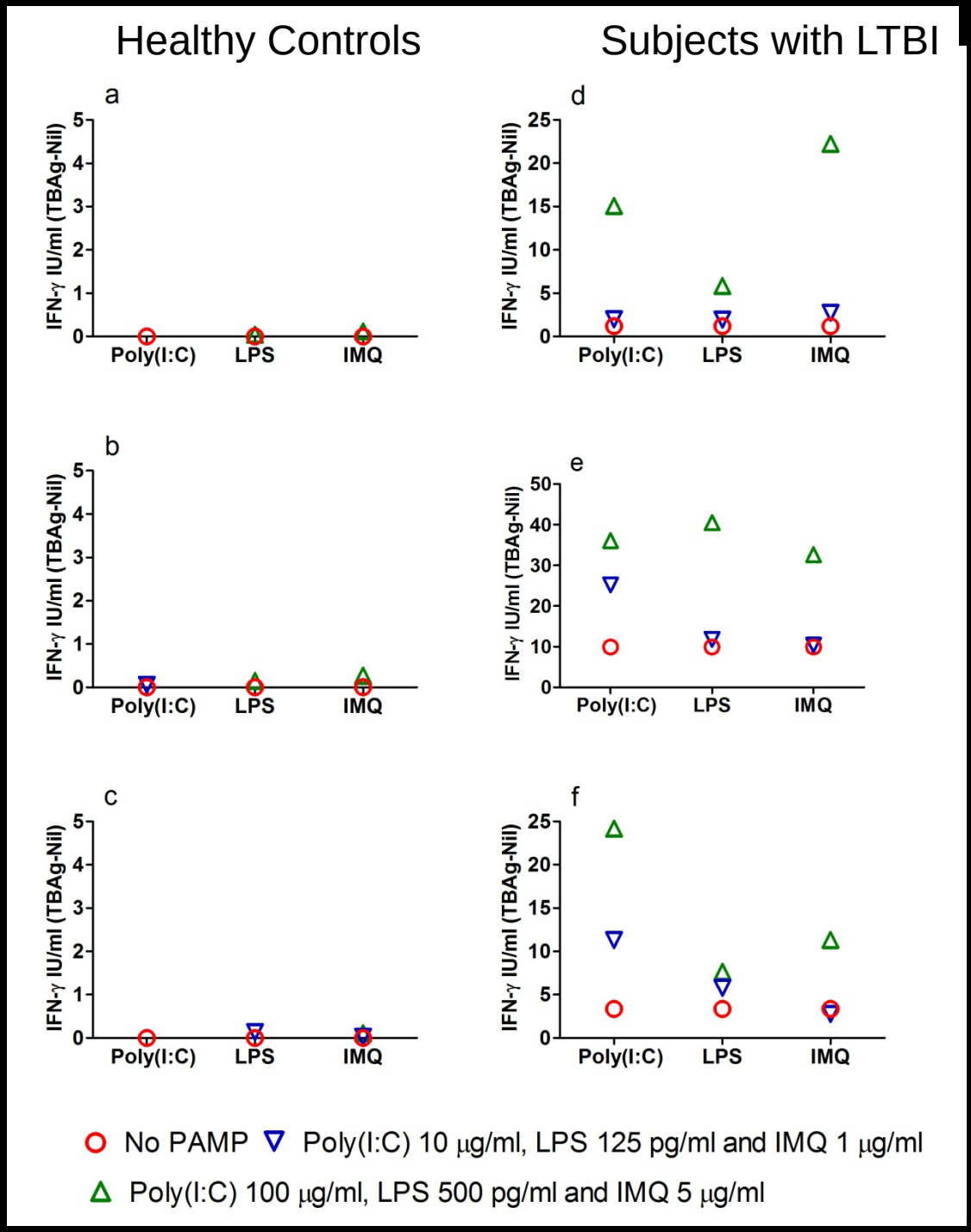
Table 1 TLR expression by human DC subsets

	Freshly isolated			*In vitro*–differentiated DCs
	Monocyte	mDC	pDC	GM-CSF + IL-4
TLR1	++	++	+	++
TLR2	++	++	–	++
TLR3	–	++	–	++
TLR4	++	–	–	++
TLR5	++	+	–	+[37] [32,36]
TLR6	++	++	++	++
TLR7	+[34,35] [17,32,33,36,37]	+[34,35] [32,33]	++	–
TLR8	++	++	–	++
TLR9	–	–	++	–
TLR10	–	+	+	
Refs.[a]	17,32–37	32–35	9,17,32–35	9,32,36,37

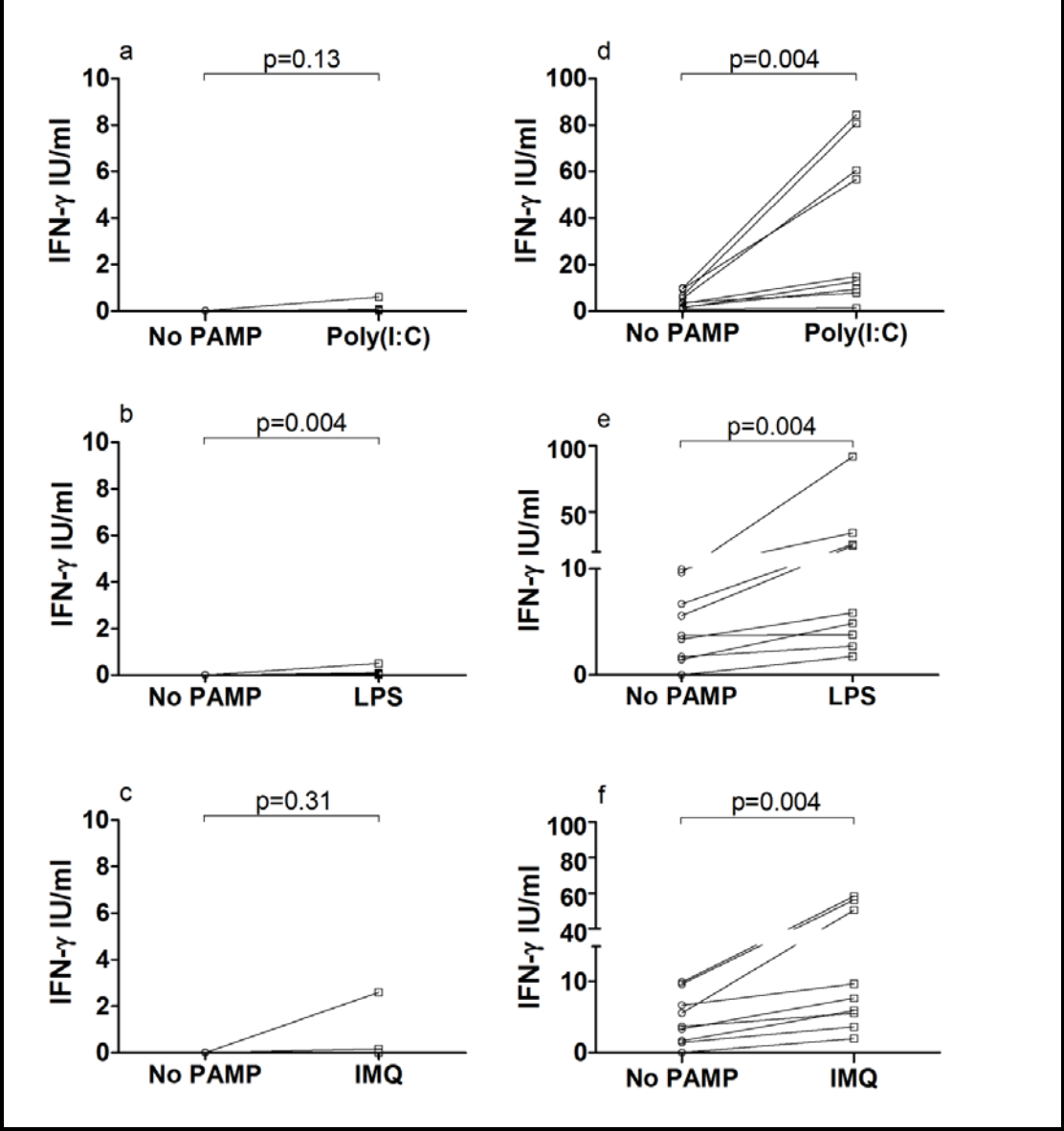
Iwasaki & Medzhitov Nat Immun 2004

PAMPs Increase TB Response in QFT-IT Assay in LTBI Subjects



N=8

PAMPs Increase TB Response in QFT-IT Assay



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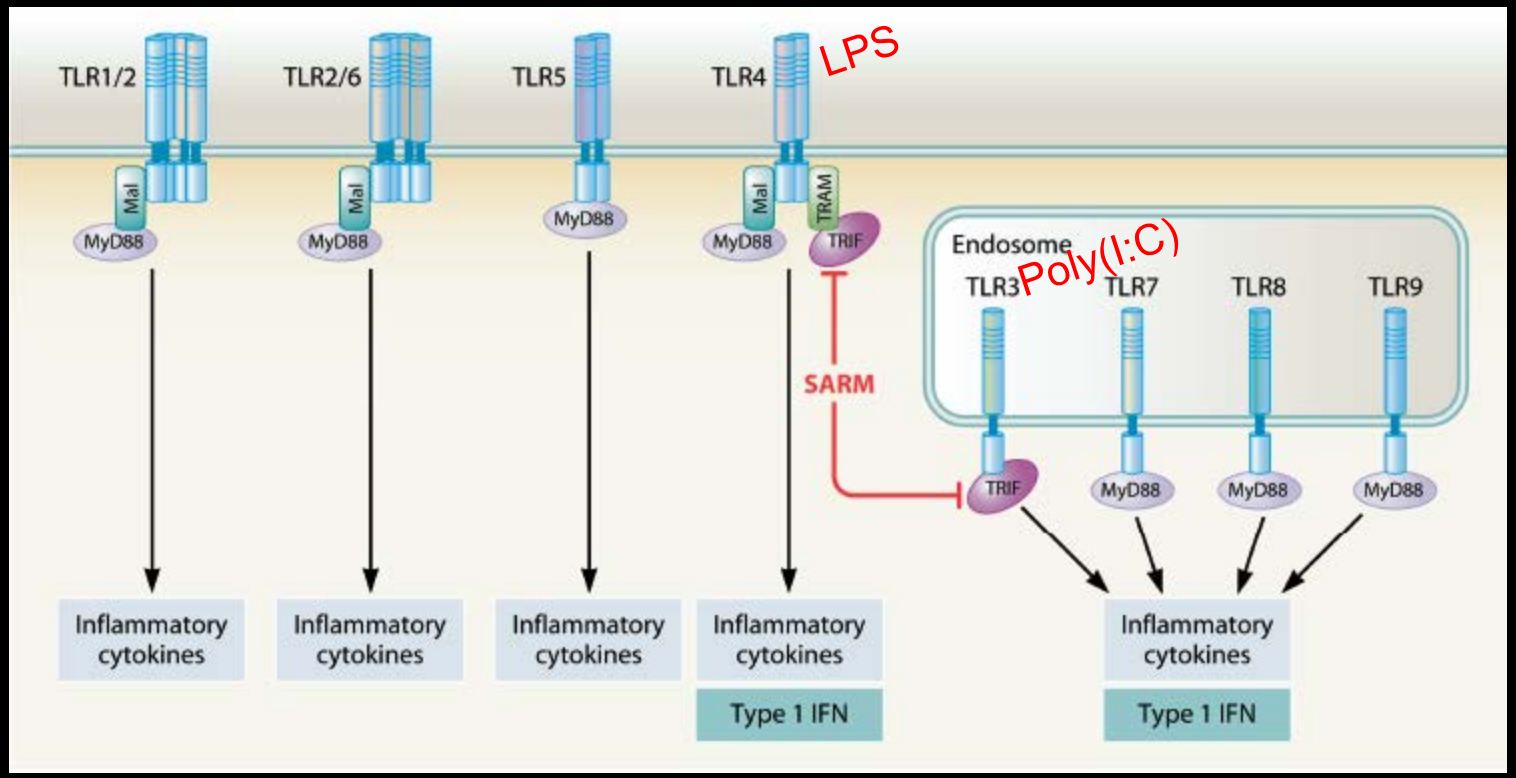
TLR Agonists Activate Adaptive Immune Responses



Naïve T-cell activation ← Antigen presentation Corss-presentation ← Cell recruitment DC maturation: ↑Costimulatory molecules, ↑MHC

Effector T-cell Activation

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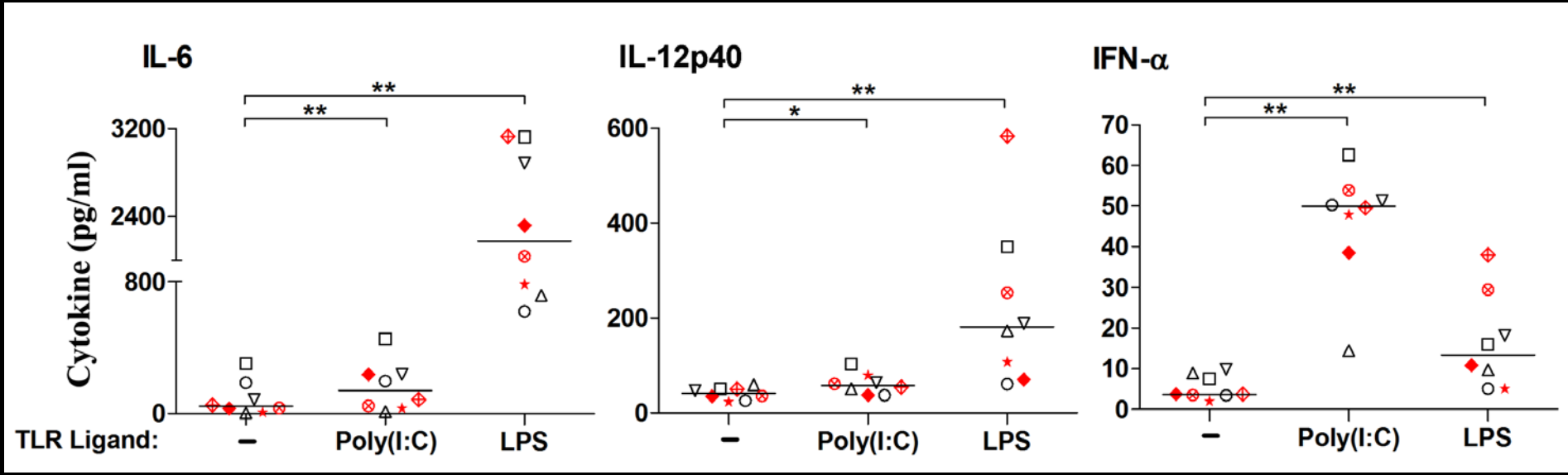
Poly(I:C) & LPS Induce Inflammatory Cytokines & IFN-α in Whole Blood in QFT Nil Tube



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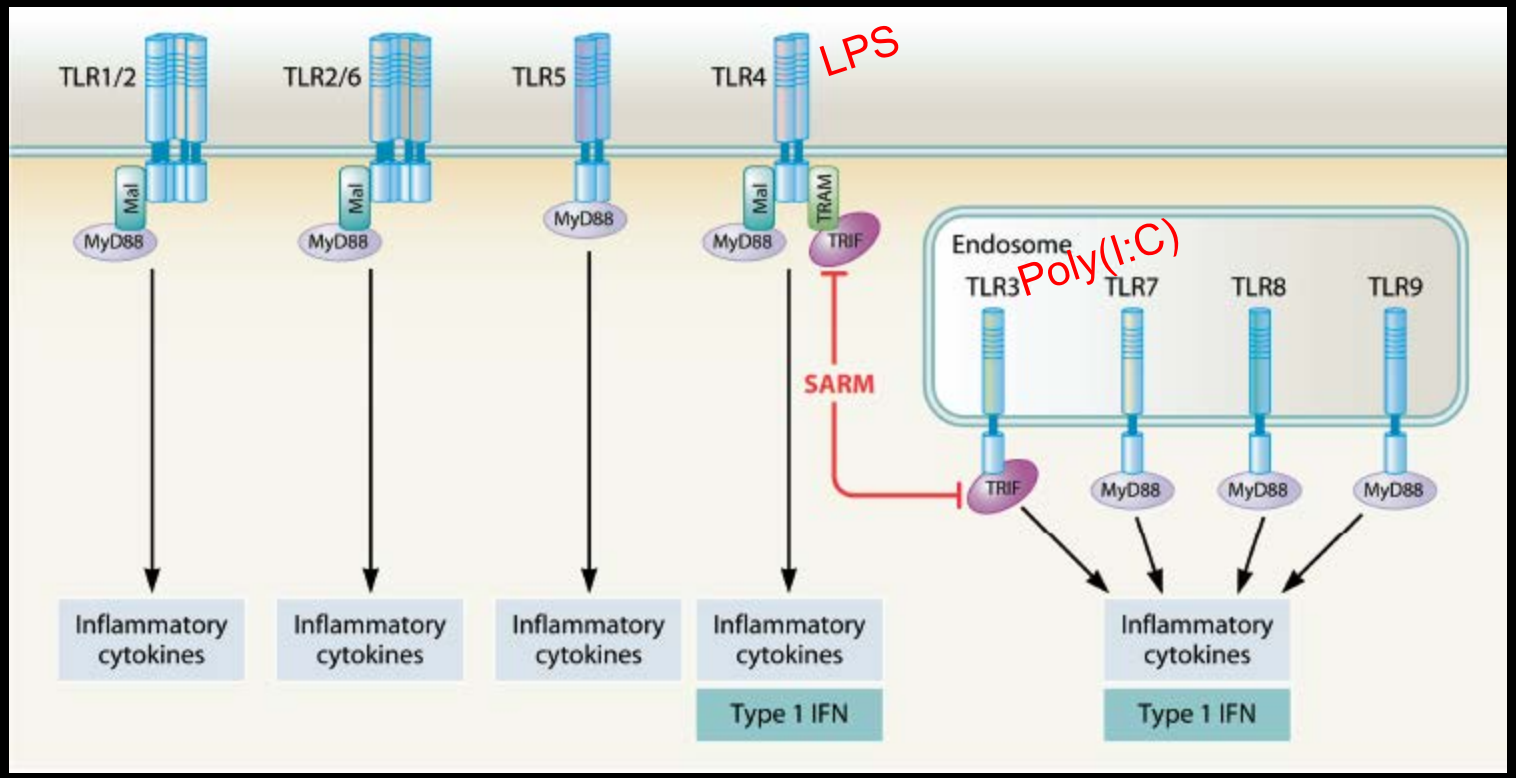
TLR Agonists Activate Adaptive Immune Responses



Naïve T-cell activation ← Antigen presentation Corss-presentation ← Cell recruitment DC maturation: ↑Costimulatory molecules, ↑MHC

Effector T-cell Activation

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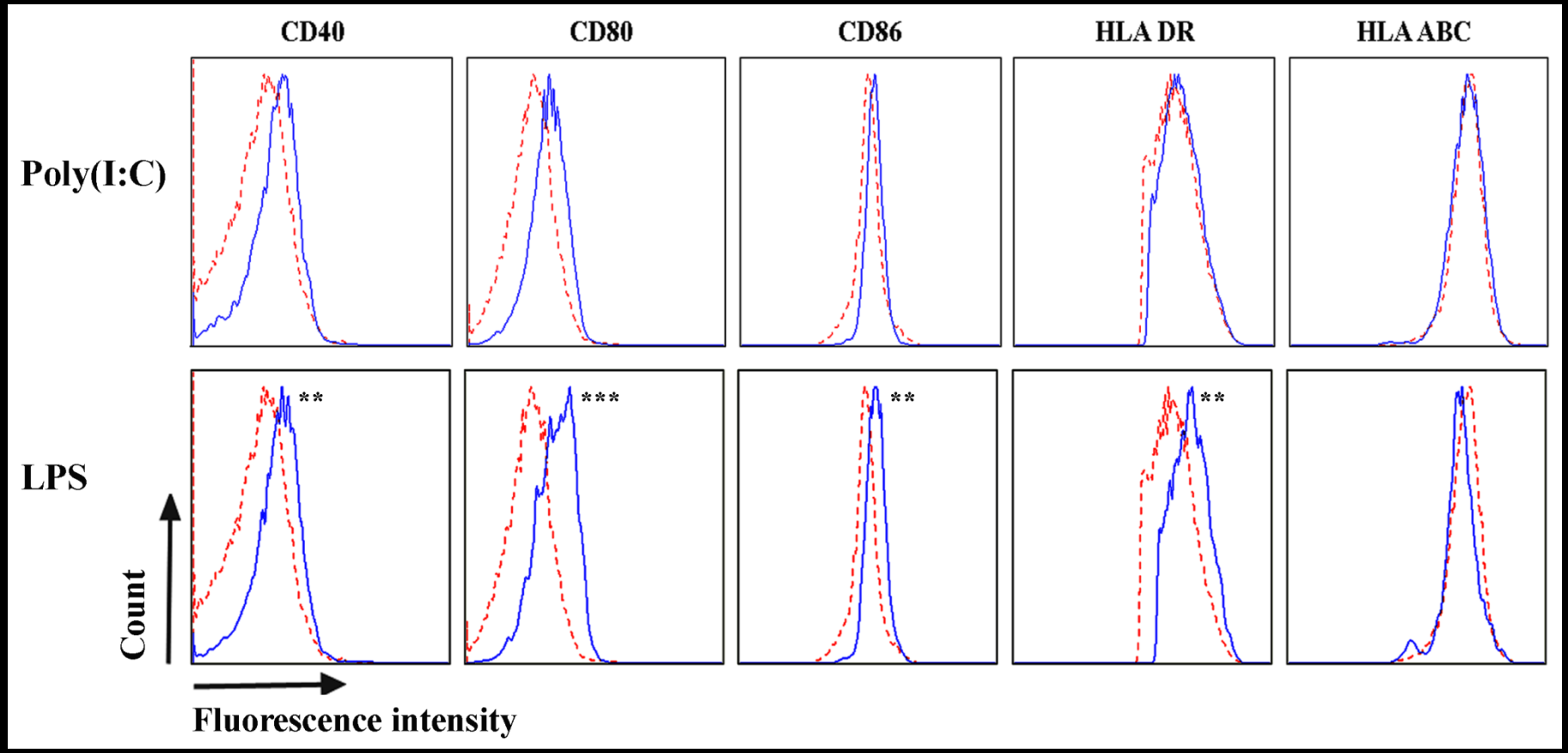
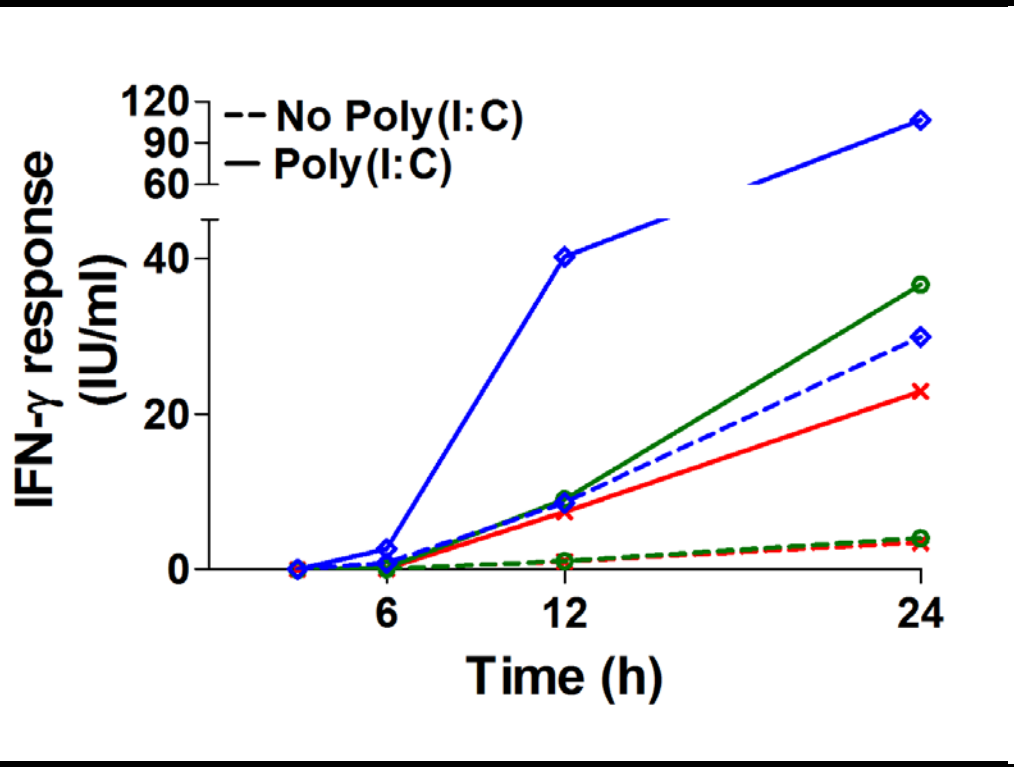
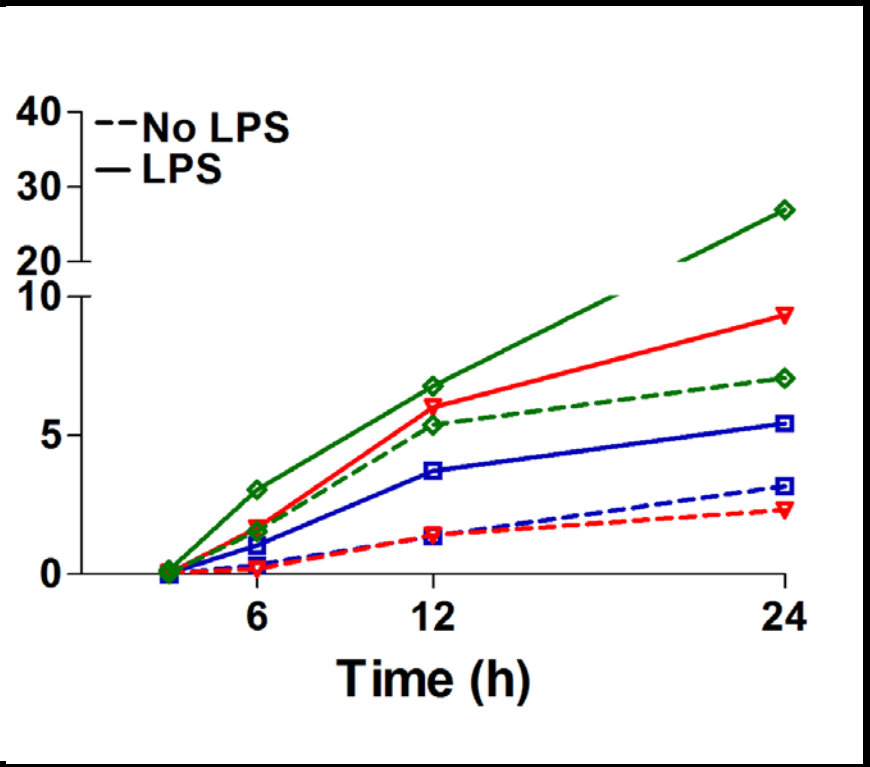
LPS Induce Maturation of Monocytes in Whole Blood in QFT Nil Tube

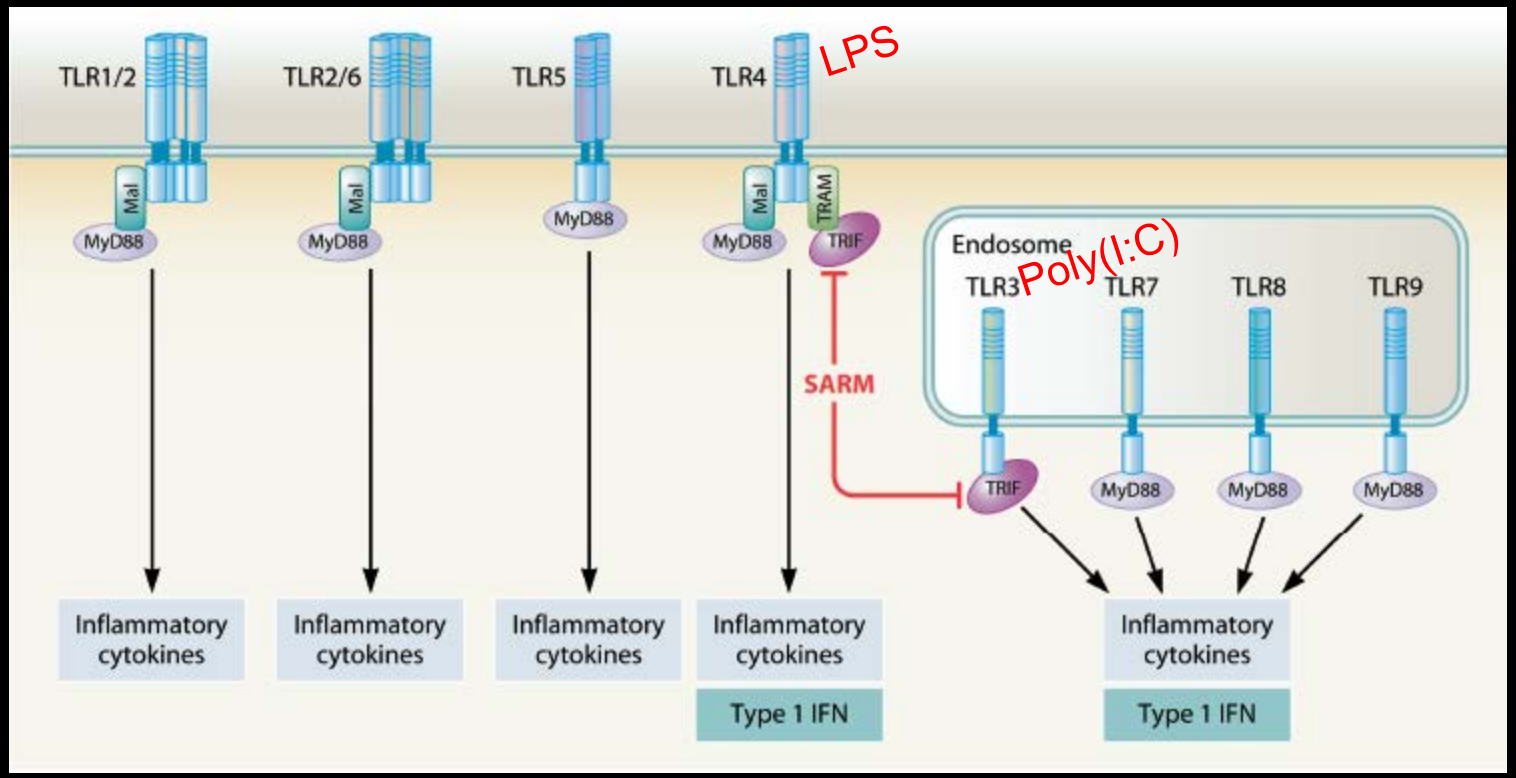


PAMPS Triger Earlier and Greater IFN-γ Release





TLR Agonists Activate Adaptive Immune Responses



Naïve T-cell activation ← Antigen presentation Corss-presentation ← Cell recruitment DC maturation: ↑Costimulatory molecules, ↑MHC

Effector T-cell Activation

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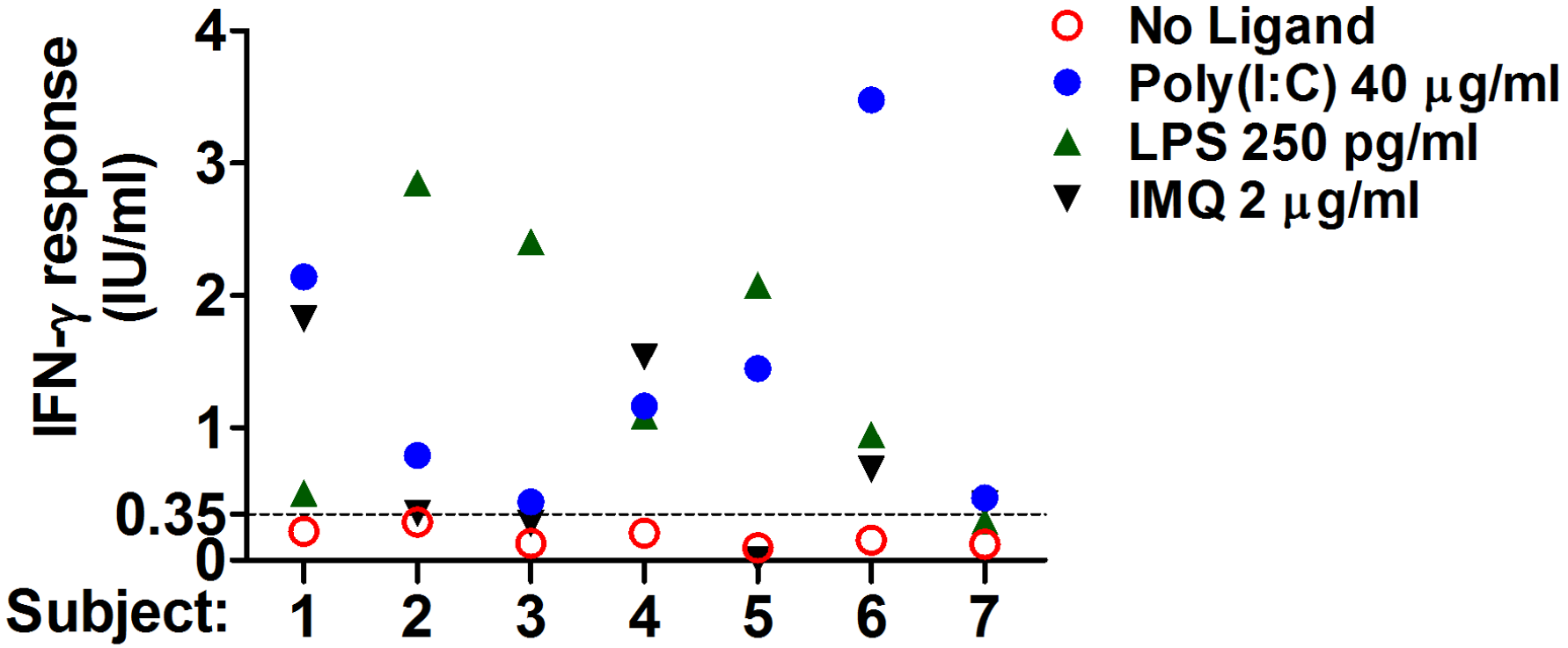
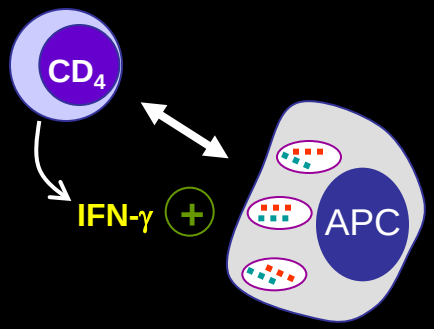
PAMPs Enhance Responses in High Risk Subjects

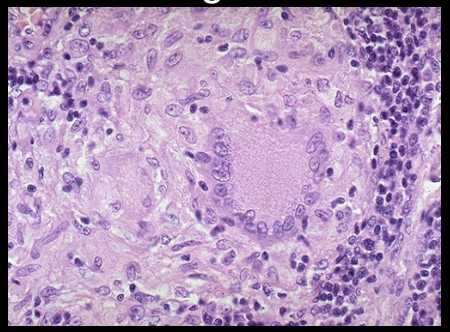


PAMPs Enhance IGRA Response

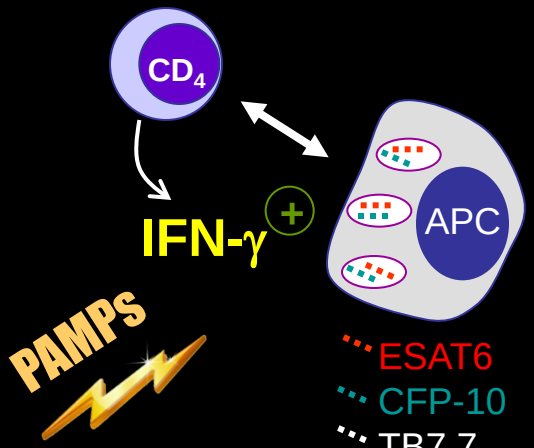
in vivo



Human granuloma



in vitro



ELISA

Reproducibility of QFT-IT in HCW

TABLE 1
Serial testing studies of interferon-gamma release assays in health care workers (HCWs) in low and intermediate incidence countries

Author (reference), year, country	Duration between testing	Conversion, n/N (%)		IGRA reversions*, n/N (%)
		Tuberculin skin test	IGRA*	
Joshi et al (15), 2012, USA	2 to 30 days	N/A	N/A	18/45 (40)
Rafiza et al (16), 2012, Malaysia	1 year	N/A	69/703 (9.8)	14/59 (23.7)
Fong et al (17), 2012, USA	1 year or 1 to 6 months for repeat of positive IGRA	N/A	52/1857 (2.8)	8/10 (80)†
Torres Costa et al (18), 2011, Portugal	1 year	61/199 (30.7)	51 /462 (11)	
Schablon et al (19), 2010, Germany	High-risk HCWs tested annually, all others evaluated every other year	Reversion rates: 4/188 (2.1)	15/245 (6.1)	46/208 (22.1)
Ringshausen et al (20), 2010, Germany	18 weeks	N/A	3/162 (1.9)	13/42 (32.6)
Park et al (21), 2010, South Korea	1 year	N/A	14/244 (5.7)	6/18 (33.3)
Lee et al (22), 2009, South Korea	1 year	N/A	21/146 (14.4)	N/A
Chee et al (23), 2009, Singapore	1 year	16/75 (21.3)	9/182 (4.9)	N/A
Yoshiyama et al (24), 2009, Japan	2 and 4 years	0/18 (Note: denominator includes only baseline concordant positives)	5/277 (1.8)	N/A
Pollock et al (25), 2008, USA	1 to 7 months	N/A	2/43 (4.6). Selected HCWs at 'increased risk' and negative at baseline	13/32 (41)

**All conversions/reversions using simple negative/positive; †Testing was performed among individuals with positive QuantiFERON-TB (Cellestis Ltd, Australia) results close to the cut-off point. IGRA Interferon-gamma release assay; N/A Not available*

Coversions 2% to 15%
Reversions 20 to 40%

Pai and Elwood Can Respir J. 2012

QFT-GIT Assay Standardization

Standardized

- Pre-analytical
  - Blood collection*
  - 37 ºC incubation
  - Plasma separation
- Analytical
  - ELISA
  - Interpretation

Not Standardized

- Pre-analytical
  - Skin preparation
  - Incubation delay
  - Incubation duration
  - Time of day
  - Day of month
  - Season
  - Diet
  - Infection
  - Antibiotics

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