

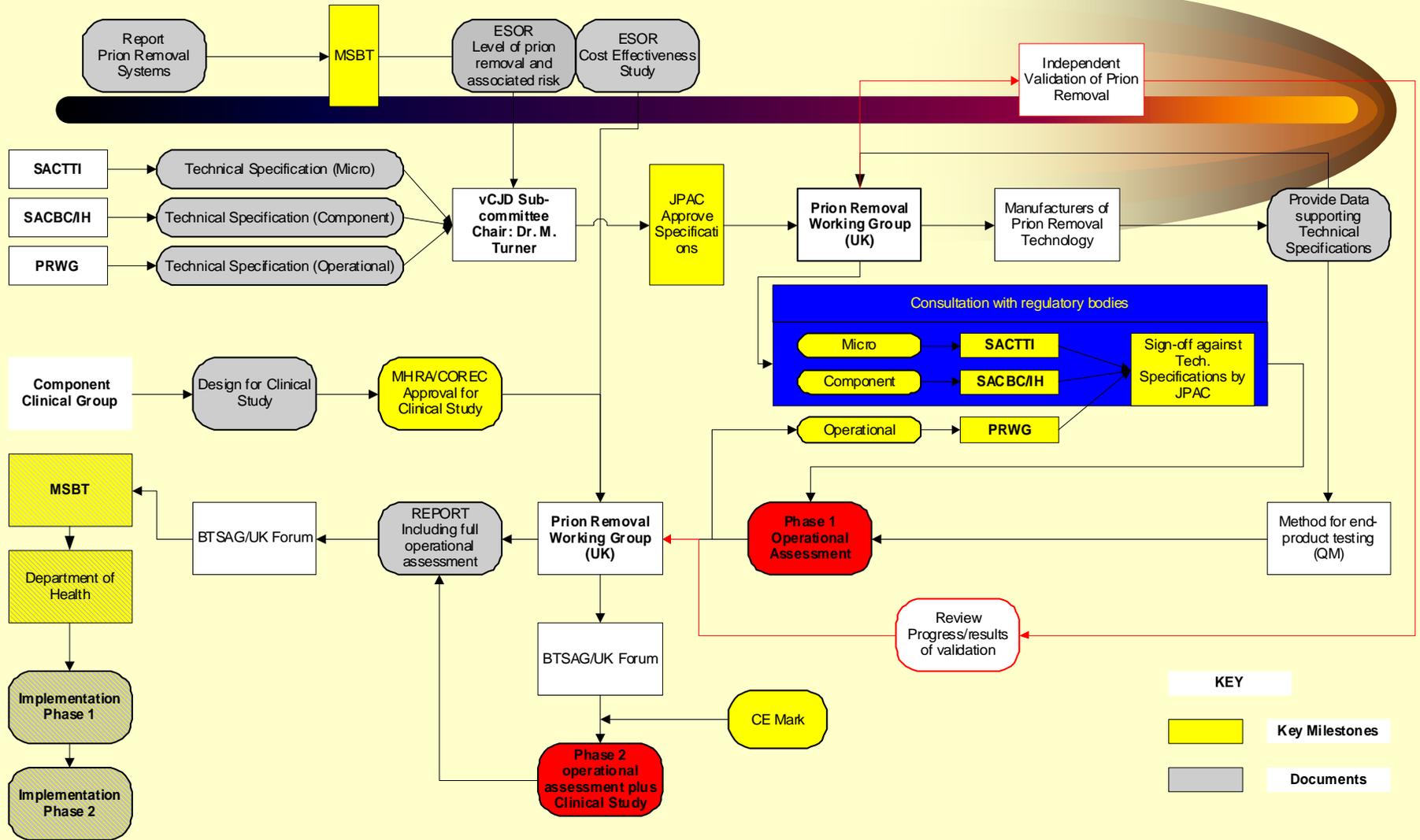
Evaluation of Prion Reduction Filters.



UK + Irish Blood Services Prion Removal Working Group

- To be the primary point of contact within UKBS for manufacturers developing prion removal technology.
- To provide expertise and advice to manufacturers on the laboratory and clinical development requirements for prion removal systems.
- To liaise with manufacturers regarding in-house operational evaluations.
- To liaise with JPAC and SAC's on all matters regarding approval of prion removal systems for UKBS use.
- To ensure that appropriate decision-making bodies are kept apprised of the technology.

Prion Removal Systems
Approval/Assessment of suitability
DRAFT 3rd December 2004 Version 5



How great a reduction in infectivity is needed to be clinically useful?

- Assumes red cell concentrates in OAS, with prior LD and 10-30 ml residual plasma
- Assumes that total residual infectivity >2 ID₅₀/whole unit will transmit for certain
- Assumes prion removal mainly from plasma

Prion reduction filters

Infectivity ID	residual leucocytes	residual plasma	Total
LD alone	0.2	130	130.2
1 log	0.2	13	13.2
2 log	0.2	1.3	1.5
3 log	0.2	0.13	0.33
4 log	0.2	0.013	0.21

Prion reduction filters

- 1-2 logs - of limited value
- 3 logs - 75-90% reduction in incidence of secondary transmission
- further reduction in residual plasma could augment reduction in infectivity and incidence of secondary transmission
- any further affect on cell-associated infectivity could be of significant additional benefit.

Prion reduction specification

- 3 log reduction by spiking to be demonstrated by Western blot and bioassay.
- reduction in endogenous infectivity up to limit of model which must be capable of demonstrating at least a 1 log reduction - demonstrated by Western blot and bioassay
- Process variables (4°C and ambient temp) by Western blot once validated
- Companies have been asked to propose surrogate markers for process monitoring.

In-process quality monitoring - what would be a suitable marker?

- Direct measurement of infectivity levels not possible.
- Need to demonstrate parallelism in reduction/removal of surrogate marker across prion removal filter
- Possible surrogates: Factor IX, PrP^c
- Challenging because so little plasma in SAGM red cells

Component quality specification

- In vitro as per UK Guidelines to day 42
- In vivo- volunteer red cell survival studies using radio-chromium-recovery must be 75% at 24 hours -also red cell survival
- Red cell membrane changes
 - expression of common red cell antigens
 - alteration in band 3 protein
 - CD47 expression
 - interaction with large panel of normal sera / plasmas

Independent evaluation study

- Requested by SEAC, MSBTO and UK Blood Service.s
- provide independent substantiation of some of the key data provided by the companies
- where possible extend that data to more clinically informative models
- Initially probable spiking studies with
 - 263K brain homogenate / microsomal / sonicated assessed by Western blot and bioassay
 - 301V spleen assessed by Western blot and bioassay.
 - Endogenous infectivity studies
- Trade off between comprehensiveness and time-lines

Clinical studies



- Primary aim of clinical studies is to look for adverse events and immune responses
- Study 0: exposure of patients to 1,2,3 units
- Study 1: complex cardiac surgery- 300 patients, all receiving PRF-treated RCC.
- Study 2: transfusion dependent (probably MDS) randomised: PRF-treated vs control RCC - 150 in each arm.